Ozone and Chlorine Treatment of Minimally Processed Lettuce

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ABSTRACT: The objectives of this research were to determine the effectiveness of ozone in combination with chlorine on the microbiological and sensory attributes of lettuce as well as the quality of the water used for processing commercial lettuce salad. Iceberg lettuce was inoculated with 8.0 log CFU/g microorganisms isolated from spoiling lettuce, treated with combinations of chlorine and ozone, and analyzed microbiologically. Commercial salads rinsed with chlorine, ozone, or a mixture were evaluated for sensory acceptability. Chlorine, ozone, and chlorine-ozone reduced aerobic plate count up to 1.4, 1.1, and 2.5 log, respectively. As determined by appearance, commercial lettuce salads treated with chlorine, ozone, or a combination had a shelf life of 16, 20, or 25 d, respectively. Using an ozone-chlorine or ozone rinse for commercial lettuce salads, there were no visible turbidity changes in process water. The quality of the water remained constant for longer periods of time making it available for longer reuse. Therefore, chlorine-ozone combinations may have beneficial effects on the shelf life and quality of lettuce salads as well as on the water used for rinsing or cleaning the lettuce.

Keywords: ozone, chlorine, lettuce, fresh-cut, salad

Introduction

Minimally processed fruits and vegetables have increased in popularity because of potential health benefits and a trend toward consumer desires for "fresh" foods (Alzamora and others 2000). In 1998, the sales volume of minimally processed fruits and vegetables in the United States was estimated to be approximately $6 billion, and was expected to increase to about $20 billion during the next 3 to 5 y (Reyes 1996). The increasing demand for fresh-cut products presents a challenge for researchers and processors to make the products more stable and safe from a microbiological standpoint.

Sources of microbial contamination for minimally processed fruits and vegetables include raw materials, plant workers, processing environment, and handling equipment (Alzamora and others 2000). Traditionally, processors have used water with or without chemical sanitizing agents to rinse fresh-cut and minimally processed produce. Chlorine has been the most widely used sanitizer. It is used routinely in wash, spray, or flume waters for fresh fruits and vegetables (Beuchat 2000). However chlorine has a limited effect in reducing microorganisms on fruit and vegetable surfaces (Beuchat 2000; Sapers 2001). In addition, concerns have been raised about the residual chorine by-products that may be generated, such as trihalomethanes, in the wastewater (Simpson and others 2000).

In 2001, gaseous and aqueous ozone was approved by the U.S. Food and Drug Administration for application as an antimicrobial agent to foods (FDA 2001). Research and commercial applications have indicated that ozone may be a suitable replacement for chlorine as a sanitizer (Graham 1997). However, limited studies have been done to determine the ozone effect as a disinfectant in the fresh-cut produce industry (Achen and Yousef 2001; Kim and others 1999a, 1999b; Liew and Prange 1994; Singh and others 2002a, 2002b). None have evaluated the potential for synergistic effectiveness of ozone and chlorine combinations to treat fresh-cut produce to reduce contaminating microorganisms.

The objectives of this study were: (1) to determine the sanitizing efficacy of ozone and chlorine, alone or in combination, on microbial reduction on fresh-cut salads, and (2) to determine the effects of ozone, chlorine, and ozone-chlorine commercial treatments on the sensory characteristics of these products and the quality of water used for processing the products.

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Material and Methods

Production of aqueous ozone-chlorine solutions

Ozonated water was generated by bubbling gaseous ozone into 1 L of distilled water. Ozone gas mixture was produced using an active oxygen generator machine with 2 UV lamps (Active Oxygen Generator, Golden Buffalo, Orange, Ca., U.S.A.). The gas was then pumped into the system using an aquatic air pump (Tetratec, deep water, DW 96-2, Blacksburg, Va., U.S.A.) at a flow rate of 4 L/min with an internal pressure in the system of 215 Pa. A level of greater than 10 mg/mL ozone was produced in the water. Ozonated water was held in a sealed container at 4 °C until stable and consistent ozone concentrations were measured. Ozone concentration was then measured immediately before use with a commercially available ozone test kit (CHEMetrics, Vacu-vials, Ozone K-7403, Calverton, Va., U.S.A.).

