THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Food Science)

DEGREE

Food Science | Food Science and Technology

FIELD | **DEPARTMENT**

TITLE: Effect of Cultivar and Ripening Stage on the Quality and Microstructure of Frozen Mangoes (*Mangifera indica* L.)

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THESIS

EFFECT OF CULTIVAR AND RIPENING STAGE ON THE QUALITY AND MICROSTRUCTURE OF FROZEN MANGOES (*Mangifera indica* L.)

KANNIKA RIMKEEREE

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

กรรณิการ์ ริมคีรี 2555: ผลของพันธุ์และระดับความสุกต่อคุณภาพและโครงสร้างระดับจุลภาคของมะม่วงแช่เยือกแข็ง ปริญญาวิทยาศาสตรมหาบัณฑิต (วิทยาศาสตร์การอาหาร) สาขาวิทยาศาสตร์การอาหาร ภาควิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร อาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก: รองศาสตราจารย์สงวนศรี เจริญเหรียญ, Ph.D. 121 หน้า

งานวิจัยนี้ศึกษาการเปลี่ยนแปลงทางเคมีกายภาพและโครงสร้างระดับจุลภาคในระหว่างกระบวนการสุก และศึกษาผลของพันธุ์และระดับความสุกของมะม่วงที่มีต่อคุณภาพของมะม่วงแช่เยือกแข็ง พันธุ์ที่ใช้ได้แก่ มหาชนก น้ำดอกไม้ และโชคอนันต์ การทดลองเริ่มจากนำมะม่วงในแต่ละพันธุ์ไปบ่มให้สุกและแบ่งระดับความสุกเป็น 6 ระดับ โดยใช้ความแตกต่างของสีเปลือกมะม่วงเป็นเกณฑ์ ในระหว่างกระบวนการสุกมะม่วงทั้งสามพันธุ์มีการเปลี่ยนแปลงของสีเปลือกอย่างชัดเจนจากสีเขียวเป็นสีเหลืองถึงเหลืองส้ม ค่าความแน่นเนื้อลดลงพร้อมๆ กับการลดลงของปริมาณของแข็งที่ไม่ละลายในแอลกอฮอล์ ส่วนปริมาณเพคตินที่ละลายในน้ำมีค่าเพิ่มขึ้นเมื่อระดับความสุกเพิ่มขึ้น ยังพบว่าเซลล์พาเรนไคม่ามีลักษณะที่ผิดไปจากรูปร่างเดิมและสูญเสียการยึดเกาะกันระหว่างเซลล์ มีการสลายของมิดเดิ้ลลาเมลล่าที่บริเวณผนังเซลล์ ขนาดของเซลล์ในมะม่วงพันธุ์มหาชนกและโชคอนันต์มีขนาดเล็กกว่าและอยู่รวมกันแน่นกว่าพันธุ์น้ำดอกไม้ สอดคล้องกับค่าความแน่นเนื้อที่สูงกว่า เมื่อนำมะม่วงทั้งสามพันธุ์ที่ความสุกระดับ 3, 4 และ 5 มาแช่เยือกแข็งด้วยเครื่องไครโอจินิก หลังทำละลายพบว่าที่ความสุกระดับ 3 มะม่วงทุกพันธุ์มีค่าความแน่นเนื้อและคะแนนความเข้มของลักษณะเนื้อสัมผัสสูงที่สุด ปริมาณของเหลวที่สูญเสียหลังการละลายน้ำแข็งและปริมาณเพคตินที่ละลายในน้ำมีค่าต่ำที่สุด การแช่เยือกแข็งมีผลทำให้เกิดการบวมของผนังเซลล์และพับทบไปมา ในพันธุ์น้ำดอกไม้และโชคอนันต์ที่ความสุกระดับ 3 ผนังเซลล์เกิดความเสียหายน้อยกว่าที่ความสุกระดับ 5 นอกจากนี้มะม่วงพันธุ์น้ำดอกไม้ที่ความสุกทุกระดับผนังเซลล์เกิดความเสียหายมากกว่าพันธุ์มหาชนกและโชคอนันต์ ซึ่งผลสอดคล้องกับปริมาณของเหลวที่สูญเสียหลังการทำละลายที่น้ำดอกไม้มีค่าสูงที่สุด การสูญเสียความแน่นเนื้อของมะม่วงหลังการแช่เยือกแข็งเป็นที่ชัดเจนว่า เนื่องมาจากการเปลี่ยนแปลงของมิดเดิ้ลลาเมลล่า ผนังเซลล์ และองค์ประกอบของผนังเซลล์ มะม่วงพันธุ์มหาชนกและโชคอนันต์ที่ระดับความสุก 3 พบว่ามีความเหมาะสมมากกว่าพันธุ์น้ำดอกไม้สำหรับใช้ในกระบวนการแช่เยือกแข็ง เพื่อลดการสูญเสียความแน่นเนื้อและความเสียหายบริเวณผนังเซลล์

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ลายมือชื่อนิสิต ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก

Kannika Rimkeeree 2012: Effect of Cultivar and Ripening Stage on the Quality and Microstructure of Frozen Mangoes (*Mangifera indica* L.). Master of Science (Food Science), Major Field: Food Science, Department of Food Science and Technology. Thesis Advisor: Associate Professor Sanguansri Charoenrein, Ph.D. 121 pages.

The objective of this study was to investigate the physicochemical and microstructural changes during mango ripening and the effect of cultivar and ripening stage on the quality and microstructure of frozen mangoes. Three mango cultivars (Maha Chanok, Nam Dok Mai, and Chok Anan) at different stage of ripening were observed. The ripening stage was categorized into six stages, which were based on the color index of mango peel color. The color of mango fruits changes noticeably from green to yellow-orange in all cultivars. A significant decrease in firmness was accompanied by a decrease in alcohol-insoluble residues and an increase in water-soluble pectin content throughout the ripening stages. The microstructure which was examined with light microscope revealed that during ripening of all cultivars, parenchyma cells became irregular in shape and loss of cell-to-cell contact. Maha Chanok and Chok Anan were found to exhibit smaller cell sizes and more compact cells as well as a greater firmness than Nam Dok Mai. Transmission electron micrographs illustrated that the cell walls of all cultivars were found to be intact at the stage 3, whereas the middle lamella was found to dissolve at ripening stage 5. Three mango cultivars with three different ripening stages were frozen in a cryogenic freezer. After freezing and thawing, the mangoes at ripening stage 3 exhibited the highest firmness value, highest firmness sensory scores, lowest drip loss and lowest water-soluble pectin. The microstructure study using light and transmission electron microscopy showed that all frozen-thawed mangoes exhibited a swelling and folding of cell wall due to freezing damage. The cell walls at the ripening stage 3 of Nam Dok Mai and Chok Anan cultivars appeared less damaged than in the other stages, while severe cell wall damage was found in the ripening stage 5. Frozen samples of Nam Dok Mai at all ripening stages appeared to have more cell wall damages than Maha Chanok and Chok Anan samples. These damages correlated with the extremely high drip loss of frozen-thawed Nam Dok Mai. It is clear that the loss in firmness of mango tissues after freezing and thawing are due to the changes in middle lamella, cell walls and cell wall composition. The use of Maha Chanok and Chok Anan mangoes at the ripening stage 3 is recommended, to preserve the cell wall damages and a loss in firmness, which occur during the freezing process.

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Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I would like to thank Assoc. Prof. Dr. Sanguansri Charoenrein my thesis advisor for her kindness, guidance and assistance during my study. I am very grateful for her advises, encouragement and valuable suggestions without which this study could not have been successfully completed. I would sincerely like to thank to my committee for devoting their precious time to this research study and giving comments, Assoc. Prof. Dr. Wunwiboon Garnjanagoonchorn, and Prof. Dr. Jingtair Siriphanich.

Special thanks are extended to Prof. Dr. Vanna Tulyathan, who kindly served as a graduate representative and provides valuable comments.

This research was supported by the Graduate School Kasetsart University (research grant for the international publication) and was partially funded by the Thailand Research Fund under project RSA 5480020.

In addition, I would like to thank all stuffs member of Food Science and Technology for their assistance. I also thank all my friends for their help and support.

I am very grateful to my parents and my sister for their love and encouragement.

Kannika Rimkeeree

April 2012

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EFFECT OF CULTIVAR AND RIPENING STAGE ON MICROSTRUCTURE AND THE QUALITY OF FROZEN MANGOES (*Mangifera indica* L.)

INTRODUCTION

Mango is a tropical fruit generally harvested at the mature-green stage to minimize over-ripening and losses in quality during postharvest handling and transportation. The stage of ripeness and eating quality of mango fruit is judged by a variety of attributes evaluated by various methods in the supply chain. The determination of fruit ripening stage is important for fresh cut and processed products, and to provide a consistent supply of good quality fruit for retail marketing. For most mango cultivars, ripening is associated with textural loss, decrease of chlorophyll content in the peel and decline of flesh acidity, accompanied by increasing in carotenoids contents and total soluble solids. Firmness has been considered a reliable indicator of mango maturity at harvest and ripeness during commercial mango handling, and an important tool for growers, importers, retailers and consumers (Padda *et al.*, 2011). Fruit firmness changes noticeably from very hard at harvest to very soft at the fully ripe stage.

During the peak harvesting periods due to excess mango fruits in the markets, their prices are very low. Also, fresh mangoes presently have limited potential to compete on the world market due to the short shelf life. Mangoes can be stored without perishing for 4 to 8 days at room temperature and 2-3 weeks in cold storage at 13 ºC and 85-90% relative humidity (Carrillo-Lopez *et al*., 2000). Thus, preservation of the fruit can help to extend shelf life and support the growing popularity of off-season consumption. Processing is considered as an extension of storage life or improving the value of raw produce. Freezing is one of the best methods for long-term storage of fruits. Freezing preserves the original color, flavor, and nutritive value of most fruits. The freezing process reduces the rate of these degradation reactions and inhibits the microbiological activity. The quality of the frozen fruits is very

dependent on other factors such as the type of fruit, varietal characteristics, stage of maturity, pretreatments, and the rate of freezing (De Ancos *et al.*, 2006). However, this process can cause texture degradation by causing freezing injuries to cell tissues. The influence of freezing, frozen storage, and thawing on fruit quality has been extensively reviewed (Reid, 1996; Skrede, 1996; Hui *et al*., 2004). Marin *et al*. (1992) have studied the chemical and biochemical changes that take place during freezing and frozen storage of four Spanish mango cultivars: Smith, Lippens, Palmer and Davis-Haden. Rattanapanone *et al.* (2007) studied the biochemical changes during ripening of mango fruit and frozen storage of mango flesh cultivar Chok Anan and Maha Chanok. The activities of peroxidase and polyphenol oxidase were also investigated.

Microscopy techniques are increasingly being used to study the influence of processing conditions and ingredients on food structure. Structural research on some fruits affected by the freezing process has been reported in many studies. Shomer *et al*. (1998) observed ultrastructural injuries in both the exocarp and the mesocarp cells of thawed Madjhoul dates as view by transmission electron microscopy (TEM). Otero *et al*. (2000) also found major damage in mango tissues due to the formation of ice crystals which observed by Light microscopy (LM). Fava *et al*. (2006) investigated the ultrastructural cell changes in blueberries during freezing-thawing process. LM and TEM images demonstrated folding and compression of the cell wall of the epicarp which occurred during the freeze-thaw process. Furthermore, Ramirez *et al*. (2011) studied the microstructure damage in frozen-thawed apple slices. LM images showed the highest microstructure damage in frozen tissue as compared to unfrozen and the other pre-treatments studied.

Several studies have investigated the changes in physicochemical properties during the ripening of mangoes. Some researchers determined the stage of ripening but no reports have been available on Thai mango cultivars. Also, there have been no reports of using the color index to identify the different ripening stage of Thai mangoes. Moreover, the combination studies on how cultivar type and ripening stage affect the microstructure and textural quality of frozen mangoes are not available. To

improve the high quality frozen mangoes, the knowledge of suitable selection ripening stages of raw material and the changes that will occur during freezing process is required. Thus, the objective of this study was to investigate the effect of cultivar and ripening stage on the quality and microstructure of frozen mangoes. Three mango cultivars; Maha Chanok, Nam Dok Mai, and Chok Anan which commonly exported were analyzed in the present study. The three mango cultivars and three ripening stages of mango were used to determine the conditions most resistant to freezing damage. The qualities of frozen-thawed mangoes were evaluated. Also, cell wall damages were examined by LM and TEM.

The hypotheses of this research were that (1) the cultivar differences and differences in the ripening stage might affect the microstructure and quality of frozen mango and (2) the partially ripe mango might preserve the better texture quality of frozen-thawed mango than ripe and fully ripe mango.

It is expected that the results obtain from this study will be benefit both agriculture and industry. The practical applications are that the selection of suitable cultivar and ripening stage of mango can reduce tissue damages which occur during freezing and thawing process. It also addresses the possibility of using the partially ripe mango to make high quality frozen mango pieces and consumer acceptability.

OBJECTIVES

1. To study the physicochemical and microstructural changes during ripening of Thai mangoes.

2. To study the effect of cultivar and ripening stage on microstructure and quality of frozen mangoes.

3. To find the suitable cultivar and its ripening stage to minimize the freezing damage of frozen mangoes.

LITERATURE REVIEW

1. Mango

Many mango (*Mangifera indica* L.) cultivars are commercially grown in Thailand. The area under mango cultivation continues to expand considerably over recent years. The area was increased from 1,860,005 to 1,906,960 and 1,925,164 Rai in 2007, 2008 and 2009 respectively, with an increase of the production from 2.30 to 2.37 and 2.47 million tons, respectively (Thai Mango Growers Association, 2010).

1.1 Thailand's export markets

Mango is one of economically important fruits grown in Thailand. Further investigations reveal that although Thailand was the third largest of mango producers in the world in 2010, the export was relatively small, represented 1.69% of total mango production in Thailand (Food and Agriculture Organization [FAO], 2012). Main export markets highly concentrate in regional markets; Japan, Malaysia, Korea, Singapore, etc. In 2011, fresh mangoes in particular accounted for 35% of total export value of mangoes. There are about 3 product types; canned (44%), frozen (16%) and dried (5%) mangoes, which hold the majority of the export market share (Figure 1).

Comparing between export volumes of Thai fresh mango exports and Thai processed mango exports (Figure 2), indicating that the export of processed mangoes show an upward trend whereas the export of fresh mangoes fluctuated during the past several years and show downward trend from 2001 to 2005, then the trend dramatically went rise in 2006 and show an upward trend until 2011.

Fresh mangoes export has been limited by harvesting season, highly perishable nature and susceptibility to postharvest disease, extremes of temperature and physical injury (Pantastico *et al.*, 1984). Therefore, processed mangoes enable exporters to serve their markets even during off-season periods for fresh mangoes.

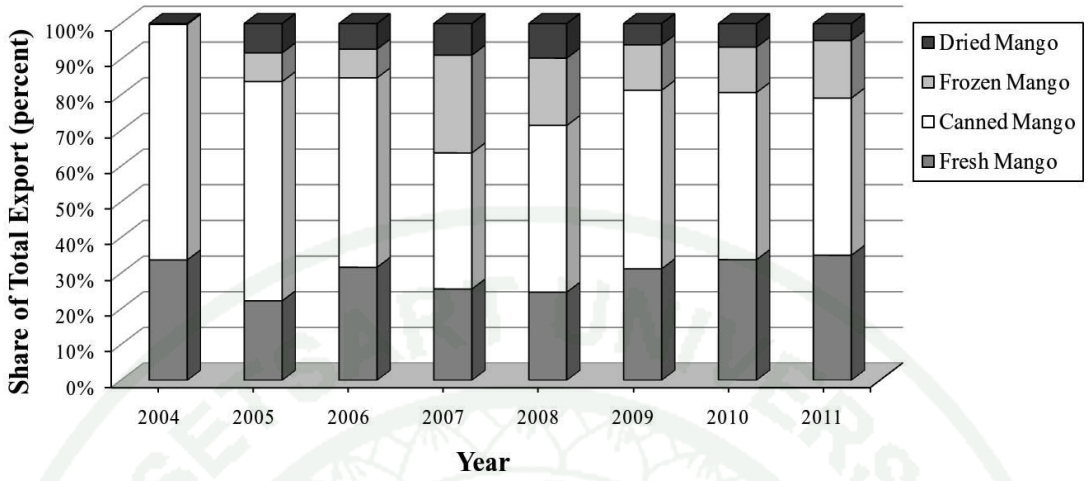

Figure 1 Mango exports from Thailand by product type, share in % of value.

Source: Office of Agricultural Economics [OAE] (2012)

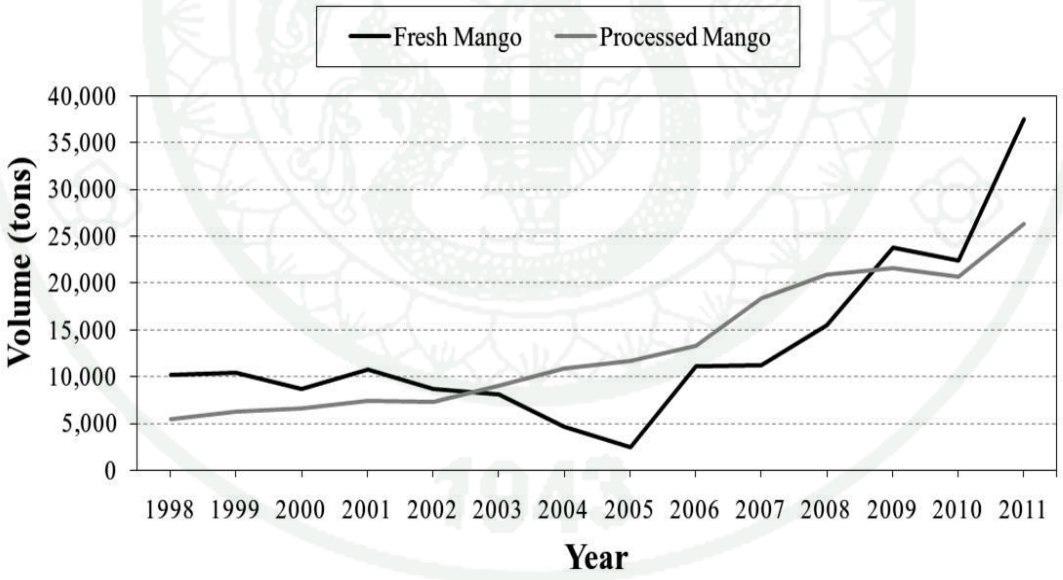

Figure 2 Volume of fresh and processed mango in tons.

Source: OAE (2012)

1.2 Mango cultivars

Although more than a hundred cultivars exist in Thailand, only 6-7 cultivars are grown on a commercial scale. Mango cultivars commonly exported includes Nam Dok Mai, Maha Chanok, Chok Anan, Rad, Nang Klangwan, and Pimsaen. Because of its sweet aroma and delicate texture, Nam Dok Mai is currently the most popular cultivar and its production ranks first among Thai export mango cultivars (Postharvest Technology Research Institute [PHTRI], 2009).

1.1.1 Maha Chanok cultivar

Maha Chanok cultivar is a hybrid mango between Maxican's and Thai's local mangoes; Sunset and Nang Klarngwun. The fruit shape is elongated as shown in Figure 3A which is similar to Nang Klarngwun cultivar. Maha Chanok mangoes have thick skin with thin seed and smell like mango Sunset (Chunwongse, 1999; Phumichai, 2000). The color turns from green to yellow-orange or orange-red when ripen (Figure 4A). Maha Chanok mango is known to have delicious taste; sweet and sour.

1.1.2 Nam Dok Mai cultivar

The cultivar of Nam Dok Mai is the most famous and widely cultivated in Thailand. Consumers usually prefer to consume Nam Dok Mai mango at the ripe stage (Nontri, 2002). Nam Dok Mai has an oval shape with a sharp-pointed tip as shown in Figure 3B. It is a thin skin and a fibreless mango with a smooth, silky texture and a deliciously unique flavor. A great feature of this cultivar is the ability to produce off-season flowering, giving an extended ripening season (PHTRI, 2009). It can sometimes be found in specialty markets in Japan, Europe, and rarely the United States. The ripe fruit range from bright-green to yellow color (Figure 4B).

1.1.3 Chok Anan cultivar

Chok Anan cultivar is mainly produced in the northern part of Thailand for the domestic fresh market and small scale processing (Spreer *et al*., 2009). It has an oval shape with tapered tips (Figure 3C). The skin of this cultivar is thick. During ripening, the green peel color turns to yellow (Figure 4C). It has a sweet taste and firm texture. Chok Anan cultivar has higher ability to produce off-season flowering without chemical induction than the other cultivars (Titiprasert *et al*., 2001). This generates additional income for farmers by reducing the labor peak at harvest.

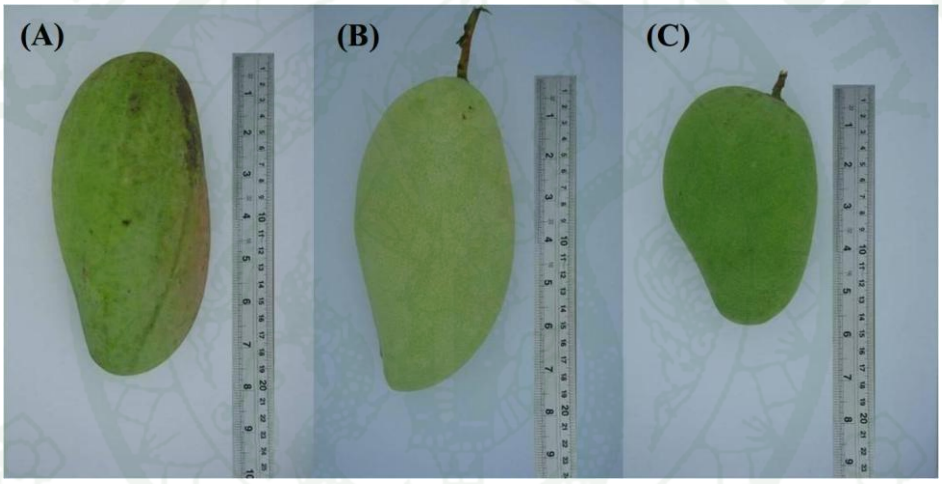

Figure 3 Three different mango cultivars at mature-green stage. (A) Maha Chanok, (B) Nam Dok Mai, and (C) Chok Anan.

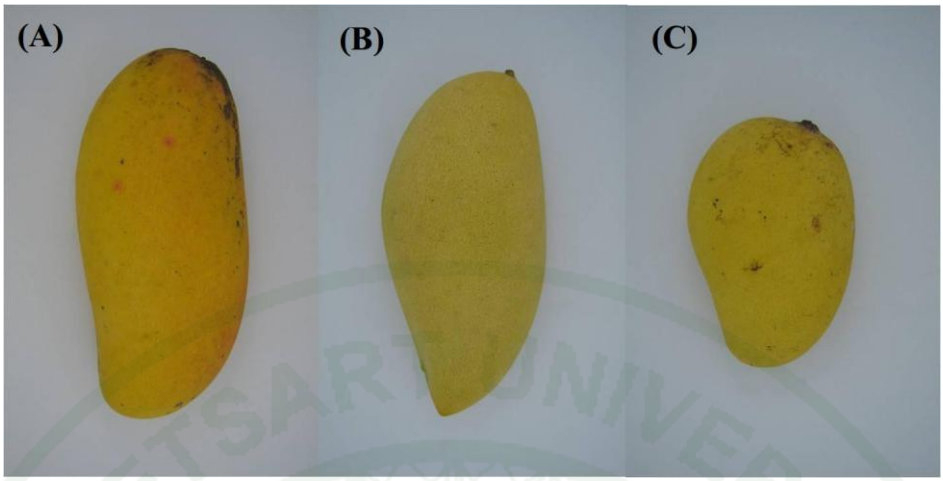

Figure 4 Three different mango cultivars at fully ripe stage. (A) Maha Chanok, (B) Nam Dok Mai, and (C) Chok Anan.

2. Fruit ripening

The ripening process of the mango fruit involves physical and biochemical changes which alter their respiration, ethylene production, flavor, texture, aroma and nutritional values (Lizada, 1993). The change in color that occurs as fruits ripen is caused by the degradation of chlorophyll and the accumulation of carotenoids. A rapid destruction of chlorophyll was observed in the Tommy Atkins mango cultivar. Carotenoid levels increased during ripening whereas anthocyanin levels gradually decreased (Medlicott et al., 1986). The chlorophyll content in the peel of Nam Dok Mai decreased concomitantly with an increase in β-carotene (Kasantikul et al., 1984; Ketsa et al., 1999b). Previous study reported on mango fruit cultivars Kaew, Maha Chanok, Chok Anan, and Nam Dok Mai developed bright yellow-orange mesocarp coloration at their fully ripe stage (Vásquez-Caicedo et al., 2005). Also, carbohydrates and starch converted into sugars, which result in an increase of sweetness. The total soluble solids and total soluble sugars increased during the ripening of Alphonso mangoes (Yashodo et al., 2006), Keitt mangoes (Medlicott and Thompson, 1985), and Tommy Atkins mangoes (Vásquez-Caicedo et al., 2006) while acidity and organic acids decreased as the ripening progressed.

Commonly, fruit cell walls contain a large amount of pectins which are the major components of the primary cell wall and middle lamella and thus contributing to the texture and quality of fruits. As in other fruits, the mango tissues softening are primarily due to depolymerization and solubilization of polyuronide in the middle lamella of cell walls (Huber, 1983). The cell walls lose their integrity and disintegration, thus leading to the loss of fruit firmness. Fruit firmness changes from very hard at harvest to very soft at the fully ripe stage. The relationship between the polyuronide degradation and the softening has been extensively studied in many fruits. For some tropical fruits the alcohol-insoluble residue (AIR) and texture declined rapidly during ripening (El-Zoghbi, 1994). The increase in total pectin of ripe mangoes was a consequence of higher free carboxylic groups due to de-esterification of pectins (Yashoda et al., 2006). In raspberries, although the amount of total pectin in the AIR measurements did not change dramatically during ripening, there were modifications in the relative solubility of the pectin polymers. The amount of water-soluble pectin (WSP) increased as the fruit ripen (Vicente *et al*., 2007a).

3. Plant cell walls

3.1 Cell walls

Plant cell walls are distinguished from animal cells by the presence, around the plasmalemma, of a wall within which complex physicochemical and enzymatic phenomena progress. In the course of cell growth, the dimensions of the cell wall vary according to the type of macromolecule of which it is composed. The cell wall is approximately 30% cellulose, 30% hemicelluloses, 35% pectin and 5% protein in dicotyledonous plants (Fry, 1988). In fruit cell wall, pectin content is higher and protein content lower (Knee and Bartley, 1981). The schematic of plant cell wall is shown in Figure 5.

