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# The effect of postharvest calcium application in hydro-cooling water on tissue calcium content, biochemical changes, and quality attributes of sweet cherry fruit

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ABSTRACT

To improve storage/shipping quality of sweet cherry (*Prunus avium* L.), the effect of calcium chloride ($CaCl_2$) added to hydro-cooling water on physiological and biochemical processes related to fruit and pedicel quality was investigated on two major cultivars. The fruit tissue Ca content increased up to 29–85% logarithmically for 'Sweetheart' and 39–188% linearly for 'Lapins' as $CaCl_2$ rate increased from 0.2% to 2.0% at 0 °C for 5 min. The increase of fruit tissue Ca content was accompanied by reductions in respiration rate, ascorbic acid degradation, and membrane lipid peroxidation, which enhanced total phenolics content and total antioxidant capacity, and resulted in increases in fruit firmness and pitting resistance and decreases in titratable acidity loss and decay of both cultivars. Pedicel browning was inhibited by $CaCl_2$ at 0.2% and 0.5%, but increased by higher rates at 1.0% and 2.0%, possibly via modifying membrane lipid peroxidation.



## 1. Introduction

Sweet cherries (*Prunus avium* L.) have a high respiration activity and are highly susceptible to mechanical damage, therefore they have a short shelf-life even under strict cold chain management, including rapid elimination of field heat after harvest and low temperature control during storage/shipping (Kupferman & Sanderson, 2001). The major postharvest deteriorations are surface pitting resulting from impact damage, loss of flavour, darkening of fruit skin colour, pedicel browning, and decay development (Bai, Plotto, Spotts, & Rattanapanone, 2011; Mattheis, Buchanan, & Fellman, 1997).

Calcium (Ca) plays an extremely important role in the fruit for cell wall structure and strength, plasma membrane structure and integrity, and cellular signalling responses (Poovaiah & Reddy, 1993). However, fruit are often deficient in Ca due to its low mobility in plants (Conway, Sams, & Hickey, 2002). Enhancing Ca content can be extremely beneficial in reducing disorders and maintaining quality of fruit during storage. Although pre-harvest sprays with calcium salts have been effective in controlling physiological disorders of fruit, direct application of Ca solution to harvested fruit is the most successful method to increase fruit tissue Ca content (Conway, 1982; Conway et al., 2002; Tsantili et al., 2007). Increase in tissue Ca content by postharvest Ca treatment reduces disorders and maintains storage quality of whole fruit including apple (Saftner, Conway, & Sams, 1998), strawberry (Hernandez-Munoz, Almenar, Del Valle, Velez, & Gavara, 2008), peach (Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007), honeydew melon (Lester & Grusak, 1999) and fresh-cut produce (Saftner, Bai, Abbott, & Lee, 2003; Silveira, Aguayob, Chisaric, & Artésb, 2011).

In addition to improving shelf life, postharvest Ca treatment improves quality attributes and enhances nutritional quality of pomegranate (Ramezanian et al., 2010) and cornelian cherry (Aghdama, Dokhaniehb, Hassanpourc, & Fard, 2013) through maintaining higher levels of nutraceutical compounds.

Calcium chloride ($CaCl_2$) is naturally occurring, edible, inexpensive, and has been approved by the US Food and Drug Administration for postharvest use (Saftner et al., 1998). Postharvest application of $CaCl_2$ at appropriate rates imparts no detrimental effect on consumer acceptance of treated fruit (Lester & Grusak, 2001; Saftner, Conway, & Sams, 1999). Three main ways of postharvest Ca application in fresh produce have been reported: dipping/washing in warm or hot (60 °C) solutions, vacuum/pressure infiltration, and mixing with wax coatings (Conway et al., 2002; Hernandez-Munoz et al., 2008; Silveira et al., 2011). A postharvest dip in warm $CaCl_2$ solutions (21 °C) increased fruit firmness and reduced pitting of 'Van' cherries (Lidster, Porritt, & Tung, 1978).

