Comparison of Aqueous Ozone and Chlorine as Sanitizers in the Food Processing Industry: Impact on Fresh Agricultural Produce Quality

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The effects of ozone treatment on fresh strawberry and shredded lettuce food quality were tested by varying applied ozone concentration, contact time, pH and temperature to assess ozone a potential food sanitizer. The produce quality was assessed by comparing the changes in texture firmness, browning and decolorization, oxygen consumption and carbon dioxide respiration after the post-treatment storage from 0 to 21 days. The effectiveness of ozonation on natural microfloras including mesophiles, psychrotrophes, yeasts and molds, was also evaluated for the improvement in produce shelf-life. As compared to chlorine treatments, ozone treatments slightly increased the lettuce browning but substantially retarded its respiration rates and firmness deterioration even after 21 days of storage. For strawberry, no significant difference in food quality was observed between ozone and chlorine treatments. Finally, ozone treatments at the doses below 10 mg/L were found not effective in killing natural microfloras grown on the produce surfaces.

Keywords Ozone, Disinfection, Food Quality, Natural Microfloras, Ozonation, Water Treatment

INTRODUCTION

Recently, great concerns have been raised about food quality and microbial safety for fresh agricultural produce. This is particularly serious for those minimally processed produce because they are commonly subject to very limited cleaning and sanitizing prior to consumption. Besides, the mechanical damage after processing may cause an increase in respiration, surface dehydration, moisture loss and oxidative browning, and provide attachment sites and nutrients for microbial contamination and growth (Brackett, 1987; Priepke et al., 1976). To prevent the potential contamination and extend the shelf-life of agricultural produce, various chemical treatments have been suggested. Among them, chlorine and associated compounds remain to be the most commonly used sanitizers.

However, their use could result in the formation of various chlorinated by-products, some of which are considered to be carcinogenic (Kim et al., 1999a; Zhou 1995). As well, some of concerned foodborne pathogens were found resistant to chlorination (Beuchat and Brackett, 1990; Graham, 1997). Consequently, ozone has been proposed as an alternative sanitizer due to its powerful disinfection potential (Khadre et al., 2001; Kim et al., 1999b) and spontaneous decomposition to non-toxic products such as oxygen and water (Zhou, 1995). Wang et al. (2004) observed that cilantro treated with ozonated water for 5 minutes had the best overall quality as compared to tap water, acidic electrolyzed water (AEW), chlorinated water and aqueous ozone followed by AEW.

The ozone treatment also effectively maintained the typical cilantro aroma during storage at 0°C for 14 days. Kim et al. stated that the counts of mesophilic and psychrotrophic microorganisms on shredded lettuce were reduced by approximately 4 and 4.6-log-units respectively after ozone (1.3 mM) was bubbled in washing water for 5 minutes (Kim et al., 1999a). Similarly, Rodgers et al. (2004) obtained around 4 to 5-log-units reduction of mesophilic microorganism for sliced apple, lettuce, strawberry or cantaloupe after a 3 ppm ozone treatment for 5 minutes.

In 2001, the U.S. FDA approved the petition to consider ozone as generally recognized as safe (GRAS) for the treatment of food and approved it as an antimicrobial agent. However, Spotts et al. (1992) reported that
washing by ozonated water did not reduce the decay rates of pears as compared to those washed with water alone. Baur et al. (2004) pre-washed shredded iceberg lettuce with 1 mg/L ozonated water at 4°C for 2 min and then rinsed by tap water at pH 8 for 90 s. Ozone treatment caused less improvement in produce shelf-life than the treatment with 200 mg/L chlorinated water (Baur et al., 2004).

Klaiber et al. (2004) also found that the pre-washing of uncut carrots with ozonated water at 4°C, pH 8 for 2 min was not effective in reducing the microbial counts. The discrepancy may be partially contributed to different types of agricultural produces tested and different ozonation conditions. As well, few studies have been reported about the effects of ozonation on natural microfloras, even though they have been considered to play critical role in produce shelf-life. Thus, the objective of this study was to examine the effects of ozone on important food quality and natural microfloras under well-controlled conditions. For comparison purpose, a series of chlorine treatments were also conducted.