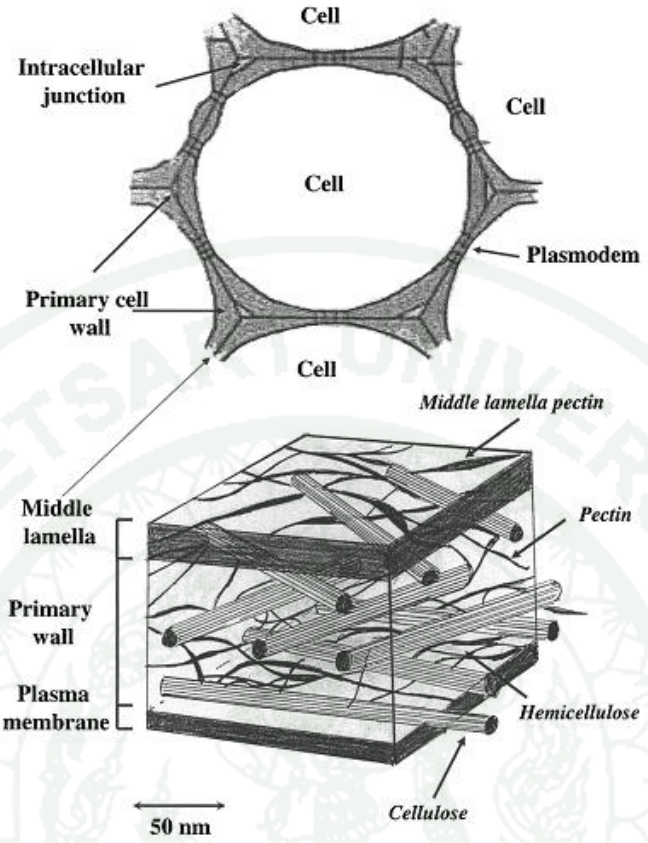

Figure 5 Schematic representation of the plant cell walls along with the location of the main polysaccharides components.

Source: McCann *et al*. (1990)

The cell wall is a complex laminate structure can be divided into three district zones; middle lamella, primary cell wall, and secondary cell wall, and the amount of pectin present decreases in this order (Northcote, 1986). The first wall deposited after cell division is called the ‘‘middle lamella’’ and is essentially composed of pectic material (Perez and Mazeau, 2005).

3.2 Pectic substances

Pectic substances are a group of closely associated polysaccharides from the primary cell walls and intercellular regions of higher plants. They are deposited mainly in the early stages of growth when the area of the wall is increasing. Meristematic and parenchymous tissues are therefore particularly rich in pectic substances (Northcote, 1986). The term pectic substances are commonly used to encompass the methoxyl ester, pectin, the de-esterified pectic acid, pectates, and neutral polysaccharides (arabinans, arabinogalactans, galactans).

Pectins are the common and major components of primary cell wall and middle lamella. Pectin possesses a negative charge, which enables it to bind easily with calcium and other divalent cations. The dominant feature of pectins is a linear chain of α-(1→4)-linked D-galacturonic acid units (Figure 6). The varying proportions of the acid groups are present as methoxyl esters. In common understanding this polysaccharide is considered as a heterogeneous linear chain with rhamnose in the backbone that is interrupted by short segments of the galacturonan chain. The rhamnose causes a kink in the regular chain conformation and prevents crystallization. The model of heterogeneity in pectin is shown in Figure 7A. It is also the unit to which the highly branched ‘‘hairy region’’ is anchored (Saulnier *et al*., 1988; Voragen and Pilnik, 1989; Will and Dietrich 1992). The detailed structure of the hairy region is shown in Figure 7B.

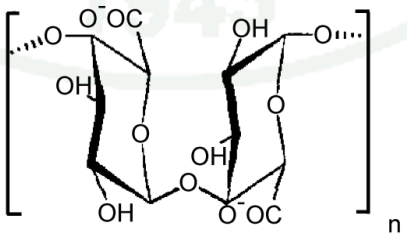

Figure 6 Chemical structure of pectin.

Source: Yi and Neufeld (2005)

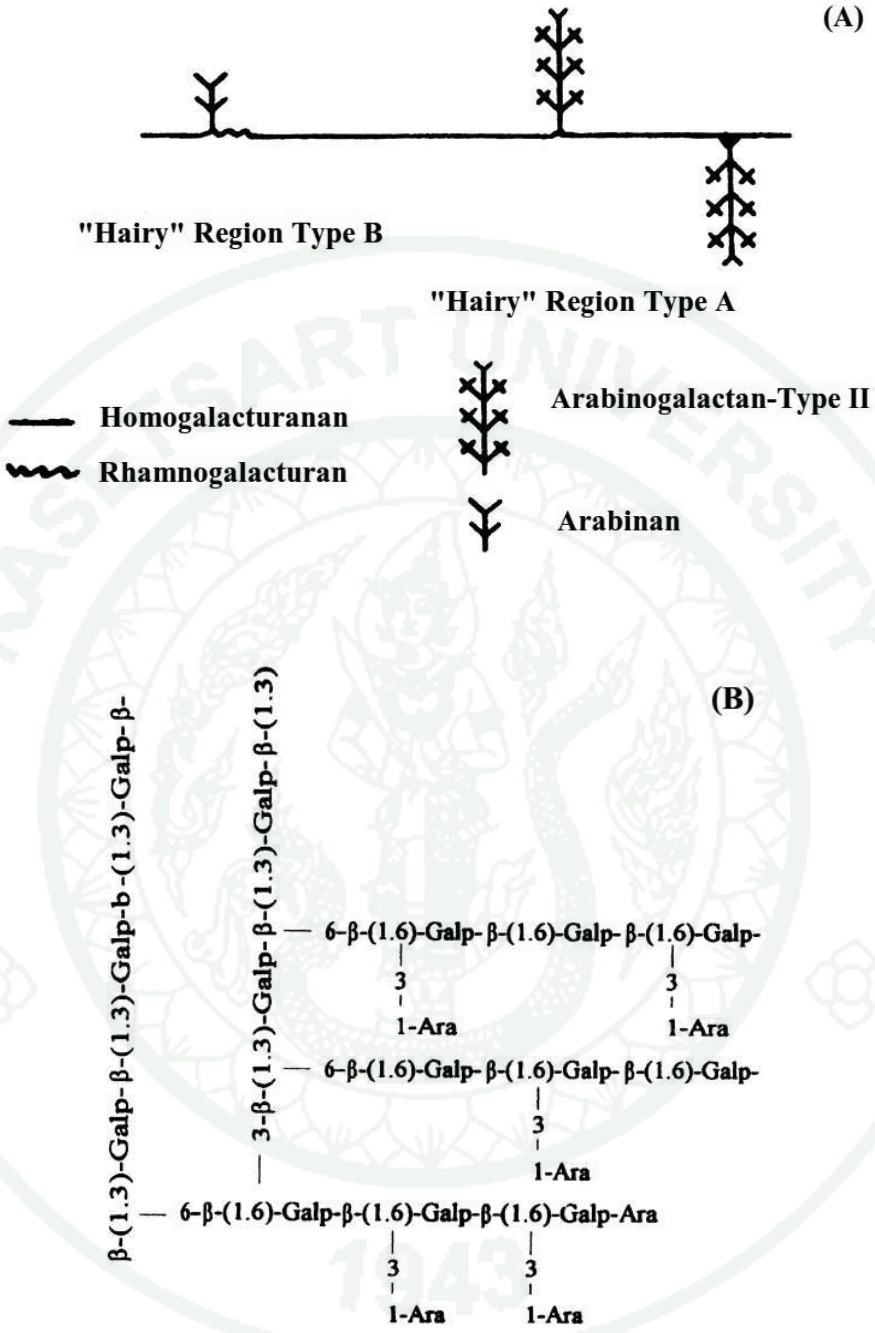

Figure 7 Model of heterogeneity in pectin (A), Detailed structure of the hairy region (B).

Source: Saulnier *et al*. (1988); Voragen and Pilnik (1983); Will and Dietrich (1992)

Several examples of active pectin degradation are known. The technical pectins extracted from apples, sugar beet, or citrus fruits are water-soluble, due to the initiation of protopectin's enzymic degradation. In overripe pears and snow berries, a complete soften of their tissues was observed (Spiridon and Popa, 2005). The texture of fruits and vegetables during growing, ripening, and storage is strongly influenced by the amount and nature of the pectin. Important changes in the properties of fruit products during storage and processing are related to changes in the pectic component (Voragen *et al*., 1995).

3.2.1 Pectin changes with ripening

One of the most important changes that occur during ripening of fruits is a softening of their fleshy tissues. The evolution of the texture is mainly due to structural changes in the cell wall, as other factors such as osmotic properties of the cells and turgor pressure usually stay constant upon ripening (Knee and Bartley, 1981; Bartley and Knee, 1982; Huber, 1983). The major and most common changes in the cell wall structure during ripening are related to pectins. There have been numerous studies on the evolution of pectins during fruit ripening and their correlation with softening. For economic reasons, more data are available on major crops, like tomato, pome fruits (apple, pear), and fruits that soften dramatically, leading to commercial problems, like mango and kiwi (Voragen *et al*, 1995).

A decrease in the proportion of galacturonic acid per gram of fruit (Brinson *et al*., 1988; Mitcham and McDonald, 1992) or per gram cell wall material (Ahmed and Labavitch, 1980; Bartley *et al*., 1982) at the very last stages of ripening is generally accompanied by an increase in soluble pectins. The cell wall can be thoroughly modified as in banana (Wade *et al*., 1992) or mango (Mitcham and McDonald, 1992) where softening is accompanied by an extensive loss of galacturonic acid and solubilization of the remaining galacturonic acid. Besides, more extensive changes seem indeed to be associated with fruits that soften more upon ripening, such as mangoes, tomatoes, and kiwi (Voragen *et al*., 1995).

3.2.2 Pectin changes with processing

The processing and the quality of products is strongly influenced by the types of tissue present, the polysaccharide composition, ultrastructure of their cell wall (McNeil et al., 1984; Selvendran, 1985), the chemical, physical, and enzymatic changes that may occur. These changes include depolymerization, de-esterification, and solubilization of pectin. The preservation and preparation of pectin-containing foods frequently involves in heating process, which often results in texture loss, a serious quality defect.

4. Fruit Freezing

Freezing is one of the best methods for long-term storage of fruits. Freezing preserves the original color, flavor, and nutritive value of most fruits. Fresh fruits, when harvested, continue to undergo chemical, biochemical, and physical changes, which can cause deterioration reactions such as senescence, enzymatic decay, chemical decay, and microbial growth. The freezing process reduces the rate of these degradation reactions and inhibits the microbiological activity. However, it should be recognized that freezing and frozen storage is not a reliable biocide.

The quality of the frozen fruits is very dependent on other factors such as the type of fruit, varietal characteristics, stage of maturity, pretreatments, and the rate of freezing (De Ancos *et al.*, 2006). The freezing process reduces the fruit temperature to a storage level (–18 °C) and maintaining this temperature allows to preserve the frozen product for 1 year or more (De Ancos *et al.*, 2006). The influence of freezing, frozen storage, and thawing on fruit quality has been extensively reviewed (Reid, 1996; Skrede, 1996; Hui *et al*., 2004).

4.1 Freezing principles

The freezing process reduces food temperature until its thermal center reaches –18 °C, with the consequent crystallization of water, the main component of plant tissues. Water in fruit and fruit products constitute 85-90% of their total composition (De Ancos *et al.*, 2006). Crystallization of water during freezing reduces water activity (a_w) in these tissues and consequently produces a decline in chemical and biochemical reactions and microbial growth.

Figure 8 shows typical freezing curves at different freezing rates. When the product is cooling down to 0 °C, ice begins to develop (section A-S, Figure 8). The exact temperature for the formation of first ice crystal depends on the type of product and is a consequence of the constituents concentration independent of water content (De Ancos *et al.*, 2006). Ice formation takes place after the product reaches a temperature below its freezing point (−5 °C to −9 °C) for only a few seconds. This process is known as super-cooling (position S). After that, due to heat release during the first ice formation, the temperature increases until the freezing point is reached (position B). Section B-C corresponds to the freezing of most of the tissue water at a temperature that is practically constant, with a negative slope from a decline of the freezing point due to solute concentration. Section C-D corresponds with the cooling of the product until the storage temperature.

4.2 Factors affecting frozen fruit quality

Two principles dominate the control of quality and safety in frozen foods is the product-process-package factors (PPP) and the time-temperature-tolerance factors (TTT) (De Ancos *et al.*, 2006). PPP factors need to be considered at an early stage in the production of frozen fruits and they are the bases of commercial success of the product. The product factor requires high quality raw materials and ingredients to produce high quality frozen food. The process factor is the speed and effectiveness of the freezing operations and the use of additional processes. The package factor should offer physical and chemical barriers. TTT factors maintain the quality and

safety during storage. The concepts refer to the relationship between storage temperature and storage life (IIR, 1986).

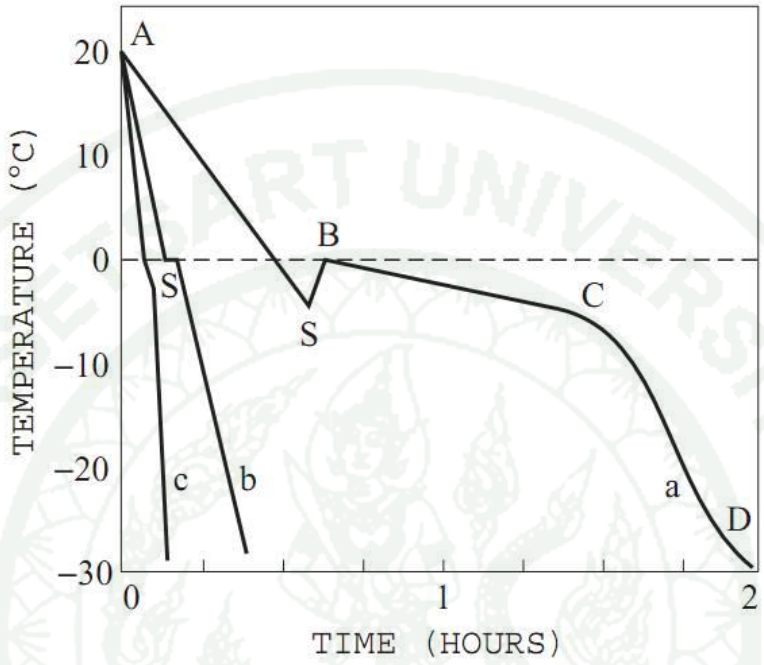

Figure 8 Typical freezing curves of foods at different rates. (a) very slow, (b) fast, and (c) very fast.

Source: Fennema (1976)

The safe and high quality frozen fruits can be produced by follow the directions; the selection of suitable product for freezing, PPP factors, knowledge of the effect of freezing, frozen storage, and thawing on the fruit tissues that causes physical, chemical, and biochemical changes, stability of frozen fruits (TTT factors), thawing, and microbiological quality (De Ancos *et al.*, 2006).

The selection of suitable product is necessary for freezing process. The differences of frozen fruit quality exist between fruit varieties and cultivars based on chemical, biochemical, and physical characteristics that determine the sensory and nutritional quality. Freezing potential of fruit varieties or cultivars are evaluated with

practical trials after freezing, frozen storage, and thawing of the fruit products. The suitability of varieties or cultivars for freezing can be studied on the basis of physical (texture and color), physical-chemical (pH, acidity, and soluble solids), chemical (volatile, pigments, and polyphenol compounds), nutritional (vitamins and dietary fiber content), and sensory aspects (firmness, color, and taste). These kinds of studies have been done with different fruits such as kiwi (Cano and Marin, 1992; Cano *et al*., 1993), mango (Marin *et al*., 1992; Cano and Marin, 1995), pineapple (Bartolome *et al.*,1996a, 1996b, 1996c), papaya (Cano *et al*., 1996; Lobo and Cano, 1998), raspberry (De Ancos *et al.*, 1999, 2000a, 2000b; Gonzalez *et al*., 2002), strawberry (Castro *et al*., 2002), and other fruits.

4.3 Effect of freezing and frozen storage on fruit tissues: physical, chemical, and biochemical changes

4.3.1 Plant cell structure

Understanding the effect of freezing on fruit requires a short review of plant cell structure. A relationship between cell structure properties and freezing cell damage has been extensively reviewed (Reid, 1996; Skrede, 1996). Plant cells are surrounded by a membrane and interspersed with extensive membrane systems that structure the interior of the cell into numerous compartments. The plasmalemma or plasmamembrane encloses the plasma of the cell and is the interface between the cell and the extracellular surroundings. Contrary to animal cells, plant cells are surrounded by a cell wall and many of them contain a special group of organelles inside: the plastids (chloroplasts, leucoplasts, amyloplasts or chromoplasts) as shown in Figure 9.

The cell wall of plants consists of several stacked cellulose microfibrils embedded in a polysaccharide matrix able to store water thereby increasing the cell volume. According to their capacity to bind or store water, the polysaccharides involved in the matrix can be classified as follows; pectin > hemicelluloses > cellulose > lignin (De Ancos *et al.*, 2006).

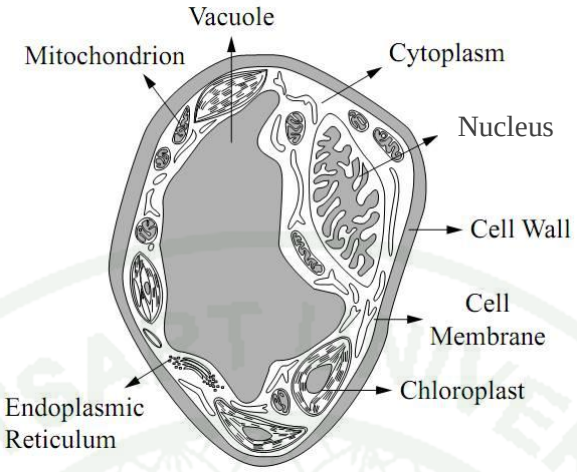

Figure 9 Cross-section of a plant cell.

Source: De Ancos *et al.* (2006)

4.3.2 Physical changes and quality

The first factor that produces mechanical damage to the cell is the volume expansion due to the formation of ice that affects the integrity of cell membrane. During frozen storage, retail-display, or the carry-home period, the fluctuations in product temperature produces ice recrystallization which affects the number, size, form, and position of the ice crystal formed during freezing. The large fluctuation frequent produces partial fusion of ice and the reforming of large and irregular ice crystals that can damage cellular membranes and produce a freeze-dried product, allowing sublimed or evaporated water to escape (De Ancos *et al.*, 2006). The sublimation of the ice may occur during frozen storage if the packaging product is unsuitable. The recrystallization and freezer burn dehydration increase with temperature fluctuations, but the harmful effect of these two processes on frozen fruit quality can be decreased by lowering the storage temperature below –18 °C (IIR, 1996).

4.3.3 Chemical and biochemical changes and quality

Quality changes, such as loss of the original fruit color or browning, developing off-odor and off-taste, texture changes, and oxidation of ascorbic acid, are the main changes caused by chemical and biochemical mechanisms that affect fruit quality. Texture of frozen fruits is dependent on chemical and biochemical modifications of the cell wall and middle lamella components (pectins, hemicellu-loses, and celluloses). In fact, a decrease of the total pectin during freezing and frozen storage has been related to a reduction of firmness in different fruits (Lisiewska and Kmiecik, 2000). The size and location of ice crystals cause cell membrane rupture that promotes pectic enzymes and/or chemical activity and contributes to mechanical damage in cell wall material (De Ancos *et al.*, 2006). The influence of freezing rate on tissue integrity, texture, and drip loss has been reviewed by different authors (Cano, 1996; Reid, 1996; Skrede, 1996).

5. Transmission electron microscope

The transmission electron microscope (TEM) operates on the same basic principles as the light microscope but uses electrons instead of light. TEM uses electrons as light source and their much lower wavelength makes it possible to get a resolution a thousand times better than with a light microscope. The objects can be seen to the order of a few angstrom (10^{-10} m). The possibility for high magnifications has made the TEM a valuable tool in both medical, biological and materials research (Williams and Carter, 2009). A light source at the top of the microscope emits the electrons that pass through vacuum in the column of the microscope. Instead of glass lenses focusing the light in the light microscope, the TEM uses electromagnetic lenses to focus the electrons into a very thin beam. The electron beam then passes through the specimen. Depending on the density of the material present, some of the electrons are scattered and disappear from the beam. At the bottom of the microscope the unscattered electrons hit a fluorescent screen, which gives rise to a shadow image of the specimen with its different parts displayed in varied darkness according to their density (Williams and Carter, 2009).

6. Related research literature review

6.1 Mango and other fruits ripening: changes in cell wall components in relation to fruit softening

Although fruit softening can be affected by several factors, fruit softening is probably caused by the cumulative effect of modifications occurring in the primary cell wall, all of which contribute to a loss of firmness and changes in the textural qualities (Payasi *et al*., 2009). Biochemical studies of cell wall changes during fruit ripening indicate that structural changes in pectin, hemicelluloses and cellulose together are responsible for changing in cell wall structure (Paull *et al.*, 1999).

Mitcham and McDonald (1992) studied on the cell wall modification during ripening of mango fruit. Keitt and Tommy Atkins mango fruit were evaluated for selected ripening criteria at six ripening stages, from mature green to over ripe. They reported that the cell wall material (dry weight per gram fresh weight of mesocarp) was consistently higher in Tommy Atkins than in Keitt, although the amount of cell wall material decreased during ripening in both cultivars. The firmness between mature green and ripe stage was found to decrease sharply, while the soluble uronides was found to increase significantly. However, Keitt accumulated more soluble polyuronides at the ripe stage than Tommy Atkins. They suggested that these textural differences may be the result of differences in cell wall composition and a greater amount of cell wall material in Tommy Atkins.

Ketsa *et al*. (1999a) investigated how the rate of softening related to changes in the amounts of pectic cell wall components. Mangoes were divided into three groups. One group was the control and was placed at 25 °C and 75% RH for ripening. The other two groups were placed at 4°C and 95% RH for 1-3 weeks and then transferred to 25°C for ripening. They observed that the levels of water-soluble pectin in Nam Dok Mai mango increased progressively over ripening period. Moreover, during ripening the water-soluble pectin in the chill-stressed fruits increased more slowly than the non-stored fruits, though by day 4 the amount of

water-soluble pectin was similar in all fruits. The conversion of protopectin to water-soluble pectin in ripening mango fruits is accompanied by polymer degradation. In this study, the lower concentration of water-soluble pectin in low-temperature stressed mango fruits correlated with delayed softening. This suggests that softening of ripening mango fruits results from an increase in the degradation and depolymerization of cell wall pectin.

Yashoda *et al*. (2006) examined the extent of correlation with textural softening in Alphonso mango during ripening, in relation to changes in cell wall constituents. Mature, freshly harvested mango fruits were taken to denote unripe stage, while the subsequent stages of ripening were picked from the fruits kept for normal ripening. The texture analysis showed progressive decrease in the force at different stages of ripening. The decrease in alcohol-insoluble residues was found during ripening indicated that large alcohol-insoluble polymers are being degraded to shorter alcohol-soluble polymers. Water-soluble pectin was found to undergo drastic depolymerization during mango ripening, which contributes to tissue softening and textural changes. It is known that fruit softening generally is accompanied by a decrease in the content of insoluble pectic substance with a concomitant increase in soluble polyuronides.

6.2 The microstructural changes as a result of fruit ripening.

Alphonso mango were observed with a light microscopic. Yashoda *et al*. (2006) suggested that the more compact and rigid cell wall at the unripe stage appeared loosely structured and expanded with considerable degree of swelling at the end of ripening which eventually resulted in a loss of fruit firmness.

Ben-Arie and Kislev (1979) also reported that the softening of apple and pear fruits was associated with the dissolution of the middle lamella during fruit ripening, which were observed by using TEM. Microstructural differences observed with a microscopy can give an insight into the contributions of different tissues within the fruit to the overall texture. Additionally, the integration of physicochemical data

and microstructure can help to identify causes of these textural changes among cultivars and provide new understanding into the preservations of mango fruit quality.

6.3 Effect of freezing on quality of frozen mango

Freezing has been shown to induce significant changes in the texture, color and soluble solids of mango and apple. Also, the values of the studied texture parameters (firmness and Young's modulus) decreased significantly after freezing (Chassagne-Berces *et al*., 2010). The tissue damage can be due to the presence of large amounts of water in fresh fruits which turn into ice crystals after freezing, leading to loss of firmness. To limit these problems, some research projects have attempted to improve the texture quality of frozen mango by using various pretreatments.

Marin *et al*. (1992) have studied the chemical and biochemical changes that take place during freezing and frozen storage of four Spanish mango cultivars: Smith, Lippens, Palmer and Davis-Haden. Mango was air-blast-frozen as slices without any previous treatment and stored at –18 °C for a 4-month period. The results showed that freezing preservation of mango slices did not lead to changes in moisture content. The titratable acidity of the slices decreased as the pH increased in all cultivars due to the freezing process, no changes were found in these parameters throughout frozen storage. The behavior of soluble solids and reducing and total sugar contents showed that no important changes appeared in the cultivars considered. Ascorbic acid and β-carotene contents decreased considerably after 120 days of storage. Peroxidase (POD) and polyphenoloxidase (PPO) activities showed down after freezing, and no regeneration was found throughout frozen storage. The final PPO activity was low (< 20% as compared to the raw fruit) whereas the POD activity was higher (> 40% raw fruit). The remaining POD activity might cause deteriorative changes in the final quality of frozen mango slices. It has been demonstrated that the composition of the cultivars throughout this work was quite similar. The pattern of chemical and biochemical changes was also similar in the four cultivars, being Davis-Haden slices those that maintained a nutritional value (ascorbic acid and β-carotene

content) closest to the raw fruits. However, after freezing, Lippens and Smith mango slices showed the highest inactivation rates of both POD and PPO activities. Nevertheless, in order to determine which of the four cultivars is the most suitable for freezing, studies about sensorial acceptability of the products must be done.

