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Effect of Chemical Treatment with Citric Acid or Ozonated Water on Microbial Growth and Polyphenoloxidase Activity in Lettuce and Cabbage

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Abstract

Effects of chemical treatment with a citric acid solution or ozonated water on microbiological changes in lettuce and cabbage during storage were studied. Fresh lettuce and cabbage samples were cut into small pieces and treated by soaking in either ozonated water or a citric acid solution. After treatment, populations of total bacteria, yeast and mold, and *E. coli* were determined. Numbers of microorganisms increased during storage, but ozonated water and citric acid treatments retarded the increase in microbial growth. Among treatments, 1% citric acid treatment was the most effective in terms of microbiological change and inhibition of polyphenoloxidase (PPO). For lettuce, citric acid treatment decreased the microbial growth overall by 1.5 log CFU/g and inhibited the PPO activity by 80%. These results indicate that chemical-treated lettuce and cabbage retained a better quality than those of the control during storage.

Key words: chemical treatment, lettuce, cabbage, microbiological change

INTRODUCTION

Consumption of minimally processed salad vegetable has increased in recent years, partly due to its nutritional value and presumed beneficial health effects (1). The convenience of shredded, packaged lettuce and cabbage as typical vegetable benefits to consumers provides the industry with considerable savings in transportation and storage costs (2). However, minimally processed salad vegetables are subject to microbial decay largely caused by the growth of microorganisms originating from preharvest environments, with Gram-negative bacteria and yeasts making up the bulk of the initial microbial load (3,4). Although minimally processed vegetables are usually washed in cold water before packaging, the antimicrobial effectiveness of this treatment is nonetheless limited (5). Chemical treatment is an alternative treatment to thermal processing, because it is effective in controlling microbial levels in ready-to-eat vegetables. Among chemical treatments, chlorinated water is widely used to sanitize fresh-cut produce, but this treatment even at low concentration has a detrimental effect on taste and odor (6). Therefore, there is a great interest in developing alternative sanitizers for washing fresh vegetables.

Polyphenoloxidase (PPO) is responsible for browning of damaged fruits and vegetables by catalyzing hydroxylation of monophenols to *o*-diphenols and dehydrogenation of *o*-diphenols to *o*-quinones in the presence of oxygen (7,8). Since the enzymatic browning causes deterioration of nutritional quality and affects appearance and organoleptic properties, inactivation of PPO is desirable for preservation of foods (9). The use of thermal processing for inactivation of PPO is limited because it often degrades the sensory and nutritional quality of food products. Therefore, non-thermal processing techniques have been tried and used in food industry (7-9). As a non-thermal processing, chemical treatment could extend the shelf life of food products and provide better quality control by inactivating pathogenic microorganisms and PPO.

Therefore, the objectives of this study were to examine the effect of chemical treatment on the microbiological change and PPO inactivation of fresh lettuce and cabbage during storage, and to provide suggestions for optimal processing for extending the shelf-life and improving quality of fresh salad vegetables in the marketplace.

MATERIALS AND METHODS

Sample preparation and chemical treatment

Fresh lettuce and cabbage were purchased from a local

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market in Daejeon, Korea. Samples were cut into 2×2 cm pieces and treated by dipping in a solution of either 3 ppm ozone or 1% citric acid. Ozone concentration was determined according to the method of Singh et al. (10). After treatment, samples were transferred aseptically to a sterile polypropylene container and stored at 4°C.

Microbiological analysis

After chemical treatment, samples were ground in the phosphate buffer (pH 7.0) using a blender, filtered through a sterile cheese cloth, and diluted with peptone water for microbial count. Serial dilutions were performed in triplicate on each selective agar plate. Total bacterial counts were obtained by plating the appropriately tenfold diluted samples onto plate count agar (Difco, USA). Samples were evenly spread on the surface of the plates with a sterile glass rod. Yeast and mold were plated on potato dextrose agar (Difco, USA). Both plates were incubated at 35°C for 48 hr. For *E. coli*, chromogenic *E. coli* / coliform medium (OXOID, Basingstoke, Hampshire, England) was used. The plates were incubated at 35°C for 24 hr and colony forming units (CFU) were determined. During storage at 4°C, changes of residual total bacteria, yeast and mold, and *E. coli* counts were investigated. All samples were tested in duplicate and each microbial count was the mean of three determinations. Microbial counts were expressed as log CFU.

