Journal of Food Protection, Vol. 81, No. 5, 2018, Pages 795–798
doi:10.4315/0362-028X.JFP-17-361

Research Note

Reduction of Environmental *Listeria* Using Gaseous Ozone in a Cheese Processing Facility

SOFRONI EGLEZOS[1]* AND GARY A. DYKES[2]

[1]*IEH Laboratories, 2 Darnick Street, Underwood, Queensland 4119, Australia; and* [2]*School of Public Health, Curtin University, Bentley, Western Australia 6102, Australia (ORCID: http://orcid.org/0000-0001-5014-9282)*

MS 17-361: Received 24 August 2017/Accepted 13 December 2017/Published Online 9 April 2018

ABSTRACT

A cheese processing facility seeking to reduce environmental *Listeria* colonization initiated a regime of ozonation across all production areas as an adjunct to its sanitation regimes. A total of 360 environmental samples from the facility were tested for *Listeria* over a 12-month period. A total of 15 areas before and 15 areas after ozonation were tested. *Listeria* isolations were significantly ($P < 0.001$) reduced from 15.0% in the preozonation samples to 1.67% in the postozonation samples in all areas. No deleterious effects of ozonation were noted on the wall paneling, seals, synthetic floors, or cheese processing equipment. The ozonation regime was readily incorporated by sanitation staff into the existing good manufacturing practice program. The application of ozone may result in a significant reduction in the prevalence of *Listeria* in food processing facilities.

Key words: Cheese; Environmental; Good manufacturing practice; *Listeria*; Ozone; Sanitation

Contamination from processing environments is the primary reason for the broad distribution of *Listeria monocytogenes* in commercially produced ready-to-eat foods *(19, 20)*. The failure to break the *Listeria* contamination cycle indicates that the food industry is using imperfect sanitation protocols within their good manufacturing practice.

Ozone is one of the technologies that may be used to enhance sanitation protocols in food processing environments. Ozone is a strong oxidizing agent, and its disinfecting activity is a result of nonspecific oxidation of organic molecules *(4)*. Excess ozone autodecomposes rapidly to produce oxygen, and thus, it leaves no residues in food *(13)*. The sanitizer is active against all forms of microorganisms at relatively low concentrations *(13)*.

Ozone has been used to reduce bacteria in food. Gaseous ozone treatment (5 to 30 mg/L) has a bactericidal effect on *Salmonella* Enteritidis inoculated on the surface of tomatoes and can be used for surface sanitation of *Salmonella* Enteritidis on tomatoes before storage under different conditions *(7)*. Treating shell eggs with ozone significantly ($P < 0.05$) reduced *Salmonella* on shell eggs. Contaminated eggs treated with ozone at 4 to 8°C and 15 lb/in^2 (103 kPa) gauge for 10 min at 22 to 25°C for 5 min produced 5.9 log or greater microbial reductions *(17)*. The continuous sparging of alfalfa seeds with ozonated water (initial ozone concentration of 21.3 ± 0.2 μg/mL) for 20 min significantly reduced *L. monocytogenes* by 1.48 log CFU/g *(18)*. The treatment (2 min) of inoculated alfalfa sprouts with water containing 5.0 ± 0.5, 9.0 ± 0.5, or 23.2 ± 1.6 μg/mL of ozone resulted in significant ($P \leq 0.05$) reductions of *L. monocytogenes* (0.78, 0.81, and 0.91 log CFU/g, respectively), compared with populations detected on sprouts treated with water *(22)*.

Cheeses, especially soft cheeses, are prominent vehicles of *Listeria (6)*. A commercial cheese (acid curd) made from pasteurized milk caused a large listeriosis outbreak in Germany from October 2006 through February 2007, affecting 189 people *(14)*. *L. monocytogenes* contamination of Asadero, a Mexican-style cheese made from pasteurized milk, was responsible for a multistate outbreak and recall in the United States; eight affected people, seven of them pregnant, were reported in five states *(11)*. A widespread listeriosis outbreak attributable to pasteurized cheese, which led to extensive cross-contamination affecting cheese retailers in Canada, was responsible for confirmed illnesses in 38 people, 16 of them maternal-neonatal *(9)*.

