International Journal of Food Microbiology 297 (2019) 21–26

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Short communication

Ozone-based treatments for inactivation of *Salmonella enterica* in tree nuts: Inoculation protocol and surrogate suitability considerations

Jennifer J. Perry[*,1], Marilia Peña-Melendez, Ahmed E. Yousef

Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Ct., Columbus, OH 43210, United States of America

ARTICLE INFO

Keywords:
Almond
Pistachio
Decontamination

ABSTRACT

The feasibility of using gaseous ozone, alone or in combination with other treatments, to decontaminate in-shell almonds and pistachios, prepared under different pathogen-inoculation conditions, was explored. Nuts were inoculated with either *Salmonella enterica* serovar Enteritidis or a potential *Salmonella* surrogate, *Enterococcus faecium* OSY 31284. The effect of inoculation method (with or without vacuum application), and of drying inoculated nuts (up to three days) on treatment efficacy was investigated. Inoculated nuts were subjected to gaseous ozone alone (almonds, pistachios) or ozone in combination with heating in brine solution (pistachios). Ozone treatment included application of vacuum (10 in Hg), followed by vessel pressurization to 12.5 psig with ozone-oxygen mixture (160 g ozone/m^3 gas mixture) and holding for 30 min. Heating was conducted in a brine solution (5% NaCl) at 70 °C, for 10 min. Ozone-based treatments were significantly more effective ($P < 0.05$) on almonds than pistachios, with maximum *S.* Enteritidis reduction of 2.9 vs. 0.8 log CFU/g, regardless of inoculation method or the drying time. Treatment of inoculated pistachios with heated brine and gaseous ozone reduced *S.* Enteritidis population by 5.0 to 7.0 log CFU/g and was not significantly more effective than treatment with heated brine alone (reduction of 4.8 to 7.1 log CFU/g). Application of vacuum during inoculation increased bacterial population on nut kernels by approximately 1.2 log CFU/g, but the increase in inoculum population had no effect on inactivation of either species of inoculated bacteria. Decontamination treatments were less effective against both bacteria by up to 2 log CFU/g when drying time of inoculated nuts increased. *E. faecium* was significantly more resistant to heat and ozone treatment ($P < 0.05$) than was *S.* Enteritidis on pistachios, but not on almonds. Results of this study show that laboratory methodology affects observed treatment effectiveness. Considering its high resistance to the heat-ozone combination, *E. faecium* may not be a suitable surrogate for *S.* Enteritidis during processing of pistachios by this treatment. Efficacy of ozone gas to decontaminate *S.* Enteritidis-inoculated nuts depends heavily on the type of nut. Although reductions of *S.* Enteritidis populations on in-shell pistachios are low, treatment of in-shell almonds resulted in greater reductions, indicating the promise of this technology to enhance the safety of specific nut products.

1. Introduction

Recent foodborne outbreaks associated with low-moisture products, such as a 2016 incident involving pistachios contaminated with *Salmonella* Montevideo, have raised concerns about the safety of this category of foods (CDC, 2016). Survival of *S.* Enteritidis for over 500 days on almond kernels has been demonstrated (Uesugi et al., 2006). Additionally, the ability of *Salmonella* to contaminate nut kernels by migration through the shells of almonds and pecans was reported (Beuchat and Mann, 2010; Danyluk et al., 2008). Gaseous treatment with propylene oxide (PPO) has been used in the food industry to pasteurize raw almonds (Almond Board of California, 2008; Grocery Manufacturers Association, 2010) and the treatment can achieve ≥5 log reduction in *S.* Enteritidis population (Danyluk et al., 2005). Although PPO has been approved as an effective decontamination treatment, it presents disadvantages including: (i) some countries may not have regulations for PPO, limiting the export of nuts (such as almonds) that are produced in the US and underwent this type of treatment; (ii) PPO does not appear on the list of nonagricultural substances allowed in organic products (Code of Federal Regulations, 2011a); and (iii) concern has arisen regarding reports of PPO being a probable human carcinogen, based on animal studies (US-EPA, 2010). Such findings triggered consumer backlash which may encourage producers to find alternative decontamination methods. Thermal treatment

* Corresponding author at: 5735 Hitchner Hall, Orono, ME 04469, United States of America.
E-mail address: jennifer.perry@maine.edu (J.J. Perry).
[1] Present address: School of Food and Agriculture, University of Maine, 5735 Hitchner Hall, Orono, ME 04469, United States of America.

https://doi.org/10.1016/j.ijfoodmicro.2019.02.025
Received 1 August 2018; Received in revised form 24 January 2019; Accepted 28 February 2019
Available online 01 March 2019
0168-1605/

may also be used to enhance nut safety, but researchers have consistently highlighted the increase in heat resistance of *Salmonella* serovars in low water activity products (Beuchat and Mann, 2011; Izurieta and Komitopoulou, 2012; Melendez-Pena, 2011), suggesting that this approach alone may not be sufficient to offset the threat.

