LWT - Food Science and Technology 97 (2018) 555–560

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Hot water treatment as a kill-step to inactivate *Escherichia coli* O157:H7, *Salmonella enterica*, *Listeria monocytogenes* and *Enterococcus faecium* on in-shell pecans

Karuna Kharel[a], Veerachandra K. Yemmireddy[a], Charles J. Graham[b], Witoon Prinyawiwatkul[a], Achyut Adhikari[a,*]

[a] School of Nutrition and Food Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA, 70803-4200, USA
[b] Red River Research Station, Louisiana State University Agricultural Center, Bossier City, LA, 71112, USA

ARTICLE INFO

Keywords:
Pecans
Microbial contamination
Thermal treatment
D-value
Kill-step

ABSTRACT

In-shell pecans are susceptible to microbial contamination. This study was performed to investigate feasibility of using hot water treatment as a kill-step for food-borne pathogens during pecan shelling. In-shell pecans were subjected to hot water at 70, 80 or 90 °C for 1, 2, 3, 4 or 5 min. The time-temperature treatments to achieve a 5-log reduction of *Salmonella enterica*, *Escherichia coli* O157:H7, *Listeria monocytogenes,* and non-pathogenic *Enterococcus faecium* were determined. Thermal death values were determined for each tested condition. *L. monocytogenes* was most susceptible to heat treatment and were reduced by 4.6 ± 0.35 log CFU/g at 70 °C for 5 min, while 3–5 min at 80 and 90 °C treatments was required to achieve a similar reduction level for *S. enterica*, *E. coli* O157:H7, and *E. faecium*. *S. enterica* were most resistant and required 4 min treatment time to achieve a 5-log reduction at 80 and 90 °C. The D-values ranged from 1.15 to 1.72, 0.83 to 1.19, and 0.41–0.92 min at 70, 80 and 90 °C, respectively. *E. faecium* had the highest D-value (1.72 min at 70 °C), indicating a potential surrogate for process validation for pecan industries. Utilizing proper hot water treatment during pecan shelling could reduce food safety risk.

1. Introduction

Low-moisture foods such as tree-nuts with water activity lower than 0.7 are presumed to be low-risk food (Blessington, Theofel, & Harris, 2013; Harris, 2012). However, in the past few years tree nuts such as pecans, almonds, walnuts, pine nuts, pistachios, and mixed nuts have frequently been associated with various recalls and outbreaks due to contamination with foodborne pathogens such as *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* (Zhang et al., 2017). Even at low level of contamination (10–100 cells/gm), *S. enterica* have been reported for outbreaks associated with high fat and low moisture foods such as chocolate and peanut butter (Kapperud et al., 1990). Studies have shown that infectious dose was low possibly due to the high fat and low moisture in foods like nuts that protects organisms from the highly acidic condition of the stomach (Aviles, Klotz, Smith, Williams, & Ponder, 2013).

Pecans are one of the several most favored tree nuts consumed worldwide in different forms. The microbial food safety of pecans depends on the pre and post-harvest production and processing practices (Beuchat & Pegg, 2013). A quantitative risk assessment study by Farakos et al. (2017) shows that there is a possibility of risk of salmonellosis in U.S. population on consumption of *Salmonella* contaminated pecan. They reported that the shelling process of pecans during post-harvest treatments and acquiring illness at home by consuming uncooked pecans are well correlated. Post-harvest practice during pecan shelling includes conditioning of pecans to facilitate kernel separation, minimize kernel breakage and increase the shelling efficiency and can help to reduce the microbial levels from pecans (Beuchat & Pegg, 2013). Some of the conditioning methods currently used by industries are: (i) soaking in hot water at > 81 °C for 1–8 min or steam processing for 6–8 min; (ii) immersing in cold water (usually chlorinated) for 8 h and then draining for 16–24 h; or (iii) soaking in chlorinated water with a minimum free chlorine concentration of 200 ppm at 15–30 °C for 2 min (Beuchat & Mann, 2011; Farakos et al., 2017). However, as per our knowledge, none of the methods are scientifically validated as a "kill-step" which requires a 5 log reduction for a combination of potential pathogens such as *E. coli* O157:H7, *L. monocytogenes,* and *S. enterica*. Farakos et al. (2017) reported that hot conditioning, in comparison to