MATERIALS AND METHODS

Produce Samples

Shredded lettuce samples were kindly provided by Pride Pak Canada Ltd., Mississauga, Ontario from its commercial processing line prior to any treatment. After being shipped to the laboratories of the Food Research Program, Agriculture and Agri-Food Canada (AAFC) at Guelph, the samples were further sorted to discard the outer and core leaves, and other discolored and wilted pieces to ensure the test samples representative. The sorted samples were aseptically re-cut into 3.5 x 3.5 cm and sealed into sterile plastic bags (Fisherbrand, Fisher Scientific) with an approximate weight of 185 g. Fresh strawberry samples were harvested directly from a local farmer (Fergus Strawberry Farm, ON.). Only those strawberries with similar size, ripeness and color and without any lesions on the surface were randomly picked and placed into sterile plastic bags. Each of the sample storage bags containing lettuces or strawberries was kept in a cold room at 4°C and 80% relative humidity prior to use.

Ozonation

Ozone stock solution was prepared by continuously bubbling the ozone gas generated from extra dry oxygen gas by an ozone generator (Lab2B, Ozonia, Elmwood Park, NJ, USA) into a 500 mL gas wash bottle filled with Milli-Q water. During bubbling, the gas wash bottle was immersed in an iced water bath for over 20 min to achieve the equilibrium of saturation. The exhaust gas from the gas wash bottle was introduced into the container containing oversaturated Na2S2O3 solution to completely and absorb decompose any gaseous ozone prior to discharge into the atmosphere.

Figure 1 shows a schematic of the batch ozone reactor specifically designed for this project. It was made of a 4-liter glass beaker covered with a Teflon cover floating on the water surface to eliminate any headspace that could cause ozone loss due to volatilization. A stainless steel baffle was placed inside the reactor to provide complete mixing while minimizing the potential vortex effects. Prior to ozonation tests, the reactor containing 2500 mL phosphate buffer solution (see Table 1) was filled with a preset amount of test produce samples and mixed by a Teflon-coated magnetic stirring bar for 3 to 5 s. The ozone treatment was started by adding an aliquot of prepared ozone stock solution as specified in Table 1.

After a preset period of contact time, a water sample was collected to measure pH and ozone residual. Immediately, 15 mL sterile sodium thiosulfate solution (1M) was injected into the reactor to completely quench the ozone residual left behind. The treated produce samples were placed in a sterile salad spinner for 2 min to drain off any remaining water on the sample surfaces and packed in the plastic bags for food quality analyses. Throughout ozonation, the treatment temperature was controlled by immersing the reactor into a constant-temperature water bath. In similar manner, additional comparison tests were conducted by using 5.65–6.00% sodium hypochloride (Purified Grade, Fisher Scientific) as a chlorine stock solution to treat raw produce samples. The detailed experimental arrangements are summarized in Tables 1 and 2.

Note that any glassware contacted with ozone, chlorine or phosphate buffer solutions was thoroughly cleaned to ensure that it was ozone-demand-free or chlorine-demand-free. The glassware was wrapped with aluminum foil to autoclave at 121°C for 15 min prior to its use. The phosphate buffer solutions (0.01M and 0.05M for lettuce and strawberry tests, respectively) were prepared by adding monobasic sodium phosphate and dibasic potassium phosphate into Milli-Q water, and then was ozonated for 1 hour followed by autoclave at 121°C for 15 min.

Food Quality and Chemical Analyses

Most of the food quality parameters were determined according to the Official Methods of Analysis (AOAC,
Specifically, lettuce browning was determined by taking digital images of the lettuce samples randomly picked into a Petri dish after 0, 7, 14, 21 days of the treatment. The resultant images were analyzed for percent browning area using an image analysis program (Northern Eclipse, Mississauga, Ontario). Each of these measurements was made in triplicate to obtain average percent browning area. The colorization measurements were made using a Minolta CR-200 chroma meter (Minolta, Osaka, Japan) calibrated with standard white tile for the strawberry samples after 0, 5, 7, 12 days of the treatment. The colorimetric values from five strawberries were averaged to obtain chroma, hue and total color difference. The firmness of shredded lettuce was determined using a TAXT2i texture analyzer (Technologies Corp. New York, U.S.A.) equipped with a modified 3-blade Kramer shear cell. The measurements were repeated three times for each sample and averaged in kgf/10g. The firmness for strawberries was measured using a 2-mm-diameter punch probe (Technologies Corp. New York, U.S.A.). Oxygen and carbon dioxide concentrations inside the packages of lettuce or strawberry samples were monitored using an O2 and CO2 meter (PBI Dansensor, Checkmate 9900, Rønnedevej 18, DK-4100 Ringsted, Denmark). The gas samples were taken using a ½” 26G needle penetrated into the rubber septum on each packing bag prior to opening the packages. The concentrations of dissolved ozone and free chlorine in water were measured using indigo method and iodometric method, respectively (APHA- AWWA-WEF, 1995).

