Effects of Chitosan Combined with Chitosan-Montmorillonite Nanocomposites Coating

on Postharvest Quality of 'Hom Thong' Banana Fruit

Miss Arisa Wantat

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

A Dissertation Submitted in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in Biotechnology

Common Course

FACULTY OF SCIENCE

Chulalongkorn University

Academic Year 2020

ผลของการเคลือบด้วยไคโทซานร่วมกับไคโทซาน-มอนต์มอริลโลไนต์นาโนคอมพอสิตต่อคุณภาพ
หลังการเก็บเกี่ยวของผลกล้วยหอมทอง

น.ส.อริสา วันทัศน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ ไม่สังกัดภาควิชา/เทียบเท่า
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2563
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

Thesis Title	Effects of Chitosan Combined with Chitosan-Montmorillonite Nanocomposites Coating on Postharvest Quality of 'Hom Thong' Banana Fruit
By	Miss Arisa Wantat
Field of Study	Biotechnology
Thesis Advisor	Assistant Professor Kanogwan Seraypheap, Ph.D.
Thesis Co Advisor	Associate Professor Pranee Rojsitthisak, Ph.D.

Accepted by the FACULTY OF SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

.... Dean of the FACULTY OF SCIENCE
(Professor POLKIT SANGVANICH, Ph.D.)

DISSERTATION COMMITTEE

.... Chairman
(Assistant Professor Jittra Piapukiew, Ph.D.)

.... Thesis Advisor
(Assistant Professor Kanogwan Seraypheap, Ph.D.)

.... Thesis Co-Advisor
(Associate Professor Pranee Rojsitthisak, Ph.D.)

.... Examiner
(Associate Professor SEHANAT PRASONGSUK, Ph.D.)

.... Examiner
(Assistant Professor VARAPHA KONGPENSOOK, Ph.D.)

.... External Examiner
(Emeritus Professor Saichol Ketsa, Ph.D.)

อริสา วันทัศน์ : ผลของการเคลือบด้วยไคโทซานร่วมกับไคโทซาน-มอนต์มอริลโลไนต์นาโนคอมพอสิตต่อคุณภาพหลังการเก็บเกี่ยวของผลกล้วยหอมทอง. (Effects of Chitosan Combined with Chitosan-Montmorillonite Nanocomposites Coating on Postharvest Quality of 'Hom Thong' Banana Fruit) อ.ที่ปรึกษาหลัก : ผศ. ดร.กนกวรรณ เสรีภาพ, อ.ที่ปรึกษาร่วม : รศ. ดร.ปราณี โรจนสิทธิศักดิ์

ไคโทซานได้ถูกนำมาใช้ในการเคลือบผิวผลไม้เพื่อยืดอายุหลังการเก็บเกี่ยวของผลิตผลทางการค้าอย่างแพร่หลาย ในงานวิจัยนี้ได้ศึกษาการยืดอายุกล้วยหอมทองซึ่งเป็นผลไม้ส่งออกที่มีความสำคัญของประเทศไทยโดยการเคลือบผิวของผลกล้วยด้วยสารละลายไคโทซานน้ำหนักโมเลกุลต่ำ (LMW-CTS: 65 กิโลดาลตัน) น้ำหนักโมเลกุลปานกลาง (MMW-CTS: 265 กิโลดาลตัน) และน้ำหนักโมเลกุลสูง (HMW-CTS: 540 กิโลดาลตัน) ความเข้มข้น 1 เปอร์เซ็นต์โดยมวลต่อปริมาตร เป็นเวลา 1 นาที พบว่าการเคลือบ HMW-CTS ทำให้มีค่าความหนาของฟิล์มสูงที่สุดและมีประสิทธิภาพในการขวางกั้นการซึมผ่านของไอน้ำและแก๊สออกซิเจนได้ดี ผลที่เคลือบด้วย HMW-CTS ยังสามารถชะลออัตราการหายใจและการผลิตเอทิลีนได้อีกด้วย ดังนั้นจึงมีผลทำให้เกิดการชะลอการอ่อนนิ่มของผล การสูญเสียน้ำหนัก การเปลี่ยนแปลงสีของผล และปริมาณของแข็งที่ละลายน้ำได้ นอกจากนี้ผลที่เคลือบด้วย HMW-CTS ยังมีการลดกิจกรรมของเอนไซม์ที่ย่อยสลายผนังเซลล์ ได้แก่ พอลิกาแลกทูโรเนส (PG) และ เพกเทตไลเอส (PL) และนอกจากนี้ พบว่าผลที่เคลือบด้วย HMW-CTS ยังสามารถเพิ่มประสิทธิภาพในการต้านอนุมูลอิสระ ลดความเสียหายที่เกิดขึ้นกับเยื่อหุ้มเซลล์ และลดความรุนแรงของโรคได้อีกด้วย จากผลการศึกษานี้แสดงให้เห็นว่าการเคลือบผิวของผลกล้วยหอมทองด้วย HMW-CTS แสดงประสิทธิภาพสูงที่สุดในการชะลอการอ่อนนิ่มของผล และสามารถยืดอายุการเก็บรักษาได้นานที่สุดอีกด้วย

การพัฒนาผงไคโทซาน-มอนต์มอริลโลไนต์นาโนคอมพอสิต (CTS-MMT) เพื่อผสมกับสารละลายไคโทซานและจำลองเป็นฟิล์มบาง โดยทำให้เป็นกลางด้วยสารละลายโซเดียมไฮดรอกไซด์ที่ความเข้มข้น 4 เปอร์เซ็นต์โดยมวลต่อปริมาตร เมื่อนำไปวิเคราะห์คุณลักษณะของฟิล์ม พบว่าฟิล์มที่ผสม 2% CTS-MMT มีความสามารถในการต้านน้ำสูงขึ้น โดยแสดงค่ามุมสัมผัสของหยดน้ำมากขึ้น และยังแสดงค่าการดูดซับน้ำและการละลายน้ำลดลง เมื่อเปรียบเทียบกับฟิล์มไคโทซานแบบไม่ผสม (CTS) สารละลายไคโทซานที่ผสมผง CTS-MMT ที่ความเข้มข้น 1 2 และ 4 เปอร์เซ็นต์โดยมวลต่อปริมาตร ได้ถูกนำมาใช้ในการเคลือบผิวของผลกล้วย โดยทำการเคลือบผิวเป็นเวลา 1 นาที และเก็บรักษาที่อุณหภูมิ 25 องศาเซลเซียส เป็นเวลา 15 วัน พบว่าผลกล้วยหอมทองที่เคลือบด้วย 2% CTS-MMT สามารถลดอัตราการหายใจและการผลิตเอทิลีน ชะลอการเปลี่ยนแปลงสีของผล ลดค่าการรั่วไหลของอิเล็กโตรไลต์ และลดปริมาณมาลอนไดอัลดีไฮด์ ซึ่งแสดงให้เห็นว่ามีการรั่วไหลของเยื่อหุ้มเซลล์ที่ลดต่ำลง นอกจากนี้ 2% CTS-MMT ยังสามารถชะลอกระบวนการสุกของผล โดยเพิ่มประสิทธิภาพการขวางกั้นแก๊สออกซิเจนของฟิล์ม ซึ่งเป็นคุณลักษณะที่สำคัญในการรักษาคุณภาพหลังการเก็บเกี่ยวของผลกล้วยหอมทอง

