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Quality and Cell Wall Components of 'Anna' and 'Granny Smith' Apples Treated with Heat, Calcium, and Ethylene

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Abstract. 'Anna' and 'Granny Smith' apples *(Malus domestics* Borkh.) that were held at 38C for 4 days before storage at 0C not only were firmer than controls upon removal from storage, but also softened **more slowly during shelf life at 17C. Skin yellowing and loss of acidity attendant upon the heat treatment were not prevented by dipping fruit in 2% $CaCl_2$ before heating. Both heat-treated and control fruit softened at the same rate upon exposure to ethylene at 100 µl·liter^{-1} upon removal from storage. The insoluble pectin content of cortical tissues was higher in heat-treated fruit than in controls after 10 days at 17C, while soluble pectin levels were lower. Arabinose and xylose levels were lower in cell walls from heat-treated cortical tissue, but the treatment had no effect on loss of galactose residues during shelf life.**

Previous researchers have noted that heating apples at 38C for 4 to 6 days after harvest retarded or inhibited fruit softening while maintaining most other aspects of storage quality (Liu, 1978; Porritt and Lidster, 1978). The exception was a slight yellowing of the skin and a decrease in titratable acidity in comparison with unheated fruit. Calcium treatments also extend the storage life of apples, primarily by reducing the rate of metabolic activity (Bangerth et al., 1972}, thus reducing senescence phenomena such as fruit softening, yellowing, and loss of acidity (Ferguson; 1984; Glenn et al., 1988). Since uptake of Ca by pear cells was specifically enhanced by heating cells at 38C (Klein and Ferguson, 1987), we endeavored to determine if heating Ca-treated apples could synergistically inhibit loss of fruit firmness in storage, without associated problems of acid loss and skin yellowing.

The specific objectives of this research were to increase the effectiveness of postharvest Ca dips by use of a heat treatment, while simultaneously counteracting the disadvantages of heat treatment alone (loss of acidity and yellowing). Since heat treatment inhibits ethylene production by apples (Klein, 1989), we investigated whether softening could be reinduced by exposing heat-treated fruit to ethylene at 100 µl·liter^{-1} upon removal from storage. We also studied whether the decrease in apple fruit softening after a prestorage heat treatment is due to changes in the neutral sugar and pectin composition of the cell wall in cortical tissue.

Materials and Methods

'Granny Smith' and 'Anna' apples were heated for 4 days at 38C after a 5-min dip in 20C water or 2% $CaCl_2$ prior to being placed in storage. 'Granny Smith' fruit were also dipped in 38C solutions of water or 2% to $CaC1_2$ before storage. Control fruit were stored at 0C immediately after harvest.

Ripeness criteria (firmness, background color, percent titratable acidity, and soluble solids concentration) of 'Granny Smith' apples were examined upon removal from storage after 2, 4, and 6 months at 0C and during 10 days of shelf life at 17C. Data from the 4-month removal from storage are presented as being representative of treatment trends after 2 and 6 months of storage. 'Anna', which softens quickly even at 0C, was examined only after 1 month of storage. At each removal, fruit lots were subdivided in two. One subsample was exposed to 100 µl ethylene/liter for 18 hr at 17C before further holding at 17C to determine shelf life, after which the quality characteristics of ethylene-treated fruit also were evaluated.

Background color of the fruit was determined with a Techwest apple color-meter (TechWest Enterprises, Vancouver, B.C.), using a scale on which 1 indicates green and 10 indicates yellow. Firmness was measured with a Hunter-Spring penetrometer (Hunter Spring Corp., Warminster, Pa.) equipped with an 11-mm-diameter probe. The soluble solids concentration (SSC) and percent titratable acidity (calculated as malic acid) in expressed juice were measured, respectively, with a hand-held refractometer and by titrating a 2-ml aliquot to pH 8.2 with 0.1 N NaOH. Means of four replicates of at least five fruits per replicate are presented.

The effect of the prestorage treatments on Ca concentrations in various tissues was determined with 'Anna' and 'Granny Smith' fruit after 1 and 4 months of storage, respectively. Samples of skin and two layers of flesh 0.5 and 1 cm below the skin were frozen, lyophilized, acid-digested, and the content of Ca measured by atomic absorption spectrophotometry. Again, means of four replicate samples of at least five fruits each are presented.

