Efficacy of Sodium Hypochlorite and Acidified Sodium Chlorite in Preventing Browning and Microbial Growth on Fresh-Cut Produce

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Abstract

The use of suitable sanitizers can increase the quality of fresh-cut produce and reduce the risk of foodborne illnesses. The objective of this study was to compare the washing effects of 100 mg/L sodium hypochlorite (SH) and 500 mg/L acidified sodium chlorite (ASC) on the prevention of enzymatic browning and the growth of microbial populations, including aerobic plate counts, *E. coli*, and coliforms, throughout storage at 4°C and 10°C. Fresh-cut zucchini, cucumbers, green bell peppers, and root vegetables such as potatoes, sweet potatoes, carrots, and radishes were used. Compared to SH washing, ASC washing significantly ($p < 0.05$) reduced microbial contamination on the fresh-cut produce and prevented browning of fresh-cut potatoes and sweet potatoes during storage. More effective inhibition of aerobic plate counts and coliforms growth was observed on fresh-cut produce treated with ASC during storage at 10°C. Polyphenol oxidase (PPO) activity of fresh-cut potatoes and sweet potatoes was more effectively inhibited after washing with ASC. The use of 500 mg/L ASC can provide effective antimicrobial and anti-browning treatments of fresh-cut produce, including processed root vegetables.

Key words: fresh-cut vegetables, fresh-cut root vegetable, acidified sodium chlorite, browning, microbial growth

INTRODUCTION

Minimally processed or fresh-cut produce is becoming more popular with consumers who perceive these products as fresh, healthy, and convenient. During the peeling, cutting, and shredding of produce, the surface is exposed to air, which results in rapid deterioration in quality and reduced shelf-life compared to whole fruits and vegetables (1). In addition, fresh-cut produce always has the possibility of contamination with foodborne pathogens during any stage of product handling, from harvest to the point of sale (2,3). Since fresh-cut produce is not subject to further microbial killing steps, effective application of sanitizing agents to remove microorganisms and to inhibit the growth and cross-contamination of pathogens is critical to ensure its quality and safety (4,5). It is well known that washing with sanitizer is one of the most critical processing steps in fresh-cut produce production (6).

Chlorine (sodium hypochlorite) is one of the most widely used sanitizers of fruits, vegetables and fresh-cut produce. It maintains quality by reducing microbial populations and controlling their growth (7). The recommended concentrations of chlorine range from 50 to 200 mg/L for washing fresh vegetables (8). However, the efficacy of chlorine on pathogen reduction is limited under commercial washing conditions (9). The reduced effi-

cacy of chlorine may be caused by organic matter in recycled wash water, insufficient cooling, or exposure to light or air. High concentrations of chlorine may additionally cause product tainting, sodium residue on products and equipment, and damage to produce tissue (10). Additionally, chlorine can react with organic matter to form carcinogenic products (11). Therefore, research has focused on finding alternative sanitizers for improved produce quality and safety (12).

The use of acidified sodium chlorite (ASC) has been approved by the US FDA for the purpose of sanitizing food products, including processed fruits and vegetables, as a component of a spray or dip solution at levels of 500 to 1,200 mg/L (13). ASC is a combination of citric acid and sodium chlorite in aqueous solution with a mostly oxidative mode of action (4). The bactericidal effect of ASC has mainly been demonstrated on pathogenic organisms such as *E. coli* O157:H7 and *Listeria monocytogenes* in previous studies (14-16). A few studies have been performed with ASC for surface washing of fresh-cut carrots (4,14), fresh-cut cilantro (15), and fresh-cut jalapeno peppers (16). Although the quality of fresh-cut produce is influenced by the microbiological load and physiology of the produce (17), there have been limited reports dealing with the washing effects of ASC on reducing microbial loads including aerobic plate counts, *E. coli*, and coliforms, as well as on the an-

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ti-browning of fresh-cut root vegetables, such as potatoes and sweet potatoes.

Therefore, the objectives of this study were to compare the effects of sodium hypochlorite and acidified sodium chlorite on reductions of microbial loads on fresh-cut vegetables and to evaluate their effects against growth of microbial population on fresh-cut vegetables, as well as their anti-browning effects on fresh-cut potatoes and sweet potatoes during storage at 4°C and 10°C.

