# Combining Lactic Acid Spray with Near-Infrared Radiation Heating To Inactivate *Salmonella enterica* Serovar Enteritidis on Almond and Pine Nut Kernels

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**The aim of this study was to investigate the efficacy of near-infrared radiation (NIR) heating combined with lactic acid (LA) sprays for inactivating *Salmonella enterica* serovar Enteritidis on almond and pine nut kernels and to elucidate the mechanisms of the lethal effect of the NIR-LA combined treatment. Also, the effect of the combination treatment on product quality was determined. Separately prepared *S.* Enteritidis phage type (PT) 30 and non-PT 30 *S.* Enteritidis cocktails were inoculated onto almond and pine nut kernels, respectively, followed by treatments with NIR or 2% LA spray alone, NIR with distilled water spray (NIR-DW), and NIR with 2% LA spray (NIR-LA). Although surface temperatures of nuts treated with NIR were higher than those subjected to NIR-DW or NIR-LA treatment, more *S.* Enteritidis survived after NIR treatment alone. The effectiveness of NIR-DW and NIR-LA was similar, but significantly more sublethally injured cells were recovered from NIR-DW-treated samples. We confirmed that the enhanced bactericidal effect of the NIR-LA combination may not be attributable to cell membrane damage *per se*. NIR heat treatment might allow *S.* Enteritidis cells to become permeable to applied LA solution. The NIR-LA treatment (5 min) did not significantly ($P > 0.05$) cause changes in the lipid peroxidation parameters, total phenolic contents, color values, moisture contents, and sensory attributes of nut kernels. Given the results of the present study, NIR-LA treatment may be a potential intervention for controlling food-borne pathogens on nut kernel products.**

Consumption of edible nut kernels has shown an upward trend worldwide, as consumers have taken a profound interest in health and nutrition. Although nut-associated illness outbreaks are relatively uncommon, recent outbreaks of salmonellosis related to consumption of nut kernels, including almonds and pine nuts, have raised awareness of nuts as a potential vehicle for food-borne illness [1–4]. Two hundred five cases of salmonellosis associated with consumption of whole raw almonds in Canada and the United States have been attributed to *Salmonella enterica* serovar Enteritidis phage type 30 (PT 30) [3]. More recently, the Centers for Disease Control and Prevention released a report stating that at least 53 people in 5 states of the United States had become infected during a non-PT 30 *S.* Enteritidis outbreak that was linked to contaminated Turkish pine nuts [2]. *Salmonella* spp. cannot multiply on nuts but have the ability to survive on and in dry nut kernels for more than 1 year [5]. In light of this, research regarding enhancing the microbial safety and quality of nuts potentially offers great health and economic benefits to the general public and to food processors [6].

Existing disinfection practices for all low-moisture foods, including nuts, are currently under evaluation. Several processes for eliminating *Salmonella* from almond surfaces have been investigated, including propylene oxide fumigation [7], chlorine dioxide gas [8], and various heat processes involving steam [6, 9]. However, few treatments are available for the decontamination of almond kernels that are consumed raw due to limitations of these interventions. The major drawback of using fumigants, such as propylene oxide, is the potential presence of residues and their negative impact on export marketing [10]. Chlorine dioxide is an effective gaseous alternative to propylene oxide for inactivating *Salmonella* contamination on raw almonds, but it can lead to discoloration of the kernel surface at high concentrations [8]. Steam

is more effective than dry heat, but prolonged exposure causes quality loss and requires additional processing to remove condensed moisture before storage [6, 10]. Furthermore, to date, there have been no published reports that focused on controlling food-borne pathogens on pine nut kernels, despite the recent *S.* Enteritidis outbreak [2]. Consequently, the development of new technologies that can effectively reduce *Salmonella* spp. on nut kernels without compromising product quality is needed.

Recently, infrared (IR) heating has been gaining wider acceptance because of its higher heat transfer capacity and high energy efficiency compared with conventional heating. Brandl et al. [10] employed catalytic IR heating for reducing *Salmonella* populations on almond kernels. In our previous studies, near-IR radiation (NIR; 0.76 to 2 μm) was shown to be more effective than conventional heating for inactivating pathogens on solid foods [11], and NIR heating combined with a nonthermal technology such as UV-C radiation showed significant synergistic lethal effects [12, 13]. On the other hand, a broad spectrum of chemical compounds and their usages has been developed by the food in-

dustry for minimizing microbial contamination on surfaces of raw agricultural commodities. Pao et al. (14) reported on the potential use of acid sprays for eliminating *Salmonella* spp. on raw almonds. An estimated 5-log reduction can be achieved using combinations of spraying with 10% citric acid, shelling, and storage. However, considering nut quality, acidic solutions should be applied at lower concentrations.

Since there are no published data on the behavior of *Salmonella* on the surfaces of nut kernels during simultaneous treatment with organic acid sprays and NIR heating, we chose to combine antimicrobial treatments to achieve maximal effectiveness against *S.* Enteritidis on nut kernels, using both chemical (spraying with organic acid solution) and physical (NIR heating) interventions. The essence of this approach is that nuts can remain microbiologically safe and are acceptable organoleptically and nutritionally due to the mild heating applied. Among various organic acids of identical concentration and similar pH, the use of lactic acid offers the best antimicrobial potential based on our previous study (15). Therefore, lactic acid was chosen for this investigation.

