Journal of Food Protection, Vol. 69, No. 4, 2006, Pages 712–718

Growth of *Salmonella* Enteritidis Phage Type 30 in Almond Hull and Shell Slurries and Survival in Drying Almond Hulls

AARON R. UESUGI AND LINDA J. HARRIS*

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

MS 05-462: Received 20 September 2005/Accepted 20 November 2005

ABSTRACT

Traceback investigation of a 2000 to 2001 outbreak of salmonellosis associated with consumption of raw almonds led to isolation of the outbreak strain *Salmonella enterica* serovar Enteritidis phage type (PT) 30 on three geographically linked almond farms. Interviews with these growers revealed that significant rain fell during the 2000 harvest when many almonds were drying on the ground. The objectives of this study were to document weather conditions during the 2000 harvest, determine the potential for growth of *Salmonella* Enteritidis PT 30 in hull or shell slurries, and evaluate survival of *Salmonella* Enteritidis PT 30 on wet almond hulls during drying. Dry almond hulls and in-shell kernels wetted for 24 h increased in weight by 250 to 300% and 100%, respectively. Both hull and shell slurries supported rapid growth of *Salmonella* Enteritidis PT 30 at 24°C; slurries containing hulls also supported growth at 15°C. Maximum *Salmonella* Enteritidis PT 30 concentrations of 6.2 and 7.8 log CFU/ml were observed at 15 and 24°C, respectively. *Salmonella* Enteritidis PT 30 grown in wet hulls that were incubated at 24°C survived drying at either 15 or 37°C. Reductions of 1 to 3 log CFU/g of dry hull were observed during drying; reductions generally declined as incubation time increased from 2 to 7 days. Evaluation of shipping records revealed that approximately 60% of outbreak-associated almonds had not been exposed to rain, eliminating this factor as the sole cause of the outbreak. However, the data provide evidence that wet almonds may be a greater risk for high concentrations of *Salmonella*, and specific guidelines should be established for harvesting and processing almonds that have been exposed to rain or other water sources.

Tree nuts have rarely been associated with outbreaks of foodborne illness and are generally considered low-risk foods because they are consumed in a dried state. Target water activity for stored tree nuts is usually <0.65 (approximately 6% moisture) *(6).* This level of water activity eliminates growth of bacteria and fungi, including *Aspergillus flavus* and *Aspergillus parasiticus,* which produce aflatoxin.

Almond harvest occurs after the kernels have reached maturation and the hulls begin to split and dry. Mechanized shakers are used to drop the almonds to the orchard floor, where they are left to dry. Although hand ''knocking'' the almonds onto tarps was a common practice 40 years ago, it is used today only in very young or small orchards or when mechanical shakers cannot access the trees. Drying times on the ground depend on the moisture content of the nuts and may be 1 to 2 weeks when the crop is harvested early or 4 to 7 days when the drying process has begun on the trees *(12).* An optimum hull moisture of 8 to 12% ensures efficient hulling *(15).* After the drying period, the almonds are swept from around the trees and piled into windrows between the rows of trees. The windrows are mechanically collected from the orchard floor, and pressurized air is used to remove light debris. Almonds may be temporarily stored in stockpiles on the farm or at the hulling-shelling facilities. Stockpiles are sometimes covered with plastic for fumigation with phosphine to control insect damage *(7).* The hulls or both the hulls and shells are removed through a series of sheer rollers prior to sending the in-shell almonds or kernels to an almond processor. The high soluble sugar content of almond hulls make them an excellent nutrient source for animals, and the majority of the hulls produced in California are sold for dairy cattle feed *(1).* Shells are sold primarily for use as livestock bedding, artificial fire logs, or other energy sources.

Once almonds are shaken to the ground, they are usually protected from water sources so that they can dry efficiently. Although rain is relatively uncommon, it may fall during the harvest period, resulting in some rehydration of almonds on the ground. Before harvest, some almonds may drop prematurely from the trees and become soaked with irrigation water.

From late fall 2000 to spring 2001, a salmonellosis outbreak occurred that was associated with the consumption of raw almonds *(5).* Almonds had not previously been reported as a source of salmonellosis. *Salmonella enterica* serovar Enteritidis phage type 30 (PT 30), a rare phage type unknown in the United States, was identified as the outbreak strain. *Salmonella* Enteritidis PT 30 had been isolated in other countries from chicken, frozen chicken livers, chicken feed, poultry sewage, raw liquid eggs, and human diarrhea *(4).* Traceback investigations of the almond outbreak 6 to 9 months after the outbreak-associated almonds were harvested led to isolation of *Salmonella* Enteritidis PT 30 from swabs of equipment surfaces at both the processor

* Author for correspondence. Tel: 530-754-9485; Fax: 530-752-4759; E-mail: ljharris@ucdavis.edu.

and huller-sheller and from swabs of the floor of the almond orchards. Subsequent interviews with the growers revealed that two separate and significant rainfall incidents occurred in October 2000 when shaken or windrowed almonds were on the orchard floor. Because harvesting equipment could not enter the orchards while the predominantly Cerini clay loam soil was wet, almonds were not collected for drying until 1 to 2 weeks after the first rainfall. A second rain event 2 weeks after the first further complicated the harvest. Growers indicated that the rained-upon almonds were dried in the field or in stockpiles with makeshift drying devices and in a few cases with commercial dryers.

