**Effect of Ozone and Ultraviolet Light on *Listeria monocytogenes* Populations in RTE Chill
Brines**

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ABSTRACT

The efficacy of ozone and ultraviolet light (UV) treatment as hurdles against *Listeria monocytogenes* suspended in fresh (9% NaCl, 91.86% transmittance) and spent brines (20.5% NaCl, 0.01% transmittance) was evaluated. Brines were inoculated with a cocktail of *L. monocytogenes*- strains N1-227, N3-031, and R2-499. Ozonation was performed by sparging gaseous ozone into brine. This was followed by UV irradiation (253.7 nm) of the brine in sterile quartz cuvettes. Enumeration was performed by spread plating on modified Oxford medium and Trypticase Soy agar supplemented with yeast extract. In fresh brines containing *L. monocytogenes*, ten minutes of ozonation lead to a 7.44 ± 0.13 log CFU/ml mean reduction and 10 minutes of UV radiation caused a 1.95 ± 0.41 log CFU/ml mean reduction. Ten minutes of ozonation and UV exposure in combination resulted in > 9 log CFU/ml reduction in *L. monocytogenes* populations in fresh brine. Sixty minutes of ozonation of spent brines resulted in a 4.85 ± 0.61 log CFU/ml mean reduction of *L. monocytogenes* populations. Ten minutes of UV exposure in spent brines resulted in 0.49 ± 0.14 log CFU/ml mean reduction in *L. monocytogenes*. A combination of 60 minutes ozonation and 10 minute UV exposure resulted in an excess of 5 log CFU/ml reduction in *L. monocytogenes* cells in spent brine. Ozonation did not cause a significant increase in the transmittance of the spent brine to aid UV penetration but resulted in color change. Ozonation in combination with UV treatment may serve as an effective treatment in reducing *L. monocytogenes* in chill brines.

50 1. INTRODUCTION

51 *Listeria monocytogenes* is a foodborne pathogen that causes illness ranging from mild
52 gastroenteritis to severe invasive disease (Carpentier & Cerf, 2011). Clinical manifestations of
53 invasive listeriosis include abortion, sepsis, meningoencephalitis and death (Schlech & Acheson,
54 2000). Those at high risk for listeriosis include pregnant women, neonates, the elderly and the
55 immunocompromised (Allerberger & Wagner, 2010). Foodborne outbreaks associated with *L.*
56 *monocytogenes* often have serious implications because of high case fatality rate (19%) and
57 hospitalization rate (94%) (Scallan, Hoekstra, Widdowson, Hall, & Griffin, 2011). *L.*
58 *monocytogenes* can survive and grow in refrigerated foods and foods with high salt content,
59 making food contamination a serious food safety concern (Cole, Jones, & Holyoak, 1990;
60 Larson, Johnson, & Nelson, 1999). The robustness and ubiquity of *L. monocytogenes* in the
61 environment makes it a post processing contamination hazard in the food industry (Tompkin,
62 2002).

63 Non-reheated frankfurters and deli meats have been classified as “high risk foods” for *L.*
64 *monocytogenes* contamination and a “Zero tolerance policy” for *L. monocytogenes* in RTE
65 (ready-to-eat) foods exists in the United States (Swaminathan & Gerner-Smidt, 2007). Product
66 recalls associated *L. monocytogenes* contamination also cost deli meat manufacturers millions of
67 dollars (Gottlieb et al., 2006) making it imperative to develop robust mitigation strategies that
68 prevent contamination of product in the post processing environment. *L. monocytogenes* is a post
69 processing contaminant threat capable of existing in various niches of the food production
70 environment (Tompkin, 2002). The halotolerance and psychrotolerance of *Listeria*

monocytogenes increases the risk of its survival in brines used to chill RTE (Ready to eat) foods after the heat processing step (Gailey, Dickson, & Dorsa, 2003).

