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Additive Effects of Postharvest Calcium and Heat Treatment on Reducing Decay and Maintaining Quality in Apples

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Abstract. **'Golden Delicious' apples *(Malus domestics* Borkh.) were treated with heat or $CaCl_2$ solutions or a combination thereof to determine the effects of these treatments on decay and quality of fruit in storage. Heat treatment at 38C for 4 days, pressure infiltration with 2% or 4% solutions of $CaCl_2$, or a combination of both, with heat following $CaCl_2$ treatment affected decay and firmness during 6 months of storage at 0C. The heat treatment alone reduced decay caused by Botrytis *cinerea* (Pers.:Fr.) by ≈30%, while heat in combination with a 2% $CaCl_2$ solution reduced decay by ≈60 %. Calcium chloride solutions of 2% or 4% alone reduced decay by 40 % and 60 %, respectively. Heat treatments, either alone or in combination with $CaCl_2$ treatments, maintained firmness (80 N) best, followed by fruit infiltrated with 2% or 4% solutions of $CaCl_2$ alone (70 N) and the nontreated controls (66 N). Instron Magness-Taylor and Instron compression test curves show that heat-treated fruit differed qualitatively and quantitatively from nonheated fruit. Heat treatment did not increase the amount of infiltrated Ca bound to the cell wall significantly, and a combination of heat treatment after $CaCl_2$ infiltration increased surface injury over those fruit heated or infiltrated with $CaCl_2$ solutions alone.**

The use of synthetic chemicals to control plant diseases, especially for postharvest treatments, is becoming more restricted. Prestorage heat treatment of apples has been shown to affect fruit quality during storage beneficially. In 'Spartan' and 'Golden Delicious' apples exposed to 38C for 4 to 6 days and stored subsequently at –1C for 4 to 7 months (Porritt and Lidster, 1978), fruit softening was suppressed and naturally occurring decay, largely due to *Corticium* and *Penicillium* sp., was reduced. Similarly, exposure at 40C for 2 to 4 days maintained firmness of stored 'Golden Delicious' fruit (Liu, 1978). Recently, Klein et al (1990) found that, for 'Anna' or 'Granny Smith' fruit, the best heat treatment was exposure to 38C for 4 days, and that a $CaCl_2$ dip after the heat treatment augmented the effect of the heat treatment appreciably. In further work with 'Anna', Ca content generally did not increase as much from a dip following heat treatment as from a dip of nonheated apples (Lurie and Klein, 1992).

Increasing Ca in tissue of apple fruit alone also maintains firmness and reduces decay caused by *Penicillium expansum* Link, *Botrytis cinerea,* and *Glomerella cingulata* (Stoneman) Spauld. & Schrenk (Conway and Sams, 1983; Sams and Conway, 1984; Conway et al., 1991).

In a recent study (Sams et al., 1993), 'Golden Delicious' apples were heat-treated at 38C for 4 days and then infiltrated with *4%* $CaCl_2$ solutions before storage for 6 months at 0C. Heat treatment and Ca infiltration maintained fruit firmness and reduced decay induced by inoculation with *P. expansum* after storage. However, heat treatment affected the surface of the fruit so that there was a smaller increase in Ca in heated than in nonheat-treated fruit. Heat treatment, however, caused no injury.

There is increasing evidence that polyamides maintain postharvest quality in fruits and vegetables. Temperature preconditioning inhibits the development of chilling injury with corresponding increases in polyamine levels in zucchini squash *(Cucurbita pepo* L.) (Kramer and Wang, 1989). Pressure infiltration of 'Golden Delicious' and 'McIntosh' apples with polyamides resulted in an immediate increase in firmness (Kramer et al., 1991). The effect of heat on polyamine levels is not known.

The objectives of our investigation were to determine the effect of infiltration of $CaCl_2$ solutions into fruit before prestorage heat treatment on decay caused by the important postharvest pathogen *Botrytis cinerea* **on** firmness, cell wall-bound Ca, polyamine levels, and fruit injury.

