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Effects of Different Sanitizers on the Quality of 'Tah Tasai' Chinese Cabbage (*Brassica campestris* var. narinosa) Baby Leaves

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살균소독제가 다채 어린잎채소(*Brassica campestris* var. narinosa)의 수확 후 품질에 미치는 영향

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Abstract

The demand of packaged baby leaves has been increased for its convenient use as fresh-cut produce. This investigation was aimed to explore the effects of different sanitizers on the quality parameters of 'Tah Tasai' Chinese cabbage (*Brassica campestris* var. narinosa) baby leaves. Thirteen days old baby leaves were harvested and washed in tap water (TW), 100 ppm chlorine solution (Cl), 2 ppm ozonated water (O_3), 15 ppm chlorine dioxide solution (ClO_2) and washing with 0.2% citric acid solution followed by 50% ethanol spray (CA+Et). The samples were then packaged in 50 μm polyethylene bags and stored at 5℃ for 10 days. Off-odor of packaged baby leaves was not detected during storage. There was no significant difference in color parameters among the treatments. Samples treated with O_3 showed substantially higher electrolyte leakage throughout the storage. This treatment also rendered a higher accumulation of CO_2 in the packages. Samples treated with Cl and CA+Et maintained good overall visual quality with higher scores compared to that of O_3 and ClO_2. Although Cl treatment showed lower number of total aerobic count at the beginning of storage, citric acid in combination with ethanol treatment was more effective until the end of storage. The combined treatment also showed comparatively lower coliform plate count. This result indicates that citric acid wash followed by ethanol spray could be an alternative to chlorine for environment friendly sanitization of baby leaves.

Key words : baby leaves, microbial, packaging, quality, sanitation, washing

Introduction

'Tah Tasai' Chinese cabbage (*Brassica campestris* var. narinosa) is a popular baby leaves in Korea which is used for fresh-cut salad mix. This product is marketed fresh in polyethylene bags and has become increasingly popular in the past few years (1). However, the rapid quality deterioration and reduced shelf life of these type of fresh-cut produce compared to whole vegetables is a major challenge to the industry (2,3). Although different packaging procedures have shown to be increased postharvest qualities, fresh produce can modify the atmosphere in their packages as a result of respiratory O_2 consumption and CO_2 evolution (4). This physiological process greatly depends on temperature where the products are stored. Although the optimum storage temperature of baby leaves depends on the type and cultivar, it is often recommended to store the product slightly higher or near to 0℃ (5). However, the actual temperature in storage, transportation or market display is higher than the recommended temperature which plays a major role in the preservation of produce quality (6,7). Produce quality is a complex term that includes many aspects of produce like appearance, color, feeling and taste as well as its contamination with microorganisms. The consumers can only assess the sensory appearance. Hence, there is an increasing

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demand for the development of improved methods that guarantee a high produce quality until the end of shelf life.

Washing is an important step that has been widely adopted by the industry to remove microorganisms and other foreign materials. Therefore, washing process and the proper application of different sanitizing agents should be highly optimized to guarantee a minimal number of spoilage microorganisms. Chlorine has been widely used as a sanitizer during produce washing in many countries. However, the effectiveness of chlorine is highly dependent on the concentration and pH of the solution. Chlorination also causes the formation of hazardous byproducts such as chloroform, haloacetic acids or other trihalomethane that have known or suspected carcinogenic or mutagenic effects (8-11). Although ozonated water alone or in combination with other sanitizers showed a better effect on quality maintenance in many fresh-cut produce, the application of ozone is little complicated as its solubility is affected by the temperature, pH and organic matter present in water (12). Moreover, the people who might have contact with ozone may face illness in their respiratory tract or in other parts of the body (13). Although, many other sanitizing agents are available for sanitizing fresh produce, their efficacies vary and none of them are able to ensure complete elimination of pathogen. Organic acids are most important sanitizers that have been applied largely for preserving physicochemical qualities (14) and for preventing microbial growth at levels that did not adversely affect taste and flavor (15), but leaving no effect on environment. Citric acid washing can extend the shelf life of fresh-cut produce by reducing the loss of eating quality and disease development (16). For instance, in green celery, Gomez and Artes (17) found that 0.5 M ascorbic and 0.1 M citric acid washing was as effective as 100 ppm chlorine washing for reducing microbial counts and improving consumer acceptability. Leaves of some selected vegetables decontaminated with 5% citric acid or ethanol showed a considerable decrease in microbial count compared to water washing (18). Ibrahim et al. (18) also concluded that the combination of these agents could be a potential sanitizer for fresh vegetables. However, our preliminary study reflected that the application of these acids as sanitizers at higher concentration reduce the overall quality and produce off flavor in leafy vegetables after few days of storage (unpublished). Hence, the objective of this study was to evaluate the efficacy of the combined effects of citric acid and ethanol as an alternative sanitizer to chlorine or other sanitizers in fresh baby leaves during storage at 5℃. Some storage qualities like changes in color, electrolyte leakage, off-odor and overall quality were also evaluated.