A better understanding of the effect of environmental conditions and almond handling practices during harvest on the growth and survival of *Salmonella* Enteritidis PT 30 is essential for understanding the possible causes of the 2000 to 2001 outbreak and for developing research-based and almond-specific good agricultural practices *(16).* Thus, the objectives of this study were to (i) document the weather and orchard conditions of the year 2000 almond season at the outbreak-associated farms, (ii) determine the potential for growth of *Salmonella* in almond hulls and/or shell slurries, and (iii) determine the ability of *Salmonella* Enteritidis PT 30 to survive during drying of water-saturated hulls.

MATERIALS AND METHODS

Weather data documentation. Precipitation data, soil and air temperatures, and storm report data collected from weather stations in regions near the outbreak-associated farms were accessed online from the California Irrigation Management Information System (CIMIS) *(3)* and the National Climatic Data Center (NCDC) of the National Oceanic and Atmospheric Administration (NOAA) *(10).* CIMIS data (precipitation and soil and air temperatures) for the year 2000 were accessed for station 124 Panoche, located in Fresno County in the San Joaquin Valley region, which has been active since 27 July 1995 *(3).* NCDC data (precipitation and storm reports) for 2000 were accessed for station Panoche 2 W, which is located in San Benito County and has been active since 1 November 1949 *(10).* These two stations were the stations closest (within 30 km) to the farms associated with the outbreak that could provide the desired statistical information.

Almonds. Outbreak-associated almond varieties were primarily Carmel and Monterey. Nonpareil almonds represent approximately 36% of the annual crop in California. Therefore, these three varieties were selected for use in this study. Whole unhulled Carmel, Monterey, and Nonpareil almonds were collected from University of California, Davis, trial orchards by Dr. Bruce Lampinen (Department of Pomology, University of California, Davis). Hulls were removed by hand to produce in-shell kernels. Additional Nonpareil almond hulls and shells (without kernels) were collected from a huller-sheller facility. Samples were stored at ambient temperature (24 ± 2°C) in sealed plastic bags (30.5 by 30.5 cm; Com-Pac Int., Carbondale, Ill.).

Determination of water uptake by whole almond hulls and in-shell kernels. Whole unbroken Carmel, Monterey, and Nonpareil almond hulls and in-shell kernels were individually weighed. Each in-shell kernel was visually inspected for an intact shell and the absence of any visible cracks. Each whole hull or in-shell kernel was placed into a separate glass beaker, deionized water was added to cover, and samples were allowed to soak; hulls were soaked for 0.5, 1, 2, 3, 8, or 24 h, and in-shell kernels were soaked for 1 to 2 h or from 1 to 7 days. After the appropriate time interval, the hull or in-shell kernel was removed from the water and shaken vigorously over a paper towel until no new water droplets could be seen on the paper towel. The sample was weighed to determine the water weight gain. Each in-shell kernel was then opened to visually determine whether the inner shell and/or kernel were wet.

Culture and growth conditions. A stepwise exposure procedure *(11)* was used to isolate separate rifampin- and nalidixic acid–resistant variants (*Salmonella* Enteritidis PT 30 Rif^r and *Salmonella* Enteritidis PT 30 Nal^r, respectively), which are capable of growing in the presence of 80 μg/ml rifampin (Sigma, St. Louis, Mo.) or 50 μg/ml nalidixic acid (Sigma), respectively, from *Salmonella* Enteritidis PT 30 (LJH 608, ATCC BAA-1045) isolated from recalled 2000 to 2001 outbreak-associated almonds. Isolates were stored at −80°C in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) supplemented with either 80 μg/ml rifampin (TSBR) or 50 μg/ml nalidixic acid (TSBN) and 15% glycerol (Fisher, Fair Lawn, N.J.). Prior to each experiment, *Salmonella* Enteritidis PT 30 Rif^r and *Salmonella* Enteritidis PT 30 Nal^r were streaked onto tryptic soy agar (TSA; tryptic soy broth plus 1.5% granulated agar; Difco, Becton Dickinson) supplemented with 80 μg/ml rifampin (TSAR) or 50 μg/ml nalidixic acid (TSAN) and incubated at 37°C for 24 h. An isolated colony was transferred to TSBR or TSBN, respectively, and incubated at 37°C for 24 h. At two consecutive 24-h intervals, a single loopful of the culture was transferred into fresh medium.

Inoculation of almond hull and shell slurries with *Salmonella* Enteritidis PT 30 Rif^r. *Salmonella* Enteritidis PT 30 Rif^r cells were collected from the overnight culture in TSBR by centrifuging 1 ml of the broth (10,000 rpm for 2 min; Microcentrifuge 5417, Eppendorf, Westbury, N.Y.). The broth was decanted, and the cells were suspended in 0.5 ml of 0.1% Bacto peptone (Difco, Becton Dickinson) to give an inoculum of approximately 10^9 CFU/ml. The inoculum concentration was confirmed by making serial 10-fold dilutions in 0.1% peptone and plating the inoculum onto TSAR and bismuth sulfite agar (BSA; Difco, Becton Dickinson) or BSA supplemented with 80 μg/ml rifampin (BSAR). The inoculum was diluted 10,000-fold in 0.1% peptone to give a final concentration of 10^5 CFU/ml for inoculating the hull and shell slurries.

