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THE INFLUENCE OF POSTHARVEST CALCIUM CHLORIDE TREATMENTS ON APPLE (*Malus domestica* Borkh) FRUIT QUALITY AND POLYPHENOL OXIDASE, PEROXIDASE, AND PECTIN METHYL ESTERASE ACTIVITIES

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ABSTRACT

The purpose of this study was to determine the following: (1) optimum postharvest, pressure infiltrated calcium chloride ($CaCl_2$) treatments for fresh and processed 'Golden Delicious' and 'Delicious' apple (Malus domestica Borkh.) fruit quality, and (2) to examine the effect of increased calcium concentrations on enzymes associated with softening (pectin methyl esterase), browning (polyphenol oxidase), and senescence (peroxidase). Apple tissue calcium concentrations were determined and fresh and processed fruit qualities were evaluated at harvest and after three and six months in storage at 1°C. PPO and POD activities were determined at harvest and after three and six months in storage at 1°C. PME activity was measured at harvest and after two, four, and six months in storage at 1°C.

Firmness, ascorbic acid content (mg·100g fresh wt^{-1}), and total phenols ($\mu g \cdot g$ fresh wt^{-1}) were maintained at levels comparable to initial values as calcium concentrations increased for both testing intervals. Soluble solids (%) and titratable acidity (% malic acid) were not significantly affected by any calcium treatment for either cultivar or storage interval.

Sauce made from apples with calcium concentrations of 2000 $\mu g \cdot g$ dry wt^{-1} for 'Golden Delicious' and 1200 $\mu g \cdot g$ dry wt^{-1} for 'Delicious' tended to flow less (lower consistency values) and have less free liquid. Sauce made from 'Golden Delicious' after six months of storage did not meet USDA requirements for sauce grades probably because the

apples were in an advanced stage of senescence. A decrease in luminosity (lower 'L' Hunter Colorimeter values) was observed in applesauce made from calcium treated apples at both time intervals. Apple juice produced from calcium-treated fruit reflected a lighter colored juice at both time intervals as expressed by higher 'L' values.

PPO activity, ($\blacktriangle$OD) min^{-1} mg $protein^{-1}$, decreased as calcium concentrations increased at both testing intervals. PPO activity, after six months in storage, was higher than that at three months. POD activity, (also measured as $\blacktriangle OD \cdot min^{-1} \cdot mg\ protein^{-1}$) increased as calcium concentration was increased at both time intervals, and activity in both cultivars was higher after six months than after three months in storage. PME activity (meq $H^{+} \cdot min^{-1} \cdot mg\ protein^{-1}$) decreased as calcium concentrations were increased at all three testing intervals and reached highest activity after approximately four months in storage.

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INTRODUCTION

Profitable apple marketing includes the maintenance of eating and/or processing quality until sales can be made at the highest price in light of production, harvesting, and storage costs. Calcium is presently used in the apple industry to control bitter pit (Drake et al., 1966; Bangerth et al., 1972; Shear, 1975 and Bangerth, 1979), to inhibit fungal invasion (Sharples and Johnson, 1977 and Conway and Sams, 1983), and to retard senescence during storage (Bangerth et. al., 1972; Mason et al., 1975; Scott and Wills, 1977; and Sams and Conway, 1984), thereby extending storage life.

Postharvest storage life of apples stored in 0°C storage is limited to approximately six months if controlled atmosphere (CA) storage is not available (Lutz and Hardenburg, 1968; Massey, 1989). In South Africa, Australia, and New Zealand, postharvest calcium treatments are presently being used to extend apple storage life during storage and transport (Scott and Wills, 1979). Implementation of a postharvest calcium treatment may extend storage life not only for fresh market apples but also for apples held for processing. The effect of a 3% $CaCl_2$ (w/v) postharvest pressure infiltration treatment on fresh market and processing qualities was investigated by Drake and Spayd (1983). Fresh market quality of $CaCl_2$ treated apples was superior to the untreated apples after five months in storage. Processed product quality of $CaCl_2$ treated apples was better than CA or cold stored untreated apples after five months in storage.

The thesis that follows consists of three sections. Section one

will consist of a literature review. Sections two and three are prepared in a format suitable for submission to the Journal of the American Society for Horticultural Science. Section two is a report of the effect of calcium concentration on fresh fruit, saucing, and juicing quality. The effect of calcium concentration on the activity of enzymes that affect fresh fruit and processing quality is examined in section three. The objectives of these investigations were as follows: (1) to determine an optimum calcium treatment and concentration within apple tissue for maintenance of quality in fresh and processed apple products, and (2) to examine the effect of increased calcium concentrations on enzymes associated with softening (pectin methyl esterase), browning (polyphenol oxidase), and senescence and degradation after processing (peroxidase).

I. LITERATURE REVIEW

Calcium Research

In recent years, calcium research has progressed beyond the role of calcium as a macronutrient. Calcium has been reported to act as a major metabolic and developmental controller involved in the mediation of various physiological processes in plants (Hepler and Wayne, 1985; Poovaiah, 1985). Among the physiological processes influenced or mediated by calcium are the following: senescence and ripening (Poovaiah, 1979), physiological disorders (Bangerth, 1979, and Hopfinger and Poovaiah, 1979), plant growth and hormone action (Thimann and Schneider, 1938; Dela Fuente and Leopold, 1973; Cohen and Nadler, 1976) and membrane structure and function (Morre and Bracker, 1976). A complete and thorough discussion of calcium involvement in these and other processes can be obtained through recent reviews (Hepler and Wayne, 1985; Poovaiah, 1985; Poovaiah and Reddy, 1987).

Calcium and Cell Wall Integrity

The importance of calcium in cell-to-cell cohesion has been well recognized, and the cementing effect is primarily due to calcium pectate in the middle lamella (Demarty et al., 1984; Ferguson, 1984; Poovaiah and Reddy, 1987). The major function of calcium in cell wall structure is to link pectic polymers that consist of galacturan chains in which the carboxyl groups located on the side chains are linked

together via calcium ions (Ferguson, 1984). The 'egg-box' model of the polygalacturan chains describes how calcium forms bridges between the polygalacturan chains, resulting in a pectin-like cement (Grant et al., 1973). The ability to form gels is the most characteristic physical property of these polymers (Grant et al., 1973). The degree of calcium bonding is dependent on the degree of methyl esterification (Ferguson, 1984), that ultimately determines cell wall rigidity (Evans, 1985; Demarty et al.,1984; Dey and Brinson, 1984). If side chains are highly esterified, then calcium is unable to form covalent linkages between pectic polymers (Jarvis, 1984). The lack of these linkages results in a less rigid cell wall.

Pectins are synthesized in the fully esterified form and are soluble and readily transported (Jarvis, 1984). They are then transported to the middle lamella and de-esterified. This de-esterification allows free calcium ions to form linkages between polysaccharide side chains which results in a more rigid cell wall and a less soluble pectic gel.

Most of the calcium introduced into fruit tissues accumulates in the middle lamella region, and it is there that calcium is thought to have antisenescent effects (Ferguson et al., 1980, Ferguson, 1984). A time course study used to examine the changes in cell ultrastructure in apples with differing calcium concentrations revealed greatest structural differences after 99 days in storage (Fuller, 1976). Electron micrographs show intact plasma membranes, little leakage of cytoplasm, and relatively intact functional cells in fruit with higher calcium content (7.7 to 6.4 mg·100g fresh wt^{-1}). Separation of plasma membranes

from cell walls, solute leakage, and disorganization of cell walls was characteristic of low calcium (3.1 to 3.7 mg·100g fresh wt^{-1}) fruit (Fuller, 1980). Electron microscopy demonstrated extreme dissolution of the middle lamella when apple tissue was treated with polygalacturonase (PG), produced from a fungal source (Ben-Arie and Kislev, 1979).

The dissolution of the middle lamella is caused by the depolymerization or metabolic turnover of the pectic fraction. Pectic depolymerization or degradation is the cleavage of polysaccharide chains by enzymatic action. The cleavage can occur either within the chain (endo) or at the ends (exo). Presently, rates of metabolic turnover in relation to depolymerization have not been determined, but it is hypothesized that most fruits soften due to pectic depolymerization, with resynthesis and metabolic turnover limited (Dey and Brinson, 1984; Labavitch, 1981; and Huber, 1983). Both processes have been studied extensively in tomatoes by Wallner and Bloom (1977), Gross and Wallner (1979), and Themmen et al., (1982). Pectin solubilization during ripening due to depolymerizing enzymes appears to be regulated by calcium in the middle lamella and cell wall (Buescher and Hobson, 1982, and Powell et al., 1982). Concentrations of 5 mM $CaCl_2$ significantly inhibited PG activity in tomatoes during ripening (Buescher and Hobson, 1982). Knee (1978) measured the incorporation of methyl-labeled ^{14}C-methionine and ^{3}H-inositol into apple tissue pectic fractions, suggesting a metabolic turnover of pectins. Knee and Bartley (1981) further suggested that tissue softening was initiated by methylation of pectin in the middle lamella. Esterified carboxyl groups are unable to

participate in divalent cation-stabilized junctions and cell separation and subsequent pectin solubilization occurs.

Metabolic turnover associated with fruit ripening is the continual resynthesis of soluble pectic polysaccharides. Knee et al. (1977) reported the synthesis of new cell wall polysaccharides, using ^{14}C labeled glucose, CO_2, and proline in developing strawberry fruits. They further reported that 70% of the polyuronides in the cell wall and middle lamella became solubilized upon ripening and attributed this solubilization to hydration of the polysaccharide.

Postharvest Calcium Treatments in Apples

Many physiological disorders are caused by low calcium concentrations within apples (Delong, 1936; Drake et al., 1966; Bangerth et al., 1972; and Shear, 1975). High concentrations of calcium in the flesh can prevent the occurrence of bitter pit, whereas, low concentrations predispose the fruit to this postharvest storage disorder (Shear 1975; Ferguson et al., 1979; Mason, 1979).

Postharvest diseases decrease in intensity when a postharvest calcium treatment is implemented (Sams and Conway, 1985; Sams and Conway, 1987). Apple tissue pressure infiltrated with a 4% $CaCl_2$ solution as a postharvest treatment was 29 to 47% less susceptible to decay caused by Penicillium expansum Lk. ex Thom.

Apart from the preventative action of calcium on the occurrence of specific senescent disorders in apples, there is evidence for an effect in retarding the rate of fruit ripening. This phenomenon is manifes-

ted in a general delay of the respiratory climacteric (decreased CO_2 and ethylene production) and maintenance of fruit firmness (Ferguson, 1984). Treatment of apple tissue and whole fruits with calcium sprays, dips, and various infiltration processes resulted in lower rates of CO_2 and/or ethylene production (Bangerth et al., 1972; Liebermann and Wang, 1982; Ferguson, 1983; Sams and Conway 1984; Conway and Sams, 1987).

Enzymatic Softening of Fruits

Softening is an integral part of ripening of most fruits. Such softening has extensive commercial importance because the postharvest life of the fruit is limited to a large extent by an increase in softness, bringing with it a decrease in physical integrity and an increase in disease susceptibility.

Softening is normally accompanied by an increase in soluble pectin (Bartley and Knee, 1982; Huber, 1983) and is often correlated with an increase in pectolytic enzyme activity. Polygalacturonase activity was correlated with the rate of tissue softening in tomatoes (Brady et al., 1982; Brady et al., 1985) avocadoes, (Awad and Young, 1979) and pears (Ben-Arie and Kislev, 1979). In apples pectin solubility increased but endo-polygalacturonase activity was not detected (Liang et al., 1982). However, pectin methyl esterase and B-galactosidase (other pectin degrading enzymes) have been reported (Wallner and Bloom, 1977).

The role of calcium as a possible inhibitor of these enzymatic reactions is not known, but there is evidence that calcium limits cell wall hydrolysis (Brady et al., 1985; Buescher and Hobson, 1982). The

appearance of a 70 Kdal cell wall polypeptide was delayed three months in calcium-treated fruits (Glenn et al., 1988). Calcium has also been shown to promote the activity of β-glucan synthase, a key enzyme involved in cell wall synthesis (Poovaiah and Reddy, 1987).

Effect of Calcium on Fruit Quality and Processing of Apples

Calcium chloride vacuum infiltration treatments on peeled apple slices were effective in firming fresh, canned, and frozen products (Hills et al. 1947; Collins and Wiley, 1967). Calcium treatments have also been reported to improve apple slices prepared for pie making without affecting flavor (Kertesz, 1947; Stirtin and Hills, 1950).

The use of externally applied $CaCl_2$ to whole fruits has been shown to help overcome deterioration and maintain firmness of apples in storage (Mason et al., 1975; Poovaiah et al., 1978; Hardenburg and Anderson, 1981; Drake and Spayd, 1983; Hopfinger et al., 1984; Conway and Sams, 1987). $CaCl_2$ treatment to improve quality usually involves one of the following methods: dips alone (Betts and Bramlage, 1977), dip in a pressurized vessel that forces calcium into the apples (Scott and Wills, 1975; Poovaiah et al., 1978; and Conway and Sams, 1987), and vacuum infiltration (Scott and Wills, 1975; Poovaiah et al., 1978). All of these treatments have proven successful at increasing calcium concentrations within apple tissue, however, pressure infiltration is more effective at getting calcium into the tissues (Scott and Wills, 1977; Scott and Wills, 1979). Vacuum and pressure infiltration treatments were similar in their control of bitterpit during storage,

and both were superior to dipping (Scott and Wills, 1979). Pressure infiltrated 'Golden Delicious' and 'Lord Lambourne' apples were firmer and stored better than apples treated with a $CaCl_2$ dip (Drake and Fridlund, 1986).

Fresh market and processing parameters measured five months after a 3% $CaCl_2$ pressure infiltration treatment revealed calcium treated apples maintained firmness whereas the control fruit softened. Applesauce made from $CaCl_2$ treated apples after five months in storage was more yellow than applesauce made from controlled atmosphere (CA) or cold stored apples. Consistency and syneresis values of applesauce made from $CaCl_2$ treated apples, after five months in storage, were lower than applesauce made from CA stored apples (Drake and Spayd, 1983).

Enzymes That Affect Fruit Quality and Processing

Three of the major enzymes that affect fruit quality and pre- and post-processing qualities of apples are polyphenol oxidase (PPO), peroxidase, and pectin methyl esterase (PME). PPO is involved in the tissue browning reaction (Vamos-Vigyazo, 1981). Peroxidase is involved in deteriorative changes in processed fruit flavor, texture, and color (Blundstone et al., 1971; Haard, 1977). Reportedly, PME is involved in cell wall degradation associated with fruit softening at harvest and during storage (McColloch and Kertesz, 1946; Pollard and Kieser, 1951; Lee and Wiley, 1970).

Pectin methyl esterase (E.C. 3.1.1.11):

PME demethylates methyl ester groups of the galacturonic residues off the polygalacturonic acid component of pectin, thereby producing a carboxyl group that may form linkages with free calcium ions (Goodwin and Mercer, 1983). Bartley and Knee (1982) suggested that increasing divalent cations (i.e Ca^{+2}) could increase the crosslinkages between the nonesterified carboxyl groups, thus limiting enzymatic degradation.

PME occurs commonly in various parts of higher plants, including the fruit. Tomatoes, in particular, are a rich source of the enzyme (Kertesz, 1938). The activity of PME varies during ripening (Kertesz, 1938; Hamson, 1952; Hall and Dennison, 1960; Hobson, 1963). The number of isozymes of PME and their relative levels in tomatoes, vary among cultivars and stages of ripeness (Pressey 1977). For example, the heterogeneity of PME has been demonstrated by thin-layer isoelectric focusing in which at least eight isozymes were detected (Delincee, 1976).

The enzyme systems involved in apple softening and overall textural changes are still uncertain. Bartley (1978) has reported the occurrence of an exo-PG in apples; however, no further reports of endo- or exo-type PG's in apples have been cited in the literature. Lee and Wiley (1970) measured PME activity by gas liquid chromatography (GLC) and have shown PME activity to increase in storage up to 60 days and then leveled off. This leveling off of PME activity was attributed to the accumulation of products or limitations in substrate level, which may have an inhibiting effect on enzyme activity. Lee (1969) has shown PME activity to be approximately two times higher in 'Golden Deli-

cious', apples than in 'York Imperial', a firmer cultivar.

PME activity in other fruits is variable. PME activity decreased during ripening in avocadoes (Zauberman and Schiffmann-Nadel, 1972) and increased tenfold in bananas during ripening (Hultin and Levine, 1965).

PME activation was enhanced by addition of divalent cations (one of which was Ca^{+2}) (MacDonnel et al., 1945); however, activation was in the extracting media only and not associated with native calcium levels in the tissue. The optimum pH range for PME activity in most plants is 6.0 to 7.0 (Whitaker, 1972). The optimum pH of 6.6 for apple PME activity was shown by Pollard and Kieser (1951). The molecular weight of apple PME was determined by Miyairi et al. (1975) and found to be 27,000 by gel filtration on Sephadex G-100 and 28,000 using polyacrylamide gel electrophoresis (PAGE).

Polyphenol oxidase (E.C. 1.14.18.1):

Polyphenol oxidase (PPO) is also known as tyrosinase, polyphenolase, catechol oxidase, cresolase, and catecholase. Scott (1975) reported that the enzyme was first associated with browning in mushrooms over 100 years ago. In the presence of molecular oxygen activity of PPO brought about the aerobic oxidation (browning) of certain plants. PPO is present in particularly high concentrations in mushrooms, potato tubers, peaches, apples, bananas, avocadoes, tea leaves, and coffee (Vamos-Vigyazo, 1981).