The concentrations of natural microfloras were assayed by adding 10g lettuce or 60g strawberry into 100 mL sterile peptone water (0.1 wt%) and smashed by a stomacher (Stomacher 400, Seward, U.K.) at a high speed for 2 min. Serial dilutions of the stomached samples were made with 0.1% peptone water, and 0.1mL of the appropriate dilutions was spread onto the surface of the selective media. Tryptic Soy Agar (TSA) medium was used for both mesophiles and psychrotrophs. Their colony counts were enumerated after incubation at 35°C for 48 h and 7°C for 10 days, respectively. In similar manner, the colony counts of yeasts and molds were enumerated on Dichloran-Rose Bengal-chloramphenicol Agar (DRBC) medium after incubation at 22°C for 5 days.

**RESULTS AND DISCUSSION**

**Respiration**

Figures 2 to 5 show typical variations in respiration in terms of O2 and CO2 concentrations for the lettuces treated by ozone and chlorine, respectively. Similar trends were obtained under other treatment times, pH values and temperatures. At each of the storage times, the O2 concentration only slightly decreased as the applied ozone concentration increased. However, as the storage time increased, more O2 concentration was reduced. For
example, at an applied ozone concentration of 10 mg/L, the O$_2$ concentration was decreased by 35% after 1 week of storage and 55% after 2 weeks’ storage as compared to its initial levels. Afterwards, the O$_2$ concentration in the treated lettuce packages generally remained around 6% (v/v).

Meanwhile, the CO$_2$ concentration increased substantially when the ozone concentrations were increased up to 1 mg/L, above which the ozone concentrations posed little additional effects on CO$_2$ concentration. The increase in CO$_2$ concentration occurred largely within the first week, and then the CO$_2$ concentration became almost constant for each of the ozone concentrations. In addition, the pH showed little effect on O$_2$ and CO$_2$ concentration as long as it was kept below 7 regardless of different temperatures and ozonation contact times. When the pH was up to 8, the O$_2$ concentration was decreased by as much as 50% and 80% after 1 week and 2 weeks of storage, respectively. Afterwards, little change in the O$_2$ and CO$_2$ concentrations was observed during the storage.

In contrast, after chlorine treatment the O$_2$ concentration had dramatically declined while the CO$_2$ concentration had rapidly increased. For example, at a chlorine concentration of 100 mg/L, the O$_2$ concentration decreased by 50% after the first week of storage. This decrease was even faster during the second week. After 3 weeks of storage, the O$_2$ concentration was reduced to less than 0.5%. Meanwhile, the CO$_2$ concentration was increased substantially after the first week of storage, and afterwards varied slightly and remained within a range of 6.5–10%. This observation was similar to the reports from Odumeru et al. (2003) and Baur et al. (2004).

Almost identical respiration rates were observed for the strawberries after ozone treatment or chlorine treatment. Regardless of their different treatment conditions, the O$_2$ concentration was decreased by about 60% while the CO$_2$ concentration was increased by approximately 25% to 35% after the first 5 days of storage. As compared with lettuce, strawberries had higher respiration rates. This observation is consistent with Hardenburg et al. (1986), who found the respiration rates of strawberries were usually high and could be inclined to be perishable.

As physiological deterioration and microbial growth would cause an increase in respiration for the cut tissues,
the rate of respiration could be used to assess their shelf-life (Watada et al., 1996). Depletion of oxygen rather than rise in carbon dioxide triggered the product deterioration (Barry-Ryan et al., 2000). It is suggested that to prolong post-harvest shelf-life, the respiration should be reduced, and at least 1 to 3% O₂ concentration is necessary to maintain aerobic respiration and to avoid off-flavor development in anaerobic conditions (Rolle et al., 1987).

Based on the preceding guidelines, ozone treatment could greatly extend the shelf-life of post-harvested produces beyond 21 days, as is evident by the lower respiration rates. In comparison, chlorine treatment would result in much faster respiration rates. Consequently, chlorine treatment could be deemed to be inappropriate for preserving the lettuce for more than 14 days. In comparison with ozone treatments at lower pH and temperature, the respiration rates of the lettuce treated at a pH of 8 or 23°C would be higher.

Our results were consistent with the observations made by Cantwell (1995) that the respiration rates increased with treatment temperature. Bolin et al. (1977) also reported that lettuce dipped in the lower pH solutions would have a longer shelf-life.

