Journal of Food Protection 86 (2023) 100175

Contents lists available at ScienceDirect

Journal of Food Protection

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

Research Paper

Aqueous Ozone Efficacy for Inactivation of Foodborne Pathogens on Vegetables Used in Raw Meat-Based Diets for Companion Animals

Sahaana Chandran, Christopher A. Baker [#], Allyson N. Hamilton, Gayatri R. Dhulappanavar, Sarah L. Jones, Kristen E. Gibson [*]

Department of Food Science, Center for Food Safety, University of Arkansas System Division of Agriculture, Fayetteville, AR 72704, USA

ARTICLE INFO

Keywords:
Aqueous ozone
Batch wash
Listeria monocytogenes
Pet food
Salmonella
Spray wash

ABSTRACT

The present study evaluates the efficacy of a batch wash ozone sanitation system (BWOSS) and spray wash ozone sanitation system (SWOSS) against *Listeria monocytogenes* (two strains) and *Salmonella enterica* subsp. *enterica* (three serovars) inoculated on the surface of carrots, sweet potatoes, and butternut squash, commonly used in raw meat-based diets (RMBDs) marketed for companion animals such as dogs and cats. Produce either remained at room temperature for 2 h or were frozen at −20°C and then tempered overnight at 4°C to mimic the preprocessing steps of a raw pet food processing operation ('freeze-temper') prior to ozone treatment. Two ozone concentrations (0 and 5 ppm) were applied for either 20 s or 60 s for BWOSS and 20 s for SWOSS. Based on an ANOVA, BWOSS data showed no significant difference ($P > 0.05$) in microbial reduction between 0 and 5 ppm ozone concentration across all treatment durations for each produce type. BWOSS resulted in mean microbial reductions of up to 1.56 log CFU/mL depending on the treatment time and produce type. SWOSS data were analyzed using a generalized linear model with Quasipoisson errors. Freeze-tempered produce treated with SWOSS had a higher bacterial log reduction at 5 ppm ozone compared to 0 ppm ozone ($P = 0.0013$) whereas room temperature produce treated with SWOSS did not show any significant difference in microbial reduction between ozone concentrations. The potential to mitigate microbial cross-contamination was also investigated during SWOSS treatment. The results indicate that 5 ppm ozone decreased pathogens in the rinsate and proximal surfaces by 0.63–1.66 log CFU/mL greater than no ozone depending on the pathogen and sample. Overall, data from this study indicate that SWOSS would be more effective compared to BWOSS in reducing the microbial load present on the surface of root tubers and squash subjected to freezing and thawing and has the potential to mitigate cross-contamination within RMDB manufacturing environments.

The practice of feeding companion animals raw meat-based diets (RMBDs) has been increasing steadily worldwide (Ahmed et al., 2021). RMBDs consist primarily of raw meat followed by raw fruits and vegetables, legumes, grains, and supplements such as manganese, vitamin E, and vitamin D3 (Runesvärd et al., 2020). As the meat, fruits, and vegetables used in these RMDBs are not thermally processed, there is an increased risk of contamination with pathogenic bacteria (Runesvärd et al., 2020). To inactivate potential pathogenic and spoilage microorganisms in RMDBs, the pet food industry utilizes high-pressure processing (HPP). In a recent study assessing HPP's effectiveness in achieving a 5-log reduction of *Salmonella,* Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes*, the authors concluded that HPP, when coupled with frozen storage, successfully achieved, and maintained a 5-log reduction of *Salmonella* and STEC (Lee et al., 2023). However, *L. monocytogenes* displayed greater resistance and did not achieve a 5-log reduction in all raw pet food formulations tested. This presents a considerable pathogen exposure risk to both pets and their owners. For instance, sixty different commercially available RMBDs manufactured by 11 different brands in the greater Minneapolis area were analyzed for the presence of *Salmonella*, and four out of 11 were positive (Mehlenbacher et al., 2012). In collaboration with the Food Emergency Response Network (FERN) and their Microbiology Cooperative Agreement Program, the Veterinary Laboratory Investigation and Response Network (Vet-

Abbreviations: RMBD, Raw Meat-Based Diet; BWOSS, Batch Wash Ozone Sanitation System; SWOSS, Spray Wash Ozone Sanitation System.

* Corresponding author. Address: Department of Food Science, University of Arkansas, 1371 West Altheimer Dr., Fayetteville, AR 72704, USA.
E-mail address: keg005@uark.edu (K.E. Gibson).
Current address: U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 5001 Campus Drive, College Park, ML 20740, USA.

https://doi.org/10.1016/j.jfp.2023.100175
Received 9 August 2023; Accepted 29 September 2023
Available online 5 October 2023

LIRN) reported that out of 576 samples of raw dog and cat foods, exotic animal food, and jerky treats analyzed, 2.6% and 5.6% of samples tested positive for *Salmonella* and *L. monocytogenes*, respectively (Nemser et al., 2014). It has been reported that 35% of sick pet visits to the veterinary clinic are due to salmonellosis, and human cases of salmonellosis have also been linked to contaminated pet food (Soffer et al., 2016). Recently, the USFDA issued a cautionary advisory for pet owners who feed their pets Darwin's Natural Pet Products. This advisory came in response to a specific lot of raw cat and dog food that tested positive for *Salmonella* (USFDA, 2023). Several other recalls of raw pet food products from different companies due to contamination with *Salmonella* and *L. monocytogenes* have occurred over the past five years (USFDA, 2018a, 2018b, 2018c, 2018d, 2019, 2021a, 2021b, 2021c, 2022).

Analysis of RMBDs sold in Europe has also demonstrated contamination with enteric pathogens. van Bree and coauthors (2018) analyzed 35 Dutch commercially available RMBDs, and *L. monocytogenes* was present in more than 50% of samples (19 out of 35) and *Salmonella* in seven samples (van Bree et al., 2018). Analysis of 60 different frozen RMBDs produced by 10 different manufacturers in Sweden confirmed the presence of Enterobacteriaceae in all 60 samples, and *Salmonella* and *Campylobacter* were detected in 7% and 5% of the samples, respectively (Hellgren et al., 2019). A study in Finland analyzed 88 RMBDs from 12 producers, and enteric pathogens were present in 28% of the samples (Fredriksson-Ahomaa et al., 2017). Hence, there is a need to control the microbial safety of ingredients during the manufacturing process of RMDBs for pets.

As indicated, RMBDs include raw vegetables such as root vegetables, broccoli, and squash—all of which can be sources of microbial contamination. Vegetables can become contaminated with pathogens at multiple stages, including during field production through the introduction of contaminated water or soil amendments such as manure, fecal deposition from animals, harvesting by an infected person, and postharvest handling and processing (Hanning et al., 2009; Islam et al., 2004; Pagadala et al., 2015; Sivapalasingam et al., 2004, Strawn et al., 2013). The prevalence of pathogenic bacteria on root vegetables and tubers such as carrots, radishes, and sweet potatoes has been studied considerably less compared to vegetables with edible portions grown above ground. However, there is a higher chance of root vegetables being contaminated due to the potential colonization of pathogenic bacteria within the root zone (Warriner, 2005; Luu et al., 2020). Carrots and radishes grown in soil beds inoculated with *Salmonella* can remain contaminated for 84 and 203 days during their growing cycle, respectively (Islam et al., 2004). Internalization of *Salmonella* and *Escherichia coli* O157:H7 into carrot and radish seedlings has also been reported, although no internalization was observed in mature plants (Jablasone et al., 2005). However, both bacteria persisted on the surface of the plants throughout the cultivation period. Given that postharvest washes are not known to effectively remove field-acquired contamination, the presence of pathogens on the surface of vegetables presents substantial risks (Murray et al., 2017). Winter squash, such as butternut squash, is also commonly used in RMDB formulations. While there is limited research on the contamination of squash vegetables by pathogenic microorganisms, there was a recall of fresh-cut butternut squash products in 2021 due to *L. monocytogenes* contamination indicating a potential risk (USFDA, 2021d, 2021e). These findings emphasize the importance of implementing a preventive control step for vegetables prior to use in RMBDs to minimize the presence of potential pathogenic bacteria.

