A Comparison of Ozone and Chlorine to Reduce the Microbial Load in Fresh Lettuce

A Senior Honors Thesis

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by:

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Abstract

The effects of ozone were examined on fresh head lettuce as a means for reducing the initial microbial load (about 10^4-10^5 CFU/g) on the lettuce. Ozone concentrations used were 50 parts per million and 100 ppm. These concentrations resulted in an average reduction of both mesophilic and psychrotrophic bacteria of 2.04 and 2.57 $\log_{10}$ CFU/g on the initial load of microorganisms on the lettuce. Once the effect was established, ozone was compared to a sodium hypocholorite treatment, which is a method currently used in industry. The concentrations used for comparison of ozone and chlorine were 1 mM and 2 mM for each. The reactions were stopped in both cases by using sodium thiosulfate ($Na_2S_2O_3$) to determine the initial effect of both ozone and chlorine. By using a neutralizer, the effects due to residual ozone or chlorine on the lettuce were eliminated. At 1 mM, ozone resulted in an average inactivation of mesophilic and psychrotrophic bacteria of 1.15 $\log_{10}$ CFU/g and at 2 mM, an average inactivation of 1.68 $\log_{10}$ CFU/g. At 1 mM and 2 mM of chlorine, the average inactivation of mesophilic and psychrotrophic bacteria was 1.41 and 1.90 $\log_{10}$ CFU/g respectively.

Introduction

The consumption of fresh vegetables is, in many cases, a part of the daily diet. These fresh vegetables are minimally processed, meaning that they do not undergo a great extent of processing to ensure that the vegetables are free from microorganisms which can serve as a starting point for disease or spoilage.

The minimal processing of lettuce and other vegetables involves a chlorine dip (50-100 ppm of free available chlorine) to remove microorganisms which are present on the surface of the lettuce (1). After the chlorinated dip, the water is removed from the lettuce by centrifugation and the product is ready for packaging. Once the lettuce reaches the consumer, the average microbial count on the lettuce is around 10^4 CFU/g.

The centrifugation of the lettuce to remove the chlorinated water from the dip does not remove all chlorine present; that is, there may still be residual chlorine on the lettuce after centrifuging. This residual chlorine is considered to be a disadvantage to the use of chlorine because of its toxicity and possibility to form carcinogens with other compounds (2). However, chlorination of water is currently the only method approved for disinfection of lettuce and other vegetables.

Ozone is being examined as a potential replacement for chlorine. Although the exact mode of action is not known, ozone is lethal to many microorganisms including pathogenic and spoilage bacteria, and it is also effective against some viruses (2) . In the United States, ozone is approved for bottled water at a concentration of 0.4 ppm at the time of bottling as a disinfectant and also as a disinfectant in poultry chill water (which is recycled after treatment with ozone) (7). Other research concerning the action of ozone includes: reducing the microbial load on meat, preventing post-harvest decay and also extending the shelf life of spices an other dehydrated foods (4, 7, 8).

The projected goal of this study was to examine the effects of ozone on the initial microbial load in lettuce.

Materials and Methods

Lettuce

Fresh iceberg lettuce was obtained from a local grocery store on the day that the test was conducted. The top leaf of the lettuce was removed and the lettuce was shredded into pieces that ranged from 5-10 mm in width. From this point, duplicate 25 g samples of lettuce were weighed out and placed in sterile stomacher bags. Sterile, deionized water was added at 20x the weight of the sample for all variations and replications and duplicate samples taken for each dilution.

No Treatment

Three minutes after the addition of water to the lettuce, the sample was stomached for two minutes. Duplicate samples were taken for each dilution.