Chill brines, used to cool frankfurters once they are thoroughly cooked, are often recycled to cut down on production, waste water treatment costs and reduce water usage. The United States Department of Agriculture (USDA) requires chill brines to have a minimum NaCl concentration of 9% at -2.2°C in order for reuse of up to a week and 20% NaCl at -12.2°C for 4 weeks of reuse (USDA, 2000). *L. monocytogenes* has been known to survive in cheese brines (23.8% NaCl, pH 4.9) stored at 4°C for 259 days (Larson, Johnson, & Nelson, 1999). Palumbo and Williams, (1991), demonstrated that *Listeria* was quite resistant to inactivation or injury when suspended in ground frankfurters, surviving freezing to -18°C with a reduction of < 1 log CFU/g over a 14 week period (Palumbo & Williams, 1991). Thus the use of additional hurdles to prevent post processing contamination is necessary.

Ozone (O₃) is an allotrope of oxygen used for disinfection of bottled water and waste water treatment (Rice, Robson, Miller, & Hill, 1981). In bacteria, it may act as a protoplasmic oxidant causing progressive oxidation of vital cellular components (Khadre, Yousef, & Kim, 2001). It is approved by the United States Food and Drug Administration for use as a disinfectant or sanitizer in the gas or liquid phase on food including meat and poultry and has GRAS status (FDA, 1982). Ozone is effective against a broad range of gram-positive and gram-negative bacteria with *Listeria* showing high sensitivity to ozone (Guzel-Seydim, Greene, & Seydim, 2004; Khadre et al., 2001).

UV radiation has been used for the disinfection of surfaces, fluids and drinking water because it is germicidal to bacteria, yeasts viruses, algae and protozoa (Koivunen & Heinonen-Tanski, 2005) . The antimicrobial effect of UV radiation occurs due to the photochemical

changes (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; Koivunen & Heinonen-Tanski, 2005) that take place in proteins and nucleic acids when UV radiation is absorbed by the cell (Ferron, Eisenstark, & Mackay, 1972). Various foods and beverages have been treated with UV radiation to decrease bacterial content and eliminate pathogens such as *L. monocytogenes* (Bintsis et al., 2000).

Both ozone and UV radiation have demonstrated anti-listerial activity and do not leave harmful residue (White, 2010). Previous research has shown ozone and UV treatment to be beneficial in reducing bacterial content of poultry chill water and red meat processing waste water when used individually and in combination (Diaz, Edward Law, & Frank, 2001; Waldroup, Hierholzer, Forsythe, & Miller, 1993; Wu & Doan, 2005).

The purpose of this study was to (a) determine the time of ozonation and UV treatment to reduce *L. monocytogenes* in fresh brine, (b) determine ozonation and UV treatment time to cause decrease in *L. monocytogenes* populations in spent brine, and (c) determine the effects of these treatments on physical characteristics such as color and UV transmittance of spent brines.

2. MATERIALS AND METHODS

2.1 Ozone Equipment.

A bench top activated oxygen generator was used for ozonating the brines (Golden Buffalo; Orange, Calif., U.S.A.) generating 0.9 g ozone/h at a flow rate of 2.4 L/min. Ozone was produced by passing room air over a UV lamp (Voltarc, GPH287T5/VH-S400/CB) having a UV output of 3W and a spectral output of 185 NM and pumped into brine solutions through a delivery tube (0.25-inch inner diameter; Nalgene 180 PVC; Nalgene Nunc Int. Corp; Rochester, N.Y., U.S.A.) Ozone was sparged through a perforated tubing ring (0.18 inch inner diameter; Nalgene 890 Teflon FEP Nalgene Nunc Int. Corp; Rochester, N.Y., U.S.A.) fitted to the bottom

inside circumference of a sterile container (Nalgene 300 filter receiver (Nalgene Nunc Int. Corp; Rochester, N.Y., U.S.A.). The Nalgene container was placed on a magnetic stirrer/ hotplate (Fisher scientific) and a stir bar was placed in the center of the container to facilitate further diffusion of the gas into solution. All ozonation experiments were carried out in a chemical fume hood.

