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The effect of calcium on papaya fruit softening

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University of Hawaii, 1992
THE EFFECT OF CALCIUM ON PAPAYA FRUIT SOFTENING

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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ABSTRACT

Papaya (*Carica papaya* L. "Sunset") fruit growth had 2 main peak periods of growth. The highest mesocarp Ca uptake occurred in fruits less than 60 days post-anthesis possibly related to a high transpiration rate from fruit with a high surface area to weight ratio. Water and nutrition which were supplied predominantly through the phloem may result in low Ca uptake by the mesocarp 60 to 80 days post-anthesis when the mesocarp rapidly increases its FW and DW. Mesocarp Ca uptake rate increased again 100 to 140 days post-anthesis when mesocarp FW growth rate reduced and DW growth rate increased. Dramatic increases of sucrose in the mesocarp during this period may reduce water potential, which may bring xylem water and Ca into the mesocarp. The Ca concentration in the mesocarp was not different from the peduncle to the blossom end probably because the vascular bundles carrying Ca pass longitudinally through the whole tissue.

Soil Ca application sometimes increased mesocarp Ca concentration. Factors such as soil type, levels of other minerals in the soil, root growth, and rainfall might affect the uptake of Ca by papaya plants. Mesocarp Ca concentration fluctuated significantly throughout the year. Potassium and N (urea) fertilization decreased mesocarp Ca concentration. Attempts to increase mesocarp Ca content by spraying CaCl₂ onto papaya during growth and development and by postharvest dipping of peduncle into CaCl₂ were unsuccessful.

Mesocarp Ca concentration was positively correlated to the firmness of ripe papaya fruit. There were no correlations between the firmness of ripe fruits and Mg
concentration, or K concentration, but there were correlations between fruit firmness and the ratio of Ca concentration to Mg concentration, or to K concentration, or to Mg+K concentrations. The involvement of K or Mg appeared to be related to Ca, rather than by the effects of these minerals. Mesocarp Ca content of 150 μg g⁻¹ FW or above gave maximum delay in softening and firmest flesh.

Both the proportion of different extractable fractions and molecular size range of papaya pectin and hemicellulose were altered during fruit ripening. The pectin molecular size declined and the solubility of pectin in Ca chelating agent CDTA and Na₂CO₃ solutions increased. The molecular size range of hemicellulose also changed with an increase of solubility of hemicellulose in KOH fractions. These changes may lead to disruption and loosening of the papaya fruit cell wall structure during fruit ripening. These results suggested that pectin hydrolysis and the modification of hemicellulose were involved in papaya fruit softening and may be influenced by Ca content.

Infiltrating mesocarp tissue with CaCl₂ effectively slowed the softening rate and reduced the rates of respiration and ethylene production. Treatment with Ca chelating agent EGTA hastened softening rate and increased rates of respiration and ethylene production. Calcium probably slowed the mesocarp softening through strengthening the cell wall structure, inhibiting cell wall degrading enzyme activity, and/or reducing the metabolic rate of ripening process. Infiltration of mesocarp tissue with MgCl₂ and KCl had no effect on softening.
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<tr>
<td>ACC</td>
<td>1-aminocyclopropane-1-carboxylate</td>
</tr>
<tr>
<td>CDTA</td>
<td>cyclohexane trans-1,2-diaminetetra-acetate</td>
</tr>
<tr>
<td>CWM</td>
<td>cell wall material</td>
</tr>
<tr>
<td>DFS</td>
<td>Donnan free space</td>
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<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<tr>
<td>DPH</td>
<td>1,6-diphenyl-2,3,5-hexatriene</td>
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<tr>
<td>DW</td>
<td>dry weight</td>
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<td>EDTA</td>
<td>N,N'-1,2-ethanediyl-bis-[N-(carboxymethyl)glycine]</td>
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<td>EGTA</td>
<td>ethyleneglycol-bis-(β-aminoethyl ether)N,N,N',N'-tetraacetic acid</td>
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<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>FS</td>
<td>free space</td>
</tr>
<tr>
<td>FW</td>
<td>fresh weight</td>
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<tr>
<td>IP₃</td>
<td>inositol-1,4,5-triphosphate</td>
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<td>MES</td>
<td>2-(Morpholino)ethanesulfonic acid</td>
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<tr>
<td>PAW</td>
<td>phenol: acetic acid: water</td>
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<td>PG</td>
<td>poly(1,4-α-D-galacturonide)glycanohydrolase, EC 3.2.1.15</td>
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<td>PME</td>
<td>pectin methylesterase</td>
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<tr>
<td>Quin-2</td>
<td>(2-[(2-bis-[carboxymethyl]amino-5-methylphenooxy)methyl]-6-methoxy-8-bis-[carboxymethyl]aminoquinoline)</td>
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<tr>
<td>TSS</td>
<td>total soluble solid</td>
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<td>WLF</td>
<td>wall loosening factor</td>
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CHAPTER 1.
INTRODUCTION

Introduction

The papaya is a popular fruit crop throughout the tropics (Storey, 1969) and one of the major economic crops in Hawaii (Hawaii Dept. Agr. 1991). The Hawaii papaya industry had an income of $15 million in 1990 (Hawaii Dept. Agr. 1991). Most U.S. papayas are produced in Hawaii (Cappellini et al., 1988). However, shipments of papaya fruits to the New York Market from Hawaii have been found to have overripe and abnormally soft fruits (Cappellini et al., 1988).

Calcium plays an important role in plant growth and development (Roux and Slocum, 1982; Hepler and Wayne, 1985; Kauss, 1987; Poovaiah et al., 1988). Calcium is involved in a number of physiological processes, such as the structure of cell walls, the permeability of membranes, and the activities of enzymes, as well as a second messenger in cells. These Ca effects are intimately related to fruit quality and senescence (Poovaiah et al., 1988). Considerable evidence has been shown that increased Ca level delays fruit softening and improve fruit quality: apple (Poovaiah, 1986; Conway and Sams, 1987), tomato (Wills and Tirmazi, 1979) and avocado (Eaks, 1985). The response of fruit to temperature stress may be modified by fruit Ca level. Calcium inhibits the heat-induced membrane damage in beet root (Toprover and Glinka, 1976) and chilling injury in avocado (Chaplin and Scott, 1980). A number of postharvest disorders have been found to be related to low Ca
levels: bitter pit in apple (Drake et al., 1966), blossom-end rot in tomato (Evans and Troxler, 1953), and end spot in avocado (Haas, 1936). Increased Ca levels in fruits achieved by pre- and postharvest treatment can reduce the occurrence of disorders and decay (Shear, 1975; Ferguson and Watkins, 1989; Conway et al., 1991).

Sporadically, commercial papaya packing sheds in Hawaii have reported batches of fruit that ripened very rapidly. This so called "soft fruit" disorder leads to shipment and possibly higher disease losses in papaya. The inability to predict the occurrence of this disorder adds to uncertainty and limits the range of options available to deal with this fruit disorder. From limited survey data, soft papaya fruit is possibly related to low Ca content in the mesocarp or an imbalance in the levels of Ca, K, and possibly Mg in the fruit (Paull, 1987). High N fertilizer application may also play a role. The major objective of this study was, therefore, to determine the role of Ca in postharvest storage and ripening characteristics of papaya fruit, to reduce the economic loss and improve fruit quality.

**Literature review**

**Calcium and fruit ripening**

Calcium can slow the rate of fruit ripening and improve the quality of fruits as indicated that the fruit with higher Ca levels have a lower fruit respiration rate and ethylene production (Liberman and Wang, 1982; Ferguson, 1983; Conway and Sams, 1987; Glenn et al., 1988), slower fruit softening rate (Bangerth et al., 1972; Cooper and Bangerth, 1976; Conway and Sams, 1987; Glenn et al., 1988; Abbott et al., 1989; Stow, 1989), reduced color change rate (Ferguson, 1984; Glenn et al., 1988;
Klein et al., 1990), lower solute leakage (van Goor, 1968; Rousseau et al., 1972; Cooper and Bangerth, 1976; Poovaiah and Leopold, 1976; Simon, 1978), and reduced the senescent breakdown and decay (Weis et al., 1980; Conway, 1982; Conway and Sams, 1987; Conway et al., 1991).

Apple fruits with naturally higher concentrations of Ca or with Ca content artificially raised during development on the tree or after harvest may ripen at a slower rate than those with lower Ca concentration (Cooper and Bangerth, 1976; Conway and Sams, 1987; Abbott et al., 1989). In mature apples, Ca decreases the overall rate of respiration and the activity of malic enzyme, and stimulates higher levels of protein synthesis (Faust and Shear, 1972). These metabolic effects require at least 0.7 micromole of Ca per gram fresh weight of the fruit (Faust and Shear, 1972). Infiltration with CaCl$_2$ increases the strength of apple fruit tissue immediately and during storage (Conway and Sams, 1987; Abbott et al., 1989). The effect of Ca is less pronounced in tissues that contain adequate amount of Ca for maintaining cell integrity (Conway and Sams, 1987). Supplemental Ca not only increases firmness retention during the storage, but also induces patterns of textural change different from those that occur under the influence of endogenous Ca alone (Abbott et al., 1989).

Avocados with low levels of Ca ripen more rapidly than those with higher levels of Ca and produce more ethylene (Tingwa and Young, 1974). Vacuum infiltrating avocados with water and 0.05, 0.1, 0.2 and 0.3 M Ca solution results in delaying fruit ripening (Eaks, 1985). Days to ripen are increased and respiratory
rates and rate of ethylene evolution are reduced as the Ca concentration is increased (Eaks, 1985). At higher Ca concentrations (0.4 and 0.5 M), no detectable climacteric occurs, basically no ethylene is produced and fruits fail to ripen. The response to exogenous ethylene in fruits treated with Ca is also reduced (Eaks, 1985).

Cherry fruit firmness is increased but fruit size is decreased by increasing the amount of applied Ca (Facteau et al., 1987). Mango fruits treated with Ca result in ripening being delayed by one week compared to control fruits (Tirmazi and Wills, 1981). Dipping mature unripe pears in Ca solution under reducing pressure and subsequent storage at 20 °C delay ripening by up to 40% of the time taken for control fruit to ripen (Wills et al., 1982). Peach storage life can be extended from two to six days at room temperature (32 °C) by a field spay of Ca nitrate (Singh et al., 1982; Lill et al., 1990). Foliar application of Ca prolongs the self-life of strawberries, as indicated by a delay in accumulation of sugars, a decrease in organic acids, an increase in color, and a reduction of mold development (Cheour et al., 1991).

The mechanism of Ca action in reducing the rate of fruit ripening is complex. Effective concentrations used in artificially raising Ca content in fruits are usually much higher than those found in intact fruit. Although high concentrations of Ca are needed to move metabolically active Ca to the site of its action, most of the Ca that accumulates on exchange sites is probably metabolically inactive (Faust and Klein, 1974). Magnesium can exchange with accumulated Ca in tissue by 90%
without changing the metabolic effect of Ca (Faust and Klein, 1974). The uptake of Ca into cytoplasm is extremely restricted (Evans et al., 1991). The Ca concentration in the cytoplasm is $10^{-6}$ to $10^{-7}$ M, at least 1000 times lower than that in the apoplast (Evans et al., 1991). It has been suggested that the Ca effect on ripening may arise from an influence of Ca on substrate transport via alteration of membrane permeability (Bangerth et al., 1972). Calcium may improve the cell wall structural integrity and render the substrate less accessible to cell wall-degrading enzymes (Ferguson, 1984; Poovaiah et al., 1988; Conway et al., 1991). Some cell wall-degrading enzymes, such as polygalacturonase (Baldwin and Pressey, 1988) and xylanase (Ronen et al., 1991), and fragments of polysaccharide (Brecht and Huber, 1988; Priem and Gross, 1992) can induce the production of ethylene. Ethylene may correlate and accelerate the ripening process (Trewavas, 1981). Recent studies suggest that ethylene is necessary to trigger fruit ripening (Oeller et al., 1991; Klee et al., 1991).

**Calcium-related disorders**

1. Calcium movement in plants

Calcium generally does not move via the phloem but via the xylem (Bangerth, 1979; Clarkson, 1984). The critical factors for Ca movement and distribution in the plant are soil water status, transpiration, and growing tissue (Bangerth 1979, Clarkson 1984). Environmental conditions, such as temperature, relative humidity, levels of other minerals in the soil, and age of the plant affect plant Ca uptake (Shear, 1975; Clarkson, 1984; Ferguson, 1984; Kirkby and Pilbeam, 1984). Cultural
practices are important factors which influence uptake and distribution of Ca in plants, and eventually influence the incidence of Ca-related disorders (Bangerth, 1979).

Because the Casparian band has not fully developed in the apical region of roots, growing root tips appear to be the main site of Ca uptake (Robards et al., 1973; Russell and Clarkson, 1976). Restriction of root growth in Chinese cabbage increases the incidence of tipburn of the leaves (Aloni, 1986). Collards are susceptible to moisture stress because of small size of roots, thus increasing susceptibility to tipburn (Johnson, 1991). Root growth and root activity are an essential prerequisite for adequate Ca uptake and translocation. An adequate Ca concentration is needed to ensure good root growth (Marschner and Richter, 1973). However, Ca fertilization and conditions that favor root activity do not always help to reduce field Ca-deficiency disorders. Calcium fertilization shows beneficial in some instances, but does not consistently show positive effects in many other field experiments (van der Boon et al., 1966; Millaway and Wiersholm, 1979; Facteau et al., 1987). Active root growth and root activity might have opposite effects in certain circumstances. For example, vigorous root growth can enhance shoot growth, which accelerates the competition between leaves and storage organs for Ca (Jakobsen, 1979). Summer pruning, which can reduce bitter pit, reduces vegetative growth and probably diminishes root growth (Preston and Perring, 1974).

Calcium deficiency in plants seldom arises from the failure of Ca supply from the roots, but generally arises from its distribution and its allocation in mature and
growing regions of plant. There is a lack of direct cause and effect relationship between Ca in the soil and Ca in the fruits (Ferguson and Watkins, 1989). Calcium deficiency normally happens in organs and tissues with a low transpiration rate but a high demand for assimilates (Shears, 1975). Organs which undergo rapid growth are also susceptible to Ca deficiency (Collier and Tibbitts, 1982, 1983; Barta and Tibbitts, 1991). Growing tissues and transpiring leaves are two different kinds of Ca sinks, and they independently influence Ca movement (Clarkson, 1984). A shortage of water, or an irregular water supply results in reducing Ca translocation into storage organs and increases in Ca deficiency disorders. The competition for transpiration water of xylem between leaves and storage organs restricts the transport of Ca to the storage organs (Bangerth, 1979).

Concentration of Ca in the apple fruit flesh changes during fruit growth and development (Ferguson and Watkins, 1989). An initial increase in Ca concentration is associated with the period preceding the first stage of rapid increase in flesh weight (Jones et al., 1983). Calcium concentration in apple flesh declines as the fruit grows. This phenomena is due to the rate of fruit expansion being greater than the rate of mineral input, resulting in a dilution of the mineral concentration (Ferguson and Watkins, 1989). The distribution of Ca in apple fruit depends on the extent of the xylem system and fruit evaporative surface. Further distribution and redistribution are slow and may depend on further water input from both the phloem and the xylem, along with the concentration of other cations such as Mg\(^{++}\), K\(^{+}\) and H\(^{+}\). Secondary distribution or redistribution of Ca in a storage organ or
fruit is difficult to assess (Ferguson and Watkins, 1989). Some reports show Ca migration in the apple fruit during storage (Bramlage et al., 1979; Perring, 1984; Ferguson and Watkins, 1983), but these results are not easy to explain because of differential water loss or movement during the long storage periods, and also because Ca concentration variation between samples may be too great to determine changes quantitatively. The mechanism for cation movement in the detached fruit is unclear whether this movement happens as a response to changes in ion gradients, vascular water flow, or ion compartment in one site as distinct from another (Ferguson and Watkins, 1989).

During tomato fruit growth and development, Ca is imported into the fruits principally through the xylem (Ho and Adams, 1989), with the proportion of water imported to the fruit via the xylem falling from 8-15% at young stage to 1-2% at mature stage (Ho et al., 1987). Water is imported into the fruit mostly via the phloem: 85% in 2 weeks post-anthesis fruit and 98% in mature fruit (Ho et al., 1987). The daily Ca accumulation rates therefore decrease with the decline in the proportion of xylem water import rate during the fruit growth and development (Ho et al., 1987). The import of Ca into tomato fruits is also favored at night although the rate of Ca absorption and translocation from roots to shoots does not differ between day and night (Tachibana, 1991). When oxygen supply to the roots is limited at night, the absorption and translocation of Ca from roots to shoots is greatly inhibited (Tachibana, 1991). Increases in salinity of the nutrient solution decrease the translocation of Ca from roots to shoots (Ehret and Ho, 1986; Ho and
Adams, 1989). Maintaining relatively humid condition only at night favors transport of Ca into the fruit, due to the development of root pressure flow (Banuelos et al., 1985). This evidence suggests that root pressure at night may be very important in providing adequate Ca to tomato fruits.

2. Calcium-related disorders in fruits and vegetables

The cause of Ca deficiencies can be divided into two major categories (Ferguson and Drobak, 1988): 1) A direct result of low Ca\(^{++}\) or an imbalance between Ca\(^{++}\), and Mg\(^{++}\), K\(^{+}\), H\(^{+}\), caused by insufficient Ca\(^{++}\) on cell structure and function immediately as Ca\(^{++}\) reaches inadequate concentrations, or an imbalanced nutrition occurring with relatively high concentrations of cations such as Mg\(^{++}\), K\(^{+}\), and H\(^{+}\) and low concentration of Ca\(^{++}\); and 2) The predisposition of tissue to dysfunction with low Ca\(^{++}\) concentrations. In this case, the tissue may not show symptoms until certain physiological triggers are set off. A example is bitter pit in apple fruit, where the symptoms may be delayed, reduced, or prevented by factors that delay and reduce ripening. The tissue does not necessarily have an inadequate Ca concentration, but it has the potential to develop a Ca deficiency disorder under certain physiological conditions. This fact implies that a critical concentration of Ca in some pool is necessary to drive specific metabolic events, or to prevent disorders.

Shear (1975) listed a number of Ca deficiency disorders associated with a localized inadequacy of Ca among the fruits and vegetables. These Ca-related disorders include bitter pit (Drake et al., 1966), cork spot (Shear, 1972), cracking (Shear, 1971), internal breakdown (Bangerth et al., 1972), and water core (Perring,
1968) of apples; end spot of avocados (Haas, 1936); hypocotyl necrosis of beans (Shannon et al., 1967); internal browning of brussels sprouts (Maynard, 1972); internal tipburn of cabbage (Maynard et al., 1965) and of chinese cabbage (Hori et al., 1960); cavity spot and cracking of carrots (Maynard et al., 1961); blackheart of celery (Geraldson, 1954); cracking of cherries (Bullock, 1952); tipburn of lettuce (Thibodeau and Minoti, 1969); soft nose of mango (Young and Minor, 1961); cork spot of pears (Woodbridge, 1971); blossom-end rot of peppers (Hamilton and Ogle, 1962); cracking of prunes (Cline and Tehrani, 1973); leaf tipburn of strawberry (Mason and Guttridge, 1974); black seed (Estabrooks and Tiessen, 1972), blossom-end rot (Evans and Troxler, 1953) and cracking (Dickinson and McCollum, 1964) of tomatoes; and blossom-end rot of watermelons (Waters and Nettles, 1961).

In apple, the most studied system, Ca is the most variable element among samples within seasons and is the element most consistently relating negatively with senescent breakdown, spot, and scald (Bramlage et al., 1974). Bitter pit in apple occurs mostly during postharvest storage. The primary symptom of bitter pit is a discrete pitting of cortical flesh; the pits become brown and desiccated with time (Ferguson and Watkins, 1989). The symptom of bitter pit shows predominantly in the outer cortex of the fruit. The distribution of Ca in apple fruit is not uniform, with the highest Ca concentration in the skin and in the core, and the lowest in the cortex. The occurrence of bitter pit is principally determined by fruit mineral status, especially fruit Ca content. Analysis of cortex tissue provides a reliable relationship between Ca content and disorder incidence (Ferguson et al., 1979; Ferguson and
Increasing fruit Ca level by pre- or post-harvest Ca treatment successfully reduces the incidence of bitter pit in apples (Perring, 1986; Ferguson and Watkins, 1989; Hewett and Watkins, 1991).

Mineral composition of apples has been correlated with internal breakdown, positively for K and Mg, but negatively for Ca (Sharples, 1967; Perring, 1968). Internal breakdown is partially reduced by orchard sprays of Ca (Martin et al., 1969). Dipping apples in CaCl₂ solution prior to storage (cold or ambient temperature) greatly reduces the development of internal breakdown and retards flesh softening and senescence (Bangerth et al., 1972; Faust and Shear, 1972; Scott and Wills, 1977; Conway and Sams, 1987). Infiltration of fruit with sorbitol produces internal breakdown symptoms, whereas glucose causes a similar browning coloration, and sucrose does not cause visible damage. Infusing calcium inhibits internal breakdown symptoms and browning, retards respiration, reduces the metabolism of endogenous substrates, and increases the oxidation of exogenous substrates. These results suggest calcium inhibits internal breakdown by enhancing the uptake and compartment of substrates, particularly sorbitol. If sorbitol causes internal breakdown, then Ca may simply favor its uptake and metabolism to disallow its involvement in reactions related to internal breakdown (Bangerth et al., 1972).

Blossom-end rot, the physiological disorder of tomatoes, is associated with low fruit Ca concentration (Wiersum, 1966; Ward 1973; Ehret and Ho, 1986). Blossom-end rot is also enhanced by water deficits (Ward, 1973), by increasing the osmotic potential of the nutrient solution (Ho and Adams, 1989), or by reducing
transpiration rate during the day (Wiersum, 1966; Gerard and Hipp, 1968; Bradfield and Guttridge, 1984; Banuelos et al., 1985).

A similar disorder to blossom-end rot in tomato also occurs in cucumber fruits (Frost and Kretchman, 1989). A water soaked area with brown necrotic spots develops in Ca deficient fruit in the distal end of the fruit pericarp. There is a Ca gradient within the fruit. The proximal peduncle portion contains the highest level of Ca, while the distal section contains the lowest (Frost and Kretchman, 1989). Calcium plays an important role in susceptibility of stored avocados to chilling injury (Chaplin and Scott, 1980). Chilling injury symptom consistently occurs first in the distal end of the fruit while the proximal remains apparently normal. Analysis shows that the Ca concentration of individual fruit is always highest in the proximal end and lowest in the distal end. Furthermore, severity of chilling injury in the fruit is significantly reduced when the fruit is infiltrated with CaCl₂ solution after harvesting before storage at chilling temperature.

Tipburn in lettuce is a Ca-related disorder. The symptoms are collapse and necrosis of the apex and margins of actively growing leaves (Collier and Tibbitts, 1982). Leaves of the plants wholly or partly enclosed as a result of heading are particularly susceptible to the disorder (Collier and Tibbitts, 1982). These leaves do not freely transpire and contain relatively low levels of Ca (Collier and Huntington, 1983). A more detailed study indicates that tipburn in lettuce results from a localized deficiency of Ca (Barta and Tibbitts, 1991). The area of the leaves expressing tipburn symptoms contains Ca concentrations of 0.2 to 0.3 mg Ca g⁻¹ DW,
whereas the non-symptom area contains 0.4 to 0.5 mg Ca g\(^{-1}\) DW (Barta and Tibbitts, 1991). The critical Ca concentration for tipburn development is 0.4 mg g\(^{-1}\) DW, and is similar to the threshold for the development of blossom-end rot in tomato (Cerda et al., 1979), and of bitter pit in apple (Fallahi et al., 1988).

