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Reduction of *Salmonella* Enteritidis Population Sizes on Almond Kernels with Infrared Heat

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ABSTRACT

Catalytic infrared (IR) heating was investigated to determine its effect on *Salmonella enterica* serovar Enteritidis population sizes on raw almond kernels. Using a double-sided catalytic IR heating system, a radiation intensity of 5,458 W/m^2 caused a fast temperature increase at the kernel surface and minimal temperature differences between the top and bottom kernel surfaces. Exposure of dry kernels to IR heat for 30, 35 and 45 s resulted in maximum kernel surface temperatures of 90, 102, and 113°C, and when followed by immediate cooling at room temperature, yielded a 0.63-, 1.03-, and 1.51-log reduction in *S. enterica* population sizes, respectively. The most efficacious decontamination treatment consisted of IR exposure, followed by holding of the kernels at warm temperature for 60 min, which effected a greater than 7.5-log reduction in *S. enterica* on the kernels. During that treatment, the kernel surface temperature rose to 109°C and gradually decreased to 80°C. Similar IR and holding treatments with lower maximum kernel surface temperatures of 104 and 100°C yielded reductions of 5.3 and 4.2 log CFU/g kernel, respectively. During these treatments, moisture loss from the kernels was minimal and did not exceed 1.06%. Macroscopic observations suggested that kernel quality was not compromised by the IR-holding combination treatment, as skin morphology, meat texture, and kernel color were indistinguishable from those of untreated kernels. Our studies indicate that IR heating technology is an effective dry pasteurization for raw almonds.

Outbreaks of salmonellosis have been associated with the consumption of whole raw almonds in the United States and Canada in 2000 to 2001 *(2, 8),* in the United States in 2004 *(1),* and in Sweden in 2005 to 2006 *(11). Salmonella enterica* has the ability to survive on dry almond kernels for prolonged periods of time *(17),* and few treatments are available for the decontamination of almond kernels that are consumed raw. Consequently, there is a great interest in the development of new technologies that can improve the microbial safety of almonds without compromising their quality as a raw product.

Propylene oxide is commonly used to reduce microbial populations on bulk almonds, and recently, has been shown to be an effective treatment against *Salmonella* contamination of almonds *(3).* However, a maximum residue limit has not been established for this fumigant by foreign countries. Therefore, propylene oxide is used at present solely for the decontamination of raw almonds destined to the U.S. domestic market. This constraint implies that there is a great need for decontamination treatments for raw almonds exported to foreign countries, of which California is the largest supplier. Chlorine dioxide is an effective alternative gas to propylene oxide for the reduction of *Salmonella* contamination on raw almonds, but it can lead to discoloration of the kernel surface at high concentrations *(19).* This side effect could be circumvented when treatment with 10 mg/liter chlorine dioxide for 10 min was carried out under vacuum conditions; this yielded a reduction of 4.5 log in *Salmonella* populations on kernels *(19).* Chemical treatments that have been investigated for eliminating *Salmonella* on raw almonds also include acidic solutions sprayed onto the kernel surface. When used in a combination of one or two sprays and storage for 3 or 1 day(s), respectively, or as three consecutive sprays, 10% citric acid provided a 5-log reduction in *Salmonella* populations on raw almonds *(15).* Although a significant reduction in *Salmonella* populations on raw kernels can be achieved also through heating with steam *(12),* this approach is energy intensive. Steam pasteurization increases moisture content of the kernels and therefore, requires additional processing to remove the excess moisture before storage.

To date, recorded outbreaks of salmonellosis linked to almonds in the United States have occurred only from consumption of raw almonds. Almond kernels that are treated with high heat for the production of roasted or blanched nuts do not appear to pose any risk of contamination with *Salmonella (1).* In this study, we assessed the feasibility of using double-sided infrared (IR) heating for dry pasteurization of raw almond kernels. We determined the effect of IR radiation on the heating rate of the raw kernels, on the reduction of *Salmonella* Enteritidis population sizes on inoculated kernels, and on the macroscopic quality of this treated raw food product.

