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Postharvest Biology and Technology 28 (2003) 97–111

Postharvest Biology and Technology

www.elsevier.com/locate/postharvbio

Chemical changes in the cortical tissue and cell walls of calcium-infiltrated 'Golden Delicious' apples during storage

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Received 28 August 2001; accepted 20 July 2002

Abstract

'Golden Delicious' apples (*Malus* × *domestica* Borkh.) were either untreated or pressure-infiltrated after harvest with 0, 1, 2, 3 or 4% $CaCl_2$ solutions (w/v) and stored at 0 °C for up to 6 months. The chemical composition of the fruit cortical tissue and cell walls of the 2–4 mm layer under the epidermis was studied. Storage of untreated fruit resulted in a decrease in K, P, Mg, S and Suc content of the tissue, while Fru and Glc increased. In the cell wall, Ca, Mg and total polysaccharide content increased while S, P, total neutral sugar and protein content decreased. During storage, fruit infiltrated with 0% $CaCl_2$ showed a decrease in total polysaccharide and uronic acid content while neutral sugar content increased. After 6 months, the 0% treated fruit had higher levels of total P, Na and S compared to the untreated fruit. These changes in the cell wall of both untreated and 0% treated fruit resulted in an overall decrease in cell wall content of the apple tissue during storage. Analyses of Ca-infiltrated tissue and cell wall characteristics showed an interaction between $CaCl_2$ treatment and time in storage for total and cell wall-bound minerals, total neutral sugar (Glc, Ara, Gal and Rha), protein and cell wall content. $CaCl_2$ infiltration resulted in an increase in both total and cell wall-bound Ca of the apple tissue during storage, with a maximum reached at 2% $CaCl_2$ for fruit stored 4 or 6 months. Ca-infiltrated fruit had higher levels of total K and Na, cell wall-bound Mg, and reduced loss of Ara and Gal after 6 months storage compared to fruit treated with 0% $CaCl_2$, resulting in reduced cell wall degradation of 2% $CaCl_2$ treated fruit during storage. The major changes in the tissue and cell walls occurred after 6 months storage, indicating that this stage was critical for quality maintenance.

Keywords: Apple; $CaCl_2$; *Malus domestica* Borkh.; Cell wall; Pressure infiltration; Postharvest

1. Introduction

The multiple roles of Ca associated with the plant cell have been well documented (Ferguson and Drobak, 1988; Bush, 1995). Soluble Ca is

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0925-5214/02/$ - see front matter
PII: S0925-5214(02)00139-4

involved in protein phosphorylation via Ca-calmodulin binding (Poovaiah et al., 1988). A large portion of the Ca in plant cells is located in the cell wall and plasma membrane where it plays a major role in senescence and ripening. Concentrations of 1–5 mm Ca^{2+} occur in the cell wall region (Poovaiah et al., 1988). Cell wall-bound Ca is involved in maintaining cell wall integrity by binding carboxyl groups of polygalacturonate chains, which are mainly present in the middle lamella and primary cell wall.

Postharvest Ca treatments used to increase Ca content of the cell wall were effective in delaying senescence, resulting in firmer, higher quality fruit (Sams et al., 1993) that were less susceptible to disease during storage (Conway et al., 1991). Many techniques to increase Ca content in the cell walls of fruit tissue after harvest have been developed. These include heat treatment (Klein et al., 1997), dipping (Conway and Sams, 1983), vacuum infiltration (Rajapakse et al., 1992), pressure infiltration (Conway and Sams, 1985), surfactants or coating agents (Saftner et al., 1998), or a combination of these techniques (Klein et al., 1990). While the beneficial effects of Ca infiltration were consistently shown, some studies emphasized the potential for injury caused to the fruit resulting from these treatments (Conway et al., 1994). Fruit injury led to visible effects such as skin discoloration and/or superficial pitting in the lenticel region (Saftner et al., 1998), probably leading to additional chemical changes in the tissue and the cell wall.

Pressure infiltration of $CaCl_2$ solutions mainly affects Ca concentration of tissue near the surface of treated fruit. This effect varies depending upon the sampled tissue, with the greatest increase in Ca concentration being in the cortex near the epidermis (Conway and Sams, 1987). Calcium applied to the fruit penetrates primarily through lenticels and increases Ca content of the tissue, mainly in the middle lamella region (Tobias et al., 1993). The cell wall is a dynamic structure whose properties will change in response to variations in the cell environment. The cell wall is composed of polysaccharides (hemicellulose, cellulose and pectic substances), protein (mostly glycoprotein), ions and phenolic compounds. During ripening and storage, the increase in carbohydrate solubilization by enzymes such as polygalacturonase (Wu et al., 1993) resulted in the softening of the tissue (Sams and Conway, 1984).

Many studies have examined the effects of Ca infiltration on fruit firmness and decay after harvest, but few focused on compositional changes in cell walls of infiltrated fruit throughout storage. Therefore, the objectives of this study were to determine the effects of pressure infiltration, $CaCl_2$ concentration and storage time on fruit tissue and cell wall composition during storage.

2. Materials and methods

2.1. Fruit

'Golden Delicious' apples were harvested from a commercial orchard in southern Pennsylvania at the preclimacteric stage and randomly divided into six lots of 144 fruit.

2.2. Calcium treatment

A 20 l jacketed stainless steel pressure vessel was used to infiltrate fruit with $CaCl_2$ solutions under a pressure of 103 kPa for 3 min. Each lot was treated in one of the following ways. Control: untreated; 0%: distilled H_2O; 1, 2, 3 or 4% (w/v) solutions of $CaCl_2$ ($CaCl_2 \cdot 2H_2O$) prepared in distilled H_2O. Fruit were placed on Kraft paper and allowed to drain for 4 h, and then stored at 0 °C. From each lot, 36 fruit were removed from storage after 2 weeks, 2, 4 or 6 months and equilibrated at 20 °C for 12 h before sample preparation.

