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Environmental Friendly Sanitation to Improve Quality and Microbial Safety of Fresh-Cut Vegetables

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1. Introduction

Fresh-cut products have a limited shelf-life due to rapid deterioration caused by microbial growth as well as physiological disorder. Cutting of fruits and vegetables increases microbial spoilage of fresh-cut produce through transfer of microflora on the outer surface to the interior tissue where microorganisms have access to nutrient-laden juice (Das & Kim, 2010a). Washing with sanitizer is an important step in reducing the microbial population and quality deterioration. The use of chemical compounds to extend postharvest life of fruit and vegetables has become lesser accepted by consumers since these compounds may be contaminant of the environment or harmful to human health. Therefore, the proper application of different sanitizing agents should be highly optimized to guarantee a minimal number of spoilage microorganisms.

Chlorine has been widely used in produce washes in order to inactivate microorganisms and ensure quality and safety. However, increasing public health concerns about the possible formation of chlorinated organic compounds and the emergence of new more tolerant pathogens, have raised doubts in relation to the use of chlorine by the fresh-cut industry (Kim, 2007). The use of chlorine as a sanitizing agent is prohibited in some countries due to the hazardous byproducts formed by chlorine with process water and other organic matters. Consequently, sanitization of fresh vegetables with chlorine in the industry renders a negative impact to the environment and human health as a whole. Recently, to avoid chlorine the use of non-chemical sanitizers in fresh-cut industries is becoming the more popular trend universally. Therefore, the industry is searching for alternative environment-friendly sanitizing methods to maintain the quality of fresh-cut produce at the best level.

Ozone is a strong antimicrobial agent with high reactivity, penetrability and spontaneous decomposition to a non-toxic product (Khadre et al., 2001; Kim et al., 2007a). Ozone is found in natural form in the atmosphere or it can be produced by generators. Ozone as an aqueous disinfectant was declared to be generally recognized as safe (GRAS) for food contact applications. Ozone's primary advantages include fast decomposition in water to oxygen, no residue, and improved microbial reduction efficacy against bacteria and fungal spores

than hypochlorite. Ozone forms oxidated radicals in the presence of water that penetrate and act on cell membranes. The use of ozonated water has been applied to fresh-cut vegetables for sanitation purposes reducing microbial populations and extending the shelf-life of some of these products (Beltran et al., 2005; Hassenberg et al., 2007; Kim et al., 2007a). Ozone has been declared in many countries to have potential use for food processing including sanitation of fresh and fresh-cut vegetables. When compared to chlorine, ozone treated with optimum conditions has a greater effect against certain microorganisms and rapidly decomposes to oxygen, leaving no residues (Kim, 2007; Rico et al., 2007). Inactivation of microorganism by ozone is a complex process that attacks various cell membrane and wall constituents (e.g. unsaturated fats) and cell content constituents (e.g. enzymes and nucleic acids). The micro-organism is killed by cell envelope disruption or disintegration leading to leakage of the cell contents. Disruption or lysis is a faster inactivation mechanism than that of other disinfectants which require the disinfectant agent to permeate through the cell membrane in order to be effective (Kim et al., 1999). Bacteria are more sensitive than yeasts and fungi. Gram-positive bacteria are more sensitive to ozone than Gram-negative organisms and spores are more resistant than vegetative cells (Das & Kim, 2010b; Pascual, 2007).

Electrolyzed water (EW), the second most popular sanitizer in Korea (Kim, 2008) is considered as an environment-friendly sanitizer compared to chlorine. However, there are different opinions on the effect of EW among industrial users. EW generated by adding NaCl to pure water from non-diaphragm system is one of the major EW systems in Korea. This electrolytic process facilitates the conversion of chlorine oxidants (Cl_2 , HClO / OCl^-) which are effective for inactivating a variety of microorganisms (Kim, 2007; Yang et al., 2003). The bactericidal effect of EW have been evaluated on several fresh-cut vegetables such as lettuce, carrots, spinach, and cucumber (Izumi, 1999; Nimitkeatkai & Kim, 2009). Acidic EW has a strong bactericidal effect against pathogens and spoilage microorganisms due to its low pH, high oxidation reduction potential (ORP) and the presence of residual chlorine (Kim, 2007). Using acidic EW resulted in moderate control of aerobic bacterial growth during storage of fresh-cut cilantro (Wang et al., 2004). Acidic EW was also tested for its efficacy in inactivating *Salmonella* on fresh-cut produce. However, the concentration of acid used can influence the organoleptic quality of vegetables, i.e., loss of texture (Kim, 2007; Zhang & Farber, 1996). Electrolyzed water at high pH (pH 6.8, 20 mg/L available chlorine) was tested as a disinfectant and the research found that it did not affect tissue pH, surface color, or general appearance of fresh-cut vegetables (Izumi, 1999).

Heat treatments such as hot water and hot air are non-chemical methods that have been used to control microorganism and senescence-related symptoms of fresh produce. Recently, mild heat treatment as physical technology to extend shelf-life of fresh and fresh-cut produce have become of interest. Heat treatment using hot water was used for fungal and insect control, but has been extended to improve the storage quality of fresh-cut produce. Hot water dips to control both decay and quality change of fresh and fresh-cut produce are often applied for 30 seconds to a few minutes at temperatures of 40-60°C (Kim, 2007; Kim et al., 2011). Heat treatments combined with other agents have also been used to prevent the microbial quality, browning, and maintaining texture in various vegetables (Das & Kim, 2010a). A combination of heat treatment followed by calcium dip has also been applied for the primary purpose of controlling postharvest pests and/or diseases and has been found to have very good results in maintaining or improving the texture of various

horticultural products. Combined heat treatments with UV-C were applied to fresh-cut processed broccoli (*Brassica oleracea* L.) florets to investigate their effects on several quality and senescence parameters. Heat treatments and UV-C radiation have been utilized to extend storage quality of fresh-cut products (Lemoine et al., 2008).

Acidity is a commonly used factor to control the growth of microorganisms in foods. Organic acids such as citric acid and ascorbic acid have been applied for preserving physicochemical qualities (Rosen & Kader, 1989) and preventing microbial growth at levels that did not adversely affect taste and flavor (Yildiz, 1994). Therefore, organic acids could be a potential sanitizer for fresh-cut vegetables. However, different studies have shown that the inhibitory or bactericidal effect depends on the characteristics of the acid used to adjust the medium pH (Buchanan et al., 1993; Eswaranandam et al., 2004; Parish & Higgins, 1989). Application of organic acids as sanitizers at higher concentration can reduce the overall quality and produce off-flavor in leafy vegetables after few days of storage (Kim 2007; Chandra & Kim, 2011). The acid tolerance of fresh-cut vegetables varies among different microorganisms and products. Organic acid alone treatment is not successful sanitation in controlling pathogens and maintaining food quality during storage. Hurdle technology or combined technology, which involves simultaneous multiple preservation approaches, is generally better to control both microbial safety and food quality of fresh-cut produce.

Natural antimicrobial agents derived from fruit, herb, and shell have been investigated as preservatives. The interest in the possible use of natural alternatives to food additives to prevent bacterial and fungal growth has notably increased. Edible coatings containing natural antimicrobial agents are gaining importance as potential treatments to reduce the deleterious effects imposed by fresh-cut processing on fresh-cut fruits. However, application of natural edible coatings for fresh-cut vegetables has not received interest as much as fresh-cut fruits. Essential (volatile) plant oils occur in edible, medicinal and herbal plants which minimizes questions regarding their safe use in food products. Essential oils and their constituents have been widely used as flavouring agents in foods since the earliest recorded history and it is well established that many have wide spectra of antimicrobial action (Holley & Patel, 2005). No fresh-cut company used natural antimicrobial agent to wash or preserve fresh-cut produce in Korea due to less effects than chemical sanitizers and non-economic efficiency. However, these days natural agents are good candidate to replace tap-water washing because these agents have more potency against microorganisms. Natural agents can be used for washing microgreens and organic fresh-cut produce which are sold at a higher price. The growing demand for fresh and fresh-cut produce by consumers had led to the need for natural food preservation methods such as the use of natural antimicrobials and their combination with other hurdles, without adverse effects on the consumer or the food itself (Tiwari et al., 2009).

Although a wide range of different microbial agents are available for sanitizing fresh-cut produce, their efficacies vary and none are able to ensure elimination of pathogen completely without compromising sensory quality. In addition, recent studies have shown that chlorine lacks efficacy on pathogen reduction; the formation of the chlorine by-products are also deleterious to human health. Thus, there is much interest in developing a safer and more environmental friendly antimicrobial alternative to chlorine. Ozone, electrolyzed water, mild heat treatment, organic acid, and natural antimicrobial agents, or the combination of those sanitizing methods have been applied to various fresh-cut vegetables. It is generally accepted

that an ideal sanitizing agent should have two important properties: a sufficient level of antimicrobial activity and a negligible effect on the sensory quality of the product.