MATERIALS AND METHODS

Almonds. Raw almond kernels of the variety Nonpareil (size 27-30: 27 to 30 kernels per 28 g) were obtained from the Almond

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Board of California (Modesto). The inoculated kernels were stored at a moisture content of 5% prior to heat treatment. Raw kernels processed in the almond industry have a standard moisture content of 4.5 to 5.5% *(9).*

Strains and culture conditions. *S. enterica* serovar Enteritidis PT 30 (SEPT30, LJH 608, ATCC BAA-1045) was isolated from recalled almonds associated with a salmonellosis outbreak in 2000 to 2001, and described previously by Uesugi and Harris *(18).* All known outbreaks of salmonellosis that were linked to raw almonds grown in California were caused by *S. enterica* Enteritidis *(1, 2, 8, 11).* The Almond Board of California has requested that strain SEPT30 be used in all studies funded by its organization in order to standardize research protocols, thus allowing for a better comparison of the efficacy of sanitization methods under investigation.

A nalidixic acid–resistant mutant of SEPT30 was obtained by streaking the wild type onto Luria-Bertani agar containing 50 μg/ml nalidixic acid (Sigma-Aldrich, St. Louis, Mo.). This nearly isogenic mutant was named ''SEPT30N'' and used throughout our studies. SEPT30N survived at the same rate as the wild-type strain during postinoculation drying of the kernels, during dry storage of the inoculated kernels at 12°C, and to 45-s exposure to IR treatment, indicating that it had resistance to desiccation and heat stress similar to that of the wild type.

Inoculation of the almond kernels was performed with agar-grown cells according to the method developed by Danyluk et al. *(3),* with the exception that nalidixic acid was added at 50 μg/ml to all culture media mentioned below. Briefly, strain SEPT30N was grown overnight in tryptic soy broth, and 1 ml of the culture was spread onto each of three tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, Md.) plates. The plates were incubated for 24 h at 37°C to produce a bacterial lawn. The cells were collected from the three plates and suspended in 25 ml of 0.1% peptone, resulting in a concentration of ca. 10 log CFU/ml. The suspension was added to 400 g of almond kernels placed in a bag, and the kernels rubbed with the suspension for 60 s. The kernels were spread in a single layer onto a tray double lined with filter paper (Fisherbrand Qualitative P8, Fisher Scientific, Pittsburgh, Pa.) and incubated at 24°C for 24 h. The SEPT30N population size on the kernels after drying was ca. 8 log CFU/g almond. The inoculated kernels were stored at 12°C until used, and the SEPT30N population sizes on the kernels were determined to be very stable over time. The population size of the pathogen on the stored kernels was measured before each treatment, as described below under ''Measurement of *Salmonella* population sizes.''

Because of the high density of the kernel meat and that of the kernels in the large storage containers used by the industry, the moisture content of the kernels (maintained at 4.5 to 5.5%) will largely dictate the relative humidity experienced by *S. enterica* cells that may be present on the kernel surface before processing. Therefore, it is considered that under conditions relevant to almond processing in the industry, the physiological state of bacterial contaminants on the kernels will be relatively constant. This curtails the need to investigate the effect of a wide range of physiological states of *S. enterica* cells on the effectiveness of a given sanitization treatment.

IR heating equipment. Two different IR heaters were used for the study. Both had double-sided catalytic emitters (heaters) provided by Catalytic Infrared Drying Technologies, L.L.C. (Independence, Kans.). The first instrument was equipped with a computer that was used to automatically control the IR intensity in the instrument in order to measure the temperature profile of almonds exposed to various IR intensities. Because of the location of this instrument, only uninoculated kernels could be used in this system. Therefore, a second, small-scale IR heater was used for disinfection experiments with SEPT30N-inoculated nuts. The emitter of this instrument had a surface area of 28.26 by 28.26 cm^2 for emitting IR energy. A chicken wire mesh metal tray was used for holding the contaminated almond kernels. The tray was placed at 14 cm from the top emitter and 18 cm from the bottom emitter to achieve similar heating rates on both top and bottom surfaces of the almond kernels in this system. This instrument was not equipped with a computer to control IR intensities.

