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EDGE BROWNING AND MICROBIAL QUALITY OF FRESH-CUT ICEBERG LETTUCE WITH DIFFERENT SANITIZERS AND CONTACT TIMES

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Abstract

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This study was conducted to evaluate the effect of different washing solutions and contact times on the cut edge browning and microbial quality of fresh-cut iceberg lettuce. Samples were cut into small pieces, washed for 90 seconds and 180 seconds in normal tap water, chlorinated water (100 $\mu L \cdot L^{-1}$ & pH 6.5), electrolyzed water (with free chlorine of 100 $\mu L \cdot L^{-1}$,pH 7.2) or ozonated water (3 $\mu L \cdot L^{-1}$). Samples were then, dewatered, packaged in 30 µm polypropylene bags, and stored at 5^0C for 9 days. Various quality and safety parameters such as gas composition, off-odor, electrical conductivity, cut edge browning and microbial numbers were evaluated during storage. Results revealed no detectable off odor in all samples during the storage period. However, significant differences in gas composition and electrolyte leakage were marked in samples washed for 180 seconds. Samples washed in chlorine for longer contact time showed least cut edge browning index and lower microbial numbers than other washings initially as well as finally. Hence, concluded that 180 seconds contact time of chlorine washing was better solution to maintain the microbial quality and cut edge browning of fresh-cut iceberg lettuce.

Key words: browning, chlorinated water, electrolyzed water, ozonated water, sanitizer

Introduction

The ready-to-use fresh vegetables markets have grown rapidly in recent decades as a result of changes in consumer attitudes in convenience and health concerns, especially consumption of fresh-cut lettuce. However, increasing public health concern related to the microbial safety of vegetables has resulted increased numbers of studies that analyze the efficiency of different methods for maintaining the quality and food safety of fresh-cut products (Allende et al., 2008; Selma et al., 2008; Gomez-Lopez et al., 2007; Inatsu et al., 2005; Beuchat et al., 2004; Lukasik et al., 2003). These conclude that the simple practice of washing raw fruit and vegetables removes a portion of

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pathogenic and spoilage microorganisms, decreasing their initial levels and microbiological activity. Because bacteria cells attach in a relatively short time period to the surface of fruit and vegetables and they tend to locate in protected binding sites, they may escape contact with washing or sanitizing agents, which makes it difficult to remove all cells by vigorous washing or treatment with sanitizers (Allende et al., 2008; Mandrell et al., 2006; Wang et al., 2004). Thus, the success of the washing depends on different factors such as target microorganisms, characteristics of produce surfaces, attachment of cells to produce surfaces, formation of resistant biofilms and internalization of microorganisms, type of washing, exposure time, dose, pH, temperature, etc. Hence, maintenance of this reduction during storage is as important as initial microbial reductions after washing (Ragaert et al., 2007). 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. Cut edge browning is one of the major common defects in fresh-cut iceberg lettuce which strongly affects the decision of consumer to buy.

Currently, chlorine is the sanitizing agent most widely used by the industry for fresh-cut produce mainly due to its high antimicrobial activity and low cost (Kim et al., 2007; Singh et al., 2002; Sapers, 2001). Similarly, electrolyzed water (EW) is a relatively new concept based on previously unknown law of anomalous changes of reaction and catalytic abilities of aqueous solutions subjected to electrochemical unipolar (either anoxic or cathodic) treatment. Acidic EW has a strong bactericidal effect against pathogens and spoilage microorganisms due to high oxidation reduction potential (ORP) (Bari et al., 2003; Kiura et al., 2002). The advantage of the use of electrolyzed water at neutral pH appears good results to fresh-cut leafy vegetables (Rico et al., 2008).

Furthermore, there are numerous studies in the literature about the effect of ozone treatment on the safety and quality of iceberg lettuce (Hassenberg et al., 2007; Koseki and Isobe, 2006; Beltran et al., 2005). Due to its high biocidal efficacy, wide antimicrobial spectrum and environment friendly, it is getting more popular now-a -days. Both molecular ozone and the free radicals produced by its breakdown play a part in inactivation mechanism. The micro-organism is killed by cell envelope disruption or disintegration leading to leakage of the cell contents. Again, ozone effectiveness against micro-organisms depend the concentration and contact time of applied ozone (Olmez and Akbas, 2009).

Therefore, there is an increasing need to investigate the efficacy of commercial sanitizers and other alternative technologies. So, in the present study, to find out the best sanitizing method among tap water, chlorinated water, electrolyzed water, ozonized water with different contact times were evaluated on microbial quality and safety of fresh-cut iceberg lettuce during the storage.

