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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1   Running title:

2   Postharvest Ca treatment affects biochemical changes of sweet cherry

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5   **Tissue Calcium Content, Biochemical Changes, and Quality Attributes of**

6   **Sweet Cherry Fruit**

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24   **Abstract**

25   To improve storage/shipping quality of sweet cherry (*Prunus avium* L.), the effect of calcium

26   chloride ($CaCl_2$) added to hydro-cooling water on physiological and biochemical processes

27   related to fruit and pedicel quality was investigated on two major cultivars. The fruit tissue Ca

28   content increased up to 29% to 85% logarithmically for 'Sweetheart' and 39% to 188% linearly

29   for 'Lapins' as $CaCl_2$ rate increased from 0.2% to 2.0% at 0 °C for 5 min. The increase of fruit

30   tissue Ca content was accompanied by reductions in respiration rate, ascorbic acid degradation,

31   and membrane lipid peroxidation, which enhanced total phenolics content and total antioxidant

32   capacity, and resulted in increases in fruit firmness and pitting resistance and decreases in

33   titratable acidity loss and decay of both cultivars. Pedicel browning was inhibited by $CaCl_2$ at

34   0.2% and 0.5%, but increased by higher rates at 1.0% and 2.0%, possibly via modifying

35   membrane lipid peroxidation.

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37   *Keywords:* Sweet cherry; calcium content; senescence; lipid peroxidation; nutritional quality,

38   storage/shipping quality

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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 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

70   (Aghdama, Dokhaniehb, Hassanpourc, & Fard, 2013) through maintaining higher levels of

71   nutracuetical compounds.

72   Calcium chloride ($CaCl_2$) is naturally occurring, edible, inexpensive, and has been

73   approved by the US Food and Drug Administration for postharvest use (Saftner, Conway, &

74   Sams, 1998). Postharvest application of $CaCl_2$ at appropriate rates imparts no detrimental effect

75   on consumer acceptance of treated fruit (Saftner, Conway, & Sams, 1999; Lester & Grusak,

76   2001). Three main ways of postharvest Ca application in fresh produce have been reported:

77   dipping/washing in warm or hot (60 °C) solutions, vacuum/pressure infiltration, and mixing with

78   wax coatings (Conway, Sams, & Hickey, 2002; Hernandez-Munoz, Almenar, Del Valle, Velez,

79   & Gavara, 2008; Silveira, Aguayob, Chisaric, & Artésb, 2011). A postharvest dip in warm $CaCl_2$

80   solutions (21 °C) increased fruit firmness and reduced pitting of 'Van' cherries (Lidster, Porritt,

81   & Tung, 1978).

82   Hydro-cooling at water temperatures near 0 °C for a short period of time (i.e., 5 min) is

83   widely used shortly after harvest to eliminate field heat of sweet cherry in the US. Sweet cherries

84   should be cooled to < 5 °C by 4 h after harvest to reduce respiration rate and maintain fruit and

85   pedicel quality (Alique, Zamorano, Martinez, & Alonso, 2005; Mattheis & Fellman, 2004).

86   Although room-, forced-air, and hydro-cooling are all used commercially, hydro-cooling is the

87   most efficient method to cool sweet cherries with minimizing pedicel shrivel and browning from

88   moisture loss (Mattheis & Fellman, 2004).

89   The objective of this study was to evaluate the effect of $CaCl_2$ applied in simulated hydro-

90   cooling water on physiological and biochemical processes related to fruit and pedicel quality of

91   two major cultivars ('Lapins' and 'Sweetheart') growing in the US Pacific Northwest (PNW).

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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 $CaCl_2$ treatments. All treatments included dipping fruit in iced water (0 °C) containing $CaCl_2$ (OptiCAL^{TM}, 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 ($\mu g\ g^{-1}$). Each sample consisted of the flesh from 30 fruit.

2.3. Ethylene and respiration rate determinations

116   Thirty fruit with pulp temperature of 20 °C were placed in hermetically sealed glass

117   containers (960 mL) equipped with 2 rubber sampling ports at 20 °C. After 1 h incubation, one

118   mL of the headspace was withdrawn with a syringe and injected into a gas chromatograph

119   (Shimadzu GC-8AIF, Kyoto, Japan) equipped with a flame ionization detector and a Porapack Q

120   column (80/100 mesh, 3.0 mm i.d., 2.0 m long). The carrier gas was nitrogen at a flow rate of 40

121   mL min^{-1}, the oven temperature was 90 °C, and the injector and detector temperatures were 140

122   °C. Ethylene production rate was expressed as nL kg^{-1} h^{-1}. After ethylene sampled, headspace

123   CO_2 concentrations were determined using an O_2/CO_2 analyzer (Model 900161, Bridge

124   Analyzers Inc., Alameda, California, USA). Fruit respiration rate was expressed as mL CO_2 kg^{-1}

125   h^{-1}.