เพื่อศึกษาผลของไคโทซานและผงไคโทซาน-มอนต์มอริลโลไนต์นาโนคอมพอสิตต่อการเกิดโรคหลังการเก็บเกี่ยว โดยปลูกเชื้อ *Colletotrichum musae* ซึ่งเป็นเชื้อราก่อโรคแอนแทรกซ์โนสบนผลของกล้วยหอมทอง พบว่าการเคลือบผลด้วย CTS และ 2% CTS-MMT หลังการปลูกเชื้อ มีผลลดการก่อโรคและลดความรุนแรงของโรคอย่างมีนัยสำคัญทางสถิติระหว่างเก็บรักษาที่อุณหภูมิ 25 องศาเซลเซียส นอกจากนี้ยังพบว่า การเคลือบผลด้วยวัสดุทั้ง 2 ชนิดสามารถเพิ่มปริมาณฟีนอลิก กรดแอสคอร์บิก และกลูทาไธโอน ในผลกล้วยได้อีกด้วย ยิ่งไปกว่านั้นยังพบว่า CTS และ 2% CTS-MMT มีความสามารถในการกระตุ้นให้เกิดกระบวนการต้านเชื้อ โดยเพิ่มกิจกรรมของเอนไซม์แอนติออกซิแดนท์ ได้แก่ แอสคอร์เบต เปอร์ออกซิเดส (APX) คะทาเลส (CAT) กลูทาไธโอนรีดักเทส (GR) และดีไฮโดรแอสคอร์เบตรีดักเทส (DHAR) ดังนั้นจึงกล่าวได้ว่า CTS และ 2% CTS-MMT เป็นสารเคลือบผิวที่เหมาะสมในการลดความรุนแรงของโรคในผลกล้วยหอมทอง

สาขาวิชา	เทคโนโลยีชีวภาพ	ลายมือชื่อนิสิต ....
ปีการศึกษา	2563	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

5872860323 : MAJOR BIOTECHNOLOGY

KEYWORD: Chitosan, Montmorillonite nanocomposites, Coating, Shelf life, Banana fruit, Storage

Arisa Wantat : Effects of Chitosan Combined with Chitosan-Montmorillonite Nanocomposites Coating on Postharvest Quality of 'Hom Thong' Banana Fruit. Advisor: Asst. Prof. Kanogwan Seraypheap, Ph.D. Co-advisor: Assoc. Prof. Pranee Rojsitthisak, Ph.D.

Chitosan (CTS) has been widely applied as a fruit coating in commercial fruit crops to prolong their shelf-life. In the present research, chitosan coatings were applied on 'Hom Thong' banana fruit, an important exported cultivar of Thailand. Postharvest qualities of 'Hom Thong' banana were measured after coating with 1% (w/v) low molecular weight (LMW-CTS: 65 kDa), medium molecular weight (MMW-CTS: 265 kDa), and high molecular weight (HMW-CTS: 540 kDa) chitosan for 1 min. The application of HMW-CTS showed the thickest of coated film and had effectiveness as a barrier of water vapor and O_2 transmission. HMW-CTS treatment could retard fruit respiration and ethylene production, thus delaying fruit softening, reducing weight loss, peel color change, and TSS content. Moreover, HMW-CTS coated fruit exhibited reduced cell wall hydrolase enzyme activities, including polygalacturonase (PG) and pectate lyase (PL). Furthermore, HMW-CTS could enhance the antioxidant capacity, reduce cell membrane injury and disease severity of fruit. These results suggested that HMW-CTS was the most effective treatment in delaying fruit softening and exhibited a longer shelf life of 'Hom Thong' banana.

The development of chitosan-montmorillonite nanocomposites (CTS-MMT) nanocomposites combined with chitosan solution was further investigated. The CTS solution combined with CTS-MMT nanocomposites was casted as free-standing films and neutralized with 4% (w/v) NaOH. The combination of 2% CTS-MMT in CTS thin film had a higher water resistance ability by increasing the degree of water contact angle and lowering water absorption and water solubility values compared to CTS film. The CTS solution combined with CTS-MMT nanocomposites at 1%, 2%, or 4% (w/v) were applied as banana fruit coating. Fruit was dipped in the solution for 1 min and then stored at 25 °C for 15 days. The 2% CTS-MMT coating reduced the respiration rate and ethylene production, retarded peel color change and inhibited electrolyte leakage and malondialdehyde content of bananas, indicating a decrease in membrane leakage. Taken together, the 2% CTS-MMT could delay banana fruit ripening due to the increase in the oxygen barrier property of the film, which is an important parameter for maintaining the postharvest quality of 'Hom Thong' banana fruit.

To evaluate the effect of CTS and CTS-MMT on the development of postharvest disease, banana fruit were inoculated with *Colletotrichum musae,* which caused anthracnose in bananas. CTS and 2% CTS-MMT treatments significantly reduced the incidence and severity of the disease during storage at 25 °C. In addition, both treatments markedly increased the contents of phenolic content (TPC), ascorbic acid (AsA), and reduced glutathione (GSH) in bananas. Moreover, CTS and 2% CTS-MMT treatments had the ability to reinforce the microbial defense mechanism of bananas by enhancing the activities of antioxidant enzymes, including ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and dehydroascorbate reductase (DHAR). Therefore, the CTS and 2% CTS treatments could mitigate the disease severity in 'Hom Thong' banana fruit.

Field of Study: Biotechnology   Student's Signature ....

Academic Year: 2020   Advisor's Signature ....

                                                     Co-advisor's Signature ....

ACKNOWLEDGEMENTS

The completion of this thesis is made possible with help and support from several people. I would like to express my sincere gratitude to my thesis advisor, Assistant Professor Dr. Kanogwan Seraypheap, who always gives me her valuable time, helpful suggestion, motivation, knowledge, and support throughout my Ph.D. study and research.

I would like to express my deepest thank to my thesis co-advisor, Associate Professor Dr. Pranee Rojsitthisak, who gave her valuable time for support, guidance, and supervision.

I would like to express my appreciation to Assistant Professor Dr. Jittra Piapukiew, Associate Professor Dr. Sehanat Prasongsuk, Assistant Professor Dr. Varapha Kongpensook, and Professor Dr. Saichol Ketsa for serving on my thesis committee, their help, and valuable suggestions to complete my thesis.

This research was supported by the Center of Excellence in Environment and Plant Physiology (CEEPP), Department of Botany, Faculty of Science, Chulalongkorn University. I would like to thank all members for their excellent assistance and friendship.

I am grateful for the Resource Development in Science Project (Science Achievement Scholarship of Thailand, SAST) for supporting my thesis. I would like to thank the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) for their financial support.

Also, I would like to express my special thanks to friends for helping me with my work. Above all, I would like to express my special gratitude to my family, especially my parents, for their encouragement, understanding, and support throughout my study and life.

Arisa Wantat

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

CHAPTER I

INTRODUCTION

Rationales

Banana (*Musa acuminate* L.) is one of the major commercial fruit crops grown in tropical and subtropical areas. It is considered a high-energy food source and is easily consumed (Williams, 1995). However, banana is a typical climacteric fruit that rapidly softens and ripens after harvest. The banana fruit quality quickly declines when the fruit develops to fully ripen (Kevers et al., 2007) which is the limiting factor for exporting bananas to distant countries. In addition, changes in texture, firmness, and color of banana peel directly impact consumer preference (Goulao and Oliveira, 2008), affecting commercial banana value in the market. Thus, the development of postharvest treatment to prolong banana shelf life and maintain fruit quality during transit and storage is needed.

Coating technique is one of the most popular methods to extend plant commercial shelf life, especially fruit crops, by delaying ripening, softening, water loss, and fungal decay development (Baldwin et al., 1997). Coatings can lead to a change in the composition of the atmosphere surrounding the fruit resulting in a modified atmosphere (MA) that can act as a barrier to gas exchange, especially for O_2, CO_2, and ethylene (Amarante et al., 2001).