2.2 Ultraviolet Light Equipment and Set-up.

For UV treatment an Oriel photoreactor (Model 66901, Newport Stratford, Stratford, CT) was used. The photoreactor consisted of an aluminum arc lamp housing unit, 350-Watt mercury bulb (Model 6286, Newport Stratford, Stratford, CT), and a digital power supply unit. A reflector located parallel to the bulb focused the beam onto a condensing lens, producing a collimated beam directed horizontally into the UV chamber. A two inch, 253.7 ± 10 nm directional filter (Model 56501, Newport Stratford, Stratford, CT) was attached to the end of the sample container to exclude other wavelengths (250 to 2500 nm) produced by the mercury bulb. Maximum intensity ($350\text{--}400$ mW/cm²) of the mercury bulb and was measured using a radiant power meter with thermopile sensor (Models 70260, 70263, Newport Stratford, Stratford, CT). A fused silica filter (Model 70185, Newport Stratford, Stratford, CT) was mounted to the sensor to minimize drift in measurements, because of the sensitivity of the thermopile sensors to environmental temperatures. Constant power to the bulb was provided by a digital power supply unit that displayed wattage and approximated the bulb hours (rated at 1000 hrs maximum). A 24x12x12 inch polystyrene chamber with an opening for the condensing lens on one side with a sensor placed directly opposite, lined to prevent light penetration, was constructed to house the 4x4x2 in sample stand during UV exposure.

Before use, the quartz UV cylinders were rinsed with acetone (42324-0040 Fisher Scientific, Suwanee, GA), followed by sterile, distilled water and autoclaved for sterility. During UV treatments, a stirrer housed inside the sample stand was used to continuously stir the test liquid. Sample temperature was maintained at 10–15°C by re-circulating a 50/50 mix of water and ethylene glycol through the UV chamber and through the sample stand for one hour before and during UV application.

2.3 *Listeria monocytogenes* Cultures

Listeria monocytogenes strains N1-227, serotype 4b isolated from hotdog batter implicated in a 1998 outbreak; N3-031, serotype 1/2a, isolated from turkey franks; and R2-499, serotype 1/2a, isolated from a RTE meat product were obtained from Dr. Kathryn Boor and Dr. Martin Wiedmann, Cornell University (Fugett, Fortes, Nnoka, & Wiedmann, 2006).

Fresh Brine Preparation

Fresh brine was prepared by dissolving 90 g of Top-Flo evaporated salt (7559 Cargill, Minneapolis, MN) to a volumetric flask and bringing the volume up to 1000 ml with distilled, sterilized water. After dissolving, the brine was filter sterilized using 0.45 µm pore size filter (Whatman International Limited, Maidstone, England) and stored at 2-4°C until use. Fresh brine was prepared within 24 hours of use.

2.3 Spent Brines

Recycled spent brine was obtained from a frankfurter processor after its maximum usage. Each batch of spent brine (approximately 1000 L) was shipped via refrigerated truck and stored at 4°C until use. Twenty-four hours prior treatment, the spent brine was filter sterilized using

nylon membrane filters, 0.45µM pore size (Whatman International Limited, Maidstone, England)
to remove existing microbiota, and the filtered brine was stored at 2-4°C until use.

2.4 Inoculum Preparation

Each strain of *L. monocytogenes* was activated from stock cultures by three successive 24 h transfers in 25 ml TSBYE and incubated at 35°C for 24 hrs. After the final incubation, the cultures were centrifuged for 5 min at 10,000 x g, the supernatant decanted, and the pellet washed with sterile, distilled water. This process was repeated twice before the pellet was re-suspended in 10 ml of the fluid being treated (fresh brine or spent brine). Equal portions of each strain (6.6 ml) were added to a sterile 500 ml volumetric flask and brought up to 200 ml using the fluid being treated (fresh brine or spent brine). Each preparation was made the day ozonation and UV treatment exposure and stored at 2-4°C.

2.5 Ozone Treatment

200 ml of the *Listeria* brine suspension was added to a preautoclaved Nalgene container (Nalgene 300 filter receiver, Nalgene Nunc Int. Corp; Rochester, N.Y., U.S.A.) with a sparger fitted to the bottom inside circumference. A stirbar was placed inside the container and was activated following which the ozonation unit was turned on. On achieving required time of ozonation, the stirring of the brine suspension was stopped and the unit turned off. Samples were collected to measure residual ozone values, for further UV treatment, and for bacteriological analysis. Fresh brine samples were ozonated for 5 minutes and 10 minutes. Spent brines were ozonated for 30 minutes, 45 minutes and 60 minutes.

