Effect of Postharvest Calcium Chloride Treatments on Tissue Water Relations, Cell Wall Calcium Levels and Postharvest Life of 'Golden Delicious' Apples

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ABSTRACT. Changes in tissue water relations, cell wall calcium (Ca) levels and physical properties of Ca-treated and untreated 'Golden Delicious' apples (*Malus $\times$ domestica* Borkh.) were monitored for up to 8 months after harvest. Pressure infiltration of fruit with CaCl_2 solutions at concentrations up to $0.34 \text{ mol}\cdot\text{L}^{-1}$ reduced both fruit softening and air space volume of fruit in a concentration-dependent manner. Turgor potential-related stress within the fruit persisted during storage and was higher in Ca-treated than in untreated fruit. Fruit that were pressure infiltrated with CaCl_2 solutions between 0.14 and $0.20 \text{ mol}\cdot\text{L}^{-1}$ and then waxed to reduce water loss during storage showed no peel injury. Calcium efflux patterns from apple tissue disks indicated two distinct Ca compartments having efflux kinetics consistent with those for cell wall Donnan-phase bound and water free space soluble Ca. At Ca concentrations up to $0.20 \text{ mol}\cdot\text{L}^{-1}$, cell wall bound Ca approached saturation whereas soluble Ca showed a linear dependence. At higher external Ca concentrations, only soluble Ca in the tissue increased. During 8 months of cold storage, cell wall Ca-binding capacity increased up to 48%. The osmotic potential of apples harvested over three seasons ranged between -1.32 and -2.33 MPa . In tissue disks, turgor potential changes caused by adjusting the osmolality of the incubation solution with CaCl_2 or sorbitol were accompanied by changes in the osmotic and water potentials of the tissue. In CaCl_2 solutions up to $0.34 \text{ mol}\cdot\text{L}^{-1}$, turgor potential was $\geq 0.6 \text{ MPa}$ in tissue incubated in 0.14 or $0.17 \text{ mol}\cdot\text{L}^{-1}$ solutions of CaCl_2 and was more than 3 times higher than in tissues incubated in low ($\leq 0.03 \text{ mol}\cdot\text{L}^{-1}$) or high ($\geq 0.27 \text{ mol}\cdot\text{L}^{-1}$) concentrations of CaCl_2 . At osmotically equivalent concentrations, turgor potential was up to 40% higher in Ca- than in sorbitol-treated tissue. The results suggest that postharvest treatment with 0.14 to $0.20 \text{ mol}\cdot\text{L}^{-1}$ solutions of CaCl_2 are best for maintaining fruit water relations and storage life of 'Golden Delicious' apples while minimizing the risk of salt-related injuries to the fruit. While higher concentrations of CaCl_2 may better maintain firmness, these treatments adversely affect fruit water relations and increase the risk of fruit injury.

A close relationship has been established between fruit Ca levels and numerous physiological and pathological disorders. Increasing the Ca content of apples decreases the incidence of bitter pit, scald, water core, and internal breakdown (Yuen, 1994) and maintains fruit firmness and quality (Mason et al., 1975). Postharvest infiltration of CaCl_2 solutions can also retard postharvest decay caused by pathogens (Biggs et al., 1993; Conway et al., 1991). The various effects of Ca on fruit ripening are associated with membrane structure and function and with cell wall structure and composition (Ferguson, 1984).

The relationship between Ca and the cell wall may partially explain the maintenance of firmness and the increased resistance to invasion by certain microorganisms prompted by Ca. Most of the Ca entering apple tissue accumulates in the cell wall (Ferguson, 1984). Free carboxyl groups on pectin polymers play an important role in stabilizing the cell wall, probably through the cooperative binding of Ca (Damarty et al., 1984; Grant et al., 1973). However, Siddiqui and Bangerth (1996) found no relationship between fruit firmness and the quantity of Ca associated with free, ionically bound or covalently bound pectin fractions of purified cell walls. Unfortunately, most studies on Ca binding properties have been made on purified cell walls treated with acid, detergent or salt

before assaying for Ca binding capacity. These treatments remove cell wall components and may affect the structure and binding properties of the isolated walls for Ca (Tu et al., 1992). Compartmental analyses of radioisotope elution measurements have identified two cell wall compartments for Ca in plant tissues; one for charged surfaces that bind cations including Ca (Donnan-phase) and the other for water free space which contains unbound Ca ions (Macklon, 1975). While data from compartmental efflux studies provide indirect flux estimates, the cell walls of isolated cortical tissue used in such studies are unpurified and are more likely to approach native, Ca-binding conditions. 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 (Damarty et al., 1984; Ferguson, 1984). Unbound Ca ions are believed to have little or no direct effect on tissue strength.

