This is to certify that the

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on the Textural Quality of Fresh
and Processed Pickling
Cucumber Fruit

presented by

Regina Rosa Fernandes

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Horticulture

Irwin E. Widders
Major professor

Date Sept. 25, 1991

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INFLUENCE OF CALCIUM NUTRITION ON THE TEXTURAL QUALITY OF FRESH AND PROCESSED PICKLING CUCUMBER FRUIT

By

Regina Rosa Fernandes

A DISSERTATION

Submitted to
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for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

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ABSTRACT

INFLUENCE OF CALCIUM NUTRITION ON THE TEXTURAL QUALITY OF FRESH AND PROCESSED PICKLING CUCUMBER FRUIT

By

Regina Rosa Fernandes

The relationship between endogenous fruit calcium (Ca^{2+}) and tissue firmness and the rate of softening of fresh, stored and processed pickling cucumber (*Cucumis sativus* L. cv Castlepik 2012) fruit were investigated. The influence of low tissue Ca^{2+} levels on polygalacturonase (PG) activity in fresh and stored fruit and the effect of Ca^{2+} addition to the brine solution on texture after fresh-pack processing were also studied. Pericarp and endocarp tissue Ca^{2+} concentrations (dry wt) ranged from 0.08 % to 2.4 % Ca^{2+} and from 0.05 % to 0.5 % Ca^{2+}, respectively, when cucumber plants were cultured in nutrient solution modified for Ca^{2+}, 0.00, 0.01, 0.1, 1.0, 10.0 and 20.0 mM Ca^{2+} during the reproductive period.

PG activity and firmness of fresh fruit were not affected by the Ca^{2+} fertilization treatments. Fruit Ca^{2+} concentrations and fruit firmness were positively correlated after 5 and 24 days of post harvest storage. High Ca^{2+} concentrations in the nutrient solution increased pericarp firmness up to 3 weeks after processing, if pickles were refrigerated. High endogenous Ca^{2+} levels were negatively correlated with rates of pericarp softening in processed slices and positively correlated up to 8 mM Ca^{2+} in processed spears during accelerated aging. Ca^{2+} ammendments to the processing brine,

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5 and 20 mM Ca^{2+}, enhanced pericarp and endocarp post-processing firmness. This effect was maintained even when the spears were incubated at 46°C for 5 or 20 days. In a field experiment, foliar applications of 2.5 % (v/v) Ca^{2+}-chelate, 2,3,4,5 trihydroxypentanedoic acid (Nutri-Cal), or side-dress applications of $CaCl_2$ (62.8 Kg/ha) increased the Ca^{2+} in pericarp tissue while only the Ca^{2+}-chelate solution increased textural firmness. Xylem sap Ca^{2+} concentrations declined during plant ontogeny (14.3 - 9.4 mM Ca^{2+}), and were higher in defruited plants than in fruiting plants.

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Finally, from the deepest of my heart my gratitude to my mother Monserrat, my husband Berthier, my son Romeu and my daughter Gígi for their understanding and love. A special thank to Berthier and Gígi for their constant help during the greenhouse experiments.

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INTRODUCTION

Ca^{2+} is thought to have a physiological role in influencing textural quality and firmness of fruit (Cooper & Bangerth, 1976). Tissue firmness and integrity of both the green and processed fruit stock tissue are important quality parameters of fresh-pack pickling cucumbers.

Ca^{2+} serves as an intermolecular adhesive in plant cell walls maintaining cell cohesion (Hanson,1984) by acting as cross-linking component between polygalacturonide chains (Davis & Dennis, 1983) in the middle lamella (Ferguson, 1984). The loss of Ca^{2+} is followed by solubilization of cell wall cementing substances and by hydrolysis of the structural components of the wall (Poovaiah & Leopold, 1973). As long as Ca^{2+} is present, structural integrity of the cell wall is preserved (Glenn & Poovaiah, 1990).

Ca^{2+} has been reported to interact with polygacturonase, a cell wall degradative enzyme, by supressing its activity (Poovaiah, 1979). In mature fruit undergoing ripening, polygalacturonase activity is also thought to be influenced by the level of endogenous Ca^{2+} (Rigney & Wills, 1981). The mode of action of Ca^{2+} in the softening process involves binding to pectic polymers, reducing the number of free acid groups will reduce the rate of pectic solubilization (Ferguson, 1984).

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The strength of the cell wall structure, and the inhibition of the breakdown of pectic polysaccharides during growth or post-harvest handling (Rigney & Wills, 1981) are affected by endogenous Ca^{2+}. However, endogenous Ca^{2+} levels (% dry weight) in pickling cucumber fruit decline during fruit ontogeny (Engelkes, 1987). Limited research has been conducted on pickling cucumbers to determine the effects of a low fruit Ca^{2+} status on parameters affecting quality for processing.

Efforts have focused on maintaining the firmness of cucumbers during and after fruit processing. Ca^{2+} has been reported to be an important factor in maintaining the tissue firmness during processing of cucumbers, especially after fresh-pack processing (Fleming *et al.*,1978;Buescher *et al.*, 1981;McFeeters *et al.*,1985).

Firmness is associated with the amount of bound Ca^{2+} while the amount of bound Ca^{2+} is dependent on the supply of Ca^{2+} primarily from the brine solution in fresh-pack pickling cucumbers. McFeeters & Fleming (1989) reported, that endogenous Ca^{2+} concentrations in the cucumbers maintain fruit firmness in pieces of mesocarp tissue following blanching. In McFeeters's study, however, fruit were obtained from diverse sources and thus potentially variable in numerous physiological parameters other than Ca^{2+}.

The question is how to enhance Ca^{2+} levels within the fruit tissue. Ca^{2+} is a highly immobile macronutrient in

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plants, thus problems that arise with Ca^{2+} nutrition in plants are frequently related to redistribution and mobility (Ferguson,1984). In growing tissues most of the Ca^{2+} is translocated via the xylem through the transpiration stream (Marschner, 1986). In addition, fruit and root growth in cucumber plants compete for photosynthates. Consequently, with increasing fruit set and net growth, the rate of root growth is drastically reduced (De Stigter, 1969). Ca^{2+} uptake by the root system has been shown to decline during fruiting (Hansen, 1973). It is hypothesized that Ca^{2+} concentrations in xylem sap decline during plant ontogeny, specifically during fruit development, as a result of reduced new root growth.

The objectives of this study were: (1) to evaluate the effect of modified endogenous Ca^{2+} within fruit tissues on texture of immature, freshly harvested, pickling cucumber fruit and after post harvest storage; (2) to determine the influence of low Ca^{2+} levels in fruit tissues on the activity of polygalacturonase in freshly harvested and stored pickling cucumber fruit; (3) to verify the effect of endogenous Ca^{2+} and ammendments of Ca^{2+} to the brine solution on tissue firmness following fresh-pack processing, during accelerated aging and during refrigeration in pickled cucumber fruit; (4) to evaluate alternative Ca^{2+} fertilization strategies for enhancing the Ca^{2+} concentration of pickling cucumber fruit tissues and (5) to understand better how fruit set and growth might affect Ca^{2+} supply via the xylem to the fruit.

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LITERATURE REVIEW

Textural Quality of Fruits and Vegetables

Texture is the one of the most important factors in determining the quality of fruits and vegetables, thus control or modification of texture is a major objective in modern food technology (Van Buren, 1979). To approach problems in this area requires an understanding of the factors that influence texture. One of these factors, according to Wallner (1978) is the molecular composition and structure of the membrane and cell wall.

Plant Cell Membranes

Electron microscopic studies have shown the cell membranes as a fluid, mosaic structure of globular proteins within a matrix of phospholipid layers. The arrangement of proteins is controlled by the combined effects of hydrophilic and hydrophobic characteristics (Singer & Nicholson, 1972). According to Marschner (1986) the hydrophilic layer, charged head regions (amino, phosphate, and carboxyl) is oriented toward the membrane surfaces while the hydrophobic tails of the fatty acids are oriented inward. Proteins molecules can be either attached by electrostatic binding to the surfaces as

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membrane-bound enzymes or may be integrated into the membranes or even traverse the membranes to form "protein channels" through which the ions are transported. Functionally intact membrane systems establish and maintain the osmotic conditions that result in water uptake and turgor, an important component of texture (Bourne, 1976).

Plant Cell Wall

In fleshy fruits the contribution of the cell wall to texture is more apparent. The fleshy tissue of most fruits is characterized by parenchyma cells with relatively thin walls. In some plant tissues, cell differentiation, secondary wall thickening, and lignin deposition provide considerable additional strength (Wallner, 1978). Structural features of cells, thus, play an essential role in determining texture. The relative proportion of the fibrous collenchyma, sclerenchyma and xylem tissues to the more tender parenchyma tissues must be considered (Reeve, 1970).

The ultra-structure and molecular architecture of the cell wall has been presented by Albersheim (1974) as being made up of cellulose fibrils imbedded in a matrix consisting largely of pectic substances, hemicelluloses, proteins, lignin, lower molecular weight solutes and water. Lignin is a complex polymer derived from phenolic compounds. It replaces water in the cell wall matrix increasing the

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rigidity and cohesion of the walls (Sakakibara, 1971).

Water plays an important role in the walls as a structural component of the matrix gel, as an agent interrupting direct hydrogen bonding between polymers, cooperating in stabilizing conformation of polymers, and serving as a solvent facilitating the diffusive transport of salts, low molecular weight organic compounds and enzymes (Northcote, 1972). The "turgor pressure" exerted by the protoplast on the cell walls keep them in a state of elastic stress. The combined effects of the turgor pressure of the cell contents with the elastic cell walls determine the viscoelastic properties of the tissues in the biological material (Mohsenin, 1970).

The relative composition of the wall varies from the plasmalemma, separating the wall from the cytoplasm, to the middle lamella. The primary wall is the most important for edible fruits and vegetables with exceptions as in the case of celery in which secondary walls give to them the well-known fibrous texture (Van Buren, 1979). In most fruits, the protein comprises less than 5% of the wall weight (Wallner, 1978), and usually it binds to cell wall polysaccharides as galactose-o-serine or arabinose-o-hydroxyproline glycosidic linkages forming a protein-hemicellulose network in the cell wall matrix (Preston, 1974). Three major polysaccharides are present: cellulose, a xyloglucan (β-1,4 linked glucose with xylose residues attached), and the pectic polymers -

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rhamnogalacturonan with side branches of galactose and arabinose (Albersheim, 1975). These are extensively cross-linked to form a continuous macromolecule of exceptional strength (Wallner, 1978).

Cellulose is a rather inert material, particularly in its crystalline form. It is a β-(1-4) glucan having 8-12 thousand glucose units per chain. Its degree of hydration may influence wall properties. Hemicelluloses can be described as neutral sugar polysaccharides, and frequently, the principal chain of a particular type of hemicellulose is made up largely of one type of monosaccharide, a galactan, a xylan, or a glucan (Isherwood, 1970). It has been proposed by Albersheim (1974) that if one has a xyloglucan, then xylose is the side chain, if it is an arabinogalactan, arabinose is the side chain. The side chains influence the degree of water adsorption by the polymer, and decreased water adsorption can result in a rigid structure. However, according to Northcote (1972) a high percentage of water absorbing side chains would decrease the interpolymer complex binding in a highly hydrated matrix leading to a weak, unstable cell wall.

The middle lamella may be considered an extension of the matrix material of the primary cell wall, lacking the cellulose fibrils. It consists principally of the Ca^{2+} salts of polymers of galacturonic acid that have been partially esterified with methyl alcohol, and is known as pectic material (Bourne, 1976). The carboxyl groups of

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polygalacturonic acids may be partly or completely neutralized by one or more bases (Pilnik & Voragen, 1970). As the outermost portion of the plant cell, it plays the primary role in intercellular adhesion (Van Buren, 1979).

Pectic Substances

The chemistry of the pectic substances has been the subject of intensive study (Jarvis, 1984; Pilnik & Voragen, 1970). Pectins, according to Jarvis (1984) are polysaccharides, heavily branched, largely methyl-esterified alternate with unbranched chains of varying degrees of esterification. The unbranched, non-esterified chains can aggregate through Ca^{2+} binding to form the junction zones that hold a gel together.

Changes in the chemical nature of the pectic materials hold the key to understanding and controlling the textural properties of horticultural products. Many fruits become soft as the pectic materials in the interlamellar layer are degraded and lose their strength and cementing power (Bourne, 1976; Jarvis, 1984) which causes loosening of walls and loss of cell cohesion (Wills & Rigney, 1979).

These changes in the cell wall have been accompanied by a conversion of the insoluble pectic substances to soluble pectic substances (Lampi *et al.*, 1958; Mohsenin, 1970; Ben-Arie & Sonego, 1979; Hudson, 1984), observed largely

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during the pickling process (Pressey & Avants, 1975; Tang & McFeeters, 1983; McFeeters *et al.*, 1985). Pectic solubilization has been correlated with the progressive dissolution of the middle lamella (Ben-Arie *et al.*, 1979). Cell separation during abscission is also due to pectic solubilization and it is followed by loss of Ca^{2+}.

Experimentally, Ca^{2+} addition retards abscission and removal accelerates abscission (Poovaiah & Leopold, 1973).

In green fruits the pectic material is principally in the form of partially esterified polygalacturonic acid of very high molecular weight called protopectin. It imparts great strength to the tissue and is insoluble in water (Bourne, 1976). As the fruit ripens, the chain length of the pectin polymer decreases forming water-soluble pectin, mostly composed of colloidal polygalacturonic acids and essentially free from methyl ester groups (Pilnik & Voragen, 1970). Since this is not as strong as protopectin, the structure becomes softer and eventually mushy (Bourne, 1976).

In a very simplified sense the cellulose has the function of giving rigidity and resistance to tearing, while the pectic substances and hemicelluloses confer plasticity and the ability to stretch (Van Buren, 1979).

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proteins forming the cell wall, acting as a cross-linking component, mainly in the middle lamella (Davies & Dennis, 1983). Experimental evidence for this arises from studies of cell cohesion and cell wall extensibility. Cell cohesion is typically attributed to Ca^{2+} pectate of the middle lamella laid down during cell division (Hanson, 1984).

Various reports have appeared indicating that Ca^{2+} pectate acts as a cementing agent in the cell wall of plant cells (Jones & Lunt, 1967; Loneragan *et al.*, 1968; Mohsenin, 1970; Epstein, 1972; Poovaiah & Leopold, 1973; Clarkson & Hanson, 1980; Yamaya *et al.*, 1982; Davies & Dennis, 1983; Jarvis, 1984; Hudson & Buescher, 1986). Galacturan chains are the major pectic components in which carboxyl groups are methylated, or available for bonding. The degree of Ca^{2+} bonding will depend to some extent on the degree of pectin methylation (Ferguson, 1984).

According to Jarvis (1984), there are two types of gels which are formed by pectic polysaccharides. They include Ca^{2+} and acid gels, which form respectively low- and high-ester pectins, the latter made normally with the help of a dehydrating agent like sucrose. In a Ca^{2+} gel, the most common type in cell walls, the junction zones are built up from unbranched, non-esterified galacturonan chains bonded together non-covalently by co-ordinated Ca^{2+} ions. Whatever the exact structure of the junction zones, it requires co-operation between consecutive Ca^{2+} galacturonate units. The strength of

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the binding thus depends on the length of the uninterrupted pectate segments that can interact. The removal of Ca^{2+} ions with strong chelating agents certainly weakens cell walls (Soll & Bottger, 1982).

Changes in composition and structure in cortical cell walls in apple tissue occurres predominantly in the middle lamellar region and resultes in a loss of cell cohesive strength and textural quality. Ca^{2+} preserves structural integrity of the cell wall and maintained cell cohesiveness (Glenn & Poovaiah, 1990). The same authors have shown the distribution of Ca^{2+} along the cell wall structure, and their results confirm the role of the cell wall as a Ca^{2+} store and the importance of Ca-cell wall interaction in maintaining firmness. Ca^{2+} deprivation during cell wall development may lead to a skeletal weakness (Wilkinson, 1970). Thus, Ca^{2+} has been shown to be an integral part of cell wall struture.

Ca^{2+} as Related to Cell Membranes

The effect of Ca^{2+} on maintaining membrane integrity has been considered by several researchers (Jones & Lunt, 1967; Loneragan *et al.*, 1968; Poovaiah & Leopold, 1976; Poovaiah, 1979; Hecht-Buchholz, 1979; Hopfinger & Poovaiah, 1979; Millaway & Wiersholm, 1979; Marschner, 1986).

Ca^{2+} serves to avoid membrane damage and leakiness (Yamaya *et al.*, 1982). An increase in solute leakage indicates a loss

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of selective permeability of the cell membranes. The role of Ca^{2+} might be to retain membrane lipids in a more fluid state (Ferguson, 1984). The general disorganization of Ca^{2+} deficient cells and tissues suggests that the role of Ca^{2+} is to maintain membranes in a functional state (Epstein, 1972; Smith, 1978). Thus maintaining the cell membrane in a functional state is important for the absorption and transport of ions and other solutes (Matsumoto & Kawasaki, 1981).

The exact nature of the manner in which Ca^{2+} functions to stabilize membranes is yet to be elucidated; however it is suggested that Ca^{2+} binds ionic groups of the membrane to form structural bridges (Christiansen & Foy, 1979). These bridges are formed by Ca^{2+} binding through O^-ligands of acid groups of phospholipids and proteins at the diffuse aqueous layer of the external surface of the plasma membrane (Gillard, 1970).

Charge-neutralizing Ca^{2+} at the surface permits higher anion concentrations in the region of absorption that induces conformational or physical changes in the membrane, which facilitates the establishment of an increased proton-motive force (Smith, 1978).

Binding to the surface increases membrane hydrophobicity, reduces the conductivity of hydrated ion channels, and prevents membrane leakiness (Hanson, 1984). Thus, Ca^{2+} brings a structural and physiological stability to plant tissue , and along with other materials in the plant, regulates the influx and efflux of water and solutes (Poovaiah & Leopold, 1976).

The important point is that the binding of Ca^{2+} to the external surface of the plasma membrane tightens it against indiscriminate ion fluxes, including that of Ca^{2+} itself, making the membrane more hydrophobic (Macklon & Sim, 1981). Complexes of Ca^{2+} with surface ligands appears to be specific for Ca^{2+} in the sense that membrane dysfunction and eventual death result if Ca^{2+} is substituted by another ion (Hanson, 1984).

Ca^{2+} Related Disorders

Ca^{2+} deficiency causes a breakdown of membrane structure (Jones & Lunt, 1967; Bangerth, 1973; Hecht-Buchholz, 1979). In reproductive tissues, which are probably the largest harvest organ in the vegetable and fruit industry, Ca^{2+} deficiency takes the greatest toll. It appears in tomatoes, peppers, and melons as blossom end rot and in apple fruit as bitter pit, fruit softening, water core, cork spot, internal breakdown, and cracking (Bangerth, 1973; Shear, 1975; Mason, 1979). Several other physiological disorders are associated with inadequate Ca^{2+} nutrition as black heart of celery, and tipburn in lettuce (Kirkby, 1979). Shear (1975) has related a complete survey of disorders attributed to inadequate Ca^{2+} nutrition. The characteristic feature of all these physiological disorders is a localized inadequate Ca^{2+} level in the plant, mostly the plant parts that are naturally low in

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Ca^{2+} has been associated with post harvest storage life (Wills & Rigney, 1979; Badawi *et al.*, 1981; El-Hammady *et al.*, 1987; Abbott *et al.*, 1989; Cheour *et al.*, 1990). The addition of Ca^{2+} prevented bitter-pit symptoms on apples (Hopfinger & Poovaiah, 1979; Hewett, 1984; Fallahi *et al.*, 1985; Watkins *et al.*, 1989), storage rot on apples (Fallahi *et al.*, 1985), and on peaches (Bhullar *et al.*, 1981), breakdown after storage on apples (Bramlage *et al.*, 1985; Watkins *et al.*, 1989), and cracking of tomatoes (Dickinson, 1962).

Increasing the Ca^{2+} content of fruits by spraying several times with Ca^{2+} salts during fruit development or by post-harvest dipping in Ca^{2+} chloride solutions, leads to an increase in the firmness of the fruit (Cooper & Bangerth, 1976). Johnson *et al.* (1983) reported that foliar application of Ca^{2+} chloride effectively reduced bitter-pit in apples, and when sprayed fruit were subsequently stored in controlled atmosphere, in contrast to air, the disorder was virtually eliminated.

Ca^{2+} chloride infiltration increased all measures of apple tissue strength immediately. Relative increases persisted during storage (Abbott *et al.*, 1989), minimizing the percentage of fruit decay, perishability, loss in fruit moisture content, and consequently prolonging the storage period and shelf life in apples (El-Hammady *et al.*, 1987;

Tomala & Sadowski, 1989) and pears (Badawi *et al.*, 1981).

Electrolyte leakage of watermelon fruit was greatly reduced when tissues were incubated in an isotonic medium containing Ca^{2+} chloride (Elkashif & Huber, 1988). Increased firmness could be achieved with Ca^{2+} chloride treatment on pear tissue (Badawi *et al.*, 1981; Knee, 1982). According to Drake & Proebsting (1985) Ca^{2+} chloride also increased the firmness and reduced the number of split fruit in canned cherries. Infiltration of apricot fruits with Ca^{2+} chloride before processing resulted in a definite firming of canned apricots (French *et al.*, 1989).

Fruit firmness was correlated positively with Ca^{2+} concentration of the apple tissue (Lau *et al.*, 1983; Fallahi *et al.*, 1985) both before and after storage at 0°C (Sams & Conway, 1984). The same correlation has been found for sweet cherries (Facteau, 1982). Therefore, Ca^{2+} apparently protects cell walls from disorganization (Badawi *et al.*, 1981)

Ca^{2+} as Related to Physiological Processes

It has been widely recognized that Ca^{2+} plays a crucial role in regulating many physiological processes (Yamaya *et al.*, 1982). Ca^{2+} has been shown to be important for normal plant growth (Loneragan *et al.*, 1968; Clarkson & Hanson, 1980), and for normal fruit and seed development (Jones & Lunt, 1967; Frost & Kretchman, 1989). Plants grown under

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Ca^{2+}-deficient nutrient solution were spindly and produced small and mishapen fruit or no fruit (Staub *et al.*, 1988). Ca^{2+} stress severely reduced cucumber root growth (Matsumoto & Kawasaki, 1981; Konno *et al.*, 1984), fruit growth, and seed quality (Frost & Kretchman, 1989). Pillowing disorder has been associated with decreasing Ca^{2+} levels in the cucumber mesocarp tissue (Staub *et al.*, 1988).

Root extension ceases in the absence of an exogenous Ca^{2+} supply as well as cell division (Marschner, 1986). Ca^{2+} has also been reported to be required for auxin transport (de la Fuente & Leopold, 1973). However, auxin treatment of growing shoot tissue results in Ca^{2+} efflux or redistribution. There is some evidence that these auxin-mediated Ca^{2+} fluxes may be based in membrane transport (Hanson, 1984).

Ca^{2+}, auxin (IAA), and extension growth seem to be interrelated. Auxin is involved in Ca^{2+} transport within plant tissue, and the inhibition of Ca^{2+} transport or a decline in the level of auxin induces Ca^{2+}-deficiency which in turn interferes with cell extension. At the same time, Ca^{2+} stabilizes the cell walls, an action that is contrary to auxin-induced extensive growth (Marschner, 1986).

Most of the requirements for Ca^{2+} are based on macromolecular structures that bind Ca^{2+}. Those external to the protoplast require millimolar free Ca^{2+}, whereas those within the protoplast are stabilized at micromolar levels of free Ca^{2+}. Proteins are the only known cellular components

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that effectively bind Ca^{2+} at micromolar levels (Kretsinger, 1977).

Normal cell functions requires maintenance of low cytosolic Ca^{2+} concentrations, in order to prevent precipitation of Pi, competition with magnesium for binding sites and inactivation or uncontrolled activation of certain enzymes (Marschner, 1986), with the cell working to keep Ca^{2+} out in the extracellular, or extracytosolic environment (Ferguson, 1984),

It has been reported that free cytosol Ca^{2+} concentration in higher plants is 10^{-6} M or less (Cormier *et al.*, 1980; Marme, 1982), but plastids, mitochondria and nuclei contain high concentrations of Ca^{2+}, as do vacuoles, which seem to serve as a sink for excess Ca^{2+}. Biochemically, Ca^{2+} has been shown to de-toxify the respiratory by-product, oxalate, in the cell solution, by forming the Ca^{2+} oxalate crystals which are deposited in the vacuoles (Carolus, 1975).

The plasma membrane presents an effective barrier to Ca^{2+} penetration (Marschner, 1986), however, electro-chemical gradients exist to drive passive Ca^{2+} influx into the symplast, being the exit back into the apoplast or xylem through linkage to metabolic energy (Drew & Biddulph, 1971).

Attention has been directed to a role for Ca^{2+} as a "second messenger" in plants (Cormier *et al.*, 1980; Marme, 1982). Variation in cytosol Ca^{2+} levels in response to environmental or hormonal signals mediates changes in enzyme

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activity via a Ca^{2+}-binding modulator as calmodulin. Since intermediary and biosynthetic metabolism would be adversely affected by the millimolar levels of free Ca^{2+} found externally, the messages must be transmitted by fluctuations within the "safe" micromolar range (Kretsinger, 1977).

The Ca^{2+}-binding protein calmodulin has been shown to be important in plant cells for both the regulation of free Ca^{2+} in the cytosol and enzyme activation. The formation of Ca^{2+}/calmodulin complexes with the enzymes might control Ca^{2+} transport within the cell as well as mediate Ca^{2+} transfer to the vacuoles (Marme, 1983). There is some evidence that Ca^{2+}/Calmodulin may influence the activity of lipoxygenase and superoxide dismutase in fruit tissue (Leshem *et al.*, 1982).