126   *2.4. Sample preparation*

127   After determining the respiration rate, 15 fruit of each sample were pitted and the fruit

128   tissue were cut into 2 mm small pieces and frozen in liquid nitrogen followed by storage in a

129   freezer (-80 °C). The frozen fruit tissue samples were used for ascorbic acid (AsA),

130   malondialdehyde (MDA), total anthocyanin, total flavonoids (TF), total phenolics (TP), and total

131   antioxidant capacity (TAC) determinations. Fifteen pedicels of each sample were also frozen for

132   MDA determination. Spectrophotometric measurements were performed on a model Ultrospec

133   3100 pro spectrophotometer (Biochrom Ltd, Cambridge, England).

134   *2.5. AsA, MDA, and total anthocyanin determinations*

135   Ascorbic acid (AsA) was measured based on the methods of Logan, Grace, Adams III, &

136   Demmig-Adams (1998). Briefly, 2 g of the frozen fruit tissue powder was ground in 10 mL ice-

137   cold 6% (v/v) $HClO_4$. The extract was centrifuged at 10,000 × g for 10 min at 2 °C and then the

138   supernatant was used immediately for the measurement. A portion of the extract was neutralized

139 with approximately one-third volume 1.5 M Na_2CO_3 to raise the pH to 1-2. Thirty to one

140 hundred μL of the neutralized samples were used to assay the AsA at 265 nm in 100 mM

141 potassium phosphate buffer (pH 5.6), before and after 15 min incubation with 5 units AsA

142 oxidase from _Cucurbita_ (Sigma). The AsA content was determined from the absorbance

143 difference and compared to a standard curve with the results expressed as mg 100 g^{-1} fw.

144   MDA level was measured according to the corrected TBA method (Hodges, Delong,

145 Forney, & Prange, 1999). Two grams of the frozen fruit or pedicel tissue was ground and

146 extracted in 5 mL 10% (w/v) trichloroacetic acid (TCA). After centrifugation at $10,000 \times g$ for

147 15 min, a 2 mL aliquot of the supernatant was mixed with 2 mL 10% TCA containing 0.6% (w/v)

148 thiobarbituric acid (TBA). The mixture was heated to 100 °C for 20 min, quickly cooled and

149 centrifuged at $10,000 \times g$ for 10 min. The supernatant was collected and absorbance was then

150 measured at 450, 532, and 600 nm. The MDA concentration was calculated according to the

151 formula: $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$ and the results expressed as nmol g^{-1} fw.

152   Total anthocyanin was determined according to Dekazos (1970). Two grams of fruit

153 tissue powder was diluted with 20 mL of acidified methanol (1% HCl). The resultant dispersion

154 was vortexed and the supernatant filtered through Waterman #4 filter paper. The residue was re-

155 extracted two more times using the same procedure. The combined supernatants were

156 centrifuged at $10,000 \times g$ for 5 min and measured at 530 nm, after dilution when necessary. Total

157 anthocyanin contents were calculated based on the molar-extinction coefficient of $E = 3.43 \times 10^4$

158 L mol^{-1} cm^{-1} and the results expressed as mg 100 g^{-1} fw.

159 *2.6. TF, TP, and TAC determinations*

160   For each sample, 1.0 g fruit tissue powder was added in 10 mL of Ethanol-Acetone

161 (EtOH-ACE) solvent (7:3) with constant stirring at 37 °C for 1 h. After cooling, the solution was

162   centrifuged at $10,000 \times g$ for 30 min at 4 °C. The supernatant was stored at -20 °C until use.

163   Total flavonoids (TF) content was determined following the method described by Du, Li,

164   Ma, & Liang (2009). In a 10 mL Eppendorf tube, 0.3 mL cherry fruit extract, 3.4 mL 30%

165   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.

