ผลของการเคลือบผิวด้วยไคโตซานต่อคุณภาพหลังการเก็บเกี่ยวและอายุการเก็บรักษาผลมะม่วงน้ำดอกไม้

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EFFECTS OF CHITOSAN COATING ON POSTHARVEST QUALITY AND STORAGE LIFE OF 'NAM DOK MAI' MANGO FRUIT

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พรจันทร์ จงศรี : ผลของการเคลือบผิวด้วยไคโตซานต่อคุณภาพหลังการเก็บเกี่ยวและอายุการเก็บรักษาผลมะม่วงน้ำดอกไม้ (EFFECTS OF CHITOSAN COATING ON POSTHARVEST QUALITY AND STORAGE LIFE OF 'NAM DOK MAI' MANGO FRUIT) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.กนกวรรณ เสรีภาพ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร.ปราณี โรจน์สิทธิศักดิ์, ดร.ธีรดา หวังสมบูรณ์ดี, 171 หน้า.

มะม่วงน้ำดอกไม้ (*Mangifera indica*L. cv Nam Dok Mai) เป็นผลไม้ส่งออกที่มีความสำคัญของประเทศไทย อย่างไรก็ตามปัญหาหลังการเก็บเกี่ยวถือเป็นปัญหาหลักที่เป็นสาเหตุของการสูญเสียคุณภาพของผลมะม่วงระหว่างเก็บรักษาและการขนส่ง การศึกษาคุณภาพของผล 'มะม่วงน้ำดอกไม้' หลังการเคลือบผิวมะม่วงด้วยไคโตซานน้ำหนักโมเลกุลสูง (HM-CTS: 360 กิโลดาลตัน) ไคโตซานน้ำหนักโมเลกุลปานกลาง (MM-CTS: 270 กิโลดาลตัน) และไคโตซานน้ำหนักโมเลกุลต่ำ (LM-CTS: 40 กิโลดาลตัน) พบว่า ไคโตซานน้ำหนักโมเลกุลสูงแสดงประสิทธิภาพของฟิล์มไคโตซานที่ดีที่สุด โดยมีค่าความหนาของฟิล์มไคโตซานมากที่สุด (3.47±0.50 ไมโครเมตร) และฟิล์มปกคลุมผิวของผลมะม่วงได้อย่างสมบูรณ์ ทำให้ไม่ปรากฏปากใบบนผิวมะม่วงภายหลังการเคลือบผิวและลดการแลกเปลี่ยนแก๊สของผล ไคโตซานน้ำหนักโมเลกุลปานกลางมีค่าความหนาของฟิล์มไคโตซานที่น้อยกว่า (1.29±0.45 ไมโครเมตร) และไคโตซานน้ำหนักโมเลกุลต่ำแสดงค่าความหนาของฟิล์มไคโตซานที่น้อยที่สุด และพบปากใบบนผิวมะม่วงเช่นเดียวกับผลมะม่วงที่ไม่ได้เคลือบผิวด้วยไคโตซาน (ชุดควบคุม) ผลที่มีการเคลือบผิวด้วยไคโตซานน้ำหนักโมเลกุลสูงยังสามารถชะลออัตราการหายใจ การผลิตเอทิลีน การนิ่มของผล และการเปลี่ยนแปลงทางเคมีภายในผล (กรดและน้ำตาล) เมื่อเปรียบเทียบกับชุดการทดลองอื่น นอกจากนี้ ผลมะม่วงยังแสดงค่าการต้านอนุมูลอิสระที่สูงโดยแสดงปริมาณวิตามินซี ความสามารถในการต้านอนุมูลอิสระ (DPPH inhibition) ปริมาณฟลาโวนอยด์ การทำงานของเอนไซม์คะตะเลสและแอสคอร์เบทเปอร์ออกซิเดส และปริมาณไฮโดรเจนเปอร์ออกไซด์ที่ต่ำ ยิ่งไปกว่านี้ผลมะม่วงที่เคลือบผิวด้วยไคโตซานน้ำหนักโมเลกุลสูง ไม่แสดงการเกิดโรคเลยจนกระทั่งสิ้นสุดอายุการเก็บรักษา ดังนั้น การเคลือบผิวมะม่วงด้วยไคโตซานน้ำหนักโมเลกุลสูงจึงเป็นชุดการทดลองที่เหมาะสมที่สุดในการนำมาใช้เคลือบผล 'มะม่วงน้ำดอกไม้' ภายหลังการเก็บเกี่ยว การเติมสเปอร์มิดีน (0.1 1 และ 10 ppm) ในไคโตซานน้ำหนักโมเลกุลสูง เพื่อศึกษาผลของสเปอร์มิดีน พบว่า มะม่วงที่เคลือบผิวด้วยไคโตซานน้ำหนักโมเลกุลสูงเพียงอย่างเดียวและไคโตซานน้ำหนักโมเลกุลสูงร่วมกับสเปอร์มิดีน 0.1 ppm แสดงค่าความหนาของฟิล์มไคโตซานที่มากกว่าไคโตซานน้ำหนักโมเลกุลสูงร่วมกับสเปอร์มิดีน 1 และ 10 ppm (3.05±0.06 และ 3.07±0.06 ไมโครเมตร ตามลำดับ) ผลมะม่วงที่เคลือบด้วยไคโตซานน้ำหนักโมเลกุลสูงร่วมกับสเปอร์มิดีน 0.1 ppm สามารถปกคลุมปากใบบนผิวมะม่วง ผลมะม่วงที่เคลือบผิวด้วยไคโตซานน้ำหนักโมเลกุลสูงร่วมกับสเปอร์มิดีนสามารถชะลออัตราการหายใจและการผลิตเอทิลีน และผลมะม่วงที่เคลือบผิวด้วยไคโตซานน้ำหนักโมเลกุลสูงร่วมกับสเปอร์มิดีน 0.1 ppm ยังสามารถคงน้ำหนักสด ความแน่นเนื้อ สีผิว และควบคุมการเจริญของเชื้อได้อีกด้วย โดยทั่วไป การนิ่มของผลเกิดขึ้นเมื่อผลสุกโดยการทำงานของเอนไซม์ที่ย่อยสลายผนังเซลล์ (พอลิกาแลกทูโรเนส (PG) และเพกทินเมทิลเอสเตอร์เลส (PME)) และปริมาณของเพกทินที่ละลายในน้ำได้ที่เพิ่มขึ้น ผลมะม่วงที่เคลือบผิวด้วยไคโตซานน้ำหนักโมเลกุลสูงร่วมกับสเปอร์มิดีนมีปริมาณของเพกทินที่ละลายในน้ำได้ต่ำ และการทำงานของเอนไซม์ PG และ PME ต่ำกว่าชุดควบคุม (ไคโตซานน้ำหนักโมเลกุลสูง) โดยเฉพาะอย่างยิ่งในผลมะม่วงที่เคลือบผิวด้วยไคโตซานน้ำหนักโมเลกุลสูงร่วมกับสเปอร์มิดีน 0.1 ppm มีค่าที่ต่ำที่สุด นอกจากนี้ ปริมาณสเปอร์มิดีนภายในผลยังถูกกระตุ้นให้เพิ่มขึ้นในผลมะม่วงที่เคลือบผิวด้วยไคโตซานน้ำหนักโมเลกุลสูงร่วมกับสเปอร์มิดีน 0.1 ppm ดังนั้น ไคโตซานน้ำหนักโมเลกุลสูงร่วมกับสเปอร์มิดีน 0.1 ppm เป็นสารเคลือบผิวที่เหมาะสมที่สุดในการคงคุณภาพของผล 'มะม่วงน้ำดอกไม้'ภายหลังการเก็บเกี่ยว

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PORNCHAN JONGSRI: EFFECTS OF CHITOSAN COATING ON POSTHARVEST QUALITY AND STORAGE LIFE OF 'NAM DOK MAI' MANGO FRUIT. ADVISOR: ASST. PROF. KANOGWAN SERAYPHEAP, Ph.D., CO-ADVISOR: ASST. PROF. PRANEE ROJSITTHISAK, Ph.D., TEERADA WANGSOMBOONDEE, Ph.D., 171 pp.

'Nam Dok Mai' mango (*Mangifera indica*L. cv Nam Dok Mai) is an important exported fruit of Thailand. However, postharvest problems are the main problem which cause the reduction of fruit qualities during transportation and storage. Qualities of 'Nam Dok Mai' mango fruit were investigated after coating with high molecular weight chitosan (HM-CTS: 360 kDa), medium molecular weight chitosan (MM-CTS: 270 kDa) and low molecular weight chitosan (LM-CTS: 40 kDa). Our results demonstrated that HM-CTS had the best chitosan coating film properties by representing the thickest of coating film on mango peel (3.47±0.50 µm) and it completely covered mango surface. Stomata were not observed after coating hence reducing gas exchanges. Fruit coated with MM-CTS showed thinner film (1.29±0.45 µm) than HM-CTS while LM-CTS showed the least thickness of chitosan film among treatments. Stomata of LM-CTS fruit were detected on mango peel as same as uncoated fruit (control). Fruit coated with HM-CTS could delay respiration rate, ethylene production, weight loss, fruit softening and changes of physicochemical (TA and TSS) when compared with other treatments. Moreover, chitosan coated fruit exhibited high antioxidant contents by presenting high contents of ascorbic acid, DPPH inhibition, free flavonoids, high activities of catalase and ascorbate peroxidase and low content of H_2O_2. Furthermore, fruit coated with HM-CTS did not show any disease symptom until the end of storage life. Therefore, HM-CTS was the optimum treatment for using as fruit coating in 'Nam Dok Mai' mango after harvest. Combination of HM-CTS and spermidine (SPD) (0.1, 1 and 10 ppm) were applied on 'Nam Dok Mai' mango to evaluate the effect of SPD addition. HM-CTS alone and HM-CTS combined with 0.1 ppm SPD showed thicker chitosan film than HM-CTS combined with 1 and 10 ppm SPD (3.05±0.06 µm and 3.07±0.06 µm, respectively). Fruit coated with HM-CTS combined with 0.1 ppm SPD fully covered stomata on mango peel. Delayed respiration rate and ethylene production were obtained in fruit coated with HM-CTS combined with SPD. Fruit coated with HM-CTS combined with 0.1 ppm SPD also maintained fresh weight, fruit firmness, peel color and controlled disease development. Fruit softening usually occurs during ripening stage caused by increased cell wall degrading enzyme activities (polygalacturonase (PG) and pectin methyl esterase (PME)) and soluble pectin content. An addition of SPD resulted in lower soluble pectin content and PG and PME activities than control treatment (HM-CTS alone), especially fruit coated with HM-CTS combined with 0.1 ppm SPD showed the lowest values. In addition, endogenous SPD was induced in fruit coated with HM-CTS combined with 0.1 ppm SPD. Therefore, HM-CTS combined with 0.1 ppm SPD was the optimum coating for improving qualities of 'Nam Dok Mai' mango after harvest.

Department: Botany	Student's Signature ----------
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CHAPTER I

INTRODUCTION

RATIONALES

Mango (*Mangifera indica* L.) is one of the most popular and widely distributed fruit in the world (Kim, Brecht and Talcott, 2007) because of its excellent flavor and good source of ascorbic acid, carotenoids, phenolic compounds and vitamin A (Pott et al., 2003; Ulloa et al., 2009). More than 70% of mango production is from Asia which include Thailand (Malik and Singh, 2005). Mango is the major important export fruit of Thailand which had export statistics at 33,000 tons in 2015 (Office of Agricultural Economics (OAE), 2016). However, mango is a climacteric fruit which emits ethylene during ripening thus accelerating ripening and softening processes after harvest (Baldwin et al., 1999; Mahto and Das, 2013). Moreover, anthracnose disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is the major postharvest disease of mango (Jitareerat et al., 2007). These are serious postharvest problems of mango fruit that cause reduction in qualities of fruit during storage, transportation, and marketing period (Mitra and Baldwin, 1997; Jitareerat et al., 2007; Abbasi et al., 2009). Fungicides are used prior to harvest and after harvest to reduce the disease problems which lead to many concerns over toxic residues in the produces (Dodd, Jeffries and

Jeger, 1989). Hence, it is necessary to develop postharvest technologies as alternatives to better maintain overall mango fruit quality during the export chain or market period.

Nowadays, edible coating is one of the popular technique that has been used on fruits and vegetables to extend shelf life and maintain fruit qualities during storage, transportation and marketing period (Min and Krochta, 2007; Pereda, Aranguren and Marcovich, 2008; Abbasi et al., 2009; Yang et al., 2014). Fruit coatings can generate a modified atmosphere (MA) of fruit by limiting gas permeability characteristics, especially to O_2, CO_2, humidity and ethylene resulted in reduced fruit respiration, retarded fruit ripening, water loss, and reduced decay (Baldwin et al., 1997; Amarante and Banks, 2001). Therefore, fruit coating is a proper technique which modifies the composition of the atmosphere surrounding the fruit to improve shelf life (Nisperos-Carriedo, Shaw and Baldwin, 1990; Baldwin et al., 1995). Coatings have long been used on tomatoes (mineral oil), citrus, pomegranate and apples (shellac and carnauba wax) and cucumbers (various waxes) (Abbasi et al., 2009; Barman, Asrey and Pal, 2011).

Chitosan (poly-β-(1,4)-D-glucosamine), a deacetylated form of chitin (a biodegradable polysaccharide), has film-forming property (Shahidi, Arachchi and Jeon, 1999) which can be applied as fruit coating. Previous studies indicated that chitosan coating had the potential to prolong storage life in many fruits and vegetables by controlling decay, reducing respiration rates, ethylene production, and transpiration (Ribeiro et al., 2007; Duan et al., 2011). Moreover, chitosan can created MA by

performing as gas barrier leading to limited gas exchange and fungal growth (Jiang and Li, 2001; Chien, Sheu and Lin, 2007). Furthermore, chitosan as an exogenous elicitor can affect the activities of several pathogenic defense-related enzymes and induce the accumulation of special substances in many plants (Cabrera et al., 2006; Trotel-Aziz et al., 2006; Meng et al., 2010), which are known to participate in defense mechanisms and prevent pathogen infections. Meng et al. (2008) indicated that preharvest chitosan spray or/and postharvest chitosan coating induced the activities of defense-related enzymes (polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL)), thus promoting protection of latent infection of pathogens in grape fruit. In addition, the extension of the storage life of 'Nam Dok Mai' mango were reported by coating 'Nam Dok Mai' mango fruit with 1% chitosan (Mw 350000 Dalton) that could maintain ascorbic acid content, weight loss, peel color change, and total titratable acidity. The coating could also reduce respiration rate and ethylene production and increase chitinase and β-1,3-glucanase activities (Jitareerat et al., 2007). However, differences in molecular weight of chitosan coating were not considered as an important role that might create the various effects of chitosan coating on 'Nam Dok Mai' mango fruit after harvest.

Another popular postharvest treatment is using polyamines to delay fruit ripening. Polyamines (PA), including spermidine (SPD, a triamine), spermine (SPM, a tetramine), and their obligate precursor putrescine (PUT, a diamine), are aliphatic

amines widely present in living organisms (Bouchereau et al., 1999; Malik and Singh, 2003; Takahashi et al., 2003). It has been illustrated that polyamines also play pivotal roles in plant physiological and developmental processes, such as morphogenesis, pollen viability, fruit ripening and senescence (Pandey et al., 2000; Ziosi et al., 2006). Exogenous PAs could induce endogenous PAs in litchi fruit leading to inhibited ethylene production, peroxide level and fruit browning so it could prolong shelf life of fruit (Jiang and Chen, 1995). Moreover, previous study in 'Lisbon' lemon treated with exogenous PUT and SPD showed higher firmness than control fruit and prevented chilling injury under cold storage (Safizadeh, 2013). Polyamines treatment correlated with cell wall degrading enzymes activity leading to maintain structure of membrane and fruit firmness and inhibit fruit softening which was reported in apple and strawberry fruits treated with SPM and SPD (Kramer, Wang and Conway, 1991; Ponappa, Scheerens and Miller, 1993). (Santivipanond, Jantaro and Seraypheap (2012)) reported that 'Hom Thong' banana treated with 0.1 ppm SPD could maintain fruit firmness, fresh weight and ascorbic acid content better than untreated fruit and treatment could retard shelf life of banana fruit. However, there is few research thoroughly study the effect of PAs on 'Nam Dok Mai' mango fruit qualities after harvest. In addition, the responses of endogenous PAs on exogenous spermine in 'Nam Dok Mai' mango should be examined. Therefore, we developed the best formulated mango fruit coating by combining chitosan and spermidine which could improve 'Nam Dok Mai' mango fruit

qualities. The responses of ‘Nam Dok Mai’ mango fruit under commercial storage condition were investigated.

Objectives

1. To develop and select the appropriate chitosan coating to prolong shelf-life of mango fruit during storage.
2. To investigate the effects of the appropriate chitosan coating combined with spermidine on qualities of mango fruit.
3. To determine the effect of the appropriate chitosan coating combined with spermidine on anthracnose disease in mango fruit.

Expecting benefits

1. This research will lead us to further understanding the effects of chitosan coating combined with spermidine on shelf-life and quality of ‘Nam Dok Mai’ mango.
2. Chitosan coating combined with spermidine can also be beneficial coating for commercial use in postharvest storage of ‘Nam Dok Mai’ mango fruit.

Contents of the thesis:

1. Literature reviews
2. Development and selection of an appropriate chitosan coating to prolong shelf-life of mango fruit during storage.
3. Investigation of the effects of the appropriate chitosan coating combined with spermidine on qualities of mango fruit.
4. Determination of the effect of an appropriate chitosan coating combined with spermidine on anthracnose disease in mango fruit.
5. Results and discussions
6. Conclusions

CHAPTER II

LITERATURE REVIEWS

2.1 Mango

Thailand is one of the top five producers of mango (Food and Agriculture Organization Corporate Statistical Database (FAOSTAT), 2012). The export of mango reached 33,000 tons in 2015 (Office of Agricultural Economics (OAE), 2016). 'Nam Dok Mai' mango (*Mangifera indica* L. cv. 'Nam Dok Mai') is one of the major export varieties of mangoes from Thailand. It presents a high source of nutritionals such as ascorbic acid and vitamin A (Pott et al., 2003; Ulloa et al., 2009). However, mango is climacteric fruit which exhibit high respiration rate and ethylene production after harvest (Abbasi et al., 2009; Mahto and Das, 2013). Therefore, mango fruit always exhibits loss of fresh weight, fruit softening and fruit qualities during ripening stage and marketing period leading to short shelf life after harvest (Jitareerat et al., 2007; Barbosa-Martínez, Ponce de León-García and Pelayo-Saldivar, 2009). Moreover, anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is the major postharvest disease of mango. In general, fruit infection occurs in the orchard and pathogen can attack various parts of mango plant and immature fruit. Mango fruit commonly displays disease symptom when entering fruit ripening stage (Muirhead and Gratitude, 1986; Dodd, Prusky and Jeffries, 1997; Barkai-Golan, 2001; Jitareerat et al., 2007). These

problems of postharvest mango are considered seriously because they cause reduction in qualities of fruit during storage, transport, and market period (Wills and Scott, 1972; Mitra and Baldwin, 1997). Different treatments have been alternatively used to inhibit losses and extend the shelf life of mango fruit.

2.1.1 Loss of mango fruit qualities after harvest

2.1.1.1 Fruit ripening

The major factor which correlated with climacteric fruit ripening is ethylene production. Ethylene is gaseous plant hormone which can induce physiological and developmental processes in plants especially fruit ripening. Ethylene biosynthesis is started by using methionine as the precursor. Methionine is changed to S-adenosylmethionine (SAM) by methionine adenosyltransferase. Then, the SAM converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by action of ACC synthase and ethylene is finally obtained from ACC using the enzyme ACC oxidase (Figure 1). Fruit ripening is the necessary physiological change which usually happened during the latest stage of fruit development and the earliest stage of senescence (Asif and Nath, 2005). Ripening process is generally changing of physiological and biochemical characters such as texture, aroma and flavor. Since, many metabolic pathways occur during ripening stage resulted in changing of biochemical mechanisms of fruit (Goulao and Oliveira, 2008). Fruit ripening will be started when ethylene concentration reaches

the threshold level, fruit will be at fruit maturity stage. However, fruit will be in this stage earlier, if the fruit is in the stresses condition such as pathogen infection, water limitation or physical damages during handling and marketing period (Thompson, 2003). Exogenous ethylene induced the ripening and endogenous ethylene production. Ethylene molecule can bind with ethylene receptor in the cell of fruit peel then elicit the signal transduction leading to induce ripening processes (Johnson et al.; Fluhr and Mattoo, 1996) (Figure 1).

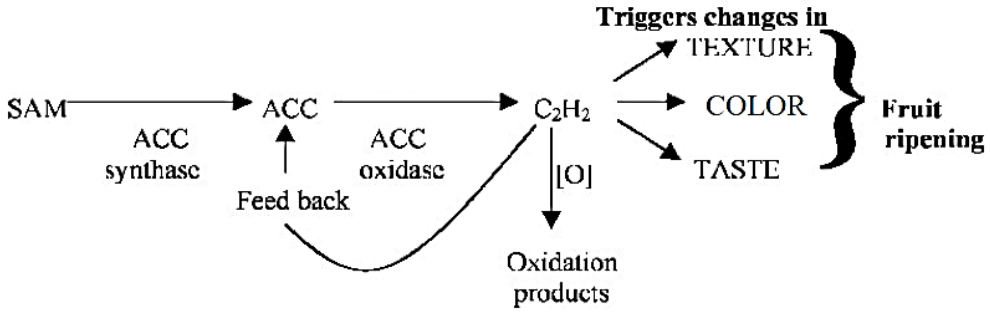


Figure 1. Ethylene biosynthesis pathway related to fruit ripening (Prasanna, Prabha and Tharanathan, 2007).

Peel color is one of the major parameter which is easily observed during ripening process. Changing of peel color during ripening process is related to degrading of chlorophyll that presents pigments of fruit. Peel color generally changes from green

to yellow or degreening when fruits are kept for a long time. It is the main external characteristic which occurred during ripening stage of fruit (Lizada et al., 1990; Siriphanich, 1995; Li et al., 1997). Peel color of mango fruit is related to the number of pigments in fruit peel, mainly chlorophylls and carotenoids (Meddlicott et al., 1992). The degreening of mango peel occurs during ripening stage due to chlorophyll breakdown. During fruit ripening, chlorophyll is degraded by catalytic enzyme leading to colorless and unmasked carotenoid pigment in plastids (Matile, Hortensteiner and Thomas, 1999).

Flavor is one of the parameters that usually reported with fruit taste and texture during ripening. Increasing of soluble solids and volatiles compounds and decreasing of organic acids and phenolic compounds are normally found during fruit ripening. Climacteric peak of respiration and ethylene productions resulted in production of volatile compounds leading to various aromatic compounds and changing of starch to sugars which make fruit sweet and soften (Manrique-Trujillo et al., 2007). The development of sweetness is mainly due to the hydrolysis of starch to sugar (Pantastico, 1975). Moreover, decreasing of acid was found during fruit ripening (Palmer, 1971). Generally, many acids are used as substrates in Krebs cycle (respiration process) therefore high respiration rate of climacteric fruit leading to the reduction of acid content in fruit (Seymour et al., 1990).

2.1.1.2 Fruit softening

Fruit ripening is closely associated with increases in cell wall degrading enzymes (Manrique-Trujillo et al., 2007). Changing of fruit texture during ripening process is related to fruit firmness and softening and changing of metabolic metabolism including loss of cell wall turgidity, degradation, and physiological changes in membrane, and modification of cell wall structure (Goulao and Oliveira, 2008). Cell wall shows complex network structure which supports structural of plant cell. It contains various polysaccharides, such as pectin, hemicelluloses, and cellulose microfibrils (Prasanna et al., 2007) (Figure 2). Fruit softening which is a necessary characteristic of the fruit ripening is related to structural changing within pectin matrix. Previous report indicated that cell wall changes associated with fruit softening during ripening usually occur in pectin, hemicelluloses, and cellulose polysaccharides (Seymour et al., 1990). Pectin is the most abundant class of polysaccharides in the cell wall matrix and the middle lamella between the primary cell walls (Willats et al., 2001). Many reports revealed that water-insoluble pectin is converted to water-soluble pectin during softening process by cell wall degrading enzyme leading to loosening of cell wall structure and textural changes (Majumder and Mazumdar, 2002; Manrique and Lajolo, 2004; Yashoda, Prabha and Tharanathan, 2005).

Polygacturonase (PG) is pectin degrading enzyme which associated with fruit softening, catalyzes the hydrolysis of the glycosidic bonds in galacturonans (Figure 3).

PG controls intercellular connections and have influenced on fruit texture (Wei et al., 2010). Effect of PG activity on fruit softening was reported in many plants. High activity of PG presented high soluble pectin and fruit softening and PG was the primary enzyme that responded to fruit ripening (Crookes and Grierson, 1983; Fischer and Bennett, 1991).

Pectin methyl esterase (PME) catalyzes the hydrolysis of methyl esters from the C6 position of galacturonic acid residues leading to pectin deesterification and available for degrading by PG (Koch and Nevins, 1989; Sozzi, 2004) (Figure 3). PME activity had been presented in many processes such as maturation, abscission and pathogen infection (Blumer et al., 2000). Moreover, PME activity was also found during ripening process in fruits such as mango, papaya and banana (Goulao and Oliveira, 2008). The study in tomato indicated that PME activity was presented during fruit development and ripening. Level of PME activity increased in ripening stage and showed the highest level in early stage of fruit ripening (Harriman, Tieman and Handa, 1991; Tieman et al., 1992).

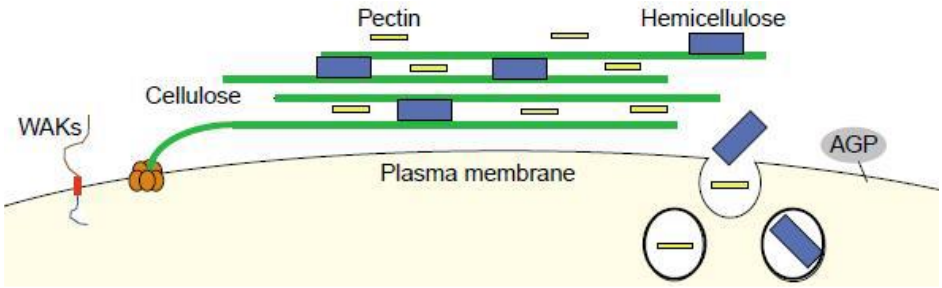


Figure 2. Cell wall structure (Kohorn, 2001).

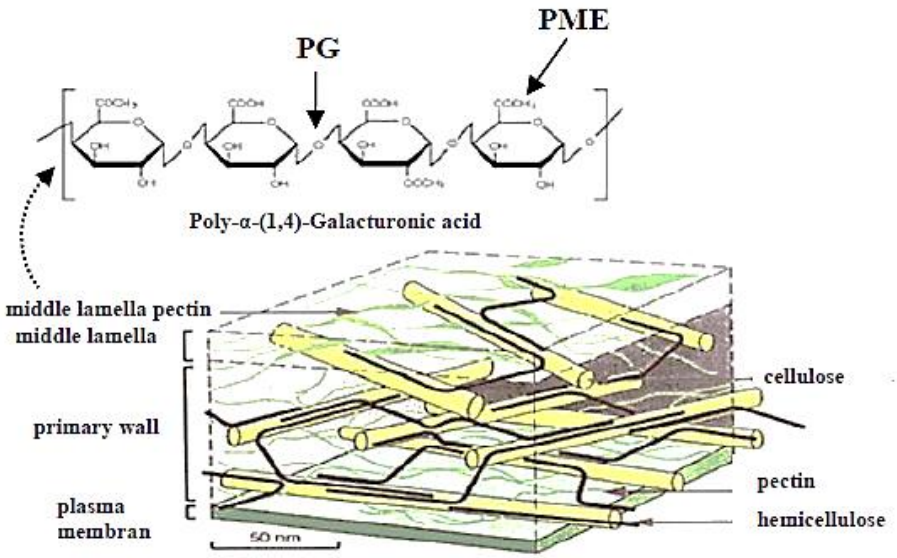


Figure 3. Function sites of polygalacturonase (PG) and Pectin methyl esterase (PME).

(International pectin producers association (IPPA), 2016).

2.1.1.3 Anthracnose of mango

Anthracnose is the main pre and postharvest disease on mango fruit. Anthracnose is caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph: *Glomerella cingulata*) (Dodd et al., 1997). In Thailand, anthracnose is the important disease of commercial mango in many cultivars especially mango cv. 'Nam Dok Mai', causing yield loss and losing of quality during marketing period and export chain (Sangchote, 1987; Ploetz and Freeman, 2009). Disease presents as quiescent infections on immature fruit then the disease symptom occurs in the postharvest period (Muirhead and Gratitude, 1986; Dodd et al., 1997).

1) Disease symptom

The fungus infects various parts of mango such as leaves, flower clusters (panicles), and fruits. On leaves, disease symptom occurs as small, angular, brown to black spots then spots develop and dead areas are increased. The start symptoms on panicles are small black or dark-brown spots then the sports merge together and flowers are destroyed before fruits are produced, and significantly decrease fruit yield. Disease symptom occurs in ripening stage of fruits by developing of sunken, dark brown to black spots during pre or postharvest. The small fruit spots can combine together and penetrate deep into the fruit pulp leading to be fruit rotting. Mature green infected fruit show latent infection and no disease symptom until ripening period. Therefore,

fruits remain appeared healthy at harvest time and anthracnose symptoms occur swiftly at ripening stage (Ploetz, 1999; Nelson, 2008). Mostly, lesions on stems and fruits produce obvious pinkish-orange spore masses under wet and humid conditions. In general, high moist and humid conditions are good conditions for anthracnose infection and development (Ploetz and Prakash, 1997; Tarnowski and Ploetz, 2008).

2) Anthracnose cycle

Spores can disperse by splashing rain or irrigation water under high humidity (more than 95%) and moisture then spores land on infection sites (Figure 4). Spores germinate and penetrate through the cuticle and epidermis by penetration peg that the appressorium produces (Jeffries et al., 1990). The fungus presents quiescent infection. Disease symptoms develop when concentration of fungal inhibitors in fruit decrease during the ripening stage of the climacteric fruit began. Mango shows small black spot and sunken in the early stage of disease development, lesions rapidly develop on fruit surface then expand through flesh and damage whole fruit. Sticky masses of conidia are produced within fruiting bodies (acervuli) on tissue, particularly during high moist and humid conditions. Cycle of disease can continuously occur during the season (Nelson, 2008).

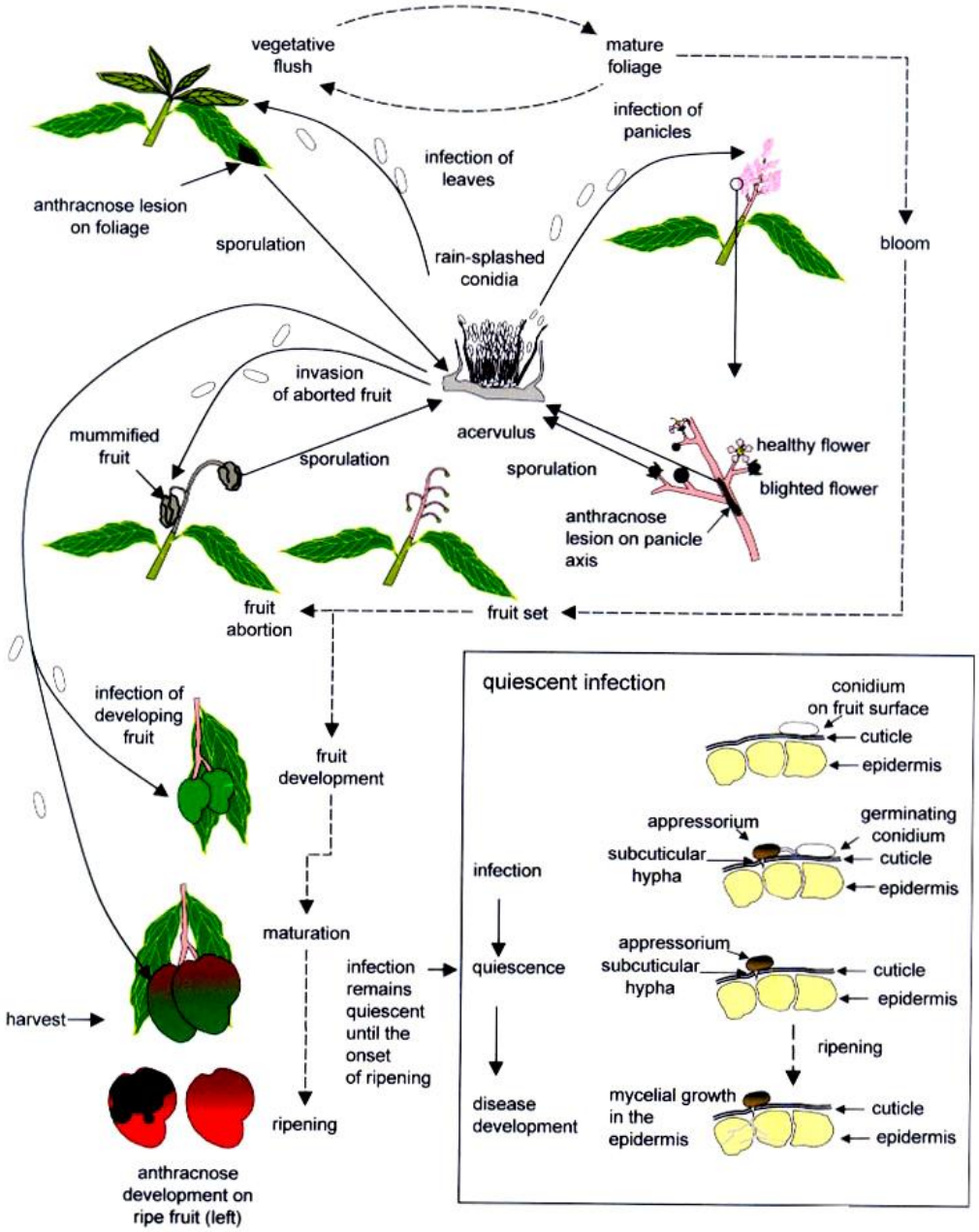


Figure 4. Anthracnose disease cycle (Arauz, 2000).