Ozone-chlorine solutions were prepared by adding 5.25% sodium hypochlorite to prepared concentrations of ozonated water. Ozone-chlorine combinations were 0-0, 0-100, 0-150, 0-200, 2.5-0, 2.5-100, 2.5-150, 2.5-200, 5-0, 5-100, 5-150, 5-200, 7.5-0, 7.5-100, 7.5-150, and 7.5-200 mg ozone/L-mg chlorine/L, respectively. Ozone concentration was measured after ozonation and prior to addition of specific concentrations of chlorine to give the desired concentration. The chlorine concentrations (± 0.2 mg/L) were verified using a commercially available chlorine test kit (CHEMetrics, Vacu-vials Chlorine K-2513 kit).

Preparation of inoculated fresh-cut lettuce

Iceberg lettuce (*Lactuca sativa*) was purchased at a local wholesale market. The lettuce was cored and cut into 2- × 3-cm strips with a sanitized, sharpened knife. The strips were placed in plastic freezer bags (Ziploc/reg/; 26.8 cm × 27.8 cm, 3.78 L capacity) and held at 6 °C for no longer than 10 min prior to inoculation.

A mixture of natural microflora was isolated from aseptically cut iceberg lettuce by incubating at 4 °C for approximately 2 wk. One milliliter of fluid from the spoiled lettuce was inoculated into trypticase soy broth (TSB; BD Diagnostic Systems, Sparks, Md., U.S.A.) and incubated at 10 °C for 3 d. The culture was transferred in TSB every 3 d until use. Nine milliliters of the inoculated TSB (about 9.8 log CFU/mL) was mixed with 491 mL of sterile distilled water and the inoculum mixture poured over 500 g of chopped lettuce placed in a 1-gallon plastic bag. The mixture was agitated on a wrist-action shaker and after 1 h, the excess water was drained from the lettuce. Inoculated samples were stored at room temperature (approximately 20 ° C) for 24 h to allow the attachment of cells to the lettuce surface. Initial counts on the lettuce ranged from 7.6 to 8.4 log CFU/g for aerobic plate count (APC) and 7.5 to 8.6 log CFU/g for psychrotrophic plate counts (PPC) depending upon the replication.

Treatments and microbiological analysis of inoculated fresh-cut lettuce

The inoculated fresh-cut lettuce (100 g) was spun-dried (Delux Flow-Thru Salad Spinner, Progressive Intl. Corp., Kent, Wash., U.S.A.) and placed into a 4-L glass beaker. One liter of each sanitizing solution was added to the beaker. A stainless steel cover was placed over the lettuce to keep it submerged in the solution. The sanitizing solution was then stirred on a magnetic stirrer at room temperature (approximately 20 °C) for 10 min at 6 rpm. The sanitizing solution was then decanted and the lettuce spun-dry for 10 s (Progressive Intl. Corp.). A 25-g sample of the dried lettuce was used for microbiological analysis.

Aerobic and psychrotrophic plate counts were performed by placing 25 g lettuce into 225 mL sterile 0.1% peptone and homogenizing in a stomacher blender (Seward, Norfolk, U.S.A.) for 1 min. Dilute lettuce samples were serially diluted in peptone and pour-plated. For the APC, plate count agar (PCA; Difco, Sparks, Md., U.S.A.) was used and plates were incubated at 37 °C for 48 h. For the PPC, PCA was incubated at 4 °C for 10 d.