Ca^{2+} increases the activity of a few enzymes, as α-amylase, phospholipases, and ATPases (Jones & Lunt, 1977) while Ca^{2+} starvation was reported to cause a remarkable increase in phosphatase activity in cucumber root cell walls (Yamaya *et al.*, 1982). Also, the basal activity of membrane associated APTase increased during Ca^{2+} starvation in cucumber roots (Matsumoto & Kawasaki, 1981). Most enzymes require high Ca^{2+} concentrations (2-3 mM Ca^{2+}) to be activated as the lipid-degrading enzyme phosphatidylserine (Moore, 1975), but there are instances, however, where low Ca^{2+} appears to activate the enzyme, as is the case of the membrane-bound protein kinase of peas which has been activated at 3 μM Ca^{2+} (Hetherington & Trewavas, 1982).

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Ca^{2+} as Related to Fruit Ripening and Senescence

Ripening results from a series of coordinated changes in the biochemistry of fruits which affect their color, texture, and flavor (Tucker & Grierson, 1982), it occurs after certain stage of maturity and it is not related with the chronological age. However, it is determined by developmental processes which are under genetic control (Grierson, 1983).

Ca^{2+} has been associated with ripening and senescence processes of fruits (Poovaiah, 1979; Leshem *et al.*, 1982). Pigment changes, protein decrease, and a hydraulic permeability increase associated with ripening are suppressed by Ca^{2+} (Poovaiah, 1979). Respiration rates, and thus rates of ripening, were reduced in tissue having high Ca^{2+} concentrations (Faust & Shear, 1972; Bangerth, 1979).

Relatively low concentrations of Ca^{2+} have a depressant effect on senescence in detached cucumber cotyledons (Ferguson *et al.*, 1983). The beginning stages of cytoplasm degeneration and the rather rapid breakdown of cell compartmentation in Ca^{2+} deficient tissues are very similar to symptoms of senescence. This implies that the lack of Ca^{2+} does not primarily affect specific metabolic processes, but leads rather to rapid autolysis of the cell due to increased membrane permeability (Hecht-Buchholz, 1979).

Normal cell function requires the maintenance of low concentrations of free Ca^{2+} in the cell cytosol (Marschner,

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1986), however, high external concentrations of Ca^{2+} are advantageous in reducing the rate of senescence or ripening (Lieberman & Wang, 1982).

Thus, the compartmentalization of Ca^{2+} is crucial. Leshem *et al.* (1986) have confirmed the same findings. When Ca^{2+} is externally situated in intact cells, senescence is delayed. However, upon entry into the cytosol it exerts the opposite effect. Increasing cytosolic Ca^{2+} promotes senescence in pea foliage, probably, by activating calmodulin, which in turn mediates the action of phospho-lipase A2 on membranes (Leshem *et al.*, 1984). Ca^{2+} can reduce and delay ethylene production by affecting the synthesis of ACC (1-amino-cyclopropane-1-carboxylic acid) in senescent membrane systems from fruit tissue (Lieberman & Wang, 1982; McGlasson, 1985). As polygalacturonase activity increases subsequent to ethylene production (Tucker & Grierson, 1982), there is evidence for a regulatory role for Ca^{2+} in hormonal action during the ripening and senescence of fruits (Leshem *et al.*, 1982). Furthermore, high levels of Ca^{2+} in the non-ripening RIN tomato fruit may be involved in suppressing polygalacturonase activity, as suggested by a high correlation between the Ca^{2+} status of the tissue and senescence development (Poovaiah, 1979).

The changes in soluble and bound Ca^{2+} during ontogeny have indicated that Ca^{2+} was solubilized during the early stages of ripening. The solubilizing and movement of Ca^{2+} from the cell

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wall into the cytoplasm might account for many of the major events associated with the onset of natural ripening of tomatoes (Rigney & Wills, 1981).

Application of Ca^{2+} inhibits normal ripening and senescence of several fruits. Reduced ethylene production has been found in apple fruit tissue when Ca^{2+} concentrations have been raised by sprays during fruit development resulting in retarded ripening and reduced senescent breakdown (El-Hammady *et al.*, 1987).

Post-harvest Ca^{2+} treatments also have shown to be efficient in retarding the rate of ripening in addition to a specific effect of Ca^{2+} on preventing the characteristic tissue breakdown, which in turn also delays senescence (Badawi *et al.*, 1981).

Softening Enzymes

Fruit softening is characterized by the solubilization of pectin (Ben-Arie *et al.*, 1979; Bartley *et al.*, 1982), and is accompanied by a rise in the free galacturonic acid during maturation (Ben-Arie *et al.*, 1979). Such changes in the amount and character of the cell middle lamella polysaccharides have been described by Pilnik & Voragen (1970) and McFeeters & Lovdal (1987) during softening of fruits as they ripen.

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activity of cell wall-degrading enzymes (Dilley, 1970), and perhaps with some non-enzymic mechanisms (Rushing & Huber, 1984). Non-enzymatic reactions, such as occur with type of acid, concentration of acid, storage time, salt content, and storage temperature have been demonstrated to be related to texture deterioration of pickled products (Bell & Etchells, 1961; Bell *et al.*, 1972).

According to Bell *et al.* (1951) and Pilnik & Voragen (1970) there are two main groups of enzymes responsible for pectin degradation and softening of fruits and vegetables : saponifying and depolymerizing enzymes.

The saponifying enzyme is a specific pectin methyl esterase which splits the methylester group of polygalacturonic acids and which is commonly called Pectin Esterase or Pectin Methyl Esterase and abbreviated to PE or PME, respectively. The depolymerizing enzymes have been classified as Polygalacturonases, abbreviated PG, with specific activities pertaining to the degree of esterification of the substrate and to random (endopolygalacturonase) or terminal (exopolygalacturonase) cleavages which splits the glycosidic bonds between galacturonic monomers in pectic substances.

Pectin methyl esterase and polygalacturonase are associated with cell walls and are believed to be involved in the softening process of many fruits such as tomatoes (Tucker & Grierson, 1982; Grierson & Tucker, 1983; Barkai-Golan &

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Kopeliovitch, 1983), pears (Bartley *et al.*, 1982), cucumbers (McFeeters *et al.*, 1980), and other fruits (Van Buren, 1979).

Many researchers (Bell *et al.*, 1951; Ben-Arie *et al.*, 1979; Wills & Rigney, 1979; Rigney & Wills, 1981; Jarvis, 1984) have observed that the glycosidic hydrolysis of pectin must first be preceded by deesterification of the pectin to pectinic acids. Thus, PME action is a prerequisite for PG activity (Ben-Arie *et al.*, 1979). Since 1950 it was suggested (Bell *et al.*, 1950) that the simultaneous action of polygalacturonase and pectin methyl esterase will speed the glycosidic hydrolysis of the pectin.

Fruit pectin methyl esterase has been found in bananas, currants, papaya, cherries, apples, strawberries, citrus, peaches, (Pilnik & Voragen, 1970), tomatoes (Wills & Rigney, 1979; Rigney & Wills, 1981; Tucker & Grierson, 1982), cucumbers (Bell *et al.*, 1951; McFeeters *et al.*, 1985; Hudson & Buescher, 1986), and pears (Ben-Arie *et al.*, 1979; Ben-Arie & Sonego, 1979).

Pectin methyl esterase activity of the cucumber fruit decreases rapidly with an increase in size and during fruit development. In contrast, the PE enzyme content increases rapidly in tomato fruit during development (Bell *et al.*, 1951). Plant PE is almost completely specific for methyl esters of polyuronides. For all fruit PE, a pH optimum between 7 and 8 has been described (Pilnik & Voragen, 1970).

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present in several fruit species, as avocados, ananas, cherries (Pilnik & Voragen, 1970), peaches (Pressey & Avants, 1973), pears (Ben-Arie & Sonego, 1979), papayas (Lazan *et al.*, 1989), strawberries (Davies & Dennis, 1983), cucumbers (Bell *et al.*, 1950; Lampi *et al.*, 1958; Pressey & Avants, 1975; Buescher *et al.*, 1979; Buescher *et al.*, 1981; Buescher & Hobson, 1982), persimmons (Matsui & Kitagawa, 1989), and in tomatoes, where PG is the most thoroughly investigated fruit pectin depolymerase (Dickinson, 1962; Hobson, 1965; Wills & Rigney, 1979; Poovaiah, 1979; Rigney & Wills, 1981; Tucker & Grierson, 1982; Grierson & Tucker, 1983; Barlai-Golan & Kopeliovitch, 1983; Rushing & Huber, 1984; Brady *et al.*, 1985; Grierson *et al.*, 1985; Giovannoni *et al.*, 1989; Ahrens & Huber, 1990).

The polygalacturonase in cucumbers is an exo splitting enzyme which exhibits a high affinity for large substrate molecules which cleaves most rapidly (Pressey & Avants, 1975).

Endopolygalacturonase is unusually active in tomatoes, and it has been found also in peaches, avocados, dates, pears, pineapples, and suggested in cherries and strawberries (Pilnik & Voragen, 1970). Also, in apples, raspberries, plums, red currants, gooseberries, apricots (Van Buren, 1979), and in pears (Bartley *et al.*, 1982) the enzyme has been reported. An endopolygalacturonase has been found in mature pickling cucumbers, and it is likely that this enzyme plays a significant role in tissue breakdown during the latter stages

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The key enzymes that degrade tomato pectins are endopolygalacturonases termed PG1 and PG2 (Jarvis, 1984), with high and low molecular weight, respectively. According to Brady *et al.* (1985) PG exists predominantely or entirely in the high molecular weight form (PG1) early in ripening with the PG2 forms being increasingly prominent as ripening progressed. A way in which polygalacturonases could breakdown a pectin gel "in vivo", bearing in mind that they are active only on non-esterified segments of a chain is by attacking pectic segments participating in junction zones. At the low Ca^{2+} levels in tomatoes, the segments of a chain are relatively accessible (Buescher & Hobson, 1982).

Ben-Arie *et al.* (1979) demonstrated in apples and pears that structural alterations in cell walls became apparent at advanced stages of softening showing predominantly dissolution of the middle lamella. They observed that simultaneously with the degradation of pectin there was an increase in polygalacturonase activity, but pectin methyl esterase activity declined. Cellulase activity which was also present in immature fruit increased as the fruit softened, but in the last stages of softening it decreased. Low polygalacturonase activity occurred simultaneously with delayed softening of the fruit (Lobo *et al.*, 1984).

Bell *et al.* (1950) have pointed out that the softening of brined cucumbers is enzymatic in nature and the direct result

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of hydrolytic action by polygalacturonase. They observed a correlation between the polygalacturonase activity of commercial cucumber brines and the softening of salt-stock removed from these brines.

PG activity itself is sensitive to pH with an optimum activity at pH 4.5, but activity decreases rapidly as the pH deviates from 4.5 (Wills & Rigney, 1979). Pilnik & Voragen, (1970) described a pH optimum for PGs from 3 to 5.

Other enzymes such as cellulase, β-galactosidase, and β-1,3-glucanase have been reported to be implicated in cell wall degradation. Cellulase has been found in detached avocado (Pesis *et al.*, 1978), in pears (Ben-Arie & Sonego, 1979), in cucumbers (Buescher & Hudson, 1984), and in tomatoes (Buescher & Hobson, 1982). β-galactosidase has been detected during ripening in papaya fruits (Lazan *et al.*, 1989), and in cucumber roots (Konno *et al.*, 1984), and both β-galactosidase and β-1,3-glucanase were found in tomatoes (Buescher & Hobson, 1982). Cell walls contain other enzymes, such as acid phosphatase, peroxidase, xylanase, and in very small quantities, a β(1,4)-D-glucanase which may degrade the xyloglucan fraction (Jarvis, 1984).

However, the enzyme polygalacturonase is the primary determinant of cell wall polyuronide degradation (Giovannoni *et al.*, 1989).

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Ca^{2+} as Related to Softening Enzymes

The role of Ca^{2+} in fruit softening and its interaction with the enzyme polygalacturonase have been proposed by many researchers (Poovaiah, 1979; Buescher *et al.*, 1981; Rigney & Wills, 1981; Buescher & Hobson, 1982; Tang & McFeeters, 1983; Davies & Dennis, 1983; Konno *et al.*, 1984; Brady *et al.*, 1985; McFeeters *et al.*, 1985). Poovaiah (1979) has reported that an increase in polygalacturonase activity was suppressed by Ca^{2+}.

Ca^{2+} starvation, particularly caused an increase of polygalacturonase activity which occurred accompanied by an absolute decrease in amount of pectic polysaccharides in the cell walls (Konno *et al.*, 1984).

According to Brady *et al.* (1985) cell wall uronic acids of a firm and soft cultivar were equally susceptible to hydrolysis, suggesting that differences in the digestion of the walls by polygalacturonase were dependent on differences in Ca^{2+} content or distribution. It appears that Ca^{2+} associated with the cell wall-middle lamella and its removal regulate the rate and extent of degradation by polygalacturonase (Buescher & Hobson, 1982).

Ca^{2+} chloride in brines did not alter the activity of other enzymes as catalase and lipoxygenase, but it stabilized peroxidase activity (Buescher *et al.*, 1987). It has been reported by Wills & Rigney (1979) that the presence of high concentrations of Ca^{2+} reduced the activity of both pectin

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The mechanism responsible for retarding demethylation of pectins by Ca^{2+} is probably associated with hindering pectin methyl esterase action (Hudson & Buescher, 1986).

An increase in H^+ ions could result in displacement of Ca^{2+} ions from the cross-linking of galacturonic acid residues between adjacent pectic polymers. This is the same process of wall extension during cell growth in which existing cohesive bonds must be broken and new bonds formed depending on acidification of the apoplast. Acidification due to H^+ extrusion from the protoplast results in a displacement of Ca^{2+} from cation exchange sites (Rushing & Huber, 1984). Through this mechanism which is non enzymic an increase in polyuronide solubilization can occur.

Storing and Holding Cucumbers

Pickling cucumbers are normally stored for long periods of time in brine solution. If, however, fresh fruits need to be stored or held before being processed or placed in brine solution, the cucumbers should be cooled as rapidly as possible to 7° to 10°C (Motes, 1977).

Post harvest holding of cucumbers before brining is detrimental to final salt-stock pickle quality (Lee *et al.*, 1982). The optimum conditions for holding cucumbers,

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according to Motes (1977) are temperatures between 7° and 10°C, below 90 to 95% relative humidity for no longer than 10 days. The cucumbers should be processed immediately after removal from storage. Storage and holding of fresh fruit at high temperatures after being removed from storage can result in chilling injury.

Uebersax *et al.* (1986) reported that common cold storage provided improved quality stability of processed spears, and that 5°C temperature did not increase "chilling injury" as has been reported in previous studies. These conditions allow for green-stock storage for up to 10 days. According to the same authors, temperatures within the range of 21°C to 1°C provided maximum quality of processed spears. However, texture of pickles significantly decreased with increased holding time at 20° and 30°C, even though no significant differences for holding time were detected at 5°C (Lee *et al.*, 1982).

Ca^{2+} as Related to Texture of Pickles

Retention of firmness during processing and storage of pickled cucumbers is a major quality consideration in the pickling industry (McFeeters *et al.*, 1980). In addition, the physical and chemical structures of the cell walls of processed fruits are the main determinants of textural properties (McFeeters & Lovdal, 1987).

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during post harvest processing of cucumbers (Fleming *et al.*, 1978; Hudson & Buescher, 1980; Buescher *et al.*, 1981; Tang & McFeeters, 1983; Buescher & Hudson, 1984; McFeeters *et al.*, 1985; Buescher & Burgin, 1988; McFeeters & Fleming, 1989).

Ca^{2+} has been shown to be beneficial in assuring cucumber firmness retention (Buescher & Hudson, 1984; Hudson, 1984; Fleming *et al.*, 1987; Fleming *et al.*, 1988), which was maximized in pickles processed from fermentation and storage brines containing Ca^{2+} chloride and treated with Ca^{2+} chloride after desalting (Buescher *et al.*, 1979; Hudson & Buescher, 1980; Buescher *et al.*, 1981; Buescher & Burgin, 1988).

Howard & Buescher (1990) have reported that firmness was associated with the amount of bound Ca^{2+} and the supply of Ca^{2+} in fresh-pack cucumbers. Firmness was improved by blanching, especially in Ca^{2+} chloride solution in fermented and fresh-pack pickles (Sistrunk & Kozup, 1982).

Pericarp and locular tissues of pickles were much firmer and intact after 1 and 4 months in storage when the pickles were exposed to Ca^{2+} chloride (Hudson & Buescher, 1980). Fleming *et al.* (1988) have shown that the addition of Ca^{2+} acetate to the cover brine served as a source of Ca^{2+} to help ensure cucumber firmness retention.

It has been reported by several researchers (Fleming *et al.*, 1978; Buescher *et al.*, 1979; Buescher & Hudson, 1986) that cucumber firmness is maintained even at lower concentration of NaCl in the brine solution, if Ca^{2+} salts are

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also present. The addition of Ca^{2+} to the brine solution avoids excessive shriveling of the cucumbers (Fleming *et al.*, 1988).

Ca^{2+} and Softening of Cucumbers

Excessive softening is a common problem in fermented cucumbers and is caused by solubilization of pectic substances by polygalacturonase during storage. However, breakdown of texture by PG is inhibited by Ca^{2+} (Sistrunk & Kozup, 1982). Ca^{2+} increased fruit firmness even when high levels of PG were present, and the resistance to softening persisted when the pickles were transferred to solutions without Ca^{2+} (Buescher *et al.*, 1981). Low endogenous Ca^{2+} concentrations (2-3 mM Ca^{2+}) slowed softening of blanched cucumber mesocarp tissue, even though the endogenous Ca^{2+} concentration in the cucumbers could not be controlled (McFeeters & Fleming, 1989).

The percentage of Ca^{2+}-bound is clearly substantial to control water-soluble uronic acid polymers in the cell walls (Jarvis, 1982), and the amount of bound Ca^{2+} is dependent on the degree of pectin methylation (Howard & Buescher, 1990). Demethylation is believed to change the configuration of pectin macromolecules which contributes to loosening of middle lamella-cell wall components and softening (Hudson & Buescher, 1986). Galacturonans are rapidly deesterified during cucumber fermentation (Tang & McFeeters, 1983; Hudson & Buescher,

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Ca^{2+} chloride, Ca^{2+} nitrate, Ca^{2+} acetate, Ca^{2+} hydroxide, and Ca^{2+} oxide added in the brine of processed cucumbers provided similar increases in firmness and prevention of softening by polygalacturonase (Buescher *et al.*, 1981). Ca^{2+} appears to protect against tissue softening by cross-linking of pectin macromolecules, and by reducing demethylation of pectins (Hudson & Buescher, 1986).

However, when PME is activated and demethylation of pectins takes place gives a firmer product (Tang & McFeeters, 1983). This firming effect is attributed to the crosslinking or gelation of the pectic substances which were demethylated by the action of the enzyme pectin methyl esterase with Ca^{2+} ions present in the tissue when Ca^{2+} chloride is added to the brine (Van Buren *et al.*, 1962; Van Buren, 1968).

Utilization of Ca^{2+} in fermentation brines provides the potential for reducing losses caused by pectinolytic softening (Buescher *et al.*, 1981).

Ca^{2+} Transport in Plants

The problems that arise with Ca^{2+} nutrition in plants are frequently related to redistribution and mobility (Ferguson, 1979). Ca^{2+} is a highly immobile macronutrient in plants

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(Biddulph *et al.*, 1959; Ringoet *et al.*, 1968; Hanger, 1979). Along its pathway Ca^{2+} can be fixed through absorption by subcellular organelles, adsorbed onto cation ion exchange sites within the cell walls, bound to cellular structures such as membranes and other surfaces, bound to proteins and other macromolecules, or precipitated as insoluble salts within the vacuole (Bell & Biddulph, 1963; Shear & Faust, 1970; Ferguson, 1979).

Ca^{2+} uptake is very much associated with water uptake by roots. However, within the plant the distribution is much more complex and is also dependent on hormonal effects and ion-concentration gradient effects (Kirkby, 1979). Bangerth (1976) has concluded that auxins produced by the seeds play an important role in the accumulation of Ca^{2+} within the fruits.

The uptake of Ca^{2+} by roots is generally thought to be passive (Christiansen & Foy, 1979), and appears to be restricted to a region immediately behind the root tip (Kirkby, 1979). Uptake and movement across the root cortex is largely in the free space pathway, the apoplast (Ferguson & Clarkson, 1976; Ferguson, 1979). The apparent reluctance of Ca^{2+} ions to move in the symplast of the root may be due to their low activity in the cytoplasm where most divalent cations may be electrostatically bound or sequestered in some way (Ferguson & Clarkson, 1976).

Ca^{2+} movement in plants is unidirectional, moving up from the roots and generally routed to meristematic zones and young

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tissue (Hanger, 1979). Chino (1979) reported that little accumulation of Ca^{2+} is found in the boundary region of the stele in soybean plant and within the stele in corn plant. But, Ca^{2+} is highly concentrated at the endodermis in corn plant and at the outer cell layers of the root in soybean plants where Ca^{2+} is easily translocated. However, Ca^{2+} translocation to the shoot is greatly reduced when endodermis becomes suberized (Ferguson & Clarkson, 1976), because it constitutes a barrier to apoplastic movement (Ferguson, 1979), especially if growth stops (Kirkby, 1979). Any root growth inhibiting factor as low temperature, inadequate aeration, poor nutrient status, or H^{+} ion concentration will restrict Ca^{2+} uptake and hence also impair Ca^{2+} translocation (Jacobsen, 1979; Kirkby, 1979).

Both uptake and transport processes are subject to environmental influences (Wiersum, 1979). Ca^{2+} deficiency is commonly associated with water deficiencies, however, sometimes excess water as well as water fluctuation have been contributing factors (Geraldson, 1979). Excess water resulting in rapid leaching of Ca^{2+} from the root zone and/or generation of anaerobic conditions inhibiting uptake of Ca^{2+} and water induces Ca^{2+} deficiency. Conversely, low soil moisture resulting in poor water movement through the plant also results in Ca^{2+} deficiency in crops. Other environmental factors which decrease Ca^{2+} levels are high light intensity and duration, and adverse temperature (Millaway & Wiersholm,1979).

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During rapid growth, the translocation of photosynthates and nutrients through the phloem increases rapidly. But, Ca^{2+} accumulation occurs at a relatively slow rate due to the immobility of Ca^{2+} in phloem tissues (Biddulph *et al.*, 1959; Bollard, 1970; Marschner, 1974). May be when the photosynthates moved into the organ by mass flow, there would be sufficient water delivered by the phloem to satisfy the needs of the tissue without any import through the xylem (Hanger, 1979). In addition, if phloem water exceeded the cellular and transpirational needs of the tissue, there could be a back flow of water in the xylem down a water potential gradient (Zimmermann, 1966).

Also, rapid growth causes a marked fall in the surface area to volume ratio, therefore water loss through transpiration by the fruit becomes quite low (Hanger, 1979).

Ca^{2+} is notoriously immobile in phloem tissues (Biddulph *et al.*, 1959; Biddulph *et al.*, 1961; Zimmermann, 1966; Ringoet *et al.*, 1968; Bollard, 1970; Ferguson & Clarkson, 1976; Raven, 1977; Ferguson, 1979; Hanger, 1979; Marschner, 1986). For several reasons (high pH and high phosphate concentration) the concentration of free Ca^{2+} in the phloem sap has to be very low (Raven, 1977). The high phosphate levels together with a high phloem-sap pH of 8.0-8.2 have important consequences because, if Ca^{2+} is also in high concentrations, insoluble Ca^{2+} phosphate precipitates are formed and probably could interfere with the normal functioning of the sieve tubes (Hanger, 1979).

So, there is general agreement that, in order for the requirements of growing tissue to be met, most of the Ca^{2+} has to be translocated via the xylem into the tissue (Marschner, 1986).

This phenomenon is of particular importance to developing fruit (Bollard, 1970). Fruits and seeds are often the sites where the results of Ca^{2+} immobility are manifested. The low Ca^{2+} content of these tissues is thought to arise because most of the material moving into developing fruits is carried by the phloem, particularly in the later stages of development (Ferguson, 1979). Most of the difficulty of Ca^{2+} to move in symplastic pathways is supported by the relative immobility of Ca^{2+} in the phloem, its slow rates of diffusion across layers of cells such as in fruit cortical tissue, and lack of redistribution from aging leaves to the other parts of the plant (Ferguson, 1979).

The ratio of K^{+} to Ca^{2+} in cucumber fruits is considerably higher than those for leaves, suggesting that these fruits are relatively Ca^{2+} deficient (Bollard, 1970).

The deficiency of Ca^{2+} in low-transpiring tissues has been very well documented (Bell & Biddulph, 1963; Gerald & Hipp, 1968; Shear & Faust, 1971; Tibbitts & Palzkill, 1979). According to Shear (1975) subsequent enlargement of the fruit dilutes the Ca^{2+} in the fruit, and excessive size may reduce the Ca^{2+} concentration below that necessary for normal cell

functions. This dilution effect has been reported by Widders & Price, 1984; Engelkes, 1987; and Engelkes *et al.*, 1990 to be accentuated through ontogeny, in which Ca^{2+} concentrations in pickling cucumber fruit tissues declined during fruit development. Hanger (1979) and Marschner (1986) also found a negative correlation between the growth rate and the Ca^{2+} content of growing fruits. It has been known that under conditions of high transpiration, no water enters the fruits directly from the xylem so under these conditions no Ca^{2+} is supplied to the fruits (Bollard, 1970). High transpiration rates direct the xylem volume flow to the high transpiring leaves decreasing the Ca^{2+} influx into low transpiring organs as in the case of fruits. Inhibition of transpiration (by high relative humidity or during the dark period) usually favors the direction of the xylem volume flow toward low-transpiring organs. The rate of xylem flow from the roots to the shoots under conditions of low transpiration is determined by the root pressure (Marschner, 1986).