2.1.2 Postharvest treatments of fruit and vegetable

2.1.2.1 Heat treatment

Heat treatment especially hot water (HWT) treatment was broadly used in many fruits including mango (Aveno and Orden, 2004). HWT, mainly associated with a disease control treatment, was applied in commercial fruit in some countries depending upon its efficacy (Jacobi, Wong and Giles, 1995; Anwar and Malik, 2007). Mango fruit treated with HWT at 52-55°C for 10 min after harvest could decrease 83% of the anthracnose disease and 100% of stem-end rot (Aveno and Orden, 2004). The temperature and time period of the treatment correlated with size or weight of the fruit and maturity stage (Jacobi, MacRae and Hetherington, 2001). Moreover, fruit cultivar, growing conditions and type of disease were also correlated with HWT adaptation (Anwar and Malik, 2007). Furthermore, HWT treatment showed positive effect to enhance qualities of fruit as a result of lower fruit damaging and higher marketing value (Aveno and Orden, 2004). Vapor heat treatment (VHT), as high humidity heating, the process presented heating air with high moisture content and passed the air steam through the fruit (Jacobi et al., 2001). The heat from fruit peel was transferred to fruit pulp (Jordan, 1993). VHT has been used in commercial for improving qualities of mango in many countries such as Thailand, Philippines, America and Australia (Sunagawa, Kume and Iwaizumi, 1987). In marketing period, the accepted protocol for 'Carabao' mango in Philippine was heating at 46°C for 10 min, 'Kensington' mango in

Australia was heating at 47°C for 15 min and 'Nam Dok Mai' mango in Thailand was heating at 47°C for 10 min (Heather, Corcoran and Kopittke, 1997). Forced hot air heating (FHAT), was passing of air heating to fruit at specified temperature. The difference between VHT and FHAT was fruit peel in FHAT still dry when compared with VHT (Armstrong, 1996), FHAT presented relative humidity (RH) approximately 30% (Hallman and Armstrong, 1994). FHAT was accepted to use in United States for developing mango fruit qualities (Mangan and Ingle, 1992). Moreover, the treatment was also applied in other fruit such as papaya in Hawaii (Armstrong, 1996).

2.1.2.2 Modified or controlled atmospheres (MA/CA)

The application of modified atmosphere (MA) and controlled atmosphere (CA) were wildly used in pre-harvest and postharvest technology. It could retard the storage life and maintain the overall quality of fruit. MA and CA presented higher CO_2 and lower O_2 than common air atmosphere thus delaying ripening process by reducing the ethylene production, and inhibiting the deteriorative processes of fruit leading to maintained peel and pulp color, flavor and texture (fruit firmness), and induced oxidative process for pathogens resistance (Prusky and Keen, 1993). A reduce of O_2 concentration at 3-5% and an increase of CO_2 concentration at 5-10% were the suitable atmosphere compositions for using in MA/CA system of mango fruit (Yahia, 2009). 'Tommy Atkins' mango stored in CA (3% O_2 and 10% CO_2), treatment could control disease when compared with untreated fruit (Kim et al., 2007). Application of the

microperforated bag (MP bag) with 1-methylcyclopropene (1-MCP) on ‘Manila’ mango revealed that 1-MCP treatment delayed fresh weight loss and shrivel of fruit resulted in maintaining fruit firmness which related to cell wall degrading enzyme activities (Pectinesterase (PE) and polygalacturonase (PG)) in exocarp tissue (Vázquez-Celestino et al., 2016). However, MA/CA may present the anaerobic respiration of fruit which caused the fermentation in fruit. ‘Nam Dok Mai’ mango fruit coated with chitosan at 1.5% and 2% showed discoloration of mango peel, unripe fruit and off flavor because of the formation of acetaldehyde and ethanol (Jitareerat et al., 2007).

2.2 Edible film

Nowadays, minimally-processed fruits and vegetables were extremely popular and in high demand. However, the consumers beware of toxic residue (Olivas et al., 2008), therefore, it is very important to develop a safe method for minimally-process of fruits and vegetables. One trendy method is using edible coatings. Many materials were used for producing edible coatings. Coating could maintain quality and retard storage life of fruits and vegetables (Min and Krochta, 2007; Pereda et al., 2008). Since, edible coatings exhibited forming film properties which served as a partial barrier to gases (O_2 and CO_2), water vapor and aroma compounds. Coating created a modified atmosphere (MA) within the fruit leading to reduction in rate of respiration, ethylene production, water loss, and maintaining fruit texture and flavor (Campos-Olivas, Newman and Summers, 2000; Chen and Nussinovitch, 2000). Coatings also preserved

antioxidants and nutrients in fruits (Olivas and Barbosa-Cánovas, 2005). In addition, edible coating could reduce enzymatic browning resulted in retaining of antioxidants (Vojdani and Torres, 1990).

2.2.1 Polysaccharide

Polysaccharides including cellulose and its derivatives, chitin-chitosan and starch, can be a good material for coating film which create gas and water barrier properties leading to a retardation of moisture loss from food products (Kester and Fennema, 1986; Krochta and Mulder-Johnston, 1997).

Cellulose and its derivatives

Cellulose, the most naturally occurring biopolymer, is composed of repeated D-glucose units linked through β-1,4 glycosidic bonds (Figure 5). Hydroxymethyl groups of anhydroglucose residues are settled in above and below of the polymer structure so its structure shows very tight polymer chains and stable structure in aqueous media (Olivas et al., 2008). Cellulose and its derivatives have been used as edible film for shelf life retardation during storage period in many fruits and vegetables since coating exhibited good film-forming characteristic such as adaptable, resistance to oil, water and gas transmissions (Krochta and Mulder-Johnston, 1997). Previous report in apple indicated that fresh-cut apples coated with cellulose maintained antioxidant and prolonged the shelf-life of fruit when kept in overwrapped at trays 4ºC for 1 week

(Baldwin et al., 1996). Methyl cellulous (MC) was applied as coating products and formed a barrier to lipid migration (Nelson and Fennema, 1991). 'Clemenules' mandarins fruit coated with hydroxypropyl methyl cellulose (HPMC) combined with bees wax exhibited better fruit texture and maintained higher fresh weight than uncoated fruit when stored at 4°C for 4 weeks and transferred to 4°C for 1 week (Navarro-Tarazaga and Perez-Gago, 2006).

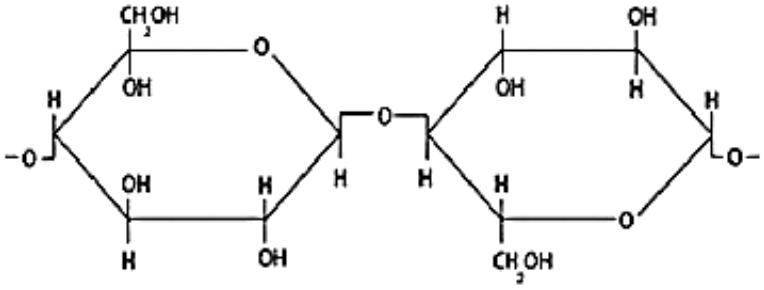


Figure 5. Structure of cellulose (Whistler and Daniel, 1985).

2.2.2 Lipid

Lipid film was generated from natural wax and surfactants and it consisted of acetylated monoglycerides. The most popular lipid materials were paraffin wax and beeswax. Lipid coating could inhibit transport of water because of the polarity. Normally, when the concentration of hydrophobic phase enhanced, the water vapor permeability was reduced. Lipid-based films were usually supported on a polymer structure for better properties (Olivas et al., 2008).

2.2.2.1 Waxes and paraffin

Paraffin wax which was produced from petroleum composed of a mixture solid hydrocarbon molecules. Paraffin wax was used to coat fruits and vegetables for prolonging shelf life. Carnauba wax was an exudate which derived from palm tree leaves (*Copoernica cerifera*) (Figure 6A). On the other hand, beewax (white wax) was produced from honeybees (Figure 6B). Nature Seal® 2020 (cellulose based polysaccharide) combined with carnauba wax coatings could provide modified atmosphere (MA), control decay and maintain qualities of mango fruit. Moreover, fruit coated with carnauba wax alone delayed water loss better than fruit coated with Nature Seal® 2020 (Baldwin et al., 1999). The report in apple indicated that combination of Semperfresh-beeswax and carnauba wax showed more effectively coating than water-based chitosan coatings (Choi et al., 2002). Moreover, vegetable oil and paraffin have presented the ability to reduce respiration rate of Golden Delicious

apples. Coated fruit improved higher firmness and titratable acidity than uncoated fruit (Conforti, 2007). Therefore, waxes have been used as barrier films to gas exchange thus reducing moisture transportation and maintaining fruit texture.

2.2.2.2 Shellac resins

Shellac resins were from the secretion of insect *Laccifer lacca* and consisted of the complex mixture of aliphatic alicyclic hydroxyl acid polymers (Figure 6C). This resin could dissolve in alcohols and in alkaline solutions. Shellac is mostly used as coatings in pharmaceutical industry and there was only few report in food product (Hernandez, 1994). Resin and its derivatives were widely used for coating in some fruit such as citrus (Hagenmaier and Baker, 1993). The coatings could completely coat and formed as an additional barrier to gas permission on fruit peel that reduced respiration rated of fruit (Hagenmaier and Baker, 1993). Moreover, commercial shellac and beeswax showed higher concentrations of aroma volatiles in orange fruit (Nisperos-Carriedo et al., 1990). However, coating fruit with shellac and wood resin-based may increase ethanol content which presented off-flavor (Ahmad and Khan, 1987; Cohen, Shalom and Rosenberger, 1990; Ke and Kader, 1990).

Figure 6. Carnauba wax (A), beewax (B) and shellac resins (C) (Bourtoom, 2008).

2.2.3 Protein film

Protein as the main structural materials in animal tissues was soluble in water or aqueous solutions of acid and exhibited widely functions in living systems (Scope, 1994). Functions of protein depended on the associated between the component of amino acid residues and their placement in polymer chain (Olivas et al., 2008). Various kinds of proteins were used as edible films. Proteins, including gelatin, corn zein, wheat gluten and soy protein were applied for fruit coating. In General, proteins can degrade in many conditions such as heat, acid, base, and/or solvent which is a critical point for film forming properties. Increased polymer interactions in protein films showed tighter film but allow less gas permission (Kester and Fennema, 1986). Moreover, protein films exhibited highly oxygen barriers function at low relative humidity.

2.2.3.1 Corn zein film

Zein was the major protein in corn that was prolamin protein. This protein could dissolved in ethanol (70-80%) (Dickey and Parris, 2001; Dickey and Parris, 2002)

(Figure 7A). Hydrophobic of zein was related with component of non-polar amino acids (Shukla and Cheryan, 2001). Moreover, zein showed a superiority film forming properties and biodegradable films. Edible films could be formed by dehydrating of ethanol solution of zein gel (Gennadios and Weller, 1990). Zein films were very brittle so adding of plasticizer was required for elasticity of film (Park, 1991). Zein films presented excellent water vapor barriers characteristic compared with other edible films. Furthermore, film showed higher water vapor barrier properties when fatty acids were added (Guilbert, 1986). Zein coatings were effective in reducing respiration rate and weight loss in Red Delicious apples after harvest (Park, Rhim and Lee, 1996). Moreover, peel color and fruit firmness of tomatoes were improved by corn-zein-based coating film (Park, Chinnan and Shewfelt, 1994). Corn-zein coatings retarded the shelf-life of tomatoes at 21°C (Tasdelen and Bayindirli, 1998).

2.2.3.2 Soy protein

Soybeans exhibited protein content approximately 38-44% that was higher than the protein content in cereal grain (8-15%). Generally, soy protein was insoluble in water whereas soluble in dilute neutral salt solutions (Olivas et al., 2008) (Figure 7B). Edible films from soy protein could be obtained from heating soymilk or solutions of soy protein isolate (SPI) (Gennadios and Weller, 1992). Using of soy protein as films or coatings in food products has been reported by Baker, Baldwin and Nisperos-Carriedo (1994) and Rhim et al. (2002). Previous report in kiwifruit indicated that fruit coated

with soybean protein showed highly longer shelf-life than uncoated fruit (Xu, Chen and Sun, 2001). In accordance with the report in apple, combination of soy protein and organic acids, such as malic and lactic acids exhibited antimicrobial property resulted in delayed deteriorative processes of whole apples (Eswaranandam, Hettiarachchy and Meulleneteffect, 2006).

A B

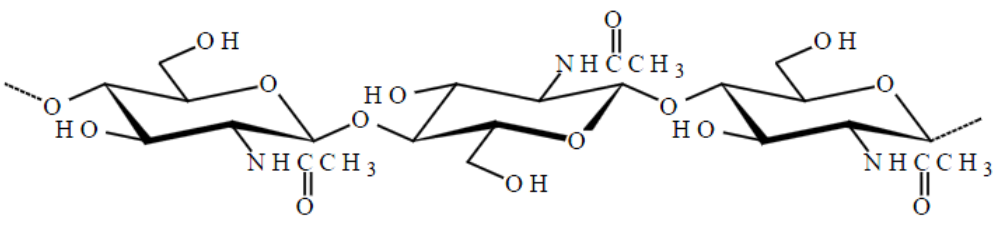
Figure 7. Corn zein (A) and soy protein isolated (B) (Bourtoom, 2008).

2.3 Chitosan

Chitosan (poly-β-(1,4)-D-glucosamine) is a deacetylated derivative of chitin (poly-N-acetylglycosamine) which is the main component of the cell walls of fungi and crustaceans (Horton et al., 1993; Chankrajang, 2000). Chitosan is obtained by partial de-

N-acetylation of chitin by the deacetylation process. Acetamide group of chitin is changed into amino group (-NH_2) thus making chitosan more soluble than chitin at the same pH (Li et al., 1997) (Figure 8). Chitosan material is not expensive, non-toxic, biocompatible and biodegradable polysaccharide (Jo et al., 2001; Yang et al., 2014). Chitosan properties are related to its molecular weight and degree of deacetylation (%DD) (Tsai et al., 2002; Kean and Thanou, 2010). Moreover, the performance of chitosan function highly depends on the source of chitosan. For instance, the antimicrobial activity of chitosan depends on many factors such as the source of chitosan and the pH of the medium (Devlieghere, Vermeulen and Debevere, 2004). Chitosan presents three reactive functional groups which are an amino group, primary and secondary hydroxyl groups at the C-2, C-3, and C-6 positions (Furusaki et al., 1996). The function groups make chitosan useful for many applications (Galed, Martinez and Heras, 2001).

(A)



(B)

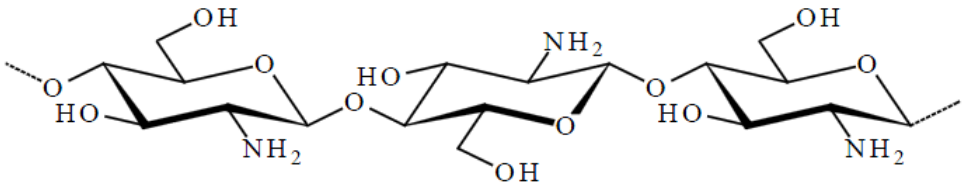


Figure 8. Structures of chitin (A) and chitosan (B) (Furusaki et al., 1996).

2.3.1 Applications of chitosan in food and medicine

Chitosan exhibits film forming property so chitosan has been used as edible film or food wrap in food industry in many country (Shahidi et al., 1999; Devlieghere et al., 2004). Coating fruits and vegetables with chitosan helps long-term storage of food (El Ghaouth et al., 1992), since chitosan film shows an active package, preservatives foods from microorganism deterioration (Chen, Liau and Tsai, 1998; Galed et al., 2001; Devlieghere et al., 2004). The chitosan film can coat on the surface of the food and inhibits fungal growth. High number of protonated amino groups (NH_2) in chitosan molecule showed higher antimicrobial activity. Liu et al. (2004) reported that the antimicrobial activity of chitosan was the result of the interaction between NH_3^+ groups of chitosan and the negative residues of the phospholipid component of the

pathogen membrane. Chitosan showed various characteristic by flexibility of its molecular structure that could apply to further improve several polymer properties such as solubility and chemical modifications (Park et al., 2010; Jarmila and Vavríková, 2011).

Chitosan is one of the popular materials that is also applied in drug delivery (Rodrigues, Costa and Grenha, 2012). Chitosan exhibits drug delivery function by its positive charge under acidic conditions. The positive charge of chitosan showed insoluble property in neutral and basic environments whereas an increase in solubility under acidic environments was observed. Therefore, for biomedical applications, chitosan molecule could maintain its structure in a neutral condition whereas it could solubilize or degraded under acidic condition (Amidi et al., 2010; Baldrick, 2010). In addition, chitosan can be used for transporting drug under acidic environment. There was a report for drug delivery which has been seen in the transport of insulin (Sunil, Mallikarjuna and Aminabhavi, 2004). Furthermore, the effect of chitosan on oil interaction was investigated in the stomach and duodenum tract. Chitosan has been shown to interact with oil inhibiting duodenal absorption and enhanced lipid waste (Rodríguez et al., 2005). However, the mechanism of interaction between chitosan and fat is still needed more investigation. In addition, drug delivery system concerns the ability of a material for its biodegradability and biocompatibility resulting in toxic degradation products (Baldrick, 2010). Therefore, chitosan was approved for these

properties by the American Food and Drug Administration (FDA) (Kean and Thanou, 2010).

2.3.2 Applications of chitosan in agriculture and plant growth

Chitosan could protect plant from environmental stress such as drought stress and improve plant yields and fruit crops (Linden and Stoner, 2007). Previous study revealed that the stomatal apertures of tomato and *Commelina communis* were reduced when the epidermis was treated with chitosan (Lee et al., 1999). Chitosan could reduce transpiration in pepper plants leading to the reduction of water use whereas the biomass production and yield still unchanged (Bittelli et al., 2001). Therefore, it is suggested that chitosan could be an effective antitranspirant to conserve water use in agriculture (Bittelli et al., 2001). Moreover, when stomatal apertures were widely opened, it was easier for plant pathogens to attack (Lee et al., 1999). Therefore, chitosan coating acted as a barrier to pathogen infection (Ribeiro et al., 2007; Duan et al., 2011). Furthermore, chitosan as an exogenous elicitor could induce plant defense mechanism in plants (Cabrera et al., 2006; Trotel-Aziz et al., 2006). Previous reports indicated that chitosan could induce activity of defense enzymes such as chitinase, β-1,3-glucanase and peroxidase (POD) in many plant species (Lin et al., 2005; Cabrera et al., 2006; Trotel-Aziz et al., 2006; Jitareerat et al., 2007; Meng et al., 2010). According to the induction of defense gene activities in several plants by chitosan treating, chitosan at 50 ppm completely controlled germination of

Botrytis cinerea conidia in vitro and also inhibited gray mold caused by *B. cinerea* in cucumber plants (Ben-Shalom et al., 2003). In addition chitosan was used as biocontrol by applying to foliage or soil to induce natural innate defense responses of plant to insects, pathogens, and soil-borne diseases (Linden and Stoner, 2005). Effects of chitosan on soil application have also been reported in many plants such as rice and lettuce (Boonlertnirun, Boonraung and Suvanasara, 2008; Muymas et al., 2011). Chitosan as soil supplementation for plant growth could increase the growth of advantageous soil microbes and nutrients in soil (Sharp, 2013). Muymas et al. (2011) revealed that growth rate of lettuce cv. 'Red Oak' was increased by growing in fermented chitin (20% (w/w)) mixed with soil which served as nitrogen source for plant. One percent of chitosan powder clearly induced vegetative and reproductive growth of *Eustoma grandiflorum* by enhancing dry weight, reducing flowering times, and maintaining cut-flower quality (Ohta et al., 1999). Moreover, previous study in *Dendrobium* orchids indicated that both chitosan solid and liquid medium could increase the growth rate and development of orchids (Nge et al., 2006; Pornpienpakdee et al., 2010). Besides, Barka et al. (2004) revealed that 1.75 % (v/v) of chitogel supplemented medium could induce CO_2 fixation of grapevine resulted in the induction of root and shoot biomass. Therefore, chitosan could increase photosynthesis, enhance nutrient uptake and induce plant growth. Moreover, chitosan was used as seed treatment or seed coating. Seeds of soybean, cotton, and many other seeds coated with chitosan showed higher root development and pathogen

growth limitation than uncoated seed (Stoner and Linden, 2006). In addition, chitosan seed coating improved the vigor of wheat (*Triticum aestivum*) (Bhaskara-Reddy et al., 1999) and the vegetative growth in pearl millet (*Pennisetum glaucum*) (Sharathchandra et al., 2004). Moreover, chitosan has been applied in tissue culture. Molecular weight and concentration chitosan had influenced bud growth of *Dendobrium phalaenopsis* (Nge et al., 2006).

2.3.3 Applications of chitosan in postharvest technology

Climacteric fruit highly presented physiological changes during ripening period that caused the reduction of fruit qualities after harvest (Giovannoni, 2001; Ampaichaichok, Rojsitthisak and Seraypheap, 2014). The effects of chitosan coating on fruit ripening have been investigated in many plants such as mango, guava and strawberry (Jitareerat et al., 2007; Hong et al., 2012). Since, chitosan showed semi-permeable film forming properties thus chitosan coating presented modified atmosphere (MA) for coated fruit leading to reduced deteriorative processes and retarded shelf life of fruits (Park et al., 2005; Duan et al., 2011). 'Nam Dok Mai' mango (*Mangifera indica* L.) coated with 1% chitosan showed highly limited anthracnose disease which caused by *Colletotrichum gloeosporioides.* Moreover, chitosan coating maintained fruit qualities by delaying respiration rate, ethylene production and inhibiting loss of fresh weight (Jitareerat et al., 2007). Chitosan treatment also increased antioxidant and antioxidant enzyme activities in many plants (Sun et al., 2010; Wang

and Gao, 2013; Zhang, Zhang and Yang, 2015). Previous report in 'Cavendish' banana indicated that fruit coated with higher degree of deacetylation (%DD) and concentration of chitosan showed higher fresh weight and vitamin C than lower concentration at 30±2°C (Suseno et al., 2014). In addition, nanochitosan coated strawberries fruit enhanced anthocyanin concentrations for 12 days of storage when compared with uncoated fruited (Eshghi et al., 2014). The result was accordant with Chiabrando and Giacalone (2015) in that chitosan coating retarded the reduction of anthocyanin content in highbush blueberry (*Vaccinium corymbosum* L. cv Berkeley and O'Neal).

Chitosan exhibited antifungal property which could apply for controlling fungal development in postharvest technology (El Ghaouth and Wilson, 1997; Bautista-Banos et al., 2006). Chitosan treatments induced pathogenesis-related (PR) proteins which were defense enzymes induced by pathogen infection (Trotel-Aziz et al., 2006; Jitareerat et al., 2007; Meng et al., 2010). After treating with chitosan in various plants, the induction of plant defense was occurred; phenylalanine ammonia-lyase (PAL), chitinase and β-1,3-glucanase was enhanced in grape berries, oranges, strawberries and raspberries (Zhang and Quantick, 1997; Romanazzi et al., 2002) leading to protection of fruits from fungal infection (Liu et al., 2007). Furthermore, strawberry sprayed with chitosan at concentration of 2, 4 and 6 g/L during pre-harvest treatment showed significant inhibition of gray mold rot caused by *B. cinerea* and fruit sprayed twice with

chitosan at concentration of 6 g/L showed higher firmness and qualities than other treatments (Bhaskara-Reddy et al., 2000). The reduction of storage rots by chitosan treatment has been reported in apple, kiwi and pear fruits (Du, Gemma and Iwahori, 1997; Bautista-Banos, De Lucca and Wilson, 2004). Moreover, chitosan treatments induced increase of phenolic compounds, flavonoid and lignin contents (Meng et al., 2008; Sun et al., 2008). Wheat seed, pea pods and rice highly presented the accumulation of H_2O_2, phenolic, lignin and phytoalexin when treated with chitosan (Walker-Simmons, Hadwiger and Ryan, 1983; Bhaskara-Reddy et al., 1999; Agrawal et al., 2002) Besides, strawberry coated with chitosan could maintain phenolic compounds and delay browning of fruit (Campaniello et al., 2008). Therefore, chitosan treatment induced all of defense resistance in plants which against pathogen attack to host plant and leading to inhibition of pathogens development.

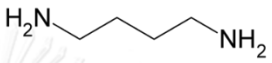
In summary, chitosan showed various properties for improving fruit qualities and agriculture technology. Therefore, application of chitosan in many ways may give the new knowledge for developing the technology that can be used in commercial products.

2.4 Polyamines

Polyamines (PA) are aliphatic amines with low molecular weight and widely discovered in all of living organisms (Galston and Sawhney, 1990; Bouchereau et al., 1999; Pandey et al., 2000). Major polyamines were putrescine (Put, a diamine),

spermidine (Spd, a triamine) and spermine (Spm, a tetramine) (Liu et al., 2006) (Figure 9). Polyamines were proposed to have many roles in plant physiological and developmental processes such as plant growth, morphogenesis, pollen viability, senescence, fruit ripening, and responses to biotic and abiotic stresses (Evans and Malmberg, 1989; Takahashi et al., 2003; Ziosi et al., 2006).

(A)



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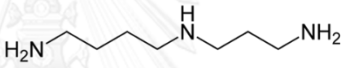

(C)

Figure 9. Structures of putresine (A), spermidine (B) and spermine (C) (Nishikawa, Tabata and Kitani, 2012).

2.4.1 Polyamines biosynthesis pathway

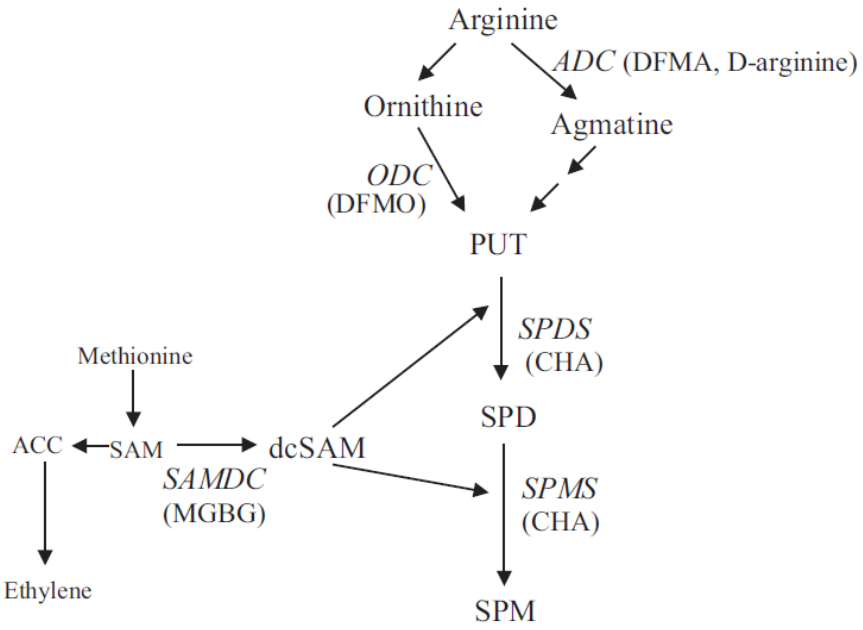

Figure 10. Polyamines and ethylene biosynthesis pathways (Liu, Honda and Moriguchi, 2006).

Polyamines biosynthesis started with the presence of the arginine decarboxylase (ADC) pathway. PUT was contributed from two pathways; the first from ornithine and was catalyzed by ornithine decarboxylase (ODC) and the second from arginine and was catalyzed by arginine decarboxylase (ADC) with two intermediate products, agmatine and *N*-carbamoyl putrescine. Spd was synthesized from Put via spermidine synthase (SPDS) by adding an aminopropyl moiety donated from decarboxylated *S*-adenosylmethionine (dcSAM) which was catalyzed by the enzyme of *S*-adenosylmethionine decarboxylase (SAMDC) using *S*-adenosylmethionine (SAM) as a substrate. Similarly, SPM was produced from SPD via spermine synthase (SPMS) with the same aminopropyl moiety rendered by dcSAM (Figure 10).

2.4.2 Relationship between polyamines and ethylene

Ethylene is the only one gaseous plant hormone presented an important role in fruit ripening. From polyamines and ethylene biosynthesis pathways, ethylene and polyamines especially SPD and SPM share a common precursor, SAM (Figure 9). Not only SAM can develop to ethylene through 1-aminocyclopropane-1-carboxylic acid (ACC) but also SAM can be decarboxylated by *S*-adenosylmethionine decarboxylase (SAMDC) into dcSAM which served as a donor of the aminopropyl group for producing SPD and SPM, respectively. Both molecules share the same substrate so they compete for this substrate during fruit development and ripening (Liu et al., 2006). The competitive relationship was investigated in many plant species.

The studies in tomato fruits revealed that ethylene production decreased whereas polyamines production increased when fruit was stored for a long time (Dibble, Davies and Mutschler, 1988). In addition, blocking of ethylene production by adding an ethylene inhibitor such as aminoethoxyvinylglycine (AVG) could enhance polyamine production (Arigita, Tamés and González, 2004). Therefore, it was proposed that ethylene and polyamines had different effects on induction of fruit ripening. Previous reports in many fruits found that exogenous polyamines could retard fruit softening on the tree such as apple and peach (Kramer et al., 1991; Bregoli et al., 2002). Therefore, exogenous polyamines possibly reduce ethylene production. In addition, Kramer et al. (1991) reported that the polyamine could retard fruit softening and might control the role in improving cell wall structure. Beyond the control of ethylene synthesis by polyamines were also controls of protein synthesis (Ke and Romani, 1988), charge neutralization of membrane, conformation changes of the membrane targets resulting in changing of membrane structure.

2.4.3 Role of polyamines in fruit development

In the early stage of fruit development, polyamines showed a high level then later decreased in the late stage during fruit development (Liu et al., 2006). In many fruits, polyamines were usually at a low level when the fruits were in ripening period such as apple and mango (Zhang et al., 2003; Malik and Singh, 2004). According to research in avocado fruit, polyamines showed the highest level in early stage of fruit

development whereas its level was declined up to 30% in maturity stage of fruit development (Kushad, Yelenosky and Knight, 1988). Since, high concentrations of polyamine may correlate with cell division and growth rate (Galston, 1983; Fraga et al., 2004). In the early stage of fruit development, fruit underwent cell division so it was possibly supported by polyamines. In the late stage of fruit development, cell enlargement was occurred so polyamine synthesis is decreased. Moreover, the reduction of polyamines at the late stage of fruit development has been reported as a signal for fruit ripening (Casas et al., 1990). However, some plant exhibited an increase of polyamines during fruit maturation and ripening stage such as citrus and tomato (Nathan, Altman and Monselise, 1984; Saftner and Baldi, 1990).

2.4.4 Exogenous polyamines in postharvest technology

Polyamines, especially SPD and SPM compete for common substrate of ethylene biosynthesis. Therefore, polyamines could apply on postharvest fruit development. There were many studies on the application of exogenous polyamines on fruit quality (Kramer et al., 1991). SPM and SPD treatments could delay softening of apple and strawberry fruits (Kramer et al., 1991; Ponappa et al., 1993). Exogenous PUT showed an increase in fruit firmness and reduction of fruit senescence in lemon, apricot and plum (Martínez-Romero et al., 1999; Martínez-Romero et al., 2002; Pérez-Vicente, 2002). Moreover, exogenous polyamines reduced browning, peroxide level and ethylene production and enhanced level of polyamines in litchi fruit kept at 5°C (Jiang

and Chen, 1995). Plum fruits treated with 1 mM PUT showed lower ethylene production, weight loss peel color change and soluble solids and higher fruit firmness when compared with untreated fruit (Valero, Martínez-Romero and Serrano, 2002; Serrano et al., 2003). The result was in accordance with previous studies in apricot and kiwi fruits, fruit treated with PUT exhibited significant reduced weight loss, color change, ethylene production and respiration rate (Martínez-Romero et al., 2002; Petkou, Pritsa and Sfakiotakis, 2004). Polyamines showed an influence on fruit texture (fruit firmness and fruit softening) therefore polyamines had mechanism for inhibiting cell wall degrading enzymes which correlated with pectic substances or it had ability to bind with cell walls and membranes leading to maintained cell wall structure (Ponappa et al., 1993; Valero et al., 1998; Martínez-Romero et al., 2002). However, high concentration of polyamines may heal the chemical injury in apple fruits. 'Red Delicious' and 'McIntosh' apples showed small black spots on fruit peel after treated with SPD and SPM at higher concentration than 1 mM (Kramer et al., 1991). Therefore, concentration of polyamines appropriated for application in various fruits has to be further investigated.