Ozone (O_3), a naturally occurring triatomic oxygen molecule that can be generated on demand (Horvath et al., 1985), is an antimicrobial gas that may be included in nut processing. It is a strong oxidizer that has been approved by the FDA for use on food and food contact surfaces (Code of Federal Regulations, 2011b) and is permitted for use on organic food products (Code of Federal Regulations, 2011a). The effectiveness of gaseous ozone against a broad range of microorganisms on food has been extensively discussed in the literature (Perry and Yousef, 2011). A limited number of studies have reported the applicability of ozone gas as a treatment for nut products, for the purpose of reducing aflatoxin levels (Akbas and Ozdemir, 2006a) or inactivation of pathogens other than *Salmonella enterica* (Akbas and Ozdemir, 2006b).

Enterococcus faecium is a Gram-positive lactic acid bacterium that has been used as a surrogate for *Salmonella* during thermal treatment of almonds (Jeong et al., 2011). According to the California Almond Board (Ly, 2014), one specific strain of *E. faecium*, NRRL B-2354, is approved for use as a surrogate for *Salmonella* in the investigation of dry or moist/steam heating processes. The designation of a suitable *Salmonella* surrogate allows for validation of decontamination treatments within food a production facility, but verification of surrogate suitability is a tedious undertaking (Kopit et al., 2014). Currently, there is no *Salmonella* surrogate for use in ozone-based treatments. This study explores the applicability of an untested *E. faecium* strain (OSY 31284) as a *Salmonella* surrogate in new decontamination processes. The study evaluates: (i) the effect of ozone-based treatments on reduction of *S*. Enteritidis populations on inoculated pistachios and in-shell almonds; (ii) the effect of inoculation method and drying interval between inoculation and treatment on the effectiveness of these treatments; and (iii) the use of a potential surrogate (*E. faecium*) when determining the effectiveness of various antimicrobial treatments on inoculated pistachios and almonds.

2. Materials and methods

All work was completed in a biosafety-II facility in the Department of Food Science and Technology, The Ohio State University. The facility is equipped with biosafety cabinets for inoculation and handling of inoculated nuts, and instruments for thermal and ozone-treatments.

2.1. Culture preparation

The following are the bacteria tested in this study: (i) *E. faecium* OSY 31284, a strain that was isolated from pistachios reported to have been inoculated with approved surrogate *E. faecium* NRRL B-2354, and (ii) *S*. Enteritidis ODA 99-30581-13, an egg isolate obtained from the Ohio Department of Agriculture, Reynoldsburg, OH. Previous work conducted with this *Salmonella* isolate has demonstrated heat resistance in excess of many other strains of this serovar (Perry and Yousef, 2013). Cultures were prepared by transferring frozen stocks into suitable broth media. These bacteria were grown in de Man, Rogosa and Sharpe (MRS) broth (for *E. faecium;* Criterion, Hardy Diagnostics, Santa Maria, CA) and Tryptic Soy Broth for *S*. Enteritidis; (TSB; Bacto™, Difco Laboratories, Sparks, MD). Inoculated broths were incubated for 24 h at 30 °C and 37 °C, respectively.

2.2. Inoculation of nuts

Unroasted, hulled and unsalted pistachios (shell-on) were obtained from Paramount Farms (San Joaquin Valley, CA) and Horizon Growers Cooperative (Tulare, CA). In-shell almonds were purchased at a local grocery store in Columbus, OH. Two inoculation methods (vacuum and no vacuum) and three post-inoculation drying times (24, 48 or 72 h) were tested. Preliminary experiments showed that inoculation without vacuum was insufficient to achieve even distribution or high enough load on nut kernels to allow for statistical analysis. A gas-tight, 300 liter, cylindrical stainless steel treatment vessel (Perry et al., 2008) was used for inoculation with vacuum. Nuts were completely submerged in a flask containing overnight culture (approximate ratio of 1:1 wt/vol), which was closed with a cotton stopper. The flask was placed in the vessel, and 5 in Hg-vacuum was applied and held for 15 min. After repressurizing the vessel to atmospheric pressure, excess liquid was decanted and nuts were transferred to sterile wire baskets. Inoculated nuts were dried in a single layer for 24, 48 or 72 h at ~25 °C inside a biosafety cabinet. Similarly, nuts inoculated without vacuum were also placed in a sterile flask, submerged in the overnight culture, and manually mixed for 15 min. Excess liquid was decanted and nuts were placed in sterile metal baskets to dry as described previously. In order to allow for differentiation of the effectiveness of treatment combinations, high levels of inocula (~8.0 log CFU/g) were targeted. Resulting counts on freshly inoculated product ranged from approximately 7.0 to 8.5 log CFU/g. Conditions employed in inoculation of nuts, (growth of cells in the presence of glucose, use of vacuum, extended drying time), were specifically intended to increase resistance of *S*. Enteritidis to treatment in order to avoid overestimation of treatment efficacy.