Chitosan can be modified as fruit and vegetable coating as it can form semipermeable film to limit fungal decay and delay the ripening as reported in many plants (Ampaichaichok et al., 2014; Jiang and Li, 2001; Jongsri et al., 2016; Petriccione et al., 2015). Chitosan-montmorillonite (CTS-MMT) nanocomposites were developed by Lertsutthiwong et al. (2012) to make them more effective as adsorbents and antimicrobial material. CTS-MMT exhibited good dry adsorption efficiency and also inhibited the growth of *E.coli*. Therefore, it could be applied as an antimicrobial coating and ethylene absorbent during postharvest storage of fruit to prolong shelf life and maintain crop quality. Montmorillonite, together with chitosan, could become an effective fruit coating to utilize in the fruit export industry. Moreover, loss of quality and shelf life of banana fruit due to postharvest diseases during postharvest storage is a serious problem of exported banana fruit that causes a reduction in the economic value of fruit during storage, transport, and marketing period. Fungicides, either preharvest or postharvest treatments, were used to reduce losses from postharvest disease. However, their uses were increasingly restricted due to public concerns over toxic residues. Thus, developing integrated postharvest technologies to maintain overall banana fruit quality during the export and marketing periods is necessary.

Montmorillonite is an alternative low-cost adsorbent which is a clay mineral with high surface areas. Chitosan-montmorillonite (CTS-MMT) nanocomposites developed by Lertsutthiwong et al. (2012) were very effective as adsorbent and antimicrobial material

and exhibits good dry adsorption efficiency, and also inhibits the growth of *E. coli*. Therefore, in this research, CTS-MMT was applied as an antimicrobial coating and ethylene absorbent during postharvest storage of banana fruit to prolong shelf life and maintain fruit quality. Montmorillonite, together with chitosan, could become an effective fruit coating due to chitosan's natural cationic, nontoxic, and antimicrobial properties and montmorillonite as adsorbent property.

To date, chitosan-montmorillonite nanocomposites have never been applied as fruit coating, especially in 'Hom Thong' banana which is an important export cultivar. Thus, the framework of this study was to develop a chitosan coating combined with chitosan-montmorillonite nanocomposites to prolong shelf life and maintain the quality of 'Hom Thong' banana fruit which help to increase the economic value of exported 'Hom Thong' banana. Physiological and biochemical parameters of banana fruit were also be analyzed to better understand the effect of chitosan-montmorillonite nanocomposites on fruit responses during storage.

Objectives

1. To develop an effective coating by using chitosan combined with chitosan-montmorillonite nanocomposites for prolonging the shelf life of 'Hom Thong' banana

2. To determine the effects of chitosan combined with chitosan-montmorillonite nanocomposites coating on postharvest qualities, physiological characteristics, and anthracnose disease on banana fruit

Expecting benefits

The obtained banana coating developed in this finding can be applied for banana shelf-life extension in commercial production.

Contents of the thesis

1. Literature reviews

2. Effects of chitosan coating on physiological responses of banana fruit during storage at 25 ± 2 °C

3. Determination of the appropriate chitosan coating containing chitosan-montmorillonite nanocomposites to prolong the shelf life of banana during storage

4. Determination of anthracnose disease development in banana fruit coated with chitosan and chitosan containing chitosan-montmorillonite nanocomposites

5. Results and discussions

6. Conclusions
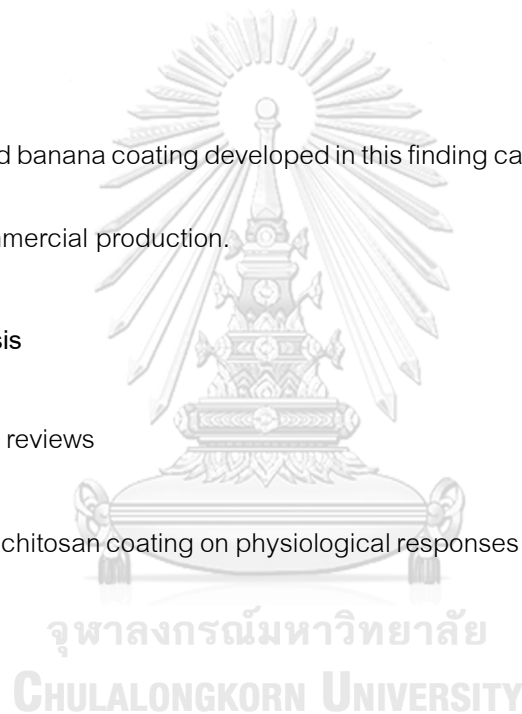
CHAPTER II

LITERATURE REVIEWS

2.1 Banana

Banana belongs to the family of *Musaceae* originated in Southeast Asia. There are several cultivars, and the main genome groups are AA, AB, AAA, AAB, and ABB. Almost all the edible bananas come from the two wild species *Musa acuminata* (AA) and *Musa balbisiana* (BB) (Singh et al., 2014). 'Hom Thong' banana (*Musa acuminata*) belongs to AAA Group and Gros Michel subgroup; it is a significant tropical fruit crop in Thailand (Daungban et al., 2017).

Banana provides a good source of nutrients and energy. It contains carbohydrates, sugar, protein, lipid, and minerals (Florent et al., 2015). Banana is a climacteric fruit that increases respiration rate and ethylene production during ripening (Prasanna et al., 2007). After harvest, biochemical processes in banana fruit are changed rapidly, and fruit is developed to ripening during storage. These changes influence its appearance, texture, flavor, and nutritive value. After harvest, the water content of banana fruit is reduced; thus, fruit face shriveling and weight loss leading to the reduction of marketable weight and their visual quality. In addition, banana is very susceptible to the

pathogens; thus, the pathogenic infections usually occur during storage, and the attraction of microorganisms can advance the rapid deterioration of fruit (Lim et al., 2002).

2.1.1 Anthracnose of banana

Anthracnose, the most postharvest disease of bananas globally, is caused by *Colletotrichum musae*. The disease brought about the loss of marketable value up to 30-40% per year (Ranasinghe et al., 2003). Anthracnose is a latent infection, it invades the immature fruit in the field, but the disease symptoms appear at the ripening stage. However, If the fruits get wounding, the disease can develop before ripening (Meredith, 1960). Anthracnose symptoms caused the sunken covered with salmon-colored brown lesion area as shown in Figure 1 (Lim et al., 2002).

Figure 1. Anthracnose symptoms of banana caused by *Colletotrichum musae*

2.1.2 Physicochemical and physiological changes during banana ripening

2.1.2.1 Fruit softening

Fruit softening is a significant change during ripening. The degradation of starch leads to a texture change and softening. Finney Jr et al. (1967) reported that loss of firmness related to the chemical structure of starch grain changes which showed a decrease during ripening. In addition, fruit softening is closely linked with cell wall component changes, which are composed of polysaccharides including pectin, hemicellulose, and cellulose (Figure 2) (Keegstra, 2010). Among polysaccharides, pectin is the most abundant in plant cell wall matrix and middle lamella (Malinovsky et al., 2014). Kojima et al. (1994) found that banana fruit softening was associated with decreasing pectin and hemicellulose content. Numerous studies reported that water-insoluble pectin was converted to soluble pectin when the ripening was in progress (Amnuaysin et al., 2012; Liu et al., 2016).
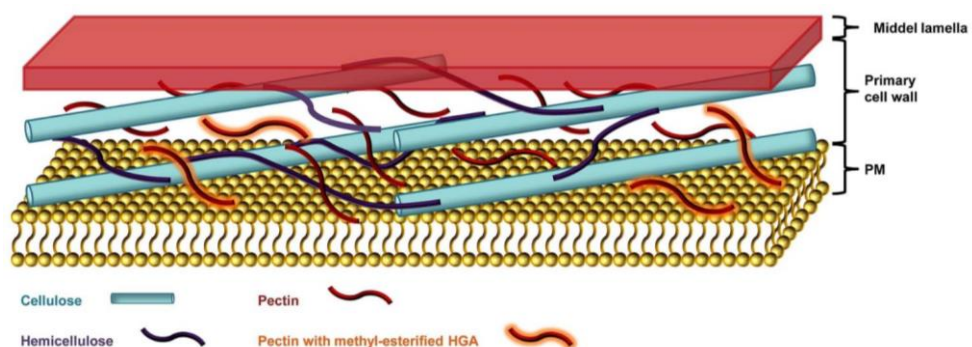
Figure 2. Cell wall structure in plant cell

