THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Food Science)
DEGREE

Food Science
FIELD

Food Science and Technology
DEPARTMENT

TITLE: Effects of Calcium Chloride on the Qualities of Frozen Fruits

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THESIS

EFFECTS OF CALCIUM CHLORIDE ON THE QUALITIES OF FROZEN FRUITS

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Food Science)
Graduate School, Kasetsart University
2012

Arpassorn Sirijariyawat 2012: Effects of Calcium Chloride on the Qualities of Frozen Fruits. Doctor of Philosophy (Food Science), Major Field: Food Science, Department of Food Science and Technology. Thesis Advisor: Associate Professor Sanguansri Charoenrein, Ph.D. 115 pages.

In the first experiment, the effect of the freezing process on the freezing characteristics of apple, mango, cantaloupe, and pineapple cubes were investigated. The results confirmed the negative correlation between the freezing point and total soluble solidss content of fruit samples. Mango had the highest total soluble solidss content and the lowest freezing point, while pineapple showed the highest freezing rate. In the second experiment, the roles of calcium on the physico-chemical properties of frozen fruits were studied by immersing fruit sample cubes in a 1% calcium chloride solution before frozen at -40°C. The presence of calcium in these fruits did not significantly affect the microstructure and total pectin content both before and after the freezing and thawing process, whereas it caused a decrease in the water soluble pectin content of all fresh and frozen-thawed fruits except for pineapple. For the fresh fruit, calcium significantly increased the firmness of cantaloupe and mango. However, subsequent to freezing and thawing, only the frozen-thawed calcium treated mango had a significantly higher firmness and lower drip loss than the untreated samples. The firmness scores of the fruits tested by trained panelists corresponded with the results from the instrument measurements and the panelists did not detect the bitterness of calcium chloride in the frozen-thawed samples. In the third experiment, the effect of pectin methylesterase and calcium infusion on the improvement of the texture of both fresh and frozen-thawed mango cubes was investigated. The weight gain, moisture content, calcium content, and pectin methylesterase activity of the mango samples were greater at relatively high vacuum levels (10 kPa). The fresh samples infused with pectin methylesterase and calcium at 10 kPa had significantly higher firmness than that of the fresh control mango. For the frozen-thawed mangoes, the samples infused with both pectin methylesterase and calcium at 50 kPa and at atmospheric pressure were found to be of superior texture and microstructure in comparison with the control frozen-thawed samples.

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ACKNOWLEDGEMENTS

This thesis could not complete without the guidance and the help of several individuals who contributed their valuable assistance in the preparation and completion of this study. First and foremost thanks to my advisor, Assoc.Prof. Sanguansri Charoenrein, for her great advice and assistance. I would like to gratefully thank Dr. Sasitorn Tongchitpakdee, my thesis co-adviser, Asst. Prof. Wannee Jirapakkul, examination chairperson, and Assoc.Prof. Thanong Pukrushpan, external examiner, for their valuable comments and suggestion and Dr. Diane M. Barrett for her kindly suggestion and supervision during I worked at University of California, Davis.

I would like to sincerely thank for all of my friends and staffs at Kasetsart University and at University of California, Davis for the friendship and all of their help.

This research was supported by grant funds under the program of Strategic Scholarships for Frontier Research Network for the Joint Ph.D. Program Thai Doctoral degree from the Office of the Higher Education Commission Thailand.

The last special thanks to my family. My graduation would not be achieved without their encouragements.

Arpassorn Sirijariyawat

January 2012

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INTRODUCTION

One of the greatest challenges for food technologists is to maintain the quality of food products for an extended period. A decrease in temperature generally decreases the rate of chemical reactions that are responsible for the deterioration in food quality over time, therefore freezing is frequently used to extend the shelf life of food products (Lim *et al.*, 2004). Many fruits are frozen to extend their availability throughout the year, and to further shelf through lengthy distribution. Frozen fruits have an excellent safety record and there have never been any cases of food poisoning, because microorganisms do not grow when the temperature is -10°C or lower (Torreggiani and Maestrelli, 2006).

However, freezing is not a perfect method of preservation, because even at low temperatures deterioration of quality may still occur. The formation of ice results in textural changes and disruption of cell compartments that cause the release of chemically reactive components (Lim *et al.*, 2004). Although increasing the freezing rate can reduce the possibility of the formation of large ice crystals in fruits, tissue damage is still unavoidable due to the presence of large amounts of water. Fruits contain more water than meat and have a cellular structure of the cell wall which is less elastic than the cell membrane, which sensitive to the growth of large ice crystals during freezing. Fruits are the most difficult of all products to freeze without causing changes in their appearance, texture, flavor, and color. A major result of fruit freezing is a loss of tissue firmness (Coggins and Chamul, 2004). Loss of water holding capacity is also a problem for many frozen fruits. Such fruits exhibit excessive drip loss on thawing, and may lack proper juiciness when chewed (Kerr, 2004). Several factors can influence the quality of frozen fruit and two important factors are the raw material and pretreatments (Torreggiani and Maestrelli, 2006).

Calcium plays a paramount role in maintaining fruit quality. Calcium is reported to preserve firmness by cross-linking with the cell wall and the middle lamella pectins, thus stabilizing the cell walls and affecting the cell turgor potential (Mignani *et al.*, 1995; Sato *et al*., 2006). Previous research has revealed that calcium pretreatment is able to improve the quality of some frozen fruits, but was found to have no effect on other types of fruit. Such findings are potentially influenced by both the immersion condition and the properties of the fruit. Alonso *et al.* (1997) reported that combined thermal/calcium pretreatments are able to prevent the loss of firmness of frozen sweet cherries. Conversely, Suutarinen *et al*. (2000) reported calcium chloride pretreatment did not significantly affect the drip loss of thawed strawberries. Galetto *et al*. (2010) found freezing by immersion in a calcium chloride solution did not provide a significant benefit in maintaining firmness but did reduce the drip loss of strawberries in comparison to slow freezing. However, none of the available research has reported on the effects of calcium on frozen apple, mango, cantaloupe, and pineapple. Particularly lacking are the effects of calcium on dissimilar fruit types all consistently examined in the same study. The relationships between the physico-chemical properties of different fruits and the effects of calcium on the textures of frozen fruits have also not been reported previously.

Apple (*Malus domestica* Borkh.), mango (*Mangifera indica* L.), cantaloupe (*Cucumis melo*), and pineapple (*Ananas comosus* L.) were chosen for the present study due to the variation in their physicochemical properties, especially in regard to texture and pectin content. The interactions between calcium ions and carboxyl groups of the pectin are described by an egg box model. The egg box structure has been suggested to provide stability to the middle lamella in the plant cell wall and improve firmness of fruits (Thakur e*t al*., 1997; Sila *et al*., 2009). Therefore, in the present study pectin content in fruits was hypothesized to have an effect on the effectiveness of calcium to improve firmness of fruits.

Apple is grown in temperate regions and is now grown in almost all continents of the world. Apple is an important source of dietary fiber. Fuji apple (*Malus domestica* Borkh.) is reported as a crunchy and juicy apple that stores well

(Mehinagic *et al.*, 2004). Alandes *et al.* (2006) reported that Fuji apple had a firmness around 10.15 N as measured using a puncture test. Baker (1997) reviewed the pectin levels in some fruit and vegetable samples and reported that apple had pectin contents of 0.71-0.84% fresh weight. Whereas, Gheyas *et al.* (1997) studied the dietary fiber content of thirteen apple cultivars and they found that uronic acid represented an average of 5.8 ± 1.2 g/kg apple flesh.

Mango (*Mangifera indica* L.) is grown in several countries, but only a few specific cultivars are commercialized according to preferences of different regions of the respective countries. Mango of the cultivar 'Nam Dok Mai' is one of the economically important fruit crops in Thailand. This fruit is normally eaten when fully ripe. Similar to the other types of fruit, mango contains pectin as a major component of the cell wall and middle lamella, which is closely related to the texture of the fruit. Varanyanond *et al.* (1999) reported that mango of the cultivar 'Nam Dok Mai' had a total pectin content 221 mg/100 g flesh and had a firmness of 2.71 kg as measured by the puncture test.

Cantaloupe (*Cucumis melo*) is a tropical plant which has been cultivated in Thailand for a long time. Cantaloupe is normally eaten as a fresh fruit, a salad, or a dessert with ice cream or custard. Chisari *et al*. (2009) reported that cantaloupe had a firmness of 6.65 N as measured by the puncture test. Whereas, Simandjuntak *et al*.(1996) reported that cantaloupe had a firmness of 189 g as measured by a penetrometer and had a total pectin content of 443 mg /g alcohol insoluble residue.

Pineapple (*Ananas comosus* L.) is one of the most important commercial fruits of the world. It is widely cultivated throughout the tropics and subtropics. Thailand is the largest exporter of pineapple. Canned pineapple is the main type of pineapple that is exported. Most canned and fresh pineapples came from the cultivar 'Smooth Cayenne'. Pineapple has a total pectin content of 4.5 g/100 g alcohol insoluble residue (Yapo, 2009) and had a resistance to shear of 32.69 N/g fresh weight as measured using the Kramer Shear test (Bartolomé *et al*., 1995).

These summaries show that, these four fruits have different physico-chemical properties especially texture and pectin content that cover the range from a low to high level. To compare results from different research projects is difficult because each researcher used different preparation and analytical methods. So, it is beneficial to study the use of calcium as a pre-freezing treatment for different fruit types and find the relation between the physico-chemical properties of fruits and effect of calcium.

The effects of enzyme pectin methylesterase and calcium to maintain the firmness of fruit products have been studied. The deesterification of pectin by pectin methylesterase and subsequent chelation of calcium by ionized carboxylic acid groups on adjacent pectic acid chains is typically depicted in the form of an 'egg-box' structure (Baker and Wicker, 1996). Due to limitation on the molecular size of enzymes and the rate of calcium to diffuse into the fruit, vacuum infusion can thus be used to add exogenous enzyme to fruit pieces more rapidly and more homogeneously than soaking (Guillemin et al., 2006). Banjongsinsiri *et al*. (2004a) used vacuum to infuse pectin methylesterase into strawberry halves. They reported that the firmness of the pectin methylesterase infused fruit was about twice more than that of the water infused control, however, the value was not significantly different from the non-infused fruit. Degraeve *et al*. (2003) reported the synergistic action of pectin methylesterase and calcium used in the vacuum infusion solution. The vacuum assisted procedure was observed to be able to accomplish an uptake of infusion solution (pectin methylesterase and calcium) and hence be capable of improving the firmness of the fresh strawberries (Duvetter *et al*., 2005; Fraeye *et al*., 2010). However, the large pressure difference created by rapid vacuum release can reduce the effectiveness of the process by crushing some tissues. Moreover, water logging may occur in the fruit tissue subsequent to a vacuum infusion (Baker and Wicker, 1996).

Because of the lack of research on frozen fruits, it is interesting to apply the combination of pectin methylesterase and calcium to study the improvement in the texture of frozen fruit.

The addition of calcium into fruit pieces by the immersion in a calcium chloride solution has been hypothesized to improve the firmness of fruit pieces by an interaction between calcium and free carboxyl groups of the pectin in fruit tissue. This interaction could provide stability to the middle lamella in the fruit cell wall and result in an increase in firmness. Moreover, the vacuum infusion of combined solution of pectin methylesterase and calcium chloride was aimed to increase the efficiency of calcium. The deesterification of pectin by pectin methylesterase generates free carboxyl groups which are the binding site for calcium to create calcium bridges between adjacent pectin molecules. This process can result in an overall increase in the firmness of fruit products.

OBJECTIVES

1. To investigate the freezing characteristics (i.e. freezing point, and freezing rate) of apple, mango, cantaloupe, and pineapple

2. To study the effect of calcium chloride as a pre-freezing treatment on the physicochemical properties and microstructure of apple, mango, cantaloupe, and pineapple and establish the relationships between the properties of these fruits and the effects of calcium.

3. To investigate the effect of vacuum infusion of pectin methylesterase and calcium chloride on the texture and microstructure of fresh and frozen mango.

LITERATURE REVIEW

1. Freezing process

The process of food freezing has gained widespread attention. Reduction of available water by ice crystal formation, and subzero temperatures, provides an environment that favors reduced chemical reactions and microbial growth, hence leading to increase storage stability. Ice crystallization can cause extensive microstructural changes to food tissue during freezing. The extent of these changes though is largely a function of the location of the ice crystals, which mainly depends on the freezing rate.

Freezing involves different factors in the conversion of water to ice. The freezing process includes two successive processes: the formation of ice crystals (nucleation), and the subsequent increase in crystal size (growth) (Zaritzky, 2006).

1.1 Homogeneous and heterogeneous nucleation

Nucleation refers to the process by which an initial crystal is formed with a critical radius which can then expand and grow. During nucleation the latent heat of solidification is released; molecules aggregate into an ordered particle of a sufficient size to survive and serve as a site for further crystal growth. At the surface of the crystals, there is a constant interchange of water molecules between the solid and liquid phases. Nucleation may be homogeneous or heterogeneous: homogeneous ice nucleation is produced in water free of impurities, and heterogeneous nucleation (catalytic nucleation) takes place when water molecules aggregate in a crystalline arrangement on nucleating agents such as active surfaces; this type of nucleation predominates in food systems.

1.2 Crystal growth

As long as a stable ice nucleus is formed, further growth is possible by the addition of molecules to the solid–liquid interphase. Growth is not instantaneous, and is controlled by the rate of removal of the latent heat released during the phase change, as well as by the rate of mass transfer in the case of solutions (diffusion of water molecules from the surrounding solution to the surface of the ice crystals and counter-diffusion of solutes away from the growing crystal surface). Crystal size varies inversely with the number of nuclei formed. At high freezing rates, a high number of nuclei are formed and the mass of ice is distributed in a large number of small crystals. At low freezing rates fewer nuclei are formed leading to large crystal sizes.

1.3 Freezing curve

Figure 1 shows the freezing profile of small samples (without thermal gradients) of pure water (Figure 1a) and of an aqueous solution (Figure 1b). For pure water, the removal of sensible heat (4.18 kJ/kg °C) occurs in the first stage. Nucleation is necessary for freezing to initiate, and the temperature can fall below 0°C without the formation of ice crystals. Point “S” indicates the supercooling of the liquid before crystallization begins. Once the critical mass of nuclei is reached, the system nucleates at point “S” and releases its latent heat faster than heat can be removed from the system. The abrupt increase in temperature (point “S” to point “B”), because of the liberation of the heat of solidification after initial supercooling, represents the onset of ice crystallization. Once crystallization begins, the temperature reaches point “B”, the freezing point of pure water (0°C). While the solid and liquid are in equilibrium, the temperature remains at the freezing point until all of the water has been converted to ice (point “C”). In pure water, the plateau from “B” to “C” represents the time during which crystal growth occurs. On completion of solidification, further removal of heat results in a decrease in temperature towards “D” (Zaritzky, 2006).

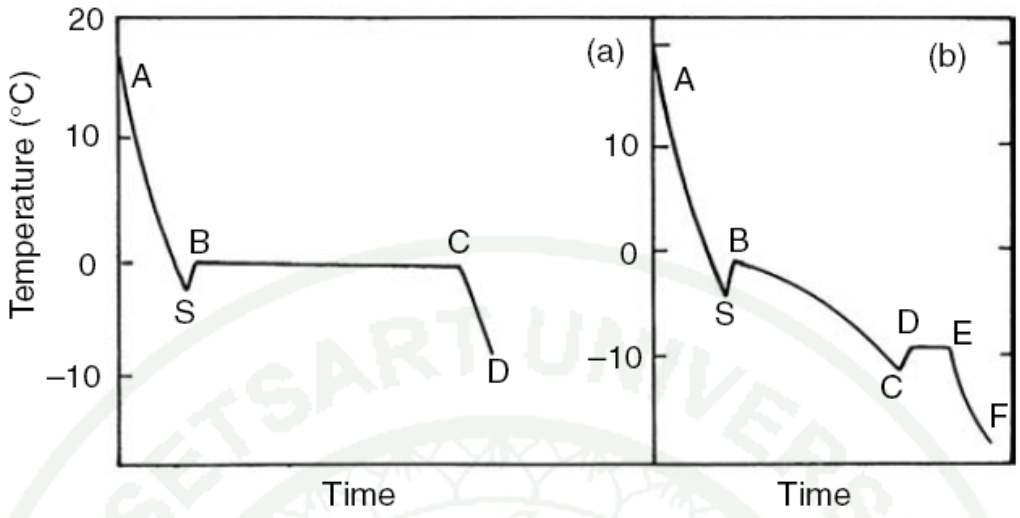

Figure 1 Freezing profiles of (a) pure water, and (b) an aqueous solution.

Source: Zaritzky (2006)

The freezing of food systems is more complex than the freezing of pure water. Food systems contain water and solutes, and the behavior is similar to that of an aqueous solution. When freezing aqueous solutions (Figure 1b), the cooling curve also shows supercooling (point "S"). Nucleation occurs at point "S" and the released heat of crystallization raises the temperature from "S" to "B". The point "B" represents the freezing point of the solution, which is lower than the freezing point of pure water. The freezing point depression is determined by the number of dissolved solute molecules. Further cooling from "B" to "C" results in the growth of ice crystals and substantial ice formation. A gradual increase in solute concentration is produced as water is separated in the form of relatively pure ice crystals, and the declining freezing point (negative slope of "B"–"C" in Figure 1b) reflects the change in concentration. Solute concentration increases during the freezing process and eventually reaches its eutectic temperature. Supersaturation, indicated by point "C" in Figure 1b, can be observed before the crystallization of the solute. Latent heat of the solute crystallization is released at "C", causing a slight increase of temperature from "C" to "D". At temperature "D", the solution assumes the eutectic equilibrium composition that remains constant during eutectic solidification and constant

temperature (“D”–“E”). Cooling below “E” is produced after the solution is solidified completely (Zaritzky, 2006).

1.4 Fruit freezing

Many fruits are frozen to extend their availability throughout the year, and to further shelf life through lengthy distribution. In general, fruits have less fibrous structure than vegetables and therefore often suffer more textural changes during freezing and storage. The quality of raw ingredients is important to the texture of frozen/thawed fruit. In some cases, the freezing of slightly unripe fruit results in a firmer texture with lower drip loss. The fruit variety may also be a factor in freezing performance. However, the maturity and grade of the fruit are probably more important factors than cultivar differences on the texture of thawed fruit.

Fruits are seldom blanched before freezing. Any benefits by blanching are outweighed by the significant cellular damage it causes. Fruits do not have the more extensive fibrous network of vegetables, and therefore become mushy or even disintegrate with heating. There are some exceptions to the rule, however. For example, polyphenol oxidase can be inactivated in bananas by blanching, without causing unacceptable changes in firmness.

Many fruits are cut or diced prior to freezing, and have pits or stones removed. As the freezing rate is of particular importance to the quality of frozen fruit, pieces with the shortest dimension greater than 2–3 cm are not typically frozen (Kerr, 2004). In addition, when fruit is used for further processing, fruit that was cut prior to freezing is often easier to cut and of better quality than fruit cut after freezing and thawing.

During freezing, the formation of extracellular ice concentrates the solutes in the surrounding aqueous phase, which in turn drives water from the cells. The higher concentration of intercellular solutes, coupled with the lessened flexibility of the frozen phase, produces cell and cell membrane damage. In addition, cell wall

substances, particular pectins, may be extracted or solubilized from the cell walls, reducing structural rigidity (Kerr, 2004). Various ingredients may be added to enhance the quality of frozen fruits. Sugar is often used to improve color, texture, and flavor. Water is drawn out of the cells by osmosis, which leads to a lower freezing point and a decreased fraction of ice at a given frozen storage temperature. An extension of this is osmotic dehydration in which high concentrations of sugar solutions are used. With this approach, fruit cells may lose up to 40–70% of their water (Kerr, 2004). Fruits such as strawberries, apricots, cherries, and pineapple have been subjected to osmotic drying prior to freezing. In general, such fruits are sweet and firm and have lower drip loss.

Various freezing methods, including air-blast freezing, fluidized-bed freezing, and cryogenic freezing have been used successfully with fruits. Individual quick frozen (IQF) methods are preferred. Contact plate freezing of fruit in blocks is not often used in current practice. A significant body of work exists to show that rapid freezing results in the best retention of quality in thawed foods. This is especially important in frozen fruits, which are subject to greater textural changes in the freezing process. Rapid freezing results in smaller ice crystal size, less cell dehydration, and less cell damage. Cryogenic freezing with liquid nitrogen often results in the best texture. However, prolonged immersion in cryogens may cause ‘‘freeze fracturing’’ at the surface. Sprays of liquid nitrogen generally cause less freeze-fracture damage than full immersion (Kerr, 2004).

1.5 The freezing damage

The freezing process can cause quality damage due to several mechanisms which were compiled by Reid and Barrett (2005).

1.5.1 Osmotic damage

When heat is removed rapidly, ice forms rapidly. This produces ice crystals which tend to be small. Because the ice grows rapidly, the concentration of

the external unfrozen matrix rises rapidly. Osmotic transfer of water is limited: the cells freeze internally, and little water translocates. In slow cooling, the ice forms slowly, external to the cells, and there is sufficient time for a large amount of osmotic transfer of water from the cells. This results in cell shrinkage that can damage the membranes (Reid and Barrett, 2005). A considerable amount of water translocates due to cell wall damage consequent upon the freezing process, and this water does not return to the cells on thawing but, rather, becomes drip loss.

1.5.2 Solute-induced damage

The high-solute concentrations of the unfrozen matrix, in particular high salt concentrations, can cause damage to many polymeric cell components and may kill the cell (Reid and Barrett, 2005). To prevent this, some form of solution-based protection might be needed. A typical method for reducing salt concentration-induced damage is to add sugars to the aqueous phase that is undergoing freeze-concentration. The concentration effect is present whether freezing is fast or slow.

1.5.3 Structural damage

For fast freezing, in addition to the concentration effect, the formation of ice within the cell may cause damage to the delicate organelle and membrane structures of the cell. One consequence of this process is that enzyme systems may be dislocated. This may result in uncontrolled enzyme action, leading to a variety of effects, including the production of off-flavors (Reid and Barrett, 2005). Prevention of such enzyme-mediated damage can be achieved by utilizing blanching, a pre-freezing heat treatment that denatures the enzymes and, hence, terminates their catalytic activity; however, it has to be remembered that blanching will influence the semipermeable properties of the cell membrane and may also damage cell turgor. Cell turgor is an important component of the eating quality of many fruits. It is produced by the internal pressure of the cell contents. Lack of turgor is perceived as softness and lack of crispness and juiciness. Where turgor is an important product characteristic, blanching may not be an acceptable procedure, and other steps may be

necessary to control enzymatic initiated degradation processes. Blanching is not the only cause of reduced turgor. If cells become leaky or lose some of their content, turgor may also be reduced or destroyed, and the texture of the fruit can become much softer. Loss of turgor due to processing procedures is of most relevance for fruits that are customarily eaten raw, rather than fruits that are customarily cooked. Cooking, a more severe thermal treatment than blanching, destroys turgor so that the retention of turgor through earlier processing procedures is not necessary.