Multiple studies have shown the efficacy of ozone in reducing bacteria present on the surface of fruits and vegetables (Alexopoulos et al., 2013; Chen et al., 2019; Gibson et al., 2019; Han et al., 2001; Ölmez & Akbas, 2009). Immersion of fresh-cut lettuce and bell peppers in continuously ozonated water (0.5 mL/L) resulted in a 1.5 and 3 log CFU/g reduction of total microbial load after 15 and 30 min, respectively (Alexopoulos et al., 2013). Ozonation of lettuce leaves inoculated with *L. monocytogenes* for 2 min at 1 ppm ozone concentration lead to approximately 1.5 log CFU/g reduction (Ölmez & Akbas, 2009). Gibson et al. (2019) studied the effect of ozone on inactivating *E. coli, Salmonella* Typhimurium, and *L. innocua* inoculated on the surfaces of cilantro, strawberries, romaine lettuce, and tomatoes. A reduction of nearly 4 log CFU/mL or greater across all produce types was reported for all microorganisms when exposed for 30 min to ozone between 0.5 and 1 ppm (Gibson et al., 2019).

Studies analyzing the efficacy of aqueous ozone against pathogens on root vegetables, tubers, and winter squash are very limited. Root vegetables have surfaces that are rough and porous (Bermúdez-Aguirre & Barbosa-Cánovas, 2013). These surfaces could harbor pathogens, and thus, different methods of ozone application need to be considered. In a study by Singh and coauthors (2002), 9.7 and 16.5 mg/L of ozonated water was used to treat baby carrots inoculated with *E. coli* O157:H7 resulting in a 1.7 log CFU/g reduction (Singh et al., 2002). Bridges and collaborators (2018) treated baby carrots inoculated with Shiga toxin-producing *E. coli,* multiple serovars of *Salmonella enterica,* and *L. monocytogenes* with 1.71 μg of gaseous ozone per gram of carrot for 5 h and observed a log CFU/g reduction of 1.2, 0.5, and 0.8, respectively (Bridges et al., 2018).

Though previous studies have shown the effectiveness of ozone against pathogenic bacteria, the observed log reductions were relatively low. Moreover, studies examining the efficacy of ozone on sanitizing root vegetables and tubers used in RMDB formulations are very limited. The present study was performed to investigate the impact of ozone application method (batch and spray), ozone concentration (0 and 5 ppm), treatment time (20 s and 60 s), produce type (butternut squash, carrot, and sweet potato), and produce preparation method (room temperature and freeze-temper) on inactivating pathogenic bacteria (*L. monocytogenes* and *Salmonella)* present on the surface of the produce. Additionally, the efficacy of ozone treatment in minimizing microbial cross-contamination during SWOSS treatment was also studied.

Materials and Methods

Ozone water generation system. The ozone water generation system utilized was developed by Advanced Ozone Integration (Atascadero, CA) under the trade name EcoPrO3^{TM}. The EcoPrO3 unit fabricated for this study contains a corona discharge ozone generator with an output rating of 10 g/h, a venturi ozone injection system, a dissolved ozone balance tank, and a process transfer pump (Supplemental Fig. S1). The EcoPrO3 includes a dissolved ozone meter (Model Q46, ATI, Collegeville, PA) which provides setpoint control of the dissolved ozone concentration in the balance tank, which was corroborated by the indigo trisulfonate method (SM 4500-OS3 B) using a Hach Pocket Colorimeter II (Hach Company, Loveland, CO) and Ozone AccuVac Ampules (Hach Company).

Batch wash ozone sanitation system (BWOSS). The BWOSS utilized in the present study was developed by Recycled Hydro Solutions (Rogers, AR) under the trade name RinseWell®. The BWOSS unit fabricated for this study contains a one-compartment, 16-gauge stainless-steel sink measuring 43 cm^2 with a depth of 30 cm. The sink holds approximately 34.1 L (9 gal.) of water. The ozonated water filling the BWOSS was generated by the EcoPrO3 (Fig. 1).

Spray wash ozone sanitation system (SWOSS). The spray apparatus consisted of four stainless-steel nozzles configured in a square. The nozzle flow rate was 2400 mL/min, and the pressure was approximately 15.5 psi. Each nozzle sprayed a solid cone shape with a nine in (22.86 cm) diameter. The produce was kept on a stainless-steel screen platform which was placed on top of the 16-gauge stainless-steel sink. The distance between the nozzle tip and the screen platform was 11.75 in (29.85 cm), and the produce were approximately 9.50–10.75 in (24.13–27.30 cm) from the nozzle tip. To prevent water

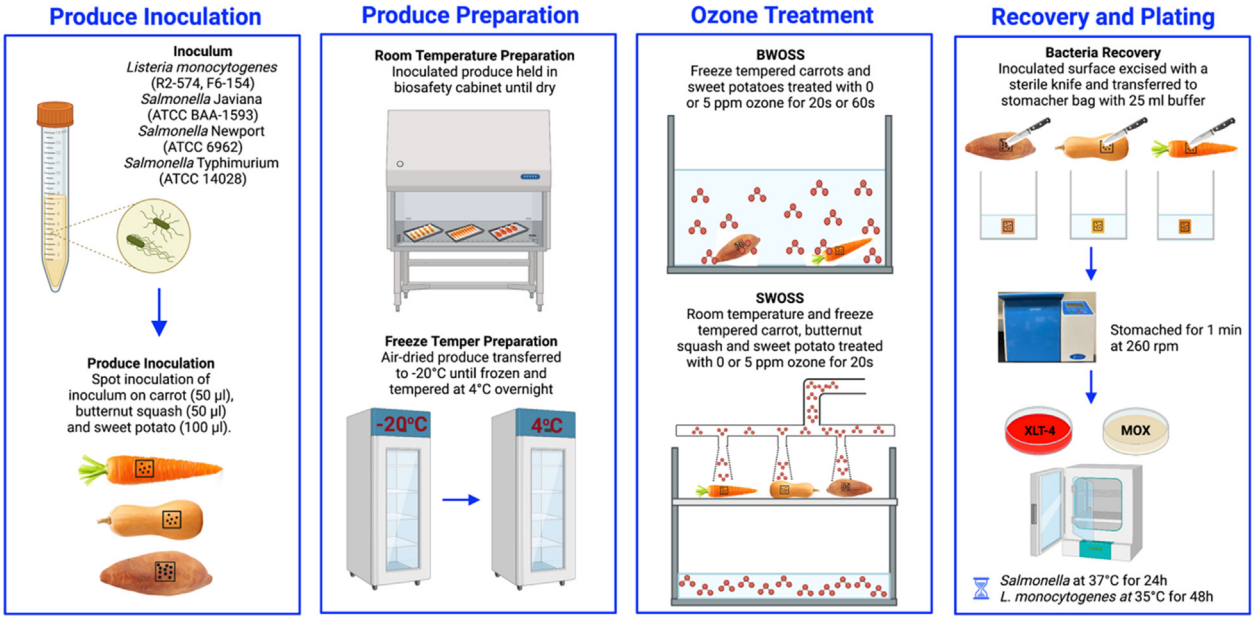

Figure 1. Produce inoculation and preparation for treatment with aqueous ozone. Created with BioRender.com.

droplets from spraying outside, a transparent plastic cover was used to cover both the screen platform and the nozzles (Fig. 2).