Materials and Methods

'Golden Delicious' apples were harvested in the preclimacteric stage (ethylene production was <0.1 μl·liter^{-1} and the climacteric rise in the CO_2 level had not yet begun) from a commercial orchard and randomized. The apples were then treated in the following manner: 1) control (no treatment), 2) heat (4 days, 38C), 3) pressure infiltration of distilled water (3 rein, 103 kPa), 4) pressure infiltration with distilled water before heat treatment, 5) pressure infiltration with a 2% solution of $CaCl_2$ (w/v, $CaCl_2 \cdot 2H_2O$), 6) pressure infiltration of a 2% $CaCl_2$ solution before heat treatment,

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7) pressure infiltration of a 4% solution of $CaC1_2$, and 8) pressure infiltration of a 4% solution of $CaC1_2$ before heat treatment. Fruit that were heat-treated were placed in tray-packed boxes with perforated polyethylene bags as liners prior to treatment. All fruit were stored at 0C following treatment. Apples were removed from storage after 6 months and tested for disease resistance, firmness, total Ca content, cell wall-bound Ca content, and polyamine content. The experiment was repeated.

Disease resistance. Twenty fruit from each treatment were wounded on two sides to a depth of 2 mm by pressing them down on the head of a nail 2 mm in diameter. The fruit were then immersed for 15 s in a conidial suspension (105 spores/ml) of *B. cinerea.* The area of decay was calculated from the means of the diameter of the width and length of lesions after 5 days at 20C.

Firmness measurements. Fruit were placed at 20C overnight. One tray of apples (20 fruit) from each replication of each treatment was measured with a manually controlled digital pressure tester (EPT-1; Lake City Technical Products, Kelowna, B.C., Canada). A second tray of similarly treated fruit was measured with a computer-controlled Instron (Canton, Mass.).

The EPT-1 was set in the Magness–Taylor (MT) mode and interfaced to a personal computer. Firmness (bioyield force) was measured at two opposite points on the equator of each fruit after removal of a 2-mm slice with a fixed-blade slicer.

Instron firmness measurements were made on two opposite manually pared surfaces using a standard 11.l-mm Magness–Taylor probe mounted on an Instron Universal Testing Machine interfaced with a personal computer to record force deformation curves (Abbott et al., 1982, 1984). Crosshead speed was 127 $mm.min^{-1}$. Apples were measured immediately after storage at 0C in order to minimize changes during the time required for the Instron-MT measurements. The probe was programmed to travel 7.94-mm after contact with the flesh, regardless of the depth of actual penetration. Firm apples will probably be penetrated to the full 7.94 mm, but dehydrated or spongy apples maybe deformed somewhat before actual penetration so that the probe does not penetrate the full amount. Complete force/deformation curves were recorded by the computer and analyzed later for maximum forces and other data (Abbott et al., 1984).

Compression tests. Immediately after the above measurements, two radial cylinders of apple flesh were removed at 90° from the MT-I sites using a 15-mm-diameter cork borer. A 2.5-mm slice of the cylinder, including the skin, was discarded and the next 10-mm segment was tested by compressing between flat plates at 127 $mm{\cdot}min^{-1}$. The Instron was programmed to detect contact with the specimen, travel 7.5 mm, reverse, and continue to collect force/deformation data for another 2.5 mm (Abbott et al., 1982, 1984).

Total calcium content. A 2-mm layer of the peel and outer flesh of each fruit was removed with a mechanical peeler and discarded. The next 2 mm was cut with the peeler from four apples, and three such samples from each treatment were divided into two parts, one for total Ca analysis and the other for cell wall-bound Ca analysis. A small sample (2 g) of fresh tissue from each treatment was also used to determine total polyamine content. For total Ca analysis, the tissue was frozen immediately in liquid N_2, freeze-dried, and ground. Dried material (1 g) was ashed, dissolved in 5 ml of 2 N hydrochloric acid, and filtered. The samples were then analyzed for Ca content by plasma emission spectrometry.

