

# Effects of Activated Ozone, As a Decontamination Intervention, When Applied to Hides, Carcasses, and to Ground Beef During Blending

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## SUMMARY

This study was conducted to evaluate the effectiveness of activated ozone, as a decontamination intervention, when applied to beef hides, carcasses, and to ground beef. Hide samples were immersed in either distilled water or ozonated water. Excision samples of beef lean and fat tissue were inoculated with either *Listeria monocytogenes* or *Salmonella* spp., then immersed in either distilled water or ozonated water. Ground beef samples were treated with ozone vapor during blending. Activated ozone was not effective (at the concentrations tested) as a decontamination strategy for the reduction of bacterial contamination on beef hides, beef carcasses, or in ground beef.

**Key Words:** decontamination, ozone, beef carcasses

## INTRODUCTION

Recently, the Food and Drug Administration approved the use of ozone as a disinfectant or a sanitizer in food processing, clearing the way for the red meat industry to utilize this technology (Kim *et al.*, 1999). Meanwhile, the increasing need for Multiple-Hurdles in food processing is occurring concurrently with a need to reduce chemical residues in the air, in water, and in our food supply. Ozone (O<sub>3</sub>) is a strong oxidant that has characteristics making it attractive as a potential sanitizer for use in food processing systems. Purizer Corporation recently received approval to test a proprietary system involving an activated chemical compound (the Deligen II system) for use in beef harvesting Multiple-Hurdles systems. The Deligen II system uses UV light to activate ozone, creating an extremely fast-reacting vapor that is a more

effective decontaminant than either ozone or UV light alone. Deligen II offers extreme flexibility in the beef plant because of the options that exist for applying the compound (liquid or gas).

## PROJECT OBJECTIVES

1. To test whether or not Deligen II, as a decontamination treatment, reduces bacteria plate counts when applied as a liquid to samples of beef hides.
2. To test whether or not Deligen II, as a decontamination treatment, reduces bacteria plate counts for inoculated pathogens when applied as a liquid to the exterior surface of carcass samples.
3. To determine if Deligen II, when applied as a vapor to ground beef during grinding/blending, reduces plate counts for indicator organisms.

## MATERIALS AND METHODS Decontamination Of Hide

**Surfaces.** Hide swatches (N = 90) were obtained from a commercial beef packing facility and immediately transported to laboratories located in the Colorado State University Center for Red Meat Safety. Effectiveness of treatment with Deligen II was evaluated on hide surfaces (n = 30 each) characterized as being (a) "clean", (b) "medium", or (c) "dirty," based on the degree of visible fecal contamination present on the hide swatch. Hide swatches were trimmed to be 10 cm x 10 cm using a 100 cm<sup>2</sup> template and placed inside sterile Whirlpak® (Nasco, Ft. Atkinson, WI) bags. For each level of hide cleanliness characterization, a total of 10 samples were evaluated as negative controls (no treatment), 10 samples were treated by immersion in sterile water (100 ml), and 10 samples were treated by immersion in Deligen II liquid (200 ml) using the "water test cart" prototype generation system provided to researchers at Colorado State University by the Purizer Corporation.

Hide swatches were evaluated for enumerated Aerobic Plate Counts (APC), Total Coliform Counts (TCC) and generic *E. coli* counts (ECC). Laboratory analysis was conducted in the Colorado State University Center for Red Meat Safety. Resulting data were evaluated using analysis of variance in a model containing fixed

effects of treatment (control versus treated) and level of hide cleanliness, along with their interaction.

**Decontamination Of Lean and Fat Surfaces.** To test effectiveness of using Deligen II as a carcass wash, samples of lean and fat collected from the exterior surface of carcasses in a commercial beef packing plant (sample surface area = 130 cm<sup>2</sup>) were inoculated with antibiotic-resistant, pathogenic strains of *Listeria monocytogenes* or *Salmonella* spp. The pathogens were inoculated at known levels (10<sup>7</sup> CFU/cm of surface area) to estimate the pathogen-specific reductions in plate counts due to treatment with Deligen II.