(Malinovsky et al., 2014)

The pectate lyase (PL) and polygalacturonase (PG) enzymes are known as pectin degrading enzymes. However, these two enzymes have a different way of hydrolyzing a substrate. PL is the pectin depolymerizing enzyme that acts by cleaving α-1,4-galacturonosidic linkages of PGA (polygalacturonic acid) via the β-elimination reaction, a key component in pectin. It is then producing 4,5-unsaturated oligogalacturonates at the nonreducing end of the cut pectin chain. In this reaction, PGA was added as the substrate in the activity analysis, and unsaturated oligogalacturonide was produced in the reaction. Differently, PG randomly hydrolyses α-1, 4-glycosidic linkages of pectin or polygalacturonic acid chains, producing free galacturonic acid (Prasanna et al., 2007).

2.1.2.2 Carbohydrates

Starch is accumulated in banana fruit about 20-25% of fresh weight (FW), and it was converted rapidly to sugar during ripening. Green banana contains sugar only 1-2% of FW and can be reached up to 15-20% at ripening (Maduwanthi and Marapana, 2017). The sugar content of ripening fruit is mainly sucrose, glucose, and fructose. (Adao and Gloria, 2005). Glucose is the predominant sugar of bananas followed by fructose and sucrose (Florent et al., 2015).

2.1.2.3 Peel color

During ripening, banana peel changes from green to yellow. The essential substances related to color development in the peel are chlorophyll and carotenoids (Subagio et al., 1996). It is a significant indicator to determine the ripening (Li et al., 1997). The external appearance of banana fruit is associated with chlorophyll and carotenoid content. Moreno et al. (2020) exhibited that chlorophyll content in banana peel decreased during ripening, and carotenoid increased. The chlorophyll degradation correlated with chlorophyllase enzyme, which is a part of the chlorophyll degradation system in the initial step (Win et al., 2006).

2.1.2.4 Flavor

Flavor is one of the parameters changing during ripening. The increase of soluble solids and volatiles compound and the decrease of acids content are typically observed during ripening process. The sweetness of fruit mainly due to the hydrolysis of starch to sugar (Adao and Gloria, 2005). Boudhrioua et al. (2003) reported that sugar content and volatiles in banana pulp increase during ripening, while organic acids and phenolic compounds decrease. Volatile compounds play a role in determining banana flavor. Pino and Febles (2013) reported that the volatile compositions of AAA group banana fruit include 75 esters, 18 ketones, 14 phenols and derivatives, 13 alcohols, 7 aldehydes, 7

acids, and 12 miscellaneous compounds. Among compounds, ester has been found to

be a dominant substance in the aroma of fully ripe banana (Marriott and Palmer, 1980).

2.1.2.5 Ethylene production

Ethylene is a phytohormone that is a major factor correlated with the climacteric

fruit ripening process. It plays a role in the initiation of ripening and senescence. Ethylene

is an autocatalytic synthesis hormone, and it can trigger the ripening process in many

fruits such as mango (Jongsri et al., 2016; Wannabussapawich and Seraypheap, 2018)

and banana fruit (Thakur et al., 2019). Ethylene biosynthesis, the first step started by

conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane carboxylic acid

(ACC) by ACC synthase enzyme. The produced ACC is then oxidized to ethylene by the

ACC oxidase enzyme (Figure 3).
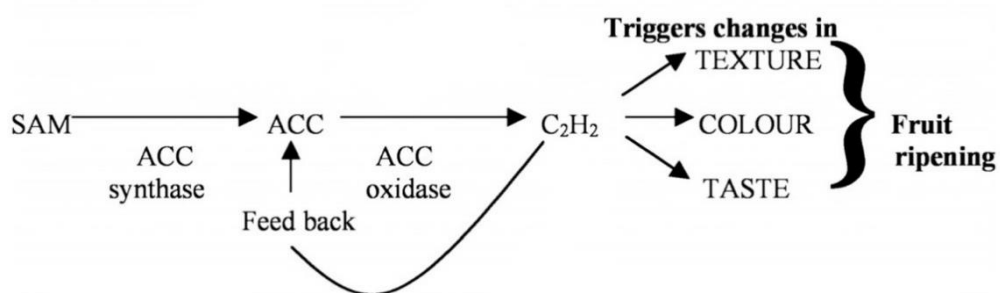
Figure 3. Ethylene biosynthesis pathway related to fruit ripening

(Prasanna et al., 2007)

2.2 Edible coatings/films

Edible coatings and films are traditionally used to cover fresh produce such as vegetables and fruit to prolong their shelf life (Bhardwaj et al., 2019). Coatings/films provide the barrier that coats the stomata leading to reduce moisture and gas transmission such as O_2, CO_2, and ethylene (Butler et al., 1996). The main purposes of fruit coating are to reduce its respiration rate, ethylene production, microbial growth, physiological disorders and maintain fruit firmness (Cakmak et al., 2018). Coatings are defined as a thin layer (< 0.3 mm) of film, and it may be consumed together with food; thus, it could be eaten. Numerous materials had been made from natural resources such as polysaccharides, lipid, and protein materials. In recent years, coating materials have been combined with other substances for developing their properties. Duran et al. (2016) found that strawberry-coated chitosan, nisin, and natamycin could maintain fruit qualities. Li et al. (2019) also reported that guava coated with chitosan and alginate could reduce the disease severity of fruit and extend its shelf up to 20 days.

2.2.1 Chitosan

Chitosan, poly-(β-(1-4)-D-glucosamine), is a polysaccharide obtained from the deacetylation of chitin, and it can be extracted from crustacean shells and fungal cell wall. Chitin, poly-(β-(1-4)-N-acetyl-D-glucosamine), which proceeded with deacetylation reaches up to about 50% is defined as chitosan (Hirano et al., 1977). The different structures of chitosan and chitin were shown in Figure 4. Chitosan material has been widely used in the food industry because it is a non-toxic, biocompatible, biodegradable polysaccharide and reasonable price (Yang et al., 2014). Chitosan coating was reported to reduce respiration rate, which degrades the organic compound accumulation in fruit, leading to prolong its shelf life such as apple (Qi et al., 2011), litchi (Lin et al., 2011), and mango (Jongsri et al., 2016).
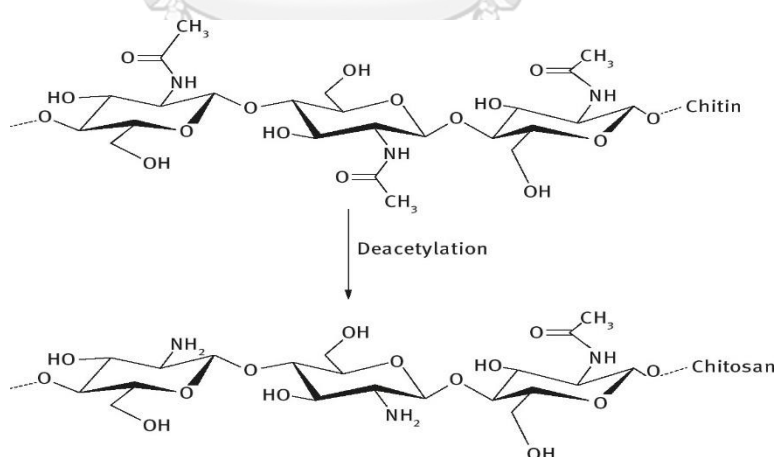
Figure 4. The structure of chitin (β-(1-4)-N-acetyl-D-glucosamine) and chitosan (Nilsen-Nygaard et al., 2015)