Nonpareil almond hulls and shells obtained from a huller-sheller facility (the only variety available at the initiation of the experiment) were separated and ground in a mill to a particle size of less than 0.624 cm. The ground hulls and shells were transferred to separate plastic bags, which were sealed and stored at ambient temperature (24 ± 2°C) for up to 12 months. Samples of ground hulls (5.0 ± 0.1 g), ground shells (5.0 ± 0.1 g), or ground hulls and shells (2.5 ± 0.1 g of each) were weighed into 50-ml conical centrifuge tubes (BD Biosciences, Bedford, Mass.). Sterile deionized water (25 ml) was added to each tube to form a slurry, and then 100 μl of the prepared inoculum (10^5 CFU/ml *Salmonella* Enteritidis PT 30 Rif^r) was added. The tubes were sealed, shaken by hand 50 times each, and held for up to 96 h at 24 ± 2°C or 15 ± 2°C.

Inoculation of water-soaked almond hulls with *Salmonella* Enteritidis PT 30 Nal^r. A second consecutive 24-h TSBN culture of *Salmonella* Enteritidis PT 30 Nal^r (approximately 10^9 CFU/ml) was used for inoculation. The inoculum concentration was confirmed by serial dilution with Butterfield's phosphate buffer (BPB) and plating onto both TSAN and BSA supplemented

with 50 μg/ml nalidixic acid (BSAN). For each inoculation sample, Carmel half-hull pieces (140) of approximately the same size and weight and 200 μl of the inoculum were added to 2 liters of sterile deionized water in a 4-liter Erlenmeyer flask. The flask was then placed on a shaker (Labline, Barnstead International, Dubuque, Iowa) and rotated at 150 rpm for 2 to 7 days. Beginning on day 2, 18 randomly selected hulls were removed daily from the flask. Half of these hulls were immediately sampled for recovery of *Salmonella* Enteritidis PT 30 Nal^r (0 h), and the other half were spread onto a single piece (46 by 57 cm) of 3-mm chromatography paper (Whatman, Clifton, N.J.) folded in half, which was placed on a metal drying rack set on a metal baking sheet. The inoculated hulls were dried at 15°C (to simulate field conditions) or 37°C to simulate a dryer; recovery of *Salmonella* Enteritidis PT 30 Nal^r was determined after 24 h of drying.

Recovery of inoculated cells. Hull and/or shell slurries were vortexed after the 96-h storage period, and 10-fold serial dilutions were made in 0.1% peptone. Samples were spread plated in duplicate (100 μl) with the Autoplater 4000 Spiral Plater (Spiral Biotech, Bethesda, Md.) or spot plated in duplicate (20 μl) onto TSA, TSAR, BSA, and BSAR. Plates were incubated at 37°C for 24 h (spiral plates) or 18 h (spot plates), and colonies were counted by hand. Counts on TSA and TSAR included all colonies regardless of appearance, but counts on BSA and BSAR included only those colonies that were typical of *Salmonella*, those with a metallic sheen and black center and surrounded by a black precipitate.

For recovery from inoculated hulls, samples consisting of three hulls were combined with 30 ml of BPB in a Whirl-Pak filter stomacher bag (19 by 30 cm; Nasco, Modesto, Calif.) and pummeled in a stomacher (Stomacher 400, Seward, Thetford, UK) for 120 s at normal speed. After stomaching, 10-fold serial dilutions were made in BPB. Samples were spot plated in duplicate (20 μl) onto TSAN and BSAN and incubated at 37°C for 18 h. Colonies were counted by hand after incubation and reported as the mean CFU per hull of duplicate plates from six three-hull samples at each time point.

Confirmation. The identities of two colonies per sample on BSA were confirmed by streaking a typical colony onto fresh BSA and incubating at 37°C for 48 h. Typical *Salmonella* colonies were inoculated onto triple sugar iron (TSI; Difco, Becton Dickinson) and lysine iron agar (LIA; Difco, Becton Dickinson) and incubated at 37°C for 24 h.

Statistics. Data were subjected to analysis of variance and Duncan's multiple range tests by statistical analysis software (SAS version 8, SAS Institute, Cary, N.C.). Differences between mean values were considered significant at $P < 0.05$.

RESULTS

Weather analysis for 2000. The average annual rainfall recorded by the Panoche 2 W weather station since 1949 is 230.11 mm; the recorded rainfall for 2000 was 249.16 mm, an 8.28% increase above normal. Although the total rainfall for 2000 did not differ greatly from annual figures, the monthly distribution was significantly different. In a typical year, 10.16, 31.49, and 35.05 mm of rainfall are recorded in October, November, and December, respectively, but in 2000, 40.13, 5.84, and 6.86 mm of precipitation were recorded for those months, respectively, which was a 295% increase above the average rainfall for October. In October 2000, the rain came from two major weather systems on 10 to 12 October (27.2 mm) and 26 to 30 October (21.9 mm), as recorded by CIMIS at the Panoche 124 weather station.