Materials and Methods

Preparation of washing solutions

Chlorine solution (100 $\mu L \cdot L^{-1}$) was prepared with food grade bleach and the pH was adjusted to 6.5 with 1N HCl. The available chlorine was determined with a portable chlorine colorimeter (Model-1200, Lamotte, Washington, USA). The solution was used immediately. EW solution was generated using by Electrolyzed Water System (HBS-500, Han Bio, Republic of Korea). The pH was adjusted to 7.2 with a residual chlorine concentration of 100 $\mu L \cdot L^{-1}$ and used within one hour. Aqueous ozone solution was prepared by continuously circulating the water by Ozone Water sterilizing System (OS-800, Advanced Scientific Technology, Republic of Korea) and a stainless steel water tank. The ozone generator was equipped with a vortexer to facilitate dissolving of gaseous ozone in the water. The concentration of dissolved ozone was determined by a dissolved ozone meter (DO3 Meter, DDK-TOA Corporation, Japan). Ozone concentration of 3 $\mu L \cdot L^{-1}$ was used immediately after the required ozone concentration reached.

Materials and treatments

Fresh iceberg lettuce was obtained from local super market, Suwon, Korea. Samples with defectives in surface color, damage, scratch etc discarded. The outer leaves were removed depending on the visual quality in each sample before processing. Then, samples were cut into small pieces (3×2 cm) using a sharp knife. This fresh-cut samples (1.4 kg in each net bag) were washed carefully with gentle agitation in separate buckets each containing 20 L solution from one of the following treatments: tap water (TW), chlorinated water (CL, 100 $\mu L \cdot L^{-1}$, pH 6.5), EW (with free chlorine of 100 $\mu L \cdot L^{-1}$, pH 7.2) and continuous flow of ozonated water (O_3, 3$\mu L \cdot L^{-1}$) for contact time of 90 seconds and 180 seconds (TW-90s, TW-180s, CL-90s, CL-180s, EW-90s, EW-180s, O_3-90s, O_3-180s) individually and separately. The washed samples were dewatered for 1 minute with a centrifugal dryer (WS 6501 T, Hanil, Republic of Korea) to remove excess water. Then these dewatered samples of 100 g each were packaged in 30 µm polypropylene (PP) bags (20 X 25 cm) sealed and stored at 5^{0}C for 9 days. Sampling for quality evaluation was at days 0, 3, 6, and 9. The experiment was conducted twice with similar trends. The results from the second experiment were reported here.

Gas composition and off-odor

Changes in O_2 (oxygen) and CO_2 (carbon dioxide) concentrations within packages were measured by a gas analyzer (Checkmate 9900, PBI Dansensor, Denmark) periodically monitored for up to 9 days of storage. Gas measurement was performed with a hypodermic needle, inserted through an adhesive septum previously fixed to the bag, at a flow rate of 1.5 mL/min for 1 minute, monitoring with a sensitivity of 0.001 (O_2) and 0.1 (CO_2). Three bags per treatment were evaluated for each experiment day. Off-odor was evaluated immediately after opening the packages by a panel of four trained personnel and scored on a five-point scale where 0 = none; 1 = slight; 2 = moderate; 3 = strong; and 4 = severe. A score of 3 was considered as non-acceptable.

Cut edge browning

Discoloration in the form of cut edge browning was evaluated using a five-point scoring system based on the degree of browning where 0 = none; 1 = slight; 2 = moderate; 3 = strong; and 4 = severe. The results are expressed as an index score calculated by taking the sum of each score multiplied by the corresponding number of pieces in four replicate samples having that score, dividing by the total number of lettuce pieces evaluated, and then multiplying by 100. Considering color variations among fresh-cut pieces within the same bag 3 readings per bag were taken to ensure that the data obtained truly represented the color of the samples.

Electrolyte leakage and microbial growth

The electrolyte leakage of fresh-cut iceberg lettuce was measured immediately after treatment and during storage to determine possible tissue deterioration. Samples of 20 g fresh-cut iceberg lettuce were submerged in 400 ml of deionized water for 30 min at 18^{0}C. The conductivity (µs/cm) of the solution was determined with a conductivity meter (Orion 4 star portable pH/ conductivity meter, Thermo Electron Corporation, USA) and was used to characterize the electrolyte leakage of plant tissues.

Samples of 20 g each were homogenized in 180 ml of 1% sterile buffered peptone water using filter stomacher bags (Stomachem 400, Seward, England) for 60 seconds at 230rpm. One milliliter of the appropriate sample dilution was pour-plated on aerobic plate count (3M Pertifilm, 3M Microbiology, St. Paul, USA) incubated at 25°C for 48 h. Simultaneously, one milliliter of the appropriate sample dilution was pour-plated on coliform plate count (3M Pertifilm, 3M Microbiology, St. Paul, USA) incubated at 25 °C for 24h for coliform counts. Only colonies showing typical coliform morphology were counted.