Although postharvest Ca treatments increase fruit firmness retention, they also may lead to fruit injury (lenticel pitting, surface discoloration) following treatment (Fallahi et al., 1987; Yuen, 1994). At least part of the Ca-induced injury appears to be a high-salt, osmotic effect since the severity of the injury increases with concentration (Sharples and Johnson, 1976) and decreases when fruit are rinsed of surface Ca (Scott and Wills, 1979) and treated with fruit coatings that reduce water loss (Saftner et al., 1998).

To our knowledge, the effect of postharvest Ca treatments on tissue water relations has not been investigated. Postharvest Ca treatments may increase fruit firmness retention through effects on turgor potential and cell shape. The objective of this study was to determine the optimal osmotic concentration range of CaCl_2 solu-

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tions for postharvest treatment of 'Golden Delicious' apples to maintain postharvest quality while minimizing the risk of fruit injury.

Materials and Methods

'Golden Delicious' apples were harvested in the preclimacteric stage (evolved ethylene production $<2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{s}^{-1}$ and the climacteric rise in CO_2 production had not yet begun) from a commercial orchard and randomized. Within 3 d after harvest, fruit were pressure infiltrated (3 min at 103 kPa) with distilled water or 0.07, 0.14, 0.17, 0.20, 0.24, 0.27, or $0.34 \text{ mol} \cdot \text{L}^{-1}$ solutions of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. Following infiltration, fruit were rinsed by dipping for $\approx 10 \text{ s}$ in distilled water at $20 \pm 1^\circ \text{C}$ before drying. Fruit used for injury analyses also were hand coated (0.4 mL per fruit) with a wax microemulsion to reduce the risk of surface injury associated with water loss/salt accumulation during storage. The wax coating has as its major ingredients, candelilla wax and water, and as its minor ingredients, oleic acid, morpholine and isopropyl alcohol. Because wax coatings increase retention of fruit firmness, tissue water relations and otherwise delay ripening during storage (Saftner et al., 1998), fruit intended for tissue analyses and tissue osmotic potential measurements were not treated with wax.

All fruit were stored for up to 8 months in air at 0°C and, as needed, removed from storage and kept at $20 \pm 1^\circ \text{C}$ until analyzed.

Waxed fruit from each treatment were rated for injury to the fruit surface and cortical tissue from 1 to 4 mm under the peel was analyzed for total Ca following 4 months of cold storage. Injury was assessed on a five-point scale based on the percentage area of the fruit surface injured. The injury ratings were 1 = $>50\%$ of fruit surface injured, 2 = 25% to 50% of fruit surface injured, 3 = 10% to 25% of fruit surface injured, 4 = fruit surface injury localized and not exceeding 10% of fruit surface, and 5 = no fruit surface injury. On this scale, an injury rating below four is unmarketable as fresh fruit due to lenticel pitting and/or surface discoloration. However, only fruit with a rating below one would be unsuitable for processing. Injury rating indicates the severity of injury on individual fruit, whereas percent fruit injured indicates the percentage of the fruit showing any indication of injury.

The preparation of fruit cortical tissue for Ca concentration analyses was as previously described (Saftner et al., 1997). Freeze-dried tissue samples were dry-ashed, dissolved 1:25 in $1 \text{ mol} \cdot \text{L}^{-1}$ HCl and analyzed for Ca concentration by inductively coupled plasma emission spectrometry (model 61E; Thermo Jarrell Ash, Franklin, Mass.). Calcium concentration is reported on a dry-mass (DM) basis ($\text{mmol} \cdot \text{kg}^{-1}$). Each sample consisted of the flesh from seven apples, and seven samples were obtained from each treatment.

