INVESTIGATION OF IN-PACKAGE OZONATION

Student Author

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Faculty Mentor

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Abstract

Food production, handling, and distribution practices pose a constant threat to the quality and safety of food products. The objective of this research is to evaluate an innovative in-package ozonation process to reduce *Salmonella enteritidis* on raw, shell eggs. Previous research has shown that in-package ozonation eliminates contaminants from outside sources, reduces pathogens, and extends shelf life. In this study, raw, shell eggs were inoculated with *Salmonella enteritidis* and exposed to ozonation treatment. Microbial recoveries were then tested to determine bacterial reductions. Measurements included: relative humidity (34 percent at 5^{o}C), surface temperatures (^{o}C), ozone concentrations, bacterial reductions of *Salmonella enteritidis*, and quality assessment of eggs (Haugh Unit [HU], color, pH, and weight). After a 24-hour storage period, all treated samples indicated 3 $\log_{10}$ reductions on average (previous research has achieved up to 6$\log_{10}$). These results show effective in-package ozonation treatment reducing *Salmonella enteritidis* on raw, shell eggs without significant effect on measured egg quality over time. Benefits of in-package ozonation include no heating, low power requirements ($\leq$ 50 Watts), short treatment time (seconds to minutes), and adaptability into existing processes. Given its ability to ensure the safety and longevity of food products, this technology has great potential for utilization in the food processing industry.

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INVESTIGATION OF IN-PACKAGE OZONATION:

The Effectiveness of Ozone to Inactivate *Salmonella enteritidis* on Raw, Shell Eggs

Austin Donner, Food Sciences

Introduction

Pathogens are disease-causing bacteria that spread from person to person and can cause a variety of illnesses. While pathogens are transmitted in many different ways, infection commonly occurs as a result of the bacteria being present in food. Foodborne illnesses cause an estimated 48 million infections and 3,000 deaths in the United States each year (U.S. Department of Agriculture, Food Safety Inspection Service, 2011a). One specific foodborne pathogen that infects many people each year is *Salmonella*.

Salmonellosis is an infection caused by the bacterium *Salmonella*, often associated with contaminated food or drink. The *Salmonella* family includes over 2,300 serotypes (one-celled microscopic organisms) of bacteria. Two serotypes, *Salmonella enteritidis* (SE) and *Salmonella typhimurium*, are the most common in the United States and account for half of all human Salmonella infections (U.S. Department of Agriculture, Food Safety Inspection Service , 2011b). Common symptoms of this disease include diarrhea, vomiting, fever, and abdominal cramps that can last four to seven days after infection. In more serious cases, a *Salmonella* infection is able to spread from the intestines to the blood stream and other body sites, making the infection potentially fatal (Centers for Disease Control and Prevention, 2010). In the United States, there are approximately 40,000 cases of salmonellosis reported annually, but it is estimated that because some of the less severe cases go undiagnosed or unreported, the actual number of infections could be at least 30 times greater (Centers for Disease Control and Prevention, 2010).

In addition to its health effects, there are substantial economic costs that result from this disease. Annual medical expenses and lost labor productivity costs due to salmonellosis are reported to be in the billions (Frenzen et al., 1999). Furthermore, according to the Food and Drug Administration (FDA), the incidence of salmonellosis appears to be rising both in the U.S. and in other industrialized nations. In 2008, the incidence of SE infection increased by 19 percent, which indicates the significance of SE as a major cause of human infection in the United States (U.S. Food and Drug Administration, 2009a).

While there are many modes of transmission and foods that are responsible for human infection, shell eggs are among the top source of *Salmonella* infection. Between 1985 and 2002, a total of 53 percent of all SE illnesses identified through CDC outbreak surveillance were attributable to eggs (FDA, 2009a). A food vehicle (a solid or liquid food that is the source of the bacterial transmission) is identified in approximately half of all SE outbreaks, with shell eggs being the principal food vehicle recognized. In a farm-to-table risk assessment of SE in eggs, which was conducted by the FDA and the U.S. Department of Agriculture's (USDA's) Food Safety and Inspection Service (FSIS), it was estimated that of the 47 billion shell eggs consumed annually as table eggs (eggs consumed directly, as opposed to eggs that are used to make egg products), 2.3 million are SE-positive, exposing a large number of people to the risk of illness (FDA, 2009a). On July 9, 2009, the FDA issued a final rule requiring shell egg producers to implement procedures to prevent SE from contaminating eggs on farms and to prevent further growth of SE during storage and distribution (FDA, 2009b).