The NOAA October 2000 *Storm Data Report and Unusual Weather Phenomena (10)* noted heavy thunderstorms in the region of interest on 10 to 12 October that moved from the west side of the San Joaquin Valley to the northeastern foothills of the southern Sierra Nevada, causing heavy crop damage from rain and hail. The second major weather system (26 to 30 October) did not cause notable property or crop damage.

The average air temperatures (and range) recorded by the Panoche 124 weather station in 2000 were 15.9 ± 3.0°C (3.9 to 32.7°C) for October and 8.6 ± 2.3°C (−1.6 to 22.6°C) for November. These air temperatures were lower than normal overall average air temperatures for these months, which are 16.8 ± 1.3 and 11.5 ± 1.5°C, respectively. The soil temperature, measured at 15 cm below the soil surface under irrigated grass, was reported as 17.8 ± 1.9 and 11.8 ± 1.6°C for October and November, respectively. Cover crops were not used in the outbreak-associated almond orchards. The average daily soil temperature between the two October storms was 14 to 17°C, and the average daily air temperature was between 10 and 17°C, with daily highs ranging from 17 to 28°C.

Water uptake by whole almond hulls and in-shell kernels. Whole almond hulls placed in water quickly absorbed moisture. Although lower water uptake was observed in Nonpareil almond hulls, especially during the first 3 h of soaking, hulls of all almond varieties reached maximum water uptake (250 to 300%) within approximately 8 h of soaking (Fig. 1a). Little difference in water uptake was observed among in-shell kernels of all varieties. In-shell Carmel, Monterey, and Nonpareil kernels gained about half their weight in water during the first hour of soaking, and all reached their maximum uptake (more than 100%) within 3 days (Fig. 1b). The in-shell kernels initially floated when placed in water and remained floating over the 7 days of the experiment.

The in-shell kernels were opened after soaking to visually note the degree of water migration. After 1 h of soaking, the inner shell surfaces of all samples ($n = 6$) from all three varieties were visibly wet, and the wet areas of Carmel, Nonpareil, and Monterey shells were estimated to cover 50, 75, and 25% of the inner surface, respectively. After 2 h of soaking, the wet inner surface area had increased to 100% for Carmel and Nonpareil shells and an estimated 75% for Monterey shells. After 24 h of soaking, 100% of the shell interior was visibly wet for all in-shell kernels examined ($n = 42$ over the 7 days). For all three varieties, the wetting appeared to radiate from the shell end opposite the peduncle (the point where the shell attaches to the hull at the stem end), but wetting was not consistent along the suture line of the shell.

Kernels removed from wet almond shells were much darker than kernels from dry shells. The same darkening of the pellicle (skin) occurs when kernels are immersed in wa-

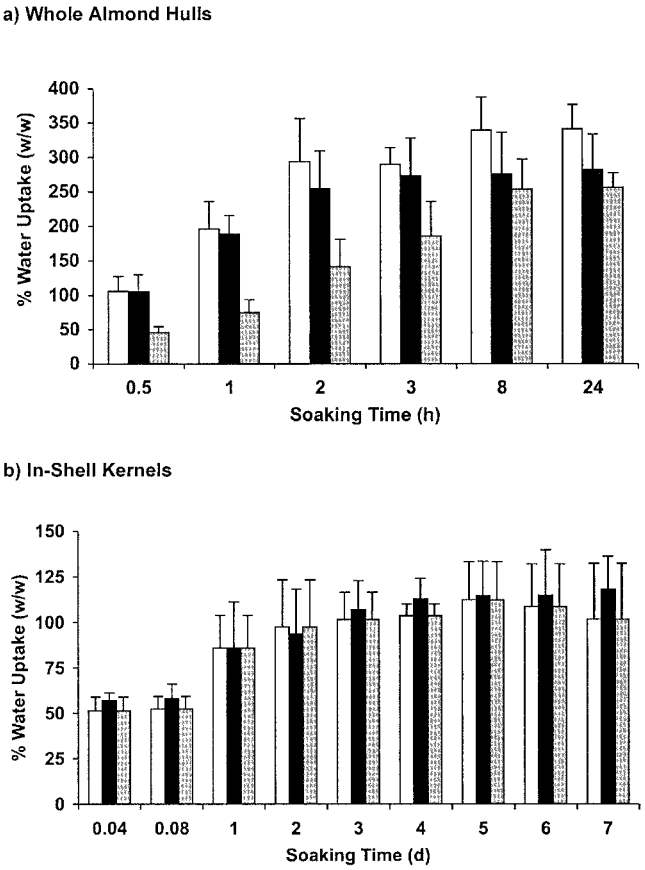

FIGURE 1. *Water uptake (wt/wt) during soaking of Carmel (white), Monterey (black), and Nonpareil (gray) almonds: (a) whole almond hulls over 24 h and (b) in-shell kernels over 7 days. Values are the average of triplicate samples from each of two experiments (*n = 6*).*

