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# The effect of postharvest calcium application on tissue calcium concentration, quality attributes, incidence of flesh browning and cell wall physicochemical aspects of peach fruits

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**Abstract**

The effects of postharvest calcium applications on cell wall properties and quality attributes of peach fruits (*Prunus persica* L. Batsch, cv. 'Andross') after harvest or cold storage up to 4 weeks were determined. The fruits were immersed in deionised water or in different calcium sources (calcium chloride, calcium lactate and calcium propionate) at two calcium concentrations (62.5 and 187.5 mM Ca). Calcium concentration profiles in fruits (peel and flesh), in cell wall and in pectin fractions were determined. The calcium content in the peel increased up to 2.7-fold, whereas flesh calcium increased up to 74%, 1 day after immersion. The increase of flesh calcium was accompanied by increase of cell wall calcium, which corresponded to a significant increase of calcium in the water-insoluble pectin fraction. However, calcium became saturated in the water-insoluble, but not water-soluble, pectin fraction with 62.5 mM Ca treatment. Treatment with 62.5 mM Ca salts was as effective as higher concentrations of calcium chloride maintaining tissue firmness during storage. Inversely, calcium lactate and calcium propionate at high concentrations (187.5 mM Ca) caused toxicity symptoms on the fruit surface, expressed as skin discoloration and superficial pitting, leading to additional chemical changes and reduced tissue firmness. Postharvest calcium applications limited the intense of chilling injury symptoms, expressed as flesh browning after 4 weeks cold storage. Peach fruits with severe flesh browning symptoms were characterized by reduced ethylene production, and reduced activities of the pectin modifying enzymes poly-galacturonase and pectin-methyl-esterase.


*Keywords:* Peach; Postharvest dip; Calcium absorption; Cell wall; Pectin; Chilling injury

## 1. Introduction

After harvest rapid ripening in peach fruits is responsible for short shelf life and represents a serious constraint for efficient handling and transportation. Ripening can be retarded by cold storage. However, cold storage life of peaches is frequently limited by chilling injury (CI) and loss of quality (Brummell, Dal Cin, Crisosto, & Labavitch, 2004; Valero, Serrano, Martinez Madrid, & Riquelme, 1997). Peach fruits after extended cold storage present symptoms of internal breakdown (IB), expressed as flesh browning (FB) and similar symptoms in other fruits have been attributed to low calcium content (Hewajulige, Wilson-Wijeratnam, Wijesundera., & Abeysekere, 2003; Thorp, Ferguson, Boyd, & Barnett, 2003).

Preharvest calcium sprays may increase slightly peach fruit calcium content (Crisosto, Day, Johnson, & Garner, 2000) and this increase may differ from year to year, highly regulated by environmental factors (Biggs, A.R., personal communication). Addition of calcium fertilizer to soil is of questionable value (Lester & Grusak, 1999). Conversely, infiltration methods under pressure or vacuum provide a rapid and effective method for increasing calcium content

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(Lara, Garcia, & Vendrell, 2004; Saftner, Conway, & Sams, 1998). However, these treatments often cause surface damage (Saftner, Conway, & Sams, 1999).

Postharvest calcium dips can increase calcium content considerably compared to preharvest sprays, without causing fruit injury, depending on salt type and calcium concentration. Postharvest calcium application maintains cell turgor, membrane integrity, tissue firmness and delays membrane lipid catabolism, extending storage life of fresh fruits (Garcia, Herrera, & Morilla, 1996; Picchioni, Watada, Conway, Whitaker, & Sams, 1998).

Many studies have examined the effects of calcium on fruit firmness and decay after harvest, but few have focused on compositional changes in cell walls of fruits throughout storage (Chardonnet, Charron, Sams, & Conway, 2003; Saftner et al., 1998). To the best of our knowledge, few data exists regarding the effect of postharvest calcium dips in cell wall physicochemical attributes of peach fruits and it has been mainly focused on qualitative characteristics (Wills & Mahendra, 1989) or fungal resistance (Conway, Sams, & Kelman, 1994).

As well as from calcium chloride, which has been extensively used in fresh fruits (Chardonnet et al., 2003; Saftner et al., 1998), calcium propionate and calcium lactate are proposed as alternative calcium sources (Buta, Moline, Spaulding, & Wang, 1995; Saftner, Bai, Abbott, & Lee, 2003). The objectives of this study were to determine the effect of postharvest fruit immersion in different calcium sources and the effect of calcium concentrations on peach fruit tissue and cell wall composition during cold storage.