2.3. Sample preparation

2.3.1. Apple tissue

Peel and outer flesh of the entire fruit was removed to a depth of 2 mm with a mechanical peeler and discarded. The next 2 mm of flesh tissue (2–4 mm) was used for analysis. Each sample was made up of the combined flesh from four apples, and nine samples from each treatment were analyzed. Each sample was divided into two equal parts: one-half was used for tissue analysis and the

second half was used for cell wall analysis. After removal from the fruit, the portion used for tissue analysis was immediately frozen in liquid nitrogen, freeze-dried, ground and stored under vacuum in desiccators until needed.

2.3.2. Apple cell walls

Following removal from the fruit as described above, cell walls were extracted as previously described (Tobias et al., 1993), freeze-dried and stored under vacuum in desiccators until needed.

2.4. Chemical and biochemical techniques for apple tissue and cell wall

Apple cortical tissue samples were analyzed for minerals, ethanol-soluble non-cellulosic neutral sugar and ethanol-soluble sugars associated with the cell wall. Cell wall samples were analyzed for minerals, total polysaccharides, non-cellulosic neutral sugars, uronic acid and protein contents.

Dried material (500 mg of apple tissue, 50 mg of apple cell wall) was ashed, dissolved in 2 N HCl and filtered through filter paper. The samples were then analyzed by inductively coupled plasma (ICP) emission spectrometry (model 61E; Thermo Jarrell Ash, Franklin, MA).

2.4.1. Total polysaccharides

Five milligrams of cell wall material was suspended in 1 ml of 100 mM potassium phosphate buffer (pH 7). To remove starch from samples, 40 μl of 0.1% (w/v) α-amylase (EC 3.2.1.1) from *Bacillus subtilis* (Sigma, St Louis, MO) was added. A drop of toluene was added to the mixture to prevent bacterial development. Tubes were incubated for 24 h at 25 °C. After centrifugation ($2500 \times g$, 10 min), the pellet was washed twice with 2 ml H_2O (Jones and Albersheim, 1972). The extract was then dried under N_2. Two hundred microliters of 95% ethanol was added, the mixture homogenized and 60% (v/v) H_2SO_4 solution was slowly added to a volume of 5 ml as described by Velichkov (1992). The homogenate was transferred to a 10 ml volumetric flask and 60% (v/v) H_2SO_4 was added to a final volume of 10 ml. Aliquots of 500 μl were analyzed according to Mokrasch (1954).

2.4.2. Non-cellulosic polysaccharides

Apple tissue samples were analyzed for soluble sugars (glucose (Glc), fructose (Fru) and sucrose (Suc)) by trimethylsilylation (TMS) as described by Li and Schuhmann (1980) and for cell wall-related sugars ((rhamnose (Rha), arabinose (Ara), xylose (Xyl), mannose (Man), glucose (Glc) and galactose (Gal)) by the analysis of their alditol acetate (AA) derivatives. Cell wall samples were analyzed for AA derivatives (Blakeney et al., 1983).

Soluble sugars were extracted by mixing 80 mg of apple tissue with 5 ml 80% ethanol for 2 h. After centrifugation ($2000 \times g$, 10 min), the supernatant was recovered and stored in Teflon-lined test tubes at −20 °C until needed. For analysis, 500 μl of 80% ethanol extract was placed in a 4 ml vial and dried down at 50 °C with N_2. After the addition of 50 μl trifluoroacetic acid (TFA), capped vials were left overnight at room temperature, the supernatant recovered and analyzed with a gas chromatograph (GC) (5890 Series II splitless mode; Hewlett-Packard (HP), Palo Alto, CA) connected to a mass selective detector (MSD) according to the method of Li and Schuhmann (1980) with the following specifications: column length, 34 m; column diameter (i.d.), 0.25 mm; carrier gas, helium; flow rate, 0.555 ml min^{-1}; split ratio, 80. β-Phenyl-D-glucoside was used as an internal standard. An 80% ethanol solution was used as a control blank.

Aliquots of 500 μl of 80% ethanol extract were placed in 4 ml vials and dried down. A solution of sodium borohydride (2 g) in anhydrous dimethyl sulphoxide (100 ml) was prepared, and 1 ml added to the dried-down ethanol extract. After 90 min at 40 °C, 0.1 ml of 18 M acetic acid was added. Acetylation was achieved by the addition of 0.2 ml 1-methylimidazole and 2 ml acetic anhydride. The final dried neutral sugar residue was suspended in 1 ml methylene chloride, transferred to GC vials and analyzed. The operating conditions described by Gross (1983) were used with the following specifications: column length, 34 m; column diameter (i.d.), 0.25 mm; carrier gas, helium; flow rate, 1 ml min^{-1}; split ratio, 41.4. Myo-inositol was used as an internal standard. An 80% ethanol solution was used as the control blank.

Twenty milligrams of de-starched cell walls were mixed with 2 ml of 2 M TFA containing 1 mg of myo-inositol. Acid hydrolysis (1 h, 120 °C) was performed according to Jones and Albersheim (1972). After centrifugation ($2000 \times g$, 10 min), the supernatant was recovered and allowed to air-dry at 40 °C to remove TFA. Tubes were then placed in a desiccator for 12 h. The dried extract was hydrolyzed using driselase (Sigma, St Louis, MO), previously purified according to Fry (1988). The AA derivatives were prepared and GC-MSD analysis was performed as described above.

2.4.3. Uronic acids

Five milligrams of cell wall material was placed in a 10 ml volumetric flask and 2 ml of cold H_2SO_4 were added while stirring, and the wall material solubilized for 2 min following the method of Ahmed and Labavitch (1977). The flasks were then placed at 4 °C and 500 μl of H_2O were added dropwise while stirring for 5 min. Another 500 μl of H_2O was added dropwise and stirring continued until the cell walls were totally dissolved. Water was added to a final volume of 10 ml. Aliquots of 200 μl of the final solution were assayed for uronic acids according to Blumenkrantz and Asboe-Hansen (1973).

2.4.4. Proteins

Ten milligrams of cell wall was suspended in 5 ml H_2O and 1 ml of 3 M NaOH was added to the mixture. Hydrolysis was performed for 4 h in a boiling waterbath (Fry, 1988). Protein content was determined according to the method of Lowry et al. (1951).