In summary, there were many studies on the relationship between polyamine and fruit development, the clear results showed the effect of polyamines on fruit development, ethylene production and rigidification of cell wall. However, in some fruits, different results and effects of exogenous polyamines application on soluble solutions and

titratable acids are still unclear. Therefore, much more work is needed for understanding these mechanisms.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

Mature green mango (*Mangifera indica* L. cv Nam Dok Mai) fruit were harvested at commercial stage (90-100 days after fruit set) from orchard in Nakornratchasrima province. Mango fruit were selected for standardization in size, shape, color and without any blemishes or disease symptoms. Mango fruit were then cleaned with tap water and air dried.

3.1.2 Chitosan material

Chitosan solutions were prepared from shrimp shells which were obtained from A.N. Lab (Samut Sakorn province). Chitosan properties were analyzed for degree of deacetylation (Muzzarelli and Rocchetti, 1985), molecular weight (Gel Permeation Chromatography (GPC, Water 600E, Waters Corp., USA)), solubility (Robert, 1992), moisture content (AOAC International, 2000) and viscosity (Brookfield Viscometer, model DV-II+, Japan). Chitosan flakes were divided into 3 groups by molecular weight: high molecular weight chitosan (HM-CTS: 360 kDa), medium molecular weight chitosan (MM-CTS: 270 kDa) and low molecular weight chitosan (LM-CTS: 40 kDa). One percent chitosan solution was

prepared in 0.5% acetic acid (w/v). The solution was stirred at 900 rpm at room temperature overnight (overhead stirrer, RW 20 digital, IKA Blueline laboratory products UK) and was then amended with 0.1% (w/v) tween® 80, stirred at 500 rpm at room temperature for 1 hour before using.

3.2 Instruments

3.2.1 Equipment for preparing chitosan solution and treating

1. Beaker
2. Cylinder
3. Overhead Stirrer (RW 20 digital, IKA Blueline laboratory products UK)
4. Balance: PG503-S (Mettler Toledo, USA)
5. Balance: U 4600 P+ (Scientific Promotion Co., Ltd., Thailand)
6. Micro pipette: Pipetman (Gilson, France)
7. Pipette tip
8. Timer
9. Shredded paper
10. Plastic basket

3.2.2 Equipment for selecting the appropriate chitosan coating to prolong shelf-life of mango fruit

1. Aluminum foil
2. Flasks
3. Cylinder
4. Beaker
5. Micro pipette: Pipetman (Gilson, France)
6. Pipette tip
7. Balance: PG503-S (Mettler Toledo, USA)
8. Spectrophotometer: 8453E UV-Vis (Agilent Technologies Inc., Germany)
9. Cuvettes
10. Parafilm
11. Glass tube 15 ml.
12. Deep freezer (-80°C)) New Brunswick Scientific, Belgium)
13. Freezer (-20°C) (Sanyo Electric Co., Ltd, Japan), Japan)
14. Refrigerated centrifuge: Universal 2R (Hettich, Germany)
15. Microcentrifuge tubes
16. Vortex mixture: Vortex-Genie 2 (Scientific Industries, Inc., USA)
17. Timer
18. Spatulas

19. Mortars and pestles

20. Gas chromatograph (GC-14, Shimadzu, Kyoto, Japan)

21. Gas chromatograph (GC-RIA, Shimadzu, Kyoto, Japan)

22. Penetrometer (Hardness tester FHM-1, Takemura, Japan)

23. Refractometer (N-1E, Japan)

24. Autoclave: TC-459 (Taichung, Taiwan)

25. Ice bath

26. Liquid nitrogen container

27. Glass jar 2.4 L

28. Forceps

29. Glass bottle 50 mL

30. Septum

31. Syringe 10 mL

32. Petri dish

33. Handed sprayers for 70% (v/v) ethanol

34. Microwave: ER-D23SC (S) (Toshiba, Japan)

35. Needle

36. Alcohol burner

37. Knife and cutting board

38. Burette

39. Colorimeter (Color Reader CR-10, Konica Minolta Sensing,Inc., Japan)

40. Scanning electron microscope (FEI Quanta 250 ESEM, Netherlands)
41. #1 Whatman filter paper
42. Digital conductivity meter: S230 SevenCompact™ conductivity (Mettler Toledo, USA)
43. Plastic baskets

3.2.3 Equipment for investigating the effects of the appropriate chitosan coating combined with spermidine on qualities of mango fruit

1. Aluminum foil
2. Flasks
3. Cylinder
4. Beaker
5. Micro pipette: Pipetman (Gilson, France)
6. Pipette tip
7. Balance: PG503-S (Mettler Toledo, USA)
8. Spectrophotometer: 8453E UV-Vis (Agilent Technologies Inc., Germany)
9. Cuvettes
10. Deep freezer (-80°C)) New Brunswick Scientific, Belgium)
11. Freezer (-20°C) (Sanyo Electric Co., Ltd, Japan), Japan)
12. pH meter (Denver Instrument Company, USA)
13. Refrigerated centrifuge: Universal 2R (Hettich, Germany)

14. Microcentrifuge tubes

15. Vortex mixture: Vortex-Genie 2 (Scientific Industries, Inc., USA)

16. Timer

17. Spatulas

18. Mortars and pestles

19. Gas chromatograph (GC-14, Shimadzu, Kyoto, Japan)

20. Gas chromatograph (GC-RIA, Shimadzu, Kyoto, Japan)

21. Colorimeter (Color Reader CR-10, Konica Minolta Sensing,Inc., Japan)

22. Penetrometer (Hardness tester FHM-1, Takemura, Japan)

23. Refractometer (N-1E, Japan)

24. Autoclave: TC-459 (Taichung, Taiwan)

25. Ice bath

26. Liquid nitrogen container

27. Forceps

28. Handed sprayers for 70% (v/v) ethanol

29. Microwave: ER-D23SC (S) (Toshiba, Japan)

30. Digital water bath (DAIHAN LABTECH CO., LTD)

31. HPLC (CTO-10AS VP column oven; Shimadzu, Japan)

32. Scanning electron microscope (FEI Quanta 250 ESEM, Netherlands)

33. Cellulose acetate membrane (0.45)

34. Septum

35. Syringe 10 mL
36. Digital conductivity meter: S230 SevenCompact™ conductivity (Mettler Toledo, USA)
37. Knife and cutting board
38. Tube for HPLC analysis
39. Plastic baskets

3.2.4 Equipment for determining the effect of the appropriate chitosan coating combined with spermidine on anthracnose disease in mango fruit

1. Flasks
2. Cylinder
3. Beaker
4. Micro pipette: Pipetman (Gilson, France)
5. Pipette tip
6. Balance: PG503-S (Mettler Toledo, USA)
7. Timer
8. Spatulas
9. Autoclave: TC-459 (Taichung, Taiwan)
10. Beaker
11. Injection needle 10 mL

12. Petri dish
13. Handed sprayers for 70% (v/v) ethanol
14. Volumetric flask 500 mL
15. Needle
16. Alcohol burner
17. Shredded paper
18. Plastic baskets
19. Hemocytometer
20. Cork borer (diameter 0.5 cm)

3.3 Chemicals and reagents

3.3.1 Chemicals for preparing chitosan solution

1. Distilled water
2. Acetic acid
3. tween® 80
4. Spermidine (SPD)

3.3.2 Chemicals for using in enzyme extraction (showed in appendix)

1. Polyvinylpolypyrrolidone (PVPP)
2. Potassium dihydrogen phosphate (KH_2PO_4)

3. Dipotassium hydrogen phosphate (K_2HPO_4)

4. Dithiothreital (DTT)

5. Ethylenediaminetetraacetic acid (EDTA)

6. Phenylmethylsulfonyl fluoride (PMSF)

3.3.3 Chemicals and reagents for evaluating antioxidant and physicochemical characteristics

1. Sodium hydroxide (NaOH)

2. Phenolphthalein

3. Titanium III sulfate ($Ti_2(SO_4)_3$)

4. Potassium dihydrogen phosphate (KH_2PO_4)

5. Dipotassium hydrogen phosphate (K_2HPO_4)

6. Sulfuric acid (H_2SO_4)

7. α, α-diphenyl-β-picrylhydrazyl (DPPH)

8. Absolute methanol

9. L (+)-ascorbic acid ($C_6H_8O_6$)

10. Rutin (RE)

11. Hydroxylamine (NH_2OH)

12. Bio-Rad D_c protein assay reagent B

13. Bovine serum albumin

14. Potato Dextrose agar (PDA)

15. Ethylenediaminetetraacetic acid (EDTA)

16. Hydrogen peroxide (H_2O_2)

17. Metaphosphoric acid

18. Acetic acid

19. 2,6-dichlorophenolindolphenol (DCIP)

20. Thiourea

21. 2,4-Dinitrophenylhydrazine (DNPH)

22. Aluminium chloride ($AlCl_3$)

3.3.4 Chemicals for using in cell wall enzyme extraction (showed in appendix)

1. Tris-HCl

2. Ethylenediaminetetraacetic acid (EDTA)

3. Triton x-100

4. Cysteine-HCl

3.3.5 Chemicals and reagents for measuring polygalacturonase (PG) activity

1. Polygalacturonic acid (PGA)

2. Sodium acetate

3. DNS reagent (showed in appendix)

4. D-galacturonic acid

5. Sodium chloride (NaCl)

6. Distilled water

3.3.6 Chemicals for measuring pectin methyl esterase (PME) activity

1. Citrus pectin

2. Bromothymol blue

3. Distilled water

4. Sodium chloride (NaCl)

3.3.7 Chemicals for measuring soluble pectin content

1. Absolute ethanol

2. m-hydroxydiphenyl

3. Sodium hydroxide (NaOH)

4. Sulfuric acid (H_2So_4)

5. Sodium tetraborate ($Na_2B_4O_7$)

6. D-galacturonic acid

7. Distilled water

3.3.8 Chemicals for studying polyamines

1. Perchloric acid ($HClO_4$)

2. Sodium hydroxide (NaOH)

3. Benzoyl chloride

4. Sodium chloride (NaCl)

5. Diethyl ether

6. Absolute methanol (HPLC grade)

7. Distilled water

8. Putresine (PUT)

9. Spermidine (SPD)

10. Spermine (SPM)

3.4 Methods

3.4.1 Development and selection of an appropriate chitosan coating to prolong shelf-life of mango fruit during storage

Fruit was dipped in 1% (w/v) chitosan solution (in 0.5% (v/v) acetic acid) amended with 0.1% (w/v) tween® 80 for 1 min. The control fruit was dipped in 0.5% acetic acid amended with 0.1% tween® 80. The total of 4 treatments was performed as describe below:

Treatment 1: 0.5% (v/v) acetic acid solution amended with 0.1% (w/v) tween® 80 (control)

Treatment 2: HM-CTS coating

Treatment 3: MM-CTS coating

Treatment 4: LM-CTS coating

The experiment was designed with Completely Randomized Design (CRD) with 4 replications. Each replication consisted of at least 2 samples. After treatment, fruit was placed at storage temperature (25±2°C) and allowed to dry before storage. Fruit was stored at storage temperature until 16 days. Every 4 days, fruit was analyzed in terms of chitosan coating film properties, postharvest qualities and physicochemical characteristics.

3.4.1.1 Analyzation of chitosan film properties by scanning electron microscopy (SEM) analysis

Thickness and surface properties of chitosan coating film were measured by using a scanning electron microscope (FEI Quanta 250 ESEM, Netherlands) with voltage set at 10 kV.

3.4.1.2 Respiration and ethylene production

Fruit was weighed and placed in 2.4 L jar at 25°C for one hour. Then, 10 mL of the internal atmosphere were withdrawn by inserting the needle of a syringe from the stilar end. The sample gas was kept in saturated saline in 50 mL glass bottle until analysis. CO_2 and ethylene were detected by Gas Chromatography (GC-RIA, Shimadzu,

Kyoto, Japan and GC-14, Shimadzu, Kyoto, Japan, respectively) at Kasetsart University (showed in appendix).

3.4.1.3 Peel color change

Peel color was evaluated by using a colorimeter (Color Reader CR-10, Konica Minolta Sensing, Inc., Japan) at three equator locations of the fruit peel (blossom end, middle, and stem end) and mean value was expressed as L (Lightless) value and hue angle.

3.4.1.4 Fruit firmness

Pulp firmness was measured by using a handheld penetrometer (Hardness tester FHM-1, Takemura, Japan) at the same regions of the peel color measurement. Firmness was recorded as kg-force in Newtons (N).

3.4.1.5 Percentage of weight loss

Percentage of weight loss was calculated as percentage loss of initial weight of fruit. Fruit were weighed to determine weight loss by using the following formula (AOAC, 1984).

$$\text{Percentage of weight loss} = \frac{\text{weight before storage - weight after storage}}{\text{weight before storage}} \times 100$$

3.4.1.6 Titratable acidity in pulp (TA)

Method to analyze titratable acidity was modified from (AOAC, 1984). Ten grams of sliced mango pulp were mixed with 100 mL of distilled water for one min and filtered. Ten mL of filtrate were added with 1% phenolphthalein indicator and then titrated with 0.1 N NaOH until the end point (pink color). The TA (%) was calculated as follows:

$$\text{TA (\%)} = \frac{\text{NaOH (mL)} \times \text{0.1 NaOH (N)} \times 0.07 \times 100}{10\ \text{g}}$$

3.4.1.7 Total soluble solid content in pulp (TSS)

One mL of mango juice was used for measuring TSS by using a hand refractometer (N-1E, Japan), and TSS content was expressed as °Brix.

3.4.1.8 H_2O_2 content

One gram of mango pulp was grinded with liquid nitrogen then 10 mL of 50 mM phosphate buffer (pH 6.5) amended with 1 mM hydroxylamine (cold solution) were added. The mixture was centrifuged at 8000×g for 25 min; 1 mL of supernatant was filled with 1 mL of 0.3% titanium III sulphate (in 20% H_2SO_4 (v/v)). After centrifuged, H_2O_2 content was measured at 410 nm by spectrophotometer following (Jana and Choudhuri, 1982). H_2O_2 content was presented as μmol/g fresh weight (FW).

3.4.1.9 DPPH inhibition (%)

The method for evaluating α, α-diphenyl-β-picrylhydrazyl (DPPH) inhibition was adapted from (Choi et al., 2006). The color of reaction was measured at 520 nm by spectrophotometer. Distilled water was used as control and 80% methanol was used as blank. The percentage of DPPH inhibition was calculated by using formula as follows:

$$\text{DPPH inhibition (\%)} = \left(\frac{\text{1- Absorbance (sample)}}{\text{Absorbance (control)}}\right) \times 100$$

3.4.1.10 Ascorbic acid content in pulp (AA)

Ascorbic acid content in pulp was determined by a colorimeter method (Shin et al., 2007). One gram of mango pulp was added to 10 mL of 6% metaphosphoric acid (in 2 mol/L acetic acid). The mixture was centrifuged at 17,600×g for 15 min at 4°C and then supernatant was filtered through #1 Whatman filter paper. One mL aliquot of the supernatant was added with 0.05 mL of 0.2% DCIP and the solution was incubated in dark room for 1 hour at room temperature. After incubating, 1 mL of 2% thiourea (in 5% metaphosphoric acid) and 0.5 mL of 2% DNPH (in 4.5 mol/L sulfuric acid) were added to the solution. After that, the solution was incubated at 60°C for 2 hours. The reaction was stopped by placing the tubes in an ice bath and added 2.45 mL of 90% sulfuric acid (cold solution). Total AA was measured by absorbance at 540

nm by using a standard curve. The concentrations were expressed as μg ascorbic acid/g FW.

3.4.1.11 Free flavonoid contents

Flavonoid contents in pulp were measured according to the method of Djeridane et al. (2006). One gram of mango pulp was extracted by absolute ethanol then centrifuged at 9000×g for 15 min. Enzyme extracts (100 μL) were mixed with 100 μL of 1% $AlCl_3$ solution for 15 min. The color was measured at 433 nm. Free flavonoid contents were expressed as μg rutin (RE)/g FW.

3.4.1.12 Catalase activity in pulp (CAT)

CAT activity was assayed by the method of Nakano and Asada (1987). The reaction mixture consisted of 1.78 mL of 50 mM sodium phosphate buffer (pH 7.0), 0.2 mL H_2O_2 (100 mM) and 40 μL enzyme extract. CAT activity was measured by the decline in absorbance at 240 nm. The activity was expressed as U/mg protein. CAT activity was calculated as follows:

$$\text{U/mg protein} = \frac{(\Delta \text{ A240/min})(1000)}{(43.6)(\mu\text{L plant extract})(\text{mg protein}/\mu\text{L plant extract})}$$

3.4.1.13 Ascorbate peroxidase activity in pulp (APX)

APX activity was evaluated by following the method of Nakano and Asada (1987). Twenty µL of enzyme extracts were mixed with 1.58 mL of 50 mM sodium phosphate buffer (pH 7.0), 0.2 mL of 100 mM H_2O_2, 20 µL of 500 mM EDTA (pH 8.0) and 0.2 mL of 2 mM ascorbic acid. The reaction rate was measured at 290 nm and expressed as U/mg protein. APX activity was calculated as follows:

$$\text{U/mg protein} = \frac{(\Delta \text{ A290/min})(1000)}{(2.8)(\mu\text{L plant extract})(\text{mg protein/ } \mu\text{L plant extract})}$$

3.4.1.14 Total protein assay

Determination of the protein content of the samples followed method of (Bradford, 1976). Fifty µL of Bradford dye reagent (BioRad) were added to test tubes containing 50 µL of enzyme extract samples and 100 µL of distilled water and then the samples were thoroughly mixed and incubated at room temperature for 5 min. After incubating, the absorbance of sample was read at a wavelength of 595 nm in a spectrophotometer. Bovine serum albumin (BSA) was used as the standard protein.

3.4.1.15 Disease incidence

Disease incidence was measured by counting number of diseased fruit in each timing and then percentage of disease incidence was calculated by using the formula following (Baldwin et al., 1999).

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased fruits} \times 100}{\text{No. of total fruits}}$$

3.4.1.16 Disease severity

Disease severity was calculated by using score 0-7 (Pedroso et al., 2011) as: 0=no disease, 1=1-2% disease, 2=5% disease, 3=10% disease, 4=20% disease, 5=40% disease, 6=60% disease, and 7=more than 80% disease. Percentage of disease index (PDI) for severity was calculated as reported by Hossain et al. (2010):

$$\text{PDI for severity} = \frac{\Sigma\ (\text{numerical ratings}) \times 100}{(\text{Total number of observations})(\text{maximum disease score})}$$

3.4.1.17 Statistical analysis

Data of each parameter in each experiment was analyzed using one-way analysis of variance (ANOVA), following by Duncan's Multiple Range Test (DMRT) using the IBM SPSS Statistic software and using significance at the $P<0.05$ level.

3.4.2 Investigation of the effects of the appropriate chitosan coating combined with spermidine on qualities of mango fruit

HM-CTS treatment was selected to study the effects of chitosan coating combined with spermidine on qualities of mango fruit. Spermidine solution at concentration of 0.1, 1.0 and 10 ppm (w/v) was added to HM-CTS coating solution. Fruit was dipped in solution for 1 min. HM-CTS solution without SPD used as control. The total of 4 treatments was performed as describe below:

Treatment 1: HM-CTS (control)

Treatment 2: HM-CTS+0.1 ppm SPD

Treatment 3: HM-CTS+1.0 ppm SPD

Treatment 4: HM-CTS+10 ppm SPD

The experiment was designed with CRD with 4 replications. Each replication consisted of at least 2 samples. Fruit was stored at 14°C for 14 days. After 14 days, fruit were transferred to storage temperature (25±2°C) until 23 days and randomly sampled fruit every 3 days at storage temperature. Fruit was measured in the term of chitosan coating film properties, physicochemical characteristics, fruit softening and polyamine analysis.

3.4.2.1 Analyzation of chitosan film properties by scanning electron microscopy (SEM) analysis

Thickness and surface properties of chitosan film were analyzed as previous describe (3.4.1.1).

3.4.2.2 Respiration and ethylene production

Respiration and ethylene production were measured and calculated as previously described (3.4.1.2.).

3.4.2.3 Peel color change

Peel color change was measured by using a colorimeter (Color Reader CR-10, Konica Minolta Sensing, Inc., Japan) and analyzed as previously describe in 3.4.1.3.

3.4.2.4 Total soluble solid content in pulp (TSS)

One mL of mango juice was used for measuring TSS by using a hand refractometer (N-1E, Japan), and TSS content was expressed as °Brix.

3.4.2.5 Disease incidence

Disease incidence was measured and calculated as previously described in 3.4.1.15.

3.4.2.6 Disease severity

Disease severity was evaluated as previously describe (3.4.1.16.).

3.4.2.7 Fruit firmness

Pulp firmness was measured by using a handheld penetrometer (Hardness tester FHM-1, Takemura, Japan) at the same regions of the peel color measurement. Firmness was recorded as kg-force in Newtons (N).

3.4.2.8 Percentage of weight loss

Percentage of weight loss was measured and calculated as previously describe (3.4.1.5).

3.4.2.9 Soluble pectin content in mango peel and pulp

Method to analyze soluble pectin content in peel and pulp was adapted from Robertson (1979). One gram of mango peel and pulp was used for analysis. For extraction, mango tissue was boiled at 85°C in 95% ethanol for 10 min and centrifuged at 10,000×g at 20°C for 15 min. Insoluble was added with 63% ethanol and boiled at 85°C for 10 min, centrifuged at 10,000×g at 20°C for 15 min. After centrifuge, insoluble was filled with 6 mL distilled water and blew for 10 min. After that, solution was centrifuged at 10,000×g at 20°C for 15 min. Five mL of supernatant were added with

0.5 mL of 1 N NaOH and 4.5 mL of distilled water and incubated at room temperature for 15 min before analysis.

For soluble pectin analysis, 100 µL of sample extract were added with 900 µL of distilled water and 5 mL of 0.0125 M $Na_2B_4O_7$ (in conc. sulfuric acid) and mixed. The reaction was stopped by boiling for 10 min and tubes were transferred to an ice bath; added 100 µL of 0.15% m-hydroxydiphenyl (in 0.5% NaOH) and mixed for 15 min. Absorbance was measured at 520 nm. D-galacturonic acid was used as standard.

3.4.2.10 Polygalacturonase activity (PG) in mango peel and pulp

PG activity was determined by following method of (Pathak and Sanwal, 1998). One hundred µL of enzyme extract were mixed with 300 µL of 1% PGA (in 50 mM sodium acetate pH 4.5), 200 µL of 0.2 M sodium acetate buffer (pH 4.5), 100 µL of 0.2 M sodium chloride and 300 µL of distilled water and then incubated at 37°C for 1 hour. For measuring the amount of reducing sugar released, 1 mL of 0.3% DNS was added to solution. After mixing, solution was boiled for 5 min and then added with 8 mL of distilled water. PG activity was measured at 520 nm by spectrophotometer. One unit of PG activity was expressed as mg D-galacturonic acid / mg protein.

3.4.2.11 Pectin methyl esterase activity (PME) in mango peel and pulp

PME activity was analyzed using the modified method of (Hangermann and Austin, 1986). One hundred µL of enzyme extract were mixed with 1 mL of 0.01 %

(w/v) citrus pectin (pH 7.5), 100 µL of 0.01% (w/v) of bromothymol blue (in 0.02 M Tris-HCl buffer pH 7.0) and 200 µL of distilled water. PME activity was measured at a wavelength of 620 nm. One unit of enzyme was expressed as nmol of methyl ester hydrolyzed/min/mg protein.

3.4.2.12 Total protein assay

Total protein was evaluated by following the method of (Bradford, 1976) as describe in 3.4.1.14.

3.4.2.13 Polyamine analysis

Extraction method and Polyamine analysis were adapted from (Flores and Galston, 1982). One gram of mango peel was extracted by 10 mL of 5% $HClO_4$ (cold solution) and then incubated on ice for one hour, sample was centrifuged at 10,000 rpm for 15 min. Five hundred µL of supernatant (PCA-soluble) were used for analysis and pellet (PCA-insoluble) was mixed with 500 µL of 5% $HClO_4$ (cold solution). After that, 1 mL of 2 N NaOH and 10 µL of benzoyl chloride were added and mixed with sample extract. The mixture was incubated for 20 min at room temperature. After incubating, 2 mL of saturated NaCl were amended and vortexed for 30 sec. Then, 2 mL of diethyl ether were added and mixed. The ether phase (upper phase) was transferred to a new tube for analyzing. Sample was evaporated and kept at -20°C until analysis.

For HPLC analysis, sample was dissolved in 1 mL of absolute methanol (HPLC grade) then filtrated through a 0.45 µm cellulose acetate membrane. Sample was injected into HPLC (CTO-10AS VP column oven; Shimadzu, Japan). Three polyamines (PUT, SPD and SPM) were measured at 254 nm. Methanol and distilled water were used in solvent system at a flow rate of 1 mL/min. Sample extract from PCA-soluble was reported as free polyamines and sample extract from PCA-insoluble was reported as bound polyamines. Polyamine values were expressed as mmol/g FW and PAs standards were prepared at 0-2 mM.

3.4.2.14 Statistical analysis

Data of each parameter in each experiment was analyzed using one-way analysis of variance (ANOVA), following by Duncan's Multiple Range Test (DMRT) using the IBM SPSS Statistic software and using significance at the $P<0.05$ level.

3.4.3 Determination of the effect of an appropriate chitosan coating combined with spermidine on anthracnose disease in mango fruit

The phytopathogenic fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. was isolated from diseased tissues of symptomatic mango fruit and identified by morphological technique (Barnett and Hunter, 1998) and molecular technique by DNA sequencing (National Center for Genetic Engineering and Biotechnology (BIOTEC)). Koch's postulate was used to confirm the causal agent of the disease (Juangbhanich,

1988). Pure culture was grown on potato dextrose agar (PDA) at ambient temperature (28±2°C).

Fruit was washed under running water and left to air dry in the laboratory. The surface of the mango fruit was sterilized by immersion in 70% ethanol for 1 min and prepared for inoculation by inflicting one 1-mm-deep wound in the three regions of each fruit with a sterile needle. Each wound was then inoculated with *C. gloeosporioides* by placing 10 µL of conidial suspension (10^4 conidia/mL) (Yenjit et al., 2010). The inoculated fruit were incubated overnight at ambient temperature before treating. The total of 4 treatments was performed as describe below:

Treatment 1: inoculation with sterile distilled water without coating

Treatment 2: fungal inoculation without coating

Treatment 3: fungal inoculation+0.5% (v/v) acetic acid solution amended with 0.1% (w/v) tween® 80

Treatment 4: fungal inoculation+(HM-CTS+0.1 ppm SPD)

Inoculated fruit was dipped in the solution for 1 min. After treatment, fruit was placed at room temperature and allowed to dry before storage. Fruit was stored at ambient temperature (28±2°C) for 10 days. The experiment was designed with CRD with 4 replications. Each replication consisted of at least 2 samples. Every 2 days, disease developments of fruit were measured.

3.4.3.1 Disease incidence

Disease incidence was measured and calculated as previously describe (3.4.1.15).

3.4.3.2 Development of lesion area

Diameter of each lesion was measured (mm). Mean was calculated and expressed as area of lesion development by following the method of (Jitareerat et al., 2007).

3.4.3.3 Statistical analysis

Data of each parameter in each experiment was analyzed using one-way analysis of variance (ANOVA), following by Duncan's Multiple Range Test (DMRT) using the IBM SPSS Statistic software and using significance at the $P<0.05$ level.

CHAPTER IV

RESULTS

4.1 Development and selection of an appropriate chitosan coating to prolong shelf-life of mango fruit during storage

4.1.1 Chitosan characteristics

Chitosan coating solutions; HM-CTS (360 kDa), MM-CTS (270 kDa) and LM-CTS (40 kDa) used in this study were characterized. Result reveals that all of chitosan materials could dissolve more than 98% in 0.5% acetic acid (Table 1). Percentage of moisture content of chitosan materials were between 10-12%; HM-CTS=12.26±0.25%, MM-CTS=10.62±0.11% and LM-CTS=12.93±0.22%. Degree of deacetylation (%DD) ranged between 85-92%; HM-CTS=84.90±0.72%, MM-CTS=90.50±0.99% and LM-CTS=91.30±0.15%. The HM -CTS presented the highest of viscosity (34.07±0.55 cps) followed by MM-CTS (29.77±0.60 cps) and LM-CTS (6.30±0.05 cps).

Table 1 Chitosan properties

Chitosan	Molecular weight (kDa)	Solubility (%) (in 0.5% acetic acid)	Moisture (%)	Deacetylation (%)	Viscosity (cps)
HM-CTS	360	99.21±0.63[a]	12.26±0.25[b]	84.90±0.72	34.07±0.55
MM-CTS	270	99.71±0.36[a]	10.62±0.11[ab]	90.50±0.99	29.77±0.60
LM-CTS	40	98.68±0.40[a]	12.93±0.22[b]	91.30±0.15	6.30±0.05

[a]Values followed by the same letter in the column were not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

4.1.2 Analyzation of chitosan film properties by scanning electron microscopy (SEM) analysis

Stomata were clearly observed on mango surface of control treatment (Figure 11). The arrows presented stomata on mango peel in control treatment. HM-CTS treatment represented the best chitosan coated film property which was completely coated on mango peel, fully covered stomata and presented continuation of film. The HM-CTS coated film also exhibited the thickest of chitosan coated film on mango peel (3.47±0.50 μm) when compared with other treatments. The cross-section images of mango peel characterized by scanning electron microscope were presented in Figure 12. The arrows indicated chitosan coated film on mango peel. Similarly, MM-CTS treatment could totally cover mango peel area but mango peel surface was still obviously exhibited more than HM-CTS treatment. MM-CTS treatment presented moderate thickness of chitosan coated film (1.29±0.45 μm). LM-CTS treatment produced the thinnest chitosan coated film. In addition, LM-CTS treatment showed the non-continuation of film on mango peel in which stomata could be observed.

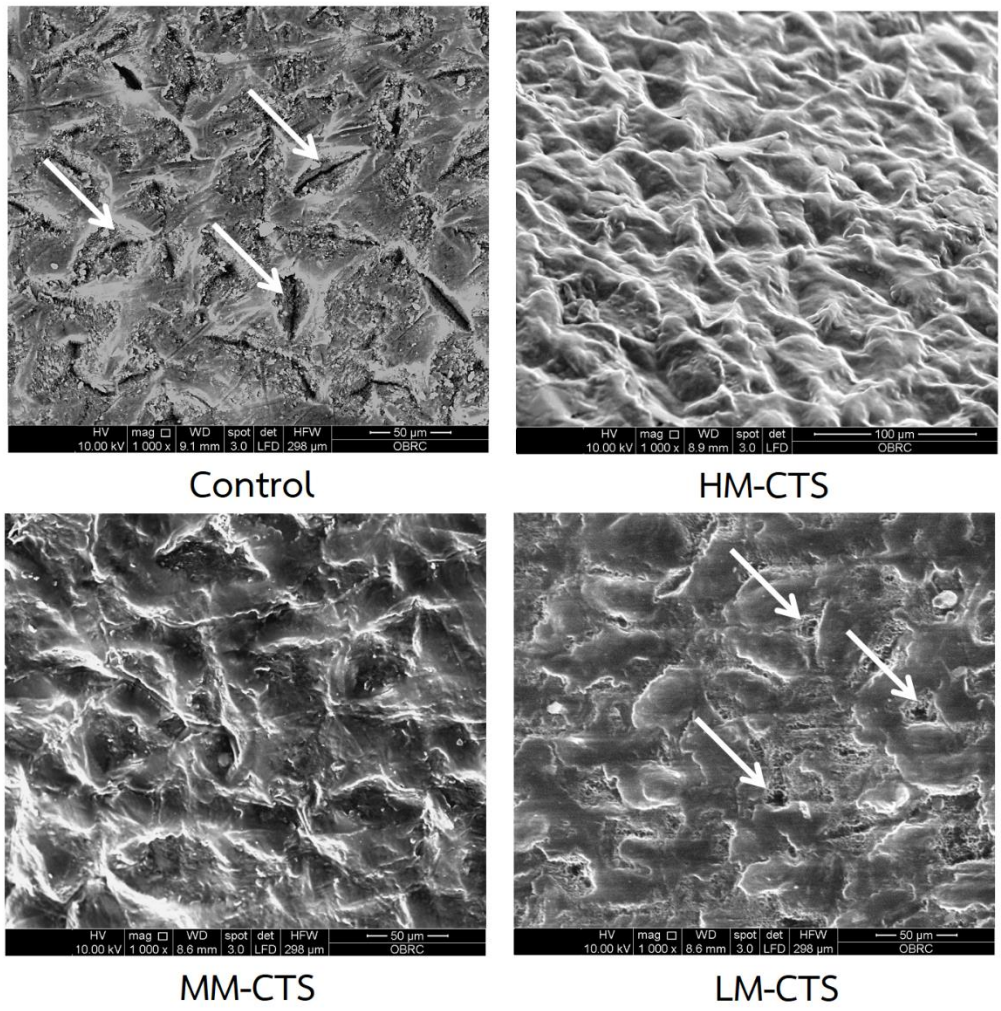

Figure 11. Surface image of mango peel coated with HM-CTS, MM-CTS and LM-CTS characterized by scanning electron microscope. The arrows indicate stomata on mango peel after coating.

