Fresh-cut Produce Wash Water Reuse Affects Water Quality and Packaged Product Quality and Microbial Growth in Romaine Lettuce

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Abstract. Washing is a critical step for maintaining quality and safety of fresh-cut produce during its preparation and is often the only measure taken to reduce microbial populations and remove tissue fluids. However, little is known about the effect of washing method on water quality or its consequence on microbial growth and finished product quality. This study was conducted to evaluate the effect of wash water reuse on changes in water quality and its subsequent effect on microbial growth and product quality of packaged fresh-cut Romaine lettuce (*Lactuca sativa* L.). Romaine lettuce leaves were sliced and washed in water with chemical oxygen demand levels ranging from 9.8 mg·L⁻¹ (fresh water) to 1860.5 mg·L⁻¹ (reused water) and product-to-water ratios of 1:20 and 1:150. The washed samples were dried and placed into packages prepared from films with an oxygen transmission rate of 8.0 pmol·s⁻¹·m⁻²·Pa⁻¹ and stored at 5 °C for 14 days. Microbial growth and product quality were monitored at days 0, 4, 8, 11, and 14 during storage. Results indicate that as the quantity of lettuce dipped in 40 L of water increased from 2.0 kg to 18.0 kg, water chemical oxygen demand increased from 124 mg·L⁻¹ to 1721 mg·L⁻¹ and biological oxygen demand increased from 140 mg·L⁻¹ to 526 mg·L⁻¹, whereas free and total chlorine levels declined from 151.5 mg·L⁻¹ to 4.7 mg·L⁻¹ and from 171 mg·L⁻¹ to 31.5 mg·L⁻¹, respectively. Thoroughly washed lettuce in clean water with a small product-to-water ratio had the least off-odor development. Samples without wash treatment and those washed with reused water had 0.8 to 1.6 log cfu·g⁻¹ higher populations of lactic acid bacteria than those washed with clean water at the end of storage.

Over the past 20 years, increasing consumer demand for foods that are fresh, healthy, and convenient has stimulated rapid development of the fresh-cut produce industry. However, the tissue injury that occurs during preparation of fresh-cut produce renders these products more vulnerable to microbial growth and quality deterioration than the whole produce. Because fresh-cut products are marketed as “ready-to-eat” with the absence of a sterilization or pasteurization step, produce washing has become a critical process in the preparation of fresh-cut produce and is often the only step that removes foreign materials and tissue fluids, and reduces microbial populations (Simons,

2001; Simons and Sanguansri, 1997). Commercial operations usually use disinfectants such as chlorine, ozone, and chlorine dioxide in the wash water to increase the rate of microbial reduction and to prevent the potential crosscontamination of human pathogens, if present, during washing (Brackett, 1992; Sapers, 2003; Sinigalia et al., 2004). Studies have shown that the rate of microbial reduction during washing is influenced by many factors, including the efficacy of sanitizers on microbial inactivation (Gonzalez et al., 2004; Rodgers et al., 2004; Zhang and Farber, 1996), the mechanical force of washing (Richard and Cooper, 1995), the attachment of microorganisms to the produce surfaces or the internal tissues (Fatemi et al., 2006; Frank, 2001; Takeuchi et al., 2000), as well as their interactions. Although studies report that organic matter in the wash water depletes the free chlorine and reduces sanitizer efficacy of pathogen reduction (Beuchat et al., 1998; Garg et al., 1990; Nguyen-the and Carlin, 1994; Pirovani et al., 2004), few studies have investigated the effects of wash operation design on the quality changes in wash water or the consequences on product quality and safety (Adams et al., 1989). In

commercial fresh-cut operations, wash system configurations vary greatly, including modifications such as open flume and closed-flume systems, wash tanks, and so on. The fresh-cut products can be single-washed, double-washed, or triple-washed, or various wash and spray combinations may be implemented. The large operational cost of water use has resulted in the industry-wide common practice of reuse or recirculation of wash water. Our surveys conducted through various fresh-cut vegetable processors found that wash water quality deteriorated rapidly during produce washing as a result of the accumulation of cut produce tissue fluids, solids, and other foreign matters. Studies conducted by Gonzalez et al. (2004) and Ruiz-Cruz et al. (2007) on shredded carrots also showed a significant reduction in the efficacy of various sanitizers on pathogen inactivation when using simulated produce wash water with a chemical oxygen demand (COD) $\approx$ 3500 mg·L⁻¹ as opposed to fresh tap water. The main objectives of this study were to investigate the effect of reusing wash water on the changes of water quality, and the effect of water quality and the product-to-water ratio on the quality and microbial growth of packaged sliced Romaine lettuce.