166   After 5 min, 1 mL of 1 M NaOH was added, and the mixture was measured at 506 nm. The total

167   flavonoids concentration was calculated from a calibration curve using rutin as standard and

168   expressed as mg 100 g^{-1} fw.

169   Total phenolics (TP) were determined according to the Folin-Ciocalteu procedure (Du, Li,

170   Ma, & Liang, 2009). In a 10 mL Eppendorf tube, 7.9 mL distilled water, 0.1 mL cherry fruit

171   extract, and 0.5 mL Folin-Ciocalteu reagent (1:1 with water) were added and mixed. After

172   exactly 1 min, 1.5 mL of Na_2CO_3 (20%) was added, and the combination was mixed and allowed

173   to stand at room temperature in the dark for 2 h. The absorbance of the solution was measured at

174   765 nm. The total phenolics concentration was calculated from a calibration curve of gallic acid

175   and expressed as mg 100 g^{-1} fw.

176   TAC was determined according to the method of Prieto, Pineda, & Aguilar (1999) with

177   modification. An aliquot of 20 µL cherry extract was introduced into an Eppendorf tube and

178   diluted with 80 µL EtOH-ACE solvent. One mL of reacting reagent (0.6 M sulfuric acid, 28 mM

179   sodium phosphate, and 4 mM ammonium molybdate) was added to the diluted extract and

180   thoroughly mixed. The tube was capped and incubated in a thermal block at 95 ℃ for 90 min.

181   After the mixed solution had cooled to room temperature, the absorbance of the solution was

182   measured at 695 nm. Results were calculated from a standard curve of AsA and expressed as mg

183   100 g^{-1} fw.

184   *2.7. Color, firmness, soluble solid content (SSC), and titratable acidity (TA) determinations*

185   Twenty-five fruit of each replication were held in the laboratory at 20 °C for 4-5 h (until

186   condensation on fruit surface evaporated) before quality evaluations. Fruit skin color was

187   determined using a colorimeter (Model CR-2500d, Minolta, Tokyo, Japan) and expressed as CIE

188   a, b, L* values. Measurements were taken on opposite sides of each fruit, midway between the

189   pedicel and calyx. The results were expressed in L* and hue angel ($h°$). After color evaluation,

190   fruit firmness was measured using a FirmTech 2 Fruit Firmness instrument (BioWorks Inc.,

191   Stillwater, OK) and expressed as g mm^{-1}. After firmness determinations, fruit juice was prepared

192   for SSC and TA measurements using a juicer (Acme Model 6001, Acme Juicer Manufacturing

193   Co., Sierra Madre, CA) equipped with a uniform strip of milk filter (Schwartz Manufacturing

194   Co., Two Rivers, WI). SSC was determined using a refractometer (Model N1, Atago, Tokyo,

195   Japan). TA was determined by titrating 10 mL juice plus 40 mL distilled water to pH 8.1 using

196   0.1 N NaOH with a commercial titration system (Model T80/20, Schott-Gerate, Hofheim,

197   Germany) and expressed as the equivalent percentage of malic acid.

198   *2.8. Pitting susceptibility determination*

199   Fruit for induced-pitting evaluation were immediately stored at 4.5 °C for 4 h before

200   inducing pitting. Surface pitting was induced by the method of Toivonen, Kappel, Stan,

201   McKenzie, & Hocking (2004). A force was applied to the fruit using an instrument fabricated to

202   drop a 10 g stainless steel rod, 2.5 mm in diameter from a height of 60 mm onto the surface of

203   the fruit. Thirty to 40 fruit from each replicate were treated, and 25 fruit free of visual skin injury

204   following pit induction were selected for evaluation. After a cold storage period at 0 °C for 2

205   weeks, subjective and objective analyses of pits were performed according to a visual damage

206   rating and measurement of pit diameter, respectively.

207   *2.9. Fruit decay, pedicel moisture content, and pedicel browning evaluations*

208   One hundred fruit of each sample were used for pedicel quality and decay evaluations.

209   Pedicel browning was expressed as the percentage of fruit with >30% stem surface discoloration.

210   The pedicels then were weighed and dried at 80 °C until the final weights were stable and

211   recorded. The pedicel moisture content was calculated using the initial fresh weight and the final

212   dry weight. After pedicel evaluations, the fruit were kept in the laboratory at 20 °C for 2 days

213   and then fruit decay was calculated as percentage of fruit with any rot symptoms.