2. Ozonated water washing

Ozonated water washing is getting more popular now-a-days due to its high biocidal efficacy, wide antimicrobial spectrum and environment friendly. Research has shown that treatment with ozone appears to have a beneficial effect in extending the storage life of fresh produce such as cucumber, apples, grapes, oranges, pears, raspberries and strawberries by reducing microbial populations and by oxidation of ethylene (Kim, 2007). The effect of ozonated waters with different concentrations and contact times on the quality attributes and microbial population of fresh-cut produce were studied. Two types of ozone generators were used to investigate the efficacy of microbial reduction and quality maintenance of fresh-cut lettuce, cilantro, carrot, broccoli, and paprika. One aqueous ozone solution was prepared by continuously circulating the water through an ozone generator and a stainless steel water tank. The circulating type ozone generator was equipped with a vortexer to facilitate dissolving of gaseous ozone in the water, and a de-gassing system to remove the undissolved ozone. The other ozone solution was prepared by flowing ozonated water into plastic bucket through an ozone generator. The flowing type ozone generator was equipped with a cylinder used as compression tank to facilitate high concentration of ozone. Both circulating type and flowing type ozone solutions were used immediately after the required ozone concentration were reached.

2.1 Sanitation with low ozone concentration (≤ 1 ppm)

Ozonated water using circulating type with low ozone concentration (less than 1 ppm) and insufficient contact time was not much effective compared to 100 ppm chlorine in reducing microbial population and maintaining quality of fresh-cut cilantro, iceberg lettuce, romaine lettuce, and baby leaves. Fresh-cut cilantro was washed in tap water, 100 ppm chlorine solution (pH 7), and 0.7 ppm ozonated water for 1 minute separately. The initial total aerobic plate count (APC) on the unwashed cilantro leaves was 6.45 log CFU/g. There was a significant decrease in APC between washed cilantro and unwashed sample after washing. However, no significant difference was found in microbial reduction of fresh-cut cilantro between tap water and ozonated water throughout 6 days storage at 5°C. The chlorine treatment maintained a low level of microbial count compared to other treatments. Fresh-cut romaine lettuce, spinach, microgreens, and baby leaves sanitized with 0.5-0.8 ppm of ozonated water had higher microbial population compared to samples washed in 50-100 ppm of chlorine. Ozonated water sanitation with low ozone concentration (less than 1 ppm) is not inadequate to be used in fresh-cut industry practically because the ozonated water was not effective in microbial decontamination and maintaining storage quality of fresh-cut vegetables (Kim, 2007).

Continuous flowing type ozonated water containing 1 ppm of ozone concentration was also used to sanitize fresh-cut iceberg lettuce if the sanitation could get much effect in reducing microbial population. The ozonated water was compared with tap water and 100 ppm chlorine solution (pH 6.5). During storage at 5 °C, there was a significant increase in APC among all treatments. The highest numbers of APC and coliform plate count (CPC) were observed in tap water washing followed by ozone washing. Chlorine treatment had the

most reduction on microbial population on fresh-cut iceberg lettuce throughout storage. In fresh-cut lettuce, cut edge browning commonly occurs during storage making unsuitable for consumers. Discoloration occurred in all fresh-cut iceberg lettuces on day 6. Samples washed in ozonated water had lower degree of cut edge browning index score than samples washed in tap water. However, chlorine solution showed the lowest degree of cut edge browning. Though ozonated water containing 1 ppm of ozone concentration was effective in delaying discoloration and reducing microbial population the effectiveness was lower compared to 100 ppm chlorine solution (Kim, 2007).

2.2 Sanitation with high ozone concentration (>1 ppm)

2.2.1 Washing fresh-cut vegetables with 1.5 ppm of ozone

Flowing ozonated water washing was also used to sanitize 'Tah Tasai' Chinese cabbage baby leaves and fresh-cut romaine lettuce. Those fresh-cut products were washed in tap water, 100 ppm chlorine (pH 7.0), and continuous flow of 1.5 ppm ozonated water for 2 minutes separately. Samples treated with the ozonated water had lower APC compared to those washed in tap water. Ozonated water containing 1.5 ppm of ozone concentration reduced APC on fresh-cut baby leaves and romaine lettuce by 0.3 and 0.6 log CFU/g, respectively, on day 0. On the other hand, 100 ppm chlorine solution treatment reduced APC on fresh-cut baby leaves and romaine lettuce by 0.5 and 0.9 log CFU/g on day 0. Fresh-cut produce washed in ozonated water had lower microbial population than samples washed in tap water until middle period of 9 days-storage at 5 °C. Ozonated water washing containing 1.5 ppm of ozone concentration and washed for 2 minutes was not sufficient to decontaminate microorganism of fresh-cut romaine lettuce and baby leaves as much as effectiveness of 100 ppm chlorine solution washing. Therefore, it has been required to find optimum ozonated water washing conditions for improving storage quality and microbial food safety of each fresh-cut product to apply to fresh-cut industry as a chlorine alternative.

2.2.2 Washing fresh-cut carrots with initial 2 ppm ozone

Higher ozone concentration with longer contact time has been applied to get much effect in reducing microbial population and maintaining quality of fresh-cut produce. Fresh-cut carrot shreds were washed in tap water for 1 minute, 50 ppm chlorinated water (pH 6.3) once or two times for each 1 minute, or initial 2 ppm ozonated water using circulating type at varying times (1, 5, and 20 minutes). The samples were then centrifuged to remove excess water, packaged in 50 µm PE film bags, and stored at 5°C. Different ozonated water washing time affected microbial growth, off-odor development, color, and overall quality of carrot shreds. A single chlorine wash and 20 minutes ozonated water wash treatments had lower APC and lactic acid bacteria compared to other washing treatments until 2 weeks storage (Fig. 1). The 20 minutes ozone treatment reduced APC on carrot shreds by 1.4 and 1.1 log CFU/g on week 1 and week 2, respectively.

Ozonated water washing for 20 minutes maintained quality by inhibiting off-odor and high overall quality score due to less whiteness development (Fig. 2). The single chlorine water wash was effective and resulted in better quality compared to two time chlorine water wash. However, samples washed for 20 min in ozonated water had better quality with less off-odor and higher overall visual quality scores than samples washed in chlorine water washed once. The efficacy of optimum ozonated water washing on microbial reduction and quality of those fresh-cut produce was similar to chlorine or better than chlorine. Ozonated water containing

initial 2 ppm ozone concentration with sufficient washing time would be effective in reducing microbial population and maintaining quality of fresh-cut carrot and could be an alternative method to maintain quality and shelf-life of fresh-cut carrot shreds (Kim et al., 2007a).

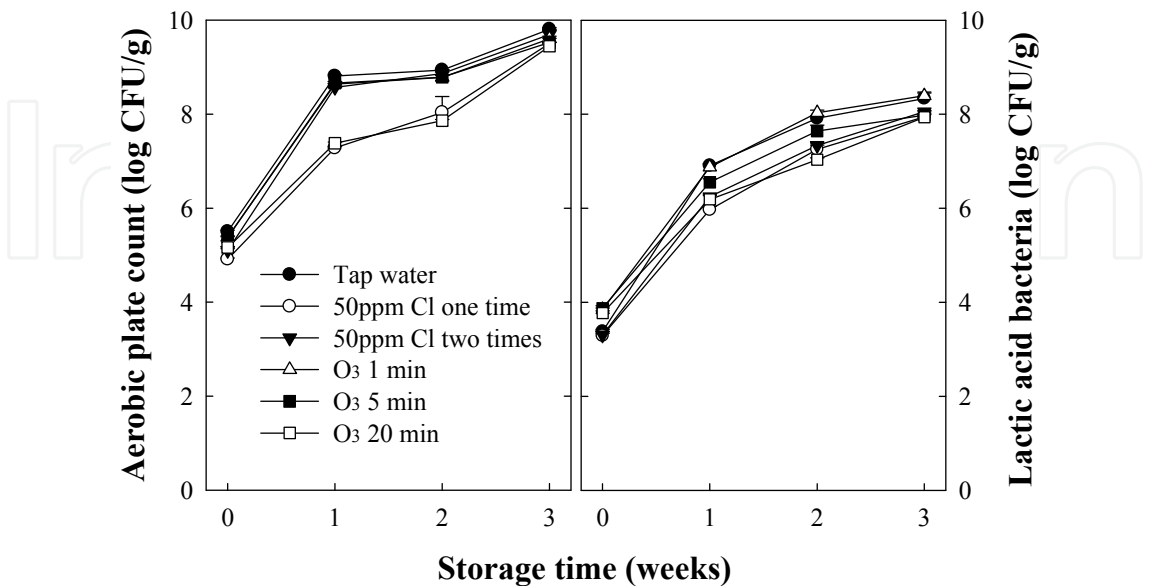


Fig. 1. Aerobic plate count and lactic acid bacteria of fresh-cut carrot shreds washed in different sanitizers and stored at 5°C for up to 3 weeks.

