Effects of Pre- and Postharvest Calcium Treatments on Shelf Life and Postharvest Quality of Broccoli Microgreens

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Abstract. We reported previously that the preharvest treatment of broccoli microgreens with 10 mmol·L⁻¹ calcium chloride (CaCl₂) increased the yield and postharvest quality. The objective of this study was to investigate whether other calcium forms have the similar effect, in particular, after postharvest dip in calcium solution. Our results are as follows: 1) Preharvest spray without postharvest dip: Both 20 mmol·L⁻¹ calcium lactate (Ca lactate) and calcium amino acid (Ca AA) chelate significantly improved broccoli microgreens quality and inhibited microbial populations as compared with the water-only control during storage at 5 °C for 21 days. However, they were less effective than 10 mmol·L⁻¹ CaCl₂. 2) Postharvest dip without preharvest spray: The microgreens sprayed with water-only control were dipped in 0, 25, 50, or 100 mmol·L⁻¹ Ca lactate solution containing 100 µL·L⁻¹ chlorine immediately after harvest. During storage at 5 °C for 14 days, 50 mmol·L⁻¹ Ca lactate dip showed the highest overall quality and lowest tissue electrolyte leakage. 3) Preharvest spray and postharvest dip: Combined preharvest 10 mmol·L⁻¹ CaCl₂ spray and postharvest 50 mmol·L⁻¹ Ca lactate dip resulted in better postharvest quality than individual pre- or postharvest calcium treatments. However, the preharvest 10 mmol·L⁻¹ CaCl₂ spray without postharvest dip displayed a best overall visual quality and longest storage life. Our data indicate that pre- and postharvest calcium treatments have positive effect on maintaining the microgreens quality and extending shelf life. However, current postharvest dip/spinning/drying method profoundly reduces the shelf life due to mechanical damages. Technologies to optimize microgreens wash are needed to provide ready-to-eat product. Alternatively, the wash step can be avoided when the microgreens are grown under controlled settings.

Microgreens are cotyledonary-leaved seedlings harvested within 10–20 d after vegetable seed germination. In recent years,

growing microgreens have become a more common practice for urban farming because of their easiness to handle indoors, and their high nutritional value and sensory appeal (Kou et al., 2013, 2014a; Xiao et al., 2012). However, their commercial production and marketing is limited by their short shelf life due to rapid quality deterioration (Berba and Uchanski, 2012; Chandra et al., 2012; Kou, et al., 2013). Furthermore, since fresh-cut products are marketed as “ready-to-eat” with no need for a sterilization or pasteurization step, wash is a critical process in the preparation of fresh-cut produce and is often the only step. Wash removes foreign materials as well as tissue fluids and reduces microbial

populations (Simons, 2001). To provide more affordable ready-to-eat microgreens to a broader market, it is necessary to develop chlorine wash methods, which can maintain postharvest quality and extend shelf life.

Calcium is important for plant growth and development by maintaining and modulating various cellular functions (Conway et al., 2002; Palta, 1996). Calcium alters intracellular and extracellular processes, resulting in retarded ripening as exemplified by lower rates of color change, softening, and CO₂ and ethylene production, increase in sugar, and reduction in total acid content (Conway, 1987; Manganaris et al., 2005; Raese and Drake, 1993). The pre- and postharvest application of calcium salts has been used successfully in many fresh fruits to reduce loss of firmness and slow down the ripening process (Floros et al., 1992; Holb et al., 2012; Mohammed et al., 1991; Saftner et al., 1998; Souty et al., 1995). CaCl₂ has been primarily used for preharvest treatment. When it is used in fresh-cut products, it may cause a bitter aftertaste in foods (Bolin and Huxsoll, 1989). However, Ca lactate treatment does not show negative effect on flavor. Therefore, Ca lactate has been suggested as a potential alternative firming additive for use in fresh-cut fruits (Luna-Guzmán and Barrett, 2000; Yang and Lawsless, 2005). Martín-Diana et al. (2005) also compared the efficacies of Ca lactate and chlorine wash treatments of fresh-cut lettuce and carrots during storage at 4 °C over 10 d and found that there was no significant differences between treatments. Ca AA chelate formulations represent another Ca source that has been used in the food and/or nutritional industries (Saftner et al., 2003). Ca AA chelate is not corrosive to processing equipment and is more likely to penetrate deeply into plant tissues. A postharvest Ca AA dip maintained firmness and doubled the shelf life of intact honeydew fruit (Lester and Grusak, 2001, 2004). Currently, the potential for use of Ca lactate and Ca AA chelate in the microgreens industry has not been explored. Compared with fruits and mature green leaves, fresh-cut microgreens are very tender and subject to much more stress, leading to rapid senescence and a very short shelf life (Watkins and Nock, 2012). For example, the shelf life of broccoli microgreens (*Brassica oleracea* L. var. *italica*) is 7–10 d at 5 °C. However, if treated by 10 mmol·L⁻¹ CaCl₂ before harvest, the shelf life can be extended to 14–21 d due to stimulated superoxide dismutase and peroxidase activities, lowered tissue electrolyte leakage, improved overall visual quality, and reduced microbial growth during storage (Kou et al., 2014a). A chemical composition comparison showed that glucosinolates, a very important group of phytochemicals, were the major compounds enhanced by preharvest treatment with 10 mmol·L⁻¹ CaCl₂ (Sun et al., 2015). This study compares the effects of preharvest spray with CaCl₂, Ca lactate, or Ca AA chelate, and a postharvest dip in Ca lactate on the quality and shelf life of broccoli microgreens.