Sripattanapipat (1997) investigated the effects of preparation, type of freezer and frozen storage on the quality of sliced mangoes. Two cultivars of mangoes, Nam Dok Mai and Chok Anan were studied. The mangoes with optimum maturity selected by using specific gravity of 1.01-1.03 were peeled and sliced in halves, dipped in antibrowning agent. The optimum condition to inhibit browning was dipping the mangoes in the mixture of 0.5% of citric acid and 0.25% ascorbic acid for 5 minutes. The mangoes were incubated at room temperature in the environment of calcium carbide for 3 and 4 days, then dipped in calcium chloride ($CaCl_2$) solution before freezing. It was found that 3-days incubated Nam Dok Mai had higher firmness and better texture than the 4-days incubated mangoes while Chok Anan could be incubated for 3 or 4 days without changing in qualities. Optimum condition for $CaCl_2$ treatment before freezing in both mangoes cultivars was dipping sliced mangoes in 1.0% $CaCl_2$ for 10 minutes. Type of freezer (air blast freezer, cryogenic freezer) treatment of mangoes with antibrowning agent and $CaCl_2$ were studied and compared with the control samples. Mangoes were frozen, vacuum packing and storage at –18 °C for 25 weeks. It was found that freezing at –90 °C by cryogenic freezer resulted in less thawing loss and browning whereas higher in values of firmness, vitamin C content, color, texture and overall acceptability scores than freezing by air blast freezer. Treatment of mangoes at optimum condition before freezing resulted in less thawing loss and browning but higher loss of acid, sugar and vitamin C than the control samples. The qualities of mangoes decreased during frozen storage. After storage for 25 weeks, vitamin C content of Nam Dok Mai and Chok Anan decreased 85% and 75% respectively, while β-carotene decreased 14% and 13% respectively. Flavor score did not change while color, texture and overall acceptability scores of the products decreased. However, the sensory qualities were still in acceptable level.

Rattanapanone *et al.* (2007) studied the changes in frozen storage of mango flesh cultivar Chok Anan and Maha Chanok. The inactivation of peroxidase (POD) in mango flesh by hot water and citric acid treatment before freezing was investigated. It was found that a hot water treatment at 85-90 °C for 90 seconds can reduce the POD activity more than 50% while dipping mango flesh in citric acid solutions did not reduce POD activity. The physicochemical changes of frozen ripened mango flesh, that had been treated with hot water at 85-90 °C for 30 and 90 seconds and kept at –18 °C for 6 months, were determined throughout the storage period. The results showed that the L*, a*, b*, C*, H° values and chemical composition of frozen mango flesh decreased during the storage period. These results were similar for the mangoes treated with hot water and the untreated mangoes (control). The control samples had higher values for the chemical composition than the treated samples. The total acidity, reducing sugar, total sugar, total soluble solids, total carotenoids and carotenes decreased, while the pH values increased. In addition, the inhibition of POD activity by dipping mango flesh in 1.0% citric acid containing $CaCl_2$ solutions was investigated. The results showed that sample dipped in 1.0% citric acid containing 2.0% $CaCl_2$ had the lowest POD activity. The treated mango flesh was then frozen at –40 °C and stored for 6 months. The results showed that the L* value did not change and the a* and H° values slightly decreased during storage, while the b* and C* values markedly decreased during the first month storage and slightly decreased during the rest of the storage period. The b* and C* values in the treated samples were significantly higher than in control mango flesh. The POD and polyphenol oxidase (PPO) activities of the frozen mango flesh decreased during the first 4 months storage, and then increased in the remaining period of storage. The POD and PPO activities in both control and treated mango flesh did not show significantly differences (P = 0.05) over storage time. The sucrose, total sugar, total carotenoids and carotene of the mango flesh decreased while the total acidity, pH values, total soluble solids, reducing sugar did not change during the frozen storage. Sensory evaluation of the frozen mango flesh for color, texture, odor, sweetness, sourness and overall acceptability were acceptable to panelists (score more than 6). However, the sensory results did not show any significant differences (P = 0.05) between the control and treated mango flesh.

Rincon and Kerr (2010) investigated the effect of osmotic drying on mango slices of different ripeness, subsequently frozen and stored at –18 °C during 20 weeks. Osmotic treatments decreased moisture content, titratable acidity, vitamin C levels, lightness (L*) and firmness, while increasing total soluble solids. Subsequent freezing resulted in further decreases in acidity, vitamin C and firmness. However, samples treated with higher concentrations of sucrose showed less change in properties during frozen storage. The results demonstrated that the initially less ripe mango could be softened somewhat by osmotic treatment, with firmness and cohesiveness maintained through frozen storage. Treated less ripe fruit also had lower acid while picking up sugar and had higher vitamin C levels than more mature fruit.

Recently, Sriwimon and Boonsupthip (2011) evaluated the effects of vacuum infusion of a mango juice-sugar mixture into partially ripe mangoes as a freezing pretreatment step. In this study, the partially ripe mango cultivar Nam Dok Mai #4 was used as an alternative for ripe mango. Based on its stronger structure, partially ripe mango could better withstand texture damage by ice crystals. The flavor and color of partially ripe mango could be enhanced by impregnation of mango juice and sugars (natural flavor) into the mango matrix. This investigation found that vacuum infusion was more effective than natural mass diffusion in incorporation of mango juice-sugar mixture. The vacuum infusion method appeared to have a shorter soaking time and better mango qualities (texture, solid content and color sensory scores). After freezing (air-blast, jet-impingement and cryogenic freezing) and thawing, the juice/sugar-infused partially ripe mangoes were high in sensory scores (7-8 in a traditional 9-point hedonic scale) and low in drip loss (9-12 g/100g) values. The texture and drip loss of partially ripe samples were improved, possibly due to its higher initial integrity and the positive influence of the additional sugars. The partially ripe mangoes better tolerated freezing damage than did ripe mango. Also, the texture can better withstand freezing at various rates.

6.4 Effect of freezing on pectic substances

Pectin is also an important contributor to the texture of tissues which have been frozen. Changes of pectic substances are seen in the pectic material as a consequence of freezing and frozen storage. Reid *et al.* (1986) investigated the effects of freezing and frozen storage on the characteristics of pectin extracted from cell walls. The change in uronide content of the fractions obtained from the cell wall material as a function of the storage time at −20 °C for the strawberry variety, Aiko. The WSP fraction, which assumed to be the fraction most loosely associated with the cell wall, showed the most dramatic change. There was a decrease in the uronic acid content of WSP fraction, paralleling a decrease in firmness. This might be expected, if uronic acid containing wall components are constituents of the material lost as drip. Therefore, they compared the cell wall fractions obtained by homogenizing thawed tissue, from which the drip has been lost, and frozen tissue, which has lost no material. The analysis of cell wall showed that soluble uronide was indeed lost in the drip of thawed tissue, leaving the cell wall material depleted of material which would otherwise be included in the water-soluble fraction. Moreover, the compositional studies of WSP fraction were characterized by subjecting it to fractionation using a column chromatography eluted by an increasing ionic gradient. The results suggested that the changes include some associated with the pectin rhamno-galacturonan backbone, as indicated by the significantly higher proportion of rhamnose found in DEAE column. To further study on the WSP fraction changes, gel filtration analyzes were performed to obtain preliminary indications of molecular weights. WSP materials collected from unfrozen, 1 day frozen, and 4.5 month frozen strawberry samples all voided a P100 column, indicating molecular weights in excess of 100,000. Interestingly, material precipitated from the drip loss fraction with ethanol also displayed a major high molecular weight peak, with in addition about 20% of the total uronide containing material eluting with the totally included volume of the column. This would correspond to a molecular weight around 10,000. The pectic material in the drip loss was a significant part of the pectin lost from the cell wall in long term storage.

Alonso *et al*. (2005) analyzed the changes in pectin composition of sweet cherry from the application of prefreezing treatments. Pectic material was analyzed after freezing, and the results therefore reflect the overall changes brought about by pretreatment and freezing. Total uronic acid levels obtained ranged from 300 to 450 µg/mg of alcohol-insoluble solids (AIS). The addition of calcium to the thermal pretreatments at 70 °C, 2 min increased significantly the total concentration of uronic acid levels in AIS. Both thermal and calcium pretreatment reduced the degree of esterification of the total pectic material. The WSP fraction accounted for 35% of total uronic acids in cherries. The samples heated at 70 °C, 2 min not immersed in 100 mM $CaCl_2$ exhibited a sharp and significant decrease in the WSP fraction. However, a significant increase in the WSP fraction and a significant decrease in esterification were observed in the same cherries after immersion in $CaCl_2$. At the higher treatment temperature (70 °C), the uronic acids in the EDTA-soluble fraction increased significantly at 256% compared with the control (untreated frozen cherry) concentration, and there was a 30% to 45% decrease in esterification. Levels of pectinase-soluble uronic acids decreased significantly with the higher treatment temperature, although this decrease was attenuated by subsequent immersion in $CaCl_2$. Pretreatments caused no significant change in the degree of esterification of the pectinase-soluble pectin fraction. The use of calcium and thermal pretreatments at low temperature had a synergic effect and decreased the degree of esterification of water-soluble and EDTA-soluble pectins, thereby favoring the formation of calcium bridges and preventing the depolymerization of pectins. Accordingly, the use of both treatments together can be recommended in the freezing process of cherries.

Chassagne-Berces *et al*. (2010) studied the changes in cell wall composition in apple tissue as a result of freezing. Three freezing protocols were applied; at –20 °C in a cold chamber, at –80 °C in gas nitrogen convection, and by immersion in liquid nitrogen. The identification and quantification of neutral sugars were carried out by gas-liquid chromatography after sulphuric acid degradation of alcohol insoluble material. Uronic acids in acid hydrolyzates were quantified using the metahydroxydiphenyl colorimetric method. Uronic acids were assumed to be composed solely of galacturonic acid. The degree of pectin-esterification by methanol

and acetic acid in alcohol insoluble materials was measured by HPLC after alkaline hydrolysis. The main neutral sugars of apple cell wall were glucose (29-32%) and arabinose (9-11% dry weight). Uronic acid content accounted for 27 to 31% of dry weight. After freezing, the proportion of neutral sugars and uronic acids were lower in cell walls of frozen-thawed apples than in cell walls of fresh apples. The alcohol insoluble materials from thawed apples after freezing at –20 °C or immersion in liquid nitrogen were significantly ($P < 0.05$) poorer in total sugars than those from fresh apples. These levels reflected a lower content in uronic acids and neutral sugars in thawed apples after freezing. Among neutral sugars, the amount of arabinose was lower in thawed apples after freezing at –20 °C than in fresh apples, whereas the amount of mannose was richer after freezing –20 °C. Rhamnose was slightly lower in apples frozen at –80 °C but the variation was extremely limited in all treatments. The other neutral sugars as well as the degrees of methyl- and acetyl- esterification did not exhibit significant variations between the fresh and the frozen-thawed sample. The main sugars, the amounts of which were modified after freezing at –20 °C, concern arabinose representative of pectin and mannose, which is cellulosic-hemicellulosic sugar. Moreover, the lowest arabinose content could be explained by arabinan losses from the rhamnogalacturonan I domains of pectin. As a consequence, modification of pectins and hemicelluloses may contribute to the collapse of the cell walls, resulting in cell separation with the presence of larger intercellular spaces. The degradation of cell walls seems to be higher for slow freezing rates (at –20 °C) than for fast freezing rates. The combined effect of turgor pressure decrease and cell wall alteration may be responsible for tearing of tissue associated with the softening.

Galetto *et al.* (2010) studied the freezing of strawberries by immersion in $CaCl_2$ solutions. In addition, the effect of immersion in pectin methylesterase (PME) solutions prior to immersion freezing was analyzed. The treatments consisted of immersion freezing in 30% (w/v) $CaCl_2$ solution, fruit immersion in 0.05% PME and then immersion freezing in $CaCl_2$, slow freezing in a chamber, and fresh fruits. The frozen fruits were stored for 55 days at –22 °C, thawed at 5 °C, and used for further analysis. The total pectin content in the AIS and the degree of esterification of the pectins did not show any significant differences between fresh and thawed

strawberries, regardless of the freezing method and the pretreatment used. Consequently there was no loss of pectic material after the freezing process (freezing, frozen storage and thawing) and no de-esterification of pectins due to the freezing process or PME pretreatment.

6.5 Effect of freezing on microstructure/ultrastructure of frozen fruit

Microscopy is increasingly being used to study the influence of processing conditions and ingredients on food structure and structural research on some fruits affected by the freezing process has been reported in many studies.

Shomer *et al*. (1998) studied the morphological, structural and membrane changes in frozen tissues of Madjhoul date fruits. Freezing injury was investigated by light and transmission electron microscopy. Light microscopy observations revealed that the morphology of thawed fruit tissue resulted in cell separation and formation of large intercellular spaces. The different degrees of cell separation were detected in the pericarp. The ultrastructural injuries were observed in both the exocarp and the mesocarp cells of Madjhoul date fruits. The damaged cell wall appeared torn or crushed, resulting in the formation of intracell-wall spaces.

Otero *et al*. (2000) analyzed the effect of freezing on the microstructure of two whole fruits of large size by comparing traditional methods and high-pressure-shift freezing (HPSF). Commercially matured peach and mango were used in this study. Two traditional freezing methods and HPSF were used. The results showed that air-blast freezing had the lowest freezing rate of the three processes studied. This was consistent with the major damage found in the fruit tissues due to the formation of large ice crystals. Light microscope observation showed that there was also damage in the surface zone of the samples, where there were broken cells. The damage increased from the surface towards the center of the product. This effect is attributed to the observed thermal gradients manifested in bigger characteristic times in the center than in the surface of the fruits. Although one of the faster freezing methods, with a freezing rate twice that of air-blast, freezing with liquid nitrogen is also negatively

affected by thermal gradients. The cell wall disruptions were appeared in the central tissue of fruits frozen with liquid nitrogen. Large numbers of small intra- and extracellular ice crystals appear at the surface of the product, but fewer large extracellular ice crystals form at the center, disrupting the cell walls. Also, whole fruit freeze-cracking was observed during the freezing of both peach and mango with liquid nitrogen, resulting in irreversible macroscopic damage. In HPSF, expansion was followed by uniform supercooling in the sample, which inducing pervasive nucleation of a large number of small intra- and extracellular crystals without this significantly affecting the microstructure. HPSF produced no appreciable damage at the surface of the sample. Also, the damage in the inner zones was negligible. Moreover, freeze-cracking was not observed in any case of HPSF.

Fava *et al*. (2006) also found the structure and ultrastructural cell of blueberries changes during freezing-thawing process. Under light microscopy, in transverse sections of frozen-thawed fruits, the folded epicarp presented tangential compression and scarce epicuticular materials. The mesocarp appeared partially collapsed. Epidermal cells showed disrupted membranes, collapsed cytoplasm, thick tangential cell walls and thin folded radial walls. Transmission electron microscope (TEM) observation of the outer tangential epidermal cell wall revealed that the presence of a thin epicuticular wax layer separated from the cuticle. The cuticle was amorphous and with a general aspect similar to the raw fruits (control). Frozen-thawed blueberries exhibited a compressed and folded epicarp, cell dehydration and plasmolysis. Probably propagation of extracellular ice caused disruption and destabilization of membranes and consequent loss of the capability of the plasma membrane to act as an efficient barrier against the propagation of extracellular ice.

Ramirez *et al*. (2011) investigated the effect of four pre-treatments (immersion in boiling water, vacuum impregnation, freezing-thawing, and uni-axial compression) on apple microstructure characterized by cell cavities size parameter. The frozen-thawed apple slices presented the highest damage in apple microstructure. The mean and most frequent values of size measurements in the cell cavities of frozen-thawed samples were higher than those of the control and the other pre-

treatments studied. The results indicate that freezing-thawing and immersion in boiling water are the most aggressive pre-treatments. During an inappropriate freezing condition in qualitative terms, it is common to find cellular damage due to ice crystals formation which break cell membranes and cell walls. Hence, the cells appeared irregular in shape and tissue distortion was observed due to the growing of ice crystals with cell separation.

Several studies have investigated the changes in physicochemical properties during the ripening of mangoes. Some researchers determined the stage of ripening but no reports have been available on Thai mango cultivars. Also, there have been no reports of using the visual peel color to identify the different ripening stage of Thai mangoes. Moreover, the combination studies on how cultivar type and ripening stage affect the microstructure and textural quality of frozen mangoes are still not available. To improve the high quality frozen mangoes, the knowledge of suitable selection ripening stages of raw material and the changes that will occur during freezing process is required. Thus, the experiments in this research were divided into two parts, Part I: Physicochemical and microstructural changes of Thai mangoes during ripening and Part II: Influence of cultivar and ripening stage on microstructure and quality of frozen mangoes.

MATERIALS AND METHODS

Materials

1. Raw material

Three mango (*Mangifera indica* L.) cultivars were used in this experiment. Maha Chanok mangoes were purchased from a commercial orchard in Nakhon Pathom province, Nam Dok Mai #4 mangoes were purchased from a commercial orchard in Nakorn Ratchasima province and Chok Anan mangoes were purchased from a commercial orchard in Suphan Buri province during the fruit season from March to June 2010 and 2011. In addition, only Nam Dok Mai #4 fruits were bagged with brown paper bags during fruit developing.

2. Chemicals

2.1 Sodium Hydroxide (Analytical grade, Merck, Germany)
2.2 Potassium hydrogen phthalate (Analytical grade, Fisher Scientific, UK)
2.3 Phenolphthalein (Ajax Finechem, Australia)
2.4 Ethanol (Analytical grade, Merck, Germany)
2.5 Sulfuric acid (Analytical grade, Merck, Germany)
2.6 Acetone (Analytical grade, Merck, Germany)
2.7 D-(+)-Galacturonic acid (Analytical grade, Sigma-Aldrich, Slovakia)
2.8 Sodium tetraborate (Analytical grade, Ajax Finechem, Australia)
2.9 3-phenyl-phenol 85% (Analytical grade, Sigma-Aldrich, Germany)
2.10 Sodium Chloride (Food grade, Prung Thip, Thailand)

3. Apparatus

3.1 Light microscopy (Leica DME, Leica Microsystems Inc., New York, USA)

3.2 Transmission electron microscopy (JEOL−JEM−1220 TEM, Tokyo, Japan)

3.3 Water bath (model WB 22, Memmert GmbH and CO. KG, Schwabach, Germany)

3.4 Pocket digital refractometer (model PAL-1, Atogo, Tokyo, Japan)

3.5 Low temperature incubator (model IPP 400, Memmert GmbH and CO. KG, Schwabach, Germany)

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

3.7 Chest freezer (Sanyo refrigerator, model SF-C1497, Sanyo electric Co., Ltd, Osaka, Japan)

3.8 Refrigerator centrifuge (model RC-5C Plus, Sorvall, USA)

3.9 UltraScan XE (HunterLab, Reston, VA, USA)

3.10 UV-visible spectrophotometer (Genesys 10 UV Biomate 3, USA)

3.11 Texture Analyzer (TA.XT. plus, Stable Micro Systems, UK)

3.12 Commercial blender (model HGB2WT, Waring, USA)

3.13 Magnetic stirrer with heating (model C-MAG HS7, Ika, Germany)

3.14 Digital camera (Canon, IXUS 86015, Japan)

3.15 Vortex (model G-560E, Scientific Industries, Inc., USA)

3.16 Hot air oven (model ULE 500, Memmert GmbH and CO. KG, Schwabach, Germany)

3.17 Balance 2 decimal (model BP 3100S, Sartorius, Germany)

3.18 Balance 4 decimal (model AP 210-0, Ohaus, Switzerland)

3.19 Thermocouple and data-logger

3.20 Nylon/Linear Low Density Polyethylene pouch (thickness 70 µm, size 100 x 150 mm.)

3.21 Hand operated impulse sealer (PFS-300, DB, Taiwan)

3.22 Glassware

Methods

Part I: Physicochemical and microstructural changes of mangoes during ripening

1. Fruit selection

Three mango (*Mangifera indica* L.) cultivars Maha Chanok, Nam Dok mai #4, and Chok Anan were assessed in the present study. The fruits were selected for uniformity of size and color. The uniform maturity index was selected by floatation of the mangoes in NaCl solutions (Kasantikul *et al*. 1984). The immature mangoes floated in water, while the mature mangoes sank in water. Only mature green mangoes which sank in 1-2% NaCl solution and floated in 3% NaCl solution were selected for ripening experiment.

2. Ripening process

Mango fruits were placed in a plastic container which was double wrapped with newspaper (Appendix Figure A9). The size of plastic container was 32 x 52 x 32 cm^3, which is commercially used for transportation and ripening process of various fruits. The fruits weight of approximately 20 kg per container was allowed to ripen naturally at room temperature (32 ± 2 °C). The characteristics of mango fruit were observed after 0, 2, 3, 4, 5, and 7 days of ripening and 10-15 fruits were randomly sampled.

The ripening stage was categorized into 6 stages by visual skin color, using the color index shown below.

Ripening stage	1	(100% green) Mature green
	2	(1-30% yellow) Unripe
	3	(31-60% yellow) Partially ripe
	4	(61-99% yellow) Ripe
	5	(100% yellow) Fully ripe
	6	Over ripe

Peel and mesocarp images of the six ripening stages were taken using a digital camera (Canon, IXUS 86015, Japan) without flash light.

3. Sample preparation

Mangoes were washed, then peeled and de-seeded. To minimize any variations within the sample, only the central parts (60%) of the fruits were used to analyze. Approximately twenty percent from both the stem and the blossom end of the fruit were discarded, as those parts were highly diverse in terms of fruit properties.

4. Physicochemical properties

4.1 Color measurement

The color was measured at the surface of peel and mesocarp of mangoes in terms of L* (lightness), a* (+ redness and – greenness) and b* (+ yellowness and – blueness) using an UltraScan XE (HunterLab, Reston, VA, USA) based on the CIELAB color system. L*, a* and b* readings were taken at three locations on each fruit and averaged for the fruit.

4.2 Total soluble solids (TSS)

Mango flesh was homogenized and the puree was filtered through cheesecloth. Total soluble solids (°Brix) of the filtrate was measured by using a pocket digital refractometer (Type PAL-1, Atago, Tokyo, Japan).

4.3 Titratable acidity was determined according to the AOAC methods (AOAC, 1995). The details are shown in Appendix B1.

4.4 Moisture content was determined according to the AOAC methods (AOAC, 2000). The details are shown in Appendix B2.

4.5 Textural analysis

Samples were cut into cubes of 1.5 x 1.5 x 1.5 cm. Firmness of the samples was measured by a compression test using a Texture Analyzer (TA.XT. plus, Stable Micro Systems, UK) with a cylindrical probe of 36 mm diameter and 50% strain. The speed of pre-test, test, and post-test were 1.5, 1.5, and 10 mm/s, respectively (Sirijariyawat and Charoenrein, 2012). Firmness was determined by mean of the maximum peak force. At least twelve samples were analyzed for each treatment.

4.6 Cell wall extraction and cell wall fraction

4.6.1 Alcohol-insoluble residue

The alcohol-insoluble residue (AIR) was extracted from samples as described by McFeeters and Armstrong (1984). Thirty grams of fruit tissue was homogenized with 150 mL of 95% ethanol for 2 min. The insoluble material was filtered through a Whatman no.1 filter paper and sequentially washed with 75 mL of 70% ethanol and 100 mL of acetone, yielding the crude cell wall extract (AIR). The

AIR was dried in a hot air oven 40 °C overnight and weighed. All measurements were done in triplicate.

4.6.2 Total pectin content

Ten milligrams of AIR was weighed into a beaker containing a magnetic stirring bar. The volume of 4 mL of chilled concentrated sulfuric acid was added and swirled gently. The beaker was placed in a water-ice bath. The sample was stirred gently during approximate 1 mL of distilled water was added and then stirred for 5 min. The procedure was repeated once again and subsequent transferred the soluble sample to a 50 mL volumetric flask. The beaker was rinsed well with distilled water and then adjusted the volume to 50 mL (Ahmed and Labavitch, 1978). The sample was filtered through a Whatman no.1 filter paper. The filtrate was designated the total pectin and then assayed colorimetrically using m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). The volume of 4.8 mL of 0.0125 M sodium tetraborate in sulfuric acid solution was added to 0.8 mL of filtrate sample. The tube was placed in water-ice bath. The sample was shaken and then heated in a water bath at 95 °C for 5 min. Sample was subsequent cooled in water-ice bath. The volume of 80 µL of the m-hydroxydiphenyl reagent was added to the sample (the blank prepared with 80 µL of 0.5% NaOH). The sample was shaken and assayed colorimetrically for the total pectin. Absorbance readings were evaluated against a standard curve of galacturonic acid at 520 nm wavelength (Appendix B3). The results were expressed as micrograms of galacturonic acid per milligram of AIR.

4.6.3 Water-soluble pectin content

Water-soluble pectin (WSP) was extracted from AIR (Majumder and Mazumdar, 2002). Approximately 50 mg of AIR from each sample was suspended in 25 mL of water and stirred at room temperature for 30 min. The samples were then centrifuged at 7000 xg for 15 min at 4 °C, the supernatant was then filtered through Whatman no.1 filter. The procedure was repeated three times. The filtrate was designated the water-soluble fraction and assayed colorimetrically for water-

soluble pectin as method described above (Blumenkrantz and Asboe-Hansen, 1973). The results were expressed as micrograms of galacturonic acid per milligram of AIR.

5. Microstructural study

For light and transmission electron microscopy observations, sections were prepared at the Scientific Equipment and Research Division, Kasetsart University Research and Development Institute (KURDI).

Sections of mango mesocarp (1 x 1 x 2 mm) were obtained from the inner part of the fruit at a distance of ~1.5 cm from the center of the seed with a razor blade. The samples were prepared according to Ben-Arie et al. (1979). The samples were prefixed with 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2) at 4 °C overnight. Subsequent to washing with a 0.1 M phosphate buffer, the samples were then postfixed with 2% osmium tetroxide for 2 h at room temperature. The fixed tissues were washed three times with distilled water. Then the tissues were dehydrated in graded ethanol starting with 30%, 50%, 70%, 90% and 100% for 10 minutes each. End with 1 rinse in 100% ethanol for 10 minutes. After that the dehydrated tissues were rinsed in propylene oxide, two times for 10 minutes each. The tissues were infiltrated with propylene oxide / Spurr's resin (2:1, 1:1, and 1:2, respectively), overnight each on a rotator. Then they were infiltrated three times with 100% Spurr's, overnight each. The tissues were polymerized in Spurr's resin at 80 °C for 7 h.

Sections of a thickness of 1.5 µm were cut with an ultramicrotome (Leica Ultracut UCT, Vienna, Austria) and subsequently stained with 1% toluidine blue for observation under a light microscope (Leica DME, Leica Microsystems Inc., New York, USA). Five specimens from each ripening stage of all cultivars were examined. At least 500 cells of each sample were viewed under a light microscope. Mean cell size in length and diameter were measured on 50 cells of partially ripe mango in both cultivars.