Firmness, internal air space and relative turgor potential-induced fruit stress were determined on the same 40 fruit sets of unwaxed, Ca-treated and untreated fruit. Internal air space (IAS) was calculated as a proportion of total volume according to the procedure of Hatfield and Knee (1988). For this calculation the specific gravity of 'Golden Delicious' apple juice was measured directly by weighing a 100-mL aliquot of juice from individual fruit.

Turgor potential-induced stress within fruit was determined using the procedure of Skene (1980) as modified by Hatfield and Knee (1988). Turgor potential of tissue cells causes a stress within the fruit, which can be relieved by cutting it half-through along the pedicel-calyx axis with a scalpel. One hour later the width of the slit at the widest point was measured to within 0.1 mm with a gap tester.

Fruit firmness was measured with a manually controlled digital penetrometer (EPT-1 with an 11.1-mm tip; Lake City Technical Products, Kelowna, B.C., Canada) set in the Magness-Taylor mode. Firmness was measured at two opposite points on the

equator of the fruit after removal of a 2-mm slice with a fixed-blade slicer at the end of an 3-d period at 20°C .

The osmotic potentials of sap from freshly isolated cortical tissue were determined using a vapor pressure osmometer (model 5500; Wescor, Logan, Utah) as previously described (Saftner, 1992). To obtain sap, the tissue was frozen in liquid N_2 , stored at -80°C for up to 5 d, thawed, and the sap collected after centrifugation at $3000 g_n$ for 5 min. The osmotic and water potentials of sap from cortical tissue (disks 2 cm in diameter and 2 mm thick) incubated at $20 \pm 1^\circ \text{C}$ for 1 h in 0.03 to $0.34 \text{ mol} \cdot \text{L}^{-1}$ solutions of CaCl_2 or equivalent osmolalities of sorbitol (≈ 0.08 to $0.82 \text{ mol} \cdot \text{L}^{-1}$ D-sorbitol) or in distilled water were similarly determined. Corrections for free-space contamination of collected sap with CaCl_2 were made when calculating tissue osmotic potentials (Baker and Saftner, 1984). Briefly, free space solutes were measured from the free-space volume of apple cortical tissue and the osmolality of the tissue incubation solution. The free space volume was assessed by incubating tissue disks in symplast-impermeable [^{14}C]-dextran solutions of known specific activities followed by measuring the ^{14}C in the free space (Parr and Edelman, 1976). The osmotic potentials of the tissue symplast were expressed by subtracting the osmotic contribution of the free space solutes from the osmolality of the collected sap. Cortical tissue equilibrates with tissue incubation solutions in less than an hour (Meyer and Boyer, 1981; R. Saftner, unpublished data). At equilibrium, the osmotic potential of the external solution is equal to the intracellular water potential, and tissue turgor potential can be estimated from the difference between the extracellular and intracellular osmotic potentials (Li and Delrot, 1987; Oparka and Wright, 1988).

The localization of Ca within the cell wall was determined using the Ca efflux method of Macklon (1975). Cortical tissue disks (1-mm thick, 1 cm in diameter) were incubated (10 disks in 3 mL) for 2 h at 4°C in 0.001 to $0.34 \text{ mol} \cdot \text{L}^{-1}$ solutions of $^{45}\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (specific activity between 0.24 and $0.52 \text{ GBq} \cdot \text{mmol}^{-1}$; DuPont NEN, Boston, Mass.) containing $0.01 \text{ mol} \cdot \text{L}^{-1}$ L-malic acid adjusted to pH 5.5 with KOH. The radiolabeled solutions were replaced after 30, 60, and 90 min. After incubation in ^{45}Ca solutions, each set of tissue disks was removed from the radiolabeled Ca solutions by filtration. Successive 2-mL volumes of ice-cold efflux solution (unlabeled incubation solution) were added to each set of tissue disks for two time periods during a 1 h efflux period. The elution period was 1 min for the first 20 min and 2 min for the remainder of the efflux period. After the washout, each set of disks and each efflux solution was prepared for liquid scintillation counting (Baker and Saftner, 1984). In a few experiments, tissue disks were frozen in liquid nitrogen and thawed before initiation of experimentation. Since less than 1% of the radiolabeled Ca was associated with the intracellular compartments of the tissue, no corrections were made for the small amount of efflux that occurred from these compartments. Not correcting for intracellular ^{45}Ca efflux may lead to an overestimation ($\approx 1\%$) of the Donnan-phase bound Ca in the cell wall. Fruit disks were collected from 20 fruit per experiment and there were three replications for all treatments. Two experiments were done at monthly intervals from harvest through 8 months of storage.