The objectives of this research were to evaluate the efficacy of lactic acid spray along with NIR heating for inactivating *Salmonella enterica* serovar Enteritidis on almond and pine nut kernels and to determine the effect of the combination treatment on quality factors of the nut kernel product. Also, we explored the mechanism of inactivation.

## MATERIALS AND METHODS

**Bacterial strains.** Three strains each of *S. enterica* serovar Enteritidis (NCCP 12236, NCCP 12243, and NCCP 14771) obtained from the National Culture Collection for Pathogens (NCCP; Osong, South Korea) and *S.* Enteritidis PT 30 (ATCC BAA-1045) obtained from the Bacterial Culture Collection of Seoul National University (Seoul, South Korea) were used in this experiment. Stock cultures were kept frozen at −80°C in 0.7 ml of tryptic soy broth (TSB; Difco Becton Dickinson, Sparks, MD, USA) and 0.3 ml of 50% glycerol (vol/vol). Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37°C for 24 h, and stored at 4°C.

**Preparation of pathogen inocula.** All strains of *S.* Enteritidis were cultured individually in 5 ml of TSB at 37°C for 24 h, harvested by centrifugation (4,000 × *g* for 20 min at 4°C), and washed three times with 0.2% peptone water (PW; Difco). The final pellets were resuspended in PW, corresponding to approximately $10^7$ to $10^8$ CFU/ml. Subsequently, suspended pellets of *S.* Enteritidis NCCP 12236, NCCP 12243, and NCCP 14771 were combined to produce mixed-culture cocktails, while PT 30 was prepared as a single-strain inoculum. These cell suspensions with a final concentration of approximately $10^8$ CFU/ml were used in this study. To analyze the mechanism of inactivation, a final pellet of non-PT 30 *S.* Enteritidis was resuspended in 3 ml of phosphate-buffered saline (PBS; 0.1 M) and inoculated onto a sterile glass petri dish (16 mm [height] by 90 mm [inside diameter]). The cell suspensions were thoroughly dried inside a biosafety hood prior to each treatment.

**Sample preparation and inoculation.** Raw shelled 'Nonpareil' almonds (California, USA) and pine nuts (Hongcheon, South Korea) were purchased at a local grocery store (Seoul, South Korea). For inoculation, 5 ml of prepared inoculum was added dropwise to 200-g samples (*S.* Enteritidis PT 30 to almonds and non-PT 30 *S.* Enteritidis cocktail to pine nuts) inside sterile high-density polyethylene (HDPE) bags (300 mm by 450 mm). The inoculated samples were thoroughly mixed by hand massaging for 3 min to produce a homogeneous dispersal of inoculum throughout the nut kernels and dried for 24 h inside a biosafety hood (21 ± 2°C) with the fan running until the moisture content (dry basis) of the samples equaled that of uninoculated samples (ca. 4.8 and 2.5% for almonds and pine nuts, respectively). The final cell concentration was $10^5$ to $10^6$
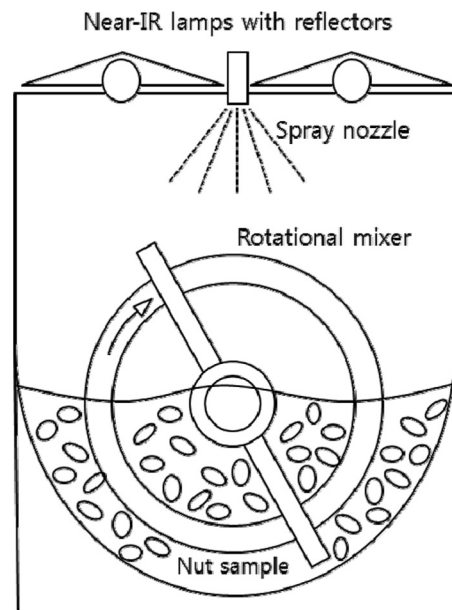


FIG 1 Schematic diagram of the NIR-LA combined treatment system used in this study.

CFU/25 g. Inoculated nut kernel samples were then immediately used in each experimental batch.

**Preparation of lactic acid solution.** Lactic acid (LA, above 90.0%; Daejung Chemical Co., Siheung-si, South Korea) was mixed with enough sterile distilled water (DW) to make a 2% (vol/vol) solution, and the solution was prepared within 1 h before experiments. The pH for DW and 2% LA solution was 6.86 and 2.03, respectively. The concentration and volume of LA applied to the sample were chosen after preliminary experiments were performed.