Cell wall-bound calcium. About 100 g of apple tissue was homogenized in 100 ml of cold 80% ethanol in a Waring blender and filtered through two layers of Miracloth (Calbiochem, La Jolla, Calif.). The residue was washed with 50 ml of 80% ethanol and used for cell wall extraction following a modification of the procedure of Tong and Gross (1990). The residue was extracted in sequence in 100 ml of 80% ethanol at 80C for 5 tin, rinsed in water, suspended in 200 ml 20 mM Hepes buffer, pH 7.0, homogenized (Polytron, Brinkmann Instruments, Westbury, N. Y.) for 1 rein, and filtered through Miracloth at each stage. The residue was suspended in 100 ml of Hepes buffer in a pressure bomb at 14.1 MPa for 10 min and then stirred for 60 min with 100 ml phenol/Tris, pH 7.5 (Huber, 1991), 10 min with 200 ml 1 chloroform: 1 methanol (v/v), and 10 min with acetone with filtration through sintered glass at each stage. The wall material was air-dried overnight and dried *in vacuo* over P_2O_5 at *40C* for 48 h. Cell wall Ca was then determined using the same procedures as for total Ca.

Polyamine analysis. Polyamides in fresh apple tissue and in cell walls were determined by dansylation and HPLC separation according to the method of Kramer et al. (1989). Polyamides in the cell walls were analyzed after hydrolysis of 20 mg of cell wall material with 2 ml of 2 N trifluoroacetic acid at 100C for 2 h. The. extracts were evaporated to dryness under N_2 and redissolved in 5% $HClO_4$ before analysis.

Injury rating. Forty fruit per treatment were rated visually by two observers for Ca injury at the time of the firmness and disease-severity ratings. The fruit were assigned a value of between 1 (severe injury) and 5 (no injury).

The experimental design was a completely randomized two-factor arrangement (two prestorage heat treatments × four infiltration treatments × three replications), with heat treatments being 0 or 4 days at 38C and infiltration treatments being none, distilled water (0% $CaC1_2$), and 2% or *4%* $CaC1_2$. The effect of water infiltration vs. no infiltration was tested by contrasting treatments 1 and 2 against 3 and 4. The effects of $CaC1_2$ were tested by regression analysis of decay, MT firmness, tissue Ca_2 or cell-wall Ca vs. Ca levels in treatments 3,5, and 7 or 4,6, and 8.

Results

Decay. Heat-treated fruit (treatments 2 and 4) had 30% less decay area due to *B. cinerea* than did the control (treatment l)(Fig. 1). Water infiltration (treatment 3) had no effect on decay, but infiltration of a 2% solution of $CaC1_2$ (treatment 5) resulted in

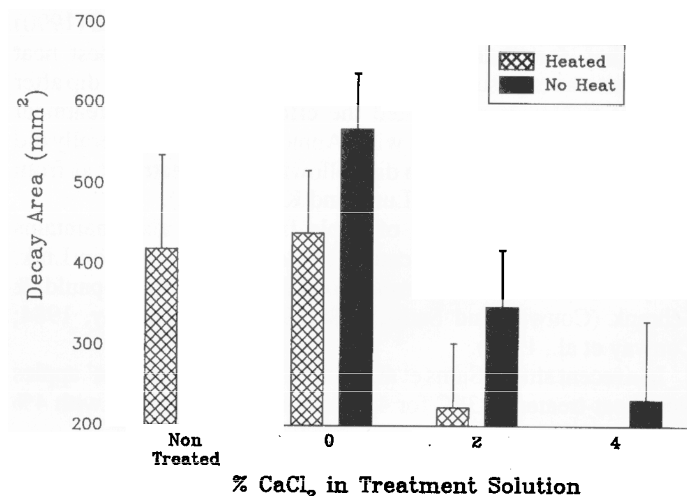

Fig. 1. Relationship between treatment and area of decay on 'Golden Delicious' apples. Fruit were either nontreated or pressure-infiltrated (3 min; 103 kPa) with 0%, 2%, or 4% solutions of calcium chloride ($CaCl_2$ and then placed immediately at 0C or heat-treated at 38C for 4 days before storage at 0C. Area of decay was determined by inoculating fruit after 6 months storage at 0C and measuring decay following 5 days at 20C. Vertical bars represent SE of means.