2.5. Statistics

Data presented are the means of nine samples, and are presented on a dry weight basis. Analysis of variance was performed using orthogonal comparisons for interaction between calcium treatment and time in storage (SAS Institute, 1996). Contrast analysis was used to determine linear and quadratic significance for each compound during storage at each $CaCl_2$ treatment. Significant interactions would be reflected in differences in linear or quadratic trends across levels of either storage or $CaCl_2$, indicated in tables as positive (+) or negative (−) slope ('0' indicates non-significance). Untreated control and 0% $CaCl_2$-treated fruit were compared using Duncan's multiple range test ($P < 0.05$). Unless otherwise stated, only significant results are discussed.

3. Results

3.1. Mineral content

Total Ca content of the cortical tissue of untreated fruit was not significantly different than that of fruit treated with 0% $CaCl_2$ (not shown), and remained stable during storage at 237 ± 46 μg g^{-1} dry weight. The increase in total Ca of apple tissue treated with $CaCl_2$ and stored for up to 6 months was due to both $CaCl_2$ treatment and time in storage (Fig. 1A). In addition, an interaction between $CaCl_2$ and storage time was found. For fruit stored 4 or 6 months, highest Ca accumulation in cortical tissue was reached with 2% $CaCl_2$ and further increases in $CaCl_2$ did not result in additional Ca accumulation in the fruit. After 4 months storage, Ca content of fruit treated with 2% $CaCl_2$ was 30% higher compared to fruit treated with 1% $CaCl_2$. Ca content in fruit treated with 2% $CaCl_2$ was 65% higher after 4 months storage compared to fruit stored for 2 weeks. Levels of cell wall-bound Ca of untreated and 0% $CaCl_2$ treated fruit were not significantly different and increased up to three-fold after 6 months storage (Fig. 1B). The increase in cell wall-bound Ca of apple tissue treated with Ca was due to both the concentration of $CaCl_2$ used and time in storage. The highest levels of wall-bound Ca in fruit stored for 4 or 6 months were measured in fruit treated with 2% $CaCl_2$. Higher concentrations of $CaCl_2$ did not result in additional Ca accumulation in cell walls. Cell wall-bound Ca in fruit treated with 0% $CaCl_2$ was three times higher after 6 months storage compared to 2 weeks storage whereas for fruit treated with 4% $CaCl_2$, cell wall-bound Ca was twice as high at 6 months compared to 2 weeks storage, and with 2% $CaCl_2$, the increase was 3.5 times.

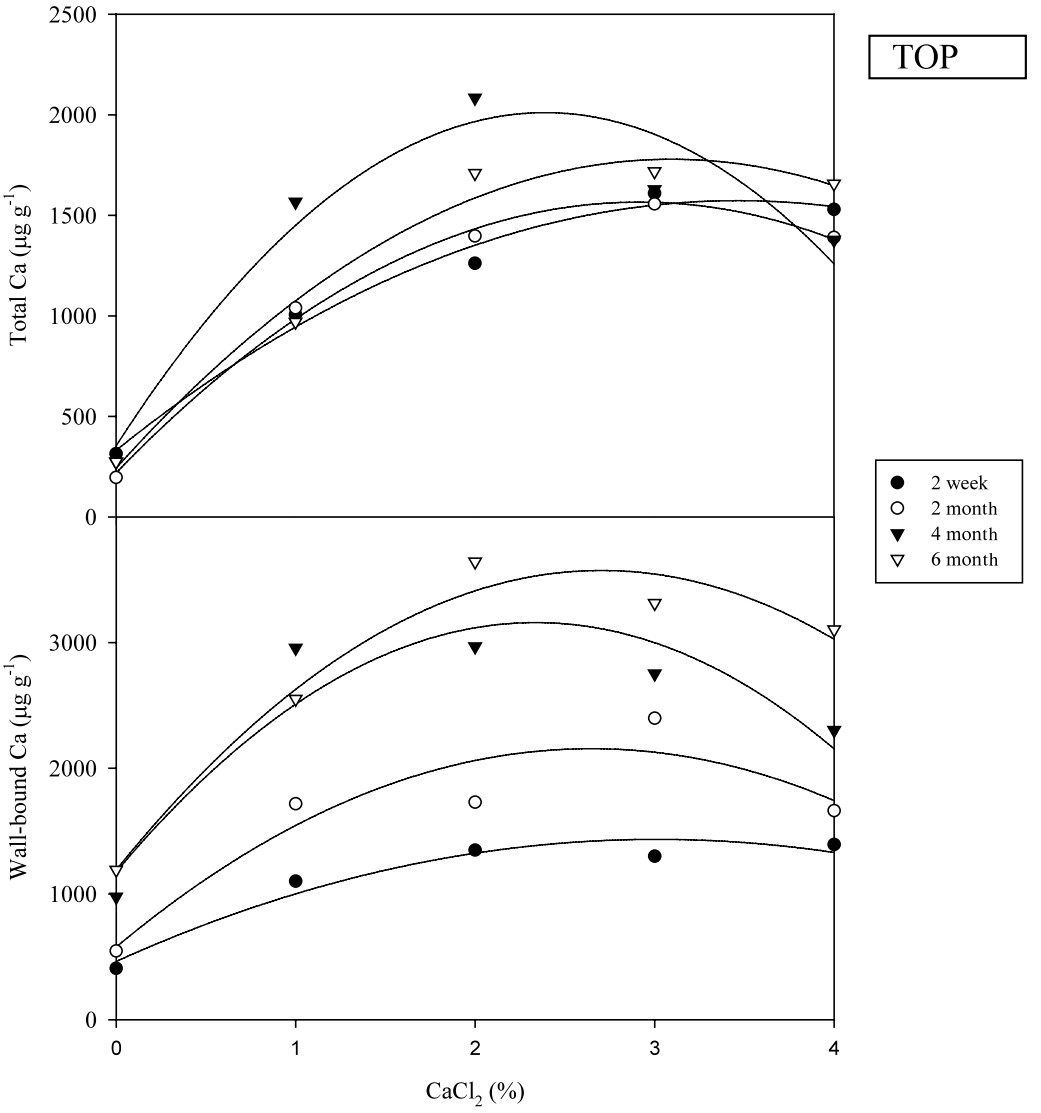

Fig. 1. Total (A) and cell wall-bound (B) calcium content of apple cortical tissue from fruit stored from 2 weeks to 6 months following infiltration of various $CaCl_2$ concentrations. The 2–4 mm layer of flesh tissue under the epidermis was used to determine both total and bound Ca contents. Part (A) is based on total tissue dry weight and (B) is based on dry weight of cell walls. Data are the means of nine replications. The interaction between $CaCl_2$ and storage time for total Ca and wall-bound Ca was significant at $P < 0.05$ and $P < 0.01$, respectively.

