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Effect of ozone and ultraviolet light on *Listeria monocytogenes* populations in fresh and spent 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*, 10 min of ozonation lead to a 7.44 ± 0.13 log CFU/ml mean reduction and 10 min of UV radiation caused a 1.95 ± 0.41 log CFU/ml mean reduction. Sequential exposure of 10 min of ozonation and UV 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 sequential treatment of 60 min ozonation and 10 min UV 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. Sequential treatments of Ozonation and UV maybe effective in reducing *L. monocytogenes* in chill brines.

1. Introduction

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

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

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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 et al., 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 (O_3) 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 and spent brine and (b) 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 3 W 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 (250–2500 nm) produced by the mercury bulb. Maximum intensity (350–400 mW/cm^2) 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 h maximum). A 24 × 12 × 12 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 4 × 4 × 2 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 1 h before and during UV application.

2.3. Fresh brine preparation

Fresh brine was prepared by dissolving 90 g of Top-Flo evaporated salt (7559 Cargill, Minneapolis, MN) in a volumetric flask and bringing the volume up to 1000 ml with distilled, sterilized water. The prepared 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 h of use.

2.4. 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, 0.45 μM pore size (Whatman International Limited, Maidstone, England) to remove existing microbiota, and the filtered brine was stored at 2–4 °C until use.

2.5. Inoculum preparation

L. 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). Each strain of *L. monocytogenes* was activated from stock cultures by three successive 24 h transfers in 25 ml Tryptic Soy Broth (TSB; all media unless stated were from Difco, Becton Dickinson, Sparks, Md.) supplemented with 0.6% Yeast