The Effect of Postharvest Calcium Application in Hydro-Cooling Water on Tissue Calcium Content, Biochemical Changes, and Quality Attributes of Sweet Cherry Fruit

Yan Wang a,*, Xingbin Xie a, Lynn E. Long b

a Department of Horticulture, Oregon State University, Mid-Columbia Agricultural Research and Extension Center, 3005 Experiment Station Dr., Hood River, OR 97031

b Oregon State University Extension, The Dalles, OR 97058

* Corresponding author:

Phone: +1 541 386 2030

Fax: +1 541 386 1905

Email address: yan.wang@oregonstate.edu
Abstract

To improve storage/shipping quality of sweet cherry (*Prunus avium* L.), the effect of calcium chloride (CaCl₂) 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% to 85% logarithmically for ‘Sweetheart’ and 39% to 188% linearly for ‘Lapins’ as CaCl₂ 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 CaCl₂ at 0.2% and 0.5%, but increased by higher rates at 1.0% and 2.0%, possibly via modifying membrane lipid peroxidation.

**Keywords:** Sweet cherry; calcium content; senescence; lipid peroxidation; nutritional quality, storage/shipping quality
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 flavor, darkening of fruit skin color, 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 signaling 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, Sams, & Hickey, 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
Calcium chloride (CaCl₂) is naturally occurring, edible, inexpensive, and has been approved by the US Food and Drug Administration for postharvest use (Saftner, Conway, & Sams, 1998). Postharvest application of CaCl₂ at appropriate rates imparts no detrimental effect on consumer acceptance of treated fruit (Saftner, Conway, & Sams, 1999; Lester & Grusak, 2001). 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, Sams, & Hickey, 2002; Hernandez-Munoz, Almenar, Del Valle, Velez, & Gavara, 2008; Silveira, Aguayob, Chisaric, & Artésb, 2011). A postharvest dip in warm CaCl₂ solutions (21 °C) increased fruit firmness and reduced pitting of ‘Van’ cherries (Lidster, Porritt, & Tung, 1978).

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 minimizing pedicel shrivel and browning from moisture loss (Mattheis & Fellman, 2004).

The objective of this study was to evaluate the effect of CaCl₂ 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, fertilizer, 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 color and freedom from defects, sound fruit with pedicels were divided into 5 treatments × 3 replications = 15 lots (3 kg/lot) of each cultivar for CaCl₂ treatments. All treatments included dipping fruit in iced water (0 °C) containing CaCl₂ (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 analyzed 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 (µg 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 ionization 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 CO₂ concentrations were determined using an O₂/CO₂ analyzer (Model 900161, Bridge Analyzers Inc., Alameda, California, USA). Fruit respiration rate was expressed as mL CO₂ 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 III, & Demmig-Adams (1998). Briefly, 2 g of the frozen fruit tissue powder was ground in 10 mL ice-cold 6% (v/v) HClO₄. 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 neutralized
with approximately one-third volume 1.5 M Na₂CO₃ to raise the pH to 1-2. Thirty to one
hundred µL of the neutralized 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×(A₅₃₂–A₆₀₀) – 0.56×A₄₅₀ 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 × 10⁴
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, & 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 NaNO₂ and 0.15 mL of 0.3 M AlCl₃·6H₂O 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.

Total phenolics (TP) were determined according to the Folin-Ciocalteu procedure (Du, Li, Ma, & Liang, 2009). In a 10 mL Eppendorf tube, 7.9 mL distilled water, 0.1 mL cherry fruit extract, and 0.5 mL Folin-Ciocalteu reagent (1:1 with water) were added and mixed. After exactly 1 min, 1.5 mL of Na₂CO₃ (20%) was added, and the combination was mixed and allowed to stand at room temperature in the dark for 2 h. The absorbance of the solution was measured at 765 nm. The total phenolics concentration was calculated from a calibration curve of gallic acid and expressed as mg 100 g⁻¹ fw.