Harvests were made from 1994 to 1996 from the same commercial orchard. In the first year, only tissue osmotic potentials were measured. In the second year, tissue osmotic potential, firmness, and cell wall Ca measurements using individual fruit were made. In the third year, all treatments as described above were made and, unless stated otherwise, representative results from the third year are presented.

Data were analyzed as a one-way ANOVA (SigmaStat, Jandel

Table 1. Quality attributes of Ca-treated and untreated 'Golden Delicious' apples after a 3-d period at 20 °C following 4 months of storage at 0 °C.

Treatment	Parameter ^z					
	Internal Ca (mmol·kg ⁻¹ dry mass)	Firmness (N)	Gape (mm)	IAS	Injury rating ± SD ^x	Fruit injured ^y (%)
None	5.5 a ^w	51 a	1.09 a	0.235 a	5.0 ± 0.1	2
Distilled water	4.8 a	50 a	1.07 a	0.233 a	1.9 ± 0.7	94
CaCl ₂ (mol·L ⁻¹)						
0.07	16.5 b	58 a	1.27 ab	0.228 b	4.1 ± 0.6	60
0.14	22.5 c	68 b	1.45 b	0.221 c	5.0 ± 0.2	2
0.17	25.5 c	70 b	1.50 b	0.220 c	5.0 ± 0.1	4
0.20	28.5 c	73 b	1.47 b	0.220 c	4.8 ± 0.3	6
0.24	32.5 cd	73 b	1.55 b	0.216 d	4.0 ± 0.5	72
0.27	35.8 cd	74 b	1.60 b	0.217 d	2.5 ± 1.0	80
0.34	38.8 d	76 b	1.62 b	0.214 e	2.2 ± 1.1	88

^zEach value of firmness, gape, and internal air space (IAS) is the mean of 80, 40, and 40 readings, respectively, from 40-fruit sets while each value of internal Ca is the mean of seven, seven-fruit replications. Gape is the width of the slit that develops 1 h after cutting a fruit half-through along the pedicel–calyx axis. At harvest, firmness, gape, and internal air space were 85 N, 1.18 mm, and 0.213, respectively.

^yPercent fruit injured indicates the percent of the 50 fruit evaluated showing any sign of injury.

^xInjury rating: 5 = no fruit surface injury; 3 = 10% to 25% of fruit surface injured; 1 = >50% of fruit surface injured. Each value is the mean ± SD for 50 fruit.

^wMeans within a column followed by the same letter are not significantly different by Tukey's multiple range test ($P \leq 0.05$).

Corp., San Rafael, Calif.). The treatment means were separated at the 5% significance level using the Tukey multiple range test. Some of the data were also analyzed by simple linear regression analyses. Injury data were not statistically analyzed since variance heterogeneity of such data could not be corrected by data transformation. Except for the injury data, only results significant at $P \leq 0.05$ are discussed.

Results

Fruit injury in the form of skin discoloration and/or lenticel pitting occurred after treating the fruit with <0.14 or >0.20 mol·L⁻¹ solutions of CaCl₂ even though fruit were protected from desiccation and salt stress during storage by waxing the fruit after treatment (Table 1). Calcium chloride treatments between 0.14 and 0.20 mol·L⁻¹ followed by fruit waxing resulted in no apparent injury to the fruit. These same treatment concentrations increased the internal Ca concentration to between 20 and 30 mmol·kg⁻¹ dry mass (Table 1). At CaCl₂ concentrations up to 0.34 mol·L⁻¹, the Ca treatments also increased turgor potential-induced fruit stress (Table 1). Correspondingly, pressure infiltration of fruit with CaCl₂ solutions at concentrations up to 0.34 mol·L⁻¹ reduced both fruit softening and internal air space volume (Table 1). The correlation coefficients from simple linear regression analyses between the Ca treatments and internal Ca, firmness, gape, and internal air space are 0.99, 0.95, 0.96, and 0.97, respectively. The beneficial effect of Ca treatments on the physical characteristics of the fruit were less pronounced at CaCl₂ concentrations greater than 0.20 mol·L⁻¹.