2.3. Decontamination treatments: almonds

An ozone decontamination treatment was used for in-shell almonds. Ozone treatment was based on a protocol which had previously been demonstrated to produce significant reduction of *Salmonella* population in shell eggs (Perry, 2010). Inoculated, dried almonds were treated with gaseous ozone as follows: nuts (single layer) in wire mesh baskets were placed in the treatment vessel (previously described) where vacuum of 10 in Hg was applied. Vessel was repressurized with gaseous ozone added to a final concentration of 160 g/m^3 (at 12.5 psig), then nuts were held in static treatment under pressure for 30 min. Residual ozone was exhausted to a thermal destruct unit (Ozonia, Elmwood Park, NJ) by cycling with air over the course of an additional 30 min. Gaseous ozone was produced using pure oxygen and a corona discharge ozone generator (Ozonia Triogen Ltd., Zurich, Switzerland). Ozone concentration was monitored throughout the experiment with the use of an ultraviolet ozone monitor (Mini-Hicon, IN USA, Inc., Norwood, MA).

2.4. Decontamination treatments: pistachios

Treatments of pistachios consisted of (i) heated brining, during which nuts were immersed in sterile, 5% NaCl solution at 70 °C for 10 min with constant agitation; heating effect was quenched by immersion heated samples in cold diluent; (ii) ozonation (as previously described); or (iii) a combination of these steps applied sequentially as heat followed by ozone or ozone followed by heat. As these steps were meant to simulate a semi-continuous process, no cooling step was applied to nuts undergoing the combination treatment. The method of heating pistachios in brine was based on current industry practice as described in a personal communication with Jareer Abu-Ali, formerly with Paramount Farms Co., CA. Because authors were unaware of industry members using this process for almonds, it was applied to pistachios only.

2.5. Microbiological analysis

Almond shells and kernels were analyzed separately, but pistachios were analyzed whole. In-shell almonds were aseptically transferred from wire baskets to sterile polyethylene bags where they were cracked gently using a hammer and separated by manual manipulation outside of the bag. Shells and kernels, as well as in-shell pistachios were then analyzed as follows. Treated nuts (and untreated controls) were placed

in a sterilized blender (Waring Commercial Blender 51BL32, Torrington, CT) and 0.1% peptone water was added. Samples were blended for 25–60 s and aliquots of the slurry were removed in order to carry out serial dilutions. Initial investigation revealed the presence of natural bacterial flora below plating detection limit; therefore, non-selective media were used to prevent underestimation of surviving populations due to cell injury during treatment. Appropriate dilutions were plated onto MRS agar, for *E. faecium* OSY 31284, or tryptic soy agar (TSA), for *S.* Enteritidis. Plates were incubated for 48 h at 30 °C for *E. faecium* OSY 31284 and 37 °C for *S.* Enteritidis. Incubated plates were examined and populations of survivors were determined.

2.6. Statistical analysis

All experiments were repeated three times as true independent replicates. Sampling was conducted in duplicate. Statistical analysis was performed using SAS v. 9.2 (SAS Institute, Inc. 2009). A mixed model was used to compare log reductions among treatments. The following model was used for almond data, with nut portion (shell or kernel), vacuum, and drying time classified as class variables:

$$\text{Log reduction} = \mu + \text{Nut portion} + \text{Vacuum} + \text{Drying time} + (\text{Nut portion} * \text{vacuum}) + (\text{Vacuum} * \text{drying time}) + (\text{Nut portion} * \text{drying time}) + (\text{Nut portion} * \text{vacuum} * \text{drying time}) + \text{error}$$

where μ represents the regression mean.

The similarly constructed model was used to analyze pistachio data, with treatment, vacuum, and drying time classified as class variables.

Means were compared using least square means comparisons. Probability value of < 0.05 was considered significant.

3. Results

3.1. Efficacy of ozone-based treatments for inactivation of Salmonella Enteritidis on nuts

Inactivation of *S.* Enteritidis with ozone gas alone was highly variable between nut types (Fig. 1). The highest observed reduction was on almond shells (2.3–2.9 log CFU/g). Drying of nuts post-inoculation and prior to treatment reduced ozone efficacy, particularly in almond shells. Although reductions on almond kernels were lower than those observed on shells, these differences (within inoculation method and drying time) were not significant, likely due to higher variability (represented as standard deviation) in survivor population on kernels. Reductions observed on whole, in-shell pistachios were minimal, not exceeding 1-log, regardless of inoculation protocol or drying time.