PPO catalyzes two quite different types of reactions, both of which involve phenolic compounds: (1) the hydroxylation of monophenols to produce O-diphenols, and (2) the removal of hydrogens from O-diphenols to produce O-quinones. The oxygen for the hydroxylation

reaction comes directly from O_2, not from water. PPO from apples (Harel et al., 1966), potatoes, sugar beet leaves, and broad bean leaves posses both types of activity (Scott, 1975).

The molecular weight of PPO is 34,000 with a copper concentration of 0.2%; the enzyme contains one atom of copper per molecule. The optimum pH range is 5 to 7. During PPO activity, oxidation of the substrate terminates long before all the substrate is utilized. The reaction terminates because the enzyme is inactivated by the product, o-benzoquinone, which forms a covalent linkage in or near the active site of the enzyme (Scott, 1975).

Apple PPO activity decreased when apples were pressure infiltrated with a 3% $CaCl_2$ solution (Hopfinger et al., 1984). Drake and Spayd (1983) reported a decrease in total browning in apples treated with a 3% $CaCl_2$ postharvest treatment.

Peroxidase (E.C. 1.11.1.7):

Peroxidase was discovered in mushroom extracts in 1855 (Scott, 1975). Since this time this enzyme has been the subject of interest, experimentation, and speculation. Peroxidase appears to be ubiquitous in many organisms including many animal, plant, microbial, and viral systems (Haard, 1977). It is a common constituent of higher plants and occurs in large concentrations. Extensive experimental studies on peroxidase have been published, but the function(s) and metabolic control(s) of the enzyme *in vivo* are poorly understood (Haard 1977). Peroxidase is linked with the following important functions: (1) respiratory control, (2) gene regulation, (3) hormone metabolism, (4) biosynthesis and degradation of secondary plant metabolites (Haard,

1977), and (5) ethylene biosynthesis (Vamos-Vigyazo, 1981).

Besides the physiological role of peroxidase, the enzyme contributes to deterioration of flavor, texture, color, and nutritional quality in processed fruits and vegetables (Blundstone et al., 1971; BenAziz et al., 1971). Elevated temperature, freezing, dehydration, and ionizing radiation can reduce peroxidase activity, but peroxidase is capable of regeneration after the fruit or vegetable is processed using these conditions. This capability can pose a serious problem in food processing because over-processing by extremes in temperature or freezing to reduce peroxidase activity can lead to deterioration of color, consistency and flavor, as well as losses of vitamins, proteins, and amino acids (Vamos-Vigyazo, 1981). Regeneration can occur within hours, days, or longer following thermal processing or thawing. This regeneration appears to involve the refolding of the polypeptide chain (Wang and DiMarco, 1972).

Peroxidase has been classified as an iron-containing enzyme. It is further broken into these two subgroups: (1) ferriprotoporphyrin peroxidase, and (2) verdoperoxidase. Ferriprotoporphyrin peroxidase, which is common in higher plants, contains ferriprotoporphyrin III as a prosthetic group and exhibits a red-brown color when purified. Verdoperoxidase occurs primarily in milk products and microorganisms (Haard, 1977), and differs from ferriprotoporphyrin by containing an iron porphyrin group that cannot be removed when treated with acidic or acidified acetone.

Peroxidase exhibits four types of catalytic activity: (1) peroxidatic, (2) oxidatic, (3) catalytic, and (4) hydroxylation (Haard,

1977). The peroxidatic reaction is thought to be the most significant and has been studied more extensively than the others. The overall equation of the peroxidatic reaction can be given as follows (Scott, 1975):

$$ROOH + AH_2 \xrightarrow{POD} H_2O + ROH + A$$

where: R = H^+, CH_3, or C_2H_5
AH = hydrogen donor in the reduced form
A = hydrogen donor in the oxidized form

Peroxidatic reactions occur in the presence of a wide variety of hydrogen donors, i.e. phenols (P-cresol, resorcinol, guaiacol), aromatic amines (benzidine and aniline), reduced NAD and NADP, glutathione, and cytochrome C (Haard, 1977; Vamos-Vigyazo,1981).

The oxidatic reaction of peroxidase may take place in the absence of hydrogen peroxide. The reaction requires O_2 and cofactors: Mn^{2+} and a phenol (Vamos-Vagyazo, 1981). A wide variety of substrates can be utilized in this reaction such as oxalate, oxaloacetate, ketomalonate, dihydroxyfumarate, or indoleacteic acid (IAA) (Kenten and Mann, 1953; Kay et al., 1967). The reaction is illustrated below (Whitaker, 1972):

$$\text{substrate} + O_2 \xrightarrow{POD} \text{product} + 2H_2O$$

The catalytic decomposition of H_2O_2 occurs in the absence of a hydrogen donor according to Whitaker (1972):

$$2H_2O_2 \xrightarrow{POD} 2H_2O + O_2.$$

The rate of this reaction in comparison to peroxidatic and oxidatic reactions is negligible (Whitaker, 1972).

The hydroxylating reactions produced o-dihydroxy phenols from monophenols and O_2, similar to PPO. However, peroxidase required a hydrogen donor similar to those used in the oxidatic reaction for this reaction.

The occurrence of isozymes has been documented by many researchers. Reportedly, the cellular location of peroxidase is in ribosomes (Penon et al., 1970), mitochondria (Darimont and Baxter, 1973), cell walls (Ridge and Osborne, 1970), and intercellular spaces (Haard, 1977). Molecular weight of POD from various fruits and vegetables ranges from 30,000 (Japanese radish) to 54,000 (fig) (Vamos-Vigyazo, 1981).

In fruit, peroxidase has been implicated as playing an important role in senescence. Senescent events thought to be regulated are lignin synthesis (Ranadive and Haard, 1973), ethylene biosynthesis (Vamos-Vigyazo, 1981) membrane integrity (Dilley, 1970), and respiration (Haard, 1977). Peroxidase activity appears to increase during ripening and senescence of pears (Ranadive and Haard, 1972) and mangoes (Mattoo and Modi, 1969).

Peroxidase activity was reported to increase in apples during ripening (Gorin and Heidema, 1976). Peroxidase activity was reported to reach maximum activity in 'Golden Delicious' apples at the climacteric peak. Activity was also shown to increase during CA storage (Gorin and Heidema, 1976). An increase in activity was found also in

cold storage in normal atmospheres with other cultivars (Vamos-Vigyazo, 1981).

Reports of the effect of calcium on peroxidase activity and yield have been highly variable. In wheat (Jeanjean et al., 1975) and in pears (Ranadive and Haard, 1973) calcium in the extracting media acted as an activator of peroxidase, whereas in peas, calcium in the extracting solution acted as a deactivator (Penel and Greppin, 1979).

In conclusion, the effect of calcium on fresh and processed fruit quality and the enzymes that affect quality warrants further investigation. The interactions between calcium and enzyme activity and calcium and fruit quality (fresh and processed) can provide useful and pertinent information for the storage and processing of apples.

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II. THE EFFECT OF CALCIUM INFILTRATION ON FRESH AND PROCESSING QUALITY OF 'GOLDEN DELICIOUS' AND 'DELICIOUS' APPLES

Abstract

'Golden Delicious' and 'Delicious' (Malus domestica Borkh) apples were pressure infiltrated at 68.95 KPa with a 0, 1, 2, 3 or 4% ('Golden Delicious') or 0, 2, 4, 6, or 8% $CaCl_2$ ('Delicious') solution (w/v) and placed in 1°C cold-storage. Fresh and processing parameters of the apples were measured after three and six months in storage. Apples were mechanically peeled removing the peel and the outer 0.1 to 0.2 cm of flesh. The next 0.2 to 0.4 cm of flesh was analyzed for calcium. Calcium ranged from 258.0 $\mu g \cdot g$ dry wt^{-1} (0% $CaCl_2$) to 2450.0 $\mu g \cdot g$ dry wt^{-1} (4% $CaCl_2$) in 'Golden Delicious' apples and 244.0 $\mu g \cdot g$ dry wt^{-1} (0% $CaCl_2$) to 1913.0 $\mu g \cdot g$ dry wt^{-1} (8% $CaCl_2$) in 'Delicious' apples. Firmness, ascorbic acid, and total phenols were maintained at higher levels in calcium-treated than in untreated apples at both time intervals. Soluble solids and titratable acidity concentrations were not significantly affected by calcium treatments at either time interval. Applesauce produced from calcium-treated fruit had lower consistency values (less flow) than untreated fruit and consistency decreased as calcium concentrations increased. Free liquid of applesauce produced from calcium-treated fruit increased as calcium con-

centration was increased at both time intervals. Applesauce color was darker (lower Hunter Colorimeter 'L' values) from calcium-treated fruit than from untreated control fruit at both time intervals. Juice produced from calcium-treated fruit was lighter and less green and yellow (higher 'L'- and 'a'-values and lower 'b'-values) at both testing intervals.

Introduction

In recent years 'Golden Delicious' apple production has increased steadily, and the surplus has surpassed the early season demand (Drake and Spayd, 1983), making it necessary to store the surplus for an extended period of time. Extending apple storage times without affecting the quality could benefit the producers of fresh-market and processing apples. The fresh-market producers would benefit from the extended market window, and the processors would benefit from having more time available for processing the apples into sauce and juice before the apples deteriorate.

Currently, 'Golden Delicious' apples are stored in controlled atmosphere storage (CA) (Massey, 1989). CA storage is preferred because of extended storage life of the fruit. However, CA storage is expensive, and there are flavor, color, and aroma problems in processed apple products associated with CA storage (Anderson and Penny, 1973; Drake et al., 1979).

Postharvest calcium dips and pressure infiltration techniques to overcome the deterioration of apples in storage have been suggested by many researchers (Bangerth et al., 1972; Mason, 1976; Betts and Bram-

lage, 1977; Conway, 1982; and Conway and Sams, 1983). Calcium treatments increase firmness (Bangerth et al., 1972; Scott and Wills, 1977; Conway and Sams, 1983; and Sams and Conway, 1987), delay senescence (Bangerth et al., 1972; Reid and Padfield, 1975; Ferguson, 1984; Bramlage et al., 1985; and Poovaiah, 1988), and control the invasion of Penicillium expansum Lk. ex Thom during storage (Sharples and Johnson, 1977; Conway, 1982; Conway and Sams, 1983; and Sams and Conway, 1985).

Drake and Spayd (1983) suggested that a 3% $CaCl_2$ treatment on 'Golden Delicious' apples improved the keeping qualities of apples in cold storage and did not detract from the quality of the processed product (applesauce). However, tissue damage has been observed in 'Golden Delicious' apples that received higher $CaCl_2$ (3% to 4%) treatments (Conway and Sams, 1985). Textural attributes of 'Golden Delicious' apples treated with postharvest calcium were optimum for fruit pressure infiltrated with 2% $CaCl_2$ when apples were stored six-months (Abbott et al., 1989). Because of the expense of CA storage and the problems associated with processing after CA storage, calcium treatment may be a viable alternative to CA storage. However, due to tissue damage associated with higher $CaCl_2$ treatments, the objective of this study was to investigate the effects of various calcium concentrations within the tissue on fresh and processed quality parameters of two apple cultivars to determine an optimum treatment.

Materials and Methods

The two apple (Malus domestica Borkh) cultivars used in this study were 'Golden Delicious' and 'Delicious'. 'Golden Delicious'

apples were obtained from a Tennessee grower; 'Delicious' apples were obtained from a grower in south-central Pennsylvania. Two groups of 'Golden Delicious' apples, representing two sizes, 7.94 cm ±0.64 (3 1/8 in ±1/4 equalling a 100 count box) and 8.28 cm ±0.64 (3 1/4 in ±1/4 equalling a 88 count box) in diameter were used in these analyses. However, similar results were found in fruit of both sizes and, unless indicated, the data presented consisted of the smaller and less mature 7.94 cm 'Golden Delicious' and of the 'Delicious' apples (8.26 cm in diameter). Production rates of CO_2 and ethylene (Table A.1) indicated that the smaller 'Golden Delicious' fruit had just begun the climacteric rise, the larger 'Golden Delicious' were further into the climacteric rise, and the 'Delicious' apples were preclimacteric when they were treated.

Apples were pressure infiltrated (68.95 KPa) with 0, 1, 2, 3, or 4% ('Golden Delicious') or 0, 2, 4, 6, or 8% $CaCl_2$ (w/v) ('Delicious') for two minutes and allowed to dry. They were then placed in boxes lined with perforated plastic and held at 1°C.

Calcium content of the flesh was determined at the three-month testing interval by removing the outer peel with a mechanical peeler and using the outer 0.2 to 0.4 cm of flesh for analysis. The tissue was frozen, freeze-dried, and ground. From each sample, 0.5 g of the dried tissue was ashed at 500°C for 8 to 10 hr, dissolved in one ml of 2N HCl, and diluted to appropriate levels for analysis. Calcium was analyzed using an atomic absorption spectrophotometer (Perkin-Elmer 5000). All calcium values were reported on a $\mu g \cdot g$ tissue dry weight basis.

Quality parameters were measured after three and six months in storage. The following fresh fruit quality parameters were measured: (1) firmness (on opposite precut sides of room temperature apples) measured as pounds force with an Effegi pressure tester equipped with a 1.1 cm Magness Taylor probe (expressed in Newtons (N)), (2) soluble solids (Atago refractometer) expressed as percentage (%), (3) ascorbic acid (AOAC, 1980) expressed as mg·100 g fresh weight^{-1} (peeled), (4) total phenols (Swain and Hillis, 1959) expressed as μg·g fresh wt^{-1}, and (5) titratable acidity (titration to endpoint of 7.2 with 0.1 N NaOH) expressed as percentage (%) malic acid.

Applesauce was prepared using modifications of the procedure described in the Ball Blue Book (Ball Corp.,1982). The modifications consisted of the following: 460 g apple tissue, and 414 ml water cooked at 98°C for 20 min, 37.4 g sugar added after 20 min of cooking, with an overall cooking period of 23 min. Cooked apples were drained for 30 sec before finishing. A collander (sieve) with perforations of 0.16 cm diameter was used to finish the cooled apples.

The parameters measured on finished applesauce included: (1) color (Hunter colorimeter) at 24 and 48 hr after finishing, (2) consistency and free liquid (Bostwick and Adams centerosis consistometers) at room temperature approximately two hr after finishing and (3) syneresis (liquid drained off apples for 30 sec through the sieve after cooking 23 min, prior to finishing).

Apple juice was produced using an Oster Automatic Pulp Ejector juicer and then filtered through Whatman #4 filter paper. The following processing quality parameters were measured on apple juice: (1)

color (Hunter colorimeter) at one and 24 hr after processing, and (2) calcium content (Perkin-Elmer 5000 Atomic Absorption spectrophotometer).

The statistical design consisted of a randomized complete block design with five treatments and three replications for each apple size and cultivar. Data were analyzed using the general linear models (GLM) procedures of the statistical analysis system (SAS Institute Inc., 1985) using the University of Tennessee Computer Center (UTCC) facilities, located on the University of Tennessee, Knoxville campus. The r^2 and best fit quadratic regression equation for each variable versus the calcium concentration of the tissue was determined. For firmness and soluble solids, 15 to 20 individual apples served as one replication. Five apples per replication were used in the determination of ascorbic acid and total phenols. Titratable acidity and juice parameters were measured for five peeled and cored apples per replication.

Results and Discussion

Fresh Fruit Quality Parameters

Fruit firmness was maintained as the calcium concentration within the tissue increased (both cultivars and both time intervals) (Fig. II.1). These results confirm the reports of other researchers (Bangerth et al., 1972; Mason et al., 1975; Mason, 1976; Sams and Conway, 1984; Sams and Conway, 1987). At the six-month testing interval the 'Golden Delicious' apples treated with the higher treatments were comparable to the control apples after three months in storage. The

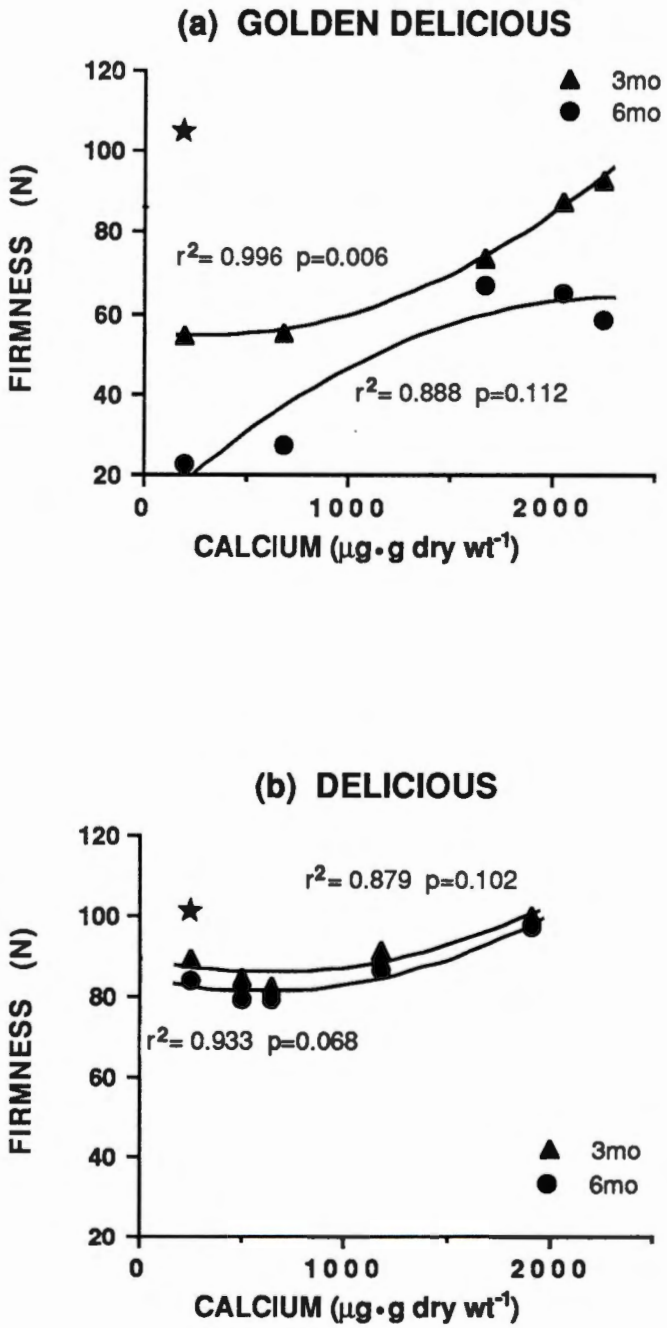


Fig. II.1: The effect of increasing calcium concentration in 'Golden Delicious' and 'Delicious' apples on firmness. ★ signifies firmness of apples at harvest. (a) 'Golden Delicious' apple firmness (N) after three and six months in storage. (b) 'Delicious' apple firmness (N) after three and six months in storage. The best fit quadratic equations were as follows: 1a) 3mo: $y=1.04*10^{-5}x^2 - 6.33*10^{-3}x + 55.30$; 6mo: $y=-1.02*10^{-5}x^2 + 4.74*10^{-2}x + 8.88$; 2b) 3mo: $y=9.13*10^{-6}x^2 - 1.17*10^{-2}x + 89.50$; 6mo: $y=9.69*10^{-6}x^2 - 1.17*10^{-2}x + 84.59$.