The challenge in preventing bacterial growth in food products lies in enhancing food safety without compromising the quality or desirability of food products. One such method is nonthermal processing using ozone for the destruction of pathogens. Given its antimicrobial properties and lack of residual substances, ozone is both effective and safe (Guzel-Seydim, Greene, & Seydim, 2004). In-package ozonation is a patent-pending technology, developed by Dr. Kevin Keener of Purdue University's Department of Food Science, for generating ozone inside a sealed package environment, as shown in Figure 1. Ozone is created in a simple process between two electrodes operated at a particular voltage, frequency, and geometry. Reactive oxygen species are generated, which react with one another and with oxygen molecules, resulting in the formation of ozone. Most of the reactive oxygen species have very short half-lives (in the range of milliseconds), making them difficult to work with. Ozone, however, has a much longer half-life, ranging from minutes to days depending on conditions (MKS Instruments, 2004). In 1999, Kim, Yousef, & Dave concluded that ozone is more efficient at lower concentrations and treatment times than more standard sanitizers, such as chlorine. Previous studies on the effects of ozone on foods have also shown promising results. Rodriguez-Romo and Yousef showed that ozone treatment conducted at atmospheric pressure for three minutes reduced SE on eggshells by 3.1 log units compared with the untreated control (2004). Since each log unit represents a 90 percent reduction in microbial population, a process shown to achieve a "5-log reduction" (10^{-5}) will reduce a population from a hundred thousand organisms (10^5) to very close to zero, theoretically. Klockow and Keener showed that spinach leaves treated with ozone and stored for half an hour, two hours, and five days gave average log reductions of .68, 2.97, and 5.61, respectively (2009). While shell eggs are the focus for this study, numerous other food products have been treated using Keener's ozonation system, including raw chicken, lettuce, spinach, tomatoes, and cranberries, all of which have displayed bacterial reductions. Past research conducted by Keener treated in-package tomatoes using the ozonation system and yielded reductions of SE > 5 $\log_{10}$.

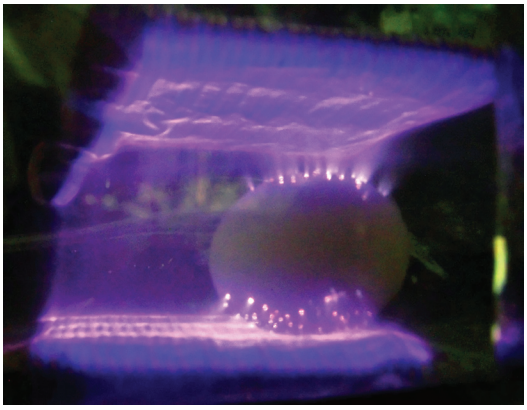

Figure 1. Treatment of inoculated egg generates a purple plasma field of ozone.

Methods

The objective of this research was to evaluate the in-package ozonation process intended to reduce SE on raw, shell eggs. In addition to evaluating the inactivation of SE on the shell eggs, quality measurements were recorded over a three-week period using a pH meter and an egg multitester unit.

In-package ozonation uses only 40 watts of power, the equivalent to a weak lightbulb. This ozonation process generates reactive oxygen species that react with one another and with the present oxygen molecules to form ozone. Oxygen species generated as a result of this process can include: ozone (O_3), oxides (O_{2-}), singlet oxygen (O or O^-), peroxides (H_2O_2), and hydroxyl radicals (OH^-). The procedure for conducting an in-package treatment on various packages includes placing a sealed package between two electrodes and creating an ionization field, as shown in Figure 2. As a current is run through the two electrodes, the ionization field creates the reactive oxygen species mentioned earlier.

The elements of the experimental design for this study are summarized below in Table 1.