Inoculum preparation. Bacterial inocula were prepared as described in Association of Official Analytical Collaboration (AOAC) International 960.09 with modifications. The bacterial strains used in this study are listed in Table 1. An isolated colony of bacteria from a streak plate was inoculated into 10 mL of tryptic soy broth (TSB; BD Difco™, Franklin Lakes, NJ) (*Salmonella*) or brain heart infusion (BHI; BD Difco™) broth (*L. monocytogenes*) for each bacterial strain and incubated at 35°C for 20–24 h, 125 rpm. Cultures were centrifuged for 10 min at 3000 × *g*, 4°C. The supernatant was discarded, and the bacterial pellets were resuspended in 10 mL sterile buffered phosphate water (BPW; pH 7.2). Centrifugation was repeated twice, and each bacterial pellet was resuspended with 2 mL BPW, vortexed, and added (0.5 mL) to 7.5 mL of BPW to obtain the inoculum cocktail (10 mL), which was vortexed and held on ice until inoculation. A cocktail of bacteria was made to inoculate on the produce surface to simulate real-world conditions where the produce would be colonized with a variety of microorganisms including pathogens from different sources; thus, testing with a mixture of pathogens would provide insights into how ozone treatment would perform under more realistic conditions. The bacterial cocktail was diluted and plated on selective agar prepared with Oxford Medium (BD Difco™) which was supplemented with Oxford Listeria Selective Supplement (Millipore Sigma, St. Louis, MO, USA) (MOX) and a xylose lysine tergitol-4 (XLT4) base (Hardy Diagnostics) which was supplemented with XLT4 supplement (Hardy Diagnostics) (XLT4) for *L. monocytogenes* and *Salmonella*, respectively. Plates were incubated at 35°C for 48 h for MOX and 37°C for 24 h for XLT4. The concentration of both *Salmonella* and *L. monocytogenes* in the final inoculum was approximately log 9 CFU/mL.

Produce preparation and inoculation. Fresh produce including carrots, butternut squash, and sweet potatoes was obtained from local retailers or a wholesale distributor (KT Produce, Lowell, AR) in Northwest Arkansas. Carrots were approximately 3.8 cm in thickness whereas sweet potatoes and butter squash were about 7.6 cm thick. Carrots were stored at 4°C, while sweet potatoes and butternut squash were stored at 22°C until use. Carrots were removed from 4°C 1 h prior to preparing produce. For each produce type, a 2.5 cm^2 area was marked on produce with a sharpie pen based on the previous methodology for bacterial recovery from inoculated produce (Dong & Li, 2021). Marked areas were free from scrapes and abrasions. Produce was placed in trays of size l = 29.9 cm, w = 54.6 cm, and h = 6.4 cm (1020 No Drainage Black Plastic Carrier Trays) and held in a Class II biosafety cabinet.

Prepared inoculum was spot inoculated on carrots (50 µL, 6–8 spots), butternut squash (50 µL, 6–8 spots), and sweet potatoes (100 µL, 10–12 spots due to the higher limit of detection) within the marked areas of produce and held in the biosafety cabinet with air flowing until dry (~1 h). Once dry, the produce was either treated with aqueous ozone within 2 h (room temperature analyses) or transferred to −20°C for 2 h, after which produce was tempered at 4°C overnight until treatment to mimic the preprocessing steps of a raw pet food manufacturing facility (freeze/temper analyses) (Fig. 1). Positive controls were performed by inoculating produce with the bacterial cocktail as previously described followed by recovery from produce without water treatment (see 'Recovery of bacteria from produce'). For negative control, each produce type was inoculated with BPW only (no pathogens) and prepared as previously described for each trial. The parameters evaluated in this study are listed in Table 2.

Ozone treatment schema. Initially, the BWOSS was used to evaluate the efficacy of ozone treatment against carrots and sweet potatoes inoculated with pathogens followed by freeze and temper preparation. Carrots and sweet potatoes were treated with either 0 or 5 ppm ozone for 20 s or 60 s. Selected treatment times were based on acceptability to RMBD pet food manufacturers.

SWOSS treatment was also used to evaluate the efficacy of ozone treatment against root tubers and butternut squash. Butternut squash was selected for inclusion in the SWOSS treatment studies, as carrots and sweet potatoes that were used in BWOSS have a rough, porous surface, and the authors hypothesized that the nature of the surface might have an effect on ozone efficacy. Therefore, the authors chose to include a vegetable that has a smooth surface and was also used in raw meat-based pet food. Butternut squash fit these criteria and was therefore selected for use in the SWOSS treatment study. In addition, the room temperature preparation of produce was added to the SWOSS treatment scheme to determine if different produce preparations influenced the efficacy of ozone treatment. The inoculated and prepared produce was treated with either 0 or 5 ppm ozone for 20 s based on the results from the BWOSS treatment.

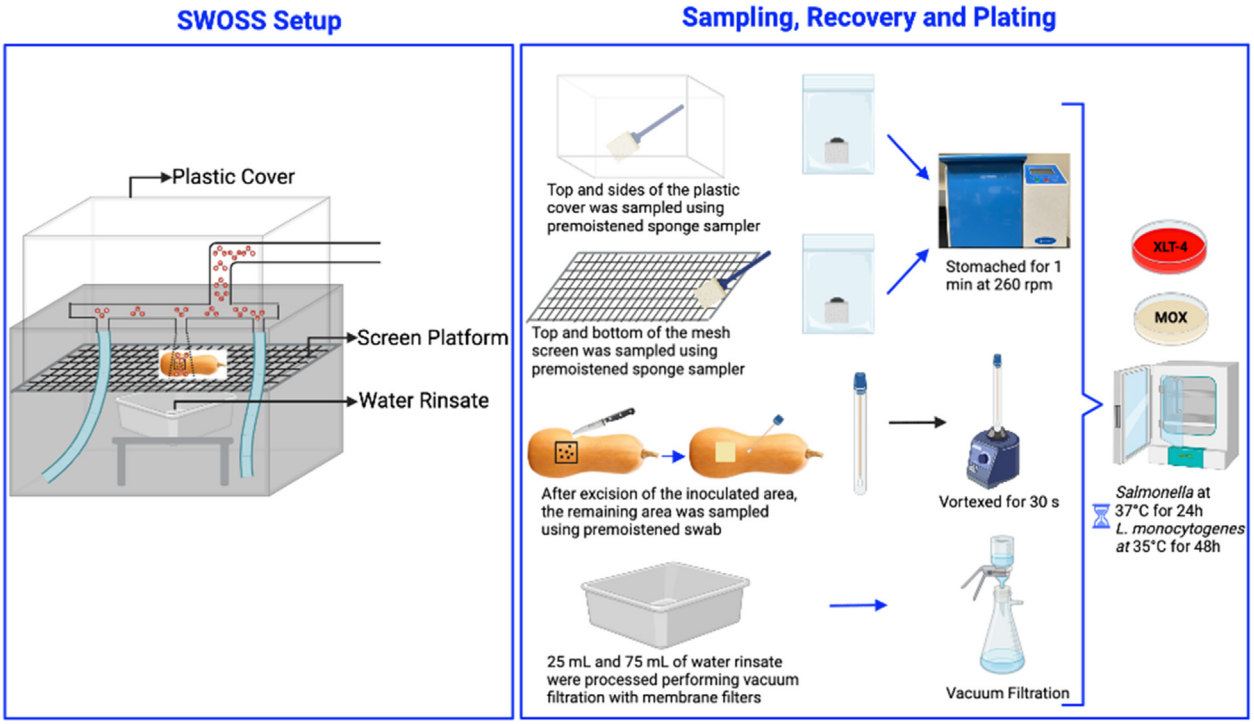

Figure 2. Recovery of bacteria from SWOSS equipment and water rinsate. Created with BioRender.com.

Table 1
Bacterial strains used in this study

Pathogen (serotype)	Strain	Source Information	Received From
Listeria monocytogenes (4b)	R2-574/F2365	Food, epidemic, L.A. (1985)	Cornell, Ithaca, NY[a]
Listeria monocytogenes (1/2a)	F6-154/J2818	Food, epidemic (sliced turkey) (2000)	Cornell, Ithaca, NY[a]
Salmonella Javiana	-	Human stool, outbreak associated with tomatoes (2004)	ATCC® (BAA-1593™)
Salmonella Newport	NCTC 129	Food poisoning fatality	ATCC® (6962™)
Salmonella Typhimurium	CDC 6516-60	Tissue from pools of heart and liver from 4-week-old chickens	ATCC® (14028™)

[a] The strains were received from the Institute for the Advancement of Food and Nutrition Sciences *L. monocytogenes* collection at Cornell University in Ithaca, NY.