Materials and Methods

Plant material. Romaine lettuce (*Lactuca sativa* L.) was purchased from a produce wholesale market (Jessup, MD) on the day of its arrival from the grower. The product was transported (within 30 min) under refrigerated conditions to the Product Quality and Safety Laboratory (Beltsville, MD) and used within 24 h after storage at 5 °C.

Washing operation on water quality. Fresh Romaine lettuce leaves were cut into $\approx$ 2.5 cm $\times$ 2.5-cm slices. The slices (2.0 kg) were contained in a nylon mesh bag (Linens N’ Things, Clifton, NJ) and dipped in 40 L of tap water with gentle agitation. The samples were removed from the water after 1 min and drained. This process was repeated for a total of nine times with fresh water added to replace the water lost during washing to simulate the commercial wash operation where numerous batches of freshly cut lettuce slices are washed in the same tank of water. Water quality was analyzed after each wash during the preliminary experiment. In the final experiment, water quality was evaluated after one, five, and nine washes or an equivalent of 2.0, 10.0, and 18.0 kg of produce washed in 40 L of water. The final experiment was repeated three times.

Chemical analyses. The changes in quality of the wash water were determined as follows: COD was determined by the reactor digestion method (HACH, 2002; Jirka and Carter, 1975), approved by the Environmental Protection Agency (EPA method 410.4) (EPA, 1993); biological oxygen demand (BOD) was determined according to Clescerl et al. (2005); the free and total chlorine were measured using a Chlorine Photometer (CP-15; HF Scientific Inc., Ft. Myers, FL);

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the pH was determined with a digital pH meter (Oakton Instruments, Vernon Hills, IL); conductivity was determined with a conductivity meter (model 135A; Orion Research Inc., Beverly City, MA); and brix was measured using a refractometer (PR-101; Spectrum Technologies, Plainfield, IL).

Wash water quality on product quality. Fresh Romaine lettuce leaves were cut into ≈ 2.5 cm $\times$ 2.5-cm slices and washed according to the following treatment conditions: fresh water/low ratio (FL)—2.0 kg of lettuce slices were washed in an industrial size washer (model MPW 800; Meyer Machine Co., San Antonio, TX) with a low product-to-water ratio at 1:150. The wash water contained ≈ 100 mg $\cdot$ L $^{-1}$ of sodium hypochlorite with pH adjusted to 6.5; fresh water/high ratio (FH)—2.0 kg of lettuce slices were washed in 40 L of tap water containing ≈ 100 mg $\cdot$ L $^{-1}$ of sodium hypochlorite with pH adjusted to 6.5 using HCl; reused water/high ratio (RH)—2.0 kg of lettuce slices were washed in 40 L of water with a COD level of ≈ 2000 mg $\cdot$ L $^{-1}$; the water was prepared by dipping 2.0-kg batches of sliced lettuce in the same tank of water until the water COD level reached a predetermined value (≈ 2000 mg $\cdot$ L $^{-1}$). The same amount of sodium hypochlorite as in the FH treatment was added to the wash water and the pH was adjusted to 6.5 using HCl. Water quality parameters, including COD, BOD, brix, and free chlorine, were measured before and after washing using the same method as described previously. Lettuce samples without any wash treatment (NW) were also prepared.

All washed samples were centrifuged using a salad centrifugal dryer (model T-304; Meyer Machine Co.) at 650 rpm (≈ 111 g) for 2.5 min to remove excess water. Romaine lettuce samples (140 g each) were transferred into packages (19 cm $\times$ 19 cm) prepared from film of 8.0 pmol $\cdot$ s $^{-1}$ $\cdot$ m $^{-2}$ $\cdot$ Pa $^{-1}$ oxygen transmission rate and the packages were flushed with N $_2$ to reach a 3.0-kPa initial O $_2$ partial pressure, sealed, and stored at 5 °C for 14 d.