VARIABLE	LEVELS/[CONC]
Ozone (Air-based Ozonation)	1400-2000 ppm range
Ozone Treatment Time	5 minutes
Salmonella entiritidis E190:88	8 Log_{10}
Background Bacterial Load of eggshell	$\leq$ 1 Log_{10}
Temperature	5° C
Relative Humidity	37-39%
MEASUREMENTS	**METHODS**
Ozone (Air-based Ozonation)	Drager tubes
Salmonella entiritidis E190:88 Plate counts	XLD Agar—Selective
Background Bacterial Load of eggshell	PCA Plate Count Agar — Total Plate Counts
pH/Temperature	pH meter and probe
Relative Humidity	Temp/RH Gauge
Egg Quality—Haugh Units (HU)	Egg Multi Test Unit: EMT-5200
Egg Color	Albumin/Yolk by Observation

Table 1. Summary of experimental design

Experiments were conducted in triplicate replications and included inoculated, treated eggs. Positive and negative controls were conducted in duplicate. Eggs at room temperature (23°C) were spot inoculated and injected with 20 µL SE (strain 190:88) within a one-inch diameter circle template on the blunt end of the egg (see Figure 3). Inoculum on the eggs was allowed to dry for two hours at 22˚C. After drying, the eggs were placed in a refrigerator for up to 24 hours to reach treatment temperature of 5˚C.

The egg quality experiments were carried out in triplicate, each over a period of 21 days. Quantitative observations were used to measure the yolk and albumin color. The pH of the yolk and albumin were measured using a pH meter, and the Haugh Unit (HU, a measure of egg quality: A, AA, etc.) was measured using an egg multitester unit.

Inoculated eggs were removed from 5˚C storage after 24 hours, placed into plastic one-gallon Ziploc freezer bags, which were filled with compressed air, and placed into weigh boats. One-gallon Ziploc freezer bags were loaded with three raw inoculated eggs each in plastic weigh boats for five minutes of ozone treatment and were double bagged after ozonation. The package was exposed to the ionization treatment process at 18KV (variable autotransformer and copper plates) for five minutes. The surface temperatures (˚C) of the bags, eggs, and copper plates were recorded. The ozone levels were measured using Drager Tubes (20-300 ppm) immediately after treatment and again at 24 hours using Drager Tubes (0.05-1.4 ppm).

All packages were stored at 5˚C for 24 hours after treatment. After 24 hours, the inoculated section (1.5 ±.2 g) of the raw, shell egg was extracted using sterile methods. Post treatment recoveries of surviving bacterial cells were performed using Shell Crush Method (Musgrove et al., 2005) and Spread Plate technique.

After respective holding storage time, each egg was pulled from its bag. The 1-2 gram shell template section was excised with a sterile scalpel, its underside rinsed with DDI (distilled de-Ionized) water to remove any adhering albumin, and then transferred to a 50mL sterile conical containing 20 mL of 0.1 percent peptone. The shell section was crushed in the conical using a sterile glass rod (one minute) and was then mixed by vortexing and immediately plated in duplicate on XLD agar (specific for *Salmonella*) using the spread plate method. Serial dilutions were carried out to 10^{-4} for positive controls, 10^{-1} for negative controls, and 10^{-2} for treatments. Positive control dilutions (10^{-2}, 10^{-3}, and 10^{-4}) and treated sample dilutions (10^{-1} and 10^{-2}) were plated using Spread Plate Technique. Plates were incubated for 24 hours at 37°C. The colony-forming units (cfu) were manually counted after a 24-hour incubation period at 37° C, and the results were quantified. A colony-forming unit is a cell or group of cells that reproduce on a plate, resulting in a visible colony to be observed in order to quantitate the number of bacteria present. The contents of each egg, albumin and yolk, were evaluated on the Egg Multi-Test Unit—EMT 5200 for egg quality based on HU. Visual inspection of the post-treatment color/clarity of albumin and yolk of