Cortical tissue samples of 'Granny Smith' at the 4-month removal from storage and after shelf life were frozen and lyophilized for subsequent preparation of cell walls for analysis of neutral sugar concentrations according to the method of Gross et al. (1986). Means presented are from duplicate analyses of triplicate samples. Acetone powders were prepared from cortical tissue of 'Anna' fruit upon removal from 1 month of storage and after 10 days of shelf life. The powder (100 mg) was serially extracted in water, 0.5% EDTA, and 0.5 N NaOH. The galacturonic acid concentration in each filtrate was measured by the method of Blumenkrantz and Asboe-Hansen (1973) and expressed as water-soluble pectin, Ca pectate, and insoluble pec-

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tin, respectively. Duplicate readings were made of four replicates for each treatment, and the experiment was conducted twice. Data are presented as percentages of the total pectin fraction.

Analyses of variance and mean separations" were calculated using SAS software (SAS Institute, 1985).

Results

Dipping 'Granny Smith' apples in a 20C solution of 2% $CaC1_2$ increased Ca content in epidermal and cortical tissues by 44% compared with controls dipped in water (Table 1). Fruit dipped in a 38C solution of 2% $CaC1_2$ had 50% more epidermal Ca than fruit dipped in 38C water only, and 75% more Ca than fruit dipped in 20C water. The dip in 38C 2% $CaC1_2$ also promoted a 20% increase in epidermal Ca over the level obtained from a 2% $CaC1_2$ dip at 20C. The levels of cortical Ca in fruit from the 20 and 38C Ca dips were similar. There was no evidence of a Ca gradient in the first 10 mm of cortical tissues below the epidermis (data not shown). Apples dipped in 20C 2% $CaC1_2$ before holding 4 days at 38C did not show significant increases in epidermal or cortical Ca, compared to fruit dipped in water alone prior to heating. Similar results to these were obtained with 'Anna' (data not shown).

Both 'Anna' and 'Granny Smith' apples that were held at 38C for 4 days before storage were yellower than control fruit after 10 days of shelf life (Table 2). Ground color readings of both heated and control fruit were generally unaffected by treatment with Ca. The exception was with 'Granny Smith' fruit that was heated for 4 days at 38C after Ca treatment; these fruits actually yellowed more during shelf life than those that were held at 38C without previous Ca treatment.

There was no discernible effect of Ca on titratable acidity of either cultivar (Table 2). 'Granny Smith' fruits that had been heated for 4 days at 38C were lower in titratable acidity upon removal from storage than untreated fruit, but differences mostly disappeared by the end of shelf life. Unheated Ca-treated 'Anna' fruits were higher in titratable acidity than heated fruit upon removal from storage, but, again, these differences were transient. Neither heating nor Ca treatment had an effect on SSC of 'Granny Smith' and 'Anna' apples (data not shown). Mean SSC of 'Granny Smith' was 13.3, while that for 'Anna' was 12.0.

A prestorage dip in 38C Ca led to enhanced retention of fruit firmness in 'Granny Smith' during shelf life (Table 3). Holding fruit at 38C for 4 days before storage, however, was more effective than Ca treatment on retention of 'Granny Smith' firmness, both at removal from storage and during subsequent shelf life. The effect of dipping in Ca on fruit firmness was minimal compared with that of heating. The rate of softening of heat-treated 'Granny Smith' over 6 months of storage was only 5% to 35% of that of control fruit (Fig. 1). Heated 'Anna' fruit also showed enhanced retention of firmness by 5 days of shelf life (Table 3, Fig. 2).