The osmotic potential of prestorage fruit harvested over 3 seasons ranged between -1.32 and -2.33 MPa which is osmotically equivalent to CaCl₂ solutions of 0.20 and 0.35 mol·L⁻¹, respectively. While the fresh mass of fruit decreased up to 4.8%, due primarily to water loss (Hatfield and Knee, 1988) during 8 months of storage, no change in tissue osmotic potential during storage was detected (data not shown).

The water status of apple tissue disks equilibrated in CaCl₂ solutions up to 0.34 mol·L⁻¹ and in osmotically equivalent solutions of D-sorbitol is shown in Fig. 1. As the CaCl₂ concentration in the tissue bathing solution increased, the water and osmotic potentials of the tissue became more negative. Tissue incubated in osmotically equivalent solutions of D-sorbitol responded similarly

(Fig 1b). At CaCl₂ concentrations up to 0.34 mol·L⁻¹, turgor potential was ≥ 0.6 MPa in tissue incubated in 0.14 or 0.17 mol·L⁻¹ solutions and was more than 3 times higher than in tissue incubated in low (≤ 0.03 mol·L⁻¹) or high (≥ 0.27 mol·L⁻¹) concentrations of CaCl₂. Tissue disks generally became flaccid when incubated in CaCl₂ solutions above 0.24 mol·L⁻¹ and the turgor potential in flaccid tissue approached zero. The turgor potential was up to 40% higher

Fig. 1. Relationships between turgor potential, osmotic potential and water potential following equilibration of apple disks in solutions osmotically adjusted with CaCl₂ or D-sorbitol. The water status of tissue disks was determined following tissue incubation for 1 h at 20 °C in distilled water or solutions containing various concentrations of CaCl₂ (closed symbols) or D-sorbitol (open symbols). Symbols for water potential of CaCl₂- and D-sorbitol-treated tissue overlap. Means of four measurements made 1 month after harvest, SDs were smaller than symbol size.

Table 2. Calcium efflux characteristics of the cell wall compartments in 'Golden Delicious' apple disks.

Tissue status and compartment	K^2 (min^{-1})	$t_{1/2}^2$ (min)
Live tissue at 4 °C		
Donnan free space	3.0×10^{-2}	22.8 ± 2.1
Water free space	3.5×10^{-1}	2.0 ± 0.3
Freeze-thawed tissue at 4 °C		
Donnan free space	2.9×10^{-2}	23.6 ± 1.8
Water free space	3.0×10^{-1}	2.3 ± 0.4

²Each value is the mean or mean $\pm$ SD from 60 measurements (12 measurements from each of five experiments).

in Ca-treated than in D-sorbitol-treated tissue at osmotically equivalent concentrations (Fig. 1a).

Calcium efflux patterns from apple disks are consistent with two distinct Ca compartments. The $t_{1/2}$ for Ca efflux was 22.8 ± 2.1 (SD) and 2.0 ± 0.3 min for the slow and fast releasing compartments, respectively (Table 2). The rate constants for the slow and fast releasing compartments were 3.0×10^{-2} and $3.5 \times 10^{-1} \text{ min}^{-1}$, respectively. The kinetic values did not change in tissue collected from fruit that had been stored for up to 8 months at 0 °C (data not shown) or in fruit that had been freeze-thawed before experimentation (Table 2). From these results, we identified the Ca compartments as being extracellular, the slow releasing Ca compartment as cell wall Donnan-phase bound Ca and the fast releasing Ca compartment as soluble Ca in the water free space of the cell wall. Figure 2 shows the levels of cell wall bound and soluble Ca in tissue disks collected from fruit stored for up to 8 months at 0 °C and then incubated with various concentrations of CaCl_2 . At concentrations up to $0.27 \text{ mol} \cdot \text{L}^{-1}$, soluble Ca increased in an essentially linear manner (Fig. 2a) whereas wall-bound Ca appeared to approach saturation at $0.20 \text{ mol} \cdot \text{L}^{-1}$ (Fig. 2b).