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Hydro-cooling at water temperatures near 0 °C for a short period of time (i.e., 5 min) is widely used shortly after harvest to eliminate field heat of sweet cherry in the US. Sweet cherries should be cooled to <5 °C by 4 h after harvest to reduce respiration rate and maintain fruit and pedicel quality (Alique, Zamorano, Martinez, & Alonso, 2005; Mattheis & Fellman, 2004). Although room-, forced-air, and hydro-cooling are all used commercially, hydro-cooling is the most efficient method to cool sweet cherries with minimising pedicel shrivel and browning from moisture loss (Mattheis & Fellman, 2004).

The objective of this study was to evaluate the effect of $CaCl_2$ applied in simulated hydro-cooling water on physiological and biochemical processes related to fruit and pedicel quality of two major cultivars ('Lapins' and 'Sweetheart') growing in the US Pacific Northwest (PNW).

## 2. Materials and methods

### 2.1. Fruit materials

Sweet cherry fruit were harvested at commercial maturity in a research plot of 'Lapins' and 'Sweetheart' trees at the Mid-Columbia Agricultural Research and Extension Center (MCAREC), Hood River, Oregon, USA. Both cultivars were 15-years old and on Mazzard rootstock. Fruit trees were maintained with standard cultural, fertiliser, herbicide and pesticide practices. Cherries were picked in the morning and immediately transported to the lab at MCAREC. After sorting for uniformity of size and colour and freedom from defects, sound fruit with pedicels were divided into 5 treatments × 3 replications = 15 lots (3 kg/lot) of each cultivar for $CaCl_2$ treatments. All treatments included dipping fruit in iced water (0 °C) containing $CaCl_2$ (OptiCAL™, Pace International LLC., Seattle, Washington, USA) at 0%, 0.2%, 0.5%, 1.0%, and 2.0% for 5 min. Treated fruit were allowed to drain and dry and were then packed in commercial zipper-lock polyethylene bags (~1 kg) with a perforation ratio at ~2%. Packed fruit were stored in a cold room at 0 °C and 90% RH for 2 and 4 weeks. Physical and biochemical determinations were carried out after 3–4 h at 20 °C in the lab upon removal from hydro-cooling water or cold storage.

### 2.2. Tissue Ca content determination

Fruit samples were washed, oven-dried at 65 °C, and ground to pass through a 1-mm sieve. The samples were then digested in a MARS Express CEM microwave using nitric acid and hydrogen peroxide. Prepared samples were analysed for Ca content by a Thermo 6500 duo ICP (Thermo and Fisher Scientific, Waltham, Mass.). Tissue Ca content is reported on a dry mass basis ($\mu g\, g^{-1}$). Each sample consisted of the flesh from 30 fruit.

### 2.3. Ethylene and respiration rate determinations

Thirty fruit with pulp temperature of 20 °C were placed in hermetically sealed glass containers (960 mL) equipped with 2 rubber sampling ports at 20 °C. After 1 h incubation, one mL of the headspace was withdrawn with a syringe and injected into a gas chromatograph (Shimadzu GC-8AIF, Kyoto, Japan) equipped with a flame ionisation detector and a Porapack Q column (80/100 mesh, 3.0 mm i.d., 2.0 m long). The carrier gas was nitrogen at a flow rate of 40 mL $min^{-1}$, the oven temperature was 90 °C, and the injector and detector temperatures were 140 °C. Ethylene production rate was expressed as nL $kg^{-1}$ $h^{-1}$. After ethylene sampled, headspace $CO_2$ concentrations were determined using an $O_2/CO_2$ analyser (Model 900161, Bridge Analyzers Inc., Alameda, California, USA). Fruit respiration rate was expressed as mL $CO_2$ $kg^{-1}$ $h^{-1}$.