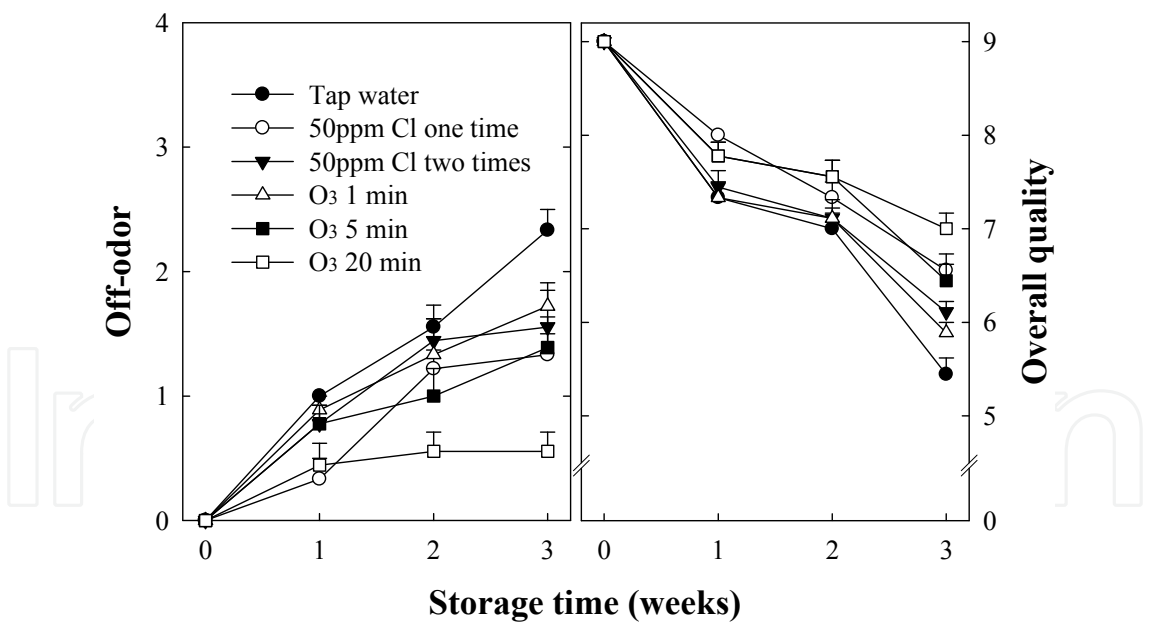


Fig. 2. Off-odor development and Overall quality of fresh-cut carrot shreds washed in different sanitizers and stored at 5°C for up to 3 weeks.

2.2.3 Washing fresh-cut broccoli with 2 ppm ozone

Ozonated water washing using flow type with different contact times on storage quality and microbial growth in fresh-cut broccoli was conducted to compare ozone with chlorine.

Fresh-cut broccoli samples were washed each for 90 and 180 seconds in normal tap water, 100 ppm chlorinated water (pH 7), and 2 ppm ozonated water separately and respectively. Then, samples were packaged in 30 μ m polyethylene bags and stored at 5°C for 9 days. No significant differences were observed in gas composition and color among different sanitizers with contact times. No off-odor was detected during the 9 days storage. Sanitizers affected microbial population of fresh-cut broccoli. In the color characteristics no difference were marked in L* and a* value and hue angle of the samples among different washing solutions and contact times during the storage period. It was found that electrical conductivity increased with the longer contact time in all washing solutions compared to shorter contact time. Electrolyte leakage is generally considered as an indirect measure of plant cell membrane damage. Ozonated water washing for 180 seconds contact time initially showed highest electrical conductivity probably due to its highly oxidizing nature than chlorine and tap water washing, but at the end of the storage the value is low and nearly equal to the above washings (Fig.3, left). It may be due to quality maintenance without texture damage or decay. Electrical conductivity is relatively high immediately after fresh-cut processing and decrease rapidly, then either decrease gradually or remain relatively stable until the samples have good quality in many fresh-cut produce (Kim et al., 2005a; Kim, 2007). This typical response pattern to processing and storage is similar to the result of fresh-cut broccoli sanitized in ozonated water and stored for 9 days at 5°C. No color difference was found among the treatments during 9 days storage (Fig. 3, right).

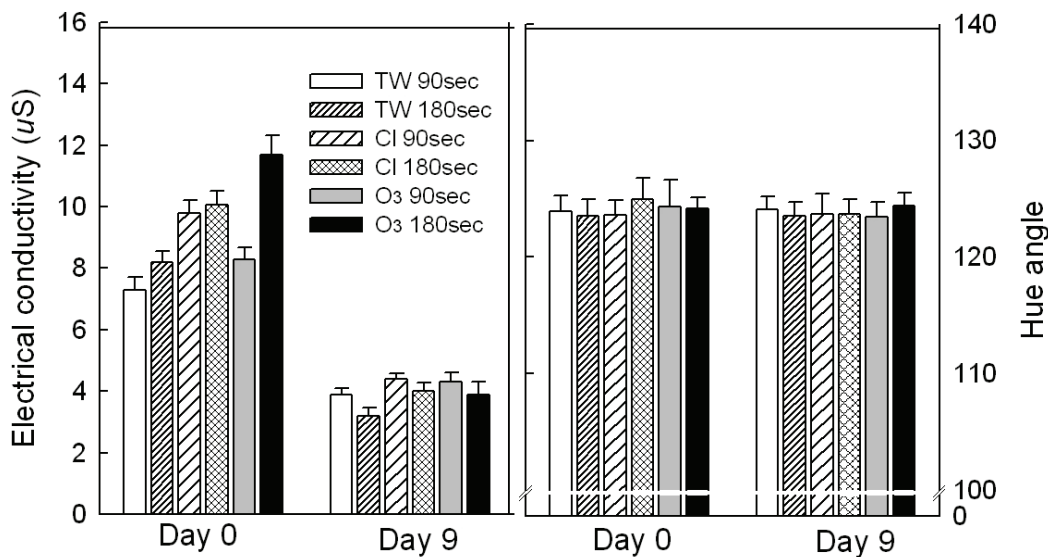


Fig. 3. Electrical conductivity and hue angle of fresh-cut broccoli washed in different sanitizers and stored for 9 days at 5°C. In figure, samples washed for 90 and 180 seconds in tap water (TW), 100 ppm chlorinated water (Cl), and 2 ppm ozonated water (O₃).

Among the sanitizers, ozonated water with 180 seconds maintained the lowest numbers of aerobic plate count throughout the storage days in comparison with others (Fig. 4). Ozonated water with 90 seconds was not much effective in reducing microbial population compared to chlorine. However, samples washed with ozonated water for 180 seconds showed the lowest coliform count. Absolutely no coliform were observed in ozonated water with 180 seconds washing treatment on day 0. The result reveals that longer contact time of ozone affects positively whereas other sanitizers don't affect on the microbial quality and

safety aspects of fresh-cut broccoli. The difference of microbial population is may be due to the following causes; the surface wash off might be attached to the samples again during washing time, higher electrical conductivity observed during storage and the reactivity against pathogen may be less effective in comparison with 2 ppm of ozone. Ozone effectiveness against microorganisms depends not only on the amount applied, but also on the effectiveness of ozone delivery method, type of material, the target microorganisms, physiological state of the bacteria cells at the time of treatment (Das & Kim, 2010b).

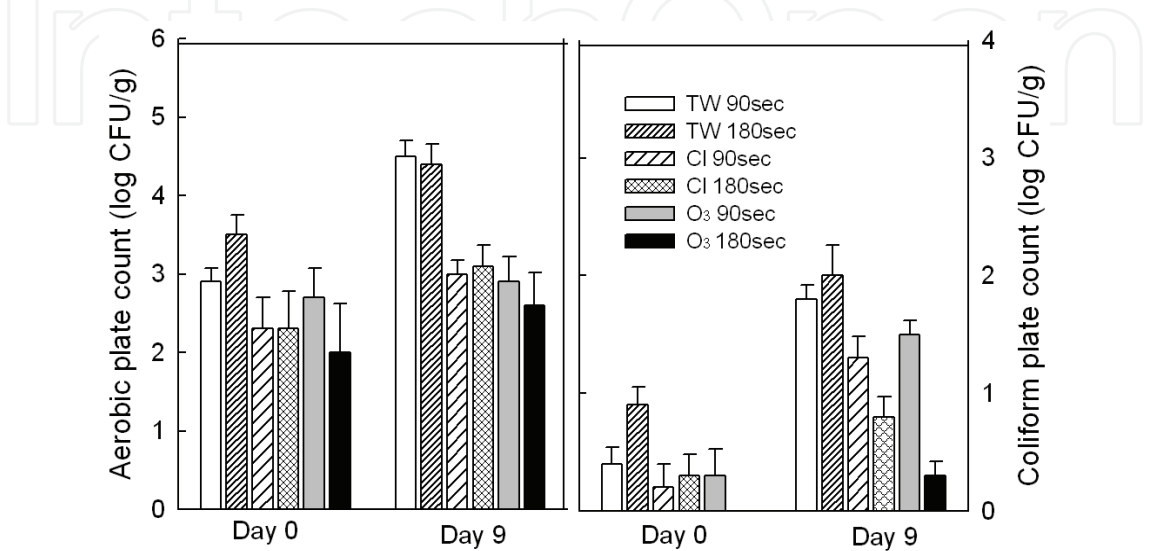


Fig. 4. Aerobic plate count and coliform plate count of fresh-cut broccoli washed in different sanitizers and stored for 9 days at 5°C. In figure, samples washed for 90 and 180 seconds in tap water (TW), 100 ppm chlorinated water (Cl), and 2 ppm ozonated water (O₃).

2.2.4 Washing fresh-cut vegetables with 3 to 4 ppm ozone

Ozonated water treatment containing 3 ppm of ozone with 3 minutes washing reduced APC and coliform/E. Coli count in both fresh-cut paprika and iceberg lettuce, similar to 100 ppm chlorine at day 0 (Table 1). Treatment with the ozonated water showed the lowest numbers of aerobic plate count and coliform/E. Coli count on day 6 in fresh-cut paprika. There was no difference in quality parameters such as color, off-odor, and visual quality among treatments. The highest numbers of aerobic and coliform/E. Coli were observed in tap water washing. Fresh-cut broccoli washed in 4ppm ozonated water for 5 minutes had lower microbial populations than samples washed in 100 ppm chlorine. No quality deterioration

Sanitizer	Fresh-cut paprika				Fresh-cut iceberg lettuce			
	APC		Coliform/E. Coli		APC		Coliform/E. Coli	
	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6
Tap water	2.3	5.3	1.5	1.2	3.6	5.3	0.8	0.9
100ppm Chlorine	2.0	4.3	0.5	1.0	3.1	4.7	0.2	0.7
3ppm Ozone	2.1	3.5	0.7	0.7	3.0	4.7	0.2	0.7

Table 1. Microbial population (log CFU/g) of fresh-cut paprika and iceberg lettuce washed in different sanitizers for 3 minutes, packaged, and stored for 6 days at 5°C.

or side effects of higher ozone concentration were found in fresh-cut broccoli. These results showed that ozonated water reduced microbial growth more effectively than 100 ppm chlorine solution. Therefore, ozonated water washing with optimum ozone concentration and sufficient contact time could be a favorite alternative sanitation to chlorine.

