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Effectiveness of superheated steam for inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis phage type 30, and *Listeria monocytogenes* on almonds and pistachios

Ga-Hee Ban [a], Dong-Hyun Kang [a,b,*]

[a] Department of Agricultural Biotechnology, Center for Food and Bioconvergence, and Research Institute for Agricultural and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea
[b] Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, 25354, Republic of Korea

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ABSTRACT

This study was undertaken to evaluate the effectiveness of superheated steam (SHS) on the inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis phage type (PT) 30 and *Listeria monocytogenes* on almonds and in-shell pistachios and to determine the effect of superheated steam heating on quality by measuring color and texture changes. Almonds and in-shell pistachios inoculated with four foodborne pathogens were treated with saturated steam (SS) at 100 °C and SHS at 125, 150, 175, and 200 °C for various times. Exposure of almonds and pistachios to SHS for 15 or 30 s at 200 °C achieved >5 log reductions among all tested pathogens without causing significant changes in color values or texture parameters ($P > 0.05$). For both almonds and pistachios, acid and peroxide values (PV) following SS and SHS treatment for up to 15 s and 30 s, respectively, were within the acceptable range (PV < 1.0 meq/kg). These results show that thermal application of 200 °C SHS treatment for 15 s and 30 s did not affect the quality of almonds and pistachios, respectively. Therefore, SHS treatment is a very promising alternative technology for the tree nuts industry by improving inactivation of foodborne pathogens on almonds and pistachios while simultaneously reducing processing time.

1. Introduction

In recent years, concerns about foodborne outbreaks involving low water activity (a_w) foods have increased (Scott et al., 2009), because salmonellosis has been known to be linked to diverse dry foods such as almonds, peanuts, and peanut butter (CDC, 2004, 2007; Isaacs et al., 2005; Palumbo et al., 2015). More recently, *Escherichia coli* O157:H7 illnesses have been epidemiologically linked to consumption of in-shell hazelnuts (FDA, 2011). In 2010 and 2014, walnuts were recalled after isolation of *Salmonella* (FDA, 2010) and *Listeria monocytogenes* (FDA, 2014). Cross contamination of raw almonds can readily occur under typical harvesting, drying, and hulling-shelling practices (Isaacs et al., 2005). Furthermore, foodborne pathogens are able to survive in dry environments such as almond kernels and pistachios for prolonged periods of time (Kimber et al., 2012; Uesugi et al., 2006).

In the US, almonds are required to be pasteurized to achieve a 4-log reduction in *Salmonella* bacteria (7 CFR Part 981). To inactivate *Salmonella* on almonds, several methodologies such as propylene oxide fumigation (Danyluk et al., 2005), infrared heat (Brandl et al., 2008), hot oil (Du et al., 2010), high hydrostatic pressure (Willford et al., 2008), acidic sprays (Pao et al., 2006), chlorine dioxide (Wihodo et al., 2005), and steam (Chang et al., 2010; Lee et al., 2006) have been evaluated. However, a maximum residue limit of propylene oxide fumigant has not been established (Brandl et al., 2008) and chlorine dioxide can lead to discoloration of almond surfaces at high concentrations (Wihodo et al., 2005). In particular, saturated steam (SS) pasteurization increases moisture content of the nuts and thus, requires additional processing to remove excess moisture before storage (Brandl et al., 2008).

Superheated steam (SHS) is steam which is given additional heat to raise its temperature above the saturation temperature at a constant pressure, and a drop in temperature of SHS will not result in condensation unless the temperature is decreased to below the saturation temperature point corresponding to the processing pressure (Cenkowski et al., 2007). SHS has long been known as a safe, non-polluting technology with low energy consumption (Chou and Chua, 2001). SHS transfers a larger amount of heat to the subject of treatment than SS (James et al., 2000; Topin and Tadrist, 1997). However, the inactivation of foodborne pathogens by SHS has rarely been studied, only for *Salmonella* on almonds (Bari et al., 2010).

Therefore, the purpose of this study was to compare and evaluate the effectiveness of SS and SHS for inactivating four foodborne pathogens on the surface of almonds and in-shell pistachios. In addition,

* Corresponding author at: Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, South Korea.
E-mail address: kang7820@snu.ac.kr (D.-H. Kang).

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the effect of SHS treatment on the quality of almonds and pistachios was determined by measuring the color change, texture, acid value (AV), and peroxide value (PV).