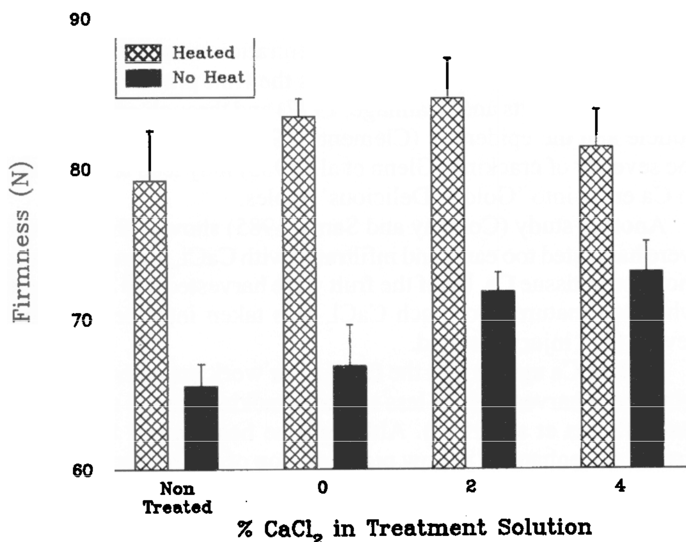

Fig. 2. Relationship between firmness and treatment of 'Golden Delicious' apples. Fruit were either nontreated or pressure-infiltrated(3 min; 103 kPa) with 0%,2%, or 4% solutions of calcium chloride ($CaCl_2$ and then placed immediately at 0C or heat-treated at 38C for 4 days before storage at 0C. Fruit firmness was determined after 6 months storage at 0C. Vertical bars represent SE of means.

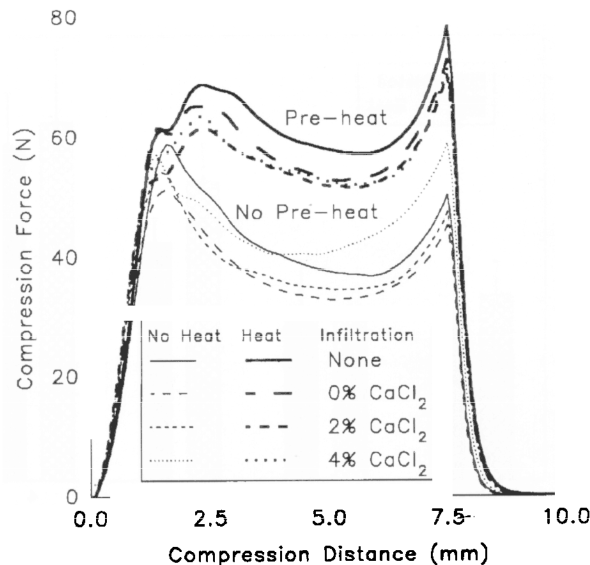

Fig. 4. Compression test curves for 'Golden Delicious' apples. Fruit were either nontreated or pressure-infiltrated (3 min; 103 kPa) with 0%, 2%, or 4% solutions of calcium chloride ($CaCl_2$) and then placed immediately at 0C or heat-treated at 38C for 4 days before storage at 0C. Measurements were determined after 6 months storage at 0C. Each curve represents the mean of 120 compression tests (three replications × 20 apples × two sides).

>40% less decay than in the control, and subsequent heat treatment (treatment 6) resulted in 60% less decay. Infiltration with 4% $CaCl_2$ (treatment 7) also led to 60% less decay than in the control, but subsequent heat treatment (treatment 8) caused injury; this fruit was not tested for decay resistance.

Firmness. Fruit infiltrated with water (treatment 3) had the same EPT-1 firmness as the control fruit (treatment 1) (Fig. 2). Fruit infiltrated with either 2% $CaCl_2$ (treatment 5) or 4% $CaCl_2$ (treatment 7) was slightly firmer than the control. The heated fruit (treatments 2 and 4) were significantly freer, but infiltration with a 2% (treatment 6) or 4% (treatment 8) solution of $CaC1_2$ before heat treatment had no effect on firmness.

These effects were confirmed in Instron-MT firmness profiles (Fig. 3). All treatments that included preheating (treatments 2,4, 6, and 8) were firmer than those that did not. Fruit treated with 2% or 4% $CaCl_2$ without heat (treatments 5 and 7) appeared as firm as the preheated fruit on the basis of maximum force, but showed a decline after initial failure, which was not seen in the other treatments.