3. The relationship between Ca and other minerals

Although Ca is found to be the prime factor in bitter pit development, other minerals, such as K, Mg, N, P, and B, have been implicated (Faust and Shear, 1968). The involvement of these minerals seems to be related to Ca (Ferguson and Watkins, 1989). Garman and Mathis (1956) suggested that the balance between Ca and Mg or K might be as important as the Ca content itself. Applications of Mg and K salts to fruit on the tree cause bitter pit or bitter pit-like symptoms (Cooper and Bangerth, 1976). However, Perring (1986) suggested that if the Ca content was high enough (about 5 mg per 100 g FW), a high concentration of Mg and K did not result in bitter pit development. Magnesium and K have little harmful effect on the development of disorders if Ca concentrations in the tissues are high (Cooper and Bangerth, 1976; Martin et al., 1975). The replacement of Ca by other cations in membranes is significant only when the Ca concentration falls below a "critical" level (Bangerth, 1979).

In "Jonathan" apples, the incidence of senescent breakdown after 5 months of storage at 0°C is not correlated with total or water-soluble Ca content at harvest, but water-extractable Ca and K/Ca from stored fruit are negatively and positively correlate with the incidence of senescent breakdown, respectively (Saks et al., 1990).
The balance between Ca and K or Mg may sometimes explain the results, but the ratios of these elements in various combinations do not usually provide a better predictive measure than Ca alone (Ferguson and Watkins, 1989). The mechanism of the relationship between Ca and Mg, K is unknown. In most plant tissues, the high concentrations of Mg\(^{++}\), K\(^+\), and H\(^+\) are able to replace Ca and are potentially antagonistic to the effect of Ca\(^{++}\) (Bangerth, 1974), thus resulting in increased permeability of the membrane. In apple fruit tissue, Ca and Mg can exchange for each other, and Mg can reduce Ca uptake into the tissue (Ferguson and Watkins, 1981a, b). It is thought that high concentrations of these cations may compete with Ca for binding sites on the cell walls or membranes, and inhibit Ca uptake into cells, which leads to the development of Ca deficiency symptoms (Ferguson and Watkins, 1989).

The relationship between N and Ca is complicated. Some experimental results show that N increases bitter pit (Faust and Shear, 1968), but others indicate that long term applications of N resulted in reduced bitter pit and higher Ca concentrations in apple fruits (Goode et al., 1978). The intensity of N metabolism and the rate of protein synthesis appear to control the import of N by different parts of the plant (Mengel and Kirkby, 1982). Nitrogen usually promotes vegetative growth, that may compete for Ca with storage organs. Nitrogen may have its influence on crop size, fruit size, and fruit/shoot ratio, and thus interfere with Ca level in the fruits (Ferguson and Watkins, 1989). Fertilization of NH\(_4\)-N results in restricted uptake of metabolic cations, especially Ca, reduced growth, causing root
injury, and increased root resistance to water uptake along with decreased leaf water potential (Pill and Lambeth, 1977; Pill et al., 1978).

Calcium and cell wall structure

1. Calcium and cell wall structure

The plant cell wall consists of two major fractions: cell wall carbohydrate and protein. The carbohydrate fraction is 90-95% of the cell wall and protein 5-10% (Gross, 1990). From studies of cell walls isolated from suspension-cultured sycamore cells, Albershiem and his coworkers proposed one of the most developed models of dicotyledon plant cell wall structure (Keegstra et al., 1973): xyloglucan (hemicellulose) binds to the cellulose microfibrils by non-covalent bonds, and coheres to the pectin matrix polymers by covalent bonds. Albersheim's group subsequently modified the model with respect to polysaccharide-protein linkages (Albersheim, 1978) and the degree of xyloglucan-arabinogalactan interlinkage (Darvill et al., 1980). Albersheim's model of cell wall structure has not been found to be universal in dicotyledon primary cell walls. Carpita (1987) proposed an alternative model of primary cell wall with cellulose microfibrils covered by hemicellulose being tightly held through hydrogen bonds. This framework is embedded in a gel matrix of pectic polysaccharides that is cross-linked in part by Ca++. Side-groups of neutral sugars are attached to the chains of pectic polysaccharide. In the study of pea cell wall structure, Talbott and Ray (1992) suggested non-covalent associations existed among the pectic polysaccharides, between pectin and extensin, and between hemicellulose and cellulose microfibrils.
The dicotyledon primary wall contains about 35% pectic polysaccharides (Darvill et al., 1980; Moore, 1986) that are particularly abundant in the middle lamellar region (Hall, 1976). The matrix of pectic polysaccharides consists of a rhamnogalactosyluronic backbone with covalently linked side chains of arabinose and galactose (Dey and Brinson, 1984). The pectin gel is created by cross-bridging with Ca\(^{++}\) and the (1-4)-\(\alpha\)-D-galactosyluronic acids, forming an "egg-box" rigid structure (Grant et al., 1973; Rees, 1977). Grant et al., 1973). Treatments with Ca\(^{++}\)-chelating agents are able to dissolve these immobilized gels. Chelating agents such as N,N'-1,2-ethanediyl-bis-[N-(carboxymethyl)glycine] (EDTA), ethyleneglycol-bis-(\(\beta\)-aminoethyl ether)N,N,N',N'-tetraacetic acid (EGTA), and cyclohexane trans-1,2-diaminetetra-acetate (CDTA) and ammonium oxalate are used (Selvendran and O'Neill, 1987). Once pectic substances are extracted exhaustively by a Ca\(^{++}\)-chelating agent, additional uronic acid-rich material is still retained by the matrix and is only removed by dilute alkali, such as Na\(_2\)CO\(_3\), NaBH\(_4\)-Na\(_2\)B\(_4\)O\(_7\), or 0.1 M KOH solutions (Selvendran and O'Neill, 1987). This second group of pectic polymers may be immobilized by alkali-labile ester linkages, probably through cross-bridging via hydroxycinnamic acid (Jarvis, 1982). Fry (1986) identified hydroxycinnamic acids, such as ferulic acid, that may participate in cross-bridging through the formation of ester bonds with neutral sugars of the side branches of rhamnogalacturonans and subsequent biphenyl or ether formation of the these aromatics. The extent of Ca\(^{++}\) cross-bridging, of esterification through aromatic linkages, and even of branching and size of neutral sugar side-chains can each
influence gel flexibility, porosity, interaction with hemicellulosic polymers, and perhaps mobility of protein components (Biggs and Fry, 1987). The fact that dialysis against NaCl solution induces partial dissociation of the pectin polymers suggests that cell wall pectin is an aggregated mosaic, held together partially through non-covalent interactions (Fishman et al., 1989). Addition of Ca inhibits the growth of the tissue and cell wall extensibility (Virk and Cleland, 1988; 1990). The rate of fruit softening is reduced by Ca treatment (Poovaiah, 1986; Conway and Sams, 1987). This evidence suggests that addition of Ca\(^{++}\) may create abundant ionic cross linkages between pectin and other polymers, and make the cell wall structure more rigid.

2. Mechanisms of cell wall loosening

Three mechanisms are possible for the wall loosening events that occur during cell wall extension (Cleland, 1987): (1) Bond breakage, load bearing bonds in the wall are broken either enzymatically or non-enzymatically. Four types of bonds are possible candidates: glycosidic bonds, bonds within or between peptide chains (including iso-tyrosine bonds), hydrogen bonds and Ca bridges between pectic chains. The participation of enzymes is required to break the first two types of bonds, while for the latter two, it could be non-enzymatic; (2) Chemical creep, load-bearing glycosidic bonds are broken enzymatically and subsequently reformed with new partners in a non-stressed configuration; and (3) Wall synthesis might simply release the stress on load-bearing regions of the wall by insertion of new pieces of polysaccharide, followed by viscoelastic extension of that area of wall.
Plant growth substances exert an influence on cell expansion. Using plant growth substances to manipulate plant cell expansion has revealed some important properties of the cell wall. Cleland (1986) proposed that the cells excreted (or took up) a wall loosening factor (WLF), whose presence or absence in the wall was required for wall loosening and wall extension. Two WLFS, $H^+$ and $Ca^{++}$ have been identified (Cleland, 1986). The evidence for $H^+$ as a WLF has been summarized (Rayle and Cleland 1977; Taiz 1984). Three criteria have been proposed to determine whether $H^+$ is a WLF in a particular system (Cleland, 1986). First, it must be shown that the growth substance causes the cells to excrete $H^+$. Secondly, it must be shown that exogenous acid is capable of inducing significant cell enlargement in the absence of growth substance. Thirdly, the most diagnostic test is probably that neutral buffers inhibit growth substance-induced growth when infiltrated into the cell wall. These three criteria have been met with auxin-induced growth of coleoptiles and stems (Cleland 1980). It appears with other growth substances that $H^+$ is not the WLF (Stuart and Jones, 1978; Ross and Rayle, 1982; Brock, 1985; Craker et al., 1978). Wall loosening would occur when $Ca^{++}$ is removed from the wall or from the region where the loosening occurs. The extensibility of the cell wall is controlled by three sets of load-bearing bonds, and $Ca$ bridge is one set of load-bearing bonds (Virk and Cleland, 1988, 1990). Calcium bridges contribute to the strength of the cell wall. The extensibility of soybean hypocotyl section is significantly increased by treatment with $Ca$ chelators, such as EGTA and Quin-2 (2-[(2-bis-[carboxymethyl]amino-5-methylphenooxy)methyl]-6-

A comparison of Ca++ effects on cell wall extension and fruit tissue softening would be helpful in searching for the mechanism of fruit softening. During fruit ripening, cell wall loosening results in fruit softening (Ferguson, 1984). The changes in the cell wall polysaccharides of many fruits are thought to play an important role in causing changes in fruit texture during ripening (Seymour et al., 1990). Studies have shown that the soluble polyuronides are depolymerized during ripening (Huber, 1983; Seymour et al., 1987). Polyuronide molecular weight average decreases from ca 160,000 to 96,000 (Seymour and Harding, 1987). Biochemical studies of cell wall related to fruit softening indicate that the structural changes of pectin, hemicellulose and cellulose together are the cause of the alteration of cell wall structure (Pesis et al., 1978; Huber, 1983; Tong and Gross, 1988; Fishman et al., 1989; McCollum et al., 1989; Seymour et al., 1990). Modification of hemicellulose structure associated with fruit ripening has been reported for tomato (Huber, 1983), strawberry (Huber, 1984), and muskmelons (McCollum et al., 1989). The size of hemicellulose polymers decreases during ripening of these fruits. A change of cellulose has also been documented in ripening avocado (Pesis et al., 1978), pear (Ahmed and Labavitch, 1980), and apple (Bartley, 1976). Recent evidence indicates that cell wall synthesis during the ripening period of the fruit could alter overall wall strength and reduce fruit firmness (Mitcham et al., 1989, 1991; Greve and Labavitch, 1991).
3. The enzymology of softening

Specific enzymes are synthesized during fruit ripening. In tomato, at least 19 new mRNAs have been identified to be expressed at higher levels during ripening than at the mature green stage (Slater et al., 1985; Mansson et al., 1985). One of the mRNAs, pTOM 6, has been identified by sequence analysis as coding for polygalacturonase (poly(1,4-α-D-galacturonic acid)glycanohydrolase, EC 3.2.1.15) (PG) (Grierson et al., 1986). Using techniques of tissue blotting and immunocytology, Tieman and Handa (1989) were able to demonstrate that during tomato fruit ripening PG protein appeared first in the columella region followed by a sequential appearance in the exocarp and endocarp.

Tomato fruit PG contains three isozymes: PG1, PG2A and PG2B (Pressey and Avants, 1973). PG2A and PG2B appear to function both as monomeric catalytic subunits of 45 KD and 46 KD or 43 KD, respectively, and as subunit of PG1 (DellaPenna and Bennett, 1988; Pogson et al., 1991). PG1, approximately 120 KD, consists of polypeptides 45, 43, and 38 KD (Pogson et al., 1991). The 43 and 45 KD polypeptides correspond to PG2A and PG2B in size and can be detected by antisera raised against PG2A. The 38 KD polypeptide is immunologically distinct. Both PG2A and PG2B appear to be the products of a single gene and are synthesized as a large molecular precursor processed to the mature protein by a series of events. The removal of a hydrophobic signal sequence and the glycosylation of asparagine occur in the endoplasmic reticulum (ER) (DellaPenna and Bennett, 1988), and post-translational modification of the core oligosaccharides
in Golgi (Moshrefi and Luh, 1983). The accumulation of PG enzyme is regulated by post-transcriptional events (Speirs et al., 1989).

PG1, PG2A and PG2B accumulate in different patterns during tomato fruit ripening. PG1 accumulates before PG2 (Brady et al., 1983). Pectin degradation begins as the appearance of PG1, and continues after the accumulation of PG2 has begun (Pressey, 1986). Therefore, two hypothesis have been made. Smith et al. (1990) proposed that the PG1 and PG2 isozymes were responsible for solubilization and depolymerization of polyuronide degradation, respectively, based on the analysis of PG isozyme accumulation in transgenic tomato fruit. DellaPenna et al. (1990) suggested that PG1 alone catalyzed polyuronide degradation *in vivo* based on a similar analysis in transgenic *rin* tomato fruit. Osteryoung et al. (1990) supported the later result by their analysis of tomato PG expression in transgenic tobacco.

The major structural change during tomato fruit ripening is the degradation of polyuronides (Huber, 1983b). Polygalacturonase has been implicated as the primary enzyme catalyzing polyuronide degradation during tomato fruit ripening (Wallner and Bloom, 1977; Themmen et al., 1982; Huber, 1983a and 1983b; Brady et al., 1982). Considerable correlative evidence has been accumulated to support this hypothesis (Bennett and DellaPenna, 1987; Giovannoni et al., 1989; Gross, 1990): (1) a general correlation between potential PG activity and softening; (2) an increase in soluble polyuronide during ripening; (3) the concomitant absence of appreciable levels of PG and softening in non-ripening tomato mutants; and (4) the ability of crude PG preparations to hydrolyse isolated cell wall material *in vitro.*
However, other results indicate that PG may not regulate exclusively the softening
process (Gross, 1990): (1) some fruits are without detectable PG, such as strawberry,
and soften during ripening (Huber, 1984; Brady, 1987); (2) some tomato fruit can
soften before the appearance of detectable PG activity (Brady, et al. 1985; Hall,
1987); (3) the tomato mutant dg contains a normal level of PG but does not soften
substantially (Jarret et al., 1984; Tong and Gross, 1989), and expression of a chimeric
PG gene in transgenic rin tomato fruit results in polyuronide degradation but not
fruit softening (Giovannoni et al., 1989); (4) when residual cell wall enzyme activity
is inhibited, PG activity is not closely correlated with soluble polyuronide (Seymour
et al., 1987); (5) tomato cell wall hydrolysis by PG may be limited in vivo by the
number of PG binding sites or other factors (Brady et al. 1987); (6) reducing 95 to
99% PG activity by antisense RNA, the transformed tomato fruits soften similarly
to normal tomatoes (Smith et al., 1988, 1990). The following facts should be also
considered in order to understand the role of PG in fruit softening (Speirs and
Brady, 1991): the tomatoes with antisense PG constructs accumulate PG to the
levels comparable to or greater than that found in some other fruits, such as papaya
(Paull and Chen, 1983) and peach (Pressey and Avants, 1978), which soften
appreciably; and several aspects of fruit ripening in rin tomato, a pleiotropic
mutation, are also affected (Speirs and Brady, 1991).

Xylanase activity has been reported in several fruits during ripening, such as
pear (Ahmed and Labavitch, 1980), papaya (Paull and Chen, 1983), avocado (Ronen
et al., 1991). During papaya fruit ripening, xylanase activity is very low in the pre-
climacteric period, and increases to the peak at the same time as the maximum increase in the rate of respiration and \( \text{C}_2\text{H}_4 \) production occur (Paull and Chen, 1983). The peak activity of xylanase happens when the fruits are 40 to 60% yellow (Paull, 1990). The firmness of the fruits also decreases rapidly at the same period. The increase of xylanase and xylosidase activities is correlated with the increase in \( \text{C}_2\text{H}_4 \) production and the reduction of fruit firmness in avocado (Ronen et al., 1991). Ethylene biosynthesis is induced by an endoxylanase isolated from fungus (Dean et al., 1991). Xylanase and xylosidase in avocado fruits may play a role in \( \text{C}_2\text{H}_4 \) production and cell wall hydrolysis during fruit ripening (Ronen et al., 1991). Glucanases and xyloglucanase may be related to fruit softening in tomato because they increase in activities at the same time when fruit softening occurs most rapidly (Maclachlan and Brady, 1992).

In avocado, cellulase has been suggested to play a role in cell wall modification during fruit softening. Initial enzyme activity is low or absent in unripe fruit and increases rapidly during the fruit ripening process (Awad and Young, 1979). However some researchers have suggested that cellulase plays a minor role during fruit ripening based on the fact that little or no cellulosic glucan is lost (Ahmed and Labavitch, 1980). Cellulase purified from avocado fruit extensively hydrolyses carboxymethylcellulose and (1-3), (1-4)-\( \beta-D \) glucans, and is not able to solubilize appreciably the cellulosic components of cell wall of avocado fruit (Hatfield and Nevins, 1986). The role of cellulase in avocado fruit softening may be hydrolysis of specific regions of xyloglucans or cellulose which could lead to
changes in cellulose fibrillar orientation (Hatfield and Nevins, 1986). This change may also cause a disruption of hydrogen bonding to other matrix polymers (Hatfield and Nevins, 1986).

Ethylene may be the trigger of fruit ripening (Oeller et al., 1991; Klee et al., 1991). Fruit ripening is significantly inhibited in transgenic fruits by either the expression of antisense RNA to the rate-limiting enzyme in the biosynthetic pathway 1-aminocyclopropane-1-carboxylate (ACC) synthase (Oeller et al., 1991) or the expression of the gene of ACC deaminase introduced from bacteria (Klee et al., 1991). In tomato, pTOM 13, a ripening specific mRNA, is correlated to ethylene production (Hamilton et al., 1990). Introduction of a chimeric pTOM 13 antisense gene to a normal plant reduces fruit ethylene synthesis in a gene dosage-dependent manner (Hamilton et al., 1990). The transgenic fruits are less ripe by comparison with the control fruit (Hamilton et al., 1990). Davies et al. (1990) used silver that blocks the hormone receptor sites and prevents ethylene perception (Sisler, 1982) to investigate the importance of ethylene in coordinating tomato ripening and its role in controlling gene expression. Their results indicate that the accumulation of some ripening related mRNA is strongly reliant on increased concentrations of ethylene, especially the PG mRNA, while the productions of other ripening related mRNAs occur independently of ethylene perception or action. During tomato fruit ripening, the products of cell wall degradation may be involved in the stimulation and coordination of ethylene production, and promote ripening (Brecht and Huber, 1988; Priem and Gross, 1992). Vacuum infiltrating preclimacteric (green) fruits with
solutions containing pectin fragments enzymically released from cell wall of ripe fruit accelerates fruit ripening as measured by initiation of climacteric CO₂ and C₂H₄ production and the appearance of a red color. The pectin fragments generated by PG treatment of pectin isolated from green fruit have little or no effect on ripening. Mannosyl- and xylosyl- containing glycans promote tomato fruit ripening as measured by red coloration and ethylene production (Priem and Gross, 1992).

4. The relationship between Ca and cell wall degradation

Calcium may inhibit fruit softening in two ways. Firstly, a greater degree of Ca²⁺ binding in pectic polymers, with concomitantly fewer free acid groups, may reduce the rate of pectic solubilization; secondly, Ca may limit the secretion or the activities of cell wall hydrolysase (Ferguson, 1984). Major changes occurring in cell wall composition of senescent apple fruit include a decrease in galactose and arabinose moieties of the water-insoluble cell wall fraction, followed by an increase in soluble polyuronide (Knee, 1973; Seymour et al., 1990). Calcium inhibits solubilization of polyuronide and arabinose moieties and reduces the loss of galactose content in the fruit during storage (Glenn and Poovaiah, 1990). Cell-to-cell contact is maintained in the cell wall region of Ca-treated fruit, whereas the region of middle lamella is separated in untreated fruit (Glenn et al., 1988). It has been suggested that fruit softening occurs either by movement of Ca²⁺ from the middle lamella or by the loss of Ca²⁺ attachment sites (Knee and Bartley, 1981). In fact, both Ca²⁺ movement from the middle lamella and loss of Ca²⁺ binding sites happen during apple fruit softening (Stow, 1989). Calcium level in tomato pericarp...
cell wall increases during fruit development to a high level in the fully grown immature fruit, but decreases just before the onset of ripening (Rigney and Wills, 1981). The proportional amount of Ca in the cell wall changes from more bound to more soluble during the early stage of fruit ripening. The rin mutant fruit, that fails to soften, contains a higher level of bound Ca during the advanced stages of fruit development compared with normal fruit (Suwanan and Poovaiah, 1978).

Increase in apoplastic Ca$^{++}$ level not only affects cell wall structure but also alters intracellular processes that may be involved with senescence (Paliyath and Poovaiah, 1985). Distinct and specific changes in polypeptide and phosphoprotein patterns are observed in Ca treated as compared with control apple fruits (Glenn et al., 1988). Phosphorylation of membrane proteins also decrease during senescence (Paliyath and Poovaiah, 1985). Calcium infiltration treatment is effective in maintaining the phosphorylation of proteins (Glenn et al., 1988). Pectic substances in the cell wall and the middle lamella serve as binding sites for Ca, and the complex is resistant to be attacked by PG (Wills and Rigney, 1979). Calcium cross-linking inter- and intra-molecularily in pectic substance limit PG attack (Buescher and Hobson, 1982). In fruits containing high levels of cell wall and middle lamella bound Ca, normal tissue softening can be achieved by removal of substantial amounts of Ca (Buescher and Hobson, 1982). In the presence of sufficient citrate, cell wall uronic acids of a firm and a soft cultivar are equally susceptible to hydrolysis, suggesting that differences in the digestion of walls by PG are dependent upon differences in Ca content or distribution (Brady et al., 1985).
Ben-Shalom (1986) assumed that cellulose and hemicellulose were sterically masked by the pectic substances. Therefore, removal of the pectin from cell wall of grapefruit segments by pectinase enhances the enzymatic hydrolysis of cellulose and hemicellulose (Ben-Shalom, 1986).

5. Methodology of cell wall analysis

Selvendran and O'Neill (1987) discussed the methods available for isolation and analysis of higher plant cell walls. Cell wall preparation is critical in evaluating component polysaccharide structure (Selvendran and O'Neill 1987; Koch and Nevins, 1989). Chemical treatments and the presence of active cell wall hydrolase may modify cell wall polymers. Alkaline pH can deesterify methyl groups from polysaccharides (Wood and Siddiqui, 1971). Organic solvents such as chloroform and acetone dehydrate cell wall, and modify the cell wall physical characteristics (Koch and Nevins, 1989; Huber, 1991). Koch and Nevins (1989) studied cell wall isolation methodology to determine the extent that physical changes occurring during cell wall isolation affected the physiological interpretation of pectin chemistry. The procedure used to prepare cell wall material without inactivation of enzymes (Gross and Wallner, 1979; Huber, 1983) might result in wrong conclusions (Kock and Nevins, 1989). Preparation of pectin rich cell wall material avoiding enzymatic activity usually involves procedures that may modify carbohydrate structure such as heating or inactivation with phenol: acetic acid: water (PAW) (Selvendran, 1975; Huber, 1991). Cell walls treated with PAW reduce Ca content about 35 to 50%, which is likely brought about by the low pH of PAW preparations,
and enhances greatly susceptibility to hydrolysis by PG (Huber, 1991). An alternative to PAW is buffered phenol (Huber, 1991). The heat-inactivated cell wall extraction procedure appears superior to other procedures because it provides optimum conditions for the preservation of cell wall structure by inactivating constitutive enzymes that may modify pectin structure during extraction steps (Koch and Nevins, 1989). Extraction of the cell wall in 50% ethanol inactivates PG and prevents β-elimination reaction of pectin during subsequent heat-inactivation (Koch and Nevins, 1989).