PPO assay

Lettuce (10 g) and cabbage (5 g) samples were ground in the phosphate buffer (pH 7.0) using a blender, and filtered through a cheese cloth. The filtrate was used for PPO assay according to the method described previously (11). To 0.3 mL of the sample solution, 2 mL of 50 mM potassium phosphate (pH 6.5) solution with 0.3 mL of 0.2 M catechol solution were added. One unit of PPO activity was defined as an increase in absorbance of 0.001/min at 420 nm. Enzyme activities were measured three times and expressed as a relative percentage of the activity of the control.

RESULTS AND DISCUSSION

Mioobiological changes of lettuce and cabbage on chemical treatments

Minimally processed vegetables are susceptible to enzymatic browning when they are cut. During cutting, the products are also easily exposed to possible contamination with bacteria, yeast and molds, and the cut surfaces can provide suitable conditions for the growth of microorganisms (12,13).

The initial populations of total bacteria, yeast and mold, and *E. coli* of fresh lettuce were around 10^5 CFU/g (Fig. 1). Chemical treatment effectively reduced microbial populations. In particular, citric acid (1%) treatment decreased the microbial numbers by more than 1.5 log cycle for all the microorganisms studied. For total viable bacteria, both ozone and citric acid treatments effectively reduced microbial populations in lettuce during

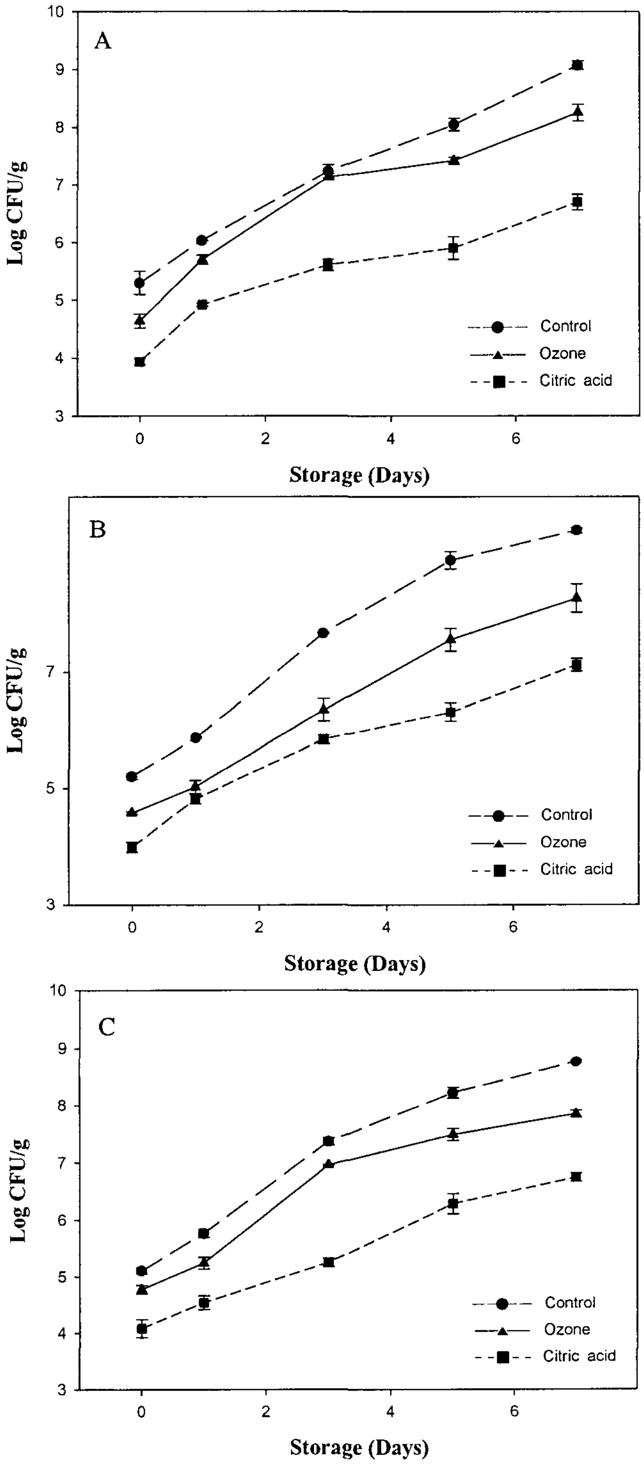

Fig. 1. Changes in populations of (A) total bacteria, (B) yeast and mold, (C) *E. coli*, in fresh lettuce by chemical treatment. Bars represent standard error.