2.6 UV treatment

One hour before treatment, the re-circulating refrigeration system was adjusted to 4–10°C and allowed to stabilize. Thirty minutes before treatment, the photoreactor was turned on, allowing the bulb to reach maximum intensity and for output to stabilize. UV intensity was recorded before and after UV exposure. After ozonation, 14 ml of the ozone treated brine was aseptically transferred into a sterile quartz cylinder (14-385-930E Fisher Scientific, Suwanee, GA). Fresh brine samples were exposed to UV radiation for 5 min and 10 min durations. Spent brines were exposed to UV radiation for 10 minutes. After the UV treatment, brine samples were collected for bacteriological analysis.

2.9 Microbiological evaluation.

L. monocytogenes was enumerated before and after ozonation and UV treatment from brine solutions by serial dilution in 0.1% peptone (211677 Becton, Dickinson and Co, Sparks, MD) and spread plating 0.1 ml onto duplicate MOX and TSAYE plates, and incubating at 37°C for 48 hrs. The dual plating system was employed in order to determine the extent of cell injury. Additionally, 3 ml of each ozone-UV treated sample was enriched in 45 ml of Brain Heart Infusion broth (BHI, 237500 Becton, Dickinson and Co, Sparks, MD) and incubated at 37°C for 48 hrs. If turbid after 48 hrs, the BHI sample was streaked onto TSAYE and incubated at 37°C for 24–48 hrs for confirmation with *Listeria* API (10-300 Biomerieux, l'Etoile, France).

2.10 Residual Ozone Measurement

Ozone in fresh and spent brines was measured spectrophotometrically for a concentration range of 0.01 to 0.1 mg O³/L using the Indigo Colorimetric Method (Standard methods for the examination of waters and waste waters, 20th edition).

2.11 Analysis of Color and Transmittance

The transmittance of fresh brine, untreated spent brine and spent brine ozonated for 30, 45 and 60 minutes was measured at 262 nm using a Shimadzu spectrophotometer (Model UV-2101PC). Colorimetric analysis of spent brine ozonated for 30 minutes was performed using a Minolta Chromometer (CR 300 series, Ramsey, NJ, USA) to determine the L^* (lightness), a^* (redness), b^* (yellowness). Measurements were carried out in triplicate.

2.12 Statistical analysis

All ozonation and UV treatment experiments of fresh and spent brines were carried out in triplicate. The experiment for the studies was a randomized blocked design factorial treatment arrangement, repeated measures with sampling, blocked on replication. Means were separated using Least Square Means, SAS version 9.1 (SAS Institute, Cary, NC); significant differences are defined at $P < 0.05$ and highly significant differences are defined as $P < 0.0001$.

3. RESULTS

3.1 Ozone (O₃) and Ultra Violet treatment of fresh brines (9% NaCl).

Fresh brine (9% NaCl) was treated with ozone (O₃) and Ultra Violet radiation (UV) for the following time intervals: 5 min ozone/ 5 min UV, 5 min ozone / 10 min UV, 10 min ozone / 5 min UV and 10 min ozone / 10 min UV. Sparged air was used as a control. Sparging of air for 5 min and 10 min through the fresh brine resulted in nominal reductions of *L. monocytogenes* of 0.04 ± 0.03 and 0.09 ± 0.02 mean log CFU/ml, respectively. To account for cell injury, plating was performed on both TSAYE and on MOX. The differences in populations on selective and non-selective media was used to determine cell injury. Results of ozone/UV treatment of fresh brines

