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Efficacy of Gaseous Ozone Against Generic *E.coli* in Ground Beef

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Abstract: The use of gaseous ozone was evaluated as an antimicrobial intervention against Escherichia coli (*E.coli*) in ground beef. This research took place in two parts. Phase 1 initially inspected for color and appearance change due to ozone gas oxidizing the beef. Phase 2 tested for antimicrobial action due to the ozone against *E.coli* bacteria. Ozone treatment to the ground beef showed no color or flavor change at ozone levels that were effective at reducing E.coli in the ground beef. Raw, ground beef inoculated with generic *E.coli* was treated with gaseous ozone at various levels it was determined that approximately 95.8% of the *E.coli* in the ground beef was killed when the ozone concentration approached 200 ppm.

Introduction: Ground beef recalls are commonly caused by unsafe levels of *E.coli* bacteria found in the product that is distributed to stores. Specifically the O157:H7 strain of Escherichia coli (*E.coli*) is found in the recalled beef. Currently there are very few antimicrobial intervention options available for the ground beef process. This research was performed to evaluate if there is a possibility that Ozone gas may be an effective antimicrobial intervention in the ground beef process.

Ozone has proven to be a very effective antimicrobial intervention in many food processing applications (Rice 1983), including red meat and beef applications. Ozone has proven to be an effective antimicrobial intervention against *E.coli*,(Akbas and Ozdemir 2006) and specifically O157:H7 *E.Coli* (Kim and Yousef, 2000). However, ozone is traditionally used in the aqueous phase for surface sanitation and general disinfection. Aqueous ozone is very convenient as an antimicrobial agent, water is the carrier of the ozone. Typically water is already in use in the washing of surfaces and produce, so adding ozone to existing processes is very convenient and cost effective.

While aqueous ozone has proven effective in other beef processing applications, the use of aqueous ozone is not possible in most ground beef processes as water would need to be added to the ground beef mixture, doing so is undesirable as that ground beef will then loose the *100% ground beef* rating.

Ozone gas may be an alternative to aqueous ozone in ground beef processing as an antimicrobial intervention. Ozone gas is used as an antimicrobial intervention in other processes with great success, (Rice 1983) and it has been very briefly tested in beef storage application in the past with good results in reducing some strains of bacteria (Fournaud & Lauret 1972). During this past testing, discoloration of the red meat was analyzed. With 100 PPM of ozone exposure at up to 30 minutes, no change in color was noticed. With 500 PPM of ozone exposure for the same time period, undesirable color and odor changes were noticed in the meat samples. The apparent odor change of the meat found in this test provides justification to test for flavor changes in cooked ground beef after ozone treatment.

Due to the lack of conclusive evidence that ozone gas will be effective on ground beef, and limitation of using only ozone gas as an antimicrobial intervention on ground beef, further research is necessary. The previous color and flavor questions that have been raised require testing to ensure that ozone will not affect these qualities of the product. This current two-part research is aimed at evaluating the effect of ozone gas on color and flavor, and then evaluating the effectiveness of ozone gas as an antimicrobial intervention on a ground beef product.

This research was performed as a joint effort between Ozone Solutions, Inc., Synergy Environmental, Inc., and Kraft Science Consulting. Each party contributed considerable time and expense to complete this research and provide the findings.

Equipment and Setup

It is theorized that ozone gas could be used as an antimicrobial intervention in the ground beef process during the mixing or grinding processes. Due to this fact, this test used a small stainless steel meat mixer manufactured by LEM Products. This mixer was used to simulate the mixing process of ground beef. Six ozone gas inlet ports were installed in the sides of this mixer to inject the ozone gas and distribute it evenly throughout the mixer.

Illustration 1: LEM Meat Mixer

Illustration 2: Meat Mixer in Action

The top of the mixer was covered with a clear Plexiglas cover that was held firmly to the top of the mixer. There was no seal on this surface. Excess ozone gas escaped from this area and the crankshaft area in the front and rear of the mixer.