TAC was determined according to the method of Prieto, Pineda, & Aguilar (1999) with modification. An aliquot of 20 μL cherry extract was introduced into an Eppendorf tube and diluted with 80 μL EtOH-ACE solvent. One mL of reacting reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added to the diluted extract and thoroughly mixed. The tube was capped and incubated in a thermal block at 95 °C for 90 min. After the mixed solution had cooled to room temperature, the absorbance of the solution was measured at 695 nm. Results were calculated from a standard curve of AsA and expressed as mg 100 g⁻¹ fw.

2.7. Color, firmness, soluble solid content (SSC), and titratable acidity (TA) determinations
Twenty-five fruit of each replication were held in the laboratory at 20 °C for 4-5 h (until condensation on fruit surface evaporated) before quality evaluations. Fruit skin color was determined using a colorimeter (Model CR-2500d, Minolta, Tokyo, Japan) and expressed as CIE a, b, L* values. Measurements were taken on opposite sides of each fruit, midway between the pedicel and calyx. The results were expressed in L* and hue angle (\( h^\circ \)). After color evaluation, fruit firmness was measured using a FirmTech 2 Fruit Firmness instrument (BioWorks Inc., Stillwater, OK) and expressed as g mm\(^{-1}\). After firmness determinations, fruit juice was prepared for SSC and TA measurements using a juicer (Acme Model 6001, Acme Juicer Manufacturing Co., Sierra Madre, CA) equipped with a uniform strip of milk filter (Schwartz Manufacturing Co., Two Rivers, WI). SSC was determined using a refractometer (Model N1, Atago, Tokyo, Japan). TA was determined by titrating 10 mL juice plus 40 mL distilled water to pH 8.1 using 0.1 N NaOH with a commercial titration system (Model T80/20, Schott-Gerate, Hofheim, Germany) and expressed as the equivalent percentage of malic acid.

2.8. Pitting susceptibility determination

Fruit for induced-pitting evaluation were immediately stored at 4.5 °C for 4 h before inducing pitting. Surface pitting was induced by the method of Toivonen, Kappel, Stan, McKenzie, & Hocking (2004). A force was applied to the fruit using an instrument fabricated to drop a 10 g stainless steel rod, 2.5 mm in diameter from a height of 60 mm onto the surface of the fruit. Thirty to 40 fruit from each replicate were treated, and 25 fruit free of visual skin injury following pit induction were selected for evaluation. After a cold storage period at 0 °C for 2 weeks, subjective and objective analyses of pits were performed according to a visual damage rating and measurement of pit diameter, respectively.

2.9. Fruit decay, pedicel moisture content, and pedicel browning evaluations
One hundred fruit of each sample were used for pedicel quality and decay evaluations. Pedicel browning was expressed as the percentage of fruit with >30% stem surface discoloration. The pedicels then were weighed and dried at 80 °C until the final weights were stable and recorded. The pedicel moisture content was calculated using the initial fresh weight and the final dry weight. After pedicel evaluations, the fruit were kept in the laboratory at 20 °C for 2 days and then fruit decay was calculated as percentage of fruit with any rot symptoms.

2.10. Statistical Analyses

There were three replications per treatment at each evaluation period. The experimental design was completely randomized and the data were subjected to analysis of variance (ANOVA) using StatSoft® Statistica version 6 (StatSoft, Tulsa, OK). When appropriate, means were separated by Fisher’s Protected LSD test at $P < 0.05$.