In Instron compression tests (Fig. 4), preheated fruit had higher maximum force values than nonheated fruit. A second peak force following the rupture peak was seen only in the heat-treated apples, and could result in increased toughness, rather than increased firmness or crispness; but, sensory tests have not been done to confirm this hypothesis.

Color observation. Although heat-treated fruit were firmer,

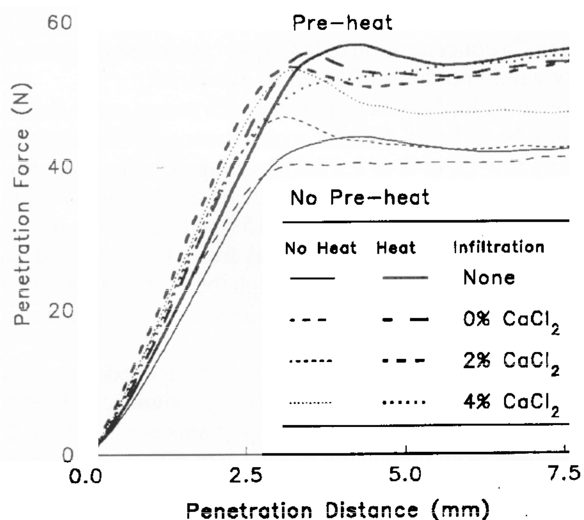

Fig. 3. Instron Magness–Taylor curves for 'Golden Delicious' apples. Fruit were either nontreated or pressure-infiltrated (3 min; 103 kPa) with 0%, 2%, or 4% solutions of calcium chloride ($CaCl_2$) and then placed immediately at 0C or heat-treated at 38C for 4 days before storage at 0C. Measurements were determined after 6 months storage at 0C. Each curve represents the mean of 120 Instron Magness–Taylor measurements (three replications × 20 apples × two sides).

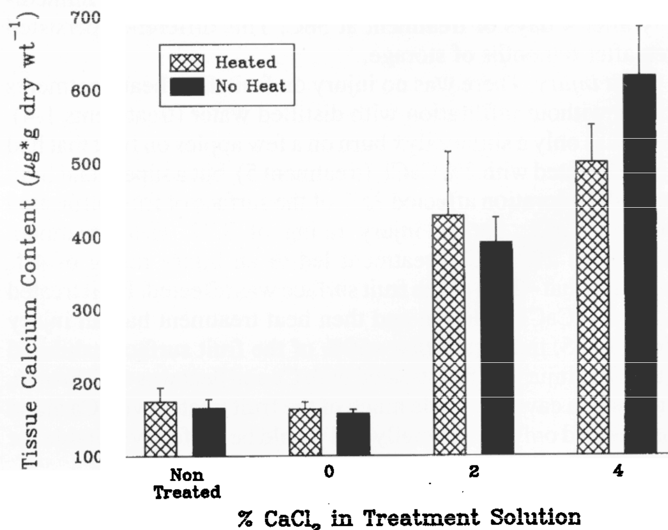

Fig. 5. Relationship between treatment and total tissue calcium content of 'Golden Delicious' apples. Fruit were either nontreated or pressure-infiltrated (3 min; 103 kPa) with 0%, 2%, or 4% solutions of calcium chloride ($CaCI_2$ and then placed immediately at 0C or heat-treated at 38C for 4 days before storage at 0C. Total tissue Ca content was determined after 6 months storage at 0C. Vertical bars represent SE of means.