Sensory (visual) analysis

A single lot of a salad mixture containing chopped lettuce plus shredded carrots and red cabbage was treated in a commercial processing facility (Strickland Produce, Inc., Nashville, Tenn., U.S.A.). The salad mixture was subdivided and treated with chlorine, ozone, or a combination of both. The salad mixture was continuously metered into recycled rinse water that had been chilled to < 4 °C, filtered, and ozonated and/or chlorinated over a 5-h period. Ozone was continuously added to produce a residual greater than 1.0 mg/L after the ozone had reacted with the organic material in the rinse water for all the ozone-treated salads. Chlorine was continuously added to produce a residual of 25 to 50 mg/L in the rinse water; then, the water was removed from the salad and was recycled. The salad mixture from each sanitizing treatment was packaged in polypropylene plastic bags on the same processing line. Packages were held at 4 °C during sensory evaluation that was performed after 4, 16, 21, and 25 d. A random, untrained panel (n = 30) was selected for the study. Panelists were asked to evaluate the lettuce packages visually to indicate their likelihood of purchasing the package of fresh-cut salad and to comment on the visual sensory qualities (appearance) taking into account color, texture, and integrity.

Turbidity analysis

Water samples (200 mL) were taken each hour for 5 h from a processing line surge tank of the commercial salad manufacturing facility during the process for each treatment (chlorine, ozone, or chlorine-ozone). The samples were treated with 0.1 mL of BSP Captor (30% wt/vol calcium thiosulfate) (Pollardwater.com, New Hyde Park, N.Y., U.S.A.) to inactivate residual chlorine and ozone immediately after collection. The turbidity of the water samples used in the commercial processing facility was measured in a quartz cuvette at 840 nm, using a HP Spec-20 spectrophotometer (Hewlett Packard 8452A, Diode Array spectrophotometer, San Jose, Ca., U.S.A.). The percentage transmittance at 840 nm of the samples of rinse water from the 3 treatments was compared. A wavelength of 850 nm is required by the Intl. Organization for Standardization (1990) in the Intl. Standard (ISO 7027) for measuring turbidity measurements.

Statistical analysis

For the microbiological part of the study, statistical analyses were performed for results generated by APC and PPC (dependent variables) using a randomized block design with 3 replications (P < 0.05), in a factorial treatment (SAS 8.1, Cary, N.C., U.S.A.). Blocking on replicas was performed to account for the variation of the initial lettuce quality and the initial microbial load. The independent variables in the study were chlorine (0, 100, 150, 200 mg/L) and ozone (0, 2.5, 5, 7.5 mg/L). The 16 treatment combinations were used in a random order (assigned through the use of a random number table generated by Microsoft Excel 2000). Surface response analyses were performed for the microbial data to be able to estimate the treatment response.

For the sensory part of the study, a Chi-square analysis was used to predict the distribution of the preference of the panelist within each replica, followed by an analysis of variance in a complete randomized design to determine significant differences among treatments. The interaction between shelf life and treatment was determined using a complete randomized design, with a factorial treatment. Treatments were compared using least square means.

Results and Discussion

Microbiological

Lettuce treated with 2.5, 5, and 7.5 mg/L of ozone had a 0.6- to 0.8-log reduction in the APC compared with rinsing with distilled water (P < 0.05); however there was no significant difference among the concentrations of ozone tested (Figure 1). Chlorine (100 to 200 mg/L) caused an approximate 0.9- to 1.2-log decrease in the APC and again, there was no significant effect of chlorine concentration on microbial destruction at the dosages used (Figure 1). The greatest measured reduction was achieved by combining 7.5 mg/L ozone and 150 mg/L chlorine. This ozone/chlorine treatment reduced the microbial load on the lettuce by 1.4 log CFU/g, whereas in the lettuce washed with distilled water only, a reduction of 0.3 log CFU/g was achieved. All treatments enhanced APC reduction compared with distilled water (P < 0.05). Lettuce treated with ozone only had significantly lower microbial reduction compared with lettuce treated with 200 mg/L chlorine alone or with chlorine plus all concentrations of ozone: 150 mg/L chlorine plus 5 and 7.5 mg/L ozone and 100 mg/L chlorine plus 2.5 mg/L ozone. The use of only 100 mg/L chlorine in combination with 2.5 mg/L ozone was not significantly different (P > 0.05) than the 150- or 200-mg/L chlorine solutions combined with ozone. Therefore a lower chlorine concentration in combination with ozone could be recommended.