3. Results and discussion

3.1. Effect of application rates of CaCl$_2$ on fruit tissue Ca content

A significant increase of fruit tissue Ca content was recorded in both cultivars when dipped for 5 min in cold water (0 °C) containing CaCl$_2$. While tissue Ca content increased in a logarithmic manner in ‘Sweetheart’, it increased linearly in ‘Lapins’ by increased CaCl$_2$ rates from 0.2% to 2.0% (Fig. 1). The tissue Ca content increased 29%, 37%, 71%, and 85% for ‘Sweetheart’ and 39%, 55%, 112%, and 188% for ‘Lapins’ at CaCl$_2$ rates at 0.2%, 0.5%, 1.0%, and 2.0%, respectively. Ca ions likely enter the fruit primarily through the lenticels and through cracks in the cuticle and epidermis (Glenn & Poovaiah, 1985). The characteristic cuticle structure of each cultivar may account for the difference of Ca absorption between the two cultivars.
3.2. Fruit respiration and ethylene production rates

The initial respiration rates of ‘Sweetheart’ and ‘Lapins’ were 26.0 and 30.5 mL CO₂ kg⁻¹ h⁻¹, respectively, and were similar to those reported by Toivonien, Kappel, Stan, Mckenzie, & Hocking (2004). Cherry fruit have moderate respiration rates and are non-climacteric (Kader, 1992). Both ‘Sweetheart’ and ‘Lapins’ exhibited increasing respiration rates over time and reached 33.9 and 38.2 mL CO₂ kg⁻¹ h⁻¹ after 4 weeks of cold storage (Fig. 2 A&B). A similar increase in respiration rate during storage was reported in other cherry cultivars (Tsantili et al., 2007). Ca treatments inhibited the respiration rates during cold storage in both cultivars compared to the control, and the respiration rate decreased with an increasing Ca treatment rate (Fig. 2 A&B). After 4 weeks of storage, at the Ca treatment rate of 0.5%, for example, the respiration rate was reduced by up to 15% and 11% compared to the control for ‘Sweetheart’ and ‘Lapins’, respectively. The reduction in respiration rate is linked to the delay of ripening and senescence of fruit and this delay in senescence by the Ca treatment was postulated to have a direct effect in maintaining the functionality of the cell membranes and fruit quality during storage (Lester & Grusak, 1999). Postharvest Ca dip treatments resulting in reduced respiration rate have been reported for both climacteric and non-climacteric fruits (Saftner, Conway, & Sams, 1998; Tsantili, Konstantinidis, Athanasopoulos, & Pontikis, 2002).

‘Sweetheart’ and ‘Lapins’ produced very low ethylene (i.e., < 100 nL kg⁻¹ h⁻¹) after harvest and during cold storage (Fig 2 C&D), which is characteristic of cherries (Kader, 1992). Ca treatments did not affect ethylene production rates of either cultivar during cold storage (Fig. 2 C&D).

3.3. AsA and cell membrane peroxidation
AsA decreased rapidly in control fruit and only 48% and 41% of the initial content for ‘Sweetheart’ and ‘Lapins’, respectively, remained after 4 weeks of cold storage. All the Ca treatments slowed the loss of AsA significantly ($p < 0.05$) (Fig. 2 E&F). After 4 weeks of storage, ‘Sweetheart’ and ‘Lapins’ fruit treated with Ca at 0.5%, for examples, maintained 70% and 66% of the initial AsA concentration, therefore 22% and 25% higher than control, respectively. There was no significant difference ($p < 0.05$) in AsA content among the different Ca treatment rates.

Tian, Jiang, Xu, & Wang (2004) reported sweet cherry lost AsA dramatically during cold storage.

Postharvest Ca treatment enhanced AsA content of cornelian cherry (Aghdama, Dokhaniehb, Hassanpourc, & Fard, 2013)

MDA concentration is a widely used indicator of lipid peroxidation resulting from oxidative stress in biological material (Hodges, Delong, Forney, & Prange, 1999). MDA content in control fruit increased 1.4 and 1.5 fold after 4 weeks of storage for ‘Sweetheart’ and ‘Lapins’, respectively. MDA also increased in Ca treated fruit, but levels remained significantly lower ($p < 0.05$) than those in control fruit (Fig. 2 G&H). Ca treatment rates did not affect MDA content significantly ($p < 0.05$).