2. Pectin

The edible portions of fruits and vegetables generally consist of the parenchyma, which is an unspecialized tissue arranged into parenchyma cells and intercellular spaces. Parenchyma cells are approximately 50 to 500 μm across and polyhedral in shape (Van Buggenhout *et al*., 2009). Parenchyma cells are, from the inner to the outer part, composed of (1) one or more vacuoles, (2) cytoplasm, (3) the plasma membrane, (4) the cell wall, and (5) the middle lamella (Figure 2). Usually, parenchyma cells contain a single large vacuole that accounts for most of the cell volume and that is responsible for the osmotic potential of the cell. The cytoplasm contains different organelles and is connected to the cytoplasm of adjacent cells by strands of cytoplasm through the primary cell wall, called plasmodesmata. The plasma membrane has a semipermeable nature which makes osmosis possible, but it is the cell wall that provides the rigidity to allow a build-up in (turgor) pressure. The cell wall of the average parenchyma cell is thin (0.1 to 10 μm) but strong. Adjacent cells are glued together by the middle lamella, which is largely composed of pectic material (Van Buggenhout *et al*., 2009).

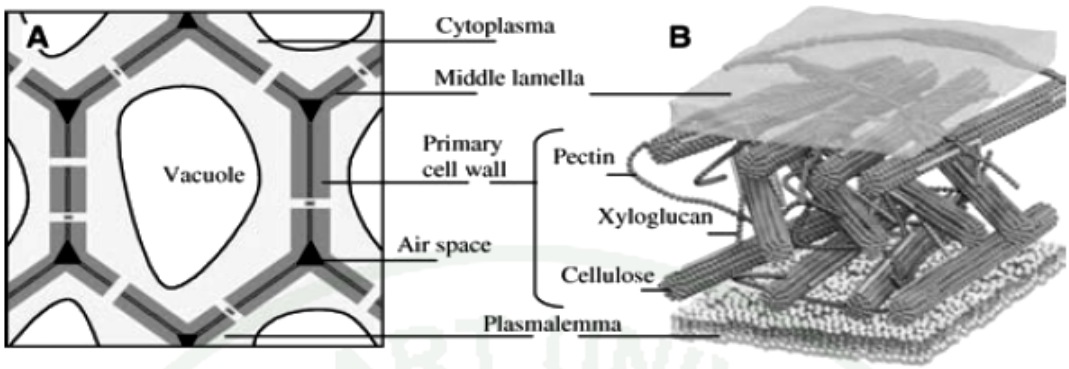

Figure 2 Schematic presentation of (A) parenchyma tissue and (B) plant cell wall.

Source: Van Buggenhout *et al*. (2009)

Pectins are a class of complex polysaccharides found in the cell walls of higher plants that function as a hydrating agent and cementing material for the cellulosic network. They are commonly produced during the initial stages of primary cell wall growth and make up about one third of the cell wall of dry substances of dicotyledonous and some monocotyledonous plants. The highest concentration of pectins in the cell wall is seen in the middle lamella, with a gradual decrease from the primary cell wall toward the plasma membrane. Pectins are found in relatively large amounts in soft plant tissues under conditions of rapid growth and higher moisture contents. The strength of the plant cell wall depends on the orientation, mechanical properties, and links between pectic substances and cellulose fiber. Some pectin molecules are glycosidically linked to xyloglucan chains that can bind covalently to cellulose (Thakur *et al*., 1997).

The firming effect of pectin in tissues involves two separate phenomena: in fresh tissue, the formation of free carboxyl groups increases the possibility and the strength of calcium binding between pectin polymers, and in heated tissues there is a combination of increased calcium binding and a decrease in the susceptibility of the pectin to depolymerization by β-elimination. Softening during the ripening of fleshy fruits is attributed to enzymatic degradation and solubilization of the protopectin (Thakur *et al*., 1997).

2.1 Structure of pectin

The composition of pectin varies with the source and conditions of extraction, location, and other environmental factors. Pectins are primarily a polymer of D-galacturonic acid and rhamnogalacturonan, making it an α-D galacturonan.

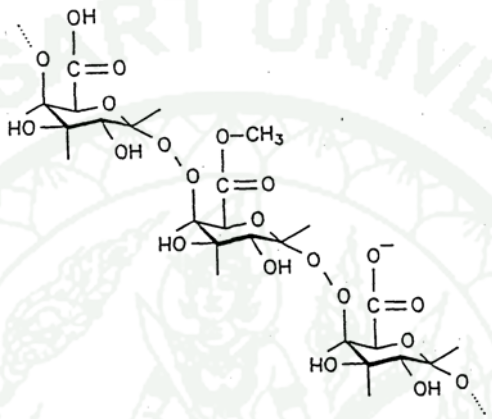

Figure 3 A repeating segment of the pectin molecule.

Source: Thakur *et al.* (1997)

The pectin molecule is formed by L-1,4- glycosidic linkages between the pyranose rings of D-galacturonic acid units. As both hydroxyl groups of D-galacturonic acid at carbon atom 1 and 4 are on the axial position, the polymer formed is a 1,4- polysaccharide (Figure 3). Pectins are block copolymers, that is, branched blocks containing a main galacturonan chain interrupted and bent by frequent rhamnose units (many of them carrying side chains) alternating with unbranched blocks where rhamnose units are rare (Figure 4) (Thakur *et al.*, 1997; Sila *et al.*, 2009). However, the middle lamella is pectin rich and is composed almost entirely of homogalacturonan (a large linear unsubstituted polymer of galacturonic acid) (Toivonen and Brummell, 2008).

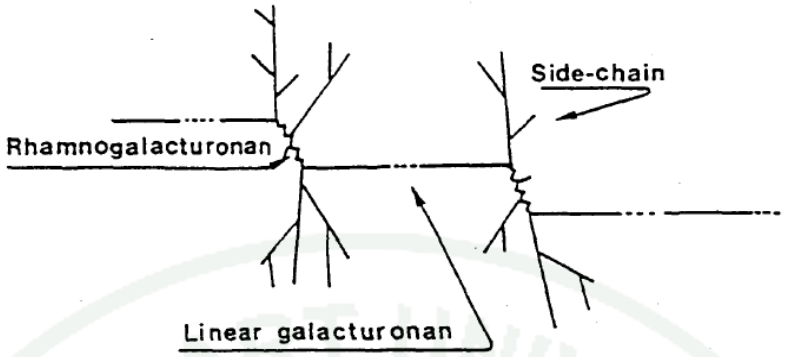

Figure 4 Schematic representation of the pectin backbone showing the "hairy" regions (rhamnogalacturonan and side chains) and the "smooth" regions (linear galacturonan).

Source: Thakur *et al.* (1997)

2.2 Gelation of low methoxyl pectins

The interactions between Ca^{2+} ions and carboxyl groups of the pectin are described by the egg box model involving a two stage process of initial dimerization and subsequent aggregation of preformed egg boxes (Figure 5). The egg box structure has been suggested to provide stability to the middle lamella in the plant cell wall. The size of the egg box junction zones is limited by the presence of sequences containing mannuronate residues, which interrupt the polyguluronate blocks. The pH is higher in the gelation of LM pectin because only dissociated carboxylic groups take part in the cross-linkages. The junctions are formed between unbranched nonesterified galacturonan blocks bound together noncovalently by coordinated calcium ions. Calcium is particularly effective when it is in a complex with carbohydrates, in large part because the ionic radius, 0.1 nm, is large enough to coordinate with oxygen atoms spaced as they are in many sugars and because of flexibility with regard to the directions of its coordinate bonds (Thakur e*t al*., 1997). The presence of methyl groups prevents the formation of junction zones in the interjunction segments of molecules, making them more flexible. Side chains on the molecule prevent their aggregation. The greater the number of reactive carboxyl groups that can form salt

linkages, the more likely it is that the bridge will be formed. In addition, the molecules with an increased number of charged groups and lower degree of methoxylation (DM) are straighter than esterified ones, and hence more likely to form a Ca^{2+} bridge (Thakur e*t al*., 1997).

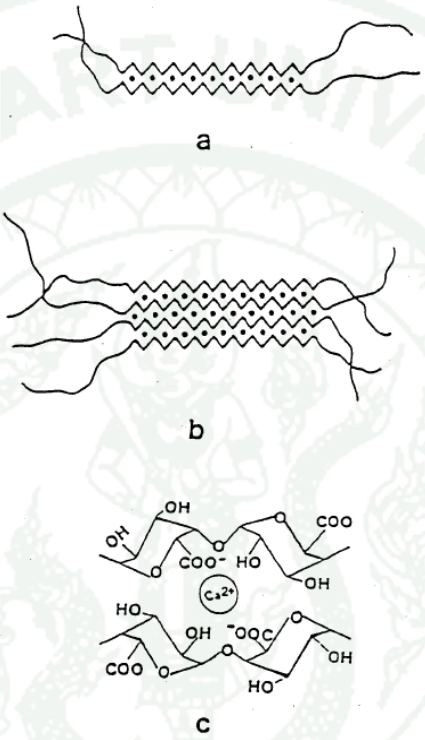

Figure 5 Schematic representation of calcium binding to polygalacturonate sequences. (a) Egg-box dimer, (b) aggregation of dimmers, (c) an egg box cavity.

Source: Thakur *et al*. (1997)

2.3 Pectin conversion reactions

Pectin can be digested in plants by endogenous and/or exogenous (pathogenic) enzymes, as well as by postharvest and/or processing dependent nonenzymatic conversion reactions (Figure 6). Despite the possibility of many pectin conversion reactions, only the well-known homogalacturonan conversion reactions are indicated in Figure 6. Knowledge of the bio-chemical basis of pectin modification/degradation in plant based foods is still limited.

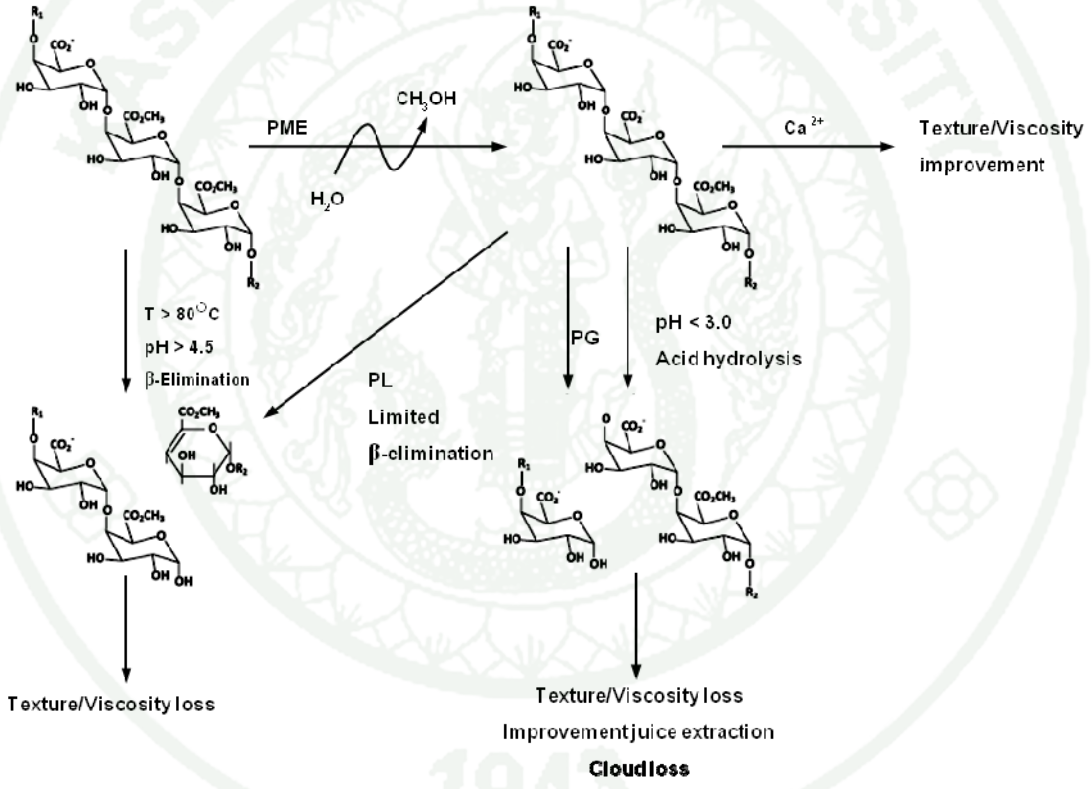

Figure 6 Schematic presentation of possible pectin (only homogalacturonan) conversion reactions in fruit and vegetable products and possible routes for tailoring quality parameters: PME = pectin methylesterase, Ca^{+2} = calcium cross linking, PG = polygalacturonase, PL = pectate lyase, T = temperature, R1/R2 = initial/terminal fragment of the pectin polymer.

Source: Sila *et al*. (2009)

Both enzymatic and chemical pectin changes play a role in process induced textural changes: (1) enzymatic degradation by the successive demethoxylation and depolymerization by pectin methylesterase (PME) and polygalacturonase (PG), respectively, and (2) chemical degradation via a β-elimination reaction or acid hydrolysis. The most convenient way to direct pectin changes during processing to a certain point is to control the enzymatic pectin changes by inactivating the undesired enzymes and boosting the activity of the functionally important ones.

2.3.1 Enzymatic pectin conversion

A wide range of endogenous and exogenous enzymes can synergistically modify and degrade pectin smooth and hairy regions. In the smooth region, the enzymes involved can either be esterases or depolymerases. Pectin methylesterases catalyze the specific demethoxylation of homogalacturonan within plant cell walls, releasing methanol and protons (and creating negatively charged carboxyl groups). The demethoxylated homogalacturonan can (1) cross-link with divalent ions (such as Ca^{2+}, Mg^{2+}), important for engineering texture/rheological properties, and (2) form a substrate for pectin depolymerizing enzymes, associated with texture/viscosity loss, although it is useful in increasing juice extraction yields and in controlling cloud stability. The depolymerizing enzymes include polygalacturonases (PG) and pectate lyase (PL). The PG cleave glycosidic bonds via acid/base-assisted catalysis, while the lyases fragment the polymers via a β-elimination reaction mechanism resulting in the formation of a double bond between C-4 and C-5 at the newly formed nonreducing end. They can be endo- or exo-acting. PL has previously been thought to be of exogenous origin in plants; however, the recent discovery of abundant PL-like sequences in many plant genomes contradicts this (Sila *et al*., 2009).

2.3.2 Nonenzymatic pectin conversion

Pectin is very stable around a pH of 3.5, its pKa value (Sila *et al*., 2009). Nonetheless, a number of reactions have been proposed for its nonenzymatic degradation. First and most important is the base-catalyzed splitting of pectin chains via the β-elimination reaction, a process that occurs in parallel with deesterification and proceeds even when pectin is heated at neutral or weakly acidic pH. Most fruit and vegetable products have a pH above 4.5 and are processed at 80°C or higher making them very susceptible to the β-elimination reaction (Figure 6).

The second mechanism leading to pectin degradation during thermal processing is acid hydrolysis (pH < 3.0). In acidic conditions, pectin with low degree of methoxylation (DM) hydrolyzes faster and the reaction is boosted by the simultaneous chemical demethoxylation of the polymer under such conditions. Acid hydrolysis is of less importance during regular food processing (Sila *et al*., 2009).

3. Pectin methylesterase (PME)

Both the number and distribution of free carboxyl groups along the pectin molecule are regulated by the activity of PME. PME catalyses the demethylesterification of galacturonic acid units of pectin, generating free carboxyl groups and releasing protons (Figure 7). Demethylesterified pectin may undergo depolymerisation by glycosidases. PME has been found in all higher plant examined, a few yeasts and insects and is also produced by phytopathogenic fungi and bacteria (Giovane *et al*., 2004; Jolie *et al*., 2010).

PME have been identified in all higher plants examined—in their fruits, leaves, flowers, stems as well as roots. Plant PME are mainly associated with the cell wall by ionic interactions, and hence released during treatment with high ionic strength solutions or shifting the pH towards the alkaline region. However, some soluble forms have been detected as well. A study to visualize PME in situ indicated a heterogeneous distribution of PME and a co-localization of PME with acidic pectins,

but could not confirm whether the PME are directly attached to the pectins or only situated in their neighborhood (Jolie *et al*., 2010).

Generally, PME are enzymes with a molar mass in the range of 25 to 54 kDa (Jolie *et al*., 2010). The isoelectric pH (pI) of PMEs varies from as low as 3.1 for fungal PME to 11 for tomato PME. Most plant PME present a pH optimum between a pH of 6 and 8, whereas for microbial PME it is between pH 4 and 9 (Jolie *et al*., 2010). PME appears to be very sensitive to the ionic environment and is usually closely regulated by pH. Generally, PME activity increases with increasing cation concentration up to an optimal value above which activity usually decreases. Optimal concentrations depend on the nature of the cation. Salt addition allows a shift of the pH optimum (Duvetter *et al*., 2009; Jolie *et al*., 2010). In many plants, multiple isoforms of PME are found. PME isoforms all catalyze the same reaction. However, they differ in isoelectric pH, molar mass, thermostability and catalytic properties. Relative proportions of PME isoforms may vary greatly according to the developmental stage and the organ considered.

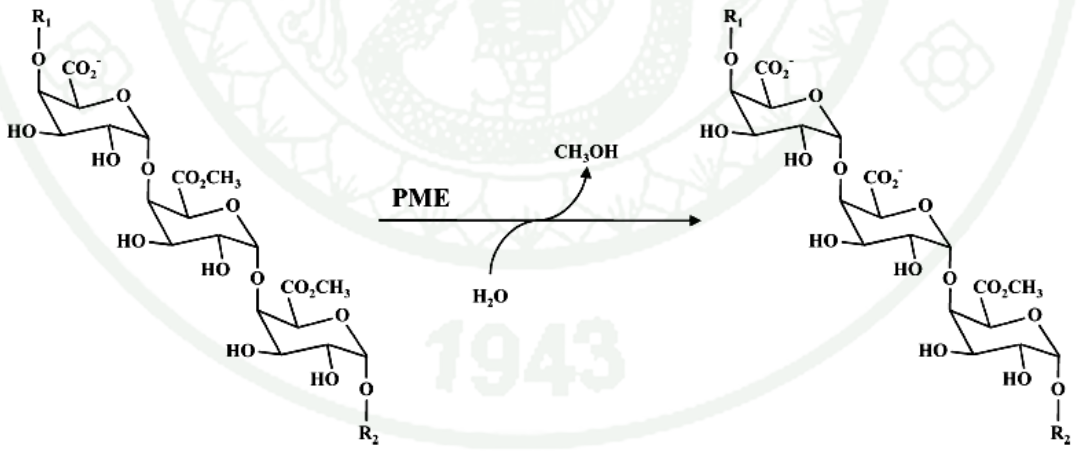

Figure 7 The demethoxylation reaction of the homogalacturonan chain of pectin catalysed by PME (R1/R2 = initial/terminal fragment of the pectin polymer).

Source: Jolie *et al*. (2010)

Most purified plant PME have neutral or alkaline isoelectric points, and are bound to the cell wall via electrostatic interactions. However, the presence of neutral or acidic PME in the soluble fraction of plant tissues has also been detected. Until a few years ago, it was generally assumed that plant PME could act blockwise on pectin galacturonic acid residues, thus producing clusters of free carboxyl groups, whereas fungal PME could have a random demethylation activity resulting in a rather random distribution of the deesterified galacturonic acid units. Recently it has been shown that some plant PME isoforms may exhibit both mechanisms depending on pH (Giovane *et al*., 2004; Jolie *et al*., 2010). PME action on the components of the plant cell wall may produce two opposite effects. On one hand, it contributes to the stiffening of the cell wall by producing blocks of unesterified carboxyl groups that can interact with calcium ions forming a pectate gel. On the other hand, the proton release may stimulate the activity of cell wall hydrolases, contributing to cell wall loosening. Manipulation of PME expression has been shown to influence physiological processes such as stem elongation and tuber yield, root development and fruit softening (Giovane *et al*., 2004).

3.1 Effect of thermal process on pectin methylesterase

Traditionally, thermal processing is the most commonly applied preservation technology since it allows efficient inactivation of both pathogenic and spoilage microorganisms and quality related enzymes. Thermal stability of PME is well documented, mainly from inactivation kinetic studies. Temperature stability of PME varies for different sources of PME and can be different for isoforms from the same source. Generally, PME are rather thermolabile, inactivating readily at temperatures below 70°C. Increasing the temperature increases the rate of thermal inactivation. However, thermostable forms occur as well and dependent on environmental factors, such as pH, ionic strength, additives, and the degree of purity. PME in tissue-type systems is less vulnerable to thermal inactivation as opposed to the purified form. On the other hand, increasing the temperature also enhances pectin demethoxylation by PME, like other enzyme catalyzed reactions, until the point is reached where protein denaturation exist. Reported optimal temperatures for PME

activity vary between 45 and 55°C, depending on the enzyme source and environment (Duvetter *et al*., 2009; Jolie *et al*., 2010).

3.2 Effect of low temperature and freezing process on pectin methylesterase

Low temperature has not been reported to affect PME activity. Terefe *et al.* (2005) studied the kinetics of the PME catalyzed deesterification of pectin in the presence of maltodextrin and sucrose at different concentrations in a temperature range between 20 and -4 °C. This temperature range was selected to avoid freezing. They reported that these low temperatures did not affect the specific activity of the enzyme through conformational changes. The researchers also studied the effect of freezing on PME activity in the presence of 40% sucrose at 25 and -4.5 °C. They concluded that freezing may affect the structural conformation of the enzyme and its catalytic efficiency. The presence of ice may also cause additional obstruction to the diffusion of the reacting molecules and may contribute to the decrease in rate of activation with freezing.

3.3 Relevance of pectin methylesterase in food industry

PME is one of the key enzymes within the context of fruit and vegetable processing. Endogenously present plant PME can positively or negatively affect the structural quality of fruit and vegetable products (cloud stability, viscosity, and texture). The enzyme should be activated or inactivated depending on the desired intention for that particular product (Duvetter *et al*., 2009).

3.3.1 Pectin methylesterase in relation to cloud stability

It is well established that endogenous plant PME decreases the cloud stability of many fruit and vegetable juices. By its action, blocks of free carboxylic acids of adjacent pectin molecules are formed, which can be cross linked by endogenous divalent cations (mostly calcium), leading to precipitation of the pectins. This precipitation entrains and removes the pulp particulates. Cloud loss is

considered a serious quality defect of juices, determining consumer acceptance, not only because of the appearance but also since sensory properties such as color, flavor and texture, associated with the cloud particles, are reduced. Cloud stabilization is traditionally accomplished by heating the juice to inactivate PME (Jolie *et al*., 2010).

