



## Comparison of different washing solutions and contact times on the quality maintenance and microbial reduction of fresh-cut iceberg lettuce

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### ABSTRACT

A 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 sec. and 180 sec. in normal tap water, chlorinated water (100 ml/l, pH 6.5), electrolyzed water (with free chlorine of 100 m/l, pH 7.2) or ozonated water (3 ml/l). Samples were then, dewatered, packaged in 30 µm polypropylene bags, and stored at 5°C for nine days. Various quality and safety parameters such as gas composition, off-odour, electrical conductivity, cut-edge browning and microbial numbers were evaluated during storage. Results revealed no detectable off odour in all samples during the storage period. However, significant differences in gas composition and electrolyte leakage were marked in samples washed for 180 sec. 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 sec. 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

The increasing public health concern related to the microbial safety of fresh-cut vegetables has focused on the efficiency of different methods for maintaining the quality and food safety of fresh-cut products (Das *et al.* 2011a, 2011b, Kim *et al.* 2010, Selma *et al.* 2008, Gómez-López *et al.* 2007). Simple practice of washing raw fruit and vegetables removes a portion of pathogenic and spoilage microorganisms, decreasing their initial levels and microbiological activity. Because microbes 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 (Das and Kim 2010a, b). 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 (Das *et al.* 2010, 2011a). 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 affecting the decision of consumer (Das *et al.* 2011a, b).

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 (Das *et al.* 2011a, 2011b, Kim *et al.* 2007). 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) (Rico *et al.* 2008). Furthermore, numerous studies on the effect of ozone treatment (Das and Kim 2010a, Das *et al.* 2011b; Hassenberg *et al.* 2007) 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

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leading to leakage of the cell contents (Das 2010). Again, ozone effectiveness against micro-organisms depends on the concentration and contact time of applied ozone (Das *et al.* 2011a, b; Olmez and Akbas 2009). So, to find out the best sanitizing method among tap water, chlorinated water, electrolyzed water, ozonated water with different contact times were evaluated on microbial quality and safety of fresh-cut iceberg lettuce during storage.

## MATERIALS AND METHODS

Chlorine solution (100 ml/l, pH 6.5) was prepared with food grade bleach and the solution was used immediately. EW solution (pH 7.2 with a residual chlorine concentration of 100 ml/l) was generated using by electrolyzed water system and used within one hour. Aqueous ozone solution was prepared by continuously circulating the water by ozone water sterilizing system and a stainless steel water tank. The concentration of dissolved ozone of 3 ml/l was determined by a dissolved ozone meter and was used very immediately. Fresh iceberg lettuce was obtained from local super market, Suwon, Korea. Samples with defectives in surface colour, 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) and 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 ml/l, pH 6.5), EW (with free chlorine of 100 ml/l, pH 7.2) and ozonated water (O<sub>3</sub>, 3ml/l) for contact time of 90 sec. and 180 sec. (TW-90s, TW-180s, CL-90s, CL-180s, EW-90s, EW-180s, O<sub>3</sub>-90s, O<sub>3</sub>-180s) individually and separately. The washed samples were dewatered (WS 6501 T, Hanil, Republic of Korea) for 1 min. and 100 g each were packaged in 30 µm polypropylene (PP) bags (20 × 25 cm) sealed and stored at 5° C for nine days. Sampling for quality evaluation was investigated on days 0, 3, 6, and 9. Changes in O<sub>2</sub> and CO<sub>2</sub> concentrations within packages were measured by a gas analyzer on evaluation days. 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 min., monitoring with a sensitivity of 0.001 (O<sub>2</sub>) and 0.1 (CO<sub>2</sub>). Three bags per treatment were evaluated for each experiment day. Off-odour 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 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. At least 3 readings per bag were taken to ensure that the data obtained truly represented the color of the samples. Electrical conductivity (EC) was quantified by immersing fresh-cut sample (20 g) in 400 ml of deionized water for for 30 min. at 18;É. The EC of the solution was measured using a conductivity meter (Orion 4 star portable pH/ conductivity meter. Samples of 20 g each were homogenized in 180 ml of 1% sterile buffered peptone water using filter stomacher bags for 60 sec. at 230rpm. One milliliter of the appropriate sample dilution was pour-plated separately on each coliform plate and aerobic plate and incubated at 25 °C for 24 hr and 48 hr respectively.