'Golden Delicious' apples after six-months in storage, exhibited very dry and soft flesh (along with several rotting fruit) in the 0% $CaCl_2$ apples and intact, but browning of the top most fleshy tissue of the 4% $CaCl_2$ treated fruit. 'Delicious' apples treated with 8% $CaCl_2$ maintained firmness levels at comparable harvest firmness levels (Fig. II.1), and did not exhibit tissue browning due to treatment damage after three or six months in storage. 'Delicious' apples treated with 0% $CaCl_2$ after six months in storage, were still firm indicating the better storage capability of this cultivar. Soluble solids content was not affected by the amount of calcium in the tissue for either cultivar (Fig. II.2).

The maturity of these apples at harvest, as indicated by CO_2 and ethylene readings, may be responsible for the loss in firmness. If the apples had been less mature at treatment time, they would not have been into the climacteric rise and the capability of calcium to retard softening might have been greater. Conway and Sams (1985) report that fruit maturity at harvest and treatment time can significantly affect the benefits achieved from the calcium treatment. If fruit is harvested and treated too early, $CaCl_2$ is not taken into the fruit, and if harvested too late, severe fruit damage may result.

Ascorbic acid levels were maintained as the calcium concentration within the tissue was increased at both time intervals (Fig. II.3). These results are consistent with those of Drake and Spayd (1983). Average ascorbic acid levels of all cultivars of apples at harvest are 4 mg·100 g fresh wt^{-1} (USDA, 1982a), however, concentrations of 8 to 15 mg·100g fresh wt^{-1} have been reported in 'Delicious' apples (Lee and

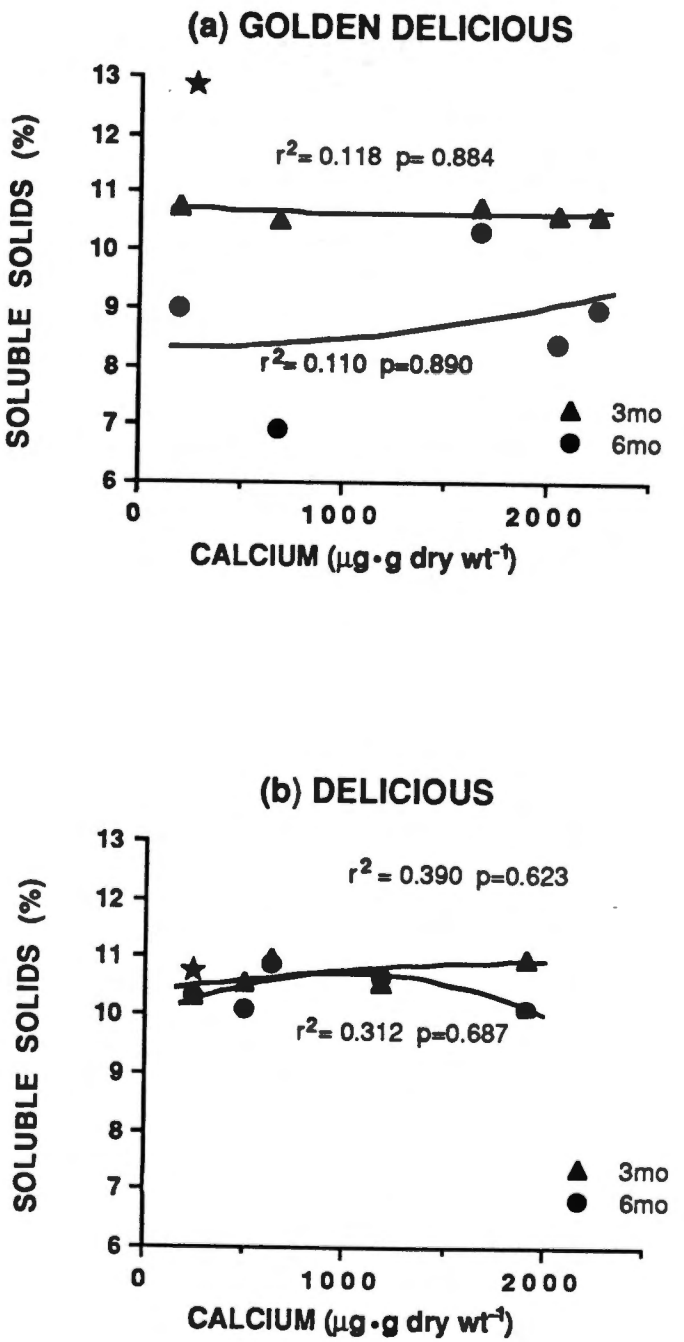


Fig. II.2: The effect of increasing calcium concentration in apple tissue on soluble solids content in 'Golden Delicious' and 'Delicious' apples. ★ represents soluble solids of apples at harvest. (a) 'Golden Delicious' apple soluble solids (%) after three and six months in storage. (b) 'Delicious' apple soluble solids (%) after three and six months in storage. The best fit quadratic equations were as follows: 2a) 3mo: $y=5.41*10^{-8}x^2 - 1.64*10^{-4}x + 10.7$; 6mo: $y=2.04*10^{-7}x^2 - 4.89*10^{-5}x + 8.29$; 2b) 3mo: $y=-1.10*10^{-7}x^2 + 5.08*10^{-4}x + 10.4$; 6mo: $y=-5.84*10^{-7}x^2 + 1.14*10^{-3}x + 10.1$.

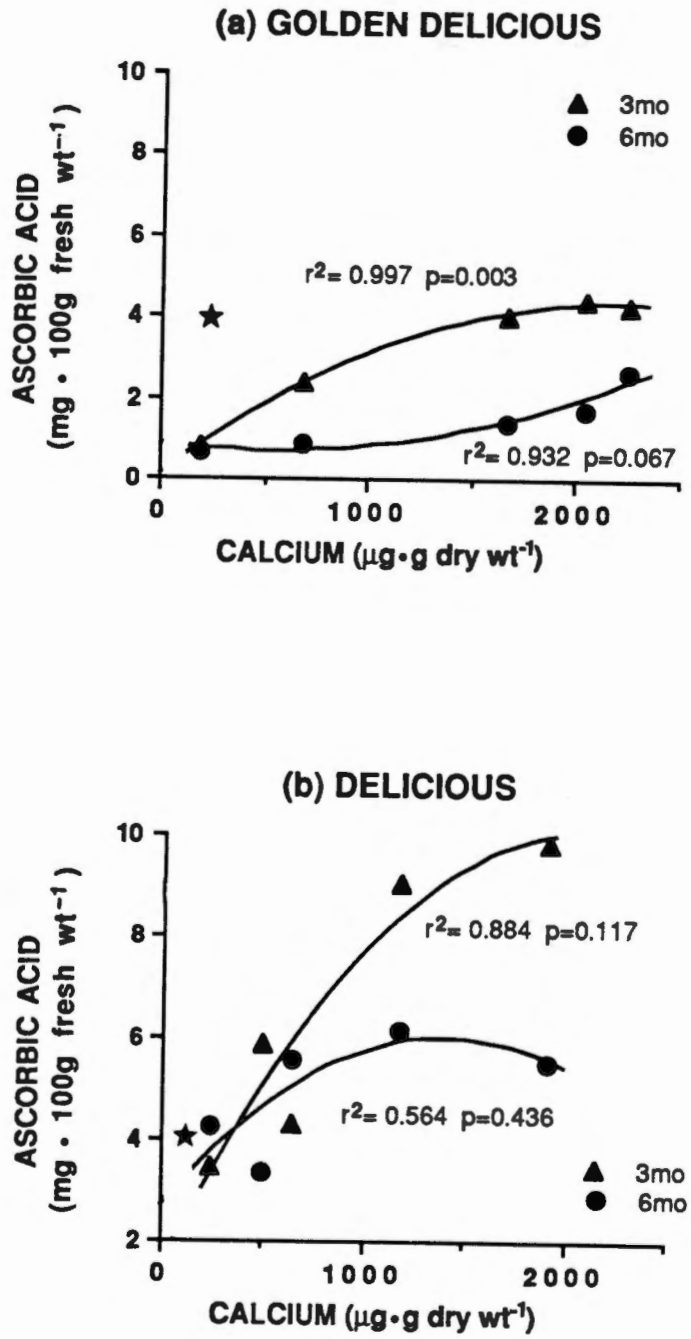


Fig. II.3: The effect of increasing calcium concentration in apple tissue on ascorbic acid content of peeled 'Golden Delicious' and 'Delicious' apples. ★ represents average ascorbic acid levels in peeled apples (USDA., 1982a). (a) ascorbic acid content of 'Golden Delicious' apples three and six months in storage. (b) ascorbic acid content of 'Delicious' apples after three and six months in storage. The best fit quadratic equations were as follows: 3a) 3 mo: $y=-8.91*10^{-7}x^2 + 3.89*10^{-3}x + 8.26*10^{-2}$; 6mo: $y=5.99*10^{-7}x^2 - 6.70*10^{-4}x + 8.65*10^{-1}$; 3b) 3mo: $y=-1.92*10^{-6}x^2 + 8.14*10^{-3}x + 1.45$; 6mo: $y=-1.68*10^{-6}x^2 + 4.73*10^{-3}x + 2.68$.

Mattick, 1989). The higher calcium levels in the 'Golden Delicious' apple tissue maintained the ascorbic acid levels at 4.3 mg·100g fresh wt^{-1} and 2.6 mg·100g fresh wt^{-1} at three and six months respectively (Fig. II.3). 'Delicious' apples exhibited the same maintenance of ascorbic acid levels (Fig. II.3); however, levels were higher than in the 'Golden Delicious' apples. Ascorbic acid content decreases during storage due to tissue deterioration and senescence (Smock and Neubert, 1950). Lower r^2 values at the six-month testing interval were probably due to deterioration of the tissue and general senescence.

Total phenols were maintained as the calcium concentration within the tissue was increased up to approximately 2000 $\mu g \cdot g$ dry wt^{-1} in 'Golden Delicious' and 'Delicious' apples (Fig. II.4). In general, phenol levels decreased above 2000 $\mu g \cdot g$ dry wt^{-1} (Fig. II.4). High amounts of phenols (especially catechins and proanthocyanidins) may manifest themselves as an astringent taste (Vamos-Vigyazo, 1981). Sensory evaluations will be required to determine if this maintenance of phenolic compounds is undesirable.

Some plant phenols, (like quercetin and caffeic acids) are anti-oxidants and have been shown to contribute to the stabilization of ascorbic acid (Vamos-Vigyazo, 1981). Thus, phenol maintenance in the apple tissue may be involved in the maintenance of ascorbic acid. Ascorbic acid is used as a pre- and post-processing treatment in apples to prevent browning of the fruit caused by polyphenol oxidase (PPO) (Smock and Neubert, 1950). The use of calcium may retard PPO activity and calcium content has been correlated with soluble PPO activity and the browning of macerated fruit tissue (Drake and Spayd,1983). The

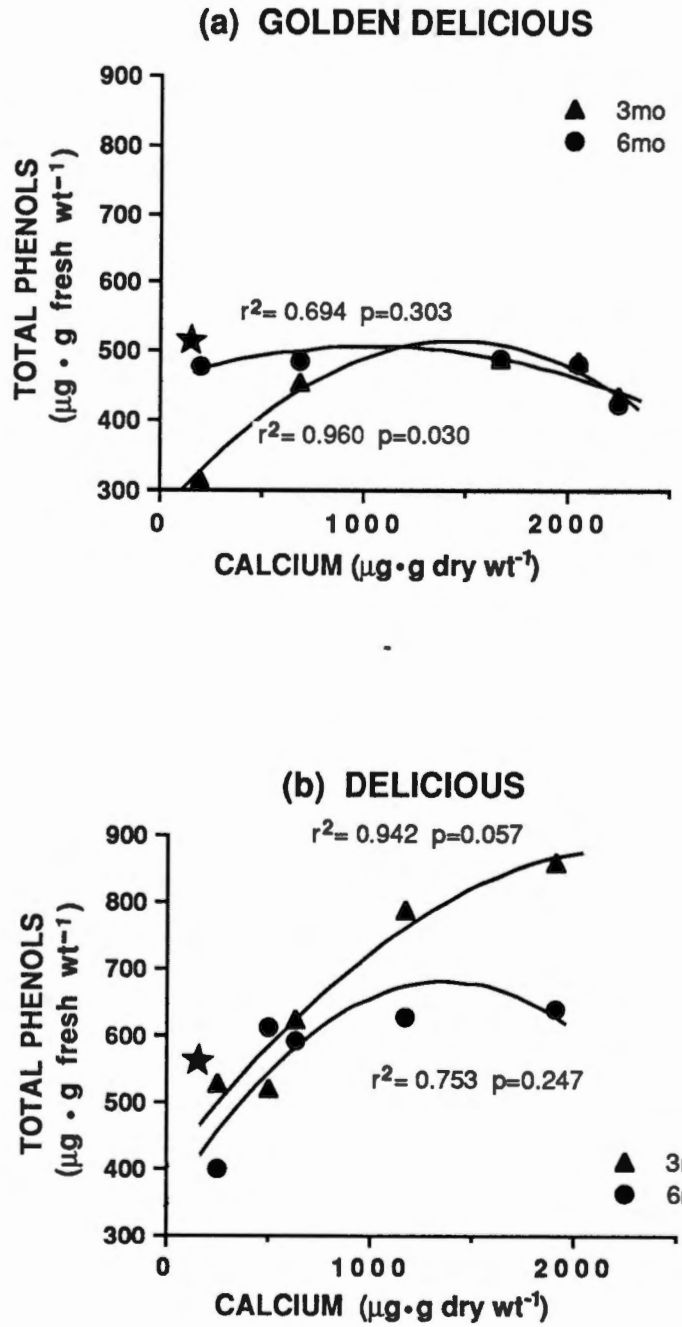


Fig. II.4: The effect of increasing calcium concentration in apple tissue on total phenol content of peeled 'Golden Delicious' and 'Delicious' apples. ★ represents total phenols at harvest. (a) total phenols of 'Golden Delicious' apples three and six months in storage. (b) total phenols of 'Delicious' apples after three and six months in storage. The best fit quadratic equations were as follows: 4a) 3mo: $y=-1.20*10^{-4}x^2 + 3.48*10^{-1}x + 260$; 6mo: $y=4.56*10^{-5}x^2 + 9.63*10^{-2}x + 455$; 4b) 3mo: $y=-8.76*10^{-5}x^2 + 4.14*10^{-1}x + 395$; 6mo: $y=-1.79*10^{-4}x^2 + 4.94*10^{-1}x + 339$.

decrease in browning exhibited by calcium treated fruit may be due to the maintenance of phenols in an oxidized state, which in turn may have affected the maintenance of ascorbic acid.

Titratable acidity (% malic acid) was not affected by calcium concentration in the tissue (Table I).

Processed Quality Parameters of Calcium Treated Apples

Color analyses were performed on applesauce using the Hunter colorimeter, standardized against a yellow tile (L=78.2 a=-3.4 b=22.0). The results were as follows: (1) L-values (luminosity) tended to be lower than the controls (in both cultivars), (2) L-values at the three-month testing interval were higher than the L-values at the six-month testing interval (in both cultivars) (Fig. II.5a and II.6a). Drake and Spayd (1983) reported higher 'L' values on applesauce made from apples treated with 3% $CaCl_2$ (pressure infiltration at 34.47 KPa for five minutes) than the control apples after five months in storage. The decrease in luminosity of applesauce tends to contradict data reported by Drake and Spayd (1983) and data obtained from color analysis of apple juice produced from identical fruit. Factors producing this darkening may include: (1) non-enzymatic oxidation of phenols; (2) sugar added during cooking reacting with the calcium in the tissue, producing a brown color within the sauce; (3) over-cooking of apples (Ball Corp., 1982); and/or (4) inclusion of some damaged tissue in the higher $CaCl_2$ treated fruit decreasing the luminosity of sauce made from these fruit. Enzymatic browning resulting in darker tissue is most likely not a factor involved in this

Table I: Titratable acidity of 'Golden Delicious'(GD) and 'Delicious' (D) apples treated with various calcium chloride concentrations after three and six months in storage.