The mixer was turned with a variable speed drill to allow for adequate mixing and speed. This allowed for higher mixing speeds which ensured the ozone gas would be thoroughly contacted with the ground beef.

Ozone gas was created by an OZV-4 Ozone Generator supplied by Ozone Solutions. This ozone generator was fed with 10 LPM of dry compressed air as a feed gas. Additional dry air was by-passed around the ozone generator to supply a total air flow of ozone + dry air of 1 CFM (30 LPM).

A side stream of the final ozone gas stream was plumbed to a Model 106 Ozone Analyzer, from 2B Technologies. This analyzer measured the ozone level in PPM to ensure a constant ozone level was used throughout this test.

The ozone gas/dry air mixture flowed into the mixer at a flow rate of 1 CFM for a 3 minute time period. The 1 CFM flow rate and 3 minute time period were used for each of the samples throughout this research.

The 3 minute ozone dosage time period was chosen to simulate the ground beef mixing time at a specific processing plant which was toured during the initial research portion of this testing.

The 1 CFM flow rate was used to ensure the ozone gas concentration inside the chamber would not be consumed by the organics. By using a high flow rate into the chamber it was theorized that the ozone level inside the chamber would remain very consistent during the 3 minute time period.

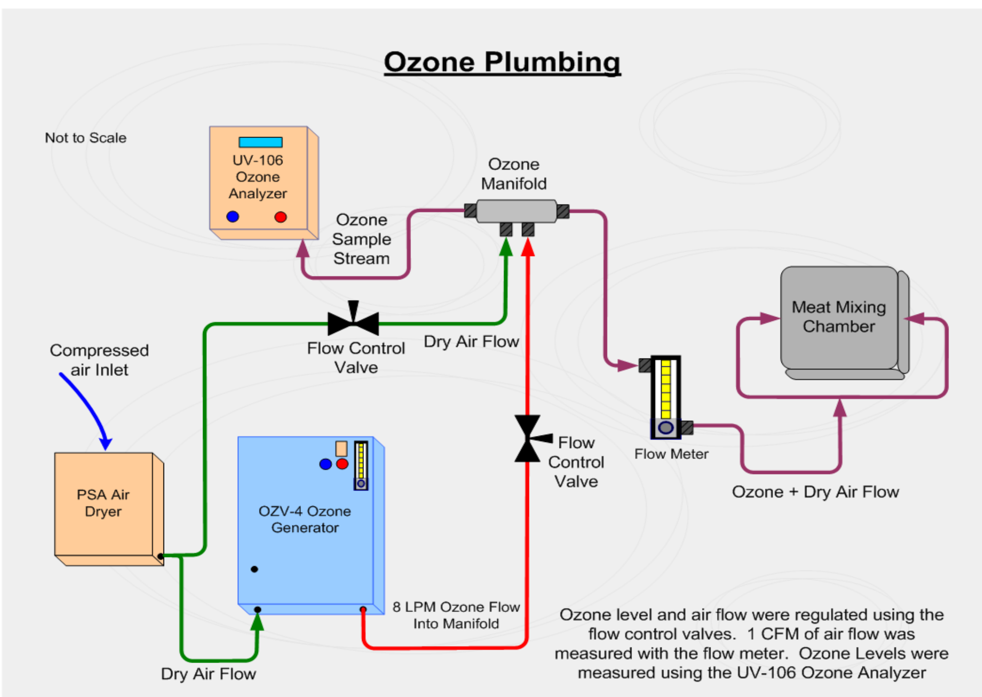

Illustration 3: Ozone Plumbing Diagram

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Color and Taste Testing – Phase 1

Methods:

During the first phase of testing, 15 lbs of ground beef was purchased from one single batch of ground beef. This ground beef was split into equal 5 lb samples and treated in the meat mixing chamber for each test, since this quantity was sufficient to be mixed by the paddles very thoroughly. The ground beef used was purchased from a local meat market the same day of the testing, with complete batches used for each phase of testing to ensure there was complete consistency in the ground beef sample.