Root pressures are known to develop in plants when transpiration is slowed to a rate that is less than the rate of water influx into the roots. As root pressure builds up, a positive pressure develops in the xylem causing the flow of liquid through the xylem ducts resulting in guttation from hydathodes (Tibbitts & Palzkill, 1979). Thus, to prevent a Ca^{2+} deficiency caused by a lack of root pressure, conditions must be altered to reduce transpiration and increase Ca^{2+} and

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Strontium (which is equally immobile in the phloem as Ca^{2+}) moved into potato tubers only when the foliar transpiration rates were lowered and the water potential gradients were more favorable for water flow to the tubers (Bell & Biddulph, 1963). Thus, water availability in the rooting medium particularly during the dark period is crucial for the long-distance transport of Ca^{2+} into low-transpiring organs, because high osmotic potential of the soil solution decreases both root pressure and Ca^{2+} influx into young leaves or fruits and induces Ca^{2+} deficiency symptoms (Marschner, 1986).

Increasing Ca^{2+} Mobility

The upward movement of Ca^{2+} in the transpiration stream through the xylem shows that Ca^{2+} ions do not move by mass flow, but by a series of exchange reactions along negatively charged sites on the walls of the xylem vessels (Biddulph *et al.*, 1961). So, movement can be promoted by the presence of divalent cations and by chelation of the Ca^{2+} ion (Hanger, 1979). Sprays of Ca^{2+} salts may supply sufficient added Ca^{2+} to control Ca^{2+} deficiencies in leafy vegetables. However, the Ca^{2+} from sprays must move into the fruits through their surface, and only very limited quantities can be supplied in this way (Shear, 1975).

Chelators of Ca^{2+} in the sap reduces the degree of adsorption of Ca^{2+} to negatively charged sites within the cell wall, potentially promoting its mobility in the xylem (Bradfield, 1976). So, when the ionic charge of the Ca^{2+} ion is neutralized by chelation with either synthetic (e.g. ethylene diamine tetra acetic acid) or natural (citric and malic acids) chelators, Ca^{2+} is moved more freely through the plant (Hanger, 1972).

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THE RELATIONSHIP OF ENDOGENOUS Ca^{2+} AND POLYGALACTURONASE ACTIVITY WITH TEXTURAL QUALITY OF PICKLING CUCUMBERS

Introduction

Ca^{2+} is thought to play a physiological role in influencing fruit quality, besides improved growth and development, by reducing respiration (Faust & Shear,1972), increasing fruit firmness (Cooper & Bangerth,1976; Sams & Conway,1984), and delaying fruit ripening and senescence (Poovaiah,1979; Leshem *et al.*,1982).

Limited research, however, has been conducted on pickling cucumbers to determine the effects of a low fruit Ca^{2+} status on parameters affecting quality for processing. An important quality parameter in fresh-pack pickled cucumber products is the firmness and integrity of both the green and processed fruit stock tissue.

The strength of the cell wall structure , and the inhibition of the breakdown of pectic polymers during growth or post harvest handling (Rigney & Wills, 1981) are affected by endogenous Ca^{2+}. However, the endogenous Ca^{2+} concentration in pickling cucumber fruit decline during fruit ontogeny (Engelkes,1987). Although plant environment and genotype have been shown to significantly affect the Ca^{2+} status of developing fruit (Engelkes *et al.*,1990) it was not known whether the variability in various fruit quality parameters

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such as fruit firmness observed among cultivars or from different fields might be ultimately due to excessively low Ca^{2+} tissue levels.

Ca^{2+} has been shown to be important serving as an intermolecular adhesive in plant cell walls maintaining cell cohesion (Hanson, 1984) by acting as a cross-linking component between polygalacturonide chains (Davis & Dennis, 1983). The major structural change associated with softening is concerned with the loosening of the cell wall by loss of cell cohesion (Ferguson, 1984; Wills & Rigney, 1979). These changes in the cell wall have been accompanied by a conversion of the insoluble pectic substance to soluble pectic substance (Lampi *et al.*, 1958; Mohsenin, 1970) and have been related with the progressive dissolution of the middle lamella (Ben-arie *et al.*, 1979). It has been reported by Poovaiah & Leopold (1973) that the loss of Ca^{2+} was followed by solubilization of cell walls cementing substances and by hydrolysis of the structural components of the wall while cell cohesiveness and structural integrity of the cell wall were preserved by Ca^{2+} (Glenn & Poovaiah, 1990).

Loss in firmness, however, has been associated with the activity of the cell wall-degrading enzymes (Dilley, 1970). Polygalacturonase (Pilnik & Voragen, 1970; Pressey & Avants, 1975; Ben-Arie & Sonego, 1979; Buescher *et al.*, 1981; Tucker & Grierson, 1982), pectin methyl esterase (Bell *et al.*, 1951; McFeeters *et al.*, 1985; Hudson & Buescher, 1986), cellulase

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(Pesis *et al.*, 1978), β-galactosidase (Konno *et al.*, 1984; Lazan *et al.*, 1989), β-1,3-glucanase (Buescher & Hobson, 1982), acid phosphatase, peroxidase, xylanase, and β(1,4)-D-glucanase (Jarvis, 1984) have been reported to be implicated in cell wall degradation. However, polygalacturonase has been shown to be the primary determinant of cell wall polyuronide degradation (Buescher & Hobson, 1982; Giovannoni *et al.*, 1989).

Ca^{2+} has been reported to have an interaction with the enzyme polygalacturonase (Buescher *et al.*, 1981; Rigney & Wills, 1981; Davies & Dennis, 1983; Konno *et al.*, 1984; Brady *et al.*, 1985; McFeeters *et al.*, 1985) suppressing its activity in plant cell walls (Poovaiah, 1979). Polygalacturonase has been also thought to be influenced by the level of endogenous Ca^{2+} in mature fruit undergoing ripening (Rigney & Wills, 1981).

It has been shown by Brady *et al* (1985) that cell wall uronic acids of a firm and soft cultivar were equally susceptible to hydrolysis, suggesting that differences in the digestion of the walls by polygalacturonase were dependent on differences in Ca^{2+} content or distribution. It appears that Ca^{2+} associated with the cell wall-middle lamella and its removal regulate the rate and extent of degradation by polygalacturonase (Buescher & Hobson, 1982). Both, the increase in polyuronide solubilization and the loosening of wall matrix via Ca^{2+} removal could reduce the physical

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resistance encountered by the enzyme as it migrates to potential sites of hydrolysis, increasing the efficiency of enzymic activity (Rushing & Huber, 1984). In fact, Ca^{2+} appears to protect against tissue softening by cross-linking of pectin macromolecules (Hudson & Buescher, 1986) providing the potential for reducing losses caused by pectinolytic softening.

The objectives of the present study, therefore, were: a) to evaluate the effect of modified endogenous Ca^{2+} within fruit tissues on texture of immature freshly harvested pickling cucumber fruit and after post harvest storage, b) to determine the influence of low Ca^{2+} levels in fruit tissues on the activity of polygalacturonase in freshly harvested and stored pickling cucumber fruit, and c) to verify the effect of post harvest storage duration and temperature on fruit firmness and polygalacturonase activity.

Materials and Methods

Three experiments were conducted in this study. Experiments 1 and 2 were carried out during April through July, and July through October, 1985, respectively, in the Plant Science Greenhouses at Michigan State University. Experiment 3 was conducted in the Fitotecnia Greenhouse at Universidade Federal do Paraná in Brazil, South America, during the months of March through June of 1989.

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Plant Culture. Pickling cucumber (*Cucumis sativus* L.) plants (cv.Castlepik 2012) were grown in sand culture (Experiments 1 and 2) and in pure silica quartz stone (Experiment 3) in 8 liter plastic containers. In the first two experiments, the nutrient treatment solutions were supplied directly to the plants from a reservoir tank via a network of tubing and a timer controlled pumping system while in Experiment 3 the nutrient treatment solutions were supplied by hand. Plants were irrigated daily until anthesis with a modified Hoagland nutrient solution (Johnson *et al.*, 1957) containing (mM): 3.0 KNO_3, 1.0 $Ca(NO_3)_2$, 1.0 NaH_2PO_4, 0.5 $MgSO_4$, 0.05 KCl, 0.025 H_3BO_3, 0.002 $MnSO_4$, 0.002 $ZnSO_4$, 0.0005 $CuSO_4$, 0.0005 H_2MoO_4, and 0.15 Fe-chelate (DTPA - disodium ferric diethylene triamine penta-acetate from Miller Chemical & Fertilizer Corporation). The pH of the nutrient culture solution was 5.0.

At flowering, the Ca^{2+} concentrations in the nutrient culture solutions were modified (macronutrients) to contain in Experiments 1 and 2, 0.01, 1.0 and 20.0 mM Ca^{2+}, and in Experiment 3, 0.00, 0.01, 0.1, 1.0, 10.0 and 20.0 mM Ca^{2+} (Table 1). These treatments were continued throughout the reproductive period so as to modify predominately the Ca^{2+} nutrition of the developing fruit. Female flowers were hand-pollinated daily as soon as the flowers opened, from 10 a.m. until noon (Experiments 1 and 2), and from 9 a.m. until noon (Experiment 3). Average greenhouse temperatures ranged from

Table 1 -

Experiment and 2
Salts[2]
NaH_2PO_4
$Ca(NO_3)_2 \cdot 4H$
KNO_3
$MgSO_4$
$CaCl_2$
$Mg(NO_3)_2$
NH_4NO_3

Experiment
Salts
NaH_2PO_4
$MgSO_4 \cdot 7H_2O$
$Ca(NO_3)_2 \cdot 4H$
$CaCl_2 \cdot 2H_2O$
KNO_3
KCl
NH_4NO_3

[2] Micronutr
al., 1957.

Table 1 - Composition of Ca^{2+} treatment solutions applied to pickling cucumber plants beginning at anthesis.

Experiment 1 and 2	Concentration (mM)		
	Ca^{2+} treatments (mM)		
Salts[z]	20.0	1.0	0.01
NaH_2PO_4	1.0	1.0	1.0
$Ca(NO_3)_2.4H_2O$	1.0	0.5	0.01
KNO_3	3.0	3.0	3.0
$MgSO_4$	1.0	0.5	0.5
$CaCl_2$	19.0	0.5	-
$Mg(NO_3)_2$	-	0.5	0.5
NH_4NO_3	1.0	1.0	1.5

Experiment 3	Concentration (mM)					
	Ca^{2+} treatments (mM)					
Salts	20.0	10.0	1.0	0.1	0.01	0.00
NaH_2PO_4	1.0	1.0	1.0	1.0	1.0	1.0
$MgSO_4.7H_2O$	0.5	0.5	0.5	0.5	0.5	0.5
$Ca(NO_3)_2.4H_2O$	10.0	5.0	1.0	0.1	0.01	-
$CaCl_2.2H_2O$	10.0	5.0	-	-	-	-
KNO_3	-	3.0	3.0	2.8	3.0	3.0
KCl	3.0	-	-	0.2	-	-
NH_4NO_3	-	3.5	7.5	8.5	8.5	8.5

[z] Micronutrient salt concentrations as specified by Johnson *et al.*, 1957.

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23-30°C during the day and 15-20°C during the night (Experiments 1 and 2), and from 25-32°C during the day and 20-25°C at night (Experiment 3). No supplimental lighting was provided.

Sample Collection

In the experiment 1, an average of three 4.5 cm diameter fruits were collected from each plant. At harvest, 100 µl of the pedicel exudate from the excised fruit and 30 µl of extracellular solution from the endocarp region of the fruit were collected. To obtain extracellular solution, fruits were fractured without the use of a knife, rinsed with deionized water, gently blotted dry and the subsequent exuding sap collected with micropipettes. All exudate samples were stored at -20°C in plastic vials for Ca^{2+} analysis.

Two cross-sectional 0.5 cm thick slices from the middle part of each fruit were measured for fruit firmness immediately after harvest, and the average of the two measurements used in the statistical analysis. Firmness of pericarp, mesocarp, and endocarp tissues (Fig. 1) was measured using an Instron Universal Machine with a probe of 32 mm in diameter, 20 cm/min. From the remaining portion of the individual fruits, approximately 25 g samples of pericarp tissue, fruit wall tissue excluding the carpel, (Frost & Kretchman, 1989) and endocarp tissue were collected, and

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placed into plastic trays, and weighed fresh (g). The samples were dried in a forced-air oven at 60°C for 72 hr, weighed, and ground in a mortar and pestle prior for tissue Ca^{2+} analysis.

In Experiment 2, five fruits were collected from each plant. Individual fruit were subjected to the five following post harvest storage treatments prior to textural analysis:

- 0 days
- 3 days at 5°C plus 24 hr at 25°C
- 5 days at 5°C plus 24 hr at 25°C
- 3 days at 25°C
- 5 days at 25°C

Textural firmness of pericarp and endocarp tissues for freshly harvested and stored fruits were measured as previously described in the Experiment 1 (see Figure 1 for green stock tissue identification for texture evaluation). Tissue samples were also collected from individual fruits for Ca^{2+} analysis.

For assay of polygalacturonase (PG) activity, samples (50 g fr wt) of both pericarp and endocarp tissues (Experiment 1) and of endocarp tissue only (20 g fr wt; Experiment 2) were excised from each fruit following the post-harvest treatments. The fresh fruit was then frozen at -70°C (Experiment 1) or -20°C (Experiment 2) for approximately 4 weeks prior to analyses.

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GREEN STOCK TISSUE IDENTIFICATION FOR TEXTURE EVALUATION

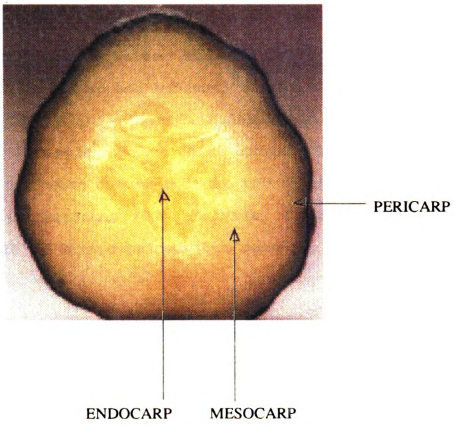

FIGURE 1. Illustration of Cucumber Tissue (0.5 cm cross-sectional slices) for cylindrical probe punch evaluation.

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In Experiment 3, one fruit, 4.5 cm diameter, was harvested from each plant and wrapped immediately in a plastic wrap to minimize loss of moisture, and to reduce respiration. All fruits were then stored at 1°C for 20-24 days. Although fruits were generally in excellent condition, those fruits which had fruit rot after storage were discarded. Fruit firmness of pericarp and endocarp tissus of the stored fruit was measured by an Autograph Model Apparatus - AG 5,000 A Shimadzu. A single, 0.5 cm thick cucumber slice was cut from the center of each cucumber fruit for firmness measurements. For polygalacturonase (PG) analysis, fresh endocarp tissue samples (20 g) were obtained from each fruit and stored at -22°C.

Ca^{2+}analysis

Aliquots of dried ground tissue, 0.2 g dry wt, were weighed and placed into 50 ml volumetric flasks. Following the addition of 10 ml concentrated nitric acid, the flasks were topped with a marble and the tissues were permitted to oxidize overnight at 25°C. Oxidation was completed by gently boiling and evaporating off the nitric acid on an electric hot plate. Just before achieving dryness, hydrogen peroxide was added a drop at a time until the color disappeared. Tissue digests were then partially diluted with deionized water and subsamples stored in sealed 20 ml plastic vials at room

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temperature. $LaCl_3$ was added as an internal standard to achieve a concentration of 1000 μg/ml. Ca^{2+} concentrations were determined in fruit tissue extracts and in fruit exudates by atomic absorption spectrophotometry (IL Video 12).

In Experiment 3, strontium chloride ($SrCl_2$) instead of lanthanum chloride ($LaCl_3$) was added to all diluted solutions (1000 μg/ml) to avoid background interference. Ca^{2+} concentrations in the unknown samples of both fruit tissues, pericarp and endocarp were determined by a Perkin-Elmer Model 2380 atomic absorption spectrophotometer.

Polygalacturonase Assay

The assay of polygalacturonase activity is based on the hydrolytic release of reducing groups from polygalacturonic acid using 2-cyanoacetamide which was analyzed spectrophotometrically (Gross, 1982). 2-cyanoacetamide has shown to be effective for spectrophotometric quantification of nanomole amounts of reducing carbohydrate in solution resulting in the formation of ultraviolet-absorbing products that fluoresce intensely after their direct condensation and cyclization with reducing sugars (Honda *et al.*, 1982).

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Enzyme extraction

The extractability of polygalacturonase described by Gross (1982) was modified for cucumber fruit, because in contrast to the general behavior of pectic enzymes, it has been shown that cucumber polygalacturonase is readily extracted by water (Pressey & Avants, 1975). Partially thawed cucumber tissues were placed in a beaker, and to each sample was added sodium chloride to achieve a concentration of 0.2 M. The tissue with NaCl was homogenized using a tissumizer, and placed into culture tubes in an ice bath. The tissue was then centrifuged at 17,000 x g for 10 min. An aliquot (1.0 ml) of the clear supernatant was taken, and placed into a 12,000 to 14,000 MW cut off membrane (spectrapor) and a number of five samples were dialyzed in each 1,000 ml erlenmeyer with continuous stirring for 12 hr at 4°C. The dialysis solution consisted of 36 mM maleic acid, 0.33 M NaCl, and 0.02% sodium azide to avoid microbial growth. The pH was adjusted to and maintained at 6.2 with 1 N NaOH. The desalted extract was used for polygalacturonase assay. All extraction procedures were carried out at 4°C.

Reaction mixtures (0.2 ml total volume) containing 37.5 mM Na-acetate (pH 4.4), 0.2% polygalacturonic acid (PGA), washed with 80% ethanol, and 30 µl of enzyme extract were incubated at 30°C for up to 3 hr. Different amounts of enzyme extract were previously tested, and the amount which showed

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the best linearity was chosen. For quantifying released reducing groups with 2-cyanoacetamide, reactions were terminated with 1.0 ml of cold 100 mM borate buffer (pH 9.0). Then, 0.2 ml of 1% 2-cyanoacetamide was added, yieldind a final volume of 1.4 ml in each tube. The samples were mixed and immersed in a boiling water bath for 10 min. to terminate the reactions. After equilibration to 25°C, the samples were placed into quartz cuvettes, and the absorbance at 276 nm was determined using a Beckman spectrophotometer (Gross, 1982).

Triplicates analysis were made of each sample extract. Amount (nanomoles) of galacturonic acid was then estimated from a standard curve in which absorbance at 276 nm was linearly related to galacturonic acid concentration up to 60 nanomoles.

In Experiment 3, a Van Potter Homogenizer was used and absorbance was measured by a DMS 80 spectrophotometer.

Statistical Analysis

Randomized complete block designs with 8, 12 and 4 blocks were used in Experiments 1, 2, and 3, respectively. Plants were sorted in blocks according to size with 1 plant per treatment. Analysis of variance was conducted on fruit tissue Ca^{2+} concentration, on firmness of pericarp, mesocarp, and endocarp tissues, on fruit exudates, and on PG activity of freshly harvested 4.5 cm diameter fruit. The Least Significance Difference (LSD) test was used to compare means. Correlation analysis were conducted where appropriate.

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Results

Plants in all 3 experiments exhibited vigorous vegetative growth prior to flowering. The Ca^{2+} treatments applied during reproductive development to the cucumber plants modified the tissue Ca^{2+} concentrations (dry weight basis) in the fruit at harvest in all experiments (Table 2).

In experiment 1, pericarp tissue Ca^{2+} concentrations expressed on a dry weight basis ranged from 0.21% to 0.95% Ca^{2+} while Ca^{2+} levels in endocarp tissue ranged from 0.08% to 0.21% Ca^{2+}. In both experiments (2 and 3) pericarp tissue Ca^{2+} concentrations ranged from approximately 0.1% to 1.8% Ca^{2+}, and from 0.08% to 2.4% Ca^{2+}, respectively, while endocarp tissue Ca^{2+} concentrations which were much lower ranged from approximately 0.05% to 0.3% Ca^{2+}, and from 0.05% to 0.5% Ca^{2+}, respectively.

The symptoms of Ca^{2+} deficiency did not appear until 2 to 3 weeks after the onset of fruit development. The deficiency of Ca^{2+} was first observed in the youngest leaves of the plants which were poorly supplied with Ca^{2+} (0.01 mM Ca^{2+}). The leaves became curled, and at more advanced stages necrosis occurred at the leaf margins. However, Ca^{2+} treatments did not affect the rate of fruit growth. The period of time from planting through harvest varied from 43-50 days, and from pollination until harvest from 10 to 11 days. The nutrient

Table 2 -

Ca^{2+} Treatments (mM)	
0.01	
1.0	
20.0	
LSD (0.05)	

Ca^{2+} Treatment (mM)	
0.01	
1.0	
20.0	
LSD (0.01)	

0.00	
0.01	
0.1	
1.0	
10.0	
20.0	
LSD (0.05)	

NS nonsi

Table 2 - Effect of Ca^{2+} treatments in the nutrient culture solution on Ca^{2+} concentrations of fruit tissues and in exudates from excised pedicels and from the seed cavity region of fractured 4.5 cm diameter pickling cucumber fruit in experiments 1, 2, and 3, respectively.

Ca^{2+} Treatments (mM)	Experiment 1 Tissue Ca^{2+} (% dry wt)		Ca^{2+}(mM)	
	pericarp	endocarp	Seed Cavity	Pedicel
0.01	0.21	0.08	3.34	28.81
1.0	0.37	0.13	4.63	30.12
20.0	0.95	0.21	4.26	42.30
LSD (0.05)	0.13	0.047	NS	9.76

Ca^{2+} Treatment (mM)	Experiment 2 Tissue Ca^{2+} (% dry wt)	
	Pericarp	Endocarp
0.01	0.13	0.05
1.0	0.48	0.18
20.0	1.83	0.38
LSD (0.01)	0.16	0.05
	Experiment 3	
0.00	0.11	0.05
0.01	0.08	0.05
0.1	0.16	0.15
1.0	0.31	0.24
10.0	1.38	0.54
20.0	2.47	0.50
LSD (0.05)	0.81	0.35

NS nonsignificant

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Firmness

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culture solution Ca^{2+} treatments affected pedicel exudate, but not the extracellular sap Ca^{2+} concentrations from the seed cavity. Pedicel Ca^{2+} concentration was much higher than within fruit ranging from 28 to 42 mM Ca^{2+} depending on the Ca^{2+} treatment while Ca^{2+} concentration in the apoplastic (extracellular sap) solution of fruit endocarp tissue ranged from 3.3 to 4.6 mM Ca^{2+} (Table 2). No significant correlations were found between fruit firmness and Ca^{2+} concentrations of both pedicel exudate and extracellular sap Ca^{2+} concentration from the endocarp tissue .

Firmness of Fresh and Stored Fruit

Firmness of pericarp, mesocarp, and endocarp tissues in fresh fruit was not affected by nutrient solution Ca^{2+} concentration in Experiment 1 (Table 1, Appendix) . However, Ca^{2+} treatments in the nutrient solution significantly affected the firmness of the pericarp tissue, but not of endocarp tissue after 3 and 5 days of storage in Experiment 2 (Table 3). The low Ca^{2+} treatment (0.01 mM Ca^{2+}) fruit showed the lowest firmness at 5 days of storage (0.62 Kg), suggesting a greater amount of softening. Consistent with this observation, the rate of softening in the pericarp tissue between 3 and 5 days of storage was also significantly affected by the Ca^{2+} treatments (Table 3). Softening was

Table 3 -

Ca^{2+} Conc. (mM)	
	P
0.01	
1.0	
20.0	
LSD(0.05	

[z] Signif
(NS).

Table 3 - Effect of Ca^{2+} treatment level on firmness and on the rate of softening between 3 and 5 days of storage of 4.5 cm diameter pickling cucumber fruit tissues (Experiment 2).

Ca^{2+} Conc. (mM)	Fruit Firmness (kg)				Rate of Softening ($days^{-1}$)	
	Storage Period (days)					
	3		5		3 - 5	
	Pericarp	Endocarp	Pericarp	Endocarp	Pericarp	Endocarp
0.01	0.77	0.18	0.62	0.13	0.030	0.010
1.0	0.81	0.18	0.67	0.15	0.028	0.006
20.0	0.74	0.16	0.70	0.14	0.008	0.004
LSD(0.05)[z]	0.052	NS	0.054	NS	0.014	NS

[z] Significant LSD at $P<0.05$ or the F value was non-significant (NS).

Table 4 -

Storage Temp. (°C)	
5°	
25°	
F test sig	
Fruit Tiss	
Pericarp	
Endocarp	
PG activi (unitsx10	

***, *, nonsignif

Table 4 - Influence of storage temperature and duration on firmness and PG activity of pickling cucumber fruit tissues in Experiment 2.

Storage Temp. (°C)	Fruit Firmness (Kg)		PG activity (units$\times 10^3$/g)
	Pericarp	Endocarp	Endocarp tissue
5°	0.72	0.17	1.66
25°	0.71	0.15	1.98
F test sign.	NS	*	***

Fruit Tissue	Storage Period (days)			LSD (5%)
	0	3	5	
Pericarp	0.93	0.77	0.65	0.030
Endocarp	0.19	0.17	0.14	0.010
PG activity (units$\times 10^3$/g)	1.82	1.71	1.93	0.180

***, *, NS significant at the 0.1% level, 5% level and nonsignificant.

Figure 2. Correlation between Ca^{2+} concentration in pericarp tissue and the rate of softening of pericarp tissue between 3 and 5 days of storage of pickling cucumber fruit.

significantly higher in the lower Ca^{2+} treatments (0.01 and 1.0 mM Ca^{2+}) than in the higher Ca^{2+} treatment (20.0 mM Ca^{2+}) as shown in Table 3. Although a similar relationship between Ca^{2+} concentration in nutrient solution and rate of softening was observed in endocarp tissue, the difference between the means was not significant (Table 3).