**Spraying with LA and near-infrared heating.** Near-infrared (NIR) heating and spraying with LA were carried out in a previously described apparatus (13). A stainless steel chamber (concave-upwards base, 380 by 205 by 158 mm) with a rotational mixer was used for combined NIR heating and LA spray treatment (Fig. 1). For spraying the 2% LA solution or DW on nut kernel samples, a hand-operated sprayer (650 ml, Apollo, Siheung-si, South Korea) was used. The sprayer was held on the top of the treatment chamber (at a distance of 13 cm from the samples). An approximate volume of 10 ± 0.5 ml of 2% LA or DW was sprayed evenly over inoculated almonds or pine nuts (200 g) during simultaneous operation of the rotational mixer (23 rpm). This was followed immediately with NIR heating applied for a maximum of 5 min. Two quartz halogen infrared heating lamps (NS-104, 350 mm; NSTECH, South Korea), with a maximum power of 500 W (radiation intensity of 141.75 $\mu$W/cm$^2$/nm at the sample location) at a 230-V input, were used as a NIR-emitting source. The maximum wavelength ($\lambda_{max}$) generated from the infrared heater used in this study was about 1,300 nm, which is in the near-infrared wave range. The radiation intensity generated from the NIR heater was measured and recorded with a NIR fiber optic spectrometer (AvaSpec-NIR256-1.7; Avantes, Eerbeek, Netherlands). Since both lamps radiate in all directions, two aluminum reflectors were installed behind the emitters to redirect the radiation waves and enhance the efficiency of NIR (Fig. 1). After the outputs of the NIR lamps were stabilized (following 2 min of run time), the two lamps were placed on the treatment chamber for the inactivation experiments (NIR with 2% LA spray, NIR with DW spray, NIR without supplemental spray, and 2% LA spray without NIR). All treatments were accompanied by stirring (23 rpm) by means of the rotational mixer. For the inactivation mechanism study, 3-ml cell suspensions dried inside glass petri dishes were treated with LA, NIR, NIR-DW, and NIR-LA

for 4 min under identical conditions. The treatment time (4 min) was selected based on the temperatures of nut samples observed during NIR treatment.

**Bacterial enumeration.** At selected time intervals, 25-g treated samples were removed and immediately transferred into sterile stomacher bags (Labplas Inc., Sainte-Julie, Quebec, Canada) containing 100 ml of 0.2% PW (detection limit, 5 CFU/g) and homogenized for 2 min with a stomacher (Easy Mix; AES Chemunex, Rennes, France). After homogenization, 1-ml aliquots of sample were 10-fold serially diluted in 9-ml blanks of PW, and 0.1 ml of sample or diluent was spread-plated onto selective medium, xylose lysine desoxycholate agar (XLD; Difco), for the enumeration of *S.* Enteritidis cells. None of the uninoculated control nuts yielded black colonies characteristic of *Salmonella* spp. on XLD agar (data not shown). Where low numbers of surviving cells were anticipated, 250 μl of sample was spread-plated onto each of four plates to lower the detection limit. All agar media were incubated at 37°C for 24 h before counting. To confirm the identity of *S.* Enteritidis, random colonies were selected from the enumeration plates and subjected to the *Salmonella* latex agglutination assay (Oxoid, Ogdensburg, NY), a serological test.

**Enumeration of injured cells.** The overlay (OV) method was used to enumerate injured cells of *S.* Enteritidis ([16](#)). TSA was used as a nonselective medium to repair injured cells. One hundred microliters of appropriate dilutions were spread-plated onto TSA medium in duplicate, and plates were incubated at 37°C for 2 h to allow injured cells to resuscitate ([17](#)). Plates were then overlaid with 7 to 8 ml of the selective medium XLD agar. After solidification, plates were further incubated for an additional 22 h at 37°C. Following incubation, presumptive colonies of *S.* Enteritidis with typical black colonies were enumerated.

**Temperature measurement.** In order to measure the surface temperature of treated samples during single NIR heating and NIR heating combined with DW or 2% LA sprays, a Raytek infrared precision thermometer (STProPlus; Raytek Co., Santa Cruz, CA) was used. Temperatures were recorded at selected treatment times, and all experiments were replicated three times.

**Measurement of extracellular UV-absorbing substances.** Cell membrane damage induced by each treatment was quantitatively assessed by determining the release of UV-absorbing materials from injured cells. Untreated and treated *S.* Enteritidis cells were resuspended using 10 ml of PBS and centrifuged at $10,000 \times g$ for 10 min. UV absorbance of sample supernatants at 260 and 280 nm was measured with a spectrophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA). Absorbance values presented are the means of triplicate measurements.

**Acid value, peroxide value, and total phenolic content measurement.** To evaluate the effect of NIR-LA treatment for a maximum of 5 min on nutritional quality of nut kernels, the acid value, peroxide value, and total phenolic concentration were monitored under accelerated conditions during storage. Treated samples (30 g each) were placed in 50-ml vial tubes, which were sealed with paper to facilitate gas exchange before being placed in an air convection oven at 60°C in the dark. After storage for 0, 9, and 18 days, about 5 g each of almond or pine nut sample was chopped and extracted with absolute ether (for acid value and peroxide value) or acetone (for total phenolic content) in a solvent recovery extractor (Soxhlet method, 4002842; JP Selecta S.A., Barcelona, Spain). The acid value and peroxide value in the oil extracted from the nut samples were determined by AOCS official methods Cd 3a-63 and Cd 8-53, respectively ([18](#)). Total phenolic contents in the extracts were estimated by a colorimetric assay based on the procedure described by Singleton et al. ([19](#)) with some modifications. Briefly, a 1-ml aliquot of the extract was mixed with 1 ml of Folin and Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, MO). After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction mixture was kept in the dark for 90 min, after which the absorbance was read at 725 nm using a spectrophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA). A blank devoid of any extract was used
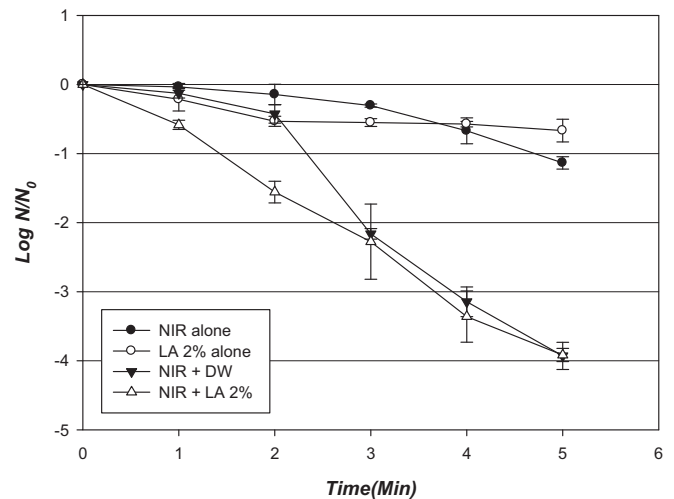