Materials and Methods

Preparation of washing solution

As a standard industrial disinfection treatment, chlorine solution (100 ppm) was prepared with food grade NaClO and the pH was adjusted to 7.0 with 1 N HCl. The available free chlorine was determined with a portable chlorine colorimeter (Model-1200, LaMotte, USA). The solution was used within 30 min of preparation. Ozonated water was prepared using an ozone generator (OS-800, AST Advanced Scientific Technology, Republic of Korea) through an ozone water sterilizing system by continuous supplying of water in a stainless steel water tank. The machine was equipped with a vortexer in order to dissolve gaseous ozone into water. The concentration of dissolved ozone was adjusted and checked by an ozone measuring meter (DO_3 Meter, DDK-TOA Corporation, Japan). Ozone solution was also used immediately when it reached at a concentration of 2 ppm. Chlorine dioxide water was prepared using an automatic ClO_2 generator (ECO-002, Ecomaster, Ecosia Co Ltd, Republic of Korea) adjusting the concentration of 15 ppm and used immediately after preparation. These concentrations of ozone and ClO_2 were chosen following the effectiveness in other study (19) and as an ideal industrial practice. A citric acid solution of 0.2% (w/v) was prepared using reagent grade citric acid (Sigma Chemical Co, USA). Reagent grade ethyl alcohol (Sigma Chemical Co, Republic of Korea) was used to prepare 50% (v/v) ethanol solution. The concentrations of citric acid for washing and ethanol for spraying were selected based on our preliminary studies with leafy vegetables including baby leaves.

Sample preparation

'Tah Tasai' Chinese cabbage (*Brassica campestris* var. narinosa) baby leaves were harvested from a commercial farm after 13 days of sowing and transported (within one hour) to our laboratory under cool condition. The required amount of samples for each treatment was separately weighed in a netted bag. The samples were prewashed in tap water for 1 min to remove the dirty materials. Then the samples were washed separately in different washing solutions such as tap water (TW, as control), 100 ppm chlorine solution (Cl), 2 ppm ozonated water (O_3), 15 ppm chlorine dioxide solution (ClO_2) and 0.2% citric acid solution with gentle agitation for 1 min. Sample washed in 0.2% citric acid solution was followed by 50% ethanol spray for 3-4 times in a netted tray (CA+Et). The excess surface water remaining in the samples was removed by centrifugation for 1 min using a dryer (WS 6501 T, Hanil, Republic of Korea). Each 80 g samples were packaged aseptically in 50 μm polyethylene bag (20 x 20 cm) and thermally sealed. Three replicates of each bag per treatment and storage duration (washing day and 3, 7 and 10 days of storage) were prepared and stored in a dark cold room at 5℃.

Gas composition within packages

Gas compositions (O_2 and CO_2) in the package headspace of baby leaves were monitored using a gas analyzer (PBI Dansensor, CheckMate 9900, DK-4100 Ringsted, Denmark) at each sampling day until the end of 10-day storage. Gas samples were taken from the bag using a hypodermic needle, inserted through an adhesive septum previously fixed to the bag. The volume taken from the package headspace for gas analysis was about 5 mL. To avoid modifications in the headspace gas composition due to gas sampling, each package was used only for single determination. Triplicate samples were measured for each evaluation day.