Ca^{2+} concentration in pericarp tissue was found to be negatively correlated (r=-0.43) with the rate of softening between 3 and 5 days of storage (Fig. 2). Also, a positive correlation was observed between fruit Ca^{2+} concentration (% dry weight) and fruit firmness after 5 days at 5°C (Fig. 3).

Fruit firmness of the endocarp tissue was influenced by the storage temperature (5° vs. 25°C) showing a significantly lower firmness at 25°C (Table 4). But, storage temperature did not affect pericarp and endocarp firmness or rate of softening during 3 and 5 days of storage, even though softening continued to occur during that period of time (data not presented). However, a significant effect of storage period (0-5 days) on fruit firmness of both pericarp and endocarp tissues was observed (Table 4).

Fruits stored at 1°C for 24 days showed a positive correlation between the fruit Ca^{2+} concentrations and fruit firmness (r=0.68, Fig. 4) compared to the correlation coefficient found for the same parameters after 5 days storage at 5°C (r=0.61, Fig. 3).

It was observed during the period of storage, especially when fruit were stored for 24 days, that the fruits grown at the higher Ca^{2+} concentration came out of storage in much better condition, exhibiting good color and without any visible symptoms of rot.

PG Activity in Fresh and Stored Fruit

The levels of enzyme activity (PG) found in the fresh fruit (Experiment 1) ranged from 0.96 to 1.29 units x 10^{-3}/g (Table 1, Appendix) while in the stored fruit the levels were observed to be higher ranging from 1.39 to 2.71 units x 10^{-3}/g (data not shown), and from 0.92 to 2.92 units x 10^{-3}/g (Fig. 7) in Experiments 2 and 3, respectively.

Polygalacturonase (PG) activity in the fresh fruit tissues was not affected by the Ca^{2+} treatments in Experiment 1 (Table 1, Appendix). However, PG activity was negatively correlated ($r=-0.46$) with Ca^{2+} concentrations in the pericarp tissue of fresh pickling cucumber fruit (Fig. 5). Endocarp PG activity was significantly higher at 25°C than at 5°C and increased significantly after 5 days of storage (Table 4). Consistent with these findings, a negative correlation was found between the endocarp tissue firmness and PG activity in the endocarp tissue after 3 days of storage (Fig. 6).

Figure 3. Correlation between Ca^{2+} concentration in endocarp tissue and fruit firmness of endocarp tissue of pickling cucumber fruit after 5 days of storage at 5°C.

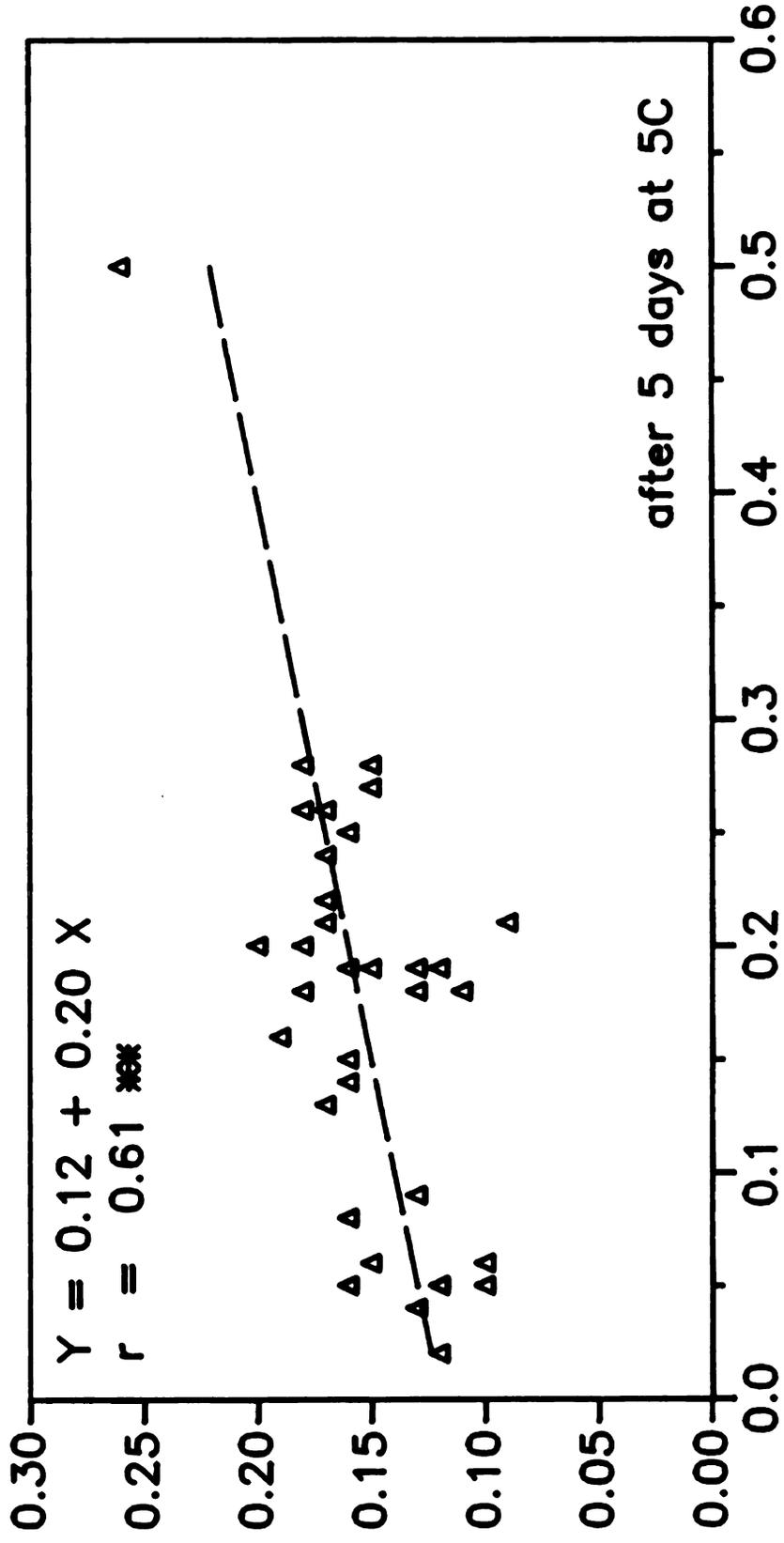
FRUIT FIRMNESS (Kg)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Y = 0.12 + 0.20 X
r = 0.61 ***
after 5 days at 5C
0.0
0.1
0.2
0.3
0.4
0.5
0.6
CA2+ CONC. (% DRY WT)

Figure 3

Figure 4. Correlation between Ca^{2+} concentration in pericarp tissue and firmness of pericarp tissue in the 24 days at 1°C stored pickling cucumber fruit.

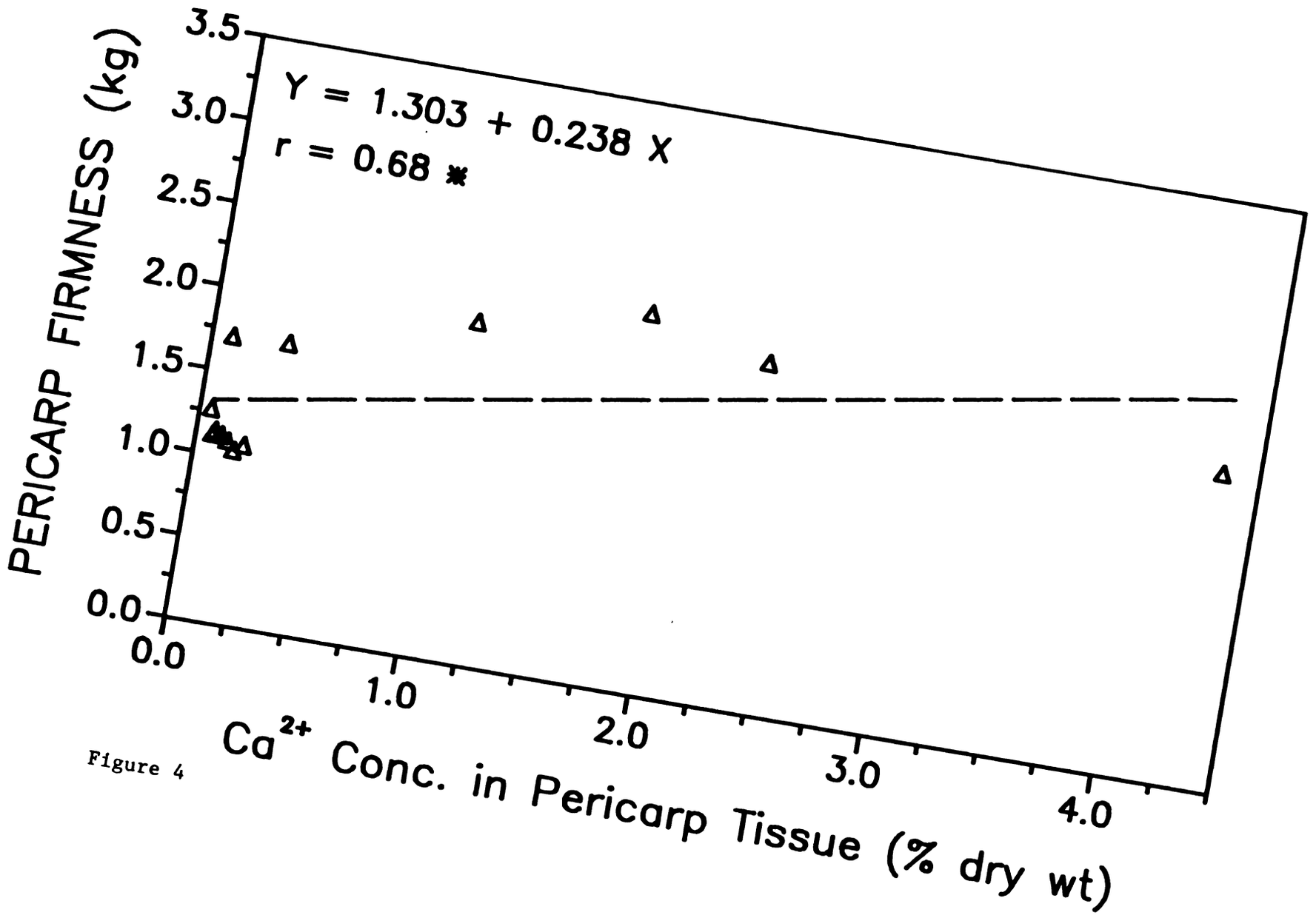
PERICARP FIRMNESS (kg)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Y = 1.303 + 0.238 X
r = 0.68 *
0.0
1.0
2.0
3.0
4.0
Ca2+ Conc. in Pericarp Tissue (% dry wt)

Figure 4

Figure 5. Correlation between Ca^{2+} concentration in the pericarp tissue and polygalacturonase activity of 5.0 cm diameter fresh pickling cucumber fruit.

Figure 6. Correlation between polygalacturonase activity and fruit firmness in the endocarp tissue of pickling cucumber fruit after 3 days at 5°C.

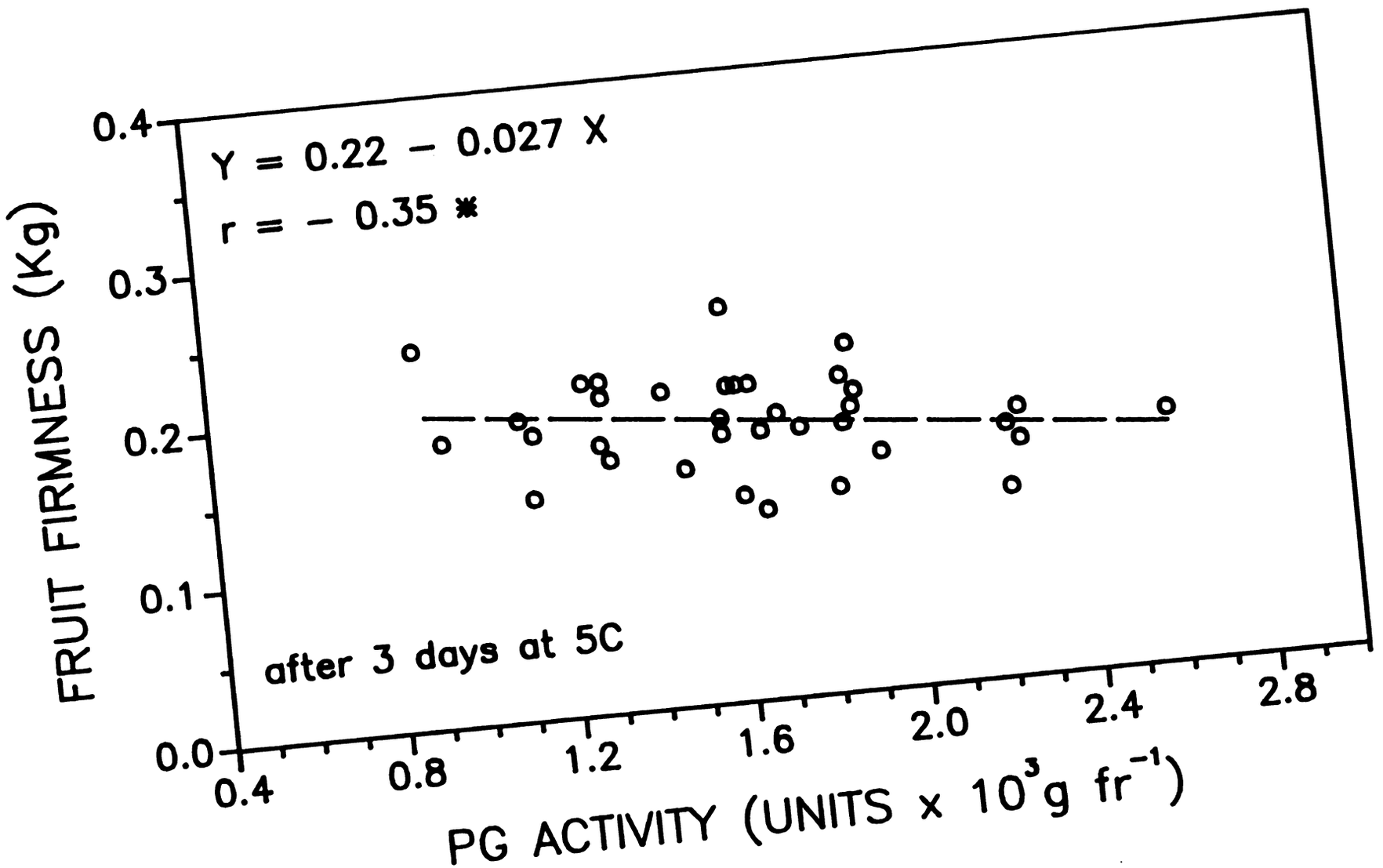
Y = 0.22 − 0.027 X
r = − 0.35 *
after 3 days at 5C
FRUIT FIRMNESS (Kg)
PG ACTIVITY (UNITS x 10³g fr⁻¹)
0.0
0.1
0.2
0.3
0.4
0.4
0.8
1.2
1.6
2.0
2.4
2.8

Figure 6

Figure 7. Correlation between Ca^{2+} concentration in endocarp tissue and polygalacturonase activity in the endocarp tissue in pickling cucumber fruit stored at 1°C for 24 days.

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A significant negative correlation (r=-0.58) also was found between Ca^{2+} concentration in endocarp tissue and PG activity of fruits stored at 1°C for 24 days (Fig. 7).

Discussion

In the present study, the endogenous Ca^{2+} concentration in the cucumber fruit was modified by the Ca^{2+} concentration in the nutrient solution. The range of tissue Ca^{2+} concentrations obtained in the fruit were quite broad in comparison with the range of concentrations typically found in field-grown pickling cucumbers (Engelkes *et al.*, 1990). Ca^{2+} concentrations in field produced 4-4.5 cm diameter cucumbers vary from 1.0% to 0.7% dry weight in pericarp tissue and 0.65% to ≈0.2% dry weight in endocarp tissue. The Ca^{2+} concentrations found in pericarp tissue in Experiments 1, 2, and 3 (Table 2) ranged from 2.4% to 0.08% of dry weight while in the endocarp tissue ranged from 0.5% to 0.05% dry weight.

A Ca^{2+} gradient typically exists within cucumber fruit (Frost & Kretchman, 1989) extending from the proximal to the distal end. The significantly lower endocarp tissue Ca^{2+} concentrations in comparison with the pericarp tissue Ca^{2+} concentrations (Table 2) might be related to a similar causal factor. There is general agreement that, in order for the requirements of growing fruit to be met, most of the Ca^{2+} has to be translocated via the xylem into the tissue (Marschner,

1986). The low endocarp vs. pericarp tissue Ca^{2+} concentrations could be in part due to a slow rate of import of Ca^{2+} via the xylem into the endocarp as it has been reported by Engelkes *et al.*(1990). The same authors have pointed out that the imported Ca^{2+} passes first through the pericarp which in turn would deplete the Ca^{2+} from the apoplastic solution entering the endocarp, the seed cavity of the cucumber fruit. Also, endocarp tissue may contain fewer vascular tissues than the pericarp tissue (Engelkes, 1987). Pericarp would be supplied mostly by the xylem while endocarp by the phloem. As Ca^{2+} is not readily translocated in phloem tissues the rate of Ca^{2+} import within the endocarp would be lower.

Xylem Ca^{2+} concentrations of 2-3 mM Ca^{2+} (White *et al.*, 1981), 4.7 mM Ca^{2+} (Marschner, 1986), and 4 mM Ca^{2+} (Wilcox *et al.*, 1977) in herbaceous plants, and 4.5 mM Ca^{2+} in apples (Bradfield, 1976) have been reported. The xylem exudate has also been measured in cucumber plants, and Ca^{2+} concentrations of 4 mM Ca^{2+} have been found (Engelkes *et al.*, 1990). These results are similar to what it was observed in the present study in the extracellular sap collected from the endocarp tissue of the cucumber fruit (Table 2), thus confirming the apoplastic (extracellular) origin of this sap.

However, values in the xylem exudate as high as 5.7 mM Ca^{2+} (control plants) and ranging from approximately 7 to 14 mM Ca^{2+} in field-grown cucumber plants sprayed with foliar fertilizers have been observed (unpublished data).

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Pedicel exudate, believed to have originated from phloem vascular tissues, was found to be extremely high in Ca^{2+} concentration (Table 2). Marschner (1986) showed a Ca^{2+} concentration around 2.08 mM Ca^{2+} in the phloem exudate of *Nicotiana* plants while Van Die & Tammes (1975) found concentrations as low as 0.25 and 0.3 mM Ca^{2+} in the phloem exudate of *Yucca flaccida* and *Arenga sacchárifera*, respectively. In cucumber (*Cucumis sativus* L.) plants the total endogenous Ca^{2+} found in phloem exudate as reported by McEuen *et al.* (1981) was 0.08 mM Ca^{2+}. However, it has been reported that an accumulation of Ca^{2+} occurs to a very large extent in the pedicels while only limited amounts reach the fruit (Wieneke, 1969; Wieneke, 1974; Terblanche *et al.*, 1979; Hanger, 1979). Terblanche *et al.*(1979) found values of 140 mM Ca^{2+} in the pedicels of apple tissues. According to them, Ca^{2+} is accumulated in the phloem parenchyma of the pedicel in the form of rectangular crystals. This might be a contributing factor to low fruit Ca^{2+}.

However, the results from the present study are consistent with what Hanger (1979) reported: during the period when the Ca^{2+} concentrations in the pedicel rose sharply, proportionally more Ca^{2+} entered the fruit (Experiment 1, Table 2).

Ca^{2+} and Fruit Firmness

The role of Ca^{2+} in fruit firmness has been widely reported (Cooper & Bangerth, 1976; Badawi *et al.*, 1981; Buescher *et al.*, 1981; Sams & Conway, 1984; McFeeters *et al.*, 1985; Abbott *et al.*, 1989). However, in fresh pickling cucumber fruit, Ca^{2+} treatments were observed not to significantly affect the firmness of the various fruit tissues in Experiment 1 (Table 1, Appendix). Supporting this result Ca^{2+} ion concentration in the mesocarp tissue showed no significant correlation with tissue firmness of fresh cucumber fruit as McFeeters & Lovdal (1987) reported.

Ca^{2+} has been associated with the regulation of ripening and senescence processes of fruits during post-harvest storage. Membrane structure and function, and cell wall structure are thought to be involved in the action of Ca^{2+} (Ferguson, 1984). The role for Ca^{2+} in maintaining fruit firmness during post-harvest processing of cucumbers (Hudson & Buescher, 1980; Tang & McFeeters, 1983) has also been reported.

However, the physiological processes involving Ca^{2+} during storage and post-harvest processing do not take place in freshly harvested fruit in the immature stage of development. For this reason, endogenous Ca^{2+} may not have a major role in influencing texture of fresh pickling cucumber fruit.

Alternatively, Ca^{2+} concentrations as low as 0.21% and 0.08% of dry weight in pericarp and endocarp tissues, respectively might be sufficient for maintaining structural integrity in the cell wall of fruit tissues (Table 2).

The length of storage affects the physical properties of the fruits (Bourne, 1983). A decrease in firmness has been observed from 0 to 5 days of storage for both pericarp and endocarp fruit tissues (Table 4). The significantly higher firmness and lower rate of softening found in the pericarp tissue of the high Ca^{2+} treatment (20.0 mM Ca^{2+}) after 5 days of storage (Table 3) in Experiment 2 suggests that Ca^{2+} might have an important role as a firmness maintaining agent during storage. Such a role for Ca^{2+} in maintaining firmness of fruit tissue during storage has been reported for other fruit crops (Cooper & Bangerth, 1976; Badawi *et al.*, 1981; El-Hammady *et al.*, 1987; Abbott *et al.*, 1989; Tomala & Sadowski, 1989).

Observing Table 3 in Experiment 2, it can be noted that there was a large amount of variability in the rate of softening at any level of Ca^{2+} in the tissue. The same variability was observed in Fig. 2 indicating that other factors besides Ca^{2+} are probably also influencing the rate of pericarp softening. Anatomical characteristics of the cucumber mesocarp tissue have been analysed by Goffinet (1977) and the average cell number (density) has been found to have a significant relationship to texture. Cell density in the cucumber tissues at harvest could be a factor influencing the

pericarp firmness during storage. Also, differences in turgor are largely responsible for the variations encountered in relation to rigidity and crispness of the plant tissue, considering that water is a structural component of the matrix gel (Bourne,1983). Water content of the fruits at harvest time can also account at least in part for the variability observed in the rate of pericarp softening in Fig. 2. Temperatures during the storage period (3 to 5 days) can not be discounted as a factor having a role in the variability observed in Fig. 2, since the fruits in this study were stored either at 5°C or 25°C. Fruit softening during storage has long been associated to changes in the amount and character of the cell wall middle lamella polysaccharides (Pilnik & Voragen, 1970). Van Buren (1979) reported that fruit softening is accompanied by increased pectic substance solubility during storage. Several enzymes have been identified as being capable of hydrolyzing the glycosidic linkages of cell wall polymers including principally polygalacturonases, β-galactosidases, and cellulases (Pilnik & Voragen,1970). Thus, cell density, water content of fruits at harvest, different temperatures during the storage period, as well as the activity of softening enzymes are factors which might be influencing in addition to endogenous Ca^{2+} the rate of pericarp softening (Table 3 and Fig. 2). Bourne (1983) reported that fruits soften at a slower rate in cool storage than at ambient temperature. Bourne (1982) also showed that fruit firmness decreases with

increasing temperatures over the range 0-45°C. The results reported here indicate that fruit firmness in the endocarp tissue was significantly lower at 25°C than at 5°C storage temperature (Table 4). However, no significant differences in fruit firmness were found in relation to storage temperature for pericarp tissue. Perhaps, if the storage period had been extended for a longer period of time, greater differences in fruit tissue firmness might have been manifested between the storage temperature treatments.

The positive correlations between fruit Ca^{2+} concentration (% dry weight basis) and fruit firmness (r=0.61) after 5 days of storage (Fig. 3) and firmness of fruits stored at 1°C for 24 days (r=0.68, Fig. 4) add support for endogenous fruit Ca^{2+} having a role in fruit softening during storage. Increasing the Ca^{2+} content of fruit leads to an increase in firmness retention of fruits. Enhanced Ca^{2+} content was also reported to minimize fruit decay, perishability, loss in fruit moisture content, and ultimately extend the storability of fruit (Tomala & Sadowski, 1989). Van Buren (1979) reported that the addition of Ca^{2+} to fruits increased their firmness and decreased softening during storage. Fruit firmness was correlated positively with tissue Ca^{2+} concentrations (Lau *et al.*, 1973; Fallahi *et al.*, 1985) both before and after storage at 0°C (Sams & Conway, 1984). The same results have been found by Facteau (1982). In contrast, low Ca^{2+} levels in fruits have been associated with poor keeping quality during

storage (Bramlage *et al.*, 1974). Thus, considerable evidence has been presented that Ca^{2+} influences firmness in the fruit tissues during storage.

Ca^{2+} and PG Activity

The interaction of Ca^{2+} with the enzyme polygalacturonase has been very well documented (Buescher & Hobson, 1982; Tang & McFeeters, 1983; McFeeters *et al.*, 1985). The mechanism by which Ca^{2+} ions has been shown to be effective in suppressing polygalacturonase activity (Poovaiah, 1979) seems to involve the crosslinking of pectin molecules by eletrostatic interactions between adjacent negatively charged carboxyl groups of the pectin (Van Buren, 1979) conferring strength to the cell walls, and protecting them from degradation by polygalacturonase (Buescher & Hobson, 1982).

The range of polygalacturonase (PG) activity levels found in the present study were much higher (Table 1, Appendix; Fig. 7) in comparison with the levels of polygalacturonase found by Pressey & Avants (1975) for the small,medium,large and mature cucumbers which were 0.29, 0.63, 0.87, and 1.56 units x 10^{-3}/g, respectively.