Results

Gas composition and off-odor

The O_2 partial pressure decreased and reached 6.4 to 10.2 kPa levels (Figure 1 Top). In contrast to O_2 levels, CO_2 partial pressures rapidly increased and slightly increased or maintained the equilibrium levels (6.0-7.5 kPa) after 3 days of storage (Figure 1). Samples washed for 90s reached similar O_2 levels (8.9-9.5 kPa) and CO_2 levels (6.5-6.6 kPa) with no significant differences among treatments. However samples washed for 180s reached O_2 levels (6.4-10.2 kPa) and CO_2 levels (6.0-7.5 kPa) with significant differences among treatments. Simultaneously no off-odor was detected (data not shown) throughout the storage period.

Cut edge browning

There was no cut edge browning until day 3 (data not shown). Discoloration occurred in all samples on day 6. Samples washed for 180s observed lower degree of cut edge browning index score than 90s washing on day 6 and day 9 (Figure 2). In either on day 6 or day 9, CL-180s showed the lowest degree of cut edge browning index score. The highest browning was observed in tap water washing followed by ozone washing.

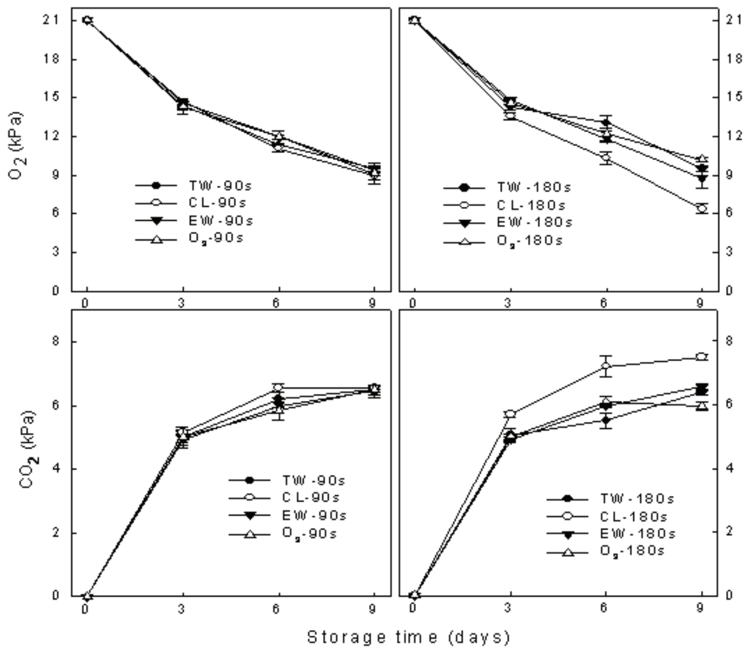

Fig.1. O_2 (Top) and CO_2 (Bottom) composition in fresh-cut iceberg lettuce during storage period. In figure, samples washed for 90 seconds and 180 seconds in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water (O_3) respectively and separately. Data are the mean of three replications with ±S.E.

Electrolyte leakage

The changes in electrolyte leakage of fresh-cut iceberg lettuce depend upon the type of washing solution and on the contact time. Initially, the highest electrolyte leakage was observed in CL and EW washing, respectively either for 90 seconds or 180 second washing. Finally, all the treatments observed nearly equal value on the 9th day of in-

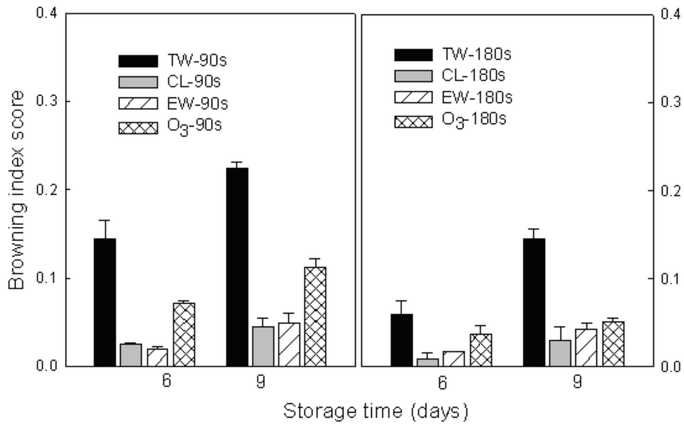

Fig. 2. Cut edge browning index of fresh-cut iceberg lettuce during storage period. In figure, samples washed for 90 seconds and 180 seconds in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water (O_3) respectively and separately. Discoloration was evaluated using a five-point scale based on the degree of discoloration where 0 = none, 1 = slight, 2 = moderate, 3 = strong, and 4 = severe. Results are expressed as an index score calculated by taking the sum of each score multiplied by the corresponding number of pieces having that score, dividing by the total number of lettuce pieces evaluated, and then multiplying by 100. Each data point represents the average of three replications ± SE.