Generally, chitin is extracted from crustacean shells such as crab and shrimp shells via acid treatment followed by alkaline treatment. The steps of chitin extraction compost of 3 major steps: demineralization, deproteinization, and depigmentation (Figure 5) (Kumar et al., 2019).
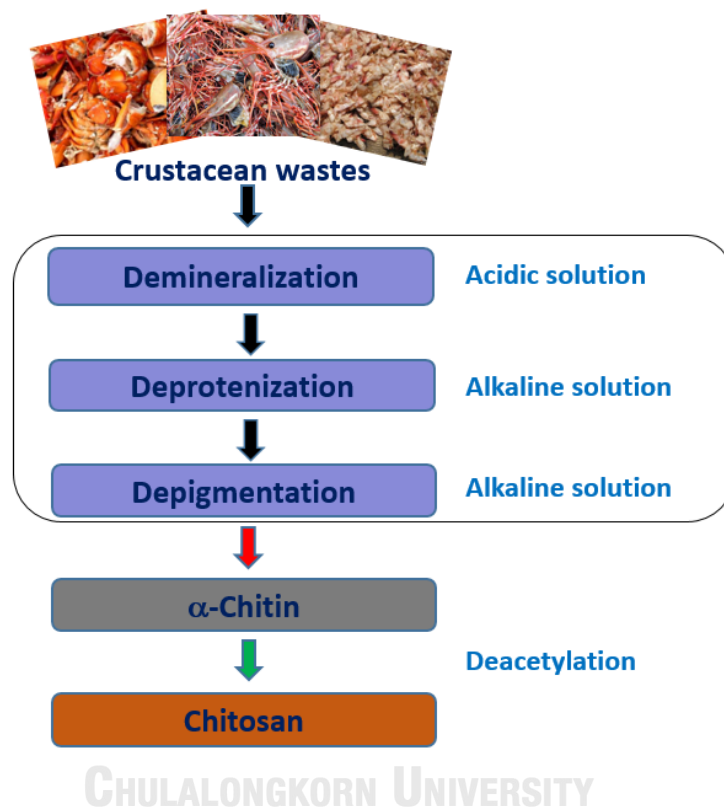
Figure 5. Preparation of chitosan from crustacean

(Kumar et al., 2019)

2.2.1.1 Properties of chitosan

Degree of deacetylation (DD): DD of chitosan indicates the number of amino groups ($-NH_2$) in the polymer chains. The deacetylation is resulting in a copolymer of N-acetyl-glucosamine and D-glucosamine. The copolymer forms that compost D-glucosamine more than 50% are defined as chitosan (Hirano et al., 1977). DD has been reported to affect chitosan properties such as solubility, charge, mechanical, thermal, and barrier properties (Paul et al., 2015). Previous studies reported that chitosan film prepared with 92.3% DD had better barrier and mechanical properties than 78.9% DD chitosan (Kim et al., 2006). In addition, Zhuang et al. (2019) supported that the films prepared with 85.2% DD chitosan had better water vapor properties and tensile strength than 81.0% DD chitosan.

The molecular weight (MW): MW of chitosan is also reported to influence its properties. Jongsri et al. (2016) reported the effects of MW chitosan on their coating properties. They found that chitosan with MW of 360 kDa had the highest viscosity and the highest thickness resulting in delaying the ripening of mango fruit compared to the chitosan solution of 270 kDa and 40 kDa. Zhong et al. (2019) also reported that chitosan film with increased MW increased surface tension and contact angle. Moreover, when increased, the MW, water vapor barrier property, and tensile strength of the film were improved. The viscosity of chitosan solution is also affected by its MW and concentration.

The solution with high concentration and high MW results in high viscosity (Jongsri et al., 2016; Zhong et al., 2019).

Antimicrobial Properties: Chitosan has been found to act against pathogens such as bacteria and fungus which causing plant disease. Chitosan has been used as a fruit coating since it can form semipermeable film to limit fungal decay. It was found to strongly inhibit *Alternaria kikuchiana* Tanaka and *Physalospora piricola* Nose mycelial growth in pear fruit (Meng et al., 2010) and *Fusarium oxysporum* in potato (Ren et al., 2021). In addition, chitosan effectively enhanced the activities of chitinase and β-1,3- glucanase and retard mango ripening (Jitareerat et al., 2007; Jongsri et al., 2017). Limon et al. (2021) found that mango fruit coated with chitosan solution also reduced the size of *C. gloeosporioides* lesions compared to uncoated mango. Chitosan coating also delayed the activities of chitinase (CHI), peroxidase (POD), and polyphenol oxidase (PPO), which preventing flesh browning as well (Jiang and Li, 2001; Meng et al., 2010). In addition, chitosan also reinforces the microbial defense mechanism by delaying the reduction of total polyphenol, anthocyanin, and flavonoid contents and enhancing catalase (CAT) and ascorbate peroxidase (APX) activities (Petriccione et al., 2015). Additionally, chitosan acts as a natural elicitor that can induce plant defense mechanisms and decrease the severity of disease symptoms (Bautista-Banos et al., 2003; Jongsri et al., 2017). These specific properties of chitosan may depend on its type, degree of deacetylation, and molecular

weight. The previous report revealed that the antimicrobial activity increased with the molecular weight of chitosan increase (Kulikov et al., 2006).

2.3 Addition of nanoparticles in the edible coatings and films

Edible coatings and films have been developed to increase the effectiveness of postharvest technology. Chitosan solutions are the proper material for embedding various nanoparticles. Numerous studies have attempted to incorporate nanocomposites into chitosan coatings and films. Yadav et al. (2021) found that the addition of zinc oxide nanoparticles to chitosan-based films enhanced its mechanical properties and increased the water vapor barrier value of the film. Additionally, antioxidant and antibacterial properties of films were reported when gallic acid was loaded in the films. Also, Kumar et al. (2020) developed edible coating by adding zinc oxide nanoparticles in chitosan matrix. According to the results, edible films were significantly against *Escherichia coli* and *Staphylococcus aureus*. Both microbials were also reported in the study of Xing et al. (2020), in which titanium dioxide (TiO_2) nanoparticles (NPs) were combined with chitosan film and had an effect of exhibiting their growth. In addition, the combination coating exhibited excellent physical properties. Salama and Aziz (2020) also reported that chitosan film enriched with titanium oxide nanoparticles and carboxymethyl cellulose could improve thermal properties and enhanced antimicrobial activity. Additionally, green

bell paper coated with this solution had a longer shelf life and lower mass loss than uncoated fruit.