Cultivar	Storage (mo)	%$CaCl_2$	Calcium ($\mu g \cdot g$ dry wt^{-1})	Initial pH	% Malic	r^2	p =
GD	3	0	193.0	4.03	0.2077		
GD	3	1	687.0	4.03	0.1832		
GD	3	2	1676.0	3.82	0.2311	0.643	0.10
GD	3	3	2050.0	3.71	0.2394		
GD	3	4	2250.0	3.66	0.2309		
D	3	0	244.0	4.13	0.2157		
D	3	2	496.0	4.09	0.2021		
D	3	4	638.0	4.12	0.1796	0.413	0.24
D	3	6	1177.0	4.06	0.1831		
D	3	8	1913.0	3.99	0.1845		
D	6	0	244.0	4.14	0.1720		
D	6	2	496.0	4.11	0.1711		
D	6	4	638.0	4.07	0.1800	0.394	0.26
D	6	6	1177.0	4.05	0.1746		
D	6	8	1913.0	4.02	0.1630		

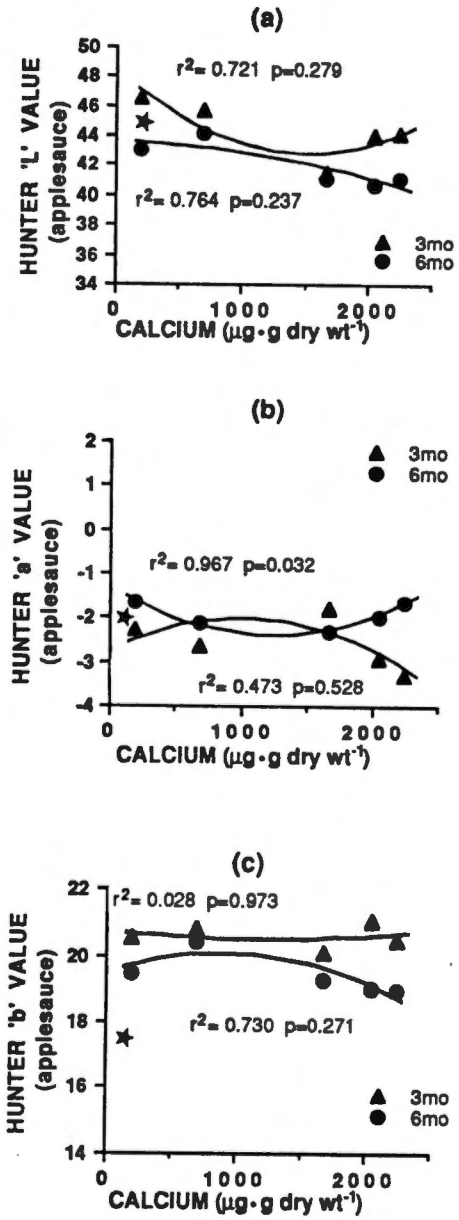


Fig. II.5: Hunter Colorimeter values of applesauce made from 'Golden Delicious' apples with varying calcium concentrations after three and six months in storage. ★ represents color values of applesauce made form untreated fruit at harvest. (a) 'L' value of applesauce after three and six months in storage, (b) 'a' value of applesauce after three and six months in storage, (c) 'b' value of applesauce after three and six months in storage. The best fit quadratic equations were as follows: 5a) 3 mo: $y=2.39*10^{-6}x^2 - 7.33*10^{-3}x + 48.41$; 6mo: $y=-5.22*10^{-7}x^2 - 1.81*10^{-4}x + 43.58$; 5b) 3mo: $y= -7.20*10^{-7}x^2 + 1.46*10^{-3}x - 2.78$; 6mo: $y=7.03*10^{-7}x^2 - 1.74*10^{-3}x - 1.33$; 5c) 3mo: $y=1.49*10^{-7}x^2 - 3.71*10^{-4}x + 20.72$; 6mo: $y=-7.39*10^{-7}x^2 + 1.35*10^{-3}x + 19.45$.

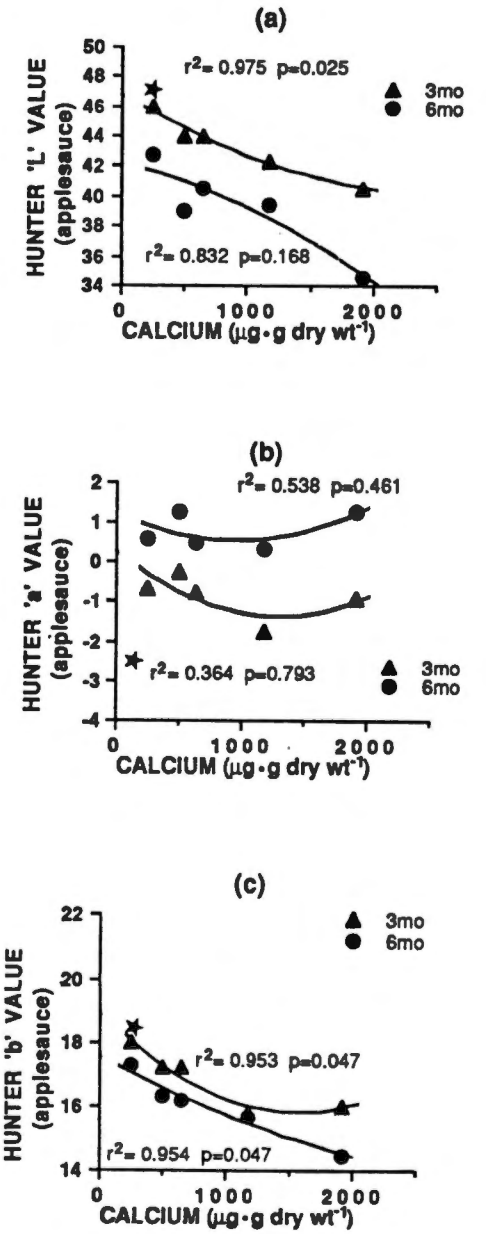


Fig. II.6: Hunter Colorimeter values of applesauce made from 'Delicious' apples with varying calcium concentrations after three and six months in storage. ★ represents color values of applesauce made form untreated fruit at harvest. (a) 'L' value of applesauce after three and six months in storage, (b) 'a' value of applesauce after three and six months in storage, (c) 'b' value of applesauce after three and six months in storage. The best fit quadratic equations were as follows: 6a) 3mo: $y=9.62*10^{-7}x^2 - 5.11*10^{-3}x + 46.85$; 6mo: $y=-9.49*10^{-7}x^2 - 2.04*10^{-3}x + 42.21$; 6b) 3mo: $y=9.94*10^{-7}x^2 - 2.60*10^{-3}x + 2.98*10^{-1}$; 6mo: $y=7.54*10^{-7}x^2 - 1.45*10^{-3}x + 1.22$; 6c) 3mo: $y=1.31*10^{-6}x^2 - 4.11*10^{-3}x + 19.04$; 6mo: $y=2.74*10^{-7}x^2 - 2.12*10^{-3}x + 17.58$.

darkening because the enzyme responsible for browning (polyphenol oxidase) is deactivated at 96°C (Wiley and Binkley, 1989). In order to differentiate between these mechanisms, research is needed into color analysis on sauce produced from apples with varying cooking periods and sauce made without added sugar. Presently, USDA (1982b) does not have color value requirements for sauce grading, and further research is required in order to determine if this slight decrease in lightness of sauce is unacceptable for marketing.

The Hunter 'a' and 'b' values changed very little as the calcium concentration was increased within the tissue (Fig. II.5b, II.5c, II.6b, and II.6c). Color values taken at 48 hours revealed the same results (Table A.2). A color comparison of commercial applesauce with experimental sauce is shown in Table II.

Applesauce consistency measurements taken with the Adams centerosis consistometer and the Bostwick consistometer (Table A.3) were similar, and the data presented here are the readings obtained from the Adams centerosis consistometer. Calcium-treated fruit tended to flow less (lower consistency readings) up to approximately 2000 $\mu g \cdot g$ dry wt^{-1} in 'Golden Delicious' at both storage intervals and up to approximately 2000 $\mu g \cdot g$ dry wt^{-1} in 'Delicious' after three months and up to 1200 $\mu g \cdot g$ dry wt^{-1} after six months in storage (Fig. II.7). All treatments were within the acceptable USDA Grade A rating for sauce consistency (flow less than 6.5 cm) (USDA 1982b).

The free liquid (solid/liquid separation) measurements (taken with the Adams centerosis consistometer) revealed an increase in separation

Table II. Comparisons of commercial applesauce with applesauce made from 'Golden Delicious'(GD) and 'Delicious' (D) calcium treated apples.

BRAND/ CULTIVAR	TMT (%$CaCl_2$)	STORAGE (mo)	HUNTER COLOR VALUES 'L'	'a'	'b'	CONSISTENCY (cm·30 sec^{-1})	FREE LIQUID (cm·30 sec^{-1})
Kroger™	–	–	49.1	-3.4	17.4	3.3	0.0
Musselman's™	–	–	49.7	-6.5	18.1	2.4	0.5
GD	0	3	46.5	-2.3	20.5	2.2	0.0
GD	1	3	45.8	-2.7	20.8	2.0	0.0
GD	2	3	41.5	-1.8	20.1	1.4	0.4
GD	3	3	44.0	-2.9	21.1	1.3	0.5
GD	4	3	44.2	-3.3	20.5	1.6	1.8
GD	0	6	43.0	-1.7	19.5	1.3	0.0
GD	1	6	44.2	-2.1	20.4	1.1	0.1
GD	2	6	41.1	-2.3	19.3	0.8	1.8
GD	3	6	40.7	-2.0	19.0	1.0	2.9
GD	4	6	41.1	-1.6	19.0	1.0	1.7
D	0	3	46.0	-0.7	18.0	0.6	0.0
D	2	3	44.0	-0.3	17.3	1.7	0.0
D	4	3	44.0	-0.8	17.3	1.4	0.0
D	6	3	42.4	-1.8	15.8	1.3	0.3
D	8	3	40.5	-0.9	16.0	0.6	1.2
D	0	6	42.8	0.6	17.3	1.1	0.2
D	2	6	39.0	1.3	16.3	1.0	0.0
D	4	6	40.6	0.5	16.2	1.4	0.0
D	6	6	39.5	0.3	15.7	1.1	0.3
D	8	6	34.6	1.3	14.5	1.5	1.2

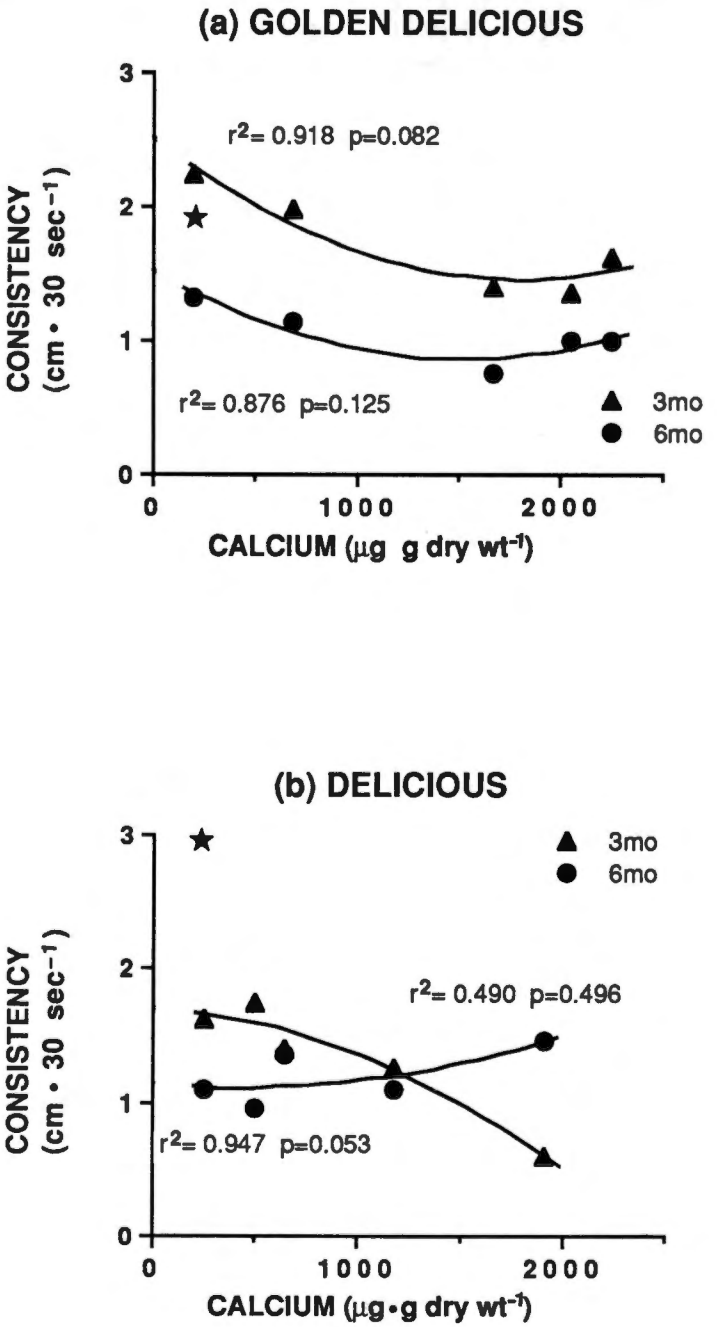
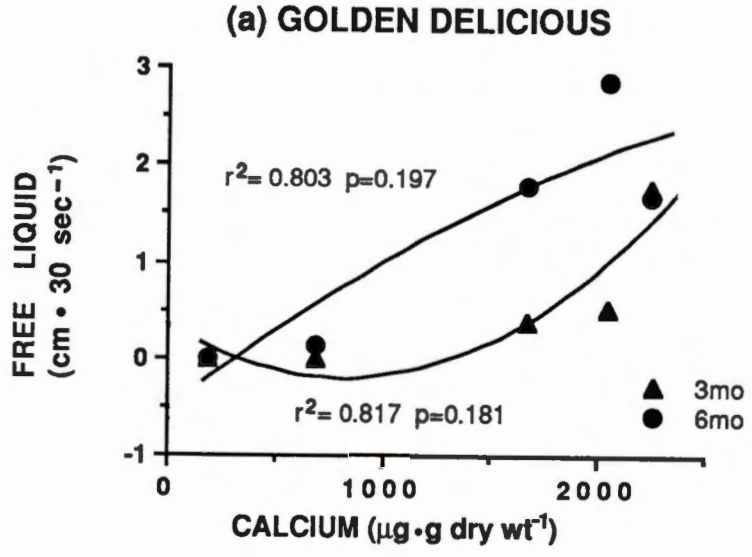


Fig. II.7: Consistency of sauce made from various calcium concentrations in 'Golden Delicious' and 'Delicious apple fruit tissue. represents sauce consistency of untreated apples at harvest. (a) 'Golden Delicious' sauce consistency after three and six months in storage. (b) 'Delicious sauce consistency after three and six months in storage. The best fit quadratic equations were as follows: 7a) 3 mo: $y=3.26*10^{-7}x^2 - 1.20*10^{-3}x + 2.52$; 6mo: $y=2.84*10^{-7}x^2 - 8.66*10^{-4}x + 1.52$; 7b) 3mo: $y=-2.56*10^{-7}x^2 - 7.97*10^{-5} + 1.69$; 6mo: $y=1.59*10^{-7}x^2 - 1.27*10^{-4}x + 1.14$.

of solids and liquids in sauce made from the higher calcium concentrations (3% and 4% treatments in 'Golden Delicious' and 8% treatment in 'Delicious') (Fig. II.8) (see Table A.3 for free liquid measurements taken with the Bostwick consistometer). The USDA grade A requirements for free liquid require a free liquid flow of not more than 0.7 cm to qualify for grade A (USDA., 1982b). 'Golden Delicious' apples processed at three months from 0, 1, 2, and 3% $CaCl_2$ treatments were acceptable as Grade A sauce. The 4% treatment at three months and the 2, 3, and 4% treatments at six months did not meet Grade A or Grade B (flow less than 1.0 cm) standards (Fig. II.8). 'Delicious' apples processed at three and six months were all acceptable as USDA Grade A sauce except the 8% $CaCl_2$ treatment at both testing intervals. The increase in flow at the highest calcium concentrations indicates a solid/liquid separation due to tissue damage at higher calcium levels. This damage was visible in the sauce as hard, brittle, particles that were not cohesive. Finishing of the sauce became very difficult with the higher calcium fruit because the cooked apples would not pass through the holes in the finisher. Maturity of the 'Golden Delicious' apples may have been the cause of the poor quality of sauce obtained at the six-month testing interval. These apples may have reached advanced stages of senescence, and at this stage calcium had increased free liquid measurements.