During storage, K, P, Mg and S content decreased in both untreated and 0% $CaCl_2$ treated fruit (Table 1). For the first 2 months of storage, K, Mg and S concentrations in the 0% $CaCl_2$-treated tissue were significantly greater than that of untreated fruit. After 6 months storage, fruit treated with 0% $CaCl_2$ had higher levels of P, Na and S than the untreated fruit. Changes in mineral content of Ca-infiltrated fruit were due to both $CaCl_2$ treatment and time in storage. K content increased with $CaCl_2$ at 2, 4 and 6 months storage. K content of fruit treated with 0% $CaCl_2$ decreased by 14% from 2 weeks to 6 months storage but increased by 22% over the same time period in

Table 1
Effect of Ca infiltration on K, P, Na, Mg and S content of apple cortical tissue during storage

Mineral element[a] (μg g^{-1})	$CaCl_2$ (%)	Storage time				Mean significance	
		2 weeks	2 months	4 months	6 months	Linear	Quadratic
K	Untreated[b]	4670 b	4248 b	4498 a	3905 a	− −	0
	0%[b]	5153 a	4712 a	4597 a	4451 a	−	0
	1%	4490	5012	4591	4968	0	0
	2%	4694	4930	4895	5752	++	0
	3%	4770	5151	4934	5139	0	0
	4%	4852	4938	5293	5146	0	0
Mean significance	Linear	0	+	+	++		
	Quadratic	−	0	0	− −		
P	Untreated[b]	438 a	395 b	366 b	363 b	− −	+
	0%[b]	456 a	483 a	448 a	431 a	−	0
	1%	450	395	372	422	0	++
	2%	445	398	406	450	0	+
	3%	447	397	424	446	0	+
	4%	441	416	444	468	0	0
Mean significance	Linear	0	− −	0	0	−	−
	Quadratic	0	++	+	0	−	−
Na	Untreated[b]	381 a	136 a	225 a	142 b	− −	++
	0%[b]	169 b	125 a	211 a	160 a	++	− −
	1%	511	204	194	165	− −	++
	2%	565	203	199	198	− −	++
	3%	560	271	191	193	− −	++
	4%	573	203	209	190	− −	++
Mean significance	Linear	++	++	0	++	−	−
	Quadratic	− −	− −	0	− −	−	−
Mg	Untreated[b]	221 b	187 b	197 a	196 a	− −	+
	0%[b]	259 a	204 a	209 a	211 a	− −	++
	1%	219	239	256	249	+	0
	2%	257	205	234	232	0	++
	3%	240	217	201	225	0	++
	4%	234	188	199	220	0	++
Mean significance	Linear	0	−	−	0	−	−
	Quadratic	0	− −	− −	−	−	−
S	Untreated[b]	188 b	138 b	12 a	94 b	− −	+
	0%[b]	206 a	154 a	138 a	111 a	− −	+
	1%	185	149	152	127	− −	0
	2%	200	137	152	141	− −	++
	3%	194	153	152	139	− −	++
	4%	199	151	148	133	− −	++
Mean significance	Linear	0	0	0	++	−	−
	Quadratic	0	0	−	− −	−	−

0, +, ++, −, − −: Non-significant, significant positive slope at $P < 0.05$, significant positive slope at $P < 0.01$, significant negative slope at $P < 0.05$, significant negative slope at $P < 0.01$, respectively.

[a] Analysis of variance showed an interaction between $CaCl_2$ treatments and storage time ($P < 0.01$). Untreated fruit were not considered in mean significance determination within each storage time.

[b] For each individual storage time, means of untreated fruit and fruit treated with 0% $CaCl_2$ were compared using Duncan's multiple range test. Means of nine replications followed by the same letter within a column are not significantly different ($P < 0.05$).

fruit infiltrated with 2% $CaCl_2$. Phosphorous content in tissue of fruit infiltrated with 1, 2 or 3% $CaCl_2$ had a positive quadratic relationship with storage time. In fruit treated with 1% $CaCl_2$, P content was 17% lower after 4 months storage compared to fruit stored for 2 weeks. For fruit stored for 2 months, P content was 18% lower with the 1% $CaCl_2$ treatment compared to the 0% $CaCl_2$ treatment. Total Na content of Ca-infiltrated fruit decreased with storage time but generally increased with $CaCl_2$ concentration. Perhaps due to variation in the data, Na content did not vary with $CaCl_2$ concentration after 4 months storage. For fruit infiltrated with 4% $CaCl_2$, Na content decreased to 67% from 2 weeks to 6 months. Na content of fruit stored for 2 weeks was threefold higher with 4% $CaCl_2$ than with 0% $CaCl_2$. Mg content in tissue of fruit infiltrated with 2–4% $CaCl_2$ had a positive quadratic relationship with storage time. At 2 and 4 months storage, increasing $CaCl_2$ concentration resulted in decreasing Mg content. Fruit treated with 0% $CaCl_2$ and stored for 6 months was 18% lower in Mg content than fruit stored for 2 weeks. After 2 months, fruit treated with 4% $CaCl_2$ had 21% less Mg compared with fruit infiltrated with 1% $CaCl_2$. Total S content decreased with increasing storage time. Fruit infiltrated with 0% $CaCl_2$ showed a 46% loss in S from 2 weeks to 6 months. S content of fruit stored for 6 months was 27% greater in fruit infiltrated with 2% $CaCl_2$ compared with fruit treated with 0% $CaCl_2$.