In the present study, PG activity was not affected by the Ca^{2+} treatments in freshly harvested pickling cucumber fruit (Table 1, Appendix). It was noted though that the PG activity levels in these fresh cucumber fruits were relatively low due

to the fact that 5 cm fruit are quite immature and still rapidly growing prior to harvest. According to McFeeters & Lovdal (1987), a positive correlation was observed between the concentration of total cell wall sugars and mesocarp tissue firmness of fresh cucumber fruit accounting for 73% of the firmness variation. This suggests that a physical characteristic such as tissue firmness may be influenced as much by the concentration of cell wall polysaccharides in a tissue as the detailed molecular structures of those polysaccharides. In addition, a decrease in rhamnose, arabinose, and galactose has been observed in fresh cucumbers during fruit development (Wallner,1978; McFeeters & Lovdal, 1987).

Since these sugars are known to be associated with polysaccharides other than the pectin substances (McFeeters & Lovdal,1987), other enzymes than polygalacturonase might be involved in the softening process of fresh pickling cucumber fruit. Wallner (1978) has supported this idea suggesting that the removal of neutral sugar polymers which serve as cross-links could weaken the complex network and then contribute directly to a loss of fruit firmness. In addition, the modification of neutral sugar side chains may affect the activity of PG on the polyuronide main chains. Another interesting finding supporting the results in Table 1 (Appendix) is that the degree of pectin methylation in the mesocarp tissue of fresh cucumber fruit increases during fruit

development (McFeeters,1986; McFeeters & Lovdal,1987). Pectin methylation decreases the degree of Ca^{2+} bonding (Ferguson,1984). A negative correlation, however, was observed between PG activity and fruit Ca^{2+} concentration (Fig. 5). No significant correlation was found with endocarp Ca^{2+} concentration.

Table 4 shows that a significantly higher PG activity was present in the fruit stored for 5 days at 25°C. The action of softening enzymes in stored fruits can be controlled by low temperature. Fresh fruits soften as much in an hour at 32°C as in a day at 10°C, or in a week at 0°C (Mohsenin, 1970).

Endocarp tissue firmness appeared to decline with an increase in PG activity after 3 days of storage (Fig. 6). The correlation (r=-0.58) between endogenous Ca^{2+} concentrations and PG activity in the endocarp tissue of fruits stored at 1°C for 24 days (Fig. 7) also adds support for endogenous Ca^{2+} having an influence on PG activity. Ca^{2+} binding and release from the cell wall-middle lamella appears to regulate the rate and extent of degradation by polygalacturonase (Buescher & Hobson, 1982). This effect is apparent at chilling temperatures (0°or 5°C) by the increase in pectin demethylation (McFeeters, 1986). Subsequently, more free carboxyl groups of pectin will be available for Ca^{2+} cross-linking (Hanson,1984).

While PG is the most widely described enzyme to be implicated in the fruit softening process, the variability

observed in the correlation shown in Fig. 7 and the complexity of the cell wall suggest that other enzymes might be involved. Ca^{2+} has been reported to limit the loss of galactose suggesting an involvement of β-galactosidase (Huber,1983). McFeeters *et al.*(1985) reported that the effectiveness of Ca^{2+} in maintaining firmness at high levels of methylation in acid conditions suggests that other types of polysaccharide/Ca^{2+} interactions may have an important role, and that the ocurrence of Ca^{2+}/galactose interactions in plant cell walls should be considered.

In conclusion, endogenous Ca^{2+} in fruit tissue appears to have a role in causing more rapid softening during post harvest storage. However, the results are not conclusive. It is difficult to affirm that much of the variability in firmness found in commercially produced cucumbers can be attributed to low Ca^{2+} levels, considering the great differences observed between the range of Ca^{2+} concentrations found in the fruit tissues from all experiments and the range normally found in pickling cucumbers grown in the field.

Other factors such as fruit growth, fruit anatomy and water content of fruits at harvest, and the activity of softening enzymes other than polygalacturonase, such as β-galactosidase, might be influencing texture of the fresh pickling cucumber fruit during storage. As Ca^{2+} is widely recognized to have a role in regulating numerous physiological processes it is possible that it might be involved in several

of these factors. Future research is needed to verify if these factors are really important in improving the texture of fresh cucumbers, and what are the interactions between Ca^{2+} with them, and how they might be controlled.

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Chapter II

INFLUENCE OF ENDOGENOUS Ca^{2+} ON TEXTURAL QUALITY OF FRESH-PACK PROCESSED CUCUMBERS

Introduction

Firmness is a critical parameter of quality of pickled cucumbers. Efforts have focused on maintaining the firmness of cucumbers during and after fruit processing. Ca^{2+} has been reported to be an important factor in maintaining the tissue firmness during processing of cucumbers (Hudson & Buescher, 1980; Tang & McFeeters, 1983; Buescher & Hudson, 1984; Buescher & Burgin, 1988; McFeeters & Fleming, 1989), especially after fresh-pack processing (Fleming *et al.*, 1978; Buescher *et al.*, 1981; McFeeters *et al.*, 1985).

The cucumber firmness retention was maximized in pickles by processing and storing in brines containing Ca^{2+} chloride or treating with Ca^{2+} chloride after desalting (Buescher *et al.*, 1979; Buescher *et al.*, 1981). Howard & Buescher (1990) have reported that firmness was associated with the amount of bound Ca^{2+} and the supply of Ca^{2+} in fresh-pack cucumber pickles. The role of Ca^{2+} in maintaining the strength of the cell wall structure is believed to be through binding to the pectic polysaccharide matrix. Thus, the percentage of Ca^{2+}-bound is clearly substantial to control water-soluble uronic acid polymers in the cell walls (Jarvis, 1982), considering that a decline in the content of bound Ca^{2+} is associated with fruit maturation (Marschner, 1986) and with an increase in polygalacturonase activity (Poovaiah, 1979).

Demethylation is believed to change the configuration of pectin macromolecules which contributes to loosening of middle lamella-cell wall components and softening (Hudson & Buescher, 1986). Although galacturonans are rapidly deesterified during pickle processing (Tang & McFeeters, 1983; Hudson & Buescher, 1985), Ca^{2+} maintained firmness, regardless of degree of pectin methylation in the tissue (McFeeters *et al.*, 1985).

It has been reported by McFeeters & Fleming (1989) that high endogenous Ca^{2+} concentrations in the cucumbers are involved in maintaining fruit firmness in pieces of mesocarp tissue after blanching. In McFeeters's study, however, fruit were obtained from diverse sources and thus potentially variable in other physiological parameters.

In light of these observations, the objectives of this study were: a) to modify the Ca^{2+} content of cucumber fruit in a culturally controlled manner in order to evaluate the influence of endogenous fruit tissue Ca^{2+} concentrations on fruit firmness prior and subsequent to fresh-pack processing; b) to determine the role of endogenous Ca^{2+} concentrations on post-processing softening of cucumber fruit tissues during accelerated aging at 46°C; c) to verify the effect of Ca^{2+} added to the brine solution on tissue firmness after fresh-pack processing of pickling cucumber fruit; d) to evaluate the effects of refrigeration at 4.4°C on firmness of fresh-pack processed cucumber spears and slices.

Materials and Methods

Plant culture. Pickling cucumber (*Cucumis sativus* L.) plants (CV. Castlepik 2012) were cultured hydroponically in sand within plastic containers during the months of July through October of 1990 in the Plant Science Greenhouses at Michigan State University. The vines were grown upright on string, and the plants were irrigated daily with a modified Hoagland's nutrient solution containing the following nutrient concentrations (mM) until anthesis: 3.0 KNO_3, 1.0 $Ca(NO_3)_2$, 1.0 NaH_2PO_4, 0.5 $MgSO_4$, and micronutrients according to Johnson *et al*. (1957). The Ca^{2+} concentrations in the nutrient culture solution were modified from anthesis through harvest to contain 0.01, 0.1, 1.0, 10.0, and 20.0 mM Ca^{2+} (Table 1).

Pistillate flowers were hand-pollinated daily as soon the flowers opened, from 9 a.m. until noon. The average greenhouse temperature ranged from 25 to 32°C during the day and 18 to 23°C during the night while the relative humidity ranged from 65 to 75% during the day and 75 to 85% during the night.

Fruit sampling

Approximately 11 days after pollination, 9 fruits per treatment were harvested when they attained a diameter of 4.0-

4.5 cm. Three slices, 0.7 cm thick were cut from the center of each cucumber. One was used for fresh fruit firmness analysis, while the other two were fresh-pack processed. The remaining halves were cut into 8 spear segments, with 2 spear segments being separated into pericarp and endocarp tissue for Ca^{2+} concentration determination, and the remaining 6 spear segments of each fruit fresh-pack processed together with the 2 slices. Leaf samples (lamina and petiole) were also collected from the fifth node from the apex of each plant. One combined sample was collected from five plants per treatment per block, and analysed for Ca^{2+} concentration for both lamina and petiole.

The two half spears were separated into pericarp and endocarp tissue for Ca^{2+} concentration determination, placed into pre-weighed trays, and the fresh weight (g) of each tissue determined. The samples were dried in an oven at 60°C for 72 hr, weighed again, and ground in a mortar and pestle. Leaf tissues were ground in a Wiley mill to pass a 20-mesh screen.

Table 1 - Composition of Ca^{2+} treatment solutions applied to pickling cucumber plants beginning at anthesis.

Salts[z]	Concentration (mM)				
	Ca^{2+} treatments (mM)				
	20.0	10.0	1.0	0.1	0.01
NaH_2PO_4	1.0	1.0	1.0	1.0	1.0
$MgSO_4$	1.0	1.0	0.5	0.5	0.5
$Ca(NO_3)_2$	8.0	8.0	1.0	0.1	0.01
$CaCl_2$	12.0	2.0	-	-	-
KNO_3	-	-	6.0	5.8	6.0
KCl	6.0	6.0	-	0.2	-
NH_4NO_3	-	-	2.0	3.0	3.0
$NaNO_3$	-	-	3.0	3.0	3.0
$Mg(NO_3)_2$	-	-	0.5	0.5	0.5

[z] Micronutrient salt concentrations as specified by Johnson *et al.*, 1957.

Ca^{2+} analysis

Tissue samples of 0.1 g previously dried at 60°C from pericarp and endocarp tissues, respectively were weighed and put in labeled digestion tubes. Then, 0.5 ml of 30% hydrogen peroxide (H_2O_2) was added to each digestion tube, and the tubes were swirled gently. Subsequently, 1 ml of perchloric acid ($HClO_4$) was added to each tube and small funnels were placed on top of the digestion tubes to act as a condenser. The tubes were heated in a Tecator digestor for 5 min. at 300°C in a perchloric acid hood. After cooling for 10-15 min., 1 ml of 30% hydrogen peroxide was added to each tube and heated again on digestor for 30 min. without the funnels.

The volume of the solution was made to 50 ml with deionized water, the sample was mixed and stored in a 20 ml plastic vial at 5°C. Following the same procedures in relation to Ca^{2+} standard solutions and dilutions for the unknown samples of pericarp and endocarp tissues were done as in Chapter 1. The Ca^{2+} concentrations in the digests were determined by atomic absorption spectrophotometry using an IL Video 12 Model.

Fresh-Pack Processing and Textural Evaluation

Six spear segments and two slices from each fruit were fresh-pack processed. The following treatment $CaCl_2$ concentrations; 0.0, 5.0, and 20.0 mM Ca^{2+}, were applied to the brine solution prior to fresh-pack processing. In addition, post-processing softening of the spear and slice tissues was evaluated by measuring firmness at three times, 0, 5, and 20 days after incubation at 46°C to accelerate aging and thus the softening processes.

Fruit firmness measurements of pericarp and endocarp tissue were conducted of fresh fruit, immediately after fresh-pack processing, and following two incubation periods at 46°C. Firmness measurements were also taken from all jars before refrigeration, and after 3 and 6 weeks refrigeration at 4.4°C. Illustration of cucumber segments used for green stock analyses and fresh-pack processed spears are shown in Fig. 1. Textural firmness was determined using the Kramer Shear Press, Model TMS-90, Food Technology Corp. located in the Food Science laboratory.

A FTA-300 (136.1 Kg) transducer with a speed from 0.503 to 0.549 cm/sec, and a 0% to 2% graph scale ranging from 0. N to 20. N was used. The maximum penetration force expressed in newtons was the only parameter used for firmness evaluation. One measurement for each tissue, pericarp and endocarp, was taken from each of the 6 spear segments and from the 2 slices

FRESH-PACK SPEAR

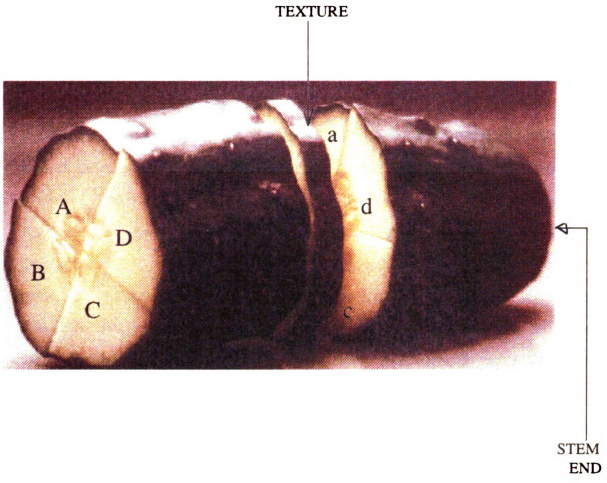

FIGURE 1. Illustration of Cucumber Segments Used For Green Stock Analysis and Fresh-Pack Processed Spears. 1) Green Stock Texture, 0.7 cm Cross-Sectional Slice Prepared For Probe Analysis. 2) Green Stock Calcium Analysis Segments A and a. 3) Fresh-Pack Spears Segments B and b + C and c + D and d.

from each jar. The average was used for statistical analysis for either spears or slices.

The cucumber fruits were processed at the Food Science laboratory according to the following procedure:

From each fruit 6 half spears were packed in 12-oz jars (350ml), and covered with brine solution. The jars were loosely closed, and passed through a steam (86°C) tunnel line for 5 minutes to blanch the fruit. After processing the jars were tightly closed and boiled in water for 10 min., cooled in tap water, and held at 24°C for 4 days for equilibration. Occasionally, the jars were inverted to help assure equilibration of soluble components (McFeeters *et al.*, 1989).

The brine contained 1.5 M NaCl, 0.2 M acetic acid and 4 mM SO_2, added as sodium bisulfite. Ca^{2+} was added to the cover brines as Ca^{2+} chloride ($CaCl_2$) to achieve 5.0 and 20.0 mM Ca^{2+} brine treatments. Measurements of the pH of brines after equilibration with cucumber tissue were conducted using an Fisher Accumet Model 810 pH meter with a combination electrode.

Following 4 days at 24°C either fruit firmness measurements were taken from the spear and slice tissues, or firmness measurements were taken after 5 and 20 days incubation at 46°C. The jars were equilibrated at room temperature before firmness measurement.

Statistical Analysis

A randomized complete block design with 4 blocks was arranged by plant size, with 5 treatments per block, and 5 plants per treatment. 9 fruits were analysed per treatment, with 45 fruits per block, totaling 180 fruits for the entire experiment.

For the analysis from the fresh-pack processed slices, 4 Ca^{2+} treatments (20.0, 10.0, 1.0, 0.1 mM Ca^{2+}), and 2 incubation periods at 46°C (5 and 20 days) were evaluated.

Analysis of variance using a RCBD with 1 factor (Ca^{2+} concentration) were conducted on Ca^{2+} concentration of fruit and leaves, and firmness of fresh pickling cucumber fruit tissues. Analysis of variance was conducted to evaluate the potential treatment effects on texture and the rate of tissue softening of pickle fruit tissues following fresh-pack processing, during accelerated aging and refrigeration.

The effect of Ca^{2+} concentration in brine solution on fruit firmness and rate of tissue softening of fresh-pack processed spears and slices during accelerated aging and refrigeration was also investigated.

The means were compared using the LSD test. Correlation analysis were also investigated between the dependent variables; pericarp and endocarp endogenous Ca^{2+}, fruit tissue firmness, and rate of tissue softening, following fresh-pack

processing, during accelerated aging, and during refrigeration of processed spears and slices. The relationships between the dependent variables were investigated either by linear regression (tissue Ca^{2+} x fruit Firmness) or by non-linear regression (tissue Ca^{2+} x tissue softening rates) analysis.

Results

Plant growth was vigorous with no stress symptoms observed on the foliage. The period of fruit growth, from pollination through harvest varied between 10 and 11 days with Ca^{2+} treatments having no effect. Ca^{2+} treatments in the nutrient culture solution applied to the plants at anthesis significantly modified the fruit and leaf endogenous Ca^{2+} concentrations (Table 2).

Pericarp tissue Ca^{2+} concentrations expressed on a dry weight basis ranged from 0.46 to 1.15 % Ca^{2+} while Ca^{2+} levels in endocarp tissue ranged from 0.15 to 0.26 % Ca^{2+}. Ca^{2+} concentrations in the petiole tissue ranged from 0.99 to 3.86 % dry weight while Ca^{2+} levels in the lamina tissue ranged from 2.14 to 5.14 % (Table 2). Firmness of both pericarp and endocarp tissues in freshly harvested cucumber fruit, however, were not affected by the Ca^{2+} treatments (Table 2).

Table 2 - Influence of Ca^{2+} concentration in nutrient culture solution on fruit and leaf tissue Ca^{2+} concentrations and on tissue firmness of fresh pickling cucumber fruit.

Ca^{2+} Conc. (mM)	Ca^{2+} concentration (% dry wt)				Fruit Firmness (newtons)	
	Fruit		Leaf			
	pericarp	endocarp	petiole	lamina	pericarp	endocarp
0.01	0.48	0.15	0.99	2.14	16.9	4.4
0.1	0.46	0.16	1.02	1.87	17.2	4.2
1.0	0.56	0.18	1.59	2.81	17.9	4.6
10.0	0.95	0.25	2.99	5.24	17.5	4.4
20.0	1.15	0.26	3.86	5.14	17.3	4.5
LSD(0.01)	0.09	0.02	0.44	1.53	NS	NS

NS nonsignificant

Incubation of Fresh-Pack Processed Spears

The brine pH after equilibration with the cucumber spears and slices was 2.5 in 0 mM Ca, 3.0 in 5 mM Ca, and 3.2 in 20 mM Ca^{2+} processing brine solution.

In fresh-pack processed spears, the Ca^{2+} fertilization treatments had a significant effect on pericarp tissue firmness immediately following processing. This firming effect on pericarp tissue was still apparent even when averaged over all Ca^{2+} brine treatments (Table 3).

The pericarp tissue firmness was significantly higher in plants fertilized with 20 mM Ca^{2+} as compared to the 0.01 mM Ca^{2+} treatment. Significantly higher firmness was also found in the pericarp tissue of plants fertilized with 20 and 10 mM Ca^{2+} in relation to the 0.1 and 0.01 mM Ca^{2+} treatments following fresh-pack processing. Consistent with these results endogenous Ca^{2+} concentration in the pericarp tissue expressed as mM was positively correlated with pericarp tissue firmness following fresh-pack processing and (Fig. 2).

No differences were found in endocarp tissue firmness as a result of the Ca^{2+} treatment in the nutrient culture solution in freshly processed pickles. However, endogenous Ca^{2+} concentration in endocarp tissue, expressed as mM, showed a

Figure 2. Correlation between Ca^{2+} concentration (mM) in pericarp tissue and firmness of pericarp tissue following fresh-pack processing at 46°C of cucumber spears in 0 mM Ca^{2+} brine solution.

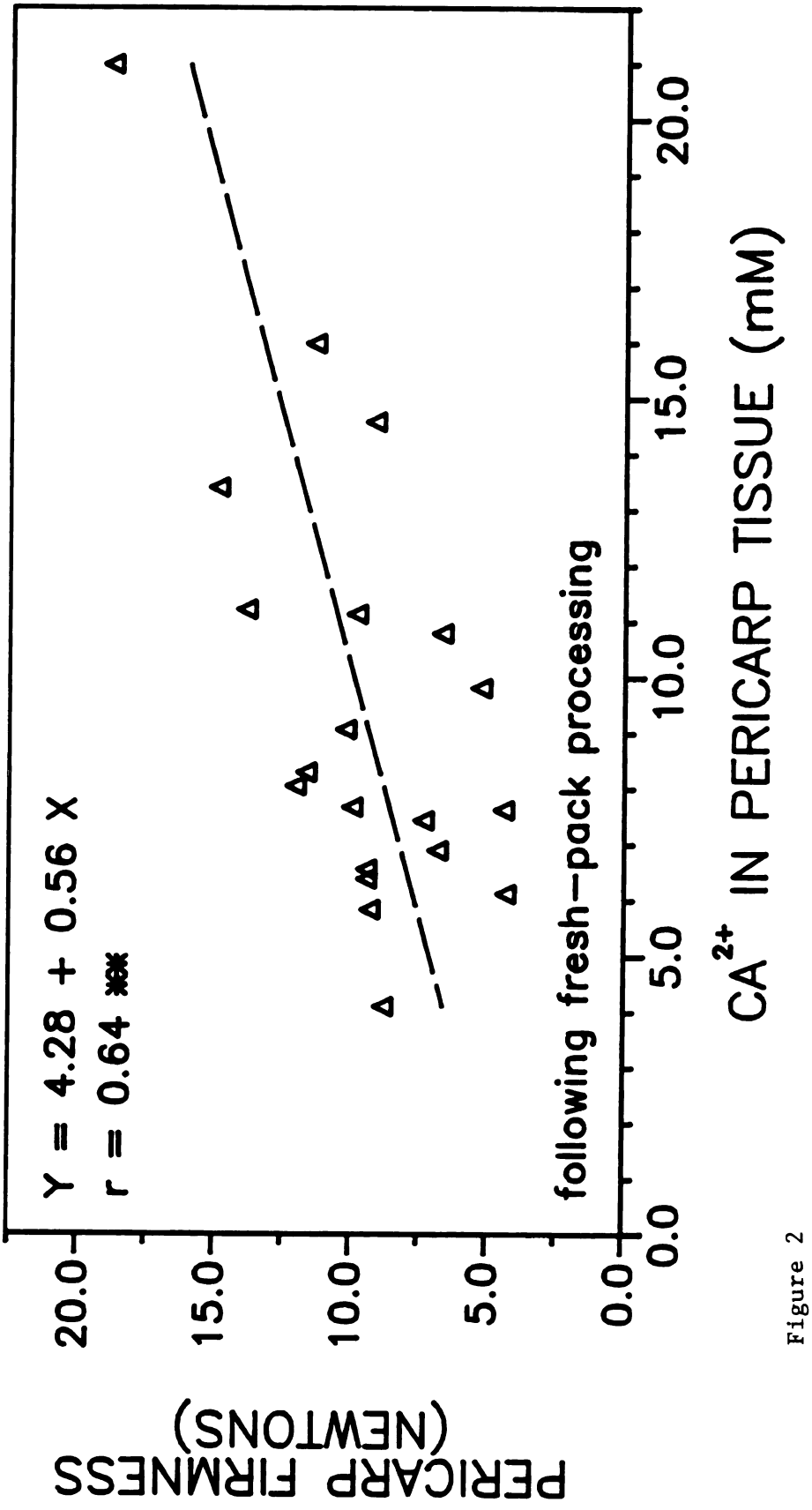
PERICARP FIRMNESS
(NEWTONS)
20.0
15.0
10.0
5.0
0.0
Y = 4.28 + 0.56 X
r = 0.64 ***
following fresh-pack processing
0.0
5.0
10.0
15.0
20.0
CA2+ IN PERICARP TISSUE (mM)

Figure 2

Figure 3. Correlation between Ca^{2+} concentration (mM) in endocarp tissue and firmness of endocarp tissue after 5 days incubation at 46°C of cucumber spears in 0 mM Ca^{2+} brine solution.