Package atmosphere and product quality assessment. The partial pressures of O $_2$ and CO $_2$ within packages of fresh-cut Romaine lettuce were measured using a gas analyzer system (Combi Control 9800-1; PBI Dansensor Co., Ringsted, Denmark). All quality evaluations were performed in a temperature-controlled room at 5 °C to minimize the effect of temperature variation during testing. Tissue electrolyte leakage was determined by immersing 50-g samples of fresh-cut Romaine lettuce in 500-mL aliquots of distilled water at 5 °C for 30 min and inserting the probe of a conductivity meter (model 135A; Orion Research) into the sample solutions to measure electric conductivity. Total sample electrolyte levels were determined on the same sample after freezing at -20 °C for 24 h and then thawing. Electrolyte leakage (at 30 min) was then expressed as the percentage of total electrolytes (Kim et al., 2005).

Visual appearance of the packaged products was evaluated by a panel of three trained

personnel. The samples were coded with three-digit numbers to mask the treatment identity in an effort to minimize the test subjectivity and to ensure test accuracy. Off-odor was evaluated immediately after opening the packages and scored on a 5-point scale in which 0 = none, 1 = slight, 2 = moderate, 3 = strong, and 4 = severe. Overall visual quality was assessed with a 9-point hedonic scale in which 9 = like extremely, 7 = like

moderately, 5 = neither like nor dislike, 3 = dislike moderately, and 1 = dislike extremely (Kim et al., 2005; Meilgaard et al., 1991). In addition to samples stored at 5 °C for 14 d, the evaluation of visual quality was also performed on samples stored at 5 °C for 11 d followed by an additional 3 d at 10 °C to simulate the storage temperature often found in commercial settings where temperature abuse occurs.

Fig. 1. Changes in (A) wash water chemical oxygen demand (COD), biological oxygen demand (BOD), total dissolved solids (TDS); (B) salinity (Sal), brix, conductivity (Cond); and (C) free and total chlorine as influenced by the amount of lettuce slices dipped in the wash water. Each symbol is the mean of three replications; vertical lines represent SE. SE bars were not shown when masked by the symbol.

Microbial enumeration. Microbial growth on lettuce slices was assayed following a procedure from Luo et al. (2004) and Allende et al. (2004). The lettuce samples (30 g) were macerated for 2 min at 260 rpm in 270 mL of 0.1% sterile peptone water, pH 7.4, with a model 400 Laboratory Stomacher (Seward Medical, London, UK). The supernatant for each sample was filtered through sterile glass wool and serially diluted with peptone water. Appropriate dilutions were spread onto selected culture media plates with an Auto-plate Model 3000 Spiral Plater (Spiral Bio-tech, Bethesda, MD). Samples for total aerobic plate count (APC) were plated on tryptic soy agar (Difco Laboratory, Detroit, MI), yeast and molds on potato dextrose agar (Difco Laboratory) with chloramphenicol (200 mg·L⁻¹), and lactic acid bacteria (LAB) on Lactobacilli Man-Rogosa-Sharpe agar (Difco Laboratory). APC and yeast and mold plates were incubated at 28 °C for 2 d and 28 °C for 3 d at ambient air respectively, and LAB plates were incubated at 30 °C for 3 d under 20 kPa CO₂ and 5 kPa O₂ in a water-jacketed incubator with automatic gas control (Forma Scientific, Marietta, OH). Microbial colonies were counted using a Protos Colony Counter (model 50000; Synoptics Ltd, Cambridge, UK) and reported as log cfu·g⁻¹ of tissue.

Experimental design and statistical analyses. The experiment was conducted using a completely randomized design. A preliminary experiment was run before the experiment reported here. Data were analyzed as a two-factor linear model using the PROC MIXED procedure (SAS Institute, Cary, NC) with storage time and wash treatment as the factors.

Results and Discussion

Effect of wash operation on water quality.