Oxidative stress induces an increase of active oxygen species (AOS) and active antioxidant systems can remove AOS and therefore play a crucial role in delaying senescence of fruit (Shewfelt & del Rosario, 2000). While both enzymatic and non-enzymatic antioxidants are implicated in oxidative stress responses, the non-enzymatic antioxidant AsA plays a key role in detoxification of AOS (Shewfelt & del Rosario, 2000). In sweet cherry, Ca reduction of AsA degradation may have resulted in reduced lipid peroxidation as indicated by a reduced accumulation of MDA (Fig. 2 E&F&G&H). Ferguson (1984) reported that Ca directly influences membrane lipid peroxidation by lowering the concentration of AOS during fruit ripening. In
addition to reducing lipid peroxidation, Ca’s beneficial effect on maintaining membrane integrity may be multifunctional such as through regulating the expression and synthesis of proteins and enzymes (Poovaiah & Reddy, 1993) and slowing down catabolism of total phospholipids and delaying an increase in the total free sterol to total phospholipid ratio (TFS:TPL) (Lester & Grusak, 1999; Picchioni, Watada, Conway, Whitaker, & Sams, 1998).

3.4. Fruit firmness and pitting susceptibility

Fruit firmness is an important quality attribute of sweet cherry for both consumer acceptance and shipping purposes (Bai, Plotto, Spotts, & Rattanapanone, 2011). Compared to control fruit, firmness was increased up to 4%, 5%, 5%, and 7% immediately after dipping and 7%, 9%, 15%, and 19% after 4 weeks of cold storage for ‘Sweetheart’; 3%, 5%, 9%, and 10% immediately after dipping and 8%, 14%, 17%, and 25% after 4 weeks of storage for ‘Lapins’ as a result of Ca treatment rates of 0.2%, 0.5%, 1.0%, and 2.0%, respectively (Fig. 3 A&B). There was a trend that fruit with higher tissue Ca content were firmer after Ca treatments and during storage. Both control and Ca treated fruit increased firmness during 4 weeks of cold storage.

Increase or decrease in cherry firmness during cold storage have been reported by others for different cultivars (Bai, Plotto, Spotts, & Rattanapanone, 2011; Kappel, Toivonen, MacKenzie, & Stam, 2002; Wang & Long, 2014). Factors determining cherry firmness development during storage warrant further study. Fruit firmness increase following a postharvest Ca treatment was reported for ‘Van’ cherry (Lidster, Porritt, & Tung, 1978) and other fruit (Conway, Sams, & Hickey, 2002; Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007).

Sweet cherry softening was related to the enzymatic degradation of the middle lamella and cell walls, and enzymes such as polygalacturonase (PG), pectin methylesterase (PME), and β-galactosidase (β-Gal) play an important role in sweet cherry fruit softening (Wei et al., 2011).
Firming and resistance to softening resulting from addition of Ca have been attributed to the formation of Ca pectate, which increases rigidity of the middle lamella and cell walls, leading to increased resistance to PG, PME, and β-Gal activities (Mignani et al., 1995). The Ca ions form intermolecular bridges between pectin molecules by interaction with free carboxyl groups of pectic acid polymers (Kays & Paull, 2004). Ca also contributes to firmness by stabilizing the cell membrane and reducing tissue water loss, thereby increasing cell turgor pressure (Mignani et al., 1995; Picchioni et al., 1998).

Sweet cherry pitting is characterized by one or more irregular hardened depressions on the fruit surface caused by mechanical impact and compression damage occurring during harvest and postharvest practices. Damage is expressed after 1-2 weeks of cold storage by the collapse of the injured cells (Einhorn, Wang, & Tuner, 2013). Pitting continues to be the leading cause of postharvest deterioration of sweet cherries grown in the US PNW. The present research demonstrated that the postharvest Ca treatment reduced pitting susceptibility ($p < 0.05$) for ‘Sweetheart’ and ‘Lapins’ (Fig. 3 C&D). A postharvest Ca solution ($21{\degree}C$) dip was also reported to reduce pitting of ‘Van’ cherries (Lidster, Porritt, & Tung, 1978). Fruit firmness was negatively correlated to pitting susceptibility (Einhorn, Wang, & Tuner, 2013); therefore, one possible reason why Ca reduces pitting susceptibility may be due to firming the fruit tissue.