Discussion

Two general mechanisms have been suggested to account for the influence of Ca on postharvest changes in fruit firmness and quality (Ferguson, 1984). One mechanism is based on Ca binding to cell walls inducing a greater stability in cell wall structure. The other mechanism is associated with Ca interactions with membrane structure and functioning. In the research reported herein, fruit pressure-infiltrated with a $0.20 \text{ mol} \cdot \text{L}^{-1}$ solution of CaCl_2 retained firmness more than untreated fruit and cell wall binding sites for Ca approached saturation when tissue disks were treated with the same concentration of CaCl_2 . Similar, though less detailed, results were found for whole fruit infiltrated with Ca (Conway et al., 1995). When higher concentrations of CaCl_2 were infiltrated into apples, fruit firmness retention was increased, albeit not significantly, and total Ca levels increased. Since the cell walls are essentially saturated with a $0.20 \text{ mol} \cdot \text{L}^{-1}$ solution, the additional Ca is probably soluble and would thermodynamically redistribute within the fruit. Due to exchange adsorption (Donnan accumulation), Ca movement to low Ca-containing wall spaces would result in further Ca binding which may result in firmer fruit. However, high soluble Ca levels can negatively affect cell membranes and/or enter the cytosol resulting in tissue injury.

During storage, firmness of untreated fruit decreased whereas the Ca-binding capacity of tissue collected from untreated fruit increased. These results may be due to increased synthesis or decreased degradation of various Ca-binding, cell wall components, so that synthesis exceeds degradation during storage. Previ-

ous studies also have shown increased recovery of cell wall components from Ca-treated compared to untreated fruit following storage (Conway et al., 1995). However, the Ca content of intact fruit remains essentially constant once harvested. With increased Ca-binding capacity and constant total Ca, the soluble Ca of fruit should decrease during fruit storage and that is what has been reported (Saks et al., 1990). As such, fruit softening during storage could be related to the ratio of wall-bound to total Ca-binding capacity of the cell wall, not to bound Ca per se. Regardless of the mechanism(s) involved, available binding sites in the wall of apple tissue are essentially saturated with a $0.20 \text{ mol} \cdot \text{L}^{-1}$ solution of CaCl_2 , a level that reduces fruit softening during storage.

Calcium treatments also alter tissue water relations and membrane functioning. Previous studies have shown that placing apple disks in hypotonic solutions above -0.8 MPa leads to solute leakage and to impaired physiological functioning (Bir and Bramlage, 1973; Burg and Thimann, 1960). In hypertonic solutions, plant cells plasmolyze and membrane damage can occur (Prat, 1972). In the research reported herein, the osmotic potential of 'Golden Delicious' fruit was variable at harvest and ranged between -1.32 and -2.33 MPa . The osmotic potential of tissue

Fig. 2. Calcium content in the Donnan and water free space of apple disks collected from fruit stored from 0 to 8 months and following incubation in various concentrations of CaCl_2 . Tissue disks (1 mm thick, 1 cm in diameter) collected from fruit stored from 0 to 8 months were incubated (10 disks from 10 fruit in 3 mL) for 2 h at 4 °C in 0.001 to $0.34 \text{ mol} \cdot \text{L}^{-1}$ solutions of $^{45}\text{CaCl}_2$ containing $0.01 \text{ mol} \cdot \text{L}^{-1}$ L-malic acid adjusted to pH 5.5 with KOH. Radioactivity associated with water free space soluble (A) and Donnan free space bound (B) Ca in the cell wall was determined by compartmental analysis. Means of three replications.