Effectiveness of hot brining, alone or in combination with gaseous ozone treatment, was investigated in pistachios (Fig. 2). Although an inverse relationship between inactivation and drying time can be observed for all treatment combinations, this effect was not statistically significant. The hot brining treatment was particularly effective against inoculated salmonellae, resulting in inactivation of approximately 5 log CFU/g or greater, regardless of inoculation protocol. Combination treatments were slightly, but not significantly, more effective than heating alone ($P \geq 0.05$), regardless of the order in which processing steps were applied.

3.2. Effect of inoculation method and drying on S. Enteritidis populations on almonds

Nut portion (shell or kernel), application of vacuum, and drying time post inoculation significantly affected the population of *S.* Enteritidis on untreated almonds (Fig. 3). At 24 h post-inoculation, the use of vacuum resulted in a more uniform population distribution across shells and kernels. At 72 h post-inoculation, however, this difference was ameliorated by population decreases being observed on both shells and kernels. At 72 h post-inoculation there were no significant differences in the level of *S.* Enteritidis cells on nut shells or kernels, regardless of whether vacuum was used for inoculation. With regard to the effect of treatment on inoculated populations, drying time exerted the most substantial effect. This trend was observable, if not always significant, across all products and treatments (Figs. 1 and 2).

3.3. Suitability of Enterococcus faecium OSY 31284 as a surrogate

E. faecium has been used as a *Salmonella* surrogate in investigation of almond pasteurization processes. In comparison with *S.* Enteritidis, *E. faecium* OSY 31284 exhibited slightly higher treatment resistance; reductions were 1-log lower than those for *S.* Enteritidis (data not shown). When inoculated pistachios were treated with heat or heat-ozone combinations in this study, *S.* Enteritidis and *E. faecium* OSY 31284 responded to treatment quite differently. Although *E. faecium* was slightly, but not significantly, less resistant to treatment with ozone gas, it was significantly ($P < 0.05$) more resistant to all processes involving application of heat. Greater than 2-log difference was observed between

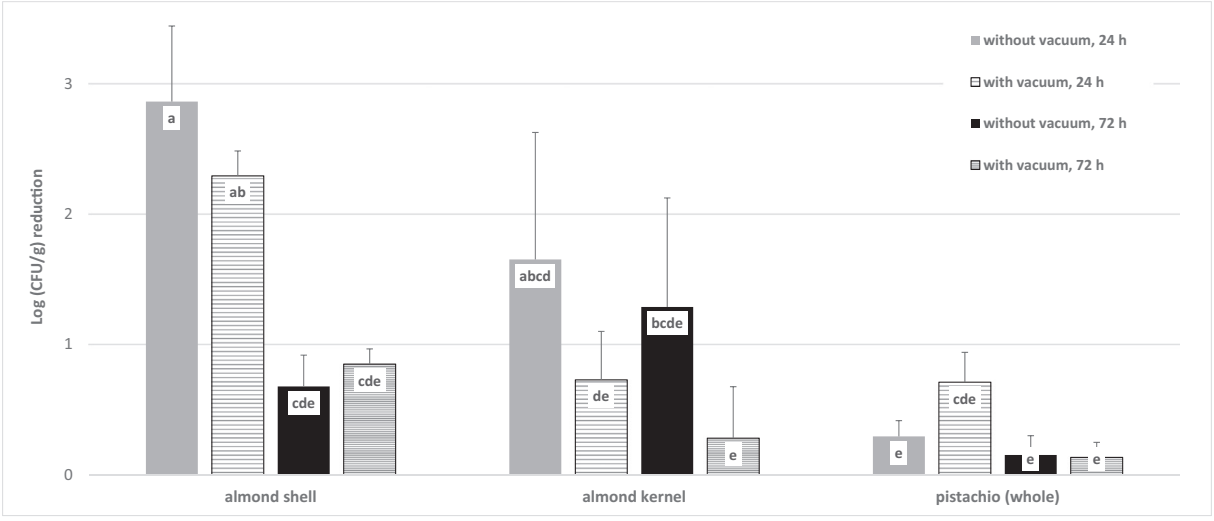

Fig. 1. Reduction in *Salmonella* Enteritidis on inoculated, in-shell nuts after treatment with gaseous ozone (10 in Hg vacuum, 160 g/m^3 ozone, 12.5 pisg, 30 min). n = 3, error bars represent standard deviation, superscripts represent significant differences ($P < 0.05$), initial inoculum level approximately 5.5–8.0 CFU/g.