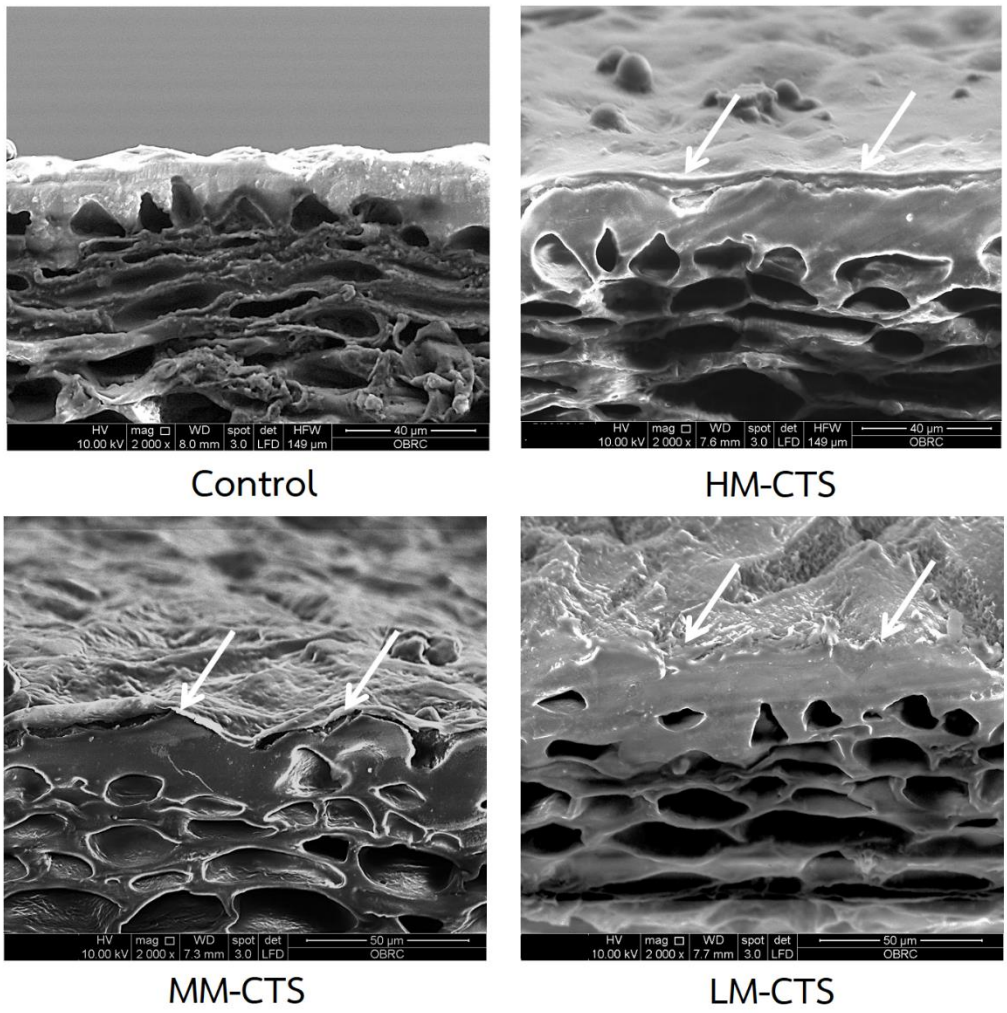

Figure 12. Cross-section image of mango peel coated with HM-CTS, MM-CTS and LM-CTS characterized by scanning electron microscope. The arrows indicate thickness of chitosan film on mango peel.

4.1.3 Physicochemical characteristics and antioxidant properties of mango fruit during storage

4.1.3.1 Ethylene production and respiration rate

Mango fruit coated with HM-CTS and MM-CTS showed significantly lower the rate of ethylene production than uncoated fruit and LM-CTS on day 4, 8, and 12 (Figure 13A). Moreover, HM-CTS and MM-CTS could reduce CO_2 production during storage and treatments showed significant difference from control and LM-CTS treatments on day 8 (Figure 13B). The result found that fruit coated with HM-CTS and MM-CTS achieved to inhibit ethylene production and respiration rate of the 'Nam Dok Mai' mango fruit during storage resulting in delayed ripening process of fruit.

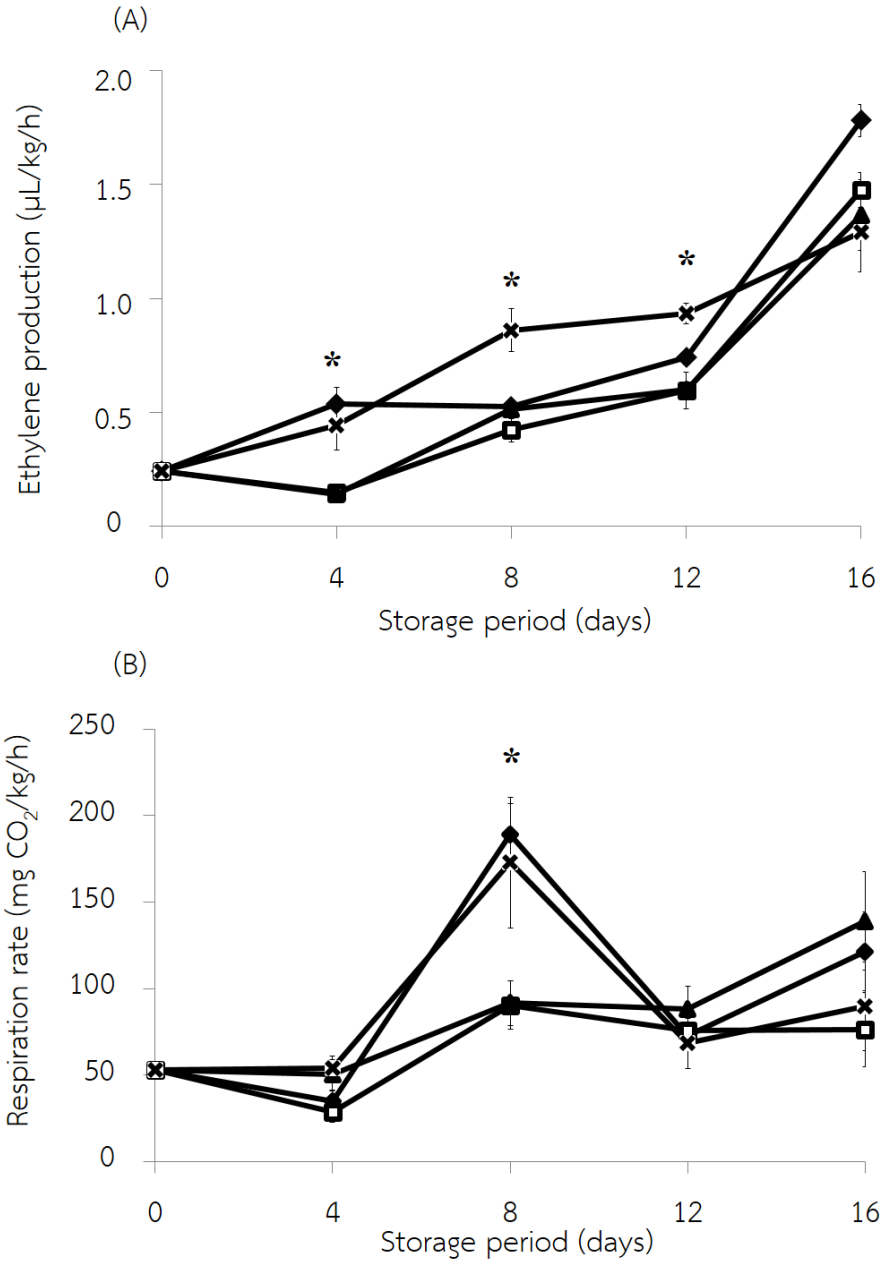

Figure 13. Ethylene production (A) and respiration rate (B) of control and chitosan coated fruit during storage at storage temperature (25±2°C) for 16 days. Each data point represents the mean of eight fruit. Vertical bars represent ±SE.

(◆) Control; (□) HM-CTS; (▲) MM-CTS; (✕) LM-CTS.

4.1.3.2 Peel color change

L value (lightness) and hue angle have been used as good indicators for measuring peel color change after harvest. Generally, peel color changes from green to yellow when mango ripens. L value increases and hue angle decreases when fruit is entering to ripening stage. L value gradually increased in uncoated fruit while mango coated with HM-CTS and MM-CTS were observed to delay peel color change on day 8, 12 and 16 when compared with control fruit (Figure 14) (Table 2).

4.1.3.3 Fruit firmness and percentage of weight loss

Fruit firmness always decreases when fruit begins to ripen. Fruit firmness sharply decreased in uncoated fruit. HM-CTS and MM-CTS could inhibit fruit softening and maintain fruit firmness. Mango fruit coated with HM-CTS showed the highest value of fruit firmness on day 12 (7.26±0.03) and day 16 (6.67±0.00) (Table 2).

After harvest, loss of fresh weight was obviously observed leading to peel wilting and fruit softening. The result was significant in HM-CTS treatment. HM-CTS treatment could inhibit loss of fresh weight during storage. Mango fruit coated with HM-CTS represented the lowest percentage of weight loss on day 12 (11.61±0.28) and day 16 (13.59±0.59). Uncoated fruit and fruit coated with LM-CTS showed high percentage of weight loss on day 12 (13.63±0.47 and 13.81±0.46) and day 16 (15.15±0.5 and 15.54±0.54), respectively (Table 2).

4.1.3.4 Titratable acidity (TA) and total soluble solid content (TSS)

TA decreased during ripening stage. The result indicated that fruit coated with HM-CTS achieved the highest of TA on day 8, 12 and 16 which was significant different from uncoated treatment. Uncoated fruit showed the lowest value of TA during storage (Table 2).

TSS content slightly increased during ripening stage. Mango fruit coated with HM-CTS showed the lowest value of TSS on day 4, 8 and 12 (Table 2). Uncoated fruit and LM-CTS treatment obtained higher level of TSS on day 8 (17.63±0.47 and 18.00±0.49) and day 12 (18.94±0.29 and 19.19±0.68), respectively. The results exhibited significant differences between HM-CTS and uncoated fruit.

Figure 14. Peel color change of mango in control and chitosan coated fruit during storage at storage temperature (25±2°C) for 16 days.

Table 2 Effects of chitosan coating on L value, hue angle, firmness, weight loss (%), TA (%) and TSS of fruit during storage at storage temperature (25±2°C) for 16 days.

Storage time (days)	Treatment	L value	Hue angle	Fruit firmness (N)	Weight loss (%)	TA (%)	TSS (°Brix)
0	Control	62.87±0.59^{a}	96.84±0.30^{a}	9.41±0.01^{a}	-	1.65±0.14^{a}	9.63±0.31^{a}
	HM-CTS	62.87±0.59^{a}	96.84±0.30^{a}	9.41±0.01^{a}	-	1.65±0.14^{a}	9.63±0.31^{a}
	MM-CTS	62.87±0.59^{a}	96.84±0.30^{a}	9.41±0.01^{a}	-	1.65±0.14^{a}	9.63±0.31^{a}
	LM-CTS	62.87±0.59^{a}	96.84±0.30^{a}	9.41±0.01^{a}	-	1.65±0.14^{a}	9.63±0.31^{a}
4	Control	63.98±0.37^{a}	93.62±1.88^{a}	9.22±0.01^{a}	5.22±0.25^{a}	1.44±0.03^{a}	14.00±0.23^{b}
	HM-CTS	62.91±0.59^{a}	95.86±0.59^{a}	9.32±0.01^{a}	4.77±0.25^{a}	1.51±0.11^{a}	11.63±0.84^{a}
	MM-CTS	63.02±0.62^{a}	95.57±0.43^{a}	9.02±0.02^{a}	5.21±0.24^{a}	1.40±0.09^{a}	14.25±0.94^{b}
	LM-CTS	63.89±0.38^{a}	95.72±0.55^{a}	9.22±0.00^{a}	5.47±0.25^{a}	1.31±0.07^{a}	15.38±0.25^{b}
8	Control	66.07±0.26^{c}	84.26±1.97^{c}	7.45±0.01^{b}	10.39±0.36^{a}	0.53±.005^{b}	17.63±0.47^{b}
	HM-CTS	63.88±0.51^{a}	95.18±0.71^{a}	7.65±0.03^{ab}	9.39±0.44^{a}	1.49±0.14^{a}	15.75±0.49^{a}
	MM-CTS	64.46±0.51^{ab}	90.91±1.13^{b}	8.14±0.01^{a}	10.30±0.44^{a}	1.36±0.03^{a}	17.25±0.49^{b}
	LM-CTS	65.33±0.48^{bc}	85.44±1.16^{c}	7.45±0.01^{b}	10.49±0.34^{a}	0.79 ±0.15^{b}	18.00±0.49^{b}
12	Control	68.38±0.26^{b}	77.35±1.53^{c}	6.08±0.03^{b}	13.63±0.47^{b}	0.12±0.01^{b}	18.94±0.29^{b}
	HM-CTS	65.08±0.59^{a}	93.11±1.32^{a}	7.26±0.03^{a}	11.61±0.28^{a}	0.28±0.09^{a}	16.88±0.47^{a}
	MM-CTS	65.92±0.59^{a}	84.27±1.48^{b}	6.57±0.01^{b}	13.33±0.57^{b}	0.20±0.03^{ab}	18.44±0.91^{ab}
	LM-CTS	67.84±0.39^{b}	78.46±0.98^{c}	6.37±0.02^{b}	13.81±0.46^{b}	0.16±0.03^{ab}	19.19±0.68^{b}
16	Control	71.44±0.44^{c}	73.82±0.97^{c}	6.28±0.02^{ab}	15.15±0.52^{ab}	0.08±0.00^{b}	19.94±0.38^{a}
	HM-CTS	66.85±0.52^{a}	91.00±1.83^{a}	6.67±0.00^{a}	13.59±0.59^{a}	0.22±0.01^{a}	19.88±0.47^{a}
	MM-CTS	67.84±0.28^{ab}	79.79±1.65^{b}	6.10±0.02^{b}	14.97±0.64^{ab}	0.09±0.02^{b}	19.75±0.34^{a}
	LM-CTS	69.07±0.52^{b}	73.61±0.63^{c}	6.37±0.01^{ab}	15.54±0.54^{b}	0.08±0.00^{b}	19.88±0.52^{a}

[a] Values followed by the same letter in the column on the same day were not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

4.1.3.5 H_2O_2 and antioxidant contents

H_2O_2 was significantly induced in uncoated fruit during storage whereas fruit coated with HM-CTS presented the lowest H_2O_2 content (Figure 15). Moreover, fruit coated with LM-CTS showed higher H_2O_2 content than fruit coated with MM-CTS. Therefore, our result indicated that HM-CTS treatment was the most effective treatment to inhibit H_2O_2 production of mango fruit during the ripening stage.

In addition, the result of the DPPH inhibition of the mango pulp extract showed a slight decrease of antioxidant capacity during ripening period. Fruit coated with HM-CTS showed the highest of DPPH inhibition during storage (Figure 16A) while control fruit showed the lowest of DPPH inhibition on day 12 (59.50%) and day 16 (57.68%). The result was significant on day 12. The ascorbic acid content highly decreased during ripening stage. Coated fruit presented higher ascorbic acid content than uncoated fruit during storage and the result showed significant difference on day 4 (Figure 16B). Mango fruit coated with chitosan resulted in the delay the reduction of ascorbic acid content after harvest. Furthermore, HM-CTS presented the highest level of free flavonoids content during storage and fruit coated with HM-CTS and MM-CTS showed significant result to retainfree flavonoids on day 12 when compared with uncoated fruit and fruit coated with LM-CTS (Figure 16C). Free flavonoids values were rapidly decreased during the first 4 days of storage then free flavonoids gradually increased after day 8.

CAT activity sharply decreased on day 4 of storage whereas APX activity rapidly increased on day 4 of storage and then reduced (Figure 17A and B). However, HM-CTS treatment showed the highest of CAT and APX activities on day 4 when compared with control treatment. Fruit coated with HM-CTS showed significant results in delaying the reduction of CAT and APX activities.

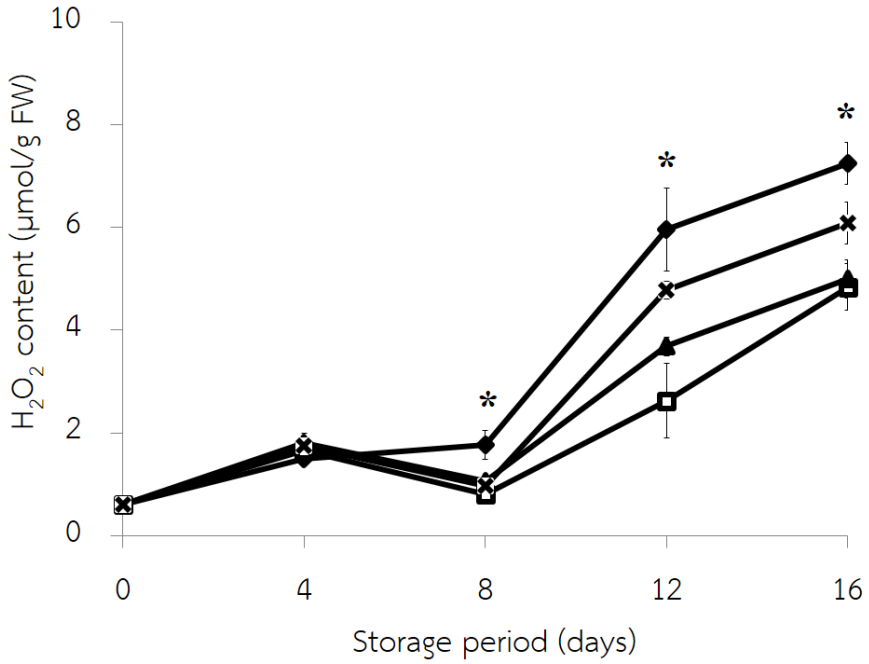

Figure 15. H_2O_2 content of control and chitosan coated fruit during storage at storage temperature (25±2°C) for 16 days and sampling every four days. Each value is the mean of eight fruit. Vertical bars indicate ±SE.

(◆) Control; (□) HM-CTS; (▲) MM-CTS; (✕) LM-CTS.

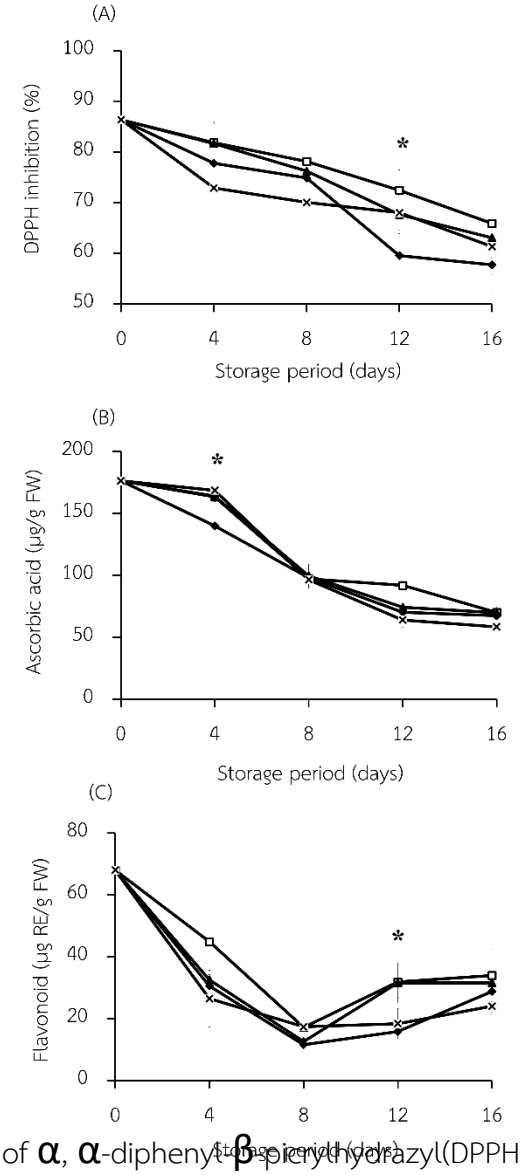

Figure 16. Percentage of α, α-dipheny-β-picrylhydrazyl(DPPH) inhibition (A), ascorbic acid (B) and free flavonoids (C) of control and chitosan coated fruit during storage at storage temperature (25±2°C) for 16 days and sampling every four days. Each value is the mean of eight fruit. Vertical bars indicate ±SE.

(◆) Control; (□) HM-CTS; (▲) MM-CTS; (✕) LM-CTS).

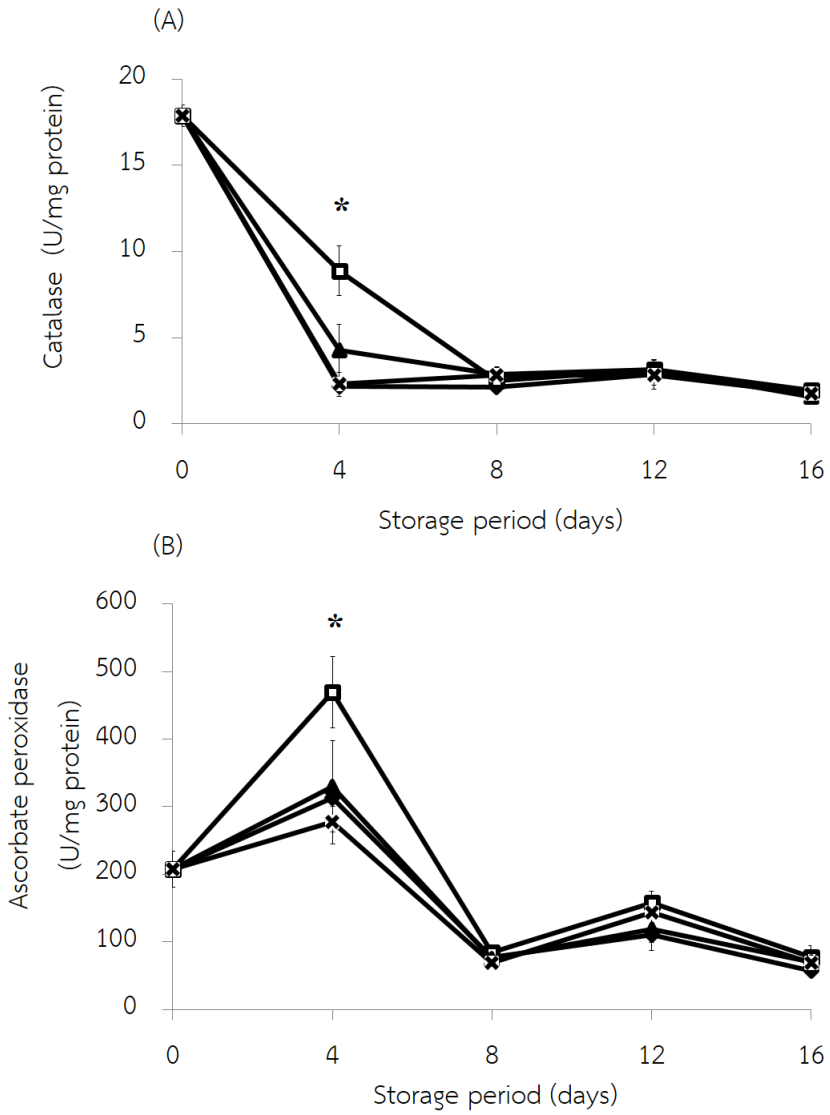

Figure 17. Catalase (A) and ascorbateperoxidase (B) activities of control and chitosan coated fruit during storage at storage temperature (25±2°C) for 16 days and sampling every four days. Each data point represents the mean of eight fruit. Vertical bars represent ±SE.

(◆) Control; (□) HM-CTS; (▲) MM-CTS; (✕) LM-CTS.

4.1.3.6 Disease incidence and disease severity

Mango fruit presented disease symptom on day 8 and sharply increased until the last day of storage. Uncoated fruit showed the highest of disease incidence and disease severity (75% and 35.9%, respectively) in the last day of storage whereas mango fruit coated with HM-CTS did not showed any disease incidence and disease severity until the end of storage period (Figure 18A and B).

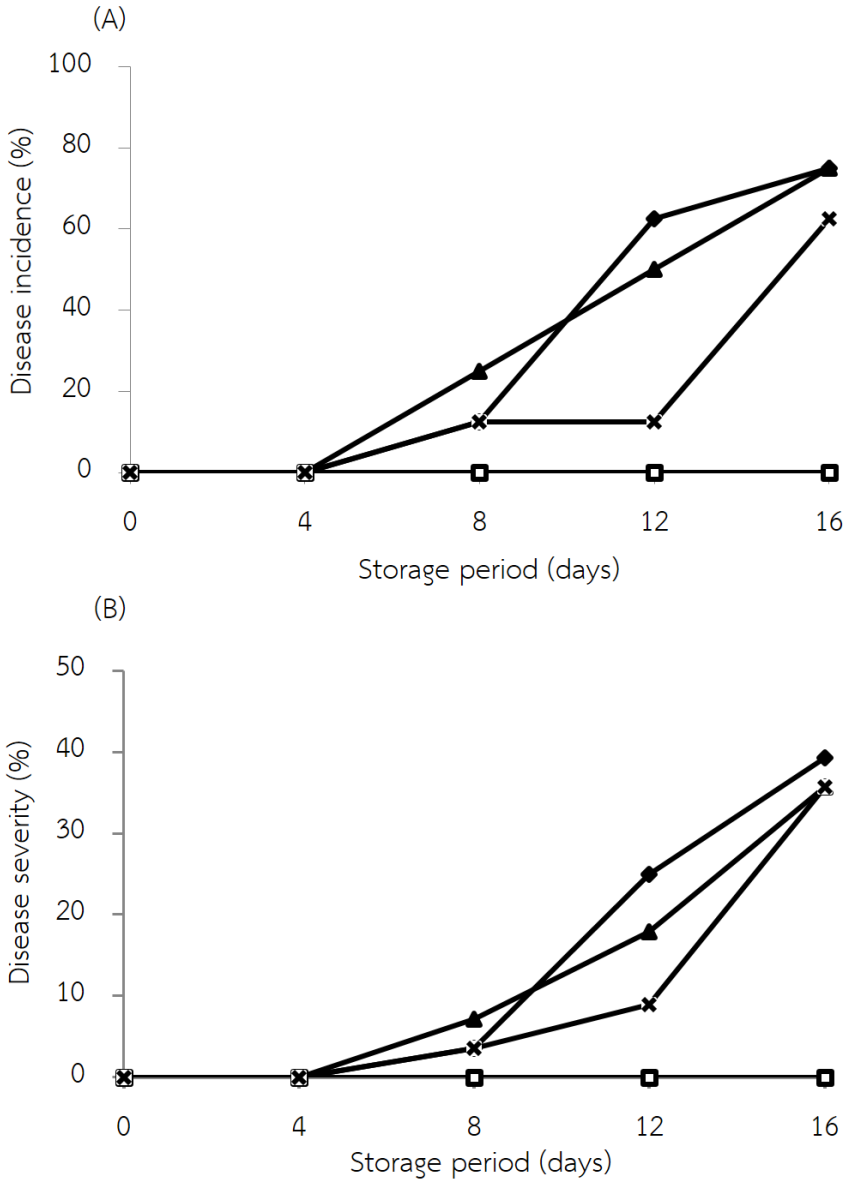

Figure 18. Disease incidence (A) and disease severity (B) of control and chitosan coated fruit during storage at storage temperature (25±2°C) for 16 days and sampling every four days. Each data point was calculated from four replications.

(◆) Control; (□) HM-CTS; (▲) MM-CTS; (✕) LM-CTS.

4.2 Investigation of the effects of the appropriate chitosan coating combined with spermidine on qualities of mango fruit

4.2.1 Chitosan coated film properties

Our result found that chitosan demonstrated a film forming properties by completely coating on the mango peel and fully covered stomata. The result was clearly showed by scanning electron microscope in mango fruit coated with HM-CTS combined with 0.1 ppm SPD and control treatments (Figure 19). In contrast, stomata were still observed in mango fruit coated with HM-CTS combined with 1 and 10 ppm SPD on mango peel (the arrows in Figure 19).

In addition, the result from the cross-section SEM images indicated that HM-CTS combined with 0.1 ppm SPD and control treatments obtained thicker chitosan film on mango peel than HM-CTS combined with 1 and 10 ppm SPD treatments (the arrows in Figure 20). Nevertheless, the result did not show significant differences of chitosan film thickness between HM-CTS combined with 0.1 ppm SPD treatment (3.07±0.06 μm) and HM-CTS treatment alone (3.05±0.06 μm). Mango fruit coated with HM-CTS combined with 1 and 10 ppm SPD treatments presented very thin chitosan film on mango peel so the thickness of chitosan film could not be determined.

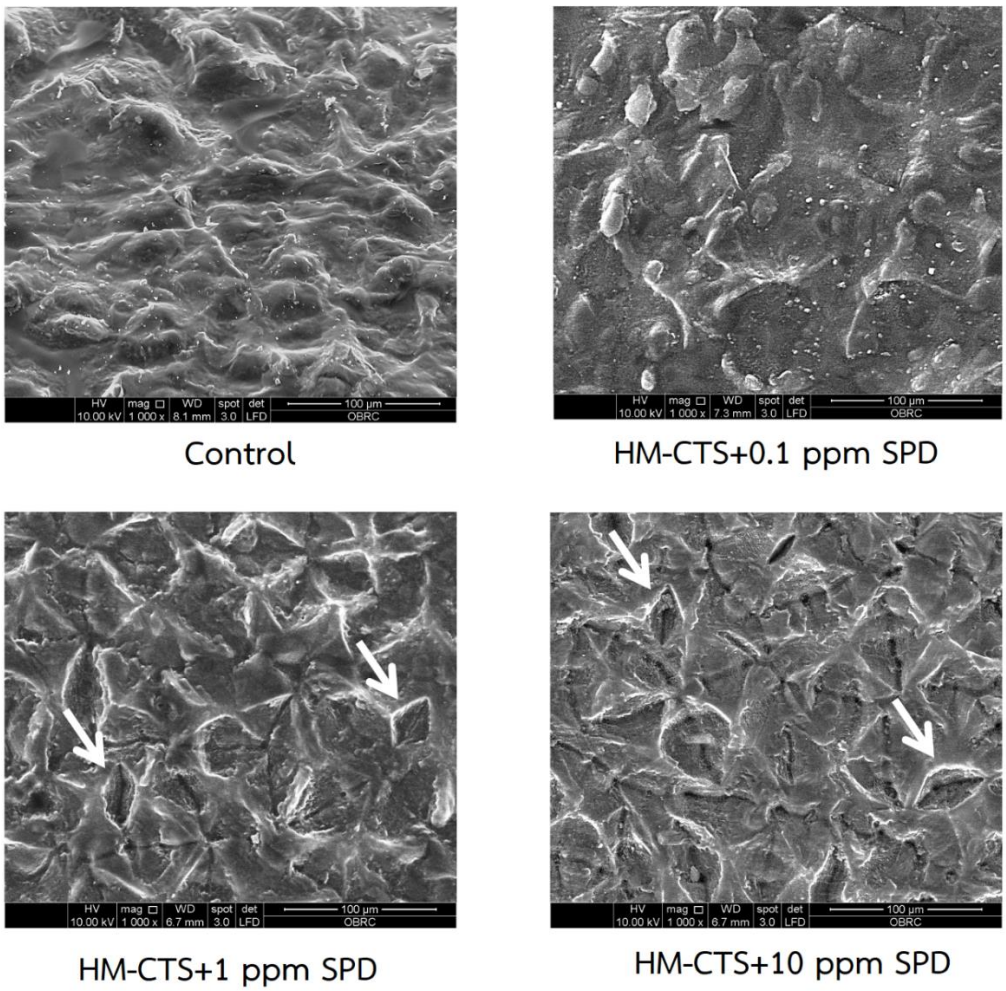

Figure 19. Surface image of mango peel coated with HM-CTS, HM-CTS+0.1 ppm SPD, HM-CTS+1 ppm SPD and HM-CTS+10 ppm SPD by scanning electron microscope. The arrows indicate stomata on mango peel after coating.