2. Materials and methods

2.1. Almonds and pistachios

Raw (untreated) almonds (*Prunus dulcis*), 'Nonpareil' cultivar, used in this study (size 27–30: 27 to 30 kernels per 28 g) were provided by Hilltop Ranch (Ballico, CA). Raw in-shell pistachios (*Pistacia vera*) used in this study were large-sized U.S. Extra number 1 grade, obtained from Setton International Foods Inc. (Terra Bella, CA).

2.2. Bacterial strains and inoculum preparation

Three strains each of *E. coli* O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890), *S.* Typhimurium (ATCC 19585, ATCC 43971, DT 104), *S.* Enteritidis PT 30 (ATCC BAA-1045) and *L. monocytogenes* (ATCC 15315, ATCC 19114, ATCC 19115) were obtained from the bacterial culture collection at Seoul National University (Seoul, Korea) and used in this study. The *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* strains were isolated from humans and animals and these ones have been used in various research studies involving inactivation of foodborne pathogens on foods. *S.* Enteritidis PT 30 (ATCC BAA-1045) was isolated from raw almonds associated with the 2000 to 2001 outbreak. Stock cultures were stored 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. Working cultures were streaked onto tryptic soy agar (TSA; Difco), incubated at 37 °C for 24 h, and stored at 4 °C.

Before each treatment, bacterial strains were prepared according to the method of Danyluk et al. (2005) with minor modifications. For growth experiments, the inoculum consisted of stationary phase cells that were obtained by inoculating TSB with a single colony from a TSA plate and incubating at 37 °C for 24 ± 2 h. A loop of this culture was transferred into TSB and incubated at 37 °C for 18 ± 2 h to ensure healthy cell growth. This overnight culture (1 ml) was spread onto TSA plates which were then incubated at 37 °C for 24 ± 2 h to produce a bacterial lawn. Four plates were prepared per 400 g almond and pistachio samples. Following incubation, bacterial cells were collected with a sterile cotton swab and suspended in 25 ml of 0.2% peptone water. The cell suspensions were pooled and thoroughly mixed for 1 min with a magnetic stir bar and stir plate. Inoculum levels were determined by tenfold serial dilution of inoculum in 0.2% peptone water and spread plating onto TSA, Sorbitol MacConkey Agar (SMAC; Difco) for *E. coli* O157:H7, Xylose Lysine Desoxycholate Agar (XLD; Difco) for *S.* Typhimurium and *S.* Enteritidis PT 30, and Oxford Agar Base (OAB; Difco) with antimicrobic supplement (Difco) for *L. monocytogenes*. Plates were incubated at 37 °C for 24 ± 2 h.

2.3. Inoculation procedure

Each almond (400 g) and in-shell pistachio sample was weighed into a plastic polyethylene bag (30 × 25 cm) and 25 ml of inoculum was added. The bag was sealed and thoroughly mixed by hand massaging for 60 s. Almonds and pistachios were poured out of the bag and spread onto filter paper and dried overnight at room temperature (22 ± 2 °C). In a preliminary study, it was found that moisture contents of samples prior to inoculation were not significantly different from those following inoculation and drying, but not yet processed.

2.4. Saturated steam and superheated steam treatment

SS at 100 °C, produced by a SS generator, was introduced into a SHS steam generator through a flexible tube. SS was converted into SHS by heating with an electrical resistance heater in the SHS generator. The maximum temperature of SHS generated in this study was about 200 °C. During these experiments, the SS and SHS temperature was controlled automatically by means of a temperature sensor and intelligent power module in each of the steam generators.

Dried inoculated almonds and pistachios were spread into a single layer on a stainless steel treatment grid and placed in an insulated steam treatment chamber (external diameter 23 cm; external height, 32 cm; internal diameter, 17 cm; internal height, 22.5 cm). A valve placed on top of the treatment chamber was used to control steam flow. Steam passed through the flexible hose and chamber by opening the steam valve. Almonds and pistachios were steam treated for 1, 5, 10, 15, and 20 s and 1, 5, 10, 20, and 30 s, respectively. SS treatment was performed at 100 °C while SHS treatments were performed at 125, 150, 175, and 200 °C. The basket was immediately removed from the chamber after each treatment, and almonds or pistachios were then placed in a stomacher bag (Labplas Inc., Sainte-Julie, Quebec, Canada).