The effectiveness of gaseous ozone has been demonstrated in a number of studies. These include in vitro studies in which, for example, the bactericidal effect of gaseous ozone (0.05, 0.1, and 2 ppm) was recently evaluated against aerosolized bacteria exposed to ozone; there was a significant ($P < 0$ .05) up to 3-log time-dependent reduction at all concentrations *(2)*. Similarly, aqueous ozone is known to kill surface-attached *L. monocytogenes* biofilms on stainless steel chips *(18)*. In addition, the application of gaseous ozone has been shown to be effective in situ; for example, gaseous ozone application in cleaned and sealed rooms has been shown to effectively kill or neutralize bacteria and help prevent nosocomial infections *(8)*. Furthermore, ozone, applied as a commercial antimicrobial

* Author for correspondence. Tel: +61 7 3841 2232; Fax: +61 7 3841 2241; E-mail: sofroni@biotestlab.com.au.

agent, was shown to be effective in reducing contamination of *Campylobacter* spp. during the processing of broiler chickens *(15)* and in the inactivation of yeast and mold spores from the air in food processing plants *(18)*. The on-site generation of ozone has been shown to be effective as a terminal sanitation step in bottled water production as well as for postharvest sanitation and decay control of fruits, vegetables, and their products during handling, processing, and storage *(12)*.

Gaseous ozone, used as an adjunct to the existing good manufacturing practice cleaning regime, may be effective in removing *L. monocytogenes*. This may be due to the ability of ozone to penetrate into food hygiene surfaces that are difficult to reach and sanitize. In this study we tested this hypothesis by ozonation of cheese processing rooms and determining the prevalence of *Listeria* in the rooms before and after application of the intervention.

MATERIALS AND METHODS

Context. A cheese processing facility based in Queensland, Australia, was operating an externally audited hazard analysis and critical control point food safety system and was listed for export under the jurisdiction of the Australian Department of Agriculture. The facility has a corporate zero tolerance policy for *Listeria* contamination on finished product (over the period of this trial there were no product isolations of *Listeria* spp.) and a corporate target of <1% environmental *Listeria* contamination. An adjunct to sanitation procedures was sought to help the facility meet their environmental *Listeria* contamination goal.

Cheese processing rooms. The facility has four areas: boot wash and staff entry (5.5 by 1.0 by 2.4 m), slice room (15.4 by 28.6 by 3.9 m), packaging and propac room (13.2 by 5.5 by 3.0 m), and shredding and mezzanine room (17.6 by 20.9 by 3.9 m). Floors are covered in a two-component high-build epoxy resin. Room wall panels are 100-mm insulated refrigeration panels consisting of a 1.6-mm sheet over an expanded polystyrene core.

Ozonation. Ozone Generator Specialists (Queensland, Australia) installed an ozone generator system (model Sanitation Pro 4) that uses a two-step process to produce ozone. Specifically, 95% purity oxygen from an oxygen generator is passed through the ozone generator. This process converts oxygen (O_2) into ozone (O_3), and the ozone gas is then distributed into an approximately 2-million-L space, at the rate of 5 L/min over 2 h with the aid of refrigeration units and industrial wall-mounted fans. The concentration of ozone at the discharge point was 20 g/min (as stated by the manufacturer). No other changes were made to the contamination control regime during this test period. This ozonation takes place for 15 min Monday to Friday and 120 min Saturday and Sunday. Treatment automatically commences using timers when staff are not present so as to provide the maximum window between treatment and ventilation purge of the ozonated air via multiple active air changes. There is a minimum 1.5-h exclusion period at the conclusion of treatment. Although unable to make routine ozone measurement due to the presence of an interfering airborne free-flowing agent used in the facility, the treatment was calibrated to hold at least 2 ppm over 60 min on each of the weekend treatments using the average of ozone readings from Aeroqual Series 500 portable ozone meter and Aeroqual Series 930 ozone transmitter (Aeroqual Ltd, Auckland, New Zealand).