214   *2.10. Statistical Analyses*

215   There were three replications per treatment at each evaluation period. The experimental

216   design was completely randomized and the data were subjected to analysis of variance (ANOVA)

217   using StatSoft[®] Statistica version 6 (StatSoft, Tulsa, OK). When appropriate, means were

218   separated by Fisher's Protected LSD test at $P < 0.05$.

219

220   **3. Results and discussion**

221   *3.1. Effect of application rates of CaCl$_2$ on fruit tissue Ca content*

222   A significant increase of fruit tissue Ca content was recorded in both cultivars when

223   dipped for 5 min in cold water (0 °C) containing CaCl$_2$. While tissue Ca content increased in a

224   logarithmic manner in 'Sweetheart', it increased linearly in 'Lapins' by increased CaCl$_2$ rates

225   from 0.2% to 2.0% (Fig. 1). The tissue Ca content increased 29%, 37%, 71%, and 85% for

226   'Sweetheart' and 39%, 55%, 112%, and 188% for 'Lapins' at CaCl$_2$ rates at 0.2%, 0.5%, 1.0%,

227   and 2.0%, respectively. Ca ions likely enter the fruit primarily through the lenticels and through

228   cracks in the cuticle and epidermis (Glenn & Poovaiah, 1985). The characteristic cuticle

229   structure of each cultivar may account for the difference of Ca absorption between the two

230   cultivars.

231   *3.2. Fruit respiration and ethylene production rates*

232   The initial respiration rates of 'Sweetheart' and 'Lapins' were 26.0 and 30.5 mL CO_2 kg^{-1}

233   h^{-1}, respectively, and were similar to those reported by Toivonien, Kappel, Stan, Mckenzie, &

234   Hocking (2004). Cherry fruit have moderate respiration rates and are non-climacteric (Kader,

235   1992). Both 'Sweetheart' and 'Lapins' exhibited increasing respiration rates over time and

236   reached 33.9 and 38.2 mL CO_2 kg^{-1} h^{-1}after 4 weeks of cold storage (Fig. 2 A&B). A similar

237   increase in respiration rate during storage was reported in other cherry cultivars (Tsantili et al.,

238   2007). Ca treatments inhibited the respiration rates during cold storage in both cultivars

239   compared to the control, and the respiration rate decreased with an increasing Ca treatment rate

240   (Fig. 2 A&B). After 4 weeks of storage, at the Ca treatment rate of 0.5%, for example, the

241   respiration rate was reduced by up to 15% and 11% compared to the control for 'Sweetheart' and

242   'Lapins', respectively. The reduction in respiration rate is linked to the delay of ripening and

243   senescence of fruit and this delay in senescence by the Ca treatment was postulated to have a

244   direct effect in maintaining the functionality of the cell membranes and fruit quality during

245   storage (Lester & Grusak, 1999). Postharvest Ca dip treatments resulting in reduced respiration

246   rate have been reported for both climacteric and non-climacteric fruits (Saftner, Conway, &

247   Sams, 1998; Tsantili, Konstantinidis, Athanasopoulos, & Pontikis, 2002).

248   'Sweetheart' and 'Lapins' produced very low ethylene (i.e., < 100 nL kg^{-1} h^{-1}) after

249   harvest and during cold storage (Fig 2 C&D), which is characteristic of cherries (Kader, 1992).

250   Ca treatments did not affect ethylene production rates of either cultivar during cold storage (Fig.

251   2 C&D).

252   *3.3. AsA and cell membrane peroxidation*

253   AsA decreased rapidly in control fruit and only 48% and 41% of the initial content for

254   'Sweetheart' and 'Lapins', respectively, remained after 4 weeks of cold storage. All the Ca

255   treatments slowed the loss of AsA significantly ($p < 0.05$) (Fig. 2 E&F). After 4 weeks of storage,

256   'Sweetheart' and 'Lapins' fruit treated with Ca at 0.5%, for examples, maintained 70% and 66%

257   of the initial AsA concentration, therefore 22% and 25% higher than control, respectively. There

258   was no significant difference ($p < 0.05$) in AsA content among the different Ca treatment rates.