The COD and BOD levels in the wash water increased significantly ($P < 0.0001$) as the weight of sliced lettuce dipped in the same tank of water increased (Fig. 1A). When the total weight of lettuce dipped in the water reached 18.0 kg, water COD and BOD levels reached to 1721.0 mg·L⁻¹ and 526.0 mg·L⁻¹, respectively. Total dissolved solids (TDS) increased in a more gradual pattern, changing from 520.0 mg·L⁻¹ to 719.0 mg·L⁻¹ when the total amount of sliced lettuce changed from 2.0 kg to 18.0 kg. Water salinity and conductivity increased rapidly with the increase in the amount of sliced lettuce dipped, whereas brix level increased gradually (Fig. 1B). The free chlorine and total chlorine levels in the water decreased rapidly, changing from 151.5 mg·L⁻¹ and 171.0 mg·L⁻¹ to 4.3 mg·L⁻¹ and 31.5 mg·L⁻¹, respectively, when the amount of lettuce dipped in the water increased from 2.0 kg to 18.0 kg (Fig. 1C). These findings indicate a rapid decline in water quality with the increase in the amount of sliced lettuce dipped in the same tank of water and are generally in agreement with the results from our surveys from a number of fresh-cut processors in which we found a

rapid increase in COD and BOD and a rapid decline in free chlorine in the wash water resulting from the repeated use of wash water with limited fresh water replenishment.

Effect of water quality on product quality and microbial growth. As shown in Figures 2

and 3, there were substantial increases in water COD, BOD, and brix and a decrease in free chlorine level after washing for all of the wash treatments. However, the magnitude of the changes in each parameter is dependant on different wash treatment. When

Fig. 2. Comparison of (A) chemical oxygen demand (COD); (B) biological oxygen demand (BOD); and (C) brix in the water before and after produce washing. RH = reused water/ high product-to-water ratio; FH = fresh water/high product-to-water ratio; FL= fresh water/low product-to-water ratio.

comparing FH and FL, water COD and BOD levels in FH increased more than 50 and five times after washing, respectively, whereas the COD and BOD levels in FL increased only 20- and onefold attributable to the much smaller product-to-water ratio in FL (1:150) than in FH (1:20) treatments. Similar changes in free chlorine levels were observed in both FH and FL treatments. Although the percent changes in all of the measured water quality parameters were generally smaller in RH than in FH treatment as a result of the large background values, the water quality was considerably poorer in the RH than in the FH treatment.

During storage, there was a significant ($P < 0.0001$) decrease in O_2 and increase in CO_2 in the headspace of the packaged lettuce with O_2 declining to 0.15 kPa and CO_2 reaching 13.8 kPa after 14 d of storage (Fig. 4). This finding is in agreement with our other packaged Romaine lettuce studies under a similar packaging configuration (Kim et al., 2005). However, there were no significant ($P > 0.05$) differences in O_2 or CO_2 among treatments, which suggests that the wash water quality did not significantly impact the respiration rate of the produce.

All treatments maintained good visual quality of the lettuce samples between d 0 and 4 (Fig. 5A). Product quality started to decline between d 4 and 8 and continued to decline throughout the remaining storage period. Among all treatments, RH-washed samples had the lowest visual quality at the end of storage. Unwashed samples maintained a good appearance because of lack of tissue damage from washing and drying. However, after samples were stored at 10 °C for 3 d after 11 d at 5 °C, the visual appearance of unwashed lettuce deteriorated significantly, whereas the visual quality of FH- and FL-washed samples only declined slightly (data not shown).

All samples had trace amount of off-odor after 8 d of storage (Fig. 5B). Unwashed and RH-washed samples had more rapid increases in off-odor than the samples washed with FH and FL. In addition to the intensity of off-odor, the panelists also noticed the variations of off-odors among those samples. Unwashed and RH-washed samples tended to have more silage or sour odor, whereas FH- and FL-washed samples had sweeter, more fruity aromas. Lopez-Galvez et al. (1997) compared the quality of fresh-cut lettuce prepared from several major commercial processors and found that the off-odor was different from the different processors. Observations made at the processing plants revealed large differences in wash system designs and water reuse practices. The findings reported in this current study may explain the differences in the samples from different processors reported by Lopez-Galvez et al. (1997).