Fractionation studies with cell wall preparation and extraction can help us to understand the changes of wall structure and the metabolism of cell wall polyuronides during ripening. Galactose is one of the cell wall components that is lost during tomato fruit ripening (Gross, 1984). Fractionation studies indicate that galactose is lost from the pectin fractions solubilized by CDTA and dilute sodium carbonate, and from the pectic material associated with the α-cellulose residue (Gross, 1984). Seymour et al. (1990) investigated in more detail the type of cell wall polysaccharides presented in tomato and, in particular, those which undergo changes during ripening. It is found that ripe fruit contains much less pectic galactans compared with the unripe fruit, and there is a significant decrease in the content of galactan side-chains in ripe fruit. Hemicelluloses of the unripe and ripe fruit show negligible difference in composition. However, gel filtration analysis of hemicellulose (alkali-soluble wall polymers) from tomato fruit indicates that the size of hemicellulose decreases markedly from high-molecular-weight (>3.5 x 10^5) to low-
molecular-weight (<4.0 x 10³) polymers during ripening (Huber, 1984). Similar change of hemicellulose is observed in ripening strawberry fruits (Huber, 1983).

**Calcium and membrane integrity**

Three types of evidence indicate that Ca has a role in membranes (Poovaiah, 1986): (1) the deterioration of membranes occurs during Ca deficiency (Marinos, 1962); (2) Ca alters the actual architecture of natural or artificial membranes (Gary-Bobo, 1970; Paliyath et al., 1984), resulting in enormous changes in fluidity and permeability; (3) Ca can influence membrane physiological function (Hanson, 1983).

The major phospholipid components of membrane are phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, and phosphatidyl inositol (Galliard, 1968). Calcium interacts with phospholipids that have anionic head groups, resulting in high membrane rigidity and surface tension (Landau and Leshem, 1988). Electron microscopy has revealed the unique importance of Ca for the stabilization of membranes (Marinos, 1962). Calcium at physiological concentrations significantly reduces the fluidity of plant protoplast membranes (Boss and Mott, 1980). Membrane fusion events, that are important in the turnover of membrane constituents, deposition of cell wall precursors, and many other cellular processes, may also depend on Ca (Papahadjopoulos, 1978). Concentrations of 1 to 5 mM Ca are required to protect the plasma membranes from the deleterious effects of low pH, salinity, toxic ions, and nutrient imbalance (Hanson, 1983). High external Na⁺ concentration causes displacement of Ca²⁺ from plasma membrane and depolarization of membrane potential, and this membrane potential
depolarization can be reduced by high Ca\(^{++}\) supply (Lauchli, 1990). Without the protection of Ca, the plasma membrane fails to discriminate between ions, the proton pump is dysfunctional, and senescence is accelerated (Poovaiah and Leopold, 1973; Paliyath et al., 1984).

Calcium is an essential element in ensuring the maintenance of selective permeability (Ferguson and Drobak, 1988). An increase in solute leakage and a loss of selective permeability are the most obvious changes in membrane found during senescence (Ferguson, 1984). The major structural change in membrane during senescence is probably an increase in microviscosity or decrease in fluidity (Ferguson, 1984). Increase in membrane microviscosity has been reported in senescing fruits (Ben-Arie et al., 1982; Legge et al., 1982a), leaves (McKersie and Thompson, 1978), and flowers (Borochov et al., 1976; Legge et al., 1982b). Use of fluorescence polarization of 1,6-diphenyl-2,3,5-hexatriene (DPH) as a deep membrane probe to examine the membrane fluid properties, indicates that the senescence-associated increase in microviscosity of apple membranes is reduced by postharvest Ca treatment (Paliyath et al., 1984). In microsomal preparations, fluorescent probes indicate that pretreatment of apple fruit tissue with 10 mol m\(^{-3}\) CaCl\(_2\) before membrane preparation results in a reduction in microviscosity (Ben-Arie et al., 1982). These results suggest that the effect of Ca in delaying senescence is partly due to reductions in microviscosity of membranes associated with senescence (Poovaiah, 1986). Apple tissue slices incubated in a 0.4 M sucrose solution for 24-hour period is characterized by a rapid decline in ethylene formation
(Liberman et al., 1977). The addition of Ca first inhibits and then stabilizes the ethylene production probably because Ca preserves the integrity of the membrane of apple slice (Lieberman and Wang, 1982).

Apart from an effect on solute permeability, high affinity binding of Ca\(^{++}\) to membrane proteins may also lead to direct effects of Ca\(^{++}\) on enzyme activity (Ferguson, 1984). Lipid peroxidation and an increase in free radical activity are major events during senescence (Frenkel, 1978; Leshem, 1981; Dhindsa et al., 1982). Calcium reduces peroxide accumulation in senescing cotyledons (Ferguson et al., 1983). It seems that any membrane-related Ca\(^{++}\) effects on senescence are associated with structural modification caused by Ca\(^{++}\) binding (Ferguson, 1984).

**Calcium and cytoplasmic metabolism**

The status of Ca\(^{++}\) in the cytoplasm of both animal and plant tissue show two key characteristics (Ferguson and Drobak, 1988): (1) free Ca\(^{++}\) in the cell cytoplasm must be maintained at extremely low concentration; and (2) the presence of Ca\(^{++}\)-binding proteins play an important role in intercellular metabolic responses. This divalent cation acts as a second messenger in regulating metabolic events (Poovaiah et al., 1987), therefore fluctuations in cytoplasmic Ca\(^{++}\) concentration may represent an important link in signal transduction. In fact, the control of Ca concentration and flow is essential for normal cell function (Hepler and Wayne, 1985). The evidence suggests that Ca acts as a secondary messenger in the response to external signals in plant tissue as in animal tissue (Hepler and Wayne, 1985). The phosphoinositides, the enzymes, and a Ca-regulated system responsive to inositol-
1,4,5-triphosphate (IP₃), are present in plants. Calcium dependent protein kinase is suggested to be involved in signal transduction in plants (Kauss, 1987). However, this system in plants is somewhat different from animals. The evidence indicates that multifaceted pathway for inositol metabolism, the relative amounts of the lipids and their sensitivity to stimuli and hormones in plant tissue are distinctly different from animals (Boss, 1989).

In plants, the concentration of free Ca²⁺ in the cytoplasm is very low, 10⁻⁸ to 10⁻⁶ M (Cheung, 1980; Hanson, 1983). In contrast, apoplast, vacuoles and ER contain much higher (at least 1000 times) concentration of Ca (Evans et al., 1991). With much higher organelle and extracellular Ca²⁺ level in the millimolar concentration range, a continuous process must occur to transport Ca²⁺ out of the cytoplasm (Briskin, 1990). There two types of Ca²⁺ transporters in plant cells (DuPont et al., 1990; Evans et al., 1991). One is the Ca⁺⁺/nH⁺ antiporter, which is dependent on the formation of a pH gradient across the membrane and is insensitive to ATPase inhibitors. The other is Ca⁺⁺-ATPase which does not require a pH gradient across the membrane and is insensitive to vanadate. The Ca⁺⁺/nH⁺ antiporter is associated with tonoplast, while the Ca⁺⁺-ATPase is associated with ER and plasma membrane (Blumwald and Poole, 1986; Giannini et al., 1987; DuPont et al., 1990; Evans et al., 1991). The Ca⁺⁺-ATPase has been purified from maize coleoptile (Robison et al., 1988). This Ca pumping ATPase has a molecular weight of 140,000, and is activated by calmodulin. Generally, active Ca transport of plasma membrane is of key importance for long-term regulation of cytosolic free Ca (Evans
et al., 1991). Endoplasmic reticulum and tonoplast may operate for short-term regulation of cytosolic free Ca concentration (Hepler and Wayne, 1985; Evans et al., 1991). The ER possibly functions in buffering of cytosolic Ca concentration as well as a source of Ca for transient fluxes (Evans et al., 1991). Calcium concentration of vacuole is much higher than that of cytosol, ranging from 0.1 to 10 mol m$^{-3}$ (Macklon, 1984). Vacuolar Ca is separated from cellular metabolism as chelators and precipitates such as oxalate and phosphate (Hanson, 1983). Vacuole may serve as a potential reservoir for Ca release during signal transduction (Evans et al., 1991). Any disfunction of this Ca regulation system will result in disruption of growth and cellular damage or death (Ferguson and Drobak, 1988). At millimolar levels, Ca$^{++}$ reacts with inorganic phosphate to form an insoluble precipitate, thus cytoplasmic Ca$^{++}$ concentration phosphate-based energy metabolism would be seriously inhibited (Poovaiah, 1988).
CHAPTER 2.
DEVELOPMENT OF HYPOTHESES

Calcium is an important factor in delaying fruit ripening, preventing physiological disorders, and reducing disease infestation. However, there is a lack of information about Ca effect on papaya fruit ripening and disease control. Previous observations have indicated that abnormal, rapid papaya fruit softening may be associated with low Ca, or an imbalance between Ca, K and possibly Mg levels in the fruit. High N fertilizer has also been suggested to play a role. This abnormal ripening leads to loss of fruit during marketing and disrupts postharvest fruit handling.

Three hypotheses of this research are:

1. Mesocarp Ca level is an important factor controlling the rate of papaya fruit softening;
2. Calcium influences the rate of softening via interaction with cell wall polymers;
3. High levels of K, Mg, and N do not cause abnormal soft fruit if Ca level is high enough in the mesocarp.

The following specific objectives will be addressed to test these hypotheses:

1. Determine the effects of Ca, K and N fertilizer applications on mesocarp Ca level;
2. Determine the effect of mesocarp Ca concentration on fruit ripening characteristics;
(3) Determine the effect of interactions among Ca, Mg, and K in mesocarp on fruit softening;

(4) Determine the effect of Ca on the degradation of mesocarp cell wall during fruit ripening.
CHAPTER 3.
THE ACCUMULATION AND DISTRIBUTION OF Ca, Mg, AND K
IN PAPAYA FRUIT DURING GROWTH AND DEVELOPMENT

Introduction

Calcium is involved in a number of plant physiological processes, such as cell wall structure, membrane permeability, and enzyme activity, as well as a second messenger in cells (Hepler and Wayne, 1985; Kauss, 1987; Poovaiah et al., 1988). These physiological effects are directly related to fruit quality and senescence (Poovaiah et al., 1988). Increased Ca level in fruits delay fruit softening and improve fruit quality in apple (Poovaiah, 1986; Conway and Sams, 1987), tomato (Wills and Tirmazi, 1979) and avocado (Eaks, 1985). Physiological disorders associated with inadequate Ca nutrition occur in many plants. The most well known examples in fruit are bitter pit of apple (Ferguson and Watkins, 1989) and blossom end rot of tomatoes (Evans and Troxler, 1953). Many physiological disorders are characterized by localized inadequate Ca level (Bangerth, 1979). The deficiency often occurs to those plant parts that are naturally low in Ca, such as fruits and storage organs (Bangerth, 1979).

Calcium may be an important factor involving in papaya fruit quality (Paull, 1987). However, there is very little information about the effect of Ca on papaya fruit quality. In this chapter, the patterns of Ca, Mg, and K uptake and distribution in papaya fruit during fruit growth and development is reported. This information
provides an essential knowledge base for further investigation of Ca effects on papaya fruit ripening.

Materials and Methods

Plant materials

Papaya (Carica papaya L. cv "Sunset") fruits were harvested from Poamoho Experiment Station in central Oahu. Fruits from 19-month old trees were used to determine the uptake patterns of Ca, Mg and K. Two to three phyllotaxic spirals existed on the tree, and three trees with only two spirals were used in this study. Fruits from each tree were divided into two groups based on the phyllotaxic position on the spiral. Fruit number was marked according to the leaf number starting from the apex. Fruit age was obtained by dividing days after anthesis of some tagged companion fruits by leaf number. The fruits were weighed, and fruit volume was measured by water displacement. Fruits, approximately 20 days different in age, were chosen from each spiral on the three trees. The fruit was divided into skin, mesocarp and seeds. Fresh weight (FW) of the skin, the mesocarp and the seeds was measured. Fresh weight (10 g) of the skin and seeds, and 20 g FW of the mesocarp were taken to determine DW and percentage DW of the fruit (DW/FW x 100%). The tissue was dried at 60°C for 7 days.

Mature fruits were used to determine Ca, Mg and K distribution. The fruit was divided into three parts: skin, mesocarp and seeds. The mesocarp from the equatorial section of the fruit was further horizontally divided into two parts: outer and inner part. The mesocarp was divided transversely into three parts from the
peduncle to the blossom end. Each part was analyzed separately. The tissues were stored at -20°C until analyzed.

**Transpiration of fruit surface**

Fruit surface transpiration was measured with a LI-COR model LI-700 transient automatic diffusion porometer. The hinge pin of the sensor head (3.18 x 9.8-mm aperture) was removed. The sensor head was gently placed onto a fruit surface equilibrated to the laboratory ambient condition (22°C, 75% RH) with ca 1.1 µmol s⁻¹ m⁻² mix of fluorescent light and day light. The medium cycle between 35% and 55% was used. The time taken (seconds) to cycle through this range was used to determine fruit water diffusive resistance (s cm⁻¹).

**Analysis of total Ca, Mg and K**

The skin (10 g FW), seeds (10 g FW) and the mesocarp (50 g FW) were used to determine total Ca, Mg and K. For young fruits, the whole fruit was used. Tissue was homogenized in a blender with 100 ml deionized water. The homogenate (10 ml) was mixed with 10 ml of 12 M HCl and heated at 60°C for 30 minutes. The solution was filtered through a Whatman #42 ashless filter paper into a 50 ml volumetric flask and made up to the volume with deionized water. The extract was further diluted with deionized water. For samples of Ca and Mg determination, lanthanum oxide (in 1 M HCl, containing 5% La) was added during dilution and the final La concentration was 0.5%. Total Ca, Mg and K concentrations were determined by an atomic absorption spectrophotometer.
Data analysis

Data were analyzed by Duncan multiple range test where appropriate.

Results

Fruit growth pattern

Growth patterns of both fruit weight and fruit volume were similar (Fig. 3.1 A). The pattern of growth rate changes in weight and volume were synchronous (Fig. 3.1 B). There were 2 major peaks in weight and volume growth. The first period of rapid growth occurred 60 to 75 days post-anthesis, the second 90 to 105 days post-anthesis (Fig. 3.1 B).

The mesocarp showed the greatest increase in FW (Fig. 3.2 A) and DW (Fig. 3.2 B) especially after 80 days post-anthesis when compared to the skin and the seeds (Fig. 3.2). The skin gradually increased in both FW (Fig. 3.2 A) and DW (Fig. 3.2 B) up to 100 days post-anthesis, and then stopped growing in both FW and DW. The seeds gradually increased in FW (Fig. 3.2 A) and DW (Fig. 3.2 B) during the whole period of fruit growth and development.

The Ca, Mg and K accumulation

Total Ca (Fig. 3.3 A), Mg (Fig. 3.3 B) and K (Fig. 3.3 C) continuously increased in the mesocarp during growth and development. The mesocarp had the highest Ca, Mg and K contents because of its large proportion in weight (Fig. 3.2). The skin had the lowest Ca and Mg contents, and the seeds the lowest K content.

The rate of uptake patterns for Ca (Fig. 3.4 C), Mg (Fig. 3.4 D) and K (Fig. 3.4 E) by the whole fruit were similar and paralleled closely to the rate of DW (Fig.
Figure 3.1 The growth pattern (A) and growth rate (B) of fruit fresh weight (♦) and fruit volume (○). Each data point is the mean of 6 observations. Fruit weight: \( Y=6.722+0.044X-0.00002X^2 \), \( R=0.927^{***} \); fruit volume: \( Y=6.315+0.041X-0.00002X^2 \), \( R=0.947^{***} \).
Figure 3.2 The growth pattern in fresh weight (A) and dry weight (B) of whole fruit (●), the skin (▲), the mesocarp (○) and seeds (□). Each data point is the mean of 6 observations. Fresh weight: fruit weight $Y = 6.722 + 0.044X - 0.00002X^2$, $R=0.927^{***}$; skin $Y = 4.462 + 0.831X - 0.007X^2$, $R=0.845^{***}$; mesocarp $Y = 8.483 + 0.039X - 0.000002X^2$, $R=0.941^{***}$; seed $Y = 8.385 + 0.253X - 0.0004X^2$. Dry weight: fruit weight $Y = 8.042 + 0.428X - 0.002X^2$, $R=0.957^{***}$; skin $Y = 6.622 + 6.780X - 0.434X^2$, $R=0.832^{***}$; mesocarp $Y = 9.628 + 0.519X - 0.0017X^2$, $R=0.944^{***}$; seed $Y = 9.128 + 2.045X - 0.053X^2$, $R=0.924^{***}$.
Figure 3.3 The accumulations of Ca (A), Mg (B) and K (C) in whole fruit (•), the skin (△), the mesocarp (○) and seeds (□) during growth and development. Each data point is the mean of 6 observations. Calcium content: skin \( Y=1.778+0.264X, \) \( R=0.723^{* * * *} \); mesocarp \( Y=-5.371+2.723X, \) \( R=0.805^{* * * *} \); seed \( Y=8.988+0.502X, \) \( R=0.657^{* * * *} \). Magnesium content: skin \( Y=-0.044+0.106X, \) \( R=0.659^{* * * *} \); mesocarp \( Y=-1.487+1.549X, \) \( R=0.809^{* * * *} \); seed \( Y=1.772+0.341X, \) \( R=0.792^{* * * *} \). Potassium content: skin \( Y=11.951+2.018X, \) \( R=0.926^{* * * *} \); mesocarp \( Y=-28.915+12.865X, \) \( R=0.716^{* * * *} \); seed \( Y=-5.119+1.921X, \) \( R=0.669^{* * * *} \).
Figure 3.4 The growth rate of fruit fresh weight (A) and dry weight (B), and the uptake rate of Ca (C), Mg (D) and K (E) during fruit growth and development. Each data point is the mean of 6 observations.
3.4 B) from day 40 onwards, but not to the rate of FW (3.4 A). The three ions had high rates of uptake when fruits were less than 40 days old, and declined 60 to 80 days post-anthesis just before fruit started to rapidly increase in FW (Fig. 3.4 A) and DW (Fig. 3.4 B). All three uptake rates increased to the peak 10 days after maximum FW growth rate, and at the same time as the DW growth rate peaked.

Skin growth rate changes in FW (Fig. 3.5 A) and DW (Fig. 3.5 B) were the same, both showed paralleled increases 40 to 100 days post-anthesis with the rate declining 100 to 120 days post-anthesis, and then increased again. Calcium uptake rate increased to a maximum of 112 µg day\(^{-1}\) during the period of 80 to 120 days post-anthesis (Fig. 3.5 C). Magnesium uptake rate continuously increased during the period of 80 to 140 days post-anthesis (Fig. 3.5 D). Potassium uptake rate was highest during the period of 40 to 60 days post-anthesis, at 933 µg day\(^{-1}\), then dropped rapidly to 309 µg day\(^{-1}\) in the next 20 days, and remained in the range of 276 to 451 µg day\(^{-1}\) for the rest of growth period (Fig. 3.5 E).

Mesocarp FW growth rate increased 60 to 100 days post-anthesis to a maximum of 5.6 g day\(^{-1}\), then declined to 4.2 to 4.3 g day\(^{-1}\) (Fig. 3.6 A). Mesocarp DW growth rate gradually increased during the period of 40 to 100 days post-anthesis, then rapidly increased to the highest level (0.7 g day\(^{-1}\)) 120 to 140 day post-anthesis (Fig. 3.6 B). Calcium uptake rate was high, at 365 µg day\(^{-1}\), 40 to 60 days post-anthesis (Fig. 3.6 C), then declined during the period of 60 to 80 days post-anthesis, increasing again to the highest rate of 570 µg day\(^{-1}\) during the last phase of growth (80 to 140 days post-anthesis). Magnesium uptake rate was 454 µg day\(^{-1}\)
Figure 3.5 The skin growth rate in fresh (A) and dry weight (B), and the skin uptake rate of Ca (C), Mg (D) and K (E) during fruit growth and development. Each data point is the mean of 6 observations.
Days after anthesis: Days after anthesis.
Figure 3.6  The mesocarp growth rate in fresh (A) and dry weight (B), and the mesocarp uptake rate of Ca (C), Mg (D) and K (E) during fruit growth and development. Each data point is the mean of 6 observations.
40 to 60 days post-anthesis, and decreased to 107 \( \mu g \) day\(^{-1}\) 60 to 80 days, and then increased to the highest level of 1000 \( \mu g \) day\(^{-1}\) 80 to 100 days post-anthesis followed by a rapid decrease (Fig. 3.6 D). Potassium uptake rate had similar pattern to Mg uptake rate (Fig. 3.6 E). Potassium uptake highest rate was 4432 \( \mu g \) day\(^{-1}\) 120 to 140 days post-anthesis.

While FW and DW of mesocarp increased about 10-fold (Fig. 3.2) and total Ca content in mesocarp increased about 4-fold (Fig. 3.3 A), Ca concentration based on FW and DW decreased (Table 3.1). Calcium concentration declined rapidly from 405 to 135 \( \mu g \) g\(^{-1}\) FW during the period of 40 to 80 days post-anthesis (Table 3.1) when the mesocarp increased more than 4-fold in FW (Fig. 3.2 A). Mesocarp FW continuously increased after 80 days post-anthesis (Fig. 3.2 A, Fig. 3.6 A), but Ca concentration did not change significantly (Table 3.1). Calcium concentration based on DW decreased quickly from 5340 to 1899 \( \mu g \) g\(^{-1}\) DW during 60 to 80 days when DW percentage was in the lowest level (6.85%), and then remained in the range of 1836 to 2553 \( \mu g \) g\(^{-1}\) DW in the rest of growth period (Table 3.1).

Seed FW growth rate increased during the period of 40 to 100 days post-anthesis, and decreased rapidly during the period of 100 to 120 days (Fig. 3.7 A). The FW dramatically increased again during the period of 120 to 140 days possibly because of seed coat growth (Fig. 3.7 A). Seed DW growth rate constantly increased during the whole growth and development period. Seed Ca uptake rate was highest (469 \( \mu g \) day\(^{-1}\)) 40 to 60 days post-anthesis, and then declined rapidly to 95 \( \mu g \) day\(^{-1}\) during the period of 60 to 80 days, and remained in the low range in the
Figure 3.7  The seed growth rate in fresh (A) and dry weight (B), and the seed uptake rate of Ca (C), Mg (D) and K (E) during fruit growth and development. Each data point is the mean of 6 observations.
Days after anthesis
Table 3.1 The changes of Ca concentrations in papaya mesocarp during fruit growth and development

<table>
<thead>
<tr>
<th>Days after anthesis</th>
<th>Dry weight (%)</th>
<th>Ca(^z) (µg g(^{-1}) FW(^{y}))</th>
<th>Ca (µg g(^{-1}) DW(^x))</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>8.90</td>
<td>405 a</td>
<td>4506 a</td>
</tr>
<tr>
<td>60</td>
<td>7.73</td>
<td>256 b</td>
<td>5340 a</td>
</tr>
<tr>
<td>80</td>
<td>6.85</td>
<td>135 c</td>
<td>1899 b</td>
</tr>
<tr>
<td>100</td>
<td>7.04</td>
<td>180 c</td>
<td>2511 b</td>
</tr>
<tr>
<td>120</td>
<td>7.13</td>
<td>163 c</td>
<td>2748 b</td>
</tr>
<tr>
<td>140</td>
<td>8.58</td>
<td>158 c</td>
<td>1677 b</td>
</tr>
</tbody>
</table>

\(^z\). Means with same letters in the same column were not significantly different at 5% level, n=6;

\(^y\). FW = fresh weight;

\(^x\). DW = dry weight.
rest of growth period. The pattern of seed Mg uptake rate was similar to that of Ca uptake. Seed K uptake rate was very high 472 μg day⁻¹ during the period of 40 to 60 days post-anthesis, and dropped to 12 μg day⁻¹ during the period of 60 to 80 days, and then increased gradually to the highest (498 μg days⁻¹) during the period of 80 to 140 days.

**The transpiration of fruit surface**

The water diffusive resistance of fruit surface increased during fruit growth and development (Table 3.2). The water diffusive resistance was 0.5 s cm⁻¹ when the fruit was 60 days old and increased to 11.3 s cm⁻¹ when the fruit was 120 days old. The water diffusive resistance in mature fruit (140 days old) was 37 times higher than that of the young fruit (60 days old).