MATERIALS AND METHODS

Materials and sample preparation

Zucchinis, cucumbers, green bell peppers, and root vegetables such as potatoes, sweet potatoes, carrots, and radishes, which are the most common vegetables used in school foodservice (18), were purchased from a wholesale market in Seoul, Korea. The items were transported to the laboratory and processed within an hour according to the pre-processing steps (Fig. 1). Each produce item was prewashed with regular tap water to remove dirt, followed by cutting and peeling. After the trimming steps, the fresh produce underwent three washing steps. The primary step was a washing with tap water (10°C) for 3 min. The second step was washing with two sanitizer solutions (10°C) for 5 min with either (a) 100 mg/L sodium hypochlorite (SH, pH 6.5～7.0) (Doctorchlo Q, Hansonhigen, Chungnam, Korea) or (b) 500 mg/ L acidified sodium chlorite (ASC, pH 2.5～2.9). The 100 mg/ L sodium hypochlorite was used according to
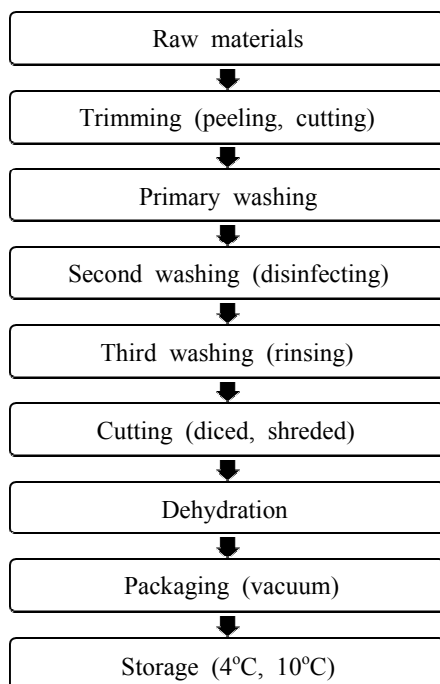
the school foodservice hygiene guidelines (19). The ASC was prepared by mixing sodium chlorite (Kanto Chemical Co. Inc., Tokyo, Japan) and citric acid at a 50:50 (w/w) ratio (Duksan Pharmaceutical Co. Ltd., Gyeoggido, Korea) (20). The final washing step used tap water (10°C) for 5 min to reduce chlorine odor. The ratio of sample to washing water was 1:10 (w/v). After washing, the washed produce was sliced ($2.0 \times 2.0 \times 1.0$ cm) automatically with a vegetable cutter (H.M.V-200, Hwa-Jin junggong™, Gyeoggi, Korea). The sliced produce was drained for 10 min using an aseptic basket. The drained fresh-cut produce was vacuum packaged in PE (poly-ethylene) bags using a vacuum packaging machine (IS-100, Zeropack, Gyeoggi, Korea). All samples were stored at 4°C and 10°C. Microbial and chemical analyses of samples were performed during 5～12 days of storage, according to the characteristics of fresh-cut produce and the results of preliminary studies (21-23).

Color measurements

Changes in the surface color of samples were measured using a colorimeter (Color JC 801, Color Techno System Co. Ltd., Tokyo, Japan). Before each measurement, the apparatus was calibrated using a standard white plate (X = 82.62, Y = 85.15, Z = 97.68) provided by the manufacturer. The results were expressed as the CIELAB ($L^*a^*b^*$) color space. L^* defines lightness and a^* and b^* define red-greenness and blue-yellowness, respectively. Numerical values of L^* were used to obtain ΔL ($L_{initial}$-L_{test}), according to the study of Sapers and Douglas (24). ΔL is the difference between the L at time t and the value at time 0 (start of experiment).

Polyphenol oxidase (PPO) activity assay

PPO activity was measured by a modified method based on the work of Dörnenburg and Knorr (25) and Hwang et al. (26). Ten grams of fresh-cut potato or sweet potato was homogenized with 10 mL of 0.1 M phosphate buffer (pH 7.0). The homogenate sample was centrifuged at $15000 \times g$ for 10 min to obtain enzyme extraction. The reaction mixture contained 0.2 mL of enzyme extract and 2.8 mL of substrate solution (0.2 M catechol in 0.1 M phosphate buffer, pH 7.0). The rate of catechol oxidation was measured at 420 nm for 3 min at 25°C using an Elisa reader (BIO-TEK Instrument Power Wave XS, Winooski, VT, USA). An enzyme activity unit was defined as an increase of 0.001 in absorbance per minute. Three independent trials were carried out.