There was no marked tendency for ethylene-treated fruit to 'be softer than non-ethylene-treated fruit at the end of shelf life (Table 3). Ethylene-treated nonheated 'Anna' fruit did, however, initially soften more rapidly than nontreated fruit, although such differences were negligible by day 5 of shelf life (Fig. 2). The softening rate of heated 'Anna' fruit was unaffected by ethylene application. As storage proceeded; control 'Granny Smith' apples became more sensitive to ethylene treatment and softened more readily, while heated fruit were similar in firmness regardless of ethylene treatment (Fig. 3). However, by the end of 10 days of shelf life, treatment with ethylene diminished the residual effect of prestorage heating on inhibition of fruit softening sufficiently that ethylene-treated heated fruit had firmness values similar to those of non-ethylene-treated controls (Figs. 2 and 3).

The pectic fractions of cell walls of control and heated 'Anna' apples were similar in composition upon removal from storage (Table 4). During shelf-life, however, when differences in softening became more apparent, solubilization of the insoluble pectic fraction was retarded in the heat-treated fruit compared with controls. This was further reflected in the lesser amounts of water-soluble pectin and Ca pectate present in heated tissues after shelf life.

The major neutral sugar residues present in cell walls from 'Granny Smith' cortical tissue were xylose, arabinose, and galactose, which comprised 38%, 27%, and 1970 of the total neutral sugars, respectively (Table 5). Analysis of variance indicated that there was a slight but significant decrease in arabinose and xylose residues in heated apples compared with controls, and a slight increase in glucose. Galactose residues decreased significantly during shelf life, but there were no differences between control and heat-treated fruit. There were no significant differences between treatments or between the beginning and end of shelf life in levels of rhamnose or mannose.

Table 1. Calcium concentrations in epidermis and cortical tissue of 'Granny Smith' apples held 4 months at 0C. Fruit were dipped before storage in solutions of 0% or 2% $CaC1_2$ held at 20 or 38C and stored immediately or after being heated 4 days at 38C (20C dip only). Means of four replicate samples of five fruits each.

Solution temperature (°c)	Epidermis Ca concn (%) 0	Epidermis Ca concn (%) 2	Cortex Ca concn (%) 0	Cortex Ca concn (%) 2
	Ca concn (mg/100 g fresh wt)[z]			
20	9.7	14.0*	3.8	5.4*
38	11.2	16.8*	4.2	5.8*
20C (4-day delay at 38C)	11.4	11.2	4.8	5.1*
Analysis of variance[y]				
Temperature		*		NS
Ca concn		***		***
Temperature × Ca concn		*		NS

[z]Within solutions and tissues, values followed by an asterisk differ at $P = 0.05$ by t test.

[y]Nonsignificant (NS) or significant at $P = 0.05$ **(*)** or 0.001 (***).

Discussion

Epidermal tissues of fruit dipped in 38C Ca solution had higher Ca concentrations than those of fruit from other treatments, which may indicate that heat and Ca must be present simultaneously for enhanced Ca uptake, as was the case with fruit cells in suspension culture (Klein and Ferguson, 1987). There was no evidence that application of heat enhanced Ca uptake into apple cortical tissues.

Calcium treatments were essentially ineffective in maintaining acidity levels and inhibiting skin yellowing due to heat treatment. Other researchers, who have noted delayed yellowing and better retention of acidity in Ca-treated fruit, used pressure or vacuum infiltration, rather than dipping, to enhance the Ca content of fruit tissues (Conway and Sams, 1983; Glenn et al., 1988; Scott and Wills, 1979).

Firmness was affected more by heating fruit for 4 days at 38C before storage than by any other treatment. 'Anna', a cultivar that softens rapidly even in cold storage, showed enhanced

Table 2. Effect of Ca dips and heat treatment on ground color and percent titratable acidity (as malic acid) of 'Anna' and 'Granny Smith' apples upon removal from storage at 0C and after 10 days of shelf life at 17C. Means of four replicate samples of five fruits each.