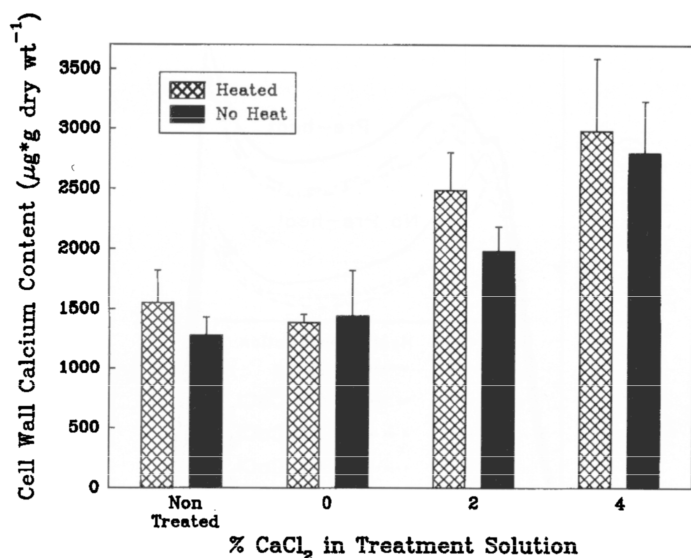

Fig. 6. Relationship between treatment and cell wall calcium content of 'Golden Delicious' apples. Fruit were either nontreated or pressure-infiltrated (3 min; 103 kPa) with 0%, 2%, or 4% solutions of calcium chloride ($CaCl_2$) and then placed immediately at 0C or heat-treated at 38C for 4 days before storage at 0C. Cell wall Ca content was determined after 6 months storage at 0C. Vertical bars represent SE of means.

visually determined background color change from green to yellow was enhanced.

Calcium content. As expected, Infiltration with a 2% or 4% $CaC1_2$ solution resulted in fruit with a higher Ca concentration (treatments 5-8) than in noninfiltrated fruit (treatments 1-4) (Fig. 5).

Cell wall-bound Ca also increased as the concentration of $CaCl_2$ in the treatment solution increased (Fig. 6). Heat treatment, however, did not increase the amount of Ca associated with the cell wall.

Polyamine content. The spermidine or spermine concentrations in fresh apple tissue and in the cell wall were similar for heated and nonheated fruit (data not shown). However, putrescine levels in the fresh tissue were higher in the nonheated (16.7 nmol·g^{-1} fresh weight) than in heated (5.1 nmol·g^{-1} fresh weight) fruit immediately after 4 days of treatment at 38C. This difference persisted even after 6 months of storage.

Fruit injury. There was no injury on fruit after heat treatments with or without infiltration with distilled water (treatments 1-4). There was only a slight calyx burn on a few apples on fruit that had been infiltrated with 2% $CaC1_2$ (treatment 5), but a superificial light brown discoloration affected 25% of the surface of fruit infiltrated with 4% $CaCl_2$, for an injury rating of 3.77. Heat treatment following a 2% $CaCl_2$ treatment led to an injury rating of 4.1, indicating that ≈20% of the fruit surface was affected. Fruit treated with a 4% $CaCl_2$ solution and then heat treatment had an injury rating of 2.5, indicating that ≈50% of the fruit surface exhibited injury. The injury on fruit treated with Ca and heat was dark brown, with sunken cavities, while much of the fruit treated with Ca alone was affected only superficially and would be perfectly suitable for processing.

Discussion

The results show that heat treatment before storage maintains fruit firmness and reduces decay caused by *B. cinerea* by ≈30%, which is similar to the decay reduction observed with *P. expansum* in earlier work (Sams et al., 1993). Calcium chloride solution infiltration had only a slight effect on maintaining firmness, presumably because Ca tissue concentration was not increased sufficiently. Calcium probably enters the fruit primarily through the lenticels (Betts and Bramlage, 1977) and through cracks in the cuticle and the epidermis (Clements, 1935). Annual variation in the severity of cracking (Glenn et al., 1985) may lead to variation in Ca entry into 'Golden Delicious' apples.

Another study (Conway and Sams, 1985) showed that, if fruit were harvested too early and infiltrated with $CaCl_2$, there was little increase in tissue Ca, but if the fruit were harvested and infiltrated when too mature, too much $CaC1_2$ was taken into the fruit and severe fruit injury resulted.

Lack of Ca uptake into the fruit in our work may have resulted from early harvest and/or less surface cracking than in a previous study (Sams et al., 1993). Although the heat-treated fruit were firmer, the enhanced yellow pigmentation of the peel in the heat-treated fruit compared to the controls gave the impression that the heated fruit were riper, a condition observed-in a previous study (Lurie and Klein, 1992).