Recovery of bacteria from produce. Bacteria were recovered from the produce surface as described previously (Dong & Li, 2021). Briefly, following ozone treatment, bacteria were recovered from produce surfaces by excising the inoculated surface with a sterile, stainless-steel knife, after which the produce sample was transferred with sterile tweezers to a sterile stomacher bag containing 25 mL of either BPW (sweet potato) or 1X phosphate-buffered saline (PBS, pH 7.4) + Tween 0.2% (butternut squash and carrot) and stomached at 260 rpm for 1 min (Stomacher 400 Circulator; Seward, Worthing, United Kingdom). Different elution buffers were used for each produce based on preliminary studies indicating that specific elution buffers yielded better recovery of bacteria based on produce type. Stomached solution (1.9 mL) was transferred to a sterile 2 mL microcentrifuge tube and serially diluted in PBS. Dilutions were plated in duplicate on respective selective agar and incubated as previously described in 'Inoculum preparation'. The assay limit of detection was calculated assuming plating 500 µL in duplicates would give us each 1 CFU, and hence, the detection limit is 2 CFU/mL or 50 CFU total in the 25 mL.

Recovery of bacteria from rinsate and SWOSS equipment. In the third SWOSS experimental trial, prepared butternut squash at room temperature was inoculated and subjected to SWOSS treatment as described above in 'Ozone treatment schema'. To understand the potential of ozonated water to aid in the prevention of cross-contamination within the system, additional samples were collected for analysis (Fig. 2). After each treatment, the water rinsate was collected and processed by performing vacuum filtration using 250 mL Nalgene filter cups (Thermo Fisher Scientific) fitted with S-Pak membrane filter (0.45 µm pore size and 0.47 mm filter diameter) (Millipore Sigma) set-up on a stainless-steel vacuum filtration manifold (Thermo Fisher Scientific).

For each sample, 75 mL and 25 mL of water rinsate were passed through separate membrane filters (Millipore Sigma) which were then

Table 2
Parameters evaluated in this study

Parameter	Description
Produce type	Butternut squash
	Carrot
	Sweet Potato
Produce preparation method	Room Temperature
	Freeze-Temper
Ozone application method	Batch Wash Ozone Sanitation System (BWOSS)
	Spray Wash Ozone Sanitation System (SWOSS)
Ozone concentration	0 ppm
	5 ppm
Treatment time	20 s
	60 s
Pathogens	*Listeria monocytogenes*
	Salmonella enterica subsp. *enterica*

plated in duplicate on selective agar. The inoculated area of the butternut squash was excised and processed as described previously. The remaining area of the butternut squash was sampled using a PUR-Blue Dry Swab (World Bioproducts) premoistened with 2 mL of BPW. The swab was vortexed for 30 s after sampling, and the collected eluent was plated in duplicate on respective selective media. The screen platform and plastic cover were sampled separately using an EZ-Reach Polyurethane Sponge Sampler (World Bioproducts) premoistened in 8 mL of BPW. For sample collection, the saturated sponge was squeezed into the sample bag to remove excess liquid and was then pressed and dragged across the entire screen platform (top and bottom) and plastic cover (top and sides). After sampling, the sponge was returned to the sample bag, and the handle was removed before stomaching at 260 rpm for 1 min. The eluent was collected and plated in duplicate on selective media as described under 'Inoculum Preparation'.

Statistical analysis. Two experimental trials were performed for BWOSS and three experimental trials for SWOSS. All samples were plated in technical duplicates. Log reductions (CFU/mL) were calculated by subtracting the log recovery of the treatment samples from the log recovery of the control samples. A completely randomized design was employed to investigate the effect of ozone concentration on the log reduction of bacteria present on the surface of different types of produce. Statistical analysis was performed individually for BWOSS data (freeze-temper analyses) and SWOSS data (room temperature analyses and freeze-temper analyses) to determine whether ozone concentration, produce type, pathogen type, and exposure time are significant predictors of log reduction. All data were first analyzed using a linear model; however, only BWOSS data met the assumptions of normality and heteroscedasticity. SWOSS data were then analyzed using a generalized linear model (GLM) with Poisson errors; however, the residual deviance was greater than the residual degrees of freedom. Therefore, a GLM with Quasipoisson errors was used as it was a more appropriate approximation. The log link function was used to relate the log reduction to ozone concentration, produce type, pathogen type, and exposure time. The treatment means and their 95% confidence intervals were calculated using estimated marginal means. Multiple pairwise comparisons were performed to identify statistical differences at $P = 0.05$. For the bacterial recovery from rinsate and SWOSS experiment data, no statistical analysis was performed as the sample size was 2. The data were analyzed in R (R core team, 2022) using *base*, *ggplot2* (Wickham, 2016), *ggpubr* (Kassambara, 2020), *emmeans* (Length et al., 2021), *multcomp* (Hothorn et al., 2008), *multcompView* (Graves et al., 2019), *lme4* (Bates et al., 2015), and *lmertest* (Kuznetsova et al., 2017) packages.

Results

Experimental parameter values. The mean starting concentrations for each produce type based on each pathogen for both freeze-tempered produce and produce kept at room temperature are listed in Supplemental Tables S1 and S2. For SWOSS treatments, the mean oxidation-reduction potential (ORP) values at 0 and 5 ppm were 581 ± 26 mV and 884 ± 6 mV, respectively, with similar values observed with BWOSS treatments at 605 ± 12 mV and 874 ± 7 mV, respectively. Water temperatures ranged from 13.4 to 27.2°C across all treatments. Mean free chlorine values at 0 ppm for SWOSS and BWOSS treatments were 0.63 ± 0.24 and 1.04 ± 0.25 ppm, respectively, while treatment with ozone (5 ppm) averaged 0.37 ± 0.20 for SWOSS and 0.41 ± 0.15 ppm for BWOSS treatment.

Efficacy of BWOSS in pathogen reduction. The estimated log reductions of bacteria after statistical analysis for BWOSS data are plotted in Figure 3 to show the effect of ozone (5 ppm) when applied for two different treatment times (20 s and 60 s) to inoculated produce (carrot and sweet potato) that were freeze-tempered. There was no significant difference observed in the log reduction of both *Salmonella* and *L. monocytogenes* when treated with 5 ppm ozone compared to 0 ppm (Fig. 3). The highest log reduction achieved through BWOSS treatment was 1.56 CFU/mL for carrots and 0.67 CFU/mL for sweet potatoes for *L. monocytogenes*. Meanwhile, for *Salmonella*, the maximum log reduction was 0.66 CFU/mL for carrots and 0.51 CFU/mL for sweet potatoes. Also, treating the produce for a longer time (60 s) with ozone did not have any significant effect on the log reduction of either of the bacteria compared to 20 s (Fig. 3).

Efficacy of SWOSS in pathogen reduction present on produce. As there was no significant bacterial reduction between 20 s and 60 s treatment time, only 20 s was considered for SWOSS treatment. For freeze-tempered produce data, the main effects, produce type ($P < 0.001$) and ozone concentration ($P = 0.0013$), were significant predictors of both *Salmonella* and *L. monocytogenes* log reduction. When treated with 5 ppm ozone, all three produce types resulted in a significantly higher estimated log reduction of each of the bacteria compared to 0 ppm ozone treatment (Fig. 4A). Butternut squash had the highest estimated log reduction of 4.67 (95% CI: 3.90, 5.59) and 4.90 (95% CI: 4.10, 5.85) for *Salmonella* and *L. monocytogenes*, respectively, when treated with 5 ppm ozone. Sweet potato had the lowest estimated log reduction of 1.32 (95% CI: 1.00, 1.73) for *Salmonella* and 1.38 (95% CI: 1.05, 1.81) for *L. monocytogenes*. For carrots, the estimated log reduction for *Salmonella* and *L. monocytogenes* was 2.38 (95% CI: 1.91, 2.96) and 2.49 (95% CI: 2.01, 3.10), respectively.