3.3.2 Pectin methylesterase in relation to texture of fruit and vegetable products

The texture of fruits, vegetables and derived products is a major quality attribute and determinant of consumer acceptance and preference. In addition to turgor pressure and the structural characteristics of cell wall polymers, pectin is believed to play a central role, since changes in pectin content, composition and fine structure occur during events that modulate texture, such as fruit ripening and thermal processing. Consequently, the action of PME on pectin affects the textural quality of fruit and vegetable products either favorably or deleteriously. Several different essential mechanisms have been distinguished which affect pectin content. First, demethoxylated pectin becomes a substrate for PG (and PL), enzymes that catalyse the depolymerisation of the pectin chain, inducing loss of textural integrity of tissue systems, also thought to occur during fruit ripening. This pectin fragmentation may also cause a viscosity or consistency loss in, for instance, pastes and purees of plant sources with high levels of endogenous PG (Jolie *et al*., 2010).

Second, pectin with a low degree of methyl esterification can cross link via divalent ion bridges to form intermolecular networks, promoting gel formation. In intact plant tissues, cell wall reinforcement and, hence, an increase in firmness can take place (Jolie *et al*., 2010). This strengthening effect of the middle lamella matrix is often deliberately sought through controlled stimulation of endogenous PME activity, for example, by means of a mild temperature or hydrostatic pressure pretreatment, prior to the actual conservation process. Supplementing these pretreatments by calcium soaking can further increase the firmness improvement. In cases where a (thermal) pretreatment would activate detrimental enzymes or where only low amounts of endogenous PME are present, application of exogenous PME

through enzyme infusion can also be performed. Formation of calcium pectin cross links may also improve or preserve the viscosity of liquid plant based products.

Third, demethoxylation of pectin reduces its sensitivity to chemical β-eliminative depolymerisation which can occur during heating and is associated with heat induced softening of many fruits and vegetables. Thus, a reduction in DM by PME stimulation through pretreatment has a second texture conserving effect, reducing the vulnerability to thermal softening (Jolie *et al*., 2010).

3.4 Exogenous pectin methylesterase

Mostly, pectinases derived from food grade fungi (*Aspergillus niger* and *Aspergillus aculeatus*) are used. The largest industrial application of exogenous PME and other pectinases as processing aids is in the fruit juice industry. The purpose of these pectinases are to increase extraction yields and reduce extraction times. Additionally, PME and other pectinases are employed in related processes, such as juice clarification and the production of wine, cider, fruit concentrates, and jams, where a complete degradation of the cell wall is desired. Exogenous PME may be applied in texture improvement of thermally processed fruits and vegetables, for example, via enzyme infusion. As is generally known, pectin is often used as a food additive, mainly because of its gelling properties. However, its functionality is highly dependent on the degree and pattern of methoxylation, which can be controlled by PME with specific modes of action under controlled reaction conditions (Jolie *et al*., 2010).

4. Calcium Pretreatment

Calcium immersion treatments are commonly used for fresh products, especially those which are more perishable. This treatment usually consists of the soaking of the product, applying or not applying mechanical agitation, followed by the removal of excess solution. This treatment is gentler to the product than the impregnation techniques which can cause tissue damage and metabolic stress.

The main purpose of calcium immersion is to improve the texture of fruit products. The interactions between calcium ions and carboxyl groups on the adjacent pectin molecules are described by the egg box model. The egg box structure has been suggested to provide stability to the middle lamella in the plant cell wall and results in an overall increase in firmness (Thakur *et al.*, 1997) (as described in section 2.2).

Different factors (calcium concentration, immersion time, and temperature) can affect product integrity.

4.1 Calcium chloride concentration

The concentrations of the calcium salts used as immersion treatments are usually within a range of 0.5-3% (Martin-Diana *et al.*, 2007).

Sousa *et al*. (2007) studied the effect of calcium chloride (1, 10 and 100 mM) pretreatments on texture parameters of frozen raspberries and blackberries. The samples were sprayed with a pretreatment solution before freezing. They suggested that a pretreatment with calcium chloride (100 mM; 1.1 %w/v) was sufficient to prevent a loss of firmness in frozen raspberries and blackberries. However, panelists did not detect the effect of firmness caused by this pretreatment in the frozen raspberry, whereas they scored the firmness of the frozen blackberry pretreated with calcium chloride higher than those of the untreated control sample.

Galetto *et al*. (2010) studied the freezing of whole strawberries by immersion in 30% (w/v) calcium chloride solution comparison with slow freezing. They found that the immersion in calcium chloride reduced the drip loss of thawed strawberries but did not provide a significant benefit for maintaining firmness and did not present a significant difference on the total pectin content, when compared to slow freezing.

4.2 Immersion time

Immersion times in the range of 1 – 15 min were previously reported. Aguayo *et al.* (2008) used a 1 min period to dip fresh-cut melon. Luna-Guzman *et al.* (1999) used period of 5 min for immersion of fresh-cut cantaloupe. Whereas, Suutarinen *et al.* (2000) used the immersion period of 0.25 - 15 min for strawberries.

4.3 Immersion temperature

The effect of temperature has been shown to be of major importance in the results of calcium immersion treatment on fresh-cut fruits. Aguayo *et al.* (2008) immersed fresh-cut melon in a 0.5% calcium chloride solution at 5°C and 60°C for 1 min. At the end of 8 days storage, the firmness loss was 21% and 3% for the immersion at 5 and 60°C, respectively. They concluded that the immersion temperature affected firmness loss of fresh-cut melon.

Previous research also reported the combined effect of calcium and immersion temperature on the properties of frozen fruits. Alonso *et al*. (1997) determined the effect of combined thermal/calcium pretreatments on changes in the texture of frozen sweet cherries. Before freezing, the samples were blanched at 50˚C/10 min or 70˚C/2 min and were immersed for 10 min in a cool solution with or without 100 mM calcium chloride. They found that the thermal pretreatment at 50°C for 10 min followed by immersion in 100 mM calcium chloride and thermal pretreatment at 70˚C/2 min with or without immersion in 100 mM calcium chloride prevented freezing induced loss of firmness of frozen sweet cherries. Immersion in

calcium chloride increased the concentration of calcium cations in the cell wall and enhanced the effect of thermal pretreatments on pectinesterase activity.

Alonso *et al.* (2005) studied the effect of calcium chloride and thermal treatment on the microstructure and pectin composition of frozen cherry. The samples were preheated at 50 °C for 10 min or 70 °C for 2 min. After preheating, each group was immersed for 10 min in a cool solution at 0 °C with or without 100 mM calcium chloride before freezing. They found that pretreatment at 70 °C for 2 min produced serious damage in the delicate tissues of this fruit and was therefore not recommended. They suggested that calcium chloride and thermal treatments at 50 °C had a synergic effect which decreased the degree of esterification of pectin, thereby favoring the formation of calcium bridges and preventing the depolymerization of pectins.

The combined effect of calcium immersion and other factors on fruit firmness have also been reported. Suutarinen *et al*. (2000) studied the effect of calcium chloride concentration (1 - 10 g/L), immersion time (0.25 – 15 min) and immersion temperature (25 - 50˚C) of the solution on the quality of frozen strawberries. They found that calcium chloride pretreatment did not significantly affect the drip loss of thawed strawberries compared to drip loss of untreated reference samples. The effect of calcium treatment on the texture of strawberries depended on the treatment condition. The condition that gave the highest firmness was the immersion in 5.5 g/L calcium chloride solution for 7.6 min at 37.5˚C.

Chauhan *et al*. (2009) studied the optimization of pretreatment using a blanching medium (10 – 20 ˚Brix sucrose solution) and additive soak treatment comprising solution of calcium chloride (1000–2000 ppm) and ascorbic acid (200–400 ppm). The pineapple slices were blanched in sugar solutions of different concentrations at 95 ˚C for 2 min followed by additive soak treatment comprising a solution of calcium chloride and ascorbic acid for 10 min at 40 ˚C. They found that the calcium chloride pretreatment prior to freezing was more effective than sugar blanching on minimizing drip losses and maintaining the texture of frozen and thawed

pineapple slices. However, calcium chloride pretreatment was found to have a negative effect on sensory acceptability (color, aroma, taste, and overall acceptability). They suggested that the compromised optimum values for blanching medium, calcium chloride and ascorbic acid levels were found to be 17 ˚Brix, 1493 ppm and 300 ppm, respectively.

5. Improving fruit texture by vacuum infusion of pectin methylesterase and calcium

PME infusion has been defined as the application of exogenous PME specifically to alter characteristics of intact tissue. Both pressure assisted and vacuum assisted infusion have been used to infuse enzymes into fruit tissue. These methods are only practical in those instances where significant gas voids exist within the tissue. Thus, the uptake of the enzyme solution is largely limited by the displacement of gases within these voids.

The main purpose of PME and calcium infusion is to improve the firmness of fruit tissues. The deesterification of pectin by PME and the chelation of calcium ions by ionized carboxylic acid groups on adjacent pectin chains are manifest in the form of ‘egg-box’ structures. Such a phenomenon results in an overall increase in firmness of fruit products (Baker and Wicker, 1996).

As for pressure assisted infusion, any gases contained in the porous fruit or vegetable will be compressed into the interior voids of the tissue and displaced by the enzyme solution. Upon release of the pressure, such gases reoccupy their former volume, and may force much of the liquid back out of the tissue resulting in less water logging and uptake of treatment solution. The subjection of some porous tissues to sudden excessive pressure can also cause irreversible tissue collapse, preventing the entry of the enzyme solution (Baker and Wicker, 1996).

Under vacuum conditions, when the porous tissue is immersed in the solution, the air is extracted from the pores; when the atmospheric pressure is restored, the solution penetrates into the intracellular spaces by capillary and pressure gradients. The partial substitution of the internal gas by the new liquid phase allows the reformulation of the food by the modification of the solid matrix, avoiding eventual stress due to long exposure to gradient solute concentration as in osmotic process (Martin-Diana *et al*., 2007). An advantage of this procedure is that it allows the removal of almost all of the interior gases; thus, the enzyme solution remains within the tissue after infusion. In addition, because the driving force for solution entry is limited to normal atmospheric pressure, there is somewhat less potential for tissue compression than if high-pressure infiltration were used. Nevertheless, even with vacuum infusion, the large pressure differential affected by rapid vacuum release can reduce the effectiveness of the process by crushing some tissues. Moreover, even when the vacuum is released gradually, tissues can be seen to compress. Ideally, the vacuum release should be sufficiently slow to permit the porous tissue that is being infused to rebound to its original shape while absorbing the enzyme solution. Complete saturation of a tissue with enzyme solution may be an advantage for certain processes, such as the peeling of citrus fruit, where the infused material is discarded. However, water logging of tissues may be an adverse effect in other applications; thus, in such cases, milder vacuum conditions would be necessary to avoid the uptake of excess water. The porosity of fruit, which is related to ripeness, also influences infusion success. Fruit and vegetables with a relatively impenetrable skin, such as cherries, blueberries, peas and corn, are more difficult to infuse (Baker and Wicker, 1996).

There are some previous research projects which studied the use of PME and calcium to improve the texture of fruit products. The improvement in texture was described by the deesterification of pectin by PME and the subsequent chelation of calcium by ionized carboxylic acid groups on adjacent pectin chains.

Since infusion into the fruits is limited by the molecular size of enzymes and the rate of passive calcium diffusion, vacuum infusion can be used to infiltrate the exogenous enzyme into fruit pieces more rapidly and homogeneously, compared to merely soaking. Guillemin *et al*. (2006) studied the penetration of 27 U/g fungal pectin methylesterase which was added by soaking and by vacuum impregnation (0.05 bar) in 1.5 cm apple cubes. They reportedly obtained a homogenous distribution of enzyme activity through all of the apple cubes after vacuum impregnation, while the penetration of the enzyme was limited at less than 2mm depth after atmospheric soaking for 1 h.

Moreover, Duvetter *et al*. (2005) compared passive osmotic infusion, vacuum assisted infusion, and pressure assisted infusion of 100 U/mL fungal pectin methylesterase and 0.5% calcium chloride into strawberry halves with the aim to improve the firmness. They reported that the vacuum assisted procedure was the only method able to accomplish an uptake of infusion solution and hence capable of improving the firmness of the strawberries. Their result showed a reduced firmness increase when tomato pectin methylesterase was vacuum infused instead of fungal pectin methylesterase. They also suggested that the vacuum level of infusion was an important factor determining the final firmness. From their study, the infusion at 1 kPa yielded firmer fruits compared with 30 kPa.

Some other previous research projects showed the effect of only PME infusion on the texture of fruit products. Banjongsinsiri *et al*. (2004a) used vacuum (at 85 kPa) to infuse Valencia orange pectin methylesterase (14-18 U/mL) into fresh-cut strawberry halves. The activity of pectin methylesterase infused fruit was about twice that of the noninfused control or water-infused control. They reported the increase of firmness of the pectin methylesterase infused fruit was about twice in comparison with the water infused fruit, however, the value was not significantly different from the noninfused fruit.

The synergistic effect of PME and calcium has been reported to improve firmness of thermal processed fruits, and frozen fruits.

For thermally processed fruits, Degraeve *et al*. (2003) studied the infusion of apple cubes, strawberry halves, and whole raspberries with only calcium or a combined solution of 0.6% w/w pectin methylesterase and 1%calcium. They found that the vacuum pretreatment (at 50 mmHg) limited the loss in fruits firmness following pasteurization. They reported the synergistic action of pectin methylesterase and calcium used in the vacuum infusion solution. Their results also confirmed that the effect of vacuum assisted impregnation strongly depended on the plant tissue considered. Apple cubes have a highly porous structure so that PME and calcium penetration is possible both by classical infusion and by the vacuum-assisted procedure. Raspberries are not very porous, do not contain occluded air, and the peel surrounding their tissue constitutes an efficient barrier to mass transfer. Conversely, strawberries halves have a side without an external peel and their tissue is relatively porous. Therefore, they suggested that strawberry halves were a good candidate for vacuum assisted impregnation of pectin methylesterase to improve their firmness.

Guillemin *et al*. (2008) used vacuum impregnation (0.05 bar) to impregnate pectin methylesterase (27 U/g) and calcium chloride (0.5% w/w) into apple pieces prior to pasteurization. They reported that pasteurized apple pieces impregnated with pectin methylesterase and calcium showed a significantly higher firmness than that of non impregnated samples.

Fraeye *et al*. (2009) studied the vacuum infusion (0 kPa) of fungal pectin methylesterase (10 U/mL) and/or calcium chloride (0.5%) on firmness of strawberry halves after thermal processing. Their results showed that when infused with only pectin methylesterase, the pectin chains remained water soluble, indicating that no cross linking took place. This resulted in a firmness decrease after thermal processing which was comparable to non infused processed strawberries. On the other hand, they reported a significant decrease in processing related tissue damage when a combination of both pectin methylesterase and calcium was infused.

Fraeye *et al*. (2010) studied the effect of the vacuum infusion (0 kPa) with 10 U/mL fungal pectin methylesterase and 0.5% calcium chloride, followed by a thermal (70 °C, 0.1 MPa), a high pressure (25 °C, 550 MPa) or a combined thermal-high pressure (70 °C, 550 MPa) process on the properties of strawberry samples. They found that the processing of strawberries caused a decrease in firmness, which was limited by infusion of pectin methylesterase and calcium chloride, although the extent of the beneficial effects depended on the type of processing.

For the case of frozen fruits, Van Buggenhout *et al*. (2006) investigated vacuum infusion (1 kPa) of 100 U/mL pectin methylesterase and 0.5% calcium on texture of frozen strawberry halves. They reported that the efficiency of the infusion process in improving the textural quality of strawberries subsequent to rapid freezing process. On the other hand, a combination of pectin infusion with immersion in the pectin methylesterase and calcium solution resulted in a limited solution uptake and hence in a limited firmness increases of the fruit halves.

Charoenphan (2009) studied the use of osmodehydration with 50% sucrose, the addition of calcium (0.5% w/w) into osmotic solution, and the addition of both calcium (0.5% w/w) and pectin methylesterase (0.12% v/v) onto the osmotic solution to improve the firmness of frozen cantaloupe. In this research, cantaloupe cylinders were immersed in the osmotic solution at atmospheric pressure for 1 hr. The result showed that the addition of calcium or both calcium and pectin methylesterase into the osmotic solution improved the firmness and reduced the drip loss of frozen-thawed cantaloupe. The synergistic effect of calcium and pectin methylesterase was evidenced on the short term frozen storage.

Since the large pressure difference caused by rapid vacuum release may reduce the effectiveness of this process, some tissues may be subject to sudden compression and cell rupture. Moreover, the fruit tissue may be subject to water logging subsequent to a vacuum infusion. Therefore, the vacuum levels during the infusion process should be considered.

These previous research projects showed that vacuum infusion was the most suitable method to infuse calcium and pectin methylesterase into fruit pieces. The use of combined calcium and pectin methylesterase solution showed more efficiency to improve the texture of fruit products (fresh-cut fruit, thermal process fruit, and frozen fruits) than the use of calcium or pectin methylesterase alone.

MATERIALS AND METHODS

Materials

1. Raw materials

1.1 Ripe apples (cv. Fuji), mangoes (cv. Nam Dok Mai), cantaloupes (cv. Sunlady), and pineapples (cv. Smooth Cayenne) were purchased from Si Mum Muang central market in Bangkok, Thailand.

1.2 Ripe mangoes (cv. Kent) were purchased from a local supermarket in Davis, California, U.S.A.

2. Chemical substances

2.1 3-Phenyl-phenol ($C_6H_5C_6H_4OH$, Sigma, St. Louis, MO, USA)

2.2 Acetone (CH_3COCH_3, Merk, Darmstadt, Germany)

2.3 Acetylacetone ($CH_3COCH_2COCH_3$, Sigma, St. Louis, MO, USA)

2.4 Alcohol oxidase (from *Pichia pastoris*, Sigma, St. Louis, MO, USA)

2.5 Ammonium acetate ($CH_3CO_2NH_4$, Sigma, St. Louis, MO, USA)

2.6 Calcium chloride ($CaCl_2 \cdot 2H_2O$, Ajax Finechem Pty Ltd., Taren Point, Australia)

2.7 Calcofluor white stain (Sigma, Buchs, Switzerland)

2.8 D-(+)-Galacturonic acid ($C_6H_{10}O_7 \cdot H_2O$, Sigma, St. Louis, MO, USA)

2.9 di-Potassium hydrogen orthophosphate (K_2HPO_4, Ajax Finechem Pty Ltd., Taren Point, Australia)

2.10 Ethanol (C_2H_5OH, Merk, Darmstadt, Germany)

2.11 Glacial acetic acid (CH_3CO_2H, Sigma, St. Louis, MO, USA)

2.12 Lanthanum chloride ($LaCl_3$, Fisher Scientific, Leics, UK)

2.13 Methanol (CH_2OH, Merk, Darmstadt, Germany)

2.14 Nitric acid (HNO_3, J.T. Baker, Bangkok, Thailand)

2.15 Pectin methylesterase (commercial PME from *Aspergillus aculeatus*, Novoshape, Novozymes, Bagsvaerd, Denmark)

2.16 Potassium dihydrogen orthophosphate (KH_2PO_4, Ajax Finechem Pty Ltd., Taren Point, Australia)

2.17 Purpald ($C_2H_6N_6S$, Sigma, St. Louis, MO, USA)

2.18 Sodium hydroxide (NaOH, Merk, Darmstadt, Germany)

2.19 Sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$, Ajax Finechem Pty Ltd., Taren Point, Australia)

2.20 Sulfuric acid (H_2SO_4, Merk, Darmstadt, Germany)

2.21 Whatman no.1 filter paper (Whatman International Ltd, Maidstone, England)

3. Instruments

3.1 Blender (Waring, USA)

3.2 Centrifuge (CN-1050, Hsiangtai, Taiwan)

3.3 Chest freezer (SF-C1497, Sanyo, Japan)

3.4 Confocal laser scanning microscope (LSM 5 PASCAL, Carl Zeiss, Germany)

3.5 Cryogenic freezer (Minibatch 1000 L, Bangkok Industrial Gas Co., Thailand)

3.6 Hand refractometer (Digital hand-held pocket refractometer, PAL-1, Atago, Japan)

3.7 Hot air oven (ULE500, Memmert, Germany)

3.8 Laboratory balance (AP210-0, Ohaus, Switzerland)

3.9 Laboratory balance (BP 3100S, Sartorius, Germany)

3.10 Low temperature incubator (IPP400, Memmert, Germany)

3.11 Muffle furnace (Tactical 308, Gallenkamp, Leicestershire, UK)

3.12 Spectrophotometer (GENESYSTM10, Thermo Electron Corporation, USA)

3.13 Stirrer (IKA C-MAG HS7, IKA, Germany)

3.14 Texture analyzer. (TA.XT2, Stable Micro Systems, UK)

3.15 Vacuum oven (Eyela vacuum oven, VOS-450SD, Tokyo Rikakikai, Japan)

3.16 Vortex mixer (Vortex-Genie2, Scitific Industries Inc., New York, USA)

3.17 Water bath (Schutzart DIN40050-IP20, Memmert, Germany)

University of California at Davis

3.18 Air blast freezer (Conrad freezer, Barber Colman company, Rockford, Illinois, USA)

3.19 Confocal laser scanning microscope (Olympus IX 71, Olympus Inc., Center Valley, PA)

3.20 Microplate Photometer (Multiskan FC, Thermo Fisher Scientific, USA)

3.21 Texture Analyzer (TA.XTplus, Stable Micro Systems, UK)

3.22 Vacuum oven (Isotemp Vacuum Oven Model 280A, Fisher Scientific, Pittsburgh, PA, USA)

Methods

1. Fruit properties and freezing characteristic of fruits

1.1 Apples (cv. Fuji), mangoes (cv. Nam Dok Mai), cantaloupes (cv. Sunlady), and pineapples (cv. Smooth Cayenne) were selected for uniformity in size, the maturity based on the peel and flesh color, and the total soluble solids content. The total soluble solids content were in the range of 11 – 14 °Brix for apples, 16 – 19 °Brix for mangoes (cv. Nam Dok Mai), 9 – 11 °Brix for cantaloupes, and 13 – 15 °Brix for pineapples.

1.2 Twenty percent of total length from both the stem end and the blossom end of fruits were discarded, as those parts are highly diverse in fruit properties, particularly firmness and sweetness. Only the central part of the fruits was used to minimize any variation within the samples. All fruits were washed, peeled, and cut into 1.5 cm cubes. Eight fruit cubes were packed per one plastic bag (7.5 x 20 cm).

1.3 All fruit cubes were frozen at -40°C in a cryogenic freezer until the central temperature of the samples reached -25°C. The freezing rate of the samples was expressed as the rate of temperature decrease from the initial temperature (25˚C) to -18˚C per minute (˚C/min). The frozen samples were stored at -18°C in a chest freezer for 30 days and thawed at 8°C in a low temperature incubator prior to analysis for frozen-thawed samples. The central temperature of the samples during the freezing and thawing process were recorded every 1 min using thermocouples and data logger. The freezing time was 30 – 45 min and the thawing time was 120 min. All of these processes were repeated for 2 replications.