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

260   Postharvest Ca treatment enhanced AsA content of cornelian cherry (Aghdama, Dokhaniehb,

261   Hassanpourc, & Fard, 2013

262   MDA concentration is a widely used indicator of lipid peroxidation resulting from

263   oxidative stress in biological material (Hodges, Delong, Forney, & Prange, 1999). MDA content

264   in control fruit increased 1.4 and 1.5 fold after 4 weeks of storage for 'Sweetheart' and 'Lapins',

265   respectively. MDA also increased in Ca treated fruit, but levels remained significantly lower ($p <$

266   0.05) than those in control fruit (Fig. 2 G&H). Ca treatment rates did not affect MDA content

267   significantly ($p < 0.05$).

268   Oxidative stress induces an increase of active oxygen species (AOS) and active

269   antioxidant systems can remove AOS and therefore play a crucial role in delaying senescence of

270   fruit (Shewfelt & del Rosario, 2000). While both enzymatic and non-enzymatic antioxidants are

271   implicated in oxidative stress responses, the non-enzymatic antioxidant AsA plays a key role in

272   detoxification of AOS (Shewfelt & del Rosario, 2000). In sweet cherry, Ca reduction of AsA

273   degradation may have resulted in reduced lipid peroxidation as indicated by a reduced

274   accumulation of MDA (Fig. 2 E&F&G&H). Ferguson (1984) reported that Ca directly influences

275   membrane lipid peroxidation by lowering the concentration of AOS during fruit ripening. In

276 addition to reducing lipid peroxidation, Ca's beneficial effect on maintaining membrane integrity

277 may be multifunctional such as through regulating the expression and synthesis of proteins and

278 enzymes (Poovaiah & Reddy, 1993) and slowing down catabolism of total phospholipids and

279 delaying an increase in the total free sterol to total phospholipid ratio (TFS:TPL) (Lester &

280 Grusak, 1999; Picchioni, Watada, Conway, Whitaker, & Sams, 1998).

281 *3.4. Fruit firmness and pitting susceptibility*

282   Fruit firmness is an important quality attribute of sweet cherry for both consumer

283 acceptance and shipping purposes (Bai, Plotto, Spotts, & Rattanapanone, 2011). Compared to

284 control fruit, firmness was increased up to 4%, 5%, 5%, and 7% immediately after dipping and

285 7%, 9%, 15%, and 19% after 4 weeks of cold storage for 'Sweetheart'; 3%, 5%, 9%, and 10%

286 immediately after dipping and 8%, 14%, 17%, and 25% after 4 weeks of storage for 'Lapins' as

287 a result of Ca treatment rates of 0.2%, 0.5%, 1.0%, and 2.0%, respectively (Fig. 3 A&B). There

288 was a trend that fruit with higher tissue Ca content were firmer after Ca treatments and during

289 storage. Both control and Ca treated fruit increased firmness during 4 weeks of cold storage.

290 Increase or decrease in cherry firmness during cold storage have been reported by others for

291 different cultivars (Bai, Plotto, Spotts, & Rattanapanone, 2011; Kappel, Toivonen, MacKenzie,

292 & Stam, 2002; Wang & Long, 2014). Factors determining cherry firmness development during

293 storage warrant further study. Fruit firmness increase following a postharvest Ca treatment was

294 reported for 'Van' cherry (Lidster, Porritt, & Tung, 1978) and other fruit (Conway, Sams, &

295 Hickey, 2002; Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007).

296   Sweet cherry softening was related to the enzymatic degradation of the middle lamella

297 and cell walls, and enzymes such as polygalacturonase (PG), pectin methylesterase (PME), and

298 β-galactosidase (β-Gal) play an important role in sweet cherry fruit softening (Wei et al., 2011).

299 Firming and resistance to softening resulting from addition of Ca have been attributed to the

300 formation of Ca pectate, which increases rigidity of the middle lamella and cell walls, leading to

301 increased resistance to PG, PME, and β-Gal activities (Mignani et al., 1995). The Ca ions form

302 intermolecular bridges between pectin molecules by interaction with free carboxyl groups of

303 pectic acid polymers (Kays & Paull, 2004). Ca also contributes to firmness by stabilizing the cell

304 membrane and reducing tissue water loss, thereby increasing cell turgor pressure (Mignani et al.,

305 1995; Picchioni et al., 1998)

306   Sweet cherry pitting is characterized by one or more irregular hardened depressions on

307 the fruit surface caused by mechanical impact and compression damage occurring during harvest

308 and postharvest practices. Damage is expressed after 1-2 weeks of cold storage by the collapse of

309 the injured cells (Einhorn, Wang, & Tuner, 2013). Pitting continues to be the leading cause of