2.5. Bacterial enumeration

Treated almond kernels and pistachios were placed in stomacher bags along with 50 ml of 0.2% peptone water. Almond samples were homogenized for 2 min with a mechanical stomacher (EASY MIX, AES Chemunex, Rennes, France). Pistachio samples were shaken for 30 s, rubbed by hand for 15 s, and then shaken for an additional 30 s. After homogenization, 1 ml aliquots of samples were 10-fold serially diluted with 9 ml of sterile 0.2% peptone water, and 100 μl of appropriate dilutions were spread-plated onto SMAC, XLD, and OAB with antimicrobic supplement to enumerate surviving populations of *E. coli* O157:H7, *S.* Typhimurium and *S.* Enteritidis PT 30, and *L. monocytogenes*, respectively. When low bacterial numbers were anticipated, 1 ml was distributed over four Petri dishes (0.25 ml each). As a control (time-zero survival), untreated almonds and pistachios inoculated with the four pathogens were stomached and shaken, respectively, diluted and plated. All plates were incubated at 37 °C for 24 h, and then colonies enumerated. To confirm pathogen identity, presumptive colonies were randomly selected from selective media and subjected to biochemical and serological tests. These tests consisted of the *E. coli* O157:H7 latex agglutination assay (Oxoid, Basingstoke, UK), the *Salmonella* latex agglutination assay (Oxoid, Basingstoke, UK), and the API Listeria test (BioMérieux, Hazelwood, MO). D-values were calculated from the negative inverse slope of the log survival (CFU/g) versus time plot (Murphy et al., 2000).

2.6. Color and texture measurement

Color assessments were measured using a Minolta colorimeter (Model CR-400; Minolta Camera Co. Ltd., Osaka, Japan). Measurements were taken from SS and SHS treated and untreated samples measured at random locations on almonds and pistachios and averaged. L* (intensity of lightness), a* (intensity of redness), and b* (intensity of yellow color) values were measured in triplicate for each treatment.

Changes in texture of SS and SHS treated almonds and pistachios were evaluated with a texture analyzer (TA-CT3, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a blade set probe. After treated samples were dried, a sample was placed onto the press holder, and a blade was moved down at 2 mm/s. Maximum force was recorded using Texturepro CT software (Brookfield Engineering Laboratories, Inc.). Three measurements were performed for each treatment with independently-prepared samples.

2.7. Acid value and peroxide value

Indicators of lipid oxidation in SS and SHS treated almonds and pistachios were measured by AV and PV. AV and PV were determined using

official methods Cd 3d-63 and 8b-90 of the American Oil Chemists' Society (AOAC, 1998, 2007). Analyses were done in triplicate.

2.8. Statistical analysis

All experiments were repeated three times with duplicate samples. Triplicate data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and the separation of means was tested by Duncan's multiple-range test at a probability level of $P < 0.05$.

3. Results

3.1. Inactivation of pathogenic bacteria on almonds

Survival (log CFU/g) of *E. coli* O157:H7, *S.* Typhimurium, *S.* Enteritidis PT 30, and *L. monocytogenes* on almonds and pistachios after SS and SHS treatment are shown in Figs. 1 and 2. Initial inoculum levels of *E. coli* O157:H7, *S.* Typhimurium, *S.* Enteritidis PT 30, and *L. monocytogenes* on almonds were 6.5, 6.8, 7.0, and 6.0 log CFU/g and those on pistachios were 6.4, 6.6, 6.7, and 5.8 log CFU/g, respectively. Significant ($P < 0.05$) log reductions of the four pathogens were observed as the duration of SHS treatment increased. SHS treatment at 200 °C for 15 s achieved 6.2, 6.5, 6.7, and 5.7 log reductions in *E. coli* O157:H7, *S.* Typhimurium, *S.* Enteritidis PT 30, and *L. monocytogenes* on almonds, respectively, whereas SS treatment at 100 °C attained 3.9, 3.8, 2.9, and 2.4 log reductions for each pathogen. Levels of the four pathogens on pistachios were reduced by 6.1, 6.3, 6.4, and 5.5 log for *E. coli* O157:H7, *S.* Typhimurium, *S.* Enteritidis PT 30, and *L. monocytogenes*, respectively, after SHS treatment at 200 °C for 30 s, whereas these pathogens experienced log reductions of 3.0, 3.3, 2.7, and 2.7 after SS treatment at 100 °C for the same time interval. SHS treatment caused an additional 1.8–4.2 and 2.1–3.6 log reduction of the three pathogens on almonds and pistachios, respectively, compared to SS treatments. Populations of the four pathogens on almonds were reduced to below the detection limit (0.3 log CFU/g) when subjected to heating for 15 s at 175 °C and for 10 s at 200 °C, for 15 s at 175 and 200 °C, for 15 s at 200 °C, and for 20 s at 175 °C and for 15 s at 200 °C for *E. coli* O157:H7, *S.* Typhimurium, *S.* Enteritidis PT 30, and *L. monocytogenes*, respectively.