**FIG 2** Survival curves of *Salmonella* Enteritidis PT 30 on almond kernels treated with NIR alone or 2% lactic acid sprays and NIR heating combined with distilled water or 2% lactic acid sprays. The error bars indicate standard deviations calculated from triplicates.

for background subtraction. Gallic acid (Sigma-Aldrich) was used as the reference standard, and the results were expressed as milligrams of gallic acid equivalents (GAEs) per gram of extract.

**Color/posttreatment moisture content measurement and sensory evaluation.** To determine the effect of NIR-LA treatment for 5 min on the color of nut kernel skin, a Minolta colorimeter (model CR400; Minolta Co., Osaka, Japan) was used to measure color changes of treated samples. The color attributes were quantified from the values of $L^*$, $a^*$, and $b^*$, which indicate the color lightness, redness, and yellowness of the sample, respectively, and which were measured at identical locations on surfaces of each almond and pine nut kernel. After 5 min of NIR-LA treatment, the posttreatment moisture content (dry basis) was measured immediately with a halogen moisture analyzer (HB43-S; Mettler Toledo, Columbus, OH). All measurements were taken in triplicate.

Sensory evaluation was performed to determine how specific attributes (texture and flavor) varied over NIR-LA-treated nut samples (5-min exposure) compared to those of a nontreated control. In all sensory tests, the panels consisted of 13 members (6 men and 7 women; age range, 25 to 31 years) from the Department of Food and Animal Biotechnology, Seoul National University, and scores were obtained by rating the sensory attributes using the following 7-point hedonic scales: 7, very good; 6, good; 5, below good/above fair; 4, fair; 3, below fair/above poor; 2, poor; 1, very poor. Samples, labeled with three-digit random numbers, were placed on white paper plates and presented after being cooled to room temperature. The presentation order was randomized, and the panelists were asked to use water to clean their palates between samples.

**Statistical analysis.** All experiments were repeated three times with duplicate samples. Data were analyzed by the analysis of variance (ANOVA) procedure of the Statistical Analysis System (SAS Institute, Cary, NC, USA). Means were separated using Tukey-Kramer's multiple-range test, and a $P$ value of $<0.05$ was used to indicate significant differences.

## RESULTS

**Survival curves of food-borne pathogens.** Viable-count reductions of *S.* Enteritidis PT 30 on almond kernels and *S.* Enteritidis cocktail on pine nut kernels during single NIR or 2% lactic acid (LA) spray treatment and NIR heating combined with distilled water (DW) or 2% LA sprays are depicted in [Fig. 2](#) and [3](#), respec-
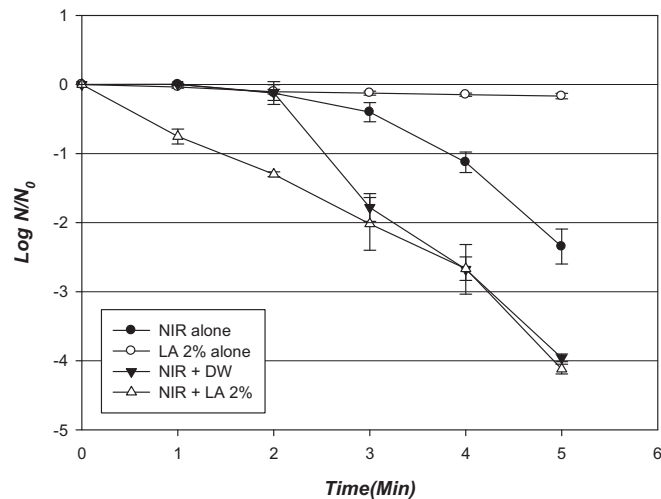
FIG 3 Survival curves of *Salmonella enterica* serovar Enteritidis on pine nut kernels treated with NIR alone or 2% lactic acid sprays and NIR heating combined with distilled water or 2% lactic acid sprays. The error bars indicate standard deviations calculated from triplicates.