Color, sensory quality and electrolyte leakage

Color reading of baby leaves was performed on the surface of the packages after sealing of the respective samples, using a chromameter (Minolta CR-400, Osaka, Japan). A total of 10 sampling points were selected from both sides of each bag. Color changes were quantified in the L^*, a^*, b^* color space and a total of 30 readings from each treatment were obtained and averaged the value. The meter was calibrated using the manufacturer's standard white plate (Y 93.5, x 0.3155, y 0.3320). L^* refers to the lightness and ranges from black = 0 to white = 100. A negative value of a^* indicates green, while a positive number indicates red color. Positive and negative b^* indicate yellow and blue color, respectively (20). The color values of a^* and b^* were further converted into hue angle (hue = $180° + \tan^{-1}(b^*/a^*)$).

The sensory analyses of baby leaves were carried out by a six member expert panel. The members of the panel were trained to recognize and score off-odor and overall quality of baby leaves prior to the test. Off-odor was evaluated immediately after opening the package and scored on a 0 to 4 scale, where 0 = none, 1 = slight, 2 = moderate, 3 = strong, and 4 = severe (21); a score of 3 was considered not acceptable. Overall visual quality was evaluated with another nine-point hedonic scale where: 9 = like extremely; 7 = like moderately; 5 = neither like nor dislike; 3 = dislike moderately; and 1 = dislike extremely (22); a score of 6 was considered the limit of marketability (21). These sensory qualities were evaluated on the processing day and after 3, 7, and 10 days of storage at 5°C. For measuring electrolyte leakage, electrical conductivity of tissues was measured using 20 g baby leaves submerged in 400 mL deionized double distilled water at 20±1℃ for 30 min. The conductivity was measured using a conductivity meter (Orion 4 star portable pH/conductivity meter; Thermo Electron Corporation, USA) by inserting the probe into the sample solution.

Microbial enumeration

Total aerobic plate counts was carried out to determine the microbial load of the samples. At each sampling time, baby leaves bags were aseptically opened using a sterilized scissors dipped in 95% ethanol and then ignited in the flame of a Bunsen burner. Twenty gram sample was weighed out from each bag and placed in a stomacher bag with 180 mL double distilled water. The mixture was homogenized in a stomacher (Seward Stomacher 400C, Brinkmann, USA) for 1 min. Appropriate serial dilutions were made from the homogenate and inoculated onto Petrifilm™ Aerobic Count Plates (3M Microbiology Products, USA) . The plates were incubated at 35℃ for 48 h. After incubation, plates with 25 to 250 colony forming units (CFU) were enumerated with the assistance of a microbial colony counter supplied by the same company. Similarly, for coliform count, samples were plated onto coliform count plates (same company). The inoculated plates were incubated for 24 h at 35℃. After incubation, bright red colonies associated with entrapped gas were enumerated as coliform count. All microbial counts were reported as log colony forming units per gram (log CFU g^{-1}).

Results and Discussion

The gas composition (O_2 and CO_2) from the headspace of packaged baby leaves were monitored on the evaluation day during storage at 5℃. Oxygen level decreased gradually throughout the storage, whereas CO_2 level increased sharply on the third day of storage and remained stable or slightly declined near the end of storage (Fig. 1). However, samples treated with tap water and chlorinated water showed a lower decline in O_2 and smaller increase in CO_2 level compared to other treatments. At the end of the storage, O_2 and CO_2 level reached at about 7.5 kPa and 3.1 kPa, respectively for TW and Cl treated samples, whereas these levels were about 5-6 kPa and 3.6 kPa for other treatments. The decline in O_2 and increases in CO_2 is a major physiological attributes as observed in many vegetables and fresh-cut produce due to the evolution of CO_2 through respiratory activities (19,23-25). Several studies also showed that different packaging materials significantly affect the gas composition of many fresh vegetables (22,25). Since we used the same packaging film, the differences in gas composition attributed to the variation in sanitizers used for washing of baby leaves. The maximum level of CO_2 was recorded in O_3 treated sample followed by ClO_2 and CA+Et treatments. As a consequence, significantly higher electrolyte leakage was recorded in O_3 treated sample throughout the storage with an increasing tread until day 7 (Fig. 2). Except for Cl treatment, electrolyte leakage of other treatments slightly decreased on the third day of storage and increased thereafter until the end of storage. Being an indirect measurement of quality, electrolyte leakage reflects the damage and integrity of tissue and membrane, therefore has been used in many studies (19,24-26). Marangoni et al. (27) reported that lower values of electrolyte leakage are obtained when the sample's membranes are intact. The substantially higher reading (about 5-fold) of electrolyte leakage of O_3 treated sample might be due to the highly oxidizing nature of O_3 that caused severe oxidative stress to the immature and delicate natured baby leaves. Das and Kim (19) also found that O_3 caused a significant increase in electrolyte leakage immediately after washing and the beginning of storage of fresh cut broccoli. Other treatments exhibited no difference in electrolyte leakage value suggesting a little deterioration of baby leaves subjected to these sanitizers. Unlike other fresh-cut produce (19,24), we did not find remarkably higher values of electrolyte leakage immediately after washing. The reason may describe as our samples were young individual plant with a single cut for harvesting from the field rather than pieces of leaves or several cuts for processing. However, the small increase in electrolyte leakage at the end of storage was perhaps due to cell damage caused by microbial growth.