## RESULTS AND DISCUSSIONS

### Gas composition and off-odour

The O<sub>2</sub> partial pressure decreased and reached 6.4 to 10.2 kPa levels, whereas CO<sub>2</sub> level rapidly increased or maintained the equilibrium levels (6.0–7.5 kPa) after three days of storage (Fig. 1). Samples washed for 90s reached similar O<sub>2</sub> levels (8.9–9.5 kPa) and CO<sub>2</sub> levels (6.5–6.6 kPa) with no significant differences among treatments. However, samples washed for 180s reached O<sub>2</sub> levels (6.4–10.2 kPa) and CO<sub>2</sub> levels (6.0–7.5 kPa) with significant differences among treatments (Fig 1). More significantly, CL-180s showed the lowest O<sub>2</sub> and highest CO<sub>2</sub> values at the end. Low O<sub>2</sub> MA packaging is widely used to slow down plant metabolism and respiration. For fresh-cut iceberg lettuce, recommended O<sub>2</sub> and CO<sub>2</sub> concentrations are 3% and 5–10%, respectively (Kader 2002). In this experiment, O<sub>2</sub> (6–10 kPa) levels in all treatments from day 3 to the end of

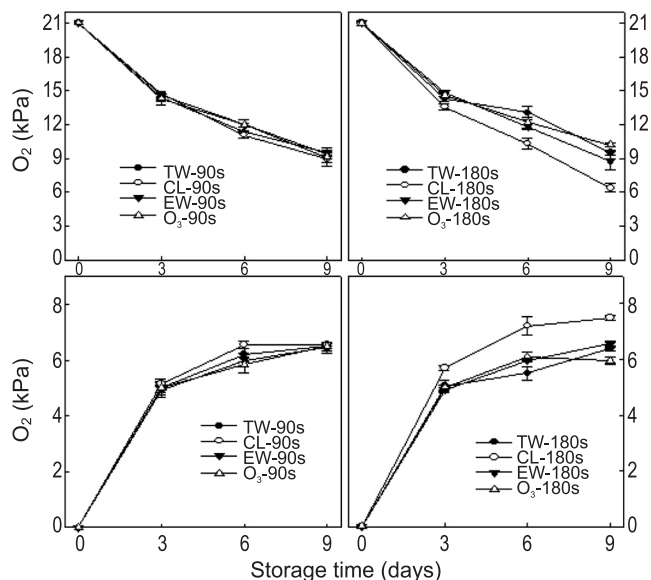


Fig 1 O<sub>2</sub> (top) and CO<sub>2</sub> (bottom). Composition in fresh-cut iceberg lettuce during storage period. Samples washed for 90s and 180s in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water (O<sub>3</sub>) respectively and separately. Data are the mean of three replications with ±SE

storage were higher than recommended levels. There was no detectable off-odour in packaged fresh-cut iceberg lettuce during storage, irrespective of washing solutions and contact time as off-odour 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 (Fig 1). It is 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-odour 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 the investigation (Das and Kim *et al.* 2010a, b; Das *et al.* 2011a,b, Kim *et al.* 2007). 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* (Fig 1).

#### Cut-edge browning

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: (i) hydroxylation of monophenols to diphenols and (ii) 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 coloured. Subsequent reactions of the quinones lead to melanin accumulation, which is the brown or black pigment associated with 'browning' in plant tissues. Samples washed for 180s observed lower degree of cut-edge browning index score than 90s washing on day 6 and day 9 (Fig 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 2). This indicates, chlorine washing had good effects on preventing cut-edge browning of fresh-cut iceberg lettuce as studied earlier by some researchers (Das *et al.* 2010a, Jinhua *et al.* 2009, Baur *et al.* 2005).

#### Electrolyte leakage

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 discolouration 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

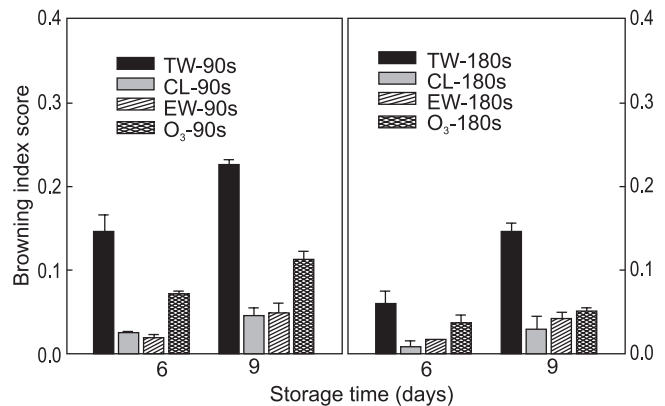


Fig 2 Cut-edge browning index of fresh-cut iceberg lettuce during storage period. Samples washed for 90s and 180s in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water ( $O_3$ ) respectively and separately. Discolouration was evaluated using a five-point scale based on the degree of discolouration 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  $\pm$  SE.