Ethylene and CO_2 data indicated that $CaCl_2$ treatments may have been performed later than desirable for long-term storage. This phenomenon may indicate that more research into optimum maturity for $CaCl_2$ treatment is needed. The increase in free liquid at the higher



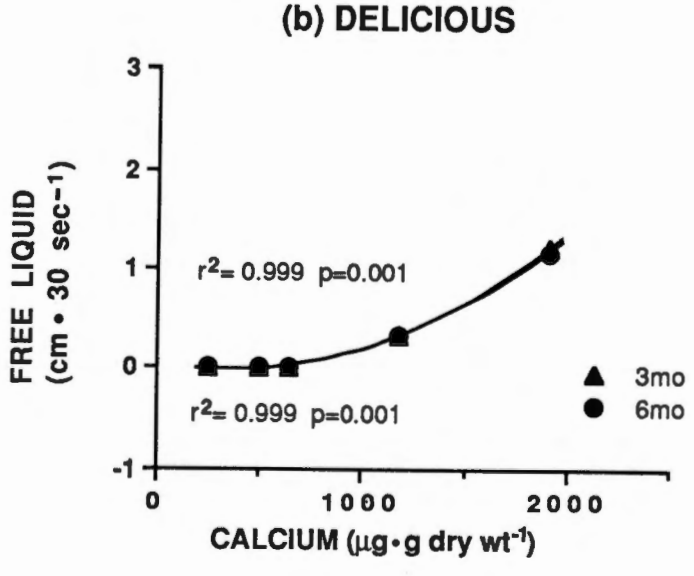


Fig. II.8: Free liquid of sauce made from various calcium concentrations in 'Golden Delicious' and 'Delicious' apple fruit tissue. (a) 'Golden Delicious' free liquid after three and six months in storage. (b) 'Delicious' free liquid after three and six months in storage. The best fit quadratic equations were as follows: 8a) 3mo: $y=8.03*10^{-7}x^2 - 1.33*10^{-3}x + 0.340$; 6mo: $y=-2.11*10^{-7}x^2 + 1.7*10^{-3}x - 0.55$; 8b) 3mo: $y=5.11*10^{-7}x^2 - 3.77*10^{-4}x + 5.53*10^{-2}$; 6mo: $y=4.87*10^{-7}x^2 - 3.44*10^{-4}x + 4.64*10^{-2}$.

calcium treatments is most likely due to pre-processing tissue damage and this would limit the calcium treatments to less than 4% in 'Golden Delicious' and less than 8% in 'Delicious'.

Commercial applesauce production generally involves blending of different cultivars to obtain a desirable flavor and texture (Ball Corp., 1982). Blending other cultivars with $CaCl_2$ treated 'Golden Delicious' and 'Delicious' apples may decrease this solid/liquid separation.

Sauce made from the three-month old control 'Golden Delicious' apples tended to exhibit greater syneresis than the calcium-treated apples, with the 2 and 3% treatments exhibiting the least syneresis (Fig. II.9). The same general trend was observed at the six-month testing interval except that the control apples had the least amount of syneresis (Fig. II.9). This decrease can be explained by tissue dryness due to senescence; the control apples, after six months in storage, were very dry and absorbed much of the water added during cooking.

Apple Juice Parameters

Apple juice produced from fruit treated with $CaCl_2$ were lighter, less green and yellow as expressed by higher L- and a-values, and lower b-values, 24 hr after juicing (Fig. II.10 and Fig. II.11). Hunter colorimeter values for juice color one hr after juicing were similar and are presented in Table A.4. This trend was expressed in both cultivars at both time intervals. Although the differences were detectable by the colorimeter, only the difference in luminosity was

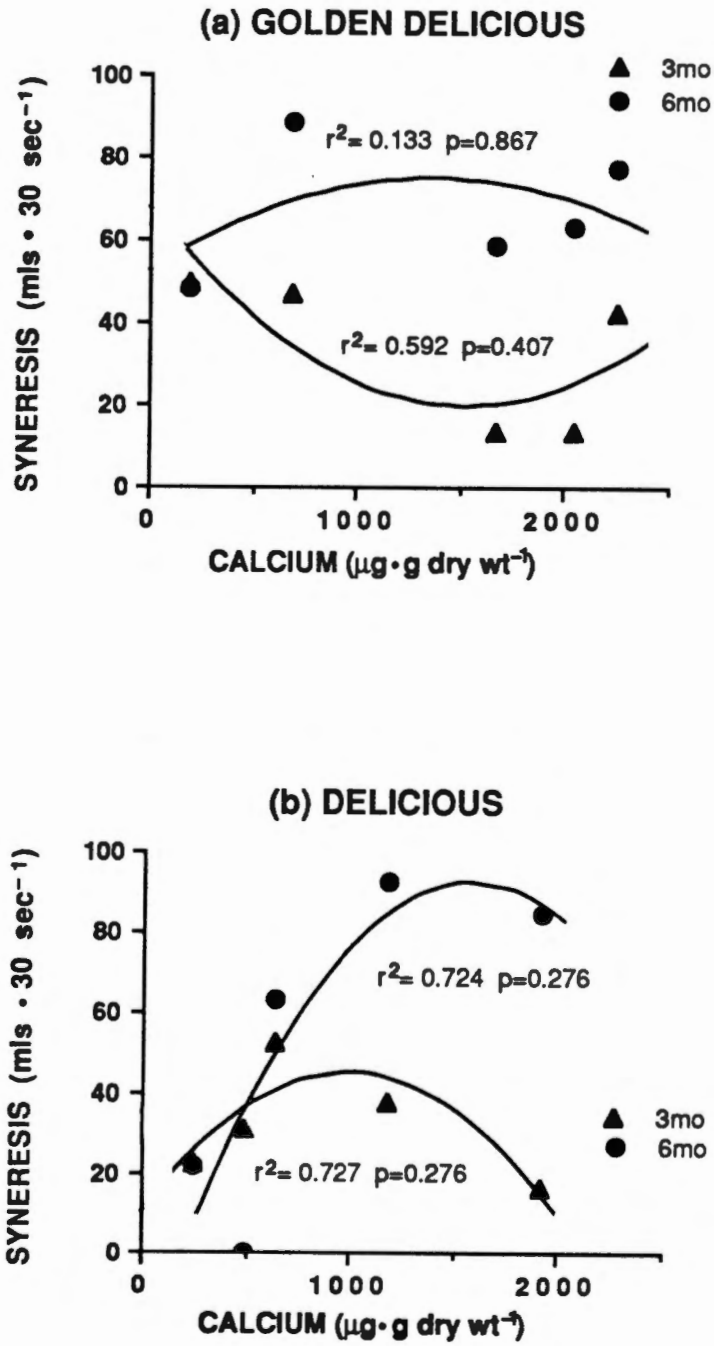


Fig. II.9: Syneresis of cooked apples from various calcium concentrations in 'Golden Delicious' and 'Delicious' apple fruit tissue. (a) 'Golden Delicious' after three and six months in storage. (b) 'Delicious' after three and six months in storage. The best fit quadratic equations were as follows: 9a) 3mo: $y=2.07*10^{-5}x^2 - 6.33*10^{-2}x + 68.1$; 6mo: $y=-1.17*10^{-5}x^2 + 3.23*10^{-2}x + 52.5$; 9b) 3mo: $y=-3.57*10^{-5}x^2 + 7.10*10^{-2}x + 9.91$; 6mo: $y=-4.81*10^{-5}x^2 + 1.52*10^{-1}x - 28.2$.

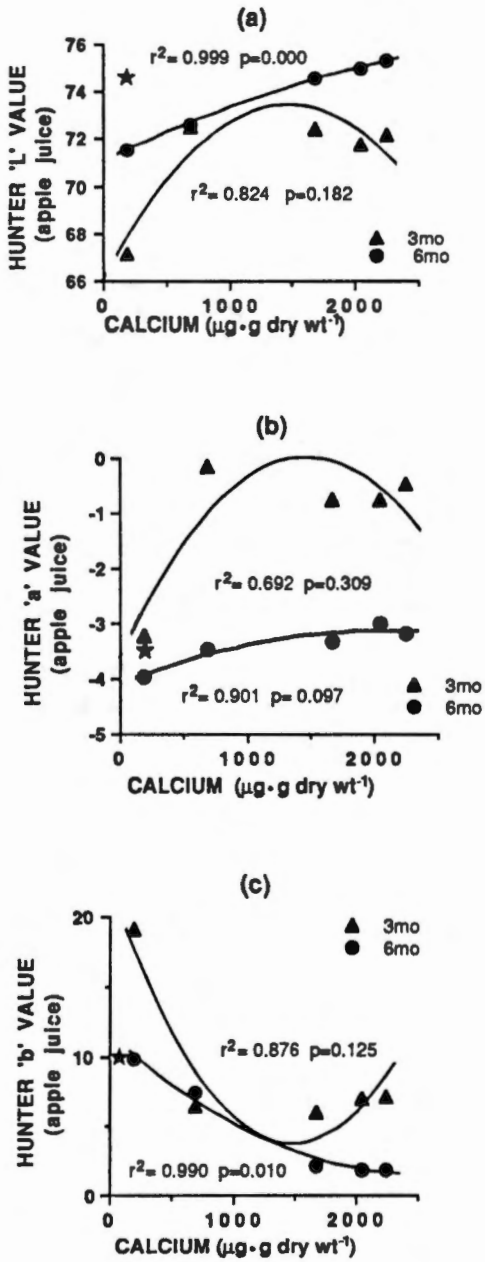


Fig. II.10: Hunter Colorimeter values of apple juice made from 'Golden Delicious' apples with varying calcium concentrations after three and six months in storage. ★ represents color values of apple juice made from untreated fruit at harvest. (a) 'L' value of apple juice after three and six months in storage, (b) 'a' value of apple juice after three and six months in storage, (c) 'b' value of apple juice after three and six months in storage. The best fit quadratic equations were as follows: 10a) 3 mo: $y=3.44*10^{-6}x^2 + 1.01*10^{-2}x + 65.98$; 6mo: $y= -3.08*10^{-7}x^2 + 2.59*10^{-3}x + 71.05$; 10b) 3mo: $y=-1.66*10^{-6}x^2 + 4.91*10^{-3}x - 3.627$; 6mo: $y=-2.21*10^{-7}x^2 + 9.20*10^{-4}x -4.111$; 10c) 3mo: $y=8.40*10^{-6}x^2 - 2.50*10^{-2}x + 22.297$; 6mo: $y=1.58*10^{-6}x^2 - 8.02*10^{-3}x + 11.651$.

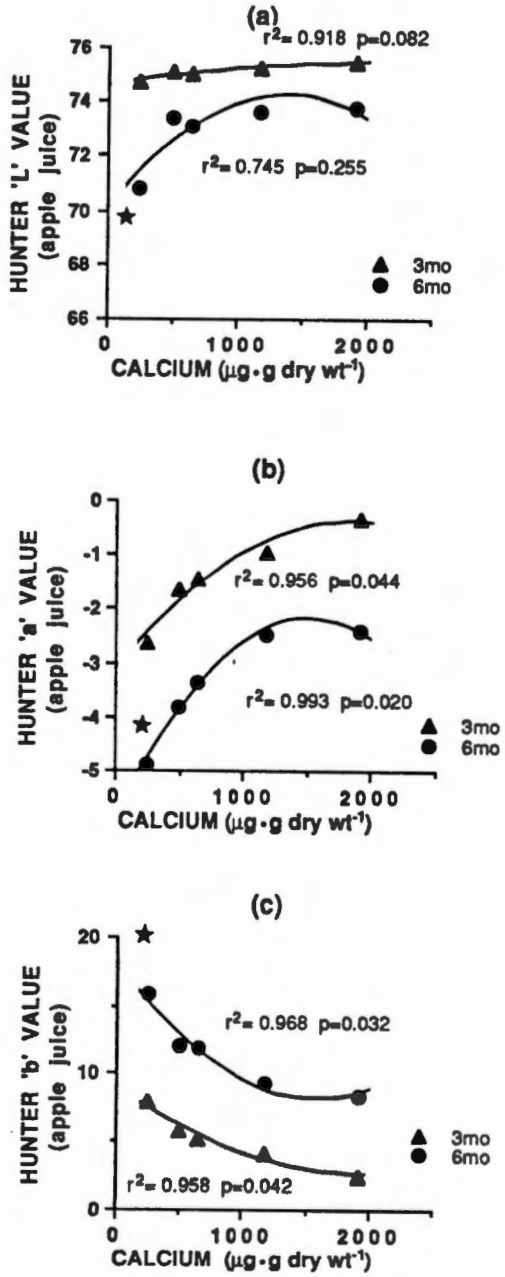


Fig. II.11: Hunter Colorimeter values of apple juice made from 'Delicious' apples after 24 hrs., with varying calcium concentrations after three and six months in storage. ★ represents color values of apple juice made from untreated fruit at harvest. (a) 'L' value of apple juice after three and six months in storage, (b) 'a' value of apple juice after three and six months in storage, (c) 'b' value of apple juice after three and six months in storage. The best fit quadratic equations were as follows: 11a) 3 mo: $y=-1.88*10^{-8}x^2 + 8.14*10^{-4}x + 74.564$; 6mo: $y=-2.14*10^{-6}x^2 + 5.98*10^{-3}x + 70.08$; 11b) 3mo: $y=-7.91*10^{-7}x^2 + 2.93*10^{-3}x - 3.116$; 6mo: $y=-1.64*10^{-6}x^2 + 5.00*10^{-3}x - 5.966$; 11c) 3mo: $y=1.66*10^{-6}x^2 - 6.52*10^{-3}x + 9.034$; 6mo: $y=3.92*10^{-6}x^2 - 1.25*10^{-2} + 18.208$.

obvious to the human eye. The higher a- and lower b-values were most likely due to the lower amounts of particulate matter in the treated fruit in relation to higher amounts of particulate matter in the controls. Commercial apple juice (Musselmans™ and Tree Top™) was similar in color to the experimental apple juice (Table III).

Added calcium has been promoted in several food products due to the increased awareness of calcium in the diet. Therefore, the apple juice was tested for calcium. Juice made from 'Delicious' and 'Golden Delicious' apples treated with calcium had 2 to 5 times and 1.8 to 2.1 times more calcium, respectively than juice made from control fruit (Fig. II.12). The lower amounts of calcium in 'Golden Delicious' apples were a result of the actual calcium treatments these apples received (1 to 4%) in relation to the calcium treatments the 'Delicious' apples received (2 to 8%). However, calcium concentrations in apple tissue was higher in 'Golden Delicious' tissue (193.0 to 2250.0 $\mu g \cdot g$ dry wt^{1}) than in 'Delicious' (245.0 to 1913.0 $\mu g \cdot g$ dry wt_{-}^{1}). A possible explanation for this occurrence is that 'Golden Delicious' apples have more bound calcium ions within the tissue which are not transferred into the soluble form in the juice.

In conclusion, postharvest calcium treatment of apples resulted in the following: (1) maintenance of fruit firmness, (2) maintenance of ascorbic acid and total phenols, which in turn may affect browning of the processed product, and (3) no consistent effect on soluble solids or titratable acidity (% malic acid). The optimum $CaCl_2$ treatment recommended to obtain the maintenance of firmness, ascorbic

Table III: Color comparisons of commercial apple juice with apple juice made from 'Golden Delicious' (GD), and 'Delicious' (D) apples three months after postharvest calcium treatments.

Brand/Cultivar	Hunter Colorimeter 'L'	'a'	'b'
Musselmans™	73.9 ± --	-3.6 ± --	7.2 ± --
Tree Top™	72.9 ± --	-4.1 ± --	9.9 ± --
GD (0%)	67.2 ± 2.0	-2.7 ± 0.3	6.9 ± 1.8
GD (1%)	72.2 ± 0.6	-1.4 ± 0.3	6.9 ± 0.9
GD (2%)	71.3 ± 2.5	-1.4 ± 0.9	7.5 ± 2.6
GD (3%)	70.8 ± 1.0	-2.1 ± 0.2	8.4 ± 1.3
GD (4%)	73.0 ± 0.8	-1.5 ± 0.4	5.6 ± 0.8
D (0%)	74.1 ± 0.7	-4.1 ± 1.6	12.4 ± 4.5
D (2%)	74.8 ± 0.2	-2.8 ± 0.9	8.5 ± 2.3
D (4%)	74.9 ± 0.8	-2.8 ± 1.5	8.6 ± 3.9
D (6%)	75.1 ± 0.2	-2.1 ± 0.8	6.5 ± 2.1
D (8%)	75.7 ± 0.0	-1.2 ± 0.6	5.1 ± 1.3

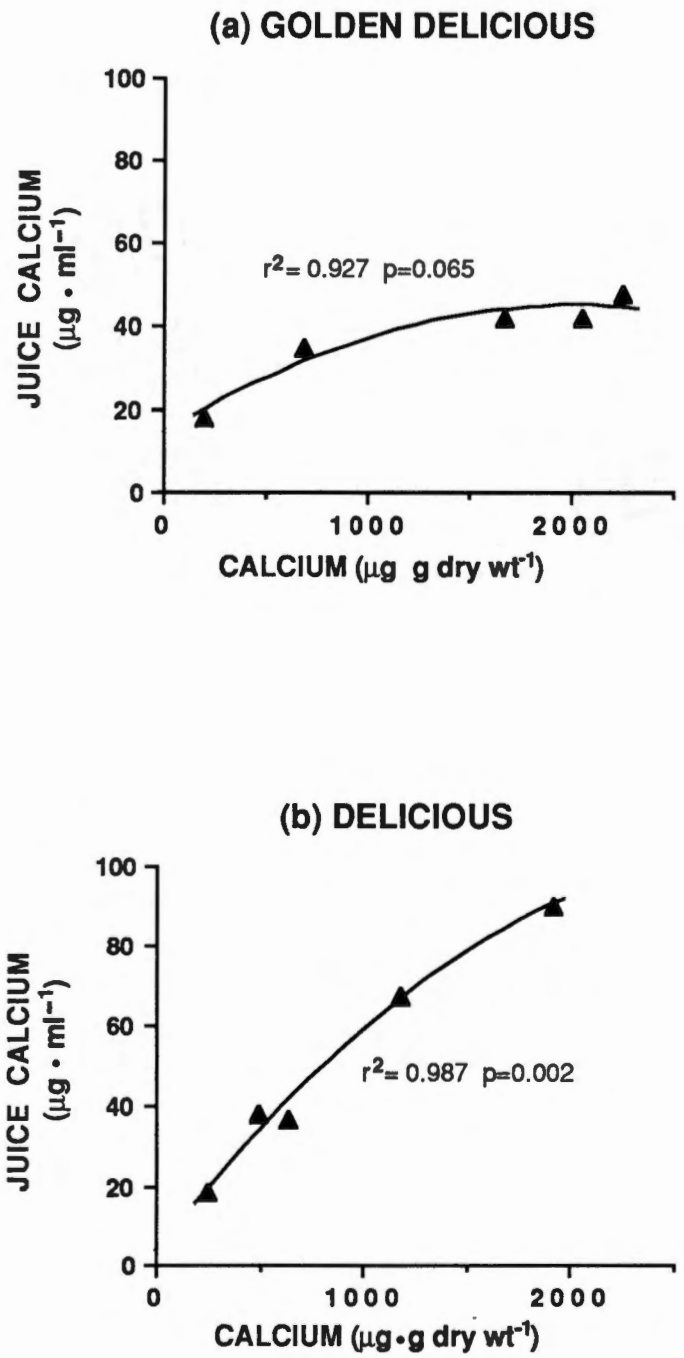


Fig.. II.12: Calcium content of apple juice produced from 'Golden Delicious' and 'Delicious' apples of varying calcium concentrations. (a) calcium content of 'Golden Delicious'apple juice after three months in storage. (b) calcium content of 'Delicious' apple juice after three months in storage. The best fit quadratic equations were as follows:
12a) 3 mo: $y=-7.51*10^{-6}x^2 + 0.031x + 14.12$;
12b) 3mo: $y=-1.09*10^{-5}x^2 + 0.062x + 3.45$.

acid, and total phenols is 2 to 3% for 'Golden Delicious', and 4 to 6% for 'Delicious' apples.