**Horizontal and longitudinal distribution of Ca, Mg and K**

The mesocarp had lowest Ca, Mg and K concentrations when compared to the skin and seeds (Table 3.3). Calcium concentration was about 9 times higher in seeds (1041 μg g⁻¹ FW) and about 3 times higher in the skin (402 μg g⁻¹ FW) than that in the mesocarp (117 μg g⁻¹ FW). The seeds and the mesocarp also had the highest (720 μg g⁻¹ FW) and the lowest (202 μg g⁻¹ FW) Mg concentration, respectively, among the three tissue types. Potassium concentration was the highest in the skin (4128 μg g⁻¹ FW), and the lowest in the mesocarp (1221 μg g⁻¹ FW).

Calcium concentration was significantly higher in the outer mesocarp (187 μg g⁻¹ FW) than in the inner mesocarp (127 μg g⁻¹ FW) (Table 3.4). While Mg concentration was higher in the inner mesocarp (261 μg g⁻¹ FW) than in the outer
Table 3.2 The transpiration rate of fruit surface during growth and development.

<table>
<thead>
<tr>
<th>Days after anthesis</th>
<th>Diffusive resistance ($s\ cm^{-1}$) $^z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>0.5 e</td>
</tr>
<tr>
<td>80</td>
<td>3.0 d</td>
</tr>
<tr>
<td>100</td>
<td>5.4 c</td>
</tr>
<tr>
<td>120</td>
<td>11.3 b</td>
</tr>
<tr>
<td>140</td>
<td>18.6 a</td>
</tr>
</tbody>
</table>

$^z$. Means with same letters in the same column were not significantly different at 5% level, n=6.
Table 3.3 Horizontal distribution of Ca, Mg, and K in papaya fruit. Fruits were harvested at 10% yellow stage.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Content (µg g(^{-1}) FW(^\text{2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>Skin</td>
<td>402 b</td>
</tr>
<tr>
<td>Mesocarp</td>
<td>117 c</td>
</tr>
<tr>
<td>Seed</td>
<td>1041 a</td>
</tr>
</tbody>
</table>

\(^2\) Means with same letters in the same column were not significantly different at 5% level, n=5.
Table 3.4  Horizontal distribution of Ca, Mg, and K in papaya mesocarp. Fruits were harvested at 10% yellow stage.

<table>
<thead>
<tr>
<th>Position</th>
<th>Content (μg g⁻¹ FW)²</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>Mg</td>
</tr>
<tr>
<td>Outer mesocarp</td>
<td>187 a</td>
<td>150 b</td>
</tr>
<tr>
<td>Inner mesocarp</td>
<td>127 b</td>
<td>261 a</td>
</tr>
</tbody>
</table>

². Means with same letters in the same column were not significantly different at 5% level, n=5.
(150 μg g⁻¹ FW), K concentration was higher in the outer mesocarp (1347 μg g⁻¹ FW) than in the inner mesocarp (817 μg g⁻¹ FW).

Mesocarp Ca concentrations were in the range of 117 to 143 μg g⁻¹ FW and were not significantly different from the peduncle to the blossom end (Table 3.5). The equatorial zone had the highest (202 μg g⁻¹ FW), and the peduncle end the lowest (92 μg g⁻¹ FW) Mg concentrations. The K concentration was 3084 μg g⁻¹ FW at the peduncle end, 2.5 times higher than at the equatorial zone (1221 μg g⁻¹ FW) and blossom end (1213 μg g⁻¹ FW).

Discussion

The "Sunset" papaya fruit growth pattern was similar to other papaya varieties, such as "Sunrise", "Washington" and "Thailand" (Selvaraj et al., 1982). The rate of fruit growth showed 2 peak growth periods (Fig. 3.1 B): the first was observed between 60 to 75 days post-anthesis and the second between 90 to 105 days post-anthesis. In other varieties the first phase of rapid growth was between 50 to 90 days post-anthesis and the second phase of rapid growth between 100 to 140 days post-anthesis (Selvaraj et al., 1982).

Calcium uptake by the mesocarp and seeds was high (Fig. 3.6 C and Fig. 3.7 C) before 60 days post-anthesis when the fruit was small (Fig. 3.1 A). Newly growing organs are strong sinks for minerals (Clarkson, 1984). The transpiration through the fruit surface was high during this period (Table 3.2). The cuticle of papaya fruits is not well developed in immature fruits (Sanxter, 1989). The
Table 3.5 Longitudinal distribution of Ca, Mg, and K in papaya mesocarp. Fruits were harvested at 10% yellow stage.

<table>
<thead>
<tr>
<th>Position</th>
<th>Content (µg g⁻¹ FW)²</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>Mg</td>
<td>K</td>
</tr>
<tr>
<td>Peduncle end</td>
<td>143 a</td>
<td>92  c</td>
<td>3084 a</td>
</tr>
<tr>
<td>Equatorial zone</td>
<td>117 a</td>
<td>202 a</td>
<td>1221 b</td>
</tr>
<tr>
<td>Blossom end</td>
<td>133 a</td>
<td>143 b</td>
<td>1213 b</td>
</tr>
</tbody>
</table>

². Means with same letters in the same column were not significantly different at 5% level, n=5.
transpiration on fruit surface is an important motive force for water and Ca ion flow into fruit (Bangerth, 1979; Ferguson and Watkins, 1989).

Calcium uptake by the mesocarp and the seeds was low during period of 60 to 100 days post-anthesis (Fig. 3.6 C and Fig. 3.7 C). The changes of predominant supply pathway from xylem to phloem has been associated with the cessation of cell division and the onset of cell expansion (Ferguson and Watkins, 1989). The main source of nutrition and water is possibly imported through phloem during which period when mesocarp and seeds rapidly increased their FW and DW (Fig. 3.6 A, B and Fig. 3.7 A, B). The phloem is not considered to be the pathway of Ca translocation since Ca concentration is restricted in $10^{-6}$ to $10^{-7}$ mM range in the cytoplasm (Evans et al., 1991). When the proportion of water imported to tomato fruit via the xylem decreases and via phloem increases, fruit Ca accumulation rate also decreases (Ho et al., 1987). The competition between fruit and leaf for water may also reduce the Ca transport to the fruit during this period. The leaf normally has a higher transpiration rate than fruit (Clarkson, 1984). Therefore, most of xylem water together with the Ca may be transported mainly towards the leaves.

Calcium uptake by mesocarp increased after 100 days post-anthesis (Fig. 3.6). Sucrose in papaya mesocarp rapidly increased about 4 times during 110 to 140 days of post-anthesis (Chan et al., 1979). High sucrose accumulation in mesocarp during this period may decrease water potential and increase the xylem water flow towards the mesocarp. Root pressure may also provide a certain amount of Ca to papaya fruit. Calcium import into tomato fruits is favored at night although the rate of Ca
absorption and translocation from roots to shoots does not differ between the day and the night (Tachibana, 1991). The maintenance of relatively humid condition only at night favors transport of Ca into the fruit, due to the development of root pressure flow (Banuelos et al., 1985). This evidence suggests that root pressure may be important in providing adequate Ca to tomato fruits. The fact that Ca uptake by mesocarp did not relate precisely to mesocarp growth (Fig. 3.7) indicates that Ca uptake is not determined by growth alone.

Although mesocarp continuously took up Ca during fruit growth and development (Fig. 3.3 and Fig. 3.6), mesocarp Ca concentration based on FW and DW decreased (Table 3.1). This indicates that mesocarp growth rate is faster than its Ca uptake rate, causing a decrease of the Ca concentration. Calcium concentration in apple flesh also changes during fruit growth and development. The rate of apple fruit expansion is greater than the rate of mineral uptake, resulting in a dilution of mineral concentration (Ferguson and Watkins, 1989).

The changes of mesocarp Mg uptake rate were associated with the changes of mesocarp DW growth rate after 60 days post-anthesis (Fig. 3.6). The mesocarp is likely a sink for nutrients when it undergoes rapid growth. Since Mg can be transported through the xylem and the phloem (Mengel and Kirkby, 1982), mass flow may bring considerable Mg to the growing mesocarp. Similar to mesocarp Mg uptake, K uptake rate changes were also related to the changes in mesocarp DW growth rate (Fig. 3.6). The highest mesocarp K uptake rate and the highest mesocarp DW growth rate occurred at the same period, 120 to 140 days post-
anthesis (Fig. 3.6). Potassium concentration is high in phloem sap (Mengel and Kirkby, 1982), and organs that are preferentially supplied with phloem sap, such as fruits, are high in K (Mengel and Kirkby, 1982).

Calcium distribution in the mesocarp is not significantly different among the peduncle, equatorial and blossom regions (Table 3.5). In contrast, there is a longitudinal Ca gradient in avocado (Chaplin and Scott, 1980) and tomato (Ehret and Ho, 1986). Avocado fruit has the lowest Ca level in the blossom end (Chaplin and Scott, 1980). Blossom-end rot in tomato is associated with low Ca concentration (Ehret and Ho, 1986). The vascular network of the tomato fruit has increased branching from the proximal to the distal half, with the ratio of xylem to phloem area decreasing with branching (Walker, 1976). This anatomical feature may increase the resistance to xylem water movement from the proximal to the distal half. This may cause a low Ca content in the distal half (Ehret and Ho, 1986). The vascular bundles in papaya mesocarp extends longitudinally through the mesocarp (Paull, unpublished data). Calcium may be transported by these vascular bundles and distributed relatively even from the peduncle to the blossom end.
CHAPTER 4.
THE EFFECT OF Ca SPRAYING, DIPPING AND FIELD FERTILIZATION ON FRUIT SOFTENING AND OTHER RIPENING CHARACTERISTICS

Introduction

It normally takes up to 2 weeks to transport papaya fruit to the U.S. mainland, this is followed by a period in the wholesale and retail level (An and Paull, 1990). Papaya shipments to the New York market have been found to contain over ripe fruits in nearly one half of the inspections (Cappellini et al., 1988). Sporadically, commercial papaya packing sheds in Hawaii have reported batches of fruit that ripened very rapidly. This so called "soft fruit" disorder leads to shipment and possibly higher disease losses in papaya. The inability to predict the occurrence of this disorder adds to the uncertainty and limits the range of options available to deal with this fruit disorder.

There are many reports showing the importance of Ca in maintaining fruit quality. Increasing Ca concentration in apple fruits, either by pre-harvest spray or by postharvest infiltration or dipping, has been shown to delay the rate of fruit softening (Bangerth et al., 1972; Conway and Sams, 1987; Abbott et al., 1989), maintain vitamin C content (Poovaiah, 1986), reduce carbon dioxide and ethylene evolution (Faust and Shear, 1972; Conway and Sames, 1987), alleviate physiological storage disorders (Bramlage et al., 1974; Perring, 1986), and reduce the losses due
Calcium treatments of mangoes (Tirmazi and Wills, 1981), pears and bananas (Wills et al., 1982), sweet cherry (Factean et al., 1987) and strawberry (Cheour, 1991) also delay fruit ripening and senescence.

From the limited available survey data, abnormal soft papaya fruit is possibly related to low Ca content or an imbalance in levels of Ca, K, and possibly Mg in the mesocarp. High N fertilizer application may also play a role (Paull, 1987). These preliminary results suggest that the effect of increasing papaya fruit Ca level by spraying, dipping and field fertilization need to be determined. The major objective of this study was to determine the effect of Ca and the interaction effect of Ca, Mg, K and N on papaya fruit softening.

Materials and methods

Plant materials

Papaya (Carica papaya L. cv "Sunset") fruits grown at Poamoho Experiment Station in central Oahu were used for treatments of pre-harvest CaCl₂ spray and post-harvest CaCl₂ dip. The commercial variety "Kapoho Solo" was used for the field fertilization experiment on the island of Hawaii. The fruits were harvested and taken to the laboratory on the same day. Fruits were sorted by skin color and stored at 25°C until fruits reached the desired stage of ripeness as required for the experiment. The degree of skin and mesocarp color and the deformation force of the fruit were determined. The skin color and mesocarp color were subjectively evaluated by estimating the per cent yellowing of fruit surface and mesocarp vertical
section, respectively. Deformation force of fruits was measured with a penetrometer
(Accu Force, CADET) fitted with a 1.6 cm diameter tip. The penetrometer was
pressed 2 mm into fruit at the fruit equator. Mesocarp Ca, Mg and K
concentrations were measured by atomic absorption spectroscopy.

Pre-harvest CaCl₂ treatment

The fruits of 10 trees were sprayed with 2 liters of deionized water (control)
or 2% (w/v) CaCl₂ solution every 2 weeks. Fruits were harvested following 6 spray
applications. Fruits were harvested at the stage of 10% yellow skin and stored until
a majority of the fruits had at least 95% yellow skin. The experiment was repeated
3 times.

Post-harvest CaCl₂ treatment

Fruits were harvested with a knife to leave as long peduncle as possible.
Fruits with 10% yellow skin were dried for 24 hours with a fan at 22°C. The fruit
peduncle was trimmed at the end and dipped immediately into deionized water or
2% (w/v) CaCl₂ solution for an additional 48 hours while continuously exposed to
the wind from a fan. The fruits were stored until fruits of the deionized water
treatment were ripe with 95% yellow skin color. The experiment was repeated 3
times.

Field fertilizer treatment

Papaya variety "Kapoho Solo" was grown in the island of Hawaii. The
seedlings were transplanted into the field in 1987. The soil type belonged to the
Papai series of Typic Tropofolists. A randomized complete block design was used
with five treatments: control, Ca, Ca+K, K, and N. Commercial fertilization rates, used as the control level, were applied to the whole 26 acre field including the experimental block. The amount of fertilizer (N:P:K=14:14:14) applied was: 15 kg acre\(^{-1}\) month\(^{-1}\) in 1988, 36 kg acre\(^{-1}\) month\(^{-1}\) in 1989, and 24 kg acre\(^{-1}\) month\(^{-1}\) in 1990. For the other treatments, extra fertilizer was applied when the trees were 6 month old and had begun to flower in the spring of 1988. Trees were side dressed with Ca carbonate (containing 48.5% CaO) at the rate of 192 g tree\(^{-1}\) month\(^{-1}\) in Ca treatment, muriate of potash (containing 61% K\(_2\)O) at the rate of 197.5 g tree\(^{-1}\) month\(^{-1}\) in K treatment, and urea (containing 46% N) at the rate of 158.7 g tree\(^{-1}\) month\(^{-1}\) in N treatment. In the Ca+K treatment, trees had the same rate of Ca and K fertilizer as indicated above. The trees started to produce fruits in the fall of 1988. The experimental fruits were harvested for the first time in the September of 1989. The fruits were harvested monthly and air-shipped on the same day to Honolulu where the test was installed. Newly matured leaf petioles were harvested once from treatment plots in October 1990. The leaf petioles were frozen at -20°C until use.

Fruits with color break to 15% yellow were used. The fruits were stored until the skin color of the majority of fruits in the control treatment reached 85% yellow. Data were collected for 11 months.

**Analysis of total Ca, Mg, K, and N**

The mesocarp (50 g FW) was used to determine total Ca, Mg and K. The tissue was homogenized, extracted and analyzed by the same procedure outlined in
chapter 3. For leaf petiole, total Ca, Mg and K concentration were determined on ashed, dry ground tissue.

Mesocarp tissue from fruits harvested in October 1989 and newly matured leaf petioles harvested in October 1990 were used to determine total N. Total N of mesocarp and leaf petiole was measured as $\text{NH}_4$-N in acid digests using colorimetric procedure of Isaac and Johnson (1976). The tissue was dried in the oven at 70°C and ground into a powder (< 20 mesh). Dry tissue (250 mg) was transferred into a 75 ml digestion tube in which 1/2 teaspoon Na$_2$SO$_4$ was added. Acid digestion mixture (5 g SeO$_2$ and 65 g salicylic acid crystals in 2.2 l of concentrated H$_2$SO$_4$) were added into the digestion tube. The mixture was allowed to stand at least 2 hours, and then 3 drops of Na$_2$S$_2$O$_3$ solution (200 g Na$_2$S$_2$O$_3$ 5H$_2$O in 200 ml deionized water) were added. The mixture was allowed to stand for another hour. Two ml increments of H$_2$O$_2$ (30%) were added twice. Then the mixture was digested for 40 minutes at 410°C on a block digester. After cooling, deionized water was slowly added down the side of the tube. Then the contents were transferred to a 25/50 ml Folin Wu tube and the volume was brought to 25 ml with deionized water. The solution was stirred completely and allowed to stand overnight. The digested sample was measured for $\text{NH}_4$-N with segmented flow autoanalyzer (Technicon AAII).

Data analysis

Data were analyzed by "t" test or Duncan multiple range test and regression where appropriate.
Results

Pre-harvest Ca chloride treatment

There was no significant effect on mesocarp Ca concentration when fruits were sprayed 6 times with 2% CaCl$_2$ (Table 4.1). Calcium concentration was 115 $\mu$g g$^{-1}$ FW in control fruit, and 117 $\mu$g g$^{-1}$ FW in CaCl$_2$ treated fruit. Calcium chloride spray had no effect on mesocarp Mg and K concentration. The rate of fruit ripening, time to 95% skin yellow and fruit firmness, was not significantly different between control and the CaCl$_2$ treatment. Green spots (ca. 1 to 2 mm dia.) appeared on the fruit surface following CaCl$_2$ spraying. These spots remained green on ripe fruits.

Post-harvest CaCl$_2$ treatment

Dipping papaya fruits in 2% CaCl$_2$ did not significantly increase mesocarp Ca concentration (Table 4.2). Mesocarp Ca concentration was 159 $\mu$g g$^{-1}$ FW in the CaCl$_2$ treatment, and 171 $\mu$g g$^{-1}$ FW in the control treatment. Calcium chloride treatment had no effect on mesocarp Mg and K concentration. The final fruit firmness and post-harvest life were not affected by CaCl$_2$ treatment.

Field fertilization on Ca, Mg, K and N concentrations in the leaf petioles

Calcium fertilizer treatment significantly increased Ca concentration in leaf petioles above the control (Table 4.3). The Ca+K treatment had a tendency to increase leaf petiole Ca concentration while K treatment decreased it. Nitrogen treatment had no effect on leaf petiole Ca level.
Table 4.1 Effects of pre-harvest CaCl$_2$ applications to "Sunset" papaya fruit in Ca, Mg and K concentrations in the mesocarp and fruit ripening characteristics. The fruits of 10 trees were sprayed with deionized water (control) or 2% (w/v) CaCl$_2$ solution every 2 weeks. Fruits were harvested at the stage of 10% yellow skin color following 6 spray applications. Fruits were ripened at 25°C.

<table>
<thead>
<tr>
<th>CaCl$_2$ (%)</th>
<th>Concentration (µg g$^{-1}$ FW)</th>
<th>Deformation force (N)</th>
<th>Storage days$^y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>115 a 235 a 1125 a 34 a 3.5 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>117 a 222 a 1218 a 36 a 4.0 a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^z$. Data was analyzed with "t" test and means with same letters in the same column were not significantly different at 5% level, n=20;

$^y$. Storage days of fruits were subjectively evaluated by external skin color from 10 to 95 % yellow.
Table 4.2 Effects of post-harvest CaCl$_2$ application to "Sunset" papaya fruit on Ca, Mg and K concentration in the mesocarp and fruit ripening characteristics. Fruits were harvested at 10% yellow skin color and dried for 24 hours with a fan. Fruit peduncle was trimmed and dipped immediately into deionized water or 2% (w/v) of CaCl$_2$ solution for an additional 48 hours exposure to the fan. The fruits were stored until fruits of the deionized water treatment were ripe with 95% yellow skin color.

<table>
<thead>
<tr>
<th>CaCl$_2$ Concentration (µg g$^{-1}$ FW)</th>
<th>Deformation force (N)</th>
<th>Storage days</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)</td>
<td>Ca$^2$</td>
<td>Mg</td>
</tr>
<tr>
<td>0</td>
<td>171 a</td>
<td>203 a</td>
</tr>
<tr>
<td>2</td>
<td>159 a</td>
<td>198 a</td>
</tr>
</tbody>
</table>

$^2$. Data was analyzed with "t" test and means with same letters in the same column were not significantly different at 5% level, n=20.
Table 4.3 The effect of fertilization on leaf petiole Ca, Mg and K concentrations. Newly matured leaf petioles were harvested and stored at -20°C until use.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ca</th>
<th>Mg</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.98 bc</td>
<td>0.65 a</td>
<td>1.89 b</td>
</tr>
<tr>
<td>Ca</td>
<td>1.29 a</td>
<td>0.47 b</td>
<td>1.93 b</td>
</tr>
<tr>
<td>Ca+K</td>
<td>1.15 ab</td>
<td>0.29 c</td>
<td>3.80 a</td>
</tr>
<tr>
<td>K</td>
<td>0.84 c</td>
<td>0.41 bc</td>
<td>3.74 a</td>
</tr>
<tr>
<td>N</td>
<td>0.99 bc</td>
<td>0.76 a</td>
<td>1.21 c</td>
</tr>
</tbody>
</table>

*z. Data was analyzed with Duncan multiple range test and means with same letters in the same column were not significantly different at 5% level, n=6.*
The Ca treatment did not affect leaf petiole K level. Calcium plus K and K fertilizer treatments significantly increased leaf petiole K concentration. Nitrogen fertilizer treatment significantly decreased K level in the leaf petioles.

Calcium, Ca+K and K treatments significantly decreased leaf petiole Mg concentration (Table 4.3). Nitrogen fertilizer treatment had no effect on leaf petiole Mg concentration.

The N fertilizer treatment significantly increased leaf petiole N level; 52% higher N than that of the control treatment (Table 4.4). Other treatments had no effect on leaf petiole N level.

Field fertilization on fruit ripening

Except for N fertilizer treatment, all fertilizer treatments had no significant effect on ripe fruit skin color (Table 4.5 and Table 4.6). The N treatment significantly delayed both skin and mesocarp color development. Calcium fertilizer treatment and Ca+K fertilizer treatment slightly delayed mesocarp color development, while the K treatment had no effect.