Microbiological analysis

The aerobic plate counts and coliforms were analyzed by microbiological testing methods (27). Duplicate samples (25 g) were homogenized with 225 mL of 0.1% ster-

Fig. 1. The pre-processing steps of fresh-cut produce.

ile peptone water (Difco, Laboratories, Sparks, MD, USA) in a stomacher (Interscience, Saint Nom, France) for 2 min. The mixtures were serially (1:10) diluted with 0.1% sterile peptone water. One mL of the appropriate dilutions was dropped into sterile petri dishes and $15 \sim 20$ mL of molten ($45 \sim 50^{\circ}$C) plate count agar (PCA, Difco Lab., Sparks, MD, USA) was poured into the plates for aerobic plate counts. The plates were incubated at $36 \pm 1^{\circ}$C for $24 \sim 48$ hr. One mL of each appropriate dilution was aseptically dropped onto *E. coli*/Coliform Count Plate Petrifilm[TM] (3M, St. Paul, MN, USA) for coliforms, spread evenly over the surface of the agar, and incubated at $36 \pm 1^{\circ}$C for 24 hr. Colonies were enumerated by counting the number of blue colonies producing gas for *E. coli* and red colonies producing gas for coliforms. The colonies on the PCA plates and petrifilm were then counted, and bacterial counts from duplicate plates were converted to log numbers.

Statistics analysis

The experiment was repeated three times with 2 replicates per experiment. The obtained results were analyzed by analysis of variance (ANOVA) and the means (n=3) were also separated Duncan's multiple range test at $p < 0.05$ using the Statistical Analysis System, SAS V 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Washing effects of sodium hypochlorite (SH) and acidified sodium chlorite (ASC) on reductions of initial microbial populations of fresh-cut produce

Trimming processes like peeling and cutting are important steps that determine the shelf-life of minimally processed foods (28). Furthermore, the microbiological quality of fresh-cut produce is the most important consideration (29), thus initial microbial populations must be reduced as much as possible during the washing process. Fig. 2 and Fig. 3 show the washing effects of sodium hypochlorite (SH) and acidified sodium chlorite (ASC) on reductions of aerobic plate counts and coliforms in fresh-cut produce, respectively. Fresh-cut produces include potatoes, sweet potatoes, carrots, radishes, cucumbers, zucchinis, and green bell peppers, which were compared to unwashed produce. High initial contamination levels of aerobic plate counts were detected in unwashed carrot (5.57 log CFU/g), zucchini (5.69 log CFU/g), and green bell peppers (5.71 log CFU/g) (Fig. 2). The largest reduction in aerobic plate counts was observed in zucchini (2.29 log) with SH and carrots (2.76 log) with ASC. Overall, superior microbial reductions in both aerobic plate counts and coliforms were observed
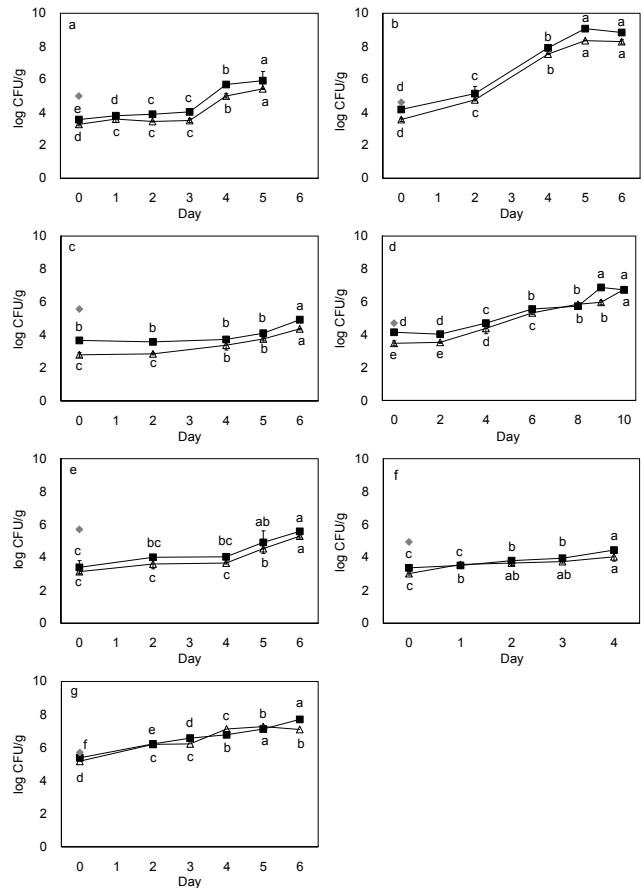