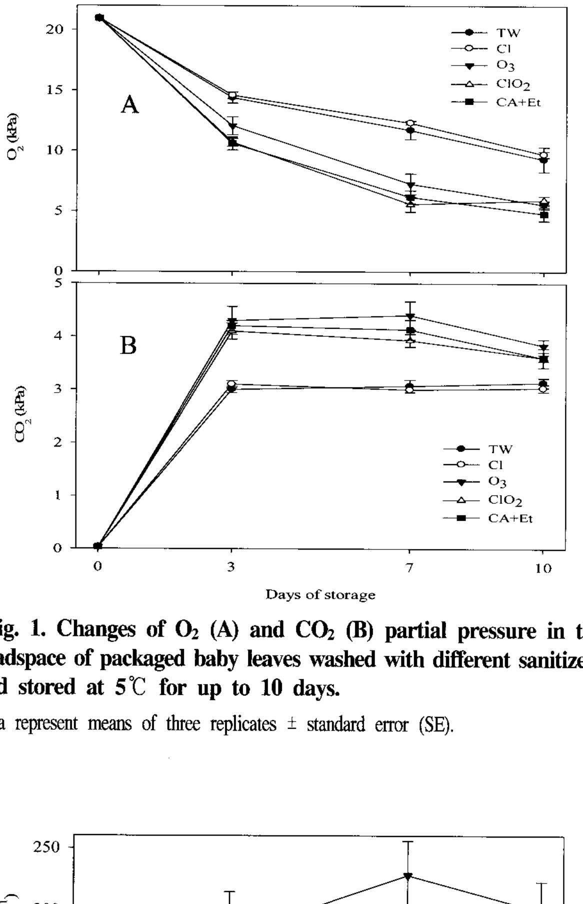

Fig. 1. Changes of O_2 (A) and CO_2 (B) partial pressure in the headspace of packaged baby leaves washed with different sanitizers and stored at 5℃ for up to 10 days.

Data represent means of three replicates ± standard error (SE).

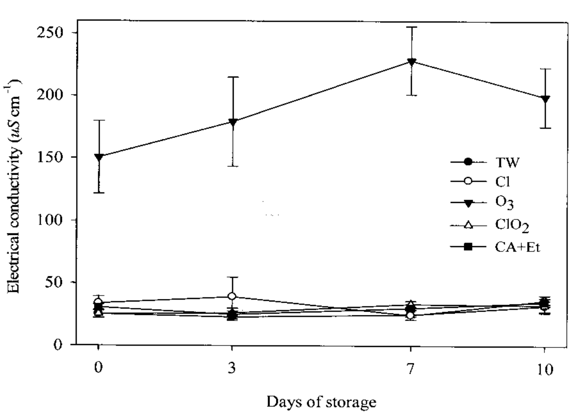

Fig. 2. Electrical conductivity of baby leaves treated with different washing solutions during storage at 5℃ for up to 10 days.

Values are the mean of three replicates ± standard error (SE).