Determination of kernel heating rates. To determine the effect of IR intensity on the heating rate of almond kernels, single-layered kernels were heated by the double-sided catalytic IR emitters with four different levels of radiation intensity: 5,458, 5,000, 4,000, and 3,000 W/m^2. All treatments in the above experiments were stopped when the surface temperature of the kernels was higher than 90°C. A radiation intensity of 5,458 W/m^2 was the highest radiation intensity that could be achieved with this IR device. Both the top and bottom surface temperatures of the kernels were measured with a Handspring Visor Deluxe PDA (Handspring, Inc., Mountain View, Calif.) and the data recorded with an IQ3000 Thermometer data logger (IQ Scientific Instruments, San Diego, Calif.). Kernels were also wetted by quick immersion in distilled water and then treated with the four radiation intensities. For each treatment condition, the temperature profiles of 10 replicate kernels were obtained, but only the average value of the closest six temperature profiles was plotted. In addition to the temperature profile, the color and appearance of almonds treated with different processing times and IR intensities were assessed visually by the investigators and members of the Almond Board of California.

Treatment of SEPT30N-inoculated almonds. In order to determine the effectiveness of different disinfection treatments, three types of tests were conducted. First, inoculated almonds were treated with IR heat for various times up to 45 s, and then cooled at room temperature for 15 min before the reduction in SEPT30N population sizes was measured. Second, the effect of kernel wetness on disinfection efficacy was determined by wetting the almonds before heating, and then repeating the wetting and heating cycle two times. Third, the almonds were heat treated for different times to achieve different kernel surface temperatures, and then were held at temperatures above 80°C for periods up to 60 min.

For each replicate treatment, 12 kernels inoculated with SEPT30N were placed on a metal wire tray in the IR heater at the start of the heating treatment. Two of these kernels each had a temperature probe to measure the temperature at the surface of the kernel. These kernels were pierced through with a T thermocouple, such that the tip of the needle was at the very surface of the kernel. The needle was connected to an Omega HH506RA High-Accuracy Data Logger/Thermometer (Omega Engineering, Inc., Stamford, Conn.), and the kernel surface temperature was recorded every 5 s.

At the end of the IR heating period, the tray was removed from the instrument, and the 10 kernels that did not have a temperature probe were processed for measurement of the bacterial population sizes. In the experiments where the almonds were wetted before treatment with IR, the entire tray containing the kernels was submerged for 2 s under water, and then placed in the IR instrument to start the heating treatment. For repeated cycles of wetting and IR exposure, the kernels were cooled to 50°C after IR heating and before subsequent wetting.

To determine the effect of holding at warm temperature on

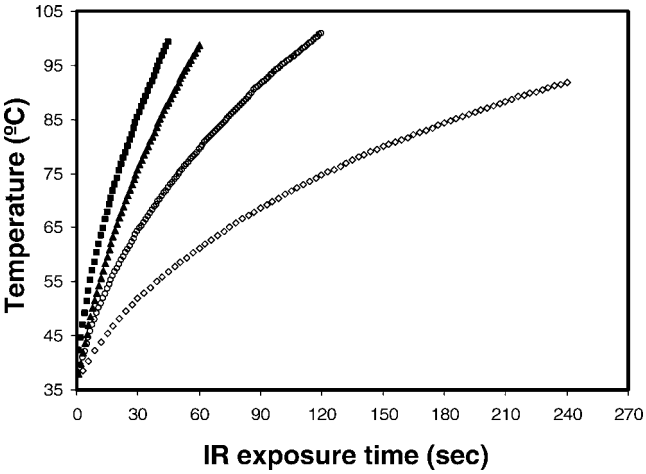

FIGURE 1. *Temperature profiles of the surface of dry almond kernels treated with IR heat at different radiation intensities in an instrument with double-sided catalytic emitters. Radiation intensities were (■) 5,458, (▲) 5,000, (○) 4,000, and (◇) 3,000 W/m^2. Each data point represents the average temperature of the top and bottom surfaces of six almond kernels.*