Firmness

Figures 6 and 7 compare typical trends of produce firmness after ozone and chlorine treatments for 5 minutes at 4°C and a pH 7, respectively. In general, ozone treatment caused little change in lettuce firmness throughout 21 days of storage, regardless of difference in ozone concentrations. The firmness values varied within a range of 11 to 13 kgf/10g. An exception was that after treated by ozone at pH 8, the firmness of treated samples remained almost the same over 2 weeks period in storage but rapidly declined by about 25% of initial level. Nevertheless, the firmness values of treated lettuces after 21 days of storage were still 8.5 to 10.5 kgf/10g regardless of different applied ozone concentrations.

In contrast, chlorine treatment had an adverse impact on lettuce firmness. A slight decline in firmness occurred in the first week, followed by a rapid decline in firmness during subsequent storage. After being stored for 3 weeks, the firmness of lettuce samples treated with more than 200 mg/L chlorine was, on average, 1 to 2 kgf/10g.

Similar trends were observed for strawberry samples treated with ozone and chlorine, respectively. After 5 days of storage, the firmness of strawberry treated with ozone was approximately 40% higher than those measured on the 0-day storage. Little decline in the firmness was observed even after 12 days’ storage, regardless of applied ozone concentrations. However, when strawberries were treated by chlorine, their firmness after being stored for 3 weeks was decreased by approximately 20%.

Similar results have been reported by Baur et al. who found the crispness of lettuce samples generally dropped with increase in storage but no significant (P > 0.05) difference in texture measurement was shown between chlorine treatments at 200 mg/L and ozone treatment at 1 mg/L followed by tap water rinsing during 9-day storage at 4°C (Baur et al., 2004). However, Delaquis et al. (2000) reported that the texture (crispness) of iceberg lettuce pieces was slightly improved by treatment with warm 100 ppm chlorinate solution for 3 minutes at 47°C. In contrast, our results showed that the temperatures between 4 and 23°C had little impact on lettuce texture. But the treatment at a pH 8 resulted in both higher respiration rates and substantial decline in firmness. Thus, it would not be recommended to treat lettuce at a pH higher than 7.

Ozone treatment of strawberries resulted in an increase in firmness during the first 5 days of storage and then remained almost the same for the following 12 days, which may be a consequence of water loss rather than retention of flesh firmness and of relatively less growth of molds on the strawberry surfaces. With regard to the effect of chlorine treatment on the firmness of strawberry, the results obtained from this study were similar to those
reported by Yu et al. (1996), who also noted that fresh strawberry firmness slightly increased during the first 2 days and decreased afterwards. As compared to ozone treatment, the samples treated with chlorine were softer, which could make them more prone to mechanical injury and subsequently decrease their shelf-life.

It is well recognized that the crispy texture of fruits and vegetables is an important quality attribute to be considered as freshness by consumers. Adverse impact on produce texture would result in the quality deterioration and consequently shorten the shelf-life (King and Bolin, 1989; Rolle et al., 1987). In comparison with chlorine treatment, ozone treatment at appropriately controlled conditions may be more helpful to keep texture crisp.

**Colorization**

Figures 8 to 9 show typical browning variations for the lettuce as a function of ozone and chlorine concentration, respectively. Similar trends were observed for the treatments under different temperature, pH and treatment duration. In general, the lettuce browning increased greatly with ozone concentration and storage time. The browning was less affected by ozone treatment time (data not shown). Nevertheless, the browning was less than 2.5% for a storage period of one week. When the ozone concentration was increased up to 10 mg/L, the browning was increased up to about 6% and 9% after two and three weeks of storage, respectively.

In comparison, chlorine treatment caused less browning for lettuce. The browning increased with storage time while little impact was observed for the chlorine concentrations used. After 21 days of storage, the browning was increased by only 3 to 4%. By changing pH, contact time and temperature for chlorine treatments, no obvious difference in browning was observed. These results were consistent with the observations by Baur et al. (2004), who also reported as compared with ozone treatment, the browning of shredded lettuce was delayed by washing with chlorinated water at 4°C.

Browning is used as one of the major indicators for the loss of quality of cut-lettuce. The lower browning for the lettuce treated by chlorine treatment may be partially attributed to higher CO2 developed during storage, which would prevent the browning of damaged plant tissues by blocking production of phenolic compounds and inhibiting polyphenol-oxidase (PPO) activity (Siriphanich and Kader, 1985). As O2 is a necessary substrate for reactions of enzymatic discoloration (Kader, 1980), dissolved O2 produced from the decomposition of ozone during the treatment may also promote initial enzymatic browning occurring on the surface of produce samples.