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Materials and Methods

Plant materials. Broccoli (*Brassica oleracea* var. *italica*) cultivar Arcadia seeds were purchased from Living Whole Foods, Inc. (Springville, UT). Hydroponic pads (20.8 × 25.4 cm; Growers Supply, Dyersville, IA) were made from biodegradable wood fibers. One hydroponic pad was set evenly in one 54 × 28 × 6 cm tray (vacuum-formed standard 1020 open flats without holes). The pad was soaked in the 600 mL tap water (pH 5.5–6.0). The seeds (≈37.8 g) were spread evenly on the wet pad. The trays were kept in the dark in a growth chamber at 25 °C for the first 4 d, and then exposed to light with an irradiance of 42 μmol·s⁻¹·m⁻², of 12 h/12 h (light/dark) for the next 6 d (Kou et al., 2013).

Preharvest calcium treatments. The trays were sprayed once a day with ≈200 mL H₂O (tap water, pH 5.5–6.0) only; 1, 10, or 20 mmol·L⁻¹ Ca AA (Albion Laboratories, Inc., Clearfield, UT); 1, 10, or 20 mmol·L⁻¹ Ca lactate; or 10 mmol·L⁻¹ CaCl₂ (Sigma-Aldrich, Inc., St. Louis, MO) after sowing the seeds (Table 1). Ten-day-old broccoli microgreens were harvested with a pair of sterilized scissors by cutting at the bottom of the hypocotyls. No damaged leaves were used for analyses. The broccoli microgreens (10 g each) were packaged in sealed bags (10 cm × 10 cm) prepared with polyethylene films (Pacific Southwest Container Inc., Modesto, CA) of 16.6 pmol·s⁻¹·m⁻²·Pa⁻¹ oxygen transmission rate. Samples were stored at 5 °C in the dark for 21 d, with quality evaluation performed on Days 0, 4, 7, 14, and 21.

Postharvest calcium treatments. Ten-day-old seedlings treated with water-only during preharvest were moved to the washroom across the aisle. Dip solutions contain 0, 25, 50, or 100 mmol·L⁻¹ Ca lactate plus 100 μL·L⁻¹ chlorine (sodium hypochlorite, pH 6.5) (Table 1). The freshly cut microgreens (200 g) were placed in predisinfected mesh bags, washed in 40 L dip solutions with gentle agitation for 30 s at room temperature, then centrifuged at 300 rpm for 3 min with a commercial salad centrifugal dryer (model T-304, Garroute Spin Dryer; Meyer Machine Co, San Antonio, TX) to remove excess water. The washed microgreens were packaged in 10 g amounts in each bag and stored at 5 °C in the dark for 14 d. Evaluations were performed on Days 0, 4, 7, 11, and 14.

