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Physiological and biochemical changes relating to postharvest splitting of sweet cherries affected by calcium application in hydrocooling water

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ABSTRACT

Hydrocooling sweet cherries shortly after harvest (4 h) and then transporting fruit in cold flume water during packing are used to maximize postharvest quality, but can cause fruit splitting. This study demonstrated that cherry fruit (two splitting-susceptible cultivars) absorbed Ca in a quadratic polynomial manner with increasing $CaCl_2$ concentration from 0.2% to 2.0% in cold water (0 °C) for 5 min, but did not take up Cl. The enhanced tissue Ca content reduced splitting potential by decreasing fruit soluble pectin release and increasing the splitting threshold. In contrast, depleting Ca from fruit tissue by EDTA or low pH, increased soluble pectin release and splitting potential. In a simulated commercial procedure, hydrocooling cherry fruit in appropriate $CaCl_2$ solutions (i.e., 0.2–0.5%) for 5 min and then passing the fruit in cold flume water for 15 min increased fruit firmness, retarded losses in ascorbic acid, titratable acidity, and skin color, and reduced splitting and decay following 4 weeks of cold storage.

1. Introduction

Sweet cherry (*Prunus avium* L.) has a high respiration activity and a short postharvest life. In the U.S. Pacific Northwest (PNW), sweet cherries are hydrocooled as soon as possible after harvest and transported in cold flume water during packing to reduce the respiration rate and extend storage/shipping life. However, serious splitting may occur in the hydrocooling and flume water or after packing during storage/shipping, especially with susceptible cultivars such as 'Skeena' and 'Bing' grown in the U.S. PNW. Split fruit cannot be marketed. In addition, the surface of the split becomes a site for fungal penetration and infection during storage/shipping.

Sweet cherry cracking or splitting, due to rainfall, may also occur during maturation on the trees as harvest approaches. Splitting is a serious economic problem in sweet cherry production world-wide (Simon, 2006). The disorder is characterized by cracks or splits in the fruit's cuticle which sometimes extend deep into the flesh. Three types of splitting (stem end, apical, and side cracks) are well-defined in the literature (Christensen, 1996; Simon, 2006). In certain years cracking may be as great as 90% in susceptible cultivars after rainfall in some orchards (Christensen, 1996). Considerable research effort has been devoted to identify the mechanism and practical solutions for rain-induced cracking of sweet cherries (Balbontín et al., 2013; Simon, 2006).

Opara, Studman, and Banks (1997) reviewed splitting and cracking in fruits. Splitting pre- or postharvest in sweet cherries is assumed to be caused by a rapid water uptake resulting in a turgor pressure increase to a point beyond the expansion capability of the cherry cuticle (Christensen, 1996). While the water potential in the fruit is the major driving force for splitting, many factors confer susceptibility/resistance to splitting: genetic, physiological, and cell, tissue, and organ structures (Balbontín et al., 2013). Among the critical factors, the concentration of extracellular Ca that cross-links adjacent pectin polymers may be an important determinant for splitting of fruit after water absorption (Demarty, Morvan, & Thellier, 1984; Lichter et al., 2002). A number of researchers have shown that Ca sprays reduce sweet cherry rain cracks (Wójcik et al., 2013; Yamamoto, Satoh, & Watanabe, 1992). According to Christensen (1996), the low water permeability of the fruit cuticle increases the resistance to splitting while Ca in the fruit tissue decreases permeability of the cellular membranes, reducing the water absorption rate of the fruit. Reduction of fruit splitting by Ca is also related to maintenance or strengthening of the cell wall structure (Glenn & Poovaiah, 1989). It was also reported that Ca inhibited splitting of sweet cherries immersed in a solution of an unknown temperature (Christensen, 1996; Glenn & Poovaiah, 1989).

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Calcium chloride ($CaCl_2$) is naturally occurring, edible, and has been approved by the U.S. Food and Drug Administration for postharvest use (Saftner, Conway, & Sams, 1999). The objective of this study was to evaluate the effect of $CaCl_2$ applied in cold water for 5 min (simulating the commercial hydrocooling process) on fruit tissue Ca and Cl uptake, splitting potential, physiological and biochemical changes relating to splitting, and shipping quality of two major splitting-susceptible cultivars ('Skeena' and 'Bing') grown in the U.S. PNW. The goal was to provide the sweet cherry packing industry useful scientific information on reducing postharvest splitting and decay and extending shipping life of sweet cherries.

2. Materials and methods

2.1. Fruit materials

'Skeena' and 'Bing' fruit, ~6500 fruit of each cultivar, were harvested at commercial maturity on a research farm at the Mid-Columbia Agricultural Research and Extension Center (MCAREC), Hood River, OR (lat. 45.68° N, long.121.52° W). Both cultivars were on 15-year old Mazzard rootstocks. Fruit trees were maintained using standard cultural, fertilizer, herbicide and pesticide practices. The commercial maturity was determined as color grade 5 according to the color comparator developed by CTIFL (Centre Technique Interprofessionnel des Fruit et Legumes, Paris, France), in which 1 = light pink and 7 = dark mahogany. Cherry fruit were picked in the morning and immediately transported to the laboratory at MCAREC. Fruit with healthy pedicels were sorted based on uniformity of size, color and freedom from defects for the following tests. The sorted fruit were randomly divided into 3 groups as replications. Fruit pulp temperature was equilibrated at ~20 °C in the lab before dipping.