2.4 Montmorillonite (MMT)

Montmorillonite is a natural clay with fine white powder (Figure 6). Its structure consists of tetrahedral silica sheets layered between the alumina octahedral sheets and the interlayer containing Na^+ or Ca^{2+}, which can be exchanged with cation ions (Figure 7). MMT has the ability in absorption property, cation exchange capacity, and drug delivery (Wang et al., 2008). The modification of MMT results in swelling of clay galleries; thus, it improves the polymer chain intercalation in the polymer matrix (Xia et al., 2019). Inorganic ions in the clay can be replaced by cations ions such as Ni, Co, and Zn. In addition, it can be replaced by organic cationic molecules such as chitosan via cation-exchange reactions (Hoidy et al., 2010).

Figure 6. Sodium-montmorillonite powder
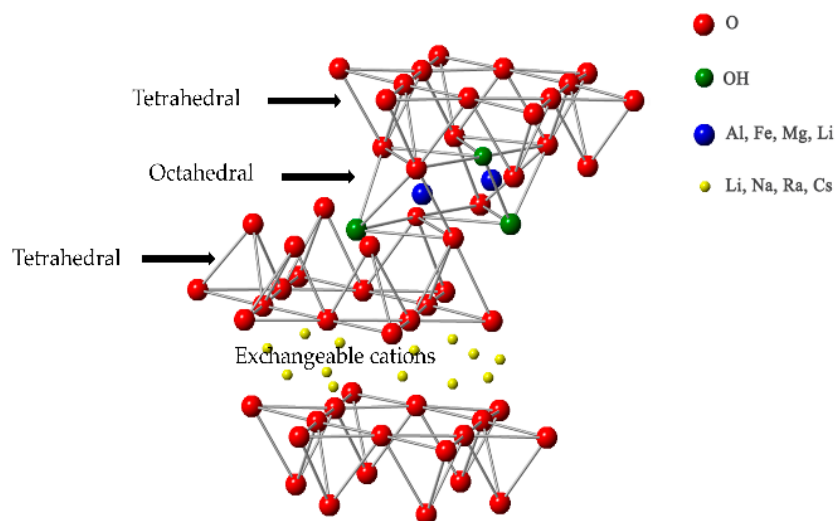
Figure 7. Structure of montmorillonite

(Kurecic and Smole, 2012)

Montmorillonite (MMT) is a nano-materials which have been widely used to improve many properties of polymers. Numerous studies reported that it could improve the gas barrier property of PET film (Ke and Yongping, 2005), improve mechanical and water vapor of alginate film (Alboofetileh et al., 2013). Moreover, montmorillonite is well known for its low cost and low toxicity. It has been widely used in the food, medicine, and chemical industry in drug delivery (Joshi et al., 2009; Salcedo et al., 2012). In addition, montmorillonite combined with silver could prolong the shelf life of fresh fruit (Costa et al., 2011). Lertsutthiwong et al. (2012) developed chitosan-montmorillonite (CTS-MMT) nanocomposites improving their properties as adsorbents and antimicrobial material. Produced CTS-MMT nanocomposites exhibited a good dry adsorption efficiency, also inhibited the growth of *E.coli*. Therefore, it might be applied as an antimicrobial coating

and ethylene absorbent during postharvest storage of fruit to prolong shelf life and maintain crop quality (Rhim et al., 2006). Montmorillonite has been reported to incorporate with other compounds such as lactobiopolymer and starch, resulting in maintaining fruit firmness and quality of fresh-cut carrot (Guimaraes et al., 2016) and strawberry (Junqueira-Gonçalves et al., 2017). Neji et al. (2020) reported that the exfoliated nanoclay in the chitosan matrix also increased their mechanical strength.

2.5 Nanoclay Polymer Composites

Polymer nanoclay composites are the interaction of polymer matrix with the nano-plates of clay. Among nano-plates, MMT is wildly used for dispersion in the polymer matrix. There are 3 different types of nanocomposites: conventional composites (microcomposites), intercalated nanocomposites, and exfoliated nanocomposites (Figure 8) which depend on the process conditions and the interaction between layered silicate and the polymer matrix.

2.5.1 Conventional composites (Microcomposites): The nanoclay retain their original structure in the poly matrix. The polymer chains are unable to penetrate the nanoclay galleries.

2.5.2 Intercalated nanocomposites: The layers of nanoclay are inserted by the polymer chains, resulting in expanded d-spacing. The characteristic peak displacement to lower angles represents intercalating of these nanosheets.

2.5.3 Exfoliated nanocomposites: The layers of nanoclay are completely

separated and randomly distributed in the poly matrix. The characteristic peak

disappears in the diffraction pattern of these nanocomposites (Usuki et al., 2005).
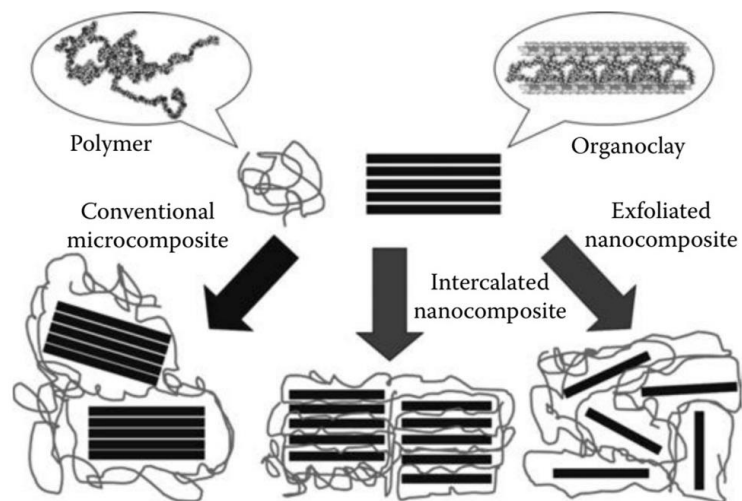
Figure 8. Schematic view of different groups of composites

(Usuki et al., 2005)

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

Banana fruit (*Musa acuminata* Colla (AAA group) cv. Hom Thong) were harvested after flowering 70-80 days from a commercial orchard in Pathumthani province, Thailand. After harvesting, fruit were transferred immediately to the laboratory within 2-3 h and selected for uniformity in size, color, shape, and lack of noticeable defects. Then, banana hands were separated into individual fruit, washed with running tap water, and dried at room temperature.

3.1.2 Chitosan material

The different molecular weight of shrimp chitosan materials (food grade) was purchased from Marine Bio Resources Co., Ltd. with degree acetylation of 90% and molecular weight of high molecular weight chitosan (HMW-CTS: 540 kDa), medium molecular weight chitosan (MMW-CTS: 265 kDa), and low molecular weight chitosan (LMW-CTS: 65 kDa).

3.1.3 Sodium montmorillonite material

Sodium montmorillonite used in this experiment was purchased from Southern Clay (Perry Hall, MD, USA), which has a cation-exchange capacity of 71 meq/100 g and a zeta potential of 39.3 mV.

3.2 Methods

3.2.1 Effects of different molecular weights of chitosan coating on physiological responses of banana fruit during storage at 25 ± 2 °C

3.2.1.1 Preparation of derived CTS films

All chitosan solution was prepared by dissolving chitosan in 0.5% acetic acid at the concentration of 1% w/v. To prepare CTS films, 150 mL of chitosan solution was poured into an acrylic tray (21 cm x 29 cm x 0.5 cm) and then dried at room temperature. The obtained films were neutralized by soaking in 4% (w/v) NaOH for 20 min and repeatedly rinsed with distilled water. The processed films were dried and stored in a desiccator before further analysis.