Na content in cell walls of untreated and 0% treated fruit remained stable throughout storage, while S and P decreased and Mg increased (Table 2). Cell wall-bound Na content of fruit infiltrated with 3 or 4% $CaCl_2$ had a positive quadratic relationship with time, and was at a minimum after 2 months in storage. Levels of cell wall-bound S and P decreased and Mg increased with time in storage. In fruit treated with 3% $CaCl_2$, cell wall-bound S decreased 82% and P content decreased 56% from 2 weeks to 6 months. P content of cell walls decreased with increasing $CaCl_2$ at 2, 4 and 6 months. Fruit stored for 2 months had 50% lower wall-bound P when treated with 3% $CaCl_2$ than with 0% $CaCl_2$. Cell wall-bound Mg of fruit infiltrated with 0% $CaCl_2$ increased 58% from 2 weeks to 6 months.

3.2. Soluble sugars

Suc content of untreated fruit tissue decreased by 30% after 6 months storage, while Fru and Glc increased by 24 and 35%, respectively (Table 3). Suc content of Ca-treated fruit appeared to decrease during storage but this decrease was not significant when fruit were infiltrated with 0 or 2% $CaCl_2$. Fruit treated with 3 or 4% $CaCl_2$ showed the highest level of Suc. Glc content was not affected by $CaCl_2$. Fru content of fruit infiltrated with $CaCl_2$ increased during storage, but this increase was not significant in fruit infiltrated with 2% $CaCl_2$.

Mannose (Man_{cw}) and glucose (Glc_{cw}) were the only cell wall-related sugars associated with the tissue samples used in our study (Table 4). Man_{cw} and Glc_{cw} content increased during storage of untreated fruit by 24 and 32% from 2 weeks to 6 months storage, respectively. When stored for 2 weeks, a 55 and 68% increase in Man_{cw} and Glc_{cw}, respectively, was found in fruit infiltrated with 0% $CaCl_2$ compared to the untreated apples, and the 1% $CaCl_2$ treatment increased this another 24%. However, this immediate increase was reversible and between 2 weeks and 2 months storage, levels of Man_{cw} and Glc_{cw} in infiltrated fruit (with or without Ca) fell to those found in untreated fruit.

A 20% decrease was measured for both sugars from 2 weeks to 6 months storage in 0% $CaCl_2$-infiltrated fruit. Man_{cw} and Glc_{cw} decreased with storage time but no significant change was found in Man_{cw} when fruit were treated with 3% $CaCl_2$. Glc_{cw} content in fruit treated with 0% $CaCl_2$ was 44% lower in fruit stored for 6 months compared to fruit stored for 2 weeks. Fruit stored for 2 weeks following infiltration with 4% $CaCl_2$ had 30% more Glc_{cw} than fruit treated with 0% $CaCl_2$.

3.3. Cell wall content

Cell wall content of untreated fruit and 0% $CaCl_2$-treated fruit decreased during storage (Table 5). Due to missing data, cell wall content was not determined 2 weeks after storage. An interac-

Table 2
Effect of $CaCl_2$ infiltration on P, Na, Mg and S content of apple cell walls during storage

Mineral element [a] ($\mu g\ g^{-1}$)	$CaCl_2$ (%)	Storage time				Mean significance	
		2 weeks	2 months	4 months	6 months	Linear	Quadratic
P	Untreated[b]	223 a	266 a	186 a	133 a	− −	− −
	0%[b]	208 a	263 a	136 b	136 a	− −	−
	1%	199	190	117	74	− −	− −
	2%	206	178	112	92	− −	0
	3%	200	133	108	88	− −	0
	4%	163	134	79	78	− −	0
Mean significance	Linear	0	− −	− −	− −		
	Quadratic	0	++	0	+		
Na	Untreated[b]	4094 a	6037 a	3619 a	5263 a	0	0
	0%[b]	4355 a	5695 a	4229 a	5403 a	0	0
	1%	6808	7218	6870	7594	0	0
	2%	7250	7426	6500	7479	0	0
	3%	7966	5635	6515	7081	0	+
	4%	6263	5073	5479	6718	0	++
Mean significance	Linear	++	0	+	0		
	Quadratic	− −	− −	− −	− −		
Mg	Untreated[b]	16 b	29 a	53 a	40 a	++	− −
	0%[b]	31 a	34 a	57 a	49 a	++	0
	1%	26	55	100	91	++	−
	2%	31	37	71	89	++	0
	3%	25	66	60	92	++	0
	4%	25	51	54	97	++	0
Mean significance	Linear	0	++	0	++		
	Quadratic	0	−	−	−		
S	Untreated[b]	1166 a	2109 a	448 a	690 a	− −	0
	0%[b]	1279 a	2157 a	458 a	611 a	− −	0
	1%	3239	2760	653	626	− −	0
	2%	3826	2360	676	570	− −	++
	3%	4387	935	775	797	− −	++
	4%	2623	428	710	425	− −	++
Mean significance	Linear	+	− −	+	0		
	Quadratic	− −	− −	−	0		

0, +, ++, −, − −: Non-significant, significant positive slope at $P < 0.05$, significant positive slope at $P < 0.01$, significant negative slope at $P < 0.05$, significant negative slope at $P < 0.01$, respectively.

[a] Analysis of variance showed an interaction between $CaCl_2$ treatments and storage time ($P < 0.01$). Untreated fruit were not considered in mean significance determination within each storage time.

[b] For each individual storage time, means of untreated fruit and fruit treated with 0% $CaCl_2$ were compared using Duncan's multiple range test. Means of nine replications followed by the same letter within a column are not significantly different ($P < 0.05$).

tion between $CaCl_2$ treatment and time in storage was found. Cell wall content only increased during storage of 2% $CaCl_2$-infiltrated fruit. For fruit treated with 2% $CaCl_2$, cell wall content was 10% higher in fruit stored for 6 months compared to fruit stored for 2 months. At 4 and 6 months, cell wall content increased with increasing $CaCl_2$. After 4 months, fruit treated with $CaCl_2$ had between 39 and 25% higher cell wall content compared to that in fruit treated with 0% $CaCl_2$.