membrane damage. Therefore, measurement of electrolyte leakage is ideal for commercially available fresh-cut vegetables (Das *et al.* 2010a, b). Initially, the highest electrolyte leakage was observed in CL and EW washing, respectively either for 90 sec. or 180 sec. washing. Finally, all the treatments observed nearly equal value on the day9 of investigation (Fig 3). 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

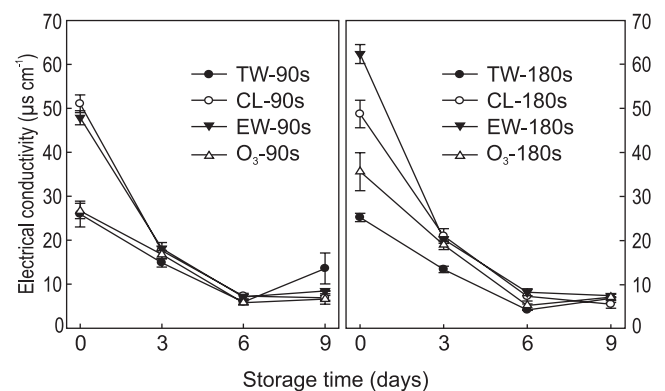


Fig 3 Electrical conductivity of fresh-cut iceberg lettuce during storage period. Samples washed for 90s and 180s in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water ( $O_3$ ) respectively and separately. Data are the mean of three replications with  $\pm$ SE

further damage during storage (Das *et al.* 2010a, 2011a, b) (Fig 3).

**Microbial growth**

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 (Figs 4, Fig 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 (Figs 4, Fig 5) (Das *et al.* 2011a, b, 2010a, b, Jinhua *et al.* 2009, Baur *et al.* 2005).

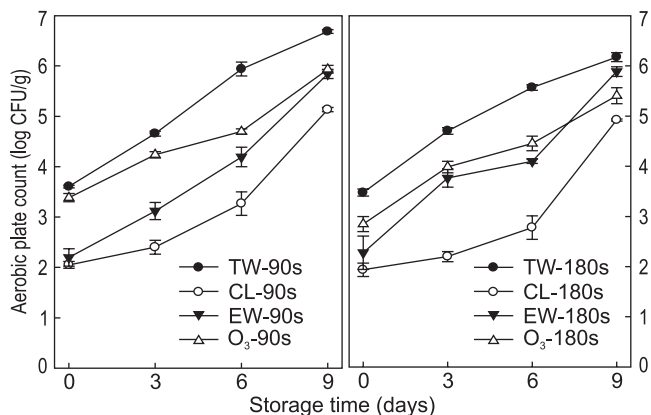


Fig 4 Aerobic plate count of fresh-cut iceberg lettuce during storage period. Samples washed for 90s and 180s in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water (O<sub>3</sub>) respectively and separately. Data are the mean of three replications with ± SE

To reduce the microbial spoilage of fresh-cut produce microorganisms for the safety Different washing solutions are applied. It is concluded that longer contact time of chlorine washing is the best among washing solutions we have tried. Chlorine (100 ml/l) 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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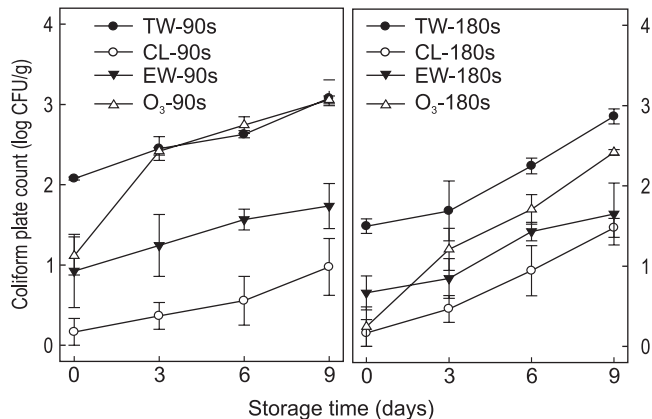


Fig 5 Coliform count in fresh-cut iceberg lettuce during storage period. In figure, samples washed for 90s and 180s in tap water (TW), chlorinated water (CL), electrolyzed water (EW) and ozonated water (O<sub>3</sub>) respectively and separately. Data are the mean of three replications with ± SE

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