For the analysis with the transmission electron microscope, ultra-thin sections (60 nm) were obtained using an ultramicrotome, stained with uranyl acetate and lead citrate, and examined using a JEOL−JEM−1220 TEM (Tokyo, Japan) at 80 kV. Six cells were randomly chosen to measure the thickness of cell walls for each ripening stage of all cultivars. The thickness of cell walls was measured from the outer part of cell to cell using three points. The average thickness was calculated as the average value of three points measurement divided by two.

Part II: Influence of cultivar and ripening stage on microstructure and quality of frozen mangoes

1. Sample preparation

In previous study (Part I), during ripening, mango was categorized into 6 ripening stages based on the peel color. Physicochemical and microstructural changes during ripening were investigated. In this part, the three mango cultivars with three different ripening stages (stages 3, 4, and 5) were selected to frozen. This selection was mainly based on flavor, taste and texture acceptance of fresh mango. Mangoes were washed, peeled, de-seed and cut into cubes of 1.5 cm in length. Approximately twenty percent from both the stem and the blossom end of the fruit were discarded, since those parts tend to be highly diverse in terms of fruit properties. Only the central parts of the fruits were used to minimize any variations within the samples.

2. Freezing and thawing

Eight cubes of sample were then packed and sealed in each plastic pouches (NY/LLDPE, 70 μm, 100 × 150 mm). The weight of samples in each pouch was 28 – 30 g. The initial temperature of the samples was controlled at 23 ± 2 °C. The samples were then frozen at – 40 °C in a cryogenic freezer (Minibatch 1000L, Bangkok Industrial Gas Co., Thailand) until the temperature of the samples reached – 25 °C. The freezing curve of frozen mango at – 40 °C is shown in Appendix Figure C1. In this study, the freezing time of frozen mango was 30 minutes and the freezing rate

was approximately 1.5 °C/min. Frozen mangoes were kept at – 20 ± 2 °C in a chest freezer (Sanyo refrigerator, model SF-C1497, Sanyo electric Co., Ltd, Osaka, Japan) for 15 days. Thawing was performed at 4 °C after 5 h in a low-temperature incubator (Memmert, model IPP 400, Germany). The experiments were done in two trials.

3. Drip loss

Drip loss was determined by a method adapted from Lowithun and Charoenrein (2009). The details are shown in Appendix B4.

4. Textural analysis

The firmness of the frozen-thawed samples was measured using a compression test Texture Analyzer (TA.XT. plus, Stable Micro Systems, UK) with a cylindrical probe of 36 mm diameter and 50% strain. The speed of the pre-test, test, and post-test were 1.5, 1.5, and 10 mm/s, respectively. Firmness was determined by the maximum peak force. At least twelve samples were analyzed for each treatment.

5. Cell wall extraction and cell wall fraction

The alcohol-insoluble residue (AIR) prepared directly from the fresh and frozen tissues, without thawing. Thirty grams of samples were extracted using 95% ethanol, 70% ethanol and acetone, respectively. The AIR was dried overnight at 40 °C and weighed. AIR was then assayed colorimetrically for total pectin and water-soluble pectin using method adapted from Blumenkrantz and Asboe-Hansen (1973). The details of the method described in previous section (Part I).

6. Microstructure

For light and transmission electron microscopy observations, frozen samples were immediately cut into 1 x 1 x 2 mm samples. The samples were prefixed with 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2) at 4 °C overnight. The details of the method described in previous section (Part I).

7. Sensory evaluation

7.1 Firmness intensity score was evaluated using a ranking test on a five-point scale (extremely soft-extremely firm) by 8 trained panelists. The details of training and score sheets are shown in Appendix D.

7.1 Sensory attributes were evaluated using a preference score (appearance, color, flavor, taste, texture and overall acceptability) on a nine-point hedonic scale (dislike extremely - like extremely) by 30 untrained panelists. The panelists were 10 male and 20 female aged between 20 and 55 years. Frozen-thawed mango samples were served to each panelist in balanced and random order at room temperature.

8. Statistical analysis

A completely randomized design was used in this experiment. Analysis of variance (ANOVA) and Duncan's new multiple range test (DMRT's test) were used to determine statistically significant differences ($P \leq 0.05$) of the mean values.

Place and Duration

1. Place

The Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University (Bangkhen campus), Thailand.

The Scientific Equipment and Research Division, Kasetsart University Research and Development Institute (KURDI).

2. Duration

From March 2009 to June 2011

RESULTS AND DISCUSSION

Part I: Physicochemical and microstructural changes of mangoes during ripening

To characterize physicochemical properties changes during mango ripening, ripening index is required to define the stage of ripening. Until now, there have not been any reports on the ripening index of Thai mangoes which based on a visual peel color. Thus, in this part we attempted to identify the ripening stage and investigate the changes in peel and mesocarp color, total soluble solids, titratable acidity, firmness, cell wall material and cell wall fraction, and microstructure during the ripening. The hypotheses of this part were (1) the different ripening stages might alter their physicochemical properties and microstructure of ripening mango and (2) loss in fruit firmness during ripening might be affected by the changes in cell wall material, cell wall fraction and cell structure.

1. Fruit color development during ripening

The fruits weight of approximately 20 kg per container was allowed to ripen naturally at room temperature (32 ± 2 °C). The ripening stage was categorized into six stages which based on the visual appearance of peel surface color of mango. The peel color varies during ripening and this trend can be used to estimate the state of ripeness. The six ripening stages are shown in Figure 10. Peel and mesocarp images of the six ripening stages were taken using a digital camera (Canon, IXUS 86015, Japan). After harvesting, the peel of mature mangoes appeared to show green color at stage 1. During ripening of all mango fruits harvested at stage 1, the color of the fruits developed rapidly from green to yellow/yellow-orange at stage 6 within 7 days.

The mangoes turned a lighter green to yellow color at stage 2 as it begins to ripen. The ripening mangoes at stage 3 appeared to show about half green and half yellow. As the ripening process continues, the mangoes turned to have more yellow at

stage 4. At this stage the fruit is ready for retail display. In addition, mangoes had turned completely yellow at stage 5 which is the fully ripe stage. The slight wrinkling of the skin were observed at stage 6 which is the over ripe stage. However, only the Maha Chanok cultivar appeared to show a yellow-orange color development. The skin color of all cultivars developed from stage 5 to 6 slower than between the other stages.

The mesocarp color of all cultivars changed noticeably throughout ripening (Figure 11 and). In both, the Nam Dok Mai and Chok Anan cultivars, the mesocarp color changed from whitish green to yellow-orange. In addition, for the Maha Chanok cultivar, the color changed from whitish yellow to orange. Color development during fruit ripening is accompanied by carotenoid biosynthesis. Previously study, Vásquez-Caicedo *et al.* (2004) observed the β-carotene content to highly correlate with the redness in fully ripe mesocarp of nine mango cultivars.

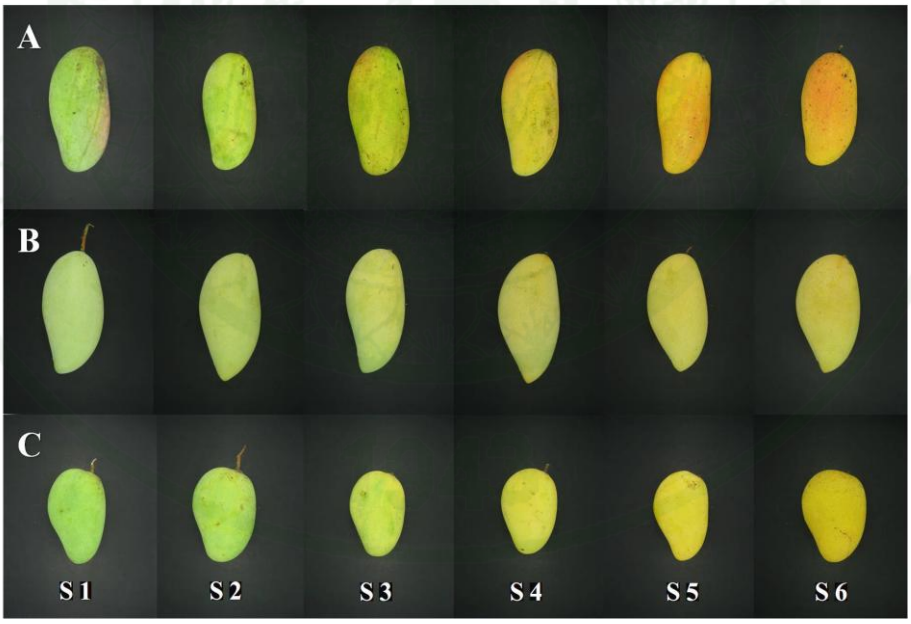

Figure 10 Changes in peel color of three mango cultivars during ripening. (A) Maha Chanok, (B) Nam Dok Mai, and (C) Chok Anan. S = the ripening stage of mango. Fruits were harvested at stage 1 and allowed to ripen to stage 6.

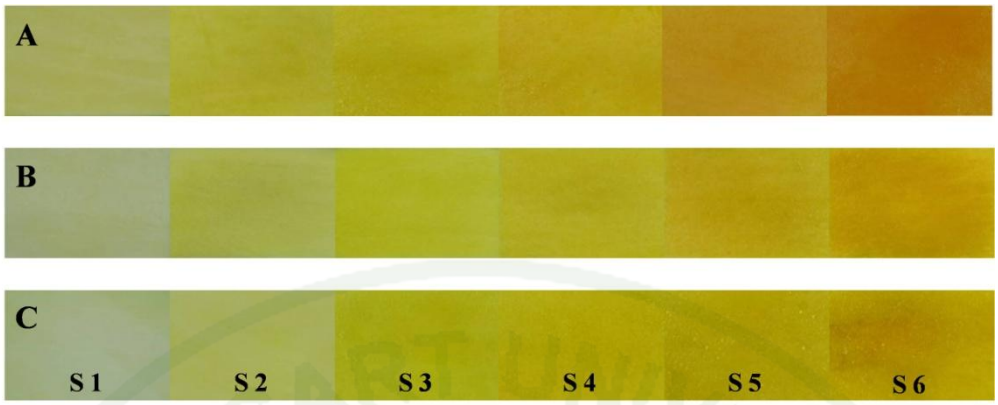

Figure 11 Changes in mesocarp color of three mango cultivars during ripening. (A) Maha Chanok, (B) Nam Dok Mai, and (C) Chok Anan. S = the ripening stage of mango. Fruits were harvested at stage 1 and allowed to ripen to stage 6.

Color changes in peel and mesocarp are presented as L*, a*, and b* values (Figure 12, 13 and Appendix Table C3, C4). L* value represents lightness, a* value represents redness, and b* value represents yellowness. During the peel color development of all cultivars, the a* and b* values were found to increase sharply from stages 1 to 4, and then to increase slightly to stage 6. However, the L* value was revealed to increase through the first two stages of ripening, with no increase afterward. The highest peel a* values were found in Maha Chanok, Chok Anan, and Nam Dok Mai, respectively, whereas the peel b* value of Nam Dok Mai was similar at all ripening stages but lower than that of the other cultivars.

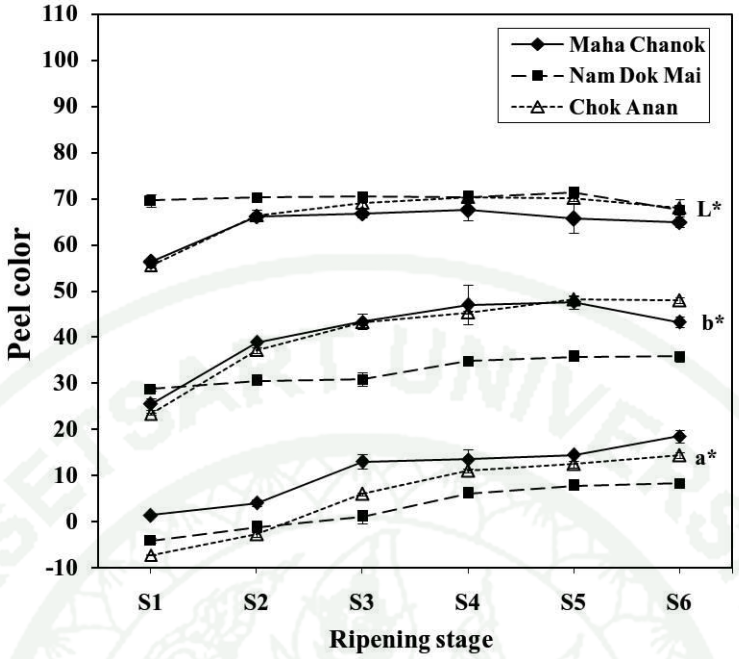

Figure 12 Changes in peel L*, a*, and b* values during fruits ripening at room temperature. Increase in the a* values indicate changes from green to red. Increase in b* values indicate changes from blue to yellow. L* values represent the lightness, 0 denotes black and 100 denotes white. The standard deviations are shown.

The colors of mesocarp of all mango cultivars are shown in Figure 13 and Appendix Table C4. For all cultivars, the L* value was found to decrease slightly during ripening while the a* and b* values increased. There was a gradual increase in the a* value as from stage 1, while the b* value revealed a rapid increase from stages 1 to 3, and a slight increase afterward. These results indicated that the mango mesocarp potentially developed a full yellow color during the early stages of ripening, however continued to accumulate red pigments (carotenoids) until the full ripe stage is attained, as indicated by its deep orange colored mesocarp (Medlicott *et al.*, 1986). The increases in the a* and b* values appeared to correlate well with the color development (Figure 10, 11).

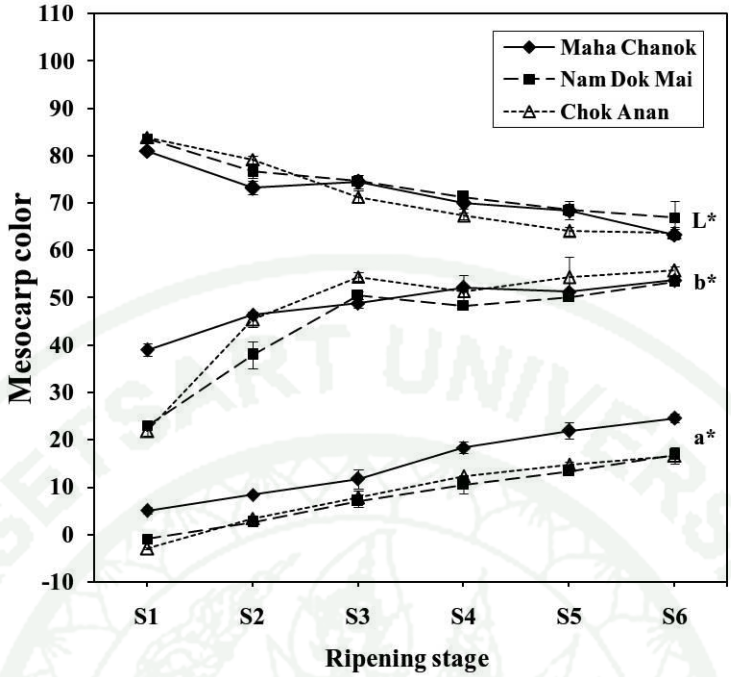

Figure 13 Changes in mesocarp L*, a*, and b* values during fruits ripening at room temperature. Increase in the a* values indicate changes from green to red. Increase in b* values indicate changes from blue to yellow. L* values represent the lightness, 0 denotes black and 100 denotes white. The standard deviations are shown.

2. Total soluble solids (TSS) and titratable acidity (TA)

The acidity was established to decrease more rapidly than the TSS increase during fruit ripening (Figure 14 and Appendix Table C1). The amount of TA was revealed to decrease significantly ($P \leq 0.05$) from approximate 2.90 to 0.20%, 2.70 to 0.10%, and 2.60 to 0.20% for Maha Chanok, Nam Dok Mai, and Chok Anan, respectively. The decrease in TA with ripeness is likely due to their utilization as substrates for respiration (Medlicott and Thompson, 1985). TSS was found to increase strongly within the stage 2 of ripening from about 8 to 15 °Brix for all mango cultivars with a subsequent slight increase to 18-20 °Brix. Brix degree is used to measure sugar, organic acid, and other components in the juice of fruit (Linskens and Jackson, 1995).

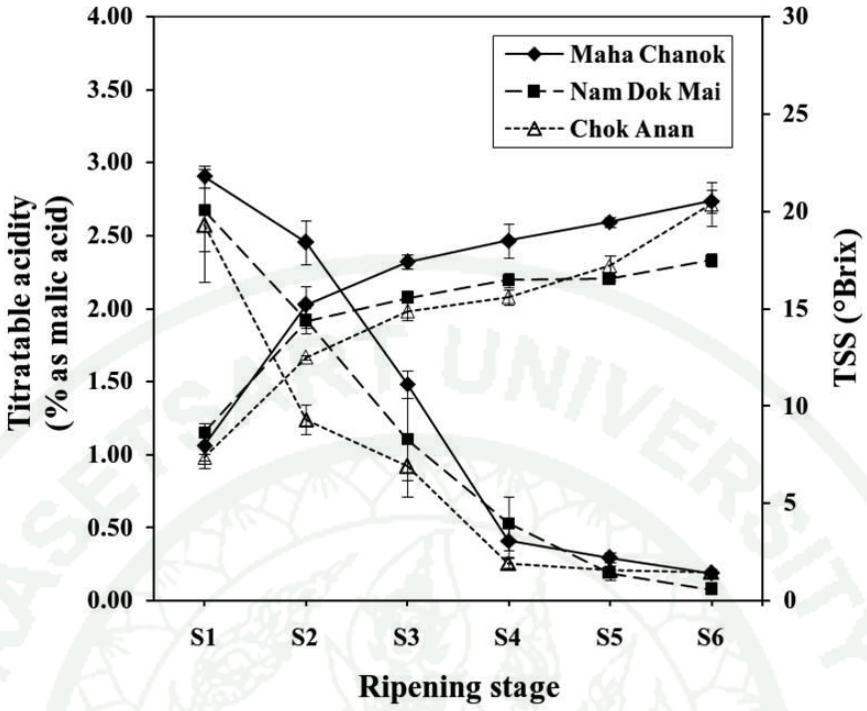

Figure 14 Changes in titratable acidity and total soluble solids (TSS) during ripening of three mango cultivars with the standard deviations are shown.

3. Firmness

The ripening process was found to be associated with the loss of fruit firmness, decrease in TA, as well as increase in TSS. For all cultivars, fruit firmness was found to decrease sharply from the initial stage to stage 3 followed by a slight decrease (Figure 15 and Appendix Table C1). In addition, loss of firmness in Maha Chanok and Chok Anan cultivars was found to be lower than that in Nam Dok Mai cultivar. However, irrespective of the mango cultivars, the firmness was found to be similar at stage 4 of the ripening process. Previously, Jarimopas and Kitthawee (2007) also found that Chok Anan cultivar was firmer than Nam Dok Mai throughout the development period. Although fruit firmness can be affected by several factors, fruit softening is probably caused by the cumulative effect of modifications occurring in the primary cell wall, all of which contribute in diverse ways to a loss of firmness and changes in the textural qualities (Payasi *et al*., 2009).

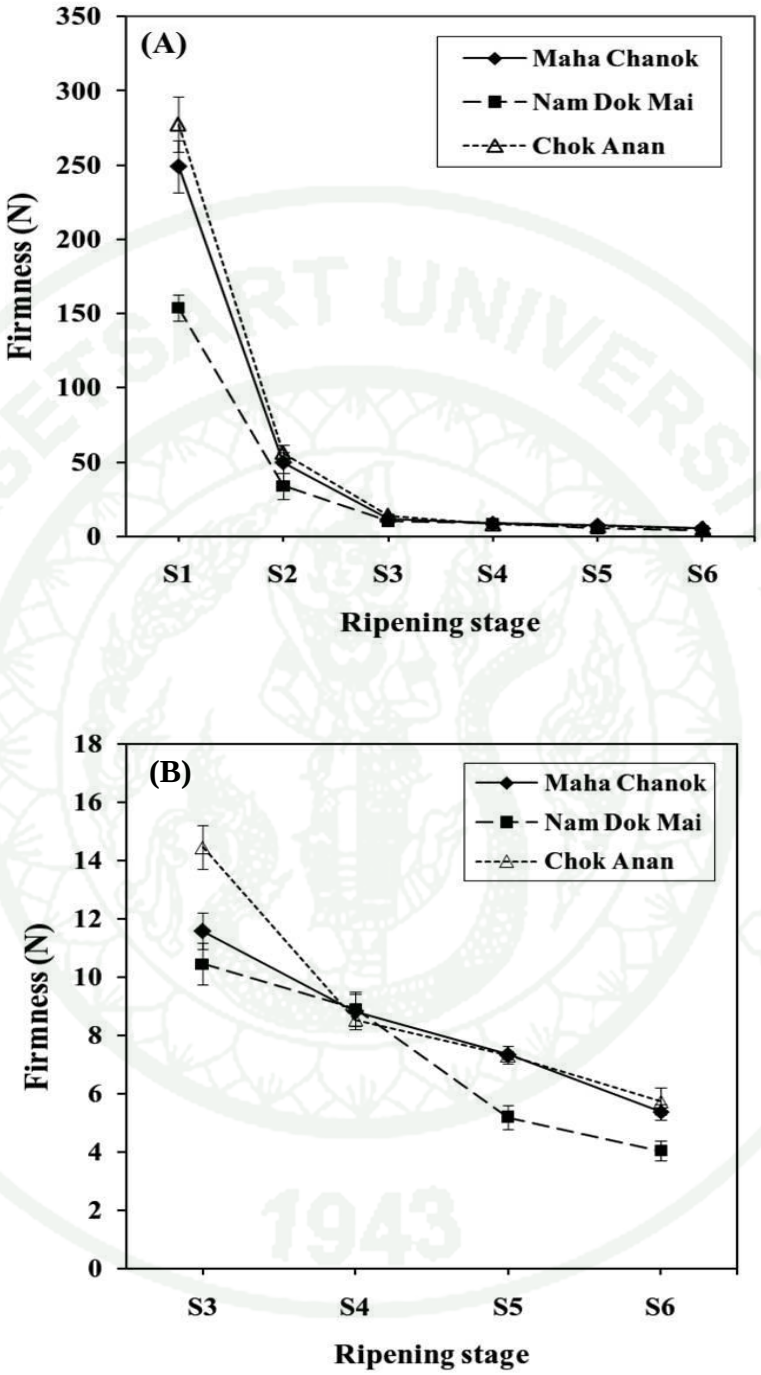

Figure 15 Changes in mango fruits firmness of three different cultivars measured during ripening. (A) fruits firmness of stage 1 to 6 and (B) the magnification of firmness from stage 3 to 6. The standard deviations are shown.

4. Cell wall material and fraction

To determine cell wall changes which occur in ripening mangoes, quantitative analysis of the fractionated cell wall material was performed. The data of cell wall material and fraction are shown in Appendix Table C2. The amount of AIR was shown to decrease markedly from stage 1 to stage 6 (Figure 16A), from 708.00 to 92.40, 513.01 to 90.93, and 640.32 to 117.43 mg/g dry basis of Maha Chanok, Nam Dok Mai, and Chok Anan, respectively. The changes in AIR are a potential indication for the degradation of large alcohol-insoluble polymers to shorter alcohol-soluble polymers during fruit ripening (Yashoda *et al*., 2006). In addition, the decrease in AIR is probably due to the conversion of starch to soluble sugars and the conversion of fiber to alcohol-soluble solids (El-Zoghbi, 1994). In the present study, the softening of mango fruits was found to be accompanied by a rapid decrease in AIR during ripening. Moreover, Maha Chanok and Chok Anan appeared to have greater firmness than that of Nam Dok Mai, which may due to the greater AIR content in both cultivars. An apparent decrease in the amount of AIR was also observed in diverse mango cultivars (Tandon and Kalra, 1983; Mitcham and McDonald, 1992; El-Zoghbi M, 1994; Yashoda *et al*., 2006). Similarly, a recent study on blueberries revealed fruit firmness and AIR to decrease as ripening progresses (Vicente *et al*., 2007b).

Total pectin was revealed to increase remarkably from stage 1 to 5 and then decline for both Maha Chanok and Nam Dok Mai cultivars (Figure 16B). However, total pectin was shown to increase noticeably from the beginning stage to stage 3 and then remain rather constant for Chok Anan cultivar. The increase in pectin content during the ripening process was a consequence of the generation of a higher proportion of free carboxylic groups due to de-esterification of pectins (Yashoda *et al*., 2006). The amount of total pectin at the end of the ripening stage in Chok Anan was proven to be higher than that of Maha Chanok and Nam Dok Mai, respectively.

Levels of WSP fraction in all cultivars were found to increase progressively through the different ripening stages (Figure 16C). In addition, all mango cultivars appeared to show a slight decrease in WSP subsequent to stage 5 as well as a decrease in total pectin content. The increase in WSP was also found in ripening Nam Dok Mai mangoes (Ketsa *et al*., 1999a). Yashoda *et al*. (2006) suggested that WSP had undergone drastic depolymerization during the fruit ripening, which contributed to tissue softening and textural changes. The higher amount of WSP was found in Chok Anan and Nam Dok Mai, while Maha Chanok was shown to have a lower WSP. In contrast, Maha Chanok and Chok Anan were proven to retain a significantly greater firmness ($P \leq 0.05$) than Nam Dok Mai at all ripening stages, except for stage 4. Such textural differences among the cultivars are likely the result of dissimilar cell wall compositions and a greater amount of the AIR in both cultivars rather than with the quantity of pectin present in the fruit.

The correlations between firmness values and peel L*, a*, b*, firmness values and mesocarp L*, a*, b*, firmness value and %TSS, firmness value and titratable acidity, firmness value and AIR, firmness value and total pectin, firmness value and WSP content of fresh mangoes during ripening were analyzed. The coefficient of correlation is shown in Appendix Figure C2-C4. Firmness was highly positively correlated to titratable acidity (r = 0.802) and AIR content (r = 0.982). Moreover, firmness was highly negatively correlated to peel L* (r = – 0.815), mesocarp b* (r = – 0.838) and %TSS (r = – 0.889).