### 2.4. Sample preparation

After determining the respiration rate, 15 fruit of each sample were pitted and the fruit tissue were cut into 2 mm small pieces and frozen in liquid nitrogen followed by storage in a freezer (−80 °C). The frozen fruit tissue samples were used for ascorbic acid (AsA), malondialdehyde (MDA), total anthocyanin, total flavonoids (TF), total phenolics (TP), and total antioxidant capacity (TAC) determinations. Fifteen pedicels of each sample were also frozen for MDA determination. Spectrophotometric measurements were performed on a model Ultrospec 3100 pro spectrophotometer (Biochrom Ltd, Cambridge, England).

### 2.5. AsA, MDA, and total anthocyanin determinations

Ascorbic acid (AsA) was measured based on the methods of Logan, Grace, Adams, & Demmig-Adams, 1998. Briefly, 2 g of the frozen fruit tissue powder was ground in 10 mL ice-cold 6% (v/v) $HClO_4$. The extract was centrifuged at 10,000*g* for 10 min at 2 °C and then the supernatant was used immediately for the measurement. A portion of the extract was neutralised with approximately one-third volume 1.5 M $Na_2CO_3$ to raise the pH to 1–2. Thirty to one hundred μL of the neutralised samples were used to assay the AsA at 265 nm in 100 mM potassium phosphate buffer (pH 5.6), before and after 15 min incubation with 5 units AsA oxidase from *Cucurbita* (Sigma). The AsA content was determined from the absorbance difference and compared to a standard curve with the results expressed as mg $100\, g^{-1}$ fw.

MDA level was measured according to the corrected TBA method (Hodges, Delong, Forney, & Prange, 1999). Two grams of the frozen fruit or pedicel tissue was ground and extracted in 5 mL 10% (w/v) trichloroacetic acid (TCA). After centrifugation at 10,000*g* for 15 min, a 2 mL aliquot of the supernatant was mixed with 2 mL 10% TCA containing 0.6% (w/v) thiobarbituric acid (TBA). The mixture was heated to 100 °C for 20 min, quickly cooled and centrifuged at 10,000*g* for 10 min. The supernatant was collected and absorbance was then measured at 450, 532, and 600 nm. The MDA concentration was calculated according to the formula: $6.45 \times (A_{532}-A_{600}) - 0.56 \times A_{450}$ and the results expressed as nmol $g^{-1}$ fw.

Total anthocyanin was determined according to Dekazos (1970). Two grams of fruit tissue powder was diluted with 20 mL of acidified methanol (1% HCl). The resultant dispersion was vortexed and the supernatant filtered through Waterman #4 filter paper. The residue was re-extracted two more times using the same procedure. The combined supernatants were centrifuged at 10,000*g* for 5 min and measured at 530 nm, after dilution when necessary. Total anthocyanin contents were calculated based on the molar-extinction coefficient of $E = 3.43 \times 10^4$ L $mol^{-1}$ $cm^{-1}$ and the results expressed as mg $100\, g^{-1}$ fw.

### 2.6. TF, TP, and TAC determinations

For each sample, 1.0 g fruit tissue powder was added in 10 mL of Ethanol–Acetone (EtOH-ACE) solvent (7:3) with constant stirring at 37 °C for 1 h. After cooling, the solution was centrifuged at 10,000*g* for 30 min at 4 °C. The supernatant was stored at −20 °C until use.

Total flavonoids (TF) content was determined following the method described by Du, Li, Ma, and Liang (2009). In a 10 mL Eppendorf tube, 0.3 mL cherry fruit extract, 3.4 mL 30% ethanol, 0.15 mL of 0.5 M $NaNO_2$ and 0.15 mL of 0.3 M $AlCl_3$. $6H_2O$ were added and mixed. After 5 min, 1 mL of 1 M NaOH was added, and the mixture was measured at 506 nm. The total flavonoids concentration was calculated from a calibration curve using rutin as standard and expressed as mg $100\, g^{-1}$ fw.