1.4 The moisture contents of all fresh fruit samples were analyzed by means of drying the samples in a vacuum oven at 70˚C until the attainment of a constant weight (AOAC., 2000). The measurements were done in triplicate for each treatment.

1.5 For total soluble solids content, the fresh fruits were blended and crushed through cheesecloth. Total soluble solids was measured from the fruit juices by a hand refractometer. The measurements were done in triplicate.

1.6 The obtained data were analyzed by a one-way analysis of variance with SPSS for Windows. Duncan's multiple range test was used to compare the means ($p<0.05$).

2. Effect of calcium pretreatment on pectin content, texture, and microstructure of four frozen fruits

2.1 Selection of calcium chloride concentration

In comparison between the four fruit samples, cantaloupe has the lightest taste in both sweetness and sourness. Therefore, ripe cantaloupe was chosen to study the suitable concentration of calcium because of the ease to detect bitterness of calcium chloride from the samples.

2.1.1 Cantaloupes were selected for uniformity in size, maturity based on peel and flesh color, and total soluble solids content (9 – 11 °Brix). Twenty percent of total length from both the stem end and the blossom end of the fruit samples was discarded to minimize any variations within the samples, as those parts are highly diverse in fruit properties, particularly firmness and sweetness. The samples were then washed, peeled, and cut into 1.5 cm cubes. Cantaloupe cubes were immersed in 0.5%, 1%, and 1.5% calcium chloride solution for 15 min.

2.1.2 The ranking test was used for this evaluation. All samples (the untreated and all three calcium treated cantaloupes) were rank for the degree of bitterness and firmness by 25 untrained panelists. The samples temperature was 25 °C.

2.1.3 The samples were ranked from "1" to "4" for the lowest to the highest degree of bitterness and firmness.

2.1.4 The data were analyzed by Friedman's test (Meilgaard *et al*., 2007) (Appendix B).

2.2 Ripe apples (cv. Fuji), mangoes (cv. Nam Dok Mai), cantaloupes (cv. Sunlady), and pineapples (cv. Smooth Cayenne) were selected for the uniformity the same with section 1.1. The samples were then washed, peeled, and cut into 1.5 cm cubes. As to decrease any potential variations of the initial sample between treatments, the fruit cubes from each fruit were segregated for each individual treatment.

2.3 For the calcium treated samples, fruit cubes were immersed in 1% calcium chloride solution (selected concentration from section 2.1) for 15 min at room temperature. Untreated fruit cubes were used as control samples.

2.4 Both control and calcium treated samples were packed in plastic bags. Eight fruit cubes were packed per one plastic bag (7.5 x 20 cm). After packing, fruit cubes were frozen at -40°C in a cryogenic freezer until the central temperature of each sample reached -25°C (around 30 – 45 min). The frozen samples were then stored at -18°C in a chest freezer for 30 days and thawed at 8°C in a low temperature incubator for 120 min prior to analysis. The central temperature of the samples during the freezing and thawing process were recorded every 1 min using a thermocouple and data logger. All treatments were repeated for 2 replications.

2.5 Fresh and frozen-thawed fruits were analyzed for their physico-chemical properties, microstructure, and sensory characteristics.

2.5.1 Moisture content

The moisture contents of both the fresh control and calcium treated samples were analyzed by the same method in section 1.4.

2.5.2 Calcium content

Fresh control and calcium treated samples (3 – 5 g) were precisely weighed and subsequently dried in an oven at 105˚C. The dry samples were incinerated in a muffle furnace at 550˚C. The ash was subsequently dissolved in 1 mL of 1N HNO_3 and 0.5 mL of 5% $LaCl_3$ and brough to 50 mL with 1 N HNO_3 (adapted from Alonso *et al*. (1997)). The calcium content of the extract solution was analyzed with the application of an atomic absorption spectrophotometer (AA 280 FS, Varian Inc., USA) at the Scientific and Technological Research Equipment Centre Foundation, Chulalongkorn University. An air-acetylene flame was used at a wavelength of 422.7 nm. The measurements were done in triplicate and the results were calculated using the following equation:

$$\text{Calcium content} = (C \times V) / (10 \times W)$$

where C is the calcium concentration obtained from an atomic absorption spectrophotometer (ppm), V is the total volume of the extract solution (mL), and W is the weight of the sample (g). The calcium content was expressed as mg/100g sample.

2.5.3 Extraction of the alcohol insoluble residue (AIR)

The alcohol insoluble residue was extracted from the fresh and frozen-thawed samples in accordance with the method outlined by McFeeters and Armstrong (1984). The samples (30 g) were blended with 150 mL of 95% ethanol for 2 min and filtered on a Buchner funnel, to which 75 mL of 70% ethanol was added and subsequently removed by suction, through a Whatman no.1 filter paper. Then the samples were washed with 100 mL of acetone. The alcohol insoluble residues were dried in a hot air oven at 40°C for 24 hr. The measurements were done in triplicate and the results were calculated using the following equation:

$$AIR = (W_t - W_i) \times 100 / W_d$$

where W_i is the initial weight of Whatman filter paper (g), W_t is the weight of Whatman filter paper and AIR after dried in a hot air oven (g), and W_d is the dry weight of the sample (g). AIR content was expressed as g/100 g dry sample.

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2.5.4 Total pectin content

Galacturonic acid content in AIR was analyzed and expressed as total pectin content. The analysis started with the addition of 4 mL of chilled concentrated sulfuric acid to 10 mg of AIR. This solution was placed in an ice-water bath, stirred gently, and mixed with 2 mL of water. The dissolved solution was then transferred to a volumetric flask and adjusted to its volume (Ahmed and Labavitch, 1978). The obtained solution was used in the assay for galacturonic acid by means of the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). A 0.0125 M solution of sodium tetraborate in concentrated sulfuric acid (4.8 mL) was added to 0.8 mL of the dissolved solution which was placed in an ice-water bath. The mixture was shaken in a Vortex mixer, and then the tube was heated in a water bath at 95˚C for 5 min. After cooling in an ice-water bath, 80 µL of the m-hydroxydiphenyl reagent was added. The tube was shaken and measured for the absorbance of the extract solution at 520 nm with the application of a spectrophotometer. The measurements were done in triplicate and the results were calculated using the following equation:

$$\text{Total pectin content} = ((Abs_s - Abs_b) \times V) / (\text{Slope} \times \text{AIR} \times 10^4)$$

where Abs_s is the absorbance of the extract solution, Abs_b is the absorbance of blank, V is the total volume of the extract solution (mL), Slope is the slope of the standard curve, and AIR is the weight of AIR (g). Total pectin content was expressed as g/100 g AIR.

2.5.5 Water soluble pectin content (WSP)

Water soluble pectin was extracted from AIR in accordance with the method as outlined by Majumder and Mazumdar (2002). The AIR (50 mg) was dispersed at room temperature in 25 mL of distilled water by stirring for 30 min. The solution was centrifuged at 3000g and the supernatant which was comprised of water soluble pectin substances was segregated. Each sample was extracted using a three fold extraction. These supernatants were collected and adjusted to 100 mL with distilled water. The galacturonic acid content of those solutions was analyzed using the method of Blumenkrantz and Asboe-Hansen (1973) and shown as the water soluble pectin content of the samples. A 0.0125 M solution of sodium tetraborate in concentrated sulfuric acid (4.8 mL) was added to 0.8 mL of the dissolved solution which was placed in an ice-water bath. The mixture was shaken in a Vortex mixer, and then the tube was heated in a water bath at 95˚C for 5 min. After cooling in an ice-water bath, 80 µL of the m-hydroxydiphenyl reagent was added. The tube was shaken and measured for the absorbance of the extract solution at 520 nm with the application of a spectrophotometer. The measurements were done in triplicate and the results were calculated using the following equation:

$$WSP = ((Abs_s - Abs_b) \times V) / (Slope \times AIR \times 10^4)$$

where Abs_s is the absorbance of the extract solution, Abs_b is the absorbance of blank, V is the total volume of the extract solution (mL), Slope is the slope of the standard curve, and AIR is the weight of AIR (g). Water soluble pectin content was expressed as g/100 g AIR.

2.5.6 Texture

The texture of fresh and frozen-thawed samples was determined by means of a Texture Analyzer with a 36 mm cylindrical flat head probe (P36). The firmness was measured with a compression at 50% strain and a compression rate of 1

mm/s. The maximum peak force was expressed as firmness in Newton. Ten pieces of fruit were used for each treatment.

2.5.7 Drip loss

The drip loss of the frozen samples was measured in accordance with the method outlined by Lowithun and Charoenrein (2009). Frozen samples were laid over absorbent paper and placed into double layered zip lock plastic bags to eliminate evaporation during thawing. Then the samples were thawed at 8 ˚C. The drip loss was evaluated with periodic weighing of the absorbent paper until a constant value was reached (6 hr for apple and pineapple, and 9 hr for mango and cantaloupe). The measurements were done in triplicate and the results were calculated using the following equation:

$$DL\ (\%) = (W_t - W_0)/W_s \times 100$$

where W_0 is the weight of the absorbent paper prior to thawing, W_t is the weight of the absorbent paper after thawing and W_s is the weight of the sample.

2.5.8 Microstructure

Both the control and calcium treated fruits were sliced to 1 mm thickness and observed with the application of a confocal laser scanning microscope with a 10x objective lens. The microscope was controlled with the LSM 5 PASCAL software. Cell walls of the samples were stained with a calcofluor white stain. The maximum emission wavelength of this fluorescent dye is 433 nm and the excitation occurs at approximately 355 nm. Subsequently to being observed the micrographs for both the control and calcium treated fruit slices were frozen in a chest freezer at -20°C for 14 days. Then, the frozen fruit slices were thawed and observed using a confocal laser scanning microscope for the frozen-thawed samples.

2.5.9 Sensory evaluation

The sensory characteristics of all samples were evaluated by 10 trained panelists. The two characteristics examined in this study were firmness, and bitterness. Agar gel and calcium chloride solutions were used as reference samples for both the selection and training sessions.

For the selection session, the ability of the panelists to detect the difference among similar samples and the ability to discriminate graded levels of intensity of a given attribute were evaluated using a duo-trio test, and ranking test, respectively. Only panelists who passed both the duo-trio test and the ranking test were selected to attend the training session (Meilgaard *et al.* 2007).

During the training session, the panelists were trained to recognize scales. Three levels of agar gels from low (0.7% w/v) to high concentrations (2% w/v) were used as reference samples and a value of 1 characterized a soft texture, a value of 3 showed a medium firm texture, and a value of 5 meant a very firm texture, respectively. In the same way, three levels of calcium chloride solution from no calcium chloride (only water) to a high calcium chloride concentration (0.3% w/v) were used as reference samples with a similar ranking system: a value of 1 which meant no bitterness, a value of 3 which meant medium bitterness, and a value of 5 which meant very bitter, respectively. The trained panelists had to recognize these scales prior to evaluating the real samples. A scoring test (5 point scale) was used to evaluate the firmness and bitterness of the real samples (all fruit samples). The samples temperature was controlled at 25 °C during the evaluation.

2.6 Statistical analysis

The obtained data were analyzed using a one-way analysis of variance with SPSS for Windows. Duncan's multiple range test was used to compare the means ($p<0.05$).

Remark: Part 1-2 study at Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand from April 2009 to June 2010.

3 Effect of vacuum infusion with pectin methylesterase and calcium on texture and microstructure of fresh and frozen mangoes

3.1 Ripe mangoes (cv. Kent) were selected for uniformity in size, and the maturity was based on flesh color, texture, and the total soluble solids content. In this study, mangoes with total soluble solids in the range of 16 – 19°Brix and firmness in the range of 23 – 30 N were selected. The firmness of mango flesh, measured after the skin was removed, was evaluated using a Texture Analyzer with a 25kg load cell and a 8 mm cylindrical flat head probe (P8). A compression depth of 8 mm and a compression rate of 1 mm/s were used. The maximum peak force was expressed as firmness in Newton. Approximately twenty percent of length from both the stem end and the blossom end of the fruit were discarded, as those parts were highly diverse in the terms of fruit properties. Only the central parts of the fruits were used to minimize any variations within the samples. The samples were washed, peeled, and cut into cubes of 1.5 cm in length. To decrease any potential variation in the initial sample between treatments, the fruit cubes from each fruit were segregated for each individual treatment.

3.2 The untreated mango cubes and mango cubes which were infused with water were used as control samples. The infusion solution under evaluation was composed of 0.001 unit of PME/mL of infusion solution and 1% calcium chloride (w/v). The optimal PME concentration (0.001 unit of PME/mL of infusion solution) was selected based on a preliminary study. A ratio of 1:3 (w/w) of the samples and the infusion solution was used to ensure complete immersion of all samples in the infusion solution. Three levels of vacuum pressure, from no vacuum (atmospheric pressure, 101.325 kPa), to a medium level of vacuum (50 kPa or 50.6 % vacuum), and the highest level of vacuum (10 kPa or 90.1 % vacuum) were used. All infusion treatments were done at room temperature (25°C).

3.3 For the infusion at atmospheric pressure, mango cubes were submersed in the infusion solution for 10 min at atmospheric pressure.

3.4 For the vacuum conditions, the mango cubes and the infusion solution were inserted into plastic containers. An aluminum mesh was used for the immersion of the sample in the infusion solution. The plastic containers were placed in a vacuum oven at controlled pressure levels as indicated by the pressure gauge of the instrument.

The samples were submersed into the infusion solution for 10 min at both 50 kPa and 10kPa. After 10 min, the vacuum was released to attain an atmospheric pressure within 1 min, with the subsequent disposal of the solution. Infusion of liquid solution occurred during the vacuum was released. The samples were then placed on paper towels for 5 min, packed in plastic bags, and kept at 25°C for 2 hr prior to analysis or freezing, to ensure adequate time for the reaction of the enzyme and calcium with the pectin present in the samples.

3.5 Samples were frozen in an air blast freezer at -50°C until the central temperature of the samples reached -25°C. Frozen samples were subsequently kept in a -20°C chest freezer for 14 days. The frozen samples were thawed at 4°C for 2 hr prior to analysis. Each experiment was repeated in triplicate.

3.6 Fresh and frozen-thawed mangoes were analyzed physico-chemical properties and microstructure.

3.6.1 Weight gain

Weight gain of the samples after infusion was calculated by means of the following equation:

$$\text{Weight gain (\%)} = (W_t - W_i) / W_i \times 100$$

where W_i denotes the initial sample weight, and W_t denotes the sample weight subsequent to infusion. The measurements were done in triplicate.

3.6.2 Moisture content

Moisture content of all fresh samples was analyzed by the same method in section 1.4. The measurements were done in triplicate.

3.6.3 Calcium content

Calcium content of all fresh samples was analyzed by the same method in section 2.5.2.

3.6.4 Pectin methylesterase activity

PME activity of fresh samples was determined by the method outlined by Anthon and Barrett (2004) with analysis of the methanol released from the samples with alcohol oxidase and acetylacetone. To allow for the determination of the release of methanol, both the control samples and the samples immediately subsequent to infusion were cut into thin slices of approximately 1mm thickness. 18 mL of water and 2 g of sliced mangoes were added to the samples in a glass bottle with a screw cap. The bottles were sealed and incubated in a shaking water bath at 25°C for 60 min. The immersion liquid (200 µL) was collected immediately after the immersion and subsequent to incubation for 60 min. To determine the methanol content, 50µL of the samples was added by pipette to a microplate. As a standard, 50µL of 0 to 2.0 mM methanol was added by pipette to the microplate. The assay was started by the addition of 50µL of 1.0 U/mL alcohol oxidase. The alcohol oxidase was prepared by dilution of the enzyme with 0.2 M phosphate buffer pH 7.0 until a final concentration of 1.0 U/mL was attained. After 20 min at room temperature, 100 µL of the acetyl acetone reagent was added. The acetyl acetone reagent was composed of 12 mL of 2 M ammonium acetate, 36 µL of glacial acetic acid, and 24 µL of acetyl acetone. After 60 additional minutes at room temperature, the absorbance were read at

405 nm on a Microplate Photometer. The measurements were done in triplicate and the results were calculated using the following equation:

$$\text{Methanol (}\mu\text{moles/g sample)} = ((Abs_s - Abs_b) \times V)/(\text{Slope} \times W \times 10^3)$$

$$\text{PME activity} = Methanol_{60} - Methanol_0$$

where Abs_s is the absorbance of the extract solution, Abs_b is the absorbance of blank, V is the total volume of the extract solution (mL), Slope is the slope of the standard curve, W is the sample weight (g), $Methanol_{60}$ is the methanol content in the immersion liquid after immersion for 60 min, and $Methanol_0$ is the methanol content in the immersion liquid which was collected immediately after the immersion. PME activity was expressed as μmoles methanol/g sample/hr.

3.6.5 Texture

The texture of both fresh and frozen-thawed samples were determined by a Texture Analyzer with a 25kg load cell, and a 50 mm cylindrical flat head probe (P50). Firmness was measured by compression at 50% strain and a compression rate of 1 mm/s. The maximum peak force was expressed as firmness. Ten replicate pieces of fruit were used for each sample.

3.6.6 Microstructure

The samples were sectioned with a razor blade. The thickness of the sections were approximately 1 mm. The microstructure of the samples was observed with the application of a confocal laser scanning microscope using a 10x objective lens and an exposure time of 5000 ms. The microscope was controlled with the Metamorph software. The cell walls of the samples were stained with a calcofluor white stain. The maximum emission wavelength of this fluorescent dye is 433 nm and the excitation occurs at approximately 355 nm.

3.7 Statistical analysis

The obtained data were analyzed by one-way analysis of variance. Duncan's multiple range test was used to compare the means ($p<0.05$).

Remark: Part 3 study at Department of Food Science and Technology, College of Agricultural and Environmental Sciences, University of California, Davis, USA from July 2010 to April 2011.

RESULTS AND DISCUSSION

1. Fruit properties and freezing characteristics of fruits

1.1 Freezing point and freezing profile

The temperature of each fruit was recorded during freezing at -40˚C. The freezing profiles of the four types of fruit are shown in Figure 8. The approximate freezing points of all fruits were estimated from the freezing profile, where the obviously change of slope was observed. The freezing points of all fruits were in the range of -1.6 to -3.0°C (Table 1). The fruit freezing point is very important for the storage and processing of fruit. There were some previous reports on the freezing point of fruits, with values between -2.20 to -2.32°C for apple (Jie *et al*., 2003) and -1.4 to -2.0°C for pineapple (Hayes, 1987). These values were slightly different in comparison to our freezing point results (-1.6 and -2.1°C for apple and pineapple, respectively) which is potentially due to the diversity of fruit varieties, maturity, or growing practices.

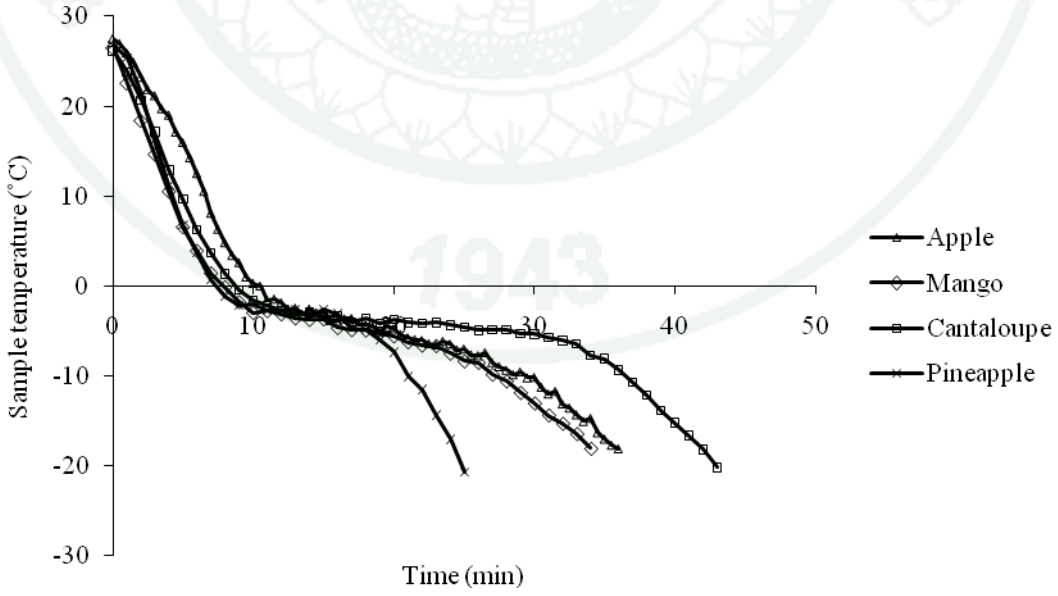

Figure 8 Freezing profile of fruit samples frozen at -40˚C. The data were averaged from 2 replications.

In the case of these four fruits, mango (cv. Nam Dok Mai) displayed the lowest freezing point (-3.0°C) followed by pineapple (-2.1°C), and the others showed the same value (-1.6°C). The total soluble solids of these four fruits (Table 1) were also significantly different ($p<0.05$). The highest total soluble solids value was found in mango followed by pineapple, apple, and cantaloupe. Higher total soluble solids contents imply a higher sugar content which results in a lower freezing point. Our results confirmed the negative correlation between the freezing point and total soluble solids content of the fruit samples (Figure 9). The mathematical model was

$$Y = 0.6807 – 0.2004X$$

Where Y is the freezing point, and X is the total soluble solids content of fruit. The coefficient of determination (R^2) was 0.72.

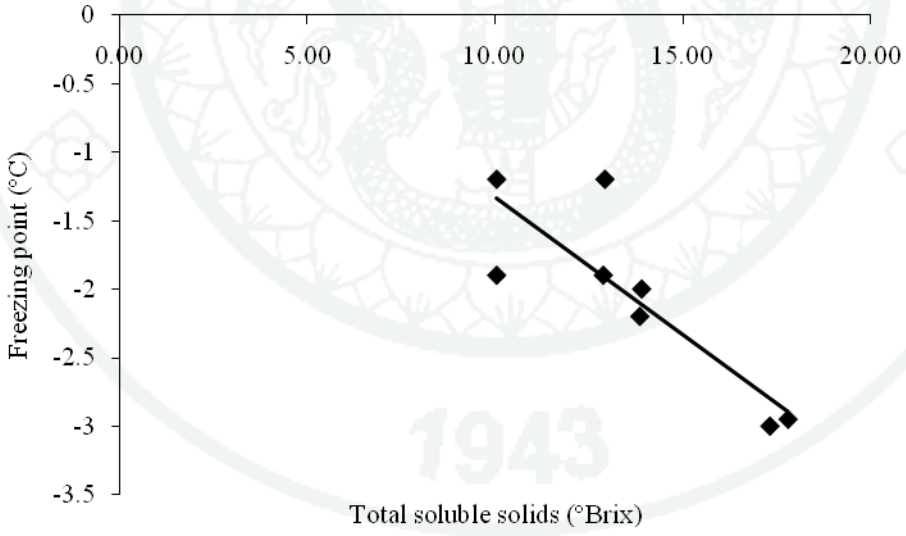

Figure 9 The correlation between freezing point (°C) and total soluble solids content of fruits.