Figure 4B shows the estimated log reduction of bacteria on produce that was kept at room temperature after inoculation and sprayed with ozone at 0 and 5 ppm for 20 s. No interaction effects were observed and only the main effect produce type [carrot ($P = 0.0232$) and sweet potato ($P < 0.001$)] was significant. The main effect pathogen (*Salmonella*) neared statistical significance (P value = 0.0534). The estimated log reduction for *L. monocytogenes* and *Salmonella* was the same for both 5 and 0 ppm ozone treatment for the same produce type (Fig. 4B). Butternut squash again had the highest estimated log reduction for both *Salmonella* [3.09 (95% CI: 2.64, 3.61)] and *L. monocytogenes* [3.62 (95% CI: 3.11, 4.21)] at 5 ppm, whereas sweet potato had the lowest estimated log reduction of 1.35 (95% CI: 1.07, 1.70) for *Salmonella* and 1.58 (95% CI: 1.26, 1.98) for *L. monocytogenes*. For carrots, the estimated log reduction for *Salmonella* and *L. monocytogenes* was 2.49 (95% CI: 2.07, 2.99) and 2.92 (95% CI: 2.44, 3.45), respectively, when treated with 5 ppm ozone for 20 s.

Efficacy of SWOSS in pathogen reduction present in rinsate and equipment. Figure 5 shows the raw data of the microbial population recovered from the plastic cover, rinsate, screen platform, and the whole produce when room temperature inoculated butternut squash was treated for 20 s with SWOSS at 0 and 5 ppm ozone. Treatment with 5 ppm ozone resulted in the recovery of at least 1 log CFU/mL less compared to 0 ppm for both *L. monocytogenes* and *Salmonella* in all samples except for *Salmonella* recovered from plastic cover (0.72 log CFU/mL recovered less with 5 ppm ozone treatment) and whole produce (nothing was recovered from either of the ozone treated butternut squash). For *L. monocytogenes*, the log recovered from the plastic cover, rinsate, screen, and whole produce after 5 ppm ozone treatment was less than 1.5 CFU/mL, whereas for *Salmonella*, it was less than 0.3 CFU/mL.

Discussion. Sanitizer efficacy against pathogens potentially present in vegetables included in RMDBs such as carrots, sweet potatoes, and winter squash, have not been adequately evaluated. Conversely, numerous studies have been performed in the past to evaluate the efficacy of ozone against microorganisms present in fresh produce commonly consumed raw. Alexopoulos and coauthors (2013) investigated the effect of ozone on aerobic mesophiles present in fresh green lettuce and green bell pepper by supplying ozone at a concentration of 0.5 ppm either continuously or at the start of the experiment. After 15 min of exposure, a log CFU/g reduction of 1.71 and 1.4 was observed in lettuce and bell pepper, respectively, for continuous

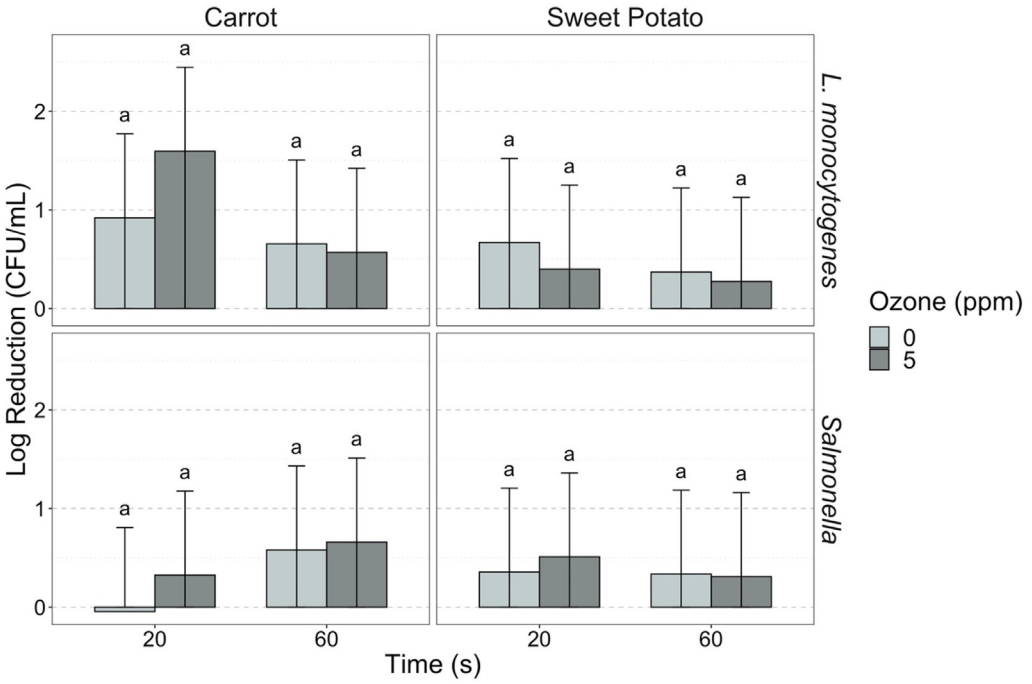

Figure 3. Log reduction obtained from analysis of variance with multiple pairwise comparisons for batch wash ozone (BWOSS) treatment on carrot and sweet potato. The mean starting concentration was 7 logs for each bacterium and produce type. Statistical difference between different treatments is denoted by compact letters over error bars at $P = 0.05$.

ozonation. However, the treatment of lettuce and bell pepper in preozonated water for 15 min resulted in a lesser log CFU/g reduction of 0.46 and 0.5, respectively (Alexopoulos et al., 2013). The authors also reported increased log reduction with increasing exposure time (30 min) for continuous ozone while only a minimal increase in log reduction was observed over time for preozonated water treatment. The present study also aimed to determine the efficacy of continuous ozonated water; however, a 10-fold higher ozone concentration (5 ppm) and lesser exposure times (20 s and 60 s) were selected here. Therefore, it is difficult to compare the studies.

In the present study, produce type was a significant predictor of log reduction in both SWOSS treatment analyses (freeze-tempered and room temperature). Treatment of freeze-tempered butternut squash with 5 ppm ozone resulted in greater than 4 log CFU/mL reduction for both *Salmonella* and *L. monocytogenes*. However, only approximately a 1–2 log CFU/mL reduction of bacteria was observed for carrots and sweet potatoes, regardless of bacteria type, under the same treatment conditions. Butternut squash has a smoother outer surface than carrots or sweet potatoes; hence, the surface smoothness could have allowed the ozone sanitation process to be more effective. Conversely, carrots and sweet potatoes have rough outer surfaces where bacteria can be harbored, making microorganisms more difficult to inactivate during the ozone sanitation procedure. The difference in produce surfaces could also be the reason for the BWOSS not being as effective compared to the SWOSS. More specifically, the water pressure during the spray method may have assisted in dislodging the bacteria from the outer surface of the carrots and sweet potatoes, allowing for inactivation by ozone.

Gibson and coauthors (2019) studied the effect of a batch wash sanitation procedure on cilantro, strawberries, romaine lettuce, and cherry tomatoes inoculated with *E. coli, S.* Typhimurium, and *L. innocua.* Cilantro, strawberry, and romaine lettuce—all of which have irregular and porous outer surfaces—had significantly lower microbial reductions (less than 3 log CFU/mL) compared to cherry tomatoes (greater than 3 log CFU/mL), which have a smooth and uniform outer surface, when treated for 2vmin with less than 1 ppm aqueous ozone (Gibson et al., 2019). In another study, apples inoculated with *E. coli* O157:H7 were either dipped in ozonated water (22–24 ppm) or washed in bubbling ozone in water (21–28 ppm) for 3 min (Achen & Yousef, 2001). Dipping the apples in ozonated water resulted in a 2.6 log CFU/g reduction of *E. coli* O157:H7 on the apple surface, whereas ozone bubbled into water led to a 3.7 log CFU/g reduction. In addition, the stem-calyx region, which has a rough surface compared to the smooth apple surface, resulted in less than 1 log reduction of *E. coli* O157:H7 when treated with both delivery methods of ozone. The results from these studies provide evidence that the surface of the produce can play a significant role in ozone sanitation efficacy.