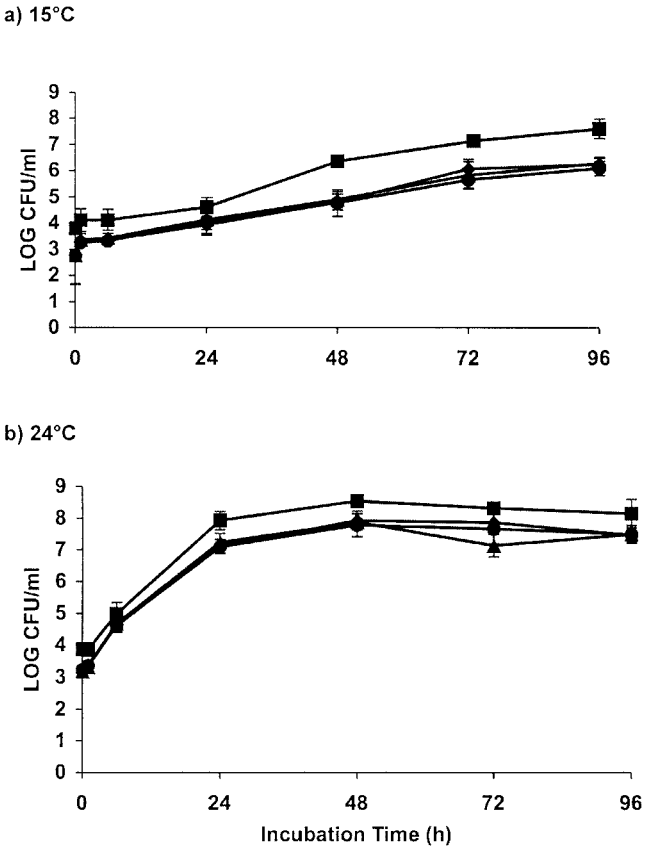

FIGURE 2. *Growth of* Salmonella *Enteritidis PT 30 Rif*^{r} *in slurries of Nonpareil almond hulls inoculated at approximately* 10^3 *CFU/ml, stored at 15°C (a) or 24°C (b), and plated onto TSA (■), TSAR (●), BSA (▲), and BSAR (♦). Values are the average of triplicate samples from each of two experiments (*n = 6*).*

ter. In some instances there were visible water droplets on the surface of the kernel.

Growth of *Salmonella* Enteritidis PT 30 compared with that of nalidixic acid– and rifampin-resistant variants. Growth of *Salmonella* Enteritidis PT 30, *Salmonella* Enteritidis PT 30 Rif^r, and *Salmonella* Enteritidis PT 30 Nal^r in TSB was followed over 48 h. Growth curves at 37°C were similar for both antibiotic-resistant variants and the parent strain (data not shown). Both antibiotics were equally effective in suppressing the background microbiota found on almonds. *Salmonella* Enteritidis PT 30 Rif^r was used in early experiments with hull and shell slurries; however, a switch was later made to *Salmonella* Enteritidis PT 30 Nal^r for consistency with other experiments ongoing in the laboratory.

Growth of *Salmonella* Enteritidis PT 30 Rif^r in almond hull and shell slurries. Colony counts made on TSAR, BSA, and BSAR, were not significantly different for any test parameter ($P > 0.05$). Nonpareil hull slurries provided an excellent environment for *Salmonella* Enteritidis PT 30 to multiply. Concentrations of *Salmonella* Enteritidis PT 30 increased slowly but significantly ($P < 0.05$) at 15°C to 6.2 log CFU/ml after 96 h (Fig. 2a). At 24°C, concentrations increased significantly within 6 h ($P < 0.05$) to a maximum of 7.8 log CFU/ml within 48 h (Fig. 2b).

Shell slurries did not support the growth of *Salmonella* Enteritidis PT 30 at 15°C; initial and 96-h concentrations were not significantly different ($P > 0.05$) on BSAR (Fig. 3a). At 24°C, however, significantly higher counts ($P < 0.05$) were obtained after 24 h with a maximum concentration of 6.7 log CFU/ml achieved after 48 h (Fig. 3b). Growth of *Salmonella* Enteritidis PT 30 in combined hull and shell slurries was between that observed in slurries of either hulls or shells alone (data not shown).

Data from BSAR were used to directly compare the growth of *Salmonella* Enteritidis PT 30 in hull, shell, and hull plus shell slurries. After 96 h of incubation at 15°C, concentrations in hull-only, hull plus shell, and shell-only slurries were all significantly different ($P < 0.05$). At 24°C, concentrations in hull-only slurries were significantly greater ($P < 0.05$) than those in either hull plus shell or shell-only slurries from 6 to 48 h of incubation. After 72 h, concentrations in hull-only and hull plus shell slurries were not significantly different ($P > 0.05$).

