I Effectiveness of Ozone for Controlling *Listeria monocytogenes* in Ready to Eat Cured Ham

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II Abstract

The muscle foods are exposed to microbial contamination during slaughter and handling. This can cause microbial spoilage and food borne illness. Newly emerging strains of *Listeria monocytogenes* need to be inactivated to render safe ready to eat ham products. We investigated the effectiveness of ozone in inactivating *Listeria monocytogenes* in ready to eat cooked cured ham (97% fat free). The effectiveness of ozone in three different environments 1) gaseous, 2) aqueous and 3) humidified (>90%) was studied. The effectiveness was studied for all environments at 3 ozone concentrations (0.2, 0.5 & 1.0 ppm), 3 exposure times (1, 15 & 30 min) and 2 temperatures (10 & 20ºC). The effectiveness was represented as %kill of *Listeria monocytogenes*. The maximum microbial inactivation for any of the treatment combinations was limited to 99.7% (i.e., < one log cycle reduction). The most effective environment was gaseous followed by aqueous and humidified. Effectiveness increased with increasing exposure time, temperature & ozone concentration. The results indicated that the presence of high organic matter (i.e. protein) might have quenched the lethal activity of ozone. The samples exposed to gaseous environment at 20ºC were also analyzed for the physical characteristics of color and shear strength. The Hunter a* values for different treatments were in the range of 10.3 to 3.2. The samples tend to have lower a* values with increased ozone concentration and exposure time. The shear strength values of ham samples under different treatment conditions were not significantly different. Ozone may be of limited use in reducing microbial population such as *Listeria monocytogenes* on cured ham products.

III Introduction

Rapidly increasing population density throughout the world and the increased need for food storage and transportation has been accompanied by increased food poisoning caused by microbial strains such as *Listeria monocytogenes.* Food borne illness costs the U.S economy billions of dollars each year in lost productivity, hospitalization, long-term disability and even death. Consumers are demanding safe, nutritious, natural foods. The government is enhancing consumer awareness by requiring proper detailed labeling, thus enabling the consumer to select the food products they desire. The accumulation of toxic chemicals in our environment has increased the national focus on the safe use of sanitizers, bleaching agents, pesticides, and other chemicals in industrial processing *(1).* There are several processing methods available for inactivation of microorganisms in foods; thermal, high pressure, pulsed electric field, oscillating magnetic field, irradiation and ozonation. Ozonation is a relatively new method for food processing. Ozone has been used safely and effectively in water treatment for nine decades, at scales from a few gallons per minute to millions of gallons per day. It is approved in the US as generally recognized as safe (GRAS) for treatment of bottled water and as a sanitizer for process trains in bottled water plants *(2).* Recent investigations involving the use of ozone dissolved in water for sanitizing surfaces of vegetables, fruits and other agricultural products support the claim that ozone is a powerful disinfectant. The Food industry in Europe has been using ozone for decades. However, the food industry in the U.S. has little or no experience with application of ozone to foods. This may be due to lack of studies concerning the efficacy of ozone in food processing, safety issues, toxicology and the impact on nutrients. In June 1997, ozone was awarded GRAS status as a disinfectant for foods by an independent panel of experts, sponsored by the Electric Power Research Institute *(3).* This action cleared the way for the use of ozone in the $430 billion food processing industry. However, the final rule of FDA has not been published.

Foods of muscle origin provide favorable conditions for microbial growth. Muscle foods may be exposed to microbial contamination during slaughter and handling and this can cause microbial spoilage and food borne illnesses. New microbiological strains of *Listeria monocytogenes* have prompted a need to improve the microbiological status of ready to eat meat products. The Food Safety and Inspection Service (FSIS) of the USDA has responded with establishment of “zero tolerance” and “clean meat” programs. Numerous studies have been

conducted in the application of different processing methods to inactivate microorganisms in various food materials. However, the application of ozone in inactivating the microorganisms in various food materials is still in the infancy stage *(4)*.