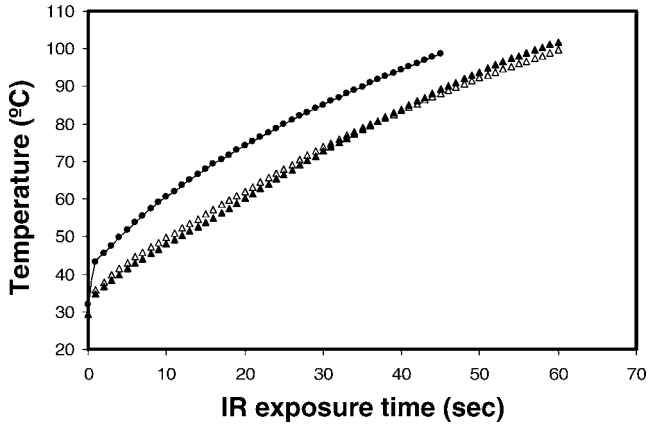

FIGURE 2. *Temperature profiles of the top (closed symbols) and bottom (open symbols) surfaces of almond kernels that were dry (circles) or briefly wetted with water (triangles) before IR heat treatment at 5,458 W/m^2 in an instrument with double-sided catalytic emitters. Each data point represents the average temperature of six replicate almond kernels. The temperature profile of the bottom surface of dry almonds is not apparent because of its great similarity to that of the top surface.*

disinfection efficacy, the kernel surface temperature was maintained below the maximum tested temperature (but above 80°C) during the holding period. This was achieved by placing almond kernels in a four-layer aluminum foil pouch in the IR heater after the gas source for the flame had been turned off to prevent overheating of the sample.

Measurement of *Salmonella* population sizes. To assess the disinfection efficacy of IR heating, the SEPT30N population sizes on the kernels were estimated before (control treatment) and after heat treatment. In each case, 10 kernels (equivalent to ca. 10 g) were placed into 20 ml of Butterfield's buffer (0.31 mM KH_2PO_4, pH 7.2) and homogenized in a Seward Stomacher model 400 for 2 min at high speed. The resulting suspension was dilution plated onto TSA containing 50 μg/ml nalidixic acid. The plates were incubated at 37°C overnight and CFU were enumerated. The average population size, expressed as CFU per gram of kernels was computed from two to four replicate samples (10 kernels per sample), and each experiment was repeated twice on separate days.

Statistical analysis. For comparison of mean bacterial population sizes, the data were analyzed statistically with the software package Prism 3.0 (GraphPad Software, Inc., San Diego, Calif.). Logistic distribution was used to approximate the curve and determine the model for the surface temperature of kernels in relation to IR intensity and heating time in Figures 1 and 2, and the Nonlinear Regression procedure in SAS (SAS Institute, Inc., Cary, N.C.) was used to estimate the model.