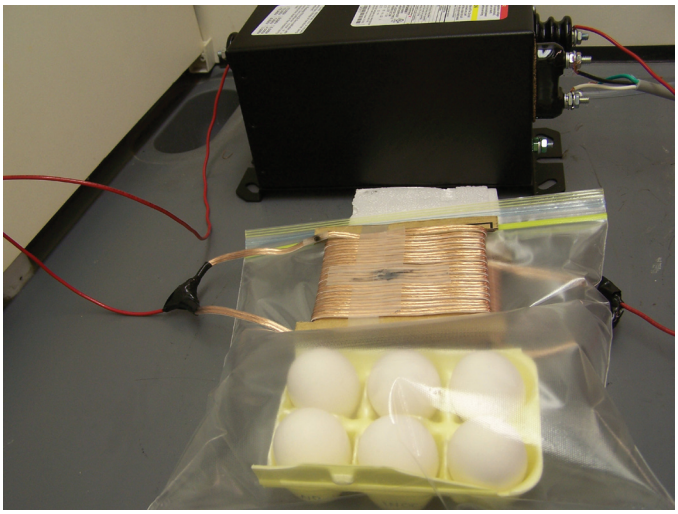

Figure 2. Example of ozone treatment of fresh, raw, shell eggs with PK-1 ozonation system.

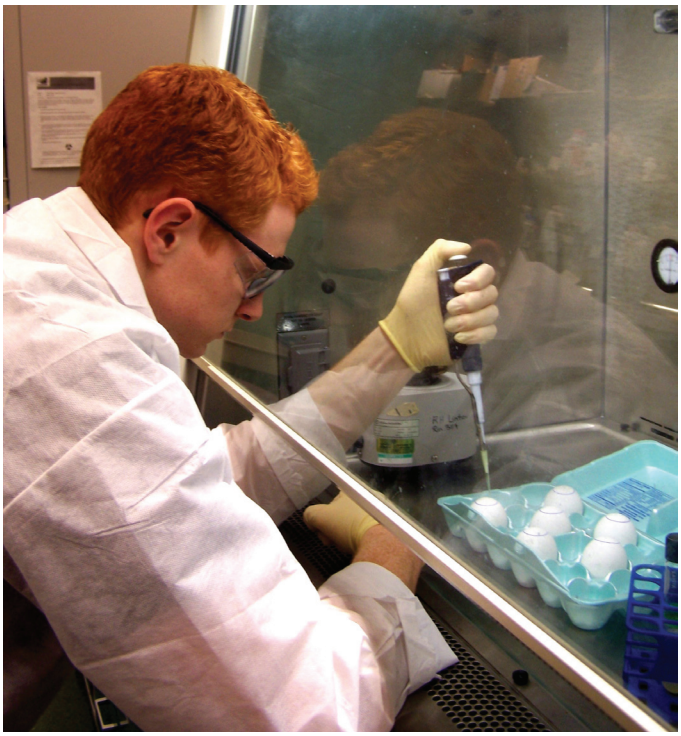

Figure 3. Inoculation of eggs with *Salmonella enteritidis*.

each egg after respective 5° C storage/holding time was recorded. pH levels and temperatures of egg albumin and yolks were measured using Spectrum ATC/pH meter and ISFET probe.

Results and discussion

Table 2 provides a brief summary of the experimental results, including average bacterial reductions from the eggshells and average egg quality after ozonation treatment.

Eggshells inoculated with SE indicated 3 $\log_{10}$ reductions on average (previous research has achieved up to 6 $\log_{10}$). Figure 4 depicts the 3 $\log_{10}$ reduction of SE after the ozonation treatment, indicating successful ozonation.

These results demonstrate effective in-package ozonation treatment reducing SE on raw, shell eggs without significant effect on measured egg quality over time (21 days). Figure 5 displays the ozone concentration (ppm) in the storage bags versus at the time of treatment. At five minutes, ozone concentrations were up to 2,500 ppm. The higher the ozone concentration, the greater the bacterial reduction over time. After 24 hours, ozone concentrations were at non-detectable levels (<0.5 ppm).

Quality assessment results indicate no change in egg quality throughout the three-week quality assessment study. Table 3 shows that over a period of 21 days, the egg quality characteristics remained uncompromised after ozonation treatment.