Ozone is a gas at ambient and refrigerated temperatures. It is soluble in water and like most gases, increases in solubility as the water temperature decreases. It has the unique property of auto-decomposition, producing numerous free radical species, the most prominent being the hydroxyl free radical (OH^-). Ozone's mode of action is through oxidation of bacterial cell wall components. There is very little information available on the most effective phase of ozone application and the effect of varying concentrations of ozone for reducing microbial levels on ready to eat pork products and also the changes in physical characteristics of the meat due to ozone treatment.

IV Objectives

1. Evaluate the effectiveness of ozone for controlling *Listeria monocytogenes* in fully cooked cured ham.
2. Determine the most effective application environment; gaseous, aqueous, humidified (> 90%RH), time, temperature and concentration level for maximum *Listeria monocytogenes* inactivation.
3. Evaluate the effects of ozone treatment on physical properties of ready to eat fully cooked cured ham.

V Procedures

***Listeria monocytogenes* – Isolation, Propagation & Inoculation:**

The *Listeria monocytogenes* organism used for this study was isolated from a food source. The active *Listeria monocytogenes* culture was tested for purity using – motility, gram staining, catalase and CAMP tests. The active culture was preserved in a frozen state; culture + 40% glycerol + Tryptic soy broth enriched with 0.6% yeast extract (TSB). For all treatments the frozen culture was activated and propagated in TSB enriched with 0.6% yeast extract at 37°C for 48 hrs. The grown culture was spindled out using a centrifuge and refrigerated, for inoculation on to the ham sample.

Approximately 0.1ml of this active culture containing a known population of *Listeria monocytogenes* was spread on a $50.0cm^2$ area of Hyvee brand Reduced fat (97% fat free) cooked cured ham slices. The inoculated samples were stored for 20 minutes under refrigerated condition, to allow the culture to absorb to the surface of the ham.

Treatments & Experimental Design:

Treatment	**Levels**
Ozone level (ppm)	0.20, 0.5, 1.0
Temperature (ºC)	10, 20
Exposure time (min)	1, 15, 30

The whole experiment was conducted under three different environments; gaseous, aqueous and high humidity (>90% RH) at all the treatment levels listed above. The ozone was generated using an ozone concentrator-generator, OZ1 BTU, manufactured by Ozotech, Inc. Ozone monitoring equipment, Model 450H, Advanced Pollution Instrumentation Inc., was used to calibrate the ozone concentration output from the generator and concentrator system. The samples to be treated under the gaseous environment were placed in a sample treatment plexiglass chamber (8.75"X 6.75"X 7.75"). The chamber was connected to the ozone generator -concentrator system via flexible tubes. To create a high humidity environment ozone from the generator was passed through an ultrasonic humidification system (Electrotech system Inc.) to create a humidified ozone environment in the sample treatment chamber. An ozone entrainment device was designed for the aqueous environment study. The samples were suspended in a water entrainment chamber and ozone was bubbled continuously through the water. The iodometric method was used to determine the concentration of ozone dissolved in the water. Two control samples were used in the study. One control was inoculated with *Listeria monocytogenes* and the other was not.

Microbial Analysis:

The enumeration of *Listeria monocytogenes* was done using the spread plate technique *(5)*. The efficacy of the above treatments was reported as the percent kill (%kill) of *Listeria monocytogenes*.