disks increased when incubated in hypotonic solutions of CaCl_2 or D-sorbitol. In CaCl_2 solutions below $0.14 \text{ mol}\cdot\text{L}^{-1}$ ($> -0.9 \text{ MPa}$), turgor potential was not maintained and fruit treated with solutions of low osmotic concentration or with water often showed signs of peel injury. In CaCl_2 solutions between 0.20 and $0.34 \text{ mol}\cdot\text{L}^{-1}$ (-1.3 and -2.2 MPa), tissue disks became flaccid, turgor potential approached zero and fruit treated with hypertonic solutions of CaCl_2 were generally injured. In tissue incubated in CaCl_2 solutions between 0.14 and $0.20 \text{ mol}\cdot\text{L}^{-1}$ (-0.9 to -1.3 MPa), turgor potential was maintained at a relatively high level even though the osmotic potential of the tissue increased somewhat during incubation. At equivalent osmolalities, the turgor potential was higher in Ca- than in D-sorbitol-treated tissue. This suggests that part of the CaCl_2 -induced maintenance of turgor potential is independent of a CaCl_2 effect on tissue osmotic potential. While Ca is known to stabilize membrane structure/functioning and to strengthen cell walls (Damarty et al., 1984; Ferguson, 1984), the cause of the CaCl_2 effect on turgor potential is unknown. Fruit pressure-infiltrated with the same concentrations of CaCl_2 were firmer and had higher turgor potential-related stress and lower air space volume than water-treated or untreated fruit. Since high turgor and low interstitial volume increase the mechanical strength of tissue cells (Niklas, 1992), Ca-induced changes in turgor and interstitial air space volume are likely to contribute, along with cell wall Ca-binding effects, to Ca-related maintenance of fruit firmness. Fruit treated with 0.14 to $0.20 \text{ mol}\cdot\text{L}^{-1}$ concentrations of CaCl_2 showed no signs of injury after 4 months of cold storage provided that the fruit were wax-coated following Ca treatment. Currently, the commercial use of fruit coatings in apples is primarily restricted to applications that increase gloss and reduce transpiration losses poststorage. If fruit infiltrated with CaCl_2 are not wax-coated to protect against significant water loss during storage, Ca salts may accumulate near the fruit surface, reducing the tissue osmotic potential and inducing surface injury to the fruit.

For commercial applications, a 0.14 to $0.20 \text{ mol}\cdot\text{L}^{-1}$ solution of CaCl_2 is the optimal Ca concentration range for treatment of 'Golden Delicious' apples from the standpoint of tissue water relations and injury risk potential. To allow for some water loss and salt buildup at the fruit surface (especially around open lenticels) during storage, a $0.14 \text{ mol}\cdot\text{L}^{-1}$ solution of CaCl_2 would be preferred to a 0.17 or $0.20 \text{ mol}\cdot\text{L}^{-1}$ solutions of CaCl_2 for postharvest treatment of 'Golden Delicious' fruit.

A $0.14 \text{ mol}\cdot\text{L}^{-1}$ solution of CaCl_2 , however, is not optimal for maintaining fruit firmness, prolonging fruit shelf life and reducing decay caused by postharvest pathogens (Conway et al., 1991; Saftner et al., 1997). While fruit firmness and decay control is best maintained at internal Ca concentrations above $50 \text{ mmol}\cdot\text{kg}^{-1}$, conventional postharvest pressure infiltration with a $0.14 \text{ mol}\cdot\text{L}^{-1}$ solution of CaCl_2 seldom increases the internal Ca concentrations above $25 \text{ mmol}\cdot\text{kg}^{-1}$. One way to increase Ca infiltration into fruit without increasing the salt concentration or the applied pressure gradient of the treatment would be through the addition of surfactants such as Silwet L-77 to the infiltration solution (Saftner et al., 1997). Preliminary results indicate that fruit pressure infiltrated with a $0.14 \text{ mol}\cdot\text{L}^{-1}$ solution of CaCl_2 containing $1 \text{ g}\cdot\text{L}^{-1}$ Silwet maintains fruit firmness better than treatment with a $0.27 \text{ mol}\cdot\text{L}^{-1}$ solution of CaCl_2 without surfactant (R. Saftner, unpublished data). Silwet L-series surfactants are cleared for use as inert ingredients for use in formulations applied to raw agricultural commodities after harvest under Environmental Protection Agency Regulation 40 CFR 180.1001 (c).

In conclusion, results of research reported herein suggest that Ca treatments of apple tissue affect both cell wall composition and mechanical strength and membrane functioning. A $0.14 \text{ mol}\cdot\text{L}^{-1}$

solution of CaCl_2 is the preferred concentration for maintenance of postharvest life while minimizing the risk of surface injury to 'Golden Delicious' fruit.

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