RESULTS AND DISCUSSION

Effect of IR intensity on heating rate. In contrast to heat transfer by conduction or convection (e.g., steam), IR travels at high speed from its source to create heat on absorption by the target object. IR radiation heat has been proposed to sterilize heat-resistant medical instruments *(7, 13)* and to eliminate pathogens from meat *(7).* The surface temperatures of almonds heated with IR rose very rapidly. The increases in kernel surface temperature over time at radiation intensities of 5,458, 5,000, 4,000, and 3,000 W/m^2 are shown in Figure 1. The rate of temperature increase correlated with radiation intensity. At high intensities, such as 5,458 W/m^2, the top and bottom surface temperature profiles were very similar (Fig. 2). A 30-s exposure to IR heat at 5,458 W/m^2 was required to increase the surface temperature of dry kernels to 85°C. When the surface of the kernels was wetted, this temperature was achieved in 45 s (Fig. 2). Thus, prewetting the almonds decreased the heating rate at the kernel surface. Compared with single-sided IR heating, the double-sided heating was much faster for delivering required radiation heat. The required processing time to reach a desired surface temperature, using IR heat compared with conventional heat, was dramatically shortened to seconds from minutes (data not shown). This suggests that the disinfection process should be done with a double-sided heating device in order to shorten the heat exposure time and minimize its effect on almond quality. In this device, the effect of IR intensity and time of exposure on the temperature of the kernel surface, as based on our data in Figure 1, is best described ($r^2 = 0.9597$) by the following model:

$$T = \frac{\exp(4.101 - 0.008 \times t + 3.12 \times 10^{-6} \times I \times t)}{1 + \exp(-0.362 - 0.008 \times t - 2 \times 10^{-5} \times I \times t)}$$

where T is the surface temperature, t the time of exposure to IR, and I the radiation intensity.

The above equation is also valid for Figure 2. In this case, the correlation coefficient for the experimental data for dry kernels and data predicted by the model is 0.9954. For wet kernels, the correlation coefficient decreased slightly to 0.9844.

Efficacy of IR treatments without holding period. The heating rate in the small IR unit used for decontamination of almonds was slightly higher than that achieved by an IR intensity of 5,458 W/m^2 in the larger unit used in the earlier part of the study. Therefore, the kernel surface temperature achieved for a same exposure time was higher

TABLE 1. *Effect of short IR exposure time on almond kernel surface temperature and SEPT30N population size*

Exposure time (s)	Final temp (°C)[a]	Population size (log CFU/g kernel)[b]
0	29 ± 1	8.07 ± 0.10
20	74 ± 2	7.73 ± 0.02
25	82 ± 2	7.47 ± 0.05
30	90 ± 2	7.44 ± 0.06
35	102 ± 5	7.04 ± 0.00
45	113 ± 5	6.56 ± 0.02

[a] Mean ± standard error of the mean kernel surface temperature. The temperature was measured on 2 of 10 kernels per each of three replicate samples, for a total of six measurements.

[b] Mean ± standard error of the mean population size of three replicate samples of 10 kernels each.

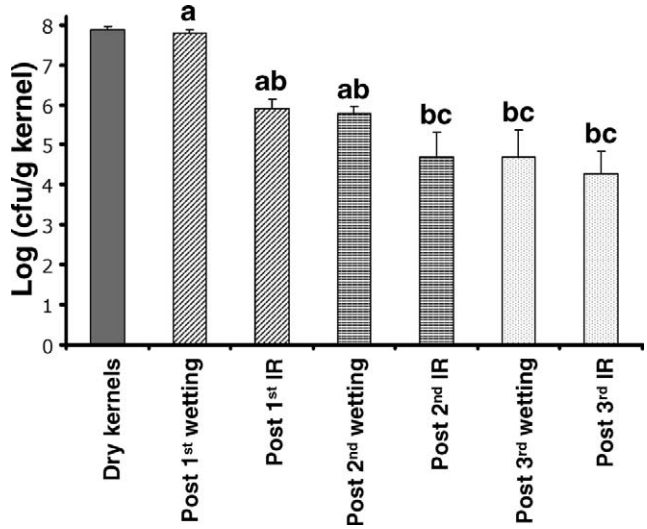

FIGURE 3. *Log value of SEPT30N population sizes on almond kernels exposed to three treatment cycles, consisting each of prewetting the kernels, followed by IR heating. IR exposure times were 45, 40 and 40 s for the first, second, and third cycle, respectively, and the maximum kernel surface temperature reached was 103°C. Kernels were cooled at room temperature to 50°C after the first and second IR heating and before wetting. Each data point represents the mean and standard error of the mean population size of three replicate samples of 10 kernels each. Means marked with different letters are considered significantly different by the Tukey-Kramer multiple comparison test at* $P < 0.05$.