Prestorage treatment	Anna[z]				Granny Smith[y]			
	Color[x]		Titr. acid (%)		Color		Titr. acid (%)	
	Removal	Shelf	Removal	Shelf	Removal	Shelf	Removal	Shelf
Control	5.0	5.4	0.49	0.44	2.3	2.4	0.67	0.56
2% Ca dip (20C, 5 min)	4.8	6.0	0.57	0.45	2.1	2.3	0.63	0.63
Hot water dip (38C, 5 min)	---	---	---	---	2.4	2.4	0.63	0.52
2% Ca dip (38C, 5 min)	---	---	---	---	2.1	2.3	0.68	0.58
4 days, 38C	5.6	6.4	0.46	0.44	2.7	2.8	0.58	0.58
2% Ca dip (20C), followed by 4 days, 38C	5.2	6.3	0.47	0.44	2.7	3.2	0.55	0.50
$LSD_{0.05}$	0.7		0.09		0.3		0.08	

[z]One month of storage.
[y]Four months of storage.
[x]Techwest color meter; 1 = green, 10 = yellow.

Table 3. Effect of prestorage Ca dips and heat treatments on firmness of 'Anna' and 'Granny Smith' apples after removal from storage at 0C and after 10 days at 17C, with and without an 18-hr exposure to ethylene at 100 µl·liter^{-1}. Means of four replicates of five fruit each.

Prestorage treatment	Firmness (N)					
	Anna[z]			Granny Smith[y]		
	Removal	Shelf life	Shelf life + ethylene	Removal	Shelf life	Shelf life + ethylene
Control	66	46	43	65	58	55
2% Ca dip (20C, 5 min)	67	45	48	65	61	58
Hot water dip (38C, 5 min)	---	---	---	64	57	58
2% Ca dip (38C, 5 min)	---	---	---	65	63	59
4 days, 38C	65	55	54	74	67	64
2% Ca dip (20C) followed by 4 days at 38C	66	55	50	72	70	72
$LSD_{0.05}$		10.1			4.5	

[z]One month of storage.
[y]Four months of storage.

retention of firmness in response to heating after just 1 month of storage (Table 3). However, the beneficial effects of prestorage heating were noted only after 4 months of storage of 'Granny Smith', which is a firm cultivar that softens slowly even at room temperature (Figs. 1 and 2).

The mechanism whereby prestorage heating leads to enhanced retention of fruit firmness is unclear. In processed foods, heating at high temperatures for short periods of time leads to increased product firmness. This is due chiefly to a remobilization of Ca (either endogenous or added during processing) to Ca pectate as a result of enhanced pectinesterase (EC 3.1.1.11) activity at high temperatures (Doesburg, 1965). However, we found less Ca pectate in heated fruits that, nonetheless, were much firmer than controls. We were also unable to find significant pectinesterase activity in our study (unpublished results), just as other researchers have previously noted (Bartley and Knee, 1982).

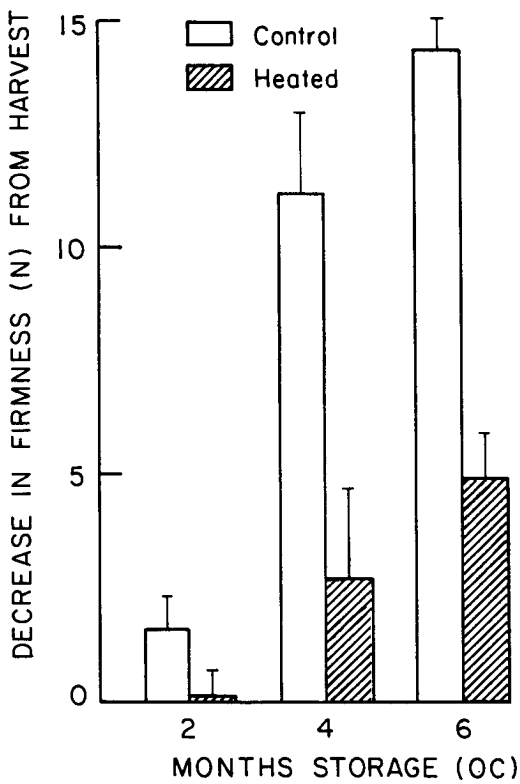

Fig. 1. Firmness (N) after 2, 4, or 6 months of storage of 'Granny Smith' apples stored at 0C immediately after harvest or after a 4-day delay at 38C. Firmness at harvest, 77.3 N. Means of four replicate samples of five fruits each. Bars indicate SE.