Table 3
Effect of Ca infiltration on ethanol-soluble non-cellulosic neutral sugars of apple cortical tissue

Non-cellulosic sugar[a] (mg g^{-1})	$CaCl_2$ (%)	Storage time				Mean significance	
		2 weeks	2 months	4 months	6 months	Linear	Quadratic
Suc	Untreated[b]	165 a	151 a	134 a	116 a	− −	0
	0%[b]	155 a	147 a	150 a	137 a	0	0
	1%	154	146	127	119	− −	0
	2%	157	148	144	133	0	0
	3%	168	163	154	124	− −	0
	4%	189	162	188	153	−	0
Mean significance	Linear	++	0	+	0		
	Quadratic	0	0	+	0		
Glc	Untreated[b]	117 a	142 a	136 a	158 a	++	0
	0%[b]	117 a	133 a	134 a	148 a	++	0
	1%	118	117	141	158	0	0
	2%	121	125	135	141	0	0
	3%	127	124	140	156	++	0
	4%	111	118	126	130	0	0
Mean significance	Linear	0	0	0	0		
	Quadratic	0	0	0	0		
Fru	Untreated[b]	277 a	293 a	281 a	345 a	++	++
	0%[b]	274 a	271 a	286 a	341 a	++	++
	1%	271	264	294	336	++	++
	2%	271	274	300	322	0	0
	3%	282	271	325	326	++	0
	4%	283	273	310	320	++	0
Mean significance	Linear	0	0	++	− −		
	Quadratic	0	0	0	0		

0, +, ++, −, − −: Non-significant, significant positive slope at $P < 0.05$, significant positive slope at $P < 0.01$, significant negative slope at $P < 0.05$, significant negative slope at $P < 0.01$, respectively.

[a] No significant interaction was shown between $CaCl_2$ and storage time for Suc, Glc or Fru.

[b] For each individual storage time, means of untreated fruit and fruit treated with 0% $CaCl_2$ were compared using Duncan's multiple range test. Means of nine replications followed by the same letter within a column are not significantly different ($P < 0.05$).

3.4. Structural compounds

Total polysaccharides, neutral sugar and protein content of cell walls decreased after 2 months in untreated fruit during storage (Table 6). During storage, total polysaccharide, uronic acid and protein content decreased in cell walls of fruit infiltrated with 0% $CaCl_2$, while total neutral sugars increased up to 4 months and then decreased. Fruit infiltrated with 4% $CaCl_2$ showed a 50% loss in cell wall protein from 2 weeks to 6 months. In general, infiltrating with Ca caused lower protein content in cell walls at each observation time irrespective of $CaCl_2$ concentration. Total polysaccharides and neutral sugar content of Ca-infiltrated fruit decreased during storage. Total protein content of Ca-infiltrated fruit decreased with $CaCl_2$ concentration as well as time. After 4 months, total protein content was 33% lower in fruit treated with 4% $CaCl_2$ compared to fruit infiltrated with 1% $CaCl_2$. Uronic acid content was stable during storage and seemingly unaffected by Ca infiltration. Neutral sugar content fluctuated during the first 4 months and then decreased drastically in all treatments between 4 and 6 months.

During storage of untreated fruit, Glc, Gal, Xyl, Rha, and Man content remained stable while Ara decreased (Table 7). In 0% $CaCl_2$-infiltrated fruit, Ara, Gal, Xyl and Man content decreased while

Table 4
Effect of Ca infiltration on ethanol-soluble sugars associated with the cell walls in apple cortical tissue samples

Cell wall associated sugar[a] (μg g^{-1})	$CaCl_2$ (%)	Storage time				Mean significance	
		2 weeks	2 months	4 months	6 months	Linear	Quadratic
Man_{cw}	Untreated[b]	258 b	265 a	269 a	319 a	+	+
	0%[b]	402 a	265 a	258 a	321 a	−	++
	1%	497	256	263	325	− −	++
	2%	495	270	252	316	− −	++
	3%	487	259	292	326	0	0
	4%	485	265	285	308	− −	++
Mean significance	Linear	0	0	++	0		
	Quadratic	0	0	0	0		
Glc_{cw}	Untreated[b]	379 b	427 a	435 a	503 a	++	0
	0%[b]	636 a	411 a	415 a	496 a	− −	++
	1%	791	378	436	528	− −	++
	2%	791	404	407	490	− −	++
	3%	817	370	484	521	− −	++
	4%	823	384	436	460	− −	++
Mean significance	Linear	++	0	+	0		
	Quadratic	0	0	0	0		

0, +, ++, −, − −: Non-significant, significant positive slope at $P < 0.05$, significant positive slope at $P < 0.01$, significant negative slope at $P < 0.05$, significant negative slope at $P < 0.01$, respectively.

[a] Analysis of variance showed an interaction between $CaCl_2$ treatments and storage time ($P < 0.01$). Untreated fruit were not considered in mean significance determination within each storage time.

[b] For each individual storage time, untreated fruit and fruit treated with 0% $CaCl_2$ were compared using Duncan's multiple range test. Means of nine replications followed by the same letter within a column are not significantly different ($P < 0.05$).

Glc content increased during storage. Changes in Glc, Ara, Gal and Rha of Ca-infiltrated fruit were due to both $CaCl_2$ treatment and time in storage. During storage, Glc, Ara and Gal content of fruit infiltrated with $CaCl_2$ decreased. Fruit infiltrated with 2% $CaCl_2$ had a 68% loss in Glc content from

Table 5
Effect of Ca infiltration on the cell wall content (% dry wt. tissue) of apple cortical tissue during storage

$CaCl_2$ (%)	Storage time			Mean significance	
	2 months	4 months	6 months	Linear	Quadratic
Untreated[a]	9.8 b	8.7 a	7.9 a	−	0
0%[a]	11.3[b] a	8.3 a	8.8 a	− −	++
1%	11.1	11.5	10.5	0	0
2%	10.8	11.0	11.9	+	0
3%	10.7	11.2	10.4	0	0
4%	9.1	10.4	10.9	0	0
Linear	−	++	++		
Quadratic	0	− −	− −		

0, +, ++, −, − −: Non-significant, significant positive slope at $P < 0.05$, significant positive slope at $P < 0.01$, significant negative slope at $P < 0.05$, significant negative slope at $P < 0.01$, respectively.

[a] For each individual storage time, means of untreated fruit and fruit treated with 0% $CaCl_2$ were compared using Duncan's multiple range test. Means of nine replications followed by the same letter within a column are not significantly different ($P < 0.05$).

[b] Analysis of variance showed an interaction between $CaCl_2$ treatments and storage time ($P < 0.01$). Untreated fruit were not considered in mean significance determination within each storage time.