in the small unit than in the large one (Table 1 and Figure 2, respectively). Table 1 shows the temperature and *S. enterica* population sizes on almond kernels exposed to various IR heating periods and then immediately cooled at room temperature. A maximum reduction of 1.51 log CFU/g kernel was achieved, with an exposure time of 45 s, during which a maximum kernel surface temperature of 112.9°C was reached. It is noteworthy that during these short exposure times, kernel surfaces and thus, SEPT30N cells, were subjected to high heat only at the end of the IR treatment. For example, within a 45-s exposure, the kernel surface temperature was in the range of 100 to 113°C for only 10 s. Despite the short-time exposure, the ability of SEPT30N cells to survive such high temperatures on the kernels suggests that their physiological state may have imparted them enhanced heat tolerance. Kirby and Davies *(10)* demonstrated that *Salmonella* Typhimurium cells dried for at least 48 h on a solid surface have increased survival to high heat. Additionally, the effect of low water availability on the heat resistance of *Salmonella* has been well documented *(14)* and is thought to have contributed to large outbreaks of salmonellosis linked to various food products such as chocolate *(5)* and peanut butter–containing products *(16)*. It is likely that *Salmonella* cells on dry almond kernels have similarly high tolerance to heat because of their dehydration.

Efficacy of IR treatment with prewetting. Wetting the kernels by brief immersion under water before a 45-s IR exposure yielded an additional reduction in SEPT30N population size of 0.43 log, despite a lower maximum kernel temperature of 103°C compared with that of 113°C for dry kernels (data not shown). Figure 3 shows the effect of three cycles of brief wetting and IR heating of almond kernels on SEPT30N population sizes. The three-cycle treatment resulted in a 3.6-log kill of SEPT30N on the kernels. It suggested that a brief wetting of the almond kernels has the potential to increase the efficacy of IR heat treatment, possibly by improving the heat transfer to the microsites where the bacterial cells are located. This combination of approaches would have the benefits of increasing bacterial kill while removing the water on the kernels during heating. Thus, it avoids the need for additional drying steps during postdecontamination processing, steps that are commonly required for other thermal treatments such as steaming *(12)* and blanching.

Efficacy of IR treatment with holding period. In view of the fact that IR heating for the decontamination of raw almond kernels is allowable only for short periods if a roasting effect is to be avoided, we tested the efficacy of short IR heat exposures, followed by holding of the kernels at a moderate temperature. Because of the simple design of the IR heating system used for treatment of inoculated kernels, holding was carried out by placing the kernels in an aluminum foil pouch in the instrument after the IR heater had been turned off. In an almond processing plant, holding could be achieved by storing the kernels after IR heating in temperature-controlled containers.

Figure 4A illustrates the kernel surface temperature profiles resulting from a 30-s IR heating (maximum temperatures just below 100°C), followed by holding times of 15, 30, and 60 min in the aluminum pouches while the IR instrument was turned off. The final kernel surface temperatures were 95, 85, and 75°C, after holding times of 15, 30, and 60 min, respectively. As revealed in Figure 4B, the effective treatments were those with holding times of 30 and 60 min, which provided a reduction of 3.46 and 3.37 log in SEPT30N population sizes, respectively. However, it should be noted that even the shortest kernel holding time of 15 min following 30-s IR heating effected a significantly greater SEPT30N reduction (100-fold) than did the same