Pre-/postharvest calcium treatments. The following combinations of pre- and postharvest calcium treatments were performed (Table 1). H₂O/Cl meant preharvest spray with water only and postharvest dip in 100 μL·L⁻¹ chlorine solution; H₂O/Ca lactate indicated preharvest water spray and postharvest dip in 50 mmol·L⁻¹ Ca lactate and 100 μL·L⁻¹ chlorine solution; CaCl₂/Cl stood for preharvest 10 mmol·L⁻¹ CaCl₂ spray and postharvest dip in 100 μL·L⁻¹ chlorine solution; CaCl₂/Ca lactate represented preharvest 10 mmol·L⁻¹ CaCl₂ spray and postharvest dip in 50 mmol·L⁻¹ Ca lactate with 100 μL·L⁻¹ chlorine solution. Microgreens (200 g) were

Table 1. List of all the treatments.

Treatment	Preharvest spray	Postharvest dip in chlorinated water
Preharvest treatment	Water only 10 mmol·L ⁻¹ CaCl ₂ 1 mmol·L ⁻¹ Ca lactate 10 mmol·L ⁻¹ Ca lactate 20 mmol·L ⁻¹ Ca lactate 1 mmol·L ⁻¹ Ca AA 10 mmol·L ⁻¹ Ca AA 20 mmol·L ⁻¹ Ca AA	No postharvest dip
Postharvest treatment	Water only	No calcium 25 mmol·L ⁻¹ Ca lactate 50 mmol·L ⁻¹ Ca lactate 100 mmol·L ⁻¹ Ca lactate
Pre/postharvest treatment	H ₂ O/Cl H ₂ O/Ca lactate CaCl ₂ /Cl H ₂ O/Ca lactate	Water only 10 mmol·L ⁻¹ CaCl ₂ No calcium 50 mmol·L ⁻¹ Ca lactate No calcium 50 mmol·L ⁻¹ Ca lactate

CaCl₂ = calcium chloride; Ca lactate = calcium lactate; Ca AA = calcium amino acid.

Fig. 1. Effects of preharvest spray with different calcium forms on (A) O₂ and (B) CO₂ partial pressure within packages of broccoli microgreens during 5 °C storage. Data presented are the means of four replications; vertical lines represent SES.

placed in mesh bags, and then washed in 40 L wash solutions with gentle agitation for 30 s. The microgreens (in mesh bags) were centrifuged at 300 rpm for 3 min with a commercial salad centrifugal dryer (model T-304, Garroute Spin Dryer; Meyer Machine Co) to remove excess water. The washed microgreens were packaged in 10 g amounts in each bag. Packaged microgreens were stored at 5 °C in the dark for 14 d and evaluations were performed on Days 0, 4, 7, 11, and 14.

Postharvest quality and microbiological assessment. The package atmospheres were measured immediately upon removal of the samples from storage. The CO₂ and O₂ in the headspace of packages containing microgreens were measured as described in Kou et al. (2014a).

Overall visual quality was evaluated with a 9-point hedonic scale following a modified procedure from Luo et al. (2004) and Meilgaard et al. (1991), where 9, 8, 7, and 6 = like extremely, strongly, moderately, and slightly,

respectively; 5 = neither like nor dislike; and 1, 2, 3, and 4 = dislike extremely, strongly, moderately, and slightly, respectively. All visual quality evaluations were carried out by three trained evaluators. All evaluators had over 5-year research experience with fresh produce, especially performing sensory evaluations of leafy greens. Before the beginning of this experiment, additional training specific to the organoleptic properties of broccoli microgreens were provided to evaluators. The acceptable range for overall visual quality was considered to be a score of 6.0 or above (Kou et al., 2014b).

The electrical conductivity of the solution was measured using the same method as described in Kou et al. (2014a). Briefly, 3 g microgreens were submerged in 150 mL deionized water at 20 °C and shaken for 30 min. The electrolyte of the solution was measured using a conductivity meter (model 135A; Orion Research, Inc., Beverly, MA). Total electrolytes were obtained after freezing the samples at -20 °C for 24 h and subsequent thawing, and expressed as a percentage of the total electrolyte. Microbial growth on broccoli microgreens was assayed following a procedure from Luo et al. (2004) with some modifications. Each 3 g microgreens was macerated in 27 mL phosphate-buffered saline, using a model 80 Laboratory Stomacher (Seward Medical, London, UK) for 2 min at high speed in filtered stomacher bags. A 50 µL sample of each filtrate or its appropriate dilution was logarithmically spread on agar plates with an automated spiral plater (Wasp II; Don Whitley Scientific Ltd., West Yorkshire, UK). Enumeration of microorganisms was performed using the following culture media and conditions: 1) tryptic soy agar (Difco Laboratory, Sparks, MD) incubated at 28 °C for 24 h for the enumeration of total aerobic mesophilic bacteria (AMB) and 2) potato dextrose agar (Difco Laboratory) supplemented with 200 g·mL⁻¹ chloramphenicol incubated at room temperature (22 °C) for 48 h for the enumeration of yeasts and molds (Y&M). Microbial colonies were counted using a ProtoCOL Colony Counter 50000 (Synoptics, Cambridge, UK) and reported as Log cfu/g (Log colony-forming unit per gram tissue).