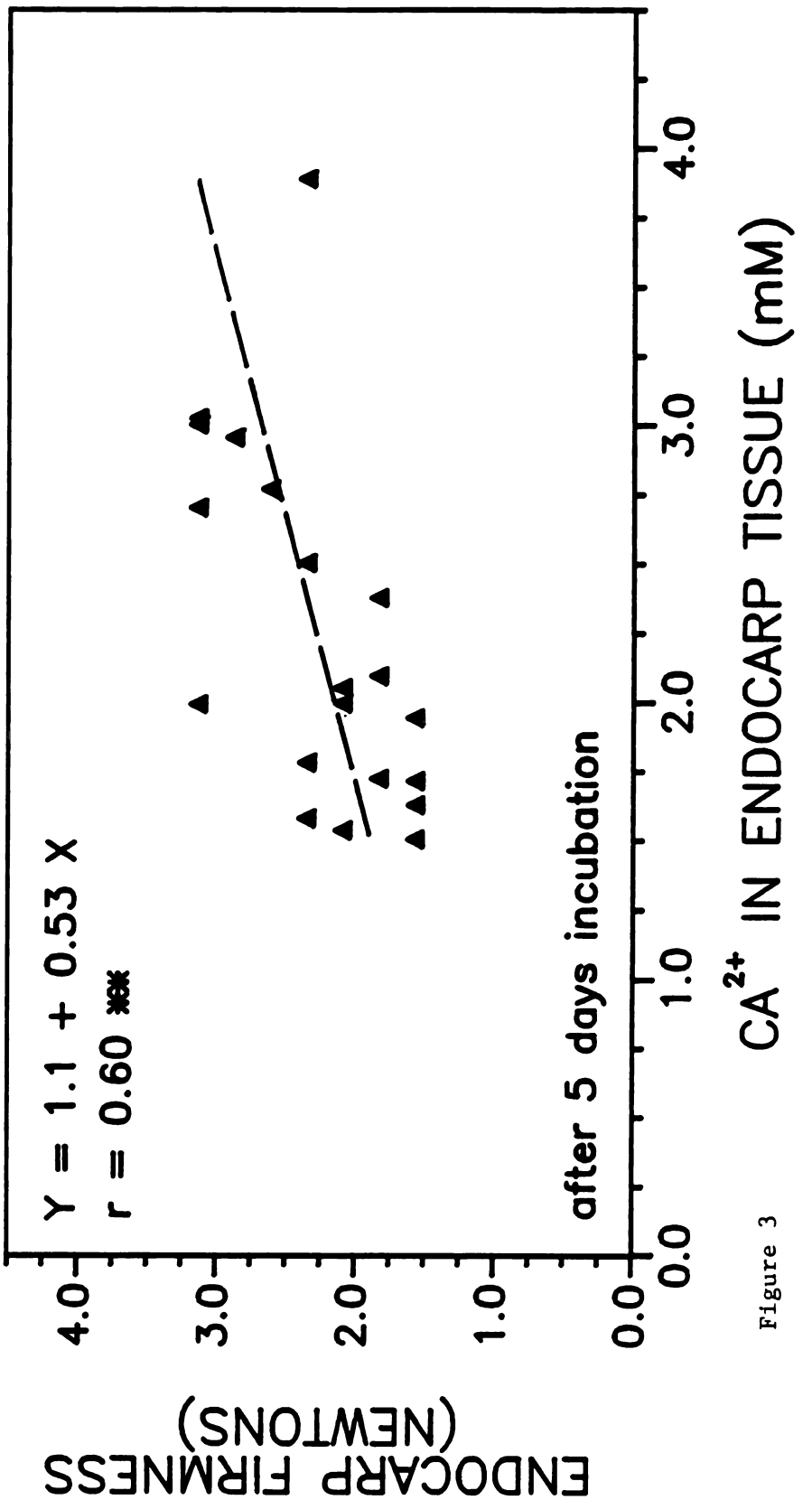
ENDOCARP FIRMNESS
(NEWTONS)
4.0
3.0
2.0
1.0
0.0
Y = 1.1 + 0.53 X
r = 0.60 ***
after 5 days incubation
0.0
1.0
2.0
3.0
4.0
CA^{2+} IN ENDOCARP TISSUE (mM)

Figure 3

Table 3 - Effect of Ca^{2+} concentration in nutrient culture solution on firmness and on the rate of pericarp softening of fresh-pack processed cucumber spears averaged over all Ca^{2+} brine treatments and in 0 mM Ca^{2+} brine during incubation at 46°C.

Ca^{2+} Conc. (mM)	Tissue Firmness (newtons)			Rate of Softening (newtons/day)	
	Pericarp			Pericarp	
	Incubation Time (days) 0	5	20	0-5 days	5-15 days
0.01	10.26	4.69	2.93	1.11	0.12
0.1	10.90	6.10	2.88	0.96	0.21
1.0	12.85	6.38	2.95	1.29	0.23
10.0	12.68	5.90	3.31	1.35	0.17
20.0	13.86	5.55	3.33	1.66	0.14
LSD(0.05)	3.16	NS	NS	0.35	NS
			0 mM Ca^{2+} brine		
0.01	7.07	2.45	2.68	0.92	-0.02
0.1	6.79	2.48	2.06	0.86	0.03
1.0	9.93	2.26	2.07	1.53	0.02
10.0	10.94	2.90	2.54	1.60	0.03
20.0	13.55	2.84	2.50	2.14	0.03
LSD(0.01)	4.60	NS	NS	0.91	NS

NS nonsignificant

positive correlation with endocarp tissue firmness after 5 days incubation at 46°C (Fig. 3).

Ca^{2+} treatments did not affect the pericarp firmness after 5 and 20 days incubation (Table 3). Following accelerated aging at 46°C for 5 or 20 days, the Ca^{2+} firming effect was lost due to the large amount of softening of spears from all treatments (Table 3).

The high levels (10 and 20 mM) of Ca^{2+} concentration in the nutrient solution showed the higher rates of pericarp softening after 5 days incubation at 46°C when averaged over all Ca^{2+} brine treatments (Table 3). Higher rates of softening were also observed in the higher Ca^{2+} treatments after 5 days incubation in pericarp tissue in 0 mM Ca^{2+} brine solution (Table 3).

Consistent with these results, the rate of pericarp softening after 5 days incubation at 46°C appears to increase as the endogenous Ca^{2+} concentration increased in the pericarp tissue up to approximately 8 mM (Fig. 4). At higher endogenous tissue Ca^{2+} levels, the rate of fruit softening is relatively constant (Fig. 4). The Ca^{2+} treatments did not affect the rate of softening of pericarp tissue between 5 and 15 days incubation at 46°C (Table 3).

No interaction was found between the effects of Ca^{2+} concentration in the nutrient solution and Ca^{2+} concentration within the brine solution on fruit tissue firmness or on the rate of softening either following fresh-pack processing or

Figure 4. Correlation between endogenous Ca^{2+} concentration and the rate of softening of pericarp tissue in spears and slices of fresh-pack processed pickling cucumber in 0 mM Ca^{2+} brine solution during 5 days incubation at 46°C.

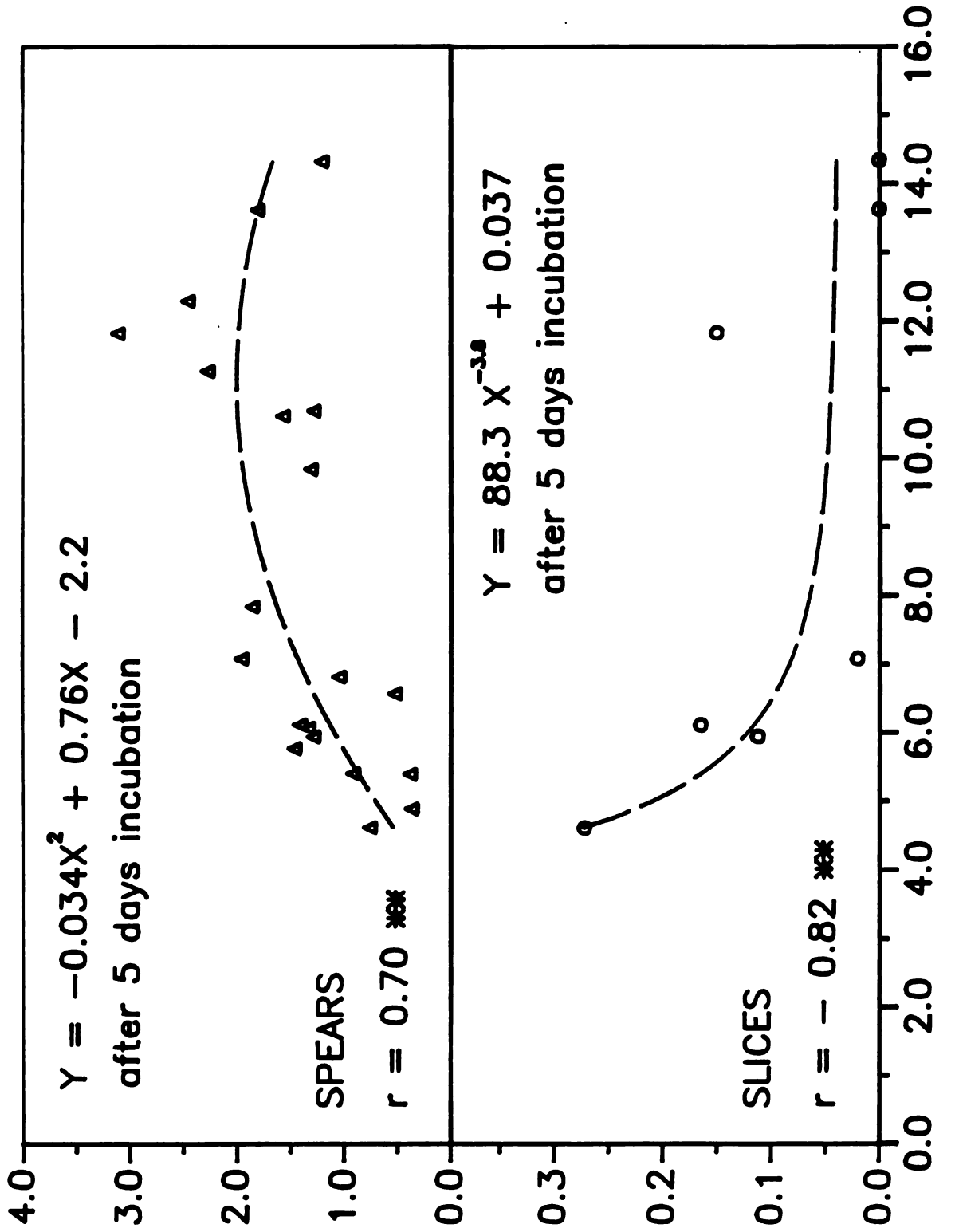
Y = −0.034X² + 0.76X − 2.2
after 5 days incubation
SPEARS
r = 0.70 ***
Y = 88.3 X^-1.8 + 0.037
after 5 days incubation
SLICES
r = − 0.82 ***
RATE OF SOFTENING (NEWTONS/DAY)
RATE OF SOFTENING (NEWTONS/DAY)
PERICARP CA2+ (mM)
4.0
3.0
2.0
1.0
0.0
0.3
0.2
0.1
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0

Figure 4

Table 4 - Effect of Ca^{2+} concentration in the brine solution on firmness and on the rate of tissue softening of fresh-pack processed cucumber spears during accelerated aging at 46°C.

Ca^{2+} Conc. (mM)	Tissue Firmness (newtons (N))						Rate of Softening ($days^{-1}$)			
	Pericarp			Endocarp			Pericarp		Endocarp	
	Incubation Time (days)									
	0	5	20	0	5	20	0-5 days	5-15 days	0-5 days	5-15 days
0	9.65	2.59	2.37	3.18	2.28	1.75	1.41	0.02	0.18	0.04
5	11.79	3.46	3.09	3.26	2.14	2.09	1.66	0.03	0.22	0.01
20	14.88	11.14	3.79	3.24	2.75	2.49	0.75	0.48	0.09	0.02
LSD(0.05)	1.83	1.14	0.39	NS	0.31	0.30	0.35	0.08	0.09	NS

NS nonsignificant

during 5 or 20 days of accelerated aging at 46°C in processed spears.

The addition of Ca^{2+} to the processing brine solution of 5 and 20 mM Ca^{2+} enhanced the post-processing firmness of both pericarp and endocarp tissues regardless of whether the spears were incubated at high temperature for 5 or 20 days (Table 4). The 20 mM Ca^{2+} brine treatment showed a significantly higher pericarp firmness following fresh-pack processing, and a significantly higher pericarp and endocarp firmness during accelerated aging as compared to the 0 and 5 mM Ca^{2+} brine treatment. Spears with 5 mM Ca^{2+} showed significantly higher pericarp firmness following fresh-pack and after 20 days incubation in pericarp and endocarp tissues than the 0 mM Ca^{2+} treatment. The Ca^{2+} brine treatments had no effect on fruit firmness of endocarp tissue following fresh-pack processing (Table 4). The firmness retention due to Ca^{2+} was much larger in pericarp than in endocarp tissue.

The effect of Ca^{2+} addition to the brine solution on the rate of softening of fruit tissues after 5 days incubation and between 5 and 15 days incubation at 46°C is also shown in Table 4. Spears with 20 mM Ca^{2+} added to the brine solution showed significantly lower rates of pericarp softening after 5 days incubation at 46°C than the spears with 0 and 5 mM Ca^{2+} in the processing brine. In contrast, higher pericarp softening was observed in the spears with 20 mM Ca^{2+} added to

Table 5 - Effect of Ca^{2+} concentration in the nutrient culture solution on pericarp firmness of unincubated fresh-pack processed cucumber spears averaged over all Ca^{2+} brine treatments and in 0 mM Ca^{2+} brine during refrigeration at 4.4°C.

Ca^{2+} Conc. (mM)	Tissue Firmness (newtons)		
	Pericarp Refrigeration Time (weeks)		
	0	3	6
0.01	10.26	11.25	9.64
0.1	10.90	11.61	10.61
1.0	12.85	13.85	11.38
10.0	12.68	12.63	10.77
20.0	13.86	13.93	12.47
LSD(0.05)	2.37	2.24	NS
	0 mM Ca^{2+} brine		
0.01	7.07	7.83	7.64
0.1	6.79	7.48	6.67
1.0	9.93	11.06	8.36
10.0	10.94	10.55	8.53
20.0	13.55	13.04	11.38
LSD(0.05)	3.28	4.05	NS

NS nonsignificant

Figure 5. Correlation between Ca^{2+} concentration (mM) in pericarp tissue and firmness of pericarp tissue of unincubated fresh-pack processed cucumber spears in 0 mM Ca^{2+} brine after 3 and 6 weeks refrigeration at 4.4°C.

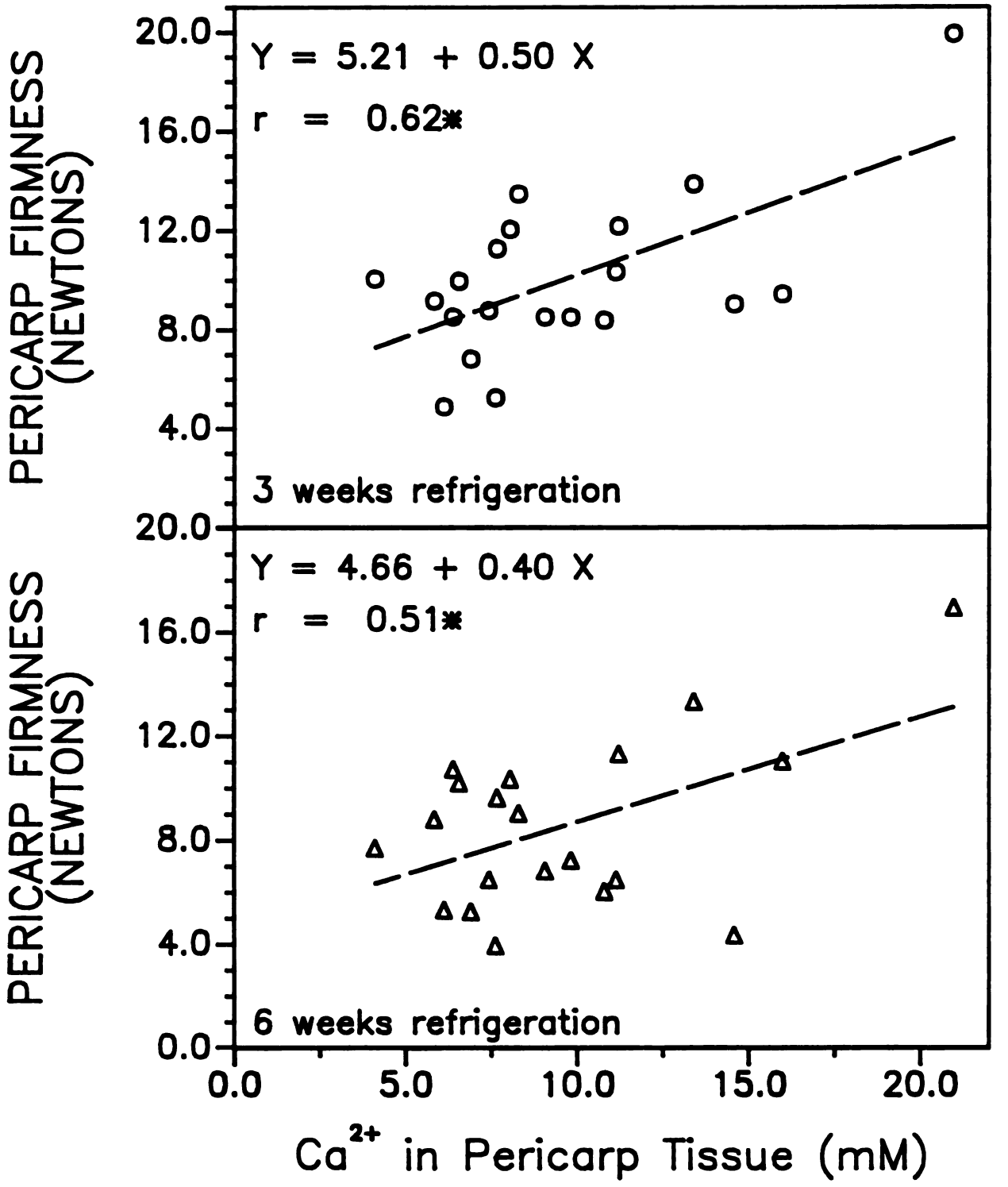
PERICARP FIRMNESS (NEWTONS)
Y = 5.21 + 0.50 X
r = 0.62*
3 weeks refrigeration
PERICARP FIRMNESS (NEWTONS)
Y = 4.66 + 0.40 X
r = 0.51*
6 weeks refrigeration
20.0
16.0
12.0
8.0
4.0
0.0
0.0
5.0
10.0
15.0
20.0
Ca2+ in Pericarp Tissue (mM)

Figure 5

the brine solution in comparison with the spears in 0 or 5 mM Ca^{2+} between 5 and 15 days incubation at 46°C.

No significant effect of Ca^{2+} concentration in the brine solution was found on the rate of endocarp softening during the same period of incubation (Table 4).

Refrigeration of Fresh-Pack Processed Spears

Unincubated Fresh-Pack Processed Spears

Ca^{2+} in the nutrient culture solution maintained the pericarp tissue texture during refrigeration of fresh-pack processed spears. The textural effect within the pericarp tissue was extended up to 3 weeks refrigeration at 4.4°C in both 0 mM Ca^{2+} brine and when averaged over all Ca^{2+} brine treatments (Table 5). After 3 weeks refrigeration at 4.4°C, firmness was significantly higher in the 1 and 20 mM Ca^{2+} treatments as compared to the 0.01 and to the 0.1 and 0.01 mM Ca^{2+} treatments, respectively. No significant effect of Ca^{2+} treatments on pericarp tissue firmness was observed after 6 weeks refrigeration either averaged over all Ca^{2+} brine treatments or in 0 mM Ca^{2+} brine (Table 5). Ca^{2+} treatments had no effect on endocarp tissue texture during refrigeration.

In 0 mM Ca^{2+} brine solution, the effects of Ca^{2+} fertilization treatments on pericarp texture at 0 and 3 weeks of refrigeration were more pronounced. Refrigeration maintained firmness of the 20 mM Ca^{2+} treatment in pericarp

tissue until 3 weeks as compared to the 0.1 and 0.01 mM Ca^{2+} treatments (Table 5).

Consistent with these results, positive correlations were found between the pericarp Ca^{2+} concentration and pericarp firmness of fresh-pack processed spears when no Ca^{2+} was added to the brine solution after 3 weeks refrigeration at 4.4°C (Fig. 5). In addition, a positive correlation was observed between the endogenous pericarp Ca^{2+} concentration and pericarp tissue firmness of fresh-pack processed spears in 0 mM Ca^{2+} brine solution after 6 weeks refrigeration at 4.4°C (Fig. 5), even though no response in firmness was found to nutrient solution Ca^{2+} treatments.

The Ca^{2+} fertilization treatments had no effect on the rate of tissue softening of unincubated fresh-pack processed spears either averaged over all Ca^{2+} brine treatments or in 0 mM Ca^{2+} brine solution during refrigeration at 4.4°C.

5 and 20 Days Incubated Spears

Refrigeration had a significant effect on pericarp tissue texture in 5 days incubated processed spears after 3 weeks refrigeration when no Ca^{2+} was added to the brine solution (Table 6). No differences in tissue texture were found due to refrigeration for 20 days incubated processed spears.

The 20 mM Ca^{2+} treatment showed a significantly higher firmness within the pericarp tissue than the 0.1 and 0.01 mM

Table 6 - Effect of Ca^{2+} concentration in nutrient culture solution on fruit firmness of 5 days incubated processed cucumber spears in 0 mM Ca^{2+} brine solution during refrigeration at 4.4°C.

Ca^{2+} Conc. (mM)	Tissue Firmness (newtons)					
	Pericarp			Endocarp		
	Refrigeration Time (weeks)					
	0	3	6	0	3	6
0.01	2.45	2.42	2.70	1.76	1.85	1.90
0.1	2.48	2.50	2.77	2.09	2.09	2.18
1.0	2.25	3.20	3.07	1.83	2.15	2.28
10.0	2.90	3.13	2.61	2.81	2.25	2.16
20.0	2.84	3.50	3.17	2.88	2.82	2.24
LSD(0.05)	NS	0.81	NS	0.47	NS	NS

NS nonsignificant

Ca^{2+} treatments in 5 days incubated spears after 3 weeks refrigeration (Table 6). No effect of Ca^{2+} fertilization treatments on pericarp firmness was observed after 6 weeks refrigeration in 5 days incubated fresh-pack processed spears.

The firming effect of 10 and 20 mM Ca^{2+} treatments as compared to the 1.0, 0.1, and 0.01 mM Ca^{2+} treatments on endocarp tissue texture before refrigeration in 5 days incubated processed spears was not extended after either 3 or 6 weeks refrigeration (Table 6).

Ca^{2+} treatments in the nutrient solution had no significant effect on the rate of tissue softening of either 5 or 20 days incubated fresh-pack processed spears after 3 weeks refrigeration or between 3 and 6 weeks refrigeration.

No interaction was found between the effects of Ca^{2+} concentration in the nutrient solution and Ca^{2+} concentration within the brine solution on fruit firmness or on the rate of softening of fresh-pack processed spears during refrigeration at 4.4°C.

The effect of refrigeration on fruit firmness of 0, 5, and 20 days incubated fresh-pack processed spears when Ca^{2+} was added to the brine solution is shown in Table 7. Regardless of storage time the Ca^{2+} concentrations in the brine solution affected firmness similarly. When 5 and 20 mM Ca^{2+} was added to the brine solution, a significantly higher pericarp firmness in the spears was observed as compared to the spears

Table 7 - Effect of Ca^{2+} brine concentration on fruit firmness and on rate of tissue softening in 0, 5, and 20 days incubated fresh-pack processed cucumber spears during refrigeration at 4.4°C.

Ca^{2+} Conc. (mM)	Tissue Firmness (newtons)						Rate of Softening (newtons/day)			
	Pericarp			Endocarp			Pericarp		Endocarp	
	Refrigeration Time (weeks)						Refrigeration Time (weeks)			
	0	3	6	0	3	6	0-3	3-6	0-3	3-6
				0 days						
0	9.65	9.99	8.52	3.17	2.37	1.96	-0.11	0.50	0.27	0.14
5	11.79	12.33	10.17	3.26	2.54	2.45	-0.18	0.72	0.24	0.03
20	14.88	15.64	14.24	3.24	3.44	3.18	-0.25	0.46	-0.07	0.08
LSD(0.01)	1.53	2.32	2.79	NS	0.54	0.53	NS	NS	0.23	NS
				5 days						
0	2.58	2.95	2.86	2.28	2.23	2.15	-0.12	0.03	0.01	0.03
5	3.38	3.90	3.22	2.14	2.40	1.89	-0.17	0.22	-0.09	0.17
20	11.15	10.74	11.03	2.75	2.36	2.38	0.14	-0.09	0.13	-0.01
LSD(0.01)	1.12	0.99	1.16	0.31	NS	0.34	0.20	0.16	0.15	0.15
				20 days						
0	2.37	2.02	2.12	1.75	1.61	1.57	0.12	-0.03	0.05	0.01
5	3.16	2.81	2.21	2.09	1.87	1.48	0.12	0.20	0.07	0.12
20	3.79	3.94	3.30	2.50	2.37	1.42	-0.05	0.21	0.04	0.31
LSD(0.01)	0.55	0.51	0.58	0.40	0.39	NS	NS	0.18	NS	0.17

NS nonsignificant

in 5 mM Ca^{2+} or without added Ca^{2+} (Table 7). This firming effect was maintained for 3 and 6 weeks by refrigeration when 20 mM Ca^{2+} was added. In addition, processed spears in 20 mM Ca^{2+} brine solution exhibited a significantly higher endocarp firmness as compared to the 0 and 5 mM Ca^{2+} brine solutions in unincubated spears (Table 7).

Significantly lower rates of softening were observed in the endocarp tissue of unincubated spears when 20 mM Ca^{2+} was added to the processing brine. The same effect was observed for both pericarp and endocarp tissue in 5 days incubated as compared to 0 and 5 mM Ca^{2+} in the brine after 3 weeks and between 3 and 6 weeks refrigeration, respectively (Table 7). However, the decrease in the rate of softening caused by the Ca^{2+} ammendments to the brine solution was reversed in both 5 and 20 days incubated spears in pericarp and endocarp tissues after 3 weeks and between 3 and 6 weeks refrigeration, respectively.

Ca^{2+} ammendments to the processing brine had no effect on the rate of softening of both pericarp and endocarp tissues of unincubated spears refrigerated for more than 3 weeks or on the rate of pericarp softening refrigerated up to 3 weeks. Also, no effect was found of Ca^{2+} ammendments to the brine solution on rate of softening of 20 days incubated spears refrigerated up to 3 weeks (Table 7).

Incubation of Fresh-Pack Processed Slices

Fruit tissues of fresh-pack processed slices were much softer immediately following blanching as compared to fresh tissue. Ca^{2+} concentration in the nutrient culture solution had little or no effect on firmness and rate of softening of processed slices in 0 mM Ca^{2+} in the processing brine. The exception was the significantly higher firmness observed in the pericarp tissue of plants fertilized with 10 mM or higher Ca^{2+} concentration as compared to 0.1 mM Ca^{2+} after 20 days of accelerated aging (Table 8). Also, adding support for Ca^{2+} having a role on fruit softening, the rate of pericarp tissue softening during aging of slices, after 5 days incubation at 46°C, was negatively correlated with tissue Ca^{2+} concentrations (Fig. 4).