Chen *et al*. (1990) studied the depression of the freezing points of mixed solutions of sugar and acid at various concentrations (0-60°Brix). Their results showed that the freezing points of these solutions decreased as concentrations (°Brix) increased. The freezing points obviously decreased when the concentration was higher than 30°Brix. Jie *et al*. (2003) measured freezing points and soluble solids of 11 fruits. They found a high negative correlation between soluble solids and the freezing point. Auleda *et al*. (2011) studied the freezing point of apple juice, pear juice and peach juice at various concentrations within the range of 10–40 °Brix. Their results confirmed that the freezing point of the juices decreased with increasing concentration.

Table 1 Properties, freezing rate, and freezing point of apple, mango (cv. Nam Dok Mai), cantaloupe and pineapple.

Fruit	Moisture content (g/100g sample)	Total soluble solids (°Brix)	Freezing rate (˚C/min)	Freezing point (°C)
Apple	86.54^{b}±0.70	12.9^{c}±0.03	1.3^{b}±0.1	-1.6
Mango	82.43^{c}±0.60	17.6^{a}±0.34	1.3^{b}±0.2	-3.0
Cantaloupe	91.64^{a}±1.12	10.0^{d}±0.00	1.0^{b}±0.1	-1.6
Pineapple	85.61^{b}±0.87	13.9^{b}±0.04	1.9^{a}±0.2	-2.1

* Data are mean ± standard deviation from 2 replications. In each column, values followed by different letters are significantly different ($p<0.05$).

1.2 Freezing rate

The freezing rate of the tested fruits is shown in Table 1. These results show that pineapple had the highest freezing rate ($p<0.05$) and cantaloupe had the lowest. The composition of fruit especially moisture content had an effect on the freezing rate. Food products with higher initial freezing points, lower initial water content and higher unfreezable water content have a shorter freezing time (Hsieh *et*

al., 1977). For that reason, the high moisture content of the cantaloupe samples was likely to be what caused the low freezing rate (Table 1).

Apple and pineapple had similar moisture contents but pineapple had a significantly higher freezing rate ($p<0.05$) than that of apple. The reason was because of the different composition and structure of the fruits which affected their thermal conductivity. The thermal conductivity is a parameter which is necessary to quantify freezing rates. Apples were reported to have a thermal conductivity around 0.43 – 0.513 W/m·°C, while the thermal conductivity of pineapples were higher than that of apples (Table 2).

Table 2 Thermal properties of apple, mango, cantaloupe and pineapple from literature reviews.

Thermal properties	Fruits	Values	Reference
Thermal conductivity (W/m·°C)	Apple	0.43 - 0.513	Donsi *et al*. (1996); Sweat (1974)
	Mango	0.562	Ikegwu and Ekwu (2009)
	Cantaloupe	na*	
	Pineapple	0.549, 0.567	Sweat (1974); Ikegwu and Ekwu (2009)
Thermal diffusivity (m^2/S)	Apple	na	
	Mango	$1.39x10^{-7}$	Ikegwu and Ekwu (2009)
	Cantaloupe	na	
	Pineapple	$1.52x10^{-7}$	Ikegwu and Ekwu (2009)

* na = not available

In comparison between mango and pineapple, the freezing rate of mangos were significantly lower ($p<0.05$) than that of pineapple samples even though the mangos also had a lower moisture content. The reason may be due to the difference in thermal diffusivity of these fruits.

Ikegwu and Ekwu (2009) estimated the thermal properties of some tropical fruits. They found that mango and pineapple had similar thermal conductivities but mango showed lower thermal diffusivity than that of pineapple (Table 2). Our study clearly shows that besides moisture content, thermal conductivity and thermal diffusivity also have an effect on the freezing profile and freezing rate of fruit samples.

2. Effect of calcium pretreatment on pectin content, texture, and microstructure of four frozen fruits

2.1 Selection of calcium chloride concentration

In comparison between the four fruit sample types, cantaloupe has the mild taste in both sweetness and sourness. Therefore, cantaloupe was chosen to study the suitable concentration of calcium chloride because of the low hiding effect from the fruit on the bitterness of calcium chloride. The samples were ranking by the panelists from “1” to “4” for the lowest degree of bitterness to the highest degree of bitterness. In the same way, the panelists also ranked firmness of the sample from “1” to “4” for the lowest degree of firmness to the highest degree of firmness. The ranking orders of all samples are shown in Figure 9. These results show the increase in bitterness and firmness levels subsequent to immersion in the calcium chloride solution. The control sample was ranked to have the lowest bitterness and firmness level whereas the sample after immersion in 1.5% calcium chloride was ranked to have the highest bitterness and firmness level. Although, the sample immersed in 1% calcium chloride was ranked to have a higher bitterness than that of the control sample, the bitterness of this sample was not significant different ($p>0.05$) from the bitterness of the sample after immersion in 0.5% calcium chloride. Moreover, the samples which were immersed in 1% calcium chloride had higher firmness values than those of the samples immersed in 0.5% calcium chloride, although there was no significant difference. Therefore, 1% calcium chloride was chosen to be used in the next experiments.

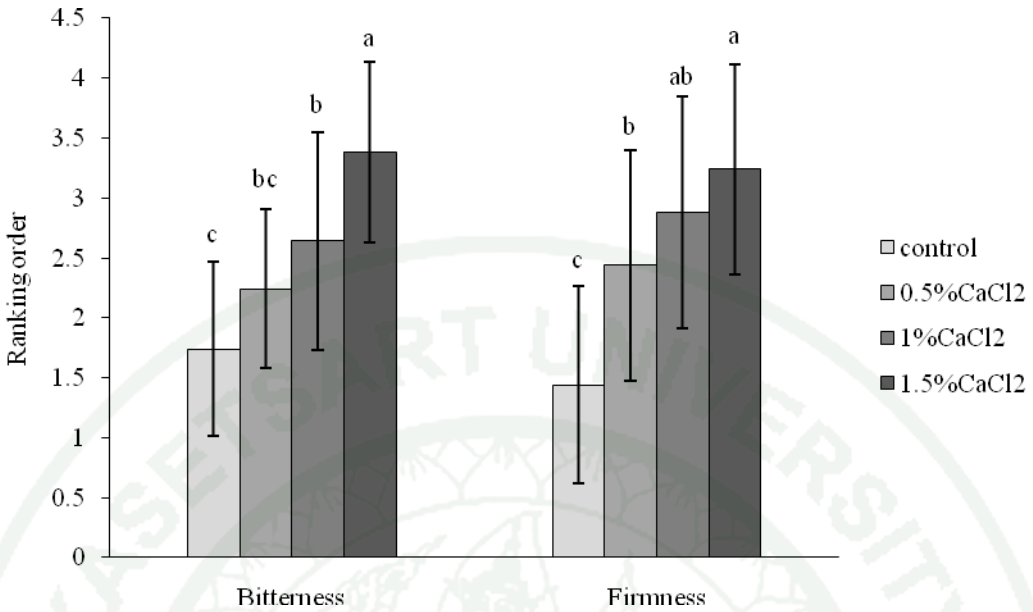

Figure 10 Ranking order of bitterness and firmness of control cantaloupe and cantaloupe after immersion in various calcium chloride concentrations. Bars indicate standard deviation with different letters denoting significant differences ($p<0.05$).

2.2 Moisture content

The moisture content of both the fresh control and calcium treated fruit samples are shown in Table 3. The calcium treated samples tended to have higher moisture contents than those of the control samples. The linkages between pectin molecules are caused by the formation of calcium bridges which potentially result in an increase in the amount of water bound within the pectin polymer and the firm tissue components (Chang *et al*., 1996). However, the immersion in calcium chloride solution for 15 min did not show a significant effect ($p>0.05$) on the moisture content of any fruit type.

Table 3 The moisture content (g/100g sample) of the fresh control and calcium treated fruits.

Sample	Moisture content (g/100g sample)	
	Control	Calcium
Apple	86.54[a]±0.70	87.61[a]±0.56
Mango (cv. Nam Dok Mai)	82.43[a]±0.60	84.54[a]±0.46
Cantaloupe	91.64[a]±1.12	91.86[a]±0.71
Pineapple	85.61[a]±0.87	87.07[a]±0.59

* Data are the mean ± standard deviation from 2 replications. Values in the columns of the control and calcium treated samples of identical fruits, followed by different letters are significantly different ($p<0.05$)**.**

2.3 Calcium content

Both the fresh control and the calcium treated samples were tested for the total calcium content in order to confirm the increase in calcium for fruit cubes subjected to the immersion process. The immersion in the calcium solution caused a significant increase ($p>0.05$) in calcium content of all fruit pieces (Table 4).

Luna-Guzman *et al.* (1999) reported the increase in calcium content in cantaloupe cylinders from 16 to 54 mg/100g sample after immersion in 2.5% calcium chloride solution for 1 min at 20°C. The cantaloupe cylinder diameter was 1.8 cm and cylinder width was 2 cm. The increasing in calcium content of their report was slightly higher than the present result which was potentially because of the high concentration of calcium (2.5%) in their study. For another fruits, Aguayo *et al.* (2008) reported the increasing in calcium content in strawberries from 12 to 20 mg/100 g sample after immersion in 1% calcium chloride. They use whole strawberries as the sample therefore the diffusion of calcium into the fruit may be blocked by a peel, thus the increasing in calcium content of their result rather low.

Table 4 The calcium content (mg/100g sample) of the fresh control and calcium treated fruits.

Sample	Calcium content (mg/100g sample)	
	Control	Calcium
Apple	2.44^{b}±0.11	27.16^{a}±1.73
Mango (cv. Nam Dok Mai)	2.18^{b}±0.43	28.43^{a}±1.15
Cantaloupe	8.78^{b}±0.23	30.07^{a}±1.39
Pineapple	2.74^{b}±0.43	40.15^{a}±0.43

* Data are the mean ± standard deviation from 2 replications. Values in the columns of the control and calcium treated samples of identical fruits, followed by different letters are significantly different ($p<0.05$).

2.4 Pectin content

Alcohol insoluble residue (AIR)

Alcohol insoluble residue extraction is a method commonly used to isolate cell wall material. During this procedure enzymes are inactivated and low molecular weight components are removed. The AIR content of all fruits is shown in Table 5 - 8. In the case of fresh control samples, the AIR values of all four fruit types were in the same 8.8 – 9.9 g/100 g dry sample range. Calcium treated samples showed significantly higher ($p<0.05$) AIR content than that of control samples both for fresh and frozen-thawed fruits. The freezing process did not have a significant ($p>0.05$) effect on the AIR values of any of the four fruit types.

Since pectins contribute to the structure of plant tissue both as a part of the primary cell wall and as the main middle lamella component, they function as a cementing material for the cellulosic network. The formation of calcium bridges between adjacent pectin molecules may result in the higher AIR extraction levels which could explain the higher yields of calcium treated fruits.

Total pectin content

Total pectin content was determined from galacturonic acid content in AIR and varied for all fruits types (Table 5 - 8). Apple showed the highest total pectin content followed by mango, cantaloupe, and pineapple. In the case of pineapple, the total pectin level was the lowest even in spite of the comparable level of AIR with respect to the other fruits. This result demonstrates that the pineapple cell wall is composed of a low pectin content with a high content of other fibers such as cellulose and hemicellulose. The total pectin content did not significantly change ($p<0.05$) subsequent to the calcium treatment or freezing process. A similar result was found in all four fruit types.

Alonso *et al*. (2005) analyzed the pectin content in frozen-thawed cherries after freezing at -70°C and storage at -24°C for 1 month. They found that the control and calcium treated (100 mM calcium chloride) frozen-thawed cherries showed no significant difference in total pectin content. Chassagne-Berces *et al*. (2009) reported the same total uronic acid content of fresh and frozen-thawed apple subsequent to being frozen at -20 or -80°C. Galetto *et al*. (2010) studied freezing by immersion in calcium chloride solutions on pectin content of strawberries. Their result showed no significant difference between the total pectin content of fresh and frozen-thawed strawberries. Moreover, the immersion freezing in a calcium chloride solution also did not affect the total pectin amount in the frozen-thawed samples.

Table 5 The pectin content of fresh and frozen-thawed apples.

Treatment	Alcohol insoluble residue (g/100g dry sample)	Total pectin (g/100g AIR)	Water soluble pectin (g/100g AIR)
Fresh control	9.83^{c}±0.59	28.52^{a}±0.05	2.79^{a}±0.29
Fresh calcium	11.90^{ab}±0.37	25.50^{a}±1.02	1.13^{b}±0.26
Frozen control	10.84^{bc}±0.63	30.30^{a}±0.00	3.16^{a}±0.06
Frozen calcium	12.90^{a}±0.64	29.12^{a}±2.03	1.57^{b}±0.00

* Data are the mean ± standard deviation from 2 replications. Values in the columns followed by different letters are significantly different ($p<0.05$).

Table 6 The pectin content of fresh and frozen-thawed mangoes (cv. Nam Dok Mai).

Treatment	Alcohol insoluble residue (g/100g dry sample)	Total pectin (g/100g AIR)	Water soluble pectin (g/100g AIR)
Fresh control	9.88^{b}±0.26	21.21^{a}±0.88	11.69^{ab}±0.61
Fresh calcium	11.52^{a}±0.51	18.27^{a}±0.05	9.85^{c}±0.78
Frozen control	9.56^{b}±0.24	21.02^{a}±1.17	12.72^{a}±0.50
Frozen calcium	11.36^{a}±0.78	18.62^{a}±1.15	10.20^{bc}±0.28

* Data are the mean ± standard deviation from 2 replications. Values in the columns followed by different letters are significantly different ($p<0.05$).

Table 7 The pectin content of fresh and frozen-thawed cantaloupes.

Treatment	Alcohol insoluble residue (g/100g dry sample)	Total pectin (g/100g AIR)	Water soluble pectin (g/100g AIR)
Fresh control	8.84^{b}±0.66	20.74^{a}±0.95	2.20^{ab}±0.19
Fresh calcium	10.30^{a}±0.08	18.76^{a}±0.45	0.32^{c}±0.02
Frozen control	8.62^{b}±0.26	21.83^{a}±0.69	2.62^{a}±0.40
Frozen calcium	9.72^{a}±0.45	22.32^{a}±2.02	1.61^{b}±0.04

* Data are the mean ± standard deviation from 2 replications. Values in the columns followed by different letters are significantly different ($p<0.05$).

Table 8 The pectin content of fresh and frozen-thawed pineapples.

Treatment	Alcohol insoluble residue (g/100g dry sample)	Total pectin (g/100g AIR)	Water soluble pectin (g/100g AIR)
Fresh control	9.42^{b}±0.16	7.79^{a}±0.54	1.09^{a}±0.14
Fresh calcium	11.83^{a}±0.64	6.61^{a}±0.76	0.65^{a}±0.30
Frozen control	8.52^{b}±0.08	9.27^{a}±0.40	0.98^{a}±0.16
Frozen calcium	11.24^{a}±0.91	7.85^{a}±0.66	0.80^{a}±0.01

* Data are the mean ± standard deviation from 2 replications. Values in the columns followed by different letters are significantly different ($p<0.05$).

Water soluble pectin content

The water soluble pectin content of frozen control apple, mango, and cantaloupe tended to increase subsequent to freezing and thawing. Nonetheless, the values were not significantly different ($p>0.05$). Calcium treatment showed different effects on the water soluble pectin of diverse fruits (Table 5 - 8). In the case of apple, mango, and cantaloupe, calcium pretreatment significantly decreased ($p<0.05$) the water soluble pectin of both the fresh and frozen-thawed fruits. However, subsequent to freezing and thawing, the increase in water soluble pectin of the calcium treated cantaloupe was more pronounced than that of the control cantaloupe. Although the calcium treatment most likely would have a negative effect on the water soluble pectin content of pineapple, the water soluble pectin content for pineapple was not significant different ($p>0.05$) for either treatment which is probably related to the low total pectin content of these fruit.

The effects of calcium pretreatment on water soluble pectin of apple, mango, and cantaloupe were potentially due to the reduction of the solubility of the cell walls and middle lamella pectin due to the cross-linking with calcium (Rico *et al*., 2007). The decrease in water soluble pectin content was found after the immersion of peach halves in 360 mg/L calcium chloride (Manganaris *et al*., 2005). Pinheiro and Almeida (2008) reported the reduction of water soluble pectin content after the immersion of tomato pericarps in 50 mM calcium chloride.

2.5 Firmness

The firmness of all samples was determined in terms of the maximum peak force and shown in Figure 11. Among these four fruits, mango had the lowest firmness which was probably related with the high water soluble pectin content. In comparison between fresh and frozen-thawed fruits as regardless on calcium treatment, the type of fruit exhibited an effect on %firmness decrease after the freezing and thawing process. In the case of apple, frozen-thawed apple cubes showed the highest level of %firmness decrease after freezing and thawing in spite of fresh

apple cubes having the highest firmness level. This phenomenon is likely because of the rigid structure, high firmness, and crunchiness of fresh apple in comparison with the others. This result show that fresh fruits with firm textures tend to have less resistance to deterioration by ice crystals during freezing and thawing than fresh fruits with a soft texture.

In consideration on calcium treatment, the calcium treatment did not significantly ($p>0.05$) increase the firmness of either the fresh or frozen-thawed apple and pineapple cubes, which may be related to the lack of a significant change in the water soluble pectin content of pineapple. The calcium treatment did not improve the firmness of both the fresh and the frozen-thawed apple samples. Most likely this is due to the high tissue firmness in fresh apple (it had the highest firmness observed in this study), thus the effect of calcium to improve the firmness of the fruit was minimal since the fruit was already firm. Severe tissue damage occurred in the frozen-thawed apple samples leading to the high firmness decrease and the high drip loss subsequent to freezing and thawing. Therefore, the treatment with calcium could not improve the firmness of frozen-thawed apple.

The effect of calcium treatment on the texture of fresh-cut apple and pineapple have also previously been reported. Alandes *et al*. (2006) studied the effect of calcium lactate (5 g/L) on the texture of fresh-cut apple cubes during storage at 4°C for 3 weeks. They found that after calcium immersion or storage for 1 week, the texture of the control and calcium treated apple was not significantly different. A significant effect of calcium to maintain texture of fresh-cut apple was observed after 2 weeks of storage when the control apple texture started to turn soft. Zhu *et al*. (2007) reported that the immersion in 1% calcium chloride did not improve the firmness of apple cubes but after blanching, calcium treated apple cubes showed a firmer texture than control samples. Antoniolli *et al*. (2003) evaluated the effects of calcium chloride on the quality of fresh-cut pineapple after being dipped in 1% and 2% calcium chloride solutions for 30 seconds. They found that being calcium dipped did not influence the texture of fresh-cut pineapple slices after being stored at 4°C for 0-12 days.

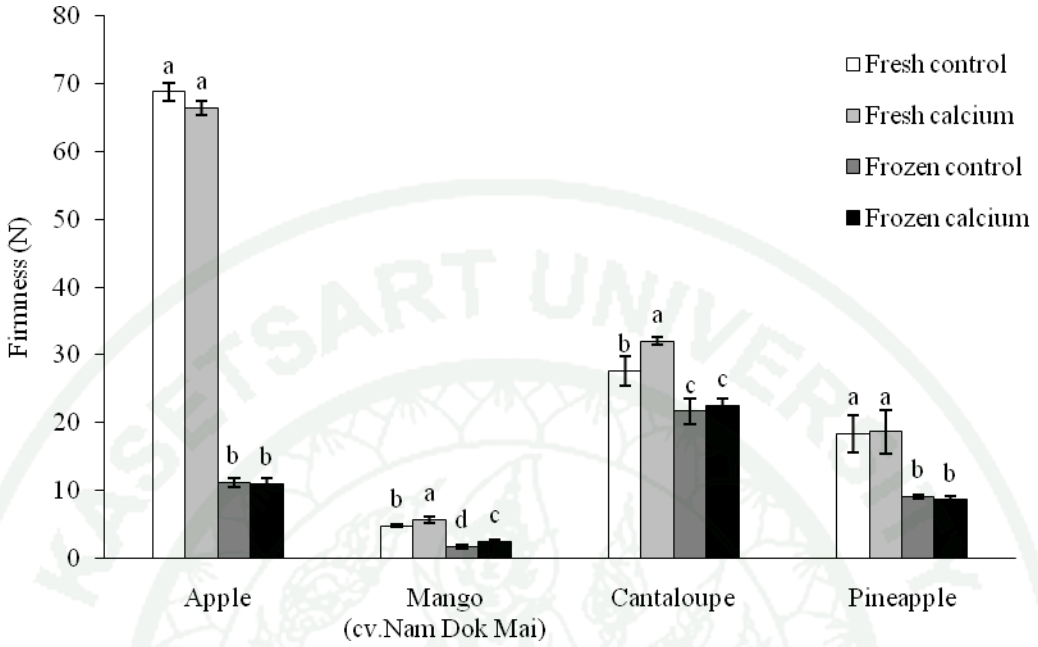

Figure 11 Firmness (N) of the control and calcium treated fruits. Bars indicate the standard deviation from 2 replications with different letters for identical fruit indicating a significant difference ($p<0.05$).

On the other hand, calcium treated fresh mango and cantaloupe were found to have significantly higher firmness values than the control group of fresh samples since calcium helps to maintain the cell wall integrity by means of interaction with the cell wall and middle lamella pectin to form calcium pectate (Rico *et al*., 2007). This effect of calcium on the texture of fresh-cut mango and cantaloupe has previously been reported. Gonzalez-Aguilar *et al*. (2008) evaluated the effect of a combined solution of 1% calcium chloride, 2% ascorbic acid, and 2% citric acid on the quality of three cultivars of fresh-cut mango cubes stored at 5°C. They reported that the use of this combined solution significantly reduced the loss of firmness of the fresh-cut mango. Luna-Guzman *et al*. (1999) studied calcium chloride dips to improve the firmness of fresh-cut cantaloupe cylinders during storage at 5°C. They found that when dipped for 1 min in 2.5% calcium chloride solutions at 20, 40 or 60°C, firmness was maintained or improved, especially at higher dip temperatures.

Subsequent to freezing and thawing, the firmness of all samples were found to decrease in comparison to the fresh samples. During freezing, any water present in the samples can partially form ice crystals which negatively affects the integrity of the cellular compartments, causing decreased levels of firmness subsequent to freezing and thawing of the samples. In comparison with the frozen-thawed control and the calcium treated samples, the decrease in firmness of the frozen-thawed control mango (66.26%) was more pronounced than that of the frozen-thawed calcium treated mango (55.66%). Moreover, the calcium treated frozen-thawed mango was found to be of significantly higher ($p<0.05$) firmness than that of the control frozen-thawed mango. Since mango has a high total pectin content, there is the potential for the extended binding sites of calcium to form calcium bridges and consequently enhance the tissue structure and decrease the firmness loss of frozen-thawed mango samples.