Fig. 2. Effect of sanitizers on the change of aerobic plate count in fresh-cut produce during storage at 10°C. (◆): initial value of unwashed produce, (■): 100 mg/L SH, (△): 500 mg/L ASC. (a): potato, (b): sweet potato, (c): carrot, (d): radish, (e): zucchini, (f): cucumber, (g): green bell pepper. Means of 3 replications $\pm$ SD followed by the different letters are significantly different ($p < 0.05$).

with the ASC washing compared to SH. In the case of sweet potatoes, ASC washing caused a 1.06 log reduction of aerobic plate counts, which was a significant ($p < 0.05$) reduction compared to washing with SH. No significant ($p > 0.05$) difference in aerobic plate count populations was observed between the sweet potatoes washed with SH and unwashed sweet potatoes. Washing fresh-cut carrots with ASC sanitizer caused a 2.76 log reduction of aerobic plate counts, compared to a 1.9 log reduction with SH washing. Our results are similar to those of Ruiz-Cruz et al. (14) who reported that fresh-cut carrots treated with 500 mg/L of ASC had a 2.3 log CFU/g reduction of total aerobic bacteria compared to unwashed carrots. In addition, washing fresh-cut radishes with 100 mg/L of SH and 500 mg/L of ASC reduced aerobic plate counts by 0.55 and 1.21 log CFU/g, respectively, compared to unwashed radishes. These results indicate that washing with ASC is more effective to re-

duce microbial contamination on fresh-cut produce, which is similar to the result of the previous study (15). ASC treatment at 100 mg/L has been reported to exhibit higher effectiveness at reducing *E. coli* O157:H7 and *Salmonella* Montevideo populations than stabilized chlorine dioxide or free chlorine on strawberries (30). Allende et al. (15) also reported that 250 mg/L ASC was significantly ($p<0.01$) more effective at reducing aerobic mesophilic bacteria, yeasts, and molds on fresh-cut cilantro compared to sodium chlorite, sodium hypochlorite, and citric acid. In the present study, there were no significant differences between washing treatments of SH and ASC for fresh-cut potatoes, zucchini, and cucumbers, although the initial aerobic plate counts of washed samples were significantly ($p<0.05$) lower than those of unwashed samples (Fig. 2).

The washing effects of SH and ASC on coliforms in fresh-cut produce are shown in Fig. 3. Significant washing effects were observed for SH and ASC against coliforms in all fresh-cut produces in this study. The initial coliform counts of unwashed radishes, zucchini, and green bell peppers were 2.89 log CFU/g, 2.77 log CFU/g, and 3.37 log CFU/g, respectively. Washing fresh-cut radishes, zucchini, and green bell peppers with SH reduced initial coliform groups by 0.76 log CFU/g, 0.79 log CFU/g, and 0.47 log CFU/g, respectively. More significant washing effects by ASC compared to SH were especially observed in radishes (1.69 log CFU/g), zucchini (1.57 log CFU/g), and green bell peppers (0.53 log CFU/g). Among the unwashed samples, the lowest level of coliforms was observed in cucumbers (1.64 log CFU/g), followed by carrots (1.74 log CFU/g). After washing, no coliforms were detected in washed potatoes, carrots, and cucumbers, regardless of the kind of sanitizer. The greatest microbial reduction by washing was observed in fresh-cut potatoes. The microbial limits for consumer consumption of uncooked foods proposed by Solberg et al. (30) are 6 log CFU/g for aerobic plate counts and 3 log CFU/g for coliforms. According to the present results, all of the fresh-cut produce samples satisfied microbial standards for aerobic plate counts and coliforms (31).