In general, sauce made from apples treated with $CaCl_2$ had lower consistency values resulting in a more cohesive sauce. All sauce was acceptable as USDA Grade A. Free liquid (solid/liquid separation) of applesauce made from calcium-treated fruit increased in the applesauce made from the higher $CaCl_2$ treated fruit. The 4 and 8% $CaCl_2$ treatment in 'Golden Delicious' and 'Delicious' apples, respectively, did not meet USDA grading standards after three months in storage. After six months in storage, the free liquid of applesauce made from the 0 to 6% $CaCl_2$ treated 'Delicious' apples still met USDA grading standards, however, 'Golden Delicious' appeared to have passed their storage life and free liquid measurements were not acceptable. Hunter 'L' values measuring luminosity were lower in the calcium treated fruit indicating a darker sauce. These data indicate a 2 to 3% $CaCl_2$ treatment in 'Golden Delicious' and a 4 to 6% $CaCl_2$ treatment in 'Delicious' apples is optimum to maintain processing quality.

Additional areas of research have become apparent during the course of this study, i.e. investigations into optimum maturity for postharvest treatment and sensory evaluation experiments. This study has narrowed the range of postharvest calcium treatments by illustrating damaging effects of some calcium treatments on some processing parameters. In order to maintain fresh and processing quality of apples treated with postharvest calcium, a 2% to 3% $CaCl_2$ treatment in 'Golden Delicious' and a 4% to 6% $CaCl_2$ treatment in 'Delicious' apples seems to be optimum.

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III. THE EFFECT OF CALCIUM ON POLYPHENOL OXIDASE, PEROXIDASE, AND PECTIN METHYL ESTERASE ACTIVITIES OF 'GOLDEN DELICIOUS' AND 'DELICIOUS' APPLES

Abstract

'Golden Delicious' and 'Delicious' (Malus domestica Borkh) apples were pressure infiltrated (68.95 KPa) with a 0, 1, 2, 3, or 4% ('Golden Delicious') or 0, 2, 4, 6, or 8% $CaCl_2$ ('Delicious') solution (w/v) and placed in 1°C cold-storage. Polyphenol oxidase (PPO) (E.C. 1.10.3.1) and peroxidase (POD) (E.C. 1.11.1.7) activities of the apples were measured after three and six months in storage (1987-1988). Apples were mechanically peeled, removing the peel and the outer 0.1 to 0.2 cm of flesh. The next 0.2 to 0.4 cm of flesh was analyzed for calcium. Calcium content ranged from 258.0 $\mu g \cdot g$ dry wt^{-1} (0% $CaCl_2$) to 2450.0 $\mu g \cdot g$ dry wt^{-1} (4% $CaCl_2$) in the 'Golden Delicious' apples and 244.0 $\mu g \cdot g$ dry wt^{-1} (0% $CaCl_2$) to 1913.0 $\mu g \cdot g$ dry wt^{-1} (8% $CaCl_2$) in 'Delicious' apples. PPO activity in 'Golden Delicious decreased from 0.956 (0% $CaCl_2$) to 0.278 (4% $CaCl_2$) $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$ after three months in storage. After six months in storage, PPO activity decreased from 1.736 (0% $CaCl_2$) to 0.582 (4% $CaCl_2$) $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$. PPO activity in 'Delicious' apples decreased from 1.587 (0% $CaCl_2$) to 0270 (4% $CaCl_2$) $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$ and 3.193 (0% $CaCl_2$) to 1.266 ($CaCl_2$) $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$ at three and six months, respectively. POD activity increased as calcium concentration in the tissue was increased. 'Golden Delicious' apple POD activity increased from 0.654 (0%

$CaCl_2$) to 1.200 (4% $CaCl_2$) $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$ after three months in storage and from 1.127 (0%$CaCl_2$) to 3.016 (4% $CaCl_2$) $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$. 'Delicious' apple POD activity revealed a similar increase in activity at both storage intervals (0.982 to 2.419 $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$ at three months and 1.942 to 3.352 $\Delta OD \cdot min^{-1} \cdot mg\ protein^{-1}$ at six months). Pectin methyl esterase (PME) (E.C. 3.1.1.11) activity was measured after two, four, and six months in storage (1988-1989). Apples were mechanically peeled, removing the peel and the outer 0.1 to 0.2 cm of flesh. The remaining flesh was analyzed for calcium. Calcium content of 'Golden Delicious' apples analyzed for PME activity, ranged from 203.0 $\mu g \cdot g$ dry wt^{-1} (0% $CaCl_2$) to 886.0 $\mu g \cdot g$ dry wt^{-1} (4% $CaCl_2$). PME activity in 'Golden Delicious' apples decreased at all three storage intervals as the calcium concentration increased within the tissue. PME activity (meq $H^+ \cdot min^{-1} \cdot mg\ protein^{-1}$) decreased from 0.078 (0% $CaCl_2$) to 0.038 (4% $CaCl_2$) at two months, 0.099 (0% $CaCl_2$) to 0.022 (4% $CaCl_2$) at four months, and 0.026 (0% $CaCl_2$) to 0.014 (4% $CaCl_2$) at six months.

Introduction

Postharvest calcium treatments cause a delay in flesh softening (Bangerth et al., 1972; Mason et al.,, 1975; Mason, 1976; Ferguson, 1984) and a delay in the occurrence of physiological disorders, including senescent breakdown (Bangerth et al., 1972; Hardenburg and Anderson, 1981; Bramlage et al., 1985; Marme, 1985; Sams and Conway, 1985). During prolonged storage (5 mo), a postharvest $CaCl_2$ (3%) treatment improved the keeping qualities of fresh apples, and did not

deter from the quality of the processed product (Drake and Spayd, 1983). Processed quality of apple products produced from a 3% $CaCl_2$ treatment produced a sauce with lower consistency values and less syneresis than controlled atmosphere (CA) or cold-stored apples (Drake and Spayd, 1983).

One of the major indicators of fresh fruit quality is firmness. Calcium treatments contribute to the maintenance of firmness in 1°C storage for six months (Mason, 1976 and Section I, p.31). Pectin methyl esterase (PME), a pectic enzyme found in many fruits, may be responsible, in part, for pectin solubilization and cell wall-middle lamella degradation that affects fruit firmness. PME activity increased in apples during storage (Lee, 1969), and increased in activity with respect to the respiratory climacteric in persimmons (Awad and Young, 1979). Calcium treatments on apples have been shown by many researchers to slow down or delay the respiratory climacteric (Bangerth et al., 1972; Faust and Shear, 1972; and Sams and Conway, 1984), and investigation of PME activity with respect to calcium concentration should prove beneficial to determine if de-esterification and subsequent degradation of pectin polymers is occurring during postharvest storage.

In fruit, peroxidase (POD) activity is involved in various processes. These processes include: (1) POD as an indicator of senescence (Ranadive and Haard, 1973; and Gorin and Heidema, 1976), (2) POD's affect on processed fruit flavor, texture, color, and nutrition (Blundstone et al., 1971; and BenAziz et al., 1971) and, (3) POD's affect on membrane integrity (Dilley, 1970) which can be associated

with a decrease in fruit firmness. POD activity reaches a maximum in 'Golden Delicious' apples at the climacteric peak (Gorin and Heidema, 1976). Because calcium has been shown to delay the respiratory climacteric, POD activity may also be affected.

Processed fruit color is a factor involved in USDA grading standards and marketability. Any discoloration of the processed product may affect the grade and ultimately the sale of the product. Polyphenol oxidase (PPO) is responsible for the majority of the browning that occurs in most fruit tissue (Vamos-Vigyazo, 1981). Calcium treatments suppress PPO activity (Drake and Spayd, 1983, and Hopfinger et al., 1984).

The objective of this study was to determine the effects of various calcium concentrations (in apple tissue) on three enzymes that have been shown to affect fruit quality. The activities of PME, POD, and PPO within the tissue can be related to some of the factors that affect fresh and processed fruit quality.

Materials and Methods

'Golden Delicious' and 'Delicious' (Malus domestica Borkh) apples were harvested in east Tennessee and south central Pennsylvania, respectively, in the fall of 1987. 'Delicious' (8.26 cm ±0.64 equalling 3 1/4 in ±1/4) and two different sizes of 'Golden Delicious' (7.94 cm ±0.64 equalling 3 1/8 ±1/4 in and 8.26 cm ±0.64 equalling 3 1/4 in ±1/4)) were used. The stages of maturity (as determined by rates of CO_2 and ethylene production at harvest Table A.1) were as follows: (1) the smaller 'Golden Delicious' apples were beginning into

the climacteric rise, (2) the larger 'Golden Delicious' apples were more advanced into the climacteric rise, and (3) the 'Delicious' apples were preclimacteric. PPO and POD activity was determined on these apples.

'Golden Delicious' apples were harvested in the fall of 1988 in east Tennessee and used for evaluating PME activity. CO_2 and ethylene production rates, at harvest, indicated that these apples were preclimacteric.

All 'Golden Delicious' apples were pressure infiltrated (68.95 KPa) with 0, 1, 2, 3, or 4% $CaCl_2$ (w/v) solution for two minutes. 'Delicious' apples were pressure infiltrated (68.95 KPa) with 0, 2, 4, 6, or 8% $CaCl_2$ (w/v) solution for two minutes. The apples were air-dried, placed in boxes lined with perforated plastic to prevent dehydration, and stored in 1°C storage.

PPO extraction was performed and activity was determined using the colorimetric method described by Shannon and Pratt (1967) and Hopfinger et al., (1984) (appendix B). POD extraction was performed and activity was determined by the method described by Ponting and Joslyn (1948), and Hopfinger et al., (1984) (appendix B).

The extraction of PPO and POD was performed at 0°C. Thirty grams of flesh were blended (using a Polytron) in 0°C 0.1M glycine buffer (pH10) for two min. The slurry was filtered through cheesecloth, Whatman #4, and Whatman #42 filter paper. The filtrate was then poured into 120 ml of acetone (-30°C) and allowed to stand for two hours at -30°C. The mixture was filtered (Whatman #42) and washed three times with 7.5 ml of 0°C 0.1M glycine buffer. The precipitate was then

resuspended in 13 ml of 0.1M citrate-0.2M phosphate buffer (pH6.2) and centrifuged at 20,000g for 20 minutes. The supernatant was used as the enzyme source.

Enzyme activity for PPO and POD was measured spectrophotometrically at 420 and 485 nm, respectively. PPO activity was measured by the following methods: one ml of enzyme source was mixed in 20 ml of 0.01M acetate buffer (pH 7.0) containing two ml of 0.5M catechol and measured at 30 sec intervals for six min. POD activity was measured by adding one ml of the enzyme source in to 20 ml of 0.01M acetate buffer (pH 7.0) containing one ml of 0.04M aqueous guaiacol and one ml of 0.1N hydrogen peroxide. Activity was measured at 30 sec intervals for six min. Ten ml of enzyme source were heated to 96°C for 30 min and used as the control. Protein was determined using the method described by Biorad (Technical Bulletin #1069). Enzyme activity is expressed as $\blacktriangle OD \cdot min^{-1} \cdot mg\ protein^{-1}$.

PME extraction and activity was determined, using a modification of the protocol described by Awad (1985) (appendix B). The modification of the procedure was as follows: one hundred grams of flesh was ground with a Polytron homogenizer for two min in two hundred ml of 5% NaCl. The solution was then brought to pH 7.0 with 1N NaOH and stirred at 2°C for 10 hr. The solution was then filtered through Miracloth and used for enzyme assay.

Five ml of enzyme solution were added to 10 ml of a 1% (w/v) solution of apple pectin (Sigma #P2157) in 5% NaCl. The mixture was brought rapidly to pH 7.0 with 0.1N NaOH. The release of carboxyl

groups by the action of PME on the pectic substrate was measured by titration using 0.01N NaOH at 25°C. PME units were expressed as meq·ester hydrolyzed·min^{-1}·mg $protein^{-1}$. Boiled enzyme solution showed no activity. Protein was determined by the method described by Biorad (Technical Bulletin #1069).

Calcium content of the apple tissue was determined (at three months for the 1987-88 apples and at four months for the 1988-89 apples) by removing the outer peel with a mechanical peeler and using the inner flesh for analysis. Tissue was then frozen (-30°C), freeze-dried, and ground into a powder. From each sample, 0.5 g of dried tissue was ashed, dissolved in one ml of 2N HCl, and diluted to appropriate levels for analysis. The samples were then analyzed for calcium, using a Perkin-Elmer model 5000 atomic absorption spectrophotometer. All calcium values are reported on a dry weight basis.

The statistical design consisted of a randomized complete block design with five treatments and three to four replications for each cultivar for both years. Data were analyzed using the general linear models (GLM) procedures of the statistical analysis system (SAS Institute, Inc., 1985) using the University of Tennessee Computer facilities (UTCC) located on the University of Tennessee, Knoxville campus. The r^2 and best fit quadratic regression equation for each variable versus the calcium concentration of the tissue was determined. Five apples served as one replication for all enzyme activities.

Results and Discussion

Polyphenol oxidase activity (PPO)

PPO activity was measured on the less mature of the two 'Golden Delicious' groups and on the 'Delicious' apples. PPO activity decreased as the calcium concentration within the tissue was increased (both time intervals and both cultivars) and was higher at the six-month testing interval than at three months (Fig. III.1). This decrease in activity may be a result of the following: (1) PPO biosynthesis and/or inactivation, (2) substrate availability, (3) available O_2, and/or (4) pH dependency.

Total phenols reportedly decrease during storage, and PPO activity and browning varies with respect to cultivar, picking maturity, and storage conditions (Vamos-Vigyazo, 1981). Substrates of the PPO reaction are phenols (chlorogenic acid and catechins in apples) (Vamos-Vigyazo, 1981). Calcium treatments have been shown to maintain phenol levels within apples (500 $\mu g \cdot g$ fresh wt^{-1} in 'Golden Delicious' and 800 $\mu g \cdot g$ fresh wt^{-1} in 'Delicious')(Section I p. 36). These data would indicate that sufficient substrate is available for the reaction to take place, assuming that the substrate is in the correct form and cellular location.