No off-odor was detected in packaged baby leaves until the end of storage in all treatments. Since off-odor is often associated with the onset of anaerobic respiration under low O_2 and high CO_2 condition, our result may indicate that no anaerobic respiration was occurred owing to the high OTR (oxygen transmission rate) film used in this study. However, overall visual quality declined slightly at the end of storage, especially for O_3 and ClO_2 treated samples (Fig. 3). Samples treated with TW, Cl and CA+Et maintained scores higher than the marketable limit at the end of storage. Although no noticeable difference was found among TW, Cl and CA+Et treatments throughout the storage, CA+Et treated sample scored 7.1 at day 10 on the 9-point hedonic scale, which was the highest score among the treatments. The decline of overall visual quality in O_3 treated sample might be a

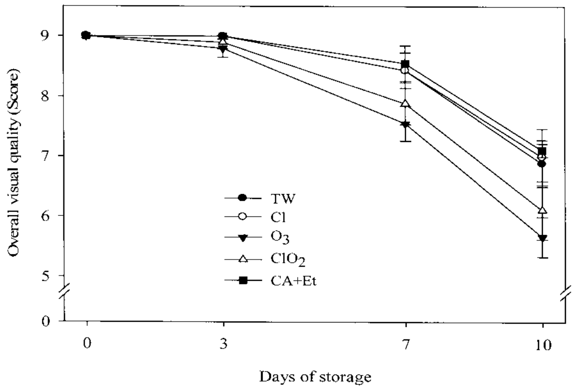

Fig. 3. Changes in overall visual quality of baby leaves washed with different sanitizers and stored at 5℃ for up to 10 days.

Values are the mean of three replicates ± standard error (SE).

consequence of tissue damage as reflected from the electrolyte leakage data (Fig. 2). The changes in color of baby leaves expressed by L^*, a^*, b^* and hue angle are presented in Table 1. Very little changes occurred in color parameters L^*, a^* and b^* throughout the storage. Hue angle value slightly decreased at the end of storage. L^* value which indicates the lightness, on the other hand, slightly increased at the end of storage. However, the color parameters of baby leaves treated with different sanitizers and over the storage time were statistically insignificant. This indicates that color of baby leaves was not affected by sanitizing agents either in washing or during the entire storage period. It appears that the sanitizers used and the storage temperature selected was appropriate for the baby leaves used in this study. The low storage temperature may have slow down the degradation of chlorophyll which is the major pigment of baby leaves. Tomas-Callejas et al. (28) found no significant difference in total chlorophyll content over an 11-day storage period of mizuna baby leaves. Our color readings were closely similar with the results of Wang et al. (26) who also found insignificant differences in L^*, a^*, b^* and hue angle values in fresh-cut cilantro.

The effects of different sanitizers were clearly distinguished in the total aerobic plate count after washing and during storage (Fig. 4). The highest number of aerobic plate count was observed in TW treated sample on washing day (6.7 log CFU g^{-1}) and successive storage until day 7. Although Cl treatment showed lower number of total aerobic count immediately after washing (6.1 log CFU g^{-1}) and the beginning of storage, citric acid in combination with ethanol spray treatment showed the lowest number until the end of storage period. All sanitation treatments were effective compared to control sample until day 7 except O_3 treatment on 7-day. However, no significant difference was found in microbial number among the treatments at the end of storage. Similar to our result, Wang et al. (26) also found no significant difference in total aerobic plate count at the end of 14 days storage of cilantro leaves. The possible reason might be due to the baby leave samples became softer with the progress in storage which caused damage in texture for all samples. Similar trends of total aerobic count were also found in case of coliform count (Fig. 5). Equal number of coliform (3.1 log CFU g^{-1}) was found in Cl and CA+Et treatments on the washing day and on day 3. Although CA+Et treatment showed the lowest number of coliform, it was statistically insignificant with Cl treatment throughout the storage; O_3 treatment on 0 and 3-day as well as ClO_2 treatment on 7 and 10-day of storage. ClO_2 treatment showed almost constant number of coliform until day 7 and increased at the end of storage. It is noteworthy that the purpose of using sanitizing agent is to control disease causing organisms present in fresh produce. However, the safety assessment and legal requirements concerning these agents have to be taken into consideration (29). Although O_3 has been used as a potential

Table 1. Changes in color parameters of baby leaves after storage at 5℃ for 10 days