An increase in soluble pectin and a decrease in insoluble pectin are characteristic of softening in many fruits (Bartley and Knee, 1982). Porritt and Lidster (1978) found less water-soluble pectin in heated 'Golden Delicious' apples than in unheated controls, but this was not the case with 'Spartan' fruit, which nonetheless were firmer after heat treatment. In the case of 'Anna' fruit, there was a relative retention of insoluble pectin by heated fruit during shelf life and, consequently, a smaller

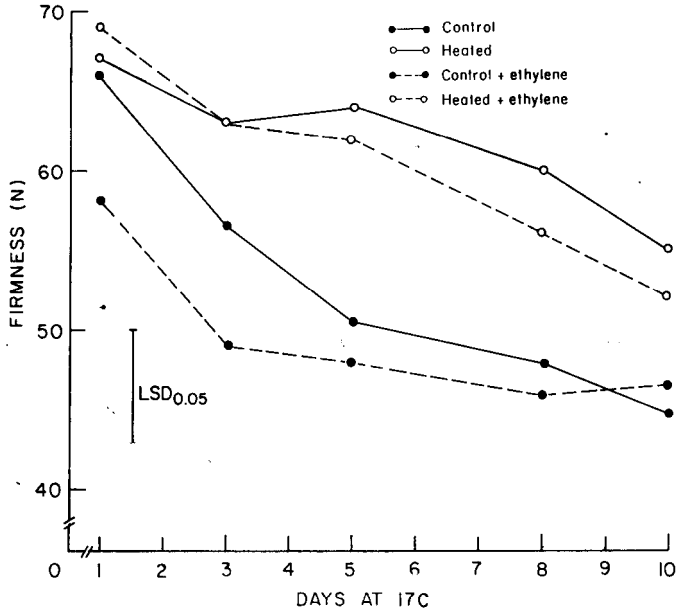

Fig. 2. Firmness (N) of 'Anna' apples during 10 days at 17C. Fruit were stored for 1 month at 0C immediately after harvest or after a 4-day delay at 38C. Broken line indicates fruit that were exposed to ethylene at 100 µl·1iter^{-1} at 17C for 18 hr at the beginning of shelf life. Data are averaged over Ca treatments and represent means of eight replicates of five fruit each.

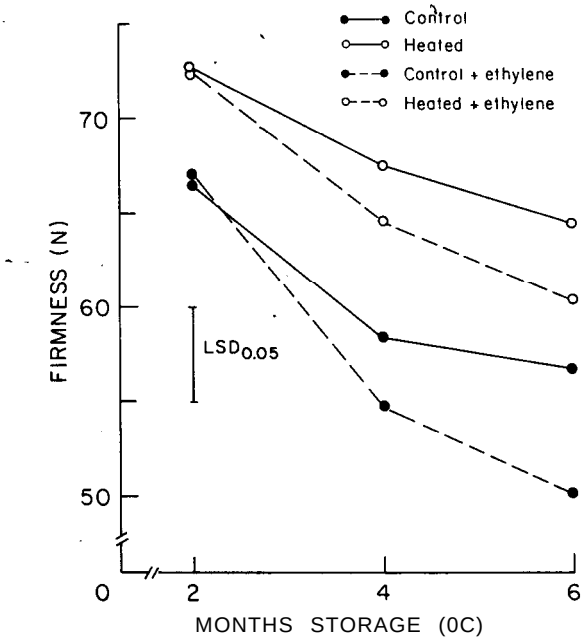

Fig. 3. Firmness **(N)** of 'Granny Smith' apples after 10 days of shelf life at 17C following 2, 4, and 6 months of storage. Fruit were stored at 0C either immediately after harvest or after a 4-day delay at 38C. Broken line indicates fruit that were exposed to ethylene at 100 µl·liter^{-1} at 17C for 18 hr at the beginning of shelf life.

amount of soluble pectin and Ca pectate compared with controls (Table 4). Since insoluble pectin itself is composed of various polyuronide fractions (Doesburg, 1965), a more detailed fractionation procedure may help further elucidate differences between pectic fractions of heated and unheated fruit.