The rates of tissue softening following blanching differed greatly between spears and slices. Fruit textural measurements in processed slices following fresh-pack processing averaged 3.3 N in pericarp tissue and 2.9 N in endocarp tissue while the average of the textural measurements of processed spears from the same fruits was 10.2 N in pericarp tissue and 3.2 N in endocarp tissue following fresh-pack processing. Thus, the magnitude in pericarp firmness in processed spears was much higher than in processed slices. Differences in textural firmness between processed spears and slices from the same fruits were as high as 14.9 N.

Table 8 - Effect of Ca^{2+} concentration in nutrient culture solution on fruit firmness of fresh-pack processed cucumber slices in 0 mM Ca^{2+} brine solution during incubation at 46°C.

Ca^{2+} Conc. (mM)	Tissue Firmness (newtons)			
	Pericarp		Endocarp	
	Incubation Time (days)			
	5	20	5	20
0.1	2.55	1.76	2.16	1.96
1.0	2.75	2.16	2.35	1.57
10.0	2.94	2.36	2.94	1.77
20.0	2.75	2.36	2.55	1.77
LSD(0.05)	NS	0.42	NS	NS

NS nonsignificant

Ammendments to the processing brine solutions of 20 mM Ca^{2+} enhanced the post-processing firmness of both pericarp and endocarp tissues regardless of whether the slices were incubated for 5 or 20 days at 46°C (Table 9). Processed slices in 20 mM Ca^{2+} brine solution showed significantly higher pericarp firmness as compared to processed slices in 0 or 5 mM Ca^{2+} brine solution after either 5 or 20 days at 46°C. However, the rate of pericarp tissue softening was higher in slices processed in 20 mM Ca^{2+} brine solution as compared to slices processed either in 5 or without added Ca^{2+} in the brine solution between 5 and 15 days incubation at 46°C (Table 9).

Slices processed in 20 mM Ca^{2+} brine solution showed significantly higher endocarp firmness as compared to slices processed in either 5 mM Ca^{2+} brine solution after 5 days incubation or 0 and 5 mM Ca^{2+} brine solution after 20 days incubation at 46°C (Table 9). Addition of 5 mM Ca^{2+} to the processing brine solution, however, did not enhance significantly the post-processing firmness of both pericarp and endocarp tissues of fresh-pack processed slices (Table 9).

Ca^{2+} added to the processing brine solution had no effect on the rate of endocarp tissue softening between 5 and 15 days incubation at 46°C.

The Ca^{2+} concentration in nutrient solution and in brine affected tissue firmness and the rate of softening during accelerated aging similarly in fresh-pack processed slices.

Table 9 - Effect of Ca^{2+} brine concentration on fruit firmness and on rate of softening of fresh-pack processed cucumber slices during incubation at 46°C.

Ca^{2+} Conc. (mM)	Tissue Firmness (newtons)				Rate of Softening (newtons/day)	
	Pericarp		Endocarp		Pericarp	Endocarp
	Incubation Time (days)				5-15 days	5-15 days
	5	20	5	20		
0	2.75	2.16	2.50	1.76	0.04	0.05
5	2.53	2.43	2.28	1.94	0.01	0.02
20	5.12	3.24	2.80	2.45	0.12	0.02
LSD(0.05)	0.82	0.37	0.35	0.34	0.06	NS

NS nonsignificant

Refrigeration of Fresh-Pack Processed Slices

Ca^{2+} concentration in the nutrient culture solution had no effect on firmness and rate of softening of processed slices during refrigeration. Ca^{2+} addition to the processing brine solution enhanced the post-processing firmness and maintained pericarp tissue texture for 6 weeks refrigeration in 5 days incubated slices and up to 3 weeks refrigeration in 20 days incubated slices (Table 10).

The effect of Ca^{2+} addition to the brine solution on endocarp tissue firmness was only apparent in 20 days incubated slices and up to 3 weeks refrigeration. In both pericarp and endocarp tissues, slices processed in 20 mM Ca^{2+} brine solution showed higher firmness as compared to slices processed in 0 or 5 mM Ca^{2+} brine solution (Table 10).

An opposite effect, however, in relation to the rate of softening was observed in the endocarp tissue in 5 days incubated slices refrigerated up to 3 weeks and in both pericarp and endocarp tissues in 20 days incubated slices refrigerated for more than 3 weeks (Table 10).

Ca^{2+} ammendments to the processing brine had no effect on the rate of softening of 5 days incubated slices either refrigerated for 3 weeks in pericarp tissue or between 3 and 6 weeks refrigeration in both pericarp and endocarp tissues. The rate of pericarp and endocarp softening in 20 days

Table 10 - Effect of Ca^{2+} brine concentration on fruit firmness and on rate of tissue softening of 5 and 20 days incubated fresh-pack processed cucumber slices during refrigeration at 4.4°C.

Ca^{2+} Conc. (mM)	Tissue Firmness (newtons)						Rate of Softening (newtons/day)			
	Pericarp			Endocarp			Pericarp		Endocarp	
	Refrigeration Time (weeks)						Refrigeration Time (weeks)			
	0	3	6	0	3	6	0-3	3-6	0-3	3-6
						5 days				
0	2.75	2.75	2.45	2.50	2.36	2.21	0.00	0.09	0.04	0.05
5	2.53	2.72	2.14	2.28	2.38	1.84	-0.06	0.19	-0.03	0.18
20	5.12	4.73	4.56	2.80	2.28	1.92	0.13	0.05	0.17	0.12
LSD(0.05)	0.82	0.51	0.87	0.35	NS	NS	NS	NS	0.15	NS
						20 days				
0	2.16	1.98	1.76	1.77	1.49	1.32	0.05	0.07	0.09	0.05
5	2.43	2.16	1.86	1.94	1.79	1.35	0.08	0.10	0.05	0.14
20	3.24	3.11	2.28	2.45	2.55	1.40	0.04	0.27	-0.03	0.38
LSD(0.01)	0.51	0.54	NS	0.47	0.55	NS	NS	0.17	NS	0.22

NS nonsignificant

incubated slices was also not affected by the Ca^{2+} brine treatments after 3 weeks refrigeration (Table 10).

Discussion

This study is thought to be relevant to production of pickling cucumbers since Ca^{2+} concentrations within commercially produced fruit are highly variable (Engelkes *et al.*,1990), thus potentially affecting textural quality. Engelkes *et al.*(1990) reported that Ca^{2+} concentrations within 150 g fresh weight fruit (similar in size to 4.5 cm diameter fruit), produced under diverse environmental conditions, varied from approximately 0.7 to 1.0 % of dry weight in pericarp tissue and from 0.2 to 0.6 % in endocarp tissue. The range of Ca^{2+} concentrations induced experimentally in this study (Table 2) exceeded those reported by Engelkes *et al.* (1990) for pericarp tissue but less for endocarp tissue.

Ca^{2+} concentrations in the leaf lamina tissue were higher than in the petiole tissue (Table 2). Engelkes (1987) found in field-grown pickling cucumbers Ca^{2+} concentrations ranging from 1.85 to 3.10 % and 1.45 to 1.84 % of dry weight in leaf lamina and petiole tissue, respectively, while in the cucumber cultivar Castlepik 2012 the Ca^{2+} concentrations found were 2.56 % in leaf lamina tissue and 1.75 % in leaf petiole tissue. Carolus (1975) also reported Ca^{2+} concentrations around 3.71 % in the foliage of cucumbers. Thus, the field leaf Ca^{2+}

concentrations were intermediate to what was induced experimentally in the present study (Table 2).

McFeeters & Lovdal (1987) reported that firmness of cucumber fresh fruit showed no relationship with Ca^{2+} concentration in mesocarp tissue. This is consistent with the results from this study (Table 2).

Consistent also with the findings of McFeeters & Fleming (1989) blanched cucumbers submitted to accelerated aging at high temperature softened rapidly (Table 3,8). High endogenous Ca^{2+} concentrations within pickling cucumber fruit were found to enhance the textural firmness of pericarp (fruit wall) tissue following blanching (Table 3).

Maximum firmness of fresh-pack processed spears was achieved at fresh pericarp tissue Ca^{2+} concentrations of approximately 0.95 % dry weight and higher while in endocarp tissue the maximum firmness was achieved at Ca^{2+} concentrations of 0.25 % dry weight and higher. In processed slices maximum firmness was achieved at similar tissue Ca^{2+} concentrations, 0.97 % dry weight for pericarp and 0.28 % for endocarp tissue.

The effect of endogenous Ca^{2+} on pericarp firmness of processed spears was apparent even when Ca^{2+} was added to the solution prior to blanching and storage (Table 3). However, after accelerated aging at 46°C for 5 or 20 days (Table 3), the textural differences attributed to endogenous tissue Ca^{2+} concentrations in pericarp tissue disappeared within the processed spears. Softening associated with aging of blanched

spear tissue also contributed to a reduction in differences among treatments.

The changes in the chemical nature of the pectic materials are the primary cause of changes that occur in the textural properties of horticultural products (Van Buren,1979). The decrease in protopectin and the increase in pectin, and thus in water insoluble and water soluble pectin, respectively, are caused by pectin methyl esterase and polygalacturonase (Pilnik & Voragen,1970; Jarvis,1984; Ben-Arie & Sonego,1979). However, according to Bourne (1983) heat can also promote the depolymerization of the protopectin and the pectin resulting in great amount of fruit softening.

The middle lamella is frequently stronger than the cell wall, with the result that when stress is applied the tissue breaks across cells walls allowing the contents of the cell to escape. In raw plant material the rupture occurs across the cell walls while in heated plant material rupture occurs in the middle lamella between intact cells (Bourne,1976). This fact could be a possible explanation to the great amount of softening observed in processed spears and slices during incubation at high temperatures.

The relationships between tissue Ca^{2+} and firmness of pericarp tissue following fresh-pack processing support the hypothesis that endogenous Ca^{2+} has a role in maintaining tissue firmness of fresh-pack processed spears.

The effectiveness of Ca^{2+} in maintaining the firmness of cucumber tissue after fresh-pack processing and during storage of pickles has been widely reported (Buescher *et al.*, 1981; Tang & McFeeters, 1983; McFeeters *et al.*, 1985; Fleming *et al.*, 1987). However, the effect of endogenous tissue Ca^{2+} concentrations previously modified in a culturally controlled manner on fresh-pack pickle firmness after blanching, and after accelerated aging at high temperatures had not been yet demonstrated. The effect of both endogenous tissue Ca^{2+} and Ca^{2+} addition to the brine solution on pickle firmness and on the rate of tissue softening during refrigeration had also not been evaluated.

McFeeters & Fleming (1989) reported that the rate of softening of pericarp tissue in slices during accelerated aging was negatively correlated with endogenous Ca^{2+} concentration. A similar relationship was observed in blanched slices after 5 days (Fig. 4), however, a much different correlation was obtained when spears sections were processed from the fruit. The rate of softening of pericarp tissue in the spears was positively correlated with endogenous tissue Ca^{2+} concentration (Fig. 4). The cause for this difference between spears and slices is not readily apparent, but it might be related to the effect of fruit section geometry on the blanching process or on the physical measurement of firmness with the shear press.

On the other hand, the endogenous Ca^{2+} concentration in the cucumbers tissue obtained by Mcfeeters & Fleming (1989) from a variety of sources and climates varied from 1.8 to 8.2 mM. In their study, endogenous Ca^{2+} seemed to be more effective in slowing softening at concentrations varying from 1.8 to 3.8 mM, that is, at very low Ca^{2+} concentrations. Evaluating the range of Ca^{2+} concentrations in the pericarp tissue as presented in Fig. 4 (4.5 to 14.0 mM), it can be observed that the endogenous tissue Ca^{2+} concentrations induced in the present study are much higher than in McFeeters & Fleming's study. Also, even in the processed slices, a high degree of variability in the correlation between pericarp Ca^{2+} concentration and the rate of pericarp softening (Fig. 4) was observed. Thus, the endogenous Ca^{2+} effect in slowing softening of either blanched spears or slices seems to occur within a relatively narrow range of Ca^{2+} concentrations within the tissue. At high tissue concentrations, variations in tissue Ca^{2+} has no effect on textural firmness.

The differences in texture observed between spears and slices following fresh-pack processing could be attributed to the larger surface area of the internal fruit exposed to the processing brine in the slices as compared to spears. In contrast, the internal tissue integrity in processed spears might have been protected during blanching, due to fruit section geometry. Such an effect has been observed by McFeeters & Fleming (1989) when pieces of mesocarp tissue

showed much higher softening rates as compared to cucumber slices. In the present study, cucumber slices exhibited higher softening rates as compared to cucumber spears.

The beneficial effect of amending the processing solution with 20 mM Ca^{2+} was apparent. This might be due to the Ca^{2+} ions saturated the binding sites that inhibited softening (McFeeters & Fleming, 1989). However, this effect was reversed in the pericarp tissue of processed spears and slices with longer period of incubation, and in pericarp and endocarp tissue during certain periods of refrigeration. This decline in texture is difficult to explain, but it seems to be related with high tissue firmness prior to incubation or refrigeration. The effect of Ca^{2+} in maintaining tissue firmness of brined cucumbers as well as in many other vegetables has been based in the cross-linking of Ca^{2+} ion to pectin molecules by eletrostatic interactions between two negatively charged carboxyl groups of pectin (Van Buren, 1979). However, blocks of at least 14 consecutive demethylated carboxyl groups on adjacent polygalacturonan molecules are required for Ca^{2+} ion cross-linking according to Kohn (1975). Pectin methyl esterase was inactivated by blanching at temperatures above 80°C, thus, maintaining high levels of methylation in the blanched cucumber tissues (McFeeters *et al.*, 1985). Also, the apparent pK's of carboxyl groups ranges from about 3.8-4.5 in dilute solutions of pectic acid as it is neutralized with NaOH (Cesaro *et al*, 1982).

In the present study, the pH of the fresh-pack processed pickles varied from 2.5 to 3.2 depending on the Ca^{2+} concentration in the processing brine. The blanching temperature was 86°C. Under these conditions, probably high levels of methylation were maintained due to the inactivation of pectin methyl esterase, and also a high level of protonation due to the pH. Thus, less negatively charged carboxyl groups on adjacent molecules would be available for Ca^{2+} cross-linking with the combination of a high degree of methylation and a high level of protonation. But, the fact that Ca^{2+} is so effective in firmness retention under these conditions, regardless of the degree of pectin methylation in the tissue (Tang & McFeeters, 1983; McFeeters & Fleming, 1989), and also at low concentrations, suggests that in addition to binding the carboxyl groups of pectin molecules, the textural effects of Ca^{2+} are also due to binding at specific texture modification sites other than pectin carboxyl groups (McFeeters & Fleming, 1989). Thus, other types of polysaccharides/Ca^{2+} interations may have an important role (McFeeters *et al.*, 1985). Losses in galactose from mesocarp cell walls have been reported by Howard & Buescher (1990) in fresh-pack pickles. Tang & McFeeters (1983) reported that galactose constitutes about 50% of the total hydrolyzable neutral sugars present in the cucumber cell wall while Wallner (1978) suggested that the removal of neutral sugars that serve as crosslinks could weaken the cell wall structure and

contribute to firmness loss, increasing also the susceptibility of pectin to degradation by polygalacturonase.

In conclusion, culturally modified endogenous tissue Ca^{2+} affected texture of fresh-pack pickles after blanching and during refrigeration, even when Ca^{2+} was added to the brine solution before blanching and storage. Ca^{2+} addition to the processing brine also improved the texture of fresh-pack processed spears and slices either after accelerated aging or during refrigeration.

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Chapter III

EVALUATION OF CALCIUM FERTILIZATION STRATEGIES FOR ENHANCING TEXTURAL QUALITY OF PICKLING CUCUMBERS

Introduction

Ca^{2+} has been shown to be important for normal cucumber plant growth and for fruit development (Frost & Kretchman, 1989) and quality (Cooper & Bangerth,1976). Cucumbers grown under Ca^{2+}-deficient nutrient solution were spindly and produced small and mishapen fruit or no fruit (Staub *et al.*, 1988). Ca^{2+} stress severely reduced cucumber root (Matsumoto & Kawasaki, 1981; Konno *et al.*, 1984). Pillowing disorder has also been associated with decreasing Ca^{2+} levels in the cucumber mesocarp tissue (Staub *et al.*, 1988).

Environmental conditions, such as mild drought stress and genotype, have been also demonstrated to influence the Ca^{2+} concentration in pickling cucumber fruit (Engelkes *et al.*, 1990). Thus, low soil moisture resulting in poor water movement through the plant can result in localized Ca^{2+} deficiency (Geraldson, 1979). Tang & McFeeters (1983) and Fernandes & Widders (1986) reported that endogenous Ca^{2+} concentrations in fresh cucumber fruit tissue were highly correlated with the rate of pericarp softening in brine following blanching or during post-harvest storage.

The question is how to enhance Ca^{2+} levels within the fruit tissue. Ca^{2+} is the most immobile macronutrient in the plants. Thus problems that arise with Ca^{2+} nutrition in plants are

frequently related to redistribution and mobility (Ferguson, 1979). Ca^{2+} is not readily remobilized from mature leaves into developing fruit such as during periods of low uptake by the plant (Shear, 1975; Hanger, 1979; Bengtsson & Jensén, 1983). Due to the immobility of Ca^{2+} in phloem tissues (Biddulph *et al.*, 1961; Zimmermann, 1966; Ringoet *et al.*, 1968; Ferguson & Clarkson, 1976), Ca^{2+} accumulation within developing fruit occurs at a relatively slow rate (Hanger, 1979).

In order to meet the requirements of growing tissues, most of the Ca^{2+} is thought to be translocated via the xylem into the fruit tissue through the transpiration stream (Marschner, 1986). Water loss by transpiration from fruit, however, is quite low and declines due to an ontogenetic decrease in the surface area to volume ratio of the fruit (Hanger, 1979). All of these factors contributes to the low tissue Ca^{2+} concentrations in fruits.

Foliar applications of nutrient solutions provide a potential means for supplying mineral nutrients directly to specific organs of a plant at critical stages of plant development (Biddulph *et al.*, 1959; Mason, 1979; Tibbitts & Palzkill, 1979; Richardson & Lombard, 1979). However, foliar application is not considered to be an effective means of supplying Ca^{2+} to plants (Hanger, 1979). Chelating compounds enhance the solubility of Ca^{2+} in solution, and thus the potential absorption of Ca^{2+} by leaf tissue following foliar application (Millikan & Hanger, 1965; Wiersum, 1979).

In cucumber plants, fruit and root growth compete for photosynthates. Consequently, during fruit set and net growth, the rate of root growth is significantly reduced (McCollum, 1934; DeStigter, 1969; Barret & Amling, 1978). The proportion of plant dry weight in the roots of fruiting plants was reported to decline during plant ontogeny from 0.38 to 0.09 while in defruited plants the proportion of roots decreases gradually from 0.37 to 0.30 (Hall, 1977). Sink competition between fruits and roots is important for the uptake of water and mineral nutrients (Stigter, 1969; Marschner, 1986). Root activity and thus nutrient uptake by the roots, in particular Ca^{2+}, declined with the onset of the reproductive stage (Marschner, 1986). Ca^{2+} uptake by the root system was lower in fruiting than in non-fruiting trees (Hansen, 1973).

It is, therefore, hypothesized that Ca^{2+} concentrations in xylem sap of pickling cucumber plants would decline during plant ontogeny, specifically during reproductive development, as a result of reduced new root growth. Thus, Ca^{2+} supply to developing fruit might be limited.

The objectives of the present study were: a) to evaluate alternative Ca^{2+} fertilization strategies for enhancing the Ca^{2+} concentration and thus textural quality of pickling cucumber fruit under field conditions, and b) to understand better how fruit set and growth might affect Ca^{2+} supply via the xylem to the fruit.

Materials and Methods

A field experiment was conducted in 1990 at the Michigan State University Horticulture Research Center, in which pickling cucumbers, cv Flurry (Asgrow Seed Company, Kalamazoo, MI) were precision planted with a Heath vaccum planter on 2 July 1990. The cucumbers were planted into 9 flat beds spaced 2.3 m between centers with 3 rows per bed. Between row spacing was 71 cm and plants within the row were thinned to 7.6 cm between plants at the cotyledonary stage.

A randomized complete block design was used for this experiment. Each block consisted of a bed of three rows, 53.5 m long,with 5 treatment plots each 7.7 m long and separated by 2.1 m of guard bed. All blocks were separated from neighboring blocks by guard rows. Complete blocks were replicated four times.

The Ca^{2+} fertilizer treatments applied were :

(1) *Nutri-Cal foliar applied:* A 2.5% solution of Nutri-Cal (25 ml/l) was prepared to which was added a surfactant, L-77 (Union Carbide) (0.5 ml/l). Only deionized water was used in preparation of these solutions. At each application time, 912 ml of this solution was sprayed unto the foliage of each plot (7.7 m of bed). This application rate is equivalent to 6 quarts Nutri-Cal/acre (1.49 Kg Ca/ha) per application.

Two applications of this formulation of Nutri-Cal were made; 9 and 16 August 1990. At these times, the cucumber

plants were at the following developmental stages, fruit set and during a period of rapid expansive fruit growth, respectively.

(2) *Nutri-Cal foliar applied:* A 1.25% solution of Nutri-Cal (12.5 ml/l) was prepared to which was added 0.5 ml/l of L-77 surfactant. At each application time, approximately 912 ml of this solution was sprayed unto the foliage of each plot (7.7 m of bed). This application rate was equivalent to 3 quarts Nutri-Cal/acre or 304 g Ca^{2+}/acre (0.745 Kg Ca^{2+}/ha) per application.

Three applications of this formulation of Nutri-Cal were made at 4 day intervals: 9, 13, and 16 August 1990. The intention was to make a total of 4 applications of the 1.25% Nutri-Cal solution so as to apply a total amount equivalent to treatment 1. The fruit however,reached harvestable maturity before the fourth application could be made. It must be noted, therefore, that the total cumulative application of Ca^{2+} was 912 g Ca^{2+}/acre (2.24 Kg Ca^{2+}/ha) for treatment 2 as compared to 1.2 Kg Ca^{2+}/acre (2.979 Kg Ca^{2+}/ha) for treatment 1.

(3) *$CaCl_2$ foliar applied*: A solution containing 9.744 g $CaCl_2$/l plus 0.5 ml/l L-77 surfactant was prepared. At each application time, 912 ml of this solution was sprayed unto the foliage of each plot (7.7 m of bed). This application rate is equivalent to 603 g Ca^{2+}/acre (1.49 Kg Ca^{2+}/ha) per application. In terms of net amount of Ca^{2+} applied, this treatment is identical to treatment 1, but without the chelate

in the solution. Two applications of this solution were made during the fruiting period; 9 and 16 August 1990.

(4) *$CaCl_2$ side dressed:* At vine tip-over, 31 July 1990, 22.7 Kg Ca/acre (62.8 Kg/ha $CaCl_2$) were side-dress applied to the pickling cucumbers. A band of $CaCl_2$ salt was placed into a furrow approximately 15.3 cm to the side of the row and 6.4 cm deep and covered up.

(5) *Control:* To all control plants, a solution containing deionized water plus 0.5 ml/l of L-77 surfactant was applied foliarly. Applications were made on 9, 13, and 16 August 1990.

Nutri-Cal (8% Ca^{2+} solution) is a commercial foliar fertilizer in which the Ca^{2+} is chelated by 2,3,4,5 trihydroxypentanedoic acid (a trihydroxygluterate).

Fruit were once-over destructively harvested from 6.11 m of bed from each treatment plot on 20 August 1990. The fruit were then taken to a grading station, graded into commercially inportant size grades (#1a, 1b, 2a, 2b, and 3) including oversize fruit (> 5 cm diameter) and misshapen fruit and then weighed.

Fruit yield of fresh cucumbers was determined for each of the size grade fractions. A subsample of ten size #3 fruit (3.8 - 5.0 cm diameter) were randomly collected and evaluated for fruit quality, including fruit L/D ratio (length/diameter), seed cavity diameter, % seed cavity,

relative seed size, internal quality defects including placental hollows and the separation of the carpels.

Fruit texture was measured on an additional sample of eight size #3 fruit. A transverse slice, 7 mm thick, was cut from the middle section of each fruit. Texture (firmness) of the pericarp (fruit wall), and the endocarp (seed cavity) tissues were measured for each slice using a shear press (TMS-90, Food Technology Corporation). A minimum of 2 replicate measurements were made for each tissue on each slice in order to obtain an accurate determination.

Fruit tissue Ca^{2+} concentration was determined for the individual fruit evaluated for texture. Both pericarp and endocarp tissues were sampled and weighed. The fruit tissue samples were then dehydrated at 60°C in a force air drying oven for a minimum of 72 h and then reweighed. The tissue were ground in a Wiley mill to pass a 20 mesh screen and an 0.1 g subsample wet ashed in H_2O_2 and $HClO_4$ after the method of Adler and Wilcox (1985). Ca^{2+} concentration in diluted extracts was determined by atomic absorption spectrophotometry (Video 12 AA, Instrumentation Laboratory).

Leaf samples (first fully expanded leaf approximately the 5^{th} leaf from the vine apex) were collected from each treatment plot at 4 times during plant development for Ca^{2+} analysis. Times of collection were prior to foliar applications (8 August), mid-fruit development period (approximately 6-8 days after 1^{st} application) (15 August), harvest time (22 August),

and approximately a week following harvest (29 August, 1990). Leaves were separated into petiole and lamina tissue, rinsed with deionized water to remove potential surface contamination, dehydrated in a forced air oven at 60°C for 72 h and then ground in a Wiley mill. Following wet ashing as described by Adler & Wilcox (1985), the extracts were analyzed for Ca^{2+} by standard procedures for atomic absorption spectrophotometry.