Percent tissue electrolyte leakage differed significantly over time ($P < 0.0001$) and among treatments ($P < 0.05$) (Fig. 6). From d 0 to d 4, there was a large decrease in percent tissue electrolyte leakage in all of the

treatments. Samples without washing had the lowest electrolyte leakage percentage between d 0 and 4 among all treatments. However, from d 4 to d 14, percent tissue

electrolyte leakage in this treatment increased rapidly, reaching more than 40% higher than the FH and FL treatments at the end of 14-d storage. Samples washed with

Fig. 3. Comparison of free chlorine in the water before and after produce washing. RH = reused water/high product-to-water ratio; FH = fresh water/high product-to-water ratio; FL = fresh water/low product-to-water ratio.

Fig. 4. Effect of wash treatment on the changes of (A) oxygen partial pressure and (B) carbon dioxide partial pressure of packaged fresh-cut Romaine lettuce stored at 5 °C. Each symbol is the mean of three replications; vertical lines represent SE. SE bars were not shown when masked by the symbol. NW = no wash; RH = reused water/high product-to-water ratio; FH = fresh water/high product-to-water ratio; FL = fresh water/low product-to-water ratio.

Fig. 5. Effect of wash treatment on the changes of (A) off-odor development and (B) visual appearance of packaged fresh-cut Romaine lettuce stored at 5 °C. Each symbol is the mean of three replications; vertical lines represent SE. SE bars were not shown when masked by the symbol. NW = no wash; RH = reused water/high product-to-water ratio; FH = fresh water/high product-to-water ratio; FL = fresh water/low product-to-water ratio.

Fig. 6. Effect of wash treatment on the changes of electrolyte leakage of packaged fresh-cut Romaine lettuce stored at 5 °C. Each symbol is the mean of three replications; vertical lines represent SE. SE bars were not shown when masked by the symbol. NW = no wash; RH = reused water/high product-to-water ratio; FH = fresh water/high product-to-water ratio; FL = fresh water/low product-to-water ratio.

RH, FH, and FL had similar electrolyte leakage on d 0 and 4. However, after 8 d in storage, the electrolyte leakage in the RH treatment increased sharply and reached a level (6.7%) similar to the NW treatment at the end of storage. Contrary to the sharp increase in electrolyte leakage in both NW and RH treatments between d 8 and d 14, the increase in electrolyte leakage was more gradual and moderate in the FH treatment, and there was only a slight increase in the FL treatment. After 14-d storage, electrolyte leakage in the FL treatment remained the smallest (4.6%) among all treatments.

Tissue electrolyte leakage level is generally considered as an indirect measure of plant cell membrane damage (Jiang et al., 2001; Marangoni et al., 1996; Murata, 1989) and has been shown to correlate well with visual quality assessments of packaged fresh-cut fruits and vegetables (Fan and Sokorai, 2002; Kim et al., 2005; Luo et al., 2004; Portela and Cantwell, 2001). Loss of membrane integrity results in an increase in ion leakage (Jiang et al., 2001). The decrease in electrolyte leakage observed from d 0 to d 4 is partly the result of the tissue recovery process from the tissue injury incited by cutting, washing, and centrifugal drying. Similar trends were observed for packaged fresh-cut cilantro (Luo et al., 2004) and lettuce (Kim et al., 2005). The NW treatment had the lowest electrolyte leakage on d 0 and d 4, probably as a result of the lack of tissue injury from the absence of washing and drying steps in this particular treatment. The highest increase observed from d 8 to d 14 in both NW and RH treatments suggested a rapid quality loss in these treatments, which concurs with the strongest off-odor detected in these two treatments at the end of storage (Fig. 5B). The lowest electrolyte leakage observed in FL followed by FH suggests that FL- and FH-washed samples maintained the first and second greatest tissue integrity at the end of storage, respectively. This analysis is supported by the least off-odor development noticed in these treatments.

Microbial growth. Aerobic plate count was significantly ($P < 0.001$) lower (a difference of 0.6 to 0.9 log cfu·g⁻¹) for all washed samples (RH, FH, and FL) than for unwashed samples on d 0 and d 4, but this difference diminished after 4 d of storage (Fig. 7A). APC increased significantly for all treatments over time ($P < 0.001$). Yeast and mold growth remained stable from d 0 through d 11 and increased after d 11. Unwashed samples exhibited significantly ($P < 0.001$) more yeast and mold growth than wash treatments until d 11, but the difference diminished by d 14 (Fig. 7B).