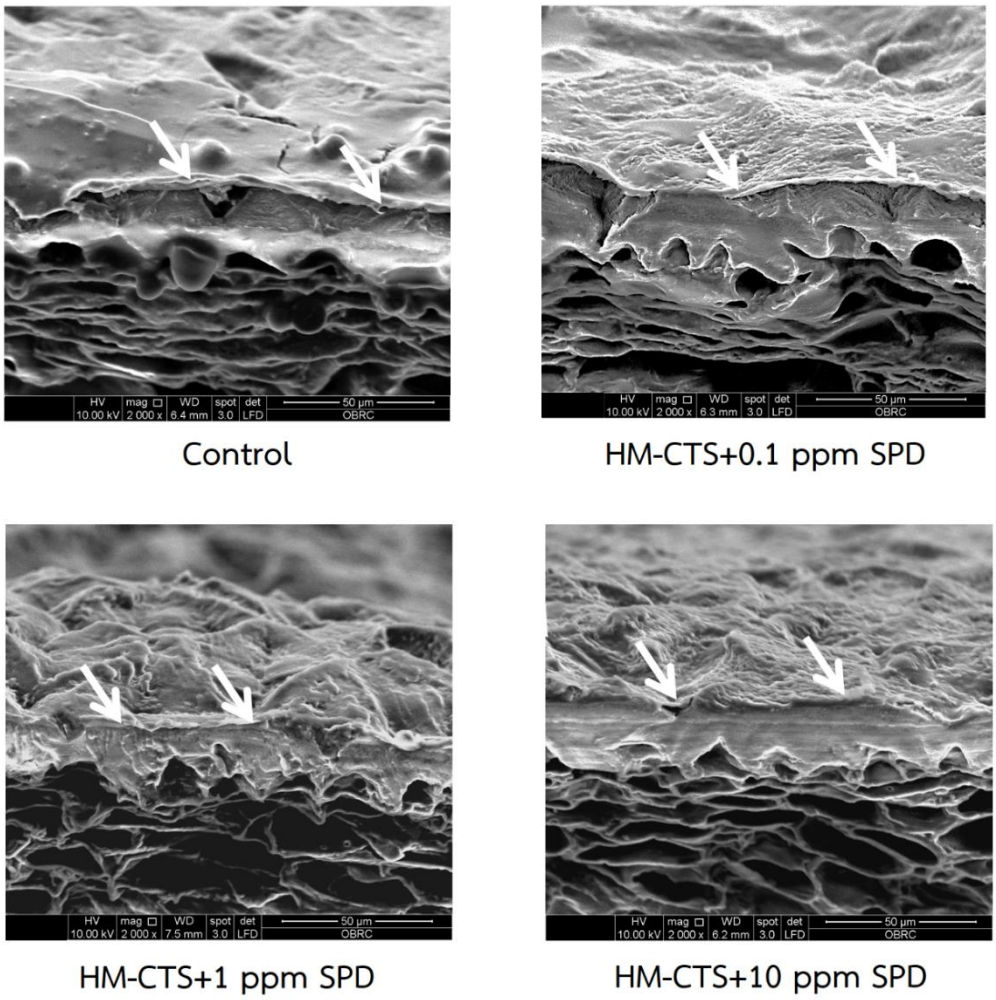

Figure 20. Cross-section image of mango peel coated with HM-CTS, HM-CTS+0.1 ppm SPD, HM-CTS+1 ppm SPD and HM-CTS+10 ppm SPD by scanning electron microscope. The arrows indicate thickness of chitosan film on mango peel.

4.2.2 Postharvest qualities and physicochemical characteristics

4.2.2.1 Respiration rate and ethylene production

Respiration rate of control fruit gradually increased during storage and then decreased after day 20 during storage (Figure 21A). Mango fruit coated with HM-CTS alone presented climacteric peak on day 20. Fruit coated with HM-CTS combined with SPD treatments showed significant result in reducing respiration rate on day 20 when compared with HM-CTS treatment. Besides, mango fruit coated with HM-CTS exhibited high ethylene production during storage on day 14 to day 20 and ethylene production decreased after day 20 (Figure 21B). Fruit coated with HM-CTS and HM-CTS combined with 10 ppm SPD showed higher level of ethylene production than fruit coated with HM-CTS combined with 1 and 1 ppm SPD. The result was significant difference on day 14, 17 and 20.

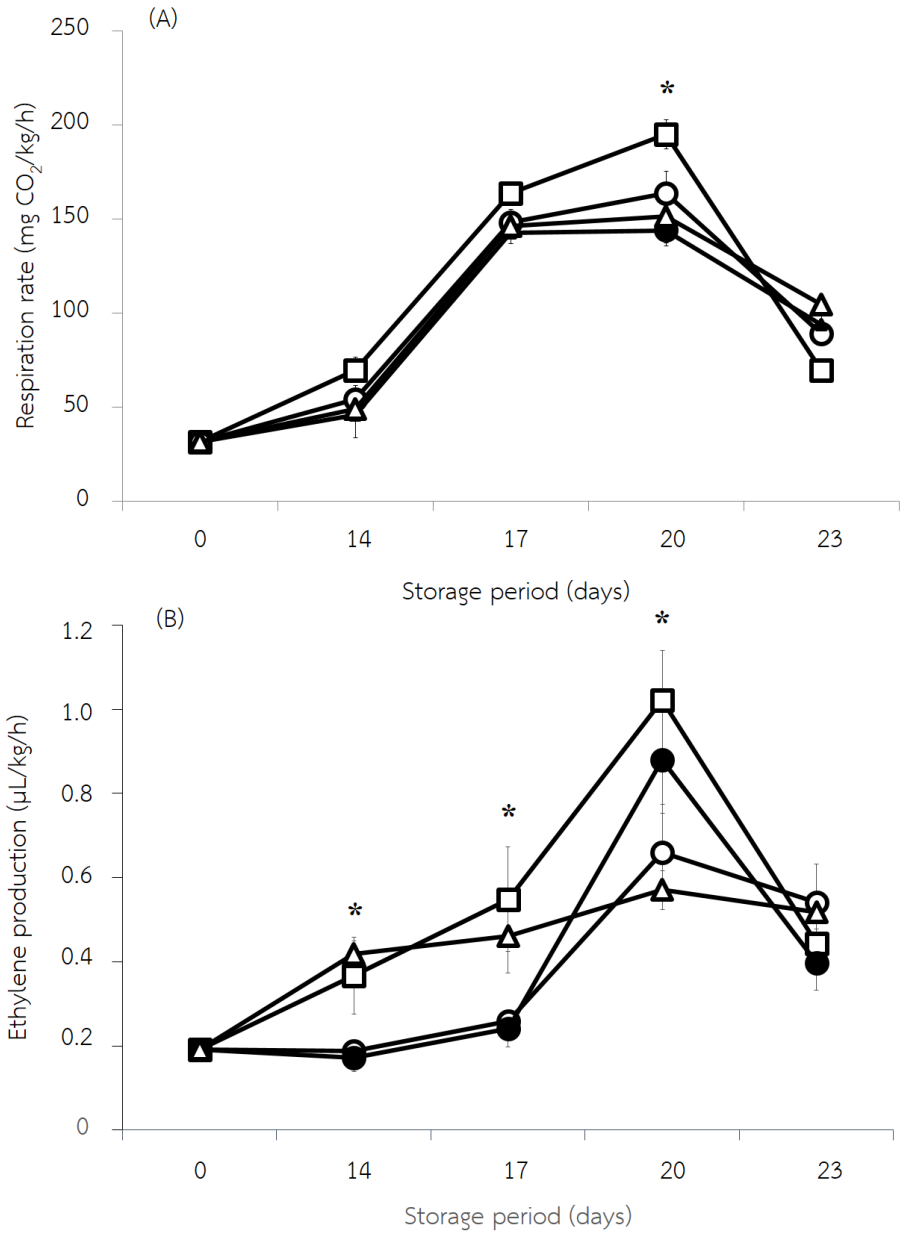

Figure 21. Respiration rate (A) and ethylene production (B) of control and HM-CTS combined with 0.1 1 and 10 ppm SPD coated fruit during storage for 23 days. Each data point represents the mean of eight fruit. Vertical bars represent ±SE.

(□) Control; (○) HM-CTS+0.1 ppm SPD; (●) HM-CTS+1 ppm SPD; (△) HM-CTS+10 ppm SPD.

4.2.2.2 Peel color change

Peel color of mango changed from green to yellow during 14 days of storage (Figure 22). L value showed a little enhancement and hue angle gradually decreased during ripening stage. Mango fruit coated with HM-CTS combined with 0.1 ppm SPD showed significant difference in delaying peel color change on day 14 when compared with other treatments (Table 3).

4.2.2.3 Fruit firmness, percentage of weight loss and total soluble solid (TSS) content

High percentage of fruit weight naturally declined during fruit ripening and storage. After harvest, due to the loss of water content within fruit, fruit firmness always decreased. Mango fruit coated with HM-CTS combined with 0.1 ppm SPD and 1 ppm SPD showed higher fruit firmness than fruit coated with only HM-CTS alone and HM-CTS combined with 10 ppm SPD. (Table 3). In addition, mango fruit coated with HM-CTS combined with 0.1 ppm SPD showed the highest of fruit firmness on day 14 and 17 and the result was significant difference on day 14.Our result found that percentage of weight loss increased during storage. Mango fruit coated with HM-CTS combined with 0.1 ppm SPD could delay percentage of weight loss during storage and fruit showed significant difference from control fruit (Table 3). TSS content increased during ripening stage. Fruit coated with HM-CTS combined with 0.1 1 and 10 ppm SPD

presented lower TSS content than fruit coated with HM-CTS alone. However, there were no differences between treatments (Table 3).

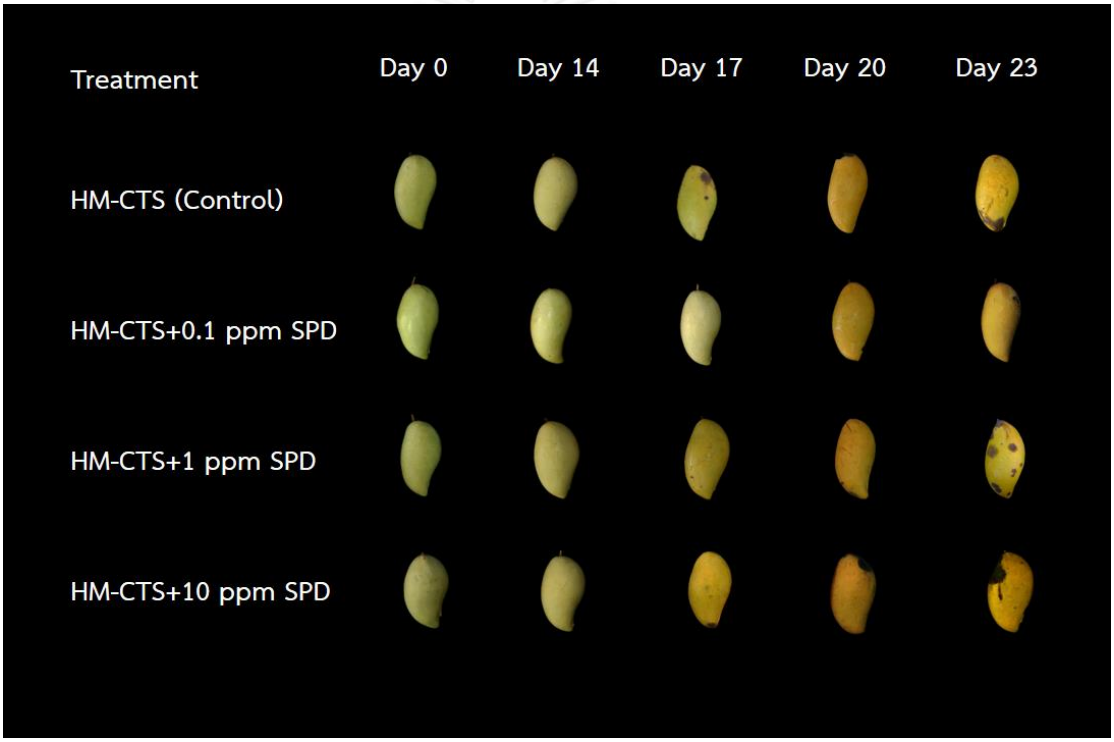

Figure 22. Peel color change of mango fruit coated with HM-CTS, HM-CTS+0.1 ppm SPD, HM-CTS+1 ppm SPD and HM-CTS+10 ppm SPD during storage for 23 days.

Table 3 Effects of the chitosan coating containing spermidine on L value, hue angle, firmness, weight loss (%) and TSS of mango fruit during storage for 23 days.

Storage period (days)	Treatment	L value	Hue angle	Fruit firmness (N)	Weight loss (%)	TSS (°Brix)
Day 0	Control	64.58±0.07^{a}	108.17±0.41^{a}	9.32±0.00^{a}	-	12.00±0.00^{a}
	HM-CTS+0.1 ppm SPD	64.58±0.07^{a}	108.17±0.41^{a}	9.32±0.00^{a}	-	12.00±0.00^{a}
	HM-CTS+ 1 ppm SPD	64.58±0.07^{a}	108.17±0.41^{a}	9.32±0.00^{a}	-	12.00±0.00^{a}
	HM-CTS+10 ppm SPD	64.58±0.07^{a}	108.17±0.41^{a}	9.32±0.00^{a}	-	12.00±0.00^{a}
Day 14	Control	67.35±0.22^{c}	106.61±0.71^{a}	6.86±0.01^{c}	7.53±0.39^{b}	15.00±0.00^{a}
	HM-CTS+0.1 ppm SPD	64.97±0.24^{a}	106.46±0.47^{a}	7.85±0.03^{a}	5.91±0.12^{a}	15.50±1.32^{a}
	HM-CTS+ 1 ppm SPD	66.54±0.40^{bc}	105.05±0.97^{a}	7.06±0.02^{bc}	7.41±0.56^{b}	15.75±0.75^{a}
	HM-CTS+10 ppm SPD	66.32±0.24^{b}	104.77±0.66^{a}	7.45±0.02^{ab}	6.85±0.59^{ab}	15.50±0.00^{a}
Day 17	Control	67.55±0.65^{a}	101.18±0.45^{a}	6.57±0.01^{a}	10.99±0.36^{b}	17.63±0.94^{a}
	HM-CTS+0.1 ppm SPD	66.72±0.21^{a}	100.80±0.56^{a}	6.96±0.02^{a}	9.37±0.24^{a}	16.13±0.72^{a}
	HM-CTS+ 1 ppm SPD	66.87±0.31^{a}	100.41±0.67^{a}	6.57±0.01^{a}	11.25±0.43^{b}	15.75±0.75^{a}
	HM-CTS+10 ppm SPD	66.85±0.39^{a}	99.58±0.60^{a}	6.77±0.02^{a}	11.42±0.42^{b}	15.75±0.75^{a}
Day 20	Control	68.00±0.73^{a}	96.31±0.75^{a}	5.20±0.02^{a}	13.77±0.40^{b}	18.00±0.00^{a}
	HM-CTS+0.1 ppm SPD	67.94±0.47^{a}	95.41±0.77^{a}	5.20±0.01^{a}	12.07±0.38^{a}	17.25±0.75^{a}
	HM-CTS+ 1 ppm SPD	68.68±0.57^{a}	94.37±0.94^{a}	5.59±0.01^{a}	12.98±0.61^{ab}	16.88±1.13^{a}
	HM-CTS+10 ppm SPD	67.72±0.59^{a}	94.21±0.65^{a}	5.10±0.01^{a}	13.31±0.38^{ab}	18.00±0.61^{a}
Day 23	Control	68.39±0.75^{a}	93.52±0.69^{a}	4.71±0.01^{a}	14.83±0.48^{b}	19.50±0.87^{a}
	HM-CTS+0.1 ppm SPD	68.15±0.44^{a}	93.15±1.11^{a}	4.41±0.02^{a}	12.98±0.21^{a}	19.13±0.72^{a}
	HM-CTS+ 1 ppm SPD	68.06±0.66^{a}	91.09±0.73^{a}	4.51±0.01^{a}	14.06±0.60^{ab}	18.00±0.00^{a}
	HM-CTS+10 ppm SPD	68.65±0.66^{a}	91.65±0.94^{a}	4.41±0.01^{a}	14.41±0.40^{b}	19.00±1.00^{a}

[a] Values followed by the same letter in the column on the same day were not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

4.2.2.4 Disease incidence and disease severity

Disease incidence on mango of control and HM-CTS combined with 1 or 10 ppm SPD treatments were obviously observed on day 17 during storage whereas fruit coated with HM-CTS combined with 0.1 ppm SPD presented disease symptom on day 20 (Figure 23). Moreover, fruit coated with HM-CTS combined with 0.1 ppm SPD showed the lowest of disease incidence (37.5%) and disease severity (19.64%).

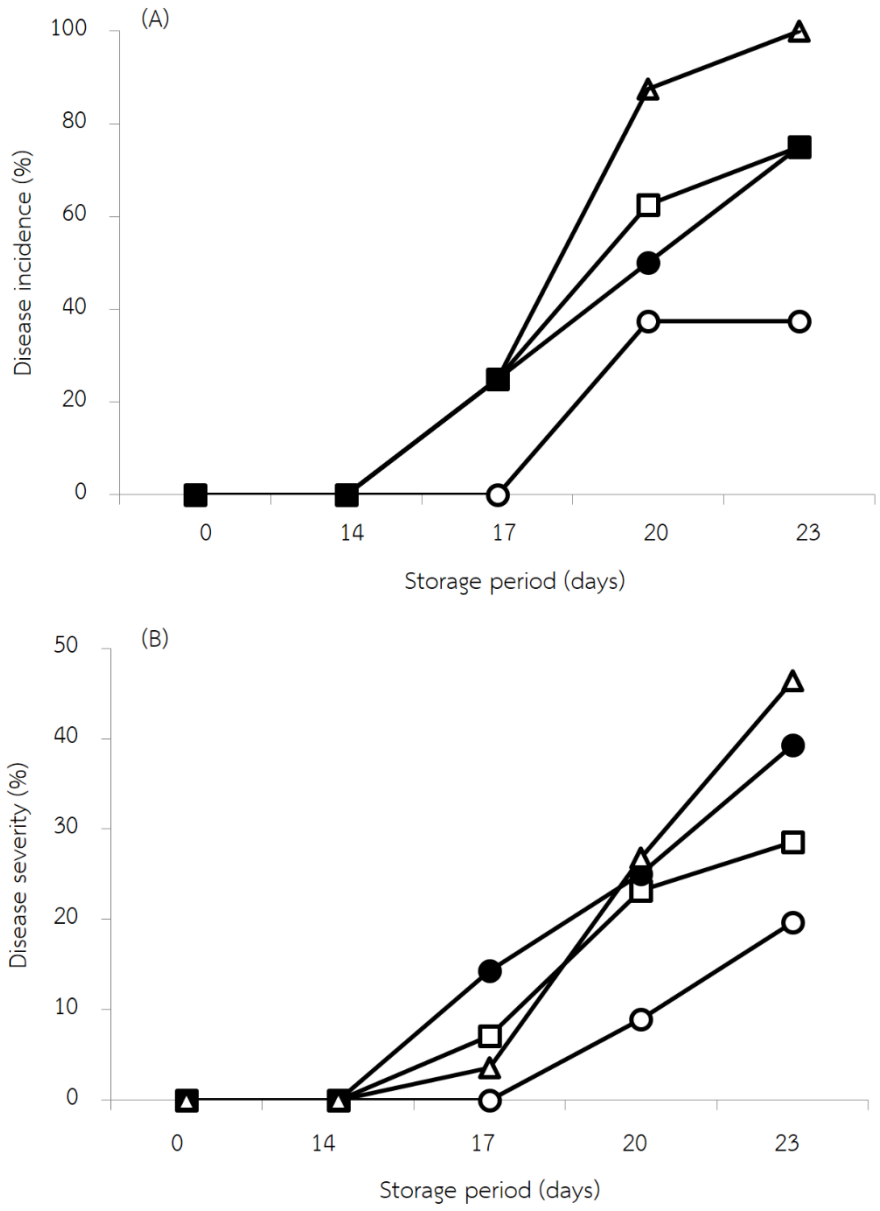

Figure 23. Disease incidence (A) and disease severity (B) of mango fruit coated with HM-CTS and HM-CTS combined with 0.1 1 and 10 ppm SPD during storage for 23 days. Each data point was calculated from four replications.

(□) Control; (○) HM-CTS+0.1 ppm SPD; (●) HM-CTS+1 ppm SPD; (△) HM-CTS+10 ppm SPD.

4.2.3 Cell wall characteristics

4.2.3.1 Soluble pectin content in mango peel and pulp

From the study in ripped mango peel, soluble pectin increased during ripening stage and fruit coated with HM-CTS combined with 0.1 ppm SPD showed the lowest soluble pectin content during storage among treatments. HM-CTS combined with 0.1 ppm SPD treatment presented significant difference resulting in reduced soluble pectin content in mango peel on day 17 20 and 23 (Figure 24A). In the same way in mango pulp, fruit coated with HM-CTS combined with 0.1 1 and 10 ppm SPD showed lower soluble pectin value than fruit coated with HM-CTS alone (Figure 24B).

4.2.3.2 Polygalacturonase activity (PG) in mango peel and pulp

PG activity was found to increase during mango fruit ripening leading to fruit softening. The result observed in mango peel showed that fruit coated with HM-CTS combined with 0.1 ppm SPD had the lowest PG activity when compared with control treatment (Figure 25A). The result in mango pulp was similar to those in mango peel. Mango fruit coated with HM-CTS alone showed the highest PG activity during storage (Figure 25B). HM-CTS combined with 0.1 ppm SPD treatment showed significant effect to inhibit PG activity during ripening period.

4.2.3.3 Pectin methyl esterase activity (PME) in mango peel and pulp

PME activity increased when mango fruit reached the ripening stage. Our study indicated that mango fruit coated with HM-CTS combined with 0.1 1and 10 ppm SPD showed low PME activity in peel and pulp during storage. In addition, mango fruit coated with HM-CTS combined with 0.1presented the lowest of PME activity in peel and pulp and fruit also showed significant difference result when compared with control fruit (Figure 26).

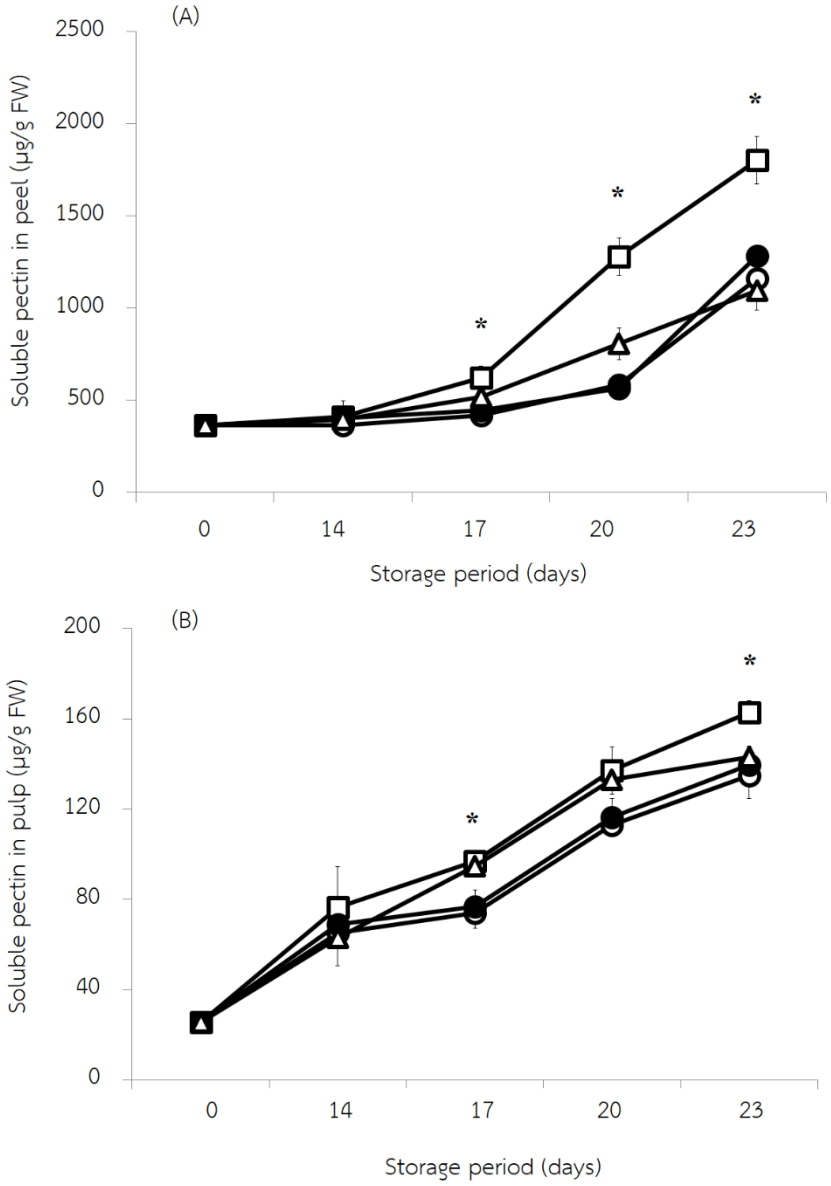

Figure 24. Soluble pectin content in peel (A) and pulp (B) of mango fruit coated with HM-CTS and HM-CTS combined with 0.1 1 and 10 ppm SPD during storage for 23 days. Each data point was calculated from four replications. Vertical bars represent ±SE. (□) Control; (○) HM-CTS+0.1 ppm SPD; (●) HM-CTS+1 ppm SPD; (△) HM-CTS+10 ppm SPD.

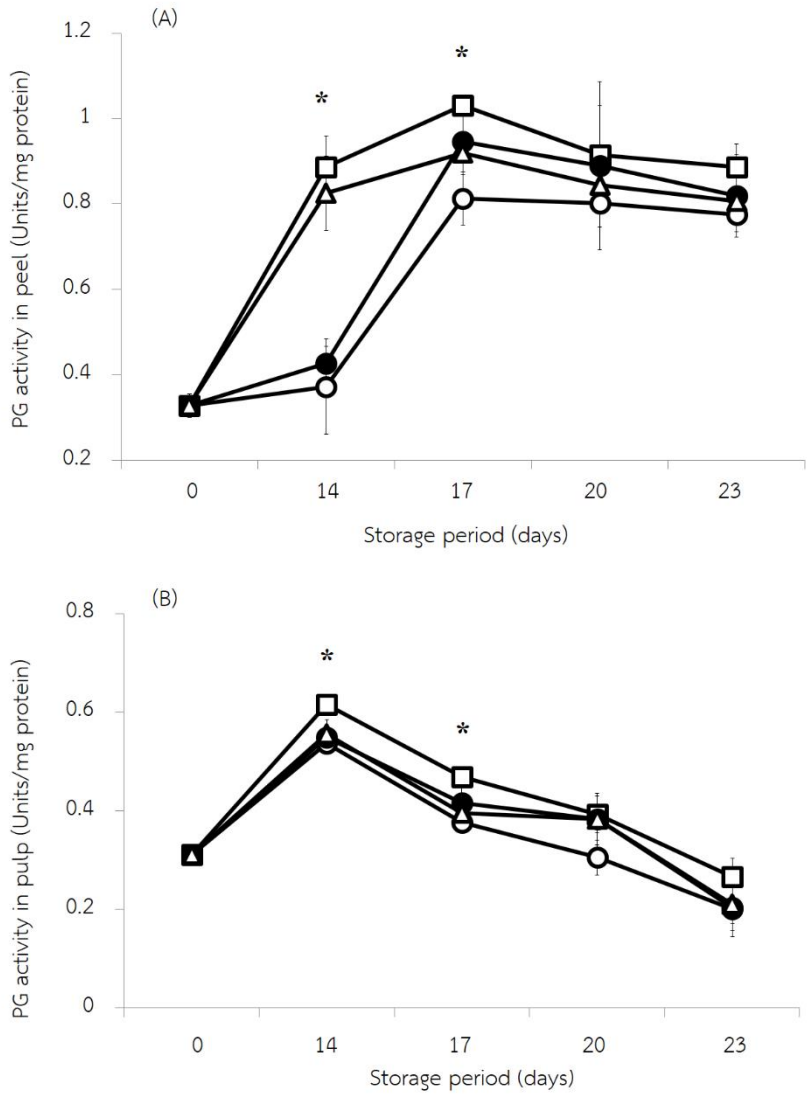

Figure 25. Polygalacturonase activity (PG) in peel (A) and pulp (B) of mango fruit coated with HM-CTS and HM-CTS combined with 0.1 1 and 10 ppm SPD during storage for 23 days. Vertical bars represent ±SE.

(☐) Control; (○) HM-CTS+0.1 ppm SPD; (●) HM-CTS+1 ppm SPD; (△) HM-CTS+10 ppm SPD.

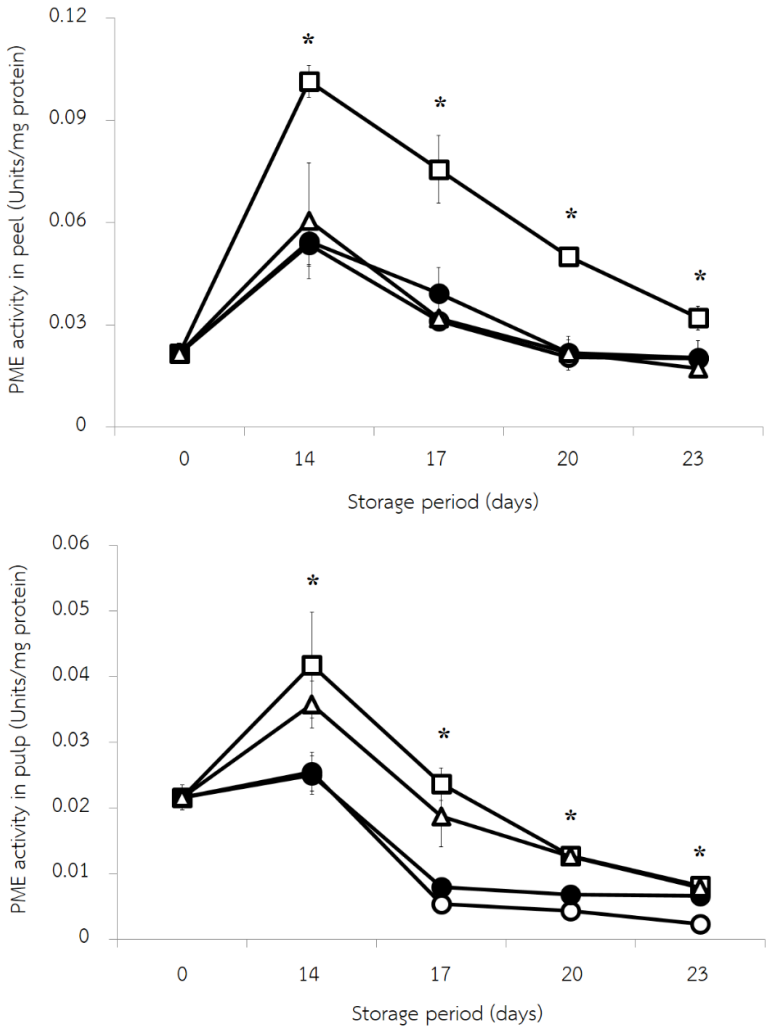

Figure 26. Pectin methyl esterase activity (PME) in peel (A) and pulp (B) of mango fruit coated with HM-CTS and HM-CTS combined with 0.1 1 and 10 ppm SPD during storage for 23 days. Each data point was calculated from four replications. Vertical bars represent ±SE.

(□) Control; (○) HM-CTS+0.1 ppm SPD; (●) HM-CTS+1 ppm SPD; (△) HM-CTS+10 ppm SPD.

4.2.4 Polyamines analysis

On day 14 of storage, free polyamines (PUT, SPD and SPM) and bound polyamines in mango peel were investigated. It was found that free PUT and SPM decreased in all treatments excepted in mango fruit coated with HM-CTS combined with 0.1 SPD. In addition, fruit coated with HM-CTS combined with 0.1 1 and 10 ppm SPD showed an increasing level of free SPD on day 14 except control fruit (Figure 27). HM-CTS combined with 0.1 ppm SPD treatment significantly induced three endogenous free polyamines (PUT, SPD and SPM) in mango peel compared with other treatments whereas HM-CTS combined with 1 and 10 ppm SPD treatments could induce only endogenous free SPD in fruit (Figure 27B). The same result was showed in endogenous bound SPD. Bound SPD decreased in all treatments except in fruit coated HM-CTS combined with 0.1 ppm SPD treatment (Figure 28). Mango fruit coated with HM-CTS combined with 0.1 ppm SPD treatment significantly induced bound SPD (0.07±0.01 mmol/g FW) at day 14. However, bound PUT and SPM were not detected in this experiment.

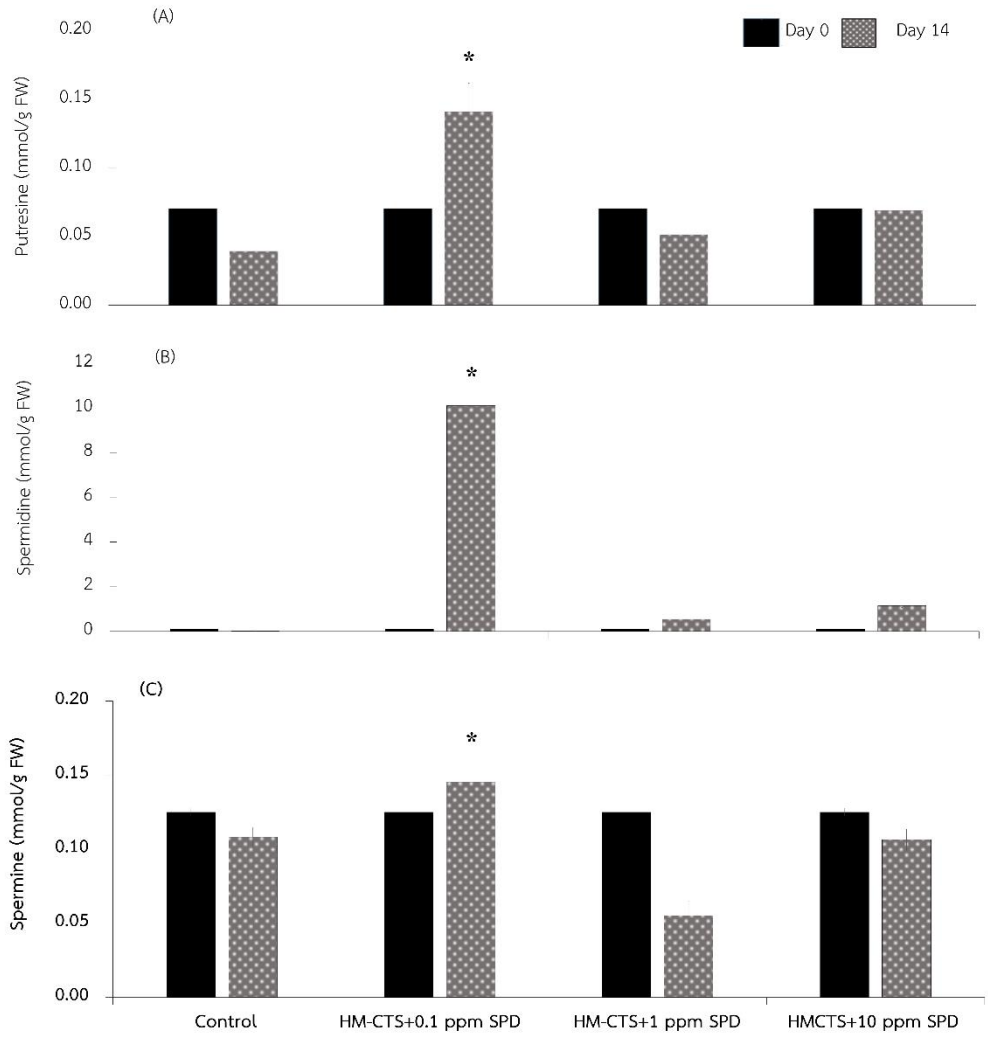

Figure 27. Free endogenous putresine (A), spermidine (B) and spermine (C) of mango peel coated with HM-CTS and HM-CTS combined with 0.1 1 and 10 ppm SPD on day 0 and day 14. Each data point was calculated from four replications. Vertical bars represent ±SE.