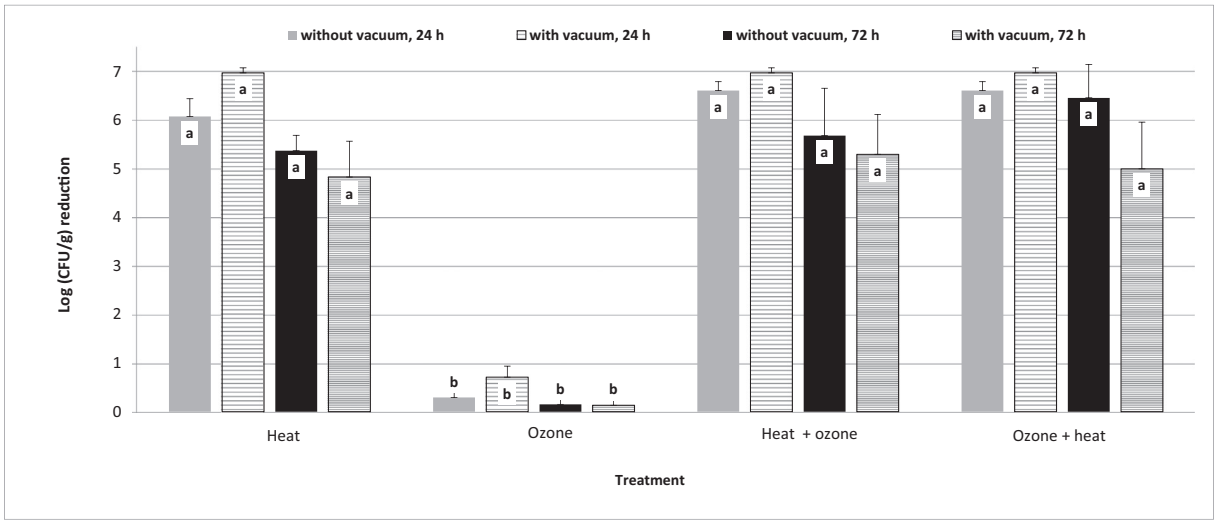

Fig. 2. Reduction in *Salmonella* Enteritidis on inoculated, in-shell pistachios after treatment with heated brine (5% NaCl, 70 °C, 10 min, constant agitation), gaseous ozone (10 in Hg vacuum, 160 g/m^3 ozone, 12.5 pisg, 30 min) or sequential combinations of these treatments.
n = 3, error bars represent standard deviation, superscripts represent significant differences ($P < 0.05$), initial inoculum level approximately 8.0 CFU/g.

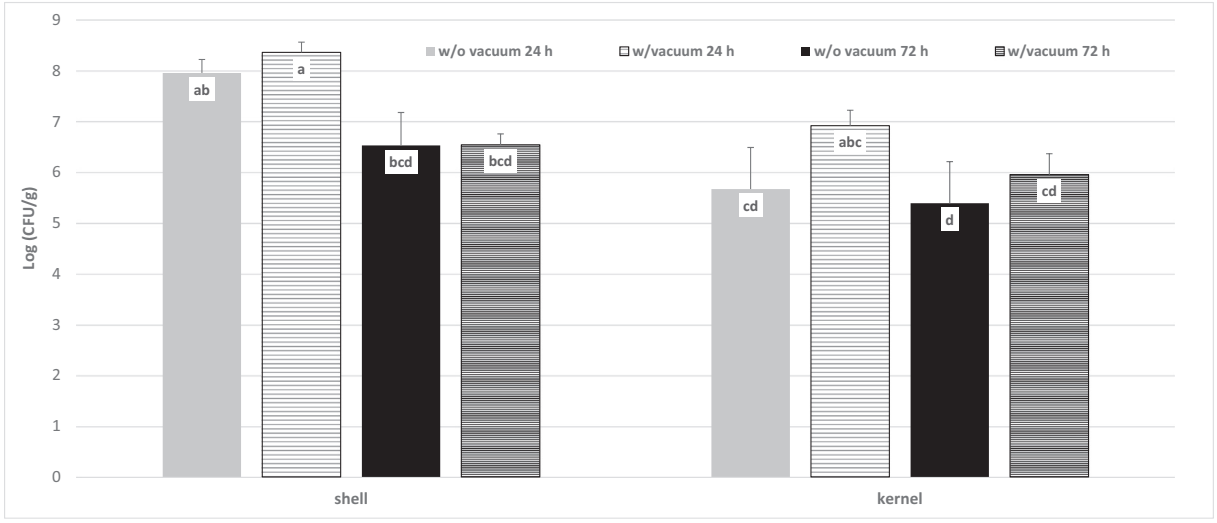

Fig. 3. Population of *Salmonella* Enteritidis on in-shell almonds inoculated with or without vacuum and stored for 24-72 h (room temperature).
n = 3, error bars represent standard deviation, superscripts represent significant differences ($P < 0.05$).

Table 1
Difference in lethality of different treatments against *Enterococcus faecium* and *Salmonella* Enteritidis on inoculated, in-shell pistachios as affected by vacuum application during inoculation, drying of inoculated pistachios at ~25 °C, and treatment method.

