Department of Biosystems and Agricultural Engineering
MICHIGAN STATE UNIVERSITY

BE 487 - Spring 2022
Biosystems Design Projects

Optimizing an Ozone Processing System Against *E. coli* on Whole-Muscle Beef (Under NDA)

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Background

E.W. Grobbel is a food processor located in Detroit, Michigan. Their notable products include corned beef, brisket, and pickles.

E. coli O157:H7 is a harmful bacteria that can be found on beef[1]. Ingestion of *E. coli* can cause severe abdominal cramps, diarrhea, and vomiting. In rare cases, *E. coli* can cause a serious and sometimes fatal illness, hemolytic uremic syndrome, which can result in kidney failure, anemia, and internal bleeding.

E.W. Grobbel strictly follows all health and sanitary guidelines in order to minimize the risk of *E. coli* contamination in their product. An ozonated water spray application system is used to reduce *E. coli* populations on product surfaces during processing. Ozonated water can inhibit growth of pathogens through the oxidation of membrane proteins. This validated *intervention* step is effective in its current state. Additionally, ozone application can also improve the shelf-life of treated foods[2].

The validated *kill* step for the beef products involves thermal processing performed by consumers by cooking to an internal temperature of 165°F.

Objectives

The problem statement is to optimize the efficacy of current ozone operations to further reduce *E. coli* on the surface of beef and recommend solutions
to improve the overall process efficiency.

The main objectives of the project are to:

- Reduce water usage by the ozone generation equipment.
- Increase the efficacy of the process for *E. coli* reduction.
- Improve Grobbel's ability to verify operation parameters.

Constraints

The ozone treatment is not a validated kill step and thus, there is not a log reduction of *E. coli* federally required to achieve. Instead, the team must:

- Optimize the efficacy of the current equipment without diminishing returns.
- Follow federal and industrial standards for product and worker safety.
- Retain current processing equipment footprints due to space limitations.
- Exclude glass components in ozone concentration measurement devices.

Design Alternatives

In order to meet the proposed objectives, three main design alternatives were considered.

Hollow cone vs Full cone spray nozzle

The first alternative was switching the spray nozzles used by the ozone applicators. E.W. Grobbel currently uses hollow cone spray nozzles, but full cone nozzles could offer advantages. A full cone nozzle has a larger spray area as well as a larger droplet size. This could lead to higher concentrations of ozone being retained in the water droplets and a greater lethality for bacteria on the surface[3].

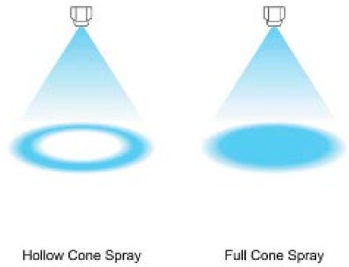


Figure 1: Hollow and full cone nozzle spray patterns

Untreated water vs ozonated water

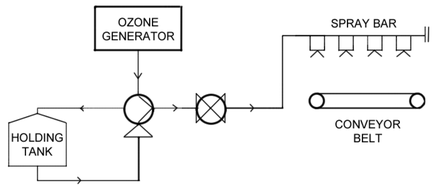
A second alternative considered was switching from ozonated water to untreated water spray. *E. coli* concentrations can be reduced by rinsing the surface of the meat products with liquid. By switching to an untreated water spray it can be determined how much of the *E. coli* reduction can be attributed to the rinsing effect versus ozone inactivation.

Ozone concentration measurement device

Finally, alternate ozone concentration measurement devices were researched. E.W. Grobbel performs hourly measurements of their ozone spray to ensure it falls within the targeted range. Currently a color comparator disk is used to perform these measurements. This visualization tool is subject to human error. E.W. Grobbel requested that digital ozone concentration meters be researched.

Figure 2: Ozone concentration color comparator disk method

Experimental Design



The experimental rigging works as follows:

- Inject ozone into water stream.
- Recirculate ozone with pump and holding tank to reach minimum 1.1 ppm ozone.
- Control flow to spray bar with ball valve.
- Spray fluid through the spray bar onto meat sample and conveyor belt within the spray chamber.

Sample identifiers were based on design alternative suggestions: water (**W**), ozonated water (**OZ**), hollow cone nozzles (**HC**), and full cone nozzles (**FC)**.

7 x 13 cm chuck and Delmonico steaks were inoculated with a 5-strain *E. coli* O157:H7 cocktail in 25 cm^2 areas, with two sample sites per steak to measure the efficacy of proposed treatments.