Experimental design and statistical analysis. Package atmospheres, tissue electrolyte leakage, and microbial data were analyzed as two-factor linear models using the PROC MIXED procedure (SAS Institute Inc., 1999, Cary, NC). The two factors were storage time and treatment type. Different samples were analyzed on each evaluation day for all studies. Four replications (four bags) per treatment per evaluation period were examined. All the experiments were repeated three times. At each time, we had four technical repeats. Data presented are the results from one representative experiment. Assumptions of normality and variance homogeneity of the linear model were checked and the variance grouping technique was used to correct for variance heterogeneity. When effects were statistically significant,

means were compared using Sidak adjusted *P* values to maintain experiment-wise error ≤0.05.

Results and Discussion

Effects of different preharvest calcium treatments on the postharvest quality of broccoli microgreens. From Day 0 to 4, oxygen partial pressures in all the samples decreased rapidly (Fig. 1A), nearly reaching equilibrium by Day 4. Water-only control exhibited the lowest O₂ level from Day 4 to 21. All the calcium treatments had a slightly higher yet constant O₂ (1–2.2 kPa) until the end of storage. In comparison, CO₂ partial pressure for all treatments increased during the first 4 d, and then declined to ≈3.7 kPa. No significant difference was observed among all the calcium treatments and water-only control (Fig. 1B). These results suggest that the preharvest calcium treatments had no significant effect on broccoli microgreens' respiration during cold storage. Other studies also show that under low temperature storage, calcium treatment did not affect the respiration rate on lettuce, carrot, 'Vogue' cherry, and apple (Duque and Arrabaca, 1999; Martín-Diana et al., 2005; Tsantili et al., 2007).

Total AMB growth of all samples increased significantly (*P* < 0.001) over storage time (Fig. 2A). However, on Day 21, the numbers of total AMB in calcium treatments were lower than water-only control. In particular, 10 mmol·L⁻¹ CaCl₂-treated samples had significantly (*P* < 0.05) lower total AMB (8.9 Log cfu/g) growth than those sprayed with H₂O (9.9 Log cfu/g) at Day 21. Nonetheless, no significant (*P* > 0.05) difference was found among all other calcium treatments. Several calcium treatments also displayed significantly inhibitory effect on Y&M growth (Fig. 2B). Among these treatments, 10 mmol·L⁻¹ CaCl₂ treatment was the most effective. On Day 21, 10 mmol·L⁻¹ CaCl₂-treated samples averaged 1.2 Log cfu/g (*P* < 0.05) fewer Y&M colonies than the water-only treatment. 1 and 20 mM Ca lactate also had obvious inhibitory effect at Day 21.

We further measured tissue electrolyte leakage because it was closely related to the tissue integrity and shelf life of fresh-cut produce (Allende et al., 2004; Kou et al., 2013). All the calcium-treated samples had lower tissue electrolyte leakage (*P* < 0.05) than water-only control samples during the entire storage period (Fig. 3A). Samples treated with higher calcium concentrations had significant lower tissue electrolyte leakage than those treated with low concentration (1 mmol·L⁻¹). At Day 21, the highest tissue electrolyte leakage (18.75%) occurred in water-only treated samples, whereas 10 mmol·L⁻¹ CaCl₂ treatment had the lowest tissue electrolyte leakage values of 3.56%. Tissue electrolyte leakage in 20 mmol·L⁻¹ Ca lactate, 10 mmol·L⁻¹ Ca lactate, 20 mmol·L⁻¹ Ca AA and 10 mmol·L⁻¹ Ca AA were 3.97%, 4.00%, 4.02%, and 10.4%, respectively. Calcium application has shown to increase

membrane integrity and stability, and decrease electrolyte leakage (Esmel et al., 2004; Poovaiah, 1986). Calcium may also be involved in regulating membrane stability and the senescence of plant cells (Rubinstein, 2000; Torre et al., 1999). Less disruption in plasma membranes led to lower tissue electrolyte leakage (Meng et al., 2009). Therefore, preharvest calcium spray might increase broccoli membrane integrity and reduce tissue electrolyte leakage.