Although Ca treatment did not always increase the firmness of the ripe fruit in each monthly tests (Table 4.5), Ca and Ca+K fertilizer treatments significantly increased the fruit firmness with an average deformation force of 76 N and 78 N, respectively versus 68 N in control treatment (Table 4.6). Potassium treatment had no significant effect on fruit firmness (69 N). Nitrogen fertilizer treated fruits were also firmer (81 N) than control fruits (Table 4.6).
Table 4.4 The effect of fertilization on leaf petiole nitrogen concentration. Newly matured leaf petioles were harvested and stored at -20°C until use.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N concentration (% DW)$^z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.98 b</td>
</tr>
<tr>
<td>Ca</td>
<td>0.98 b</td>
</tr>
<tr>
<td>Ca+K</td>
<td>1.02 b</td>
</tr>
<tr>
<td>K</td>
<td>0.95 b</td>
</tr>
<tr>
<td>N</td>
<td>1.59 a</td>
</tr>
</tbody>
</table>

$^z$. Data was analyzed with Duncan multiple range test and means with same letters in the same column were not significantly different at 5% level, n=3.
Table 4.5 Effects of fertilization on "Kapoho Solo" papaya fruits ripening. The fruits were ripened at 25 °C until the skin color of most fruits in the control treatment reached about 85% yellow.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>Initial skin color</th>
<th>Final skin color</th>
<th>Final mesocarp color</th>
<th>Deformation force (N)</th>
<th>Firmness ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>December, 1989</td>
<td>Control</td>
<td>11 a</td>
<td>91 a</td>
<td>91 a</td>
<td>87 a</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>6 a</td>
<td>83 a</td>
<td>94 a</td>
<td>92 a</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>Ca+K</td>
<td>5 a</td>
<td>90 a</td>
<td>99 a</td>
<td>93 a</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>8 a</td>
<td>89 a</td>
<td>97 a</td>
<td>86 a</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>8 a</td>
<td>86 a</td>
<td>93 a</td>
<td>78 a</td>
<td>0.88</td>
</tr>
<tr>
<td>January, 1990</td>
<td>Control</td>
<td>16 a</td>
<td>92 a</td>
<td>96 b</td>
<td>84 bc</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>6 a</td>
<td>80 b</td>
<td>86 c</td>
<td>108 a</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>Ca+K</td>
<td>5 a</td>
<td>80 b</td>
<td>90 c</td>
<td>104 ab</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>13 a</td>
<td>93 a</td>
<td>98 a</td>
<td>70 c</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>6 a</td>
<td>81 a</td>
<td>92 bc</td>
<td>86 abc</td>
<td>1.03</td>
</tr>
<tr>
<td>February, 1990</td>
<td>Control</td>
<td>2 b</td>
<td>85 a</td>
<td>93 a</td>
<td>76 bc</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>7 a</td>
<td>88 a</td>
<td>98 a</td>
<td>64 cd</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Ca+k</td>
<td>3 b</td>
<td>79 a</td>
<td>92 a</td>
<td>87 b</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>3 b</td>
<td>83 a</td>
<td>95 a</td>
<td>52 d</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1 b</td>
<td>55 b</td>
<td>81 b</td>
<td>106 a</td>
<td>1.40</td>
</tr>
</tbody>
</table>
Table 4.5 (Continue) Effects of fertilization on "Kapoho Solo" papaya fruits ripening. The fruits were ripened at 25 °C until the skin color of most fruits in the control treatment reached about 85% yellow.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>Initial skin color</th>
<th>Final skin color</th>
<th>Final mesocarp color</th>
<th>Deformation force (N)</th>
<th>Firmness ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>March, 1990</td>
<td>Control</td>
<td>0.3 a</td>
<td>73 c</td>
<td>87 ab</td>
<td>89 a</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>0.7 a</td>
<td>77 cb</td>
<td>84 b</td>
<td>87 ab</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Ca+K</td>
<td>0 a</td>
<td>87 ab</td>
<td>95 ab</td>
<td>83 ab</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0.5 a</td>
<td>91 a</td>
<td>99 a</td>
<td>69 b</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.3 a</td>
<td>82 abc</td>
<td>91 a</td>
<td>90 a</td>
<td>1.02</td>
</tr>
<tr>
<td>April, 1990</td>
<td>Control</td>
<td>2 a</td>
<td>91 a</td>
<td>96 a</td>
<td>85 ab</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>0 b</td>
<td>89 a</td>
<td>98 a</td>
<td>77 b</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Ca+K</td>
<td>0 b</td>
<td>77 b</td>
<td>86 b</td>
<td>102 a</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0 b</td>
<td>81 ab</td>
<td>92 ab</td>
<td>89 ab</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0 b</td>
<td>86 ab</td>
<td>95 a</td>
<td>77 b</td>
<td>0.89</td>
</tr>
<tr>
<td>May, 1990</td>
<td>Control</td>
<td>7 a</td>
<td>89 a</td>
<td>100 a</td>
<td>52 b</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>6 ab</td>
<td>88 a</td>
<td>100 a</td>
<td>53 b</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>Ca+k</td>
<td>3 b</td>
<td>89 a</td>
<td>99 a</td>
<td>61 ab</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>7 a</td>
<td>89 a</td>
<td>97 a</td>
<td>53 b</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>4 b</td>
<td>78 a</td>
<td>94 a</td>
<td>67 a</td>
<td>1.27</td>
</tr>
</tbody>
</table>
Table 4.5 (Continue) Effects of fertilization on "Kapoho Solo" papaya fruits ripening. The fruits were ripened at 25 °C until the skin color of most fruits in the control treatment reached about 85% yellow.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>Initial skin color</th>
<th>Final skin color</th>
<th>Final mesocarp color</th>
<th>Deformation force (N)</th>
<th>Firmness ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>June, 1990</td>
<td>Control</td>
<td>9 a</td>
<td>91 a</td>
<td>99 a</td>
<td>45 b</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>6 abc</td>
<td>90 a</td>
<td>96 ab</td>
<td>68 a</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>Ca+K</td>
<td>2 c</td>
<td>84 a</td>
<td>92 b</td>
<td>71 a</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>3 bc</td>
<td>90 a</td>
<td>98 ab</td>
<td>65 a</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>6 ab</td>
<td>86 a</td>
<td>95 ab</td>
<td>65 a</td>
<td>1.42</td>
</tr>
<tr>
<td>July, 1990</td>
<td>Control</td>
<td>4 a</td>
<td>78 ab</td>
<td>89 ab</td>
<td>63 b</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>3 ab</td>
<td>84 a</td>
<td>94 a</td>
<td>77 ab</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>Ca+K</td>
<td>0.3 cb</td>
<td>80 ab</td>
<td>94 a</td>
<td>82 ab</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>3 a</td>
<td>78 ab</td>
<td>95 a</td>
<td>73 ab</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0 c</td>
<td>64 ab</td>
<td>79 b</td>
<td>89 a</td>
<td>1.40</td>
</tr>
<tr>
<td>August, 1990</td>
<td>Control</td>
<td>10 a</td>
<td>95 a</td>
<td>99 a</td>
<td>46 c</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>8 a</td>
<td>90 a</td>
<td>97 a</td>
<td>58 abc</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>Ca+K</td>
<td>8 a</td>
<td>90 a</td>
<td>97 a</td>
<td>49 bc</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>6 a</td>
<td>91 a</td>
<td>98 a</td>
<td>62 ab</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>8 a</td>
<td>94 a</td>
<td>98 a</td>
<td>70 a</td>
<td>1.51</td>
</tr>
</tbody>
</table>
Table 4.5 (Continue) Effects of fertilization on "Kapoho Solo" papaya fruits ripening. The fruits were ripened at 25 °C until the skin color of most fruits in the control treatment reached about 85% yellow.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>Initial skin color</th>
<th>Final skin color</th>
<th>Final mesocarp color</th>
<th>Deformation force (N)</th>
<th>Firmness ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>September, 1990</td>
<td>Control</td>
<td>6 a</td>
<td>95 a</td>
<td>100 a</td>
<td>67 ab</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>2 b</td>
<td>89 b</td>
<td>96 b</td>
<td>81 a</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Ca+K</td>
<td>5 ab</td>
<td>92 ab</td>
<td>98 ab</td>
<td>72 ab</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>3 b</td>
<td>93 ab</td>
<td>98 ab</td>
<td>65 b</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>3 ab</td>
<td>92 ab</td>
<td>98 ab</td>
<td>79 ab</td>
<td>1.16</td>
</tr>
<tr>
<td>October, 1990</td>
<td>Control</td>
<td>10 b</td>
<td>97 a</td>
<td>99 b</td>
<td>66 b</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>3 c</td>
<td>88 b</td>
<td>99 b</td>
<td>89 a</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>Ca+K</td>
<td>18 a</td>
<td>98 a</td>
<td>100 b</td>
<td>64 b</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>9 b</td>
<td>96 a</td>
<td>104 a</td>
<td>73 b</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>2 c</td>
<td>89 a</td>
<td>97 b</td>
<td>76 ab</td>
<td>1.14</td>
</tr>
</tbody>
</table>

* Data was analyzed with Duncan multiple range test and means with same letters in the same column were not significantly different at 5% level, n=20;

* % yellow of the fruit surface;

* Firmness ratio = firmness of the treatment / firmness of control treatment.
Table 4.6 Effects of fertilization on "Kapoho Solo" papaya fruits ripening (summary). The fruits were ripened at 25°C until the skin color of most fruits in the control treatment reached about 85 % yellow.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial skin color</th>
<th>Final skin color</th>
<th>Final mesocarp color</th>
<th>Deformation force (N)</th>
<th>Firmness ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7 a</td>
<td>89 a</td>
<td>96 ab</td>
<td>68 b</td>
<td>1.00</td>
</tr>
<tr>
<td>Ca</td>
<td>4 b</td>
<td>86 a</td>
<td>95 b</td>
<td>76 a</td>
<td>1.13</td>
</tr>
<tr>
<td>Ca+K</td>
<td>4 b</td>
<td>86 a</td>
<td>95 b</td>
<td>78 a</td>
<td>1.16</td>
</tr>
<tr>
<td>K</td>
<td>5 b</td>
<td>88 a</td>
<td>97 a</td>
<td>69 b</td>
<td>1.02</td>
</tr>
<tr>
<td>N</td>
<td>3 b</td>
<td>80 b</td>
<td>91 c</td>
<td>81 a</td>
<td>1.20</td>
</tr>
</tbody>
</table>

* Data was analyzed with Duncan multiple range test and means with same letters in the same column were not significantly different at 5% level, n=220;

*% of yellow of the fruit surface;

* Firmness ratio = firmness of the treatment / firmness of control treatment.
Figure 4.1 Effect of fertilization on mesocarp Ca (A), Mg (B) and K (C) concentrations throughout the year. The treatments were control (●), Ca (△), Ca plus K (○), K (□), nitrogen (▲). Analysis of variance: month_Ca concentration ***, Mg concentration ***, K concentration not significant; treatment ***, month x treatment_Ca concentration ***, Mg concentration ***, K concentration not significant. Each data point the mean of 15 observations. Significant at 0.1% (***)
Field fertilization on mesocarp Ca, Mg, K and N concentrations

There was an overall increase in mesocarp Ca concentration but a decrease in Mg concentration in all treatments throughout the year (Fig. 4.1 A and B). Mesocarp Ca and Mg concentration fluctuated significantly, but mesocarp K concentration had no significant difference throughout the year (Fig. 4.1 C).

During the 11-month period, the mesocarp of the Ca fertilizer treatment had the highest Ca level in 2 months, and the mesocarp of the Ca+K fertilizer treatment had the highest Ca level for 8 months by comparison with other treatments (Fig. 4.1 A). The mesocarp of the K treatment had the lowest Ca level in 10 months out of 11 months. The N treatment also reduced Ca concentration in the mesocarp (Fig. 4.1 A). In the control treatment, mesocarp Ca concentration was at peak levels: 120, 172 and 183 μg g⁻¹ FW, in January, April and September, respectively. In the Ca fertilizer treatment, mesocarp Ca concentration reached the peak in January (129 μg g⁻¹ FW), April (156 μg g⁻¹ FW), August (176 μg g⁻¹ FW) and October (178 μg g⁻¹ FW). In Ca+K fertilizer treatment, mesocarp Ca concentration peaked in January (138 μg g⁻¹ FW), April (155 μg g⁻¹ FW) to May (157 μg g⁻¹ FW), and September (201 μg g⁻¹ FW). In the K fertilizer treatment, the peak of mesocarp Ca concentration occurred in December (108 μg g⁻¹ FW), April (131 μg g⁻¹ FW) and August (158 μg g⁻¹ FW). In N fertilizer treatment, Ca concentration in the mesocarp did not fluctuate as much as the other treatments, with mesocarp Ca concentration in the range of 115 to 117 μg g⁻¹ FW from December to February, and in the range of 149 to 158 μg g⁻¹ FW from July to October. In all treatments, mesocarp Ca
concentration had peak levels in April and had the lowest level in February and/or March. Based on 11 months data, Ca and Ca+K fertilizer treatments significantly increased mesocarp Ca concentration (Table 4.7). Both N and K fertilizer treatments significantly decreased mesocarp Ca concentration (Table 4.7).

The seasonal change in mesocarp Mg concentration varied in a similar pattern to mesocarp Ca concentration through July. In the control, mesocarp Mg concentration had peak levels in April (246 μg g⁻¹ FW) and July (220 μg g⁻¹ FW). In the Ca fertilizer treatment, mesocarp Mg concentration decreased throughout the year with the highest level in January (228 μg g⁻¹ FW) and the lowest level in October (127 μg g⁻¹ FW). In Ca+K fertilizer treatment, the peak level of mesocarp Mg occurred in February (245 μg g⁻¹ FW), April (234 μg g⁻¹ FW) and July (195 μg g⁻¹ FW). In K fertilizer treatment, mesocarp Mg concentration reached the peak in February (216 μg g⁻¹ FW), April (226 μg g⁻¹ FW) and July (192 μg g⁻¹ FW). In the N fertilizer treatment, mesocarp Mg concentration had peaks in February (209 μg g⁻¹ FW), April (202 μg g⁻¹ FW) and July (215 μg g⁻¹ FW). The variation patterns of Mg concentration were similar, with peak level in April and July, in all treatments except Ca treatment. Based on 11-month data, Ca, K and N fertilizer treatments decreased mesocarp Mg concentration (Table 4.7).

Potassium and Ca+K fertilizer treatments increased mesocarp K concentration, while N fertilizer treatment reduced mesocarp K concentration throughout the year (Fig. 4.1). Based on 11-month data, Ca fertilizer treatment had no effect on mesocarp K concentration, and Ca+K fertilizer treatments increased
about 40% mesocarp K concentration, and N fertilizer treatment decreased (17%) mesocarp K concentration (Table 4.7).

The N fertilizer treatment did not increase mesocarp N concentration (Table 4.8). The Ca fertilizer treatment significantly increased the N concentration. The K and Ca+K fertilizer treatments had no effect on mesocarp N concentration.

**Seasonal changes of rainfall and relative humidity**

The amount of rainfall was higher from November 1989 to February 1990 than in the other months (Fig. 4.2). Rainfall was highest (200 mm) in January 1990. The relative humidity was within the range of 85% to 93%, from November 1989 to March 1990.

**The relationship between Ca, Mg and K concentration and fruit firmness**

Mesocarp Ca concentration was positively correlated with ripe fruit firmness (Table 4.9). The quadratic (Firmness ratio = -0.521 + 0.022Ca - 0.00007Ca²) and cubic (Firmness ratio = -2.707 + 0.071Ca - 0.0004Ca² + 0.0000008Ca³) regression equations were significant. The development of skin and mesocarp color during fruit ripening was significantly delayed, and the softening of the fruits was significantly slower in the fruits from the N treatment (Table 4.5 and Table 4.6). The June data did not follow the same trends as other monthly data. The significance increased to 0.1% level in quadratic (Firmness ratio = -0.717 + 0.023Ca - 0.00007Ca²) and cubic (Firmness ratio = -2.780 + 0.069Ca - 0.0004Ca² + 0.0000007Ca³) regression equations if the data from the N treatment and June data were removed (Table 4.9). When
Table 4.7 Effects of fertilization on "Kapoho Solo" papaya mesocarp Ca, Mg and K concentration.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (μg g⁻¹ FW)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>Control</td>
<td>138 b</td>
</tr>
<tr>
<td>Ca</td>
<td>145 a</td>
</tr>
<tr>
<td>Ca+K</td>
<td>151 a</td>
</tr>
<tr>
<td>K</td>
<td>120 d</td>
</tr>
<tr>
<td>N</td>
<td>130 c</td>
</tr>
</tbody>
</table>

² Data was analyzed with Duncan multiple range test and means with same letters in the same column were not significantly different at 5% level, n=165.
Table 4.8 The effect of fertilization on "Kapoho Solo" papaya mesocarp nitrogen concentration.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N concentration (% DW)\textsuperscript{z}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.48 b</td>
</tr>
<tr>
<td>Ca</td>
<td>0.73 a</td>
</tr>
<tr>
<td>Ca+K</td>
<td>0.57 ab</td>
</tr>
<tr>
<td>K</td>
<td>0.47 b</td>
</tr>
<tr>
<td>N</td>
<td>0.45 b</td>
</tr>
</tbody>
</table>

\textsuperscript{z}. Data was analyzed with Duncan multiple range test and means with same letters in the same column were not significantly different at 5% level, n=3.
Figure 4.2 Seasonal changes of rainfall and relative humidity. Rainfall (▲), relative humidity (△).
Table 4.9  The relationship between Ca, Mg and K concentrations in the mesocarp and fruit firmness.

<table>
<thead>
<tr>
<th>Regression model</th>
<th>Coefficient of determination²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minus N and June data</td>
</tr>
<tr>
<td>Firmness ratio = a+bCa</td>
<td>0.246 **</td>
</tr>
<tr>
<td>Firmness ratio = a+bCa+cCa²</td>
<td>0.413 ***</td>
</tr>
<tr>
<td>Firmness ratio = a+bCa+cCa²+dCa³</td>
<td>0.429 ***</td>
</tr>
<tr>
<td>Firmness ratio = a+bMg</td>
<td>0.038</td>
</tr>
<tr>
<td>Firmness ratio = a+bMg+cMg²</td>
<td>0.128</td>
</tr>
<tr>
<td>Firmness ratio = a+bMg+cMg²+dMg³</td>
<td>0.162</td>
</tr>
<tr>
<td>Firmness ratio = a+bK</td>
<td>0.0003</td>
</tr>
<tr>
<td>Firmness ratio = a+bK+cK²</td>
<td>0.059</td>
</tr>
<tr>
<td>Firmness ratio = a+bK+cK²+dK³</td>
<td>0.059</td>
</tr>
<tr>
<td>Firmness ratio = a+b(Ca/Mg)</td>
<td>0.181 **</td>
</tr>
<tr>
<td>Firmness ratio = a+b(Ca/Mg)+c(Ca/Mg)²</td>
<td>0.202 *</td>
</tr>
<tr>
<td>Firmness ratio = a+b(Ca/Mg)+c(Ca/Mg)²+d(Ca/Mg)³</td>
<td>0.386 **</td>
</tr>
</tbody>
</table>
Table 4.9 (Continue) The relationship between Ca, Mg and K concentrations in the mesocarp and fruit firmness

<table>
<thead>
<tr>
<th>Regression model</th>
<th>Coefficient of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minus N and June data</td>
</tr>
<tr>
<td>Firmness ratio = a + b(Ca/K)</td>
<td>0.086</td>
</tr>
<tr>
<td>Firmness ratio = a + b(Ca/K) + c(Ca/K)^2</td>
<td>0.215 *</td>
</tr>
<tr>
<td>Firmness ratio = a + b(Ca/K) + c(Ca/K)^2 + d(Ca/K)^3</td>
<td>0.255 *</td>
</tr>
<tr>
<td>Firmness ratio = a + b[Ca/(Mg+K)]</td>
<td>0.114 *</td>
</tr>
<tr>
<td>Firmness ratio = a + b[Ca/(Mg+K)] + c[Ca/(Mg+K)]^2</td>
<td>0.221 **</td>
</tr>
<tr>
<td>Firmness ratio = a + b[Ca/(Mg+K)] + c[Ca/(Mg+K)]^2 + d[Ca/(Mg+K)]^3</td>
<td>0.260 *</td>
</tr>
</tbody>
</table>

Data was analyzed by the procedures of general linear model and non-linear curve fitting model; *Firmness ratio = firmness of treatment / firmness of control treatment; *, **, *** mean significant level at 5%, 1% and 0.1%, respectively.
Figure 4.3 The relationship between Ca concentration in the mesocarp and ripe fruit firmness.
mesocarp Ca concentration reached 150 μg g⁻¹ FW or above fruits could keep firmer during ripening (Fig. 4.3).

Mesocarp Mg and K concentrations did not correlated with firmness ratio of ripe fruits (Table 4.9). Mesocarp concentration ratios of Ca to Mg, Ca to K and Ca to Mg+K were correlated with firmness ratio of ripe fruits. The significance of these correlations increased if the data from the N treatment and June were removed.

Discussion

Papaya nutrition research during 1960's to early 1980's attempted to optimize tree growth and to increase the yield. Previous studies were focused on the effects of N, K, phosphorus, Mg, and boron fertilization on growth, fruiting and petiole composition of papaya plants (Awada and Long, 1969 and 1971; Wang and Ko, 1975; Cibes and Gaztambide, 1978; Parez and Childers, 1982). These studies have contributed to the growth of papaya industry in Hawaii. With the recognition of Ca as a crucial element in fruit quality, more attention has been brought to the effect of fruit nutrient status on fruit quality maintenance. In apple, fairly comprehensive information on the effect of Ca on fruit ripening and senescence has been published (Conway and Sams, 1987; Glenn et al., 1988; Poovaiah et al., 1988; Abbott et al., 1989; Stow, 1989; Ferguson and Watkins, 1989). There is no published information on the effects of Ca on papaya fruit quality.

Spraying Ca salt on apple fruits during their growth and development stage provided a safe method of supplementing endogenous Ca (Glenn et al., 1985;
Howett and Watkins, 1991). Such treatments reduce the incidence of physiological disorders, maintain fruit firmness, and reduce the rate of senescence (Conway and Sams, 1987; Glenn et al., 1988; Abbott et al., 1989; Howett and Watkins, 1991). Spraying CaCl$_2$ onto papaya during fruit growth and development did not significantly increase Ca level in the mesocarp (Table 4.1). The Ca might remain only on the surface of the fruit skin as indicated by green spots on ripe fruit skin. The fruit cuticle is the first barrier to the penetration of Ca into fruit. Calcium can diffuse through the cuticle of apple fruit (Glenn et al., 1985; Charnel, 1989). The thickness of the cuticle of papaya fruit increases from 15 to 50 $\mu$m during growth and development (Quintana and Paull unpublished data). The average thickness of the cuticle of mature papaya fruit is 41 $\mu$m (Paull and Chen, 1989) and is almost 2 times thicker than that of "Golden Delicious" apple fruit (Glenn et al., 1985). In order to penetrate the cuticular structure, the Ca ion must pass through the outer waxy epicuticle layer, the underlying cutin matrix and the hydrophilic pectin and epidermal cell wall region (Schonherr and Bukovac, 1972). The structure of lenticels, cracks, or other surface irregularities are the important pathway of Ca diffusion through the cuticle in apple (Glenn et al., 1985). In papaya fruit, there are no lenticles on the surface and the stomata on the surface of mature fruit are apparently blocked by wax (Paull and Chen, 1989). Other factors are also involved in Ca penetration, such as charged groups of the cutin polymer and the pH of the spray solution (Chamel, 1989).
Infiltration of Ca into apple fruit under vacuum or pressure improves the effectiveness of Ca dips (Ferguson and Watkins, 1989). The Ca concentration in vacuum infiltrated apple fruit is 70% higher than in untreated fruit (Ferguson and Watkins, 1989). Vacuum infiltration of dye into papaya fruits only reaches a depth of 1 to 2 mm (Paull, unpublished data). Papaya fruit exocarp is about 0.5 mm thick at the immature green stage and 1 mm thick at the mature green stage (Sanxter, 1989). Exocarp tissue has very small intercellular spaces (Sanxter, 1989). Attempts to draw Ca into the mesocarp through transpiration by dipping papaya fruit peduncle with CaCl$_2$ for 48 hours, did not increase the mesocarp Ca level in mesocarp. Transpiration in detached fruit may not be an effective motive force to move Ca into the mesocarp. Alternatively, the peduncle vascular bundle may become blocked during the 48 hours treatment.

Calcium soil fertilization did not always increase mesocarp Ca concentration (Fig. 4.1). Calcium uptake by papaya plants may be influenced by many factors. Temperature, relative humidity, levels of other minerals in the soil, and plant age all can affect the uptake of Ca by plants (Shear, 1975; Clarkson, 1984; Ferguson, 1984; Kirkby and Pilbeam, 1984). The present field fertilization experiment was carried out in a non-irrigated commercial papaya plantation with Tropofolists soil type. Tropofolists soil has a moisture retention capacity similar to a "sand type", i.e. a large quantity of water is drained at low tensions (Yaibuches, 1971). Calcium uptake is mostly associated with water uptake by roots (Bangerth, 1979; Kirkby, 1979; Clarkson, 1984). High mesocarp Ca concentration of fruits harvested in April
may be related to the high rainfall during January to February when the fruits were in the young growth and developmental stage. Mesocarp Ca uptake rate was high 60 days post-anthesis (Fig. 3.6). Mesocarp Ca concentration increased in all treatments as the plants aged (Fig. 4.1). Growing root tips appear to be the main site of Ca uptake (Robards et al., 1973). As papaya plants aged and root branching increased, Ca uptake by the plants would be expected to increase.

Calcium fertilizer treatment increased leaf petiole Ca concentration (Table 4.3). Application of Ca to papaya plants increases Ca concentration in leaf petiole, therefore, the newly mature leaf petiole were selected as index tissue for Ca fertilization (Awada and Suehisa, 1984). Growing tissues and transpiring leaves are two types of Ca sinks which influence Ca movement independently (Clarkson, 1984). After a juvenile period, the papaya plant simultaneously has both vegetative and reproductive growth throughout the year. This means that there is always competition for Ca between vegetative organs and fruits. However, when abundant Ca is loaded into the xylem of the fruit tree roots, competition between the Ca sinks had no harmful consequences (Clarkson, 1984). Calcium fertilization of the soil is necessary in order to produce papaya fruits with a higher Ca concentration.