310 postharvest deterioration of sweet cherries grown in the US PNW. The present research

311 demonstrated that the postharvest Ca treatment reduced pitting susceptibility ($p < 0.05$) for

312 'Sweetheart' and 'Lapins' (Fig. 3 C&D). A postharvest Ca solution (21 °C) dip was also reported

313 to reduce pitting of 'Van' cherries (Lidster, Porritt, & Tung, 1978). Fruit firmness was negatively

314 correlated to pitting susceptibility (Einhorn, Wang, & Tuner, 2013); therefore, one possible

315 reason why Ca reduces pitting susceptibility may be due to firming the fruit tissue.

316 *3.5. Fruit quality attributes*

317   Fruit skin luster is one of the most important parameters which determine sweet cherry

318 consumer acceptance (Bai, Plotto, Spotts, & Rattanapanone, 2011). Sweet cherry continues to

319 lose luster, which was correlated to the reduction of L* and $h°$ during postharvest

320 storage/shipping (Bai, Plotto, Spotts, & Rattanapanone, 2011). In 'Sweetheart' and 'Lapins', L*

321 and $h°$ decreased gradually and Ca treatments retarded their reductions slightly (Fig.

322 4A&B&C&D), in that 'Sweetheart' treated by Ca maintained a higher L* value than control at

323 statistically significant levels (p < 0.05) during 4 weeks of cold storage (Fig. 4 A).

324   Flavor loss due to a decline in fruit acid content shortens the potential storage/shipping

325 life of sweet cherries; therefore, reducing the rate of acidity loss is a critical objective for

326 extending the potential marketing period (Mattheis, Buchanan, & Fellman, 1997). In

327 'Sweetheart' and 'Lapins', while TA decreased during storage for both control and Ca treatments,

328 TA loss was retarded by Ca treatments ($p < 0.05$) (Fig. 4 E&F). Organic acids might be used as

329 the carbon source in the tri-carboxylic acid cycle as the major part of the respiratory process

330 (Kays & Paull, 2004), resulting in a decrease in TA concentration in sweet cherry during storage.

331 The results showed a clear effect of Ca treatments in retarding TA loss associated with

332 decreasing fruit metabolism, including respiration rate (Fig. 2 A&B), leading to maintenance of

333 respiration substances and in turn maintaining fruit flavor. SSC remained essentially unchanged

334 for both cultivars during 4 weeks of cold storage and was not affected by the Ca treatments (Fig.

335 4 G&H).

336   Cherries are rich sources of dietary phenolics with antioxidant properties that are

337 associated with a wide range of health benefits (Mulabagal, Lang, Dewitt, Dalavoy, & Nair,

338 2009). Anthocyanins and flavonoids are important phenolic compounds in cherries (Aghdama et

339 al., 2013). Ca treatments at 0.2%, 0.5%, and1.0% but not 2.0% increased total anthocyanin, TF,

340 TP and TAC of 'Sweetheart' significantly (p < 0.05) after 4 weeks of storage. For example, Ca at

341 0.5% increased total anthocyanin, TF, and TP by 21%, 45%, and 38%, respectively. Ca at 0.2%,

342 0.5%, and 1.0% increased TAC by 17%, 15%, and 21%, respectively. In 'Lapins', Ca at 1.0 and

343 2.0% enhanced total anthocyanin, TF, and TP, but only Ca at 2.0% increased TAC by 13% at a

344 statistically significant level ($p < 0.05$) (Fig. 5). Ca at 0.2 and 0.5% did not affect total

345 anthocyanin, TF, TP, and TAC of 'Lapins'. Aghdama et al., (2013) demonstrated that a

346 postharvest $CaCl_2$ treatment enhanced antioxidant capacity of cornelian cherry fruit by

347 effectively maintaining higher TP, TF, and anthocyanin contents. A postharvest $CaCl_2$ treatment

348 also maintained the nutritional quality of pomegranate fruit with higher TP and TAC

349 (Ramezanian et al., 2010). Biosynthesis of phenolics such as anthocyanins and flavonoids in

350 plant is carried out via the shikimate-phenylpropanoid-flavonoid pathways, in that phenylalanine

351 ammonia-lyase (PAL) serves as a key enzyme (Kays & Paull, 2004). It has been postulated that

352 an enhancing of TP and TAC in fruit by Ca treatment is due to stimulating the phenylpropanoid

353 pathway by increasing the PAL activity (Aghdama et al., 2013; Jacobo-Velazquez et al., 2011).