**TABLE 1** Levels of surviving cells and cells including injured *Salmonella* Enteritidis PT 30 on almond kernels following NIR heating combined with distilled water or 2% lactic acid sprays

| Treatment time (min) | Log reduction [$\log_{10} (N_0/N)$] by treatment type and selective medium[a] | | | |
| | NIR-DW | | NIR-LA | |
| | XLD | OV-XLD | XLD | OV-XLD |
|---|---|---|---|---|
| 0 | 0.00 ± 0.00 Aa | 0.00 ± 0.00 Aa | 0.00 ± 0.00 Aa | 0.00 ± 0.00 Aa |
| 1 | 0.13 ± 0.07 ABa | 0.04 ± 0.03 Aa | 0.58 ± 0.07 Aa | 0.68 ± 0.11 Aa |
| 2 | 0.43 ± 0.13 Ba | 0.33 ± 0.07 Aa | 1.56 ± 0.16 Ba | 1.51 ± 0.18 Ba |
| 3 | 2.16 ± 0.08 Ca | 1.83 ± 0.30 Ba | 2.28 ± 0.54 Ba | 2.14 ± 0.48 Ba |
| 4 | 3.15 ± 0.21 Da | 2.60 ± 0.32 Ca | 3.36 ± 0.37 Ca | 3.16 ± 0.37 Ca |
| 5 | 3.93 ± 0.20 Ea | 3.06 ± 0.26 Cb | 3.92 ± 0.10 Ca | 3.90 ± 0.11 Da |

[a] Values are means ± standard deviations from three replications. Values in the same column followed by the same uppercase letter are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row are not significantly different ($P > 0.05$). XLD, xylose lysine desoxycholate; OV-XLD, overlay XLD agar on TSA.

tively. The overall reduction patterns of *S.* Enteritidis PT 30 on almonds were similar to those of the *S.* Enteritidis cocktail on pine nuts. Significant ($P < 0.05$) log reductions of *S.* Enteritidis on almonds and pine nuts were observed after 1 min of NIR-LA combined treatment, whereas with NIR-DW combined treatment, the time to initiation of a significant ($P < 0.05$) reduction was delayed by an additional 1 to 2 min (Fig. 2 and 3). NIR-LA combined treatment for 5 min achieved 3.92- and 4.12-log reductions in *S.* Enteritidis PT 30 and cocktail, respectively. Reductions of 3.93 and 3.96 log CFU/g were observed in *S.* Enteritidis PT 30 and cocktail after NIR-DW treatment for 5 min, respectively. Especially for the NIR-DW combination, *S.* Enteritidis populations on both samples sharply declined after 3 min of treatment, and the reduction levels at 3-, 4-, and 5-min intervals were not significantly ($P > 0.05$) different from those of NIR-LA treatment (Fig. 2 and 3). NIR heating alone for 5 min reduced cell numbers of *S.* Enteritidis PT 30 and cocktail by 1.14 and 2.35 log CFU/g, respectively. After 5 min of LA spray treatment alone, levels of *S.* Enteritidis PT 30 and cocktail were reduced by 0.67 and 0.17 log CFU/g, respectively.

**Resuscitation of injured cells.** Levels of sublethally injured *S.* Enteritidis PT 30 on almonds and *S.* Enteritidis cocktail on pine nuts following NIR-DW and NIR-LA treatment are presented in Tables 1 and 2, respectively. When surface-inoculated nut kernels were subjected to NIR-DW combined treatment, smaller reductions of *S.* Enteritidis were observed by the agar OV method than by direct plating on selective agar, and statistically significant ($P < 0.05$) differences between levels of surviving cells (noninjured versus total cells, including those sublethally injured) were observed after the maximum treatment time of 5 min (Tables 1 and 2). For NIR-LA combined treatment, however, there were no significant ($P > 0.05$) differences between the reduction levels enumerated on the selective agar (XLD) versus those on the agar used for recovery (OV-XLD) during the entire treatment time.

**Average temperature-time histories of nut kernels.** Figure 4 shows average surface temperatures of almonds and pine nuts

during NIR heating alone and NIR heating combined with DW or 2% LA sprays. The surface temperature rose immediately in response to infrared waves when nut kernel samples were exposed to NIR. The initial heating rates of all treatment combinations were similar on almonds and on pine nuts. However, after 3 min of treatment, the rate of temperature increase of nut kernels treated with NIR-DW or NIR-LA declined, and these temperature disparities continued to increase with treatment time (Fig. 4). The heating rate of the NIR-LA treatment was not significantly ($P > 0.05$) different from that of the NIR-DW treatment at all treatment time intervals. At the maximum treatment time (5 min), surface temperatures of almonds and pine nuts increased to ca. 77 and 81°C, respectively, whereas during 5 min of NIR treatment alone, surface temperatures of almond and pine nut kernels reached ca. 93 and 101°C, respectively.

**Leakage of bacterial intracellular substances.** A disruption of the cell membrane or a change in membrane permeability causes an increase in the amount of intracellular substances found outside the cell. Spectrophotometric observation can detect these substances at 260 nm for nucleic acids (i.e., purines and pyrimidines) and 280 nm for proteinaceous materials (e.g., tyrosine and tryptophan) (20). Table 3 shows absorbance values at 260 and 280 nm after each treatment (LA, NIR, NIR-DW, and NIR-LA). The overall pattern for the leakage of nucleic acids (260 nm) was similar to that of proteins (280 nm). Based on trends of leaked intra-