Measurement of the Ham Color and Shear Strength:

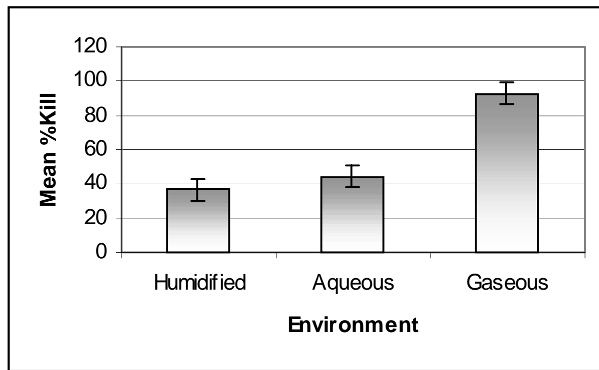
The most effective environment and temperature amongst all treatments were determined based on % kill of *Listeria monocytogenes*. The most effective environment (i.e., gaseous) and temperature treatment (i.e., 20 °C) combination, was chosen to study the effect of ozone concentration and exposure time on the physical properties of color and shear strength of ozone treated ham samples. Color was measured as Hunter a* values using a Minolta Colorimeter. Shear strength was measured as peak load using a Kramer shear cell attached to a Sintech2/D machine.

Statistical Analysis:

A total of 108 ham samples were treated under the described combinations of ozone concentration, environment, exposure time and temperature. The entire experiment was replicated twice (2 temperature X 3 time X 3 concentration X 3 environment X 2 replications). Analysis of variance was employed to separate mean values. Statistical analysis software (SAS Institute, Cary, NC) was used for the analyses.

VI Results:

The results indicated that among all the environments of ozone application tested; gaseous, aqueous and humidified (>90% RH), the gaseous environment was the most effective for inactivating *Listeria monocytogenes*, followed by the aqueous and the high humidity environment (Figure 1). All these environments were significantly different ($p<0.05$). The most effective environment resulted in about a one-log cycle reduction in microbial population. As ozone concentration increased from 0.2ppm to 0.5ppm, the microbial inactivation significantly increased (Figure 2). Above 0.5ppm, the inactivation was not significant ($p<0.05$).



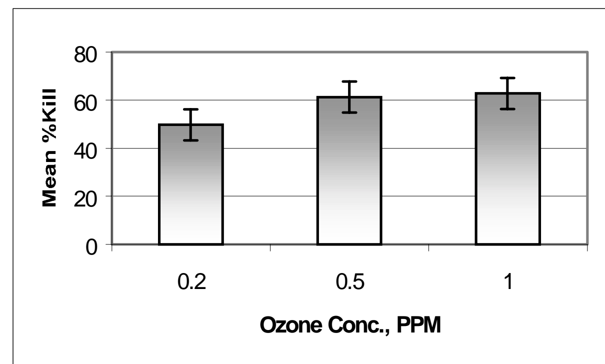
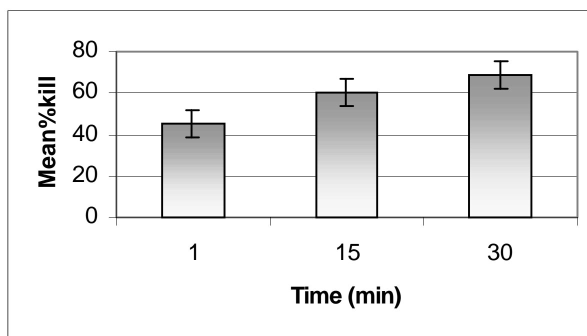


Figure 1. Effect of Environment on %Kill **Figure 2. Effect of Ozone Conc. on %Kill**

With the increase in ozone concentration, there was no significant difference in microbial inactivation for the aqueous environment ($p<0.05$). Moreover, as the exposure time increased from 1 min to 15 min and from 15 min to 30 min there was a significant increase in inactivation of *Listeria monocytogenes* ($p<0.05$) (Figure 3). The effectiveness of microbial inactivation increased significantly ($p<0.05$) as the treatment temperature increased from 10 to 20ºC (Figure 4). However, for the aqueous environment the effectiveness of microbial inactivation increased significantly as treatment temperature decreased to 10°C from 20°C.