Overall quality is an important factor influencing the marketability of food products. All calcium-treated samples retained superior quality over water-treated samples from Day 7 onwards (Fig. 3B). From Day 14 onwards, yellowing leaves and moisture accumulation were observed on broccoli microgreens, which resulted in reduced quality scores. On Day 21, the overall quality scores in all calcium-treated samples declined to 5.2–6.3. The 10 mmol·L⁻¹ CaCl₂-treated samples had the highest overall visual quality, especially from Day 14 to 21. On Day 21, 20 mmol·L⁻¹ Ca lactate also maintained a better overall quality score (scores of 5.7), while water-treated seedlings had the lowest overall quality (score of 2.9). The results indicated that the preharvest treatment with all three forms of calcium had a positive effect on postharvest quality and prolonged shelf life of microgreens. The overall quality results agreed well with those from tissue electrolyte leakage, suggesting that the loss of visual appeal was related to senescence.

Effect of postharvest calcium lactate wash/dip on the quality of broccoli microgreens. Since preharvest treatment with Ca AA was least effective, we only selected Ca lactate for postharvest treatments. After dipping, spinning, and drying, the shelf life for all samples was reduced to 14 d from 21 d. This could have been resulted from the tissue physical damage to the tissue during spinning and drying because the microgreens were very tender. During the first 3 days of storage after treatments, the headspace O₂ concentration of all the samples dropped rapidly to under 1 kPa, and slowly dropped to near 0 at the end of storage (Fig. 4A). However, the CO₂ level increased rapidly during the first 3 d, followed by a rapid decrease, then maintained a constant level (≈4 kPa) of CO₂ (Fig. 4B). No significant differences were observed between the dip in Ca lactate and dip in chlorinated water only. These results suggest that postharvest calcium treatment had no significant effect on O₂ depletion and CO₂ evolution rates for broccoli microgreens.

Significant (*P* < 0.05) differences were detected among dip treatments with different Ca lactate concentrations for tissue electrolyte leakage and overall quality (Fig. 5A and B). Broccoli microgreens treated with 50 mmol·L⁻¹ Ca lactate maintained the lowest tissue electrolyte leakage (4.2%) throughout the 14-day storage period and had the highest overall quality score (6.0) on Day 14. Water-treated samples had significantly (*P* < 0.01) higher tissue electrolyte leakage and lower overall quality than all calcium treated ones. These

Fig. 2. Effects of preharvest spray with different calcium forms on (A) aerobic mesophilic bacteria (AMB) and (B) yeast and mold (Y&M) populations of packaged broccoli microgreens during 5 °C storage. Data presented are the means of four replications; vertical lines represent ses.

Fig. 3. Effects of preharvest spray with different calcium forms on (A) tissue electrolyte leakage and (B) overall quality of packaged broccoli microgreens during 5 °C storage. Data presented are the means of four replications; vertical lines represent ses.

results suggest that 25 mmol·L⁻¹ is insufficient to act and 100 mmol·L⁻¹ is likely toxic for microgreens. Fifty mmol·L⁻¹ Ca lactate dip had a positive effect on extending the shelf life and keeping the lower tissue electrolyte leakage. However, in general, the spinning and drying after dip dramatically accelerated tissue senescence and quality deterioration.

Effect of combined pre-/postharvest calcium treatment on the quality of broccoli microgreens. We further tested the effect of combining preharvest 10 mmol·L⁻¹ CaCl₂ and postharvest 50 mmol·L⁻¹ Ca lactate treatments on the quality of microgreens. During the entire 14-day storage period, no significant differences were found in the changes in O₂ and CO₂ composition in packages between any of the treatments (Fig. 6A and B). These results were in agreement with those observed for the separate pre- and postharvest calcium treatments (Figs. 1 and 4).