3.2.1.2 Water vapor transmission rate (WVTR)

WVTR values of the film were determined according to the method based on ASTM E 96/E6M-16 (ASTM D 3985, 2002) using a water vapor permeation tester (Labthink model W3/031). The film in each treatment was tested at the same condition at 38 °C and 90% RH with 3 replications. Its weight was recorded every 20 min for 8 h. WVTR was reported as g/m^2 day.

3.2.1.3 Oxygen gas transmission rate (OTR)

OTR values of the film were measured according to the method based on ASTM D 3985-17 using an oxygen permeation tester (Illinois model 8001L). The test was performed at 23 °C and 0% RH condition with 3 replications. The OTR was reported as cc/m^2 day.

3.2.1.4 Coating treatments

All chitosan treatments (1% w/v) were prepared following the same preparation method as the coating solution in 3.2.1.1, and 0.5% acetic acid was set as the control group. After being cleaned and air dried, the fruit was dipped in each treatment for 1 min, and it was then air-dried and stored at 25 ± 2 °C (80% RH) for 15 days. The treatment was performed as describe followed:

Treatment 1: Distilled water

Treatment 2: 0.5% (v/v) acetic acid solution plus with 0.1% (w/v)

tween® 80

Treatment 3: LMW-CTS plus with 0.1% (w/v) tween® 80

Treatment 4: MMW-CTS plus with 0.1% (w/v) tween® 80

Treatment 5: HMW-CTS plus with 0.1% (w/v) tween® 80

The experiment was arranged in a Completely Randomized Design (CRD) with 4 replications, and each replication was comprised of at least 2 samples. Fruit was analyzed on days 0, 9, 12, and 15 of storage time.

3.2.1.5 SEM analysis

The chitosan-coating film was measured for thickness and anatomical characteristics of the covered surface on banana peel using a scanning electron microscope (FEI Quanta 250 ESEM, Netherlands) with the voltage set at 10 kV.

3.2.1.6 Respiration rate and ethylene production

Respiration rate and ethylene production determinations were performed following the method described by Jongsri et al. (2016). After weighted, the individual banana fruit in each treatment was kept in a 2.4 L jar and sealed with a rubber septum under a temperature at 25 °C for 1 h and 2 h for respiration rate and ethylene production analysis, respectively. After incubation, 1 mL of the internal atmosphere was collected using a syringe and injected into gas chromatograph (GC 7890B, 151 Agilent Technologies, Inc., USA). The respiration rate was measured as CO_2 production and expressed in mg CO_2/kg/h. The ethylene levels were expressed as μL/kg/h.

3.2.1.7 Weight loss

Fruit was weighted and recorded on day 0 and different record dates. Weight loss (%) was calculated using a formula described by AOAC International (2000) as follows:

$$\text{Weight loss (\%)} = [(W_1 - W_2)/W_1] \times 100$$

W_1 = The initial weight on day 0

W_2 = The final weight

3.2.1.8 Total soluble solids content

Total soluble solids content was analyzed using a hand refractometer (N-1E, Japan) following Allegra et al. (2017). Five grams of banana were homogenized with 10 ml distilled water; then, it was centrifuged at 8,000 rpm for 5 min. The supernatant was determined as °Brix and calculated following this formula:

$$\text{Total soluble solids} = °\text{Brix} \times 3 \text{ (dilution factor)}$$

3.2.1.9 Peel color changes

Peel color of banana was measured on the same three regions of fruit firmness measurement using a colorimeter (Color Reader CR-10, Konica Minolta Sensing, Inc., Japan). Color was express as Lightness (L) and Hue angle (h).

3.2.1.10 Fruit firmness

Fruit firmness was measured using a penetrometer (Hardness tester FHM-1, Takemura, Japan) at three regions of peel fruit (blossom end, middle, and stem end). Fruit firmness was recorded as Newtons (N).

3.2.1.11 Water-soluble pectin

Water-soluble pectin method of Robertson (1979) was applied with some modifications. One gram of banana peel was ground and homogenized in 4 mL of 95% ethyl alcohol, then incubated in 85 °C water bath for 10 min. After incubation, the homogenate was centrifuged at 10,000 × g, 20 °C, 15 min. The residue sediment was re-extracted with 3 mL of 63% ethyl alcohol in 85 °C water bath for 10 min. After centrifuged at 10,000 × g, 20 °C, 15 min, the residue was dissolved in 3 mL of distilled water as water-soluble pectin. Pectin solution was centrifuged at 10,000 × g, 20 °C, 15 min, then 2 mL of supernatant was mixed with 0.25 ml of 1N NaOH then added with distilled water to total volume 5 mL and kept at room temperature 15 min before analysis.

In the analyzing method, 1 mL of supernatant was mixed with 5 mL of 0.0125M $Na_2B_4O_7$ (dissolved in glacial sulfuric acid) then boiled in a water bath for 10 min. After boiling, the reaction mixture was immediately moved on ice. Then, 0.1 mL of 0.3% m-hydroxydiphenyl (dissolved in 0.5% NaOH) was added and kept on ice for 15 min. The reaction mixture was analyzed and calculated from the absorbance of 520 nm. The

mixture using 1N NaOH instead of 0.3% m-hydroxydiphenyl was used as blank. Water-soluble pectin content was compared to the standard curve of D-galacturonic acid and reported as µg/g FW.

3.2.1.12 Cell wall enzymes

Cell wall enzyme extraction was performed according to Lohani et al. (2004) method with some modifications. Briefly, 1 g of banana peel was grounded in liquid nitrogen to powder and homogenized with 5 mL of extraction buffer containing 0.2M Sodium phosphate buffer pH 7.0, 0.2M Cysteine-HCl, 0.2M EDTA, and 1% TritonX-100. The homogenate was then centrifuged at 15,000 × g 4 °C for 30 min, clearly supernatant was applied in PG and PL activities assay.

Assay of pectate lyase (PL) activity: PL activity was assayed using the method of Moran et al. (1968). The reaction mixture, a total of 1 mL contained 200 µL of 0.02M sodium acetate buffer (pH 4.5), 0.3 mL of 1% polygalacturonic acid (PGA, aqueous solution adjusted to pH 4.5), 0.4 mL of distilled water, and 0.1 mL of extraction sample. After the vortex, the mixture tube was incubated at 37 °C for 30 min, and then the reaction was stopped by boiling in the water bath for 2 min. The PL activity was measured using a spectrophotometer at the absorbance of 235 nm. D-galacturonic acid was applied as the standard. The reaction mixture without extraction sample (0.02 Sodium acetate buffer pH

4.5 was used instead of extraction sample) was defined as blank. The PL activity was expressed as units/mg protein.

Assay of polygalacturonic acid (PG) activity: Polygalacturonic acid activity assay was adjusted from Pathak and Sanwal (1998). The activity was assayed in a mixture containing 0.2 mL of 0.2M sodium acetate buffer (pH 4.5), 0.3 mL of 1% polygalacturonic acid (PGA, aqueous solution adjusted to pH 4.5), 0.5 mL of extraction sample in a total volume of 1mL. After mixing, the reaction mixture was incubated at 37 °C for 1 h followed by the addition of 0.5 mL 3,5-dinitrosalicylate reagent (DNS). After that, the reaction was stopped by boiling in the water bath for 5 min; then, it was cooled at room temperature. PG activity was calculated from reaction product as saturated oligo-galacturonates absorbs at 520 nm compared with standard curve using D-galacturonic acid. One unit enzyme is defined as the amount of PG enzyme required for liberating 1 μmol of galacturonic acid per min. The PG activity was expressed as units/mg protein.