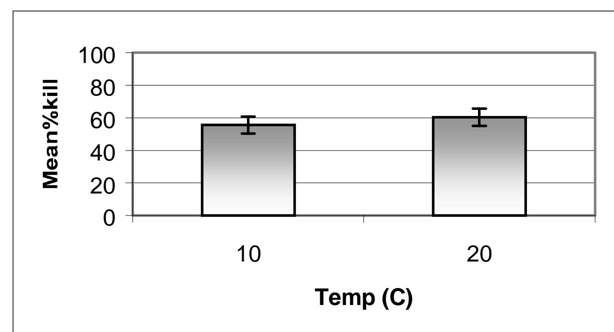
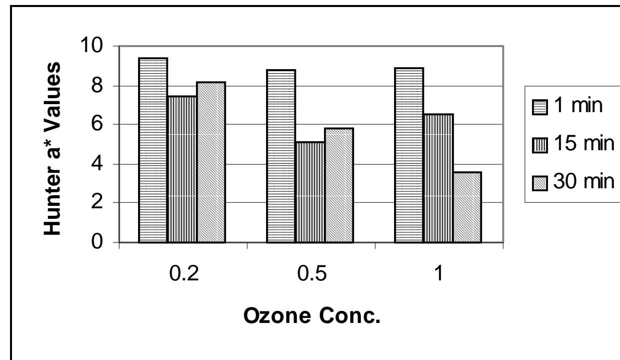


Figure 3. Effect of Exposure Time on %Kill **Figure 4. Effect of Temperature on %Kill**

We studied several physical characteristics under the most effective ozonated environment, gaseous at 20ºC. Analysis of the peak load shear strength results did not show significant differences due to ozone exposure time or concentration levels. The treatment combinations of 0.2ppm ozone for one minute and 0.5ppm ozone for fifteen minute were the

only treatment that were significantly different ($p<0.05$), for shear strength. The mean a* values of the samples treated with 0.2ppm ozone for 1 min and 15 min were significantly higher



($p<0.05$) than those treated with higher ozone concentrations of 0.5ppm for 1 min and 1.0ppm for 30min (Figure 5).

Figure 5 - Effect of Ozone Treatment on a* values at 20°C Temperature

The well known effectiveness of ozone as a sanitizing and disinfecting agent suggested its probable usage for inactivating food borne pathogens such as *Listeria monocytogenes* in ready to eat cooked cured ham. It was surprisingly, only moderately effective. Less than one log cycle reduction in *Listeria monocytogenes* counts resulted after 30 min of exposure at ozone concentration up to 1.0ppm. The aqueous and high humidity environments were less effective than the gaseous environment.

It has been previously shown that organic matter can affect the antimicrobial activity of ozone *(6,7,8)*. The antimicrobial activity of ozone has long been known. Less clear is its mode of action; suggestions for primary targets include unsaturated lipids in the cell surface, sulfhydryl groups, nucleic acids, and others. Proposed mechanisms for inactivation have been recently summarized *(9)*. The reactivity of ozone is believed to be due to the oxidizing power of free radicals formed in a chain reaction during its decomposition. Indeed, ozone molecules themselves may be relatively nontoxic to microorganisms *(10)*. Organic matter may inhibit this chain reaction *(11)*. Foods of muscle origin contain an abundance of complex organic matter as fat and proteins. The presence of this organic matter may have quenched the lethal activity of ozone. Though the exact mechanism of interaction is not known, this could be one of the most

important reasons for reduction in lethal activity of ozone *(12)*. The results of the hunter a* measurement showed that there is significant reduction in a* values of the ham sample subjected to higher ozone concentration and exposure time. This may be due to interaction of free radicals with proteins and other organic matter present in the ham. The shear strength measurements did not show any significant changes.

The results of this research seem to indicate that although ozone was effective in reducing *Listeria monocytogenes* on ready to eat fully cooked cured ham the population was not reduced more than one log. Ozone tended to reduce a* or redness values at higher concentrations and longer exposure times. Ozone had no effect on shear strength of the ham samples.

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