AMB populations for all the treatments increased significantly ($P < 0.001$) during storage (Fig. 7A). Overall, there was no significant difference between any of the different H₂O or calcium dip treatments. In comparison, samples with preharvest CaCl₂ spray but no postharvest treatment had significantly ($P < 0.01$) lower bacterial populations than those receiving other pre-/postharvest treatments. These results suggest that the increased bacterial populations resulted from the tissue damage and/or contamination during postharvest dip.

Similar to AMB populations, Y&M populations had an increasing trend during the storage (Fig. 7B). From Day 0 to 7, Y&M populations maintained stable levels (5.2–6.0 Log cfu/g). However, from Day 7 to 14, there was a rapid increase in Y&M populations for all the treatments. The preharvest treatments with CaCl₂ without postharvest treatment had significantly ($P < 0.01$) lower Y&M populations than all other combinations of pre-/postharvest calcium treatments. Lee et al. (2009) reported a similar result for ‘Tah tasai’ Chinese cabbage microgreens treated with chlorinated water. To reduce tissue damage, a slow spin speed was used to dry microgreens. However, excess moisture remaining on washed leaf surfaces might promote microbial growth in those packages.

There was no significant difference for tissue electrolyte leakage among all treatments except, from Day 11 onwards, tissue electrolyte leakage for H₂O/Cl treatment was significantly higher ($P < 0.001$) than that of other treatments and remained higher ($P < 0.01$) through the end of storage (Fig. 8A). Overall quality for all the pre- and postharvest combined treatments declined significantly ($P < 0.05$) during storage (Fig. 8B). However, preharvest CaCl₂ without postharvest dip maintained the highest overall quality score and the lowest tissue electrolyte leakage during the entire 14 d storage. In contrast, H₂O/Cl had the lowest quality score. These results suggest that preharvest calcium spray is more efficient than postharvest dip. There were a few factors influencing the results.

Fig. 4. Effects of postharvest treatment with calcium lactate (Ca lactate) on (A) O₂ and (B) CO₂ partial pressure within packages of broccoli microgreens during 5 °C storage. H₂O/H₂O + chlorine: preharvest water only and postharvest with 100 µL·L⁻¹ chlorine; H₂O/25 mmol·L⁻¹ Ca lactate + chlorine: preharvest water only and postharvest with 25 mmol·L⁻¹ Ca lactate + 100 µL·L⁻¹ chlorine; H₂O/50 mmol·L⁻¹ Ca lactate + chlorine: preharvest water only and postharvest with 50 mmol·L⁻¹ Ca lactate + 100 µL·L⁻¹ chlorine; H₂O/100 mmol·L⁻¹ Ca lactate + chlorine: preharvest water only and postharvest with 100 mmol·L⁻¹ Ca lactate + 100 µL·L⁻¹ chlorine; H₂O/no dip: preharvest water only and no postharvest dip. Data presented are the means of four replications; vertical lines represent SES.

Fig. 5. Effects of postharvest treatment with calcium lactate (Ca lactate) on (A) tissue electrolyte leakage and (B) overall quality of packaged broccoli microgreens during 5 °C storage. H₂O/H₂O + chlorine: preharvest water only and postharvest with 100 µL·L⁻¹ chlorine; H₂O/25 mmol·L⁻¹ Ca lactate + chlorine: preharvest water only and postharvest with 25 mmol·L⁻¹ Ca lactate + 100 µL·L⁻¹ sodium hypochlorite; H₂O/50 mmol·L⁻¹ Ca lactate + chlorine: preharvest water only and postharvest with 50 mmol·L⁻¹ Ca lactate + 100 µL·L⁻¹ chlorine; H₂O/100 mmol·L⁻¹ Ca lactate + chlorine: preharvest water only and postharvest with 100 mmol·L⁻¹ Ca lactate + 100 µL·L⁻¹ chlorine; H₂O/no dip: preharvest water only and no postharvest dip. Data presented are the means of four replications; vertical lines represent SES.

Fig. 6. Effects of combinations of pre/postharvest calcium treatments on (A) O₂ and (B) CO₂ partial pressure within packages of broccoli microgreens during 5 °C storage. H₂O/Cl: preharvest spray with wateronly and postharvest dip in 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine (pH 6.5) solution; H₂O/calcium lactate (Ca lactate): preharvest water spray only and postharvest dip in 50 $\text{mmol}\cdot\text{L}^{-1}$ Ca lactate plus 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine; CaCl₂/Cl: preharvest 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ spray and postharvest dip in 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine; CaCl₂/Ca lactate: preharvest 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ spray and postharvest dip in 50 $\text{mmol}\cdot\text{L}^{-1}$ Ca lactate plus 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine; CaCl₂/no dip: preharvest 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ and no postharvest dip. Data presented are the means of four replications; vertical lines represent SES.