For strawberries, different chemical concentrations of ozone and chlorine and different storage times used in this study did not result in much change in color, even though the treated strawberry was accompanied with moisture loss and ripeness. This observation disagreed with the report of Collins and Perkins-Veazie, 1993), who found the loss of brightness during storage and color shift from orange-red to red during storage. The difference may be due to different maturity of strawberry samples or different cultivars used in his experiments.

**Natural Microfloras**

Natural microfloras can cause the produce tissue breakdown, thereby, shortening the shelf-life (Bolin, et al., 1977; King and Bolin, 1989). As well, natural microfloras have also been considered one of the key indicators for microbial contamination. Because of their significance in food quality, both lettuce and strawberry samples were treated by ozone and chlorine to assess the ozone effectiveness in inactivating mesophiles, psychrotrophes and yeasts and molds. Figures 10 and 11 showed their typical reductions on lettuce at different ozone and chlorine concentrations, respectively.

As expected, the inactivation of natural microfloras increased with the ozone concentration. The resistance
to ozone in descending order was: mesophiles, psychrotrophes and yeasts and molds. After the lettuce was treated for 5 minutes at an applied ozone concentration of 10 mg/L, mesophiles, psychrotrophes, yeasts and molds were reduced from initially 4.55 cfu/g, 5.07 cfu/g and 4.48 cfu/g to 3.76 cfu/g, 3.90 cfu/g and 3.49 cfu/g, respectively. In comparison, chlorine treatment at 200 mg/L resulted in 2.1-, 1.8- and 2.5-log units reduction for each group of the microbes. Thus, as compared with chlorine treatment, ozone treatment was not effective in killing natural microfloras on lettuce. Similarly, ozone treatment was also not effective in inactivating microfloras grown on strawberry samples. After treatment at an applied ozone concentration of 20 mg/L for 3 minutes, mesophiles, psychrotrophes, and yeasts and molds on strawberry were reduced from initially 3.94 cfu/g, 5.1 cfu/g and 4.89 cfu/g to 2.66 cfu/g, 3.59 cfu/g and 4.11 cfu/g, respectively.

These results agreed with the observations of Baur et al. (2004), who found that pre-washing with 1 mg/L ozonated water was not effective against aerobic mesophiles. One possible explanation is that aqueous ozone was depleted rapidly due to the occurrence of competitive reactions with organic substances released from lettuce. In addition, the natural microorganisms could migrate into the deeper plant tissue, hindering the accessibility for ozone to attack.

CONCLUSIONS

The effects of ozone treatment on fresh fruit and vegetable quality and natural microfloras inactivation were examined at different ozone concentrations, contact times, pH values and temperatures. For comparison purpose, a series of chlorine treatments were also conducted. The following conclusions were drawn:

1. Ozone treatment at pH below 8 substantially retarded the respiration rates of lettuce, suggesting that ozone has the potential to extend the lettuce shelf-life. However, neither ozone nor chlorine treatment affected the respiration rates of strawberry because of different phytoalexin responses. In all, ozone application as a sanitizer in food processing industry should be produce-specific.

2. Ozone treated lettuce and strawberries had little change in firmness over three weeks of storage. In contrast, chlorine treatment resulted in dramatic decrease in firmness for lettuce, but had less impact for strawberries.

3. Ozone treatment caused more browning for lettuce as compared to chlorine treatment. The rates of lettuce browning increased greatly with increasing ozone concentration and storage time. For strawberry, no obvious difference in color change was observed between ozone treatment and chlorine treatment.

4. Both ozone and chlorine treatments were ineffective in killing natural microfloras. The average reduction of mesophiles, psychrotrophes, yeasts and molds on lettuce was approximately 0.73-, 1.1- and 1.2-log units after ozone treatment at 10 mg/L. The average reduction after chlorine treatment at 200 mg/L for 5 minutes was 2.1-, 1.8- and 2.5-log units, respectively.

ACKNOWLEDGMENTS

The authors would sincerely acknowledge the financial supports from Ontario Ministry of Agriculture and Food through the Food Safety Research Program, and from National Sciences and Engineering Research Council of Canada. The contributions from Pride Pak Canada Ltd. to provide the test samples and the permission to access the laboratories at Food Research Program, Agriculture and Agri-Food Canada at Guelph, are greatly appreciated. We are also grateful to Dr. R.C. McKellar, Dr. X. Li and Ms. C. Defelice from
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