## 2. Materials and methods

### 2.1. Plant material

Peach (*Prunus persica*, cv. 'Andross') fruits were harvested at firm-ripe stage from a commercial orchard (Naoussa, Northern Greece) in early morning (fruit internal temperature $23 \pm 1$ °C). After selection for uniformity of size and freedom from defects, they were divided into 6 lots of 30 fruits for each water and calcium treatment supplemented with wetting agent (0.03% Agral®600). All treatments included immersion for 5 min in deionized water (water temperature = 20 °C) (control) and three calcium sources (calcium chloride, calcium lactate, calcium propionate) at two calcium concentrations (62.5 and 187.5 mM Ca). The fruits were allowed to drain for 1 h before storage. One lot was analyzed 1 day after immersion and another lot was allowed to ripen for 5 days (shelf life) at 20 °C. The other lots were stored (5 °C, 95% R.H.) for 2 and 4 weeks and sampled both 1 day after removal from storage and after an additional 5 days at 20°C.

### 2.2. Quality parameters

Flesh firmness was measured on two opposite sides of each fruit, after the removal of a 1 mm thick disk of skin from each side of the fruit and the force in N required to insert an Effegi penetrometer fitted with an 11 mm diameter probe was recorded. Soluble solids content (SSC) and titratable acidity (TA) were assessed in juice obtained from six replicate samples of 10 fruits per treatment. SSC was determined with a refractometer, and TA by titration with 0.01 N NaOH to pH 8.2 and expressed as g $l^{-1}$ malic acid.

After removal from cold storage and after subsequent ripening at room temperature for 1 and 5 days, fruits were examined visually for storage disorder (flesh browning) and the severity index was determined as follows: ((% fruit with slight disorder × 1) + (% fruit with medium disorder × 2) + (% fruit with severe disorder × 4))/4. Slight disorder meant that less than 25% of the fruit flesh was affected, medium that between 25% and 50% of the flesh was affected and severe when more than 50% of the fruit flesh showed flesh browning symptoms.

### 2.3. Ethylene and respiration determination

Three fruits were enclosed in 3 L airtight jars for 1 h at 20 °C. Ethylene measurements were performed by withdrawing 1 ml headspace gas sample from the jars with a syringe, and injecting it into a Varian 3300 gas chromatograph, equipped with a stainless steel column filled with Porapak, length 100 cm, diameter 0.32 cm, at 50 °C and a flame-ionisation detector at 120 °C. The carrier gas was nitrogen at a flow rate of 20 ml/min. Respiration was calculated by carbon dioxide production in the gas phase of the jars, measured automatically by an infrared gas analyzer connected to a computer. Ethylene production and respiration rate were recorded daily for 1 week after immersion or removal from cold storage.

### 2.4. Extraction and fractionation of cell wall material (CWM)

A wedged-shaped slice of flesh tissue from each fruit was removed, pooled, frozen in liquid nitrogen, stored at −80 °C and subsequently used for cell wall extraction according to Campbell, Huysamer, Stotz, Greve, and Labavitch (1990).

Samples of the CWM (5 mg) were suspended in distilled $H_2O$ for 1 h and centrifuged (10,500*g*, 25 min). The supernatant was collected and the previous step was repeated once. The two supernatants were combined and designated as the water-soluble pectin fraction. The pellet was dissolved in 2 ml concentrated sulphuric acid (Selvendran & O'Neill, 1987), corresponding to the water-insoluble pectin fraction.

### 2.5. Assay of uronic acids, neutral sugars and cellulose

Aliquots of water-soluble and -insoluble pectin fractions were used for uronic acid determination by a colorimetric assay (Blumenkrantz & Asboe-Hansen, 1973), using galacturonic acid as standard.

Samples of the CWM were analyzed to determine the carbohydrates components of the cell wall material. Non-cellulosic neutral sugars were derivatized to alditol acetates by hydrolysis in 2 N trifluroacetic acid (TFA), reduction and acetylation (Blakeney, Harris, Henry, & Stone, 1983). The derivatives were identified by gas chromatography on a Dani Chromatograph 1000 (Dani Instruments SpA, Cologno Monzese, Milano, Italy) fitted with a 30 m fused silica capillary column (DB-225, J&W Scientific, Folsom, CA, USA). The chromatograph oven was held at 210°C and hydrogen was used as carrier gas. Quantitation was based on integration of the peaks from the flame ionization detector with a Shimadzu C-R3A (Shimadzu, Kyoto, Japan) chromatography data system.

Cellulose content was determined in the TFA-insoluble crude cell wall by the anthrone colorimetric assay (Dische, 1962).