Ozone Treatment

The ozone was generated by a Polyozone T-816 ozonizer (U.S. Filter/Polymetrics, San Jose, CA). Prior to treatment, the concentration (ppm) was measured using the spectrophotometric method which is based on the following principles. Ozone reacts with a neutral solution of potassium iodide to liberate iodine. In the presence of excess KI, the triiodide (I_3) complex of iodine is formed. The concentration of I_3 is determined spectrophotometrically at a wavelength of 352 nm. When a stock iodine solution (0.01 N) and neutral KI solution are combined at proper dilutions, 1 ml of the combined iodine solution (0.0004 N) will equal 0.96 µg of ozone (9). Ozone was bubbled into 500 mL of

2% KI for 10-15 seconds and then measured spectrophotometrically to determine the concentration. The time needed to obtain the correct ozone concentration (i.e. 1 mM or 2 mM) was calculated. The sample of lettuce and water in the stomacher bag were then exposed to ozone by directly bubbling ozone into the bag for the calculated time.

Stage I: When determining the initial effect of ozone on the microbial load in lettuce, concentrations of 50 ppm and 100 ppm were used. Ozone from the ozonizer was directly bubbled into the stomacher bag for the predetermined time and stomached for two minutes.

Stage II: Once the initial concentration of ozone was determined, the time was calculated so the amount of ozone generated would reach 1mM and 2mM. After these concentrations were reached, the bag was lightly agitated for three minutes and then stomached for an additional minute. After stomaching, $Na_2S_2O_3$ was added to stop the reaction (2 ml of $Na_2S_2O_3$ (1 mM) for 1 mM solution and 4 ml of $Na_2S_2O_3$ (1 mM) for 2 mM solution). After addition of sodium thiosulfate, the sample was stomached for one minute to disperse the sodium thiosulfate.

Chlorine Treatment

The chlorine used came from Chlorox bleach which was assumed to have a 5% concentration of NaOCl. Based on this assumption, 0.71 ml and 1.42 ml of bleach was added to 500 ml of sterile deionized water to obtain a 1 mM and 2 mM solution respectively. After addition of chlorinated water to the lettuce in the stomacher bag, the sample was gently agitated for three minutes and stomached for an additional minute. After stomaching, $Na_2S_2O_3$ was added to stop the reaction (1 ml of $Na_2S_2O_3$ (1 mM) for 1 mM solution and 2 ml of $Na_2S_2O_3$ (1 mM) for 2 mM solution). After addition of sodium thiosulfate, the sample was stomached for one minute to disperse the sodium thiosulfate.

Bacterial Enumeration

From the stomached samples, serial dilutions of 10^{-2} and 10^{-3} were made using sterile 0.1% peptone (Bacto Peptone, Difco Laboratories, Detroit, MI). One ml of each dilution was transferred to sterile petri plates and covered with plate count agar (PCA, Difco Laboratories, Detroit, MI). Samples were done in quadruplicate (for enumeration of mesophilic and psychrotrophic bacteria). One set of duplicate plates was incubated at 37°C for 48 hours while the other set of duplicate plates was incubated at 7°C for at least 72 hours.

Results and Discussion

Effects of ozone

For stage I of the experiment, the effect of ozone had about a 1 to 3 $\log_{10}$ CFU/g reduction from the initial microbial load on the lettuce. Both 50 ppm and 100 ppm had a similar of effect on the initial microbial load of the lettuce (Table 1 and 2). Ozone at 50 ppm had an average of 2.04 $\log_{10}$ CFU/g reduction while ozone at 100 ppm had an average of a 2.57 $\log_{10}$ CFU/g reduction from an initial average count of 1.04×10^5 CFU/g.

The initial microbial load in the lettuce will vary between samples taken. The variation may be due to the randomness of where the bacteria is actually located on the lettuce; that is, the distribution of the bacteria on the lettuce is not uniform (1).

Ozone activity can be lowered if there is a significant amount of organic matter with which it will react with readily. Therefore, the action of the ozone may be suppressed when using it to inactivate the bacteria present on lettuce. In other applications, ozone would require a longer contact time and higher concentration to be effective as compared to the times and concentrations used in this study (2).

The effects of ozone on the appearance of the lettuce was also examined. After bubbling, the water was drained and the sample was left refrigerated for three days in the stomacher bag. The sample that had been treated with ozone browned quickly which is due, presumably, to the action of the enzyme polyphenoloxidase (PPO). When a vegetable undergoes tissue damage, the browning increases due to enchanced substrate availability and/or induction of phenylalainine ammonialyase. The lettuce also had a translucent look while having a firm texture. The lettuce which did not undergo treatment also experienced browning, but not to the degree in which the lettuce treated with ozone did. If the browning which was seen can not be controlled, then the lettuce would not make an acceptable product for consumers.