In the case of cantaloupe, the firmness of the frozen-thawed calcium treated samples did not significantly change ($p>0.05$) from that of the frozen-thawed control samples, in spite of the significantly increased firmness of the fresh calcium treated samples over than that of the fresh control sample. Furthermore, after freezing and thawing, the water soluble pectin of calcium treated cantaloupe was found to be increased more than that of the control cantaloupe (Table 7). This finding is most likely due to the high moisture content of cantaloupe. During freezing, a large amount of ice crystals were formed which were responsible for extensive microstructural changes in the fruit tissue. Thus, the calcium treatment may be ineffective to maintain the cell integrity of this sample subsequent to the freezing and thawing process.

Since, we hypothesized that pectin content in fruits might affect the effectiveness of calcium in improving firmness of fruits. According to the total pectin content, the calcium pretreatment should show the highest effect to improve firmness of apple follow by mango, cantaloupe, and the lest effect on pineapple. The firmness results showed the effectiveness of calcium to improve firmness of fresh mango and cantaloupe cubes, whereas did not affect firmness of fresh pineapple cubes. Nevertheless, firmness of fresh calcium treated apple cubes which had the highest

total pectin content did not significantly different from that of the untreated sample. Moreover, for frozen-thawed fruits, only calcium treated mango cubes had significantly higher firmness than that of the untreated sample. This finding demonstrated that besides total pectin content, moisture content and firmness of fresh fruits also affected the efficiency of calcium to improve firmness of fresh and frozen-thawed fruit cubes.

2.6 Drip loss

Ice crystals which form during freezing can damage fruit cells. Therefore, during thawing, liquid can leak from within the cell to the exterior of the cells which results in drip loss.

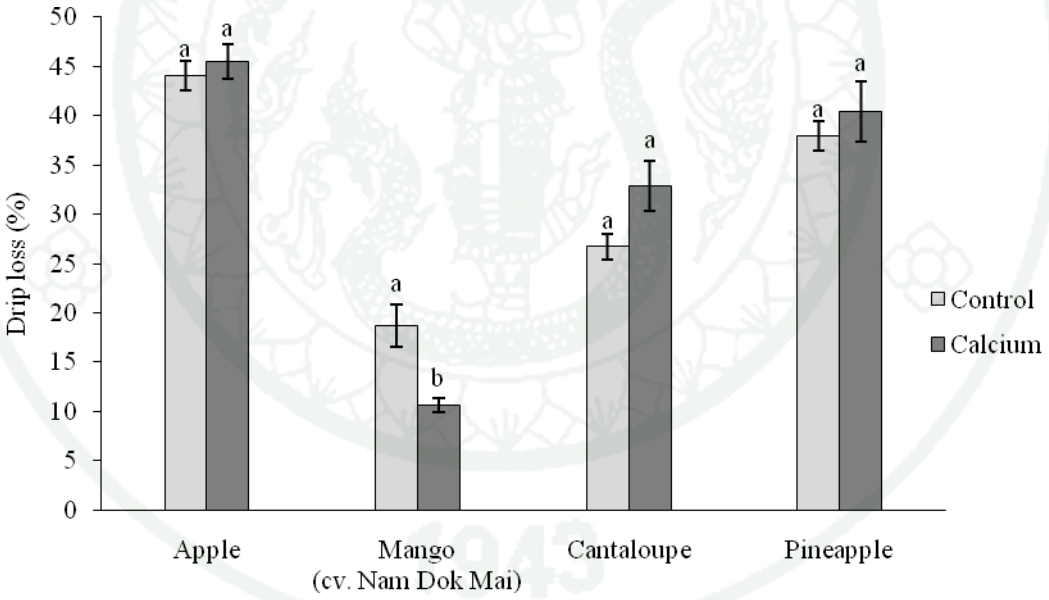

Figure 12 The drip loss (%) of frozen-thawed control and calcium treated fruits. Bars indicate the standard deviation from 2 replications with different letters for identical fruit showing a significant difference ($p<0.05$).

Among these four fruits, apple showed the highest drip loss. This result related with the decreasing in firmness after freezing of apple cubes. After freezing,

apple had the highest firmness decrease which demonstrated the high tissue damage caused by ice crystals. Therefore, drip loss of apple was the highest.

Only the calcium treated mango pieces had significantly lower ($p<0.05$) drip loss than the control sample (Figure 12). Frozen-thawed calcium treated mango pieces had significantly higher ($p<0.05$) firmness and consequently were subject to lower drip loss levels in comparison to the frozen-thawed control sample. This finding is potentially caused by the calcium which enhanced the integrity of the cells; hence the effects of the ice crystals responsible for drip loss were decreased.

2.7 Microstructure

The cell walls of all samples were stained with calcofluor and observed with a confocal laser scanning microscope. The micrographs of fresh fruits are shown in Figure 13. Control fresh apple, mango, and cantaloupe composed of well defined circular to elliptical and rather regular cells (Figure 13a, 13c, and 13e, respectively). The biggest cell size was found on cantaloupe followed by apple and mango, respectively. The fresh pineapple samples were composed of cells which were diverse in size and shape (Figure 13g). Subsequent to calcium treatment, no remarkable change in the microstructure in any fruit was observed (Figure 13b, 13d, 13f, and 13h). Although the texture result confirmed the dissimilarity of fresh control and calcium treated mango and cantaloupe, the immersion in calcium chloride solutions were not likely to alter the appearance of the cells.

The micrographs of all fruits subsequent to freezing and thawing are shown in Figure 14. The freezing and thawing was found to cause tissue damage in all samples (Figure 14a – 14h). Cells of the frozen-thawed fruits were of irregular shape, with visible loss of cell wall integrity. This observation resulted from the formation of ice crystals during the freezing process. The obvious difference in the microstructure of the frozen-thawed control and calcium treated samples were not observed in any of the fruit types.

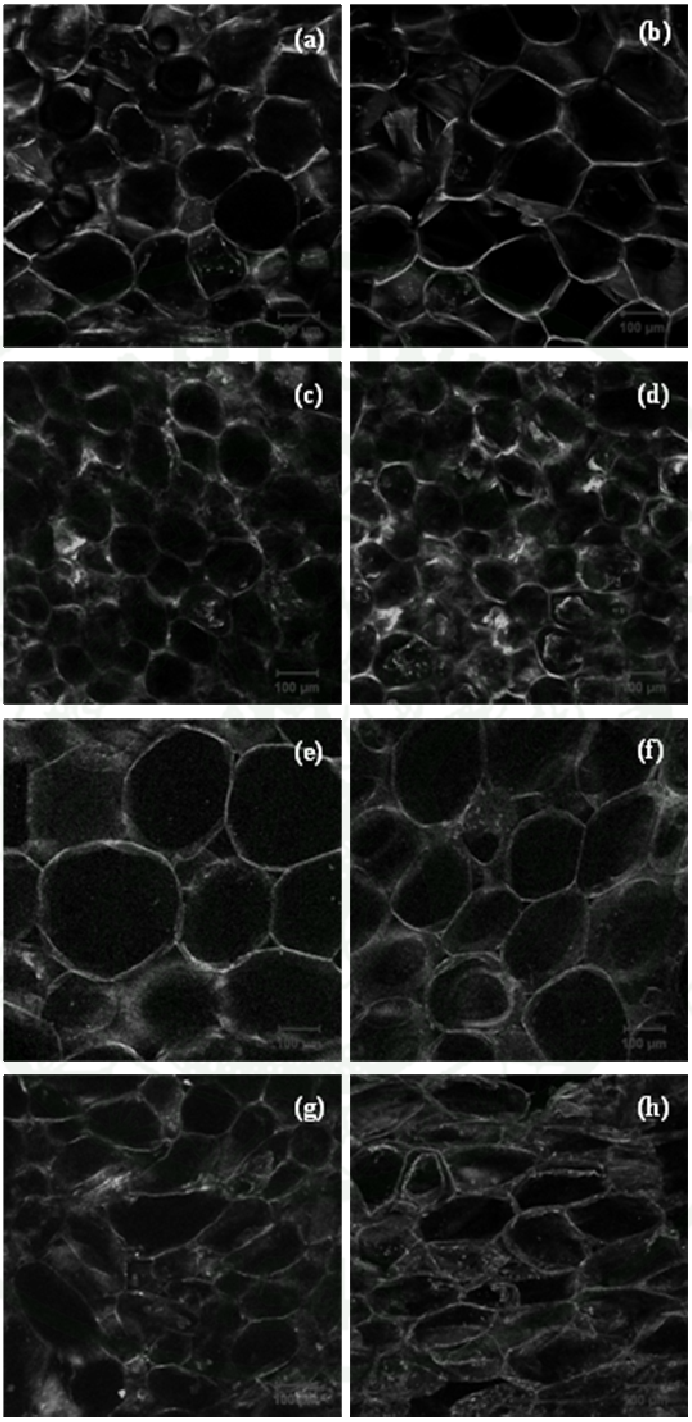

Figure 13 Micrographs of fresh fruits observed by a confocal laser scanning microscope. (a) control apple, (b) calcium treated apple, (c) control mango (cv. Nam Dok Mai), (d) calcium treated mango (cv. Nam Dok Mai), (e) control cantaloupe, (f) calcium treated cantaloupe, (g) control pineapple, (h) calcium treated pineapple

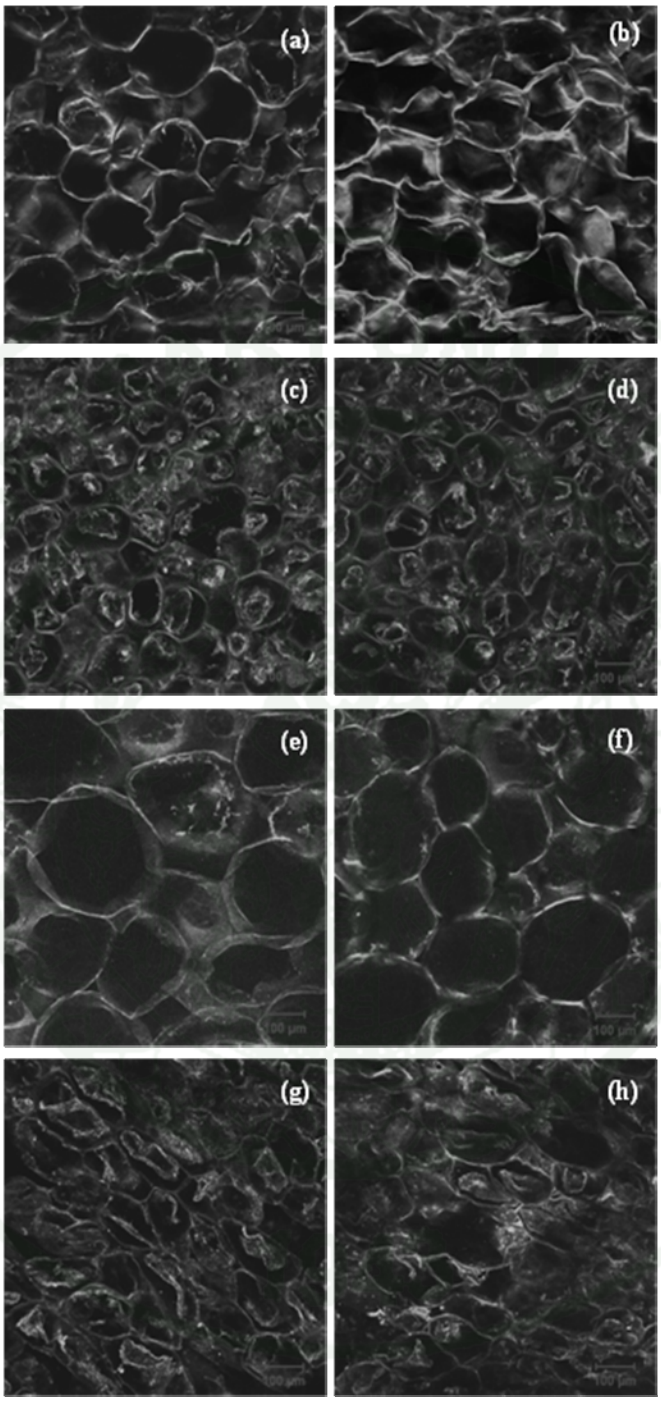

Figure 14 Micrographs of frozen-thawed fruits observed by a confocal laser scanning microscope. (a) control apple, (b) calcium treated apple, (c) control mango (cv. Nam Dok Mai), (d) calcium treated mango (cv. Nam Dok Mai), (e) control cantaloupe, (f) calcium treated cantaloupe, (g) control pineapple, (h) calcium treated pineapple

2.8 Sensory evaluation

Both fresh and frozen-thawed fruits were assessed by trained panelists. The fruits were scored for firmness and bitterness. This data is shown in Figure 15 and 16 respectively. These texture results revealed the compatibility of sensory evaluation and instrumental measurement. The correlation coefficient of these two parameters was 0.749 which was significant at the 0.01 level. The data from both the texture analyzer and the trained panelists indicated that fresh mango had the lowest firmness follow by pineapple, cantaloupe, and apple (Figure 11 and 15).

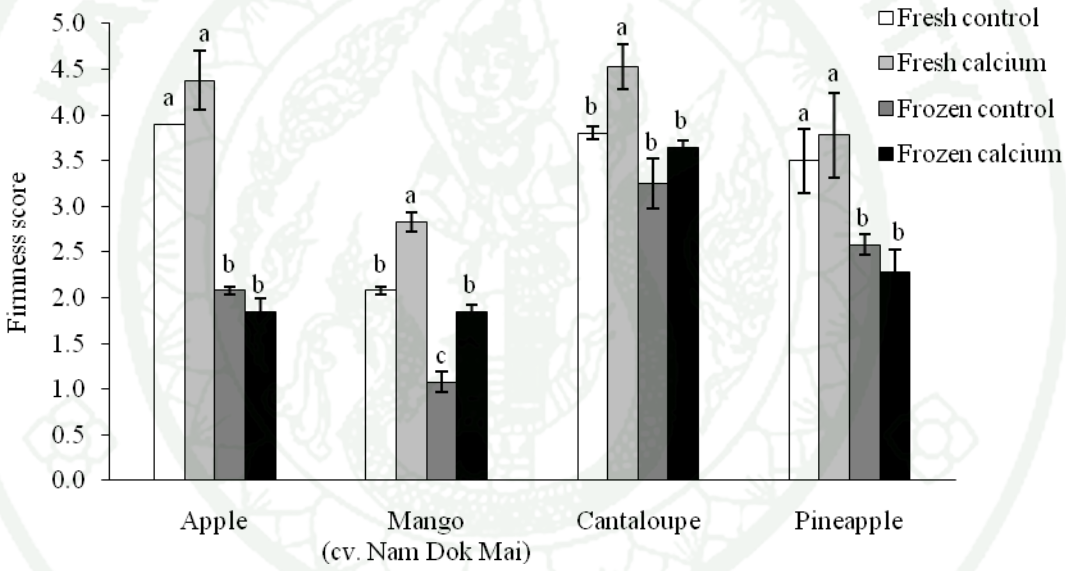

Figure 15 The firmness scores of control and calcium treated fruits. Bars indicate the standard deviation from 2 replications with different letters for identical fruit showing a significant difference ($p<0.05$).

Fresh calcium treated cantaloupe and mango had significantly higher ($p<0.05$) firmness scores than those of the fresh control samples. However, only frozen-thawed calcium treated mango had a significantly higher ($p<0.05$) firmness score than that of the frozen-thawed control sample. This result correlates well with the result yielded by the texture analyzer. This finding is an indication for the ability of the immersion in calcium chloride solution prior to freezing to decrease the loss in

texture caused by the formation of ice crystals in frozen mango pieces, however, this effect was not seen in the other fruits.

As for the levels of bitterness of the fresh fruits, the score of calcium treated samples were significantly higher ($p<0.05$) than that of the control samples. However, subsequent to the freezing and thawing process, the cells were destroyed or torn by the ice crystals. Thus, soluble substances such as sugar moved from within to the exterior of the cells, which potentially concealed the bitterness of the calcium. Furthermore, calcium was likely to be dispersed out of the fruit pieces due to the drip loss. Therefore, the trained panelists did not detect a difference in the levels of bitterness between the frozen-thawed control and the calcium treated samples.

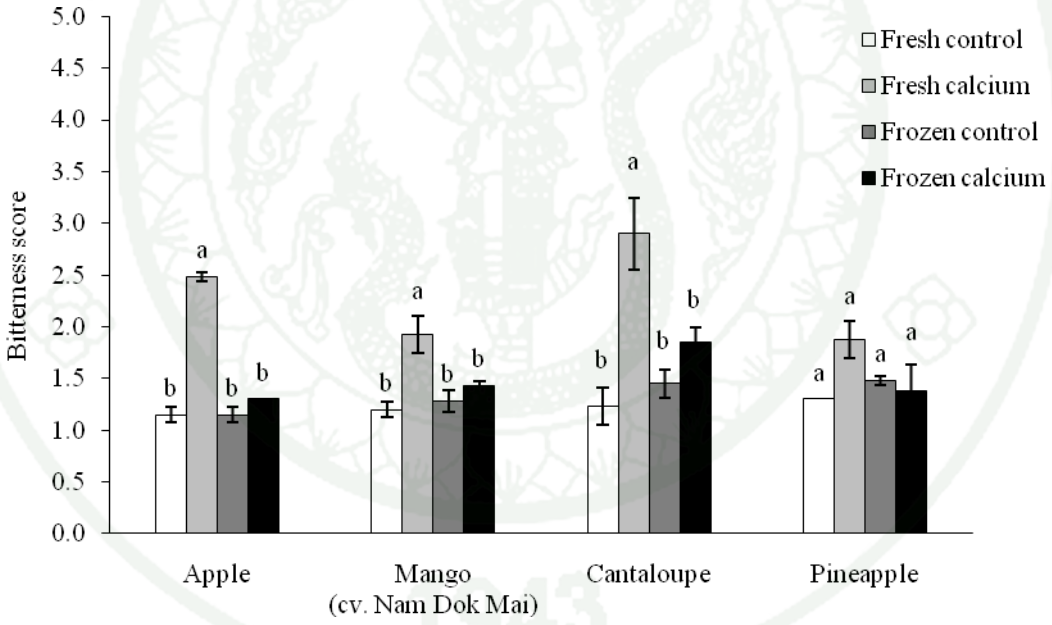

Figure 16 The bitterness scores of control and calcium treated fruits. Bars indicate the standard deviation from 2 replications with different letters for identical fruit showing a significant difference ($p<0.05$).

From these experiments (the effect of calcium pretreatment on pectin content, texture, and microstructure of four frozen fruits), the results showed that calcium showed the most effective effect on mango to improve texture and drip loss of both fresh and frozen-thawed samples. Therefore, mango was chosen to study in the next experiment (effect of vacuum infusion with PME and calcium on texture and microstructure of fresh and frozen mangoes) to increase the efficiency of calcium treatment.

3. Effect of vacuum infusion with pectin methylesterase and calcium on texture and microstructure of fresh and frozen mangoes (cv. Kent)

In this study, the efficiency of calcium to improve the texture of mango was increased by the addition of exogenous PME. Because of the low PME activity of fresh mango, the exogenous PME was needed to create the free carboxyl groups of pectins to bind with calcium. The vacuum assist method was also applied to improve the infusion of calcium and PME into the fruit pieces due to the low concentration of calcium and PME and the large size of PME. These results are reported below.

3.1 Weight gain

Figure 17 shows the percent weight gain of samples subsequent to infusion under various conditions. The weight gain of samples was greater at relatively high vacuum level (10 kPa). The weight gain of mango cubes infused with either the PME and calcium solution or just water at the highest vacuum level (10 kPa) was significantly different ($p<0.05$) in comparison with the other samples. For the medium level of vacuum (50 kPa), a lesser amount of tissue gases were drawn out, therefore when the vacuum was released, less liquid solution was infused into the sample in comparison with the infusion at 10 kPa. As for the infusion at 10 kPa, the sample which was infused with water had significantly higher ($p<0.05$) weight gains than those samples infused with PME and calcium.

During vacuum application, tissue gases are drawn out of the samples, and an infusion of the liquid solution can then occur when the vacuum is released (Baker and Wicker, 1996). Therefore, the weight gain was caused by the movement of water, calcium, and PME from the infusion solution into the fruits during the time when the vacuum was released. Similar findings to ours were reported for eggplant cylinders (Banjongsinsiri *et al*., 2004c). These authors infused eggplant cylinders at 68 kPa (32.9 % vacuum) for 15 min at 30°C. They reported percent yield (weight after process divided by initial weight) of the samples after infusion in water and combined solution of PME and calcium were 180 and 165 %w/w, respectively.

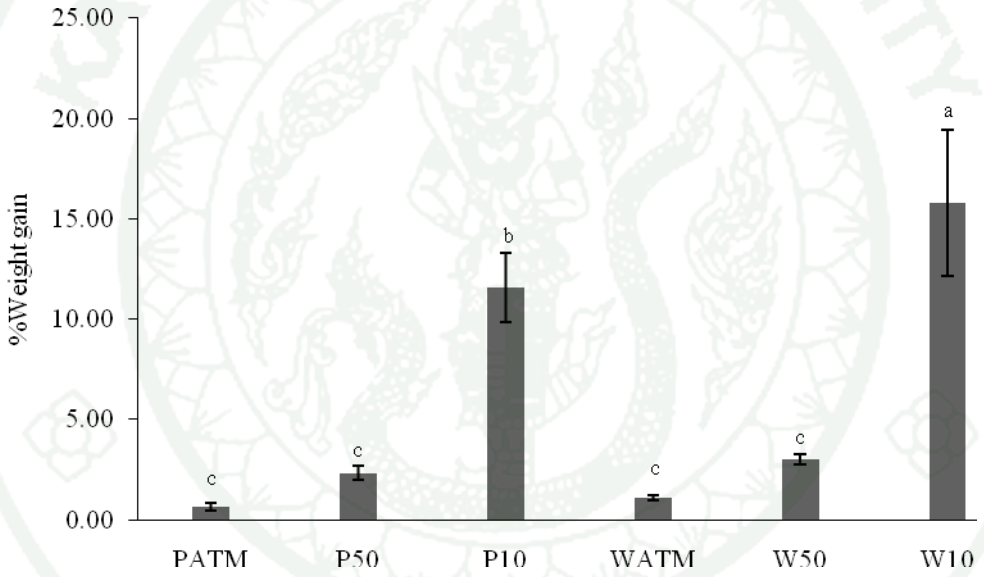

Figure 17 Weight gain (%) of mango (cv. Kent) cubes infused with pectin methylesterase and calcium at atmospheric pressure (PATM), at pressures of 50 kPa (P50), and 10 kPa (P10), and infused with water at atmospheric pressure (WATM), at pressures of 50 kPa (W50), and 10 kPa (W10). Bars indicate standard deviation with different letters to denote significant difference ($p<0.05$).