are shown in Tables 1 and 2. Combination of 5 min of ozone sparging and 5 min of UV irradiation treatment resulted in a 5.95 ± 0.22 log CFU/ml mean log reduction of *L. monocytogenes* when enumeration was performed on selective medium (MOX) and a 5.7 ± 0.07 CFU/ml mean log reduction on non-selective medium (TSAYE) ($P < 0.0001$). Combination treatments involving 5 min of ozone sparging and 10 min of UV irradiation resulted in 7.32 ± 0.24 CFU/ml mean log reduction of *L. monocytogenes* populations on MOX and 5.82 ± 0.08 CFU/ml mean log reduction of *L. monocytogenes* populations on TSAYE ($P < 0.0001$). Populations of *L. monocytogenes* decreased to undetectable levels (< 1 CFU/ml) for treatments involving 10 min of ozonation ($P < 0.0001$) on both selective and non-selective media when used in combination with 5 and 10 min UV exposure. Enrichment of treated brine in BHIB did not result in turbidity from growth indicating death of cells. Ozonation for 10 min in combination with 5 min UV exposure caused a mean log reduction of 8.83 ± 0.00 log CFU/ml of *L. monocytogenes* populations on MOX and 9.04 ± 0.00 log CFU/ml mean log reduction on TSAYE ($P < 0.0001$). Treatment of brines with ozone and UV for 10 minutes respectively resulted in a 9.03 ± 0.00 log CFU/ml mean log reduction on MOX and 9.09 ± 0.00 log CFU/ml mean log reduction on TSAYE (Table 1 and 2). Individually, 5.29 ± 0.33 CFU/ml mean log reduction in *L. monocytogenes* was attained after 5 minutes of ozonation and a 7.44 ± 1.22 log CFU/ml mean reduction was observed after 10 minutes of ozonation ($P < 0.001$). Five minutes of UV treatment caused a 1.09 ± 1.01 log CFU/ml mean reduction and 10 minutes of UV exposure caused a 1.61 ± 1.16 log CFU/ml mean reduction in *L. monocytogenes* cells suspended in fresh brine ($P < 0.0001$). Residual level of ozone in the *L. monocytogenes*- brine suspension after 5 minutes ozonation was 0.03 ppm and after 10 minutes ozonation was 0.07 ppm.

3.2 Ozone (O₃) and Ultra Violet treatment of spent brines (20.5 % NaCl)

Results of ozone/UV treatment of fresh brines are shown in Tables 3 and 4. Spent brine (20.5% NaCl) with suspended *L. monocytogenes* cells was treated with ozone for 30 minute, 45 minute and 60 minute time intervals followed by 10 minutes of UV irradiation for all three treatments. Ozonation for 30 min followed by 10 min of UV irradiation resulted 0.29±0.18 Log CFU/ml and 0.17±0.13 Log CFU/ml reduction in spent brine on MOX and TSAYE, respectively. Ozonation for 45 min resulted in a 4.03±2.61 log CFU/ml and 3.54±2.58 log CFU/ml reduction in spent brine on MOX and TSAYE respectively. Ozonation for 60 min with 10 min of UV treatment resulted in 5.53±0.68 log CFU/ml reduction on MOX and a 5.39±0.83 log CFU/ml reduction on TSAYE of *L. monocytogenes* in spent brine. Ozonation resulted in a decrease in *L. monocytogenes* population over time. Thirty, 45 and 60 minutes of ozonation resulted in *L. monocytogenes* population reduction of 0.18±0.06 log CFU/ml, 3.20±2.66 log CFU/ml, 4.97±0.61 log CFU/ml respectively. UV irradiation of the spent brines for 10 minutes resulted in 0.38±0.32 log CFU/ml decrease in *L. monocytogenes* population (P>0.05). The control treatment for 60 min (air sparging) resulted in a 0.12±0.06 log CFU/ml mean reduction in the spent brines (P>0.05). Dissolved ozone was not detected in the spent brine solution after 30 minutes, 45 minutes and 60 minutes of ozone treatment.

3.3 Change in transmittance and color of spent brine

Untreated spent brine had a transmittance of 0.01% while fresh brine (9%) had a transmittance of 91.86% at 253 nm. After 30 and 45 minutes of ozonation, transmittance changed to 0.16 % and 0.18% respectively. Transmittance of the fresh brine remained at 0.18% even after 60 min of ozonation. Turbidity of spent brine before ozonation was 17.3 NTU. Following 30 min of ozonation, turbidity of the brine was 28.3 NTU. Colorimetric analysis

revealed that at 0hr of ozonation, the color of the brine was $L^* 26$ $a^* 0.55$ $b^* 5.98$, after 30 min of ozonation the brine changed to $L^* 24.5$ $a^* 3.80$ $b^* 6.49$.