Effects of ozone compared to chlorine

The units of concentration were calculated on a molar basis in Stage II for an easier and more accurate comparison. In Stage II, the inactivation effects of ozone and chlorine were similar. Ozone at 1 mM and 2 mM exhibited a 1.15 and 1.68 $\log_{10}$ CFU/g reduction respectively. NaOCl at 1 mM and 2 mM yielded a 1.41 and 1.90 $\log_{10}$ CFU/g reduction respectively. The initial average count of the lettuce was determined to be 2.9 x 10^4 CFU/g.

The reduction of microorganisms varied with the initial count present on the lettuce (Table 1, 2, 3, 4). The psychrotrophic count was 1.36 $\log_{10}$ higher than that of the mesophilic count (Table 3). The difference in counts may have been due to a longer incubation period or simply from the distribution of the bacteria on the lettuce sampled.

The use of sodium thiosulfate to neutralize the action of ozone and chlorine does not affect the bacteria present in the medium (3). However, compared to the Stage I ozone data, sodium thiosulfate does have some bearing on the effectiveness of ozone. Since the reaction was neutralized, the action of both ozone and chlorine was stopped to get an accurate comparison of the effectiveness of both agents against bacteria. When comparing the log reductions of Stage I to Stage II, Stage I exhibits an average of a 2.57 $\log_{10}$ CFU/g reduction at 100 ppm while in Stage II, ozone at 1 mM (97 ppm) exhibits an average of a 1.15 $\log_{10}$ CFU/g reduction. The comparison of the effectiveness of chlorine and ozone at almost the same concentration further illustrates that ozone is still effective against the bacteria present even though there is a high concentration of organic matter present.

Conclusions

Both ozone and chlorine have similar inactivation effects against the bacteria present in lettuce. In the case of Stage I, ozone is more effective when given the chance to act for a longer period of time (i.e. 2 to 4 minutes). A time scale such as that just mentioned would be practical in an industrial setting. The use of ozone would be a safer alternative to the use of chlorine because of reasons mentioned earlier. Further testing with other variations, such as the absence of neutralizer should be conducted to compare the effect of chlorine and ozone over a period of time. Additional studies on the effects of ozone an browning would also be required to assess the effect of ozone on lettuce.

References

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Table 1: Ozone Inactivation to Reduce Microorganisms on Fresh Lettuce (Trial I)

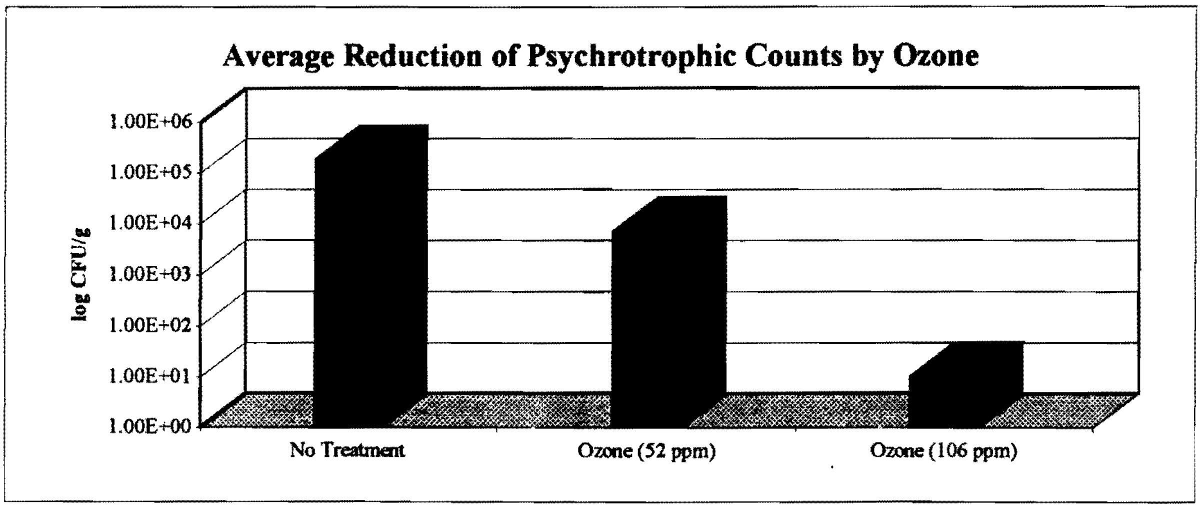
	37°C (CFU/g)	7°C (CFU/g)
No treatment	2.4×10^4	1.4×10^4
Ozone (54 mg/l)[a]	5.2×10^2	9.6×10^2
Ozone (107 mg/l)[b]	5.2×10^2	< 10 est.