Washing effects of SH and ASC on the prevention of the growth of microbial populations in fresh-cut produce during storage

Research has focused on finding alternative sanitizers to chlorine for the quality and safety of produce due to the environmental and health risks associated with the formation of carcinogenic halogenated disinfection by-products (32). As shown in Fig. 2, a greater washing effect was observed with ASC. Thus, we stored washed,

fresh-cut produce to evaluate changes in aerobic plate counts and coliforms during storage at 4°C and 10°C. *E. coli* was not detected in fresh-cut produce washed with the sanitizers during storage at 4°C and 10°C. At 4°C, there were no significant differences between SH and ASC washing treatments against the change of aerobic plate count or coliform populations during storage at 4°C (data not shown).

At 10°C, the aerobic plate counts of washed fresh-cut potatoes, regardless of the kind of sanitizer, were controlled up to 3 days, followed by a 1.5 log average increase during 5 days of storage (Fig. 2a), while populations of the aerobic plate counts of sweet potatoes washed with SH and ASC increased and reached to 8.8 and 8.2 log, respectively, at day 5 (Fig. 2b). Washing fresh-cut carrots with ASC or SH sanitizer prevented the growth of aerobic plate counts up to 4 days of storage (Fig. 2c). In addition, washing SH or ASC inhibited the growth of aerobic plates up to 2 days of storage of radishes (Fig. 2d) and 4 days of storage of zucchini (Fig. 2e). In cucumber, the least growth of aerobic plate counts was observed (Fig. 2f). Overall, the fresh-cut produce washed with ASC had lower aerobic plate counts than those washed with SH throughout the storage period, although growth was not completely inhibited at 10°C.

The greatest growth for aerobic plate counts was observed in the sweet potatoes (Fig. 2b). These results indicate that washing with ASC or SH sanitizer did not inhibit the growth of aerobic plate counts in sweet potato during storage.

The changes in coliforms for washed, fresh-cut produce stored at 10°C are shown in Fig. 3. Coliforms were not detected in washed potatoes (Fig. 3a), carrots (Fig. 3c), and cucumbers (Fig. 3f), regardless of the kind of sanitizer. However, coliforms in the washed fresh-cut potatoes significantly ($p<0.05$) increased and reached to 4.5 log CFU/g at day 5 during storage at 10°C, regardless of the kind of sanitizer. Moreover, on day 2, coliforms were still not detected in the fresh-cut potatoes washed with ASC, while a 1.5 log increase in coliforms was observed in fresh-cut potatoes washed with SH, indicating that ASC washing delayed the growth of coliforms in fresh-cut potatoes during storage at 10°C. In addition, the populations of coliforms on fresh-cut carrots treated with SH increased by 3.1 log CFU/g during 6 days of storage (Fig. 3c). Regardless of the kind of sanitizer, more rapid coliform growth was observed in fresh-cut sweet potatoes (Fig. 3b) and radishes (Fig. 3d) during storage. More significant washing effects by ASC were observed in radishes (Fig. 3d), zucchini (Fig. 3e), and green bell peppers (Fig. 3g) compared to SH during storage. Overall, similar to the growth of aerobic plate
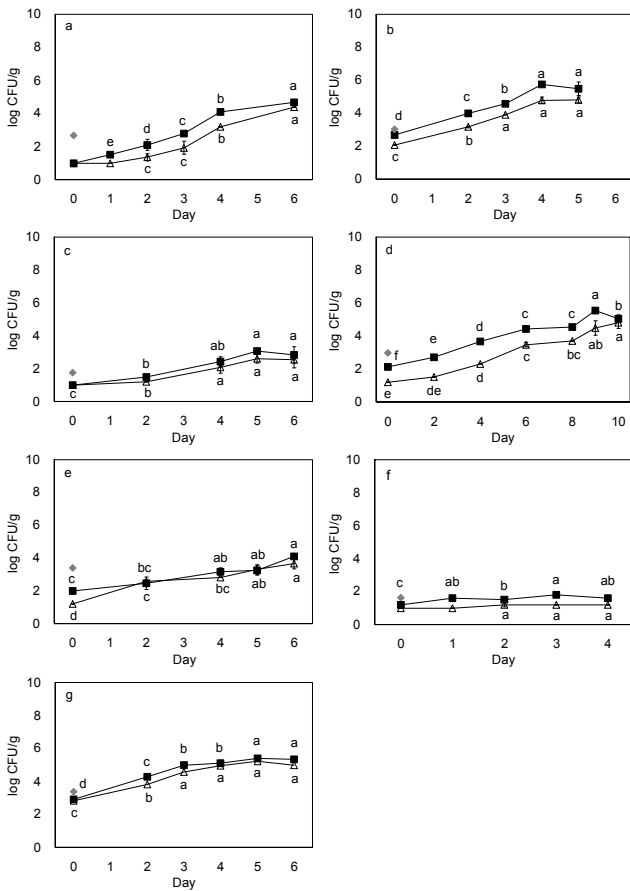
Fig. 3. Effect of sanitizers on the change of coliforms in fresh-cut produce during storage at 10°C. (◆): initial value of un-washed produce, (■): 100 mg/L SH, (△): 500 mg/L ASC. (a): potato, (b): sweet potato, (c): carrot, (d): radish, (e): zuc-chini, (f): cucumber, (g): green bell pepper. Means of 3 repli-cations±SD followed by the different letters are significantly different (p<0.05).