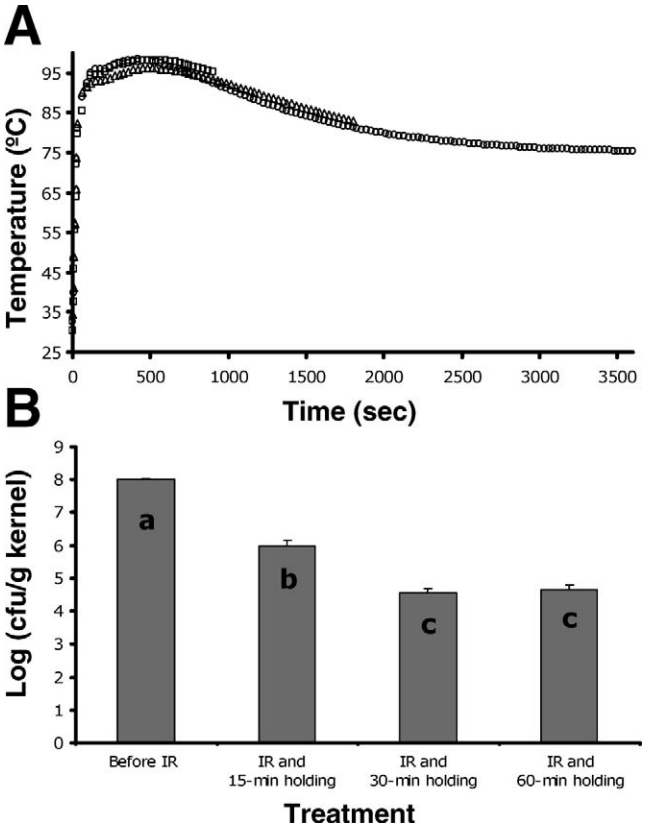

FIGURE 4. *Treatment of almond kernels with 30-s IR heating and maximum kernel surface temperatures of ca. 98°C, followed by holding at moderate temperatures. (A) Temperature profiles of kernel surface during IR and three different holding times: (□) 15 min, (△) 30 min, and (○) 60 min. (B) Log population sizes of SEPT30N on the kernels before and after the various treatments. Each data point represents (A) the mean surface temperature of two kernels, and (B) the mean and standard error of the mean population size of two replicate samples of ten kernels each. Mean population sizes marked by different letters are significantly different by the Tukey-Kramer's multiple comparison test at* $P < 0.05$.