354 *3.6. Decay*

355   Decay was reduced significantly ($p < 0.05$) by Ca treatments with no difference among

356 the Ca rates after 4 weeks of cold storage plus 2 days at room temperature for both cultivars (Fig.

357 6A&B). Ca at the rate of 0.5%, as an example, reduced decay from 9.9% of the control to 3.3%

358 for 'Sweetheart' and from 10.3% to 3.2% for 'Lapins'. Decay caused by fungal pathogens in

359 sweet cherries (Ippolito, Schena, Pentimore, & Nigro, 2005) and apples (Conway, 1982) was

360 reported being reduced by a postharvest application of Ca. The inhibitory effect of Ca on decay

361 has been related to cell wall stability by Ca ions and therefore making the cell wall less

362 susceptible to cell wall-degrading enzymes produced by fungal pathogens (Conway, Gross,

363 Boyer, & Sams, 1988).

364 *3.7. Pedicel browning*

365   The condition of the fruit pedicel often serves as an indicator of sweet cherry quality by

366 the consumer (Schick & Toivonen, 2002). Compared to the control, Ca rates of 0.2% and 0.5%

367 reduced the incidence of pedicel browning ($p < 0.05$) for 'Sweetheart' after 4 weeks and for

368 'Lapins' after 2 and 4 weeks of cold storage (Fig. 6C&D). For example, Ca at 0.5% reduced

369 pedicel browning incidence of 'Sweetheart' and 'Lapins' up to 9% and 34%, respectively, after 4

370 weeks of storage. In contrast, Ca at higher rates of 1.0% and 2.0% increased the incidence of

371 pedicel browning significantly ($p < 0.05$) for both cultivars after 2 and 4 weeks of cold storage.

372 MDA content increased with increasing browning in pedicels (Fig. 6E&F). After 4 weeks of

373 storage, MDA content had a positive relationship with pedicel browning incidence among the

374 different treatments for both cultivars. Pedicel moisture content had a negative relationship with

375 the pedicel browning incidence for both cultivars after 4 weeks of storage (Fig. 6G&H).

376 Cherry pedicel browning is a result of the loss of membrane integrity which allows

377 polyphenol oxidase and polyphenol substances to mix in the damaged cells resulting in tissue

378 browning (Schick and Toivonen, 2002). Results in this research clearly showed that Ca at

379 appropriate concentrations can maintain greener and healthier pedicels and appears to do so by

380 maintaining cellular integrity due to a reduced MDA content and enhanced moisture content. The

381 $CaCl_2$ at higher rates of 1.0% and 2.0% damaged the pedicel membrane integrity demonstrated

382 by an increase in MDA content and therefore increased the pedicel browning. Treating fruit with

383 these higher concentrations of salts increases the risk of salt-related fruit injury (Manganaris,

384 Vasilakakis, Diamantidis, & Mignani, 2007; Saftner, Conway, & Sams, 1999). Plasmolysis and

385 potentially immediate and irreversible membrane damage, as a result of osmotic effects, were

386 related to the high salt injury (Saftner, Conway, & Sams, 1998).

387

388 **4. Conclusions**

389 Hydro-cooling shortly after harvest has been widely used to eliminate field heat of sweet

390 cherries. This research demonstrated that adding $CaCl_2$ in the hydro-cooling water at 0.2% to

391 2.0% increased tissue Ca content of two major cultivars grown in the US PNW. As a result of the

392 enhanced Ca content, cherry fruit increased firmness, reduced pitting susceptibility and decay.

393 Increase in Ca content inhibited fruit respiration rate, enhanced antioxidant systems (i.e., AsA),

394 and reduced membrane lipid peroxidation, which resulted in delaying fruit senescence,

395 maintaining higher TA, inhibiting skin color darkening, and enhancing TP and TAC of sweet

396 cherries during storage/shipping. Pedicel quality was improved by $CaCl_2$ at rates of 0.2% to

397 0.5%, but damaged at 1.0 to 2.0%. Therefore, adding $CaCl_2$ to the hydro-cooling water at

398 appropriate rates (i.e., 0.2%-0.5%) has high commercial potential for improving storage/shipping

399 quality and enhancing TAC of sweet cherries.

400

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404

405 **References**

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