storage (Fig. 1A). After day 3, the number of total bacteria for the control was above 10^7 CFU/g and reached 10^9 CFU/g at day 7, which was not acceptable. However, ozone and citric acid treatments decreased the total bacterial number by 0.7 and 1.4 log cycle, respectively. After day 3, the sample treated by citric acid had 5.6 log CFU/g, while vegetables treated with ozone treatment had a similar number to the control. This strongly suggests that citric acid treatment is a better way to sanitize salad vegetables. Citric acid lowers the pH enough to prevent the microbial growth. This is well established in the literature (13,14) and our results agree with those reports. Pao and Petracek (15) reported that citric acid treatment reduced the surface pH of peeled oranges and extended shelf life.

Yeast and mold exhibited a similar pattern of inhibition as did total bacteria (Fig. 1B). The initial number of yeast and mold was 5.2 log CFU/g. Ozone and citric acid treatments decreased populations of yeast and mold to 4.6 and 4.0 log CFU/g, respectively. During storage, yeast and mold increased in a similar manner as did bacteria. After day 3, the control reached above 10^7 CFU/g, while citric acid treatment retarded the growth and only reached 5.8 log CFU/g. This result indicates that citric acid treatment could extend the shelf life of lettuce upto 3 days. Citric acid is commonly used as a preservative in foods. The beneficial role of citric acid in controlling the growth of spoilage microorganisms is well known (16).

E. coli growth was inhibited by the chemical treatments even more than was yeast and mold (Fig. 1C). After day 3, the control reached 7.4 log CFU/g, while samples treated with ozone and citric acid had 6.9 and 5.2 log CFU/g, respectively. Ozone treatment had little affect on the growth of *E. coli*, but citric acid treatment retarded the growth significantly.

For cabbage, effect of chemical treatment was similar to lettuce (Fig. 2). The numbers of total bacteria, yeast and mold, and *E. coli* were decreased by ozone and citric acid treatments. However, it should be noted that the decrease was not so great as those for lettuce. This can be explained by the difference in the commodity, where the environment for microbial growth is not the same. After day 5, all the microbial populations were below 6.5 log CFU/g for cabbage, while lettuce had above 8 log CFU/g. In particular, after day 4, chemical treatment did not affect the microbial growth for cabbage. This result suggests that ozone or citric acid treatments are not effective for cabbage samples. Other treatments such as chlorine dioxide could be a better way of reducing microbial spoilage for cabbage. Otherwise, combined treatment of non-thermal processing might be a suitable method.