3.2 Moisture content

The moisture content of all samples is shown in Figure 18. The moisture results correlated well to the weight gain in the samples. Immersion at atmospheric pressure did not affect the moisture content of the samples. The moisture content of the samples infused with either water or the PME and calcium solution were highest at the highest vacuum level (10 kPa). This finding most likely results from the movement of water into the fruit pieces after the vacuum was released.

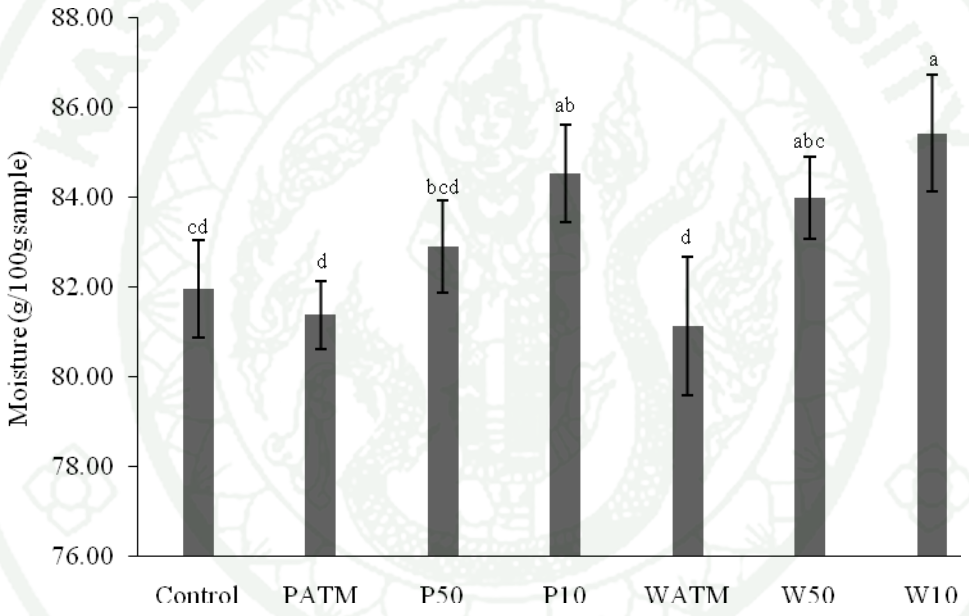

Figure 18 Moisture content (g/100g sample) of mango (cv. Kent) cubes untreated (control), infused with pectin methylesterase and calcium at atmospheric pressure (PATM), at pressures of 50 kPa (P50), and 10 kPa (P10), and infused with water at atmospheric pressure (WATM), at pressures of 50 kPa (W50), and 10 kPa (W10). Bars indicate the standard deviation with different letters to denote significant differences ($p<0.05$).

The moisture content of mango cubes which were infused at 10 kPa were significantly different ($p<0.05$) from that of the control sample, but not significantly different ($p>0.05$) from the samples treated with a medium level of

vacuum (50 kPa). Comparable results were found in both samples infused with water and the combined solution of PME and calcium. There is no comparative published information on moisture content changes in mango cubes infused with different solutions.

3.3 Calcium content

The calcium content values of all of the samples are shown in Figure 19. The immersion with water did not affect the calcium content of the mangoes. On the other hand, the infusion with PME and calcium solution significantly increased ($p<0.05$) calcium content of the infused mangoes at all pressure levels studied. Moreover, the pressure levels showed a significant ($p<0.05$) effect on calcium content.

The calcium content of samples infused with the PME and calcium solution were highest at the highest vacuum level (10 kPa). At atmospheric pressure, calcium can diffuse from the infusion solution into the fruit pieces due to the concentration gradient. For the vacuum infusion, the movement of calcium into the mango pieces occurred when the vacuum was released. In addition, the movement of gases from the mango cubes at high vacuum level (10 kPa) was more pronounced than at medium vacuum level (50 kPa). These differences could explain the significant difference ($p<0.05$) in calcium content of different vacuum infused samples.

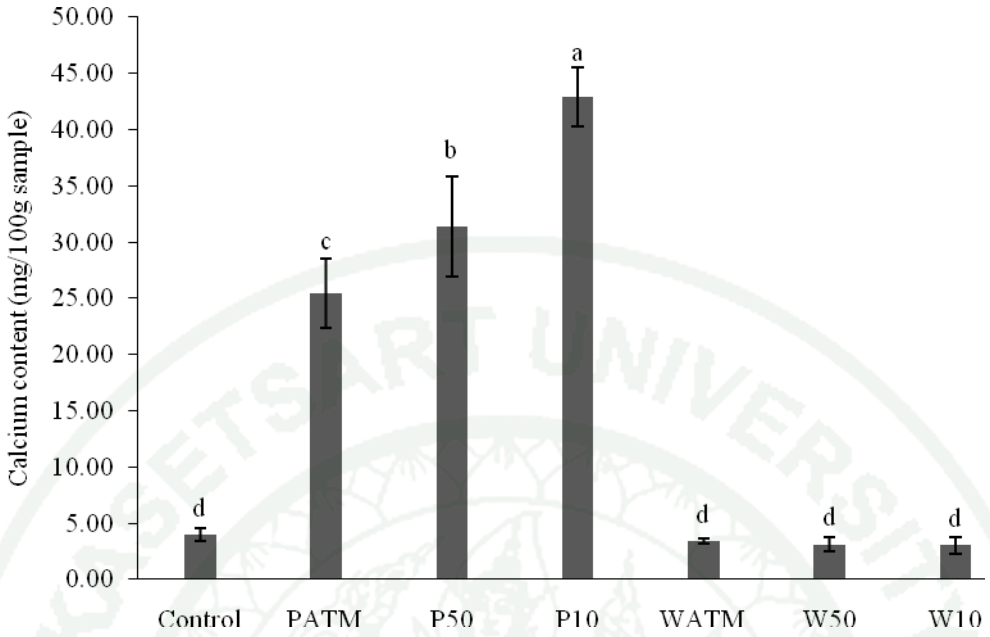

Figure 19 Calcium content (mg/100 g sample) of fresh mango (cv. Kent) cubes untreated (control), infused with pectin methylesterase and calcium at atmospheric pressure (PATM), at pressures of 50 kPa (P50), and 10 kPa (P10), and infused with water at atmospheric pressure (WATM), at pressures of 50 kPa (W50), and 10 kPa (W10). Bars indicate the standard deviation with different letters denoting a significant difference ($p<0.05$).

3.4 Pectin methylesterase activity

Infusion with water did not affect the endogenous PME activity of mango cubes at any pressure levels (Figure 20). However, the PME activity of mango cubes was significantly higher ($p<0.05$) subsequent to infusion with calcium and PME solution at all pressure levels. The specific pressure level had a strong effect on the resulting PME activity of the fruit. The samples infused with PME and calcium at the highest vacuum, e.g. 10 kPa showed the highest PME activity ($p<0.05$). The reason may have been due to the fact that at high vacuum level (10 kPa), gases present in the fruit cubes were drawn out of the tissue more effectively than at a medium vacuum level (50 kPa).

On the other hand, water in the solution was also found to infuse into the mango samples. Water logging of the tissues was apparent in the mango cubes after the vacuum was released, which was in agreement with the results of Banjongsinsiri *et al*. (2004b). Water logging likely has an effect on the translucent appearance of fresh-cut samples. Nevertheless, the visual appearance of all frozen-thawed samples was not determined to be different.

Use of vacuum infusion, as compared to atmospheric immersion, has the advantage of removing almost all of the interior gases which exist in the apoplastic spaces between cells; thus, the enzyme solution is able to replace these spaces and remains within the tissue after infusion (Baker and Wicker, 1996).

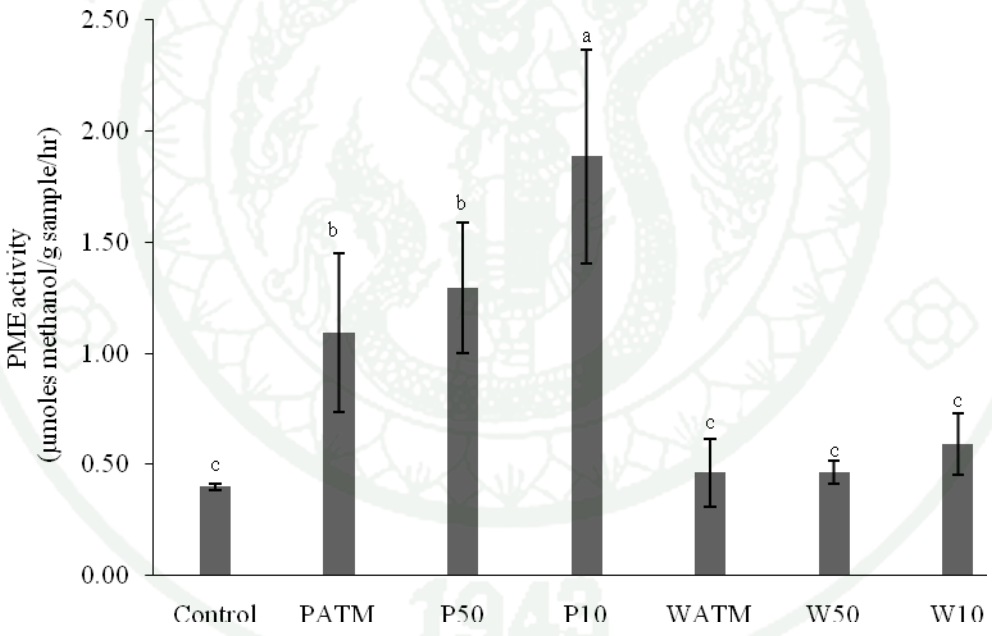

Figure 20 PME activity (µmoles methanol/g sample/hr) of fresh mango (cv. Kent) cubes untreated (control), infused with pectin methylesterase and calcium at atmospheric pressure (PATM), at pressures of 50 kPa (P50), and 10 kPa (P10), and infused with water at atmospheric pressure (WATM), at pressures of 50 kPa (W50), and 10 kPa (W10). Bars indicate the standard deviation with different letters denoting a significant difference ($p<0.05$).

An increase in PME activity after vacuum infusion was also reported in previous research on strawberries and apples (Banjongsinsiri *et al*., 2004a; Guillemin *et al*., 2006; Guillemin *et al*., 2008). The enzyme penetration is due to the hydrodynamic phenomena allowing the external solution to fill the sample pores (Guillemin *et al*., 2006). Guillemin *et al*. (2006) studied the importance of vacuum level on PME distribution in apple cubes after vacuum infusion with PME and calcium solution at different vacuum levels (50, 30, and 5 kPa or 50.6, 70.4, and 95.1 %vacuum, respectively). They similarly reported a better penetration of PME into apple cubes at higher vacuum level, resulting in a homogeneous distribution of PME in apple cubes subsequent to vacuum impregnation at 5 kPa.

3.5 Firmness

Fresh Mangoes (cv. Kent)

The firmness of all samples was determined in term of the maximum peak force and shown in Table 9. Infusion of the fresh samples with water was found to decrease the firmness of the samples, and a significant effect ($p<0.05$) was found at the highest vacuum level, e.g. 10 kPa. This result may have been caused by water logging of the cells after the vacuum was released. Conversely, samples that were vacuum infused with PME and calcium at 10 kPa attained a significantly ($p<0.05$) higher levels of firmness. This finding may be due to the accumulation of calcium and PME in the mango cubes subsequent to infusion at the high vacuum level of 10 kPa.

The deesterification of pectin by PME and the chelation of calcium ion by ionized carboxylic acid groups on adjacent pectic acid chains are manifest in the form of 'egg-box' structures. Such a phenomenon results in an overall increase in firmness (Baker and Wicker, 1996). Vacuum infusion with PME and calcium have also been reported to increase the firmness of peaches and strawberries (Kratchanova *et al.*, 1991; Banjongsinsiri *et al.*, 2004a; Duvetter *et al.*, 2005). Conversely, the study of Banjongsinsiri *et al*. (2004b) reported that the infusion with Valencia PME and calcium under vacuum (85 kPa or 16.1 %vacuum) for 10 min at room temperature did

not improve the firmness of mango cubes. They also found a heat sensitive PME inhibitor in mango which could inhibit the activity of Valencia PME. PME inhibitors have been reported to be active against several plant PME (PME from kiwi, orange, apple, tomato, apricot, carrot, potato and banana), whereas they do not inhibit fungal PME (Giovane *et al*., 2004). Consequently, since study used fungal PME, the PME was able to improve the texture of fresh mango cubes.

Table 9 Firmness (N) of mango (cv. Kent) cubes both prior to and following freezing.

Treatment[1]	Firmness[2] (N)	
	Fresh	Frozen
Control	52.53^{b}±4.68	19.81^{b}±1.76
PATM	59.70^{ab}±8.52	23.71^{a}±2.28
P50	56.83^{b}±5.88	23.83^{a}±2.05
P10	70.39^{a}±11.45	17.62^{b}±2.14
WATM	46.85^{bc}±6.33	19.50^{b}±1.77
W50	49.32^{bc}±4.65	18.57^{b}±0.35
W10	39.49^{c}±3.31	12.21^{c}±1.43

[1] Untreated (control), infused with pectin methylesterase and calcium at atmospheric pressure (PATM), at pressures of 50 kPa (P50), and 10 kPa (P10), and infused with water at atmospheric pressure (WATM), at pressures of 50 kPa (W50), and 10 kPa (W10)

[2] Data are mean ± standard deviation. Values in the columns followed by different letters are significantly different ($p<0.05$).

Frozen Mangoes (cv. Kent)

Subsequent to freezing and thawing, the firmness of all samples decreased in comparison to those of the fresh samples (Table 9). Frozen-thawed samples which were infused with PME and calcium at 50 kPa and at atmospheric

pressure had significantly higher ($p<0.05$) firmness than those of the control frozen-thawed samples, whereas the samples which were vacuum infused with water at a pressure of 10 kPa attained the lowest level of firmness ($p<0.05$).

Fresh samples which were infused with PME and calcium at 10 kPa, had superior levels of firmness, in comparison to that of the fresh control sample (Table 9). Nevertheless, subsequent to freezing and thawing, the firmness values of those samples were not significantly different ($p>0.05$) from those of the frozen-thawed control samples. During freezing, water present in the samples partially formed ice crystals which negatively affected the integrity of the cellular compartments, which caused decreased levels of firmness subsequent to freezing and thawing of the samples.

The sample that was vacuum infused at 10 kPa had a higher moisture content in comparison to that of the control sample (Figure 18). During freezing, a large number of ice crystals were formed, which were responsible for extensive microstructural changes in the fruit tissue. Thus, PME and calcium addition may be ineffective to maintain the cell integrity of this sample subsequent to the freezing and thawing process.

Rincon and Kerr (2010) studied the combined processes of partial osmotic dehydration using a 30°Brix sucrose solution followed by freezing to improve the quality of frozen mangoes. They found that after osmotic dehydration the moisture content of mangoes decreased from 0.870 to 0.793 g/g pulp. Hardness of the control sample decreased 51.21% after freezing and frozen storage for 7 weeks whereas hardness of the osmotically dehydrated sample decreased by only 16.26%. They suggested that pretreatment by osmotic drying can remove some of the available water thus leaving less available water to form ice crystals, which may damage the tissue and reduce hardness, during freezing.

The high vacuum level was hypothesized to infuse a lot of calcium and PME from infusion solution into the fruit cubes and resulted in the increasing in firmness. However, this finding showed that besides calcium and PME content, moisture content was the significant factor which had an effect on firmness of frozen-thawed mango.

Earlier research has reported a remarkable texture improvement in strawberries following the combined infusion of PME and calcium at 1 kPa for 5 min, in particular when combined with rapid (-25°C) or cryogenic freezing (-80°C), which was absent during the slow freezing condition (-18°C) typically used (Van Buggenhout *et al*., 2006).

3.6 Microstructure

Calcofluor is a non-specific fluorochrome that binds with cellulose and chitin contained in the cell walls. In plant cell biology research it is used for the staining of cell walls of both algae and higher plants. The cell walls of all samples were stained with calcofluor and observed with a confocal laser scanning microscope. The micrographs of fresh mango are shown in Figure 21. Control fresh mangoes were composed of well defined circular to elliptical and regular cells (Figure 21a). Although the texture result confirmed the dissimilarity of treatments, the infusions were not found to alter the appearance of the cells for either the infusion with PME and calcium (Figure 21b – d), or in the case of the infusion with water (Figure 21e - g). The microstructure of these samples was still composed of intact cells of rough and regular shapes comparable to those of the fresh mango samples. The effect of vacuum infusion on the microstructure of mangoes was found to be in line with that of strawberries (Van Buggenhout *et al.*, 2008; Fraeye *et al.*, 2009; Fraeye *et al.*, 2010).

Micrographs of the samples subsequent to freezing and thawing are shown in Figure 22. The freezing and thawing process was found to cause tissue damage in all samples (Figure 22a – g). Cells of the frozen-thawed mangoes were of

irregular shape, with visible loss of cell wall integrity. This effect resulted from the formation of ice crystals during the freezing process. In comparison to the control frozen-thawed samples, the microstructure of samples infused with PME and calcium at 50 kPa (Figure 22c) or at atmospheric pressure (Figure 22b) were most similar to those of fresh mango (Figure 21a), whereas the samples which were infused with water at a pressure of 10 kPa (Figure 22g) were found to be subject to severe tissue damage and considerable loss of cell integrity, which was most likely caused by the high moisture content of the samples (Figure 18). This water would change to many ice crystals during freezing causing tissue damage after thawing.

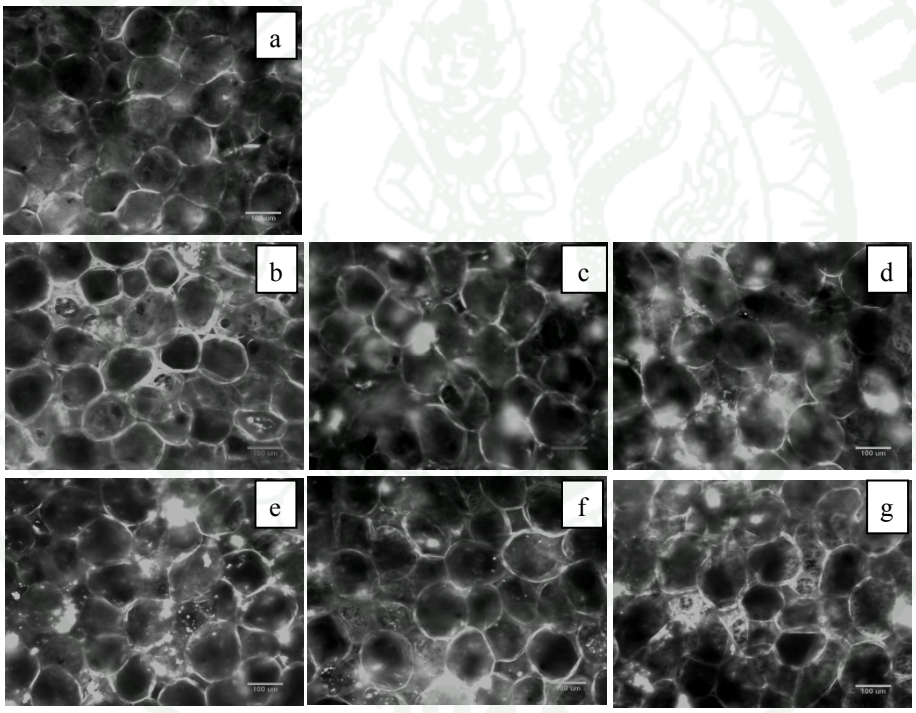

Figure 21 Micrographs of fresh mango (cv. Kent) observed by a confocal laser scanning microscope. (a) Control, (b) PATM: infusion with pectin methylesterase and calcium at atmospheric pressure, (c) P50: infusion with pectin methylesterase and calcium at 50 kPa, (d) P10: infusion with pectin methylesterase and calcium at 10 kPa, (e) WATM: infusion with water at atmospheric pressure, (f) W50: infusion with water at 50 kPa, and (g) W10: infusion with water at 10 kPa . Bars = 100 µm

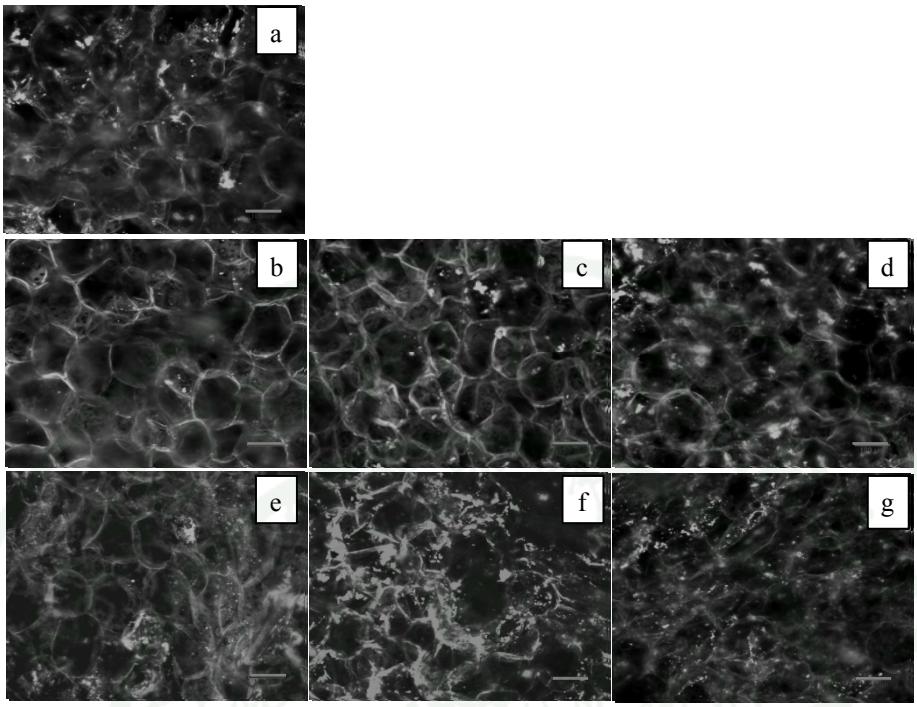

Figure 22 Micrographs of frozen-thawed mango (cv. Kent) observed by a confocal laser scanning microscope**.**(a) Control, (b) PATM: infusion with pectin methylesterase and calcium at atmospheric pressure , (c) P50: infusion with pectin methylesterase and calcium at 50 kPa, (d) P10: infusion with pectin methylesterase and calcium at 10 kPa, (e) WATM: infusion with water at atmospheric pressure , (f) W50: infusion with water at 50 kPa, and (g) W10: infusion with water at 10 kPa. Bars = 100 µm

The presence of such differences in the microstructure confirms that the infusion of PME and calcium at a pressure of either 50kPa or at atmospheric pressure was an effective process to decrease the extent of tissue damage during the freezing process. These microstructure observations correlate well with the texture results. Vacuum infusion with PME and calcium was also reported to be effective in the preservation of the structural integrity of the strawberry tissue (Van Buggenhout *et al.*, 2008).