Samples of fat (10 cm x 5 cm x 1 cm) from the exterior surface of carcasses were excised at three processing locations within a commercial beef packing plant: (a) fresh fat before carcasses were subjected to the final washing/organic acid rinsing, (b) fresh fat following the final washing/organic acid rinsing, but before chilling, and (c) refrigerated fat following carcass chilling. All samples were immediately placed in individual, sterile Whirlpak® bags and transported to laboratories located in the Colorado State University Center for Red Meat Safety for analysis.

Samples of lean tissue were obtained from the fabrication line in a commercial beef packing plant to simulate lean surfaces contaminated during fabrication. Using surgical scalpels and forceps, researchers removed 10 cm x 5 cm x 1cm samples from rib lifter meat. Samples were placed in sterile Whirlpak® bags and transported to laboratories located in the Colorado State University Center for Red Meat Safety for analysis. Multiple samples were obtained from each piece of rib lifter meat.

Upon arriving at the laboratory, all samples were inoculated with the appropriate pathogen (either *Listeria monocytogenes* or *Salmonella* spp.). Samples from the four sites (fat from three locations and lean tissue from the fabrication area) were randomized within site group and allocated into three treatment groups: (a) Control Group; (b) Water Group – 100 ml of sterile water was added to each sample, the sample bag was gently stomached (massaged to mix the contents of the bag) for 1 minute, then the tissue sample was removed and placed in a

new, sterile bag; (c) Ozone Group – approximately 200 ml of Deligen II liquid (activated ozone) was added to each sample bag, the bag was gently stomached for 1 minute, then the tissue was aseptically removed and placed in a new, sterile bag. The Diligen II liquid was produced using the “water test cart” prototype generation system provided to researchers at Colorado State University by the Purizer Corporation. The test cart was operated to produce a continuous flow of Deligen II (see Table 1 for ozone concentration and flow data).

Laboratory analysis was conducted in the Colorado State University Center for Red Meat Safety using appropriate media and plating techniques so as to isolate the antibiotic-resistant, media-selective pathogens originally inoculated onto the tissue samples. Appropriate dilutions of samples inoculated with Streptomycin-resistant *Salmonella* spp. were plated on TSA YE agar for Aerobic Plate Counts (APC); and on TSA YE with Streptomycin and on XLT4 for total *Salmonella* count. For samples inoculated with Streptomycin-resistant *Listeria monocytogenes*, appropriate dilutions were plated on TSA YE agar for APCs; and on TSA YE with Streptomycin and on PALCAM for total *Listeria monocytogenes* counts.

#### **Decontamination Of Ground Beef In Grinders/Blenders:**

To test effectiveness of using Deligen II to decontaminate samples of ground beef during the manufacturing process, we evaluated the reduction in APC, TCC and ECC following application of the deligenated ozone. Samples of 85% lean ground beef (N = 60) were collected during manufacturing in the Colorado State University Meat Laboratory (following coarse grinding and during the blending process) such that 30 samples were obtained from the blenders before application of the deligenated ozone (to serve as a negative control) and 30 additional samples were collected from the blender following treatment with the Purizer Corporation formulation for 1 minute. The experiment was conducted in three complete replicates such that 10 control samples and 10 treated samples were evaluated in each of the replicates.

Three additional ozone treatments were evaluated using different ozone vapor concentrations and/or exposure times. For each of these additional treatments, 10 samples per treatment were evaluated. Table 2 lists the ozone concentrations and exposure times for each of these treatments.

Treatment of ground beef in the blender with Deligen II was accomplished by applying the compound as a vapor using the “water test cart” prototype generation system. All samples were placed in individual, sterile sample bags and evaluated for APC, TCC and ECC at laboratories located in the Colorado State University Center for Red Meat Safety.

## **RESULTS**

**(1) Effectiveness of Deligen II in reducing bacterial contamination on hide samples.** Visual categorization of beef hide samples as clean, medium, or dirty was not very effective in stratifying the samples as to the amount of microbial contamination present on the samples. Statistical analysis showed no significant difference ( $P > 0.05$ ) between control samples and samples treated with ozone. Therefore, samples were pooled for further statistical analysis. Table 3 shows that plate counts for samples treated with ozonated water (Deligen II) were not statistically different ( $P > 0.05$ ) than the plate counts for control samples (log APC/cm<sup>2</sup>, log TCC/cm<sup>2</sup>, or log ECC/cm<sup>2</sup>). Interestingly, samples treated with distilled water had statistically higher log TCC/cm<sup>2</sup> and log ECC/cm<sup>2</sup> than control samples.