Like Ca, Mg concentration in the mesocarp also fluctuated significantly throughout the year (Fig. 4.1). The uptake of Mg by the roots is similar to Ca and its movement across the cortex largely occurs in the apoplast (Ferguson and Clarkson, 1976). In contrast to Ca, there was a decrease in mesocarp Mg concentration in all treatments throughout the year (Fig. 4.1). Calcium fertilizer
treatment decreased mesocarp Mg concentration (Table 4.7). Xylem vessels have exchange sites for Ca\(^{++}\) and other divalent cations and these sites would be close to saturation (Clarkson, 1984). The cell wall always exhibited a larger preference for Ca\(^{++}\) than for Mg\(^{++}\) (Demarty et al., 1984). It is possible that Ca occupies most of the exchange sites in the xylem vessels resulting in less Mg uptake by papaya fruits.

Mesocarp K concentration was relatively stable throughout the year within each treatment (Fig. 4.1). Potassium and Ca+K fertilizer treatments significantly increased mesocarp K concentration (Table 4.7). Potassium is taken up along the length of roots (Russell and Clarkson, 1976) and is high in the phloem sap (Mengel and Kirkby, 1982). Potassium ions can move via both symplast and apoplast systems (Kirkby, 1979; Mengel and Kirkby, 1982). Plant organs that are preferentially supplied with phloem sap, such as fleshy fruit, are therefore high in K (Mengel and Kirkby, 1982).

The N fertilizer treatment significantly increased N concentration in leaf petioles (Table 4.4) but not in the mesocarp (Table 4.8). Other research demonstrate increased N content in papaya leaf petiole in response to N fertilization (Awada et al., 1986). Nitrogen is transported up to other plant parts by the xylem vessels, and the intensity of the N metabolism, and particularly the rate of protein synthesis, control the N import by different plant parts (Mengel and Kirkby, 1982). The apex of papaya plant is actively growing throughout the year and thus appears to be a strong sink for N. The mesocarp of papaya contains small amounts of free amino acid (Chen, 1963). The nitrogen concentration is about 6 to 7 times higher

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in seeds than in flesh (Awada and Suehisa, 1970). In papaya plants, the mesocarp seems to be a weaker sink for N than leaf petioles.

Potassium fertilization reduced Ca concentration in leaf petioles (Table 4.3) and the mesocarp (Table 4.7). Other field experiments have also found that K fertilization decreases leaf petiole Ca concentration (Awada and Long, 1971; Awada, 1977; Awada et al., 1986). There are two possible reasons why high K fertilization results in low Ca uptake by plants. One possibility is that increased soil K displaces exchangeable Ca that was then leached from the root zone (Russell, 1973). The field fertilization experiment was carried out on the Papai series, Typic Tropofolists soil. The Papai series consists of well-drained, muck soil developed in organic matter and volcanic ash that is underlain by "aa" lava (Ikawa et al., 1985). Cation exchange capacities of Tropofolists are high only on the surface, but low in the subsoil (Periaswamy, 1973). The Ca ions on the soil particles of this soil type might be displaced by the K cations after K fertilization. Another possibility is that the cells produce anion equivalents that non-specifically attract cations (Kirkby, 1979). Cation species that are taken up fastest neutralize these anion equivalents first and reduce the electrostatic attraction for the uptake of other cation species (Kirkby, 1979). Potassium is taken up by the plant through both active and passive pathways (Ratner and Jacoby, 1976). Therefore, K is favored in comparison to other cation species and can compete strongly in cation uptake.

On the other hand, Ca fertilization did not affect the K concentration in leaf petiole (Table 4.3) and the mesocarp (Table 4.7). The effect of Ca++ on K+ uptake
by Ca\textsuperscript{++} is pH dependent (Marschner, 1986). The stimulation of K\textsuperscript{+} uptake increases with a decreased pH (Jacobson et al., 1960; Marschner, 1986). This effect is possibly related to the properties of Ca in membrane integrity (Poovaiah et al., 1988). Calcium ions inhibit the K\textsuperscript{+} uptake when the soil pH is above 6 (Jacobson et al., 1960). At high pH condition, cation competition occurs between K\textsuperscript{+} and Ca\textsuperscript{++} (Jacobson et al., 1960). The pH of Tropofolists soil is in the range of 4.1 to 5.8 (Periaswamy, 1973). Awada et al. (1975) found that CaCO\textsubscript{3} application (11.2 metric ton ha\textsuperscript{-1}) increased soil pH from 4.6 to 6.7 and lowered leaf petiole K concentration. The amount of CaCO\textsubscript{3} (192 g per tree) applied in the present experiment may not lead to competition with K for uptake by papaya plant because it was insufficient to increase soil pH to above 6.

Calcium plus K fertilization increased mesocarp Ca concentration (Table 4.7). Although K may compete with Ca for cation exchange sites or for uptake by the plants, the plant could still absorb a certain amount of Ca, especially when Ca fertilizer was applied at the same time. Application of K fertilizer to papaya plants increases mesocarp total soluble solids (Awada and Long, 1971). It is possible that increases in mesocarp K and assimilates reduce the water potential, resulting in water and Ca movement into the mesocarp.

Nitrogen (urea) fertilization had no effect on leaf petiole Ca concentration (Table 4.3), but decreased mesocarp Ca concentration (Table 4.7). There is a strong association between high N fertilization and Ca deficiency disorders in apple fruits (Shear, 1975; Ferguson and Watkins, 1989). Ammonium ions may compete with Ca
uptake in the root, in transport and in distribution within the plant (Fukumoto and Nagai, 1983). Because the demand for N is higher in shoots, the stimulation of shoot growth by N may divert the Ca from the fruit to the shoot. Nitrogen fertilization may also dilute the Ca concentration due to greater growth of the fruit.

The N fertilizer treatment decreased K concentration in both leaf petiole (Table 4.3) and the mesocarp (Table 4.7). The form of N applied in our experiment was urea. The relationship between NH$_4^+$ and K$^+$ uptake is not simple competition. While NH$_4^+$ effectively decreases the K$^+$ uptake, the inhibition of NH$_4^+$ uptake by K$^+$ does not occur (Rufty et al., 1982). It is assumed that a small proportion of ammonium N is taken up by the plant in the form of NH$_3$ instead of NH$_4^+$, leaving H$^+$ in the external solution (Rufty et al., 1982). Inhibition of uptake of K$^+$ by NH$_4^+$ may be a reflection of competition for negative charges within the cells (Marschner, 1986).

Calcium concentration in the mesocarp was positively correlated with the firmness of ripe papaya fruits (Table 4.9 and Fig. 4.3). Apple fruits with naturally higher Ca concentration or with Ca concentration artificially raised during development on the tree or after harvest ripe at a slower rate than those with lower Ca concentration (Cooper and Bangerth, 1976; Conway and Sams, 1987; Abbott et al., 1989). There is a positive correlation between Ca concentration and tensile strength or the firmness of apple fruit tissue (Poovaiah et al., 1988). Infiltration with CaCl$_2$ increases the strength of apple fruit tissue immediately and during storage (Conway and Sams, 1987; Abbott et al., 1989). Cherry fruit firmness is also
increased after Ca application (Facteau et al., 1987). Avocados with lower Ca concentration ripen more rapidly than those with higher Ca concentration (Tingwa and Young, 1974).

The textural changes of fruit tissue during fruit softening result primarily from changes in cell wall structure (Huber, 1983; Fischer and Bennett, 1991). The changes include the decrease in cell cohesion (Poovaiah et al., 1988) and the degradation of cell wall polymers (Huber, 1983; Seymour et al., 1987; Seymour et al., 1990). The middle lamella is rich in pectin materials that contribute to cell cohesiveness (Poovaiah et al., 1988). The primary cell wall has approximately 35% pectic polysaccharide (Darvill et al., 1980) which serves as a matrix for the microfibers of cellulose and hemicellulose (Fry, 1986). Pectin material constitutes an important site of Ca interaction. The pectin matrix is created by cross bridges between Ca²⁺ and the (1-4)-α-D-galactosyluronic acid, forming an "egg-box" rigid structure (Grant et al., 1973). Calcium cross links are load-bearing bonds which control the loosening and extensibility of the cell wall (Cleland, 1987; Virk and Cleland, 1988, 1990). Fruit softening has been considered largely to be the result of enzyme-mediated hydrolysis of the cell walls (Huber, 1983; Fischer and Bennett, 1991). Calcium seems to delay softening in apple by delaying degradation of the cell wall polymers (Sams and Conway, 1984).

When the mesocarp Ca concentration reached to 130 μg g⁻¹ FW (approximately 1.30 mg g⁻¹ DW) the "soft fruit" disorders in papaya could be effectively reduced (Fig. 4.3). The critical Ca concentration for eliminating the
development of tipburn in lettuce (Barta and Tibbits, 1991), of blossom-end rot in tomato (Cerda et al., 1979), and of bitter pit in apple (Fallahi et al., 1988) is 0.4 mg g⁻¹ DW. Papaya mesocarp Ca concentration is naturally higher than lettuce leaf, and tomato and apple fruits. Therefore, a higher threshold in papaya fruit would be expected.

There were no correlations between the firmness of ripe fruits and Mg concentration, or K concentration. There was a correlation between fruit firmness and the ratio of Ca concentration to Mg concentration, or to K concentration, or to Mg+K concentrations (Table 4.9). This involvement of K or Mg appears to be related to Ca, rather than by an effect of these minerals per se. One of the causes of fruit softening is the movement of Ca²⁺ from the middle lamella (Knee and Bartly, 1981; Knee, 1982). Infiltration of apple fruit or fruit tissue with Mg²⁺ or Sr²⁺, which have similar charge and size to Ca²⁺, increased the firmness of the fruit or the fruit tissue (Conway and Sams, 1987; Stow, 1989). Infiltration to fruit tissue with K⁺ also slightly increased the firmness of the fruit tissue (Stow, 1989). Calcium and Mg exchange for each other in apple fruit tissue (Ferguson and Watkins, 1981 a and b; Hanker and Ferguson, 1989). Even though ion exchanges between Ca and Mg or between Ca and monovalent cations are performed in the cell wall, cell walls always prefer Ca (Demarty et al., 1984). Localized deficiency of Ca or an imbalance between Ca and other minerals may be the main cause of the Bitter Pit disorder in apple (Ferguson and Watkins, 1989). Applications of Mg and K cause Bitter Pit or Bitter Pit-like symptoms (Cooper and Bangerth, 1976). However, if the Ca
concentration is high enough (about 50 μg g⁻¹ FW), then even high concentrations of Mg and K do not result in Bitter Pit (Perring, 1986).
CHAPTER 5.

CHANGES OF PECTIN AND HEMICELLULOSE DURING FRUIT RIPENING

Introduction

Fruit ripening is the last stage of fruit development which involves a major transition in metabolism. In addition to the biosynthesis of pigments and volatiles, fruit ripening is also characterized by the softening of the flesh tissue (Huber, 1983; Brady, 1987; Fischer and Bennett, 1991). Dramatic changes in pectin structure also accompany the softening of many fruits. For several decades, researches have been focused on the degradation of cell wall pectin by polygalacturonase (PG) (Gross, 1990). Polygalacturonase might be responsible for ripening-associated pectin degradation and fruit softening (Huber, 1983; Bennett and DellaPenna, 1987; Brady, 1987). However, recent studies indicate that the endo-polygalacturonase may not be the dominant factor in tissue softening of tomato (Giovannoni et al., 1989; Smith et al., 1988, 1990). Some fruits, such as strawberry (Huber, 1984) and muskmelon (McCollum et al., 1989) also soften without detectable PG during ripening.

Biochemical studies of cell wall related to fruit softening indicate that the structural changes of pectin, hemicellulose and cellulose together are the cause of the alteration of cell wall structure (Pesis et al., 1978; Huber, 1983; Tong and Gross, 1989; Fishman et al., 1989; McCollum et al., 1989; Seymour et al., 1990). Modification of hemicellulose structure associated with fruit ripening has been reported in tomato (Huber, 1983), strawberry (Huber, 1984), and muskmelons
(McCollum et al., 1989). The size of hemicellulose polymers decreased during ripening of these fruits. Changes in cellulose has also been documented in ripening avocado (Pesis et al., 1978), pear (Ahmed and Labavitch, 1980), apple (Bartley, 1976), and tomato (Maclachlan and Brady, 1992). Recent evidence indicates that cell wall synthesis during fruit ripening could alter overall wall strength and reduce fruit firmness (Mitcham et al., 1989, 1991; Greve and Labavitch, 1991).

In papaya, cell wall-degrading enzymes during fruit ripening have been investigated (Chan and Tam, 1982; Paull and Chen, 1983). These enzymes include polygalacturonase (PG), pectin methylesterase (PME), xylanase and cellulase. There is a close relationship between PG and xylanase and fruit softening (Paull and Chen, 1983). The present study investigated the mechanisms of papaya fruit softening by analyzing the changes in cell wall components. The results reported in this chapter indicate that the softening of papaya fruits involve degradation of at least two cell wall components, pectin and hemicellulose.

Materials and methods

Plant material

Papaya (Carica papaya L.) variety "Sunset" plants were grown in a field at the Poamoho Experimental Station in central Oahu. Fruits were harvested at color break to 5% yellow stage and stored at 25°C until they reached the desired ripeness stage required for the experiment. Fruits were selected based on % yellow of skin and firmness. Fruit skin color was subjectively evaluated. Fruit firmness was
determined with a penetrometer fitted with a diameter of 1.6 cm tip. The penetrometer was pressed 2 mm into fruit at the fruit equator.

**Preparation of cell wall material**

Fruits with skin color of color break, 30, 50, 60 and 100% yellow were used. Mesocarp tissue from the fruit equatorial area was taken. The skin, placenta and seeds were removed. The mesocarp tissue was sliced (about 1 mm thick) and quickly frozen with liquid N. The frozen mesocarp tissue in mortar was ground with a pestle in liquid N to a very fine powder. Twenty five g of this powder was placed in 250 ml of boiling 80% (v/v) ethanol for 20 minutes to inactivate potential wall-modifying enzymes. The insoluble material was collected by centrifugation (10 min. 628 x g). The pellet was washed 3 times with 80% (v/v) ethanol, then with acetone until the pellet was turned from yellow to white. The pellet was then extracted with 90% dimethylsulfoxide (DMSO) overnight, and then thoroughly washed with water and dialyzed against water for 12 hours. This white pellet was regarded as cell wall material (CWM). Samples were shaken constantly in every extraction steps. The extraction was carried out at 10°C. The extracts were collected and analyzed for total uronic acid, and total sugar. The extracts of each fraction except the acetone and DMSO fractions were lyophilized and determined DW.

**Sequential fractionation of cell wall material**

The cell wall material was fractionated according to the procedure of Selvendran and O'Neill (1987) and Seymour et al. (1990) with some modification. The cell wall material prepared from 25 g of fresh mesocarp tissue, ca. 0.2 to 0.4 g
DW of cell wall, was extracted twice, first with 0.1 M than 0.5 M 1,2-cyclohexanediaminotetraacetic acid (CDTA) sodium salt at pH 5.5. The remainder was extracted twice with 0.05 M Na₂CO₃ and then sequentially extracted once with 0.5 M, 1 M and 4 M KOH. The residue was regarded as α-cellulose. All extractions were carried out at 10°C. After analysis for total uronic acid and total sugar, the extracts were dialyzed against water using Spectra/Por molecular porous membrane tubing (MW cutoff 3,500) with at least 5 changes of water. The extracts were lyophilized and weighed.

**Analysis of total uronic, total sugar and the degree of methylation**

Total uronic acid was determined according to the procedure of Blumenkrantz and Asboe-Hansen (1973). Sulfuric acid (3.6 ml, 18 M) containing 0.0125 M sodium tetraborate was added quickly to 0.6 ml of extract. The test tubes were held in ice/water bath during sulfuric acid addition. Following mixing, the test tubes were heated in a water bath at 100°C for 5 minutes. After cooling in an ice/water bath, 60 μl of m-hydroxydiphenyl reagent [0.05% (w/v) of meta-hydroxydiphenyl in 0.5% NaOH] was added to each tube. The tubes were again mixed and allowed to develop color for 10 minutes, then absorbance was measured at 520 nm in a Novaspec II spectrophotometer.

Total sugar was measured using the phenol/sulfuric acid assay of Dubois *et al* (1956). One ml of extract was mixed with 25 μl of 80% (w/v) phenol, then 2.5 ml 18 M H₂SO₄ was quickly added to the test tubes and mixed. After 10 minutes, the
test tube contents were again mixed. Twenty minutes later, the absorbance was read at 485 nm in a Novaspec II spectrophotometer.

Pectin methylation was measured following the procedure of O'Connell (1989, personal communication), a modification of the procedure of Wood and Siddiqui (1971). Extract (250 μl) was gently swirled with 125 μl 1.5 M NaOH. After 30 minutes at room temperature (22°C), the contents in test tubes were acidified with 125 μl 2.75 M H₂SO₄ and cooled in an ice/water bath. The contents in the tubes were gently swirled with 100 μl of K permanganate/osmium reagent (0.125 M KMnO₄ and 100 μM OsO₄), and test tubes were gently swirled and cooled in ice/water bath for 15 minutes. Sodium arsenite (0.125 M, 400 μl) containing 0.015 M H₂SO₄ and 25 μM OsO₄ was added. Test tubes were mixed well and warmed to room temperature (22°C). One ml of 0.02 M pentane-2,4-dione agent containing 2 M CH₃COONH₄ and 0.5 M CH₃COOH was added and mixed well. The test tubes were closed with marbles and heated at 58 to 60°C in a water bath for 15 minutes, and then cooled to room temperature. The absorbance was then read at 412 nm in Shimadzu UV 160U spectrophotometer.

Analysis of molecular changes of pectin and hemicellulose

Approximately 0.2 to 0.4 g cell wall material was extracted with 0.5 M CDTA at pH 5.5 until no further uronic acid was detected in the effluent. The CDTA extract was dialyzed with 100 mM imidazole at pH 5.5 for 24 hours using Spectra/Por molecular porous membrane tubing (MW cutoff 3,500). The dialyzed extract was concentrated by covering the bag with solid polyethylene glycol (average
molecular weight 15,000 to 20,000). One ml of the concentrated extract containing 5 mg total uronic acid was applied to a Sepharose CL-4B column that had been equilibrated with 100 mM imidazole at pH 5.5. The column was eluted with 100 mM imidazole at pH 5.5. The column volume was 137 cm$^3$ (68 cm x 2.011 cm$^2$). The flow rate was 22.5 ml (cm$^2$) hr$^{-1}$. The volume of each fraction was 2.25 ml. Each fraction was assayed calorimetrically for uronic acid, total sugar, and the degree of methylation.

The pellet left from CDTA extraction was extracted with 0.05 M \( \text{Na}_2\text{CO}_3 \) until no further uronic acid was detected in the effluent. The depectinated residue was sequentially extracted twice with 1 M then 4 M KOH. The KOH-soluble fractions were dialyzed against 20 mM Na-citrate buffer with 50 mM NaCl (pH=5.5), with at least 5 changes dialyzing buffer. The dialyzed KOH-soluble fractions were adjusted in volume to yield a total sugar concentration of 2 mg ml$^{-1}$. Two ml of this solution was applied to a Sepharose CL-4B column that had been equilibrated with 20 mM Na-citrate buffer with 50 mM NaCl at pH 5.5. The column was eluted with the same buffer. The volume of column was 137 cm$^3$ (68 cm x 2.011 cm$^2$). Fractions (2.25 ml) were collected with a flow rate of 22.5 ml (cm$^2$) hr$^{-1}$. The total sugar was measured for each fraction.

Statistic analysis of data

Data were analyzed by Duncan multiple range test where appropriate.
Results

Preparation of cell wall material

Total sugar increased from 82.0 mg g\(^{-1}\) FW in color break stage to 103.5 mg g\(^{-1}\) FW in 100% yellow ripe stage (Table 5.1). The amount of ethanol-soluble uronic acid was not different among the three ripening stages (color break, 50 and 100% yellow). Total sugar and uronic acid in the acetone fraction did not change during ripening, but increasing five fold and nearly two fold, respectively, in the DMSO fraction. Water-soluble uronic acid increased significantly during fruit ripening. The uronic acid contents in the water fraction increased 6-fold from 0.7 to 4.3 mg g\(^{-1}\) FW, and the total sugar increased 3-fold between color break stage (0% yellow) and 100% yellow ripe stage. As fruits ripened, the yield of cell wall material decreased from 17.7 mg g\(^{-1}\) FW in color break fruits to 9.2 mg g\(^{-1}\) in 100% yellow ripe fruits.

Fractionation of cell wall material

Converting the yield of each cell wall fraction to percentage of total yield of all fractions allowed comparison of the three ripening stages (Table 5.2). Expressing results on tissue FW showed considerable variation. Less variation occurred when the weight of fractions was calculated on a DW of cell wall material. Data were therefore calculated on a DW of cell wall material.

The percentage yield of 0.1 M CDTA fraction decreased dramatically from 14.8% in the fruit at color break stage to 6.5% in 100% yellow fruits (Table 5.2). The uronic acid content increased during fruit ripening with 47% in color break fruits, and 69% in 100% yellow fruits. Total sugar changed little during ripening
Table 5.1 Total sugar and uronic acid solubilized during preparation of cell wall material from papaya mesocarp at color break, 50 and 100% yellow stages. Fruit ripe stage was subjectively evaluated by % yellow of fruit surface.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Ripe stage (% yellow)</th>
<th>Yield(^x) (mg g(^{-1}) FW)</th>
<th>Total sugar(^y) (mg g(^{-1}) FW)</th>
<th>Uronic acid (mg g(^{-1}) FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Color break</td>
<td>56.9 b(^x)</td>
<td>82.0 b</td>
<td>5.2 a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>67.3 b</td>
<td>96.6 ab</td>
<td>5.1 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>89.0 a</td>
<td>103.5 a</td>
<td>4.6 a</td>
</tr>
<tr>
<td>Acetone</td>
<td>Color break</td>
<td>-</td>
<td>0.8 a</td>
<td>0.7 a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>-</td>
<td>0.5 a</td>
<td>0.7 a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-</td>
<td>0.9 a</td>
<td>0.7 a</td>
</tr>
<tr>
<td>DMSO</td>
<td>Color break</td>
<td>-</td>
<td>0.3 c</td>
<td>0.06 c</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>-</td>
<td>1.1 b</td>
<td>0.08 b</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-</td>
<td>1.6 a</td>
<td>0.1 a</td>
</tr>
<tr>
<td>Water</td>
<td>Color break</td>
<td>3.0 b</td>
<td>1.1 c</td>
<td>0.7 c</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>9.0 a</td>
<td>2.5 b</td>
<td>2.5 b</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9.8 a</td>
<td>3.6 a</td>
<td>4.3 a</td>
</tr>
<tr>
<td>Cell wall*</td>
<td>Color break</td>
<td>17.7 a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>11.4 b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9.2 c</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
1. Means in the same column and the same fraction with same letter were not significantly different at 5% level. n=3.