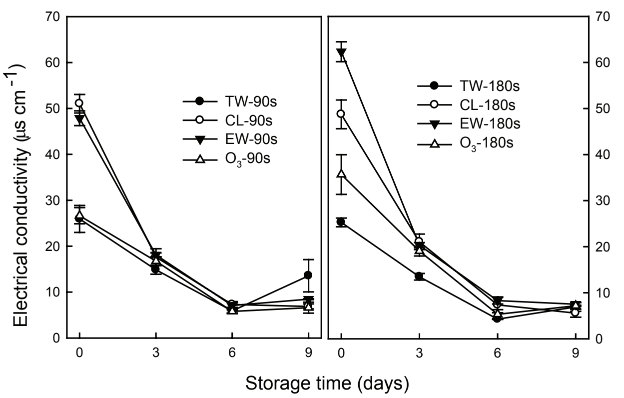

Fig. 3. Electrical conductivity of fresh-cut iceberg lettuce during storage period. In figure, samples washed for 90 seconds and 180 seconds in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water (O3) respectively and separately. Data are the mean of three replications with ±S.E.

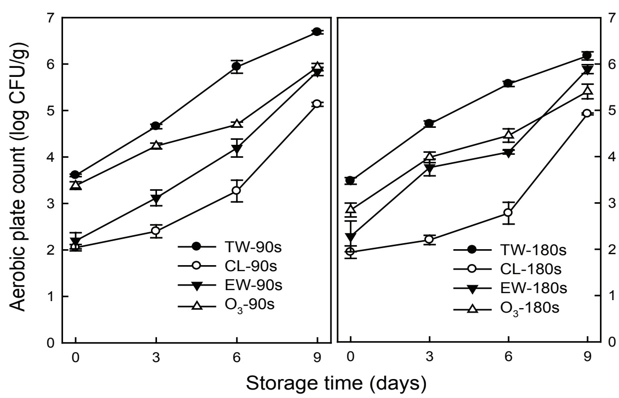

Fig. 4. Aerobic plate count of fresh-cut iceberg lettuce during storage period. In figure, samples washed for 90 seconds and 180 seconds in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water (O3) respectively and separately. Data are the mean of three replications with ±S.E.

vestigation (Figure 3).

Microbial growth

Higher numbers of aerobic and coliform bacteria observed with short contact time irrespective of washing solutions (Figures 4 and 5). Treatment with chlorine either with 90s or 180s showed the lowest numbers of aerobic and coliform plate counts than other treatments. The highest num-

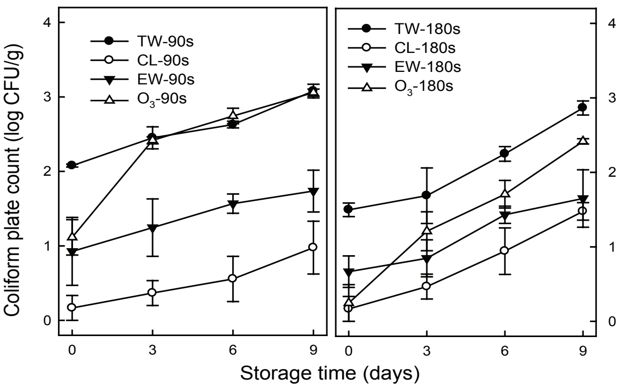

Fig. 5. Coliform count in fresh-cut iceberg lettuce during storage period. In figure, samples washed for 90 seconds and 180 seconds in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water (O_3) respectively and separately. Data are the mean of three replications with ±S.E.

bers of aerobic and coliform were observed in tap water washing followed by ozone washing. The experiment was repeated twice and the same results observed as mentioned earlier.