Fig. 2 shows that the overall reduction tendencies of *E. coli* O157:H7, *S.* Typhimurium, *S.* Enteritidis PT 30, and *L. monocytogenes* on pistachios were similar to those on almonds. However, a longer treatment time was needed for pistachios than for almonds to reduce populations to below the detection limit (0.3 log CFU/g). The four pathogens on pistachios were reduced to below the detection limit (0.3 log CFU/g) when treated for 30 s at 175 and 200 °C, for 30 s at 175 °C and for 20 s at 200 °C, for 30 s at 200 °C, and for 30 s at 200 °C for *E. coli* O157:H7, *S.* Typhimurium, *S.* Enteritidis PT 30, and *L. monocytogenes*, respectively.

Table 1 shows decimal reduction time determined from survival curves for four foodborne pathogens on almonds and pistachios. The four pathogens on pistachios showed a higher D-value (9.23–11.15 s and 2.22–4.56 s) than those on almonds (4.87–6.68 s and 1.31–2.41) treated with SS at 100 °C and SHS at 200 °C, respectively.

3.2. Effect of SS and SHS treatment on color and texture of almonds and pistachios

The L*, a*, and b* values of SS and SHS treated almonds and pistachios were not significantly ($P > 0.05$) different from those of untreated controls (data not shown). There were no significant ($P > 0.05$) differences between maximum load values of texture measurements among all tested samples, indicating that treatment with SHS at 200 °C for 15 s and 30 s did not significantly ($P > 0.05$) change the quality of almonds and pistachios, respectively (data not shown).

3.3. Effect of SS and SHS treatment on lipid oxidation of almonds and pistachios

Changes of oxidative rancidity of SS and SHS treated almonds and pistachios are shown in Tables 2 and 3. There was a slight difference

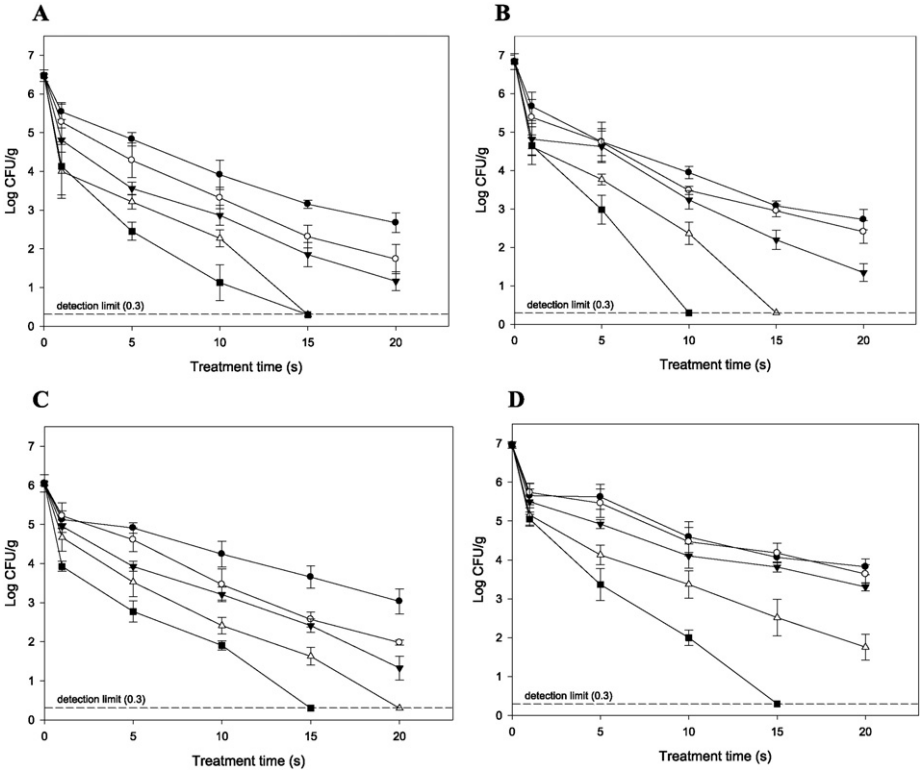

Fig. 1. Survival curves for *E. coli* O157:H7 (A), *S.* Typhimurium (B), *L. monocytogenes* (C), and *S.* Enteritidis PT 30 (D) on almonds treated with SS at 100 °C (●), SHS at 125 °C (○), SHS at 150 °C (▼), SHS at 175 °C (△), SHS at 200 °C (■).