Fig. 7. Effects of combinations of pre/postharvest calcium treatments on (A) aerobic mesophilic bacteria (AMB) and (B) yeast and mold (Y&M) populations of packaged broccoli microgreens during 5 °C storage. H₂O/Cl: preharvest spray with water only and postharvest dip in 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine (pH 6.5) solution; H₂O/calcium lactate (Ca lactate): preharvest water spray only and postharvest dip in 50 $\text{mmol}\cdot\text{L}^{-1}$ Ca lactate plus 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine; CaCl₂/Cl: preharvest 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ spray and postharvest dip in 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine; CaCl₂ / Ca lactate: preharvest 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ spray and postharvest dip in 50 $\text{mmol}\cdot\text{L}^{-1}$ Ca lactate plus 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine; CaCl₂/no dip: preharvest 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ and no postharvest dip. Data presented are the means of four replications; vertical lines represent SES.

Fig. 8. Effects of combinations of pre/postharvest calcium treatments on (A) tissue electrolyte leakage and (B) overall quality of packaged broccoli microgreens during 5 °C storage. H₂O/Cl: preharvest spray with water only and postharvest dip in 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine (pH 6.5) solution; H₂O/calcium lactate (Ca lactate): preharvest water spray only and postharvest dip in 50 $\text{mmol}\cdot\text{L}^{-1}$ Ca lactate plus 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine; CaCl₂/Cl: preharvest 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ spray and postharvest dip in 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine; CaCl₂/Ca lactate: preharvest 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ spray and postharvest dip in 50 $\text{mmol}\cdot\text{L}^{-1}$ Ca lactate plus 100 $\mu\text{L}\cdot\text{L}^{-1}$ chlorine; CaCl₂/no dip: preharvest 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ and no postharvest dip. Data presented are the means of four replications; vertical lines represent SES.

First, calcium solution was sprayed every day during preharvest treatment. However, postharvest calcium treatment consisted of only a 30 s dip. Second, postharvest dip was applied just before packaging and extra moisture on the surface was not easily removed without resulting in dehydration and or additional tissue damage to the already cut tissues. Third, spinning and drying processes caused tissue injury which might accelerate quality deterioration and encourage microbial growth. On buckwheat microgreens, we also found that unwashed samples maintained better visual quality and lower tissue electrolyte leakage than washed samples (Kou et al., 2013). Therefore, the spinning and drying steps were the major factors to reduce the microgreen postharvest quality. In order for processors to be able to provide safe ready-to-eat products, improved wash and drying technologies for microgreens need to be developed.

Conclusions

In this study, the effects of various pre/postharvest calcium treatments on the quality and shelf life of broccoli microgreens

were evaluated. Results indicated that preharvest spray with 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ without postharvest wash was the most efficient treatment for broccoli microgreens as compared with Ca lactate and Ca AA chelate. Preharvest spray with 10 $\text{mmol}\cdot\text{L}^{-1}$ CaCl₂ without postharvest wash significantly reduced tissue electrolyte leakage and microbial growth, and delayed decline of overall quality of microgreens during storage. Postharvest dip with 50 $\text{mmol}\cdot\text{L}^{-1}$ Ca lactate or combination of preharvest spray with CaCl₂/postharvest dip with Ca lactate exhibited some beneficial effects, which reduced tissue electrolyte leakage, AMB, and Y&M as compared with no pre- and postharvest calcium treatment. However, current dip/wash and drying procedures significantly reduce the quality of the broccoli microgreens since broccoli microgreens are very delicate. Improved wash/drying technologies are necessary to provide ready-to-eat microgreens with better quality and longer shelf life. Optionally, the postharvest wash step can be avoided when the microgreens are grown under controlled settings to minimize the microbial contamination. Microgreens crops usually are grown indoors. Thus, the

materials used for propagation can be easily decontaminated to maintain compliance with food safety regulations.

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