Inhibition of PPO and browning are often treated as the same subject. Browning may be prevented not only by enzyme inactivation but by available O_2. Since PPO contains copper as a prosthetic group, it can be inhibited by metal chelating agents such as cyanide and carbon monoxide (Walker, 1975). Compounds such as these do not exist in high

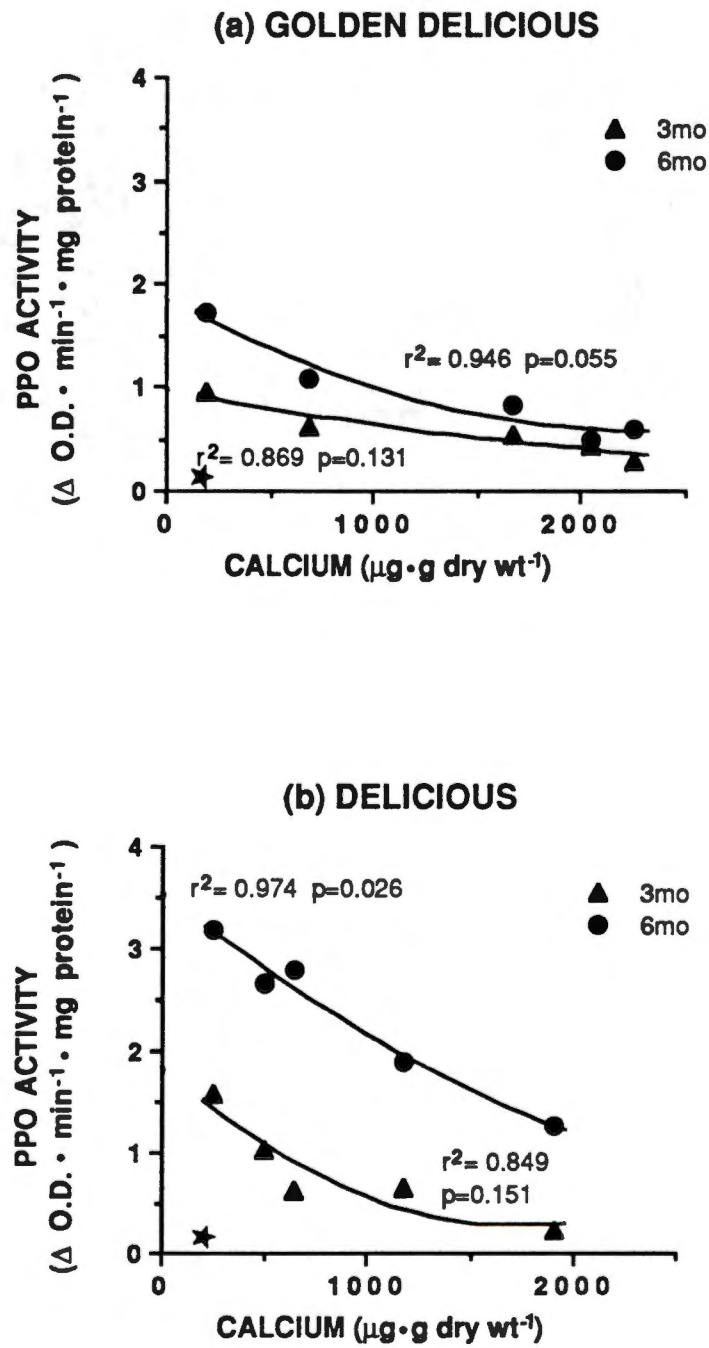


Fig. III.1: The effect of calcium concentration within apple tissue on polyphenol oxidase (PPO) activity. (a) 'Golden Delicious' apple PPO activity after three and six months in storage. (b) 'Delicious'; apple PPO activity after three and six months in storage. ★ indicates PPO activity of untreated apples at harvest. The best fit quadratic equations were as follows: 1a) 3mo: $y=4.95*10^{-8}x^2 - 3.83*10^{-4}x + 0.968$; 6 mo: $y=2.49*10^{-7}x^2 -1.14*10^{-3}x + 1.887$; 1b) 3mo: $y=5.14*10^{-7}x^2 - 1.79*10^{-3}x + 1.840$; 6 mo: $y=2.13*10^{-7}x^2 - 1.61*10^{-3}x + 3.558$.

concentrations in the flesh and most likely do not inhibit PPO activity due to chelation with the copper atom. To date, inhibition due to calcium chelation with the copper atom in PPO has not been reported.

Ascorbic acid has been reported as possibly acting directly on PPO by chelating with the prosthetic group (Pierpoint, 1966). However, the ability of ascorbic acid to inhibit browning in fruit products is primarily due to ascorbic acid maintaining the o-quinones (products) in a reduced state; thus they are unable to bind together to produce the brown pigment (Vamos-Vigyazo, 1981). Postharvest calcium treatments have been shown to maintain ascorbic acid levels (Drake and Spayd, 1983 and Section I p.34) and this may affect the activity of PPO. PPO activity is measured colorimetrically by the production of the pigments and ascorbic acid prevents the binding of o-quinones (producing the brown pigment). The total decrease in browning can also be observed by the production of lighter-colored juice from calcium-treated fruit (Section II p. 48-49).

Optimum pH for PPO activity is 7.0 (Ponting and Joslyn, 1948), with a range of 4.2 to 7.0 (Vamos-Vigyazo, 1981). Titratable acidity has been shown to increase over the control fruit, after five months in storage, when a 3% $CaCl_2$ a calcium treatment is implemented (Drake and Spayd, 1983). However, $CaCl_2$ treatments did not have an effect on titratable acidity in a previous investigation (Section II. p.38). In either case, the small increase observed by Drake and Spayd (1983), or the unchanged state observed in Section II the slight change in acidity most likely does not affect PPO activity.

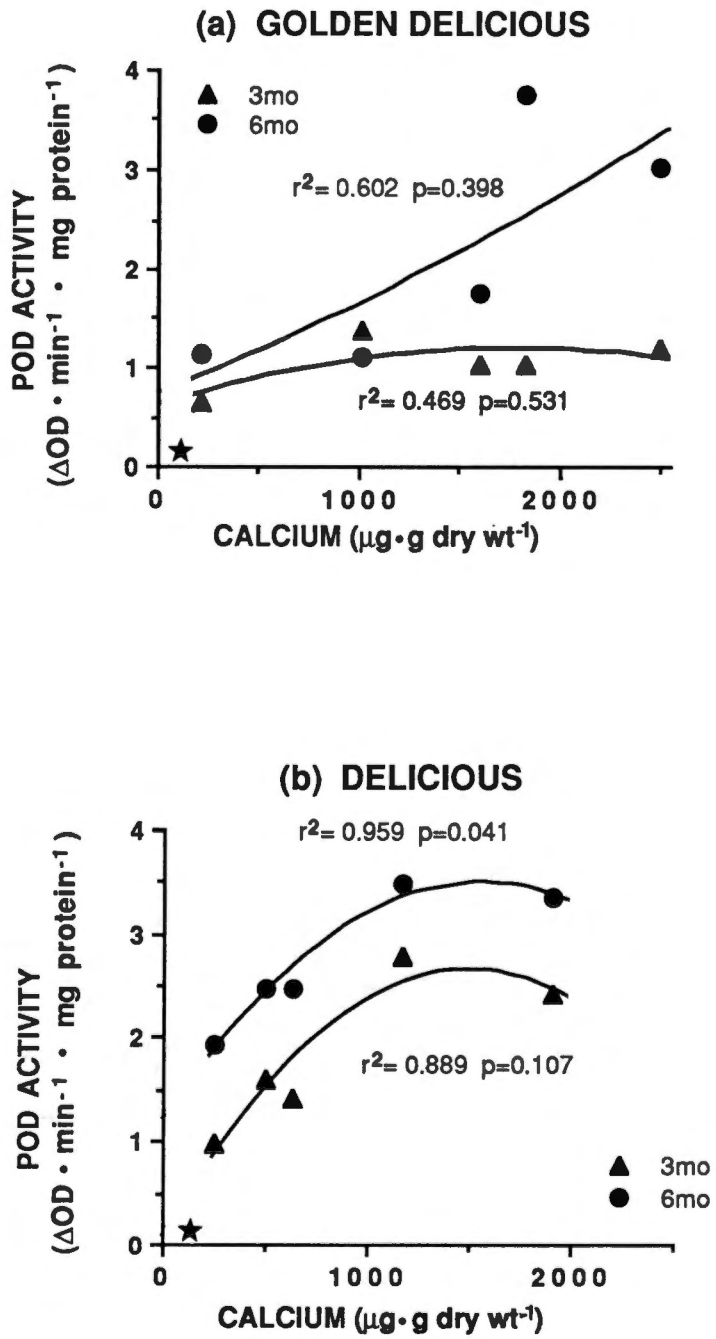


Fig. III.2: The effect of calcium concentration within apple tissue on peroxidase (POD) activity. (a) 'Golden Delicious' apple POD activity after three and six months in storage. (b) 'Delicious'; apple POD activity after three and six months in storage. ★ indicates POD activity of untreated apples at harvest. The best fit quadratic equations were as follows: 2a) 3 mo: $y=-1.82*10^{-7}x^2 + 6.51*10^{-4}x + 0.612$; 6 mo: $y=9.13*10^{-8}x^2 + 8.22*10^{-4}x + 0.732$; 2b) 3mo: $y=-1.16*10^{-6}x^2 + 3.47*10^{-3} + 0.089$; 6 mo: $y=-9.34*10^{-7}x^2 + 2.92*10^{-3}x + 1.213$.

Peroxidase (POD)

POD activity was measured on the larger, more mature 'Golden Delicious' and 'Delicious' apples. In 'Golden Delicious' apples POD in the control fruit at both time intervals. Activity of POD was higher activity in calcium-treated fruit increased slightly over that in the control fruit at the three-month testing interval (Fig. III.2). Enzyme activity in calcium-treated fruit increased at the six-month interval. POD activity in calcium-treated 'Delicious' fruit was higher than that at six months than it was at three months at all calcium concentrations.

POD activity in 'Golden Delicious' apples reaches maximum activity at the climacteric peak (Gorin and Heidema, 1976). The data obtained from 'Golden Delicious' apples in these experiments indicated that the fruits with greater calcium concentrations reached the climacteric peak after six months in storage (Fig. III.2). The lower POD activity at three months in calcium treated apples is evidence of this delay in the climacteric peak. Calcium treatments delayed the climacteric rise, and in response to this, POD activity is lower.

The higher POD activity in the calcium-treated fruit can also be a result of calcium activation of the POD reaction or increased amounts of H^+ donors (i.e. phenols and ascorbic acid) in the high-calcium tissue. In wheat (Jeanjean et al., 1975) and pears (Ranadive and Haard, 1973), calcium in the extracting medium acted as an activator of POD. During extraction and homogenization it is possible that free calcium became available (and in higher amounts as calcium concentration increases in tissue) and activated the reaction. Other pos-

sibilities include the presence of more phenols and ascorbic acid that are maintained in fruit with higher calcium content. These substances can act as H^+ donors in the reaction.

Pectin Methyl Esterase (PME)

Increasing the calcium concentration within the tissue tended to decrease the activity of PME (Fig. III.3). This decrease in activity was observed at two, four, and six-month testing intervals. However, at the six-month testing interval, calcium did not exhibit as great an effect on activity as at the two and four-month intervals.

PME hydrolyzes the methyl ester of galacturonic acid (Dey and Brinson, 1984). Since calcium links the side chains of galacturonases together (Grant et al., 1973), the action of PME would be expected to decrease because of its inability to fit into active site. Action of other pectic enzymes (i.e. endo-polygalacturonase types) would most likely decrease also. This decrease in activity could be a result of the inability of PME to first de-esterify the polysaccharide chains for further action by other enzymes due to increased cross-linkages between calcium and galacturonic acid.

In conclusion, the effect of postharvest calcium treatments on PPO is beneficial. By increasing calcium concentrations within the apple tissue, PPO activity decreased and total product browning was reduced. Postharvest calcium treatments decrease POD activity and POD activity in 'Golden Delicious' follows the delayed respiratory climacteric caused by calcium. This occurrence is supported by peroxidase activity being relatively lower in the calcium treated fruit at three months

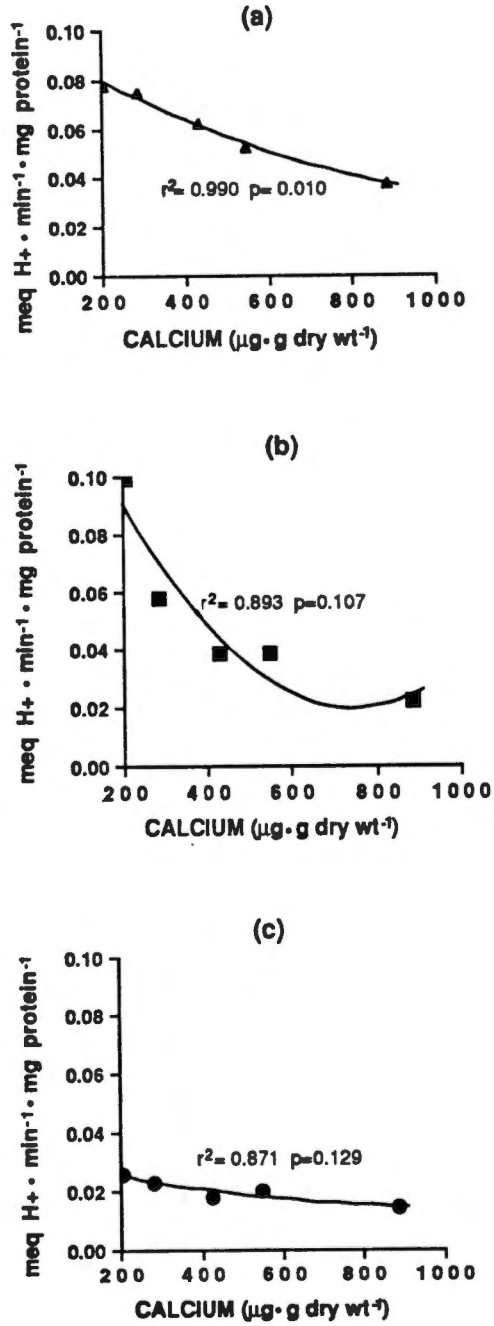


Fig. III.3: The effect of calcium concentration within 'Golden Delicious' apple tissue on pectin methyl esterase (PME) activity. (a) PME activity after two months in storage. (b) PME activity after four months in storage. (c) PME activity after six months in storage indicates PME activity of untreated apples at harvest. The best fit quadratic equations were as follows: 3a) 2mo: $y=3.47*10^{-8}x^2 - 9.93*10^{-5}x + 0.098$; 4mo: $y=2.36*10^{-7}x^2 - 3.53*10^{-4}x + 0.152$; 6mo: $y=1.57*10^{-8}x^2 - 3.27*10^{-5}x + 0.031$.

than at six months. At three months, the calcium treated fruit had not yet reached the climacteric and thus peroxidase activity was lower. However, at six months, calcium treated fruit were in the climacteric and peroxidase activity was higher. POD activity exhibited in 'Delicious' apples did not show the same delayed pattern, but the overall comparison between the two, indicating that POD activity increased with calcium concentration was similar. The increase in POD activity ex pressed in 'Delicious' may be a result of availability H^+ donors as expressed by maintenance of ascorbic acid levels at higher concentrations than 'Golden Delicious'. The general increase in POD activity (due to calcium) found in both cultivars may also be a result of the maintenance of cell wall and membrane integrity. Since POD has been found in the cytoplasm and in the cell walls of many plants (Vamos-Vigyazo, 1981), maintenance of integrity would not only preserve substrate availability for POD but also preserve the enzyme itself. In order to fully interpret the effects of the increased POD activity on quality parameters of processed fruit, investigations on the nutritive value and sensory evaluation needs to be completed. This study would determine whether the increase in activity is deleterious.

PME activity decreased as the calcium concentration in the tissue was increased. This decrease in activity in the calcium treated fruit may have been responsible for the maintenance of apple firmness in calcium treated fruit that has been shown by many researchers. PME activity reached maximum activity between two and four months in storage. Apple PME activity has been shown to reach maximum activity after 60 days in storage at 1°C (Lee, 1969), and to increase in

activity with respect to the respiratory climacteric in avocadoes (Awad, 1985). The higher activity observed after four months in storage may have indicated the climacteric peak of these apples. PME activity at the highest calcium concentration changed very little during storage and may indicate structural binding via calcium bridging of the majority of pectic polymers, resulting in the inability of PME to act on pectic polysaccharide chains.

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APPENDIXES

APPENDIX A

DATA AND STATISTICS

Table A.1: CO_2 and Ethylene production rates of 'Golden Delicious' (GD) and 'Delicious' (D) apples as measured by gas chromatography.

Cultivar	Year	Day	Tmt	CO_2	C_2H_4
			(%$CaCl_2$)	($mgCO_2 \cdot kg^{-1} \cdot hr^{-1}$)	($\mu l \cdot kg^{-1} \cdot hr^{-1}$)
GD (7.94 cm)	87	1	0	17.9 ± 3.6	4.2 ± 0.8
GD "	87	3	0	31.2 ± 4.0	13.2 ± 3.0
GD "	87	90	0	109.2 ± 11.2	84.8 ± 7.6
GD "	87	90	1	92.0 ± 11.6	81.6 ± 11.2
GD "	87	90	2	66.0 ± 15.2	60.8 ± 13.2
GD "	87	90	3	55.2 ± 15.6	72.0 ± 7.6
GD "	87	90	4	37.6 ± 36.4	74.4 ± 4.4
GD "	88	180	0	150.8 ± 28.0	197.6 ± 57.6
GD "	88	180	1	134.4 ± 16.4	106.8 ± 40.0
GD "	88	180	2	126.8 ± 19.2	157.6 ± 51.2
GD "	88	180	3	106.2 ± 6.0	100.4 ± 51.6
GD "	88	180	4	112.0 ± 9.2	75.2 ± 35.6
D (8.26 cm)	87	1	0	20.6 ± 1.5	4.3 ± 0.3
D "	87	3	0	20.0 ± 1.3	4.3 ± 0.3
D "	87	5	0	20.3 ± 1.5	4.5 ± 0.3
D "	87	90	0	--	121.3 ± 0.6
D "	87	90	2	--	79.6 ± 20.4
D "	87	90	4	--	61.6 ± 5.6
D "	87	90	6	--	53.2 ± 6.4
D "	87	90	8	--	55.2 ± 8.0
D "	87	180	0	--	20.0 ± 5.6
D "	88	180	2	--	20.0 ± 4.4
D "	88	180	4	--	14.0 ± 0.4
D "	88	180	6	--	15.6 ± 1.3
D "	88	180	8	--	17.2 ± 2.4
GD (7.94 cm)	88	1	0	--	0.1 ± 0.0
GD "	88	3	0	--	0.1 ± 0.0
GD "	88	60	0	--	121.9 ± 11.3
GD "	88	60	1	--	101.5 ± 18.9
GD "	88	60	2	--	88.2 ± 20.9
GD "	88	60	3	--	65.4 ± 32.8
GD "	88	60	4	--	35.8 ± 41.9
GD "	88	61	0	--	170.4 ± 8.8
GD "	88	61	1	--	141.9 ± 7.8
GD "	88	61	2	--	118.8 ± 15.4
GD "	88	61	3	--	86.1 ± 43.2
GD "	88	61	4	--	44.2 ± 49.8

Table A.1 cont:

Cultivar		Year	Day	Tmt ($\%CaCl_2$)	CO_2 ($mgCO_2 \cdot kg^{-1} \cdot hr^{-1}$)	C_2H_4 ($\mu l \cdot kg^{-1} \cdot hr^{-1}$)
GD	"	89	135	0	--	211.9 ± 26.8
GD	"	89	135	1	--	272.6 ± 47.8
GD	"	89	135	2	--	232.0 ± 43.7
GD	"	89	135	3	--	195.6 ± 92.5
GD	"	89	135	4	--	189.1 ±117.6
GD	"	89	136	0	--	359.3 ± 65.5
GD	"	89	136	1	--	363.2 ± 65.3
GD	"	89	136	2	--	373.3 ± 65.6
GD	"	89	136	3	--	277.9 ±116.1
GD	"	89	136	4	--	244.0 ±138.8
GD	"	89	179	0	--	297.23 ± 60.23
GD	"	89	179	1	--	313.94 ± 54.25
GD	"	89	179	2	--	280.45 ± 71.25
GD	"	89	179	3	--	226.73 ± 48.93
GD	"	89	179	4	--	210.79 ±119.12
GD	"	89	180	0	--	518.59 ± 53.51
GD	"	89	180	1	--	429.14 ± 86.98
GD	"	89	180	2	--	407.86 ±105.30
GD	"	89	180	3	--	328.93 ±152.38
GD	"	89	180	4	--	291.95 ±159.52

Table A.2: 'Golden Delicious' (GD), and 'Delicious' (D) applesauce color readings (Hunter Colorimeter) 48 hours after processing.