Table 6
Effect of $CaCl_2$ infiltration on total protein, uronic acid and polysaccharide content of apple cell walls during storage

Cell wall component[a]	$CaCl_2$ (%)	Storage time				Mean significance	
(mg g^{-1})		2 weeks	2 months	4 months	6 months	Linear	Quadratic
Total polysaccharide	Untreated[b]	588 a	633 a	594 a	505 a	++	− −
	0%[b]	612 a	612 a	567 a	500 a	− −	0
	1%	624	618	557	524	− −	0
	2%	617	620	573	446	− −	− −
	3%	618	606	596	467	− −	− −
	4%	656	639	582	502	− −	0
Mean significance	Linear	0	0	0	0		
	Quadratic	0	0	0	+		
Total uronic acid	Untreated[b]	251 a	236 a	255 a	235 a	0	0
	0%[b]	261 a	248 a	268 a	204 a	− −	− −
	1%	258	266	283	254	0	− −
	2%	250	231	261	233	0	0
	3%	237	259	275	264	0	−
	4%	259	256	246	242	0	0
Mean significance	Linear	0	0	0	0		
	Quadratic	0	0	0	0		
Total neutral sugar	Untreated[b]	400 a	404 a	379 a	177 a	− −	0
	0%[b]	356 a	391 a	397 a	125 a	++	− −
	1%	349	124	274	179	− −	0
	2%	333	270	243	170	− −	0
	3%	311	240	273	156	− −	− −
	4%	354	337	369	158	− −	− −
Mean significance	Linear	0	0	0	0		
	Quadratic	0	+	++	0		
Total protein	Untreated[b]	29 a	36 a	18 a	19 a	− −	−
	0%[b]	27 a	38 a	13 a	14 a	− −	0
	1%	20	28	12	12	− −	0
	2%	19	26	15	14	− −	− −
	3%	19	25	10	12	− −	0
	4%	17	23	8	9	− −	0
Mean significance	Linear	− −	− −	− −	− −		
	Quadratic	++	++	0	0		

0, +, ++, −, − −: Non-significant, significant positive slope at $P < 0.05$, significant positive slope at $P < 0.01$, significant negative slope at $P < 0.05$, significant negative slope at $P < 0.01$, respectively.

[a] Analysis of variance showed an interaction between $CaCl_2$ treatments and storage time ($P < 0.01$). Untreated fruit were not considered in mean significance determination within each storage time.

[b] For each individual storage time, means of untreated fruit and fruit treated with 0% $CaCl_2$ were compared using Duncan's multiple range test. Means of nine replications followed by the same letter within a column are not significantly different ($P < 0.05$).

2 weeks to 6 months, and a 38% loss in Ara and Gal. Gal content of fruit infiltrated with 4% $CaCl_2$ decreased by 42% from 2 months to 6 months. Xyl content of fruit treated with $CaCl_2$ decreased during storage but this decrease was not significant in fruit treated with 1% $CaCl_2$. During storage, Rha content had a negative quadratic relationship with time in fruit treated with 1 or 4% $CaCl_2$. Man content had a negative quadratic relationship with time for fruit treated with 2, 3 or 4% $CaCl_2$.

4. Discussion

Storage of untreated fruit was associated with a decrease in soluble and cell wall-bound com-

Table 7
Neutral sugar composition of apple cell walls as affected by $CaCl_2$ infiltration and storage

Neutral sugar[a] (mg g^{-1})	$CaCl_2$ (%)	Storage time				Mean significance	
		2 weeks	2 months	4 months	6 months	Linear	Quadratic
Glc	Untreated[b]	190 a	199 a	224 a	63 a	0	0
	0%[b]	170 a	200 a	208 a	34 a	+	− −
	1%	149	23	116	60	−	0
	2%	164	74	102	52	− −	0
	3%	132	113	99	42	− −	0
	4%	162	118	142	32	− −	0
Mean significance	Linear	0	0	0	0		
	Quadratic	0	+	++	0		
Ara	Untreated[b]	109 a	87 a	80 a	46 a	−	−
	0%[b]	95 a	82 a	88 a	36 a	− −	−
	1%	97	34	78	51	− −	0
	2%	85	73	72	52	− −	0
	3%	87	64	88	51	− −	−
	4%	90	93	112	48	−	− −
Mean significance	Linear	0	0	+	0		
	Quadratic	0	+	++	0		
Gal	Untreated[b]	57 a	45 a	57 a	28 a	0	0
	0%[b]	53 a	43 a	53 a	25 a	− −	−
	1%	53	24	42	30	− −	0
	2%	50	47	37	31	− −	0
	3%	51	39	58	32	−	−
	4%	59	57	62	33	−	−
Mean significance	Linear	0	+	+	0		
	Quadratic	0	0	++	0		
Xyl	Untreated[b]	41 a	39 a	41 a	22 a	0	0
	0%[b]	30 b	38 a	42 a	14 b	−	− −
	1%	30	23	32	19	0	0
	2%	28	28	26	17	−	0
	3%	27	22	26	17	−	−
	4%	28	32	39	13	−	− −
Mean significance	Linear	−	0	0	0		
	Quadratic	0	0	+	0		
Rha	Untreated[b]	9 b	24 a	8 a	9 a	0	0
	0%[b]	11 a	25 a	8 a	12 a	0	− −
	1%	8	16	8	12	0	0
	2%	9	22	10	12	0	− −
	3%	7	27	13	14	0	− −
	4%	8	26	13	21	++	0
Mean significance	Linear	− −	0	0	++		
	Quadratic	++	+	0	++		
Man	Untreated[b]	5 a	11 a	5 a	4 a	0	0
	0%[b]	6 a	10 a	6 a	4 a	− −	− −
	1%	6	6	7	5	0	0
	2%	5	7	7	5	0	−
	3%	5	10	8	4	0	− −
	4%	6	10	9	5	0	− −
Mean significance	Linear	0	0	++	0		
	Quadratic	0	++	0	0		

0, +, ++, −, − −: Non-significant, significant positive slope at $P < 0.05$, significant positive slope at $P < 0.01$, significant negative slope at $P < 0.05$, significant negative slope at $P < 0.01$, respectively.