2. Total sugar and uronic content in each fraction was determined without dialysis.

3. Yield of cell wall material remaining after dialysis.
Table 5.2 The yields of extracts of cell wall material in different fractions from papaya mesocarp at color break, 50 and 100% yellow stages. Fruit ripe stage was subjectively evaluated by % yellow of fruit surface.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Ripe stage (% yellow)</th>
<th>Yield&lt;sup&gt;a&lt;/sup&gt; (mg g&lt;sup&gt;-1&lt;/sup&gt; FW)</th>
<th>Yield&lt;sup&gt;b&lt;/sup&gt; (mg g&lt;sup&gt;-1&lt;/sup&gt; DW CWM)</th>
<th>Percent&lt;sup&gt;c&lt;/sup&gt; (FW tissue)</th>
<th>Percent&lt;sup&gt;d&lt;/sup&gt; (DW CWM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M</td>
<td>Color break</td>
<td>1.9</td>
<td>161.0</td>
<td>10.3</td>
<td>14.8</td>
</tr>
<tr>
<td>CDTA</td>
<td>50</td>
<td>1.8</td>
<td>117.4</td>
<td>10.6</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.1</td>
<td>106.4</td>
<td>7.1</td>
<td>6.5</td>
</tr>
<tr>
<td>0.5 M</td>
<td>Color break</td>
<td>2.6</td>
<td>149.4</td>
<td>14.5</td>
<td>13.7</td>
</tr>
<tr>
<td>CDTA</td>
<td>50</td>
<td>2.8</td>
<td>243.8</td>
<td>16.1</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.3</td>
<td>246.4</td>
<td>15.0</td>
<td>15.1</td>
</tr>
<tr>
<td>0.05 M</td>
<td>Color break</td>
<td>3.1</td>
<td>175.1</td>
<td>16.9</td>
<td>16.1</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>50</td>
<td>3.4</td>
<td>294.0</td>
<td>19.3</td>
<td>19.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.7</td>
<td>295.7</td>
<td>18.0</td>
<td>18.1</td>
</tr>
<tr>
<td>0.5 M</td>
<td>Color break</td>
<td>1.8</td>
<td>99.6</td>
<td>9.6</td>
<td>9.2</td>
</tr>
<tr>
<td>KOH</td>
<td>50</td>
<td>1.8</td>
<td>156.3</td>
<td>10.3</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.3</td>
<td>143.5</td>
<td>8.7</td>
<td>8.8</td>
</tr>
<tr>
<td>1 M</td>
<td>Color break</td>
<td>1.1</td>
<td>64.2</td>
<td>6.2</td>
<td>5.9</td>
</tr>
<tr>
<td>KOH</td>
<td>50</td>
<td>0.9</td>
<td>80.5</td>
<td>5.3</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.9</td>
<td>100.0</td>
<td>6.1</td>
<td>6.1</td>
</tr>
</tbody>
</table>
Table 5.2 (Continue) The yields of extracts of cell wall material in different fractions from papaya mesocarp at color break, 50 and 100% yellow stages.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Ripe stage (% yellow)</th>
<th>Yield$^a$ (mg g$^{-1}$ FW)</th>
<th>Yield (mg g$^{-1}$ DW CWM)</th>
<th>Percent$^b$ (FW tissue)</th>
<th>Percent (DW CWM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 M</td>
<td>Color break</td>
<td>1.8</td>
<td>98.9</td>
<td>9.6</td>
<td>9.1</td>
</tr>
<tr>
<td>KOH</td>
<td>50</td>
<td>1.8</td>
<td>156.3</td>
<td>10.3</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.2</td>
<td>233.3</td>
<td>14.2</td>
<td>14.3</td>
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<tr>
<td>Residues</td>
<td>Color break</td>
<td>6.0</td>
<td>339.6</td>
<td>32.9</td>
<td>31.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.9</td>
<td>428.2</td>
<td>28.2</td>
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<td></td>
<td>100</td>
<td>4.7</td>
<td>505.8</td>
<td>30.8</td>
<td>31.0</td>
</tr>
</tbody>
</table>

$^a$. The yields of each fraction as mg g$^{-1}$ fresh weight (FW) of tissue or mg g$^{-1}$ dry weight (DW) of cell wall material (CWM) were the remaining after dialysis.

$^b$. Percent = the yield of the each fraction / the total yield of all fractions x 100.
Table 5.3 Total sugar and uronic acid contents in extracts of cell wall material in different fractions from papaya mesocarp at color break, 50 and 100% yellow stages. Fruit ripe stage was subjectively evaluated by % yellow of fruit surface.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Ripe stage ( % yellow )</th>
<th>Total sugar (mg g⁻¹ DW)</th>
<th>Uronic acid (mg g⁻¹ DW)</th>
<th>Percent (Total sugar)</th>
<th>Percent (Uronic acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M</td>
<td>Color break</td>
<td>53.4</td>
<td>147.7</td>
<td>23</td>
<td>47</td>
</tr>
<tr>
<td>CDTA 50</td>
<td></td>
<td>56.8</td>
<td>203.5</td>
<td>21</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>49.7</td>
<td>211.8</td>
<td>25</td>
<td>69</td>
</tr>
<tr>
<td>0.5 M</td>
<td>Color break</td>
<td>49.3</td>
<td>145.4</td>
<td>22</td>
<td>46</td>
</tr>
<tr>
<td>CDTA 50</td>
<td></td>
<td>76.3</td>
<td>230.1</td>
<td>29</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>26.2</td>
<td>91.7</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>0.05 M</td>
<td>Color break</td>
<td>40.2</td>
<td>19.8</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Na₂CO₃ 50</td>
<td></td>
<td>30.6</td>
<td>19.1</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>8.6</td>
<td>4.0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>0.5 M</td>
<td>Color break</td>
<td>13.8</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>KOH 50</td>
<td></td>
<td>19.3</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>20.7</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>1 M</td>
<td>Color break</td>
<td>45.2</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>KOH 50</td>
<td></td>
<td>35.3</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>36.5</td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 5.3 (Continue) Total sugar and uronic acid contents in extracts of cell wall material in different fractions from papaya mesocarp at color break, 50 and 100% yellow stages.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Ripe stage (% yellow)</th>
<th>Total sugar (mg g⁻¹ DW)</th>
<th>Uronic acid (mg g⁻¹ DW)</th>
<th>Percent⁺ (Total sugar)</th>
<th>Percent (Uronic acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 M</td>
<td>Color break</td>
<td>26.5</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>KOH</td>
<td>50</td>
<td>46.8</td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>60.8</td>
<td>0</td>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

⁺ Total sugar and uronic acid were determined before dialysis.
⁺⁺ Percent = Individual fraction / Total of all fractions x 100.
(Table 5.3). The percentage yield of 0.5 M CDTA fraction increased slightly then decreased during ripening, the highest was in 50% yellow fruits (Table 5.2). The total uronic acid and total sugar were the lowest in 100% yellow fruits and highest in 50% yellow fruit (Table 5.3). The percentage of total sugar and uronic acid decreased as fruit ripened in the 0.05 M Na₂CO₃ fraction (Table 5.3) while the yield increased (Table 5.2). There were little yields difference between 0.5 M and 1 M KOH fractions. The 4 M KOH fraction yield increased as fruit ripened: 9.1% in color break fruits, 10.6% in 50% yellow fruits and 14.3% in full yellow fruits. Uronic acid was not detected in the KOH fractions. The total sugar content in 0.5 M and 4 M KOH fraction increased during fruit ripening. The residue yield was similar for all three ripening stages (Table 5.3).

The degradation of pectin and hemicellulose

The large molecular weight pectin molecules (in the fractions eluting between 50 ml and 80 ml) as indicated by uronic acid content decreased during fruit ripening (Fig. 5.1 A). There were concomitant increases in medium (in the fractions between elution volume 80 ml and 110 ml) and small (in the fractions between elution volume 110 ml and 150 ml) molecular size pectin (Fig. 5.1 A). Fruits at color break stage had a deformation force greater than 334 N, and most of uronic acid molecules had large molecular weights. These molecules were attached by sugar polymers as indicated by total sugar concentration (Fig. 5.1 B) and were highly methylated as indicated by methanol content released from pectin extraction after NaOH treatment (Fig. 5.1 C). Fruit deformation force had not changed (> 334 N)
Figure 5.1 Gel chromatography profiles of pectins extracted from papaya mesocarp tissue. Fractions were analyzed for uronic acid (A), total sugar (B) and methanol (C) released from pectin polymers after NaOH treatment. Pectins were prepared from color break (△), 30% (○), 50% (●) and 100% (□) yellow fruits.
at the 30% yellow stage compared to the color break stage. One third of the pectin had depolymerized from large molecular size to medium molecular size at 30% yellow stage. As fruits reached 60% yellow stage, the deformation force decreased to 98 N. Almost 50% of pectin were depolymerized to medium and small size molecules, and only the molecules eluted from 50 to 70 ml were methylated (Fig. 5.1 C). Fruit deformation force declined to 20 N as the fruits reached the 100% yellow stage. Most of pectin molecules had been degraded to medium and smaller size molecules. The large pectin molecules were much less methylated at 100% yellow stage than at color break stage. Similar changes occurred in both total sugar and uronic acid content during fruit ripening (Fig. 5.1 B).

Gel-filtration analyses of hemicellulose revealed changes in the molecular size distribution during ripening (Fig. 5.2). Hemicellulose molecules that eluted from 45 ml to 55 ml and from 115 ml to 125 ml decreased during fruit ripening, while hemicellulose molecules eluted from 90 ml to 110 ml and from 130 ml to 140 ml increased during fruit ripening.

Discussion

Papaya fruit softening was characterized by a 6-fold increase in water-soluble pectin (Table 5.1). Water soluble pectin is low methoxyl pectin (Fennema, 1985). The increase in water-soluble pectin was correlated with pectin demethylation during fruit ripening (Fig. 5.1 C). Pectin methylesterase (PME) activity increases during papaya fruit ripening (Paull and Chen, 1983). Demethylation by PME would result in greater numbers of carboxyl groups which may facilitate polygalacturonase (PG)
Figure 5.2 Gel chromatography profiles of hemicellulose extracted from papaya mesocarp tissue. Hemicellulose was prepared from color break (△) and 100% (□) yellow fruits.
activity (Huber, 1983) and binding of cations such as Ca (Burns and Pressey, 1987).

The majority of the pectin (93 to 99%) in cell wall was extracted by CDTA solutions (Table 5.3). The polymers solubilized in CDTA fraction originate mainly from the middle lamella (Selvendran, 1985). The solubility of pectin was about the same in the 0.1 M and 0.5 M CDTA fractions when the fruit was at the color break stage, with a increase in the proportion in the 0.1 M CDTA fraction as the fruit ripened (Table 5.3). This suggests that the pectin located in the middle lamella was more readily extractable as fruit ripening progressed. The increased extractibility of the pectin was probably related to pectin degradation (Fig. 5.1). A loss of cell cohesive strength occurs predominantly in the middle lamella during apple fruit softening (Glenn et al., 1988; Glenn and Poovaiah, 1990). The reduction in cell wall to cell wall adhesion and pericarp tissue rigidity associated with tomato fruit softening is related primarily to changes in middle lamella pectin (Speirs and Brady, 1991). There was an increase in Na₂CO₃-soluble pectin as indicated by uronic acid content during papaya fruit softening (Table 5.3). The Na₂CO₃-soluble polymers probably originate from the primary cell wall (Selvendran, 1985).

The pectin polymers degraded into smaller molecules during fruit ripening. The pattern of pectin degradation (Fig. 5.1) suggested possible involvement of PG in hydrolysis of pectin. A body of evidence has suggested that endopolygalacturonase [poly (1,4-α-D-galacturonide) glycanohydrolase EC 3.2.1.15] (PG) is mainly responsible for the degradation of pectin and softening during fruit ripening (Huber, 1883; Brady, 1987; Bennett and DellaPenna, 1987a; Giovannoni
et al., 1989). The following correlative evidence supports this hypothesis: 1. the ability of crude PG preparation to hydrolyse isolated cell wall material in vitro (Themmen et al., 1982; Huber, 1983), 2. a general correlation between PG activity and fruit softening (Brady et al., 1983; Ahrens and Huber, 1990), and 3. the absence of appreciable levels of PG and softening in ripening impaired tomato mutants that fail to soften (Tigchelaar et al., 1978; Brady et al., 1983). However, recent studies indicate that PG may not be the sole enzyme regulating the softening process. This is supported by, 1. reducing PG activity by 95 to 99% in tomato fruit using antisense RNA to PG does not inhibit softening (Smith et al., 1988, 1990), 2. the expression of a chimeric PG gene in transgenic rin tomato fruit results in polyuronide degradation but not fruit softening (Giovannoni et al., 1989), 3. some fruits soften without detectable PG during ripening, such as strawberry (Huber, 1984) and muskmelon (McCollum et al., 1989). In addition, the tomatoes with antisense PG constructs accumulate PG to the levels comparable to or greater than that found in some other fruits (Speirs and Brady, 1991), such as papaya (Paull and Chen, 1983) and peach (Pressey and Avants, 1978), that soften appreciably. The mutant tomato rin is a pleiotropic mutation, in which several aspects of fruit ripening are also affected other than softening (Speirs and Brady, 1991). During papaya fruit ripening, there is a good relationship between measures of ripening, respiration, ethylene production, and skin color, and wall-degrading enzymatic activity, such as PG, xylanase, and cellulase (Paull and Chen, 1983). Inhibition of papaya fruit softening caused by extended hot water treatments was correlated to a decrease in
PG activity (Chan et al., 1982). Although it is not clear whether PG is main enzyme causing papaya fruit softening, PG is most likely involved in fruit softening.

While one third of the pectin was degraded into smaller molecule fragments by the time the fruit reached 30% yellow stage, the fruits were still fairly firm (above 334 N). The firmness of papaya fruit then decreases quickly when the fruit color is between 40 to 60% yellow (Paull, 1990). If pectin degradation was the main cause of fruit softening, a threshold possibly existed beyond which pectin has been degraded to cause cell wall structural disorganization and fruit softening. Alternatively, pectin degradation alone was insufficient to cause fruit softening until other cell wall components had degraded to a certain extent.

The extractable total sugar increased in KOH-soluble fraction (Table 5.3), and the molecular size of KOH-soluble polymers decreased (Fig. 5.2) during papaya fruit ripening. The KOH fraction of papaya cell wall is mainly composed of xylose and glucose (Paull and Qiu, unpublished data), and was lacking in uronic acid residues (Table 5.3). The KOH-soluble polymers possibly originate from hemicellulose (Selvendran, 1985). Ripening-associated modification of hemicellulose structure has been reported in tomato (Huber, 1983), strawberry (Huber, 1984), and muskmelon (McCollum et al., 1989). Hemicellulose bridges cellulose microfibrils and pectic polysaccharide matrix (Albersheim, 1976; Carpita, 1987; Talbott and Ray, 1992). Although the molecular weight of hemicellulose did not change as much as that of pectin (Fig. 5.1 versus Fig. 5.2), this change could lead to disruption and loosening of the cell wall structure. The fact that extractable total sugar increased
significantly in 0.5 M and 4 M KOH fractions during ripening (Table 5.3) supports this conclusion.

In strawberry (Huber, 1984) and muskmelon (McCollum et al., 1989), the molecular weight of hemicellulose decreases during fruit ripening. In both cases, hemicellulose modification may be mediated enzymatically (Huber, 1984; McCollum et al., 1989). In papaya, xylanase activity reaches a peak at the same time as the respiration rate increases to its maximum (Paull and Chen, 1983). Xylanase may play a role in the modifications of papaya fruit hemicellulose. The correlations between the increase in the solubility of hemicellulose (Table 5.2 and Table 5.3), the increase of xylanase activity (Paull and Chen, 1983), and the decrease of fruit firmness at 40 to 60% yellow ripe stage (Paull, 1990) suggest that modification of hemicellulose may play a significant role in papaya fruit softening.
CHAPTER 6.
THE EFFECT OF Ca INFILTRATION TREATMENT ON MESOCARP RIPENING

Introduction

Calcium has been suggested to inhibit fruit softening in two ways. The first possibility is that a greater degree of pectic polymer Ca\(^{++}\) binding, with concomitantly fewer free acid groups, may improve the cell wall structural integrity and reduce the rate of pectin solubilization. The second possibility is that Ca may inhibit the activities of cell wall-degrading enzymes (Ferguson, 1984; Poovaiah et al., 1988; Conway et al., 1991).

It has been suggested that fruit softening occurs either by movement of Ca\(^{++}\) from the middle lamella or by the loss of Ca\(^{++}\) attachment sites (Knee and Bartley, 1981). The evidence indicates that both Ca\(^{++}\) movement from middle lamella and loss of Ca\(^{++}\) binding sites occurs during apple fruit softening (Stow, 1989). In papaya, the degradation of pectin and hemicellulose was associated with fruit softening, and the solubility of cell wall material was enhanced when extraction with Ca chelating agent CDTA by comparison to extraction in water (Chapter 5). If Ca is involved in papaya fruit softening during ripening, then infiltration of mesocarp tissue with Ca should maintain the firmness of the tissue to some extent. To test this hypothesis, it is necessary to increase the Ca content of mesocarp tissue. In a field fertilization experiment (Chapter 4), it was found that Ca content in mesocarp
tissue was positively related to the fruit firmness of ripe fruits, and that Mg and K contents were not related to the firmness of ripe fruits. However, Ca fertilization did not always increase the mesocarp tissue Ca level. Attempts to increase Ca levels in the mesocarp of intact fruits by pre- or post-harvest Ca treatment were unsuccessful (Chapter 4).

Excised tissues of apple and tomato have been used to study the role of various substances in fruit softening (Lieberman et al., 1977; Lieberman and Wang, 1982; Greve and Labavitch, 1991). Excised tissue from tomato provides a number of advantages for experimental analysis of ripening (Campbell et al., 1990). Use of excised tissue makes it possible to separate the various ripening processes by isolation of specific tissue from the fruit, to quantitatively add metabolic intermediates or inhibitors to these tissue, and to measure local processes through nondestructive monitoring and destructive analysis of replicated samples (Campbell et al., 1990). Calcium content in mesocarp of papaya is quite variable (103 to 183 μg g⁻¹ FW) among the fruits (Chapter 4). Use of excised tissue can reduce this variability by replicating samples from the same fruit.

In tomato, the processes characteristic of ripening in intact fruit are duplicated in excised pericarp discs, with a few exceptions (Campbell et al., 1990). In apple, the processes characteristic of ripening of excised tissue are different from that of the intact fruit (Lieberman et al., 1977). Therefore, comparison of known ripening processes in intact fruit and in excised tissue is necessary if excised tissue is to be used with confidence in studies of ripening of papaya fruits.
The first objective of this study was to compare the characteristics of ripening in whole papaya fruit described by others (An, 1990; Quintana, 1991) with similar processes in excised mesocarp plugs; the second objective was to test the effect of Ca and Ca chelators on mesocarp softening.

Materials and methods

Plant material

Papaya (Carica papaya L.) variety "Sunset" plants were grown in the field at Poamoho Experimental Station in central Oahu. Fruits were harvested at color break to 5% yellow stage and stored at approximately 22°C until they reached the desired ripeness stage required for the experiment. Fruits were selected on the basis of degree of skin and mesocarp color. Fruit skin and mesocarp color were subjectively evaluated. Total soluble solids were measured by a refractometer.

Plug preparation

Papaya fruits with color break, 10%, 30% and 50% yellow stages were used. The fruits were washed with soapy water and rinsed with tap water. The fruits were transferred to a laminar flow hood after the fruit surface was dry. The fruits were then wiped with 95% ethanol. Everything used for plug preparation and storage was sterilized. The plugs were taken with a 1.4 cm cork borer and sorted by mesocarp color. Seeds were removed with a twist. The skin and endocarp were remained in the plug. The plug was stored in a 15 ml sterile test tube covered with sterile cotton at approximately 22°C. The test tubes were then covered with a piece of wet paper towel in order to keep the plug moist. The firmness of the plugs was measured as
deformation force using a penetrometer with a 0.8 cm diameter tip. The penetrometer was pressed 1 cm into mesocarp tissue from endocarp side toward skin side.

**Vacuum infiltration of plugs**

All solutions used for infiltration were prepared in 100 mM 2-(Morpholino)ethanesulfonic acid (MES-NaOH) buffer (pH=6.2) and sterilized. The plugs were infiltrated at 500 mm Hg for 20 seconds. The treatments were MES-NaOH buffer (control), 10 mM EGTA, 50 mM sodium citrate, 50 mM CaCl₂, 50 mM MgCl₂, and 50 mM KCl (water and MES buffer treatments were compared in initial experiments and shown to have no significant effect on plug softening). The plugs were blotted dry with paper towels to remove excess solution on the surface and stored in a 15 ml test tube for 24 hours. The plug firmness was measured every 4 hours.

**Ethylene and respiration measurement**

Rates of ethylene production and respiration rate of individual plug were measured every 4 hours for the first 12 or 16 hours and also at the end of storage period (24 hours). In a laminar flow hood, the cotton covers of test tubes were removed, then test tubes were sealed with a rubber stopper. After 30 minutes, 2 ml of gas were withdrawn from the test tube. One ml of gas was analyzed for ethylene concentration, and 1 ml gas for CO₂ concentration. Ethylene production was analyzed by a gas chromatography filled with an alumina column (1.5 m x 3 mm)
and photoionization detector. Carbon dioxide was measured by an infrared gas analyzer.

**Fractionation of Ca from mesocarp tissue**

The fractionation of mesocarp tissue Ca was done according to the procedure of Ferguson *et al.* (1980). To avoid the variability of Ca content in different fruit, samples were taken from the same fruit at color break, 50%, and 100% yellow stages. Six fruits were used and 2 duplicated samples were taken from each fruit at each ripening stage. The mesocarp tissue was taken with a sterile 1.4 cm cork borer. The holes were immediately filled with sterile cotton and warm lanolin, and the fruit was returned to storage room until they reached the next required ripening stage. The plug minus the skin and placenta was quickly frozen with liquid N and lyophilized. The dry mesocarp tissue was ground in a mortar and pestle in liquid N to very fine powder. One hundred mg of this dry powder was extracted with 3.5 ml 80% acetic acid containing 10 mM cysteine, for 30 minutes at 25°C. The supernatant was removed after centrifugation for 10 minutes at 2000 g, and put into a flask. The extraction was repeated and the extract was added to the same flask. The pellet was then extracted twice with 5 ml 0.25 M HCl containing 10 mM cysteine in the same manner, and supernatant collected into second flask. The residue was digested in 25 ml 1 M HCl. The sample was vigorously shaken during all extractions. Extractions were diluted with 1 M HCl (acetic acid fraction 10 times, 0.25 M HCl fraction 7 times and 1 M HCl fraction 1 time) and added with lanthanum oxide (in 1 M HCl, containing 5% La). Calcium concentration in each
fraction (containing 0.5% La) was measured by an atomic absorption
spectrophotometer.

Statistic analysis of data

Data were analyzed by Duncan multiple range test and regression where
appropriate.

Results

Ripening characteristics of excised mesocarp tissue

Plug skin color development increased rapidly from the green to the 50% yellow stage within 3 days, and slowed between day 3 to day 6, and then increased to 100% yellow on day 12 (Fig. 6.1 A). The total soluble solids (TSS) increased from 9% to 12% in the first 3 days, and then decreased gradually for the rest of storage period (Fig. 6.1 B). The firmness of mesocarp tissue decreased dramatically in the first 3 days from 78 N to 20 N, and then slightly increased to 42 N on day 6, and decreased again (Fig. 6.1 C). Since the most rapid decrease in firmness of the tissue occurred within the first day (Fig. 6.2), the experimental storage period was shortened to 24 hours (Fig. 6.3).

The respiration rate of color break fruit plugs gradually increased during the first 8 hours, then rapidly increased after 8 hours and reached the maximum level (36 $\mu$J CO$_2$ g$^{-1}$hr$^{-1}$) at hour 16 (Fig. 6.3 A). The respiration rate of 10% yellow fruit plugs increased to the maximum (28 $\mu$J CO$_2$ g$^{-1}$hr$^{-1}$) at hour 8, and then decreased slightly and maintained constant. The respiration rate of plugs from 30% yellow fruits gradually increased from 24 $\mu$J CO$_2$ g$^{-1}$hr$^{-1}$ at the beginning to a maximum of
Figure 6.1 Changes of skin color (A), total soluble solid (B) and the firmness (C) of mesocarp plugs taken from 10% yellow fruits during 12 days. Analysis of variance: skin color ***, total soluble solid ***, firmness ***. Significant at 0.1% (***), level.
Figure 6.2 Firmness change of mesocarp plugs taken from 10% yellow fruits during 3 days. Analysis of variance: storage days ***. Significant at 0.1% (***) level. Each data point is the mean of 10 observations.
Figure 6.3 Changes of respiration (A), ethylene production (B) and firmness (C) of mesocarp plugs during 24 hours. Fruit ripe stages were color break (●), 10% yellow (△), 30% yellow (○), and 50% yellow (□). Analysis of variance: respiration, stage ***, storage hour ***, stage x storage hour ***; ethylene production, stage ***, storage hour ***, stage x storage hour ***; firmness, stage ***, storage hour ***, stage x storage hour ***. Significant at 1% (**) and 0.1% (***) level. Each data point is the mean of 10 observations.
31 μl CO₂ g⁻¹ hr⁻¹ at the end of storage period. The respiration rate 50% yellow fruit plugs remained in the range of 30 to 34 μl CO₂ g⁻¹ hr⁻¹ over 24 hours.