TABLE 1. Listeria *prevalence in cheese facility production areas before and after monthly ozone interventions February 2015 to January 2016*

	Before ozone samples		After ozone samples	
	n	Detections (%)	*n*	Detections (%)
Boot wash/entry	24	3 (12.5)	24	0 (0.00)
Slice room	60	12 (20.0)	60	2 (3.33)
Packaging/propac room	36	5 (13.9)	36	1 (2.78)
Shredding/mezzanine room	60	7 (11.7)	60	0 (0.00)
All areas	180	27 (15.0)	180	3 (1.67)

Sampling and microbiological analysis. Sampling was performed monthly on all rooms from February 2015 to January 2016. Preozonation samples were drawn Saturday morning after the end of the deep clean monthly sanitation before ozone was applied. Postozonization samples were drawn on Monday morning after the final ozone application. A broad range of sampling locations for each of the cheese processing rooms were chosen and are listed in Table 1. Separate polyurethane sponges (Whirl-Pak Speci-Sponge, Nasco, Fort Atkinson, WI), moistened with Butterfield's solution (25 mL; bioMérieux, Hazelwood, MO) were used to sample an area of approximately 25 cm^2. In total, 360 environmental samples were drawn.

Sponge samples were tested for the presence of *Listeria* using *Listeria* French Standardization Association AES 10/03-09/00 (bioMérieux SA, Marcy l'Étoile, France). Each sponge was enriched in 225 mL of half Fraser broth (Neogen, Lansing, MI) for 24 h at 37°C. Broths were streaked onto ALOA agar (Micromedia, Auckland, New Zealand) and incubated at 37°C for 24 to 28 h. Presumptive-positive samples were confirmed as per the Australian Standard method AS5013.24.1 *(1),* using isolation, catalase, and staining by Gram's method.

Statistical analysis. The relationship between *Listeria* prevalence and ozone intervention was analyzed using the CHITEST formula in Microsoft Excel 2003 (Microsoft Corporation, Redmond, WA). Significance was indicated when $P < 0.001$.

RESULTS AND DISCUSSION

The prevalence of *Listeria* in cheese processing areas pre- and postozone interventions is presented in Table 2. Facility prevalence is the sum of individual room prevalence. Overall, *Listeria* was isolated from many of the sites sampled, such as tub trolleys, floor boot scrubbers, outfeed conveyors, pallet jack, and tub storage rack. The sites providing the most frequent isolations preozonation were the traditionally difficult to sanitize floor and ledges below sliding doors, as well as drains. The gaseous ozonation treatment was able to penetrate into these areas and effect reductions in 24 of the 27 preozonation positive areas. After ozonation, *Listeria* was isolated from 1.67% of sites (reduced from 15.0% preozonation). The relationship between *Listeria* prevalence and ozonation was significant ($P < 0.001$) for all rooms.

Ozonation is the treatment of a substance with ozone, a highly reactive gas. It is relatively unstable and has a half-life of about 20 to 30 min in an aqueous solution composed

TABLE 2. *Monthly* Listeria *sampling sites within cheese facility production areas before and after monthly ozone interventions, February 2015 to January 2016*

	n	Sampling sites (detections before/detections after)
Boot wash/entry	2	Blue brushes (2/0), floor mat (1/0)
Slice room	5	Sliding door floor and ledge (7/1), tub trolleys (1/0), boot scrubber (0/0), drain (2/1), floor mats (2/0)
Packaging/propac room	3	Floor inside door to slice room (4/0), sliding door floor and ledge (1/1), inside outfeed conveyors (0/0)
Shredding/mezzanine room	5	Pallet jack (1/0), sliding door floor and ledge (1/0), tub trolleys (1/0), drain (4/0), tub storage rack (0/0)

of distilled water at 20°C *(16)*. The half-life is shorter if reducing materials are present *(10)*. The instability of ozone is a known limitation to the application of ozone in the food industry, which is overcome by on-site generation *(16)*.