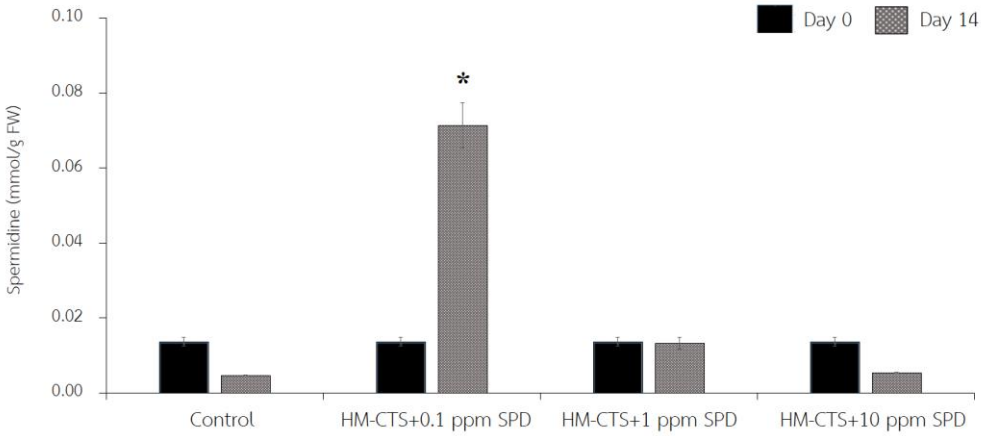

Figure 28. Bound endogenous spermidine of mango peel coated with HM-CTS and HM-CTS combined with 0.1 1 and 1 ppm SPD on day 0 and day 14. Each data point was calculated from four replications. Vertical bars represent ±SE.

4.3 Determination of the effect of an appropriate chitosan coating combined with spermidine on anthracnose disease in mango fruit

4.3.1 Disease incidence

Inoculated mango fruit presented disease symptom on day 4. Inoculated fruit and inoculated fruit dipped with 0.5% acetic acid showed disease incidence at 70% on day 4 after inoculation. However, inoculated fruit with sterile distilled water and inoculated fruit coated with HM-CTS+0.1 ppm SPD treatments did not showed disease incidence after inoculation (Figure 29 and Figure 30A).

4.3.2 Development of lesion area

Inoculated fruit showed the highest of area of lesion development during storage until the end of storage (59.22±1.62 mm) and inoculated fruit dipped with 0.5% acetic acid showed lower area of lesion development (47.50±5.02 mm) (Figure 30B). Inoculated fruit with sterile distilled water and inoculated fruit coated with HM-CTS+0.1 ppm SPD treatments did not presented any disease symptom until the last day of storage. HM-CTS combined with 0.1 ppm SPD treatment significantly controlled disease development in mango fruit.

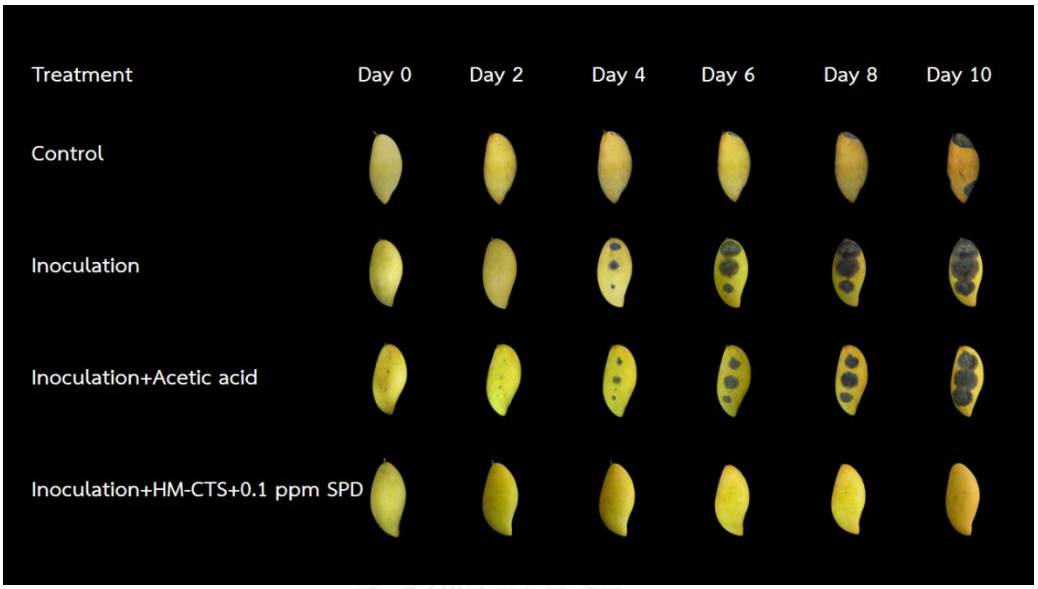

Figure 29. Disease development of inoculated fruit during storage at ambient temperature (28±2°C) for 10 days.

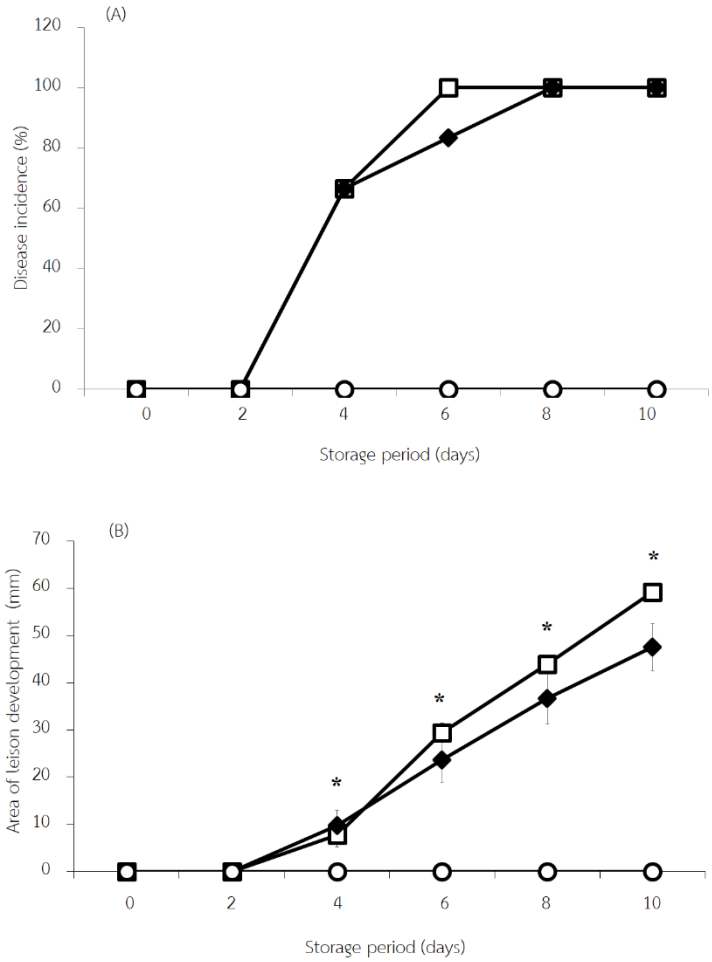

Figure 30. Disease incidence (A) and area of lesion development (B) of inoculated fruit during storage at ambient temperature (28±2°C) for 10 days. Each data point was calculated from four replications.

(△) inoculation with sterile distilled water without coating; (□) fungal inoculation without coating; (◆) fungal inoculation+0.5% (v/v) acetic acid solution amended with 0.1% (w/v) tween® 80; (○) fungal inoculation+(HM-CTS+0.1 ppm SPD).

CHAPTER V

DISCUSSION

5.1 Development and selection of an appropriate chitosan coating to prolong shelf-life of mango fruit during storage

5.1.1 Chitosan coating film properties

Our result found that HM-CTS represented the best characteristic of coating film on 'Nam Dok Mai' mango fruit since HM-CTS exhibited the thickest chitosan film and continuation of film on mango peel while mango fruit coated with LM-CTS still showed stomata on mango peel as same as control fruit (indicated by the arrows). Stomata on fruit peel can cause the reduction of fruit qualities after harvest by transpiration and gas diffusion. HM-CTS treatment could completely coat stomata on mango peel as well as waxing which affected the opening of stomatal pore on fruit surface resulting in reduced ethylene production and gas exchange (CO_2 and O_2) (Ben-Yehoshua, Burg and Young, 1985). Chitosan is the material that has film forming characteristics and it has been used as fruit coating for preserving qualities in many fruits and vegetables (No et al., 2007; Malik, Al-Thabaiti and Shiekh, 2013). In addition, properties of chitosan were associated with its molecular weight (Tsai (Tsai et al., 2002; Kean and Thanou, 2010). According to our result, HM-CTS showed the highest viscosity

of chitosan solution while MM-CTS showed moderated value and LM-CTS showed the lowest value. Likewise, (Rabea et al., 2003) revealed that the viscosity value of chitosan solution was related to the molecular weight of chitosan, the ionic strength, the concentration of solution, pH and the temperature. Therefore, molecular weight of chitosan influenced viscosity and chitosan forming film properties (thickness and texture).

5.1.2 Physicochemical characteristics

Recently, edible coating is the popular treatment for minimally-processed of fruits and vegetables (Olivas et al., 2008). Chitosan which exhibited film forming property has been successfully applied as edible coating in fruits and vegetables (Ouattara et al., 2000; Elsabee and Abdou, 2014). Our research indicated that ethylene production and respiration rate of mango were suppressed by coating with CTS during storage with HM-CTS being the most effective treatment. This was due to modified atmosphere created by chitosan film as fruit coating. The barrier film property of chitosan acted as a selective film for inhibiting gas diffusion such as C_2H_4, CO_2, and O_2 similar to other previous reports (Jitareerat et al., 2007; Ali et al., 2011). Therefore, chitosan coating could delay respiration rate and ethylene production of fruit. According to the reports in various fruits and vegetables, mango and papaya fruits coated with chitosan effectively reduced ethylene and respiration rate (Jitareerat et al., 2007; Abbasi et al., 2009; Ali et al., 2011). Moreover, 1% chitosan film combined

with 0.04% nano-silicon dioxide showed positive effect to maintain qualities of jujube fruit under ambient temperature with 85% RH for 32 days (Yu et al., 2012). These treatments could retard respiration resulted in maintain fruit fresh weigh, peel color and control decay when compared with control treatment. In addition, the result was accordance with Jitareerat et al. (2007), 'Nam Dok Mai' mango coated with 1% chitosan (Mw=350 kDa) showed lower rate of respiration and ethylene level than fruit coated with 0.5% chitosan and uncoated fruit.

Losing of fresh weight of fruits and vegetables was mainly due to the loss of water content which caused by transpiration and respiration processes (Zhu et al., 2008). From the result, since HM-CTS showed the thickest of chitosan film on mango peel and stomatal apertures were not found on mango peel, fruit coated with HM-CTS presented the lowest of percentage of weight loss during storage due to antitranspiration of chitosan film property. Similarly, fresh cut strawberries (*Fragaria ananassa* Duchesne cv. 329) coated with 1.0% chitosan showed lower fresh weight loss than fruit coated with 1% carboxymethyl cellulose (CMC) during storage at 2°C for 10 days. In addition, chitosan coating exhibited barrier for water vapor and mechanical property, thinner chitosan film showed lower water vapor permeability property (Kerch et al., 2011; Cheng, Wang and Weng, 2015). Previous study in sliced mango revealed that sliced mango coated with chitosan clearly delayed weight loss, maintained antioxidant property and prolonged shelf life (Chien et al., 2007).

Maintaining of fruit fresh weight was also related to fruit firmness. Fruit fresh weight loss during storage was mainly caused by evaporation of water content from the fruit leading to fruit shriveling (Barman et al., 2011). The ripening process of fruit clearly generated fresh weight loss and softening, fresh weight and firmness are as the main index of postharvest qualities of fruit (Bonilla et al., 2012). Mango fruit coated with HM-CTS showed higher fresh weight and fruit firmness than other treatments. This indicated that HM-CTS were completely affected water vapor transferring ability of mango fruit. Correspondingly, 'pearl' guava fruit coated with chitosan significantly maintained fruit firmness and fresh weight, delayed peel color changes compared with control fruit (Hong et al., 2012). Moreover, improving fruit firmness by chitosan coating was reported in papaya (Ali et al., 2011). Papaya fruit coated with 2% chitosan was firmer than other treatments during storage under low temperature. Furthermore, there were reports in many fruits such as tomato and mango that fruits were firmer when coated with chitosan (Kim et al., 1999; Zhu et al., 2008).

Peel color changes of fruit from green to yellow usually occurred during storage because of chlorophyll loss and high carotenoids content (Pokorny, Yanishlieva and Gordon, 2001; Ding et al., 2007). Chitosan coating exhibited significant effect in delaying a reduction of chlorophyll content in guava fruit and sweet pepper (Xing et al., 2011; Hong et al., 2012). Besides, 2.0% chitosan coating could delay peel color change of longan fruit compared with other treatments (Jiang and Li, 2001). Due to MA property

of chitosan coating, coated fruit presented high level of CO_2 which could inhibit ethylene production leading to retard fruit ripening in many fruits such as tomato and papaya (El Ghaouth et al., 1992; Martínez-Romero et al., 2006; Ali et al., 2011).

Generally, climacteric fruit exhibited chemical changes during ripening stage, mainly parameters which usually investigated were soluble solid (TSS), pH and titratable acidity (TA) (Cissé et al., 2015). Our result revealed that during storage of mango fruit, TA decreased and TSS increased. Fruit coated with HM-CTS significantly delayed TA and TSS changing compared with uncoated fruit. TSS reflected sugar content within fruit which was normally enhanced during ripening process and TA generally reduces during ripening which were important characters of climacteric fruit during ripening stage (Mahto and Das, 2013). According to Cissé et al. (2015), 'Kent' mango coated with chitosan-lactoperoxidase showed lower TSS and higher TA values than uncoated mangoes. In addition, 'Pearl' guava fruit coated with 2% chitosan showed the lowest of TA content and TA content associated with chitosan concentrations. Similar result was showed in strawberry, peach, tomato and litchi fruits coated with chitosan (Han et al., 2004). Furthermore, 'Summer Bahisht Chaunsa' mangocoated with chitosan showed low TSS content during stored at 15±1°C with 85% RH for 6 weeks whereas uncoated fruit exhibited the maximum of TSS during ripening process (Abbasi et al., 2009). Besides, Jiang and Li (2001) also indicated the influence of chitosan coating on longan fruit and reported that TA decreased during storage. Fruit

coated with chitosan completely retarded the changing of TA and TSS so it could slow ripening process (Youssef et al., 2002). An increase in TSS generated from starch and a decrease in TA showed physicochemical changes during ripening stage (Wills and Rigney, 1979). Since, chitosan coating modified the internal atmosphere and endogenous gas concentration (CO_2 and O_2) of the fruit leading to a delay of fruit ripening as well as inhibiting changes in TSS and TA.

Our result found that coating mango fruit with HM-CTS led to lower H_2O_2 content and maintained DPPH inhibition, ascorbic acid content, flavonoid content and antioxidant enzyme activities (catalase (CAT) and ascorbate peroxidase (APX)) during storage. During fruit ripening process, reactive oxygen species (ROSs) such as hydrogenperoxide (H_2O_2) and superoxide ($^{\bullet}O_2^-$) generally increases while antioxidant system decreases leading to injured plant cell structures (Kim et al., 2007). However, HM-CTS significantly decreased H_2O_2content and increased antioxidant system in mango fruit. According to (Jitareerat et al., 2007), 1% chitosan coating could induce ascorbic acid content and TA of 'Nam Dok Mai' mango when compared with 0.5% chitosan coating and control treatment. Combination of chitosan and salicylic acid could increase antioxidant enzyme activities including superoxide dismutase (SOD), CAT, APX and glutathione reductase (GR) in cucumber (Zhang et al., 2015). Moreover, it was found that production of superoxide free radicals and malondialdehyde (MDA) were significantly decreased in the plum fruit coated with combination of chitosan and

ascorbic acid (Liu et al., 2014). Our result was supported by previous study in guava fruit which chitosan coating significantly induced ascorbic acid content, increased of activities of peroxidase (POD), SOD, and CAT and limited superoxide free radical production (Hong et al., 2012). Moreover, Zeng et al. (2010) indicated that SOD and POD activities in navel orange fruit were enhanced by chitosan coating and it showed similar result in tomato and sweet cherry fruit treated with chitosan (Liu et al., 2007; Dang et al., 2010). Jujubes fruit coated with chitosan combined with nano-silicon could preserve total flavonoid content and show higher activities of scavenger antioxidant enzymes such as POD, SOD and CAT than control fruit (Yu et al., 2012). Antioxidant enzymes have an important role in oxidation resistant system. HM-CTS coating enhanced the activities of these enzymes in mango fruit so HM-CTS coating could scavenge excessive ROS molecules resulted in retarding fruit senescence and protecting plant tissues from injury.

HM-CTS completely controlled disease development in 'Nam Dok Mai' mango fruit by increasing defense enzyme activities. According to (Romanazzi et al., 2002), grape berries showed high activity of phenylalanine ammonia-lyase (PAL) after coated with chitosan and chitinase and β-1,3-glucanase activities were also induced in oranges, strawberries and raspberries treated with chitosan (Zhang and Quantick, 1997). Our result was in accordance with Jitareerat et al. (2007), 'Nam Dok Mai' mango coated with 1% chitosan could control disease development in infected fruit compared with

other treatments. POD activity increased in chitosan-treated cucumber plants and jujube fruit (Ben-Shalom et al., 2003; Yu et al., 2012) leading to enhance plant resistance from pathogen infection. In addition, Chien, F. and Yang (2007) reported that chitosan coating effectively controlled the growth of microorganisms on the sliced mango. Chitosan presented barrier film property which showed lower internal oxygen concentration in fruit therefore it was the limiting factor for fungal growth (Amarante and Banks, 2001; Jitareerat et al., 2007). According to the results, we suggest a schematic representation of HM-CTS coating for delaying the ripening of 'Nam Dok Mai' mango fruit after harvest as shown in figure 31.

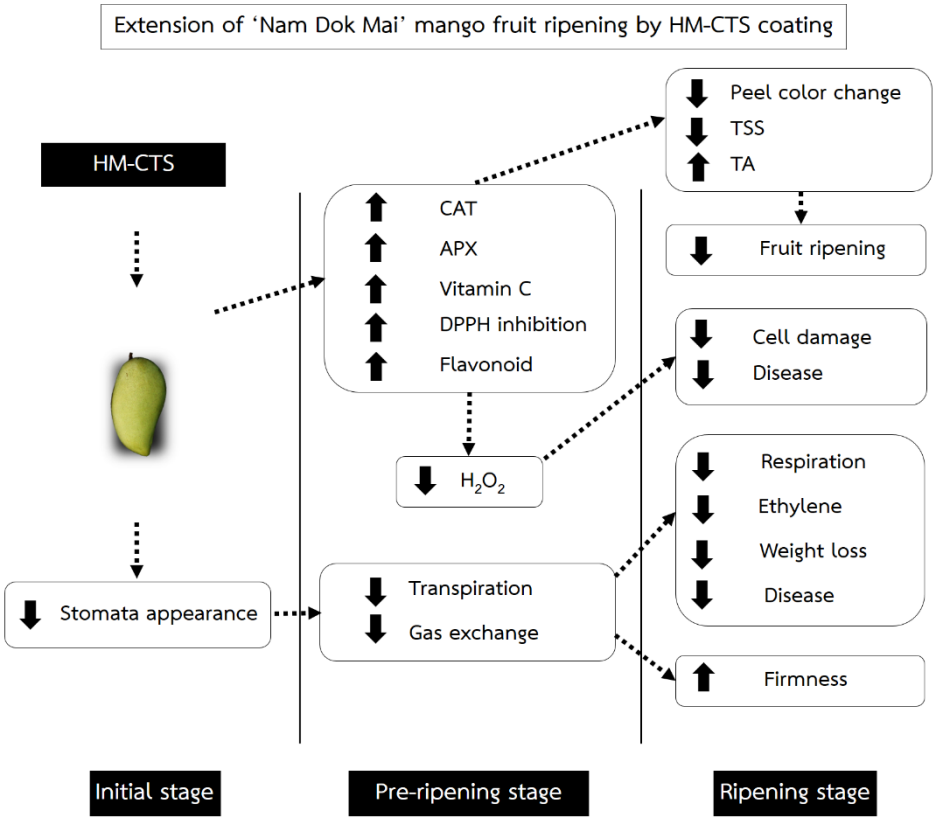

Figure 31. Schematic representation of the extension of 'Nam Dok Mai' mango fruit ripening after harvest by HM-CTS coating.

5.2 Investigation of the effects of the appropriate chitosan coating combined with spermidine on qualities of mango fruit

5.2.1 Chitosan coating film properties

Climacteric fruit presented high respiration rate and fresh weight loss by transpiration after harvest (Burg and Kosson, 1983; Ben-Yehoshua et al., 1985). Opening of stomata on fruit surface resulted in an increased of transpiration rate leading to increased loss of water content and gas diffusion. Coating could cover stomata on fruit surface and acted as barrier for gas exchange and water vapor (Ben-Yehoshua et al., 1985). Our study in 'Nam Dok Mai' mango, HM-CTS alone exhibited fully covered stomata on mango peel resulting in inhibited respiration rate and fresh weight loss. Similarly, HM-CTS combined with 0.1 ppm SPD could completely cover mango peel. Stomata were not observed on mango peel after coating whereas fruit coated with HM-CTS combined with 1 or 10 ppm SPD exhibited stomata on mango peel (indicated by arrows) indicating that higher concentration of SPD may affect film forming characteristic. In addition, HM-CTS alone and HM-CTS combined with 0.1 ppm SPD showed thicker chitosan coating film than HM-CTS combined with 1 or 10 ppm SPD. Our results were in accordance with (Barman et al., 2011) who reported that combination of PUT and carnauba wax could reduce respiration rate and ethylene levels in pomegranate fruit. Due to the antisenescence and barrier properties of the

combined treatment on fruit peel. HM-CTS combined with 0.1 ppm SPD was the best combination for fruit coating.

5.2.2 Physicochemical characteristics and fruit softening

Fruit coated with HM-CTS combined with SPD showed lower respiration rate than control fruit and fruit coated with HM-CTS combined with 0.1 and 1ppm SPD significantly reduced ethylene production during storage. Chitosan coatings served as a selective film and limited gas diffusion especially O_2 and CO_2 (Srinivasa et al., 2002). Moreover, ethylene and polyamines share the common precursor which is S-adenosyl methionine (SAM) in ethylene and polyamines biosynthesis pathways (Li et al., 1992; Mehta et al., 2002). Changing of polyamines and ethylene productions in climacteric fruits after harvest usually showed an adverse effect (Liu et al., 2006). Previous study in tomato fruit indicated that polyamine productions were enhanced whereas ethylene production was reduced after harvest (Dibble et al., 1988). In addition, Malik and Singh (2003) revealed that 'Kensington Pride' mango treated with exogenous PUT improved fruit firmness and retarded shelf life. Adding ethylene inhibitor such as aminoethoxyvinylglycine (AVG) showed significantly inhibited ethylene production and enhance polyamine production (Arigita et al., 2004).Therefore, combination of chitosan and spermidine could delay respiration rate and ethylene production resulted in the retarded shelf life of 'Nam Dok Mai' mango fruit after harvest.

In addition, our result found that fruit coated with HM-CTS combined with 0.1 ppm SPD could delay peel color change during ripening period when compared with other treatments. HM-CTS combined with 0.1 ppm SPD completely coated on mango peel and stomatal apertures on mango peel were not detected so coated fruit could inhibit gas exchange and transpiration leading to reduced respiration rate, ethylene production and fresh weight loss in ripening processes. Previous report in various fruits indicated that chitosan coating could delay chlorophyll breakdown in guava and sweet pepper leading to maintained peel color changing of fruits (Xing et al., 2011; Hong et al., 2012). Similar result was also reported in freshly peeled litchi and longan (Jiang and Li, 2001; Dong et al., 2004). Exogenous polyamines could inhibit browning and ethylene production of litchi fruits kept at 5°C (Jiang and Chen, 1995). In addition, plum fruits treated with 1 mM PUT also retarded ethylene production resulted in delayed fruit softening, TSS and TA changes and significantly maintained peel color (Valero et al., 2002; Serrano et al., 2003). Application of exogenous PAs during the latest fruit set stage of mango fruit indicated that PAs treatment effectively delayed peel color changes from green to yellow compared with control fruit (Malik and Singh, 2003; Malik and Singh, 2005). Kiwi and apricot fruits treated with PUT showed the retains of fresh weight and peel color change (Petkou et al., 2004). Similar result was shown in apricot cv. 'Bagheri' and 'Asgarabadi' treated with 1 mM PUT and SPD stored at 1°C for 21 days. These treatments could maintain fruit firmness, peel color and inhibit ethylene production. Furthermore, 'Hom Thong' banana treated with 0.1 ppm SPD had an ability

to reduce a loss of fresh weight and maintain peel color (Santivipanond et al., 2012). Therefore, combination of HM-CTS and 0.1 ppm SPD showed the best effect for maintaining peel color changes and qualities of 'Nam Dok Mai' mango fruit.

Mango fruit coated with HM-CTS combined with 0.1 ppm SPD exhibited the decreasing of disease incidence and disease severity when compared with other treatments during storage.

Chitosan widely showed bacteriostatic and fungistatic properties (Muzzarelli et al., 1990; Tomihata and Ikada, 1997). Mango fruit coated with chitosan exhibited low percentage of disease infection (6.9%) than control fruit (13.3%) after storage for 14 days (Abbasi et al., 2009). The result was supported by previous study of (Zhang and Quantick, 1997); chitinase and β-1,3-glucanase activities were induced in oranges, strawberries and raspberries coated with chitosan. Normally, fruit had higher antioxidant property by increasing activities of POD, CAT, SOD and polyphenol oxidase (PPO) during storage. Furthermore, SPD inhibited chilling injury (CI) of treated fruit compared with control fruit. PAs were reported to induce antioxidant enzyme activity leading to prolonged shelf life and delayed senescence (Malik and Singh, 2005). Correspondingly, fruit showed high performance in protecting fruit from pathogen infection. Therefore, HM-CTS combined with 0.1 ppm SPD showed synergistic effect to control disease development in 'Nam Dok Mai' mango by inducing plant defense mechanism in fruit.

Fruit softening is an important physiological change of fruit which occurs during ripening stage due to loss of water and increased activity of cell wall degrading enzyme (Asif and Nath, 2005; Prasanna et al., 2007; Barman et al., 2011). 'Nam Dok mai' mango coated with HM-CTS combined with 0.1 ppm SPD showed higher fruit firmness and lower fresh weight loss than other treatments during storage. Moreover, coated fruit showed the reduction of soluble pectin in peel and pulp during ripening period. Cell wall degrading enzymes (PG and PME) were also investigated in this study. Fruit coated with HM-CTS combined with SPD exhibited low activities of both cell wall degrading enzymes in peel and pulp when compared with control fruit. However, fruit coated HM-CTS combined with 0.1 ppm SPD presented the lowest of cell wall degrading enzymes activities during storage. Chitosan coatings exhibited barrier film property to protect fruit from water vapor and gas exchange (Ribeiro et al., 2007; Duan et al., 2011). According to (Abbasi et al., 2009), 'Summer Bahisht Chaunsa' mango coated with chitosan from crab, coating improved fruit firmness. In addition, chitosan-coated sliced mango showed the lowest weight loss resulted in maintained water in the mango pulp (Chien et al., 2007). Therefore, chitosan could avoid cell wall loosening and reduction of cell shrinkage of which in preserved cell integrity (Salunkhe and Desai, 1984; Medlicott, Bhogal and Reynolds, 1986).

Pectin is usually degraded by pectin degrading enzymes then changes to soluble pectin. Generally, soluble pectin enhances during ripening period leading to

loss of cell structure and fruit softening (Redgwell et al., 1997). Exogenous PUT and SPD significantly inhibited fruit softening, chilling injury and maintain fruit qualities of 'Lisbon' lemon fruit during storage at 1.5°C with 90% RH for 8 weeks (Safizadeh, 2013). Since, PAs interacted with pectic substances which were in cell wall structure thus inhibiting the binding of cell degrading enzyme (PME) and decreasing cell wall degrading enzyme activities (Valero et al., 2002). The result was in accordance with previous report in apricot, plum and kiwi fruits, fruit treated with PUT inhibited respiration, ethylene production, peel color change, fresh weight loss and soluble solids whereas treated fruit showed higher fruit firmness when compared with control fruit (Valero (Valero et al., 2002; Serrano et al., 2003; Petkou et al., 2004). Previous study in 'Hom Thong' banana revealed that fruit treated with 0.1 ppm SPD reduced fruit softening, fresh weight loss and peel color changes of fruit (Santivipanond et al., 2012). Besides, treating fruit with SPD or SPN could delay softening of apple and strawberry fruits (Kramer et al., 1991; Ponappa et al., 1993). Therefore, combination of chitosan and 0.1 ppm SPD could inhibit activity of cell wall degrading enzymes leading to maintained fruit firmness and fruit fresh weight and delayed softening of 'Nam Dok Mai' mango fruit.

5.2.3 Polyamine analysis

Free endogenous polyamines (PUT, SPD and SPM) and bound SPD were significantly enhanced in fruit coated with HM-CTS combined with 0.1 ppm SPD on day

14 during storage at 14°C whereas control fruit showed decreased of endogenous polyamines. Fruit coated with HM-CTS combined with 1 and 10 ppm SPD had affected on only free SPD. Endogenous polyamines were found to decrease when fruit was in the ripening stage as reported in apple, mango and peach fruits (Zhang et al., 2003; Malik and Singh, 2004; Liu et al., 2006). Our result was in accordance with Jiang, Li and Jiang (2005), exogenous polyamines could enhance endogenous polyamines concentration in litchi fruit. According to Safizadeh (2013) who indicated that 'Lisbon' lemon fruit treated with exogenous PUT and SPD could delay fruit softening. The results from the present study clearly demonstrated that PAs could interact with pectic substances in cell wall resulting in inhibiting PME activity. Therefore, exogenous polyamines induced endogenous polyamines deviating ethylene precursor to polyamines biosynthesis pathway thus reducing ethylene biosynthesis in fruits. Polyamines and ethylene competition for precursor and changing of polyamines and ethylene productions in climacteric fruit during ripening stage were reported earlier (Li et al., 1992; Mehta et al., 2002). HM-CTS combined with 0.1 ppm SPD significantly induced endogenous polyamines especially SPD in 'Nam Dok Mai' mango and it could inhibit ethylene production leading to delay further ripening parameters and prolong shelf life of mango fruit.

However, addition of 1 or 10 ppm SPD to HM-CTS did not enhance chitosan film efficiency than HM-CTS combined with 0.1 ppm SPD. Since, high concentration of

polyamines could lead to chemical injury which was reported previously in apple fruit (Kramer et al., 1991). Concentrations of SPD or SPM more than 1 mM induced the development of small black spots in 'Red Delicious' and 'McIntosh' apples. Hence, different concentration of polyamines affects film property and addition of an appropriated concentration of polyamines can improve quality of 'Nam Dok Mai' mango fruit.

