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% CaCl2 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 induced 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% CaCl2 prior to being placed in storage. ‘Granny Smith’ fruit were also dipped in 38C solutions of water or 2% to CaCl2 before storage. Control fruit were stored at 0C immediately after harvest.

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Cortex

Results

Dipping 'Granny Smith' apples in a 20C solution of 2% CaCl₂ 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% CaCl₂ 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% CaCl₂ also promoted a 20% increase in epidermal Ca over the level obtained from a 2% CaCl₂ 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% CaCl₂ 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.

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 firmness.
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 0°C and after 10 days of shelf life at 17°C. Means of four replicate samples of five fruits each.

| Prestorage treatment | Anna Color* | Titr. acid (%) | | Granny Smith 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              |

LSD0.05 0.7 0.09 0.3 0.08

*One month of storage.  
†Four months of storage.  
*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 0°C and after 10 days at 17°C, with and without an 18-hr exposure to ethylene at 100 µl·liter⁻¹. Means of four replicates of five fruit each.

| Firmness (N) | Anna  | Granny Smith |
|--------------|-------|--------------|
| Prestorage treatment | | | |
| Control | 66 | 65 | 58 | 55 |
| 2% Ca dip (20C, 5 min) | 67 | 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 | 65 | 54 | 74 | 67 | 64 |
| 2% Ca dip (20C), followed by 4 days at 38C | 66 | 66 | 55 | 54 | 72 | 70 | 72 |

LSD0.05 10.1 4.5

*One month of storage.  
†Four months of storage.

A decrease 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 17°C. Fruit were stored for 1 month at 0°C immediately after harvest or after a 4-day delay at 38°C. Broken line indicates fruit that were exposed to ethylene at 100 µl·liter⁻¹ at 17°C 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.

Table 4. Pectic fractions of ‘Anna’ apple cortical tissue after 1 month at 0°C plus 10 days at 17°C. Fruit were placed in storage immediately after harvest or after being heated 4 days at 38°C. 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 17°C          | 43.0      | 18.7          | 39.0       |
| Heat Removal               | 10.8      | 11.1          | 78.1       |
| + 10 days at 17°C          | 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.

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

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

| Neutral sugar residues (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 17°C                              | 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 17°C                           | 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 | NS |
| Temp × inspection                           | NS  | NS | NS | NS | NS | NS |

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

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 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. Avocados exposed to ethylene or propylene while held at 40°C did not soften at all, although this was not the case at 35°C (Eaks, 1978). Tomatoes held at 35°C 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 20°C (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 20°C, 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.

**Literature Cited**

Bangerth, F., D.R. Dilley, and D.H. Dewey. 1972. Effect of postharvest calcium treatments on internal breakdown and respiration of apple fruits. J. Amer. Soc. Hort. Sci. 97:679-682.

Bartley, I. and M. Knee. 1982. The chemistry of textural changes in fruit during storage. Food Chem. 9:47-58.

Biggs, M. S., W. Woodson, and A. Handa. 1988. Biochemical basis of high temperature inhibition of ethylene biosynthesis in ripening tomato fruits. Physiol. Plant. 72:572-578.

Blumenkrantz, N. and G. Asboe-Hansen. 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54:484-489.

Conway, W.S. and C.E. Sams. 1983. Calcium infiltration of Golden Delicious apples and its effect on decay. Phytopathology 73:1068-1071.

Doesburg, J.J. 1965. Pectic substances in fresh and preserved fruits and vegetables. IBVT Communication no. 25.

Eaks, I.L. 1978. Ripening, respiration, and ethylene production of ‘Hass’ avocado fruits at 20°C to 40°C. J. Amer. Soc. Hort. Sci. 103:576-578.

Ferguson, I.B. 1984. Calcium in plant senescence and fruit ripening. Plant Cell & Env. 7:477489.

Glenn, G. M., A.S.N. Reddy, and B.W. Poovaiah. 1988. Effect of calcium on cell wall structure, protein phosphorylation and protein profile in senescing apples. Plant Cell Physiol. 29:565-572.

Gross, K.C. and C.E. Sams. 1984. Changes in cell wall neutral sugar composition during fruit ripening: a species survey. Photochemistry 23:2457-2461.

Gross, K.C. and S. Wanner. 1979. Degradation of cell wall polysaccharides during tomato fruit ripening. Plant Physiol. 63:117-120.

Klein, J.D. and I.B. Ferguson. 1987. Effect of high temperature on calcium uptake by suspension-cultured pear fruit cells. Plant Physiol. 84:153-156.

Klein, J.D. and S. Lurie. 1989. Prestorage heat treatment as a means of improving poststorage quality of apples. J. Amer. Soc. Hort. Sci. 115:265-269.

Liu, F.W. 1978. Modification of apple quality by high temperature. J. Amer. Soc. Hort. Sci. 103:730-733.

Picton, S. and D. Grierson. 1988. Inhibition of expression of tomato-ripening genes at high temperature. Plant Cell & Env. 11:265-272.

Porritt, S.W. and P.D. Lidster. 1978. The effect of prestorage heating on ripening and senescence of apples during cold storage. J. Amer. Soc. Hort. Sci. 103:583-585.

SAS Institute. 1985. SAS introductory guide. 3rd ed. SAS Institute, Inc., Cary, N.C.

Scott, K.J. and R.B.H. Wills. 1979. Effects of vacuum and pressure infiltration of calcium chloride and storage temperature on the incidence of bitter pit and low temperature breakdown of apples. Austral. J. Agr. Res. 30:917-928.

Wanner, S.F. 1978. Apple fruit (3-galactosidase and softening in storage. J. Amer. Soc. Hort. Sci. 103:364-366.

Yoshida, O., H. Nakagawa, N. Ogura, and T. Sate. 1984. Effect of heat treatment on the development of polygalacturonase activity in tomato fruit during ripening. Plant Cell Physiol. 25:505-509.