* Corresponding author.
E-mail address: acadhikari@agcenter.lsu.edu (A. Adhikari).

https://doi.org/10.1016/j.lwt.2018.07.048
Received 27 May 2018; Received in revised form 22 July 2018; Accepted 24 July 2018
Available online 25 July 2018

cold, has a significant impact on reducing the potential risk of salmonellosis as it effectively reduces *Salmonella* by up to 4 log. Beuchat and Mann (2011) and Harris, Uesugi, Abd, and McCarthy (2012) demonstrated the efficacy of hot water treatment to reduce *S. enterica* by 5 log CFU/g from pecans and almonds, respectively. However, these studies do not evaluate the effect of hot water treatment on inactivation of pathogens like *E. coli* O157:H7 and *Listeria monocytogenes*.

To minimize the food safety risks, process validation should include use of various potential pathogens associated with the food or pathogens associated with known foodborne outbreaks (Swanson, 2011). Hence the main objectives of this study were to determine (i) hot water treatment conditions to achieve a 5 log reduction of *S. enterica, E. coli* O157:H7, *L. monocytogenes*, and *E. faecium* on in-shell pecans, and (ii) the rate of thermal lethality of tested organisms.

2. Materials and methods

2.1. Selection of pecans

Raw in-shell pecans (*Carya illinoinensis*) harvested from several Louisiana orchards during the October/November season of 2015–2016 were obtained from Louisiana State University Pecan Research and Extension Station at Bossier city, LA. These pecans were stored in woven polypropylene mesh bags at 4 °C, for approximately 3 months, until they were used in experiments.

2.2. Selection of bacteria

Several different outbreak strains of *S. enterica, E. coli* O157:H7, *L. monocytogenes* as well as non-pathogenic strain of *Enterococcus* spp., were used in this study. These pathogenic strains were provided by Dr. Michelle D. Danyluk at University of Florida and were similar to the ones used in their study on peanuts and pecan kernels (Brar, Proano, Friedrich, Harris, & Danyluk, 2015). *E. faecium* (ATCC 8459), a non-pathogenic organism was used as a surrogate organism for *S. enterica.* A mutant strain of *E. faecium* resistant to nalidixic acid was developed in our lab by following the method described by Parnell, Harris, and Suslow (2005).

2.3. Inoculum preparation

Frozen cultures of nalidixic acid resistant mutant of *S. enterica, E. coli* O157:H7, *L. monocytogenes,* and *E. faecium* were subcultured twice in tryptic soy broth (TSB) or TSBY (TSB with 0.6% yeast extract for *L. monocytogenes*) supplemented with nalidixic acid (TSBN) at 50 μg/ml with incubation at 37 °C for 24 h. Then, 1 ml of each overnight bacterial culture was plated on tryptic soy agar (TSA) supplemented with 50 μg/ml nalidixic acid (TSAN) and incubated at 37 °C for 24 h. Each strain was grown on TSAN plates to develop resistance towards subsequent stress conditions as suggested by Uesugi, Danyluk, and Harris (2006). The resultant lawn of bacteria on TSAN was scraped-off with a sterile glass rod using 7 ml of 0.1% sterile peptone water. In this manner, a total of 5 ml of inoculum was collected from each strain of pathogen/surrogate on TSAN plate, and separate cocktails of bacteria were prepared by mixing individual strains in a 400 ml stomacher® bag (Control Numero 5, Seward, UK). A total of 100 ml of inocula volume was maintained in 0.1% peptone water for each bacterial mixture.