One limitation to the present study includes the potential for injury to the bacterial cells during the freeze-temper procedure. However, there was no significant difference in the number of bacteria recovered from each produce when prepared at room temperature vs freeze-tempered before ozone treatment (Tables S1 and S2). Therefore, injury to the cells during the freeze-temper procedure was unlikely to have had a substantial impact on the overall results and conclusions drawn from this study. Moreover, during the freeze-temper procedure, the produce was tempered at 4°C overnight, which has been shown to assist in the recovery of injured cells (Lee et al., 2023). Previous research has indicated that *L. monocytogenes* grown at 37°C exhibits high levels of cryotolerance, even after undergoing 18 freeze-thaw cycles, with a less than 1 log decrease (Azizoglu et al., 2009). Additionally, there was an average difference of 1 log observed between populations of *S. enterica*, with no significant variation found among populations of *L. monocytogenes* when comparing their recovery from both nonselective and selective media (Azizoglu et al., 2009, Garcia et al., 2022). However, it must be noted that the serovars of *S. enterica* and strains of *L. monocytogenes* used in the above-mentioned studies differed from the ones used in the current study. As a result, the number of recovered cells in this study could potentially be slightly lower (<1 log) compared to those obtained when using nonselective media. This difference implies that the log reduction of microbial cells achieved after ozone treatment might also be lower.

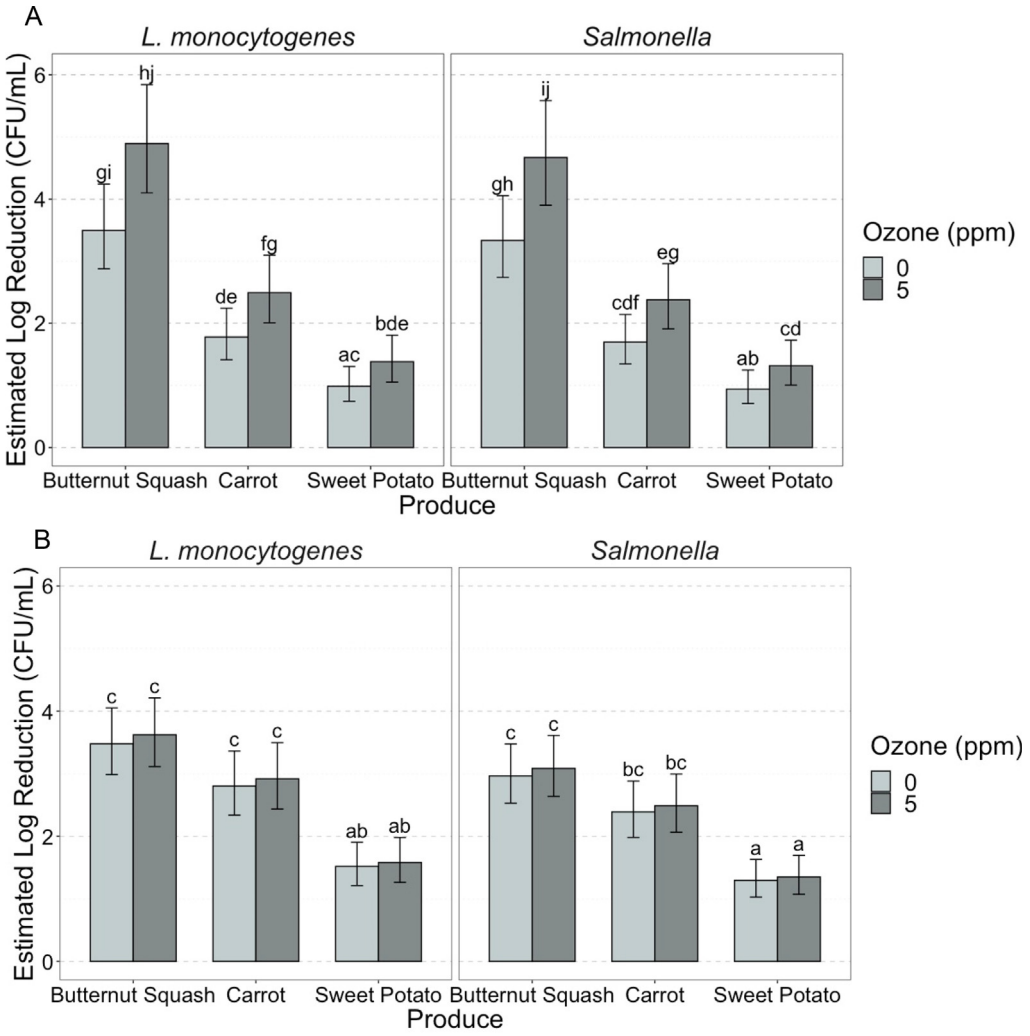

Figure 4. Log reduction obtained from Generalized Linear Model with Quasipoisson Errors for inactivation of bacteria by ozone spray (SWOSS) treatment on (A) freeze-tempered produce where the mean starting concentration was 7 logs for each bacterium and produce type and (B) room temperature produce where the mean starting concentration was between 6 and 7 logs for each bacterium and produce type. Statistical difference between different treatments is denoted by compact letters over error bars at $P = 0.05$.

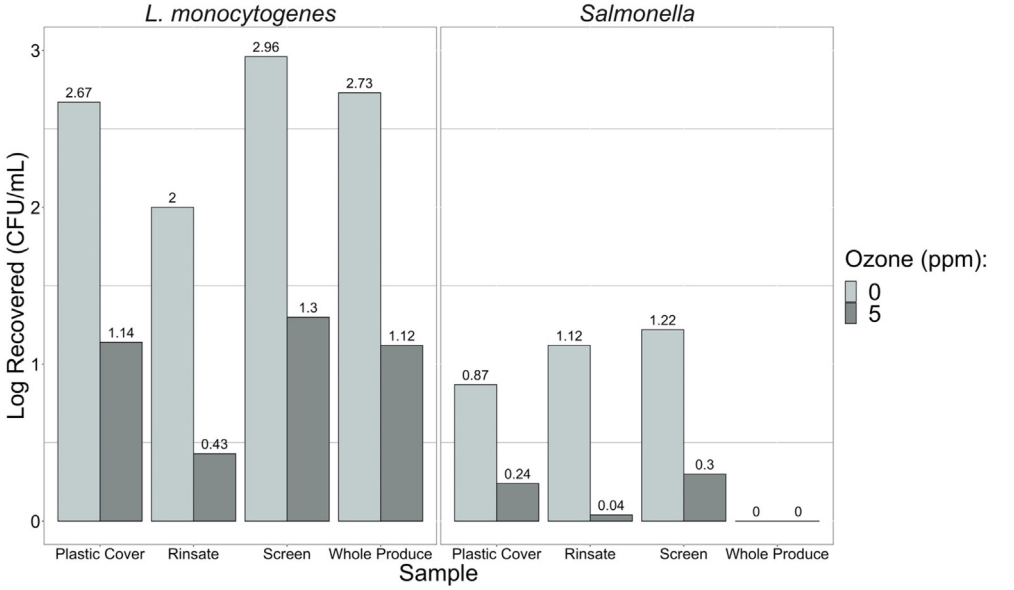

Figure 5. Raw data values of log (CFU/mL) recovered from produce and SWOSS equipment based on ozone spray treatment on whole butternut squash. The starting concentration of *L. monocytogenes* and *Salmonella* was 7 and 5 logs, respectively.

Based on the results from the present study, the BWOSS procedure using ozone may not be adequate for the sanitation of root vegetables and tubers as the treatment did not significantly reduce the counts of *Salmonella* and *L. monocytogenes* present on carrots and sweet potatoes compared to simply washing them in water. Moreover, increased exposure time (60 s) did not achieve a greater log reduction on the root vegetables and tubers suggesting a different ozone application method may be better for sanitizing these types of produce. Future studies should test higher concentrations of ozone in root vegetables; however, previous studies have reported changes in the visual and physiological properties of the carrot with increased ozone concentration and exposure time (Sarron et al., 2021).