counts, lower populations of coliforms were maintained in produce washed with ASC than in produce washed with SH during storage at 10°C. The growth of coliforms increased more rapidly during the storage period com-pared to the aerobic plate counts.

Washing effects of SH and ASC on the activity of PPO in potatoes and sweet potatoes

Color is an important sensory property that plays a role in determining product quality (33). One of the ma-jor post-harvest problems resulting in reduced commer-cial value of fruits and vegetables is enzymatic browning due to the oxidation of phenolic compounds by poly-phenol oxidase in the presence of oxygen (34,35), thus efficient ways to inhibit this chemical reaction have been studied (2). Potatoes and sweet potatoes are extremely sensitive to enzymatic browning because their surfaces contain polyphenols as substrates for polyphenol oxidase (36). In this study, we found that washing with ASC

was effective for reducing aerobic plate counts and coli-forms. Therefore, we also evaluated the effects of wash-ing with SH and ASC on inhibition of PPO activity, which inhibits the development of browning.

Furthermore, we measured ΔL and a^* values for wash-ed potatoes and sweet potatoes to determine the degree of browning over the storage period at 10°C, which is presented in Fig. 4. ΔL is the difference between L at time t and the value at time 0 (start of experiment). Overall, the ΔL values of the potatoes washed with SH and ASC showed significant increases during storage (p< 0.05). However, ΔL values were significantly (p<0.05) lower in potatoes washed with ASC (1.09) than in those washed with SH (3.22) on day 2 (Fig. 4a). The ΔL values of sweet potatoes washed with ASC and SH were similar up to 4 days, but the ΔL value of sweet potatoes washed with SH significantly were increased (p<0.05) up to 3.3 on day 5 (Fig. 4b).

We also measured a^* values for washed potatoes (Fig. 4c) and sweet potatoes (Fig. 4d) for the degree of brown-ing over the storage period at 10°C. An increase in a^* (greenness to redness) is related to the appearance of browning (37). The a^* values of fresh-cut potatoes wash-ed with SH and ASC were significantly (p<0.05) in-creased during storage. Washing potatoes with ASC and SH increased a^* values to 2.81 and 3.93, respectively, indicating superior effects of the ASC sanitizer to inhibit browning of potatoes during storage. In the case of the sweet potatoes, the a^* value of the sweet potatoes washed with ASC was lower (4.25) than that of those washed with SH (6.54) on day 4. These results show that wash-ing with ASC was more effective at preventing browning of sweet potatoes than SH washing, as indicated by the
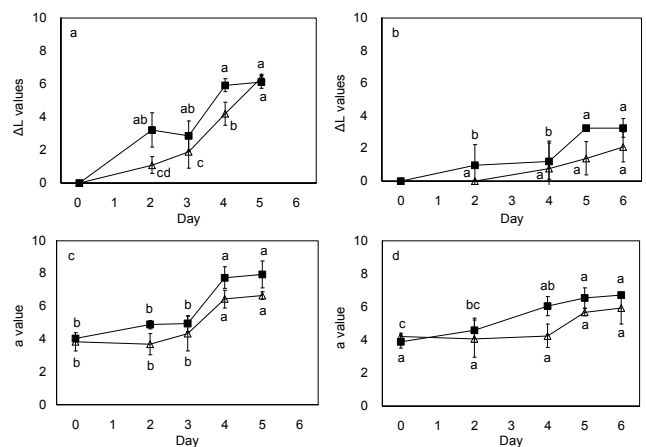

Fig. 4. Effect of sanitizer on ΔL value and a value of fresh-cut potato (a, c) and sweet potato (b, d) during storage at 10°C. (■): 100 mg/L SH, (△): 500 mg/L ASC. Means of 3 repli-cations±SD followed by the different letters are significantly different (p<0.05).

lower a* value and ΔL value.