3.5. Fruit quality attributes

Fruit skin luster is one of the most important parameters which determine sweet cherry consumer acceptance (Bai, Plotto, Spotts, & Rattanapanone, 2011). Sweet cherry continues to lose luster, which was correlated to the reduction of $L^*$ and $h^\circ$ during postharvest storage/shipping (Bai, Plotto, Spotts, & Rattanapanone, 2011). In ‘Sweetheart’ and ‘Lapins’, $L^*$ and $h^\circ$ decreased gradually and Ca treatments retarded their reductions slightly (Fig.
Flavor loss due to a decline in fruit acid content shortens the potential storage/shipping life of sweet cherries; therefore, reducing the rate of acidity loss is a critical objective for extending the potential marketing period (Mattheis, Buchanan, & Fellman, 1997). In ‘Sweetheart’ and ‘Lapins’, while TA decreased during storage for both control and Ca treatments, TA loss was retarded by Ca treatments ($p < 0.05$) (Fig. 4 E&F). Organic acids might be used as the carbon source in the tri-carboxylic acid cycle as the major part of the respiratory process (Kays & Paull, 2004), resulting in a decrease in TA concentration in sweet cherry during storage. The results showed a clear effect of Ca treatments in retarding TA loss associated with decreasing fruit metabolism, including respiration rate (Fig. 2 A&B), leading to maintenance of respiration substances and in turn maintaining fruit flavor. SSC remained essentially unchanged for both cultivars during 4 weeks of cold storage and was not affected by the Ca treatments (Fig. 4 G&H).

Cherries are rich sources of dietary phenolics with antioxidant properties that are associated with a wide range of health benefits (Mulabagal, Lang, Dewitt, Dalavoy, & Nair, 2009). Anthocyanins and flavonoids are important phenolic compounds in cherries (Aghdama et al., 2013). Ca treatments at 0.2%, 0.5%, and1.0% but not 2.0% increased total anthocyanin, TF, TP and TAC of ‘Sweetheart’ significantly ($p < 0.05$) after 4 weeks of storage. For example, Ca at 0.5% increased total anthocyanin, TF, and TP by 21%, 45%, and 38%, respectively. Ca at 0.2%, 0.5%, and 1.0% increased TAC by 17%, 15%, and 21%, respectively. In ‘Lapins’, Ca at 1.0 and 2.0% enhanced total anthocyanin, TF, and TP, but only Ca at 2.0% increased TAC by 13% at a statistically significant level ($p < 0.05$) (Fig. 5 ). Ca at 0.2 and 0.5% did not affect total
 anthocyanin, TF, TP, and TAC of ‘Lapins’. Aghdama et al., (2013) demonstrated that a
postharvest CaCl₂ treatment enhanced antioxidant capacity of cornelian cherry fruit by
effectively maintaining higher TP, TF, and anthocyanin contents. A postharvest CaCl₂ treatment
also maintained the nutritional quality of pomegranate fruit with higher TP and TAC
(Ramezanian et al., 2010). Biosynthesis of phenolics such as anthocyanins and flavonoids in
plant is carried out via the shikimate-phenylpropanoid-flavonoid pathways, in that phenylalanine
ammonia-lyase (PAL) serves as a key enzyme (Kays & Paull, 2004). It has been postulated that
an enhancing of TP and TAC in fruit by Ca treatment is due to stimulating the phenylpropanoid
pathway by increasing the PAL activity (Aghdama et al., 2013; Jacobo-Velazquez et al., 2011).