5.3 Determination of the effect of an appropriate chitosan coating combined with spermidine on anthracnose disease in mango fruit

Infected fruit coated with HM-CTS combined with 0.1 ppm SPD could suppress disease symptom until the end of storage. Our result was in accordance with Jitareerat et al. (2007) who reported that 'Nam Dok Mai' mango coated with chitosan exhibited small area of lesion development on infected fruit (less than 5%) after storage at 25°C for 10 days when compared with other treatments. Additionally, film coating property of chitosan resulted in limited gas exchange and transpiration and led to a protection of fruit from pathogen infection. Fruits were previously found to exhibit restraining factor for fungal development (Amarante and Banks, 2001; Jitareerat et al., 2007). Previous study by El Ghaouth et al. (1991) reported that chitosan could induce defense enzyme by inducing chitinase activity then it could catalyze the hydrolysis of chitin in fungal cell wall (Mauch, Hadwiger and Boller, 1984). Therefore, chitosan coating could

protect fruit form fungal infection (Hou, Chen and Lin, 1998). Chitosan also controlled fungal development and reduced the spoilage of fungi without affecting fruit ripening (Lam and Diep, 2003). In addition, chitosan coating could protect tomato fruit from pathogen attack such as *Penicillium* spp., *Aspergillus* spp., *Rhizopus stolonifer* and *Botrytis cinerea* and coated fruit exhibited low disease incidence (El Ghaouth et al., 1992). Correspondingly, chitosan treatment could induce defense responses in various fruits such as chitinase and β-1,3-glucanase in strawberries, oranges and raspberries (Zhang and Quantick, 1997) and PAL in grape berries (Romanazzi et al., 2002). Therefore, chitosan had ability to protect fruit from fungal infection.

Our study demonstrated that exogenous SPD could induce endogenous polyamines in mango fruit leading to higher firmness and delayed ethylene production which prolonged overall mango fruit qualities therefore, fruit exhibited poor condition for fungal development. Consequently, combining of HM-CTS and 0.1 ppm SPD resulted in synergistic effect to control anthracnose disease of 'Nam Dok Mai' mango. According to the results (4.2 and 4.3), we suggest a schematic representation of prolonging qualities of 'Nam Dok Mai' mango fruit after harvest by coating with the combination of HM-CTS and 0.1 ppm SPD as shown in figure 32.

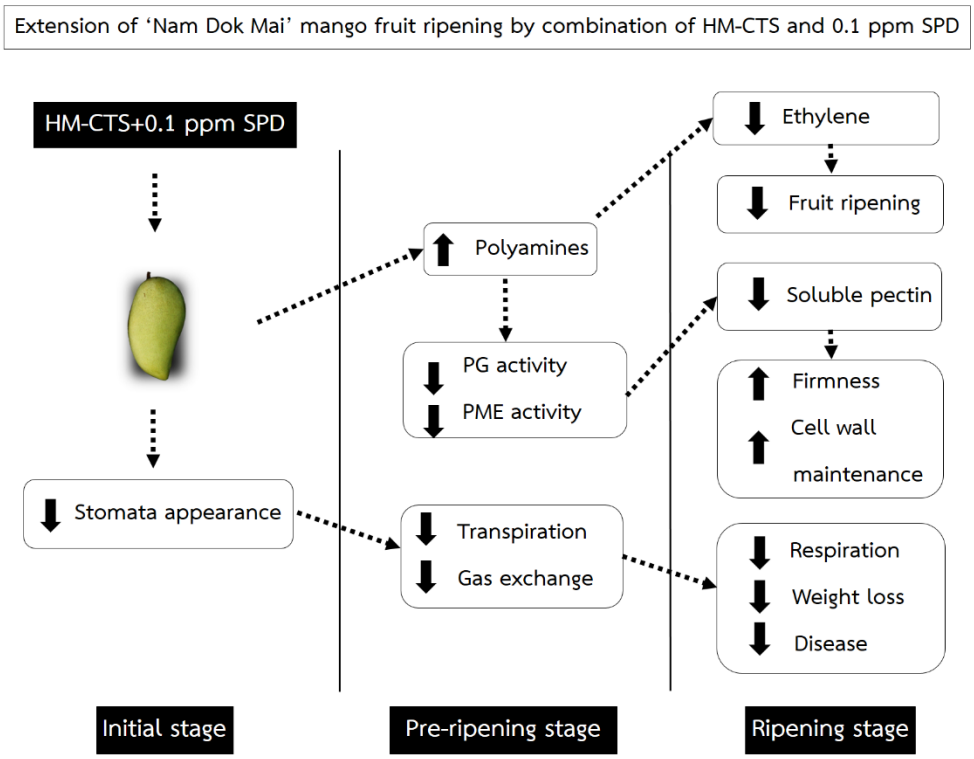

Figure 32. Schematic representation of the prolonged 'Nam Dok Mai' mango fruit qualities after harvest by combination of HM-CTS and 0.1 ppm SPD.

CHAPTER VI

CONCLUSION

6.1 Development and selection of an appropriate chitosan coating to prolong shelf-life of mango fruit during storage

6.1.1 Chitosan characteristics

HM-CTS exhibited the thickest chitosan coating film (3.47 ± 0.50) whereas MM-CTS exhibited thinner film (1.29 ± 0.45 mm). LM-CTS exhibited unmeasurable film. HM-CTS and MM-CTS completely covered stomata on mango peel surface. LM-CTS showed unthoroughly seal on mango peel and stomata were observed similar to control fruit. Therefore, HM-CTS film had the best film property for coating 'Nam Dok Mai' mango after harvest.

6.1.2 Physicochemical characteristics

Mango fruit coated with HM-CTS and MM-CTS could inhibit ethylene production and respiration during storage at storage temperature (25±2ºC) for 16 days. Chitosan coating could delay peel color changes, loss of fresh weight and maintain fruit firmness and fruit flavor by retarding the changes of TA and TSS. HM-CTS could induce antioxidant capacity by increasing ascorbic acid content, DPPH inhibition, free flavonoids content, activities of catalase, and ascorbate peroxidase and decreasing

H_2O_2 content. In addition, coated fruit with HM-CTS did not show disease symptom until the end of storage. However, LM-CTS did not delay fruit ripening. Therefore, HM-CTS could maintain physicochemical characteristics, delay ripening process and control disease of mango fruit. HM-CTS was the appropriated coating for using in 'Nam Dok Mai' mango after harvest.

6.2 Investigation of the effects of the appropriate chitosan coating combined with spermidine on qualities of mango fruit

6.2.1 Chitosan characteristics

Fruit coated with HM-CTS combined with 0.1 ppm SPD showed the thickest chitosan film (3.07±0.06 μm). There were no difference in film thickness between HM-CTS alone and HM-CTS combined with 0.1 ppm SPD. Fruit coated with HM-CTS combined with 1 or 10 ppm SPD showed very thin film and the thickness of film could not be detected. Fruit coated with HM-CTS combined with 1 or 10 ppm SPD exhibited stomata on mango peel since chitosan coating could not fully cover mango surface. While stomata on mango peel of fruit coated with HM-CTS alone and HM-CTS combined with 0.1 SPD were not observed. Therefore, HM-CTS alone and HM-CTS combined with 0.1 ppm SPD exhibited the most appropriate chitosan film property for coating mango fruit.

6.2.2 Physicochemical characteristics and fruit softening

Fruit coated with HM-CTS combined with SPD showed lower respiration rate and ethylene production than control fruit. Fruit coated with HM-CTS combined with 0.1 ppm SPD could delay peel color changes, fresh weight loss and fruit softening during storage at 14ºC for 14 days and at storage temperature (25±2ºC) until 23 days. Furthermore, coated fruit showed the lowest disease incidence and disease severity during storage. Coating fruit with HM-CTS combined with SPD led to decreased soluble pectin in peel and pulp during ripening period. Cell wall degrading enzymes (PG and PME) activities were also reduced when compared with control fruit. However, fruit coated with HM-CTS combined with 0.1 ppm SPD exhibited the lowest of cell wall degrading enzymes activities during ripening stage. Therefore, combination of HM-CTS and 0.1 ppm SPD could apply as fruit coating for maintaining physicochemical characteristics and fruit softening in 'Nam Dok Mai' mango.

6.2.3 Polyamine analysis

Fruit coated with HM-CTS combined with 0.1 ppm SPD significantly induced levels of endogenous polyamines in both forms (free and bound form), free SPD (10.10±0.42 mmol/g FW) and bound SPD (0.07±0.01 mmol/g FW) when compared with other treatments whereas coating fruit with HM-CTS combined with 1 or 10 ppm SPD influenced only free endogenous SPD (0.54±0.10 mmol/g FW). Therefore, coating 'Nam

Dok Mai' mango with HM-CTS combined with 0.1 ppm SPD could enhance endogenous polyamines level in fruit resulting in reduced ethylene production and retard fruit ripening and senescence.

6.3 Determination of the effect of an appropriate chitosan coating combined with spermidine on anthracnose disease in mango fruit

Inoculated fruit coated with HM-CTS combined with 0.1 ppm SPD could suppress disease symptom until the end of storage at ambient temperature (28±2°C) whereas inoculated fruit and inoculated fruit dipped with 0.5% acetic acid showed more area of lesion development (59.22±1.62 mm and 47.50±5.02 mm, respectively). Therefore, HM-CTS combined with 0.1 ppm SPD had ability to control anthracnose disease in 'Nam Dok Mai' mango.

REFERENCES

Abbasi, N. A., Iqbal, Z., Maqbool, M. and Hafiz, I. A. (2009). "Postharvest quality of mango (*Mangifera indica* L.) fruit as affected by chitosan coating", Pakistan Journal of Botany 41(1): 343-357.

Agrawal, G. K., Rakwal, R., Tamogami, S., Yonekura, M., Kubo, A. and Saji, H. (2002). "Chitosan activates defense/stress response(s) in the leaves of *Oryza saliva* seedlings", Plant Physiology and Biochemistry 40: 1061-1069.

Ahmad, M. and Khan, I. (1987). "Effect of waxing and cellophane lining on chemical quality indices of citrus fruit", Plant Foods Human Nutrition 37: 47-57.

Ali, A., Muhammad, M. T. M., Sijam, K. and Siddiqui, Y. (2011). "Effect of chitosan coatings on the physicochemical characteristics of eksotika II papaya (*Carica papaya* L.) fruit during cold storage", Food Chemistry 124: 620-626.

Amarante, C. and Banks, N. H. (2001). "Postharvest physiology and quality of coated fruits and vegetables", Horticultural Reviews 26: 161-238.

Amarante, C. and Banks, N. H. (2001). "Postharvest physiology and quality of coated fruits and vegetables", Horticultural Reviews 26: 161-238.

Amidi, M., Mastrobattista, E., Jiskoot, W. and Hennink, W. E. (2010). "Chitosan-based delivery systems for protein therapeutics and antigens", Advanced Drug Delivery Reviews 62: 59-82.

Ampaichaichok, P., Rojsitthisak, P. and Seraypheap, K. (2014). "Effects of different

molecular weights of chitosan coatings on postharvest qualities of 'Nam Dok Mai' mango", 2nd International Conference on Food and Agricultural Sciences 77.

Anwar, R. and Malik, A. U. (2007). "Hot water treatment affects ripening quality and storage life of mango (*Mangifera indica* L.)", Pakistan Journal of Agricultural Sciences 44: 23-30.

AOAC (1984). In Official methods of analysis of the association of official analytical chemists, (14th ed.). Washington, DC.

AOAC International (2000). Method 934.01., In Official Methods of analysis, (17th ed.), Gaithersburg, MD., AOAC Int.

Arauz, L. F. (2000). "Mango anthracnose: Economic impact and current options for integrated management", Plant Disease 84: 600-611.

Arigita, L., Tamés, R. S. and González, A. (2004). "Ethylene biosynthesis and endogenous polyamines in relation to development of in vitro cultured kiwifruit explants", Functional Plant Biology 31: 603-609.

Armstrong, J. W. (1996). "Tropical fruit, international trade and quarantine: a research perspective", Proceeding of the International Conference on Tropical Fruits, MARDI, Kuala Lumpur, Malaysia 23-26 July 1996, pp. 291-306.

Asif, M. H. and Nath, P. (2005). "Expression of Multiple forms of polygalacturonase gene during ripening in banana fruit", Plant Physiology and Biochemistry 43: 177-184.

Aveno, J. L. and Orden, M. E. M. (2004). Hot water treatment of mango: A study of four export corporations in the Phillipines Vol. 4 (1), ISSN: 1685-2044.

Baker, R. A., Baldwin, E. A. and Nisperos-Carriedo, M. O. (1994). Edible coatings and films for processed foods, In J. M. Krochta, E. A. Balwin and M. O. Niperos-Carriedo, (Eds.), Edible Coatings and Films to Improve Food Quality, Lancaster, Basel: Technomic Publishing, pp. 89-104.

Baldrick, P. (2010). "The safety of chitosan as a pharmaceutical excipient", Regulatory Toxicology and Pharmacology (56): 290-299.

Baldwin, E., Nisperos, M. O., Chen, X. and Hagenmaier, R. D. (1996). "Improving the storage life of cut apple and potato with edible coating", Postharvest Biology and Technology 9: 151-163.

Baldwin, E. A.et al. (1999). "Effect of two edible coatings with different permeability characteristics on mango (*Mangifera indica* L.) ripening during storage", Postharvest Biology and Technology 17: 215-226.

Baldwin, E. A., Nisperos, M. O., Hagenmaier, R. H. and Baker, R. A. (1997). "Use of lipids in edible coatings for food products", Food Technology 51: 56-62.

Baldwin, E. A., Nisperos, M. O., Shaw, P. E. and Burns, J. K. (1995). "Effect of coatings and prolonged storage conditions on fresh orange flavor volatiles, degrees Brix, and ascorbic acid levels", Journal of Agricultural and Food Chemistry 43: 1321-1331.

Barbosa-Martínez, C., Ponce de León-García, L. and Pelayo-Saldivar, C. (2009).

"Morphohistology of ‘Manila’ and ‘Haden’ Fruit development. A comparative study with postharvest implications", Acta Horticulturae 820: 281-288.

Barka, E. A., Eullaffroy, P., Clément, C. and Vernet, G. (2004). "Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*", Plant Cell Reports 22: 608-614.

Barkai-Golan, R. (2001). Postharvest diseases of fruits and vegetables, Elsevier publishers, USA.

Barman, K., Asrey, R. and Pal, R. K. (2011). "Putrescine and carnauba wax pretreatments alleviate chilling injury, enhance shelf life and preserve pomegranate fruit quality during cold storage", Scientia Horticulturae 130: 795-800.

Barnett, H. L. and Hunter, B. B. (1998). Illustrated genera of imperfect fungi, (4th ed.). Saint Paul, Minnnesota, America.

Bautista-Banos, S., De Lucca, A. J. and Wilson, C. L. (2004). "Evaluation of the antifungal activity of natural compounds to reduce postharvest blue mould (*Penicillium expansum* link.) of apples (*Malus domestica* borkh.) during storage", Mexican Journal of Phytopathology 22: 362-369.

Bautista-Banos, S.et al. (2006). "Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities", Crop Protection 25: 108-118.

Beers, J. R. F. and Sizer, I. W. (1952). "A spectrophotometric method for measuring the

breakdown of hydrogen peroxide by catalase", The Journal of Biological Chemistry 195: 133-140.

Ben-Shalom, N., Ardi, R., Pinto, R., Aki, C. and Fallik, E. (2003). "Controlling gray mold caused by *Botrytis cinerea* in cucumber plants by means of chitosan", Crop Protection 22: 285-290.

Ben-Yehoshua, S., Burg, S. P. and Young, R. (1985). "Resistance of citrus fruit to mass transport of water vapor and other gases", Plant Physiology 79: 1048-1053.

Bhaskara-Reddy, M. V., Arul, J., Angers, P. and Couture, L. (1999). "Chitosan treatment of wheat seed induces resistance to *Fusarium gramninearum* and improves seed quality", Journal of Agricultural and Food Chemistry 47: 1208-1216.

Bhaskara-Reddy, M. V., Belkacemi, K., Corcuff, R., Castaigne, F. and Arul, J. (2000). "Effect of pre-harvest chitosan sprays on post-harvest infection by *Botrytis cinerea* and quality of strawberry fruit", Postharvest Biology and Technology 20: 39-51.

Bittelli, M., Flury, M., Campbell, G. S. and Nichols, E. J. (2001). "Reduction of transpiration through foliar application of chitosan", Agricultural and Forest Meteorology 107: 167-175.

Blumer, J. M., Clay, R. P., Bergmann, C. W., Albersheim, P. and Darvill, A. G. (2000). "Characterization of changes in pectin methylesterase expression and pectin esterification during tomato fruit ripening", Canadian Journal of Botany 78: 607-618.

Bonilla, J., Atares, L., Vargas, M. and Chiralt, A. (2012). "Edible films and coatings to prevent the detrimental effect of oxygen on food quality: possibilities and limitations", Journal of Food Engineering 110: 208-213.

Boonlertnirun, S., Boonraung, C. and Suvanasara, R. (2008). " Application of chitosan in rice production", Journal of the Minerals Metals & Materials Society 18: 47-52.

Bouchereau, A., Aziz, A., Larher, F. and Martin-Tanguy, J. (1999). "Polyamines and environmental challenges : recent development", Plant Science 140: 103-125.

Bourtoom, T. (2008). "Edible films and coatings: characteristics and properties: Review Article", International Food Research Journal 15(3): 237-248.

Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", Analytical Biochemistry 72: 248-254.

Bregoli, A. M.et al. (2002). "Peach *(Prunus persica*) fruit ripening: aminoethoxyvinylglycine (AVG) and exogenous polyamines affect ethylene emission and flesh firmness", Physiologia Plantarum 114(Physiologia Plantarum): 472-481.

Burg, S. P. and Kosson, R. (1983). Metabolism, heat transfer and water loss under hypobaric conditions, In M. Lieberman, (Ed.), PostHarvest Physiology and Crop Preservation, Plenum Press, New York.

Cabrera, J. C., Messiaen, J., Cambier, P. and Van, C. P. (2006). "Size, acetylation and

concentration of chitooligosaccharide elicitors determine the switch from defence involving PAL activation to cell death and water peroxide production in *Arabidopsis* cell suspensions", Physiologia Plantarum 127: 44-56.

Campaniello, D., Bevilacqua, A., Sinigaglia, M. and Corbo, M. (2008). "Chitosan: antimicrobial activity and potential applications for preserving minimally processed strawberries", Food Microbiology 25: 992-1000.

Campos-Olivas, R., Newman, J. L. and Summers, M. F. (2000). "Solution structure and dynamics of the Rous sarcoma virus capsid protein and comparison with capsid proteins of other retroviruses", Journal of Molecular Biology 296: 633-649.

Casas, J. L., Acosta, M., Del Rio, J. A. and Sabater, F. (1990). "Ethylene evolution during ripening of detached tomato fruit: Its relation with polyamine metabolism", Plant Growth Regulation 9: 89-96.

Chankrajang, S. (2000). Conclusion of the use of chitin - chitosan in Thailand, In The conference and exhibition of modern agriculture with chitin-chitosan 18 February 2000, Bangkok, Thailand, pp. 1-4.

Chen, C., Liau, W. and Tsai, G. (1998). "Antibacterial effects of N-sulfonated and N-sulfobenzoyl chitosan and application to oyster preservation", Journal of Food Protection 61: 1124-1128.

Chen, S. and Nussinovitch, A. (2000). "Galactomannans in disturbances of structured

wax-hydrocolloid-based coatings of citrus fruit (easy-peelers)", Food Hydrocolloids 14: 561-568.

Cheng, S. Y., Wang, B. J. and Weng, Y. M. (2015). "Antioxidant and antimicrobial edible zein/chitosan composite films fabricated by incorporation of phenolic compounds and dicarboxylic acids", LWT-Food Science and Technology 63(1): 115-121.

Chiabrando, V. and Giacalone, G. (2015). "Anthocyanins, phenolics and antioxidant capacity after fresh storage of blueberry treated with edible coatings", International Journal of Food Sciences and Nutrition 66(3): 248-253.

Chien, P. J., F., S. and Yang, F. H. (2007). "Effects of edible chitosan coating on quality and shelf life of sliced mango fruit", Journal of Food Engineering 78: 225-229.

Chien, P. J., Sheu, F. and Lin, H. R. (2007). "Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quality and shelf life", Food Chemistry 100: 1120-1164.

Choi, W. Y., Park, H. J., Ahn, D. J., Lee, J. and Lee, C. Y. (2002). "Wettability of chitosan coating solution on 'Fuji' apple skin", Journal of Food Science 67: 2668-2672.

Choi, Y., Lee, S. M., Chun, J., Lee, H. B. and Lee, J. (2006). "Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake *(Lentinus edodes)* mushroom", Food Chemistry 99: 381-387.

Choudhury, S. R., Roy, S., Singh, S. K. and Sengupta, D. N. (2010). "Molecular

characterization and differential expression of beta-1,3-glucanase during ripening in banana fruit in response to ethylene, auxin, ABA, wounding, cold and light-dark cycles", Plant Cell Reports 29: 813-828.

Cissé, M., Polidori, J., Montet, D., Loiseau, G. and Ducamp-Collin, M. N. (2015). "Preservation of mango quality by using functional chitosanlactoperoxidase systems coatings", Postharvest Biology and Technology 101: 10-14.

Cohen, E., Shalom, Y. and Rosenberger, I. (1990). " Postharvest ethanol buildup and off-flavor in 'Murcott' tangerine fruits", Journal of American Society of Horticultural and Science 115(5): 775-778.

Conforti, F. D. (2007). "Effect of three lipid/hydrocolloid coatings on shelf life stability of Golden Delicious apples", International Journal of Food Science and Technology 42: 1101-1106.

Crookes, P. R. and Grierson, D. (1983). "Ultrastructure of tomato fruit ripening and the role of polygalacturonase isoenzymes in cell wall degradation", Plant Physiology 72: 1088-1093.

Dang, Q. F., Yan, J. Q., Li, Y., Cheng, X. J., Liu, C. S. and Chen, X. G. (2010). "Chitosan acetate as an active coating material and its effects on the storing of *Prunus avium* L.", Journal of Food Science 75: 125-131.

Devlieghere, F., Vermeulen, A. and Debevere, J. (2004). "Chitosan : antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables", Food Microbiology 21: 703-714.

Dibble, A. R. G., Davies, P. J. and Mutschler, M. A. (1988). "Polyamine content of long-keeping Alcobaca tomato fruit", Plant Physiology 86: 338-340.

Dickey, L. C. and Parris, N. (2001). "Ethanolic extraction of zein from maize", Industrial Crops and Products an International Journal 13: 67-76.

Dickey, L. C. and Parris, N. (2002). "Serial batch extraction of zein milled maize.", Industrial Crops and Products an International Journal 15: 33-42.

Ding, P., Ahmad, S. H., Razak, A. R. A., Saari, N. and Mohamed, M. T. M. (2007). "Plastid ultrastructure, chlorophyll contents, and colour expression during ripening of Cavendish banana (*Musa acuminate* 'Williams') at 18 °C and 27°C", New Zealand Journal of Crop and Horticultural Science 35: 201-210.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N. (2006). "Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds", Food Chemistry, 97: 654-660.

Dodd, J. C., Jeffries, P. and Jeger, M. J. (1989). "Management strategies to control latent infection in tropical fruits", Aspects of Applied Biology 20: 49-56.

Dodd, J. C., Prusky, D. and Jeffries, P. (1997). Fruit diseases, In R. E. Litz, (Ed.), Mango: Botany, Production and Uses, CAB International, UK, pp. 257-280.

Dong, H., Cheng, L., Tan, J., Zheng, K. and Jiang, Y. (2004). "Effect of chitosan coating on quality and shelf life of peeled litchi fruit", Journal of Food Engineering 64: 355-358.

Du, J. M., Gemma, H. and Iwahori, S. (1997). "Effects of chitosan coating on the

storage of peach, Japanese pear, and kiwifruit", Journal of the Japanese Society for Horticultural Science 66: 15-22.

Duan, J., Wu, R., Strik, B. C. and Zhao, Y. (2011). "Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions", Postharvest Biology and Technology 59: 71-79.

El Ghaouth, A., Arul, J., Ponnamapalam, R. and Boulet, M. (1991). "Chitosan coating effect on storability and quality of fresh strawberries", Journal of Food Science 56: 1618-1620.

El Ghaouth, A., Ponnamapalam, R., Castaiglne, F. and Arul, J. (1992). "Chitosan coating to extend the storage life of tomatoes", Horticultural Science 27: 1016-1018.

El Ghaouth, A. and Wilson, C. L. (1997). "Bioactive coating for harvested commodities", Patent number 5: 633.

Elsabee, M. Z. and Abdou, E. S. (2014). "Chitosan based edible films and coatings: a review", Materials Science and Engineering : C Materials for Biological Applications 33(4): 1819-1841.

Eshghi, S., Hashemi, M., Mohammadi, A., Badii, F., Mohammadhoseini, Z. and Ahmadi, K. (2014). "Effect of nanochitosan-based coating with and without copper loaded on physicochemical and bioactive components of fresh strawberry fuit (*Fragaria x ananassa* Duchesne) during storage", Food and Bioprocess Technology 7(8): 2397-2409.

Eswaranandam, S., Hettiarachchy, N. S. and Meulleneteffect, J. F. (2006). "Effect of

malic and lactic acid incorporated soy protein coatings on the sensory attributes of whole apple and fresh-cut cantaloupe", Journal of Food Science 71(3): 307-313.

Evans, P. T. and Malmberg, R. L. (1989). "Do polyamines have roles in plant development?", Annual Review of Plant Physiology and Plant Molecular Biology 40: 235-269.

Fischer, R. L. and Bennett, A. B. (1991). "Role of cell wall hydrolases in fruit ripening", Annual Review of Plant Physiology and Plant Molecular Biology 42: 675-703.

Flores, H. E. and Galston, A. W. (1982). "Analysis of polyamines in higher plants by high performance liquid chromatography", Plant Physiology 69: 701-706.

Fluhr, R. and Mattoo, A. K. (1996). "Ethylene biosynthesis and perception", Critical Reviews in Plant Sciences 15: 479-523.

Food and Agriculture Organization Corporate Statistical Database (FAOSTAT). (2012). Top production-Mangoes, mangosteen, guavas-2012, from http://faostat.fao.org/site/339/default.aspx (Retrieved 26.6.2016).

Fraga, M. F., Berdasco, M., Diego, L. B., Rodriguez, R. and Canal, M. J. (2004). "Changes in polyamine concentration associated with aging in *Pinus radiata* and *Prunus persica*", Tree Physiology 24: 1221-1226.

Furusaki, E., Ueno, Y., Sakairi, N., Nishi, N. and Tokura, S. (1996). "Facile preparation ad inclusion ability of a chitosan derivative bearing carboxymethyl-β-cyclodextrin", Carbohydrate Polymer 9: 29-34.

Galed, G., Martinez, A. and Heras, A. (2001). Preservation of citrus with chitosan Solutions : analysis by NMR imaging, In R. A. A. Muzzareli, (Ed.), Chitin Enzymology, Atec, Italy, pp. 147-153.

Galston, A. W. (1983). "Polyamines as modulators of plant development", BioScience 33: 382-388.

Galston, A. W. and Sawhney, R. K. (1990). "Polyamines in plant physiology", Plant Physiology 4: 406-410.

Gennadios, A. and Weller, C. L. (1990). "Edible films and coating from wheat and corn proteins", Food Technology 44(10): 63-69.

Gennadios, A. and Weller, C. L. (1992). Tensile strength increase of wheat gluten films: ASAE paper, In International Winter Meeting American Society of Agricultural Engineers, Nashvill, TN, pp. 92-6517.

Giovannoni, J. (2001). "Molecular biology of fruit maturation and ripening", Annual Review of Plant Physiology and Plant Molecular Biology 52: 725-749.

Goulao, L. F. and Oliveira, C. M. (2008). "Cell wall modifications during fruit ripening: when a fruit is not the fruit", Trends in Food Science & Technology 19: 4-25.

Guilbert, S. (1986). Technology and application of edible protective films, In M. Mathlouthi, (Ed.), Food packaging and preservation, London, UK: Elsevier Applied Science, pp. 371-394.

Hagenmaier, R. D. and Baker, R. A. (1993). " Reduction in gas exchange of citrus fruit by wax coatings", Journal of Agricultural and Food Chemistry 41(2): 283-287.

Hallman, G. J. and Armstrong, J. W. (1994). Heated air treatments, In J. L. Sharp and G. J. Hallman, (Eds.), Quarantine Treatments for Pests of Food Plants, Westview Press, Boulder, CO.

Han, C., Zhao, Y., Leonard, S. W. and Traber, M. G. (2004). "Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria ananassa*) and raspberries (*Rubus idaeus*)", Postharvest Biology and Technology 33: 67-78.

Hangermann, A. E. and Austin, P. J. (1986). "Continuous spectrophotometric assay for plant pectinmethyl-esterase", Journal of Agricultural Food and Chemistry 34: 440-444.

Harriman, R. W., Tieman, D. M. and Handa, A. K. (1991). "Molecular cloning of tomato pectin methylesterase gene and its expression in Rutgers, ripening inhibitor, nonripening, and never ripe tomato fruits", Plant Physiology 97: 80-87.

Heather, N. W., Corcoran, R. J. and Kopittke, R. A. (1997). "Hot air disinfestation of Australian 'Kensington' mango against two fruit flies (Diptera : Tephritidae)", Postharvest Biology and Technology 10: 99-105.

Hernandez, E. (1994). Edible coating from lipids and resins, In J. M. Krochta, E. A. Balwin and M. O. Niperos-Carriedo, (Eds.), Edible Coatings and Films to Improve Food Quality, Lancaster. Basel: Technomic Publishing, pp. 279-303.

Hong, K., Xie, J., Zhang, L., Sun, D. and Gong, D. (2012). " Effects of chitosan coating

on postharvest life and quality of guava (*Psidium guajava* L.) fruit during cold storage", Scientia Horticulturae 144: 172-178.

Horton, H. R., Moran, L. A., Ochs, R. S., Rawn, J. D. and Scrimgeour, K. G. (1993). Principles of Biochemistry, USA: Neil Patterson Publishers.

Hou, W. C., Chen, Y. C. and Lin, Y. H. (1998). "Chitinase activity of sweet potato (*Ipomoea batatas* L. Lam var. Tainong 57)", Botanical Bulletin- Academia Sinica Taipei 39: 93-97.

International pectin producers association (IPPA). (2016). "What 's pectin?", Plant Wall Cell Structure, from http://www.ippa.info/what_is_pectin.htm (Retrieved 26.6.16).

Jacobi, K. K., MacRae, E. A. and Hetherington, S. E. (2001). "Postharvest heat disinfestations treatments of mango fruit", Scientia Horticulturae 89: 171-193.

Jacobi, K. K., Wong, L. S. and Giles, J. E. (1995). "Effect of fruit maturity on quality and physiology of high-humidity hot air-treated Kensington mango *(Mangifera indica* Linn.)", Postharvest Biology and Technology 5: 149-159.

Jana, S. and Choudhuri, M. A. (1982). "Glycolate metabolism of the submerged aquatic angiosperm during aging", Aquatic Botany 12: 345-354.

Jarmila, V. and Vavríková, E. (2011). "Chitosan derivatives with antimicrobial, antitumour and antioxidant activities-A review", Current Pharmaceutical Design 17: 3596-3607.

Jeffries, P., Dodd, J. C., Jeger, M. J. and Plumbley, R. A. (1990). "The biology and

control of Colletotrichum species on tropical fruit crops", Plant Pathology 39: 343-366.

Jiang, Y. and Li, J. (2001). "Effect of chitosan coating on postharvest life and quality of longan fruit", Food Chemistry 73: 139-143.

Jiang, Y., Li, J. and Jiang, W. (2005). "Effect of chitosan coating on shelf life of cold-stored litchi fruit at ambient temperature", LWT-Food Science and Technology 38: 757-761.

Jiang, Y. and Li, Y. (2001). "Effects of chitosan coating on postharvest life and quality of longan fruit", Food Chemistry 73: 143-159.

Jiang, Y. M. and Chen, F. (1995). "A study on polyamine change and browning of fruit during cold storage of litchi (*Litchi chinensis* Sonn.)", Postharvest Biology and Technology 5: 245-250.

Jitareerat, P., Paumchai, S., Kanlayanarat, S. and Sangchote, S. (2007). "Effect of chitosan on ripening, enzymatic activity, and disease development in mango (*Mangifera indica*) fruit", New Zealand Journal of Crop and Horticultural Science 35: 211-218.

Jo, C., Lee, J. W., Lee, K. H. and Byun, M. W. (2001). "Quality properties of pork sausage prepared with water-soluble chitosan oligomer", Meat Science 59(4): 369-375.

Johnson, G. I., Sharp, J. L., Milne, D. L. and Oosthuyse, S. A. Post-harvest technology

and quarantine treatments, In R. E. Litz, (Ed.), The Mango: Botany, Production and Uses, Florida: CAB International, pp. 447-507.

Jordan, R. A. (1993). The disinfestation heat treatment process: plant qurantine in Asia and the Pacific, In A report of an Asian Production Organization Study Meeting, Taipei, Taiwan, 17-26 March 1992, Asias Productivuty Organization, Tokyo, pp. 53-68.

Juangbhanich, P. (1988). "Diagnosis of plant diseases", Principle of plant pathology Bangkok: Sanmuanchol Publishing, pp. 13-14.

Ke, D. and Kader, A. A. (1990). "Tolerance of 'Valencia' oranges to controlled atmospheres as determined by physiological responses and quality attributes", Journal of American Society of Horticultural Science 115(5): 770-783.

Ke, D. and Romani, R. J. (1988). "Effects of spermidine on ethylene production and the senescence of suspensioncultured pear fruit cells", Plant Physiology and Biochemistry 26: 109-116.

Kean, T. and Thanou, M. (2010). "Biodegradation, biodistribution and toxicity of chitosan", Advanced Drug Delivery Reviews 62: 3-11.