Inoculation method	Drying (h)	Difference in reduction (Δ log CFU/g)[a]			
		Heat[b]	Ozone[c]	Heat + ozone	Ozone + heat
No vacuum	24	2.3*	−0.3	2.6*	1.9*
Vacuum	72	2.8*	−0.4	3.4*	3.1*

[a] Difference in average log reductions (*Salmonella* Enteritidis – *E. faecium*), positive number indicates greater resistance of *E. faecium*.
[b] 70 °C, for 10 min, 5% NaCl.
[c] 160 g/m^3, 12.5 psi, 30 min holding time.
* Indicates significant difference ($P < 0.05$) between inactivation of *Salmonella* Enteritidis and *E. faecium* after identical treatments.

the reductions calculated for the two species, and data suggest that the disparity increased with drying time (Table 1).

4. Discussion

Members of the species *Salmonella enterica* require a_w of 0.94 or higher for growth; however, the pathogen survives well in drier environments (a_w below 0.94) for extended periods of time (Bell and Kyriakides, 2002; Burnett et al., 2000; Kieboom et al., 2006). Persistence of *Salmonella* at low a_w may have been a factor in outbreaks of salmonellosis associated with consumption of contaminated peanuts, pistachios and other dry products (Centers for Disease Control and Prevention (CDC), 2009a, 2009b). The risk of illness associated with such products has predicated significant investigation of "kill steps" appropriate for tree nuts. Almonds in the US must be pasteurized to achieve 4-log reduction in *Salmonella* (Code of Federal Regulations, 2009). Pasteurization of this product may be accomplished through dry blanching, oil roasting, steam pasteurization, and fumigation with polypropylene oxide. Risk assessment analysis has demonstrated that with proper application of pasteurization processes, less than one case

of salmonellosis per year in the United States should be attributable to almonds (Santillana Farakos et al., 2017). Some growers and processors of other nut and seed commodities have chosen to use similar methods for risk minimization, partially due to the significant decrease in almond-associated recalls and outbreaks since the establishment of the "almond rule". Recent estimation of *Salmonella* prevalence in US tree nuts suggests that contamination rates may be > 4% for some nuts, although populations of viable pathogen are low, approximately 0.003 most probable number per gram (Zhang et al., 2017). A risk assessment conducted on pecans predicted the rate of salmonellosis from this crop at one case per 775,193 servings, which, although a very small percentage, translates to an expected 529 illnesses per year (Santillana Farakos et al., 2017). Recent increases in demand for high protein foods are likely to increase domestic tree nut consumption, only intensifying the need for more effective pathogen control strategies.

Akbas and Ozdemir (2006b) inoculated kernels, shelled, and ground pistachios with *Escherichia coli* and *Bacillus cereus* and treated these products with ozone. The researchers reported a 3.5-log reduction of *E. coli* and a 3-log reduction of *B. cereus* in kernels and shelled pistachios and a 2-log reduction of both bacteria in ground pistachios after treatment with gaseous ozone at 1 ppm for 360 min (Akbas and Ozdemir, 2006b). Although ozone concentrations utilized in the current work are significantly higher (~10% wt/wt), an equivalent level of efficacy could not be replicated, with ozone alone treatments consistently yielding < 1-log CFU/g reduction in inoculated salmonellae. It is important to note, however that drying time in the cited study was only 1 h, as opposed to a minimum of 24 h in our study, likely contributing to the decreased resistance of bacterial inocula to the antimicrobial activity of ozone. In the current study, the use of gaseous ozone in isolation was largely ineffective against *Salmonella* on tree nuts. The exception to this trend was almond shells subjected to minimal drying before treatment, which resulted in an average reduction of 2.9 log CFU/g (Fig. 1). As such, ozone treatment could be useful against incidental contamination occurring during harvesting and/or processing, before drying. As a pretreatment for hulled, in-shell almonds, gaseous ozone could contribute to the prevention of further cross contamination during storage, or the migration of cells from the shell surface to nutmeat, where they are more difficult to inactivate.

Pistachio processing involves storage of up to two years, commonly in ventilated silos, posing significant potential for post-harvest bacterial contamination through incidental contact with insect and animal pests. Additionally, cross contamination can occur during growing, harvest or at any step during processing, transportation and packaging. The application of heat (via brining) was very effective at reducing populations of *S.* Enteritidis on pistachios. Recent work by Venkitasamy et al. (2017) suggests that this trend extends to dry heating methods as well. Our results suggest that heat would be a more suitable strategy for increasing pistachio safety than would the gaseous treatment, particularly in light of the fact that pistachios are rarely sold to consumers unroasted.

The inoculation protocols employed in this study, i.e., use or no use of vacuum and varied drying time (24 h or 72 h), illustrate the impact that laboratory methodology can have on experimental results. By inoculating with the use of vacuum, we ensured an even distribution of *Salmonella* cells inside and outside of the nut. Although even pathogen distribution is an artificial condition, it is useful for gaining a clear comparison of the inactivation potential of various technologies across all portions of the food sample. Inoculation without vacuum did not lead to an even distribution of cells between almond kernels and nutmeats, but it may better simulate cross contamination during processing and/or transportation. It was hypothesized that differences in inoculation methods would significantly influence the effectiveness of the combination treatments. However, results suggest that the drying time after inoculation had the most influence on the effectiveness of the combination treatments. This result is of particular significance due to the fact that prolonged storage in tree nut post-harvest handling is unlikely to be replicated in a laboratory setting due to time constraints, leading to analysis of artificially less-resistant pathogen populations.