3. Electrolyzed water

Strong acidic EW (pH2.7) and weak acidic EW (pH 5-6.5), which were generated by electrolysis of NaCl solution and HCl, respectively have been used as disinfectant in fresh-cut industry. Weak alkaline EW (pH 7.5-8) from non-diaphragm EW generator, recently developed is getting popular among three types of EW. In general, strong acidic EW had stronger bactericidal effect compared to alkaline EW which has high pH levels. However, strong acidic EW can cause tissue damage in some fresh-cut produce, especially leafy vegetables during storage or distribution. Little information exists on the efficacy of weak alkaline or weak acidic EW on quality and microbial reduction in fresh-cut produce.

3.1 Washing fresh-cut lettuce, sesame leaf, and strawberry

The effect of strong acidic and weak alkaline EW containing 80ppm available chlorine concentration as well as general chlorine solution on storage quality and microbial growth of fresh-cut iceberg lettuce has been studied. The effectiveness of strong acidic EW on microbial reduction was greater than weak alkaline EW at initial storage. However, strong acidic EW affected quality deterioration due to texture damage after 6 days at 10°C (Table 2). Weak alkaline EW reduced off-odor development and was as effective as chlorine in inhibiting total aerobic bacterial and coliform group on fresh-cut iceberg lettuces (cultivar; *U-lake*). Fresh-cut sesame leaves washed in weak alkaline and neutral EW had less total plate counts and better sensorial quality compared to samples washed in chlorine and strong acidic EW. The result reveals that, weak acidic EW affects positively whereas strong acidic EW was not effective in maintaining quality. Strong acidic EW may be used for sanitation of fruits which has firm texture like apple. Nimitkeatkai & Kim (2009) reported that apples washed in strong acidic EW for 5 minutes had less hue angle value throughout storage period and lower sensory evaluation score at the end of storage. However, apples washed in strong acidic EW for 2 min was effective in reducing microbial growth and maintaining sensorial quality of apples. Different result was found that microbial quality of strawberry (cultivar; *Maehyang*) washed in weak alkaline EW (pH 8.0, 60ppm HClO) was inferior compared to samples washed in chlorine (pH 6.5, 60ppm HClO). These results indicated that EW does not affect quality maintenance and microbial safety for all fresh and fresh-cut products. Though weak alkaline EW did not show the effectiveness in strawberry weak alkaline EW could be an effective alternative to chlorine for washing fresh-cut leafy vegetables.

Treatment	Gas composition (%)		Total plate count (log CFU/g)	Off-odor ^z	Discoloration ^z
	O ₂	CO ₂			
Chlorine	0	25.8 b	7.0 a	3.3 b	0.5 b
Weak alkaline EW	0	31.2 a	7.3 a	3.8 a	1.8 a
Strong acidic EW	0	23.8 b	7.1 a	2.7 c	0.7 b

^z 0 = none, 1 = slight, 2 = moderate, 3 = severe, 4 = strong

Table 2. Gas composition, aerobic plate count, and quality of fresh-cut iceberg lettuce washed in different sanitizers and stored at 10°C for 6 days

3.2 Washing fresh-cut broccoli

Weak alkaline EW was also used to sanitize fresh-cut broccoli. The fresh-cut samples were washed for 90 seconds in tap water, 80 ppm chlorinated water (pH 7), and EW (pH 7.2) containing 80 ppm free chlorine separately and respectively. Then, samples were packaged in 30 μm polyethylene bags and stored at 5°C for 9 days. No significant differences were observed in gas composition and color among different sanitizers. No off-odor was detected during the storage. Samples washed with EW showed the lowest total aerobic bacterial population and coliform count. The result reveals that weak alkaline EW affects microbial population of fresh-cut broccoli positively.

	Electrical conductivity		Aerobic plate count		Coliform plate count	
	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9
Tap water	6.1	4.2	4.28	5.52	1.39	2.20
Chlorine	8.9	4.5	3.62	4.61	-	1.89
Electrolyzed water	8.2	4.7	3.53	4.24	0.22	1.02

Table 3. Electrical conductivity and microbial population of fresh-cut broccoli washed in different sanitizers and stored at 5°C for 9 days

3.3 Combined EW washing with MA packaging

Study on effect of combined EW washing with modified atmosphere (MA) packaging was carried out to investigate the influence of the combined treatment on quality maintenance and microbial food safety of fresh-cut iceberg lettuce. Fresh-cut iceberg lettuce were washed in alkaline EW (free chlorine 80 ppm), dried, and packaged with 35 μm P-Plus film to compare with conventional technology using combination of chlorine sanitation and vacuum packaging. Samples for control treatment were prepared following industrial practices; 100 ppm chlorine (100ppm, pH 7.5) wash and vacuum packaging with 80μm Ny/PE film. Combined EW and MA technology reduced off-odor development of packaged fresh-cut iceberg lettuce during storage (Table 4). The combined technology using EW washing and MA packaging was as effective as control using chlorine in inhibiting total aerobic bacterial counts on fresh-cut iceberg lettuces. The combined EW washing and MA packaging extended two more days of shelf-life of fresh-cut iceberg lettuce compared to control treatment.

Treatment	Gas composition		Aerobic plate count (log cfu/g)	Off-odor*	Dis-coloration*
	O ₂ (%)	CO ₂ (%)			
Chlorine + Vacuum pack.	0	19.0	6.4	2.3	0.6
EW + MA packaging	1.8	11.5	6.2	0.7	0.9

* 0 = none, 1 = slight, 2 = moderate, 3 = severe, 4 = strong

Table 4. Gas composition, aerobic plate count, and quality of fresh-cut iceberg lettuce treated with hurdle technology and stored at 5°C for 12 days

4. Heat treatment

Heat treatments is one of postharvest treatments that has been used to control postharvest decay and; or to improve the storage quality of fresh-cut produce. Heat treatments alone or combined with other agents have also been used to prevent the microbial quality, browning, and maintaining texture in various fresh-cut vegetables.

4.1 Heat treatment for fresh-cut winter squash and lotus roots

The effectiveness of heat treatment for fresh-cut winter squash and lotus roots has been applied. Winter squash which had hard rinds can be treated with hot water at 60-65 °C for 2-3 minutes to reduce microbial contamination before it is fresh-cut processed (Arvayo-Ortiz et al., 1994; Hawthorne, 1989). However, other vegetables which have soft texture are not recommended to treat at high temperature like winter squash. Winter squash (cultivar; *Bouzang*) harvested in summer season was immersed in 65°C hot water for 2 minutes before it is fresh-cut processed. The heat treatment reduced microbial population and maintained good quality with bright yellow or orange with fine, moist texture and high solids, and sugar contents. The changes in the quality of fresh-cut lotus roots treated with hot water were investigated. Lotus roots washed, peeled, and sliced with 1cm-thickness was dipped in water at 30, 55, and 80°C for 45 seconds. Then, samples were air-dried at room temperature, packed in polyethylene films, and stored at 4°C for 12 days. Generally, the weight loss of the lotus roots that were treated with hot water slightly increased. The application of the heat treatment delayed the browning of the lotus roots, especially the treatment with 55°C hot water. The Hunter color 'L' and 'a' values of the lotus roots treated with 80°C hot water significantly increased during their storage. The heat treatment effectively inhibited the growth of mesophilic microorganisms. Therefore, the organoleptic quality of the lotus roots that were treated with 55°C hot water was the best among those temperatures (Chang et al., 2011).

4.2 Mild heat treatment for peeled potato

For peeled potato, the most popular method of retarding surface browning used in the Korean industry is vacuum packaging that induces high CO₂ and low O₂ levels. However, the presence of high CO₂ and low O₂ concentrations may cause off-odor development due to anaerobic respiration. Hence, heat treatment methods that reduce browning and off-odor development were investigated. Potatoes (var. 'Jopung') kept at 5°C after harvest were heat treated (30°C for 24 hours, 45°C for 3hours, or non-heated), peeled, and immersed in tap

Treatment	Gas composition (%)		Color		Off-odor ^z
	O ₂	CO ₂	Lightness (L)	Redness(a)	
Control	0.67 b	34.7 a	69.8 b	-1.61 a	3.2 a
Heat treatment at 30°C for 24 hours	2.74 a	16.8 b	71.2 a	-2.47 c	2.5 b
Heat treatment at 45°C for 3 hours	0.56 b	32.4 ab	70.3 ab	-2.15 b	1.7 c

^z 0 = none, 1 = slight, 2 = moderate, 3 = severe, 4 = strong

Table 5. Effect of heat treatment before peeling on gas composition and quality of peeled potato fresh-cut processed and vacuum packaged, and stored at 5°C for 5 days.

water at 5°C for 3hours. Samples were then vacuum-packaged with 80µm Ny/PE film and stored at 10°C for up to 5 days. Mild heat treatment (30°C) was effective in reducing CO₂ concentrations and off-odor development in the package of samples throughout storage. The 30°C mild heat treatment also delayed browning of peeled potatoes and maintained the highest overall quality score. The mild heat treatment at 30°C before peeling can be a practical method to delay browning and off-odor development of ‘Jopung’ potato (Kim et. al, 2009).