Xylem Exudate Collection

Xylem exudate collection was conducted during vegetative development (August 2), at anthesis (August 8), at mid-fruit development period (August 15), at typical time of first harvest (August 22), and a week following harvest (August 29) in 1990. Since a positive root pressure existed during the early morning hours, all collections were initiated at 8-8:30 a.m. Five vigorous healthy plants were sampled per replication. Two treatments were sampled, the control and the $CaCl_2$ side-dress treatments with three replications. Sampling was taken at two times, at the 1^{st} and at the 2^{nd} hour after decapitation of the stem.

Xylem Sap Collection Procedure

Plants were decapitated immediatly above the cotyledonary node at approximately 8-8:30 a.m. The cut surface was rinsed with deionized water to remove any potential contamination,

and then gently blotted with filter paper. A section of acid rinsed rubber tubing (≈ 15 cm long) was gently slipped over the cut end of the stem and the open end of the tube was sealed with parafilm. After one hour, the exuded sap from the plant stump was collected from the tube using a transfer pipette and transferred to a 20 ml vial. After a second hour the collection was repeated. The vials were then frozen on dry ice and stored at -20°C for Ca^{2+} analysis.

Defruiting Experiment

In order to evaluate the effects of fruiting on the ability of the plants to take up Ca^{2+}, xylem exudate was collected from fruiting and defruited plants.

Sample Collection

In the center row of the guard bed, approximately 15 to 20 plants were completely deflowered on August 16, 1990. The plants had fruit up to approximately 2.5 cm in diameter at that time. On August 31, 1990 both xylem exudate, as previously described, and leaf samples were collected from 5 plants in each of 4 replications, from fruiting and defruited plants. Xylem exudate and leaf tissue samples were analyzed for Ca^{2+} concentration as described utilizing atomic absorption spectrophotometry.

Statistical Analysis

All data were analyzed statistically by analysis of variance. The LSD test was used to compare treatment means if a significant $F<.05$ value was obtained. Correlations were investigated between tissue Ca^{2+} concentrations and fruit firmness.

Results

The 1990 growing season was ideal for pickling cucumber production. An excellent stand was obtained following precision seeding and the favorable environmental conditions during July and August resulted in good vine growth, fruit set and development. At no time was the crop exposed to a significant water stress as evidenced by the lack of foliar wilting.

The Ca^{2+} treatments did not affect yield following a once over destructive harvest. The yield varied from 12.2 to 14.7 Kg/plot (13.97 m^2). The fresh weights of each of the individual size grade fractions of marketable fruit, #1a to #3, and of total yield were similar among all the treatments.

Morphological quality parameters of #3 size grade pickling cucumber fruit were also not affected by either the foliar or the side-dress applications of Ca^{2+} as compared to the control.

The firmness of fresh pericarp (fruit wall) tissue in pickling cucumber fruit was affected by the foliar application

on a 2.5% (v/v) solution of Nutri-Cal (Table 1). These fruit had significantly higher pericarp tissue firmness as compared to fruit from non-Ca^{2+} fertilized plants (controls). The side-dress application of $CaCl_2$ did not influence fruit texture. Endocarp (seed cavity) tissue firmness was much lower, approximately 5.5 newtons, than the firmness of pericarp tissue, 22.5 - 24.8 newtons (Table 1). The endocarp tissue was not affected by Ca^{2+} fertilization, regardless of whether foliar or side-dress applications were made.

The concentration of Ca^{2+} within the pericarp tissue of size grade #3 fruit was enhanced by both the foliar application of Nutri-Cal (2.5%, v/v) solution and the side-dress application of $CaCl_2$ (22.7 Kg Ca/acre) which showed significantly higher Ca^{2+} concentrations in pericarp tissue than the foliar applied with $CaCl_2$ or non-Ca^{2+} fertilized plants (controls) (Table 1).

The application of a more diluted Nutri-Cal solution (1.25%, v/v) also appeared to increase the tissue Ca^{2+} level above the control, however, the response was not statistically significant at the 5% level. None of the Ca^{2+} fertilization treatments influenced endocarp tissue Ca^{2+} concentration. It should be noted that Ca^{2+} concentrations were also much lower in endocarp as compared to pericarp tissue. No correlation was found between tissue Ca^{2+} concentrations and fruit firmness (data not shown).

Table 1 - Effect of foliar applications of Nutri-Cal (8%) and $CaCl_2$ solutions and sidedress applications of $CaCl_2$ on Ca^{2+} concentrations of fruit and leaf tissues and on firmness of size grade #3 pickling cucumber fruit.

Ca^{2+} treatment	Application method	Ca^{2+} concentration (% dry wt)				Fresh fruit tissue firmness (newtons)	
		Fruit tissue		Leaf tissue			
		Pericarp	Endocarp	Petiole	Lamina	Pericarp	Endocarp
Nutri-Cal (2.5%)[z]	foliar	0.67	0.31	2.62	3.14	24.8	5.5
Nutri-Cal (1.25%)	foliar	0.63	0.31	2.65	3.44	23.5	5.3
$CaCl_2$	foliar	0.58	0.30	3.01	3.16	23.7	5.4
$CaCl_2$	sidedress	0.70	0.31	2.75	3.36	22.5	5.7
Control[y]		0.57	0.29	2.46	3.23	22.8	5.7
LSD (P = 0.05)		0.07	NS	NS	NS	1.5	NS

[z] A surfactant, L-77 (an organo-silicon based surfactant, Union Carbide) was added to all foliar applied solutions (0.5 ml/l).
[y] Water containing the surfactant, L-77, was applied three times to all control plants.

Leaf petiole Ca^{2+} concentrations ranged from 2.5 to 3.0% dry weight while lamina tissue concentrations were comparatively higher, 3.15 to 3.4% dry weight (Table 1). Leaf tissue Ca^{2+} concentrations were not affected by the various Ca^{2+} fertilization treatments.

Ca^{2+} concentration in the xylem sap was significantly higher in defruited plants than in fruiting (control) plants (Table 2). Ca^{2+} concentrations (% dry weight) either in lamina or in petiole tissues in defruited plants were also higher than in the plants from the control, although statistically the differences were only significant at the 7% level (Table 2). The time of sampling highly influenced the Ca^{2+} concentration in the xylem exudate showing the second hour to be significantly higher in Ca^{2+} concentration than the first hour of sample collection (Table 2).

Ca^{2+} concentrations within the xylem sap decreased during plant ontogeny (Fig. 1). Following the onset of flowering, the concentration of Ca^{2+} in the xylem sap decreased, and continued to decrease during reproductive development. A small increase was observed at 58 days, however. At this time the fruit, which had set, were approaching maturity (Fig. 1).

Discussion

Multiple foliar applications of Nutri-Cal (8% Ca) at a concentration of 2.5% (v/v) to pickling cucumber plants during

Figure 1. Changes in xylem exudate Ca^{2+} concentrations during vegetative and reproductive development of pickling cucumber plants (LSD<.01).

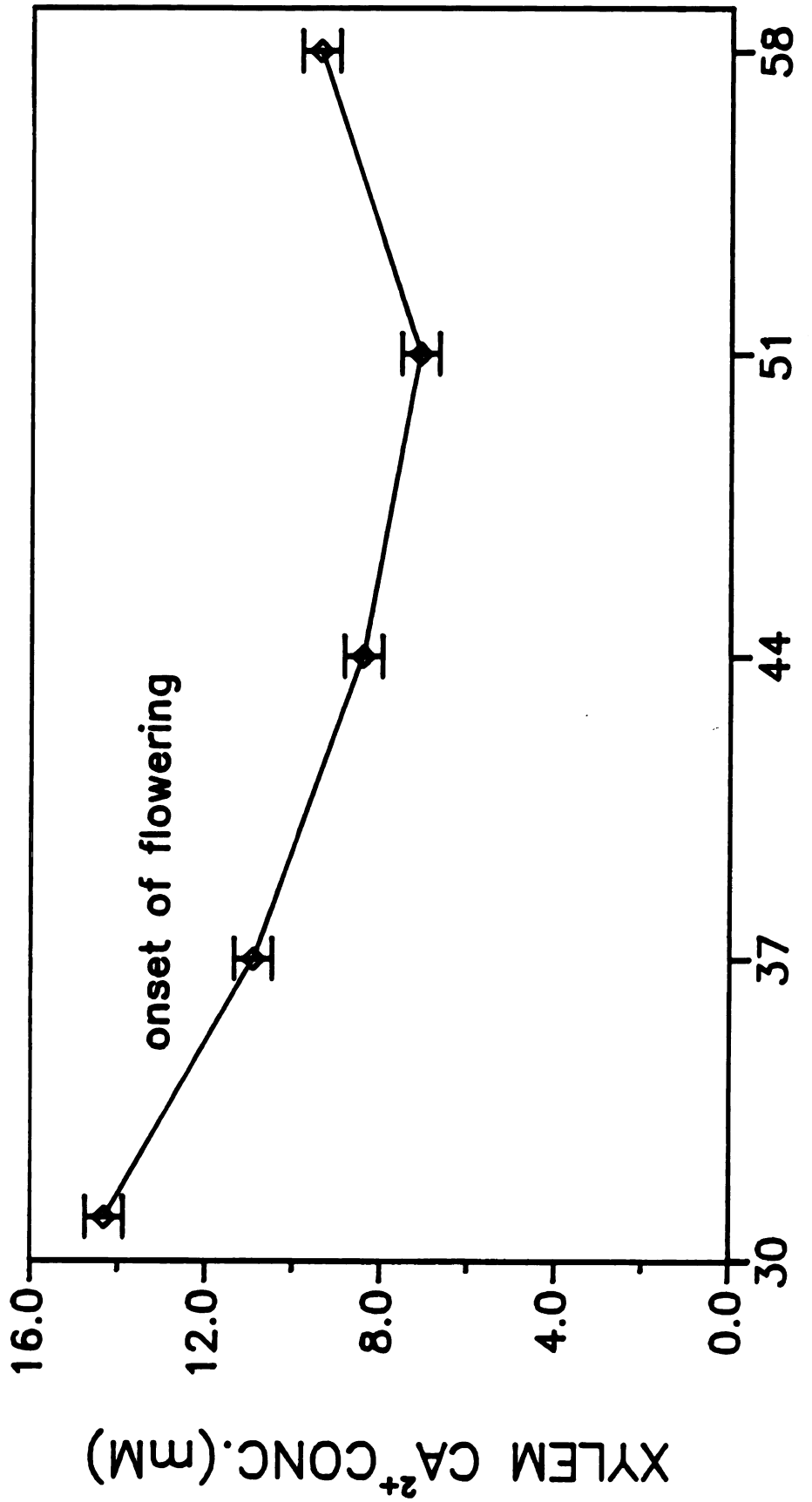
XYLEM CA^{2+}CONC.(mM)
16.0
12.0
8.0
4.0
0.0
30
37
44
51
58
DAYS AFTER PLANTING
onset of flowering

Figure 1

Table 2 - Influence of defruiting on Ca^{2+} concentrations in xylem exudate and leaf tissues and of time of sampling on xylem exudate Ca^{2+} concentrations in pickling cucumber plants.

Treatments	Xylem [Ca] (mM)	Total Ca^{2+} (% dry wt)	
		Lamina	Petiole
Control	5.72	2.66	3.05
Defruited	6.74	3.95	3.68
F test sign.	**	7%	7%

Time of sampling	Ca^{2+} concentration in xylem exudate (mM)
First hour	4.64
Second hour	7.79
F test sign.	***

***, **, significant at the 0.1% and 1% level, respectively.

fruit set and development increased the firmness of pericarp tissue in fresh #3 size grade fruit. It is not known if this effect would be apparent in the tissue following fresh-pack processing.

The physiological mechanism by which fruit firmness was increased by the foliar application of Nutri-Cal is not fully clear. The relatively high pericarp tissue Ca^{2+} concentration in fruit from plants treated with Nutri-Cal (2.5% solution) might indicate a relationship between Ca^{2+} levels in the fruit and tissue texture. However, the side-dress application of $CaCl_2$ which resulted in the highest pericarp tissue Ca^{2+} concentration, 0.70% dry weight, did not lead to firmer fruit. In addition, Ca^{2+} concentration within individual fruits was not correlated with textural firmness (data not presented). This suggests that Ca^{2+} might be involved indirectly in altering tissue texture or that there are other unknown factors which might be producing the response. Differences in apoplastic Ca^{2+} concentrations could be a factor influencing fruit firmness.

It should also be noted that although sidedress applications of $CaCl_2$ appear to have increased the pericarp tissue Ca^{2+} concentration (Table 1), there clearly was no effect of this treatment on fruit tissue texture (Table 1). This result is consistent with the notion that enhanced fruit Ca^{2+} is not a primary factor affecting textural characteristics of fresh cucumber fruit. Changes related to sugars in pectic

polymers during cucumber fruit development must be considered. The positive correlation (r=0.85) between the fresh weight concentration of cell wall sugars and cucumber firmness found by McFeeters & Lovdal (1987) suggests that firmness could be predicted by the total cell wall sugar content. Also, according to the same authors, since the galacturonic acid/sugar ratios increased during cucumber growth, this could indicate that the pectic substances with fruit development had fewer or shorter neutral sugar side chains, but it could also mean that the amounts of these sugars in the hemicellulose fraction of the wall declined. They found high positive correlations between the galacturonic acid/rhamnose and galacturonic acid/arabinose molar ratios in mesocarp cell walls and the development of the cucumber fruit (diameter) (r=0.95 and r=0.96, respectively).

It is well established from experiments in which pickling cucumber plants were cultured in nutrient solutions modified in Ca^{2+} concentration that high fruit Ca^{2+} levels were associated with firmer fruit after fresh-pack processing (Fernandes *et al.*, 1990).

One potential hypothesis which should be considered in evaluating the results of this study is that the chelator being used in the Nutri-Cal formulation, 2,3,4,5 trihydroxypentanedoic acid, might be inducing a specific physiological response that impacts ultimately upon fruit texture. At this time one can only speculate how this might

occur. Recent research has shown that applications of certain chelating compounds such as oxalate can induce a systemic resistance to foliar pathogens within cucurbit species such as cucumbers and squash (Doubrava *et al.*, 1988). An associated physiological response is increased lignification of cell walls in leaf and potentially fruit tissue (Dean & Kuc, 1987). If lignification does occur in fruit tissue, one would expect that it would alter significantly textural characteristics. Research is needed to verify if the chelator present in Nutri-Cal might have such physiological activity.

The low amount of Ca^{2+} applied to the cucumber plants by the Nutri-Cal solution also places in question any potential effect of Ca^{2+} on fruit texture. In this study, the amount of Ca^{2+} applied (1.49 Kg/ha) achieved an increase in both fruit tissue firmness and in Ca^{2+} concentrations (Table 1). However, this amount corresponds only about 37% of the total amount of Ca^{2+} which accumulates in the fruit to be harvested (4.0 Kg Ca^{2+}/ha). This assumption was made considering that a typical yield gives approximately 17,000 Kg/ha. This amount in turn corresponds to 680 Kg dry matter/ha considering that the dry weight/fruit weight ratio is 0.04 (Fernandes & Widders, 1990) and the mean Ca^{2+} concentration in whole cucumber fruit is 0.006 g/g dry weight (Engelkes,1987). The question which needs to be addressed is whether increasing the amount of Ca^{2+} supplied by the Ca^{2+} fertilizers would influence the response

of pickling cucumbers in relation to tissue Ca^{2+} concentrations and fruit firmness.

Ca^{2+} Concentrations in Xylem Exudate

The decrease observed in Ca^{2+} concentration in xylem exudate during the reproductive stage (Fig. 1) coincides with the decrease in cucumber root growth during fruit development observed by several researchers (McCollum, 1934; De Stigter, 1969; Van der Vlugt, 1986; Van der Vlugt, 1987; Van der Vlugt, 1990). Furthermore, De Stigter (1969) observed that the decline in root growth of cucumber plants continued until harvest when a recovery in growth occurred after the fruit were removed from the plant. He pointed out that it is possible that roots might have grown slightly if the fruit had been left on the plant. These findings are consistent with the results of the present study, with respect to the ontogenetic decline in Ca^{2+} concentration in the xylem exudate and the small increase at fruit maturity (Fig. 1). The question is to what degree non growing roots continue to carry on their functions of water and mineral absorption (De Stigter, 1969; Marschner, 1986). As it has been reported by Hansen (1973), Ca^{2+} uptake by roots is lower in bearing than in non-bearing trees.

It is possible that Ca^{2+} uptake and subsequent transport through the xylem is impaired during fruit development, due to

the root-fruit competition for assimilates and the associated reduction in root growth. This is supported by the observation that defruited plants had a significantly higher Ca^{2+} concentrations in xylem exudate than fruiting plants (Table 2). If one assumes that Ca^{2+} concentrations in xylem sap remains constant over time, then the level of Ca^{2+} in the fruit would be a function of the amount of time that the fruit requires to reach maturity. The longer the fruit requires to reach maturity, the higher the concentration of Ca^{2+} at harvest time. However, subsequent enlargement of the fruit dilutes the calcium in the fruit (Shear, 1975). This dilution effect has been reported by Widders & Price (1984), Engelkes, (1987), and Engelkes *et al.*(1990) to be accentuated through ontogeny, in which Ca^{2+} concentrations in pickling cucumber fruit tissues declined during fruit development.

The competition between sinks can also be observed in relation to Ca^{2+} concentrations in lamina and petiole in leaf tissues (Table 2). McCollum (1934) and Hall (1977) discussed the differential sensitivity showed by the non-fruit organs, roots and leaves, to fruit growth. In fruiting plants (Hall, 1977), the leaves exhibited slower growth rates and reduced lamina length which were not reversed until fruit growth rate declined, however, net assimilation rates of fruiting plants exceeded those of defruited ones during most of the post-anthesis period, irrespective of the use of functional or total leaf area as a basis for the calculation of this index.

In the defruited plant, according to McCollum (1934), a marked increase in development of all vegetative parts is observed, including the roots, accompanied by an increase in absorption of nutrients. The root-fruit relationships as related to the decreasing levels of Ca^{2+} in the xylem exudate during the reproductive stage of cucumber plants might provide a partial explanation for the low Ca^{2+} concentrations in pickling cucumber fruit (Engelkes, 1987; Engelkes *et al.*,1990).

In conclusion, these results seem to support the hypothesis that Ca^{2+} concentrations in xylem sap and thus Ca^{2+} uptake decline during plant ontogeny mainly during fruit development as a result of reduced new root growth. As a consequence, the availability of Ca^{2+} through the xylem to developing fruit is limited, and may account in part for the relatively low Ca^{2+} content in fruits.

Although foliar applications of Ca^{2+} fertilization treatments enhanced fruit pericarp tissue Ca^{2+} more than 15%, these changes were not related with changes in fresh tissue textural firmness.

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SUMMARY

The objectives of this study were to evaluate the effects of modified endogenous calcium (Ca^{2+}) within fruit tissues on texture and the rate of softening of fresh, stored and processed pickling cucumber fruit, to determine the influence of low tissue Ca^{2+} levels on polygalacturonase (PG) activity in fresh and stored fruit, and to verify the effect of Ca^{2+} addition to the brine solution on texture after fresh-pack processing. Alternative Ca^{2+} fertilization strategies for enhancing fruit Ca^{2+} concentrations in field- grown pickling cucumbers, and how fruit set and growth might affect Ca^{2+} supply via the xylem to the fruit were also investigated.

Firmness of freshly harvested fruit was not affected by the Ca^{2+} fertilization treatments (Table 1, Appendix), but Ca^{2+} might have an important role on firmness retention during storage, considering the higher firmness and lower rate of softening in the pericarp tissue of the high Ca^{2+} treatment (20.0 mM Ca^{2+}) after 5 days of storage (Experiment 2, Table 3). The positive correlations between fruit Ca^{2+} concentrations and fruit firmness after 5 and 24 days of storage also add support for endogenous fruit Ca^{2+} having a role in fruit softening during storage. Physiological processes involving Ca^{2+} during storage and post-harvest processing do not take place in freshly harvested fruit. Perhaps, because in the freshly

harvested fruit still at the immature developmental stage, the macromolecules structures within the protoplast are well stabilized at the micromolar levels of free calcium (10^{-6} M or less), thus maintaining the normal cell functions. Any variation in cytosolic Ca^{2+} levels in response to environment (Kretsinger, 1977) as in the case of stored fruit has physiological implications as a liquid-gel phase change in the membranes.

Leshem *et al.* (1986) reported that the passage of external Ca^{2+} across the membrane into the cytosol, or entry into the cytosol of Ca^{2+} from Ca^{2+}-containing organelles sets a "cascade effect" into motion due to the combination of Ca^{2+} with cytosolic calmodulin (CM). The complex CA:CM activates phospholipase-A_2 which causes the liquid-gel phase change, hastening the membrane deterioration process. The fatty acid tails in the bilayer become "frozen" and completely lose their motional freedom. As a result, the membrane becomes rigid and the embedded proteins are no longer able to move. Leaks are formed and overall function impaired.

External high Ca^{2+} concentrations maintain membrane integrity and consequentely firmness retention while within the cytosol Ca^{2+} exerts an opposite effect. In the present study, the culturally modified endogenous Ca^{2+} within the cucumber fruit ranged from 0.08% to 2.4% Ca^{2+} and from 0.05% to 0.5% Ca^{2+} in pericarp and endocarp tissues, respectively. It was observed in the field experiment, however, that the

foliar Ca^{2+} applied affected only slightly the Ca^{2+} concentrations within the fruit tissues as compared with the broad range of Ca^{2+} concentrations found in the fruit tissues from greenhouse grown cucumbers. Thus, the relationships between the concentrations of Ca^{2+} within compartments as the apoplast, and fruit firmness in field cultured cucumbers should be considered in a further study.

PG activity of fresh fruit was not affected by the Ca^{2+} fertilization treatments (Table 1, Appendix).

In fresh fruit the concentration of total cell wall polysaccharides influenced more texture than the molecular structures of those polysaccharides (McFeeters & Lovdal, 1987). Also, the decrease in rhamnose, arabinose, and galactose observed during fruit development of fresh cucumbers suggests that other enzymes than polygalacturonase might be involved in the softening process of fresh pickling cucumber fruit. This is also applicable to the processed fruit. Losses in galactose from mesocarp cell walls have been observed in fresh-pack pickles (Howard & Buescher, 1990). Thus, other enzymes such as β-galactosidase could be involved in the softening process of cucumbers. It is interesting to verify also how the mechanism of Ca^{2+} action in the cell wall might relate to this enzyme.

Ca^{2+} enhanced the textural firmness of fresh-pack pickles following blanching and during refrigeration, even when Ca^{2+} was added to the brine solution before blanching and storage.

Ca^{2+} added to the brine solution also improved texture of fresh-pack pickles after accelerated aging and during refrigeration.

However, higher pericarp softening was observed in the processed spears and slices with 20 mM Ca^{2+} added to the brine solution as compared to 0 or 5 mM Ca^{2+} brine treatment during incubation at 46°C. The rate of softening was also higher in the high Ca^{2+} brine treatment (20 mM Ca^{2+}) than in the 0 or 5 mM Ca^{2+} brine treatments during refrigeration of processed slices. Changes in solubility of the pectic polymers could have occurred during incubation time or refrigeration period since changes in solubility characteristics can be caused by exposure to low pH and elevated salt environment of the pickling solution (Buescher & Hudson, 1986). Acidification due to H^{+} ions in the brine solution could cause displacement of Ca^{2+} ion from the cross-linking of galacturonic acid residues. Howard & Buescher (1990) reported that firmness of fresh-pack pickles coincided more closely with the amount of Ca^{2+} bound to the cell walls than to other cell wall characteristics. In the light of these comments, changes in bound Ca^{2+} should be investigated during either the incubation time or the refrigeration period.

Foliar applications of 2.5 % Nutri-Cal (v/v) enhanced significantly pericarp firmness of fresh #3 size grade pickling cucumber fruit. The relatively high pericarp tissue Ca^{2+} concentration in fruit from plants treated with Nutri-Cal

(2.5 % solution) might indicate a relationship between Ca^{2+} levels in the fruit and tissue texture.

A potential hypothesis is that the chelator being used in the Nutri-Cal formulation, 2,3,4,5 trihydroxypentanedoic acid, might be inducing a specific physiological response that impacts upon fruit texture. An associated physiological response is increased lignification of cell walls in leaf and potentially fruit tissue which would alter significantly textural characteristics (Dean & Kuc, 1987).

Thus, investigations should be conducted to evaluate if the chelator used in the present study induces some physiological response such as increased lignification of cell walls in leaf and fruit tissues which is an important factor influencing texture.

In this study, Ca^{2+} concentrations in xylem exudate decreased during the reproductive stage with a small increase at fruit maturity. A decrease in cucumber root growth during fruit development has been observed (McCollum, 1934; Stigter, 1969; Van der Vlugt, 1990) extending until harvest when a recovery in growth occurred after the fruit were removed from the plant or at fruit maturity (Stigter, 1969). It is possible that Ca^{2+} uptake and transport through the xylem is impaired during fruit development, due to the root-fruit competition for assimilates and the reduction in root growth. Defruited plants also showed significantly higher Ca^{2+} concentrations in xylem exudate than fruiting plants.

In conclusion, the results to support the hypothesis that Ca^{2+} concentrations in xylem sap and thus Ca^{2+} uptake decline during plant ontogeny. This factor could contribute in part for the relatively low Ca^{2+} content in fruits.

Appendix

Table 1 - Effect of Ca^{2+} treatment level on firmness and PG activity of fresh 4.5 cm diameter pickling cucumber fruit tissues (Experiment 1).

Ca^{2+} treatments	Firmness (Kg)			PG activity (units x 10^3/g)
	Fruit tissue			Endocarp tissue
	Pericarp	Mesocarp	Endocarp	
0.01	0.96	0.70	0.16	1.29
1.0	0.95	0.72	0.17	0.96
20.0	0.92	0.70	0.18	1.13
F test significance	NS	NS	NS	NS

NS nonsignificant.

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