[a] Analysis of variance showed an interaction between $CaCl_2$ treatments and storage time at $P < 0.01$ for Glc, Ara, and Rha, and $P < 0.05$ for Gal. Untreated fruit were not considered in mean significance determination within each storage time.

[b] For each individual storage time, means of untreated fruit and fruit treated with 0% $CaCl_2$ were compared using Duncan's multiple range test. Means of nine replications followed by the same letter within a column are not significantly different ($P < 0.05$).

pounds. Storage also resulted in hydrolysis of sucrose, leading to an increase in the breakdown products Fru and Glc. Total Ca of untreated apple tissue remained stable, while Ca content of the cell wall increased threefold after 6 months storage, suggesting that soluble Ca of the cortical tissue was mobilized and integrated into the cell wall. Preclimacteric apples stored at 0 °C undergo ripening and this process has been attributed to cell wall solubilization by enzymes such as polygalacturonase and pectin methyl esterase (Bartley and Knee, 1981; Knee, 1977). Major changes occurring after 6 months storage include solubilization of the cell wall-related sugars Man_{cw} and Glc_{cw} and a loss of protein, S, and total neutral sugars (mainly Ara). These changes indicated a degradation of the apple cell walls and consequent reduction in fruit quality. Additionally, the loss of firmness due to cell wall carbohydrate metabolism during storage has been associated with increased susceptibility to infection by fungal pathogens (Conway et al., 1987).

Siddiqui and Bangerth (1996) suggested that storage at low temperature can lead to a loss of rigidity of the fruit due to dissolution of the middle lamella and subsequent cell separation. That would partially explain the changes detected in our study. Nevertheless, the natural increase in cell wall-bound Ca occurring during storage might slow-down cell separation since Ca has been shown to decrease cell wall susceptibility to enzymatic hydrolysis by inhibiting PG and PME activity (Poovaiah et al., 1988; Wu et al., 1993; Jauneau et al., 1994), and by cross-linking cell wall polymers through Ca bridges (Jarvis, 1984).

The infiltration process itself (0% $CaCl_2$) resulted in minor changes within the cortical tissue, mainly affecting the mineral content of the tissue, ethanol-soluble sugars from the cell wall associated with the tissue samples, and cell wall content. The process resulted in an increase in total K, Mg, S, Man_{cw} and Glc_{cw}, Rha and cell wall content, primarily after 2 weeks storage. Throughout storage, total neutral sugars in cell walls increased in fruit infiltrated with 0% $CaCl_2$, mainly due to an increase in Glc. Assuming that the tissue layers most susceptible to pressure infiltration were the cortical tissue and the epidermis of the fruit, mineral accumulation would occur from the central neighboring tissue towards the 'affected' cortical zone. The mobilization of minerals has been previously described after fruit injury or infection by a fungal pathogen and Ca was shown to migrate toward the injured zone in cucumber fruit, resulting in an increase in cell wall-bound Ca (Chardonnet and Doneche, 1995). However, our data showed that Ca was not mobilized in 0% $CaCl_2$-infiltrated apples as Ca content of apple tissue treated with 0% $CaCl_2$ was not significantly different compared to untreated fruit during storage.

Some studies have shown that postharvest treatments such as vacuum infiltration resulted in reduced internal O_2 and increased internal CO_2 levels (Rajapakse et al., 1992; Hewett and Thompson, 1992) due to air replacement by the infiltrated solution, possibly causing changes in mineral composition. Saftner et al. (1998) did not find any significant differences in the internal air space of untreated or distilled water pressure-infiltrated 'Golden Delicious' apples stored for 4 months at 0 °C. Consequently, the air replacement effect following vacuum infiltration might not occur when fruit are pressure-infiltrated. However, fruit treated with distilled water and stored for 6 months had higher total P, Na and S content compared to untreated fruit, suggesting that the infiltration process might reduce mineral loss.

Our data showed that there is an interaction between $CaCl_2$ concentration of the solution used for infiltration and time in storage for the majority of the variables studied. Ca accumulation following $CaCl_2$ infiltration resulted in fruit with higher tissue and cell wall Ca content, in agreement with previous studies (Conway et al., 1988, 1995). Our data also showed that the Ca content in the tissue and cell walls reached a maximum at 4 and 6 months storage after treatment with 2% $CaCl_2$ and no further significant increase was detected at higher $CaCl_2$ concentrations. The linear increase in cell wall content of fruit infiltrated with 2% $CaCl_2$ appeared to be mainly due to Ca accumulation in the wall material, and to a lesser extent, an increase in Mg. Pressure infiltration of 2% $CaCl_2$ was optimal for maintaining the texture of 'Golden Delicious' apples during 6 months storage

(Abbott et al., 1989), especially by maintaining fruit water relations (by decreasing air space volume) while minimizing the risk of salt-related injuries (Saftner et al., 1998). Our data did not show a significant relationship between Ca treatment and total uronic acid content of apple cell walls, in agreement with another study in which 'Golden Delicious' apples were stored for 7 months at 0 °C and equilibrated at 20 °C for 12 h before cell wall extraction (Tobias et al., 1993). When fruit were stored for 7 days at 20 °C following 5 months storage, Ca infiltration decreased polyuronide solubilization, thereby maintaining quality and firmness of ripe Ca-treated fruit (Sams and Conway, 1984). Our samples were equilibrated for 12 h prior to extraction, and this brief period for ripening may explain why we did not measure a change in uronic acid content due to Ca infiltration.

Our results suggest that changes in apple tissue and cell walls following $CaCl_2$ infiltration were due to both $CaCl_2$ and the infiltration process itself. Our study also identified an interaction between $CaCl_2$ treatment and time in storage that contributed to changes in tissue and cell wall composition. Calcium chloride concentration of 2% was sufficient for maximum Ca accumulation in the tissue and cell wall. Between 4 and 6 months storage, significant changes in the cell wall occurred, probably resulting in fruit with lower quality and increased susceptibility to fungal diseases.

Acknowledgements

We thank Dr. Arnold Saxton, Statistician, Agricultural Experiment Station, for assistance in data analysis and interpretation, and George Brown, biological laboratory technician for technical assistance.

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