Table 2: Ozone Inactivation to Reduce Microorganisms on Fresh Lettuce (Trial II)

	37°C (CFU/g)	7°C (CFU/g)
No treatment	3.7×10^4	3.4×10^5
Ozone (50 mg/l)[a]	7.9×10^2	1.3×10^4
Ozone (105 mg/l)[b]	2.5×10^1 est.	< 10 est.

[a] Ozone treatment was done for 50 seconds.

[b] Ozone treatment was done for 100 seconds.



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Ozone (50 mg/l)[a]	7.9×10^2	1.3×10^4
Ozone (105 mg/l)[b]	2.5×10^1 est.	< 10 est.

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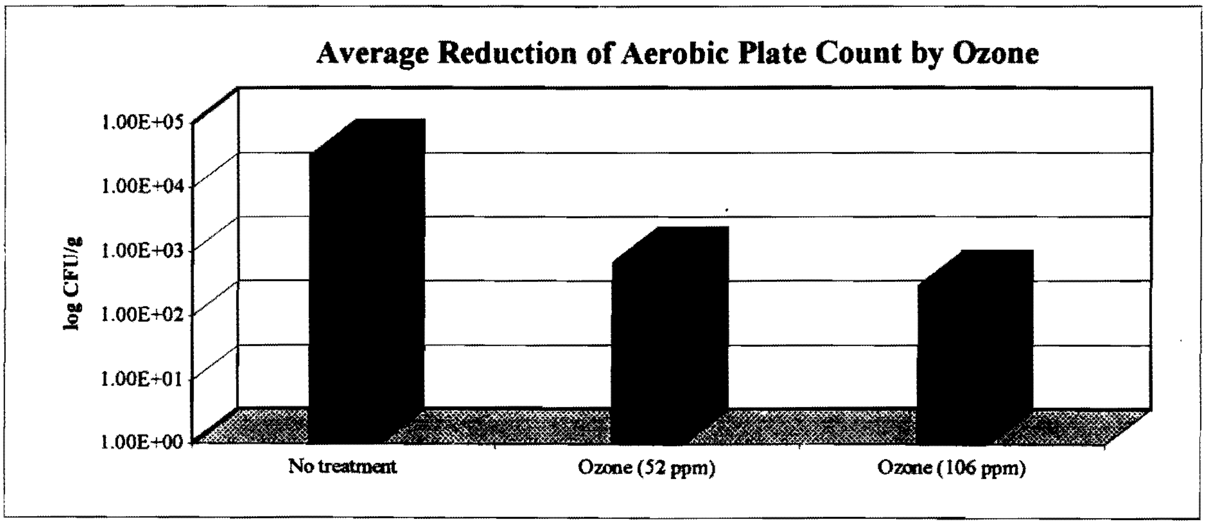

Table 3: Chlorine and Ozone Inactivation to Reduce Microorganisms on Fresh Lettuce (Trial I)

	37°C (CFU/g)	7°C (CFU/g)
No treatment	2.6×10^3	5.9×10^4
Ozone (1mM)[c]	7.0×10^3	3.2×10^3
Ozone (2mM)[d]	1.8×10^2	2.2×10^3
Chlorine (1mM)	2.7×10^2	9.0×10^3
Chlorine (2mM)	5.0×10^2	2.4×10^3