Total aerobic bacterial counts on TSA were approximately 1 to 2 and 0.5 log units higher at all time points than counts on TSAR, BSA, and BSAR at 15 and 24°C, respectively (Figs. 2 and 3). Colony morphology on TSA was highly variable compared with the uniform colony de-

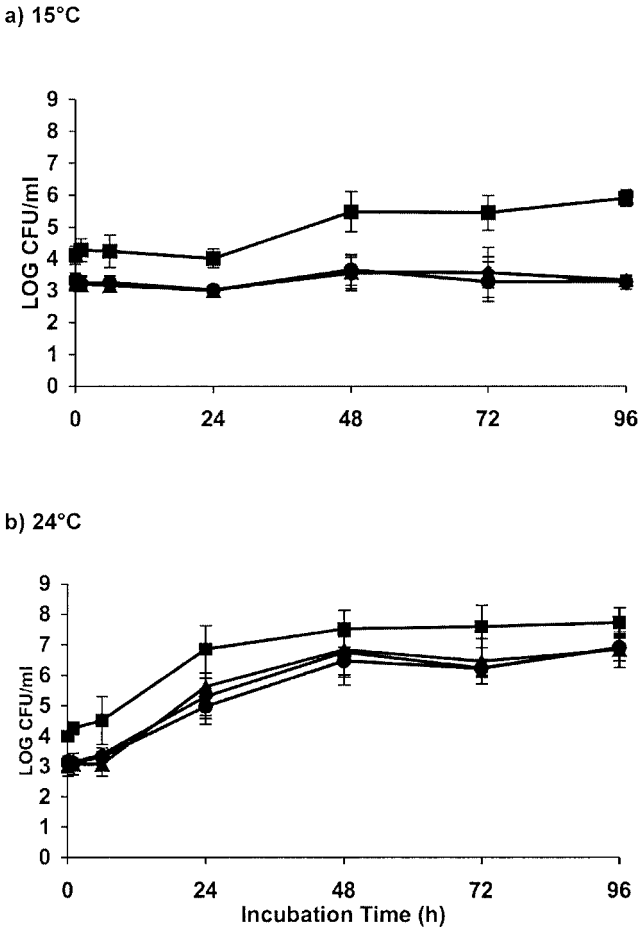

FIGURE 3. *Growth of* Salmonella *Enteritidis PT 30 Rif*^{r} *in slurries of Nonpareil almond shells inoculated at approximately* 10^3 *CFU/ml, stored at 15°C (a) or 24°C (b), and plated onto TSA (■), TSAR (●), BSA (▲), and BSAR (♦). Values are the average of triplicate samples from each of two experiments (*n = 6*).*

velopment on TSAR, and this variability was attributed to the background microbiota from the hulls and shells. All colonies selected from BSA plates produced reactions typical for *Salmonella* on TSI (red slant, yellow butt, with blackening of agar) and LIA (slant with purple and black butt).

Survival of *Salmonella* Enteritidis PT 30 Nal^r on almond hulls during drying. When almonds become wet during harvest, they may be left in the orchard to dry naturally or may be dried with commercial dryers, a considerably more expensive and less desirable option.

When dry hulls were weighed, wetted, and dried, a substantial change in appearance and a significant reduction in dry weight was observed (approximately 40%, data not shown), presumably from a loss in soluble solids. Therefore, concentrations of *Salmonella* Enteritidis PT 30 Nal^r were calculated on a per hull basis to enable a direct comparison of wet and dry samples.

With two exceptions (4- and 5-day-old hulls 24 h after drying at 15°C), there was no significant difference ($P > 0.05$) between colony counts on TSAN and those on BSAN at any time point. When *Salmonella* Enteritidis PT 30 Nal^r was incubated in solution with Carmel hulls, concentrations in the wet hulls grew to 7.8 to 8.3 log CFU per hull within the first 48 h and remained at these concentrations through day 7 (Table 1). The populations in the liquid surrounding the hulls (8 log CFU/ml) were similar to those on the hulls (8 log CFU per hull) prior to drying (data not shown). Significant reductions in the concentration of *Salmonella* Enteritidis PT 30 Nal^r during drying were observed at every time point regardless of whether the hulls were dried at 15 or 37°C. However, the degree of reduction generally declined as the incubation time increased from 2 to 7 days.

TABLE 1. *Recovery of* Salmonella *Enteritidis PT 30 Nal*^{r} *from inoculated water-soaked Carmel almond hulls after drying at 15 or 37°C and plating onto TSAN and BSAN*[a]

Drying temp (°C)[b]	Age of hull mixture (days)	*Salmonella* (log CFU/hull)					
		TSAN			BSAN		
		Initial (0 h)	After drying (24 h)	Δ[c]	Initial (0 h)	After drying (24 h)	Δ
15	2	8.3 ± 0.7	5.3 ± 1.0	3.0	8.3 ± 0.7	5.4 ± 1.1	2.9
	3	7.7 ± 0.3	5.0 ± 1.5	2.7	7.8 ± 0.2	5.4 ± 1.3	2.4
	4	7.8 ± 0.3	6.7 ± 0.1	1.1	7.9 ± 0.3	5.6 ± 1.2	2.3
	5	7.7 ± 0.3	6.8 ± 0.0	1.0	7.6 ± 0.3	5.5 ± 1.2	2.1
	6	7.7 ± 0.5	6.1 ± 0.6	1.6	7.7 ± 0.4	6.2 ± 0.5	1.6
	7	7.4 ± 0.1	6.2 ± 0.9	1.2	7.5 ± 0.1	6.1 ± 0.8	1.3
37	2	7.8 ± 0.2	5.0 ± 0.1	2.7	7.8 ± 0.2	4.9 ± 0.1	2.9
	3	7.8 ± 0.1	5.3 ± 0.4	2.6	7.9 ± 0.1	5.3 ± 0.4	2.6
	4	8.1 ± 0.1	5.9 ± 0.1	2.1	8.1 ± 0.1	5.8 ± 0.2	2.3
	5	8.0 ± 0.1	6.2 ± 0.1	1.8	8.0 ± 0.2	6.2 ± 0.1	1.8
	6	7.8 ± 0.4	6.4 ± 0.1	1.4	7.8 ± 0.4	6.3 ± 0.2	1.5
	7	8.2 ± 0.1	6.8 ± 0.2	1.4	8.2 ± 0.1	6.8 ± 0.2	1.4