**TABLE 2** Levels of surviving cells and cells including injured *Salmonella enterica* serovar Enteritidis on pine nut kernels following NIR heating combined with distilled water or 2% lactic acid sprays

| Treatment time (min) | Log reduction [$\log_{10} (N_0/N)$] by treatment type and selective medium[a] | | | |
| | NIR-DW | | NIR-LA | |
| | XLD | OV-XLD | XLD | OV-XLD |
|---|---|---|---|---|
| 0 | 0.00 ± 0.00 Aa | 0.00 ± 0.00 Aa | 0.00 ± 0.00 Aa | 0.00 ± 0.00 Aa |
| 1 | 0.00 ± 0.02 Aa | −0.08 ± 0.04 Aa | 0.75 ± 0.11 Ba | 0.88 ± 0.06 Ba |
| 2 | 0.12 ± 0.11 Aa | 0.16 ± 0.12 Aa | 1.30 ± 0.04 Ca | 1.27 ± 0.04 Ba |
| 3 | 1.78 ± 0.20 Ba | 1.35 ± 0.12 Bb | 2.02 ± 0.38 Da | 1.94 ± 0.51 Ca |
| 4 | 2.68 ± 0.36 Ca | 2.00 ± 0.07 Cb | 2.67 ± 0.17 Ea | 2.56 ± 0.14 Da |
| 5 | 3.96 ± 0.06 Da | 3.28 ± 0.13 Db | 4.12 ± 0.07 Fa | 4.02 ± 0.04 Ea |

[a] Values are means ± standard deviations from three replications. Values in the same column followed by the same uppercase letter are not significantly different ($P > 0.05$). Means with the same lowercase letter in the same row are not significantly different ($P > 0.05$). XLD, xylose lysine desoxycholate; OV-XLD, overlay XLD agar on TSA.
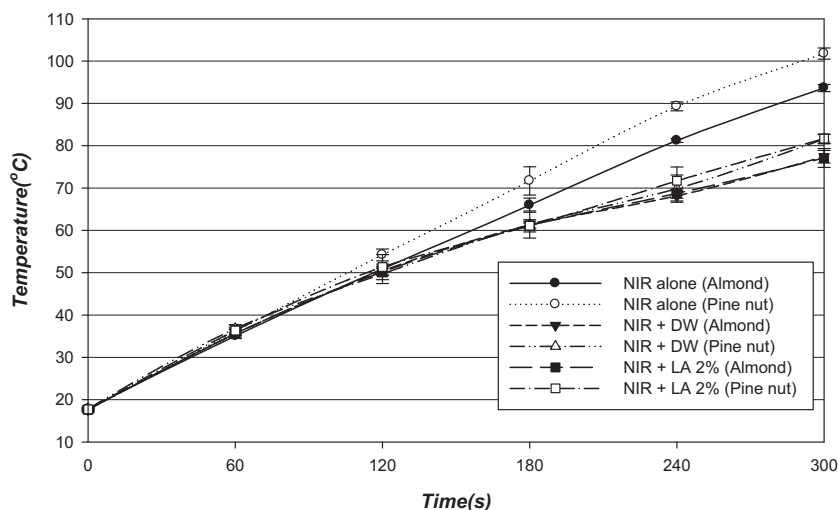
**FIG 4** Average temperature-time histories of almond and pine nut surfaces during single NIR heating and NIR heating combined with distilled water or 2% lactic acid sprays. The error bars indicate standard deviations calculated from triplicates.

cellular components, we infer that there was no significant ($P >$ 0.05) damage to cellular membranes of *S*. Enteritidis following LA spray treatment alone. Cells subjected to NIR-DW and NIR-LA treatments showed significantly ($P < 0.05$) higher leakage of UV-absorbing substances than did cells subjected to NIR heating alone. However, the degree of membrane damage between NIR-DW- and NIR-LA-treated cells was not significantly ($P > 0.05$) different (Table 3).

**Effect of NIR-LA combined treatment on product quality.** Lipid peroxidation parameters (acid value and peroxide value), total phenolic content, color values, posttreatment moisture content, and sensory attributes of almond and pine nut kernels after 5 min of NIR-LA combined treatment are summarized in Tables 4 and 5, respectively. Although the lipid peroxidation parameters and total phenolic concentrations of almonds and pine nuts varied slightly with prolonged storage time, regardless of treatment, there were no significant ($P > 0.05$) differences in acid value, peroxide value, and total phenolic content between untreated and treated samples over the entire storage time (Table 4). As shown in Table 5, color ($L^*$, $a^*$, and $b^*$) values of NIR-LA-treated (5 min) nut kernels were not significantly ($P > 0.05$) different from those of untreated samples. NIR-LA treatment for 5 min did not significantly ($P > 0.05$) change moisture content (dry basis) of almonds and pine nuts. Also, there were no significant ($P > 0.05$) differ-

ences among all tested samples scored by the hedonic scale for texture (mouth feel) and flavor, indicating that NIR heating combined with 2% LA spray for 5 min did not significantly alter the sensory quality of nut kernel products.