3.6. Decay

Decay was reduced significantly (p < 0.05) by Ca treatments with no difference among
the Ca rates after 4 weeks of cold storage plus 2 days at room temperature for both cultivars (Fig.
6A&B). Ca at the rate of 0.5%, as an example, reduced decay from 9.9% of the control to 3.3%
for ‘Sweetheart’ and from 10.3% to 3.2% for ‘Lapins’. Decay caused by fungal pathogens in
sweet cherries (Ippolito, Schena, Pentimore, & Nigro, 2005) and apples (Conway, 1982) was
reported being reduced by a postharvest application of Ca. The inhibitory effect of Ca on decay
has been related to cell wall stability by Ca ions and therefore making the cell wall less
susceptible to cell wall-degrading enzymes produced by fungal pathogens (Conway, Gross,
Boyer, & Sams, 1988).

3.7. Pedicel browning

The condition of the fruit pedicel often serves as an indicator of sweet cherry quality by
the consumer (Schick & Toivonen, 2002). Compared to the control, Ca rates of 0.2% and 0.5%
reduced the incidence of pedicel browning (p < 0.05) for ‘Sweetheart’ after 4 weeks and for
‘Lapins’ after 2 and 4 weeks of cold storage (Fig. 6C&D). For example, Ca at 0.5% reduced pedicel browning incidence of ‘Sweetheart’ and ‘Lapins’ up to 9% and 34%, respectively, after 4 weeks of storage. In contrast, Ca at higher rates of 1.0% and 2.0% increased the incidence of pedicel browning significantly ($p < 0.05$) for both cultivars after 2 and 4 weeks of cold storage. MDA content increased with increasing browning in pedicels (Fig. 6E&F). After 4 weeks of storage, MDA content had a positive relationship with pedicel browning incidence among the different treatments for both cultivars. Pedicel moisture content had a negative relationship with the pedicel browning incidence for both cultivars after 4 weeks of storage (Fig. 6G&H).

Cherry pedicel browning is a result of the loss of membrane integrity which allows polyphenol oxidase and polyphenol substances to mix in the damaged cells resulting in tissue browning (Schick and Toivonen, 2002). Results in this research clearly showed that Ca at appropriate concentrations can maintain greener and healthier pedicels and appears to do so by maintaining cellular integrity due to a reduced MDA content and enhanced moisture content. The CaCl$_2$ at higher rates of 1.0% and 2.0% damaged the pedicel membrane integrity demonstrated by an increase in MDA content and therefore increased the pedicel browning. Treating fruit with these higher concentrations of salts increases the risk of salt-related fruit injury (Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007; Saftner, Conway, & Sams, 1999). Plasmolysis and potentially immediate and irreversible membrane damage, as a result of osmotic effects, were related to the high salt injury (Saftner, Conway, & Sams, 1998).

4. Conclusions

Hydro-cooling shortly after harvest has been widely used to eliminate field heat of sweet cherries. This research demonstrated that adding CaCl$_2$ in the hydro-cooling water at 0.2% to
2.0% increased tissue Ca content of two major cultivars grown in the US PNW. As a result of the enhanced Ca content, cherry fruit increased firmness, reduced pitting susceptibility and decay. Increase in Ca content inhibited fruit respiration rate, enhanced antioxidant systems (i.e., AsA), and reduced membrane lipid peroxidation, which resulted in delaying fruit senescence, maintaining higher TA, inhibiting skin color darkening, and enhancing TP and TAC of sweet cherries during storage/shipping. Pedicel quality was improved by CaCl₂ at rates of 0.2% to 0.5%, but damaged at 1.0 to 2.0%. Therefore, adding CaCl₂ to the hydro-cooling water at appropriate rates (i.e., 0.2%-0.5%) has high commercial potential for improving storage/shipping quality and enhancing TAC of sweet cherries.

Acknowledgement

We are grateful to the Oregon Sweet Cherry Commission for their financial support of this research.

References


Einhorn, T., Wang, Y., & Turner, J. (2013). Sweet cherry fruit firmness and postharvest quality of late-maturing sweet cherry cultivars are optimized with low rate, single applications of Gibberellic Acid (GA3). *HortScience, 48*, 1010-1017.