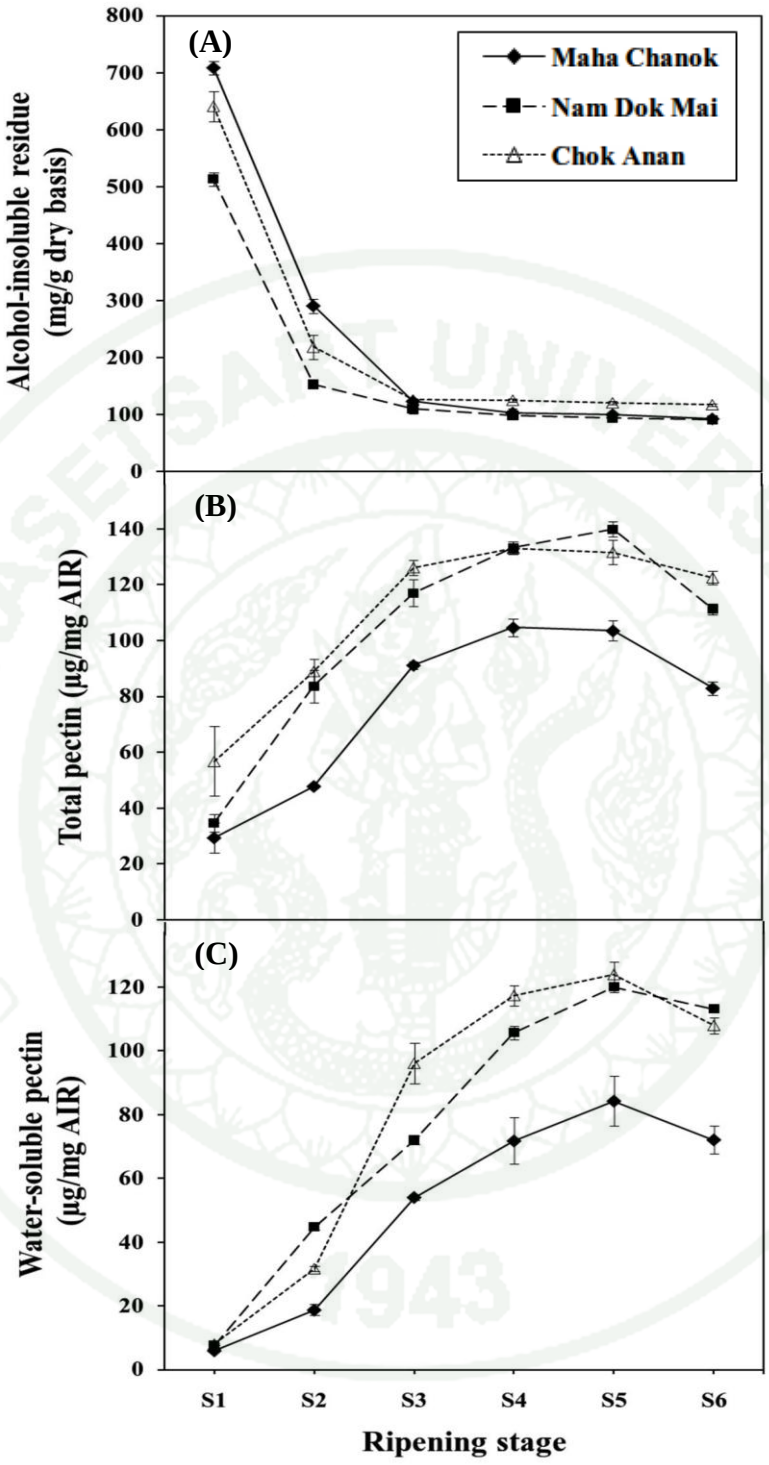

Figure 16 Changes in cell wall material and cell wall fractions during mangoes ripening. (A) alcohol-insoluble residue; AIR, (B) total pectin, and (C) water-soluble pectin; WSP. The standard deviations are shown.

4. Microstructure

The microstructure of fresh mesocarp tissues was observed with both LM and TEM. The LM observation revealed that at stage 3 of the ripening process, all mango cultivars appeared to have the initially round shaped cells as shown in Figure 17 and Figure 18 (A-1, B-1 and C-1). Parenchyma cells with a more irregular shape and with a loss of cell-to-cell contact were observed at progressed ripening stages (stage 4 and 5), which represented an increase in the intercellular area with evident cell separation as shown in Figure 17 and Figure 18 (A-2, B-2, C-2 and A-3, B-3, C-3). Moreover, all mango cultivars at the ripening stage 5 appeared to show extensive cell wall swelling and wall surfaces became partially wavy compared to the other stages. The surfaces of the cell walls became wavy possibly due to localized swelling. These results correlated well with a loss in fruit firmness as shown in Figure 15B. It suggested that a decrease in mango firmness when the ripening stage increased was accompanied by the changes in parenchyma cells of mango fruit. With the development of the ripening stage, fruits were found to exhibit progressively more cells of irregular shapes and larger intercellular areas (Redgwell et al., 1997; Nunes et al., 2009). Similar examination have previously shown considerable morphological changes in mango as a result of ripening, the more compact and rigid cell wall appeared to lose their structure and expand at the end of ripening (Yashoda et al., 2006). The structural changes which occurred in the middle lamella and primary cell wall during ripening lead to cell separation and softening of the tissues (Carrillo-lopez et al., 2002). Besides, the values of firmness and rigidity of fruits have previously been associated with the cell wall strength, cell wall adhesion, and the cell turgor (Heyes and Sealey, 1996).

Corresponding, the present study observed a decrease in firmness of all cultivars throughout the ripening stages. However, Maha Chanok and Chok Anan were shown to retain a significantly higher firmness value than that of the Nam Dok Mai cultivar ($P \leq 0.05$). These findings are likely due to the smaller cell sizes and more compact cells of both cultivars. The mean cell sizes of Maha Chanok were 124±15 µm in length and 91±11 µm in diameter. The mean cell sizes of Nam Dok

Mai were 141±26 µm in length and 101±18 µm in diameter while the mean cell sizes of Chok Anan were 124±18 µm in length and 89±16 µm in diameter. The cell sizes were measured at the partially ripening stage in all cultivars. Maha Chanok and Chok Anan cultivar which had smaller cell sizes and cells that were relatively more compact than Nam Dok Mai cultivar, also showed a higher firmness value.

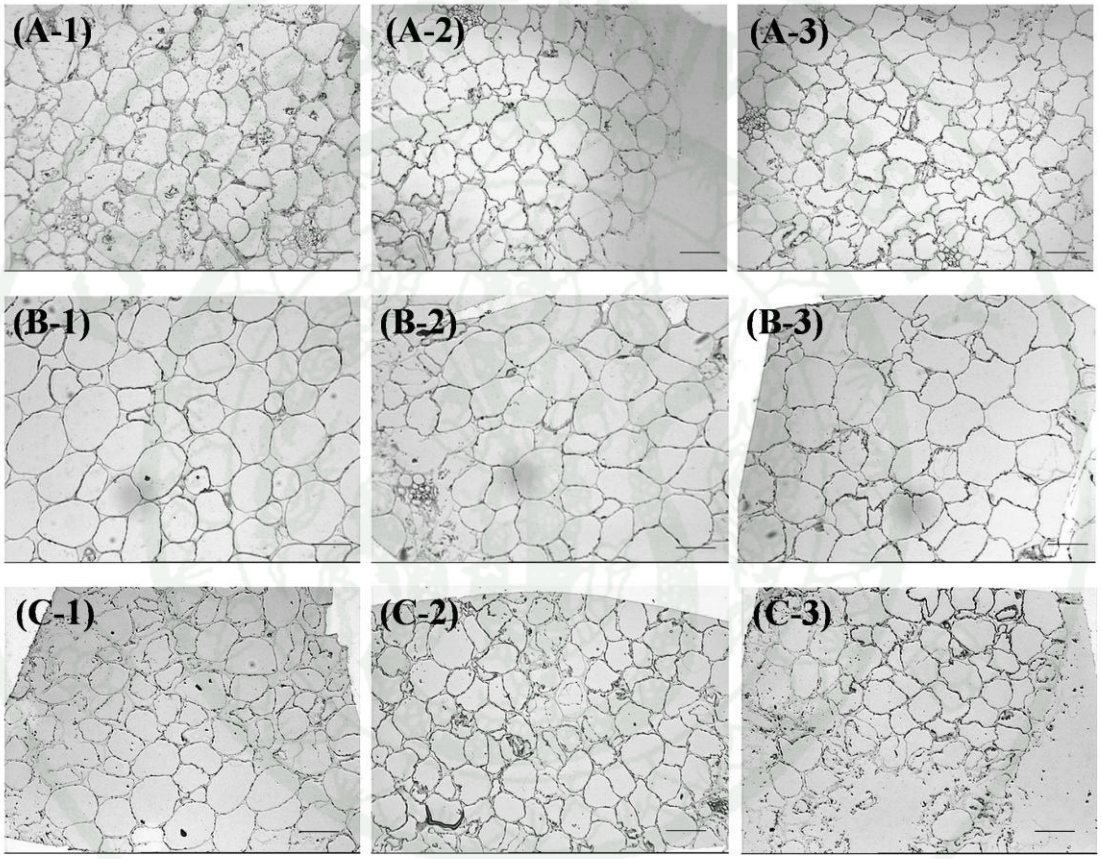

Figure 17 Microstructural changes during ripening of three mango cultivars observed with a light microscope. (A) Maha Chanok (B) Nam Dok Mai (C) Chok Anan. (1) ripening stage 3, (2) ripening stage 4, and (3) ripening stage 5. Bar = 100 µm.

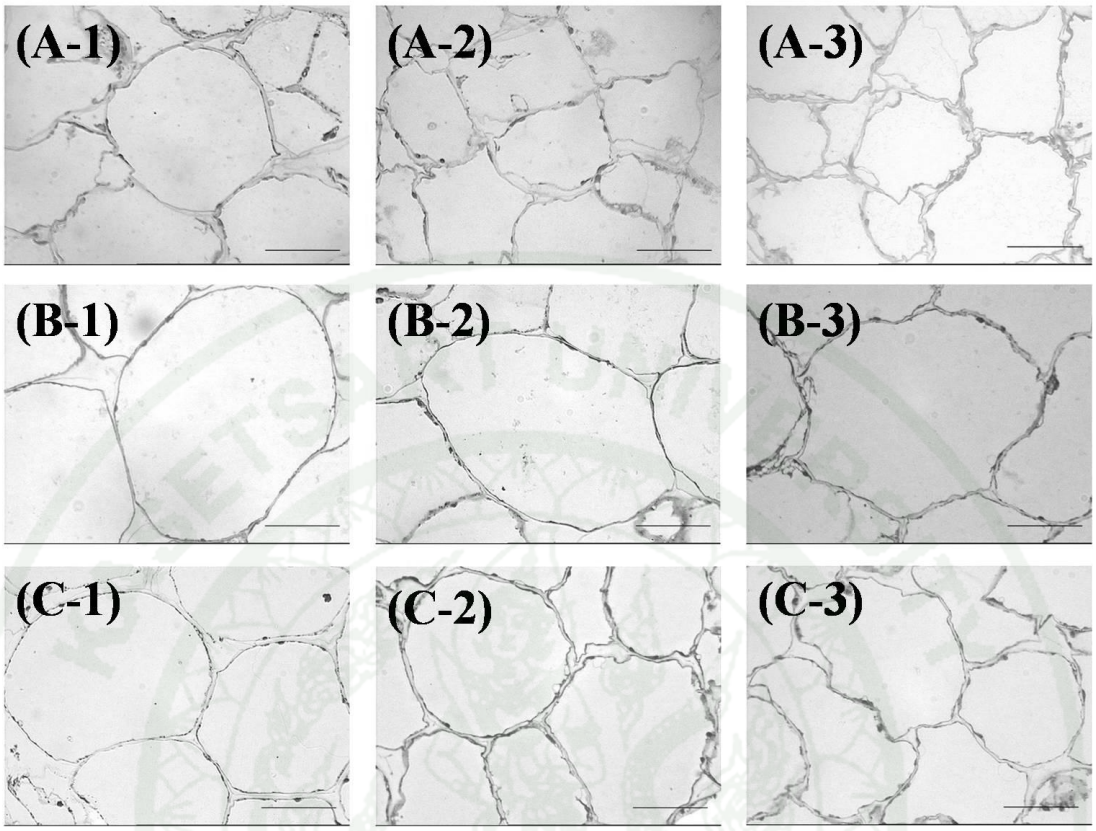

Figure 18 Microstructural changes during ripening of three mango cultivars observed with a light microscope. (A) Maha Chanok (B) Nam Dok Mai (C) Chok Anan. (1) ripening stage 3, (2) ripening stage 4, and (3) ripening stage 5. Bar = 50 µm.

The microstructural changes of the mangoes are evident in the LM images. Nevertheless, the micrographs of the TEM can be combined to describe subtle ultrastructural changes in cell walls and organelles. The TEM images of the fresh mesocarp samples are shown in Figure 19-21. The cell wall thickness of Nam Dok Mai, Maha Chanok and Chok Anan cultivar ranged from 0.7 to 1.4, 0.5 to 1.0 and 0.8 to 1.6 µm, respectively. TEM micrographs of Nam Dok Mai cultivar presented the thinner cell walls compared to the other cultivars. In parenchyma cells, the cell wall and middle lamella of all cultivars at stage 3 and 4 were found to be intact as evident in the electron-dense region between the primary cell walls of adjacent cells as presented in Figure 19-1, 19-2, Figure 20-1, 20-2 and Figure 21-1, 21-2. The dissolution of middle lamella occurred for all cultivars at stage 5 as shown in Figure

19-3, Figure 20-3 and Figure 21-3. The dissolution was likely related to an increase in WSP content during ripening progressed. A similar study reported that ultrastructural changes in the cell walls of apples and pears were appeared during ripening. Ben-Arie and Kislev (1979) observed that the structural alterations in apple cell walls became apparent at advanced stages of softening and showed pre-dominantly dissolution of the middle lamella. In pears, softening was also associated with the dissolution of the middle lamella.

Furthermore, in mango mesocarp tissues, chromoplasts was found to occur at all ripening stages studied (Figure 19-21). During mangoes ripening, numerous large plastoglobuli were presented in the chromoplasts of fruit mesophyll cells. It has been established that plastoglobuli are composed of a neutral lipid core surrounded by a monolayer of galactolipids (Smith et al., 2000). A previous study confirmed that carotenoids are mainly deposited in the plastoglobular substructures of the mango chromoplasts as well as abundance of pigment-carrying plastoglobuli in the chromoplasts (Vásquez-Caicedo et al., 2006). From the microstructure and textural results, it is clear that the loss in firmness during mango ripening can be attributed to changes in the middle lamella and cell wall.

The physicochemical properties of mango fruit have undergone rapid changes when the ripening stage increased. The loss of fruit firmness during ripening was accompanied by a decrease in AIR content and an increased in WSP content. Textural changes were also associated with the change in cell tissue and cell wall of mango fruit. The physicochemical properties and microstructural information of mango at different ripening stages obtained from this study can be used as a ripening index for the selection of suitable mango for high quality frozen fruit.

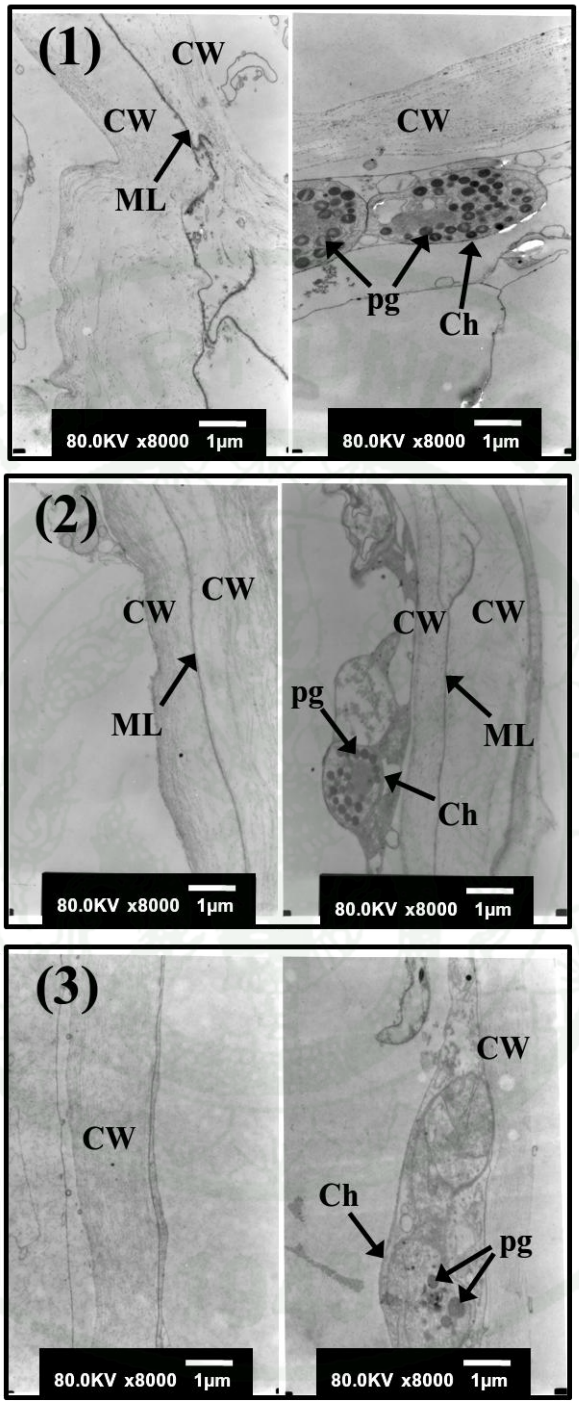

Figure 19 Transmission eletron micrograph of cell wall ultrastructure during ripening of Maha Chanok cultivars. (1) ripening stage 3, (2) ripening stage 4, and (3) ripening stage 5. CW = cell wall, ML = middle lamella, Ch = chromoplast and pg = plastoglobuli. The magnification of all micrographs is 8000x. Bar = 1 µm.

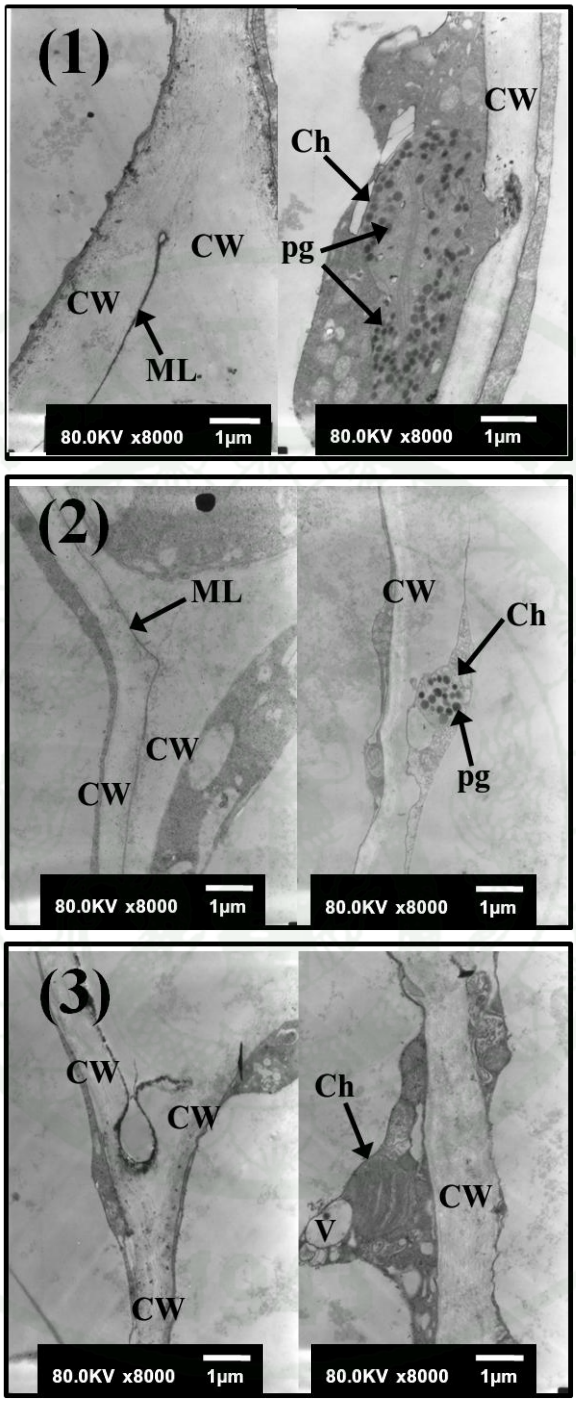

Figure 20 Transmission eletron micrograph of cell wall ultrastructure during ripening of Nam Dok Mai cultivars. (1) ripening stage 3, (2) ripening stage 4, and (3) ripening stage 5. CW = cell wall, ML = middle lamella, V = vacuole, Ch = chromoplast and pg = plastoglobuli. The magnification of all micrographs is 8000x. Bar = 1 µm.

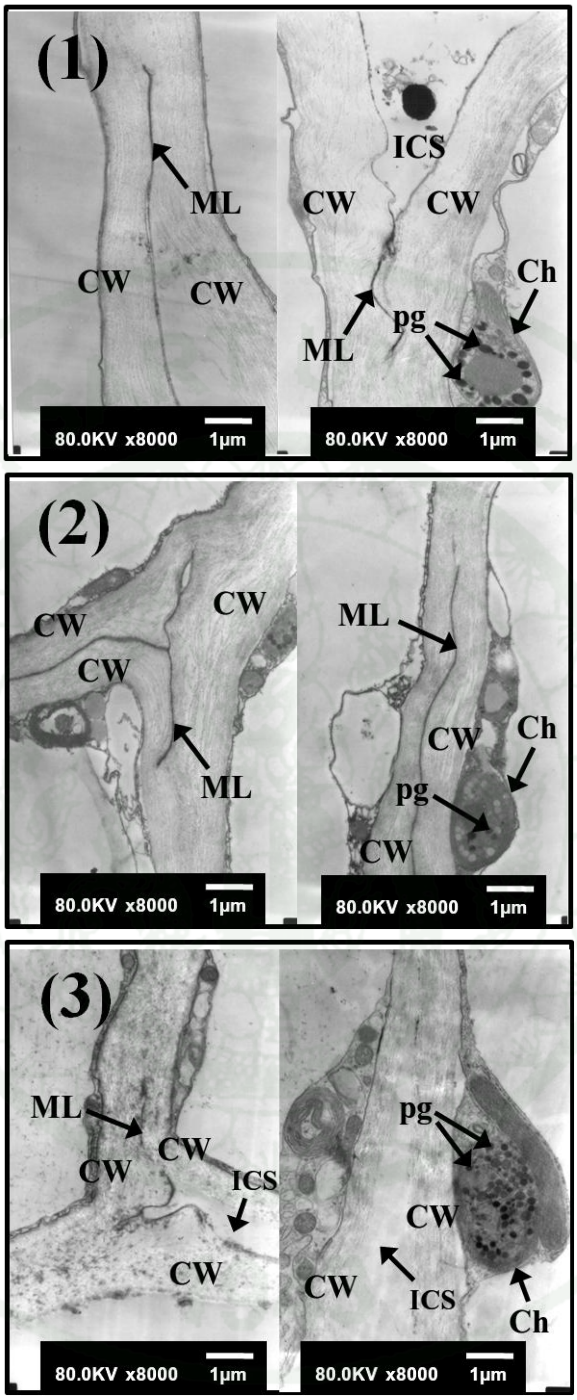

Figure 21 Transmission eletron micrograph of cell wall ultrastructure during ripening of Chok Anan cultivars. (1) ripening stage 3, (2) ripening stage 4, and (3) ripening stage 5. CW = cell wall, ML = middle lamella, ICS = intercellular space, Ch = chromoplast and pg = plastoglobuli. The magnification of all micrographs is 8000x. Bar = 1 µm.

Part II: Influence of cultivar and ripening stage on microstructure and quality of frozen mangoes

In the literature, freezing has been shown to induce significant changes in the texture of mango while there is currently no report on the optimal ripening stage of Thai mango for freezing process. To improve the high quality frozen mangoes, three mango cultivars and the three different ripening stages (stages 3, 4, and 5) of mango were selected to use in freezing process. This selection was mainly based on flavor, taste and texture acceptance of fresh mango. Mango at the earlier stage (stage 2) was not suitable for consumption due to the less mango flavor and high sourness while mango at the latest stage (stage 6) had very soft texture which was not suitable to process. The quality of frozen mango was evaluated by determining drip loss, firmness, cell wall material and fraction, and microstructure. Moreover, sensory evaluation was determined by trained and untrained panelists. The hypotheses of this part were (1) cultivar differences and differences in the ripening stage might affect the microstructure and quality of frozen mango and (2) the partially ripe mango at stage 3 might preserve the better texture quality of frozen-thawed mango than ripe mango at stage 4 or fully ripe mango at stage 5.

1. Quality of frozen mangoes

1.1 Drip loss

Drip loss is the index for measuring cell damage from freezing process (Skred, 1996). The drip loss of the frozen-thawed of all samples increased with increasing ripening stage (Figure 22 and Appendix Table C5). The frozen-thawed mangoes in all cultivars at ripening stage 5 were found to have the softest texture quality than that of the other stages, which likely contributed to the highest drip loss value. Similar results have also been found in the thawing process of thornless blackberries for which drip loss is more extensive in fully ripe than in slightly under-ripe fruit (Sapers *et al*., 1987). Wenzel *et al*. (1976) also reported that drip losses of thawing strawberries increased in riper fruits. Furthermore, Sistrunk (1963) observed

greater drip losses with over-ripe than with ripe strawberries, which he attributed to the release of pectic enzymes in the over-ripe fruit.

However, Nam Dok Mai cultivar was found to have a significant higher drip loss than Chok Anan and Maha Chanok, respectively, at all ripening stages. At the ripening stage 5, which is shown the highest drip loss, many of thawed mango pieces had collapsed, giving these samples an unattractive appearance. This result could be due to a severe tissue damages that occurred during freezing process. The higher moisture content in Nam Dok Mai cultivar may promote severe cell damages (Appendix Table C1). The extremely high drip loss about 30% of Nam Dok Mai cultivar can be more explained by microstructural study. The Nam Dok Mai at ripening stage 5 appeared to have more cell wall damage after freezing than Chok Anan samples. The cultivar may be an important factor involved in the drip loss after thawing, taking into account that size, shape, physicochemical characteristics and tissue structure varies according to mango.

1.2 Firmness

A texture measurement of the frozen-thawed mangoes showed that the firmness decreased with increasing ripening stage (Figure 23 and Appendix Table C5). The firmness value of all cultivars at ripening stage 5 was extremely low. These results suggest that the mango at ripening stage 3 (partially ripe) can maintain a greater firmness of frozen-thawed samples than the other ripening stages (stage 4, 5). Firmness values of frozen-thawed mango at ripening stage 3 and 4 were not significantly different ($P > 0.05$) between cultivars, while frozen-thawed Maha Chanok at ripening stage 5 was found to have the highest firmness. Firmness values of frozen-thawed samples were not significantly different ($P > 0.05$) between Nam Dok Mai and Chok Anan cultivar at ripening stage 5. Although the firmness values were similar between the cultivars, the drip losses were significantly differrent ($P \leq 0.05$).