**TABLE 4** Acid value, peroxide value, and total phenolic content of NIR-LA-treated almond and pine nut kernels during storage under accelerated conditions[a]

| Sample, parameter, and treatment | Value after storage time (days) at 60°C in the dark | | |
|---|---|---|---|
| | 0 | 9 | 18 |
| Almonds | | | |
| Acid value (mg KOH/g extract) | | | |
| Control | 0.97 ± 0.14 | 1.28 ± 0.03 | 1.38 ± 0.09 |
| NIR-LA | 1.08 ± 0.02 | 1.26 ± 0.08 | 1.32 ± 0.06 |
| Peroxide value (meq/kg extract) | | | |
| Control | 0.60 ± 0.17 | 0.73 ± 0.06 | 1.13 ± 0.20 |
| NIR-LA | 0.65 ± 0.06 | 0.77 ± 0.22 | 1.11 ± 0.12 |
| Total phenolics (mg GAE/g extract) | | | |
| Control | 1.81 ± 0.11 | 1.66 ± 0.13 | 1.30 ± 0.16 |
| NIR-LA | 1.76 ± 0.20 | 1.58 ± 0.05 | 1.25 ± 0.11 |
| Pine nuts | | | |
| Acid value (mg KOH/g extract) | | | |
| Control | 0.83 ± 0.02 | 0.90 ± 0.04 | 0.96 ± 0.11 |
| NIR-LA | 0.88 ± 0.05 | 0.87 ± 0.08 | 0.95 ± 0.08 |
| Peroxide value (meq/kg extract) | | | |
| Control | 0.24 ± 0.11 | 3.11 ± 0.13 | 3.17 ± 0.10 |
| NIR-LA | 0.32 ± 0.04 | 3.15 ± 0.06 | 3.24 ± 0.17 |
| Total phenolics (mg GAE/g extract) | | | |
| Control | 2.03 ± 0.08 | 1.91 ± 0.08 | 1.58 ± 0.17 |
| NIR-LA | 1.94 ± 0.09 | 1.83 ± 0.09 | 1.42 ± 0.05 |

[a] The values are means ± standard deviations from three replications. Values in the same column are not significantly different ($P > 0.05$). GAE, gallic acid equivalent.

**TABLE 3** Levels of membrane damage of LA-, NIR-, NIR-DW-, and NIR-LA-treated cells inferred from leakage of intracellular UV-absorbing substances[a]

| Treatment | Absorbance | |
|---|---|---|
| | 260 nm | 280 nm |
| None (untreated control) | 0.545 ± 0.030 A | 0.314 ± 0.017 A |
| LA | 0.537 ± 0.024 A | 0.314 ± 0.024 A |
| NIR | 1.278 ± 0.062 B | 0.622 ± 0.019 B |
| NIR-DW | 1.486 ± 0.035 C | 0.723 ± 0.024 C |
| NIR-LA | 1.502 ± 0.042 C | 0.716 ± 0.030 C |

[a] Values are means ± standard deviations from three replications. Values in the same column followed by the same letter are not significantly different ($P > 0.05$).

**TABLE 5** Color values, moisture content, and sensory attributes of nut kernels following 5 min of NIR-LA treatment (at day 0)[a]

| Sample and treatment | Color value for parameter | | | Moisture contents (%) | Sensory attribute score | |
|---|---|---|---|---|---|---|
| | L* | a* | b* | | Texture | Flavor |
| Almonds | | | | | | |
| Control | 52.53 ± 0.95 | 13.96 ± 0.75 | 36.20 ± 0.39 | 4.81 ± 0.06 | 4.83 ± 1.03 | 4.25 ± 1.48 |
| NIR-LA | 53.76 ± 0.91 | 13.87 ± 0.38 | 37.05 ± 0.66 | 5.01 ± 0.15 | 4.58 ± 1.24 | 4.00 ± 1.13 |
| Pine nuts | | | | | | |
| Control | 68.39 ± 0.83 | −0.29 ± 0.08 | 27.60 ± 1.00 | 2.53 ± 0.05 | 5.33 ± 1.07 | 4.42 ± 1.51 |
| NIR-LA | 66.90 ± 0.64 | −0.47 ± 0.16 | 27.34 ± 0.52 | 2.82 ± 0.21 | 5.08 ± 1.00 | 4.17 ± 1.11 |

[a] Values are means ± standard deviations from three replicates. Values within each column are not significantly different ($P > 0.05$). L*, lightness; a*, redness; b*, yellowness. All moisture contents are expressed on a dry basis. Sensory attributes are from panelist scorecard analysis on a 7-point hedonic scale, where 7 is very good and 1 is very poor. $n = 13$.

## DISCUSSION

The Almond Board of California has initiated several research projects addressing the lethality of dry heat processes, which revealed that typical dry roasting processes did not deliver a minimum 4-log reduction of *S. enterica* on almonds, which is the target lethality based on a prior risk assessment study (21). In our study, NIR treatment alone was also insufficient to reduce *S.* Enteritidis by the required amount (ca. 4-log-unit reduction) on almond and pine nut kernels (Fig. 2 and 3, respectively). To achieve >4-log reductions, based on the calculated parameters of the Weibull model (scale parameter = 3.367; shape parameter = 2.435; $R^2$ = 0.95; mean squared error [MSE] = 0.01), treatment for 8.4 min would be needed for *S.* Enteritidis PT 30 on almonds. However, a roasting effect was visually observed on almond kernels treated with NIR for a slightly excessive time (over 8 min [data not shown]).