### 2.6. Calcium determination

Total calcium content was determined in the peel and the flesh of peach fruits. The samples were dehydrated and ground to fine powder, which was ashed in a muffle furnace at 500 °C overnight. The ash was dissolved with 2 N hydrochloric acid and total calcium and magnesium content were determined by atomic absorption using a Perkin–Elmer 403 AA spectrophotometer (Perkin–Elmer Inc., MA, USA) at 422.7 nm and expressed as $\mu g\ g^{-1}$ dry weight (dw). Cell wall material and aliquots of pectin fractions were wet digested in a triacid solution ($HNO_3$:$H_2SO_4$:$HClO_4$, 5:1:1, v/v/v) and calcium content was measured as above and expressed as $\mu g\ mg^{-1}$ CWM.

### 2.7. Enzyme assay

Enzyme extraction was conducted according to Zhou et al. (2000). Briefly, 50 g of frozen flesh fruit, collected in the same manner as for cell wall extraction, was ground in 85 ml cold 12% PEG 4000, 0.2% sodium bisulfite for 1 min. After centrifugation (10,500*g*, 10 min) the pellet was collected and separated into two parts for extraction of each enzyme activity. Polygalacturonase (PG, EC 3.2.1.67) activity was determined according to Zhou et al. (2000) and one activity unit was defined as 1 μg galacturonic acid released per mg protein per h. Pectin methyl esterase (PME, EC 3.1.1.11) activity was determined according to Zhou et al. (2000) and one unit activity was defined as 1 mmol sodium hydroxide consumed per mg protein per h. Protein content was determined according to Bradford (1976), using bovine serum albumin (BSA) as a standard.

### 2.8. Statistical analysis

All treatments were run with at least six replicates. Data were treated for multiple comparisons by analysis of variance (ANOVA), followed by Duncan's Multiple Range Test with significance level $P < 0.05$. ANOVA was performed using the statistical software SPSS (SPSS Inc., Chicago, USA).

## 3. Results and discussion

### 3.1. Calcium

Significant increase of calcium content both in the peel and the flesh of calcium-treated peach fruits were recorded. Peel calcium increased by 2.3 to 2.7-fold and flesh calcium increased by 50% to 74% in calcium-treated peach fruits, compared to control fruits, 1 day after immersion (Table 1). Calcium source did not seem to affect calcium absorption. Calcium content was significantly higher in the high concentrations of all calcium sources applied, although it was not proportional to the

Table 1
Calcium content ($\mu g\ g^{-1}$ dw) of various tissues of peach fruits (cv. 'Andross') in relation to calcium source, concentration and storage time

| Tissue | Calcium treatment | Calcium ($\mu g\ g^{-1}$ dw) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Storage time (weeks of cold storage + days shelf life) | | | | | |
| | | 0 + 1 | 0 + 5 | 2 + 1 | 2 + 5 | 4 + 1 | 4 + 5 |
| Peel | Control[A] | 588c | 546e | 570d | 524d | 548c | 496d |
| | Calcium chloride, 62.5 mM | 1370b | 1244c | 1257c | 1166c | 1186b | 1081c |
| | Calcium chloride, 187.5 mM | 1603a | 1409b | 1512a | 1463a | 1422a | 1367a |
| | Calcium lactate, 62.5 mM | 1423b | 1215cd | 1247c | 1143c | 1197b | 1074c |
| | Calcium lactate, 187.5 mM | 1568a | 1433ab | 1419b | 1361b | 1472a | 1297b |
| | Calcium propionate, 62.5 mM | 1385b | 1157d | 1273c | 1167c | 1175b | 1078c |
| | Calcium propionate, 187.5 mM | 1598a | 1480a | 1432b | 1374b | 1434a | 1264b |
| Flesh | Control | 331d | 372e | 351e | 403d | 330d | 388d |
| | Calcium chloride, 62.5 mM | 498c | 646c | 593c | 652c | 606c | 676c |
| | Calcium chloride, 187.5 mM | 536b | 683b | 656b | 728b | 703b | 813b |
| | Calcium lactate, 62.5 mM | 511bc | 610d | 584cd | 631c | 617c | 684c |
| | Calcium lactate, 187.5 mM | 576a | 718a | 716a | 809a | 743ab | 833b |
| | Calcium propionate, 62.5 mM | 518bc | 626cd | 548d | 638c | 606c | 696c |
| | Calcium propionate, 187.5 mM | 563a | 716a | 684ab | 787a | 760a | 861a |

[A] Values within columns within tissue type followed by the same letter are not significantly different to each other at $P = 0.05$ (Duncan's Multiple Range test).

concentration of the calcium source. During cold storage a decrease of calcium in the peel and a corresponding increase of flesh calcium were recorded. These changes were further enhanced after 5 days shelf life. These may be attributed to the creation of channels of discontinuity and openings for calcium penetration, as a result of their ripening after 5 days shelf life, in relation to 1 day shelf life.