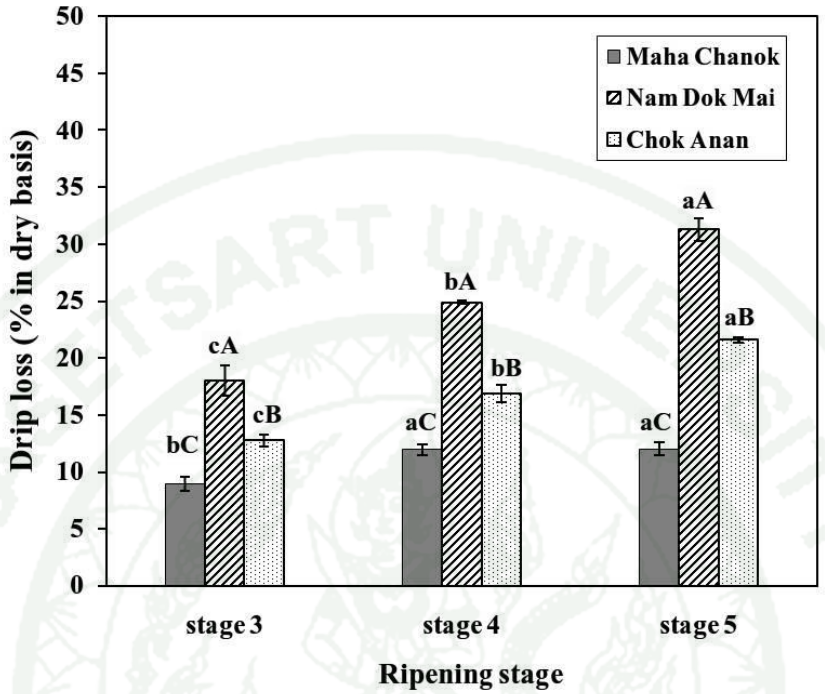

Figure 22 Drip loss of frozen-thawed mangoes. Means with the different letters (a-c) are significantly different (DMRT's test: $P \leq 0.05$) between ripening stages. Mean with the different letters (A-C) are significantly different (DMRT's test: $P \leq 0.05$) between cultivars.

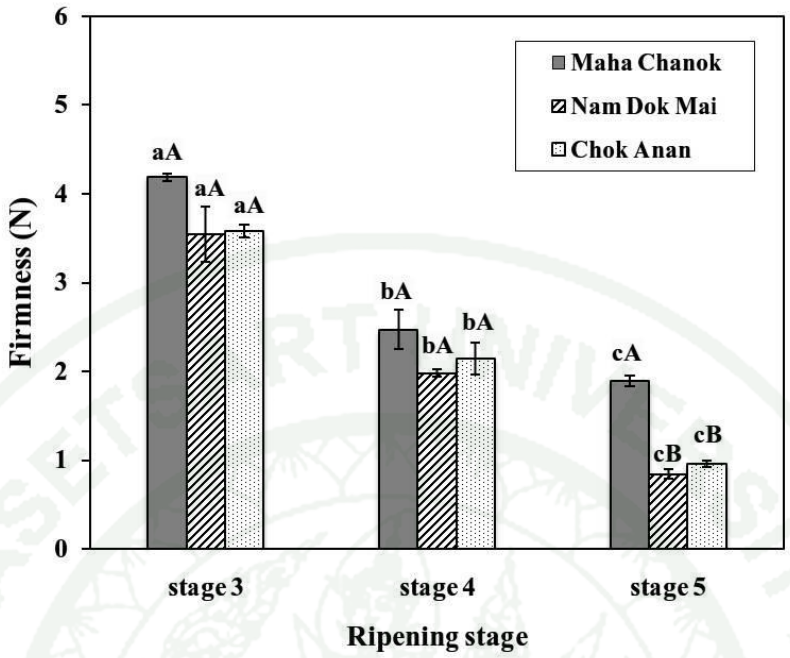

Figure 23 Firmness (N) values of frozen-thawed mangoes. Means with the different letters (a-c) are significantly different (DMRT's test: $P \leq 0.05$) between ripening stages. Mean with the different letters (A-C) are significantly different (DMRT's test: $P \leq 0.05$) between cultivars.

1.3 Cell wall material and fraction

After freezing, a slight decrease in AIR content was found in all mango cultivars (Appendix Table C2, C5). AIR content of frozen-thawed mangoes is shown in Figure 24. A decrease in AIR suggests that some changes were taking place in the cell wall material during the freezing process. It has been reported in green beans, peaches and strawberries that changes in pectic material was a consequence of freezing and frozen storage (Reid *et al*., 1986). Chok Anan cultivar maintained a higher AIR content at ripening stage 4 and 5 than Maha Chanok and Nam Dok Mai cultivar, respectively, whereas the AIR contents at ripening stage 3 were relatively similar between Maha Chanok and Chok Anan. Although Nam Dok Mai was found to have the lowest level of AIR at all ripening stages, firmness values were similar to the other cultivars at ripening stage 3 and 4. The AIR differences between cultivars of frozen-thawed mangoes were not correlated well with the different in firmness.

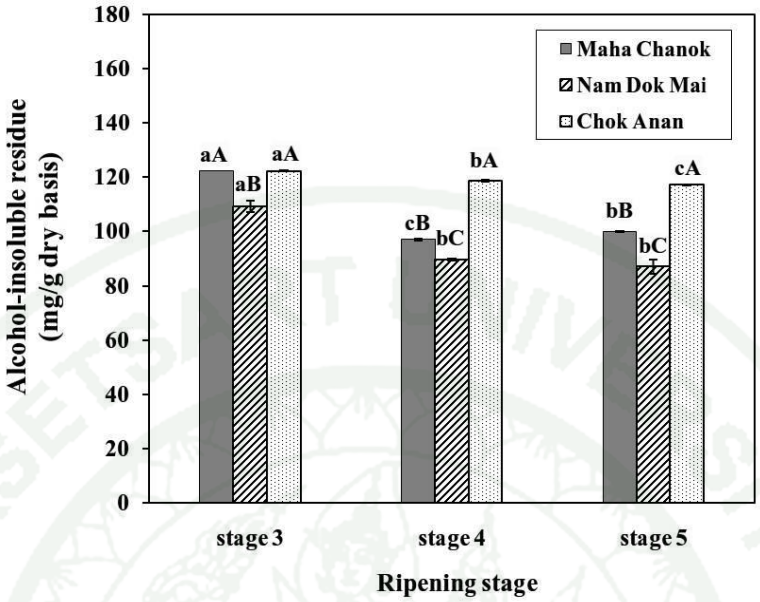

Figure 24 The amount of Alcohol-insoluble residue of frozen-thawed mangoes at three different ripening stages. Means with the different letters (a-c) are significantly different (DMRT's test: $P \leq 0.05$) between ripening stages. Mean with the different letters (A-C) are significantly different (DMRT's test: $P \leq 0.05$) between cultivars.

TP values were relatively similar between fresh and frozen-thawed samples for all ripening stages, whereas WSP values increased (Appendix Table C2, C5). This may indicate that no changes occurred in TP content. However, this does not mean that there were no changes in the cell wall composition. Figure 25 showed the total pectin content of frozen-thawed mango cultivars at different ripening stages. Maha Chanok cultivar was found to have the lowest total pectin content at all stages. In contrast to pectin content, the lowest AIR content was found in the frozen-thawed Nam Dok Mai cultivar.

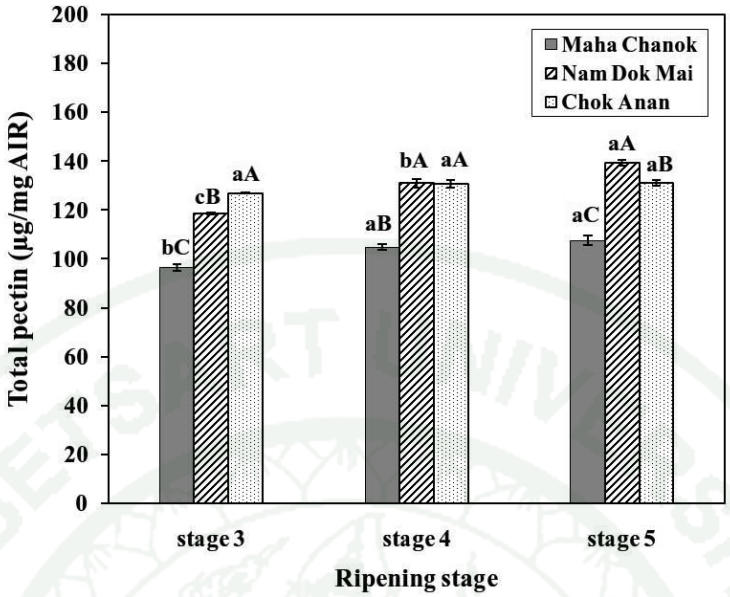

Figure 25 Total pectin content of frozen-thawed mangoes at three different ripening stages. Means with the different letters (a-c) are significantly different (DMRT's test: $P \leq 0.05$) between ripening stages. Mean with the different letters (A-C) are significantly different (DMRT's test: $P \leq 0.05$) between cultivars.

The increase in WSP may due to the solubilization or depolymerization of cell wall compositions caused by ice crystal formation during freezing process. WSP content of frozen-thawed samples at various ripening stages is shown in Figure 26. Frozen-thawed mango at the earlier stage of ripening had lower WSP content than the later stage. Corresponding, a higher firmness was found at the earlier ripening stage of all cultivars. Maha Chanok cultivar was found to have the lowest level of WSP at all ripening stages as well as the lowest total pectin content and the lowest drip loss. These results indicated that frozen-thawed Maha Chanok mango appeared to have a less tissue damages which mean Maha Chanok can maintain a firmer structure than the other cultivars. Besides, at the ripening stage 5, Maha chanok had a higher firmness than Nam Dok Mai and Chok Anan. Although Chok Anan had higher WSP values than Nam Dok Mai at ripening stage 3 and 4, the fruit firmness of the frozen-thawed mangoes had no significant differences ($P \leq 0.05$) between these two

cultivars. Alonso *et al*. (1997) explained that the texture changes in frozen cherries have been associated with the composition of pectin fractions and not with the quantity of the pectin in the fruit.

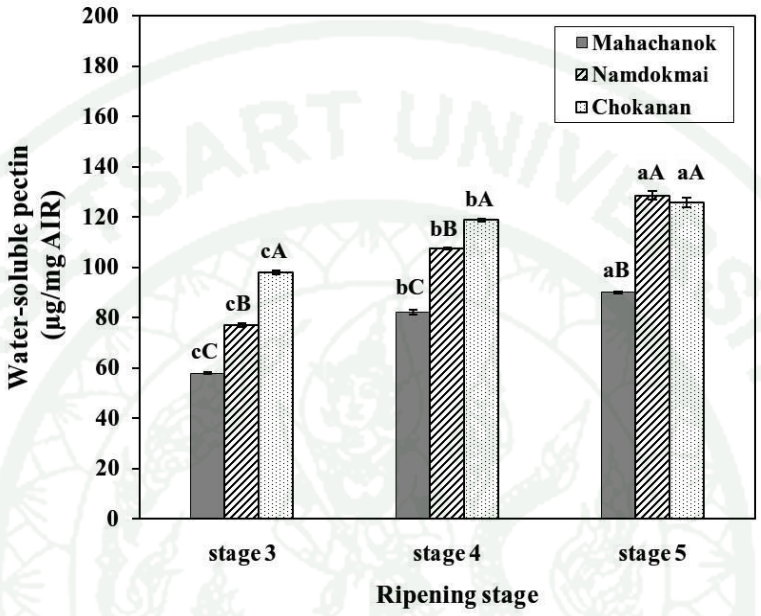

Figure 26 Water-soluble pectin content of frozen-thawed mangoes at three different ripening stages. Means with the different letters (a-c) are significantly different (DMRT's test: $P \leq 0.05$) between ripening stages. Mean with the different letters (A-C) are significantly different (DMRT's test: $P \leq 0.05$) between cultivars.

2. Microstructural changes in frozen mangoes

The microstructure of frozen mango tissues was observed by using LM and TEM. In this part of study, the microstructure and ultrastructure of Maha Chanok cultivar were examined only at the ripening stage 4. The limit of micrographs due to the fact that Maha Chanok cultivars was the first trial sample that used to prepare thick and thin sections. The observation using LM showed that after freezing process, the cell wall of all mango cultivars were swollen and folded indicated by arrow as shown in Figure 27. These irregular cell walls can clearly be observed in frozen-thawed sample at ripening stage 5. Chok Anan at ripening stage 4 and 5 appeared to show a larger swollen cell wall than the other cultivars. The dramatic cell wall damages such as cell separation in the middle lamella and cell wall rupture was found in frozen Nam Dok Mai at the stage 5. In Figure 27B-3, the arrows indicate swollen and torn cell wall. However, cell walls damages can be preserved when the earlier stage (stage 3) of ripening mango was frozen. The cell walls of Nam Dok Mai and Chok Anan at ripening stage 3 were quite similar to the cell walls of fresh samples (Figure 18). The cell wall damages can be explained by the ice crystals which formed in the extracellular medium propagating via the cytoplasm when the cell membrane lost permeability. The decompartmentalization caused by these ice crystals prevented water from returning to the intracellular medium during thawing, which caused a loss of turgidity and hence the change in fruit texture (Alonso et al., 2005).

TEM micrographs of the frozen mango are shown in Figure 28. In the unfrozen tissue (Figure 19-21), the cell wall and middle lamella were intact. After freezing, cell wall damages were observed in all cultivars. This cell wall damage can be explained by the dissolution of middle lamella which caused by ice crystals. It was correlated with an increase in WSP content of frozen mango. The dissolution of middle lamella leaded to the loss of cell adhesion and cell-to-cell contact which resulting in the formation of intercellular spaces as shown in Figure 28 (B-1, B-2, C-1 and C-2). The swelling and folding of cell walls were observed in the frozen tissue which possibly due to cell wall loosening and cell wall expansion. TEM observation revealed that the cell wall thickness of fresh Maha Chanok, Nam Dok Mai and Chok

Anan samples ranged from 0.7 to 1.4, 0.5 to 1.0 and 0.8 to 1.6 µm, respectively. After freezing, the cell wall thickness of Maha Chanok, Nam Dok Mai and Chok Anan was increased in the range from 1.0 to 3.0, 1.0 to 2.0 and 1.0 to 3.0 µm, respectively. The cell walls at the ripening stage 3 of Nam Dok Mai and Chok Anan cultivars appeared less damaged than in the other stages, while severe cell wall damage was found in the ripening stage 5 of both cultivars. Frozen samples of Nam Dok Mai at all ripening stages seemed to have more cell wall damages than Maha Chanok and Chok Anan sample. The dramatic damages in Nam Dok Mai cell walls are likely due to their moisture content which significantly higher than the other cultivars (Appendix Table C1). These cell wall damages resulted in the extremely high drip loss of frozen-thawed Nam Dok Mai sample.

From the microstructure results, it is clear that the loss in firmness in the mango tissues after freezing and thawing can be attributed to changes in the middle lamella and cell walls. The use of mangoes at the ripening stage 3 is recommended, to preserve the cell wall damages which occur during the freezing process.

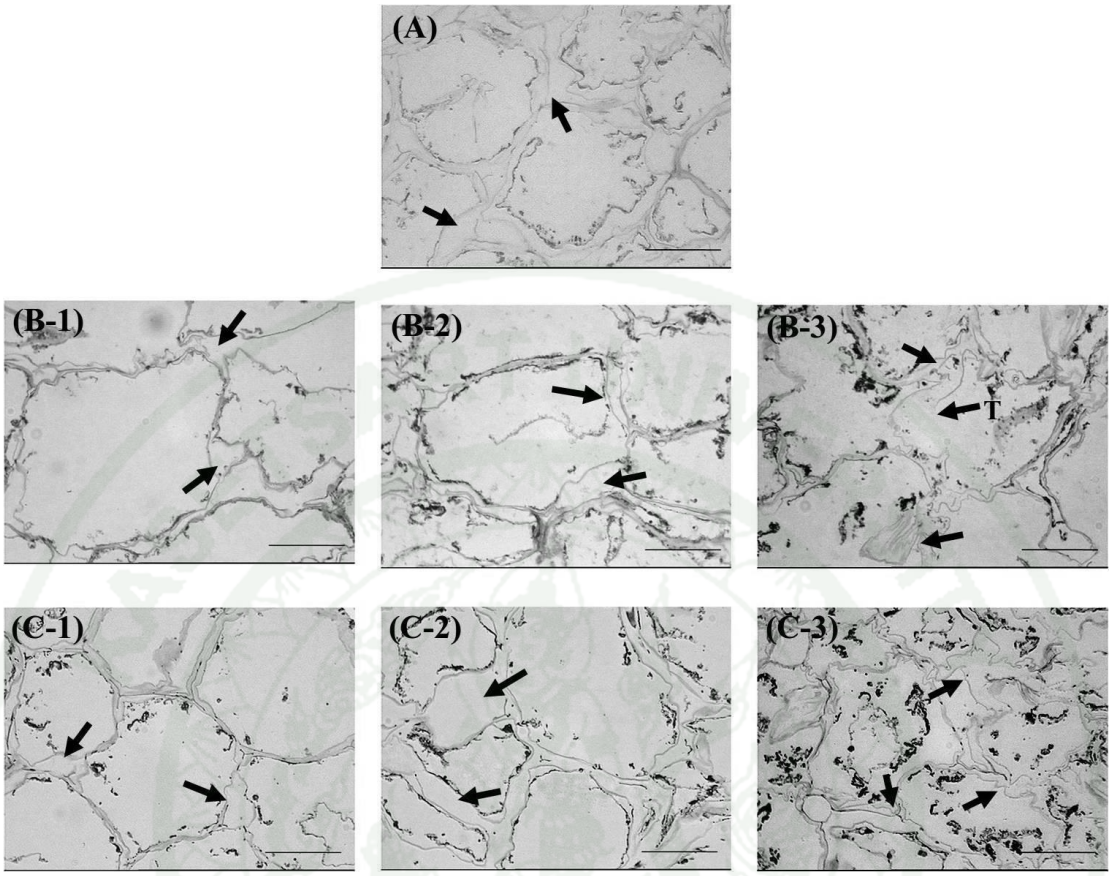

Figure 27 Light microscopy images of frozen mangoes at various ripening stages. (A) Maha Chanok at stage 4, (B) Nam Dok Mai, and (C) Chok Anan cultivars. (1) ripening stage 3, (2) ripening stage 4, and (3) ripening stage 5. Arrows indicate the swollen cell walls caused by freezing damage. T = torn cell wall. The magnification of all micrographs is 40x. Bar = 50 µm.

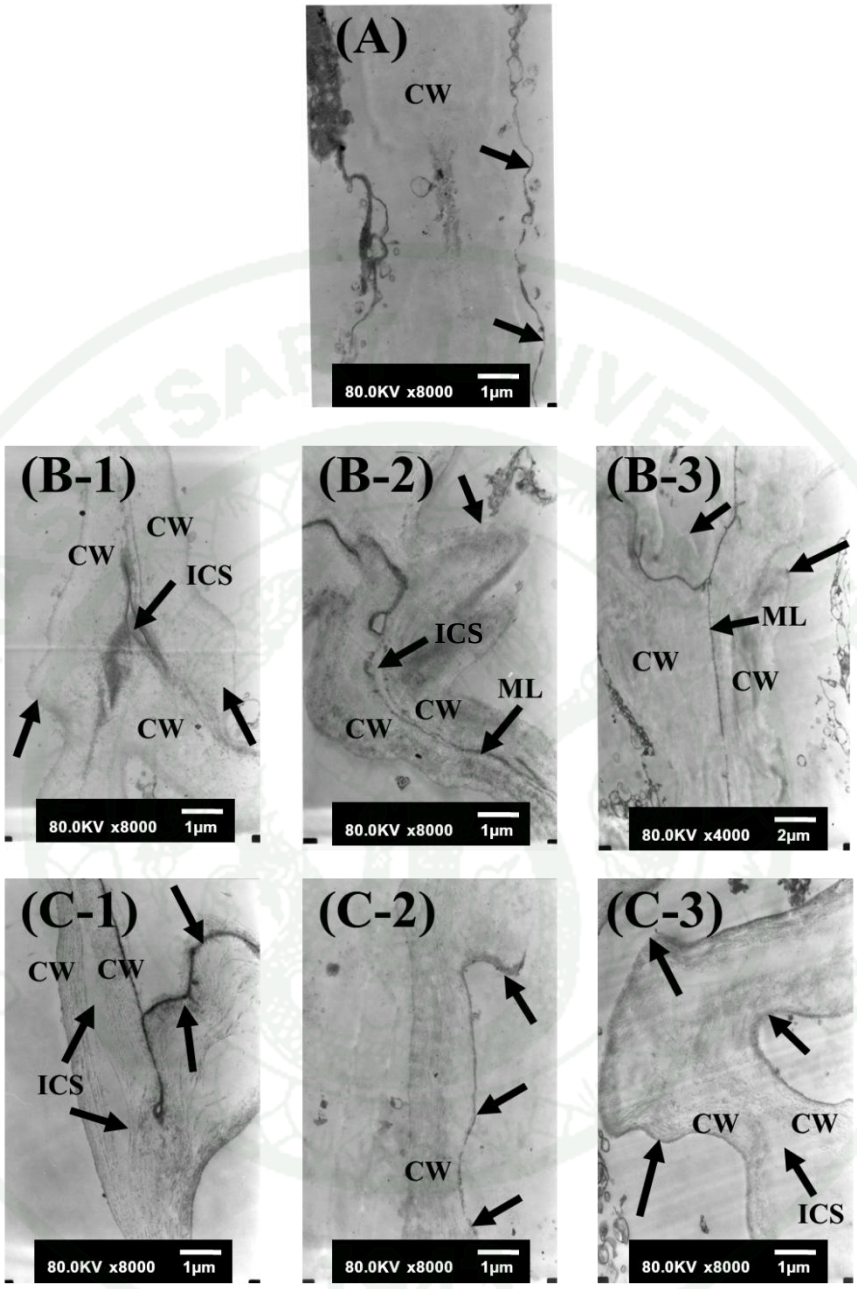

Figure 28 TEM images of frozen mangoes at various ripening stages. (A) Maha Chanok at stage 4, (B) Nam Dok Mai, and (C) Chok Anan cultivars. (1) ripening stage 3, (2) ripening stage 4, and (3) ripening stage 5. Arrows indicate the swollen and folded cell walls caused by freezing damage. CW = cell wall, ML = middle lamella, ICS = intercellular space. The magnification of micrographs is 8000x and bar = 1 µm. The magnification of image B-3 is 4000x and bar = 2 µm.

3. Sensory evaluation

Firmness intensity score was evaluated using a ranking test on a five-point scale (extremely soft - extremely firm) by 8 trained panelists. The intensity score of firmness attribute is shown in Figure 29 and Appendix Table C6. All mango cultivars, firmness intensity score of mango at the ripening stage 3 had the highest score as well as the highest firmness value which was measured by texture analyzer (Figure 23). These results correspond well to the texture measurement which confirms that the mango at ripening stage 3 can improve the texture quality of frozen-thawed mango. Frozen-thawed Maha Chanok mango appeared to have the highest firmness intensity scores at all ripening stages. The intensity scores of Nam Dok Mai and Chok Anan were relatively similar at ripening stage 3 and 5, whereas Nam Dok Mai sample had the lowest firmness at the ripening stage 4. In contrast to intensity scores, the firmness of frozen-thawed mango at ripening stage 3 and 4 which measured by texture analyzer were not significantly different ($P > 0.05$) between cultivars. Only the sample at ripening stage 5 showed the similar results.

Sensory attributes were evaluated using a preference score (appearance, color, flavor, taste, texture and overall acceptability) on a nine-point hedonic scale (dislike extremely - like extremely) by 30 untrained panelists. The preference scores are shown in Table 1. These results showed that frozen-thawed Maha Chanok mango at ripening stage 3 had the highest color scores, whereas sample at ripening stage 4 showed the highest flavor and overall acceptability scores. The taste scores were similar between stage 4 and 5 but higher than stage 3. Moreover, sample at ripening stage 3 and 4 had higher appearance scores than the stage 5. In contrast to other attributes scores, texture scores of frozen-thawed Maha Chanok were relatively similar between ripening stages. Frozen-thawed Nam Dok Mai at ripening stage 3 and 4 had higher appearance, color and overall acceptability scores than that at the ripening stage 5 ($P \leq 0.05$). The flavor and taste scores between any of the ripening stages were not significantly different ($P > 0.05$). However, the ripening stage 3 presented the highest texture scores. The color scores of frozen-thawed Chok Anan were similar between stage 3 and 4 and higher than the stage 5. Frozen-thawed Chok

Anan sample at ripening stage 3 had the highest appearance, texture and overall acceptability scores. However, flavor and taste scores between the ripening stages were not significantly different ($P > 0.05$).

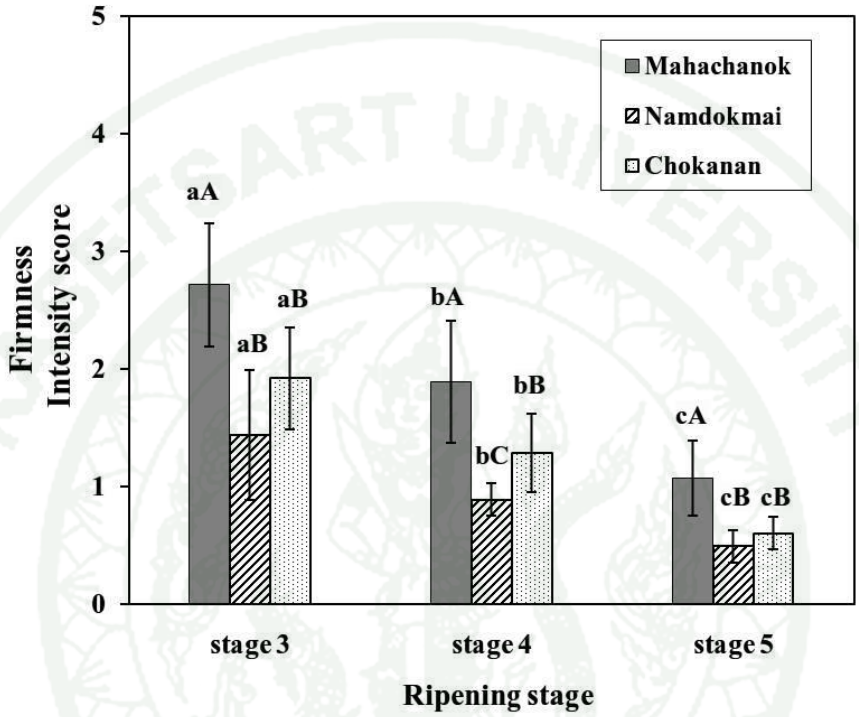

Figure 29 Intensity score (1-5) of firmness attribute of frozen-thawed mangoes. Means with the different letters (a-c) are significantly different (DMRT's test: $P \leq 0.05$) between ripening stages. Mean with the different letters (A-C) are significantly different (DMRT's test: $P \leq 0.05$) between cultivars.