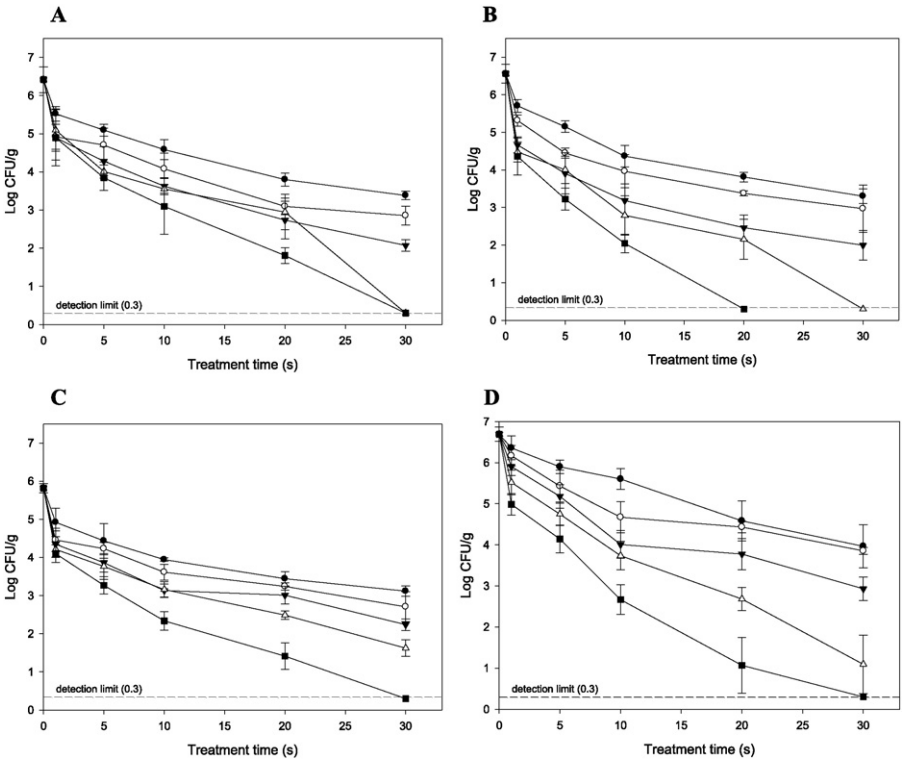

Fig. 2. Survival curves for *E. coli* O157:H7 (A), *S.* Typhimurium (B), *L. monocytogenes* (C), and *S.* Enteritidis PT 30 (D) on pistachios treated with SS at 100 °C (●), SHS at 125 °C (○), SHS at 150 °C (▼), SHS at 175 °C (△), SHS at 200 °C (■).

in AV between samples treated with SS and SHS compared to controls. After 1 s of SS and SHS heating, no significant ($P > 0.05$) differences between samples were found as temperature increased. After SS or SHS exposure for 20 s and 30 s on almonds and pistachios, respectively, there were significant differences between the untreated and treated samples in AV and PV ($P < 0.05$). The PV of almonds and pistachios treated with SS and SHS for 20 s and 30 s, respectively, decreased compared to untreated samples.

4. Discussion

Foodborne pathogens on tree nuts have been linked to large-scale outbreaks and are responsible for the recall and destruction of large quantities of nut products (Isaacs et al., 2005; Kirk et al., 2004; Miller et al., 2012). This study has evaluated the effectiveness of SHS against four pathogens on almonds and pistachios to assess its ability to control pathogens on nuts.

Table 1
D-values for *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes*, and *S.* Enteritidis PT 30 inoculated on almonds and pistachios treated with SS and SHS.

	Mean D-value (s)				
(A) Almonds	Temperature (°C)				
	100	125	150	175	200
E. coli O157:H7	5.28	4.24	3.77	2.39	1.88
S. Typhimurium	4.87	4.53	3.64	2.24	1.31
L. monocytogenes	6.68	4.91	4.25	3.40	2.41
S. Enteritidis PT 30	6.67	6.25	5.71	3.92	2.04
(B) Pistachios					
	100	125	150	175	200
E. coli O157:H7	9.88	8.44	6.91	5.83	3.26
S. Typhimurium	9.23	8.52	6.61	4.58	2.22
L. monocytogenes	11.12	9.73	8.39	7.16	4.56
S. Enteritidis PT 30	11.15	10.38	7.71	5.18	3.40

SHS heating has proven to be one of the most useful drying methods for biological or non-biological products, such as foods (Braud et al., 2001), wood chips (Johansson et al., 1997), or even porous materials (Hager et al., 1997). Furthermore, SHS combined with impingement technology, which enhances the strength of both steam blanching and impingement technology, has resulted in a uniform, rapid, energy-efficient blanching process (Xiao et al., 2014).