Despite high-temperature exposure, the ability of *Salmonella* cells to survive on kernels suggests that their physiological state may have imparted to them enhanced heat tolerance. An increase in heat tolerance of *Salmonella* spp. after exposure to low-$a_w$ environments has been well documented (22) and is thought to have contributed to large outbreaks of salmonellosis linked to various low-$a_w$ foods (23, 24). *Salmonella* cells on dry nut kernels have similarly high tolerance to heat because of their dehydration. To overcome this limitation, hurdle combinations have been performed on nut kernels by several researchers. Pretreatment of almonds with water before dry roasting provides a means to increase antimicrobial efficacy (25). Jeong et al. (26) used high-humidity hot air (moist-air impingement) to enhance the inactivation rate of *S.* Enteritidis on almonds. Brandl et al. (10) reported that wetting almond kernels by brief immersion in water before 45 s of dry heating yielded an additional reduction of *S.* Enteritidis of 0.43 log. Differences in moisture content of nutmeats used in hot air and oil roasting studies may have affected the rate of inactivation of *Salmonella*, as heat resistance of *Salmonella* was less in wet nutmeats than in dry nutmeats (27, 28). These studies suggest that a prewetting of the nut kernels has the potential to increase the antimicrobial efficacy of NIR heat treatment, possibly by improving the heat transfer to microsites where *Salmonella* cells are located. Therefore, we employed the prewetting procedures by spraying with DW or LA solution on almonds and pine nuts for increasing the moisture content of nut kernels. Also, in the present study, because a rotational mixer was used simultaneously with combined NIR-DW and NIR-LA treatments, excessive heating on one side of the nut samples was prevented and DW or LA sprays could evenly contact nut kernel surfaces. Even though surface

temperatures of almonds and pine nuts during the single NIR treatment were higher than during the NIR and DW or LA spray combined treatments (Fig. 4), NIR-DW and -LA treatments yielded greater reductions in cell numbers of *Salmonella* spp. than did NIR treatment alone (Fig. 2 and 3). This tendency was also observed in levels of membrane damage to each treated cell, inferred from leakage of intracellular UV-absorbing substances (Table 3).

Since sublethally injured food-borne pathogens, which are potentially as dangerous as their uninjured counterparts, are able to resuscitate and regain their pathogenicity under favorable conditions (29), it is essential to study the ongoing microbiological dynamics of the processed food after bactericidal treatments have been applied. In the present study, the extent to which sublethally injured pathogens survived after NIR-DW or -LA treatment was evaluated by plating on selective media with and without a resuscitation step. As treatment time increased, more sublethally injured cells were observed in the NIR-DW treatment than in the NIR-LA combination (Tables 1 and 2). In other words, the NIR-DW treatment for 5 min was not sufficient to achieve the target inactivation of *S.* Enteritidis (ca. 4-log reduction) on almond or pine nut kernels due to the resuscitation of injured cells, whereas the NIR-LA spray combined treatment effectively inactivated *S.* Enteritidis on nut samples without causing apparent sublethal injury to bacterial cells.

The difference in inactivation efficacy between NIR-DW and NIR-LA combination treatments may be attributed to the interaction of lactic acid with NIR heat. Low pH has long been recognized as one of the factors responsible for decreasing the heat resistance of bacterial spores and vegetative cells. Many studies have been published on the pH dependence of the heat resistance of *Salmonella* spp. (30, 31, 32). Low-pH exposure can cause sublethal injury to cell membranes, which in turn can cause disruption of the proton motive force across cell membranes, owing to loss of $H^+$-ATPase (33). This could make bacterial cell membranes more susceptible to heat treatment. However, in the present study, the level of membrane damage that can be inferred as a result of the leakage of intracellular substances of NIR-LA-treated cells was not significantly different from those of NIR-DW-treated cells (Table 3). Therefore, it is not a fully proven hypothesis, but NIR heat treatment of *S.* Enteritidis cells might induce a disturbance in the outer membrane, allowing the cells to become permeable to the lactic acid solution sprayed rather than damaging the cell membrane *per se*. In addition, temperature is a primary factor influencing organic acid activity, with increasing temperature enhancing the effectiveness of organic acids (34, 35). Thus,

the use of lactic acid sprays as an agent that sensitizes cells to heat could be exploited as a novel technique to reduce intensity of NIR heat and to increase the efficacy of existing thermal treatment. Due to the lower levels of NIR and applied LA, combined NIR-LA treatments did not change quality attributes of nut kernels significantly ($P > 0.05$) (Tables 4 and 5).

In conclusion, although there were small differences in inactivation levels between almonds and pine nuts due to the kernel size or morphological characteristics, about a 4-log reduction of *S.* Enteritidis can be achieved on almond or pine nut kernels by incorporating a simple LA spraying step prior to NIR radiant heat treatment without causing any deterioration in product quality. While spraying with organic acids has been widely adapted and proven effective by the meat industry for decontaminating livestock carcasses (36, 37), this is not currently used in nut processing. Given the results of the present study, the potential utilization of lactic acid sprays during NIR heating could be considered as an alternative to other interventions that are currently employed. This combination of approaches would have the benefit of increasing bacterial inactivation while removing the water presprayed on the kernels during NIR heating (Table 5). Thus, it avoids the need for an additional drying step during postprocessing. Furthermore, the effectiveness of this NIR-LA combined treatment could be further improved by refining the procedure, such as rearranging the radiation intensity of NIR emitters and spray volume or concentration of lactic acid (i.e., improving the effect of lactic acid through adjusting pH, taking into account $pK_a$).

## ACKNOWLEDGMENTS

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