4. DISCUSSION

Several studies highlight the effectiveness of ozone and UV treatment combinations (Waldroup, Hierholzer, Forsythe, & Miller, 1993; Diaz, Edward Law, & Diaz, 2002; Wu & Doan, 2005). Sensitivity of *L. monocytogenes* to ozonation was observed in experiments by Kim and Yousef (2000) during treatment of food spoilage and pathogenic bacteria. Ozone and UV were effective treatments for eliminating *L. monocytogenes* in fresh brines. Reduction exceeding 9 log CFU/ml of *L. monocytogenes* in fresh chill brine occurred when combined O₃/UV treatment was used. The efficiency of the treatments primarily depended on the time of treatment exposure and the physical characteristics of the brine used. Brine samples were inoculated in BHIB (Brain Heart Infusion broth), after treatment, as growth and turbidity in BHIB would indicate presence of damaged cells that were not killed. After, 10 min ozone/10 min UV treatment, no turbidity was observed in BHIB (Brain Heart Infusion broth), indicating no resuscitation of injured or damaged cells.

Fresh brines (9% NaCl) had a transmittance of 91.86% while spent brines (20.5 NaCl) had 0.01% transmittance. Sixty minutes of O₃/UV treatments was required to achieve 5.47±0.72 log CFU/ml reduction of *L. monocytogenes* cells. The difference in listericidal activity between the two brine matrices could have resulted because of the increased amount of organic content in spent brine that could have quenched ozone. The infusion of debris, particulate matter, nutrients and other substances during the reuse of the chill brines could increase the organic content, resulting in a higher ozone demand of the spent brine in comparison to the fresh brine solution.

Restaino et al.(1995) reported that the presence of 20 ppm of Bovine Serum Albumin in solution significantly affected the death rate of gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes*) and gram negative (*Escherichia coli* and *Salmonella Typhimurium*) bacteria in ozonated water. Exposure to ozonated water containing soluble starch caused instantaneous death of over 5 log units each of *S. Typhimurium* and *E. coli* cells per ml (time zero) , whereas equivalent levels of death for *S. Typhimurium* and *E.coli* in the presence of BSA (bovine serum albumin) occurred only after 2 and 5 min exposures, respectively. Thus the type of organic contaminant in the medium being ozonated could affect the efficiency of the treatment. The requirement of increased duration of ozonation in recycled liquids was further supported by residual ozone measurement in brines using the indigometric method. Fresh brines (200ml) had a residual ozone level of 0.03 ppm after 5 minutes of ozonation and 0.07 ppm after 10 minutes of ozone treatment. Spent brines (200ml) did not have any detectable residual ozone after 30, 45 or 60 minutes of measurement. While transmittance was not dramatically affected by the ozonation treatment, the color of the brine was considerably lighter after treatment. When colorimetrically analyzed, the redness (a^*) of the brine reduced from +0.40 to +0.27. The change in color apart from the microbicidal activity might be advantageous during recycling or treatment of the spent brines.

Transmittance and presence of leachates from constant reuse could also reduce the efficacy of UV treatments. Ten minutes of UV irradiation resulted in reduction of 1.61 ± 0.51 log CFU/ml ($P < 0.05$) and 0.38 ± 0.32 log CFU/ml ($P > 0.05$) reduction in *L. monocytogenes* population in fresh brine and spent brine respectively. The transmittance of the brine is an important factor for the effective microbicidal action of UV radiation since it cannot penetrate into food surfaces. The transmittance of the spent brines increased from 0.01% to 0.18% after 45 minutes of

ozonation and remained at 0.18% after 60 minutes of ozone treatment. The lack of an increase in transmittance of the brine might have hampered the performance of the UV treatment in the fresh brines.

The results of the study indicate that ozone and UV irradiation treatment combinations could serve as an effective measure to eliminate *L. monocytogenes* in chiller brines. The reuse of brines to cool multiple batches of product could result in infusion of ingredient component in water and hence longer treatment duration of ozonation and UV irradiation would be required with increase in recycling.