### 3.2. Cell wall calcium

Cell wall calcium was significantly increased in calcium-treated peach fruits, compared to the control fruits (Table 2), whereas no differences were observed among the different calcium sources. Additionally, during cold storage and subsequent ripening for 5 days at room temperature, an increase in cell wall calcium, mainly due to the increase of calcium in the water-soluble pectin fraction, was recorded. However, significant differences were observed in the distribution between the pectin fractions. Immersion in 62.5 mM Ca caused saturation levels for calcium in the water-insoluble pectin fraction to be reached. On the contrary, calcium in the water-soluble pectin fraction increased only with the increase of calcium concentration. The increase of cell wall calcium in calcium lactate and calcium propionate-treated fruits at 187.5 mM Ca was accompanied by significantly higher calcium levels in the water-soluble pectin fraction, whereas the calcium bound in the water-insoluble pectin fraction was significantly lower, compared to peach fruits treated with 62.5 mM Ca, due to surface damage that led to additional chemical changes.

The increase in cell wall-bound calcium of calcium-treated peaches was related to both calcium concentration and time of storage. Similar results after postharvest calcium application have been reported for apple fruits by other workers (Chardonnet et al., 2003; Saftner et al., 1998), who suggested that soluble calcium was mobilized and integrated into the cell wall.

Saftner et al. (1998) have reported that water-soluble pectin calcium does not affect flesh firmness. Furthermore, Chardonnet et al. (2003) in postharvest dips of apple fruits in concentrations up to 4% calcium chloride found that immersions in 2% calcium chloride was enough for maximum calcium accumulation in the cell wall, avoiding at the same time possible surface damage. Calcium accumulation in the cell wall facilitates cross-linking of pectic polymers leading to a cell wall network that increases wall strength and cell cohesion (White & Broadley, 2003) with unbound calcium ions to have little or no direct effect on tissue strength (Saftner et al., 1998). Consequently, cell wall calcium cannot be used as indicator of peach fruit quality. On the contrary, the calcium bound in the water-insoluble pectin fraction can be used as quality indicator.

Table 2
Calcium concentration ($\mu g\ mg^{-1}$ CWM) of cell wall material and pectin fractions of peach fruits (cv. 'Andross') in relation to calcium source, concentration and storage time

| Cell wall component | Calcium treatment | Calcium ($\mu g\ mg^{-1}$ CWM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Storage time (weeks of cold storage + days shelf life) | | | | | |
| | | 0 + 1 | 0 + 5 | 2 + 1 | 2 + 5 | 4 + 1 | 4 + 5 |
| Cell wall | Control[A] | 1.21d | 1.25d | 1.24d | 1.48f | 1.43e | 1.47d |
| | Calcium chloride, 62.5 mM | 1.66c | 2.13b | 2.22b | 2.19cd | 2.67b | 2.72b |
| | Calcium chloride, 187.5 mM | 1.98b | 2.21ab | 2.38a | 2.55a | 2.87a | 2.84a |
| | Calcium lactate, 62.5 mM | 1.60c | 1.93c | 2.09c | 2.13de | 2.58c | 2.73ab |
| | Calcium lactate, 187.5 mM | 1.94b | 2.19ab | 2.35ab | 2.26bc | 2.40d | 2.54d |
| | Calcium propionate, 62.5 mM | 1.59c | 1.96c | 2.07c | 2.07e | 2.54c | 2.62bc |
| | Calcium propionate, 187.5 mM | 2.03a | 2.29a | 2.31ab | 2.32b | 2.59bc | 2.57c |
| Water-soluble | Control | 0.18c | 0.36e | 0.24f | 0.39e | 0.36e | 0.35d |
| Pectin | Calcium chloride, 62.5 mM | 0.42b | 0.59d | 0.34de | 0.52d | 0.50d | 0.98c |
| | Calcium chloride, 187.5 mM | 0.61a | 0.68c | 0.49c | 0.79b | 0.57bc | 1.10b |
| | Calcium lactate, 62.5 mM | 0.37b | 0.58d | 0.38d | 0.62c | 0.58b | 1.00bc |
| | Calcium lactate, 187.5 mM | 0.53a | 1.05b | 1.05a | 1.21a | 1.18a | 1.29a |
| | Calcium propionate, 62.5 mM | 0.36b | 0.62cd | 0.31e | 0.61c | 0.51cd | 0.95c |
| | Calcium propionate, 187.5 mM | 0.56a | 1.16a | 0.97b | 1.26a | 1.22a | 1.23a |
| Water-insoluble | Control | 0.94c | 0.77d | 0.90e | 0.98c | 0.91e | 1.00d |
| Pectin | Calcium chloride, 62.5 mM | 1.16b | 1.33a | 1.76d | 1.53a | 1.96b | 1.48b |
| | Calcium chloride, 187.5 mM | 1.21b | 1.37a | 1.70ab | 1.55a | 2.07a | 1.54a |
| | Calcium lactate, 62.5 mM | 1.16b | 1.19b | 1.65bc | 1.44b | 1.80c | 1.48ab |
| | Calcium lactate, 187.5 mM | 1.33a | 0.91c | 1.07b | 0.85d | 1.05d | 1.03d |
| | Calcium propionate, 62.5 mM | 1.15b | 1.22b | 1.59c | 1.36b | 1.74c | 1.42b |
| | Calcium propionate, 187.5 mM | 1.31a | 0.94c | 1.15d | 0.87d | 1.05d | 1.16c |