Cultivar	$CaCl_2$ (%)	Calcium ($\mu g\ g\ dry\ wt^{-1}$)	Storage (mo.)	'L'	'a'	'b'
GD	0	193	0	43.9 ± 1.2	-2.8 ± 0.5	16.9 ± 1.0
GD	0	193	3	46.1 ± 1.2	-2.0 ± 0.5	20.3 ± 1.0
GD	1	687	3	44.5 ± 1.8	-2.6 ± 1.3	20.1 ± 1.1
GD	2	1676	3	40.3 ± 1.2	-0.5 ± 0.2	19.5 ± 0.6
GD	3	2050	3	44.2 ± 4.0	-3.3 ± 0.6	21.0 ± 1.5
GD	4	2250	3	44.5 ± 3.5	-2.8 ± 1.2	22.0 ± 1.7
GD	0	193	6	43.0 ± 0.8	-2.1 ± 0.5	19.3 ± 1.0
GD	1	687	6	44.4 ± 0.9	-2.8 ± 1.6	20.5 ± 0.7
GD	2	1676	6	41.5 ± 2.8	-0.9 ± 1.9	19.4 ± 0.6
GD	3	2047	6	39.0 ± 8.5	-0.4 ± 1.5	18.3 ± 1.4
GD	4	2250	6	40.5 ± 4.1	-0.5 ± 2.1	18.0 ± 3.4
D	0	245	0	44.9 ± 1.8	-2.7 ± 1.1	17.5 ± 0.9
D	0	245	3	45.3 ± 1.9	-0.7 ± 1.9	17.7 ± 0.8
D	2	496	3	43.6 ± 3.5	-0.3 ± 3.5	16.9 ± 0.5
D	4	638	3	43.5 ± 1.9	-0.7 ± 1.9	17.1 ± 1.4
D	6	1178	3	42.1 ± 1.6	-1.9 ± 1.6	15.5 ± 0.8
D	8	1913	3	40.0 ± 1.7	-0.9 ± 1.9	15.8 ± 0.8
D	0	245	6	41.8 ± 4.2	0.7 ± 0.7	17.0 ± 0.2
D	2	496	6	38.1 ± 4.3	1.4 ± 1.3	16.1 ± 1.7
D	4	638	6	40.1 ± 0.2	0.4 ± 0.7	16.0 ± 0.1
D	6	1178	6	39.1 ± 1.4	0.2 ± 0.2	15.5 ± 0.7
D	8	1913	6	34.9 ± 2.8	1.2 ± 1.1	14.4 ± 0.4

Table A.3: Consistency and free liquid of 'Golden Delicious' (GD) and 'Delicious' (D) applesauce using the 'Bostwick' consistometer.

Cultivar	Tmt	Calcium* ($\mu g \cdot g^{-1}$)	Storage (mo)	Consistency (cm 30 sec^{-1})	Free Liquid (cm 30 sec^{-1})
Golden	0%	193.0	0	1.5 ± 0.1	0.0 ± 0.0
Golden	0%	193.0	3	1.9 ± 0.2	0.0 ± 0.0
Golden	1%	687.0	3	1.6 ± 0.1	0.0 ± 0.0
Golden	2%	1676.0	3	0.7 ± 0.1	0.4 ± 0.4
Golden	3%	2047.0	3	0.5 ± 0.2	0.5 ± 0.4
Golden	4%	2250.0	3	0.9 ± 0.2	1.8 ± 1.0
Golden	0%	193.0	6	0.6 ± 0.2	0.0 ± 0.0
Golden	1%	687.0	6	1.6 ± 0.4	0.1 ± 0.0
Golden	2%	1676.0	6	1.0 ± 0.3	1.8 ± 0.2
Golden	3%	2047.0	6	2.5 ± 0.4	2.9 ± 0.6
Golden	4%	2250.0	6	1.6 ± 0.2	1.7 ± 0.4
Del	0%	245.0	0	1.7 ± 0.2	0.0 ± 0.0
Del	0%	245.0	3	0.7 ± 0.1	0.0 ± 0.0
Del	2%	496.0	3	0.7 ± 0.1	0.0 ± 0.0
Del	4%	638.0	3	0.5 ± 0.1	0.0 ± 0.0
Del	6%	1178.0	3	0.9 ± 0.3	1.2 ± 0.3
Del	8%	1913.0	3	0.7 ± 0.1	0.3 ± 1.7
Del	0%	245.0	6	0.5 ± 0.1	0.0 ± 0.0
Del	2%	496.0	6	0.6 ± 0.2	0.0 ± 0.0
Del	4%	638.0	6	0.7 ± 0.1	0.0 ± 0.0
Del	6%	1178.0	6	0.5 ± 0.1	0.3 ± 0.6
Del	8%	1913.0	6	0.9 ± 0.9	1.6 ± 2.0

*Calcium based on dry weight.

Table A.4: 'Golden Delicious' (GD) and 'Delicious' (D) apple juice color readings (Hunter Colorimeter) one hour after processing.

Cultivar	$CaCl_2$ (%)	Calcium ($\mu g \cdot g^{-1}$)*	Storage (mo.)	'L'	'a'	'b'
G.D.	0%	193	0	74.6 ± 2.0	-3.4 ± 0.1	10.1 ± 1.0
G.D.	0%	193	3	67.2 ± 0.3	-2.7 ± 0.3	17.6 ± 1.8
G.D.	1%	687	3	72.2 ± 0.3	-1.4 ± 0.3	6.9 ± 0.9
G.D.	2%	1676	3	71.3 ± 0.9	-2.0 ± 0.9	7.5 ± 2.6
G.D.	3%	2047	3	70.8 ± 0.2	-2.1 ± 0.2	8.4 ± 1.3
G.D.	4%	2250	3	73.0 ± 0.4	-1.5 ± 0.4	5.6 ± 0.8
G.D.	0%	193	6	72.2 ± 1.9	-3.6 ± 1.9	9.8 ± 6.8
G.D.	1%	687	6	73.8 ± 0.7	-3.3 ± 0.7	5.3 ± 1.5
G.D.	2%	1676	6	74.8 ± 0.5	-3.1 ± 0.5	2.4 ± 1.5
G.D.	3%	2047	6	75.4 ± 0.5	-3.0 ± 0.5	2.1 ± 0.7
G.D.	4%	2250	6	75.5 ± 0.5	-3.0 ± 0.5	1.8 ± 0.3
D.	0%	245	0	68.6 ± 0.5	-4.5 ± 1.0	20.9 2.5
D.	0%	245	3	74.1 ± 0.7	-4.1 ± 1.6	12.4 4.5
D.	2%	496	3	74.8 ± 0.2	-2.8 ± 0.9	8.5 2.3
D.	4%	638	3	75.0 ± 0.8	-2.8 ± 1.5	8.6 3.9
D.	6%	1178	3	75.1 ± 0.2	-2.1 ± 0.8	6.5 2.1
D.	8%	1913	3	75.7 ± 0.0	-1.2 ± 0.6	5.1 1.3
D.	0%	245	6	71.3 ± 0.9	-5.1 ± 0.4	18.3 9.3
D.	2%	496	6	73.0 ± 0.4	-3.9 ± 0.3	11.9 2.6
D.	4%	638	6	73.0 ± 0.3	-3.6 ± 0.6	11.5 1.9
D.	6%	1178	6	73.4 ± 0.2	-2.8 ± 0.1	8.8 1.5
D.	8%	1913	6	73.7 ± 0.3	-2.6 ± 0.1	7.3 0.7

*Calcium based on dry weight basis.

APPENDIX B

PROCEDURES

TOTAL PHENOLS PROCEDURE FOR APPLES

Chemicals Required:

- -Methanol
- -Folin-Denis reagent
- -Sodium Carbonate (saturated)
- -Gallic acid (as standard)

Procedure

1. Weigh 5 g apple tissue and place in tissue grinder and immediately pour 5 ml of methanol and grind. Pour off liquid and save. Repeat two times for a total of approximately 15 ml liquid.

2. Filter with Whatman #2 filter paper.

3. To 0.5 ml of filtrate, add 8 ml D.D. H_2O and 0.5 ml Folin-Denis reagent and vortex.

4. Three min later add 1.0 ml sodium carbonate and vortex.

5. One hr later measure absorbance at 725nm.

6. Prepare standard curve using gallic acid.

Reference: Swain, T. and W.E. Hillis. 1959. The phenolic constituents of Prunus domestica I. The quantative analysis of phenolic constituents. J. Sci. Food Agri. 100:63-68.

ANALYSIS PROCEDURE FOR CALCIUM

Procedure

1. Weigh 0.5 g dried ground sample into 18mm test tube.
2. Ash sample for 4 hr to overnight at 500°C.
3. Add up to 4 ml 2N HCL (add enough to dissolve sample)
4. Vortex
5. Dilute with D.D. H_2O water to exactly 10 ml (depending on amount of 2N HCL required to dissolve).
6. Using the auto-dilutor, pick up 1 ml and dispense in clean 16mm tubes.
7. Add 1 ml 5% lanthium chloride (or 0.1 ml 50% lanthium chloride) for a 0.5% lanthium chloride solution.
8. Additional dilutions may be necessary to meet specifications posted for A.A. Be sure to keep track of dilutions, and adjust for lanthium chloride in solution.

ASCORBIC ACID DETERMINATION

Reagents

-Extraction Solution: Metaphosphoric acid/acetic acid solution
In a 500 ml volumetric: 200 ml H_2O + 40 ml acetic acid. Add 15 g HPO_3 pellets and dissolve with shaking or stirring. Bring to 500 ml. volume with H_2O and filter rapidly through fluted paper (#2) into 500 ml Erlenmeyer. (Store in refrigerator, renew weekly)

-Indolephenol standard dye: Use Sigma dye
In 500 ml volumetric: 125ml H_2O + 105 mg. Sodium Bicarbonate. Add 125 mg dye and dissolve by shaking vigorously. When dye has dissolved bring to 500 ml. volume with H_2O and filter through fluted paper (#4) into a dark glass bottle. Keep closed, out of sunlight, and store in refrigerator. (To check activity, add 5 ml extraction solution containing excess ascorbic acid to 15 ml. dye. If not practically colorless, prepare fresh dye.).

-Ascorbic acid standard: Fresh each day
Place 50 mg. of USP ascorbic acid reference standard in 50 ml. volumetric. Bring to volume with extraction solution immediately before use.

Standardization of dye (each day)

In 100 ml beaker: 5 ml. extraction solution + 2 ml ascorbic acid standard solution, (2 or 3 reps.). Titrate rapidly with dye until light, but distinct, rose pink persists for 25 secs. Amount of dye may exceed 15 ml. but should not vary widely from day to day. Dye required should agree closely amoung reps. Blank: 7 ml extraction solution + vol of H_2O similar to volume of dye used in above titrations. Should require only 1-4 drops of dye for pink color. Subtract blank from standard solution and calculate dye value: 2 mg ascorbic acid/1 ml dye = 2 mg/___ml dye above, used as dye value in later calculations.

Extraction

1. Bring all solutions to room temperature.

2. Place 20 ml extraction solution in a beaker. Add 10 g apple tissue. Add another 10 ml extraction solution. Blend for 2 min. Centrifuge for 5 min at 2500 rpm.

3. Pipette 10 ml clear solution into 25 ml beakers.

4. Titrate with standardized dye to light rose pink. Record ml dye required.

Ascorbic acid calculation

$$\text{Ascorbic acid} = D \times S \times \frac{(V_1 + V_2)}{V_3} \times \frac{100}{W}$$

D = ml dye required to titrate sample
S = dye value calculated before (mg ascorbic acid/ml dye)
V_1= ml extraction solution
V_2= ml H_2O in sample (determined by drying)
V_3= ml of sliquot titrated
W = wt. of sample

Reference: A.O.A.C. 1980. Official Methods of Analysis Association of Official Analytical Chemists. 13th Edition. Determination of ascorbic acid #43.057 p. 746. AOAC Washington DC

PPO AND POD EXTRACTION AND ACTIVITY FOR APPLE

Chemicals

-Glycine buffer (0.1M, pH 10)
-Acetone (reagent grade)
-Citrate-Phosphate buffer (0.1M-0.2M respectively, pH 6.2)
-Acetate buffer (0.1M, pH 7.0)
-Catechol (0.5M)
-Hydrogen Peroxide (0.1N)

Extraction Procedure

1. Weigh 30 g prepeeled apple tissue and blend in 60 ml of glycine buffer (0°C) for 2 min (using the Polytron).

2. Filter slurry through cheesecloth, followed by Whatman #4, then Whatman #42 filter paper

3. Pour filtrate into 120 ml of -30°C acetone and allow to stand for 2 hrs.

4. Filter precipitated protein through a Buchner funnel (under vacuum) using Whatman #42 filter paper.

5. Wash precipitated protein 3 times with 7.5 ml 0°C glycine buffer.

6. Resuspend precipitated protein in 13 ml of citrate phosphate buffer.

7. Centrifuge at 20,000g for 20 min.

8. Discard pellet and use supernatant for enzyme source.

PPO Activity

1. To 20 ml of acetate buffer, add 2 ml catechol.
2. Add 1 ml of enzyme source and read the change in absorbance (at 420nm) at 30 sec intervals for six minutes.
3. Use heated enzyme source as control.

POD Activity

1. To 20 ml of acetate buffer, add 1 ml guiaicol and 1 ml H_2O_2.
2. Add 1 ml of enzyme source and read the change in absorbance (at 485nm) at 30 sec intervals for six min.
3. Use heated enzyme source as control.

References:

Hopfinger, J.A., B.W. Poovaiah, and M.E. Patterson. 1984. Calcium and magnesium interactions in browning of 'Golden Delicious' apples with bitter pit. Scientia Horticulturae 23:345-351.

Shannon, C.T. and D.E. Pratt. 1967. Apple polyphenol oxidase activity in relation to various phenolic compounds. J. Food Sci. 32:479-483.

PME EXTRACTION AND ACTIVITY

Chemicals

-Sodium Chloride (5%, pH 7.0)
-Apple pectin (Sigma #p2157)
-Sodium Hydroxide (0.1N and 0.01N)

Procedure

1. Weigh 100 g of prepeeled apple tissue and blend in 200 ml of sodium chloride (Polytron for 2 min).

2. Bring the slurry up to pH 7.0 with 0.1N NaOH and stir at 1°C for 10 hrs.

3. Filter the slurry through Miracloth and use the filtrate as enzyme source.

4. To 5 ml of pectin, add 5 ml of enzyme source and quickly bring up to pH 7.0 with 0.1N NaOH.

5. Titrate with 0.01N NaOH to keep at pH 7.0 at 2 min intervals for a total of 10 min.

6. Activity is measure as meq. of ester hydrolyzed (or H+)/min

References:

Awad, M. 1985. Persiommon pectinmethylesterase: extraction and variation during ripening. J. Food Sci. 50:1643-1645.

TOTAL ACIDITY FOR APPLES

Procedure

1. To 10 ml of apple juice add 90 ml of D.D. H_2O.
2. Take initial acidity.
3. Titrate with NaOH intil pH reaches 7.2 and record ml required.
4. Titrate with 0.1N NaOH until acidity levels off.

Calculation

$$X = \frac{a \times T \times c \times k \times 100}{e} \times 100$$

a = volume of 0.1N NaOH used to titrate to 7.2
T = correction factor for 0.1 NaOH = 0.1
c = total volume of extract (10 ml.)
e = volume of extract taken for titration (10ml.)
k = titrimetric factor (0.0067 for malic)

Reference: AOAC 1980. Official Methods of Analysis Association of Official Analytical Chemists. 13th Edition. Determination of Titratable Acidity #22.060. with conversion to malic acid. AOAC, Washington, DC

VITA

Julie Ann Eskelson Laufmann was born Dec. 10, 1959 in Sacramento, California to Bette J. and David W. Eskelson M.D.. She graduated from Meeker High School, Meeker, Colorado in 1977. Since that time she has attended Colorado Mountain College, Glenwood Springs, Colorado, Boise State University, Boise, Idaho, and graduated with a B.S. degree in Plant and Soil Science from the University of Tennessee, Knoxville in 1987. After receiving her B.S. degree she has been working toward her Master of Science degree in Plant and Soil Science at the University of Tennessee, Knoxville. She is a member of Phi Kappa Phi and Gamma Sigma Delta Honor societies. She presently lives in Knoxville with her six year old daughter, Arielle.