In conclusion, the present study evaluated various parameters that affect aqueous ozone sanitation of root vegetables and squash. The results indicate that the effectiveness of a sanitation procedure depends significantly on the type of produce; thus, it is crucial to consider the produce type when choosing a sanitation procedure. As vegetables that are used in preparing RMBDs food are usually freeze-tempered, the data from the current study indicate that SWOSS would be more effective compared to BWOSS in reducing the microbial load present on the surface of root tubers and squash and thereby contribute to reducing the contamination risk of RMBD pet foods. In addition, ozone treatment was shown to prevent microbial cross-contamination to sanitation equipment and proximal surfaces.

Funding statement

This research was supported in part by Instinct® Pet Food and Recycled Hydro Solutions; however, there is no real conflict of interest. Moreover, this manuscript is intended as a third-party evaluation of the aqueous ozone units and not a product endorsement. This work was also supported in part by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch Act.

Disclaimer

This document has not been formally reviewed by the U.S. Food and Drug Administration and should not be construed to represent Agency determination or policy.

CRediT authorship contribution statement

Sahaana Chandran: Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Christopher A. Baker:** Supervision, Project administration, Validation, Writing – review & editing, Investigation, Methodology, Data curation, Conceptualization. **Allyson N. Hamilton:** Writing – review & editing, Investigation, Formal analysis. **Gayatri R. Dhulappanavar:** Writing – review & editing, Investigation. **Sarah L. Jones:** Writing – review & editing, Investigation. **Kristen E. Gibson:** Funding acquisition, Supervision, Writing – review & editing, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Recycled Hydro Solutions and Advanced Ozone Integration for providing the BWOSS and SWOSS unit. We would also like to thank Taylor Brandt for his help with media, produce, and buffer preparation throughout this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfp.2023.100175.

References

Achen, M., & Yousef, A. E. (2001). Efficacy of ozone against *Escherichia coli* O157:H7 on apples. *Journal of Food Science, 66*(9), 1380–1384. https://doi.org/10.1111/j.1365-2621.2001.tb15218.x.

Ahmed, F., Cappai, M. G., Morrone, S., Cavallo, L., Berlinguer, F., DessÙ, G., Tamponi, C., Scala, A., & Varcasia, A. (2021). Raw meat based diet (RMBD) for household pets as potential door opener to parasitic load of domestic and urban environment. Revival of understated zoonotic hazards? A Review. *One Health, 13*. https://doi.org/10.1016/j.onehlt.2021.100327 100327.

Alexopoulos, A., Plessas, S., Ceciu, S., Lazar, V., Mantzourani, I., Voidarou, C., Stavropoulou, E., & Bezirtzoglou, E. (2013). Evaluation of ozone efficacy on the reduction of microbial population of fresh cut lettuce (Lactuca sativa) and Green Bell Pepper (Capsicum annuum). *Food Control, 30*(2), 491–496. https://doi.org/10.1016/j.foodcont.2012.09.018.

Azizoglu, R. O., Osborne, J., Wilson, S., & Kathariou, S. (2009). Role of growth temperature in freeze-thaw tolerance of Listeria spp. *Applied and Environmental Microbiology, 75*(16), 5315–5320. https://doi.org/10.1128/aem.00458-09.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models usinglme4. *Journal of Statistical Software, 67*(1). https://doi.org/10.18637/jss.v067.i01.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2013). Disinfection of selected vegetables under nonthermal treatments: Chlorine, acid citric, ultraviolet light and Ozone. *Food Control, 29*(1), 82–90. https://doi.org/10.1016/j.foodcont.2012.05.073.

Bridges, D. F., Rane, B., & Wu, V. C. H. (2018). The effectiveness of closed-circulation gaseous chlorine dioxide or ozone treatment against bacterial pathogens on produce. *Food Control, 91*, 261–267. https://doi.org/10.1016/j.foodcont.2018.04.004.

Chen, C., Liu, C., Jiang, A., Zhao, Q., Liu, S., & Hu, W. (2019). Effects of ozonated water on microbial growth, quality retention and pesticide residue removal of fresh-cut onions. *Ozone: Science & Engineering, 42*(5), 399–407. https://doi.org/10.1080/01919512.2019.1680527.

Dong, L., & Li, Y. (2021). Fate of *Salmonella* Typhimurium and *Listeria monocytogenes* on whole papaya during storage and antimicrobial efficiency of aqueous chlorine dioxide generated with hcl, malic acid or lactic acid on whole papaya. *Foods, 10*(8), 1871. https://doi.org/10.3390/foods10081871.

Fredriksson-Ahomaa, M., Heikkilä, T., Pernu, N., Kovanen, S., Hielm-Björkman, A., & Kivistö, R. (2017). Raw meat-based diets in dogs and cats. *Veterinary Sciences, 4*(4), 33. https://doi.org/10.3390/vetsci4030033.

Garcia, J. O., Wason, S., Subbiah, J., Eifert, J., Strawn, L. K., & Ponder, M. A. (2022). Media impacts recovery of *Salmonella enterica* and *Enterococcus faecium* NRRL B2354 from whole black peppercorns, Basil leaves, and chia seeds treated with antimicrobial gasses. *Frontiers in Food Science and Technology, 2*. https://doi.org/10.3389/frfst.2022.1033814.

Gibson, K. E., Almeida, G., Jones, S. L., Wright, K., & Lee, J. A. (2019). Inactivation of bacteria on fresh produce by batch wash ozone sanitation. *Food Control, 106*. https://doi.org/10.1016/j.foodcont.2019.106747 106747.

Graves, S., Piepho, H. P., Selzer, L. (2019) multcompView: Visualizations of Paired Comparisons. R package version 0.1-8. Retrieved September 8, 2023, from https://CRAN.R-project.org/package=multcompView

Han, Y., Floros, J. D., Linton, R. H., Nielsen, S. S., & Nelson, P. E. (2001). Response surface modeling for the inactivation of Escherichia coli O157:H7 on Green Peppers (Capsicum annuum L.) by chlorine dioxide gas treatments. *Journal of Food Protection, 64*(8), 1128–1133. https://doi.org/10.4315/0362-028x-64.8.1128.

Hanning, I. B., Nutt, J. D., & Ricke, S. C. (2009). Salmonellosis outbreaks in the United States due to fresh produce: Sources and potential intervention measures. *Foodborne Pathogens and Disease, 6*(6), 635–648. https://doi.org/10.1089/fpd.2008.0232.

Hellgren, J., Hästö, L. S., Wikström, C., Fernström, L., & Hansson, I. (2019). Occurrence of Salmonella, Campylobacter, Clostridium and Enterobacteriaceae in raw meat-based diets for dogs. *Veterinary Record, 184*(14), 442. https://doi.org/10.1136/vr.105199.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal, 50*(3), 346–363. https://doi.org/10.1002/bimj.200810425.

Islam, M., Morgan, J., Doyle, M. P., Phatak, S. C., Millner, P., & Jiang, X. (2004). Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. *Applied and Environmental Microbiology, 70*(4), 2497–2502. https://doi.org/10.1128/aem.70.4.2497-2502.2004.

Jablasone, J., Warriner, K., & Griffiths, M. (2005). Interactions of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* plants cultivated in a gnotobiotic system. *International Journal of Food Microbiology, 99*(1), 7–18. https://doi.org/10.1016/j.ijfoodmicro.2004.06.011.

Kassambara, A. (2020). ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0. https://CRAN.R-project.org/package=ggpubr

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmertest package: Tests in linear mixed effects models. *Journal of Statistical Software, 82*(13). https://doi.org/10.18637/jss.v082.i13.

Lee, A., Maks-Warren, N., Aguilar, V., Piszczor, K., Swicegood, B., Ye, M., Warren, J., O'Neill, E., Fleck, M., & Tejayadi, S. (2023). Inactivation of *Salmonella*, Shiga Toxin-producing *E. coli*, and *Listeria monocytogenes* in raw diet pet foods using high-pressure processing. *Journal of Food Protection, 86*(9). https://doi.org/10.1016/j.jfp.2023.100124 100124.