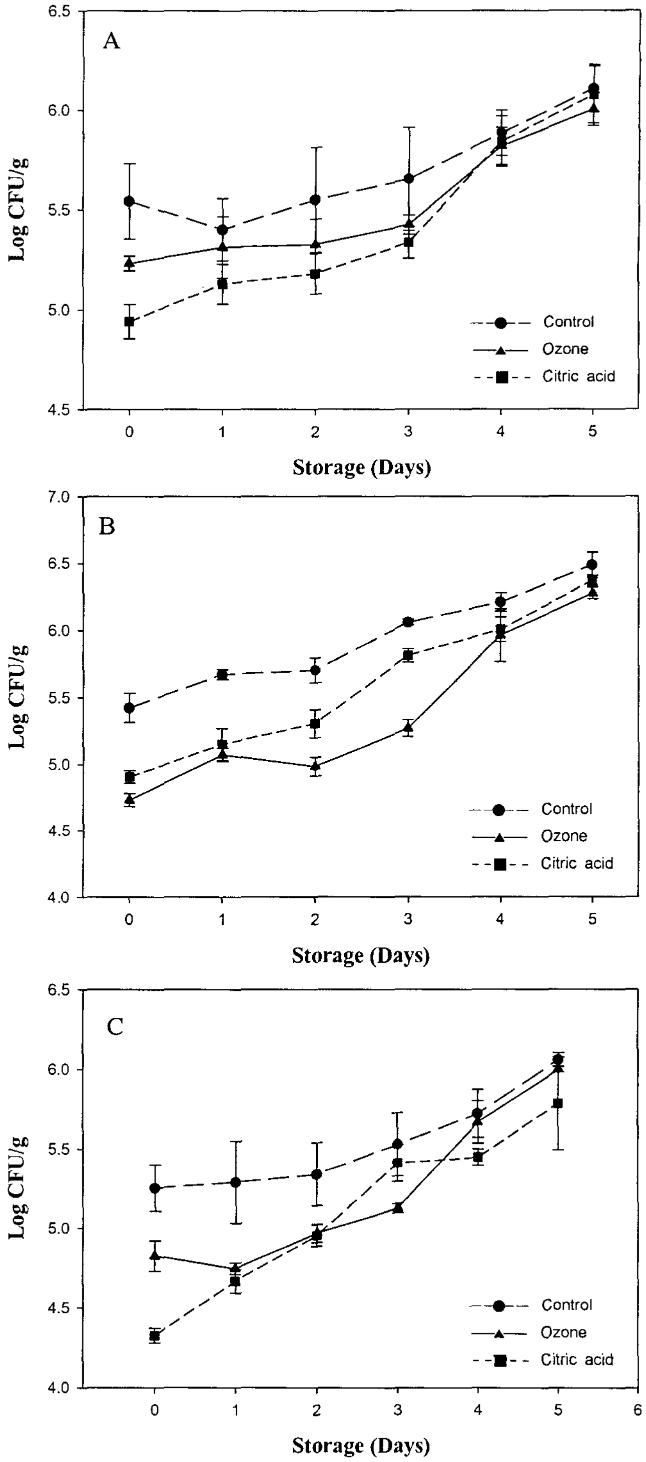

Fig. 2. Changes in populations of (A) total bacteria, (B) yeast and mold, (C) *E. coli*, in fresh cabbage by chemical treatment. Bars represent standard error.

Changes of PPO activity in lettuce and cabbage on chemical treatments

To elucidate the effect of chemical treatment on the enzymatic browning of lettuce and cabbage, PPO activity was examined during storage (Fig. 3). PPO is responsible

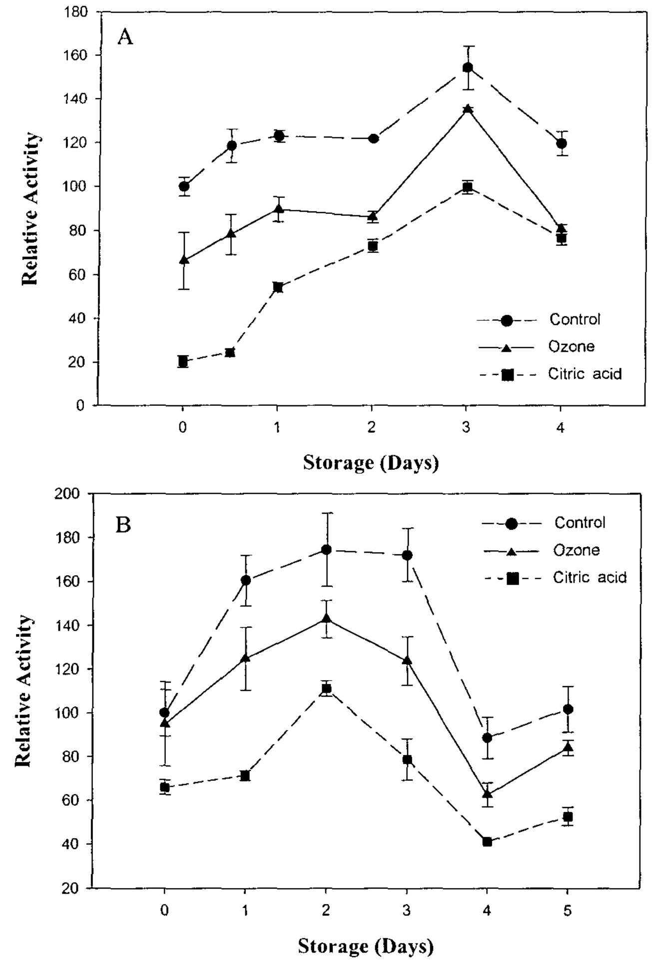

Fig. 3. Change in polyphenoloxidase activity of during storage of (A) fresh lettuce and (B) cabbage. Bars represent standard error.