Kerch, G., Sabovics, M., Kruma, Z., Kampuse, S. and Straumite, E. (2011). "Effect of chitosan and chitooligosaccharide on vitamin C and polyphenols contents in cherries and strawberries during refrigerated storage", European Food Research and Technology 233(2): 351-358.

Kester, J. J. and Fennema, O. R. (1986). " Edible films and coatings: A review", Food Technology 40: 47-59.

Kim, H. S., Son, B. Y., Park, S. M. and Lee, K. T. (1999). " A study on the properties and utilization of chitosan coating. 2. Changes in the quality of tomatoes by chitosan coating", Korean Journal of Fisheries and Aquatic Sciences 32: 568-572.

Kim, Y., Brecht, J. K. and Talcott, S. T. (2007). "Antioxidant phytochemical and fruit quality changes in mango (*Mangifera indica* L.) following hot water immersion and controlled atmosphere storage", Food Chemistry 105: 1327-1334.

Koch, J. L. and Nevins, D. J. (1989). "Tomato fruit cell wall. 1. Use of purified tomato polygalacturonase and pectinmethylesterase to identify developmental changes in pectins", Plant Physiology 91: 816-822.

Kohorn, B. (2001). "WAKs; cell wall associated kinases", Current Opinion in Cell Biology 13: 529-533.

Kramer, G. F., Wang, C. Y. and Conway, W. S. (1991). "Inhibition of softening by polyamine application in 'Golden Delicious' and 'McIntosh' apples", Journal of the American Society for Horticultural Science 116: 813-817.

Krochta, J. M. and Mulder-Johnston, C. D. (1997). " Edible and biodegradable polymer films: challenges and opportunities", Food Technology 51(2): 61-74.

Kushad, M. M., Yelenosky, G. and Knight, R. (1988). "Interrelationship of polyamine and ethylene biosynthesis during avocado fruit development and ripening", Plant Physiology 87: 463-467.

Lam, N. D. and Diep, T. B. (2003). "A preliminary study on radiation treatment of chitosan for enhancement of antifungal activity tested on fruit-spoiling strains", Journal of Nuclear Science and Technology 2(2): 54-60.

Lee, S.et al. (1999). "Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and Commelina communis", Plant Physiology 121: 147-152.

Li, N., Parsons, B. L., Liu, D. and Mattoo, A. K. (1992). "Accumulation of wound-inducible ACC synthase transcript in tomato fruits is inhibited by salicylic acid and polyamines", Plant Molecular Biology 18: 477-487.

Li, Q., Dunn, E. T., Grandmaison, E. W. and Goosen, M. F. A. (1997). Application and properties of chitosan, In F. A. G. Mattheus, (Ed.), Application of Chitin and Chitosan, Pennsylvania : Technomic publishing, pp. 3-29.

Lin, W., Hu, X., Zhang, W., Rogers, W. J. and Cai, W. (2005). "Hydrogen peroxide mediates defence responses induced by chitosans of different molecular weights in rice", Journal of Plant Physiology 162: 937-944.

Linden, J. C. and Stoner, R. J. (2005). "Proprietary elicitor affects seed germination and delays fruit senescence", Journal of Food, Agriculture and Environment 3: 184-189.

Linden, J. C. and Stoner, R. J. (2007). Pre-harvest application of proprietary elicitor

delays fruit senescence, In A. Ramina, (Ed.), Advances in Plant Ethylene Research : Proceedings of the 7th International Symposium on the Plant Hormone Ethylene, Dordrecht, The Netherlands : Springer, pp. 301-302.

Liu, H., Du, Y., Wang, X. and Sun, L. (2004). "Chitosan kills bacteria through cell membrane damage", International Journal of Food Microbiology 95(2): 147-155.

Liu, J., Nada, K., Pang, X., Honda, C., Kitashiba, H. and Moriguchi, T. (2006). "Role of polyamines in peach fruit development and storage", Tree Physiology 26: 791-798.

Liu, J., Tian, S., Meng, X. and Xu, Y. (2007). "Effect of chitosan on control of postharvest diseases and physiological responses of tomato fruit", Postharvest Biology And Technology 44: 300-306.

Liu, J. H., Honda, C. and Moriguchi, T. (2006). "Involvement of polyamine in floral and fruit development: Review", Japan Agricultural Research Quarterly 40(1): 51-58.

Liu, K., Yuan, C., Chen, Y., Li, H. and Liu, J. (2014). "Combined effects of ascorbic acid and chitosan on the quality maintenance and shelf life of plums", Scientia Horticulturae 176: 45-53.

Lizada, M. C. C., Pantastico, E. B., Abd Shukor, A. R. and Sabari, S. D. (1990). Changes during ripening in banana, In A. Hassan and E. B. Pantastico, (Eds.), Banana fruit development, postharvest physiology, handling and marketing in ASEAN, Kuala Lumpur: ASEAN Food Handling Bureau, pp. 65-72. .

Lohani, S., Trivedi, P. K. and Nath, P. (2004). "Changes in activities of cell wall

hydrolases during ethylene-induced ripening in banana: effect of 1-MCP, ABA and IAA", Postharvest Biology and Technology 31: 119-126.

Mahto, R. and Das, M. (2013). "Effect of gamma irradiation on the physico-chemical and visual properties of mango (*Mangifera indica* L.), cv. 'Dushehri' and 'Fazli' stored at 20 °C", Postharvest Biology and Technology 86: 447-455.

Majumder, K. and Mazumdar, B. C. (2002). "Changes of pectic substances in developing fruits of cape-gooseberry in relation to the enzyme activity and evolution of ethylene (*Physalis peruviana* L.)", Scientia Horticulturae 96: 91-101.

Malik, A. U. and Singh, Z. (2003). "Exogenous application of putrescine affects mango shelf life and fruit quality", Acta Horticulturae 628: 121-127.

Malik, A. U. and Singh, Z. (2004). "Endogenous free polyamines of mangos in relation to development and ripening", Journal of the American Society for Horticultural Science 129: 280-286.

Malik, A. U. and Singh, Z. (2005). "Pre-storage application of polyamines improves shelf-life and fruit quality of mango", The Journal of Horticultural Science and Biotechnology 80: 363-369.

Malik, M. A., Al-Thabaiti, S. A. and Shiekh, M. A. (2013). "Chitosan as a novel edible coating for fresh fruits", Food Science and Technology Research 19: 139-155.

Mangan, R. L. and Ingle, S. J. (1992). "Forced hot-air quarantine treatment for

mangoes infested with west Indian fruit fly (Diptera: Tephritidae)", Journal of Economic Entomology 85(5): 1859-1864.

Manrique-Trujillo, S. M., Ramirez-Lopez, A. C., Ibarra-Laclette, E. and Gomez-Lim, M. A. (2007). "Identification of genes differentially expressed during ripening of banana", Journal of Plant Physiology 164: 1037-1050.

Manrique, G. D. and Lajolo, F. M. (2004). "Cell-wall polysaccharide modifications during postharvest ripening of papaya fruit (*Carica papaya*)", Postharvest Biology and Technology 33: 11-26.

Martínez-Romero, D.et al. (2006). "Postharvest sweet cherry quality and safety maintenance by Aloe vera treatment: a new edible coating", Postharvest Biology and Technology 39: 92-100.

Martínez-Romero, D., Serrano, M., Carbonell, A., Burgos, L., Riquelme, F. and Valero, D. (2002). "Effects of postharvest putrescine treatment on extending shelf life and reducing mechanical damage in apricot", journal of Food Science 67: 1706-1712.

Martínez-Romero, D., Valero, D., Serrano, M., Martínez-Sánchez, F. and Riquelme, F. (1999). "Effects of post-harvest putrescine and calcium treatments on reducing mechanical damage and polyamines and abscisic acid levels during lemon storage", Journal of the Science of Food and Agriculture: 1589-1595.

Matile, P., Hortensteiner, S. and Thomas, H. (1999). "Chlorophyll degradation", Annual Review of Plant Physiology and Plant Molecular Biology 50: 67-95.

Mauch, F., Hadwiger, L. A. and Boller, T. (1984). "Ethylene symptoms not signal for the induction of chitinase and beta-1, 3-glucanase in pea pods by pathogens and elicitors", Plant Physiology 76: 607-611.

Meddlicott, A. P., Semple, A. J., Thompson, A. J., Blackbourn, H. R. and Thompson, A. K. (1992). "Measurement of colour changes in ripening bananas and mangoes by instrumental, chemical and visual assessments", Tropical Agriculture (Trinidad) 69(2): 161-166.

Medlicott, A. P., Bhogal, M. and Reynolds, S. B. (1986). "Changes in peel pigmentation during ripening of mango fruit (*Mangifera indica* var. 'Tommy Atkins')", Annals of Applied Biology 109: 651-656.

Mehta, R. A., Cassol, T., Li, N., Ali, N., Handa, A. K. and Mattoo, A. K. (2002). "Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality and vine life", Nature Biotechnology 20: 613-618.

Meng, X., Li, B., Liu, J. and Tian, S. (2008). "Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage", Food Chemistry 106: 501-508.

Meng, X., Yang, L., Kennedy, J. F. and Shiping Tian, B. (2010). "Effects of chitosan and oligochitosan on growth of two fungal pathogens and physiological properties in pear fruit", Carbohydrate Polymers 81: 70-75.

Min, S. and Krochta, J. (2007). "Ascorbic acid-containing whey protein film coatings for control of oxidation", Journal of Agricultural and Food Chemistry 55: 2964-2969.

Mitra, S. K. and Baldwin, E. A. (1997). Mango, In S. K. Mitra, (Ed.), Postharvest Physiology and Storage of Tropical and Subtropical Fruits, New York: CAB International., pp. 85-122.

Muirhead, I. F. and Gratitude, R. (1986). Mango diseases, In Proceedings of the First Australian Mango Research Workshop, Commonwealth Scientific and Industrial Research Organisation (CSIRO).

Muymas, P., Boon-long, P., Chadchawan, S., Pichayangura, R. and Seraypheap, K. (2011). "Effect of Biomaterial and Semi-Biomaterial on Growth and Postharvest Quality of 'Red Oak' Lettuce ", Agricultural Science Journal 42: 37-42.

Muzzarelli, R.et al. (1990). "Antimicrobial properties of N-carboxybutyl chitosan", Antimicrobial Agents and Chemotherapy 34: 2019-2023.

Muzzarelli, R. A. A. and Rocchetti, R. (1985). "Determination of the degree of acetylation of chitosans by first derivative ultraviolet spectrophotometry", Carbohydrate Polymer 5: 461-472.

Nakano, Y. and Asada, K. (1987). "Purification of ascorbate peroxidase in spinach chloroplasts: its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical", Plant Cell Physiology 28: 131-140.

Nathan, R., Altman, A. and Monselise, S. P. (1984). "Changes in activity of polyamine biosynthetic enzymes and in polyamine contents in developing fruit tissues of ‘Murcott’ mandarin", Scientia Horticulturae 22: 359-364.

Navarro-Tarazaga, M. L. and Perez-Gago, M. B. (2006). "Effect of edible coatings on quality of mandarin cv. Clemenules", Proceedings of the Florida State Horticultural Society 119: 350-352.

Nelson, K. L. and Fennema, O. R. (1991). "Methylcellulose films to prevent lipid migration in confectionery products", Journal of Food Science 56: 504-509.

Nelson, S. C. (2008). "Mango anthracnose (*Colletotrichum gloeosporiodes*)", Plant Disease: 48.

Nge, K. L., Nwe, N., Chandrkrachang, S. and Stevens, W. F. (2006). "Chitosan as a growth stimulator in orchid tissue culture", Plant Science 170: 1185-1190.

Nishikawa, H., Tabata, T. and Kitani, S. (2012). "Simple Detection Method of Biogenic Amines in Decomposed Fish by Intramolecular Excimer Fluorescence", Food and Nutrition Sciences 3: 1020-1026.

Nisperos-Carriedo, M. O., Shaw, P. E. and Baldwin, E. A. (1990). "Changes in volatile flavor components in 'Pineapple' orange juice as influenced by the application of lipid and composite films", Journal of Agricultural and Food Chemistry 38: 1382-1387.

No, H. K., Meyers, S. P., Prinyawiwatkul, W. and Xu, Z. (2007). "Applications of chitosan for improvement of quality and shelf life of food: A Review", Journal of Food Science and Technology 72(5): 87-100.

Office of Agricultural Economics (OAE). (2016). "Ministry of Agriculture and Cooperatives : Agricultural Import Export News, 2015", Export of mango

[online], from http://www.oae.go.th/oae_report/export_import/export_result.php (Retrieved 27.6.16).

Olivas, G., Dávila-Aviña, J., Salas-Salazar, N. A. and Molina, F. J. (2008). "Use of edible coatings to preserve the quality of fruits and vegetables during storage", Stewart Postharvest Review 4(3): 1-10.

Olivas, G. I. and Barbosa-Cánovas, G. V. (2005). "Edible coatings for fresh-cut fruits", Critical Reviews in Food Science and Nutrition 45: 657-670.

Ouattara, B., Simard, R. E., Piette, G., Begin, A. and Holley, R. A. (2000). "Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films", Journal of Food Science 65(5): 768-773.

Palmer, J. K. (1971). The Banana, In A. C. Hulme, (Ed.), The Biochemistry of Fruits and Their Products, London: Academic Press, Vol. 2.

Pandey, S., Ranade, S. A., Nagar, P. K. and Kumar, N. (2000). "Role of polyamines and ethylene as modulators of plant senescence", Journal of Biosciences 25: 291-299.

Pantastico, E. B. (1975). Postharvest, Handling and Utilization of Tropical and Subtropical Fruits and Vegetables, Connecticut: AVI Publishing, pp. 490-492.

Park, H. J. (1991). Edible coatings for fruit and vegetables: Determination of gas diffusivities, prediction of internal gas composition and effects of coating on shelf life: Georgia, USA: University of Georgia, Ph.D. Thesis.

Park, H. J., Chinnan, M. S. and Shewfelt, R. L. (1994). "Edible corn-zein coatings storage life tomatoes", Journal of Food Processing and Preservation 18: 317-333.

Park, H. J., Rhim, J. W. and Lee, H. Y. (1996). "Edible coating effects on respiration rate and storage life of Fuji apples and Shingo pears", Foods and Biotechnology 5(1): 59-63.

Park, J. H., Saravanakumar, G., Kim, K. and Kwon, I. C. (2010). "Targeted delivery of low molecular drugs using chitosan and its derivatives", Advanced Drug Delivery Reviews 62: 28-41.

Park, S. I., Stan, S. D., Daeschel, M. A. and Zhao, Y. (2005). "Antifungal coatings on fresh strawberries (*Fragaria×ananassa*) to control mold growth during cold storage", Journal of Food Science 7: 197-201.

Pathak, N. and Sanwal, G. G. (1998). "Multiple forms of PG from banana fruits", Phytochemistry 48: 249-255.

Pedroso, C., Lage, D. A. C., Henz, G. P. and Cafe-Filho, A. C. (2011). "Development and validation of a diagrammatic scale for estimation of anthracnose on sweet pepper fruits for epidemiological studies", Journal of Plant Pathology 93: 219-225.

Pereda, M., Aranguren, M. and Marcovich, N. (2008). "Water vapor absorption and permeability of films based on chitosan and sodium caseinate", Polymer Science 111: 2777-2784.

Pérez-Vicente, A. e. a. (2002). "Role of polyamines in extending shelf life and the

reduction of mechanical damage during plum (*Prunus salicina* Lindl.) storage", Postharvest Biology and Technology 25: 25-32.

Petkou, I. T., Pritsa, T. S. and Sfakiotakis, E. M. (2004). "Effects of polyamines on ethylene production, respiration and ripening of kiwifruit", The Journal of Horticultural Science and Biotechnology 79: 977-980.

Ploetz, R. (1999). Anthracnose: The most important disease in much of the mango-producing world, In PLP News, The Newsletter of the Plant Pathology Department, The University of Florida, Gainseville, Vol. 3(9), September 1999, pp. 1-2.

Ploetz, R. C. and Freeman, S. (2009). Foliar, floral and soilborne diseases, In R. E. Litz, (Ed.), The Mango:Botany, Production and Uses, (2nd ed.), CABI.

Ploetz, R. C. and Prakash, O. (1997). Foliar, floral and soilborne diseases, In R. E. Litz, (Ed.), The Mango:Botany, Production and Uses. , CABI, pp. 281-32.

Pokorny, J., Yanishlieva, N. and Gordon, M. H. (2001). In Antioxidants in Food: Practical Applications, Cambridge: Woodhead Publishing Limited.

Ponappa, T., Scheerens, J. C. and Miller, A. R. (1993). "Vacuum infiltration of polyamines increases firmness of strawberry slices under various storage conditions", Journal of Food Science 58: 361-364.

Pornpienpakdee, P.et al. (2010). "Improving the micropropagation efficiency of hybrid Dendrobium orchids with chitosan", Scientia Horticulturae 124: 490-499.

Pott, I., Marx, M., Neidhard, S., Mulhbauer, W. and Carle, R. (2003). "Quantitative

determination of β-carotene stereo-isomers in fresh, dried, and solar dried mangoes (*Mangifera indica* L.)", Journal of Agriculture Food Chemistry 51: 4527-4531.

Prasanna, V., Prabha, T. N. and Tharanathan, R. N. (2007). "Fruit ripening phenomena-an overview", Critical Reviews in Food Science and Nutrition 47: 1-9.

Prusky, D. and Keen, N. T. (1993). "Involvement of preformed antifungal compounds in the resistance of subtropical fruits to fungal decay", Plant Disease 77: 115-119.

Rabea, E. I., Badawy, E.T., M., Stevens, C. V., Smagghe, G. and Steurbaut, W. (2003). "Chitosan as antimicrobial agent: applications and mode of action", Biomacromolecules 4(6): 1457-1465.

Redgwell, R. J., ischer, M., Kendal, E. and MacRae, E. A. (1997). "Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls", Planta 203: 174-181.

Rhim, J. W., Gennadios, A., Weller, C. L. and Hanna, M. A. (2002). "Sodium dodecyl sulfate treatment improves properties of cast films from soy protein isolate", Industrial Crops and Products an International Journal 15: 199-205.

Ribeiro, C., Vicente, A. A., Teixeira, J. A. and Miranda, C. (2007). "Optimization of edible coating composition to retard strawberry fruit senescence

", Postharvest Biology and Technology 44: 63-70.

Robert, G. A. F. (1992). Chitin Chemistry, Themacmillian Press. London. UK.

Robertson, G. L. (1979). "The fractional extraction and quantitative determination of pectin substances in grape and musks", American Journal of Enology and Viticulture 30: 182-185.

Rodrigues, S., Costa, A. R. D. and Grenha, A. (2012). "Chitosan/carrageenan nanoparticles: Effect of cross-linking with tripolyphosphate and charge ratios", Carbohydrate Polymer 89: 282-289.

Rodríguez, J. P.et al. (2005). Interactions among ecosystem services, In S. P. Carpenter, P. L. Pingali, E. M. Bennett and M. B. Zurek, (Eds.), Ecosystems and human well-being: scenarios, Findings of the Scenarios Working Group, Millennium Ecosystem Assessment. Island Press, Washington, D.C., USA, Vol. 2, pp. 431-448.

Romanazzi, G., Nigro, F., Ippolito, A., Di Venere, D. and Salerno, M. (2002). "Effects of pre and postharvest chitosan treatments to control storage grey mould of table grapes", Journal of Food Science 67: 1862-1867.

Safizadeh, M. R. (2013). "Vacuum infiltration of polyamines reduces chilling injury and firmness loss of lemon stored at chilling temperature", International Journal of Agriculture and Crop Sciences 6(8): 445-451.

Saftner, R. A. and Baldi, G. (1990). "Polyamine levels and tomato fruit development: possible interaction with ethylene", Plant Physiology: 547-550.

Salunkhe, D. K. and Desai, B. B. (1984). Postharvest Biotechnology of Vegetables CRC Press, Inc. Boca Raton, Florida, USA, Vol. 2, pp. 70-75.

Sangchote, S. (1987). "Postharvest diseases of mango fruits and their losses", Kasetsart Journal 21: 81-85.

Santivipanond, A., Jantaro, S. and Seraypheap, K. (2012). "Utilization of polyamines to prolong postharvest storage of “Hom Thong” banana fruit *(Musa* sp., AAA group, Gros Michel subgroup, cultivar “Hom Thong”)", Thai Journal of Botany 4: 169-175.

Scope, R. K. (1994). Separation by precipitation, In R. K. Scope, (Ed.), Protein purification; Principles and practice, New York: Springer-Verlag, pp. 71-101.

Serrano, M., Martinez-Romero, D., Guille´n, F. and Valero, D. (2003). "Effects of exogenous putrescine on improving shelf life of four plum cultivars", Postharvest Biology and Technology 30: 259-271.

Seymour, G. B., Colquhoun, I. J., Dupont, M. S., Parsley, K. R. and Selvendran, R. R. (1990). "Composition and structural features of cell wall polysaccharides from tomato fruits", Phytochemisty 29: 725-731.

Shahidi, F., Arachchi, J. K. V. and Jeon, Y. (1999). " Food applications of chitin and chitosan", Trends in Food Science and Technology 10: 37-51.

Sharathchandra, R. G., Raj, S. N., Shetty, N. P., Amruthesh, K. N. and Shetty, H. S. (2004). "A Chitosan formulation Elexa™ induces downy mildew disease resistance and growth promotion in pearl millet", Crop Protection 23: 881-888.

Sharp, R. (2013). "A Review of the Applications of Chitin and Its Derivatives in

Agriculture to Modify Plant-Microbial Interactions and Improve Crop Yields", Agronomy 3: 757-793.

Shin, Y., Liu, R. H., Nock, J. F., Holliday, D. and Watkins, C. B. (2007). "Temperature and relative humidity effects on quality, total ascorbic acid, phenolics and flavonoid concentrations, and antioxidant activity of strawberry", Postharvest Biology and Technology 45: 349-357.

Shukla, R. and Cheryan, M. (2001). "Zein: The industrial protein from corn", Industrial Crops and Products an International Journal 13: 171-192.

Siriphanich, S. (1995). Physiology and postharvest technology of fruits and vegetables Thailand. Nakhon Pathom : National Agricultural Extension and Training Center Publishing, pp. 369.

Sozzi, G. O. (2004). Strategies for the regulation of postharvest fruit softening by changing cell wall enzyme activit, In R. Dris and S. M. Jain, (Eds.), Production Practices and Quality Assessment of Food Crops, Postharvest Treatment and Technology, Netherlands: Kluwer Academic, Vol.4, pp. 135-172.

Srinivasa, P. C., Baskaran, R., Ramesh, M. N. and Tharanathan, R. N. (2002). "Storage studies of mango packed using biodegradable chitosan film", European Food Research and Technology 215(6): 504-508.

Stoner, R. and Linden, J. (2006). Micronutrient elicitor for treating nematodes in field crops, USA : Patent Pending, Publishing.

Sun, D., Liang, G., Xie, J., Lei, X. and Mo, Y. (2010). "Improved preservation effects of

litchi fruit by combining chitosan coating with ascorbic acid treatment during postharvest storage", African Journal of Biotechnology 9(22): 3272-3279.

Sun, X. J., Bi, Y., Li, Y. C., Han, R. F. and Ge, Y. H. (2008). "Postharvest chitosan treatment induces resistance in potato against *Fusarium sulphureum*", Agricultural Sciences in China 7: 615-621.

Sunagawa, K., Kume, K. and Iwaizumi, R. (1987). "Vapor heat treatment of mangoes", Research Bulletin of the Plant Protection Service, Japan 23: 13-20.

Sunil, A. A., Mallikarjuna, N. N. and Aminabhavi, T. M. (2004). "Recent advances on chitosan-based micro- and nanoparticles in drug delivery", Journal of Controlled Release 100: 5-28.

Suseno, N., Savitri, E., Sapei, L. and Padmawijaya, K. S. (2014). "Improving shelf-life of cavendish banana using chitosan edible coating", Procedia Chemistry 9: 112-113.

Takahashi, Y., Berberich, T., Miyazaki, A., Seo, S., Ohashi, Y. and Kusano, T. (2003). "Spermine signaling in tobacco : activation of mitogen-activated protein kinase by spermine is mediated through mitochondrial dysfunction", Plant Journal 36: 820-829.

Tarnowski, T. L. B. and Ploetz, R. C. (2008). "First report of *colletotrichum capsici* causing postharvest anthracnose on papaya in south florida", Plant Disease 94: 1065.

Tasdelen, O. and Bayindirli, L. (1998). "Controlled atmosphere storage and edible

coating effects on storage life and quality of tomatoes", Journal of Food Processing Preservation 22: 303-320.

Thompson, A. K. (2003). Fruit and Vegetables: Harvesting, Handling and Storage, Oxford: Blackwell Publishing Limited.

Tieman, D. M., Harriman, R. W., Ramamohan, G. and Handa, A. K. (1992). "An antisense pectin methylesterase gene alters pectin chemistry and soluble solids in tomato fruit", Plant Cell Physiology 4: 667-679.

Tomihata, K. and Ikada, Y. (1997). "In vitro and in vivo degradation of films of chitin and its deacetylated derivatives", Biomaterials 18(7): 567-575.

Trotel-Aziz, P., Couderchet, M., Vernet, G. and Aziz, A. (2006). "Chitosan stimulates defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*", European Journal of Plant Pathology 114: 405-413.

Tsai, G. J., Su, W. H., Chen, H. C. and Pan, C. L. (2002). "Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation", Fisheries Science 68: 170-171.

Ulloa, J. A., Guatemala, G. M., Arriola, E., Escalona, H. B. and Díaz, L. (2009). "Estimation of the diffusivities of sodium chloride, potassium sorbate and sodium bisulphite in mango slices processed by hurdle technology", Journal of Food Engineering 91: 211-216.

Valero, D., Martínez-Romero, D. and Serrano, M. (2002). "The role of polyamines in

the improvement of the shelf life of fruit", Trends in Food Science and Technology 13: 228-234.

Valero, D., Martinez, R. D., Serrano, M. and Riquelme, F. (1998). "Influence of postharvest treatment with putrescine and calcium on endogenous polyamines, firmness, and abscisic acid in lemon (*Citrus lemon* L. Burm Cv. Verna)", Journal of Agricultural and Food Chemistry 46: 2102-2109.

Vázquez-Celestino, D., Ramos-Sotelo, H., Rivera-Pastrana, D. R., Vázquez-Barrios, M. E. and Mercado-Silva, E. M. (2016). "Effects of waxing, microperforated polyethylene bag, 1-methylcyclopropene and nitric oxide on firmness and shrivel and weight loss of 'Manila' mango fruit during ripening", Postharvest Biology and Technology 111: 398-405.

Vojdani, F. and Torres, J. A. (1990). "Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose coatings - effect of fatty-acids.", Journal of Food Science 55(3): 841-846.

Walker-Simmons, M., Hadwiger, L. and Ryan, C. A. (1983). "Chitosans and pectic polysaccharides both induce the accumulation of the antifungal phytoalexin pisatin in pea pods and antinutrient proteinase inhibitors in tomato leaves", Biochemical and Biophysical Research Communications 110(1): 194-199.

Wang, S. Y. and Gao, H. (2013). "Effect of chitosan-based edible coating on

antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries (*Fragaria x aranassa* Duch.)", LWT-Food Science and Technology 52: 71-79.

Wei, J., Ma, F., Shi, S., Qi, X., Zhu, X. and Yuan, J. (2010). "Changes and postharvest regulation of activity and gene expression of enzymes related to cell wall degradation in ripening apple fruit", Postharvest Biology and Technology 56: 147-154.

Whistler, R. L. and Daniel, J. R. (1985). Carbohydrate, In O. R. Fennema, (Ed.), Food Chemistry, New York: Marcel Dekker, pp. 69.

Willats, W. G. T., McCartney, L., Machie, W. and Knox, J. P. (2001). "Pectin: Cell biology and prospects for functional analysis", Plant Molecular Biology 47: 9-27.

Wills, R. B. and Scott, H. K. J. (1972). "Methods of increasing water loss from apples to reduce low temperature break down", Horticultural Science 47: 349-355.

Wills, R. B. G. and Rigney, C. J. (1979). "Effect of calcium on activity of mitochondria and pectic enzymes isolated from tomato fruits", Journal of Food Biochemistry 3: 103.

Xing, Y. G., Li, X. H., Xu, Q. L., Yun, J., Lu, Y. Q. and Tang, Y. (2011). "Effects of chitosan coating enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annuum* L.)", Food Chemistry 124: 1443-1450.

Xu, S., Chen, X. and Sun, D. W. (2001). "Preservation of kiwifruit coated with an edible film at ambient temperature", Journal of Food Engineering 50: 211-216.

Yahia, E. M. (2009). Introduction, In E. M. Yahia, (Ed.), Modified and controlled atmospheres for the storage, transportation and packaging of horticultural commodities, Boca Raton: CRC Press, Taylor & Francis Group, pp. 1-16.

Yang, G.et al. (2014). "Blueberry leaf extracts incorporated chitosan coatings for preserving postharvest quality of fresh blueberries", Postharvest Biology and Technology 92: 46-53.

Yashoda, H. M., Prabha, T. N. and Tharanathan, R. N. (2005). "Mango ripening chemical and structural characterization of pectic and hemicellulosic polysaccharides", Carbohydrate Research 340: 1335-1342.

Yenjit, P., Issarakraisila, M., Intana, W. and Chantrapromma, K. (2010). "Fungicidal activity of compounds extracted from the pericarp of Areca catechu against *Colletotrichum gloeosporioides* in vitro and in mango fruit", Postharvest Biology and Technology 55: 129-132.

Youssef, B. M., Asker, A. A., El-Samahy, S. K. and Swailam.H.M. (2002). "Combined effect of steaming and gamma irradiation on the quality of mango pulp stored at refrigerated temperature", Food Research International 35: 11-13.

Yu, T.et al. (2012). "Integrated control of blue mold in pear fruit by combined application of chitosan, a biocontrol yeast and calcium chloride", Postharvest Biology and Technology 69: 49-53.

Zeng, K., Deng, Y., Ming, J. and Deng, L. (2010). "Induction of disease resistance and

ROS metabolism in navel oranges by chitosan", Scientia Horticulturae 126: 223-228.

Zhang, D. L. and Quantick, P. C. (1997). "Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (*Litchi chinensis* Sonn.) fruit", Postharvest Biology and Technology 12: 195-202.

Zhang, Y., Zhang, M. and Yang, H. (2015). "Postharvest chitosan-g-salicylic acid application alleviates chilling injury and preserves cucumber fruit quality during cold storage", Food Chemistry 174: 558-563.

Zhang, Z., Honda, C., Kita, M., Hu, C., Nakayama, M. and Moriquchi, T. (2003). "Structure and expression of spermidine synthase genes in apple: two cDNAs are spatially and developmentally regulated through alternative splicing", Molecular Genetics and Genomics 268: 799-807.

Zhu, X., Wang, Q. M., Cao, J. K. and Jiang, W. B. (2008). "Effects of chitosan coating on postharvest quality of mango (*Mangifera indica* L. cv. Tainong) fruits", Journal of Food Processing and Preservation 32: 770-784.

Ziosi, V.et al. (2006). "Transcript levels of ethylene perception and biosynthesis genes as altered by putrescine, spermidine and aminoethoxyvinylglycine (AVG) during the course of ripening in peach fruit (*Prunus persica* L. Batsch)", New Phytologist 172: 229-238.

APPENDIX

1. Chemical solutions

1.1 Enzyme extraction buffer (Beers and Sizer, 1952)

50 mM Phosphate buffer (pH 6.5)	100 mL
1% PVPP*	100 g
0.1%DTT	0.1 g
100 mM PMSF (in conc. Isopropanol)	1 mL

* Add before use

1.2 Cell wall enzyme extraction buffer (Lohani, Trivedi and Nath, 2004)

20 mM Tris-HCl buffer (pH 7.0)	100 ml
20 mM EDTA	0.74 g
0.05% Triton X-100	50 mg
20 mM Cysteine-HCl	0.35 g

1.3 DNS reagent (Choudhury et al., 2010)

DNS	10 g
NaOH	16 g
Na-K tartrate	300 g
Water	1,000 mL

Store in dark bottle at room temperature.

2. Respiration rate

Percentage of CO_2 were measure by Gas Chromatography)GC-8A(.

Calculate	Air	100 mL	have	CO_2	A mL
	Air (in jar)	2,400 mL	have	CO_2	$\frac{Ax2,400}{100}$ mL
	Lime	B kg.	have	CO_2	$\frac{Ax2,400}{100}$ mL
	Lime	1 kg.	have	CO_2	$\frac{Ax2,400}{100B}$ mL

Formula PV=nRT (Boyle 's law; at 25°C)

CO_2	24,453	mL	weight	44000 mg.
CO_2	$\frac{Ax2,400}{100B}$	mL	weight	$\frac{(44000)(Ax2,400)}{(24,453)(100B)}$ mg.

VITA

Miss Pornchan Jongsri was born on February 27, 1987 in Prachuabkirikhan province. She finished the secondary school from Princess Chulabhorn's Collage Phetchaburi in 2004. Then she got scholarship from Research Professional Development Project under the Science Achievement Scholarship of Thailand (SAST) to study in Bachelor' s Degree, majored in Biology, Faculty of Science, at Silpakorn University from 2005-2008. After that she continued on Master Degree in the Department of Botany, Faculty of Science at Chulalongkorn University from 2009-2011. She has been supported by SAST to study and doing her research for the degree of Doctor of Philosophy in Botany program, Faculty of Science, Chulalongkorn University since 2012.