Although Maha Chanok sample at stage 3 had the highest firmness intensity scores (Figure 29), the highest preference scores of texture attribute was found in Nam Dok Mai sample. This may due to the variations between 30 untrained panelists and personal preference. The overall acceptability scores of Nam Dok Mai and Chok Anan sample at ripening stage 3 were higher than Maha Chanok sample while the other attributes scores were not significantly different between cultivars ($P > 0.05$). At ripening stage 4, the color score of Nam Dok Mai samples was higher than Maha Chanok. Taste and overall acceptability scores of Maha Chanok and Nam Dok Mai sample were higher than Chok Anan whereas appearance, texture and flavor scores

were not significantly different between cultivars ($P > 0.05$). At ripening stage 5, texture scores of frozen-thawed samples were significantly different ($P \leq 0.05$) between cultivars while the other attributes scores were relatively similar. Maha Chanok sample at ripening stage 5 had the highest texture score as well as the highest firmness intensity score and the highest firmness value which was measured by texture analyzer compared to Nam Dok Mai and Chok Anan sample.

These results from part II confirm that ripening stage had a significant effect on the firmness values, drip loss, WSP, microstructure and sensory attributes of frozen-thawed mango in all cultivars. The ripening stage 3 exhibited a higher firmness value, higher sensory scores and lower drip loss than the other stages. This stage also appeared to have less cell wall damages caused by the freezing and thawing process. The partially ripe mango at stage 3 can preserve the better texture quality of frozen-thawed mango than the ripe mango at stage 4 or fully ripe mango at stage 5. In addition, the results found here were relatively similar in all cultivars studied. Although Maha Chanok and Chok Anan cultivar demonstrated less cell wall damages and less drip loss as compared to Nam Dok Mai cultivar, the highest preference score of texture attribute was found in frozen-thawed Nam Dok Mai sample.

The correlations between firmness values and drip loss, firmness values and AIR, firmness value and total pectin, firmness value and WSP and firmness value and firmness intensity score by trained panelists of frozen-thawed samples were analyzed. The coefficient of correlation is shown in Appendix Figure C4-C5. Firmness was highly positively correlated to firmness intensity score ($r = 0.903$). In addition, firmness was highly negatively correlated to WSP ($r = – 0.847$).

Table 1 Preference scores of frozen-thawed mangoes at various ripening stages. Sensory evaluation were evaluated by 30 untrained panelists. (nine-point hedonic scale)

Sensory attributes	Cultivar	Ripening stage		
		stage 3	stage 4	stage 5
Appearance	MC	$6.58^{aA} \pm 1.04$	$6.28^{aA} \pm 1.24$	$5.72^{bA} \pm 1.44$
	NDM	$7.18^{aA} \pm 1.42$	$6.72^{aA} \pm 1.41$	$5.67^{bA} \pm 1.53$
	CA	$6.95^{aA} \pm 1.26$	$6.03^{bA} \pm 1.16$	$5.22^{cA} \pm 1.35$
Color	MC	$6.42^{aA} \pm 0.95$	$5.85^{bB} \pm 1.35$	$5.58^{bA} \pm 1.34$
	NDM	$6.88^{aA} \pm 1.67$	$6.93^{aA} \pm 1.11$	$5.87^{bA} \pm 1.45$
	CA	$6.59^{aA} \pm 1.44$	$6.32^{aAB} \pm 1.20$	$5.24^{bA} \pm 1.37$
Texture	MC	$5.60^{aB} \pm 1.18$	$5.76^{aA} \pm 1.37$	$5.23^{aA} \pm 1.44$
	NDM	$6.63^{aA} \pm 1.58$	$6.03^{bA} \pm 1.40$	$4.27^{cB} \pm 1.57$
	CA	$5.90^{aB} \pm 1.45$	$5.32^{bA} \pm 1.71$	$4.03^{cB} \pm 1.59$
Flavor	MC	$5.32^{bA} \pm 1.56$	$6.32^{aA} \pm 1.17$	$5.77^{bA} \pm 1.34$
	NDM	$5.78^{aA} \pm 1.63$	$6.17^{aA} \pm 1.43$	$5.57^{aA} \pm 1.19$
	CA	$5.53^{aA} \pm 1.50$	$5.68^{aA} \pm 1.37$	$5.37^{aA} \pm 1.44$
Taste	MC	$4.45^{bA} \pm 1.59$	$6.38^{aA} \pm 1.23$	$5.88^{aA} \pm 1.53$
	NDM	$5.34^{aA} \pm 1.97$	$6.23^{aA} \pm 1.51$	$5.68^{aA} \pm 1.36$
	CA	$5.44^{aA} \pm 1.48$	$5.60^{aB} \pm 1.35$	$5.15^{aA} \pm 1.47$
Overall acceptability	MC	$5.08^{cB} \pm 1.22$	$6.33^{aA} \pm 1.18$	$5.68^{bA} \pm 1.36$
	NDM	$6.26^{aA} \pm 1.55$	$6.33^{aA} \pm 1.26$	$5.20^{bA} \pm 1.34$
	CA	$6.16^{aA} \pm 1.37$	$5.55^{bB} \pm 1.48$	$4.78^{cA} \pm 1.48$

MC = Maha Chanok, NDM = Nam Dok Mai, and CA = Chok Anan

Mean values in the same row followed by different letters (a–c) are significantly different (DMRT's test: $P \leq 0.05$).

Mean values in the same column in each attribute followed by different letters (A–B) are significantly different (DMRT's test: $P \leq 0.05$).

CONCLUSIONS

The physicochemical of mango fruit has undergone rapid changes during ripening. The peel color of the fruit changed noticeably from green to yellow/yellow-orange. The loss of fruit firmness was accompanied by a decrease in the AIR content, an increase in WSP, and also associated with the microstructural changes. Additional, in-depth microstructural information can help to identify causes of textural changes during the ripening process and among the cultivars.

After freezing and thawing, the mangoes at ripening stage 3 exhibited a higher firmness value, higher firmness intensity score and lower drip loss than the other stages. This stage also appeared to have less cell walls damages which caused by freezing process. The use of mangoes at the ripening stage 3 can be recommended in the freezing process to maintain higher textural quality. Sensory evaluation showed that the mangoes at ripening stage 4 can be used to satisfy the consumers better than the other stages. The results found here were relatively similar for the three cultivars studied. However, Maha Chanok and Chok Anan demonstrated a higher resistance to freezing damage with less cell wall damages and very low drip loss as compared to Nam Dok Mai.

RECOMMENDATIONS

1. More pectin fractions and degree of polymerization or solubilization of pectin should be examined for a better understanding of firmness loss.

2. Further study on the textural quality improvement by pretreatment before freezing process should be investigated.

3. Other mango cultivars which are cheaper and high ability to produce off-season should be studied more to reduce the costs of raw material and produce a high quality products.

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APPENDICES

Appendix A

Mango and sample preparation

Appendix A

Appendix Figure A1 Mango plantation of cultivar Nam Dok Mai at Nakorn Ratchasima province.

Appendix Figure A2 Mango trees of the cultivar Nam Dok Mai.

Appendix Figure A3 Mature green Nam Dok Mai mangoes after freshly harvest.

Appendix Figure A4 Mature green Nam Dok Mai mangoes ready for transport.

Appendix Figure A5 Mango trees of the cultivar Chok Anan at Suphan Buri province.

Appendix Figure A6 Picking mature green Chok Anan mangoes.

Appendix Figure A7 Mature green Chok Anan mangoes after freshly harvest.

Appendix Figure A8 Mature green Chok Anan mangoes ready for transport.

Appendix Figure A9 Maha Chanok mangoes during ripening process in a commercial plastic container.

Appendix Figure A10 Samples preparation before analysis.

Appendix Figure A11 Samples were cut into cubes of 1.5 cm in length.

Appendix B

Chemical and physical analysis

B1. Moisture content (According to AOAC method, 2000)

1.1. Weigh moisture sample immediately and record as "wet weight of sample".

1.2 Dry the wet sample to a constant weight, at 105±2 ℃ in a hot air oven until constant weight (6 h).

1.3 Allow the sample to cool in desiccator.

1.4 Weigh the cooled sample again, and record as the "dry weight of sample". The moisture content of the sample is calculated using the following equation:

$$\text{Moisture content}(\%) = \frac{A - B}{B} \times 100$$

Where: A = Weight of wet sample (grams)

B = Weight of dry sample (grams)

B2. Titratable acidity (According to AOAC method, 1995)

Samples were homogenized and filtered through cheesecloth. Pipette 20 mL of fruit juice into a 250 mL Erlenmeyer flask. Add 50 mL distilled water. Add 3 drops of 1% phenolphthalein solution and titrate with standardized NaOH (0.1011N) to a pink color (the endpoint). The titratable acidity (% as malic acid) of the sample is calculated using the following equation:

$$\%\text{TA (wt/vol)} = \frac{N \times V_1 \times \text{Eq.wt}}{V_2 \times 1000} \times 100$$

Where: N = normality of NaOH (mEq/mL)

V_1 = volume of NaOH (mL)

Eq.wt = Equivalent weight of malic acid (Eq.wt = 67)

V_2 = volume of sample (ml)

1000 = factor relating mg to grams (mg/g)

B3. Standard curve of galacturonic acid

1.1 Weigh D-(+)-Galacturonic acid 0.0100 g into volumetric flask and adjust volume to 100 mL with distilled water in order to get 100 µg/mL.

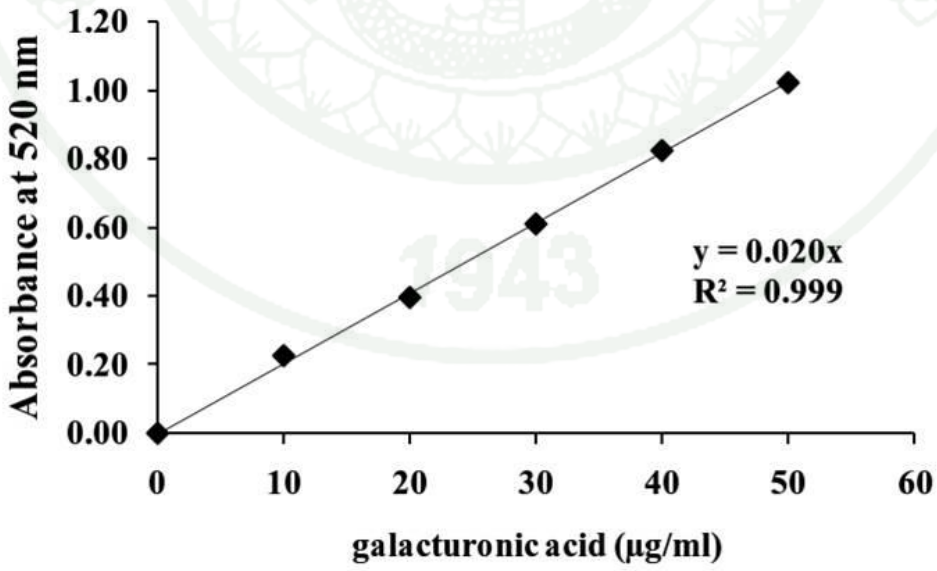
1.2 Add 4.8 ml of 0.0125M solution of tetraborate in concentrated sulfuric acid to 0.1, 0.2, 0.3, 0.4, and 0.5 mL of 100 µg/mL galacturonic acid. (The tubes were refrigerated in ice-water bath

1.3 Shake in a vortex. Heat the tube in a water bath at 95 °C for 5 min.

1.4 Cool in ice-water bath. Add 80 µL of the meta-hydroxydiphenyl solution and then mix. The blank prepared with 80 µL of 0.5% NaOH. Meta-hydroxydiphenyl solution: A 0.15% solution of meta-hydroxydiphenyl in 0.5% NaOH.

1.5 Read color absorbance at 520 nm, using the blank to zero the spectrometer.

1.6 Plot absorbance at 520 nm against galacturonic acid concentration for a standard curve. A standard curve of galacturonic acid was made between 0-50 µg/mL D-galacturonic acid.



Appendix Figure B1 Standard curve of galacturonic acid.

B4. Drip loss (A method adapted from Lowithun and Charoenrein (2009)).

Four frozen sample cubes were placed over absorbent paper in zip-lock plastic bag to eliminate evaporation during thawing. Then the samples were thawed at 4 °C. Drip loss was evaluated by periodic weighing of the absorbent paper until a constant value was reached. The preliminary results indicated that the appropriate thawing time was 5 h. Measurements were done in triplicate, and the results were calculated as drip loss on a dry basis, according to the following equation:

$$\text{Drip loss (\% in dry basis)} = \frac{W_t - W_o}{W_s \times \text{TS}} \times 100$$

Where: W_o = the weight of absorbent paper before thawing

W_t = the weight of absorbent paper after thawing

W_s = the weight of the sample

TS = the % dry matter of fruit after thawing

The dry matter of fruit after thawing was analyzed by drying the samples in a hot air oven at 105±2 °C until the samples reached a constant weight. The dry matter of the sample is calculated using the following equation:

$$\text{TS (\%)} = \frac{\text{dry weight}}{\text{wet weight}} \times 100$$

Appendix C

Results

Appendix Table C1 Characteristics of three mango cultivars during ripening.

Properties	Cultivar	Ripening stage					
		Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
%TA (as malic acid)	MC	$2.90^{aA} \pm 0.07$	$2.45^{bA} \pm 0.15$	$1.48^{cA} \pm 0.09$	$0.41^{dA} \pm 0.11$	$0.30^{deA} \pm 0.03$	$0.19^{eA} \pm 0.02$
	NDM	$2.67^{aA} \pm 0.28$	$1.92^{bB} \pm 0.09$	$1.10^{cAB} \pm 0.28$	$0.53^{dA} \pm 0.18$	$0.19^{eB} \pm 0.05$	$0.08^{eB} \pm 0.01$
	CA	$2.57^{aA} \pm 0.39$	$1.24^{bC} \pm 0.10$	$0.92^{bB} \pm 0.21$	$0.26^{cA} \pm 0.03$	$0.21^{cB} \pm 0.01$	$0.19^{cA} \pm 0.02$
TSS (°Brix)	MC	$7.97^{eA} \pm 1.16$	$15.20^{dA} \pm 0.95$	$17.40^{cA} \pm 0.36$	$18.50^{bcA} \pm 0.87$	$19.47^{abA} \pm 0.25$	$20.53^{aA} \pm 0.59$
	NDM	$8.67^{eA} \pm 0.25$	$14.37^{dA} \pm 0.35$	$15.53^{cB} \pm 0.12$	$16.47^{bB} \pm 0.35$	$16.53^{bC} \pm 0.12$	$17.50^{aB} \pm 0.36$
	CA	$7.37^{eA} \pm 0.15$	$12.50^{dA} \pm 0.10$	$14.87^{cB} \pm 0.47$	$15.60^{cB} \pm 0.40$	$17.20^{bB} \pm 0.50$	$20.37^{aA} \pm 1.12$
Moisture content (%)	MC	$84.41^{aA} \pm 0.32$	$82.36^{bB} \pm 0.24$	$82.05^{bB} \pm 0.07$	$82.45^{bB} \pm 0.48$	$82.32^{bB} \pm 0.95$	$82.53^{bB} \pm 0.27$
	NDM	$83.75^{bB} \pm 0.22$	$83.53^{bA} \pm 0.48$	$83.25^{bA} \pm 0.11$	$83.81^{bA} \pm 0.23$	$84.58^{aA} \pm 0.45$	$83.40^{bA} \pm 0.10$
	CA	$84.44^{aA} \pm 0.20$	$82.10^{bcB} \pm 0.06$	$81.82^{cB} \pm 0.66$	$82.50^{bcB} \pm 0.28$	$82.00^{bcB} \pm 0.81$	$82.71^{bB} \pm 0.17$
Firmness (N)	MC	249.23^{aB}	$49.90^{bB} \pm 6.92$	$11.59^{cB} \pm 0.62$	$8.84^{cA} \pm 0.61$	$7.35^{cA} \pm 0.32$	$5.39^{cB} \pm 0.26$
	NDM	$153.94^{aC} \pm$	$34.08^{bC} \pm 8.72$	$10.47^{cC} \pm 0.71$	$8.92^{cdA} \pm 0.58$	$5.20^{deB} \pm 0.42$	$4.06^{eC} \pm 0.35$
	CA	277.48^{aA}	$56.61^{bA} \pm 5.17$	$14.47^{cA} \pm 0.76$	$8.55^{cdA} \pm 0.32$	$7.33^{dA} \pm 0.14$	$5.76^{dA} \pm 0.47$

MC = Maha Chanok, NDM = Nam Dok Mai, CA = Chok Anan, TA = Titratable acidity, and TSS = Total soluble solids

Mean values in the same row followed by different letters (a–e) are significantly different (DMRT's test: $P \leq 0.05$).

Mean values in the same column in each attribute followed by different letters (A–C) are significantly different (DMRT's test: $P \leq 0.05$)

Appendix Table C2 Cell wall material and pectin fraction of three mango cultivars during ripening.

Properties	Cultivar	Ripening stage					
		Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**	**Stage 6**
AIR	MC	$708.00^{aA} \pm 11.91$	$290.52^{bA} \pm 12.32$	$123.06^{cA} \pm 3.63$	$102.72^{dB} \pm 7.41$	$99.87^{dB} \pm 1.23$	$92.40^{dB} \pm 2.66$
(mg/g dry basis)	NDM	$513.01^{aC} \pm 11.27$	$152.86^{bC} \pm 6.09$	$109.70^{cB} \pm 8.69$	$98.40^{cdB} \pm 2.83$	$93.37^{dC} \pm 0.93$	$90.93^{dB} \pm 1.40$
	CA	$640.32^{aB} \pm 26.13$	$218.23^{bB} \pm 21.32$	$125.78^{cA} \pm 2.84$	$125.40^{cA} \pm 2.63$	$120.76^{cA} \pm 2.93$	$117.43^{cA} \pm 2.40$
TP	MC	$29.32^{eB} \pm 5.44$	$47.76^{dB} \pm 0.56$	$91.16^{bC} \pm 1.29$	$104.62^{aB} \pm 3.11$	$103.51^{aC} \pm 3.56$	$82.93^{cC} \pm 2.42$
(µg/mg AIR)	NDM	$34.65^{eB} \pm 3.08$	$83.62^{dA} \pm 5.81$	$117.08^{cB} \pm 4.68$	$133.21^{bA} \pm 2.22$	$139.81^{aA} \pm 2.73$	$111.22^{cB} \pm 1.94$
	CA	$56.75^{cA} \pm 12.50$	$88.97^{bA} \pm 4.54$	$126.05^{aA} \pm 2.70$	$133.00^{aA} \pm 1.47$	$131.55^{aB} \pm 4.39$	$122.45^{aA} \pm 2.36$
WSP	MC	$6.03^{eB} \pm 0.11$	$18.79^{dC} \pm 1.70$	$54.07^{cC} \pm 0.91$	$71.78^{bC} \pm 7.25$	$84.25^{aB} \pm 7.78$	$72.11^{bB} \pm 4.37$
(µg/mg AIR)	NDM	$7.64^{fA} \pm 0.17$	$44.75^{eA} \pm 0.91$	$71.90^{dB} \pm 1.28$	$105.61^{cB} \pm 2.16$	$119.94^{aA} \pm 1.54$	$113.11^{bA} \pm 1.11$
	CA	$8.09^{fA} \pm 0.34$	$31.71^{eB} \pm 0.80$	$96.09^{dA} \pm 6.46$	$117.34^{bA} \pm 3.11$	$123.93^{aA} \pm 3.88$	$107.98^{cA} \pm 2.56$

MC = Maha Chanok, NDM = Nam Dok Mai, CA = Chok Anan, AIR = Alcohol-insoluble residue, TP = Total pectin, and WSP = Water-soluble pectin. Mean values in the same row followed by different letters (a–f) are significantly different (DMRT's test: $P \leq 0.05$).

Mean values in the same column in each attribute followed by different letters (A–C) are significantly different (DMRT's test: $P \leq 0.05$)

Appendix Table C3 Changes in L*, a*, and b* values of mango peels during ripening.

Properties	Cultivar	Ripening stage					
		Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
L*	MC	$56.21^{bB} \pm 0.74$	$66.21^{aB} \pm 0.74$	$66.81^{aB} \pm 0.99$	$67.52^{aA} \pm 2.19$	$65.67^{aB} \pm 3.07$	$64.96^{aA} \pm 1.19$
	NDM	$69.60^{aA} \pm 1.40$	$70.27^{aA} \pm 0.26$	$70.40^{aA} \pm 1.12$	$70.30^{aA} \pm 0.42$	$71.42^{aA} \pm 1.23$	$67.50^{aA} \pm 2.45$
	CA	$55.49^{dB} \pm 0.45$	$66.32^{cB} \pm 1.30$	$69.03^{abA} \pm 0.94$	$70.33^{aA} \pm 1.02$	$70.07^{aA} \pm 0.69$	$68.05^{bA} \pm 0.19$
a*	MC	$1.35^{dA} \pm 0.17$	$3.96^{cA} \pm 0.73$	$13.02^{bA} \pm 1.50$	$13.48^{bA} \pm 2.12$	$14.45^{bA} \pm 0.31$	$18.47^{aA} \pm 1.28$
	NDM	$-4.09^{eB} \pm 0.57$	$-1.25^{dB} \pm 0.28$	$1.06^{cC} \pm 0.36$	$6.08^{bB} \pm 0.60$	$7.79^{aC} \pm 0.12$	$8.33^{aC} \pm 0.58$
	CA	$-7.24^{fC} \pm 0.13$	$-2.81^{eC} \pm 0.67$	$6.02^{dB} \pm 0.78$	$11.04^{cA} \pm 0.20$	$12.43^{bB} \pm 0.49$	$14.38^{aB} \pm 0.30$
b*	MC	$25.47^{dB} \pm 1.25$	$38.84^{cA} \pm 0.36$	$43.36^{bA} \pm 1.61$	$46.98^{abA} \pm 4.22$	$47.56^{aA} \pm 1.39$	$43.19^{bB} \pm 1.16$
	NDM	$28.73^{cA} \pm 0.47$	$30.53^{bC} \pm 0.71$	$30.81^{bB} \pm 1.48$	$34.74^{aB} \pm 0.67$	$35.72^{aB} \pm 0.92$	$35.78^{aC} \pm 1.07$
	CA	$23.33^{eC} \pm 0.17$	$37.13^{dB} \pm 0.59$	$43.18^{cA} \pm 0.32$	$45.34^{bA} \pm 0.39$	$48.08^{aA} \pm 0.69$	$47.92^{aA} \pm 0.60$

MC = Maha Chanok, NDM = Nam Dok Mai, CA = Chok Anan

Mean values in the same row followed by different letters (a–f) are significantly different (DMRT's test: $P \leq 0.05$).

Mean values in the same column in each attribute followed by different letters (A–C) are significantly different (DMRT's test: $P \leq 0.05$).

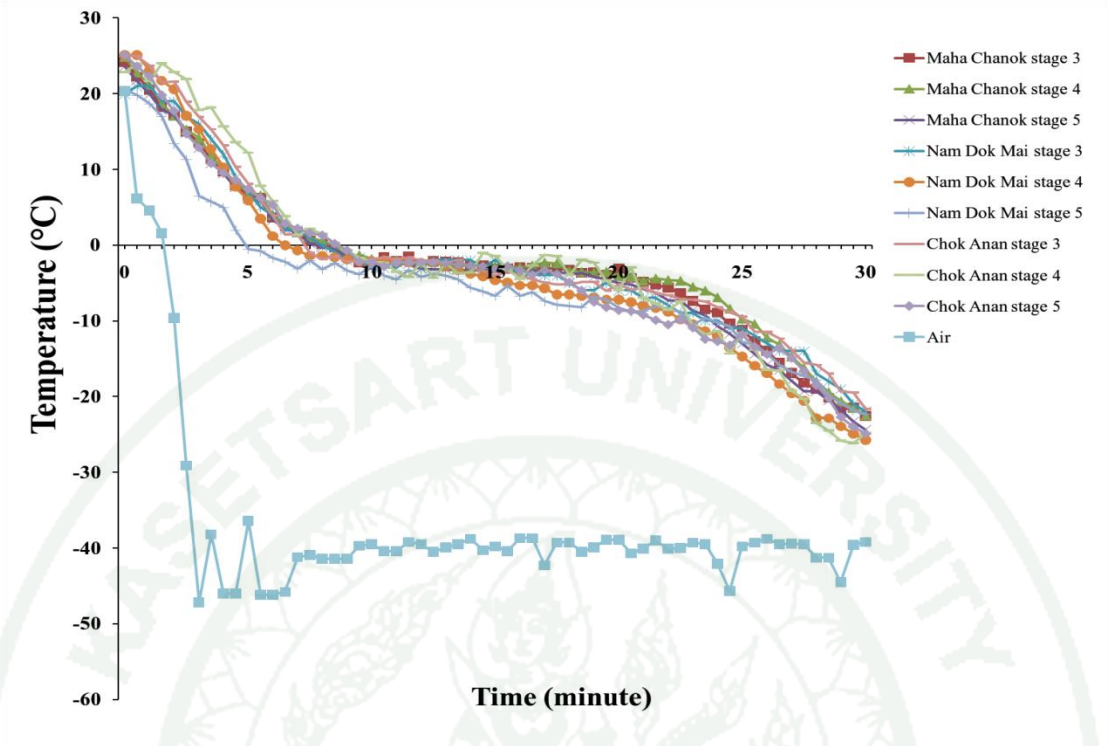
Appendix Table C4 Changes in L*, a*, and b* values of mango mesocarps during ripening.