[a] TSAN, tryptic soy agar supplemented with 50 μg/ml nalidixic acid; BSAN, bismuth sulfite agar supplemented with 50 μg/ml nalidixic acid. Values are the mean ± standard deviation of triplicate samples from each of two experiments ($n = 6$).

[b] Drying experiments at 15 and 37°C were carried out at separate times with separate hull mixtures.

[c] Difference before and after drying.

DISCUSSION

A review of weather data confirmed grower descriptions of significant rainfall during the year 2000 almond harvest in the area where outbreak-associated almonds were grown. The potential for water uptake by a hull or shell is related to the initial degree of dryness. In this study, all hulls and in-shell almonds tested were considered completely dry, with moisture at <10 and <6%, respectively, and represented a worst-case scenario for water uptake. Hulls absorbed 250 to 300% of their weight in water after a few hours of soaking. Lower uptake would be expected for hulls with more initial moisture. High-moisture hulls would be more common in almonds that had dropped prematurely or in almonds harvested earlier in the season. Rainfall is more likely to occur later in the fall when almond hulls are generally dryer when they are shaken from the tree *(12).*

Water was observed on the inside of apparently intact shells, radiating primarily from a point opposite the peduncle but in some cases also along the suture line. Moisture also was evident on the kernel surface. These observations indicate that the shell, even if it appears to be intact, is porous enough to allow water to readily pass from the external surface to the internal surface. Further research is warranted to determine whether *Salmonella* can also pass through the intact shell to the kernel under wet conditions that may exist during harvest.

When almond hulls and shells were wetted in the presence of *Salmonella,* the organism multiplied. Growth occurred more rapidly and to higher concentrations when the organism was mixed with hulls rather than shells, and this difference was particularly notable at 15°C. Almond shells provide limited soluble sugar (0.35%), fat (0.41%), protein (1.0%), and ash (0.69%) on a dry-weight basis *(14)* compared with hulls, which have higher concentrations of soluble sugar (26.55%), fat (3.34%), protein (2.7%), and ash (6.09%) *(13).* The soluble sugars found in hulls are mainly sucrose (40%), glucose (23%), and fructose (17%).

Average daily air temperatures in the almond orchards between 12 and 26 October 2000 ranged from 10 to 17°C with highs between 17 and 28°C. Almonds were reportedly left in the orchard for 7 or more days after it rained. *Salmonella* Enteritidis PT 30 was detected in the orchard soil in 2001 *(5),* and this organism probably was in the orchard in 2000. Sweepers were used to windrow the almonds before it rained, which would have resulted in some mixing and redistribution of the orchard floor. Under the harvest conditions described, there was ample time for significant increases in concentrations of *Salmonella* Enteritidis PT 30 on and around the wet almonds. King et al. *(8)* found that the amount of soil mixed with harvested almonds influenced the total aerobic bacterial plate counts for the kernels; samples containing mud balls had significantly higher concentrations of bacteria than did samples without gross soil contamination. Mud balls are more likely to be present when the orchard soils are wet during harvest.

In addition to direct contamination in the field, kernels may be contaminated during the hulling and shelling processes. In most facilities, the hulls and shells are removed at the same time, resulting in considerable mixing of the kernels with both hulls and shells. The hulling and shelling processes generate large volumes of dust that can also contribute to contamination of exposed kernels *(9).*

Wet hulls must be dried to <14% moisture for efficient removal *(12).* When kernel moisture is above 14% and the kernel is exposed to holding temperatures greater than 49°C, a condition known as concealed damage will occur as a result of the Maillard reaction *(12).* Concealed damage is rust brown to black discoloration of the nutmeat and an unpalatable flavor that cannot be detected without cutting the nut open. Storage and drying of wet almonds at 31°C can prevent discoloration from occurring. Concealed damage was not reported in the outbreak-associated almonds, so the drying temperatures probably were not greater than 49°C. In the current study, *Salmonella* was able to survive in hulls dried at 15 and 37°C. A drying temperature of 15°C is representative of field conditions in late October 2000. A drying temperature of 37°C is slightly higher than the 31°C optimum but not completely unreasonable given the relatively uncontrolled drying mechanisms reportedly used by the growers.