In recent years, investigations of inactivation efficiency utilizing SHS have been performed by a few researchers. Kondjoyan and Portanguen (2008) reported that SHS was distinctly more efficacious for inactivating *Listeria innocua* than non-SHS, leading to an average reduction of more than 5 log after 30 s treatment. Bari et al. (2010) conducted a SHS and gas catalytic infrared heat treatment study to inactivate *Salmonella* on raw almonds. SHS treatment for 70 s followed by catalytic infrared heat treatment for 70 s was able to reduce *Salmonella* populations by 5.73 log CFU/g (below the detection limit). Furthermore, no *Salmonella* was detected in the enrichment medium, suggesting that *Salmonella* cells were completely inactivated. Our previous study showed that SHS treatment effectively reduced populations of biofilm cells and reduced disinfection time compared to SS treatments (Ban et al., 2014). In that study, the numbers of biofilm cells were reduced to below the detection limit (1.48 log CFU/coupon), after exposure to 200 °C steam for 30 s or 10 s on PVC or stainless steel, respectively. In the present study, foodborne pathogens on almonds and pistachios were reduced to an undetectable level (0.3 log CFU/g) after 200 °C steam treatment for 15 s and 30 s, respectively.

There were rapid initial reductions of foodborne pathogens on samples treated with SS and SHS for 1 s and 5 s. Thereafter, reductions occurred at a slower rate, especially for those treated with SS. When SS condenses on a cooled surface, a continuous film of condensate is formed which creates a thermal barrier to the further flow of heat (Tanner et al., 1968), whereas condensation temporarily occurs and then evaporates when SHS contacts a surface (Iyota et al., 2001). The continuous film of condensate formed by SS can protect bacteria from thermal treatment and increase their heat resistance. This

Table 2
Acid values of almond (A) and pistachio (B) after exposure to SS and SHS for a range of treatment times (0–20 s).

(A) Almonds						
	Steam duration (s)					
Temperature (°C)	0	1	5	10	15	20
25	0.34 ± 0.03 A	–	–	–	–	–
100	–	0.34 ± 0.02 A	0.36 ± 0.03 A	0.34 ± 0.02 A	0.34 ± 0.02 A	0.30 ± 0.01 AB
125	–	0.33 ± 0.01 A	0.35 ± 0.01 A	0.36 ± 0.02 A	0.35 ± 0.03 A	0.29 ± 0.01 B
150	–	0.34 ± 0.03 A	0.35 ± 0.03 A	0.35 ± 0.01 A	0.34 ± 0.02 A	0.29 ± 0.02 AB
175	–	0.35 ± 0.03 A	0.35 ± 0.01 A	0.35 ± 0.03 A	0.31 ± 0.01 AB	0.29 ± 0.02 AB
200	–	0.35 ± 0.03 A	0.34 ± 0.03 A	0.34 ± 0.01 A	0.31 ± 0.02 AB	0.29 ± 0.01 B
(B) Pistachios						
	0	1	5	10	20	30
25	0.54 ± 0.02 A	–	–	–	–	–
100	–	0.53 ± 0.02 A	0.52 ± 0.02 A	0.53 ± 0.03 A	0.56 ± 0.04 A	0.53 ± 0.02 A
125	–	0.53 ± 0.02 A	0.50 ± 0.01 B	0.53 ± 0.03 A	0.55 ± 0.01 A	0.55 ± 0.04 A
150	–	0.57 ± 0.03 A	0.53 ± 0.02 A	0.53 ± 0.04 A	0.55 ± 0.02 A	0.53 ± 0.02 A
175	–	0.53 ± 0.06 A	0.52 ± 0.03 A	0.51 ± 0.02 A	0.55 ± 0.04 A	0.55 ± 0.01 A
200	–	0.55 ± 0.06 A	0.53 ± 0.01 A	0.52 ± 0.04 A	0.50 ± 0.02 B	0.57 ± 0.01 A

The values are means ± standard deviations from three replications.
Means followed by the same letter in a data series (column) are not significantly different ($P > 0.05$).

phenomenon can explain the decreasing reduction rates and tailing effect of general steam treatment (SS) for inactivation of foodborne pathogens on foods. A similar tendency of reduction rates of foodborne pathogens on foods was observed by other researchers. Kondjoyan and Portanguen (2008) observed that the overall pattern of *L. innocua* inactivation by non-SHS treatment displayed an initial pattern of rapid decline followed by much slower decline thereafter. Du et al. (2010) observed significant and rapid reductions of *Salmonella* Enteritidis on almonds in the first 30 s of hot oil heating and a slower reduction rate after 30 s.

