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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

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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,

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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