Figure 3: Beef sample with indicated swabbing sites within isolated spray chamber

Sample swabbing of the inoculated sites were based on modified USDA-FSIS beef sampling protocols for *E. coli* [4]: Pre- and post-treatment sample sites were swabbed 10 times horizontally and vertically to obtain *E. coli* from the meat sample surface.

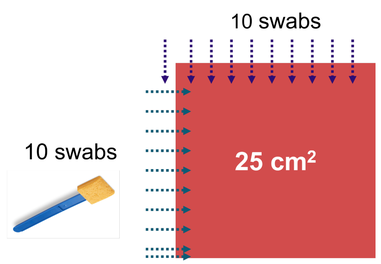


Figure 4: Swabbing protocol for inoculated beef samples

The sample sponges were diluted in buffered peptone water, mechanically stomached for homogeneity, serially diluted, and plated on SMAC medium after spray treatment. Plate counts were recorded after a 48 h incubation period at 37 °C.

Results

A one-tailed T-test was used to compare the pre and post processing values of *E. coli* concentration. Neither the water full-cone nor water hollow-cone trials had a significant reduction in *E. coli* concentration following the spray treatment. However, both ozonated water treatments had significantly smaller *E. coli* concentrations after spray processing. This indicates that ozonated water is more effective than untreated water.

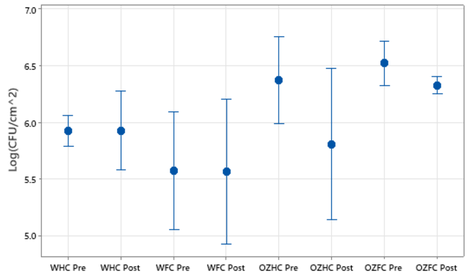


Figure 5: Average E. coli concentration and 95% confidence intervals for spray processing

Table 1: One-tailed paired T-test results for *E. coli* concentration

Comparison	T-Value	P-Value
WHC Pre vs. Post	-0.02	0.509
WFC Pre vs. Post	0.09	0.468
OZHC Pre vs. Post	4.98	0.008
OZFC Pre vs. Post	3.31	0.023

A two tailed T-test was used to gauge the impact of spray nozzle on *E. coli* reduction. As expected, neither water treatment had significantly different reductions. However, the hollow cone nozzle reduction was significantly different from the full cone for the ozonated spray. This indicates that the hollow cone nozzle was more effective.

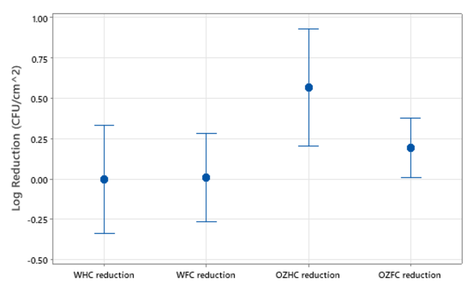


Figure 6: Average E. coli reduction and 95% confidence intervals for spray processing

Table 2: Two-tailed T-test results for *E. coli* reduction

Comparison	T-Value	P-Value
WHC vs. WFC	-0.07	0.944
OZHC vs. OZFC	2.90	0.044

Future Work

Given the time and resources allotted to the project, the experiment was successful and provided valuable information. However, there are potential sources of error that could be improved in future work with this company.

- Ozone concentration loss through the hosing prevented the experiment from reaching E.W. Grobbel's control point. This could be fixed by using shorter PVC piping rather than hosing.
- A color comparator disk was used to measure ozone concentration in the experiment. This is subject to human error. This could be fixed by purchasing a digital ozone concentration measurement device.

Recommendations

From these experimental results the following recommendations can be made for E.W. Grobbel's ozone application system.

- E.W. Grobbel continues using their current ozone concentration (minimum 1.1 ppm) and hollow cone spray nozzles.
- E.W. Grobbel purchases the Oakton DO6+ dissolved ozone meter as a supplement to the DPD color comparator disk testing kit
- Further testing should be done with variable final ozone concentration levels. This would provide insight onto whether the total processing time for a piece of meat could be reduced and retain the same level of lethality, saving water.
- Further research should be done to evaluate the plumbing used to supply ozonated water to the spray applicators. Switching from series flow to parallel flow piping may provided more consistent pressure drops and flow rates.

Select References

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3. Fujiwara, K., & Fujii, T. (2004). Research note: Effects of ozonated water spray droplet size and distance on the dissolved ozone concentration at the spray target. *Ozone: Science & Engineering*, *26*(5), 511–516. https://doi.org/10.1080/01919510490507892.
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