Illustration 4: Weighing Ground Beef Sample

Illustration 5: Ground Beef in Mixer

Both phases of testing, color/flavor and antimicrobial used the same constants of 1 CFM ozone/dry air flow, 3 minutes of mixing time, and 5 pound samples of ground beef. Only the ozone concentration was changed for each of the tests.

This first phase of the testing was performed to determine if ozone would have a negative effect on the taste or color of the red meat after cooking. To evaluate any color or taste changes, ozone concentrations of 0, 100, and 200 PPM were used. 1 CFM of ozone/dry air mixture flowed into the meat mixture during each of the tests.

After ozone treatment, the 3 ground beef samples were kept separate and formed into 0.25 lb patties using a patty maker and a digital scale. A total of 20 ground beef patties were created from each of the 5 lbs samples. From each of the 0, 100, 200 PPM batches, 3 of the completed ground beef patties were set aside for color indication. A total of 9 ground beef patties were set aside for color change evaluation.

The 9 ground beef patties set aside for color change indications were visually analyzed immediately after the patties were formed and set aside. Pictures were also taken of these patties

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for documentation. After 24 hours of refrigeration these patties were again analyzed visually with additional pictures taken for documentation.

The 17 remaining ground beef patties from each of the 3 sample batches were cooked on a similar grill and taste tested by 9 individuals. No prior information was given to these individuals and the 3 sample batches were labeled with only a 1, 2, or 3 with no indication as to what the numbers referenced.

Results:

Color results: Initially there was no visible color change between the 3 sample batches. All of the patty samples maintained the same color.

Illustration 6: Color Samples After 24 Hours of Refrigeration

After 24 hours when the same 9 ground beef patties were re-analyzed visually there was still no color change between any of the 3 sample batches.

While it was theorized that there may be some color change in the red meat after ozone treatment at levels up to 200 PPM there was no visual color change directly after ozone treatment or 24 hours later.

It is important to note that these 9 ground beef patty samples were retained for another 3 days in the refrigerator. No color change was ever evident even after this 4 days of holding time in a refrigerated environment.

Taste results: After the ground beef patties were cooked evenly they were taste tested. This was primarily an informal taste test used for general information. Each person tasting the final cooked patties were asked to rate their favored flavor on a sliding scale of best to worst.

There was no statistical difference between the results on the 0 PPM and 100 PPM sample batches. There was a noticeable statistical dissatisfaction with the flavor from the 200 PPM sample batch. There was no unanimous consensus in the favored flavor of patty, however a preference toward the 0 and 100 PPM batches was evident.

In discussion with each of the taste testers, it was revealed by the testers that the patties from the 200 PPM sample batches had a bad flavor. A few of the testers commented that there was what could be described as a slight “off flavor” to the patties from the 200 PPM sample batch, while the patties from the 0 and 100 PPM sample batches were no different in flavor, and preferences were difficult to discern.

There were a few taste testers that could discern no substantial flavor difference between any of the 3 patty samples.

Conclusion:

Due to the lack of any color, or flavor change of the ground beef patties using the 100 PPM ozone concentration it was determined that may be a feasible antimicrobial intervention in the use of ground beef processing. It was expected to see a color change in the ground beef at lower ozone concentrations rather than a flavor change. This was proved to be untrue. The flavor change occurred in the 200 PPM sample while no noticeable color change was present.

Microbiological Intervention Testing – Phase 2

Methods:

Due to the success in treating ground beef for 3 minutes with 100 PPM of ozone gas this research proceeded on to the 2nd phase of testing. For this test ground beef was inoculated with generic *E.coli* bacteria, then treated with various concentrations of ozone gas for 3 minutes inside the sealed mixing chamber.