Treatment of the lettuce with 7.5 mg/L ozone reduced the PPC on the fresh-cut lettuce by approximately 0.5 log compared with the control (Figure 2). Chlorine reduced the PPC compared with the control ($P < 0.01$), but there was no increased kill with increasing concentration. None of the ozone-chlorine treatments were significantly better than using chlorine alone at the same concentration as the combination. The addition of ozone to the treatment solutions would therefore not be necessary for controlling psychrotrophic bacteria when using at least 100 mg/L chlorine (Figure 2). Because 5 mg/L ozone and 100 mg/L chlorine was not significantly different than 200 mg/L chlorine alone, the chlorine could be reduced in the presence of ozone and equivalent sanitizing power maintained ($P < 0.05$).

Results on the efficacy of ozone are similar to those reported in previous studies. Kim and others (1999a) evaluated the efficacy of chlorine or ozone as sanitizers on shredded lettuce. They found that 1 mM and 2 mM chlorine reduced the aerobic plate count on the lettuce by 1.4 log and 2.0 log, respectively. Ozone at 1.3 mM reduced the microflora by 1.2 log after 3 min and 4 log after 5 min. However, ozone at approximately 10 mg/L (similar to the concentrations used in the present study) was much less effective against *Pseudomonas fluorescens*, reducing viable cells by only approximately 80%. Singh and others (2002b) inoculated lettuce and baby carrots with *Escherichia coli* O157:H7 and treated the products with ozone and chlorine dioxide, either alone or sequentially. Alone, 9.7 mg/L ozone reduced the foodborne pathogen by 1.5 to 2 log in 10 min. If the products were treated in sequence with ozone and chlorine dioxide, viable *Escherichia coli* O157:H7 were reduced by 3 to 4 log. The increased efficacy of the combination of ozone and chlorine dioxide compared with the present study could be because of the susceptibility of the microorganism or the use of the chlorine dioxide.

A response surface analysis was performed on replications 2, 3, and 4 (same raw material) to design quadratic regressions that would fit the APC and PPC data and predict microbial log reductions on fresh-cut lettuce treated with ozone-chlorine. The quadratic regressions accounted for 57.4% or 39.0% of the variation in the APC or PPC data, respectively. The fitted data suggests that 4.09 mg/L ozone in combination with 225 mg/L chlorine would be required to achieve the greatest log reduction of APC (Figure 3) and 6.5 mg/L and 233 mg/L, respectively, for the PPC. It should be noted that the critical chlorine value obtained from this analysis is above best use practices of 200 mg/L.

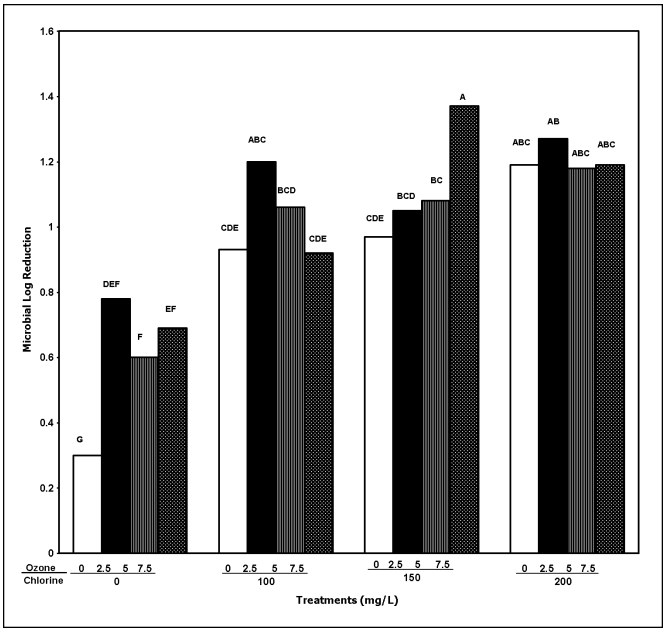

Figure 1—Least square means for the aerobic plate count microbial reduction on inoculated lettuce samples treated with ozone, chlorine, and ozone plus chlorine. Log reductions with like letters are not different ($P < 0.05$)