In the study of inactivation of biofilm cells by SHS, cells were exposed to different thermal stresses between stainless steel and PVC coupons, because thermal conductivity (k) of stainless steel (16 W/m·K) is much higher than that of PVC (0.19 W/m·K) when heat energy of SS or SHS is transferred to coupon surfaces (Ban et al., 2012). However, in the present study, thermal conductivity of the two types of nuts did not influence the inactivation of foodborne pathogens because their k values are similar (almonds, 0.64 W/m·K; pistachios, 0.66 W/m·K) (Choi and Okos, 1986). It seems to be relatively more difficult for steam to penetrate beneath the shells to inactivate foodborne pathogens on pistachio kernels compared to almonds which have no shells to interfere. For this reason, bacterial populations on pistachios did not receive the same thermal effect as on almonds. Although their exterior features are different, foodborne pathogens on pistachios within the shell were reduced to undetectable levels (0.3 log CFU/g) as well as on almonds.

E. coli O157:H7, *S.* Typhimurium, *S.* Enteritidis PT 30, and *L. monocytogenes* on almonds and pistachios were more resistant to SS compared with SHS treatments. For instance, it required 6.67 s and 4.87 s to kill 90% of *S.* Enteritidis PT 30 and *S.* Typhimurium on almonds at 100 °C, respectively, whereas it only took about 2.04 s and 1.31 s to achieve the same at 200 °C. Lee et al. (2006) observed that a higher D-value (16.13 s) was calculated for *S.* Enteritidis on Mission almonds than for the Nonpareil variety (12.22 s) after steam treatment at 93 °C. And, our previous study observed that D-values for *S.* Typhimurium biofilm cells on polyvinyl chloride and stainless steel treated with SHS 200 °C were 3.1 s and 1.34 s, respectively (Ban et al., 2014).

Bacteria can survive in the cracks, crevices, and pores of almonds and pistachios which can prevent their coming into contact with several

Table 3
Peroxide (meq/kg) values of almonds (A) and pistachios (B) after exposure to SS and SHS for a range of treatment times (0–20 s).

(A) Almonds						
	Steam duration (s)					
Temperature (°C)	0	1	5	10	15	20
25	0.24 ± 0.02 A	–	–	–	–	–
100	–	0.22 ± 0.01 A	0.22 ± 0.02 AB	0.21 ± 0.02 AB	0.22 ± 0.01 A	0.19 ± 0.01 B
125	–	0.24 ± 0.02 A	0.23 ± 0.01 A	0.21 ± 0.02 AB	0.21 ± 0.01 AB	0.20 ± 0.02 AB
150	–	0.23 ± 0.01 A	0.23 ± 0.01 A	0.21 ± 0.02 AB	0.21 ± 0.02 AB	0.20 ± 0.02 AB
175	–	0.22 ± 0.01 A	0.24 ± 0.02 A	0.22 ± 0.01 A	0.20 ± 0.01 AB	0.19 ± 0.01 B
200	–	0.24 ± 0.02 A	0.23 ± 0.01 A	0.21 ± 0.01 AB	0.21 ± 0.01 AB	0.19 ± 0.01 B
(B) Pistachios						
	0	1	5	10	20	30
25	0.25 ± 0.03 A	–	–	–	–	–
100	–	0.24 ± 0.02 A	0.24 ± 0.02 A	0.25 ± 0.02 A	0.25 ± 0.02 A	0.21 ± 0.01 AB
125	–	0.24 ± 0.02 A	0.25 ± 0.03 A	0.25 ± 0.02 A	0.22 ± 0.01 AB	0.21 ± 0.01 AB
150	–	0.25 ± 0.02 A	0.24 ± 0.02 A	0.23 ± 0.02 AB	0.22 ± 0.02 AB	0.20 ± 0.01 B
175	–	0.25 ± 0.03 A	0.25 ± 0.01 A	0.25 ± 0.01 A	0.24 ± 0.02 A	0.19 ± 0.02 B
200	–	0.25 ± 0.01 A	0.24 ± 0.02 A	0.23 ± 0.01 A	0.22 ± 0.02 AB	0.19 ± 0.02 B

The values are means ± standard deviations from three replications.
Means followed by the same letter in a data series (column) are not significantly different ($P > 0.05$).