Fig. 5 shows the changes in PPO activity for fresh-cut potatoes (Fig. 5a) and sweet potatoes (Fig. 5b) washed with SH and ASC during storage at 10℃. Overall, PPO activity increased during storage. The PPO activities of the potatoes and sweet potatoes washed with ASC were lower than those washed with SH during the storage period at 10℃. The PPO activity of potatoes washed with ASC was maintained up to 3 days in this study (Fig. 5a). Additionally, the fresh-cut sweet potatoes washed with ASC had lower PPO activity (65.17 unit less) compared to those washed with SH on day 5. These results indicate that washing with ASC reduced PPO activity and thus inhibited browning during storage. The degree of browning (ΔL value) and PPO activity were slightly increased during storage at 4℃, and thus the anti-browning effect of ASC was not noticeable (data not shown). According to the present study, ASC was more effective than SH to reduce microbial loads and the development of browning in root vegetables such as potatoes and sweet potatoes, indicating that ASC washing maintained the quality of fresh-cut produce more effectively than SH. However, Kim (38) reported that washing with ASC at 1000 mg/L reduced not only microbial growth but also hardness of texture, indicating that 1000 mg/L of ASC was efficient at reducing bacterial counts, but it could deteriorate the quality of texture. It was also reported that ASC at concentrations of 100 to 500 mg/L maintained the quality of shredded carrots stored for 17 days at 5℃ (5), while treatment of ASC at 1000 mg/L was unable to maintain the quality of shredded carrots for 4~6 days at 5℃ (4). In our preliminary test, we compared the effects of washing fresh-cut potatoes and sweet potatoes with 500 mg/L ASC (approved by the FDA at a low concentration range) to 100 mg/L SH and no significant (p>0.05) differences in texture were observed between treatments (data not shown). Thus, 500 mg/L ASC can be used as an alternative sanitizer for 100 mg/L SH in the fresh-cut produce industry based on its superior anti-browning and antimicrobial effects.
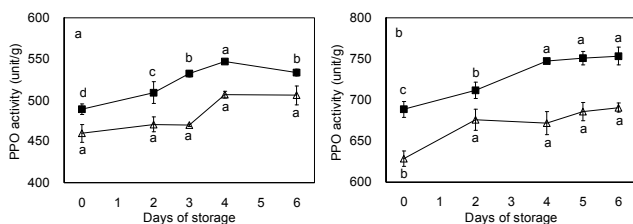

Fig. 5. Effect of sanitizer on polyphenol oxidase (PPO) activity of fresh-cut potato (a) and sweet potato (b) during storage at 10℃. (■): 100 mg/L SH, (△): 500 mg/L ASC. Means of 3 replications±SD followed by the different letters are significantly different (p<0.05).

CONCLUSION

Washing fresh-cut vegetables (potatoes, sweet potatoes, carrots, radishes, cucumbers, zucchini, and green bell peppers) with ASC resulted in more effective reductions of aerobic plate counts in the range of 0.31 to 0.86 log CFU/g and reduced coliforms by 0.31 to 1.5 log CFU/g compared to SH. Growth of microbial populations and enzymatic browning in fresh-cut produce stored at 4℃ and 10℃ were more effectively inhibited by ASC compared to SH. In addition, higher reductions in microbial load were observed in fresh-cut root vegetables including potatoes, sweet potatoes, radishes, and carrots compared to fresh-cut fruit vegetables such as cucumbers, zucchinis, and green bell peppers, regardless of the kind of sanitizer. In addition, in the case of fresh-cut potatoes and sweet potatoes, ASC washing more effectively inhibited browning and PPO activity as indicated by higher reductions in a* values (greenness to redness) and ΔL values (degree of browning) during storage at 10℃. In conclusion, ASC at a concentration of 500 mg/L is an effective alternative to SH at 100 mg/L for sanitization of fresh-cut produce to reduce microbial populations and inhibit browning of fresh cut root vegetables. These results provide new guidelines for usage of sanitizers to improve the quality and safety of fresh-cut produce.

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