Another complicating factor in the extrapolation of experimental results to production-scale settings is the frequent need to use surrogate microorganisms. Significant differences in the lethality of the combination treatments applied in this study can be observed for *S.* Enteritidis and *E. faecium* OSY 31284 on pistachios. While it is crucial that surrogates exhibit slightly greater resistance to processing compared to their pathogenic counterparts (Grocery Manufacturers Association, 2010), the disparity observed in this study would lead to extreme, unnecessary over-processing and reduced quality of the finished product. It is critical to note that treatments in this work were applied to nuts inoculated with a single strain of *S.* Enteritidis. While this isolate has been screened for heat resistance, results do not necessarily represent the "worst case scenario" for all salmonellae. These results emphasize the need for specificity of strain, process, and matrix when attempting to utilize a surrogate for actionable results.

The effects of ozone-based treatments on product quality were not assessed in this study. Because this antimicrobial is a potent oxidizer, such measurements would be critical before recommendation for use in commercial products. Results indicate that ozone gas is minimally effective against *S.* Enteritidis on stored product. Exploration of efficacy in a humidified, or aqueous treatment should be investigated, particularly for applications in which an unheated product is desired.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations of interest

None.

References

Akbas, M.Y., Ozdemir, M., 2006a. Effect of different ozone treatments on aflatoxin degradation and physicochemical properties of pistachios. J. Sci. Food Agric. 86, 2099–2104. https://doi.org/10.1002/jsfa.2579.

Akbas, M.Y., Ozdemir, M., 2006b. Effectiveness of ozone for inactivation of Escherichia coli and Bacillus cereus in pistachios. Int. J. Food Sci. Technol. 41, 513–519. https://doi.org/10.1111/j.1365-2621.2005.01099.x.

Almond Board of California, 2008. Guidelines for Validation of Propylene Oxide Pasteurization Overview.

Bell, C., Kyriakides, A., 2002. Salmonella: A Practical Approach to the Organism and Its Control in Foods. Blackwell Science, Oxford.

Beuchat, L.R., Mann, D.A., 2010. Factors affecting infiltration and survival of Salmonella on in-shell pecans and pecan nutmeats. J. Food Prot. 73, 1257–1268. https://doi.org/10.4315/0362-028X-73.7.1257.

Beuchat, L.R., Mann, D.A., 2011. Inactivation of Salmonella on in-shell pecans during conditioning treatments preceding cracking and shelling. J. Food Prot. 74, 588–602. https://doi.org/10.4315/0362-028X.JFP-10-411.

Burnett, S.L., Gehm, E.R., Weissinger, W.R., Beuchat, L.R., 2000. Survival of *Salmonella* in peanut butter and peanut butter spread. J. Appl. Microbiol. 89, 472–477. https://doi.org/10.1046/j.1365-2672.2000.01138.x.

Centers for Disease Control and Prevention (CDC), 2016. Multistate outbreak of *Salmonella* Montevideo and *Salmonella* Senftenberg infections linked ot Wonderful Pistachios (final update). Posted May 20, 2016. Available: https://www.cdc.gov/salmonella/montevideo-03-16/index.html, Accessed date: 5 March 2019.

Centers for Disease Control and Prevention (CDC), 2009a. Multistate outbreak of Salmonella infections associated with peanut butter and peanut butter - containing products - United States, 2008–2009. Morb. Mortal. Wkly Rep. 58, 1–6.

Centers for Disease Control and Prevention (CDC), 2009b. Multistate outbreak of Salmonella infections associated with peanut butter and peanut butter-containing products - United States, 2008–2009. MMWR Morb. Mortal. Wkly Rep. 58, 85–90.

Code of Federal Regulations, 2009. Almonds Grown in California; Outgoing Quality Control Requirements.

Code of Federal Regulations, 2011a. Nonagricultural (Nonorganic) Substances Allowed as Ingredients in or on Processed Products Labeled as "Organic" or "Made With Organic (Specified Ingredients or Food Group(s))".

Code of Federal Regulations, 2011b. Direct Food Substances Affirmed as Generally Recognized as Safe.

Danyluk, M.D., Uesugi, A.R., Harris, L.J., 2005. Survival of Salmonella enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. J. Food Prot. 68, 1613–1622. https://doi.org/10.4315/0362-028X-68.8.1613.

Danyluk, M.D., Brandl, M.T., Harris, L.J., 2008. Migration of Salmonella enteritidis phage type 30 through almond hulls and shells. J. Food Prot. 71, 397–401. https://doi.org/10.4315/0362-028X-71.2.397.