[A] Values within columns within cell wall components followed by the same letter are not significantly different to each other at $P = 0.05$ (Duncan's Multiple Range test).

### 3.3. Quality attributes

Peach fruits dipped in calcium salts, except those that showed surface damage, were characterized by increased flesh firmness, compared to control fruits 5 days after harvest or cold storage (Table 3), as a result of increased flesh calcium. Calcium salts can suspend or even accelerate the senescing-related processes, depending on calcium concentration (Conway et al., 1994; Saftner et al., 1998). Calcium lactate and calcium propionate (187.5 mM Ca) increased considerably the calcium content of fruits, but at the same time accelerated the process of flesh softening, as a result of surface-damage symptoms due to excessive salt concentration. Immersion of fruits in high calcium concentration salts increases the risk for salt-related fruit injury, which appears to be a result of osmotic effects (Saftner et al., 1998).

SSC and TA were not influenced by the postharvest calcium dips, and slight differences existed. However, peach fruits with increased flesh browning (FB) symptoms (control fruits) were characterized by decreased levels of SSC compared to calcium-treated peach fruits.

FB symptoms were recorded after 4 weeks cold storage and they were more intense after additional ripening for 5 days (Table 4). The high calcium concentrations resulted in decreased FB symptoms. These symptoms have been directly associated with calcium content in other fresh fruits (Hewajulige et al., 2003). Therefore, calcium dips raise the possibility of producing fruits less susceptible to flesh browning symptoms. Physiological disorders that are caused by low storage temperatures probably are related to calcium content (Hewajulige et al., 2003; Thorp et al., 2003). The same action of calcium salts has also been reported for fresh-cut fruits (Gorny, Hess-Pierce, Cifuentes, & Kader, 2002; Luna-Guzman & Barrett, 2000), where the enzymatic browning of flesh is a result of different metabolic pathways.

### 3.4. Ethylene evolution and respiration rate

A significant increase of ethylene was recorded the 3rd day after removal from 2-week cold storage (146-160 ml $kg^{-1}$ $h^{-1}$) without significant differences between the calcium-treated and control fruits (data not shown). However, significant differences were recorded in 4-week

Table 4
Flesh browning index (%) of peach fruits (cv. 'Andross') in relation to calcium source, concentration and storage time

| Calcium treatment | Flesh browning index (%) | |
|---|---|---|
| | Storage time (weeks of cold storage + days shelf life) | |
| | 4 + 1 | 4 + 5 |
| Control[A] | 16.0a | 40.0a |
| Calcium chloride, 62.5 mM | 8.3b | 19.3b |
| Calcium chloride, 187.5 mM | 7.6b | 17.4bc |
| Calcium lactate, 62.5 mM | 7.4b | 14.7c |
| Calcium lactate, 187.5 mM | 5.3b | 17.6bc |
| Calcium propionate, 62.5 mM | 4.8b | 11.4c |
| Calcium propionate, 187.5 mM | 7.4b | 11.3c |

[A] Values within columns followed by the same letter are not significantly different to each other at $P = 0.05$ (Duncan's Multiple Range test).

Table 3
Tissue firmness (N), SSC (%) and TA (g $l^{-1}$ malic acid) of peach fruits (cv. 'Andross') in relation to calcium source, concentration and storage time