2.2. Fruit tissue Ca uptake and splitting study

$CaCl_2$ solutions (0%, 0.2%, 0.5%, 1.0%, and 2.0%) were made by dissolving $CaCl_2{\cdot}2H_2O$ (EM Science, Gibbstown, NJ) in distilled water (0 °C) containing 0.05% Tween 20 (Sigma–Aldrich, St. Lois, MO) with pH of 7.0, 11.0, 11.2, 11.3, and 11.4, respectively. pH was measured by a titrator (DL-15, Mettler-Toledo, Zurich, Switzerland). Three fresh solutions (10L/replication) were made up for three replications per treatment ($CaCl_2$ concentration).

2.2.1. Ca and Cl uptake

Fruit were immersed in the $CaCl_2$ solutions for 5 or 30 min and subsequently washed, ground, and freeze-dried to pass through a 1-mm sieve. For Ca determination, the samples were digested in a MARS Express CEM microwave using nitric acid and hydrogen peroxide. Prepared samples were analyzed for Ca content using a Thermo 6500 duo inductively coupled plasma atomic emission spectrometer (ICP-AES) (Thermo and Fisher Scientific, Waltham, MA). For Cl content, the samples were extracted by 2% acetic acid and determined using a Lachat Quikchem 8000 auto-analyzer (Zellweger Analytics, Inc., Milwaukee, WI). Tissue Ca and Cl contents are reported on a dry mass basis ($\mu g\ g^{-1}$). Each sample was comprised of the flesh of 30 fruit.

2.2.2. Water absorption rate

Water absorption rate (percent weight increase per h) was determined by weighing 50 fruit individually before and after 90 min immersion in the $CaCl_2$ solutions. Fruit weight was recorded on a digital balance (XP-3000, Denver Instrument, Bohemia, NY).

2.2.3. Splitting threshold

Splitting thresholds (percent weight increase due to water absorption at which fruit split) were calculated by weighing 50 fruit individually before immersion in the $CaCl_2$ solutions and again after splitting had occurred. In this study, splitting was defined as any skin break 1.6 mm or longer (Glenn & Poovaiah, 1989).

2.2.4. Splitting potential

Splitting potential was determined according to the procedure of Christensen (1996). Fifty fruit were immersed in the $CaCl_2$ solutions at 0 °C for 6 h. At each 2 h interval, split fruit were counted and discarded. The splitting potential was calculated according to the following formula: $SP = (5 \times N_1 + 3 \times N_2 + 1 \times N_3) \times 100/250$, where N_1, N_2, and N_3 represent the number of split fruit after 2, 4, and 6 h of immersion, respectively. Splitting potential was also determined in cold (0 °C) distilled water solutions of $Ca(NO_3)_2$ and $MgCl_2$ at 0.2%, ethylenediaminetetraacetic acid (EDTA) at 5 mM (pH = 7 adjusted by 1 N NaOH), and a phosphate buffer pH = 4 (KH_2PO_4 at 25 mM + H_3PO_4).

2.2.5. Soluble pectin release rate determination

Twenty-five fruit of each replication were immersed in 500 mL of solutions (0 °C) containing $CaCl_2$ at 0.2%, 0.5%, 1.0%, 2.0%; $Ca(NO_3)_2$ or $MgCl_2$ at 0.2%; EDTA at pH = 7 or phosphate buffer at pH = 4 for 10 h. This duration was chosen because all fruit in the different solutions had split within 10 h. Pectin released into the incubation solutions was measured using the method of McComb and McCready (1952). Briefly, the incubation medium was filtered through Whatman #1 filter paper and 5 mL was saponified with 0.25 mL 1 N NaOH at 25–30 °C for 30 min. The saponified sample aliquot was added into a culture tube which contained 12 mL concentrated H_2SO_4 held in an ice bath, and mixed thoroughly. The tube and contents were heated for 10 min in a boiling water bath and immediately cooled to 20 °C. One mL of 0.15% carbazole reagent was added, mixed thoroughly, and held at 20 °C for 25 min. Uronic acid was determined at 520 nm (Model Ultrospec 3100 pro spectrophotometer Biochrom Ltd, Cambridge, England) and its content was calculated from a calibration curve using D-galacturonic acid as the standard and expressed as $ng\ g^{-1}$ FW.

2.3. Effect of adding Ca in hydrocooling water on cherry splitting and shipping quality in a simulated commercial procedure

About 160 fruits in each replication were dipped in the $CaCl_2$ solutions (0%, 0.2%, 0.5%, 1.0%, 2.0%) containing 0.05% Tween 20 at 0 °C for 5 min and then in cold water (0 °C) for 15 min to simulate commercial hydrocooling and transporting in flume water during packing, respectively. The treated fruit were allowed to drain and dry and then were packed in commercial zipper-lock polyethylene bags (~1 kg) with a perforation ratio of ~2% of the surface area. Packed fruit were stored in a cold room at 0 °C and 90% RH for 4 weeks. The bags of fruit were weighed initially and after storage. Weight loss was expressed as percentage loss of original weight.

2.3.1. Objective quality measures

Thirty fruit of each replication were randomly selected and held in the laboratory at 20 °C for 4–5 h (until condensation on fruit surface was gone) 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 angel ($h°$). After color evaluation, fruit firmness was measured using a FirmTech 2 Fruit Firmness instrument (BioWorks Inc., Stillwater, OK) and