The firmness profile comparing heat-treated and nontreated fruit indicates that the heat-treated fruit are firmer, but in a manner that makes them texturally "harder" or "tougher" and less crisp than nontreated fruit. The texture profile curves illustrate the need for measuring more than just Magness–Taylor maximum force in doing postharvest quality studies on fruit.

The mechanism by which increased tissue Ca reduces decay and maintains firmness is hypothesized to be related to Ca ions in the cell wall (Demarty et al., 1984). Cell wall pectins are composed primarily of four-linked galacturonosyl residues, with varying amounts of two-linked rhamnosyl residues interspersed in the chain (Preston, 1979). The stability of the cell wall maybe related to the cooperative binding of polygalacturonate chains with Ca ions (Grant et al., 1973; Knee, 1978), making the cell wall of the fruit less accessible to enzymes that cause softening or to cell wall-degrading enzymes produced by fungal pathogens. Increasing the Ca content of apple cell walls has been shown to inhibit maceration by polygalacturonase produced by *P. expansum* (Conway et al., 1988). To reduce apple fruit decay caused by *P. expansum* by more than 50%, fruit tissue Ca concentration must be increased to 1200-1500 μg Ca/g dry weight, but lower concentrations may inhibit progress of *B. cinerea* (Conway et al., 1991). In this study, an increase in Ca concentration to 600 μg·g^{-1} inhibited *B. cinerea,* but probably would not have affected *P. expansum.*

The relationship between maintained firmness and resistance to decay may be indirect. Similar maintenance of firmness can be attained by low O_2 (1% to 1.5% O_2) storage (Anderson, 1967) or Ca treatment, but the area of decay caused by *P. expansum* in low-O_2- stored fruit was reduced by only 15%, while Ca-treated apples had 50% less decay than nontreated fruit (Conway and Sams, 1984). Similarly, in this study, although the heat treated fruit were slightly firmer, the area of decay was less in the Ca- than the heat-treated fruit.

While the optimum amount of Ca in the fruit needed to decrease decay and maintain firmness is ≈ 1200 μg·g^{-1}, significantly exceeding this amount resulted in fruit injury (Sams et al., 1993). Little injury resulted from increasing tissue Ca to 600 μg·g^{-1}, but, when this increase is followed by heat treatment, extensive injury developed. Heat treatment following Ca infiltration, then, results in fruit that are much more easily injured by Ca and at a Ca concentration much lower than would affect nonheat-treated fruit.

The mechanism by which heat treatment reduces decay and maintains firmness through effects on the cell wall has not been explained satisfactorily. Heat inactivation of synthesis of pectin-

degrading enzymes was considered a possible mechanism, and analysis of 'Golden Delicious' fruit following storage showed significantly lower levels of soluble pectin in juice of heat-treated fruit than controls (Porritt and Lidster, 1978). In 'Spartan' apples, however, differences in soluble pectin levels were not significant, even though both cultivars were firmer as a result of heat treatment. Klein and Lurie (1990) showed that, during shelf life, the insoluble pectin fraction remained larger in heat-treated than in nonheated apples, and that the water-soluble and Ca pectate fractions did not increase as much.

Heat treatment may inhibit protein synthesis required for cell wall degradation and ethylene synthesis (Lurie and Klein, 1990). In a study comparing the effects of heat and Ca on firmness, heat treatment for 4 days at 38C before storage had the greatest effect on firmness (Klein et al., 1990). The Ca treatment was applied by dipping in a 2% $CaCl_2$ solution at 38C, and may not have increased Ca concentration of the cortex enough to influence firmness. If the Ca concentration is increased above the 1200 $\mu g.g^{-1}$ range, Ca maintained firmness better than heat treatment (Sams et al. 1993).

The heat treatment had little effect on polyamine levels in either tissue or cell walls with the exception of putrescine, The higher putrescine concentrations in the nonheated than in the heated fruit tissue immediately after heat treatment and following 6 months of storage at 0C were probably a result of low-temperature stress. The heated fruit were placed at 38C prior to storage, so may not have been affected by this stress that fruit placed immediately into 0C storage experienced, or heat treatment inhibited putrescine accumulation.

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