5. CONCLUSION

Treatment of brines with ozone and UV is effective in reducing *L. monocytogenes* populations. The efficiency of the process depends on the quality of brine used, duration of exposure and dose or intensity of the treatments based on brine parameters. Spent brines required higher ozonation times. The low transmittance of spent brines affected UV treatment efficiency. Ten minutes of ozone and UV treatments resulted in 9.06 ± 0.00 Log CFU/ml reduction of *L. monocytogenes* from fresh brines. In spent brines 60 minutes of ozonation and 10 minute UV irradiation exposure resulted in 5.46 ± 0.68 Log CFU/ml reduction in *L. monocytogenes* populations. The lighter color of the brine after treatment and reduction in *L. monocytogenes* population along with the absence of residual byproducts indicates that ozonation and UV irradiation is an effective treatment for RTE chiller brines.

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TABLES AND FIGURES

Table 1. Populations of *Listeria monocytogenes* (LM) in fresh brines (9% NaCl) before and after combined ozone (O₃) and ultraviolet light (UV) treatment as determined on Modified Oxford Agar.

Treatment time	Initial LM population (Log CFU/ml)	UV intensity (mW/cm ²)	Final ^a ozone concentration (ppm)	Final LM population (Log CFU/ml)
5minO ₃ /5minUV	9.2±0.08	11.31±0.23	0.02±0.01	3.24±0.22
5minO ₃ /10minUV	9.28±0.05	10.89±0.81	0.04±0.01	1.95±0.24
10minO ₃ /5minUV	8.83±0.52	11.36±0.28	0.08±0.01	<1
10minO ₃ /10minUV	9.03±0.09	10.85±0.34	0.07±0.01	<1

^a Measurement of ozone concentration were made at the end of treatment time.

Table 2. Populations of *Listeria monocytogenes* (LM) in fresh brines (9% NaCl) before and after combined ozone (O₃) and ultraviolet light (UV) treatment as determined on Trypticase Soy Yeast Extract Agar.

Treatment time	Initial LM population (Log CFU/ml)	UV intensity (mW/cm ²)	Final ^a ozone concentration (ppm)	Final LM population (Log CFU/ml)
5minO ₃ /5minUV	9.35±0.12	11.31±0.23	0.02±0.01	3.65±0.07
5minO ₃ /10minUV	9.36±0.08	10.89±0.81	0.04±0.01	3.53±0.08
10minO ₃ /5minUV	9.04±0.09	11.36±0.28	0.08±0.01	<1
10minO ₃ /10minUV	9.09±0.11	10.85±0.34	0.07±0.01	<1

^a Measurement of ozone concentration were made at the end of treatment time.

Table 3. Populations of *Listeria monocytogenes* (LM) in spent brines (20.5% NaCl) before and after combined ozone (O₃) and ultraviolet light (UV) treatment as determined on Modified Oxford Agar.

Treatment time	Initial LM population (Log CFU/ml)	UV intensity (mW/cm ²)	Final ^a ozone concentration (ppm)	Final LM population (Log CFU/ml)
30minO ₃ /10minUV	8.86±0.06	11.44±0.39	Not Detected	8.56±0.16
45minO ₃ /10minUV	8.86±0.06	10.36±0.22	Not Detected	4.82±2.59
60minO ₃ /10minUV	8.86±0.06	11.73±0.45	Not Detected	3.32±0.65

^a Measurement of ozone concentration were made at the end of treatment time.

Table 4. Populations of *Listeria monocytogenes* (LM) in spent brines (20.5% NaCl) before and after combined ozone (O₃) and ultraviolet light (UV) treatment as determined on Trypticase Soy Yeast Extract Agar.

Treatment time	Initial LM population (Log CFU/ml)	UV intensity (mW/cm ²)	Final ^a ozone concentration (ppm)	Final LM population (Log CFU/ml)
30minO ₃ /10minUV	8.88±0.03	11.44±0.39	Not Detected	8.71±0.19
45minO ₃ /10minUV	8.88±0.03	10.36±0.22	Not Detected	5.34±2.55
60minO ₃ /10minUV	8.88±0.03	11.73±0.45	Not Detected	3.49±0.80

^a Measurement of ozone concentration were made at the end of treatment time.