| Quality attributes | Calcium treatment | Storage time (weeks of cold storage + days shelf life) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 + 1 | 0 + 5 | 2 + 1 | 2 + 5 | 4 + 1 | 4 + 5 |
| Firmness | Control[A] | 65.7a | 45.1bc | 55.9a | 39.2bc | 50.0abc | 37.3b |
| | Calcium chloride, 62.5 mM | 65.7a | 50.0a | 58.9a | 42.2a | 54.0a | 43.2a |
| | Calcium chloride, 187.5 mM | 63.8a | 49.1a | 58.9a | 42.2a | 54.0a | 41.2a |
| | Calcium lactate, 62.5 mM | 65.7a | 47.1ab | 57.9a | 42.3a | 51.0ab | 42.2a |
| | Calcium lactate, 187.5 mM | 63.8a | 44.1c | 54.9a | 36.3c | 47.1abc | 31.4c |
| | Calcium propionate, 62.5 mM | 67.7a | 48.1ab | 56.9a | 44.1a | 53.0a | 41.2a |
| | Calcium propionate, 187.5 mM | 62.8a | 44.1c | 55.9a | 37.3c | 46.1c | 31.4c |
| SSC | Control | 10.7a | 11.5bc | 11.3c | 11.9b | 10.8d | 11.9b |
| | Calcium chloride, 62.5 mM | 10.6a | 11.6abc | 11.2c | 11.9b | 11.0cd | 12.6a |
| | Calcium chloride, 187.5 mM | 10.5a | 11.5bc | 11.3c | 11.9b | 11.4b | 12.6a |
| | Calcium lactate, 62.5 mM | 10.6a | 11.3c | 11.8ab | 11.8b | 11.2b | 12.7a |
| | Calcium lactate, 187.5 mM | 10.7a | 11.9a | 11.7ab | 12.4a | 11.9a | 13.0a |
| | Calcium propionate, 62.5 mM | 10.6a | 11.6abc | 11.5bc | 11.9b | 11.2b | 12.6a |
| | Calcium propionate, 187.5 mM | 10.7a | 11.8ab | 11.9a | 12.4a | 12.0a | 12.8a |
| TA | Control | 6.4a | 4.5ab | 4.9a | 3.5a | 4.1ab | 3.4a |
| | Calcium chloride, 62.5 mM | 6.3a | 4.5ab | 5.0a | 3.3bc | 4.3ab | 3.3ab |
| | Calcium chloride, 187.5 mM | 6.3a | 4.4ab | 4.9a | 3.2b | 4.3ab | 3.2abc |
| | Calcium lactate, 62.5 mM | 6.3a | 4.6a | 4.9a | 3.4bc | 4.4a | 3.4a |
| | Calcium lactate, 187.5 mM | 6.3a | 4.2b | 4.5a | 2.6c | 3.7cd | 2.9bc |
| | Calcium propionate, 62.5 mM | 6.4a | 4.4ab | 4.6a | 3.4bc | 4.0bc | 3.3a |
| | Calcium propionate, 187.5 mM | 6.5a | 4.3ab | 4.5a | 2.8c | 3.4d | 2.8c |

[A] Values within columns within quality attributes followed by the same letter are not significantly different to each other at $P = 0.05$ (Duncan's Multiple Range test).

cold stored fruits. The climacteric peak of control fruits was significantly smaller (61.4 ml $kg^{-1}$ $h^{-1}$) compared to calcium-treated fruits (93.7-98.4 ml $kg^{-1}$ $h^{-1}$). Peach fruits dipped in calcium lactate and calcium propionate (187.5 mM Ca) showed surface damage and produced significantly higher levels of ethylene compared to the other treatments. The ethylene climacteric peaks for the above treatments were on average 77.6, 248.3 and 174.0 ml $kg^{-1}$ $h^{-1}$ after harvest, 2 and 4 weeks cold storage, respectively and they cannot be evaluated for the effect of calcium on ethylene production.

Ethylene possesses an important role in integrating developmental signals and responses to abiotic stresses, like cold storage, and it has been suggested that calcium delays the onset of the ethylene climacteric period and climacteric peak (Ben-Arie, Mignani, Greve, Huysamer, & Labavitch, 1995). In the present work, postharvest calcium treatments do not seem to affect the time of the climacteric peak and its rise during ripening, either after harvest or after 2 week cold storage. However, differentiations in ethylene production were recorded after 4 weeks cold storage due to CI symptoms. FB influenced ethylene production, leading to a decreased climacteric peak, which has also been reported for other peach cultivars with CI symptoms (Fernández-Trujillo, Cano, & Artés, 1998; Valero et al., 1997) and limits the capacity of ethylene to create new receptors for ethylene binding after the transport of fruits at room temperature. It is therefore evident that the 4-week cold storage suppressed the feedback regulation system for ethylene production after removal of fruits at room temperature.

The respiration rate did not present climacteric peak after harvest, 2 and 4 weeks cold storage, while no significant differences were observed between calcium-treated and control fruits (7.9–8.8 ml $CO_2$ $kg^{-1}$ $h^{-1}$). Duque, Barreiro, and Arrabaca (1999) have also reported similar results for calcium-treated apple fruits. However, postharvest calcium dips in both climacteric and non-climacteric fruits have resulted in decreased respiration rate (Saftner et al., 1998; Tsantili, Konstantinidis, Athanasopoulos, & Pontikis, 2002). Peach fruits immersed in calcium lactate and calcium propionate (187.5 mM Ca) were characterized by increased respiratory activity (13.4–16.4 ml $CO_2$ $kg^{-1}$ $h^{-1}$) due to surface injury.