Air was not monitored over this study because the specific objective was to interrupt *Listeria* environmental colonization. Although there seems to be agreement that exposure to ozone reduces airborne microbes, there is some controversy about the ability of gaseous ozone to inactivate surface-attached microorganisms. A combination of ultrasound and ozone was able to remove *L. monocytogenes* biofilms from stainless steel *(3)*. In one in vivo application, a cheese ripening room was ozonated, and the effectiveness of this treatment was evaluated both in air and on surfaces through sampling on a weekly basis over a 3-month period. The results indicated that ozone treatment reduced the viable airborne mold load (species of *Penicillium, Cladosporium,* and *Aspergillus*) but did not affect viable mold on surfaces *(18)*. In another study, a portable ozone generating machine (Viroforce 1000, Viroforce Systems, Kelowna, British Columbia, Canada) was used to inactivate 13 different species of environmental fungi. These species were wild-type isolates of *Alternaria* sp., *Aspergillus* species (*flavus, niger, fumigatus*), *Aureobasidium* sp., *Botrytis* sp., *Cladosporium* sp., *Geotrichum* sp., *Mucor* sp., *Penicillium brevicompactum, Stachybotris chartarium, Trichoderma viride,* and *Ulocladium* sp. American Type Culture Collection (Manassas, VA) isolates of *Candida albicans* and *A. flavus* were also used *(10)*. Treatments could, in contrast to the earlier study, inactivate 3.0 log CFU of most of the fungi used, both in the laboratory and in simulated field conditions, on various surfaces *(10)*.

Gaseous ozone has been studied as a direct food sanitization system on baby spinach in a pilot-scale system in combination with vacuum cooling *(21)*; these processes decreased *Escherichia coli* O157:H7 populations on spinach by up to 2.4 log CFU/g. Application of low gaseous ozone level on chilled spinach simulating transport similarly effected inactivations of 4.1 to >5.0 log CFU/g, depending on the treatment time *(21)*. The application of gaseous ozone has been demonstrated to effect varying antimicrobial activities on microbes within the area treated, both in the case of airborne as well as surface microbes. There is no concern that treatment is selecting for ozone resistance because the strong oxidizing mechanism of ozone ensures that no resistant bacterial forms can be generated *(18)*.

One consideration in the use of ozone in the food industry is that ozone is a primary human irritant, causing discomfort to the eyes and the respiratory system. Most people can detect about 0.01 μmol/mol of ozone in air, where it has a very specific sharp odor somewhat resembling chlorine bleach *(5)*. Exposure of 0.1 to 1 μmol/mol produces headaches, burning eyes, and irritation to the respiratory passages *(5)*. Strict ventilation and active air change precautions were taken to remove ozone from treated areas prior to the introduction of occupants *(4)*. In our experience with this facility, monthly treatment on a weekend, with corresponding active purge and time for natural ozone breakdown to oxygen, did not result in occupant complaint levels changing from the base preozonation level. The reduction of environmental contamination in this cheese processing facility from 15.0% preozonation to 1.67% postozonation is very significant. We note that, however, even postozonation, *Listeria* was not removed entirely from this facility. *Listeria* is a pernicious, persistent environmental contaminant; in this study, there was a period of approximately 6 months of ozonation being applied before reductions began to take hold (data not shown). The nature of *Listeria* contamination is such that reintroduction will always occur, and ongoing vigilance and disruption of contamination pathways is required.

Ozonation, added as an adjunct to good manufacturing practice cleaning and sanitation protocols, has been readily taken up by facility operators. It was noted that 1 year after treatment commenced, no deleterious effects were noted on floors, walls, drains, or equipment. The application of ozone may result in significant reductions in the prevalence of *Listeria* in food processing facilities.

ACKNOWLEDGMENTS

We acknowledge Niche Food Microbiology Pty Ltd for performing the analysis and Mr. Peter Sullivan for performing the out of hours sampling. We also thank Mr. Peter Gow from Ozone Generator Specialists Pty Ltd for his expert assessment and advice on ozone equipment requirements, placement, and application.

REFERENCES

1. Anonymous. 2009. AS5013.24.1. Food microbiology—microbiology of food and animal feeding stuffs—horizontal method for the detection and enumeration of *Listeria monocytogenes*—detection method (ISO 11290-1:1996, MOD). Standards Australia, Sydney.
2. Bailey, R., L. Fielding, A. Young, and C. Griffith. 2007. Effect of ozone and open air factor against aerosolized *Micrococcus luteus*. *J. Food Prot.* 70:2769–2773.
3. Baumann, A. R., S. E. Martin, and F. Hao. 2009. Removal of *Listeria monocytogenes* biofilms from stainless steel by use of ultrasound and ozone. *J. Food Prot.* 72:1306–1309.
4. Block, S. S. (ed.). 2001. Disinfection, sterilization, and preservation, 5th ed. Lippincott Williams and Wilkins, Philadelphia.
5. Brown, T., J. Nelson, M. Stoltzfus, K. Kemp, M. Lufaso, and T. Brown. 2016. Chemistry: the central science. Pearson Education Limited, Upper Saddle River, NJ.