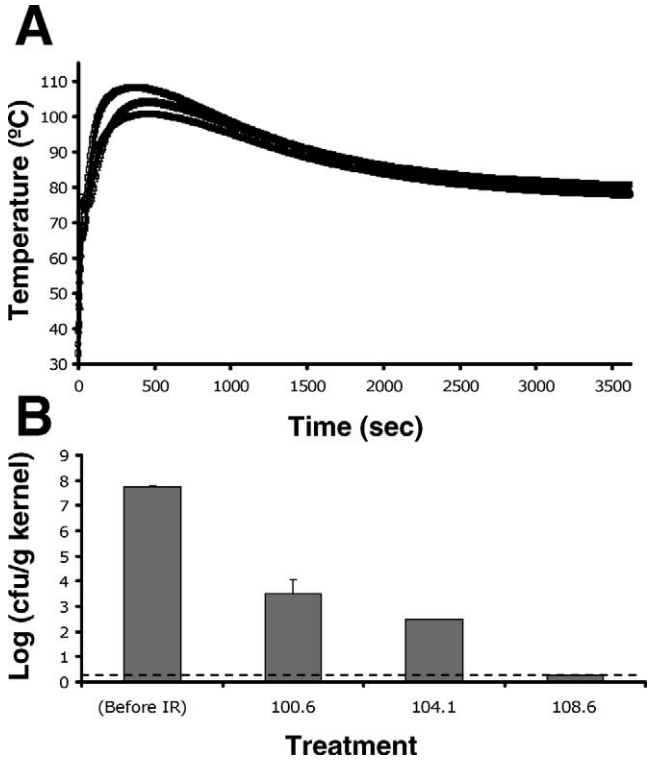

FIGURE 5. *Effect of three different treatments with IR heating, followed by a 60-min holding period, during which the maximum kernel surface temperatures varied. (A) Temperature profiles of the surface of kernels treated with maximum temperatures of 100.6, 104.1, and 108.6°C (low, middle, and high profile, respectively). (B) SEPT30N population sizes before and after each of the three treatments. Each data point represents (A) the mean surface temperature of two kernels, and (B) the mean and standard error of the mean population size of two replicate samples of 10 kernels each. The dashed line depicts the minimum detection level of 0.29 log CFU/g kernel in our assay, which corresponds to over a 7.5-log CFU/g reduction in SEPT30N population sizes on the kernels. Treatments with mean maximum temperatures of 108.6°C yielded undetectable SEPT30N populations and thus, no error bar is shown.*

IR treatment without holding (fourfold) (Fig. 4B and Table 1). Thus, maintaining the kernels at warm temperature after a short and rapid heat treatment appeared to further injure the bacterial cells and increase lethality. Studies are ongoing in our laboratory to determine the minimum holding temperature that will ensure optimal kernel quality while providing the shortest and most efficacious decontamination treatment.

The decontamination efficacy of the combined IR holding treatment was greatly influenced by the maximum surface temperature that the kernels reached before they were held at warm temperatures. With prior heating to a maximum kernel surface temperature of 80°C, a 60-min holding yielded only a 1.5-log reduction of SEPT30N (data not shown). In contrast, we observed that maximum kernel surface temperatures of 100.6, 104.1, and 108.6°C resulted in 4.2-, 5.3-, and 7.5-log reductions, respectively, using the same 60-min holding period (Fig. 5A and 5B). The latter reduction in *Salmonella* contamination of 7.5 log is comparable to the maximum reduction achieved with a 5-day process involving fumigation with propylene oxide and tempering *(3).* Thus, kernel sanitization was achieved at least as effectively in a 1-h process with IR, as with a 5-day process based on fumigation.

Quality of treated almond kernels. Macroscopic observation of the surface and internal kernel tissue of almonds treated under all reported conditions did not reveal any alterations in morphology or color due to these treatments, including those that resulted in the highest kernel surface temperatures. The high external and internal quality of these kernels was also confirmed visually by experts of the Almond Board of California. In addition, the effect of the treatment on the moisture content of the kernels was assessed by measuring the weight of 10-kernel samples (ca. 10 g) before and after treatment. In the most efficacious treatment, described in Figure 5, the kernel weight loss was 1.09%. This suggests that moisture loss during treatment was minimal. In addition, this small moisture loss could be remediated by a brief wetting of the kernels before IR heat-

ing, a treatment that increased the efficacy of decontamination, as demonstrated above.

Our results demonstrate that IR heating combined with holding at warm temperature had an efficacy at least as great as that of propylene oxide treatment for the reduction of SEPT30 populations on almonds while requiring a shorter process time *(3).* In addition, combined IR heating and holding at warm temperatures achieved better decontamination than did steam pasteurization, without the disadvantage of increasing kernel moisture *(10).* Similarly, Huang and Sites *(6)* reported that a combination of IR and holding at 85°C reduced the population size of *Listeria monocytogenes* on the surface of inoculated hot dogs by as much as 6.7 log. Based on a risk assessment study by Danyluk et al. *(4),* the Almond Board of California presently mandates the use by its processors of a decontamination treatment that results in a 5-log reduction of *S. enterica* CFU per gram of almond kernel. Thus, IR may provide a suitable dry pasteurization approach for decontamination of raw almonds. Although our results are based on a single strain of *S. enterica,* it is unlikely that strain-to-strain differences in heat resistance of *S. enterica* would significantly affect the effectiveness of our treatment, considering the over 7.5-log reduction in *S. enterica* populations that were obtained in our study, compared with the 4-log reduction mandated in the industry.

Macroscopic assessment showed that the quality of IR-treated kernels was not significantly different from that of untreated kernels. Investigation of the organoleptic properties and nutritional value of IR-treated kernels to ensure that the quality of the product is acceptable to the almond industry and its customers is ongoing in our laboratory.

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