2.4. Inoculation of pecans

Whole, undamaged in-shell pecans were selected and stored overnight inside the bio-safety cabinet at room temperature (21 °C). Pecans (n = 28) weighing 310 ± 10 g per batch were added to the stomacher bag containing 100 ml of a cocktail strain of each organism at 21 °C. Later the bags containing pecans and respective inoculums were hand massaged for a minute. The pecans in the bag were submerged in the inoculum for 1 h with frequent mixing and hand massaging. The inoculated pecans were then aseptically transferred to large petri dishes (150 × 15 mm) and air dried for 20 min inside the bio-safety cabinet. After that, pecans were placed in sterilized filter bags (T-Sac, tea filter bags, Model 1601; 2 pecans per bag) and sealed. Microbiological analysis of pecan samples at this point (as described in 2.6) before hot water treatment showed 7.88 ± 0.07 (*S. enterica*), 7.71 ± 0.07 (*E. coli* O157:H7), 7.58 ± 0.18 *(L. monocytogenes)* and 6.53 ± 0.23 (*E. faecium*) log CFU/g, respectively.

2.5. Hot water treatment of inoculated in-shell pecans

Inoculated in-shell pecans were subjected to hot water treatment in a 500 ml wide-mouthed glass bottles using a water bath (VWR, Model 10128–126, Radnor, PA, U.S.A.). Briefly, the glass bottles were first filled with sterile distilled water up to the neck (~450 ml) and then brought to a temperature of 1.5 °C higher than the set temperatures of either 70, 80, or 90 °C, respectively. This ensured that the water inside the bottles was maintained at 70, 80 and 90 °C as measured with a calibrated thermometer. Individual groups of four inoculated pecan samples (i.e., two tea filter bags) were dipped in hot water and treated for 1, 2, 3, 4 or 5 min at 70, 80 or 90 °C. Pecan processors mostly use hot water > 81 °C for 1–8 min to condition the pecans (Beuchat & Mann, 2011; Farakos et al., 2017). Thus, test temperatures were selected close to what pecan processors have in place already. In addition, preliminary trials were conducted at 70, 80 and 90 °C for 3–12 min (data not shown) which helped us to select tested time –temperature combinations.

2.6. Enumeration

Enumeration of surviving bacterial cells was performed by either crushing or using whole pecans. For organisms other than *L. monocytogenes*, four hot water treated pecans were taken in a puncture resistant stomacher® bag (Control Numero 5, Seward, UK) and crushed into pieces using a sterile pestle. After crushing, 100 mL of 0.1% peptone water was added to each bag and placed in an ice bath for 10 min to lower the temperature. Pecan samples were not subjected to crushing for the enumeration of *L. monocytogenes*.

This modification of protocol was done based on the results of our preliminary studies (data not shown) where recovery of *L. monocytogenes* cells from crushed pecans was lower than other bacteria used in this study. Few studies reported higher susceptibility of *Listeria* to bioactive compounds in pecan shells compared to other pathogens (Babu, Crandall, Johnson, O'Bryan, & Ricke, 2014; Caxambu et al., 2016; Prado et al., 2014). This might be one probable cause for the discrepancy in our preliminary study. However, understanding this mechanism is beyond the scope of the current study.

Later the pecan samples in the bag were hand massaged and shook for 1 min to dislodge the organisms. Appropriate serial dilutions of the samples were prepared, and survived organisms were enumerated by plating on Xylose Lysine Deoxycholate agar containing nalidixic acid at 50 μg/ml (XLDN) for *S. enterica*, Cefixime-Tellurite Sorbitol MacConkey Agar containing nalidixic acid at 50 μg/ml (CT-SMACN) for *E. coli* O157:H7, Oxford Listeria Agar base containing nalidixic acid at 50 μg/ml for *L. monocytogenes* and non-selective media TSAN for *E. faecium* and incubation at 37 °C for 24–48 h.

2.7. Determination of D -values

Log reduction of each organism was plotted at different temperatures on y-axis against treatment time on x-axis. D-values were calculated at each test temperature for each organism by taking the inverse of the slope of linear regression line from the log reduction graph and expressed in minutes. The D values calculated were plotted and the negative inverse slope of this curve was calculated as Z value