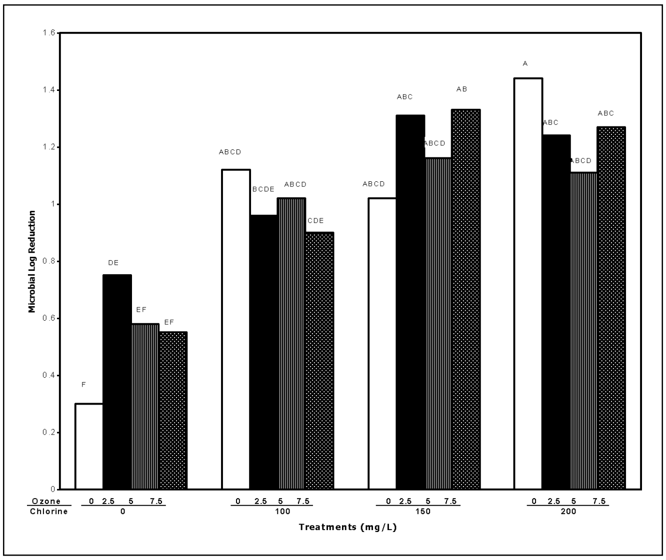

Figure 2—Least square means for the psychrotrophic microbial reduction of inoculated lettuce samples treated with ozone, chlorine, and ozone plus chlorine. Log reductions with like letters are not statistically different ($P < 0.05$).

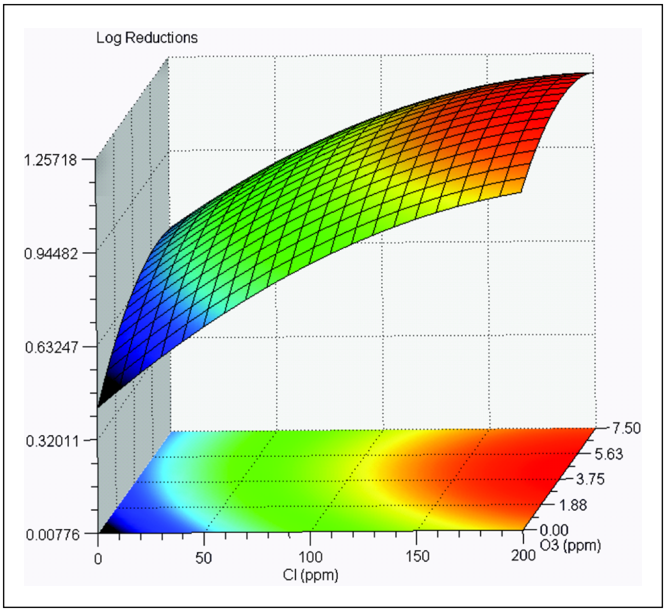

Figure 3—Quadratic regression model for the surface response for APC data, microbial log reduction compared with ozone, chlorine, and ozone-chlorine treatments (ppm = mg/L).

Sensory appearance

Treatment combinations for the commercial ready-to-eat (RTE) salads were evaluated by determining whether a panelist would be "willing to purchase" a particular sample. There was an interaction ($P < 0.05$) within the combination of ozone and chlorine, mainly because of the effects of the different treatments over storage time of the salads (Figure 4). Description by panelists of samples treated with chlorine only indicated browning, loss of integrity, color changes, water segregation, and overall poor appearance by day 16. Salads treated with aqueous ozone alone degraded more slowly and had an acceptable shelf life of approximately 21 d. The ozone and chlorine combination gave the best results achieving a shelf life of greater than 25 d. Panelist scores for the ozone- and chlorine-treated RTE salads indicated these samples had significantly better shelf life. Panelists were willing to "definitely purchase" the ozone-chlorine treated salads through day 21 of storage. By day 25, packages treated with a combination of ozone and chlorine, started to show a slight browning at the edges of the lettuce leaves and some structural breakdown, but 64.5% of the panelists would either "definitely purchase" or "probably purchase" these salads. At day 25, the RTE salads from all treatments had significantly different scores and the ozone-chlorine treated sample was the most desirable with the panelist overall rating of "probably purchase" or a least square means estimate of 2.4.