Table 4: Chlorine and Ozone Inactivation to Reduce Microorganisms on Fresh Lettuce (Trial II)

	37°C (CFU/g)	7°C (CFU/g)
No treatment	2.5×10^4	3.8×10^5
Ozone (1mM)[e]	2.1×10^3	2.7×10^4
Ozone (2mM)[f]	6.9×10^2	3.3×10^3
Chlorine (1mM)	4.6×10^2	3.1×10^3
Chlorine (2mM)	1.7×10^2	1.9×10^2

[c] 1 mM of ozone is equal to 98 ppm here
[d] 2 mM of ozone is equal to 194 ppm here
[e] 1 mM of ozone is equal to 97 ppm here
[f] 2 mM of ozone is equal to 193 ppm here

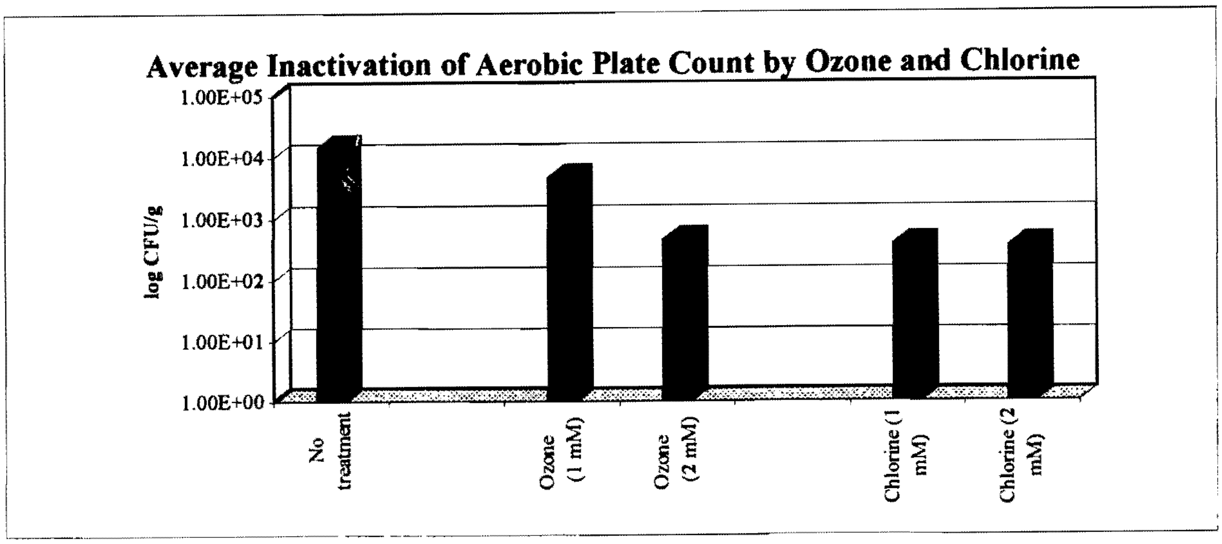

Table 3: Chlorine and Ozone Inactivation to Reduce Microorganisms on Fresh Lettuce (Trial I)

	37°C (CFU/g)	7°C (CFU/g)
No treatment	2.6×10^3	5.9×10^4
Ozone (1mM)[c]	7.0×10^3	3.2×10^3
Ozone (2mM)[d]	1.8×10^2	2.2×10^3
Chlorine (1mM)	2.7×10^2	9.0×10^3
Chlorine (2mM)	5.0×10^2	2.4×10^3

Table 4: Chlorine and Ozone Inactivation to Reduce Microorganisms on Fresh Lettuce (Trial II)

	37°C (CFU/g)	7°C (CFU/g)
No treatment	2.5×10^4	3.8×10^5
Ozone (1mM)[e]	2.1×10^3	2.7×10^4
Ozone (2mM)[f]	6.9×10^2	3.3×10^3
Chlorine (1mM)	4.6×10^2	3.1×10^3
Chlorine (2mM)	1.7×10^2	1.9×10^2

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