### 3.5. Cell wall physicochemical attributes

The increased calcium content in the water-insoluble and -soluble pectin fraction was not accompanied by a corresponding increase in uronic acid content (Tables 2 and 5). Chardonnet et al. (2003) have postulated that there is no direct relationship between uronic acid and calcium content.

'Andross' is a typical non-melting flesh peach cultivar, where the progress of ripening is gradual and slow and it does not encompass the melting stage. Uronic acid content decreased in the water-insoluble pectin fraction and increased in the water-soluble pectin fraction slowly and gradually as storage period increased, culminated after 4 weeks cold storage and 5 days shelf life. Calcium-treated peach fruits contained higher uronic acid content in the water-soluble pectin fraction compared to control fruits. Flesh browning symptoms probably indicate the impediment of solubilization of the water-insoluble pectin fraction, a fact that is already well documented for other CI symptoms in peach and nectarine cultivars (Artés, Cano, & Fernández-Trujillo, 1996; Zhou et al., 2000).

Neutral sugar and cellulose content presented minute differences between calcium treatments both after cold storage and subsequent ripening at room temperature (data not

Table 5
Uronic acid content of pectin fractions (μg $mg^{-1}$ CWM) of peach fruits (cv. 'Andross') in relation to calcium source, concentration and storage time

| Cell wall component | Calcium treatment | Uronic acid content (μg $mg^{-1}$ CWM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Storage time (weeks of cold storage + days shelf life) | | | | | |
| | | 0 + 1 | 0 + 5 | 2 + 1 | 2 + 5 | 4 + 1 | 4 + 5 |
| Water-soluble pectin | Control[A] | 85.5ab | 126.1b | 124.5a | 139.7a | 128.6b | 109.4e |
| | Calcium chloride, 62.5 mM | 83.5b | 119.7cd | 108.2cd | 110.3bc | 127.0bc | 124.3c |
| | Calcium chloride, 187.5 mM | 83.7ab | 125.2bc | 101.3d | 110.3bc | 127.0bc | 117.2d |
| | Calcium lactate, 62.5 mM | 84.6ab | 126.8b | 106.7c | 106.4c | 145.6a | 120.5cd |
| | Calcium lactate, 187.5 mM | 83.8ab | 134.8a | 120.4ab | 140.4a | 125.1bc | 142.5a |
| | Calcium propionate, 62.5 mM | 87.5a | 116.2d | 116.5b | 113.9b | 114.9d | 124.2c |
| | Calcium propionate, 187.5 mM | 87.5a | 137.6a | 125.0a | 142.9a | 120.0cd | 136.4b |
| Water-insoluble pectin | Control | 253.5a | 217.2b | 239.5bc | 213.8b | 213.8b | 200.3b |
| | Calcium chloride, 62.5 mM | 258.1a | 233.6a | 244.1ab | 219.9ab | 224.5a | 204.0ab |
| | Calcium chloride, 187.5 mM | 261.8a | 244.3a | 254.2a | 219.9ab | 228.0a | 207.9a |
| | Calcium lactate, 62.5 mM | 259.5a | 237.3a | 253.0a | 227.8a | 219.5ab | 203.5ab |
| | Calcium lactate, 187.5 mM | 256.4a | 207.9bc | 214.5d | 195.2c | 203.3c | 175.3c |
| | Calcium propionate, 62.5 mM | 255.0a | 214.4b | 231.7c | 211.7b | 213.5b | 205.7ab |
| | Calcium propionate, 187.5 mM | 255.0a | 200.4c | 219.2d | 195.2c | 210.3bc | 179.8c |

[A] Values within columns within cell wall components followed by the same letter are not significantly different to each other at $P = 0.05$ (Duncan's Multiple Range test).

Table 6
Activities of PG and PME of peach fruits (cv. 'Andross') in relation to calcium source, concentration and storage time