Color parameter	Days of storage	Treatment				
		TW	Cl	O_3	ClO_2	CA+Et
L^*	0	48.17±0.62	47.80±0.59	47.45±0.54	48.26±0.58	47.32±0.70
	10	49.65±0.60	49.01±0.61	48.70±0.63	49.59±0.75	49.27±0.58
a^*	0	-8.24±0.17	-8.10±0.23	-7.71±0.20	-7.93±0.13	7.60±0.20
	10	-7.67±0.21	-8.01±0.19	-7.68±0.17	7.63±0.16	7.63±0.14
b^*	0	12.65±0.28	12.39±0.26	11.78±0.23	12.40±0.22	11.82±0.27
	10	12.31±0.22	12.75±0.18	12.11±0.24	12.13±0.24	12.15±0.17
Hue angle	0	123.13±0.39	123.17±0.32	123.13±0.61	122.61±0.42	122.71±0.54
	10	121.84±0.49	122.10±0.45	122.38±0.50	122.19±0.47	122.13±0.61

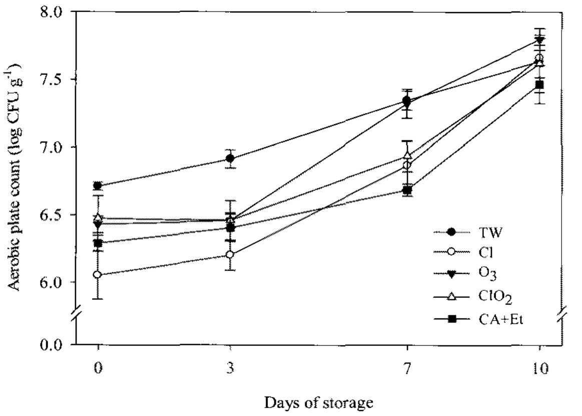

Fig. 4. Changes in aerobic plate count of baby leaves treated with different washing solutions during storage at 5℃.

Values are the mean of three replicates ± standard error (SE).

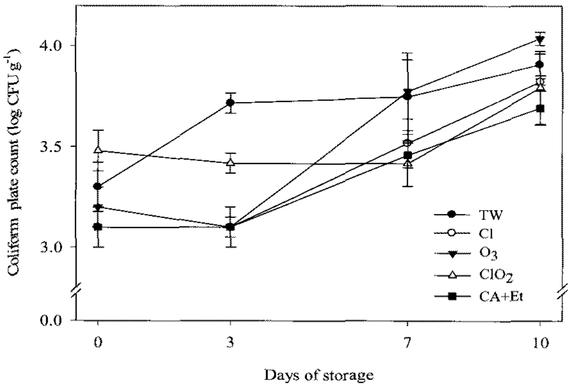

Fig. 5. Changes in coliform plate count of baby leaves treated with different washing solutions during storage at 5℃.

Values are the mean of three replicates ± standard error (SE).

sanitizer in many fresh produce, it may not be suitable for controlling microbial number in baby leaves since we used shorter contact time for washing as an industrial practice. Das and Kim (19) reported that O_3 treatment was effective in controlling microbial numbers when a longer contact time was chosen for washing. Moreover, our electrolyte leakage data (Fig. 2) reflected that O_3 caused significantly higher tissue damage compared to other sanitizers which might have favor more microbial growth. Besides, O_3 may cause some illness to people who worked with ozone especially for longer time (13). Since the visual quality and other quality parameters like off-odor retained well until the storage duration, CA+Et treatment was well comparable with Cl treatment. The inefficiency of CA+Et treatment at the beginning of storage might be due to the low concentration of citric acid used. However, we found that higher concentration of citric acid was effective in reducing microbial number similar to that of 100 ppm Cl on washing day (data not shown), but an unpleasant off-odor and softening of tissues were occurred after few days of storage. The use of Cl as a sanitizing agent is strictly prohibited in some countries due to the hazardous byproducts formed by Cl with process water and other organic matters (30,31). Consequently, sanitization of fresh vegetables with Cl in the industry renders a negative impact to the environment and human health as a whole. Citric acid and ethanol, on the other hand, both are used as anti microbial agents leaving no effect to the environment. Their combined use was almost similarly effective as of chlorine in our study possibly due to the dual sanitization effects on the sample used. The uses of citric acid alone or in combination with other sanitizers have also been reported (16,18).

Among the treatments used, CA+Et was the best treatment in terms of reducing microbial count and other parameters measured in this study. Both of citric acid and ethanol are easily available, simply applicable and overall cost effective which have no harmful effect on health or environment. Therefore, this combined treatment could be a potential alternative of using Cl in the industry. The use of this combined treatment in maintaining quality and microbial population for other vegetables may provide a new insight of this environmental friendly sanitization method.

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