Discussion

At the end of storage, samples washed for 90s reached similar O_2 levels and CO_2 levels with no significant differences among treatments (Figure 1). However samples washed for 180s reached O_2 levels and CO_2 levels with significant differences among treatments. More significantly, CL-180s showed the lowest O_2 and highest CO_2 values at the end is associated with electrolyte leakage, cut edge browning and microbial growth in the packages. Low O_2 MA packaging is widely used to slow down plant metabolism and respiration. For fresh-cut iceberg lettuce, recommended O_2 and CO_2 concentrations are 3% and 5-10%, respectively (Kader, 2002a). In this experiment, O_2 (6-10 kPa) levels in all treatments from day 3 to the end of storage were higher than recommended levels. There was no detectable off-odor in packaged fresh-cut iceberg lettuce during storage irrespective of washing solutions and contact time as off-odor is always associated the onset of an aerobic respiration under a lower O_2 and higher CO_2, which indicate that no aerobic respiration occurred (Figure 1). It has been reported that levels of O_2 below 2-4 kPa and over 10-15 kPa CO_2 induced anaerobic metabolism with generation of off flavor mainly due to ethanol, aldehyde, ethyl acetate, volatile compounds, ethylene associated compounds which are basically responsible for off-odor during the storage period either by living cells or by microbes present in the packaging bags. This might be due to the high oxygen transmission rate of the packaging film used in our investigation (Kim et al., 2004). Further the respiration rate of fresh-cut iceberg lettuce was low as per the gas analysis data. The rate of respiration is often a good indication of the storage life of a crop; higher the respiration rate, shorter the shelf life and vice versa (Figure 1).

During processing or handling of fresh-cut produce the common symptoms of the injury due to malfunction of membrane systems are water-soaked appearance, loss of turgor, leakage of electrolytes, and discoloration of tissues. The nutrients in leakage may also play a key role in the growth and development of pathogens. Electrolyte leakage is generally considered as an indirect measure of plant cell membrane damage (Bajji et al., 2002). Therefore, measurement of electrolyte leakage is ideal for commercially available fresh-cut vegetables. It is found that on day 0 higher electrolyte leakage observed with longer contact time irrespective of washing solution. It may be due to the washing time that washed off more effectively the cell sap from the cut edges of the samples and reactivity nature of the washing solutions used in our study. Furthermore, maintaining the equilibrium at the end of storage indicates the check in further damage during storage (Arvind et al., 2004; Wang et al., 2004) (Figure 3).

Fresh-cut produce are attractive and eye-catching to a large degree because of the richness of the pigment it contains, vital for quality maintenance.

In fresh-cut lettuce, cut edge browning commonly occurs during storage making unsuitable for consumers. The mechanism for cut edge browning involves the interaction of polyphenolic substrates with polyphenol oxidase (PPO) in the presence of oxygen. PPO catalyzes two reactions: (1) hydroxylation of monophenols to diphenols and (2) oxidation of diphenols to quinones.

The hydroxylation reaction is relatively slow and results in colorless products, while the oxidation reaction is relatively rapid and the resultant quinones are colored. Subsequent reactions of the quinones lead to melanin accumulation, which is the brown or black pigment associated with "browning" in plant tissues. Iceberg lettuce washed with chlorine for longer contact time (180s) showed the lowest cut edge browning index score among washing solutions (Figure 2). This indicates, chlorine washing had good effects on preventing cut edge browning of fresh-cut iceberg lettuce as studied earlier by some researchers (Jinhua et al., 2009; Baur et al., 2005; Fukumoto et al., 2002).

Except chlorine (either CL-180s or CL-90s) washing all other samples showed increasing aerobic and coliform plate count number linked to cut edge browning and electrolyte leakage which may be directly influenced by the microbial growth. Slightly, lower aerobic and coliform plate count number observed in CL-180s is due to the surface area of lettuce leaf and exposure/contact time. However, like ozone treatment in fresh-cut iceberg lettuce (Omlez and Akbas, 2009), it depends on the concentration and contact time of chlorine. This is due to the effectiveness of chlorine and its broad antimicrobial activity based on the type of material, the target microorganisms, physiological state of the bacteria cells at the time of treatment (Figures 4 and 5).

After washing, other washing solutions may control the further proliferation of microbes but chlorine washing had significantly lower number of both aerobic and coliform plate count (Figures 4 and 5) (Jinhua et al., 2009; Allende et al., 2008; Mandrell et al., 2006; Baur et al., 2005; Wang et al., 2004; Fukumoto et al., 2002).

Conclusion

Different washing solutions are applied to reduce the microbial spoilage microorganisms for the safety of fresh-cut produce. From our observation it can be concluded that longer contact time of chlorine washing is the best among washing solutions we have tried. Chlorine (100 $\mu L \cdot L^{-1}$) washing with longer contact time (180s) is good for maintaining the safety and microbial quality of fresh-cut iceberg lettuce. Hence, it is recommended that fresh-cut vegetables industries should adopt this for fresh-cut iceberg lettuce.

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