| Enzyme | Calcium treatment | Storage time (weeks of cold storage + days shelf life) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 + 1 | 0 + 5 | 2 + 1 | 2 + 5 | 4 + 1 | 4 + 5 |
| PG[A] | Control[B] | 246.2b | 369.8c | 323.1b | 415.7b | 236.6d | 227.6e |
| | Calcium chloride, 62.5 mM | 220.3c | 336.8d | 290.7c | 370.4bc | 258.1c | 282.9cd |
| | Calcium chloride, 187.5 mM | 210.9c | 312.3d | 292.5c | 364.2bc | 256.3c | 296.0c |
| | Calcium lactate, 62.5 mM | 218.3c | 334.2d | 287.6c | 384.3bc | 250.9cd | 293.3cd |
| | Calcium lactate, 187.5 mM | 277.2a | 543.1b | 360.0a | 885.2a | 349.5b | 513.0a |
| | Calcium propionate, 62.5 mM | 228.9bc | 311.9d | 302.9bc | 335.3c | 266.8c | 268.1d |
| | Calcium propionate, 187.5 mM | 291.2a | 611.9a | 353.4a | 840.7a | 368.8a | 476.3b |
| PME[C] | Control[B] | 0.540ab | 0.438ab | 0.471a | 0.348a | 0.392b | 0.319d |
| | Calcium chloride, 62.5 mM | 0.561a | 0.434ab | 0.460a | 0.356a | 0.426a | 0.375abc |
| | Calcium chloride, 187.5 mM | 0.557a | 0.450a | 0.474a | 0.356a | 0.412ab | 0.368bc |
| | Calcium lactate, 62.5 mM | 0.554a | 0.445a | 0.461a | 0.355a | 0.400ab | 0.387a |
| | Calcium lactate, 187.5 mM | 0.517b | 0.413bc | 0.461a | 0.334a | 0.400ab | 0.380ab |
| | Calcium propionate, 62.5 mM | 0.529b | 0.416bc | 0.447ab | 0.346a | 0.418ab | 0.374abc |
| | Calcium propionate, 187.5 mM | 0.519b | 0.396c | 0.420b | 0.330a | 0.412ab | 0.361c |

[A] One unit PG defined as 1 mg galacturonic acid released per mg protein:h.
[B] Values within columns of each enzyme followed by the same letter are not significantly different to each other at $P = 0.05$ (Duncan's Multiple Range test).
[C] One unit of PME defined as 1 mmole NaOH consumed per mg protein:h.

shown), although calcium treatment in apple fruits has been reported to inhibit or reduce the solubilization of the main neutral sugars (Chardonnet et al., 2003). Cellulose appears to be independent from the ripening process and the increase of calcium content does not seem to affect its content.

### 3.6. Pectin modifying enzymes

PG activity was higher in calcium-treated peach fruits compared to control fruits during their ripening after 4 weeks cold storage (Table 6). The decreased PG activity in control fruits was connected with intense FB symptoms. Abnormal ripening due to internal breakdown after 4-week cold storage plus 1 and 5 days shelf life resulted in lower polygalacturonase activity. In the present study PG activity increased gradually and culminated after 2-week cold storage and 5 days retention at room temperature, providing supporting evidence that PG increases during normal ripening but its activity decreases when abnormal ripening occurs. Additionally a certain quantity of PG activity is needed for normal fruit ripening. It is therefore evident that calcium possesses a distinguishable role in the ripening physiology of fresh fruits with an active role in the metabolic pathways that regulate the appearance of physiological disorders after extended cold storage periods. Furthermore, reduced polygalacturonase activity has been strongly associated with CI symptoms in other peach and nectarine cultivars (Artés et al., 1996; Zhou et al., 2000).

PME activity decreased during ripening of peaches at room temperature after removal from cold storage, possessing the lowest value after 4-week cold storage plus 5 days shelf life. PME activity showed significant differences between the calcium-treated and control fruits only after 4 weeks cold storage and the differences were more intense after additional 5 days ripening at room temperature.

PME activity of fruits dipped in calcium salts was higher compared to control fruits. This can be explained by the increase of sites for calcium binding in the pectin network (MacDougall, Parker, & Selvendran, 1995). Overall, the metabolic pathways that are followed in the case of fruits with intense FB symptoms are characterized by decreased activity of both PG and PME.

## 4. Conclusions

Clingstone non-melting peach fruits destined for canning are often stored for several days in cold rooms so that they can be processed gradually, according to the capacities of canning industries. Calcium chloride immersion at 62.5 mM Ca could be suggested as a potential postharvest handling of non-melting peach fruits destined for processing after prolonged cold storage, since it provides fruits with better qualitative characteristics (increased tissue firmness, less susceptibility to physiological disorders after extended cold storage) and it minimizes the risk of salt-related injuries. However, further studies should be conducted, including sensory evaluation, in order to assure that such treatments do not lead to bitter, salty or other off-flavour developments. In addition, it must be elucidated whether such calcium salts are corrosive to metal processing equipment used in processing factories for fruit sanitation and sorting.

The application of higher calcium concentration (187.5 mM Ca) does not seem to contribute to any significant quality improvement. Although calcium lactate and calcium propionate are used with great potential in the fresh fruit industry, they did not show any differences from calcium chloride.

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