Lactic acid bacterial populations were below the detection level on d 0, but grew significantly ($P < 0.0001$) during storage (Fig. 7C). LAB on samples that received NW and RH treatments grew rapidly during storage, reaching a population of over 4.0 log cfu·g⁻¹ on d 4 and over 6.0 log cfu·g⁻¹ at the end of storage. On the contrary, LAB on FH- and FL-treated samples grew at a

Fig. 7. Effect of wash treatment on the changes of (A) aerobic plate count, (B) yeast and mold count, and (C) lactic acid bacterial count on packaged fresh-cut Romaine lettuce stored at 5 °C. Each symbol is the mean of three replications; vertical lines represent SE. SE bars were not shown when masked by the symbol. NW = no wash; RH = reused water/high product-to-water ratio; FH = fresh water/high product-to-water ratio; FL = fresh water/low product-to-water ratio.

significantly ($P < 0.0001$) slower rate than the NW and RH samples, and the population remained $0.8 \log \text{cfu} \cdot \text{g}^{-1}$ (comparing RH with FH) to $1.6 \log \text{cfu} \cdot \text{g}^{-1}$ (comparing NW with FL) lower at the end of storage. Allende et al. (2004) also reported significant growth of LAB on baby spinach. LAB is a group of Gram-positive, acid-tolerant, and lactic acid-producing bacteria. In general, LAB grow

well in an environment that is rich in carbohydrates, low in O_2 , and high in CO_2 . In this study, all samples developed anaerobic conditions toward the end of storage, as indicated by the low O_2 and high CO_2 in all samples, which satisfied the anaerobic conditions required for LAB growth. However, lettuce samples washed with RH water had more nutrients remaining on the surface of the

leaves as indicated by the higher brix readings in the washed samples. NW samples had lettuce fluids remaining on the cut surface resulting from the lack of a wash treatment. As reported by Mundt et al. (1966), the tissue fluids from vegetables during processing support the growth of LAB. Therefore, the higher level of nutrient remaining on the surface of NW and RH samples may have contributed to the more rapid growth of LAB in these samples than in FH- and FL-washed samples. The correlations between the growth of LAB and off-odor development on those samples may suggest that LAB growth may have contributed to the development of off-odor under the testing conditions. This is supported by the reports that LAB are associated with the silage fermentation of grass, beans, and so on (Cai et al., 1994). Similar results were obtained with shredded carrots in which fermentation was closely associated with the growth of LAB on samples without adequate washing (data not shown). On the contrary, FH- and FL-washed samples may have had less nutrients remaining on the lettuce surface, resulting in reduced LAB growth on these treatments than on RH and NW samples. The trend in LAB growth is also consistent with the trend in electrolyte leakage. However, the changes in visual appearance of the lettuce more greatly reflected the damage caused in washing and drying the lettuce than the changes in either electrolyte leakage or LAB growth. The NW samples maintained the best visual appearance throughout storage as shown in Figure 5, but deteriorated more rapidly at 10 °C. The RH samples did show the greatest decline in visual appearance. These results suggest that LAB growth did not make a major contribution to the changes in visual appearance when packaged sliced lettuce was stored at 5 °C for 14 d.

Conclusions

Repeated use of the same tank of water to wash lettuce slices caused a rapid deterioration of water quality as indicated by the rapid increase in COD and BOD, a gradual increase in salinity, TDS, and brix and a dramatic decline in free chlorine level. The changes in water quality had a significant impact on the finished product quality and microbial growth. Samples washed with reused water with a COD level of $1860.5 \text{ mg} \cdot \text{L}^{-1}$ or those without washing had significantly more LAB growth than those washed with clean water. Although wash water quality had limited impact on the visual appearance of the finished products within 14 d storage at 5 °C, it significantly decreased lettuce tissue integrity and increased off-odor development. It is therefore important for the processors to closely monitor and maintain the quality of wash water during fresh-cut produce processing operations to effectively reduce microbial growth and improve product quality. Frequently changing wash water, instituting a wash-spray combination or a prerinsing before washing to remove the majority

of the tissue fluids should aid in the reduction of COD levels in the wash water and maintain better water quality as well as product quality.

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