Length, R. V., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2021). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.7.5.Retrieved September 8, 2023, from https://CRAN.R-project.org/package=emmeans

Luu, P., Chhetri, V. S., Janes, M. E., King, J. M., & Adhikari, A. (2020). Effectiveness of aqueous chlorine dioxide in minimizing food safety risk associated with *Salmonella, E. coli* O157:H7, and *Listeria monocytogenes* on sweet potatoes. *Foods, 9*(9), 1259. https://doi.org/10.3390/foods9091259.

Mehlenbacher, S., Churchill, J., Olsen, K. E., & Bender, J. B. (2012). Availability, brands, labelling and *Salmonella* contamination of raw pet food in the Minneapolis/St. Paul area. *Zoonoses and Public Health, 59*(7), 513–520. https://doi.org/10.1111/j.1863-2378.2012.01491.x.

Murray, K., Wu, F., Shi, J., Jun Xue, S., & Warriner, K. (2017). Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. *Food Quality and Safety, 1*(4), 289–301. https://doi.org/10.1093/fqsafe/fyx027.

Nemser, S. M., Doran, T., Grabenstein, M., McConnell, T., McGrath, T., Pamboukian, R., Smith, A. C., Achen, M., Danzeisen, G., Kim, S., Liu, Y., Robeson, S., Rosario, G., McWilliams Wilson, K., & Reimschuessel, R. (2014). Investigation of *Listeria, Salmonella*, and toxigenic *Escherichia coli* in various pet foods. *Foodborne Pathogens and Disease, 11*(9), 706–709. https://doi.org/10.1089/fpd.2014.1748.

Ölmez, H., & Akbas, M. Y. (2009). Optimization of ozone treatment of fresh-cut green leaf lettuce. *Journal of Food Engineering, 90*(4), 487–494. https://doi.org/10.1016/j.jfoodeng.2008.07.026.

Pagadala, S., Marine, S. C., Micallef, S. A., Wang, F., Pahl, D. M., Melendez, M. V., Kline, W. L., Oni, R. A., Walsh, C. S., Everts, K. L., & Buchanan, R. L. (2015). Assessment of region, farming system, irrigation source and sampling time as food safety risk factors for tomatoes. *International Journal of Food Microbiology, 196*, 98–108. https://doi.org/10.1016/j.ijfoodmicro.2014.12.005.

Runesvärd, E., Wikström, C., Fernström, L. L., & Hansson, I. (2020). Presence of pathogenic bacteria in faeces from dogs fed raw meat-based diets or dry kibble. *Veterinary Record, 187*(9). https://doi.org/10.1136/vr.105644.

Sarron, E., Gadonna-Widehem, P., & Aussenac, T. (2021). Ozone treatments for preserving fresh vegetables quality: A critical review. *Foods, 10*(3), 605. https://doi.org/10.3390/foods10030605.

Singh, N., Singh, R. K., Bhunia, A. K., & Stroshine, R. L. (2002). Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and Baby Carrots. *LWT, 35*(8), 720–729. https://doi.org/10.1006/fstl.2002.0933.

Sivapalasingam, S., Friedman, C. R., Cohen, L., & Tauxe, R. V. (2004). Fresh produce: A growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection, 67*(10), 2342–2353. https://doi.org/10.4315/0362-028x-67.10.2342.

Soffer, N., Abuladze, T., Woolston, J., Li, M., Hanna, L. F., Heyse, S., Charbonneau, D., & Sulakvelidze, A. (2016). Bacteriophages safely reduce *Salmonella* contamination in pet food and raw pet food ingredients. *Bacteriophage, 6*(3). https://doi.org/10.1080/21597081.2016.1220347.

Strawn, L. K., Gröhn, Y. T., Warchocki, S., Worobo, R. W., Bihn, E. A., & Wiedmann, M. (2013). Risk factors associated with *Salmonella* and *Listeria monocytogenes* contamination of produce fields. *Applied and Environmental Microbiology, 79*(24), 7618–7627. https://doi.org/10.1128/aem.02831-13.

USFDA. (2018a). *Blue Ridge Beef Recalls Kitten Grind Raw Pet Food Lot#GA1102 Because of Possible Contamination*. Retrieved September 8, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/blue-ridge-beef-recalls-kitten-grind-raw-pet-food-lotga1102-because-possible-contamination

USFDA. (2018b). *Steve's Real Food Voluntarily Recalls Raw Frozen Dog Food Turkey Canine Recipe Due to Possible Salmonella Contamination*. Retrieved September 8, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/steves-real-food-voluntarily-recalls-raw-frozen-dog-food-turkey-canine-recipe-due-possible

USFDA. (2018c). *Radagast Pet Food, Inc. Voluntarily Recalls One Lot of Rad Cat Raw Diet Free-Range Chicken and One Lot of Free-Range Turkey Recipe Because of Possible Health Risk*. Retrieved September 8, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/radagast-pet-food-inc-voluntarily-recalls-one-lot-rad-cat-raw-diet-free-range-chicken-and-one-lot

USFDA. (2018d). *Blue Ridge Beef Voluntarily Recalls BRB Complete Raw Pet Food Lot#GA0131 Because of Possible Contamination.* Retrieved September 8, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/blue-ridge-beef-voluntarily-recalls-brb-complete-raw-pet-food-lotga0131-because-possible

USFDA. (2019). *Go Raw, LLC Recalls One Lot of Quest Beef Because of Possible Salmonella Health Risk*. Retrieved September 8, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/go-raw-llc-recalls-one-lot-quest-beef-because-possible-salmonella-health-risk

USFDA. (2021a). *Bravo Packing, Inc. Recalls All Performance Dog and Ground Beef Raw Pet Food Because of Possible Salmonella and Listeria Monocytogenes Health Risk to Humans and Animals.* Retrieved September 8, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/bravo-packing-inc-recalls-all-performance-dog-and-ground-beef-raw-pet-food-because-possible

USFDA. (2021b). *Bravo Packing, Inc. Expands Previously Announced Voluntary Recall of Pet Food Products Due to Potential Salmonella and Listeria monocytogenes Health Risks to Humans and Animals.* Retrieved September 8, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/bravo-packing-inc-expands-previously-announced-voluntary-recall-pet-food-products-due-potential

USFDA. (2021c). *Woody's Pet Food Deli Recalls Raw Cornish Hen Pet Food for Salmonella Health Risk*. Retrieved September 8, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/woodys-pet-food-deli-recalls-raw-cornish-hen-pet-food-salmonella-health-risk

USFDA. (2021d). *Lancaster Foods recalls all conventional butternut squash items that were processed between the dates of 12/22/20 and 01/08/21 due to possible health risk*. Retrieved February 18, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/lancaster-foods-recalls-all-conventional-butternut-squash-items-were-processed-between-dates-12222

USFDA. (2021e). *Pero Family Farms Food Co.. issues voluntary recall due to possible health risk*. Retrieved February 18, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/pero-family-farms-food-co-issues-voluntary-recall-due-possible-health-risk

USFDA. (2022). *Primal Pet Foods Voluntary Recalls a Single Lot of Raw Frozen Patties Beef Formula Due to Potential Contamination with Listeria Monocytogenes.* Retrieved September 8, 2023, from https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/primal-pet-foods-voluntary-recalls-single-lot-raw-frozen-patties-beef-formula-due-potential

USFDA. (2023). *FDA Advisory: Do Not Feed Certain Lots of Darwin's Natural Pet Products for Dogs & Cats Due to Salmonella.* Retrieved September 8, 2023, from https://www.fda.gov/animal-veterinary/outbreaks-and-advisories/fda-advisory-do-not-feed-certain-lots-darwins-natural-pet-products-dogs-cats-due-salmonella

van Bree, F. P., Bokken, G. C., Mineur, R., Franssen, F., Opsteegh, M., van der Giessen, J. W., Lipman, L. J., & Overgaauw, P. A. (2018). Zoonotic bacteria and parasites found in raw meat-based diets for cats and dogs. *Veterinary Record, 182*(2), 50. https://doi.org/10.1136/vr.104535.

Warriner, K. (2005). Pathogens in vegetables. *Improving the Safety of Fresh Fruit and Vegetables, 3–43*. https://doi.org/10.1533/9781845690243.1.3.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.