Properties	Cultivar	Ripening stage					
		Stage 1	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**	**Stage 6**
L*	MC	$81.10^{aB} \pm 0.60$	$73.28^{bB} \pm 1.42$	$74.44^{bA} \pm 1.44$	$69.96^{cA} \pm 1.11$	$68.44^{cA} \pm 1.93$	$63.40^{dA} \pm 1.11$
	NDM	$83.56^{aA} \pm 0.52$	$76.69^{bA} \pm 1.42$	$74.71^{bA} \pm 0.19$	$71.29^{cA} \pm 0.41$	$68.58^{cdA} \pm 1.17$	$66.97^{dA} \pm 3.41$
	CA	$83.89^{aA} \pm 0.30$	$79.18^{bA} \pm 0.86$	$71.33^{cB} \pm 1.35$	$67.31^{dB} \pm 0.74$	$64.06^{eB} \pm 0.85$	$63.83^{eA} \pm 1.03$
a*	MC	$5.10^{fA} \pm 0.63$	$8.39^{eA} \pm 0.05$	$11.79^{dA} \pm 2.02$	$18.37^{cA} \pm 1.17$	$21.95^{bA} \pm 1.66$	$24.61^{aA} \pm 1.01$
	NDM	$-0.96^{fB} \pm 0.13$	$2.56^{eC} \pm 0.44$	$7.09^{dB} \pm 1.34$	$10.54^{cB} \pm 1.81$	$13.40^{bB} \pm 0.38$	$16.83^{aB} \pm 1.26$
	CA	$-2.94^{fC} \pm 0.18$	$3.43^{eB} \pm 0.18$	$7.81^{dB} \pm 1.43$	$12.28^{cB} \pm 0.17$	$14.66^{bB} \pm 0.77$	$16.68^{aB} \pm 1.76$
b*	MC	$39.00^{dA} \pm 1.32$	$46.36^{cA} \pm 0.49$	$48.89^{bcB} \pm 0.91$	$52.17^{aA} \pm 2.62$	$51.26^{abA} \pm 1.82$	$53.68^{aA} \pm 1.19$
	NDM	$22.97^{dB} \pm 0.29$	$37.94^{cB} \pm 2.84$	$50.61^{bB} \pm 0.82$	$48.38^{bA} \pm 0.91$	$50.18^{bA} \pm 0.84$	$53.46^{aA} \pm 0.87$
	CA	$21.83^{dB} \pm 1.07$	$45.36^{cA} \pm 1.63$	$54.45^{abA} \pm 0.89$	$51.43^{bA} \pm 1.04$	$54.33^{abA} \pm 4.29$	$55.71^{aA} \pm 0.99$

MC = Maha Chanok, NDM = Nam Dok Mai, CA = Chok Anan

Mean values in the same row followed by different letters (a–f) are significantly different (DMRT's test: $P \leq 0.05$).

Mean values in the same column in each attribute followed by different letters (A–C) are significantly different (DMRT's test: $P \leq 0.05$).



Appendix Figure C1 Freezing profile of all mango cultivars at different ripening stages at –40 °C in a cryogenic freezer.

Appendix Table C5 Properties of frozen-thawed mangoes after 15 days of storage.

Properties	Cultivar	Ripening stage		
		stage 3	stage 4	stage 5
Drip loss	MC	$8.96^{bC} \pm 0.62$	$11.93^{aC} \pm 0.49$	$12.00^{aC} \pm 0.59$
(% in dry basis)	NDM	$18.02^{cA} \pm 1.34$	$24.86^{bA} \pm 0.14$	$31.27^{aA} \pm 0.98$
	CA	$12.74^{cB} \pm 0.56$	$16.88^{bB} \pm 0.75$	$21.60^{aB} \pm 0.22$
Firmness (N)	MC	$4.18^{aA} \pm 0.04$	$2.47^{bA} \pm 0.22$	$1.89^{cA} \pm 0.06$
	NDM	$3.54^{aA} \pm 0.31$	$1.98^{bA} \pm 0.04$	$0.84^{cB} \pm 0.06$
	CA	$3.58^{aA} \pm 0.07$	$2.14^{bA} \pm 0.18$	$0.95^{cB} \pm 0.03$
AIR	MC	$122.35^{aA} \pm 0.00$	$97.09^{cB} \pm 0.36$	$99.87^{bB} \pm 0.17$
(mg/g dry basis)	NDM	$109.26^{aB} \pm 2.18$	$89.60^{bC} \pm 0.39$	$87.15^{bC} \pm 2.56$
	CA	$122.37^{aA} \pm 0.28$	$118.68^{bA} \pm 0.46$	$117.16^{cA} \pm 0.17$
TP	MC	$96.69^{bC} \pm 1.25$	$104.93^{aB} \pm 1.17$	$107.72^{aC} \pm 1.84$
(µg/mg AIR)	NDM	$118.75^{cB} \pm 0.23$	$131.12^{bA} \pm 1.74$	$139.42^{aA} \pm 1.27$
	CA	$127.06^{aA} \pm 0.29$	$130.78^{aA} \pm 1.49$	$131.17^{aB} \pm 1.11$
WSP	MC	$57.80^{cC} \pm 0.41$	$81.97^{bC} \pm 1.02$	$89.85^{aB} \pm 0.56$
(µg/mg AIR)	NDM	$76.99^{cB} \pm 0.84$	$107.38^{bB} \pm 0.45$	$128.43^{aA} \pm 1.74$
	CA	$97.84^{cA} \pm 0.85$	$118.72^{bA} \pm 0.52$	$125.66^{aA} \pm 1.95$

MC = Maha Chanok, NDM = Nam Dok Mai, CA = Chok Anan, AIR = Alcohol-insoluble residue, TP = Total pectin, and WSP = Water-soluble pectin

Mean values in the same row followed by different letters (a–c) are significantly different (DMRT's test: $P \leq 0.05$).

Mean values in the same column in each attribute followed by different letters (A–C) are significantly different (DMRT's test: $P \leq 0.05$).

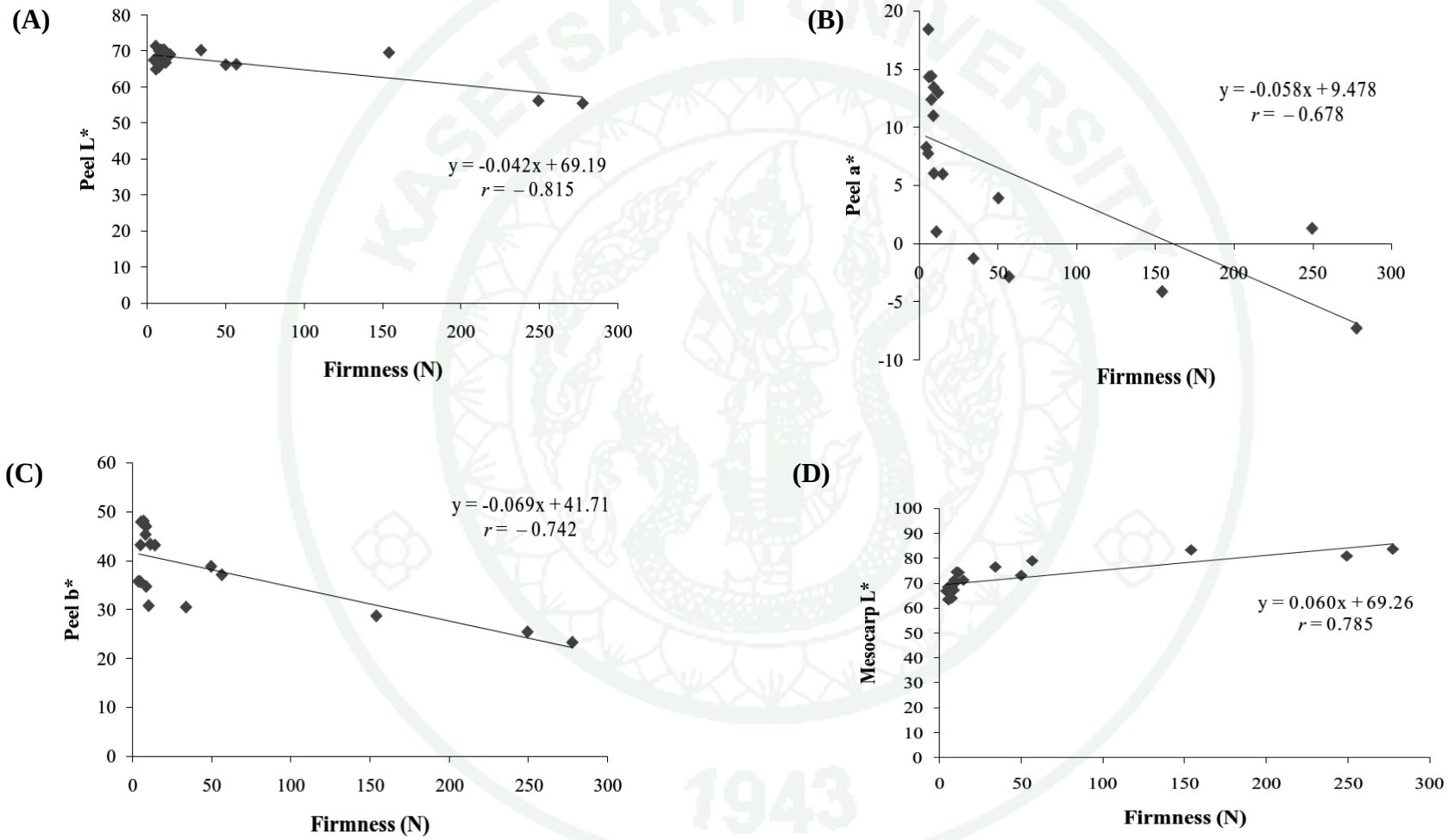
Appendix Table C6 Intensity score of firmness attribute of frozen-thawed mangoes.

Intensity score	Cultivar	Ripening stage		
		stage 3	**stage 4**	**stage 5**
Firmness	MC	$2.72^{aA} \pm 0.53$	$1.89^{bA} \pm 0.52$	$1.07^{cA} \pm 0.32$
	NDM	$1.44^{aB} \pm 0.55$	$0.89^{bC} \pm 0.14$	$0.49^{cB} \pm 0.14$
	CA	$1.92^{aB} \pm 0.43$	$1.28^{bB} \pm 0.33$	$0.60^{cB} \pm 0.14$

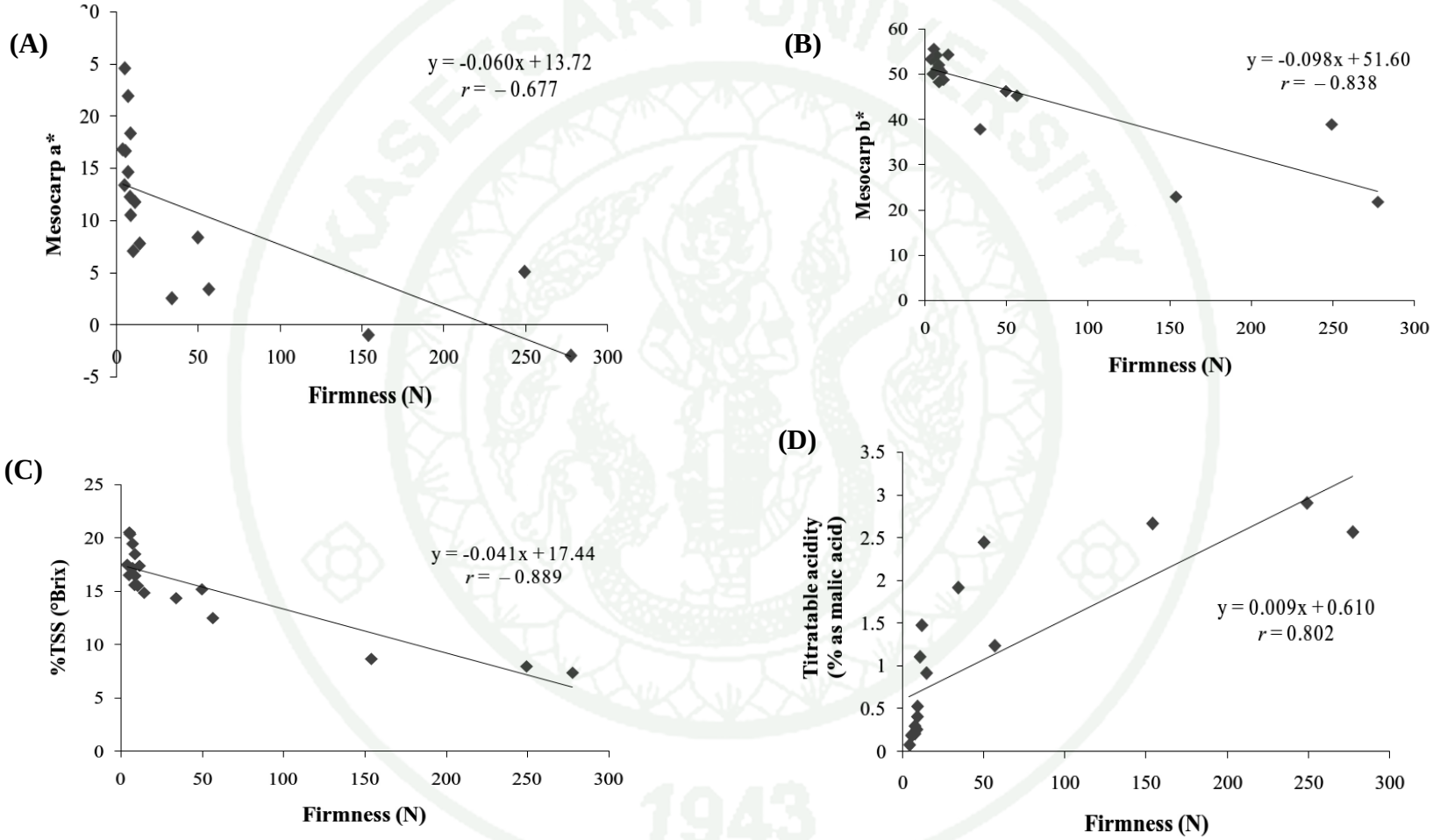
MC = Maha Chanok, NDM = Nam Dok Mai, and CA = Chok Anan

Mean values in the same row followed by different letters (a–c) are significantly different (DMRT's test: $P \leq 0.05$).

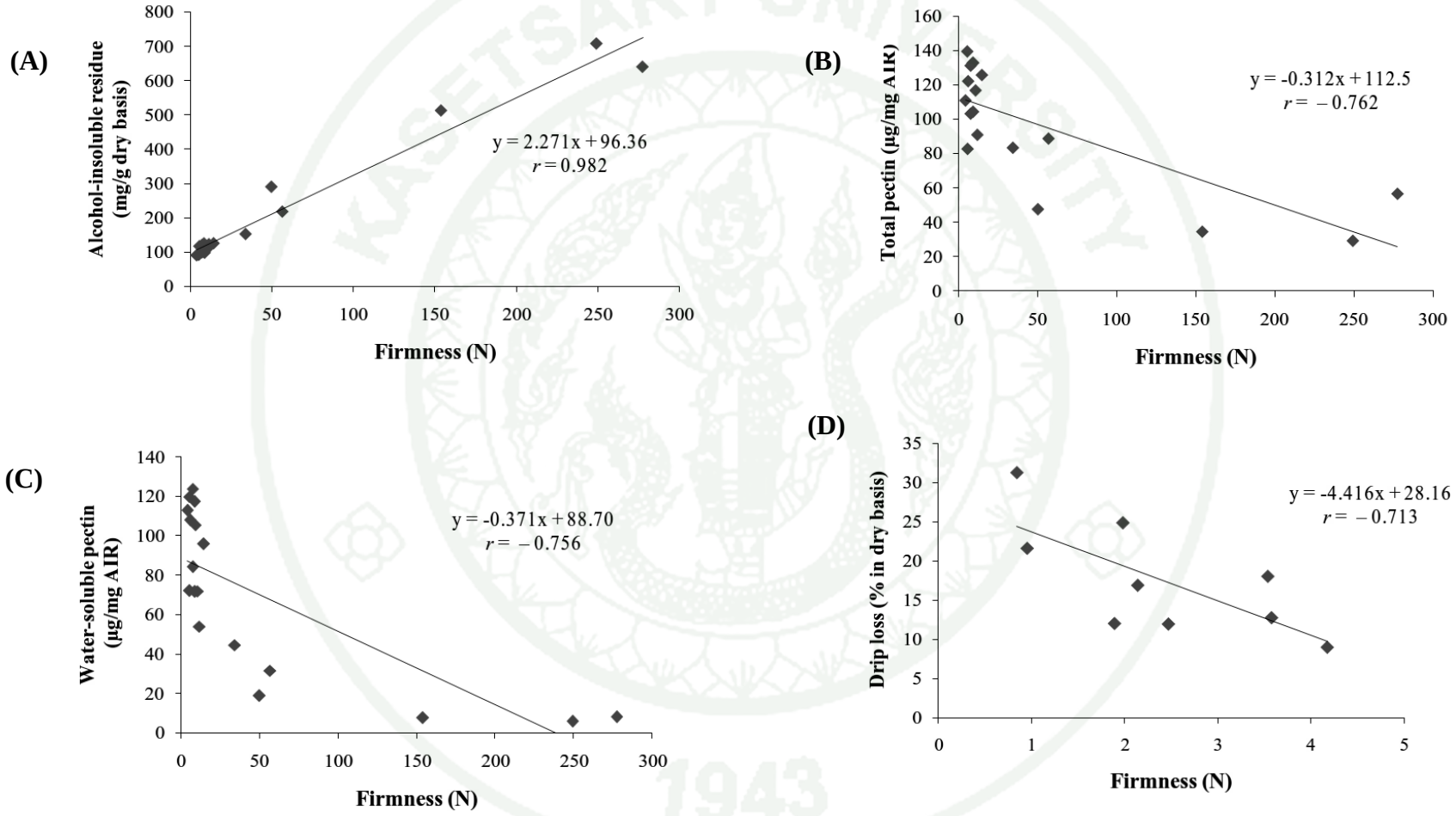
Mean values in the same column in each attribute followed by different letters (A–C) are significantly different (DMRT's test: $P \leq 0.05$).



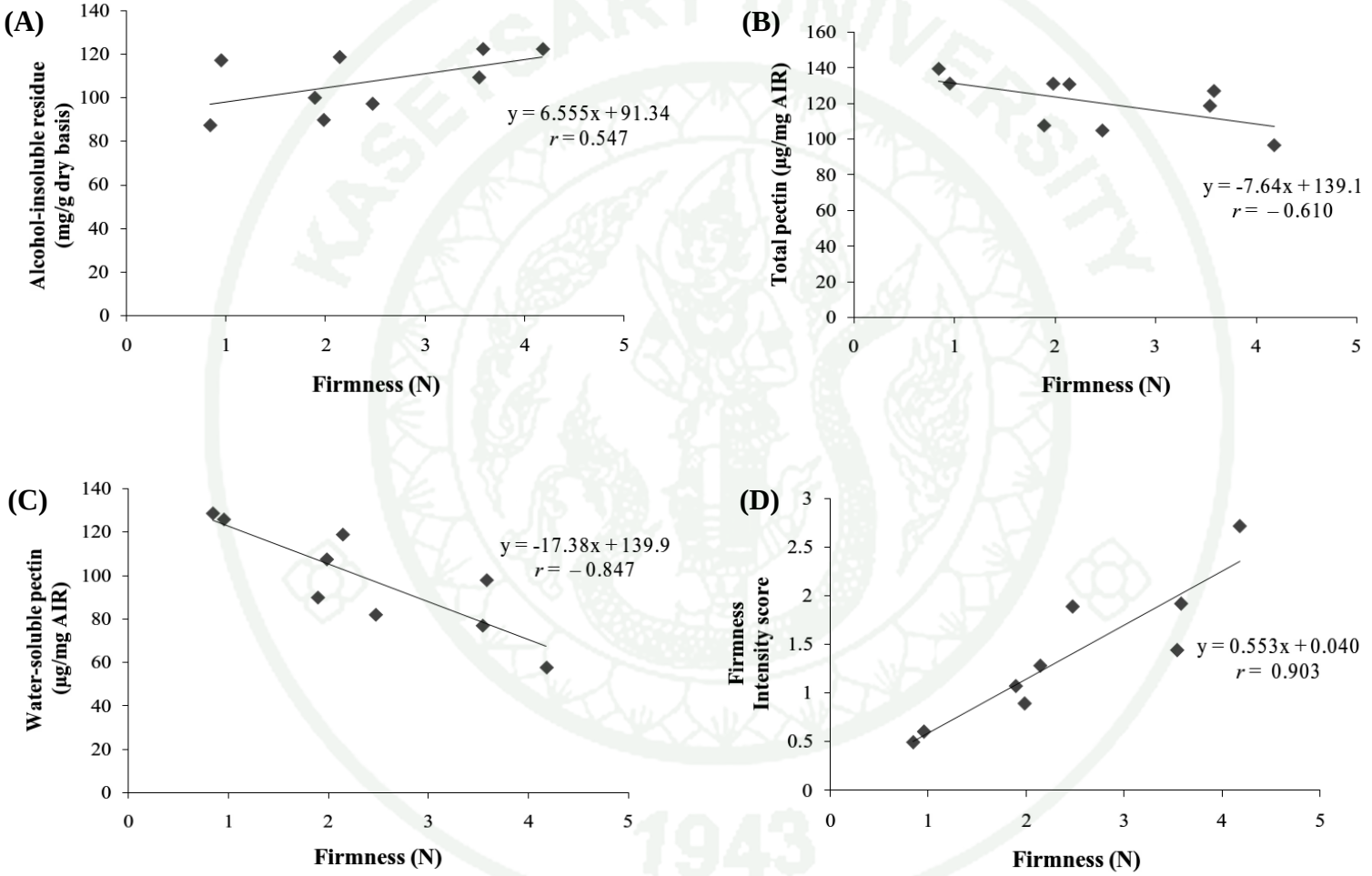
Appendix Figure C2 Correlation between firmness and peel L* (A), firmness and peel a* (B), firmness and peel b* (C) and firmness and mesocarp L* (D) of fresh mango during ripening.



Appendix Figure C3 Correlation between firmness and mesocarp a* (A), firmness and mesocarp b* (B), firmness and %TSS (C) and firmness and titratable acidity (D) of fresh mango during ripening.



Appendix Figure C4 Correlation between firmness and AIR (A), firmness and total pectin (B), firmness and WSP (C) of fresh mango and firmness and drip loss (D) of frozen-thawed mango.



Appendix Figure C5 Correlation between firmness and AIR (A), firmness and total pectin (B), firmness and WSP (C) and firmness and firmness intensity score (D) of frozen-thawed mango.

Appendix D

Sensory evaluation

D1 Sensory evaluation for trained panelists (Adapted from Meilgaard et al., 2007)

1.1 Selection

1.1 Detection tests

This selection test is used to determine a candidate's ability to detect differences among similar products with ingredient or processing variables. Duo-Trio test was used in this detection test. Present to each subject an identified reference sample, followed by two coded samples, one of which matches the reference sample. Ask subjects to indicate which coded sample matches the reference (Appendix Figure D1**)**. Count the number of correct replies. The candidates, who reply corrects more than 60%, are allowed to do next test. Agar at different concentrations was used in this test. Candidates have to repeat the test 3 times.

1.2 Ranking tests for intensity

These tests are used to determine candidates' ability to discriminate graded levels of intensity of a given attribute, which is firmness attribute. Three levels of agar concentration were use in ranking tests. Present the set of samples to each candidate in balanced, random order. Ask candidates to rank them according to the firmness attribute (Appendix Figure D2**)**. The Candidates, who reply all correct in the test, are allowed to do the training.

1.2 Training

To ensure development of a professional attitude to sensory analysis on the part of panelists, the training is conducted in a controlled professional sensory facility. Begin by presenting samples of the product. In this study a five-point scale of firmness were used to train. During training, concentrate initially on helping panelists

to understand the scope of the project and to gain confidence. Repeat the test method and allow the panel to learn through repetition until full confidence is achieved.

A five-point scale of firmness were used to train. Where 1 = extremely soft, 2 = soft, 3 = firm, 4 = very firm, 5 = extremely firm. The samples were prepared by three different concentration of agar (w/w). 0.7% agar refer to 1 score, 1% agar refer to 3 scores and 1.5% agar refer to 5 scores.

1.3 Evaluation

Present the set of frozen-thawed mangoes to each candidate in balanced, random order. Ask candidates to rank them according to the firmness intensity (Appendix Figure D3**)**.

Duo-trio test

Name:_____ Date:_____

Type of sample:_____

Instructions: Taste samples from left to right. The left hand sample is a reference. Determine which of the two sample matches the reference and indicate by placing an X.

Reference Code _____ Code_____

☒ ☐ ☐

Comments: _____

_____

Appendix Figure D1 Score sheet for duo-trio test.

Ranking Test

Name:_____ Date:_____

Type of sample:_____

Characteristic studied: _____Firmness_____

Instructions:

1. Receive the sample try and note each sample code below according to position on the tray.
2. Taste the samples from left to right and note the degree of firmness. Wait at least 30 seconds between samples and rinse palate as required.
3. Write "1" in the box of the sample which you find least firmness. Write "2" for the next, and "3" for the most firmness.

Code _____ Code _____ Code _____

Rank ☐ ☐ ☐

Comments: _____
_____

Appendix Figure D2 Score sheet for ranking test.

Sensory evaluation

Name:_____ Date:_____

Type of sample:_____

Characteristic studied: _____Firmness_____

Instructions: Taste samples from left to right and note the degree of firmness. Write "1" in the box of the sample which you find extremely soft.Write "2" or "3" or "4" for the next, and "5" for the extremely firm.

Code _____ Code _____ Code _____

Comments: _____

_____

Appendix Figure D3 Score sheet for firmness intensity test.

CURRICULUM VITAE

NAME : Miss Kannika Rimkeeree

BIRTH DATE : March 15, 1987

BIRTH PLACE : Surin, Thailand

EDUCATION :

YEAR	**INSITITUTE**	**DEGREE**
2009	Kasetsart University	B.S. (Food Science and Technology)

SCHOLARSHIP : Research Grant for International Publication, the Graduate School Kasetsart University.

PUBLICATION :

Kannika, R. and S. Charoenrein. Effect of cultivar and ripening stage on quality and microstructure of frozen mangoes (*Mangifera indica* L.). (Accept in International Journal of Food Properties)

PRESENTATION :

1. Kannika, R. and S. Charoenrein. 2010. Effect of ripening stage on textural quality of frozen mango flesh cv. Mahachanok. **Oral presentation in The 36th Congress on Science and Technology (STT 36).** BITEC Bangna Convention Centre, Bangkok, Thailand.

2. Kannika, R. and S. Charoenrein. 2011. Effect of ripening stages on quality and microstructure of frozen mango flesh (cv. Mahachanok). **Poster Presentation in the Institute of Food Technologists Annual Meeting & Food Expo (IFT 11)**. New Orleans, Louisiana, USA.

RESEARCH EXPERIENCE : Research assistant in project “Improving Thai frozen fruits to resist freeze-thaw process” funded by Thailand Research Fund.