After this research was complete, we gained access to almond shipment records indicating that almonds had been shipped to retail outlets approximately once per week from 13 October through 15 December 2000 (total of 80,859 kg) and from 16 February through 4 April 2001 (total of 54,431 kg) *(2).* Rainfall dates, shipment dates, and the estimated time to process and distribute the rain-exposed nuts (personal communication from processor) were compared. Evaluation of these records revealed that all shipments of almonds after 16 February 2001 would have included at least some rained-on almonds, but shipments prior to this date were harvested and processed before the October rainfall. Thus, although rain may have played a role in the almond salmonellosis outbreak it could not have been the only factor involved.

Almond producers, hullers-shellers, and processors should consider the increased risks of *Salmonella* contamination associated with significant wetting of almonds in the field. Almond-specific good agricultural practices should address wetting of prematurely dropped almonds with irrigation water and provide specific guidelines for harvesting rained-on almonds. Hullers-shellers and processors may want to consider segregating rained-upon almonds and increasing sanitation procedures after handling these almonds to avoid potential for cross-contamination. The higher risks associated with these nuts may also warrant their processing with a validated *Salmonella* kill step.

ACKNOWLEDGMENTS

These studies were funded by the Almond Board of California and by U.S. Department of Agriculture Cooperative State Research Education and Extension Service grant 2002-03886. We are thankful for the editing skills of Sylvia Yada, Michelle D. Danyluk, and Wen-Xian Du.

REFERENCES

1. Aguilar, A. A., N. E. Smith, and R. L. Baldwin. 1984. Nutritional value of almonds hulls for dairy cows. *J. Dairy Sci.* 67:97–103.

2. California Department of Health Services. 2002. Environmental investigation of *Salmonella* Enteritidis, phage type 30 outbreak associated with consumption of raw almonds. California Department of Health Services, Food and Drug Branch, Sacramento.
3. California Irrigation Management Information System. 2003. Home page. State of California, Department of Water Resources. Available at: http://www.cimis.water.ca.gov/. Accessed 5 December 2003.
4. Chart, H., L. Ward, and B. Rowe. 1991. Expression of lipopolysaccharide by phage types of *Salmonella enteritidis. Lett. Appl. Microbiol.* 13:39–41.
5. Isaacs, S., J. Aramini, B. Ceibin, J. Farrar, R. Ahmed, D. Middleton, M. Howes, E. Chan, A. U. Chandran, L. J. Harris, S. Pichette, K. Campbell, A. Gupta, L. Y. Lior, M. Pearce, C. Clark, F. Rodgers, F. Jamieson, I. Brophy, and A. Ellis. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *J. Food Prot.* 68:191–198.
6. Kader, A. A. 1996. In-plant storage, p. 274–277. *In* W. C. Micke (ed.), Almond production manual. Division of Agriculture and Natural Resources, University of California, Oakland.
7. Kader, A. A., and J. F. Thompson. 1992. Postharvesting handling systems: tree nuts, p. 253–259. *In* A. A. Kader (ed.), Postharvest technology of horticultural crops. Division of Agriculture and Natural Resources, University of California, Oakland.
8. King, A. D., Jr., M. J. Miller, and L. C. Eldridge. 1970. Almond harvesting, processing, and microbial flora. *Appl. Microbiol.* 20:208–214.
9. Kokal, D., and D. W. Thorpe. 1969. Occurrence of *Escherichia coli* in almonds of nonpareil variety. *Food Technol.* 23:93–98.
10. National Climatic Data Center. 2003. Home page. National Oceanic and Atmospheric Administration. Available at: http://www.ncdc.noaa.gov/oa/ncdc.html. Accessed 5 December 2003.
11. Parnell, T. L., L. J. Harris, and T. V. Suslow. 2005. Reducing *Salmonella* on cantaloupes and honeydew melons using wash practices applicable to postharvest handling, foodservice, and consumer preparation. *Int. J. Food Microbiol.* 99:59–70.
12. Reil, W., J. M. Labavitch, and D. Holmberg. 1996. Harvesting, p. 260–264. *In* W. C. Micke (ed.), Almond production manual. Division of Agriculture and Natural Resources, University of California, Oakland.
13. Saura-Calixto, F., and J. Cañellas. 1982. Chemical compositions of hulls on the sweet almond (*Prunus amygdalus*). *J. Sci. Food Agric.* 33:336–339.
14. Saura-Calixto, F., J. Cañellas, and J. Garcia-Raso. 1983. Contents of detergent-extracted dietary fibers and composition of hulls, shells, and teguments of almonds (*Prunus amygdalus*). *J. Agric. Food Chem.* 31:1255–1259.
15. Thompson, J. F., T. R. Rumsey, and J. H. Connell. 1996. Drying, hulling, and shelling, p. 268–273. *In* W. C. Micke (ed.), Almond production manual. Division of Agriculture and Natural Resources, University of California, Oakland.
16. U.S. Food and Drug Administration, U.S. Department of Agriculture, Centers for Disease Control and Prevention. 1998. Guide to minimize microbial food safety hazards for fresh fruits and vegetables. Available at: http://vm.cfsan.fda.gov/~dms/prodguid.html. Accessed 20 August 2005.