Conclusion

The in-package ozonation process evaluated in this research efficiently and safely eliminates *Salmonella* from the outside of post-processed, raw, shell eggs. The 3 Log_{10} reduction in SE is comparable to the 3.1 Log_{10} reduction seen by Rodriguez-Romo and Yousef using ozone and shell eggs (2004). In order to further validate the ozonation process, experimental parameters will need to be scaled up in continued research. To illustrate the effectiveness of this technology on a more industrial scale, future ozonation treatments will include a dozen eggs in a sealed egg carton. By showing effective treatment in a carton of raw eggs, this technology will be applicable on an industrial scale. Additional experimentation will also be needed to determine the efficacy of in-package ozonation on the pores and interior of eggs contaminated with SE. While environmental contamination is a common route for *Salmonella* infection, SE experts now believe that the predominant method through which eggs contract SE is via the transovarian route. Although the mechanism is still not well understood, SE will infect the

Bacterial Reduction on egg shells	3 $\log_{10}$ reduction
Ozonation treatment time	5 minutes
Ozone concentration at 5 min	2500 ppm
Egg quality after 3 weeks	Normal

Table 2. Summary of experimental results.

Quality Assessment Results		
Egg Parameter	Ionization Treatment Result	Experimental Averages
yolk color	no change	normal yellow
albumin color	no change	normal clear
albumin pH	no change	8.9
yolk pH	no chaneg	6.3
weight	no change	63.8 grams

Table 3. Egg quality assessment results over a 21-day period.

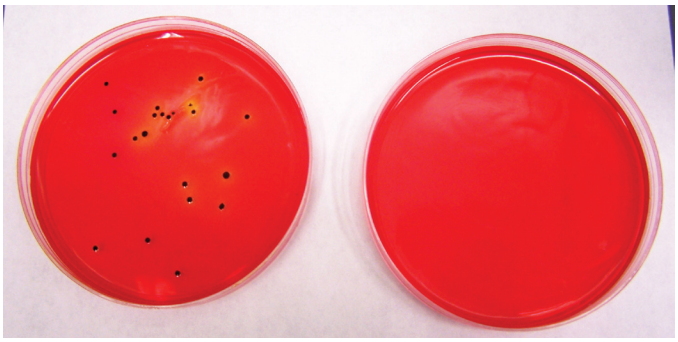

Figure 4. 10^{-5} plate of Salmonella control (left) and 10^{-1} plate of Salmonella after ionization treatment and 24 hour storage (right).

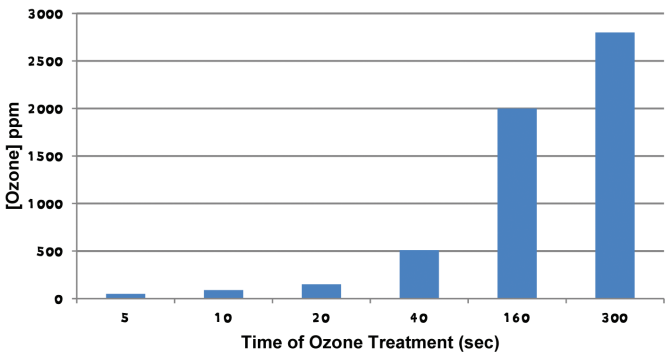

Figure 5. This graph shows the concentration of ozone (ppm) in the storage bags after varying ionization treatment times (sec).

ovaries and oviducts of some egg-laying hens, permitting transovarian contamination of the interior of the egg while the egg is still inside the hen (U.S. Food and Drug Administration, 2009). In addition to observing the effects of in-package ozonation on the reduction of SE inside of eggs, further studies need to be conducted to determine the effects of ozone on shell hardness and the possible nutritional effects, if any, on raw, shell eggs.

With the large number of bacteria-related sicknesses reported in recent years, this ionization process has the potential to provide effective treatment on a wide variety of foods. Neither the effects on durability of the eggshells after treatment nor the impact on nutritional values have yet been determined. The use of in-package ozonation provides an efficient and effective method for the reduction of *Salmonella* on raw, shell eggs. The application of this technology in the poultry industry will greatly reduce the rate of salmonellosis in the United States, as well as reduce the high economic costs associated with the disease's effects. The application of in-package ozonation can also be applied to other foods, as seen in previous studies done on vegetables, fruits, and other poultry products.

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Find out more about Dr. Kevin Keener's research in the Department of Food Sciences:

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