Neutral sugar residues in both heated and control fruit were similar to those found previously in apples during ripening (Gross and Sams, 1984), with the exception of a slight decrease in

Table 4. Pectic fractions of 'Anna' apple cortical tissue after 1 month at 0C plus 10 days at 17C. Fruit were placed in storage immediately after harvest or after being heated 4 days at 38C. Means of eight replicate samples of five fruit each.

	Fraction (% of total pectin)		
Treatment	Water-soluble	Ca pectate	Insoluble
Control			
Removal	12.6	13.6	73.8
+ 10 days at 17C	43.0	18.7	39.0
Heat			
Removal	10.8	11.1	78.1
+ 10 days at 17C	36.3	14.9	48.9
Analysis of variance			
Temperature	*	*	*
Inspection time	***	**	***
Temp × inspection	NS	NS	NS

NS,*, **,***Nonsignificant or significant at P = 0.05, 0.01, and 0.001, respectively.

Table 5. Neutral sugar residues in 'Granny Smith' cortical cell walls after 4 months at 0C plus 10 days at 17C. Fruit were placed in storage immediately after harvest or after being heated 4 days at 38C. Means of three replicate samples of five fruit each.

	Neutral sugar (mg/100 mg cell wall)					
Treatment	Rha	Ara	Xyl	Man	Glu	Gal
Control						
Removal	1.8	11.1	14.5	1.0	2.9	7.6
+ 10 d at 17C	1.9	10.7	14.9	0.9	2.8	7.0
Heat						
Removal	1.6	10.1	13.6	0.9	3.2	7.3
+ 10 days at 17C	1.7	9.4	14.4	0.9	3.1	6.9
Analysis of variance						
Temperature	NS	**	*	NS	*	NS
Inspection time	NS	NS	NS	NS	NS	*
Temp × inspection	NS	NS	NS	NS	NS	NS

NS,*,**Nonsignificant or significant at P = 0.05 or 0.01, respectively

arabinose and xylose in heated fruit tissues (Table 5). Loss of galactose residues, a general feature of apple softening (Bartley and Knee, 1982; Wanner, 1978) was not affected by heat treatment. Heated apples thus resemble *rin* tomatoes in that both remain firm despite undergoing a loss of galactose (Gross and Wanner, 1979).

Previous researchers have reported no ripening or softening response to ethylene applied while fruit were held at high temperature. Avocadoes exposed to ethylene or propylene while held at 40C did not soften at all, although this was not the case at 35C (Eaks, 1978). Tomatoes held at 35C did not ripen normally or accumulate polygalacturonase mRNA, nor were these inhibitions reversed upon application of ethylene (Picton and Grierson, 1988). Our results show that the application of ethylene did not completely reverse the effect of prestorage heating even after an intervening period of 6 months of cold storage prior to removal to 20C (Fig. 3). Both 'Anna' and 'Granny Smith' apples heated before storage softened in response to an 18-hr exposure to ethylene at 100 µl·liter^{-1} upon removal from storage, but retained firmness compared with controls. Interestingly, although ethylene production is initially reduced by heat treatment, both heated tomatoes (Biggs et al., 1988) and apples (Klein, 1989) eventually produce more ethylene than do controls upon removal to 20C, while retaining their firmness (Klein and Lurie, 1989; Yoshida et al., 1984). Possibly, the

"physiological lesion" produced by heating affects softening, which is linked with the ethylene-recognition mechanism in fruit, to a greater extent than it affects autocalaytic ethylene production.

Prestorage heat treatment shows promise both as a commercial and as a laboratory technique.' The major benefit is the retention of fruit firmness after removal from storage. Although the decrease in fruit acidity resulting from heating is not necessarily beneficial, in some tart apple cultivars, such as 'Granny Smith', consumers may find such a reduction desirable. We have also used heating as a nonchemical postharvest treatment to enhance yellowing of 'Golden Delicious' apples while retaining fruit firmness (J.D.K. and R. B.-A., unpublished). Heat treatment may also be effective in reducing the incidence of superficial scald (S.L. and J. D. K., unpublished data). In physiological studies, heat treatment may be a useful technique to separate ethylene production from physiological processes such as softening that are otherwise affected by endogenous ethylene.

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