The ozone concentration levels chosen to treat the ground beef samples were 50 PPM, 100 PPM, 150 PPM, and 200 PPM. It was elected to still use the 200 PPM concentration despite the apparent flavor change found in the phase 1 testing. This was to ensure that if no change in *E.coli* levels were found that it would be determined that ozone gas would not be a suitable antimicrobial intervention for ground beef processing. The 50 PPM, and 150 PPM ozone concentrations were chosen to ensure a multiple data points would be obtained in the reduction of *E.coli*.

All testing parameters used during Phase 1 testing were used in Phase 2 testing. The ground beef was mixed in the mixing chamber for 3 minutes of contact time with the desired ozone concentration. 1 CFM of ozone gas/dry air mixture was injected into the mixing chamber for this 3 minute time period. The same 5 pound samples of ground beef were used for each ozone concentration sample.

For this test, 25 lbs of ground beef were purchased from the same batch of ground beef as used in Phase 1. This ground beef was delivered to Kraft Science Consulting. Where it was inoculated with the E-coli bacteria for testing.

Inoculation and Testing:

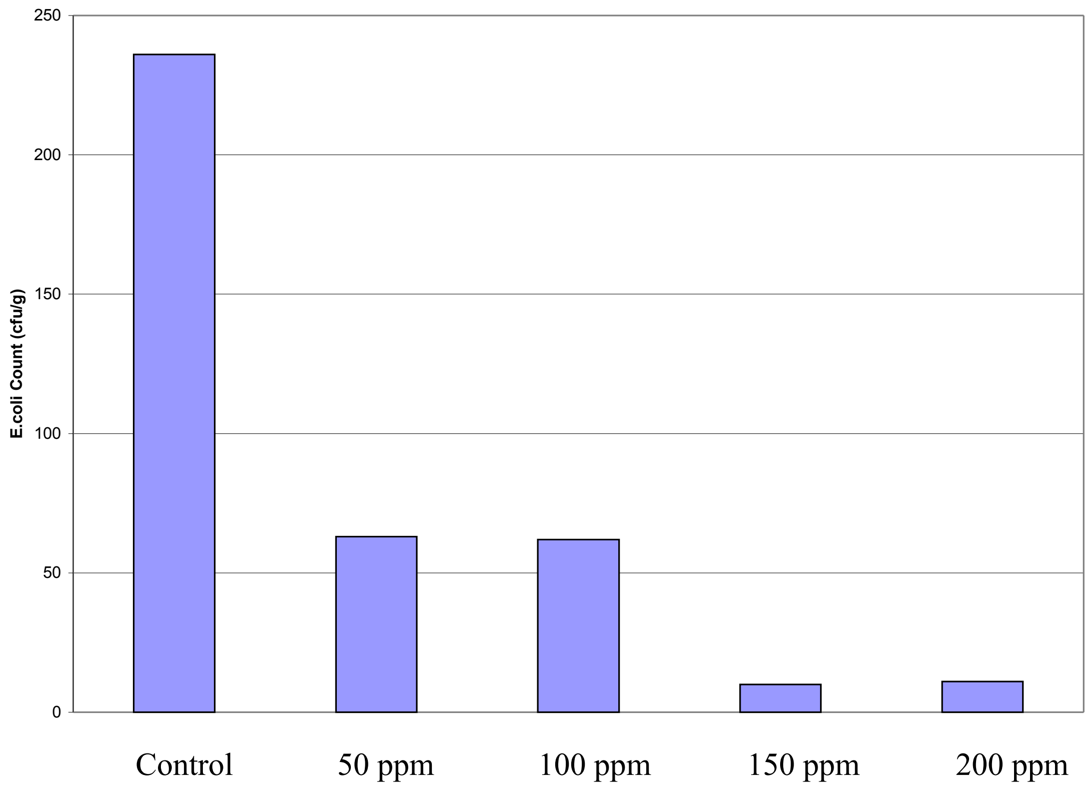
Generic *E.coli* was grown in a Tryp-Soy broth to a cell count measured to be approximately 620 cfu/ml just prior to inoculation. Twenty-five pounds of ground beef were inoculated by first dividing the beef into five 5-pound batches. Then each batch was pressed into a clean and sanitary 5 gallon container one at a time spreading 20 ml of the *E.coli* potion between each batch. Following the introduction of the potion all twenty-five pounds of ground beef were thoroughly mixed to ensure uniform dispersion.