control agents such as hot air and hot water. Steam is known to effectively penetrate cavities, crevices, and feather follicles that may provide protection for surface-attached microorganisms against water because of the high surface tension of aqueous fluids (Morgan et al., 1996). Lee et al. (2006) observed that 35 s of 93 °C steam treatment resulted in a 3.8 log reduction of *Salmonella* on 'Nonpareil' almonds and 61 s of steam duration would be required to attain 5 log reductions. Although steam treatment for 30 to 40 s effectively reduced *Salmonella* populations in almonds, the moisture content increased during the treatment time. Chang et al. (2010) ascertained that a 5 log reduction of *S.* Enteritidis was achieved following steam treatment at 95 °C for 25 s. However, visual quality degradation including detachment of almond skin, wrinkled almond surfaces and the presence of small gray spots on almond skin were observed after a 35 s steam pasteurization treatment. In our study, the same phenomenon was observed, especially after 100 °C SS treatment (data not shown). Moisture addition results in quality loss caused by loosened skin and increased mold growth as well as added costs for removing excess moisture from SS treated almonds (Almond Board of California, 2014).

In the present study, four foodborne pathogens on almonds and pistachios were decreased to detection limit (0.3 log CFU/g) inactivated after SHS treatment for 20 and 30 s, respectively. And, in our preliminary study, moisture contents in samples after treatment with saturated steam at 100 °C were increased compared with untreated samples. However, even though the moisture contents in samples after treatment with superheated steam at 200 °C were slightly increased and there were no significant difference with untreated samples (3.77 ± 0.12, 5.28 ± 0.31, and 4.08 ± 0.24% for untreated, SS at 100 °C, and SHS at 200 °C for almonds, respectively; 4.72 ± 0.19, 5.97 ± 0.34, and 5.16 ± 0.28% for untreated, SS at 100 °C, and SHS at 200 °C for pistachios, respectively). Unlike SS, a drop in SHS temperature during processing will not result in condensation of steam, as long as the temperature is still higher than the saturation temperature at the processing pressure (Pronyk et al., 2004). SHS drying has been used for various dried foods including flour, paprika powder, and onion powder (van Deventer and Heijmans, 2001). These research results imply that SHS treatment can be used as a pasteurization technology for almonds and pistachios without the need to be concerned about increasing moisture content. Before application of this technology can occur, additional researches about relevance of moisture level on efficacy of SS and SHS will need to be investigated because of heat resistance increases as the moisture contents in heating environment decrease.

In previous almond steam pasteurization studies, evaluations of quality changes were limited to subjective visual observations and statistical analyses were rarely conducted (Lee et al., 2006). For this reason, our experimental conditions focused on maintaining the quality of almonds and pistachios after SHS treatment compared to untreated samples. After SHS treatment for up to 20 and 30 s on almonds and pistachios, respectively, color values and maximum load values were not significantly ($P > 0.05$) different from untreated controls. Although L*-values (lightness) were not consistent, significant differences in color measurements were not observed. The PV of SS or SHS treated samples decreased compared to untreated samples. Hot air and RF treatments did not significantly affect the PV and free fatty acids of almonds when measured immediately after treatment (Gao et al., 2010). The mean PV of hot air and RF treated almonds after 10 and 20 day storage periods were lower than those of untreated controls (Gao et al., 2010). This might be due to possible inactivation of lipoxygenase enzymes by heat treatments (Buranasompob et al., 2001). Lipoxygenase in dry pinto beans lost all activity after 15 s at 100 °C and 93% of their initial activity after 10 min at 65 °C (McCurdy et al., 1983). For both almonds and pistachios, the AV and PV after SS and SHS treatment for up to 15 s and 30 s, respectively, fell within the acceptable range (PV < 1.0 meq/kg) for good quality used by industry (Forbus et al., 1980). Thus, short time heat treatment of almonds and pistachios did not promote rancidity. These results show that thermal application of 200 °C SHS treatment for 15 s and 30 s, respectively, did not affect the quality of almonds and pistachios.

In conclusion, this research demonstrated that SHS treatment leads to effective inactivation of *E. coli* O157:H7, *S.* Typhimurium, *S.* Enteritidis PT 30, and *L. monocytogenes* on almonds and pistachios, as well as preventing quality deterioration. SHS treatment to control foodborne pathogens on almonds and pistachios can obtain more than 5-log reduction in short treatment time and protect against increasing moisture contents compared with SS treatment. Therefore, SHS treatment is a very promising alternative technology for the tree nut industry by improving inactivation of foodborne pathogens on almonds and pistachios while reducing processing time and expense.

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