**(2) Effectiveness of Deligen II in reducing bacterial contamination on fat and lean samples.** Fat samples from the exterior surface of carcasses were inoculated with either *Listeria monocytogenes* or *Salmonella* spp. Analysis of the fat samples showed that while immersion of the sample in either distilled water or ozonated water tended to reduce the level of bacterial contamination, those reductions were too small to be biologically important. Furthermore, immersion in ozonated water did not result in statistically greater ( $P > 0.05$ ) reductions in bacterial contamination than immersion in sterile water.

Results from the analysis of lean tissue samples inoculated with either *Listeria monocytogenes* or *Salmonella* spp. were similar to the results for beef fat. No differences ( $P > 0.05$ ) in the effectiveness of the two treatments were detected. Neither treatment - immersion in ozonated water or immersion in distilled water - resulted in a biologically important reduction in bacterial contamination (Table 4).

**(3) Effectiveness of Deligen II in reducing bacterial contamination in ground beef.** Ground beef samples were treated with an ozone vapor pumped into an airtight mixer. Treatments differed by length of exposure (30 seconds or 1 minute) and ozone concentration (ppm). As Table 5 shows, bacterial contamination of the samples tended to be very low – with many samples having coliform bacteria levels at or below the threshold for detection for the laboratory methods utilized. The low level of contamination made it more difficult to detect possible differences in the effects of the different treatments. However, aerobic plate counts were higher and thus more easily compared. None of the treatments caused a biologically significant reduction in log APC/g.

## **DISCUSSION**

Results of this study indicate that activated ozone produced by use of the Deligen II system is not effective (at the ozone concentrations tested) as a decontamination strategy for the reduction of bacterial contamination on beef hides, beef carcasses (either lean or fat tissue) or in ground beef when the treatment is applied as a vapor. Researchers believe that when treating a sample that is high in organic matter, the ozone is decomposed by the organic matter before the ozone has an opportunity to inactivate the microorganisms present on the tissue sample.

## **REFERENCES**

- Kim, J., A.E. Yousef, and S. Dave. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: A review. *J. Food Prot.* 62(9):107

**Table 1. Ozone concentration (ppm) and variable flow rate of ozonated water produced with the “water test cart” prototype generation system provided by the Purizer Corporation.**

Sample	Ozone concentration (ppm)	Variable Flow Rate
Samples inoculated with <i>Salmonella</i> (lean and fat)	10.05	6.25 L/min @ 10.5 Hz
Samples inoculated with <i>Listeria monocytogenes</i> (lean and fat)	10.14	6.25 L/min @ 10.5 Hz
Hide Samples	8.13	6.43 L/min @ 10.6 Hz

**Table 2. Ozone concentration (in units of weight %) of the vapor produced with the “water test cart” prototype generation system provided by the Purizer Corporation for the treatment of ground beef.**

Treatment	Ozone Concentration (%)
Control	0
Ozone: Full Strength for 1 minute	10.05
Ozone: Full Strength for 30 seconds	10.45
Ozone: Two-thirds Strength for 1 minute	6.48
Ozone: One-third Strength for 1 minute	3.16

**Table 3. Effect of Deligen II treatment on bacterial contamination on beef hide samples.**

Treatment	n <sup>a</sup>	APC <sup>b</sup>	TCC <sup>b</sup>	ECC <sup>b</sup>
Control	30	6.92 A	4.83 B	4.78 B
Distilled water	30	6.65 A	5.34 A	5.29 A
Deligen II (Ozone)	30	6.67 A	4.65 B	4.57 B

<sup>a</sup>Number of samples analyzed.

<sup>b</sup>Least-squares means of the log values (log CFU/cm<sup>2</sup>). Within a column, means with different letters differ (P < 0.05).