6. Centers for Disease Control and Prevention. 2013. Vital signs: *Listeria* illnesses, deaths, and outbreaks—United States, 2009–2011. *Morb. Mortal. Wkly. Rep*. 62:448.
7. Das, E., G. C. Gürakan, and A. Bayındırlı. 2006. Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella Enteritidis* on cherry tomatoes. *Food Microbiol*. 23:430–438.
8. de Boer, H. E. L., C. M. van Elzelingen-Dekker, C. M. F. van Rheenen-Verberg, and L. Spanjaard. 2006. Use of gaseous ozone for eradication of methicillin-resistant *Staphylococcus aureus* from the home environment of a colonized hospital employee. *Infect. Control Hosp. Epidemiol*. 27:1120–1122.
9. Gaulin, C., D. Ramsay, and S. Bekal. 2012. Widespread listeriosis outbreak attributable to pasteurized cheese, which led to extensive cross-contamination affecting cheese retailers, Quebec, Canada, 2008. *J. Food Prot*. 75:71–78.
10. Hudson, J. B., and M. Sharma. 2009. The practical application of ozone gas as an anti-fungal (anti-mold) agent. *Ozone Sci. Eng*. 31:326–332.
11. Jackson, K. A., M. Biggerstaff, M. Tobin-D'Angelo, D. Sweat, R. Klos, J. Nosari, O. Garrison, E. Boothe, L. Saathoff-Huber, L. Hainstock, and R. P. Fagan. 2011. Multistate outbreak of *Listeria monocytogenes* associated with Mexican-style cheese made from pasteurized milk among pregnant, Hispanic women. *J. Food Prot*. 74:949–953.
12. Karaca, H. 2010. Use of ozone in the citrus industry. *Ozone Sci. Eng*. 32:122–129.
13. Khadre, M. A., A. E. Yousef, and J. G. Kim. 2006. Microbiological aspects of ozone applications in food: a review. *J. Food Sci*. 66:1242–1252.
14. Koch, J., R. Dworak, R. Prager, B. Becker, S. Brockmann, A. Wicke, H. Wichmann-Schauer, H. Hof, D. Werber, and K. Stark. 2010. Large listeriosis outbreak linked to cheese made from pasteurized milk, Germany, 2006–2007. *Foodborne Pathog. Dis*. 7:1581–1584.
15. Oyarzabal, O. A. 2005. Reduction of *Campylobacter* spp. by commercial antimicrobials applied during the processing of broiler chickens: a review from the United States perspective. *J. Food Prot*. 68:1752–1760.
16. Rice, R. G., and M. E. Browning. 1981. Ozone treatment of industrial wastewater. Noyes Data Corporation, Park Ridge, NJ.
17. Rodriguez-Romo, L. A., and A. E. Yousef. 2005. Inactivation of *Salmonella enterica* serovar Enteritidis on shell eggs by ozone and UV radiation. *J. Food Prot*. 68:711–717.
18. Serra, R., L. Abrunhosa, Z. Kozakiewicz, A. Venancio, and N. Lima. 2003. Use of ozone to reduce molds in a cheese ripening room. *J. Food Prot*. 66:2355–2358.
19. Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. *J. Food Prot*. 65:709–725.
20. Tompkin, R. B., V. N. Scott, D. T. Bernard, W. H. Sveum, and K. S. Gombas. 1999. Guidelines to prevent post-processing contamination from *Listeria monocytogenes*. *Dairy Food Environ. Sanit*. 19:551–562.
21. Vurma, M., R. B. Pandit, S. K. Sastry, and A. E. Yousef. 2009. Inactivation of *Escherichia coli* O157:H7 and natural microbiota on spinach leaves using gaseous ozone during vacuum cooling and simulated transportation. *J. Food Prot*. 72:1538–1546.
22. Wade, W. N., A. J. Scouten, K. H. McWatters, R. L. Wick, A. Demirci, W. F. Fett, and L. R. Beuchat. 2003. Efficacy of ozone in killing *Listeria monocytogenes* on alfalfa seeds and sprouts and effects on sensory quality of sprouts. *J. Food Prot*. 66:44–51.