The resulting twenty-five pounds of inoculated ground beef were then delivered to Ozone Solutions for mixing and ozone treatment. This resulted in the generation of 5 populations of ground beef (5 pounds each), each differing only in the concentration of ozone used. The five populations were then returned to the laboratory for *E.coli* quantification. To estimate the *E.coli* count of each population, three samples (approximately 10 to 20 grams each) were randomly collected from the population and using AOAC Official Method 998.08 each sample was quantified for *E.coli*. The average count of the 3 samples was calculated and used as an estimate of the actual count for that population.

Results:

As can be seen in the table and graph below, approximately 73% of the *E.coli* was killed using 50 ppm ozone for 3 minutes and approximately 96% was killed using 150 ppm when compared to the control population ("C" = no exposure).

Sample Name	Sample Weight	Buffer Volume	Total Plate Count	Count of Sample (cfu/g)	Est. Pop. Count (cfu/g)
C-1	11.4	49 ml	42	181	
C-2	14.3	48 ml	93	312	
C-3	14.4	49ml	63	214	
					"Population C" = 236
50-1	8.7	49ml	14	79	
50-2	10.9	49ml	9	40	
50-3	12.7	49ml	18	69	
					"Population 50" = 63
100-1	19.3	49ml	18	46	
100-2	10.5	49ml	16	75	
100-3	12.3	49ml	16	64	
					"Population 100" = 62
150-1	18.9	49ml	3	8	
150-2	14.8	49ml	5	17	
150-3	18.8	49ml	2	5	
					"Population 150" = 10
200-1	15.1	49ml	3	10	
200-2	17.1	49ml	8	23	
200-3	10.4	49ml	0	0	
					"Population 200" = 11



Observations

During each of the testing 1 CFM of ozone gas was introduced into the meat mixer. As there was no defined outlet port the excess gas escaped from the clear lid, and the unsealed crankshaft hole. It was observed that little to no ozone escaped with this air. This observation leads to the conclusion that almost all ozone gas was consumed by the ground beef. This may be a benefit in full scale application as potential safety risks with ozone gas in the area will be eliminated. However, this also illustrates that the ozone gas flow rate may have been lower than necessary for proper disinfection prior to the ozone gas reacting with organics. This is an area that may require more research in the future.

The ground beef that was purchased was purchased ready for use by the consumer. Further mixing in chamber did result in an over processed product that did loose some of the aesthetic properties. This also may have caused an unrealistic final product for testing.

Conclusions:

This experiment showed that gaseous ozone caused a significant kill of generic *E.coli* in ground beef when the beef is mixed/ground in a closed chamber filled with ozone gas. An approximate 73% of the *E.coli* was killed using a 50 ppm concentration of ozone with a 3 minute exposure time and an approximate 96% was killed using a 150 ppm concentration of ozone with the same exposure time.

This initial research and testing has proven that ozone gas may be a suitable antimicrobial intervention in ground beef processing. More research will be necessary to evaluate the specific ozone concentration that is most effective against *E.coli* and other possible pathogens. This initial research will aid any further testing and provide a baseline for future research using ozone gas as an antimicrobial intervention in ground beef processing.

Discussion:

Due to the apparent flavor change on the 200 PPM ozone concentration sample this is an impractical ozone concentration to use in full scale application. Due to the lack of flavor and color change in the ground beef using the 100 PPM ozone concentration and the 73% reduction in this may be a practical application for ozone. The reduction of *E.coli* increased dramatically when the ozone concentration was increased from 100 to 150 PPM. As flavor testing was not performed at this ozone concentration, additional testing should be performed to further evaluate the possibilities.

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