**Table 4. Effect of Deligen II treatment on bacterial contamination of beef tissue inoculated with streptomycin-resistant *Salmonella* spp. or *Listeria monocytogenes*.**

Treatment	n <sup>a</sup>	<i>Salmonella</i> spp.			<i>Listeria monocytogenes</i>		
		TSAYE <sup>bc</sup>	TSAYE/STREP	XLT4 <sup>be</sup>	TSAYE <sup>bc</sup>	TSAYE/STREP <sup>bd</sup>	PALCAM <sup>bf</sup>
<b>Over all sites</b>							
Control	40	7.25 A	7.07 A	6.74 A	7.12 A	7.14 A	7.09 A
Water	40	7.08 A	6.76 B	6.91 A	6.86 B	6.86 B	6.78 B
Ozone	40	7.07 A	6.93 AB	6.90 A	6.88 B	6.82 B	6.79 B
<b>Site 1 - Fat<sup>g</sup></b>							
Control	10	7.32 A	7.29 A	6.84 A	7.10 A	7.11 A	7.09 A
Water	10	7.10 A	7.06 A	7.25 A	6.75 B	7.01 AB	6.94 AB
Ozone	10	7.09 A	6.98 A	6.89 A	6.80 B	6.81 B	6.79 B
<b>Site 2 - Fat<sup>h</sup></b>							
Control	10	7.30 A	7.07 A	6.72 A	7.23 A	7.24 A	7.18 A
Water	10	7.11 AB	6.57 B	6.91 A	7.11 AB	6.88 B	6.77 B
Ozone	10	6.85 B	7.05 AB	7.03 A	6.96 B	6.76 B	6.84 B
<b>Site 3 – Fat<sup>i</sup></b>							
Control	10	7.16 A	7.00 A	6.71 A	7.17 A	7.16 A	7.10 A
Water	10	6.87 A	6.75 A	6.70 A	6.86 B	6.87 B	6.80 B
Ozone	10	7.17 A	6.82 A	6.86 A	7.01 AB	6.97 AB	6.85 B
<b>Site 4 – Lean<sup>j</sup></b>							
Control	10	7.25 A	6.93 A	6.69 A	6.95 A	7.07 A	6.96 A
Water	10	7.24 A	6.67 A	6.78 A	6.71 A	6.70 B	6.62 B
Ozone	10	7.18 A	6.88 A	6.81 A	6.74 A	6.75 B	6.68 B

<sup>a</sup>Number of samples analyzed.

<sup>b</sup>Least-squares means of the log values (log CFU/cm<sup>2</sup>). Within a column, means with different letters differ (P < 0.05).

<sup>c</sup>Trypticase soy agar with 0.6% yeast extract (TSAYE), a general purpose medium. TSAYE is used for total APC.

<sup>d</sup>Culture medium of TSAYE plus streptomycin used to isolate the streptomycin-resistant inocula.

<sup>e</sup>Selective medium used to isolate *Salmonella* spp.

<sup>f</sup>Selective and differential medium used to isolate and enumerate *Listeria* spp.

<sup>g</sup>Site 1: Samples excised from carcasses on harvest floor before carcass was subjected to the final wash/organic acid rinsing.

<sup>h</sup>Site 2: Samples excised from carcasses following the final carcass washing/organic acid rinsing.

<sup>i</sup>Site 3: Sample excised from carcasses following 24 hour chill.

<sup>j</sup>Site 4: Samples were excised from beef lifter meat on the fabrication floor.

**Table 5. Effect of Deligen II treatment on bacterial contamination in ground beef samples when applied as a vapor.**

Treatment	n <sup>a</sup>	APC <sup>b</sup>	TCC <sup>b</sup>	ECC <sup>b</sup>
Control	30	4.27 AB	1.15 AB	1.04 A
Ozone: Full Strength for 1 minute	30	4.68 A	1.08 A	1.03 A
Ozone: Full Strength for 30 seconds	10	3.86 B	1.34 B	1.26 A
Ozone: Two-thirds strength for 1 minute	10	3.95 B	1.31 AB	1.26 A
Ozone: One-third strength for 1 minute	10	3.84 B	1.02 A	0.97 A

<sup>a</sup>Number of samples analyzed.

<sup>b</sup>Least-squares means of the log values (log CFU/cm<sup>2</sup>). Within a column, means with different letters differ (P < 0.05).