4.3 Combined heat treatment for fresh-cut paprika

The combined effect of washing solutions and heat treatment were investigated as potential sanitizers for maintaining the quality and microbial safety of fresh-cut paprika. Fresh paprika shreds were washed in tap water and 1% calcium chloride combined with 19°C (normal tap water) and 50°C water temperature (heat treatment) for 2 minutes. Then, samples were packaged in 30 µm polypropylene bags and stored at 5°C for 12 days. No significant differences were observed in color and gas composition of the package among treatments and no off-odor was detected until the end of 12 days storage. However, 50°C water temperature with calcium chloride had lower microbial numbers upto the storage period in comparison with tap water (Fig. 5). The result reveals that 50 °C water temperature with calcium chloride can be used as an washing solution to maintain the microbial quality in fresh-cut Paprika (Das & Kim, 2010a).

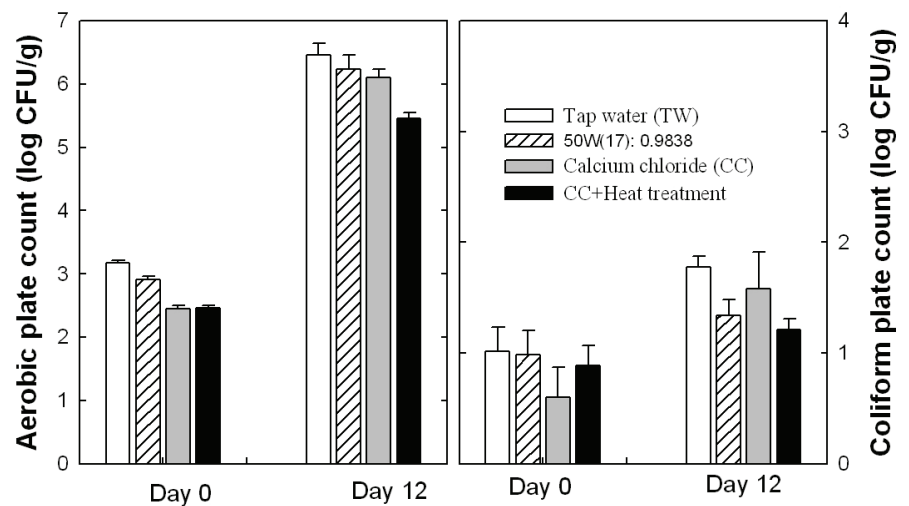


Fig. 5. Aerobic plate count and coliform plate count of fresh-cut paprika during the storage period. In figure, samples washed for 90 seconds in tap water (TW) and calcium chloride solution at 5°C.

Softening, textural changes are one of the main causes of quality losses in case of fresh-cut products. In general, fresh-cut vegetables that maintain firm and crunchy textures are highly desirable. Though there is no significant difference in gas composition and color among treatments calcium chloride and heat treatment tended to increase firmness of fresh-cut paprika during the beginning of the storage compared to tap water washing treatment. It is well known that calcium plays a major role in maintaining the quality of fruit and vegetables. Increasing the calcium content in the cell wall of fruit tissue can help delay softening of fresh-cut produce. The beneficial effects obtained with heat treatments have generally been explained in terms of pectin esterase activation. Calcium dips have been employed to improve firmness and extend the postharvest shelf-life of a wide range of fruit and vegetables.

Similarly, certain commercial additives can maintain the quality of fresh-cut products (Encarna et al., 2008; Luna-Guzman & Barrett, 2000). The changes in electrical conductivity of fresh-cut paprika depend upon the type of washing solution and heat treatment (Fig. 6). Tap water washing treatments (both TW and TW + Heat treatment) showed lower electrolyte leakage compared to calcium chloride washing during the entire storage period. A combination of heat treatment followed by calcium dip may need more research if it can be applied for the purpose of controlling postharvest pests and/or diseases and have very good results in maintaining or improving the texture of on various fresh-cut produce.

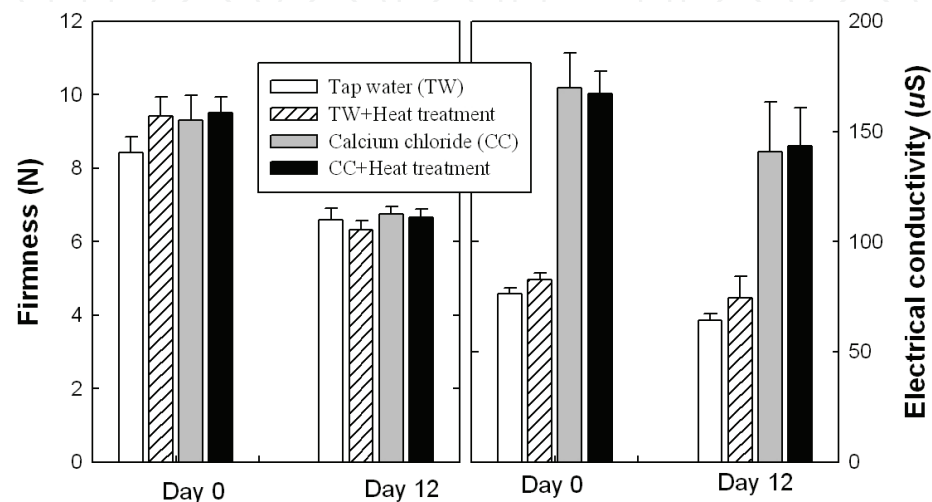


Fig. 6. Firmness and electrical conductivity of fresh-cut paprika heat treated and stored at 5°C for 12 days. In figure, samples washed for 90 seconds in tap water and calcium chloride solution at 5°C.

5. Organic acid and acid compounds

Organic acid and acid compound sanitizers have been used to sanitize fresh and fresh-cut produce. Organic acids are one of the important sanitizers that have been applied largely for preserving physicochemical qualities and for preventing microbial growth in many fresh-cut products. Organic acid with optimum condition did not adversely affect taste and flavor, but leaving no effect on environment. Citric acid can be used to extend the shelf life of fresh-cut produce by reducing the loss of eating quality and disease development. Ibrahim et al. (2009) reported that leaves of some selected vegetables decontaminated with 5% citric acid showed a considerable decrease in microbial count compared to water washing. However, the application of these acids at higher concentration may cause quality deterioration due to off-odor and texture damage in some fresh-cut leafy vegetables. Sequential treatment of citric acid and ethanol on the quality and microbial reduction of organic vegetables has also been examined. Hence, organic acid and the combined technology using have been carried out to find an alternative sanitizer to chlorine.

5.1 Washing fresh-cut lettuce with organic acid and combined acid

Fresh iceberg lettuce leaves were sanitized separately with tap water, 100 µL L⁻¹ chlorine, 0.2% citric acid, 50% ethanol, and the combination of citric acid solution and 50% ethanol spray. Samples were then dried with centrifugal dryer, packaged in 80 µm Ny/PE films, and stored for 6 days at 5°C. The 50% ethanol solution dipping was the most effective treatment to reduce

microbial population of fresh-cut iceberg lettuce (Fig. 7). However, fresh-cut iceberg lettuce sanitized in ethanol solution had severe injury with lowest visual quality score and highest electrical conductivity among treatments after 6 days storage (Fig. 8). The decline of overall visual quality in ethanol treated sample might be a consequence of tissue damage as reflected from the electrical conductivity data. Citric acid alone was not effective in reducing microbial population, similar to 100ppm chlorine solution treatment (Fig. 7). The combination of citric acid and ethanol spray reduced aerobic microbial population by 1.1 log CFU/g as compared to tap water. The combination with citric acid and ethanol spray also maintained good quality with high overall quality score at the end of 6 days storage (Fig. 8). Therefore, the combination of citric acid and ethanol spray could be an alternative to chlorine as an environment-friendly sanitizer for washing fresh-cut leafy vegetables (Kim et al., 2011).

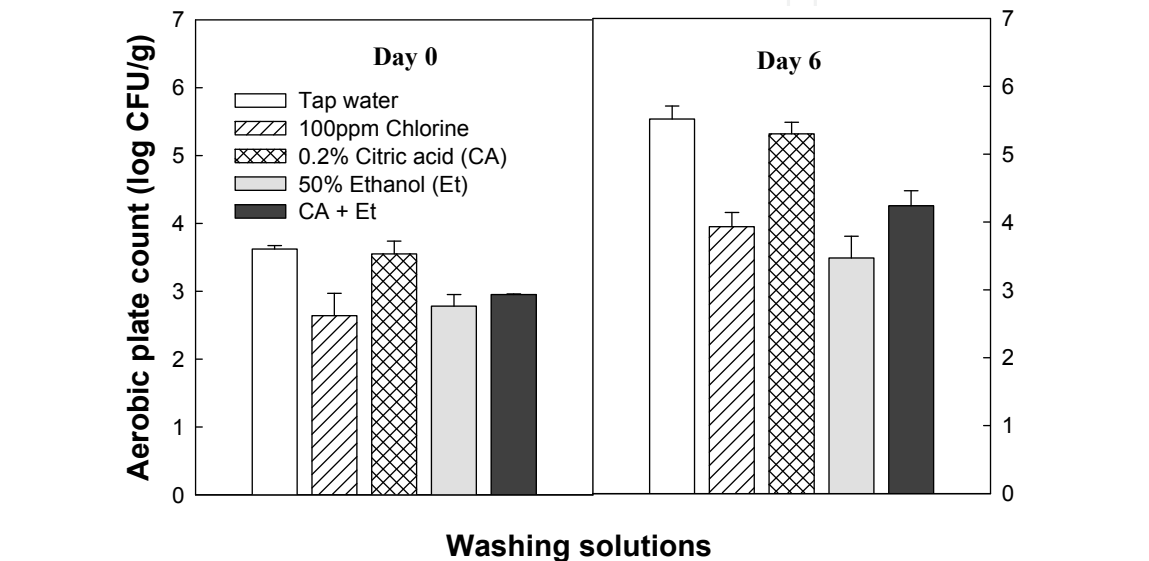


Fig. 7. Aerobic plate count of fresh-cut iceberg lettuce treated with different sanitizers and stored at 5 °C.