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study was composed of three distinct parts. The first part was aim to investigate the freezing characteristics of apple, mango (cv. Nam Dok Mai), cantaloupe, and pineapple. The type of fruit was considered to have a significant effect on the freezing characteristics and physical quality of the frozen-thawed fruits. Fruit which had low moisture content and high thermal conductivity and thermal diffusivity showed the high freezing rate. Among the four fruits studied in this research, mango (cv. Nam Dok Mai) showed the highest total soluble solids content and the lowest freezing point, whereas, pineapple showed the highest freezing rate. Total soluble solids content of fresh fruits showed the negative correlation with freezing point. The firmness of all frozen fruits decreased with different percentage as compared to the firmness of the fresh fruit. The highest firmness decrease was observed in apple followed by mango (cv. Nam Dok Mai), pineapple, and cantaloupe, respectively.

The second part was the use of calcium chloride to improve the texture of these four fruits. The effect of calcium on the quality of frozen fruits was determined by the properties of the fruits. Moisture content, total pectin content, and firmness of the fresh fruits were all found to affect the texture improvement and the drip loss reduction of frozen fruits which resulted from the calcium bridges. The calcium treatment showed significant effect on frozen-thawed fruit which had low moisture content and firmness and high total pectin content. The calcium treatment did not cause any remarkable changes in the microstructure of both the fresh and the frozen-thawed fruits. An immersion in 1% calcium chloride solution showed an effectiveness to improve the texture and reduce the drip loss of frozen-thawed mango (cv. Nam Dok Mai) with an increased calcium content and decreased water soluble pectin content of the fruit. In addition, calcium treatment did not cause bitterness in the frozen-thawed samples.

The last part was a study to improve the efficiency of calcium chloride by the use of vacuum infusion of PME and calcium. The combined effects of PME and calcium were confirmed to improve the texture and microstructure of both fresh and frozen-thawed mangoes (cv. Kent). The vacuum level during infusion was found to be a significant factor affecting the properties of infused mango (cv. Kent) cubes. Vacuum infusion with PME and calcium at 10 kPa resulted in the highest calcium content and PME activity in mangoes (cv. Kent) and this condition was established to be the most suitable condition for the preservation of the texture of fresh mangoes (cv. Kent). Calcium content, PME activity, and moisture content were the most significant factors affecting the quality of frozen-thawed mango (cv. Kent). Samples infused with PME and calcium either at 50 kPa or at atmospheric pressure had higher calcium content and PME activity than the control samples, while the moisture content of both infused samples and control samples were not significantly different. The two pressure levels used for the infusion of PME and calcium resulted in superior texture and microstructure over those of the control frozen-thawed samples.

Recommendations

For further study, an effect of infusion temperature is interested to study because it has an effect on PME activity and may improve the efficiency of PME and calcium infusion treatment.

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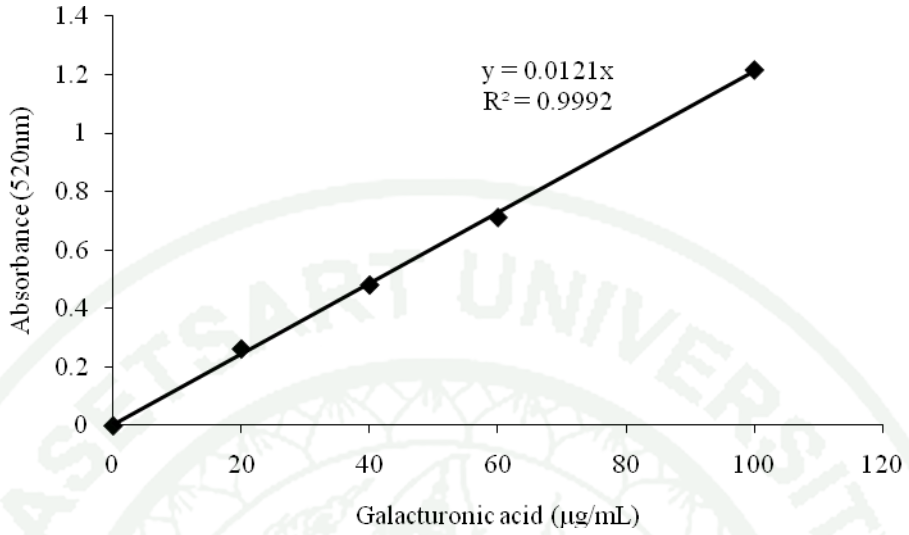
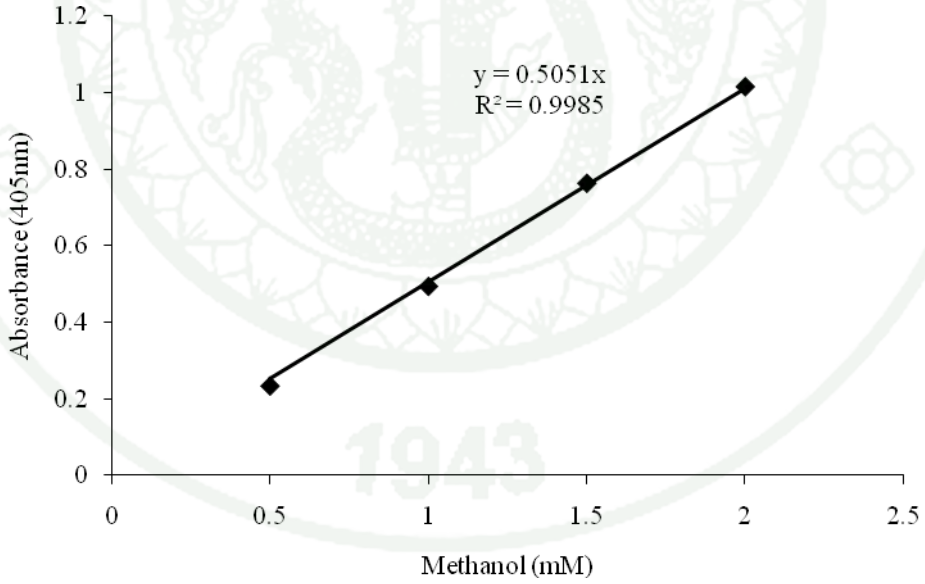
APPENDICES

Appendix A

Standard curve



Appendix Figure A1 Standard curve of galacturonic acid (µg/mL) for the calculation of total pectin and water soluble pectin content



Appendix Figure A2 Standard curve of methanol (mM) for the calculation of pectin methylesterase activity

Appendix B

Sensory evaluation and score sheet

Ranking test

Name:……..……Date:…….……

Instructions :

1. Receive the sample tray and note each sample code below according to its position on the tray.
2. Tasted the samples from left to right and rank the degree of bitterness and firmness. Rinse palate between samples.
3. Write “1” in the box of the sample which you find the least bitterness or firmness. Write “2” for the next, “3” for the next, and “4” for the most bitterness or firmness.

Characteristic	Sample code			
Bitterness				
Firmness				

Comments……

……..…….

……

Appendix Figure B1 The bitterness and firmness ranking score sheet for the study to select the optimal calcium chloride concentration.

Randomized block analysis of rank data (Meilgaard *et al.*, 2007)

If the data from a randomized block design are in the form of ranks, then a nonparametric analysis is performed using a Friedman-type statistic.

$$T = \left([12/bt(t+1)] \sum_{j=1}^{t} x_{.j}^2 \right) - 3b(t+1)$$

where "b" is the number of panelists, "t" is the number of samples, and $x_{.j}$ is the rank sum of sample "j".

The test procedure is to reject the null hypothesis of no sample differences at the α-level of significance of the value of "T" exceeds $\chi^2_{\alpha,t-1}$, and to accept H_0: otherwise, where $\chi^2_{\alpha,t-1}$ is the upper-α percentile of the χ^2 distribution with t-1 degrees of freedom. The procedure assumes that a relatively large number of panelists participate in the study. It is reasonably accurate for studies involving 12 or more panelists.

If the χ^2- statistic is significant, then a multiple comparison procedure is performed to determine which of the samples differ significantly. The nonparametric analog to Fisher's LSD for rank sums from a randomized complete block design is

$$LSD_{rank} = t_{\alpha/2,\infty} [bt(t+1)/6]^{1/2}$$

Two samples are declared to be significantly different at the α-level if their rank sums differ by more than the value of LSD_{rank}.

Duo-trio test

Name:……..……Date:…….……

Instructions : Test sample from left to right. Rinse palate between samples. The left hand sample is a reference. Determine which of the two axis samples matches the reference and indicate by placing an (X)

Test 1

Reference Code……. Code……

Test 2

Reference Code……. Code……

Comments…….……

……..…….

……

Appendix Figure B2 The duo-trio score sheet for the selection session to test the ability of the panelists to detect the difference among similar samples.

Ranking test for firmness

Name:……..……Date:…….……

Instructions :

1. Receive the sample tray and note each sample code below according to its position on the tray.
2. Tasted the samples from left to right and rank the degree of firmness. Rinse palate between samples.
3. Write "1" in the box of the sample which you find the least firmness. Write "2" for the next, and "3" for the most firmness.

Characteristic	Sample code		
Firmness			

Comments……..……

……..……

……

Appendix Figure B3 The ranking score sheet for the selection session to test the ability of the panelists to discriminate graded levels of intensity of the firmness.

Ranking test for bitterness

Name:……..……Date:…….……

Instructions :

1. Receive the sample tray and note each sample code below according to its position on the tray.
2. Tasted the samples from left to right and rank the degree of bitterness. Rinse palate between samples.
3. Write "1" in the box of the sample which you find the least bitterness. Write "2" for the next, and "3" for the most bitterness.

Characteristic	Sample code		
Bitterness			

Comments……

……..……

……

Appendix Figure B4 The ranking score sheet for the selection session to test the ability of the panelists to discriminate graded levels of intensity of the bitterness.

Scoring test

Name:……..……Date:…….……

Instructions :

1. Receive the sample tray and note each sample code below according to its position on the tray.
2. Tasted the samples from left to right and rate the bitterness and firmness intensity of the samples. Rinse palate between samples.

Bitterness	**Firmness**
1 = no bitter	1 = very soft
2 = low bitter	2 = soft
3 = medium bitter	3 = medium firm
4 = bitter	4 = firm
5 = very bitter	5 = very firm

Characteristic	Sample code			
Bitterness				
Firmness				

Comments……

……..…….

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Appendix Figure B5 The score sheet for the training session and for scoring the firmness and bitterness of fresh and frozen-thawed apple, mango, cantaloupe, and pineapple.

Appendix C

Statistical analysis

Appendix Table C1 One-Way ANOVA of moisture content (g/100g sample), total soluble solids content, and freezing rate (°C/min) in comparison between different fruit types (apple, mango, cantaloupe, and pineapple)

		Sum of Squares	df	Mean Square	F	Sig.
Moisture (g/100g sample)	Between Groups	87.469	3	29.156	40.836	.002
	Within Groups	2.856	4	.714		
	Total	90.325	7			
Total soluble solids	Between Groups	57.527	3	19.176	651.681	.000
	Within Groups	.118	4	.029		
	Total	57.645	7			
Freezing rate (C/min)	Between Groups	.861	3	.287	10.576	.023
	Within Groups	.109	4	.027		
	Total	.969	7			

Appendix Table C2 One-Way ANOVA of moisture content (g/100g sample) in comparison between fresh control and calcium treated fruits.

		Sum of Squares	df	Mean Square	F	Sig.
Moisture (apple)	Between Groups	1.166	1	1.166	2.908	.230
	Within Groups	.802	2	.401		
	Total	1.969	3			
Moisture (mango)	Between Groups	4.410	1	4.410	15.406	.059
	Within Groups	.573	2	.286		
	Total	4.983	3			
Moisture (cantaloupe)	Between Groups	.051	1	.051	.058	.833
	Within Groups	1.758	2	.879		
	Total	1.809	3			
Moisture (pineapple)	Between Groups	2.161	1	2.161	3.926	.186
	Within Groups	1.101	2	.550		
	Total	3.262	3			

Appendix Table C3 One-Way ANOVA of calcium content (mg/100g dry sample) in comparison between fresh control and calcium treated fruits.

		Sum of Squares	df	Mean Square	F	Sig.
Calcium (apple)	Between Groups	48507.860	1	48507.860	777.592	.001
	Within Groups	124.764	2	62.382		
	Total	48632.624	3			
Calcium (mango)	Between Groups	35547.332	1	35547.332	12813.312	.000
	Within Groups	5.549	2	2.774		
	Total	35552.880	3			
Calcium (cantaloupe)	Between Groups	72057.349	1	72057.349	49.137	.020
	Within Groups	2932.889	2	1466.444		
	Total	74990.238	3			
Calcium (pineapple)	Between Groups	89054.496	1	89054.496	835.899	.001
	Within Groups	213.075	2	106.537		
	Total	89267.571	3			

Appendix Table C4 One-Way ANOVA of drip loss (%) in comparison between frozen-thawed control and calcium treated fruits.

		Sum of Squares	df	Mean Square	F	Sig.
Drip loss (apple)	Between Groups	2.280	1	2.280	.871	.449
	Within Groups	5.235	2	2.617		
	Total	7.515	3			
Drip loss (mango)	Between Groups	65.044	1	65.044	25.055	.038
	Within Groups	5.192	2	2.596		
	Total	70.236	3			
Drip loss (cantaloupe)	Between Groups	38.440	1	38.440	9.322	.093
	Within Groups	8.247	2	4.124		
	Total	46.687	3			
Drip loss (pineapple)	Between Groups	5.832	1	5.832	.985	.425
	Within Groups	11.838	2	5.919		
	Total	17.670	3			

Appendix Table C5 One-Way ANOVA of alcohol insoluble residue (g/100g dry sample), total pectin content (g/100g AIR), water soluble pectin content (g/100g AIR), firmness (N), firmness score, and bitterness score in comparison between fresh control, fresh calcium treated, frozen-thawed control, and frozen-thawed calcium treated apples.

		Sum of Squares	df	Mean Square	F	Sig.
AIR	Between Groups	10.518	3	3.506	10.760	.022
	Within Groups	1.303	4	.326		
	Total	11.821	7			
Total pectin	Between Groups	25.230	3	8.410	6.489	.051
	Within Groups	5.184	4	1.296		
	Total	30.414	7			
Water soluble pectin	Between Groups	5.613	3	1.871	47.140	.001
	Within Groups	.159	4	.040		
	Total	5.772	7			
Firmness (N)	Between Groups	6406.984	3	2135.661	1992.488	.000
	Within Groups	4.287	4	1.072		
	Total	6411.271	7			
Firmness score	Between Groups	9.738	3	3.246	105.986	.000
	Within Groups	.123	4	.031		
	Total	9.860	7			
Bitterness score	Between Groups	2.468	3	.823	292.556	.000
	Within Groups	.011	4	.003		
	Total	2.480	7			

Appendix Table C6 One-Way ANOVA of alcohol insoluble residue (g/100g dry sample), total pectin content (g/100g AIR), water soluble pectin content (g/100g AIR), firmness (N), firmness score, and bitterness score in comparison between fresh control, fresh calcium treated, frozen-thawed control, and frozen-thawed calcium treated mangoes.

		Sum of Squares	df	Mean Square	F	Sig.
AIR	Between Groups	6.015	3	2.005	8.008	.036
	Within Groups	1.002	4	.250		
	Total	7.017	7			
Total pectin	Between Groups	14.374	3	4.791	5.520	.066
	Within Groups	3.472	4	.868		
	Total	17.846	7			
Water soluble pectin	Between Groups	10.691	3	3.564	10.902	.021
	Within Groups	1.308	4	.327		
	Total	11.998	7			
Firmness (N)	Between Groups	21.371	3	7.124	98.063	.000
	Within Groups	.291	4	.073		
	Total	21.661	7			
Firmness score	Between Groups	3.113	3	1.038	144.391	.000
	Within Groups	.029	4	.007		
	Total	3.142	7			
Bitterness score	Between Groups	.638	3	.213	17.462	.009
	Within Groups	.049	4	.012		
	Total	.687	7			

Appendix Table C7 One-Way ANOVA of alcohol insoluble residue (g/100g dry sample), total pectin content (g/100g AIR), water soluble pectin content (g/100g AIR), firmness (N), firmness score, and bitterness score in comparison between fresh control, fresh calcium treated, frozen-thawed control, and frozen-thawed calcium treated cantaloupes.

		Sum of Squares	df	Mean Square	F	Sig.
AIR	Between Groups	3.676	3	1.225	6.886	.047
	Within Groups	.712	4	.178		
	Total	4.388	7			
Total pectin	Between Groups	15.018	3	5.006	3.535	.127
	Within Groups	5.665	4	1.416		
	Total	20.682	7			
Water soluble pectin	Between Groups	6.032	3	2.011	40.952	.002
	Within Groups	.196	4	.049		
	Total	6.229	7			
Firmness (N)	Between Groups	139.440	3	46.480	18.514	.008
	Within Groups	10.042	4	2.511		
	Total	149.482	7			
Firmness score	Between Groups	1.718	3	.573	15.149	.012
	Within Groups	.151	4	.038		
	Total	1.870	7			
Bitterness score	Between Groups	3.306	3	1.102	22.461	.006
	Within Groups	.196	4	.049		
	Total	3.502	7			

Appendix Table C8 One-Way ANOVA of alcohol insoluble residue (g/100g dry sample), total pectin content (g/100g AIR), water soluble pectin content (g/100g AIR), firmness (N), firmness score, and bitterness score in comparison between fresh control, fresh calcium treated, frozen-thawed control, and frozen-thawed calcium treated pineapples.

		Sum of Squares	df	Mean Square	F	Sig.
AIR	Between Groups	14.262	3	4.754	15.005	.012
	Within Groups	1.267	4	.317		
	Total	15.530	7			
Total pectin	Between Groups	7.125	3	2.375	6.441	.052
	Within Groups	1.475	4	.369		
	Total	8.600	7			
Water soluble pectin	Between Groups	.230	3	.077	2.207	.230
	Within Groups	.139	4	.035		
	Total	.369	7			
Firmness (N)	Between Groups	187.479	3	62.493	13.606	.014
	Within Groups	18.372	4	4.593		
	Total	205.851	7			
Firmness score	Between Groups	3.106	3	1.035	10.131	.024
	Within Groups	.409	4	.102		
	Total	3.515	7			
Bitterness score	Between Groups	.393	3	.131	5.596	.065
	Within Groups	.094	4	.023		
	Total	.487	7			

Appendix Table C9 One-Way ANOVA of weight gain (%) in comparison between infused mangoes at various vacuum levels.

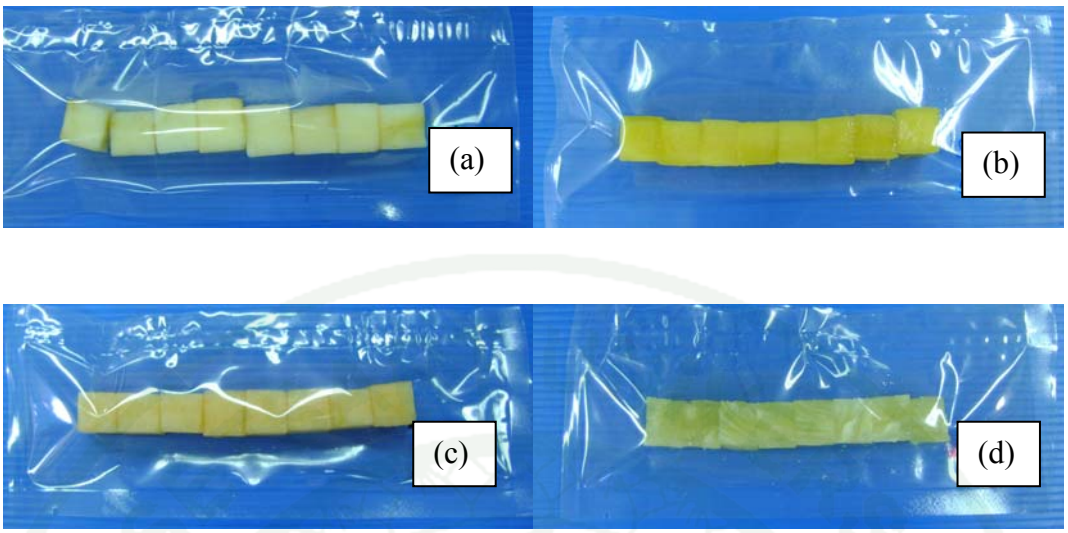
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	604.875	5	120.975	43.923	.000
Within Groups	33.051	12	2.754		
Total	637.926	17			

Appendix Table C10 One-Way ANOVA of moisture content (g/100 g sample), calcium content (g/100 g sample), pectin methylesterase activity (µmoles methanol/g sample/hr), firmness of fresh sample (N), and firmness of frozen-thawed sample (N).

		Sum of Squares	df	Mean Square	F	Sig.
Moisture	Between Groups	49.388	6	8.231	6.442	.002
	Within Groups	17.889	14	1.278		
	Total	67.277	20			
Calcium content	Between Groups	5060.990	6	843.498	159.121	.000
	Within Groups	74.214	14	5.301		
	Total	5135.204	20			
PME activity	Between Groups	5.726	6	.954	13.640	.000
	Within Groups	.979	14	.070		
	Total	6.705	20			
Firmness (fresh sample)	Between Groups	1780.257	6	296.709	6.241	.002
	Within Groups	665.613	14	47.544		
	Total	2445.870	20			
Firmness (frozen-thawed sample)	Between Groups	282.006	6	47.001	14.714	.000
	Within Groups	44.720	14	3.194		
	Total	326.726	20			

Appendix D

Pictures of fruit samples



Appendix Figure D1 Packages of fruit cubes for freezing. (a) apple, (b) mango (cv. Nam Dok Mai), (c) cantaloupe, and (d) pineapple

(a) (b) (c) (d)

Appendix Figure D2 Mango (cv. Kent) cubes after infusion process. (a) control, (b) – (d) infused with pectin methylesterase and calcium at pressures of 10 kPa, at pressure of 50 kPa, and at atmospheric pressure, respectively

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PUBLICATION

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3. Sirijariyawat, A., S. Charoenrein., and D.M. Barrett. Texture improvement of fresh and frozen mangoes with pectin methylesterase and calcium infusion. **J. Sci. Food Agr.** (submitted)

PRESENTATION

1. Sirijariyawat, A. and S. Charoenrein. 2009. Effect of calcium chloride on texture, pectin content and microstructure of frozen mango. **The 35th Congress on Science and Technology of Thailand (STT 35)**. 15-17 October 2009. The Science Society of Thailand under the Patronage of His Majesty the King, Chonburi, Thailand. (Oral)
2. Sirijariyawat, A. and S. Charoenrein. 2010. The diffusion of calcium into fresh cut cantaloupe and mango. **The IFT 2010 Annual Meeting and Food Expo.** 17-20 July 2010**.** Institute of Food Technologists**, C**hicago, IL. (Poster)