Grocery Manufacturers Association, 2010. Industry Handbook for Safe Processing of Nuts Nut Safety Handbook GMA Nut Safety Task Force 2 ACKNOWLEDGEMENTS.
Horvath, M., Bilitzky, L., Huttner, J., 1985. Ozone. Elsevier, Budapest.
Izurieta, W.P., Komitopoulou, E., 2012. Effect of moisture on salmonella spp. heat resistance in cocoa and hazelnut shells. Food Res. Int. 45, 1087–1092. https://doi.org/10.1016/J.FOODRES.2011.09.024.
Jeong, S., Marks, B.P., Ryser, E.T., 2011. Quantifying the performance of Pediococcus sp. (NRRL B-2354: Enterococcus faecium) as a nonpathogenic surrogate for Salmonella enteritidis PT30 during moist-air convection heating of almonds. J. Food Prot. 74, 603–609. https://doi.org/10.4315/0362-028X.JFP-10-416.
Kieboom, J., Kusumaningrum, H.D., Templaars, M.H., Hazeleger, W.C., Abee, T., Beumer, R.R., 2006. Survival, elongation, and elevated tolerance of Salmonella enterica Serovar enteritidis at reduced water activity. J. Food Prot. 69, 2681–2686. https://doi.org/10.4315/0362-028X-69.11.2681.
Kopit, L.M., Kim, E.B., Siezen, R.J., Harris, L.J., Marco, M.L., 2014. Safety of the surrogate microorganism Enterococcus faecium NRRL B-2354 for use in thermal process validation. Appl. Environ. Microbiol. 80, 1899–1909. https://doi.org/10.1128/AEM.03859-13.
Ly, J.U., 2014. Guidelines for Using *Enterococcus faecium* NRRL B-2354 as a Surrogate Microorganism in Almond Process Validation. Almond Board Calif, pp. 1–12.
Melendez-Pena, M., 2011. Influence of Water Activity on Processing Resistance of Salmonella Serovars and Implications on Sanitization of Pistachios by Heat and Ozone. 84.
Perry, J.J., 2010. Ozone Based Treatments for Inactivation of *Salmonella enterica* Serovar Enteritidis in Shell Eggs.
Perry, J.J., Yousef, A.E., 2011. Decontamination of raw foods using ozone-based sanitization techniques. Annu. Rev. Food Sci. Technol. 2, 281–298. https://doi.org/10.1146/annurev-food-022510-133637.
Perry, J.J., Yousef, A.E., 2013. Factors affecting thermal resistance of *Salmonella enterica* serovar Enteritidis ODA 99-30581-13 in shell egg contents and use of heat-ozone combinations for egg pasteurization. J. Food Prot. 76, 213–219. https://doi.org/10.4315/0362-028X.
Perry, J.J., Rodriguez-Romo, L.A., Yousef, A.E., 2008. Inactivation of Salmonella enterica serovar enteritidis in shell eggs by sequential application of heat and ozone. Lett. Appl. Microbiol. 46, 620–625. https://doi.org/10.1111/j.1472-765X.2008.02367.x.
Santillana Farakos, S.M., Pouillot, R., Johnson, R., Spungen, J., Son, I., Anderson, N., Doren, J.M. Van, 2017. A quantitative assessment of the risk of human salmonellosis arising from the consumption of almonds in the United States: the impact of preventive treatment levels. J. Food Prot. 80, 863–878. https://doi.org/10.4315/0362-028X.JFP-16-403.
Uesugi, A.R., Danyluk, M.D., Harris, L.J., 2006. Survival of Salmonella enteritidis phage type 30 on inoculated almonds stored at −20, 4, 23, and 35 °C. J. Food Prot. 69, 1851–1857. https://doi.org/10.4315/0362-028X-69.8.1851.
US-EPA, 2010. AEGL for Propylene Oxide.
Venkitasamy, C., Brandl, M.T., Wang, B., McHugh, T.H., Zhang, R., Pan, Z., 2017. Drying and decontamination of raw pistachios with sequential infrared drying, tempering and hot air drying. Int. J. Food Microbiol. 246, 85–91. https://doi.org/10.1016/j.ijfoodmicro.2017.02.005.
Zhang, G., Hu, L., Melka, D., Wang, H., Laasri, A., Brown, E.W., Strain, E., Allard, M., Bunning, V.K., Musser, S.M., Johnson, R., Santillana Farakos, S.M., Scott, V.N., Pouillot, R., Doren, J.M. Van, Hammack, T.S., 2017. Prevalence of *Salmonella* in cashews, hazelnuts, Macadamia nuts, pecans, pine nuts, and walnuts in the United States. J. Food Prot. 80, 459–466. https://doi.org/10.4315/0362-028X.JFP-16-396.