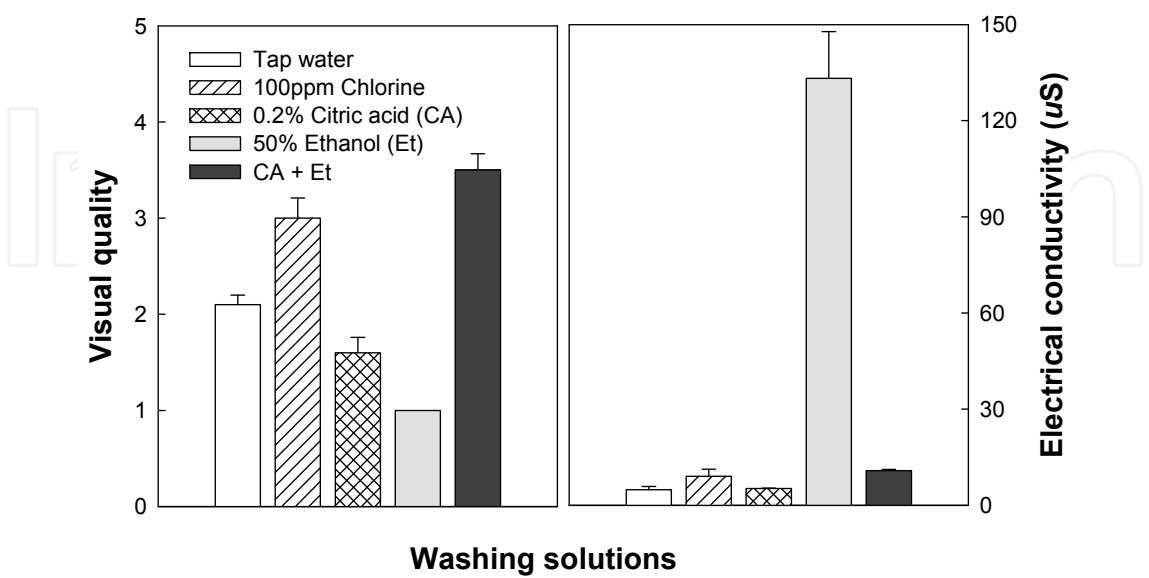


Fig. 8. Visual quality and electrical conductivity of fresh-cut iceberg lettuce treated with different sanitizers and stored for 6 days at 5 °C.

5.2 Washing fresh-cut spinach, baby leaves, and microgreens with the combined organic acid

Fresh organic vegetables such as spinach, 'Tah Tasai' Chinese cabbage baby leaves, and microgreens were also sanitized separately with tap water, 100 ppm chlorine, 0.2% citric acid (CA), and the sequential treatment of 0.2% CA solution and 50% ethanol spray (CA+Et). In case of spinach, chlorine and CA+Et increased CO₂ partial pressures in the headspace of sample packages and generally had higher electrical conductivity compared to tap water. No significant differences were observed in color among different sanitizers during storage at 5°C in fresh-cut spinach samples. The chlorine and CA+Et treatments were effective in reducing microbial population of fresh-cut spinach. However, CA+Et treatment induced off-odor of microgreens resulting more aerobic plate count compared to chlorine treatment. In case of microgreens, samples treated with CA+Et did not have good quality score, worse than score of chlorine at the end of storage probably due to severe texture damage. In 'Tah Tasai' Chinese cabbage baby leaves, sanitizer chlorine treatment showed lower number of total aerobic count immediately after washing. However, citric acid in combination with ethanol spray treatment showed the lowest number until day 7. No significant difference was found in microbial number among the treatments at the end of 10 days storage. Wang et al. (2004) also reported that 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. 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 possibly due to the dual sanitization effects on the sample used.

5.3 Acid compound sanitizers

5.3.1 Use of acidified sodium chlorite and peroxyacetic acid-based sanitizer

Acid compound sanitizers such as acidified sodium chlorite (ASC) and peroxyacetic acid-based sanitizer (PA) have been used for food safety of fresh and fresh-cut produce. Peroxyacetic acid is a strong oxidizing agent that has been used extensively to disinfect food processing equipment and has been approved by the U.S. Food and Drug Administration (FDA) as a disinfectant for fruits and vegetables (Gonzalez et al., 2004). Recent studies undertaken to determine the suitability of PA (Tsunami, Ecolab, USA) for washing fresh-cut vegetables showed it to be effective against *Listeria monocytogens*, *Salmonella* spp., and *E. coli* O157:H7 (Beuchat et al., 2004; Gonzalez et al., 2004). Acidified sodium chlorite has also been approved by the FDA for spray or dip application on various food products, including fresh and fresh-cut produce (Kim et al., 2007b). Studies have shown that ASC and PA have a strong antimicrobial efficacy against various human pathogens inoculated onto cantaloupes and asparagus (Kim 2007; Park & Beuchat, 1999). However, the effect of both ASC and PA on quality attributes such as texture and color of fresh-cut produce was not investigated with an in-depth study. It was found that carrot shreds washed in higher PA or ASC concentrations (200 and 500 ppm, respectively) lost firmness and looked melted during storage. There was a significant difference in firmness between 200 and 30 ppm PA concentration or 500 and 30 ppm ASC concentration. Therefore, high PA and ASC concentration may cause faster deterioration of carrot shreds because of inferior texture.

5.3.2 Washing fresh-cut carrot shreds

Fresh-cut shredded carrots were washed in tap water, 100 ppm chlorinated water, 30 ppm ASC, or 30 ppm PA. Samples were then packaged in 35 μ m polyethylene film and stored for 14 days at 5°C. Sanitizers affected off-odor, skin whitening, and microbial population of carrot shreds. Chlorine and PA wash maintained a lower level of microbial count compared to water or ASC treatments. Though no significant difference was found in total aerobic count between chlorine and PA until day 11, samples treated with PA had a lower APC than samples washed in chlorine at the end of storage. Chlorine caused the highest reduction (0.75 log), followed by PA (0.65 log) and ASC (0.56 log) at day 0. No significant difference in total aerobic count was found between ASC and water treatment after day 4. Therefore, 30 ppm ASC treatment was not effective in reducing microbial organism of fresh-cut carrot shreds during storage. The reason may be due to low ASC concentration (30 ppm). It was also found that 500 ppm ASC concentration was more effective in reducing microbial numbers than 50 ppm chlorine wash water. However, 500 ppm ASC concentration resulted in visual deterioration of the carrot shreds. Kim et al. (2006) reported that 30 ppm PA was effective in reducing microbial contamination as well as 100 ppm chlorine concentration. This would suggest that the residual effect of acetic acid released when PA is degraded causes reduced growth in microflora (Gonzalez et al. 2004).

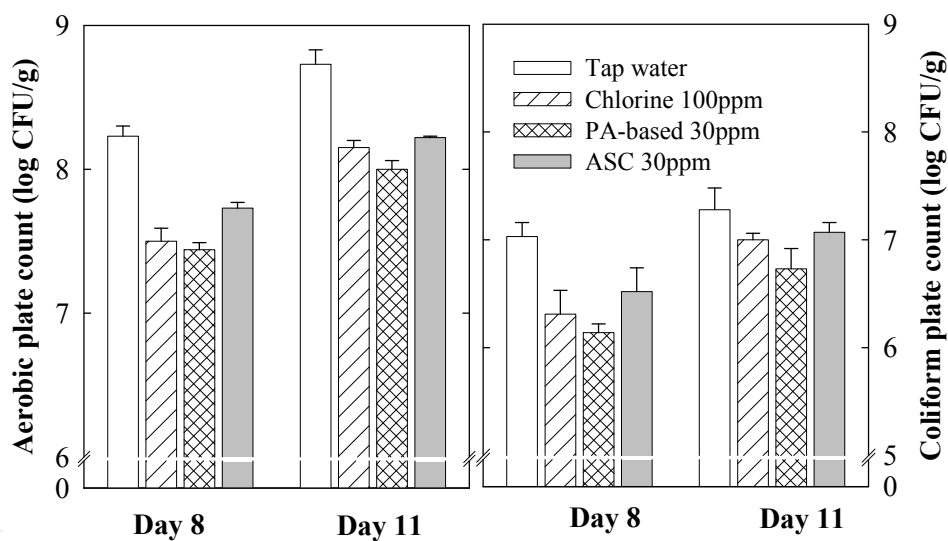


Fig. 9. Aerobic plate count and coliform plate count of fresh-cut carrot shreds. In figure, samples washed for 2 minutes in tap water, chlorinated water, peroxyacetic acid-based sanitizer (PA), and acidified sodium chlorite (ASC).