Process water

Rinse water samples collected when the salad mixture was treated with a chlorine rinse water had increased turbidity as indicated by the transmission of light as processing time progressed indicating increased product particles, soil, and pigments (Table 1). The water remained clear (translucent) for at least 1 h. After 2 to 3 h of processing the solution became cloudier. Using an ozone-chlorine or ozone rinse (Table 1), there were no visible turbidity changes. Therefore, with ozone-chlorine or ozone, quality of the water apparently remained constant for a longer period of time making it available for longer reuse. The commercial processor typically would change the rinse water after 4 h when using only the chlorine treatment but would use the rinse water for up to 8 h when ozone was added as a treatment. It is hypothesized that, because ozone has a higher oxidation potential than chlorine, it oxidizes more rapidly and more efficiently the organic matter that is suspended or dissolved in the water. It may degrade dissolved pigments in the water (chlorophyll, phenolics, carotenoids, and so on) and therefore keep the turbidity of the water lower.

Table 1—Transmission measurement at 840 nm of water used to process commercial lettuce salad mix treated with chlorine, ozone, or a mixture of both over time.

Hours of treatment	Ozone-chlorine %T	Ozone %T	Chlorine %T
0	76.1	82.3	84.7
1	73.7	81.5	84.4
2	71.4	78.3	76.3
3	75.8	79.4	75.5
4	81.8	76.6	58.5
5	76.2	80.5	62.4

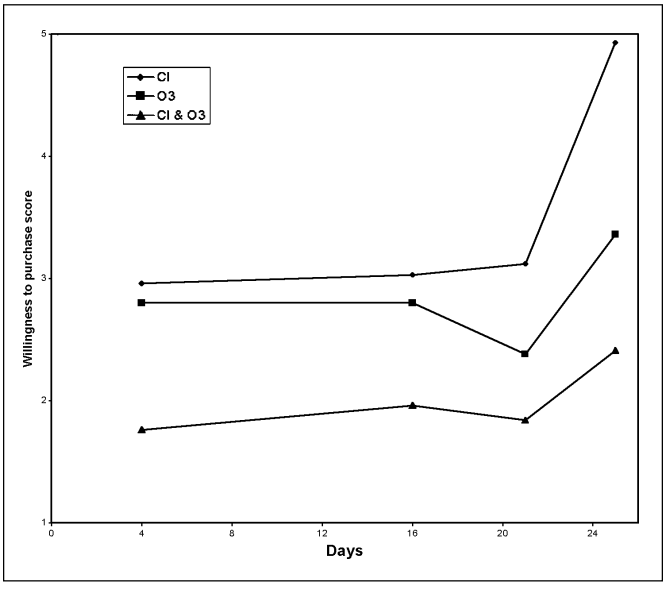

Figure 4—Panelists' willingness to purchase commercially salad samples sanitized with chlorine (Cl), ozone (O_3), or a combination of both and based on appearance of the salads. (5 = Definitely not purchase, 4 = Probably not purchase, 3 = Maybe purchase, 2 = Probably purchase, 1 = Definitely purchase).

Conclusions

In conclusion, use of a combination of ozone and chlorine resulted in equivalent or better microbial reduction, an extension of the shelf life (as determined by appearance) of a RTE salads, and a reduction in process water turbidity than chlorine alone. In addition, ozone at the concentrations used cannot totally replace chlorine. Chlorine is needed for the purpose of achieving a greater microbial reduction in RTE lettuce salads. Using a reduced chlorine concentration treatment may reduce the risk of the formation of trihalomethane compounds, which have been reported to be carcinogenic. Using a combination of 2.5 mg/L free available ozone and 100 mg/L free available chlorine is recommended for the washing/rinsing process for fresh-cut lettuce. These treatment levels were shown to be beneficial and effective in reducing the microflora of lettuce and have the potential to extend the shelf life and improve the water quality in the processing line. A higher concentration of ozone might be needed for a large manufacturing plant because the amount of organics, dissolved solids, and ozone-demanding material in the water. While chlorine levels can be reduced when used in combination with ozone, further research is needed to determine if higher ozone levels influence the quality of RTE salads.

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