The patterns of ethylene production were very different among color break, 10%, 30%, and 50% yellow stages (Fig. 6.3 B). In the color break stage, the rate of ethylene production gradually increased from non-detectable at the beginning to 3.83 nl g⁻¹ hr⁻¹ at hour 8, and remained constant over the next 8 hours, then increased to 7.15 nl g⁻¹ hr⁻¹ at hour 24. The rate of ethylene production of 10% yellow fruit plugs increased from non-detectable at the beginning to a maximum of 8.97 nl g⁻¹ hr⁻¹ at hour 8, and then declined slightly. In 30% yellow stage, the rate of ethylene production had two peaks: one (22.07 nl g⁻¹ hr⁻¹) occurred at hour 8, another (21.17 nl g⁻¹ hr⁻¹) at hour 16. The ethylene production rate of plugs from 50% yellow fruits began at a much higher level (13.65 nl g⁻¹ hr⁻¹) in comparison to other ripening stages, and reached a maximum level (27 nl g⁻¹ hr⁻¹) at hour 8, and then declined during the rest of storage period.

The plug deformation force was similar (85 N to 89 N) at the beginning in color break, 10, 30% yellow stages (Fig. 6.3 C). The softening rate of plugs from riper fruits was faster during the second 12 hours than the first 12 hours. At the color break stage, the firmness of the plugs did not change for the first 8 hours after excision from the fruits, then gradually decreased from 85 N to 69 N during the remaining 16 hours. The firmness of the plugs from 10% yellow fruit declined from 89 N to 73 N during the first 12 hours, and then declined from 73 N to 17 N during the second 12 hours. The change of plug firmness in 30% yellow stage was similar
to that in 10% yellow stage, but plug firmness decreased much faster during the second 12 hours than the first 12 hours. In 50% yellow stage, the plug firmness decreased from 13 N at the beginning to 5 N 4 hours after excision.

The effects of CaCl₂ and Ca chelators on the softening

There was a linear relationship between Ca concentration in the mesocarp tissue and Ca concentration of infiltration solution (R²=0.98 P=0.001). Calcium concentration in the mesocarp tissue increased about 100% when treated with 50 mM CaCl₂. The plug softening rate was slowed with a treatment of 50 mM CaCl₂. Variable effects on plug softening were observed at concentration less than 50 mM CaCl₂.

Calcium was more effective on reducing softening rate in plugs from color break and 10% yellow fruits than in plugs from 30% and 50% yellow fruits (Fig. 6.4). Calcium chloride infiltration treatment increased plug firmness right after the treatment and was able to maintain plug firmness in the range of 68 to 73 N for the first 8 hours at 10% yellow stage (Fig. 6.5 A). Plugs gradually decreased the firmness from 68 N to 29 N during the remaining 16 hours in CaCl₂ treatment. The CaCl₂ treatment increased plug firmness at 50% yellow stage (Fig. 6.5 B).

EGTA infiltration treatment increased plug softening rate especially 8 hours after treatment at both 10% and 50% yellow stages (Fig. 6.5). At 10% yellow stage, sodium citrate infiltration treatment decreased plug firmness 4 hours after treatment, and had no significant effect on plug firmness between 8 to 16 hours, then decreased plug firmness after 20 hour (Fig. 6.5 A). At 50% yellow stage, sodium citrate
Figure 6.4 The effect of CaCl₂ infiltration treatment on mesocarp softening. The mesocarp plugs were stored for 24 hours after treatment. The treatments were control (□) and 50 mM CaCl₂ (■). Analysis of variance: treatment *, stage ***, treatment x stage ***. Significant at 5% (*) and 0.1% (***). Each data point the mean of 10 observations.
Figure 6.5 The effects of CaCl$_2$ and Ca chelators infiltration treatments on mesocarp plug softening during 24 hours. The plugs were taken from 10% (A) and 50% (B) yellow fruits. The treatments were control (●), 10 mM EGTA (△), 25 mM sodium citrate (○) and 50 mM CaCl$_2$ (□). Analysis of variance: 10% yellow stage, treatment **, storage hour ***, treatment x storage hour ***; 50% yellow stage, treatment *, storage hour **, treatment x storage hour ns. Significant at 1% (***) and 0.1% (****) level. Not significant(ns). Each data point is the mean of 10 observations.
treatment reduced plug firmness right after treatment, and this effect was not significant after 12 hours (Fig. 6.5 B).

The effects of CaCl₂ and Ca chelators on respiration and ethylene production

Although infiltration treatments with CaCl₂ and EGTA showed a tendency to reduce and increase the respiration rate of mesocarp plugs, respectively, at 10% yellow stage, these effects were not significant (Fig. 6.6 A). The effect of sodium citrate on the respiration rate of mesocarp plugs was not significant. The patterns of ethylene production in mesocarp plugs were about the same in both the control and CaCl₂ treatments (Fig. 6.6 B), a rapid increase from hour 4 to hour 12, and then a slight reduction during the remainder of the storage period. The CaCl₂ treatment reduced the ethylene production by mesocarp plugs over 24 hours (about 50%). EGTA treatment stimulated ethylene production of mesocarp to a very high level (about 2 times as much as that of the control), with maximum level of 23.12 nl g⁻¹ hr⁻¹. Sodium citrate reduced the ethylene production.

The effects of MgCl₂ and KCl on softening, respiration and ethylene production

Infiltration treatment with MgCl₂ and KCl had no effect on the firmness of mesocarp plugs at 10% yellow stage (Fig. 6.7 A). At 50% yellow stage, Mg chloride treatment slowed mesocarp plug softening, but this effect was not significant (Fig. 6.7 B). Potassium chloride treatment had no effect on mesocarp plug softening at 50% yellow stage (Fig. 6.7 B). The MgCl₂ and KCl treatments had no effect on respiration rate of mesocarp plugs (Fig. 6.8 A). The effects of MgCl₂ and KCl treatments on ethylene production of plugs were not significant (Fig. 6.8 B).
Figure 6.6 The effects of CaCl₂ and Ca chelators infiltration treatments on respiration (A) and ethylene production (B) of mesocarp plugs taken from 10% yellow fruit during 24 hours. The treatments were control (●), 10 mM EGTA (△), 25 mM sodium citrate (○) and 50 mM CaCl₂ (□). Analysis of variance: respiration, treatment ns, storage hour ***, treatment x storage hour **; ethylene production, treatment *, storage hour ***, treatment x storage hour ns. Significant at 5% (*), 1% (**) and 0.1% (***) level. Not significant (ns). Each data point is the mean of 10 observations.
Figure 6.7 The effects of MgCl₂ and KCl infiltration treatments on mesocarp plug softening during 24 hours. The plugs were taken from 10% (A) and 50% (B) yellow fruits. The treatments were control (●), 50 mM MgCl₂ (●), 50 mM KCl (○). Analysis of variance: 10% yellow stage, treatment ns, storage hour ***; treatment x storage hour ***; 50% yellow stage, treatment ns, storage hour *, treatment x storage hour *. Significant at 5% (*) and 0.1% (***). Not significant (ns). Each data point is the mean of 10 observations.
Figure 6.8 The effects of MgCl\(_2\) and KCl infiltration treatments on respiration and ethylene production of mesocarp plugs taken from 10% yellow fruit during 24 hours. The treatments were control (●), 50 mM MgCl\(_2\) (▲), 50 mM KCl (○). Analysis of variance: respiration, treatment ns, storage hour ***, treatment x storage hour **; ethylene production, treatment ns, storage hour ***, treatment x storage hour ns. Significant at 1% (**) and 0.1% (***) level. Not significant (ns). Each data point is the mean of 10 observations.
Fractionation of Ca from mesocarp

At the three ripe stages, 82 to 90% of Ca was in the 80% acetic acid fraction (Ca bound in pectate), and with 10 to 16% of Ca in the 0.25 N HCl fraction (Ca oxalate), and little Ca in the 1 N HCl fraction (residue Ca) (Table 6.1). The Ca content of the 80% acetic acid fraction was 4.5 to 4.7 μg g⁻¹ dry weight and was not significantly different among color break, 50%, and 100% yellow fruits. The Ca contents in the 0.25 and 1 N HCl were increased during fruit ripening.

Discussion

Fruit ripening is usually accompanied by pigment biosynthesis and tissue softening (Brady, 1987). The rate of respiration and ethylene production also increase significantly in climacteric fruits (Brady, 1987). It is necessary to compare the changes of skin color, firmness, rate of respiration and ethylene production between intact fruits and excised mesocarp tissue during ripening if excised tissue is to be used with confidence in studies of ripening in papaya fruits.

The color development pattern in the skin of mesocarp plugs was sigmoid; it took 12 days to develop the color from green to 100% yellow (Figure 6.1 A). In the intact fruits, the skin color development pattern is close to linear, and it takes 8 days to develop yellow color from 5% to 90% (An, 1990). The respiration rate of mesocarp plugs from color break and 10% yellow fruits appeared to have a climacteric peak at hour 16 and hour 8 after excised, respectively (Figure 6.3 A). Mesocarp plugs from 30% and 50% yellow fruits did not show the climacteric peak in the 24 hour storage period (Figure 6.3 A). In the mesocarp plugs from 10%
Table 6.1 Calcium concentration in various fractions from mesocarp at color break, 50 and 100% stages. Dry mesocarp tissue powder (100 mg) was sequentially extracted with 80% acetic acid, 0.25 M HCl, and 1 M HCl. The Ca concentration in each fraction was determined by a atomic absorption spectrophotometer.

<table>
<thead>
<tr>
<th>Ripening stage</th>
<th>Ca(^z) (µg/mg DW)</th>
<th>80% acetic acid</th>
<th>0.25 M HCl</th>
<th>1 M HCl</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color Break</td>
<td>4.5 a</td>
<td>0.9 a</td>
<td>0.05 a</td>
<td>5.5 a</td>
<td></td>
</tr>
<tr>
<td>50% yellow</td>
<td>4.5 a</td>
<td>0.6 b</td>
<td>0.03 b</td>
<td>5.1 a</td>
<td></td>
</tr>
<tr>
<td>100% yellow</td>
<td>4.7 a</td>
<td>0.6 b</td>
<td>0.03 b</td>
<td>5.5 a</td>
<td></td>
</tr>
</tbody>
</table>

\(^z\) Means in the same column with same letter were not significantly different at 5% level. n=12.

\(^y\) *** Significant at 0.1% level.
yellow fruits, the maximum respiration rate was 28 \mu l \text{CO}_2 \text{g}^{-1} \text{hr}^{-1}, and the minimum was 19 \mu l \text{CO}_2 \text{g}^{-1} \text{hr}^{-1} (Figure 6.3 A). The respiration rate of intact fruits (harvested at color break to 10% yellow) does not start the climacteric peak until 6 days after harvest with the maximum and the minimum respiration rate of 27 \mu l \text{CO}_2 \text{g}^{-1} \text{hr}^{-1} and 17 \mu l \text{CO}_2 \text{g}^{-1} \text{hr}^{-1}, respectively (Quintana, 1991). The ethylene production in mesocarp plugs increased quickly within 8 hours after excised (Figure 6.3 B). The maximum ethylene production of mesocarp plugs from color break and 10% yellow fruits was about 9 nl g^{-1} hr^{-1}. The ethylene production in intact fruits is not detectable for 5 days after harvest, and rapidly increases to maximum level (2.5 nl g^{-1} hr^{-1}) from day 6 to day 11 (Quintana, 1991). The firmness of mesocarp plugs decreased dramatically from 89 N to 17 N in one day in 10% yellow stage (Figure 6.3 C). The mesocarp plugs from 30\% and 50\% fruits softened at a faster rate. The firmness of intact papaya fruits (harvested at color break to 10\% yellow stages) maintains at about 226 N for at least 4 days after harvest, and then decreases rapidly to 177 N on day 6 and to 39 N on day 8 (An, 1990). From these comparisons, the ripening rate of excised mesocarp plug was faster than that of intact fruit in papaya. Papaya mesocarp plugs stored for 24 hours could be considered to be undergoing accelerated ripening process according to the pattern of ethylene production and changes of firmness. The processes characteristic of ripening in intact fruit are basically duplicated in excised pericarp discs in tomato (Campbell et al., 1990), pericarp tissue color changes from green through orange to red, rates of ethylene production and respiration undergo a climacteric rise, tissue decreases in firmness.
It was found that mesocarp Ca concentration was positively related to the firmness of ripe papaya fruits (Chapter 4). Infiltration with CaCl₂ slowed the softening of mesocarp plugs (Figure 6.5). Infiltration of apple fruit with Ca immediately increases cell wall-bound Ca and firmness retention during storage (Abbott et al., 1989). The element map of the distribution of Ca shows a strong Ca signal in the sites corresponding to the cell wall region in Ca-infiltrated apple fruits (Glenn and Poovaiah, 1990).

The relationship between Ca cation and cell wall structure may partially explain the observed effects of Ca on fruit firmness. The primary wall of dicotyledons contains about 35% pectic polysaccharides (Darvill et al., 1980; Moore, 1986) which are particularly abundant in the middle lamellar region (Hall, 1976). The matrix of pectic polysaccharides consist of a rhamnogalactosyluronic backbone with covalently linked side chains of arabinose and galactose (Dey and Brinson, 1984). The pectin gel is created by cross-bridging with Ca²⁺ and the (1-4)-α-D-galactosyluronic acids, forming an "egg-box" rigid structure (Grant et al., 1973; Rees, 1977). Tomato cell wall pectins might contain a limited number of polymers with discrete size and smaller fragments rather than a continuum of species (Fishman et al., 1989). The fact that dialysis against NaCl solution induces partial dissociation of the pectin polymers suggests that the cell wall pectins are an aggregated mosaic, held together partially through noncovalent interactions (Fishman et al., 1989). When Ca concentration in the fruit is increased by Ca treatment, the degree of Ca to cross-link pectic polymers of cell wall may also increase. Extensive cross-linking
pectic polymers forms a cell wall network with a stronger mechanical strength (Dey and Brinson, 1984).

The Ca chelating agent EGTA increased the softening rate of papaya mesocarp tissue, especially after 8 hours (Figure 6.5). Extraction of papaya cell wall material with CDTA enhanced the solubility of cell wall polymers (Chapter 5). Removal of Ca from cell wall polymers may reduce mechanical strength of the cell wall and cooperate with cell wall hydrolysase because Ca may inhibit the activity of cell wall hydrolysase (Ferguson, 1984). The activities of cell wall degrading enzymes are correlated to fruit softening during papaya fruit ripening (Paull and Chen, 1983). In fruits that contain high levels of cell wall- and middle lamella-bound Ca, removal of Ca++ from pericarp tissue or from recovered cell walls by chelating agents, such as EDTA or citrate, promote extensive hydrolysis of the pectin molecules (Buescher and Hobson, 1982). In the presence of sufficient citrate, cell wall uronic acids of a firm and soft cultivar were equally susceptible to hydrolysis, suggesting that differences in the digestion of walls by PG were dependent upon differences in Ca content or distribution (Brady et al., 1985). Calcium-infiltrated apple fruits show no significant increase in soluble pectin concentration even after 7 months of cold storage (Glenn and Poovaiah, 1990). Ben-Shalom (1986) assumed that cellulose and hemicellulose were sterically masked by the pectic substances. Therefore, removal of the pectins from cell wall of grapefruit segments by pectinase enhances the enzymatic hydrolysis of cellulose and hemicellulose (Ben-Shalom, 1986).
Exogenous increase in Ca level in mesocarp of early ripe stage, such as 10% yellow, effectively slowed, but did not prevent, mesocarp softening (Figure 6.6). Additional Ca slightly increased mesocarp firmness but was not able to bring back the firmness of the tissue to the original level when infiltrated mesocarp of 50% yellow fruit with CaCl₂ (Figure 6.5). Pectin and hemicellulose underwent degradation during papaya fruit ripening (Chapter 5). These evidence suggest that loss of cell wall polymers and Ca attachment sites may result in papaya fruit softening, and that exogenous Ca application could effectively delay mesocarp softening when certain amount of Ca attachment sites exist in the cell wall.

The procedure with extraction of acetic acid and HCl gives a measurement of Ca associated with pectate and oxalate (Ferguson et al., 1980). The fractionation of Ca in papaya mesocarp tissue from color break, 50% and 100% yellow fruits indicated that the majority of Ca was in 80% acetic acid fraction (Table 6.1), implying that most of Ca in mesocarp tissue was bound to pectin in the cell wall (Ferguson, 1980). In apple and kiwifruit fruit, 76% of Ca is in the 80% acetic acid fraction (Ferguson, 1980). It has been suggested that fruit softening occurs either by movement of Ca⁺⁺ from middle lamella or by the loss of Ca⁺⁺ attachment sites (Knee and Bartly, 1981). The Ca concentration in 80% acetic acid did not change during ripening, indicating that Ca probably stayed in the cell wall during ripening. Calcium does not seem to move away from the cell wall region to the cytoplasm during ripening because the cell itself largely excludes apoplastic Ca (Evans et al., 1991). The apoplast consists of a Free Space (FS) and a Donnan Free Space (DFS).
because of high concentration of fixed cell wall anions (-COO\(^{-}\)) (Cleland et al., 1990). The exogenous Ca inhibits growth probably by releasing H\(^{+}\) from the DFS to the FS, raising the pH of DFS and inhibiting wall loosening enzymes with acidic pH optima (Cleland et al., 1990). Calcium possibly moves between the DFS and the FS during fruit ripening. If true, then Ca does not need to leave the cell wall region during fruit softening.

Papaya mesocarp tissue treated with Ca had a lower and more constant ethylene production (Figure 6.6). Calcium treatment slows the senescence processes with respect to ethylene production in apple fruit (Conway and Sams, 1987), slices of apple fruit (Lieberman and Wang, 1982), and detached cucumber cotyledons (Ferguson et al., 1983). Stable membrane integrity is required for ethylene production (Lieberman and Wang, 1982). Calcium treatment reduces the leakage of membrane in watermelon (Elkashif and Huber, 1988). Calcium interacts with phospholipids that have anionic head groups, resulting in high rigidity and surface tension of membrane (Landau and Leshem, 1988). Electron microscopy has revealed the unique importance of Ca for the stabilization of membranes (Marinos, 1962). Maintaining the membrane integrity and functions by Ca may be involved in delaying papaya mesocarp plug softening.

Mesocarp magnesium and potassium concentration of intact papaya fruits were not correlated with the firmness of ripe fruit (Chapter 4). Infiltration of mesocarp tissue with MgCl\(_2\) and KCl had no effect on tissue softening (Figure 6.7). Infiltration with magnesium in intact apple fruits (Conway and Sams, 1987) as well
as in excised tissue (Stow, 1989) increases the firmness of the fruit or the fruit tissue but has less effect than Ca. Magnesium has a relatively similar charge and size to Ca. When ion exchanges are performed in the cell walls, either between Ca\(^{++}\) and Mg\(^{++}\) or between Ca\(^{++}\) and K\(^{+}\), the cell walls have always exhibited a large preference for Ca\(^{++}\) (Demarty et al., 1984). Calcium cations are very efficient in promoting gelling in a pectin solution, while magnesium cations are practically without effect (Tefper and Taylor, 1981).
"Sunset" papaya fruit had 2 main growth peaks (Fig. 3.1). The uptake pattern of Ca, Mg and K by the whole fruit were similar (Fig. 3.4), though the uptake pattern of Ca, Mg and K by the skin, the mesocarp and the seeds were different from each other (Fig. 3.5, Fig. 3.6, Fig. 3.7). High mesocarp Ca uptake occurred when fruit was less than 60 days old and may be related to high transpiration from the fruit surface (Table 3.2). Water and nutrients are supplied predominantly through the phloem and may result in low xylem supplied Ca uptake by the mesocarp 60 to 80 days post-anthesis when the mesocarp FW and DW rapidly increased (Fig. 3.6). Mesocarp Ca uptake rate increased again 100 to 140 days post-anthesis when mesocarp FW growth rate was reduced and DW growth rate increased (Fig. 3.6). Dramatic increases in mesocarp sucrose during this period may reduce the water potential that leads to increase in water movement into the fruit and Ca uptake into the mesocarp. The Ca concentration in the mesocarp was not different from the peduncle to the blossom end (Table 3.5) probably because the vascular bundles carrying Ca pass longitudinally throughout the mesocarp tissue.

Spraying CaCl₂ on papaya during fruit growth and development did not increase mesocarp Ca concentration (Table 4.1). The Ca apparently remained on the fruit skin surface as indicated by green spots on the skin of ripe fruits. Dipping the papaya fruit peduncle in CaCl₂ for 48 hours to allow uptake of Ca by
transpiration did not increase the mesocarp Ca level (Table 4.2). Transpiration from detached fruit might not be an effective motive force to move Ca into the mesocarp. Raising the soil Ca level by fertilization did not always increase mesocarp Ca concentration (Fig. 4.1, Table 4.7). Mesocarp Ca and Mg concentration fluctuated significantly throughout the year (Fig. 4.1). Factors such as soil type, levels of other minerals in the soil, root growth and rainfall may affect the uptake of Ca by papaya plants.

Potassium and Ca+K fertilizer treatments significantly increased mesocarp K concentration (Fig. 4.1, Table 4.7). Mesocarp K concentration was relatively stable throughout the year within a single treatment (Fig. 4.1). Nitrogen fertilization had no effect on mesocarp N concentration (Table 4.8). Potassium and N fertilization decreased mesocarp Ca concentration (Table 4.7).

Mesocarp Ca concentration was positively correlated with the firmness of ripe papaya fruits (Table 4.9, Fig. 4.3). Fruit with mesocarp Ca concentration higher than 130 μg g⁻¹ FW did not show rapid softening. There was no correlation between ripe fruit firmness and Mg, or K concentration. There was correlation between fruit firmness and the ratio of Ca concentration to Mg concentration, or to K concentration, or to Mg+K concentrations (Table 4.9). This involvement of K or Mg appeared to be related to Ca, rather than by an effect of these minerals per se.

The proportion of different extractable papaya pectin and hemicellulose fractions and their molecular size range were altered during fruit ripening. The pectin molecular size declined (Fig. 5.1) and the solubility of pectin in CDTA and
Na₂CO₃ increased during fruit ripening (Table 5.2). The molecular size range of hemicellulose also changed (Fig. 5.2) with an increase of solubility of hemicellulose in KOH fractions (Table 5.2 and Table 5.3). These changes may lead to disruption and loosening the papaya fruit cell wall structure during fruit ripening. The demethylation of pectin molecules during fruit ripening resulted in more -COO⁻ groups, that may increase Ca²⁺ bridges between cell wall polymers. These results provide alternative evidence to enzymatic studies (Chan and Tam, 1982; Paull and Chen, 1983) suggesting that pectin hydrolysis and the modification of hemicellulose are involved in papaya fruit softening.

Excised mesocarp plug underwent an accelerated ripening process. This process was completed within 24 hours versus 6 to 8 days for intact fruits when the pattern of tissue softening, respiration and ethylene production were compared. Infiltrating mesocarp tissue with CaCl₂ effectively slowed the softening rate and reduced the rates of respiration and ethylene production. Treatment with Ca chelating agent EGTA had the opposite effects, hastening softening and lead to increase rates of respiration and ethylene production. Calcium slowed the mesocarp softening probably through strengthening the cell wall structure, inhibiting cell wall degrading enzyme activity and reducing the metabolic rate of the ripening process. Infiltration mesocarp tissue with MgCl₂ and KCl had no effect on softening.
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