Peroxyacetic acid-based sanitizer also maintained initial color values, inhibited off-odor development and skin whitening of samples, and achieved the highest overall quality score among those sanitizer treatments. Off-odor was detected in all samples on day 8 and day 11 (Fig. 10, left). Off-odor was lower in samples treated with PA than in any other treated samples. Fresh-cut produce is known to develop undesirable off-odors under low O₂ and elevated CO₂ atmospheres (Kim et al., 2005a). Carrot shreds treated with water, chlorine, or ASC reached score 2.0 (the limit of marketability) at the end of storage, whereas samples PA-treated had score 1.9 after 11 days of storage. In fresh-cut produce, patterns of off-odor development correlate with ethanol and acetaldehyde and there is also a strong relationship

between package atmospheric conditions and off-odor development (Kim et al., 2005b). The degree of off-odor of fresh-cut carrot shreds may be influenced by fermentation due to anaerobic microorganism. Surface whitening which is one of major postharvest quality problems in carrot was significantly retarded by applying sanitizer PA. Samples treated with PA maintained inherent color and exhibited the lowest rate of increase in whitening scores (Fig. 10, right). Surface discoloration during storage is most detrimental to the quality of shredded carrots. The possible reasons for whiteness development on carrot surface are dehydration and lignification (Kim et al., 2006). Visual observation of carrot shreds treated with PA showed a moister surface compared to other sanitizer treatments. The PA-treated samples had the highest overall quality score, with relatively low levels of whitening. Therefore, sanitizer PA treatment significantly affected quality and shelf-life of fresh-cut shredded carrots. At present, chlorine is the most practical, efficient, and low cost disinfectant available. Due to concerns about the formation of by-products, however, a safer alternative is needed. Peroxyacetic acid-based sanitizer treatment resulted in comparable antimicrobial effectiveness and sensory quality of carrot shreds throughout storage.

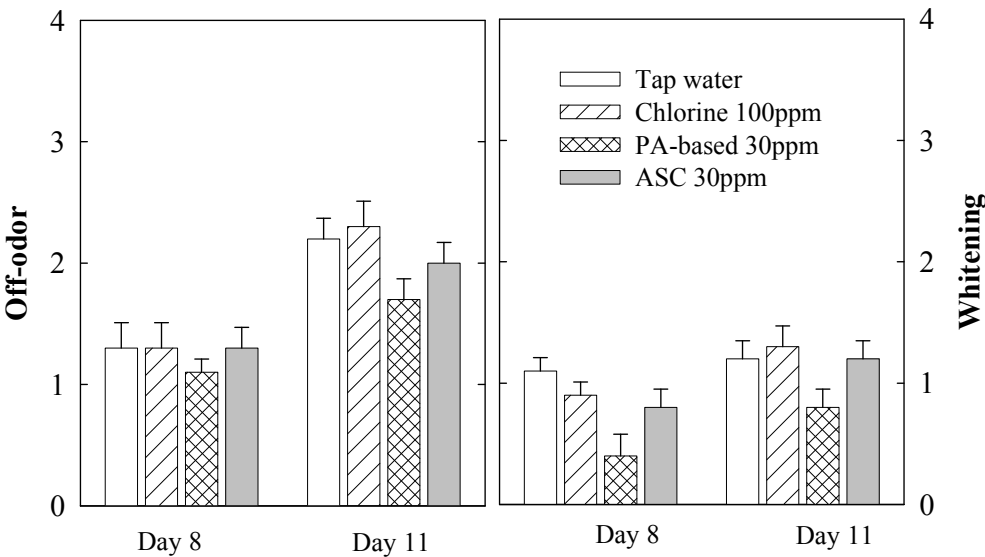


Fig. 10. Off-odor development and surface whitening of fresh-cut carrot shreds after storage at 5°C for 8 and 11 days. In figure, samples washed for 2 minutes in tap water, chlorinated water, peroxyacetic acid-based sanitizer (PA), and acidified sodium chlorite (ASC).

6. Natural antimicrobial agents

Natural compounds can serve as carriers for a wide range of food additives, including anti-browning agents, colorants, and antimicrobials that can extend product shelf-life and reduce the risk of pathogen growth on fresh-cut produce surface. In recent years there has been a considerable pressure by consumers to reduce or eliminate chemically synthesized additives in foods. Plants and plant products can represent a source of natural alternatives to improve the shelf-life and the safety of food. In fact, they are characterised by a wide range of volatile compounds, some of which are important flavour quality factors (Patrignani et al., 2008; Utama et al., 2002). A key role in the defence systems of fresh produce against decay microorganisms has been attributed to the presence of some of these volatile compounds (Patrignani et al., 2008). However, no plant volatiles have been used as natural antimicrobial

agent for fresh-cut produce practically. Recently developed natural antimicrobial agents from marine resource product have been applied to fresh-cut vegetables.

6.1 Sanitation fresh-cut lettuce with calcinated calcium

The heated scallop shell powder; calcinated calcium (CC) was investigated as potential sanitizers for maintaining storage quality and microbial safety of fresh-cut iceberg lettuce. Samples were washed in normal tap water, 50 ppm chlorinated water (pH 6.5), 1.5 g · L⁻¹ CC for 2 minutes separately. Samples were then packaged in 80 μm nylon/polyethylene bags and stored at 5°C. The initial aerobic plate count of unwashed iceberg lettuce was 6.5 log CFU · g⁻¹. The aerobic plate count on fresh-cut lettuce increased with storing time, reaching 6.05 to 7.05 log CFU · g⁻¹ on 12 days-storage. Washing in CC was effective in reducing aerobic plate count of fresh-cut lettuce samples by 0.4 to 1.0 log CFU · g⁻¹ as well as chlorine treatment throughout storage as compared to tap water (Fig. 11, left). Electrical conductivity of all samples decreased during the initial period of storage, remained stable thereafter or increased slightly at the end of storage (Fig. 11, right). Electrical conductivity of fresh-cut lettuces increased after 8 days. Electrical conductivity is generally considered as an indirect measure of plant cell membrane damage and deterioration of fresh-cut vegetables (Jiang et al., 2001; Kim et al., 2005b). Increased electrical conductivity after fresh-cut processing is a common phenomenon due to the leakage from cut ends of the samples or otherwise wounded tissues.

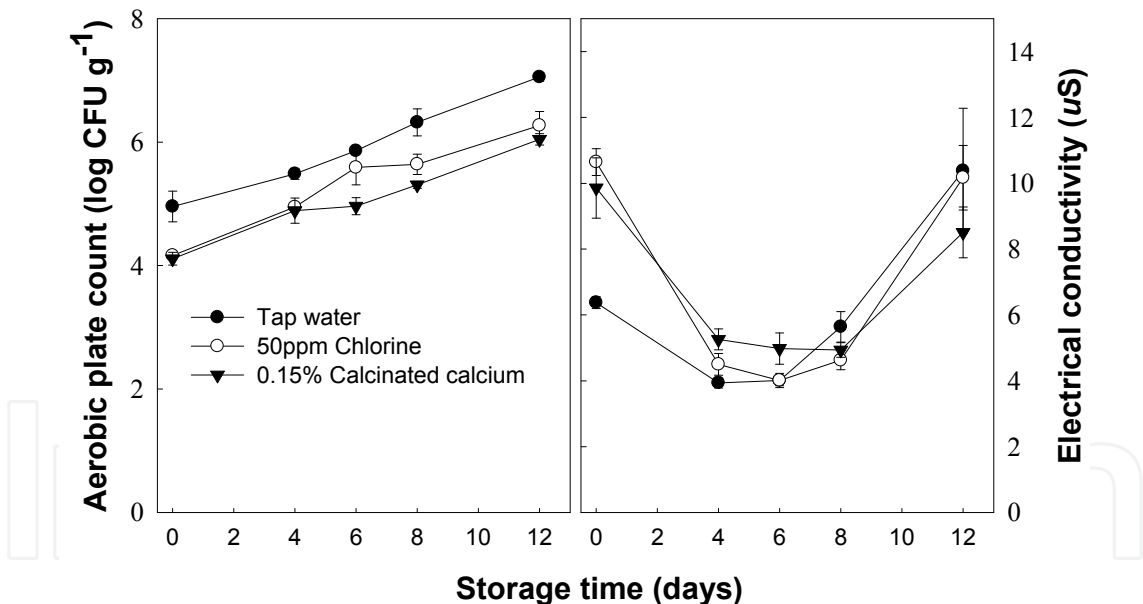


Fig. 11. Aerobic plate count and electrical conductivity of fresh-cut iceberg lettuce sanitized with different washing solutions (tap water, chlorinated water, and calcinated calcium) and stored at 5°C for 12days.