for browning of damaged and cut vegetables in the presence of oxygen. Since the enzymatic browning causes deterioration of nutritional quality and affects appearance and organoleptic properties, inactivation of PPO is desirable for preservation of foods products. As a non-thermal processing method, chemical treatment could extend the shelf life of food products and provide better quality control by inactivating PPO. After ozone and citric acid treatment, PPO activities of lettuce and cabbage were determined during storage (Fig. 3). PPO activity increased slightly during storage and had a tendency of decrease after 2 or 3 days. Increases in PPO during storage have been reported in the literature (13), with the suggestion that it is due to the ethylene produced when the vegetables were cut. However, the reason for a decrease during storage is not clear. However, it is likely due to the depletion of ethylene, which caused the increase of PPO. For both lettuce and cabbage, PPO was inactivated by ozone and citric acid treatment. In particular, citric acid treatment decreased the PPO activity for lettuce and cabbage by 80% and 34%, respectively (Fig. 3), resulting in a desirable quality in terms of color change. This is mainly due to the pH change, since citric acid shifted the pH from the optimum range of PPO. The use of citric acid treatment could prevent browning as well as microbial growth without affecting taste and odor, as occurs with chlorinated water treatment.

In conclusion, chemical treatment significantly decreased the number of microorganisms and was effective in maintaining the quality of fresh lettuce and cabbage in terms of controlling the microbial growth and preventing browning of fresh-cut vegetables. In particular, citric acid treatment was better than ozone treatment. These results provide insight into the appropriate pretreatment needed to ensure microbial safety and quality control in salad vegetables during marketing.

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REFERENCES

1. Ortega RM, Requejo AM, Sanchez-Muniz FJ, Quintas ME, Sanchez-Quiles B, Andres P, Redondo MR, Lopez Sobaler AM. 1997. Concern about nutrition and its relation to the food habits of a group of young university students from Madrid. *Z Ernahrungswiss* 36: 16-22.
2. Delaquis PJ, Stewart S, Toivonen PMA, Moyls AL. 1999. Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. *Food Res Internat* 32: 7-14.
3. Boyette MD, Ritchie DF, Carballo SJ, Blankenship SM, Sanders DC. 1993. Chlorination and postharvest disease control. *Hort Technol* 3: 395-400.
4. Carlin F, Nguyen-the C, Silva AAD. 1995. Factors affecting the growth of *Listeria monocytogenes* on minimally processed fresh endive. *J Appl Bacteriol* 78: 636-646.
5. Beuchat LR, Nail BV, Adler BB, Clavero MRS. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *J Food Prot* 61: 1305-1311.
6. Singh N, Singh RK, Bhunia AK, Stroshine RL. 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157: H7 on lettuce and baby carrots. *Lebensm-Wiss u-Technol* 35: 720-729.
7. Gomes MRA, Ledward DA. 1996. Effect of high-pressure treatment on the activity of some polyphenoloxidase. *Food Chem* 56: 1-5.
8. Beaulieu M, Bèliveau G, Daprano M, Lacroix M. 1999. Dose rate effect of γ -irradiation on phenolic compounds, polyphenoloxidase, and browning of mushrooms (*Agaricus bisporus*). *Food Chem* 47: 2537-2543.
9. Hendrickx M, Ludikhuyze L, Van Den Broeck I, Weemaes C. 1998. Effect of high pressure on enzyme related to food quality. *Trends Food Sci Technol* 9: 197-203.
10. Singh N, Singh RK, Bhunia AK. 2003. Sequential disinfection of *Escherichia coli* O157:H7 inoculated alfalfa seeds before and during sprouting using aqueous chlorine

dioxide, ozonated water, and thyme essential oil. *Lebensm-Wiss u-Technol* 36: 235-243.
11. Sun NK, Song KB. 2003. Effect of nonthermal treatment on the molecular properties of mushroom polyphenoloxidase. *J Food Sci* 68: 1639-1643.
12. Behrsing J, Winkler S, Franz P, Premier R. 2000. Efficacy of chlorine for inactivation of *Escherichia coli* on vegetables. *Postharvet Biol Technol* 19: 187-192.
13. Ihl M, Aravena L, Scheuermann E, Uquiche E, Bifani V. 2003. Effect of immersion solution on shelf-life of minimally processed lettuce. *Lebensm-Wiss u-Technol* 36: 591-599.
14. Ahvenainen R. 1996. New approaches in improving the shelf life of minimally processed fruit and vegetables. *Trends Food Sci Technol* 7: 179-187.
15. Pao S, Petracek PD. 1997. Shelf life extension of peeled oranges by citric acid treatment. *Food Microbiol* 14: 485-491.
16. Beuchat LR, Golden DA. 1989. Antimicrobials occurring naturally in foods. *Food Technol* 43: 134-142.

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