Samples treated with CC had good quality with low off-odor at the end of storage. Visual quality score of all fresh-cut iceberg lettuce samples was lower than score 3, which was considered the limit of marketability at the end of 12 days-storage (Fig. 12, left). The visual quality related to browning was probably induced by relatively high O₂ and low CO₂ concentration in sample packages. Off-odor was first detected in fresh-cut lettuce samples

treated with TW after 6 days and increased relatively until the end of storage. Off-odor of fresh-cut lettuce samples washed in chlorine and CC was lower than tap water (Fig. 12, right). Samples sanitized with chlorine or CC reached score 1.3 which was lower score than the limit of marketability on the 12 days-storage. Fresh-cut produce is known to develop undesirable off-odors under low O₂ and elevated CO₂ atmospheres (Kim, 2007). In fresh-cut lettuce, patterns of off-odor development correlated with ethanol and acetaldehyde and there was also a strong relationship between package atmospheric conditions and off-odor development (Kim et al., 2005a; Kim et al 2005b). The degree of off-odor in the packaged fresh-cut lettuce was influenced by subsequently fermentation due to anaerobic microorganism.

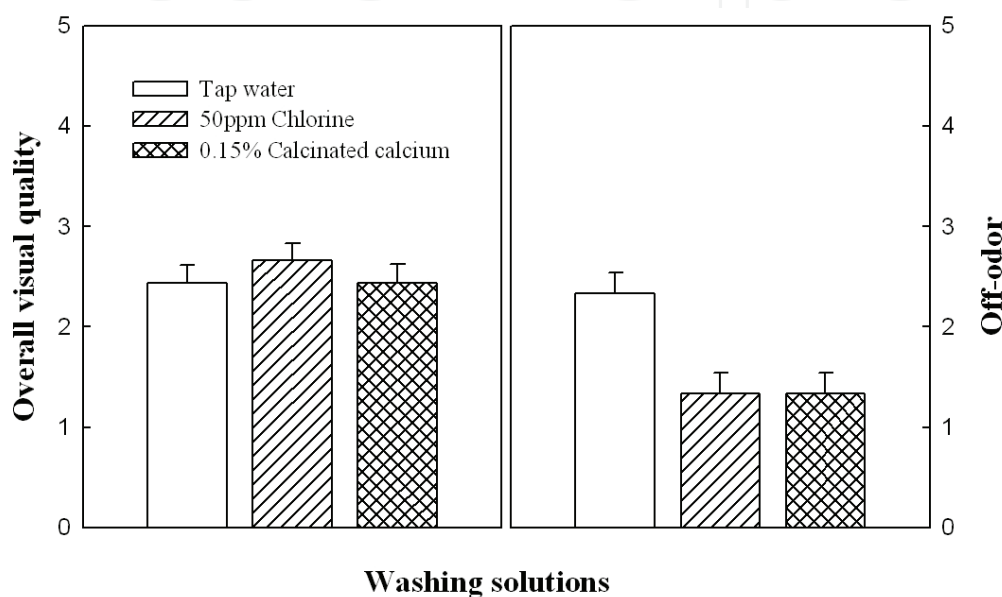


Fig. 12. Overall visual quality and off-odor development of fresh-cut iceberg lettuce sanitized with different washing solutions (tap water, chlorinated water, and calcinated calcium) after 12 days storage at 5°C.

6.2 Sanitation fresh-cut Bok choi and broccoli with calcinated calcium

Natural materials, calcinated calcium and fruit extract compound from Japanese apricot were used as sanitizer to maintain quality and reduce microbial population of bok choi with different maturities. Microgreen, baby leaf, and mature bok choies were washed in tap water, 50 ppm chlorine, and 500 fruit extract compound, for 2 min separately. Those samples were then packaged in 50 μm PE film and stored at 5°C for 6 days. One of natural compounds, the fruit extract compound was not effective significantly in reducing microbial population and quality such as off-odor. However, samples treated with CC had better quality with less off-odor until 4 to 6 days-storage in baby leaf and mature Bok choi. Calcinated calcium affected in reducing microbial population of microgreen, baby leaf, and mature Bok choi for 2, 4, and 6 days, respectively (Fig. 13). Bok choi microgreens had highest microbial population, followed baby leaves, and mature samples in terms with maturity. Therefore, mature and baby leaf Bok choi samples can have 6 and 4 days of shelf-life with CC sanitation, respectively. Fresh-cut broccoli was also washed in CC at normal tap water temperature. Broccoli samples sanitized in CC solution had good quality with lower

off-odor and microbial count at the end of 9 days-storage (Kim et al., 2010). To avoid chlorine which may lead to the formation of carcinogenic compounds, CC can be used as environmental friendly sanitizer and an alternative to chlorine washing for fresh-cut broccoli without affecting microbial and sensorial quality.

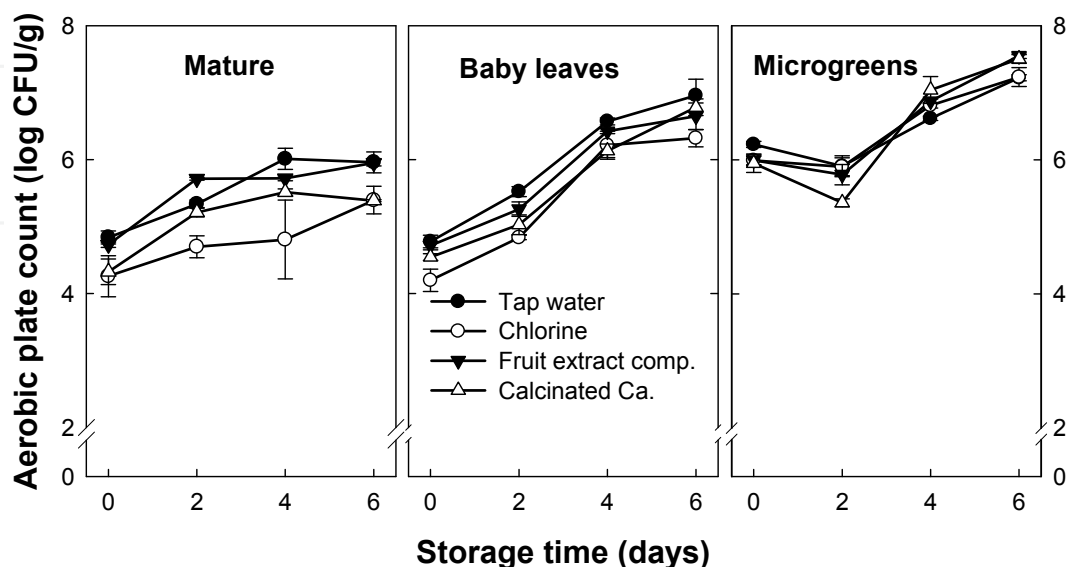


Fig. 13. Aerobic plate count of mature, baby leaf, and microgreen fresh-cut bok choy samples sanitized with different washing solutions (tap water, chlorine, fruit extract compound, and calcinated calcium).

7. Conclusion

Use of chlorine to reduce microbial populations of fresh-cut vegetables has faced with challenges to find alternatives which are more environmental friendly and not harmful to human health. Wide ranges of different agents are available for sanitizing fresh-cut produce, their efficacies vary and none are able to ensure elimination of pathogen completely without compromising sensory quality. Therefore, application of different environment-friendly sanitizing agents has been conducted to investigate highly optimized condition to guarantee a minimal number of spoilage microorganisms in many fresh-cut vegetables. As alternatives to chlorine, ozone, electrolyzed water, mild heat treatment, organic acid with ethanol, and natural antimicrobial agents, or the combination of those sanitizing methods can be used for fresh-cut vegetables. But, selection of washing solution and use of optimum condition to meet each fresh-cut vegetable should be performed to get similar or better efficacy to chlorine. Ideal sanitizing agent should have effectiveness in two important properties: antimicrobial activity and sensory quality of the fresh-cut product.

For organic fresh-cut leafy vegetables which are facing challenges to find the means to extend shelf-life and to enhance microbial food safety, combined citric acid with ethanol spray or calcinated calcium alone solution can be used in fresh-cut industry. Those environment friendly agents are good candidate to replace tap water washing or organic acid solution because these agents have more potency against microorganisms. Heat treatment without chemical use can be used for washing of fresh-cut produce which have firm texture such as winter squash, potato, lotus roots, and paprika. To get much

effectiveness in reducing microbial population of fresh-cut vegetables constant 1~4 ppm of ozonated water and weak alkaline or weak acidic electrolyzed water can be used practically. However, the concentration of ozone and contact time is very important for microbial safety of fresh-cut vegetables such as broccoli, iceberg lettuce, carrot, etc. Calcinated calcium, a natural and an environment-friendly sanitizer can be an alternative to mild heat treatment for washing of fresh-cut vegetables without affecting sensorial quality.

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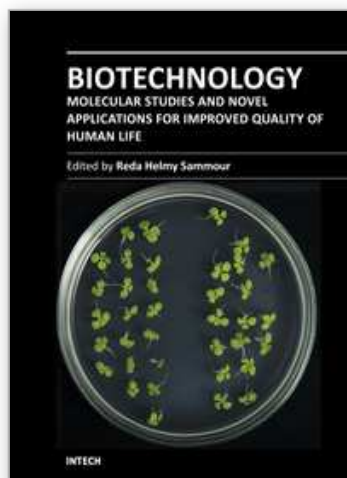
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This book deals with the importance of application of molecular biology as an approach of biotechnology for improvement of the quality of human life. One of the interesting topics in this field, is the identification of the organisms that produce bioactive secondary metabolites. It also discusses how to structure a plan for use and preservation of those species that represent a potential source for new drug development, especially those obtained from bacteria. The book also introduces some novel applications of biotechnology, such as therapeutic applications of electroporation, improving quality and microbial safety of fresh-cut vegetables, producing synthetic PEG hydro gels to be used as an extra cellular matrix mimics for tissue engineering applications, and other interesting applications.

How to reference

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