3.2.1.13 Hydrogen peroxide (H_2O_2) content

The H_2O_2 content was determined following the method described by Junglee et al. (2014). A 0.1 g of banana pulp powder was homogenized with 1 mL of a cold solution containing 0.25 mL of 0.1M potassium phosphate buffer (pH 7.0), 0.5 mL of 1M KI, and 0.25 mL of 1% TCA. The homogenate was centrifuged at 10,000 × g at 4 °C for 15 min; the supernatant was kept for further analysis. At the same time, control was prepared with distilled water instead of KI.

The analysis of H_2O_2 content, the supernatant was collected for 200 µL and placed in UV-microplate wells (protect from light) and incubated at room temperature for 20 min in darkness. The solution was then measured at 350 nm using a microplate reader (SpectraMax[®] M3, Molecular Devices, LLC., USA). The H_2O_2 content was calculated and compared with H_2O_2 standard prepared in 0.1% TCA and expressed as mmol/g FW.

3.2.1.14 %DPPH inhibition

DPPH assay was adapted from Choi et al. (2006). A 0.3 g of banana pulp powder was homogenized in 1.2 mL of cooled 80% ethanol. The homogenate was centrifuged at 9,000 x g, 4 °C for 10 min. The reaction mixture contained 25 µL of the extraction sample and 175 µL of 0.2mM DPPH (dissolved in 80% ethanol). The mixture solution was incubated under darkness for 10 min at room temperature before measuring the absorbance at 520 nm using a microplate reader (SpectraMax[®] M3, Molecular Devices,

LLC., USA). Ethanol (80%) was used instead of extraction sample serve as control and using pure 80% ethanol as the blank solution. The percentage of the DPPH radical inhibition can be calculated using the following expression:

$$\text{DPPH inhibition (\%)} = [(\text{Abs.}_{control} - \text{Abs.}_{sample})/\text{Abs.}_{control}] \times 100$$

3.2.1.15 Malondialdehyde (MDA) content

MDA content was extracted according to the modified method described by Dhindsa and Matowe (1981). A 1 g of banana pulp was ground in liquid nitrogen to the powder then, and it was homogenized with 4 mL of 0.1M potassium phosphate buffer (pH 6.8) as extraction buffer. After centrifugation at 10,000 × g, 4 °C for 15 min, the supernatant was collected and stored at 4 °C for analysis in MDA level.

MDA content was analyzed using the method described by Wang et al. (2015) with some modifications. The reaction mixture containing 0.6 mL of extraction sample and 0.6 mL of 0.5% 2-thiobarbituric acid (TBA, dissolved in 15% trichloroacetic acid (TCA)) was heated at 100 °C for 20 min and cooled on ice immediately. After cooling, the mixture was then centrifuged at 10,000 × g for 10 min. The clear solution was recorded at the 3 different absorbances, including 450, 532, and 600 nm, and it was calculated as µmol/g fresh weight (FW) by using this formula:

$$\text{MDA content} = 6.45(A_{532} - A_{600}) - 0.56(A_{450})$$

3.2.1.16 Disease severity

Disease severity was recorded as a visual estimation of % fruit area which scored

following the method described by Pedroso et al. (2011) as followed:

0 = No disease

1 = 1-2% disease

2 = 5% disease

3 = 10% disease

4 = 20% disease

5 = 40% disease

6 = 60% disease

7 = More than 80% disease

3.2.1.17 Total protein assay

Total protein assay was modified from Bradford (1976). The reaction mixture,

including 38 µL of Bradford dye (BioRad), was added with 152 µL of distilled water and

10 µL of extraction sample. The reaction was kept in darkness at room temperature for 5

min (the stability of the reaction 5-60 min), and the mixture was measured using the

absorbance of 595 nm. Total protein was calculated to compare with bovine serum

albumin (BSA) standard protein and express in mg protein/mL.

3.2.1.18 Statistical analysis

The data were subjected to statistical analysis using one-way ANOVA. The means were compared using Duncan's multiple range tests at a significance level of 0.05 using SPSS software.

3.2.2 Production of Chitosan-montmorillonite (CTS-MMT) nanocomposites

CTS-MMT nanocomposites were produced via an ion exchange reaction using the method described by Lertsutthiwong et al. (2012) with -some modifications. A 1% (w/v) of MMT powder was suspended in distilled water and stirred for 30 min. Then, 2% (w/v) chitosan (65 kDa) solution (dissolved in 1% acetic acid) was slowly added (100 mL/h) to MMT suspension under stirrer at room temperature using a peristaltic pump (SP-MiniPump, Baoding Shenchen Precision Pump Co., Ltd, China) to obtain the ratio of 2: 1 (Chitosan: MMT). After continuously stirred at 1,000 rpm for 60 min, the resultant mixture was then centrifuged at 6,000 x g for 10 min. The precipitate was then washed with distilled water twice for removal of the excess chitosan. Finally, it was dried at 70 °C overnight before powdering using a pistil and mortar, then sifted through a sieve size of 200 mesh.

3.2.3 Determination of the appropriate chitosan coating containing chitosan-montmorillonite (CTS-MMT) nanocomposites to prolong shelf life of banana during storage at 25 ± 2 °C

3.2.3.1 Preparation of coating solution and treatment

Chitosan powder (540 kDa and degree acetylation of 90%) was dissolved in 0.5% (v/v) acetic acid at the concentration of 1% (w/v), and it was then shaken at 150 rpm overnight. After that, 0.1% tween 80® was added following CTS/MMT powder at the various concentrations (0%, 1%, 2%, and 4% w/v) under stirring for 1 h then stored at room temperature 2 h for removing bubbles before using.

Coating treatment, banana fruits were groups into 5 groups (5 treatments) with 120 fruits per group. In each group, fruit were dipped in the solution for 1 min as described below:

Treatment 1: Distilled water (D.W.)

Treatment 2: CTS (CTS)

Treatment 3: CTS + 1% CTS-MMT (1% CTS-MMT)

Treatment 4: CTS + 2% CTS-MMT (2% CTS-MMT)

Treatment 5: CTS + 4% CTS-MMT (4% CTS-MMT)

After treatment, fruit was then air-dried at room temperature and placed in the bucket with shredded paper before storing at 25 ± 2 °C (80% RH).

3.2.3.2 Scanning Electron Microscopy (SEM) analysis of coated thin film

After treatment, film surface morphology was observed under a scanning electron microscope (FEI Quanta 250 ESEM, Netherlands). The chitosan film samples were cut and observed with a voltage set at 10 kV.

3.2.3.3 Weight loss

Weight loss of fruit was determined as previously described in 3.2.1.7.

3.2.3.4 Fruit firmness

Fruit firmness was measured at 3 points in each fruit using a penetrometer (Hardness tester FHM-1, Takemura, Japan) and calculated as previously described in 3.2.1.10.

3.2.3.5 Total soluble solids (TSS) content

TSS content was analyzed using a hand refractometer (N-1E, Japan) and calculated as previously described in 3.2.1.8.

3.2.3.6 Peel color changes

Peel color was determined at 3 regions in each fruit and reported as previously described in 3.2.1.9.

3.2.3.7 Respiration rate and ethylene production

Respiration rate and ethylene production determinations were performed following the method described by Jongsri et al. (2016) as previously described in 3.2.1.6.

3.2.3.8 Malondialdehyde (MDA) content

The method for determining the MDA content in bananas was extracted as described by Dhindsa and Matowe (1981) with some modifications as previously described in 3.2.1.15.

3.2.3.9 Electrolyte leakage (EC)

EC of banana peel was measured according to the method described by Anthony et al. (2017). The banana peel was cut into the size of 1 cm x 1 cm and soaked in 18 mL distilled water in a test tube. After incubation at room temperature for 2 h, the EC value was measured and recorded as E_0. The sample was then boiled for 20 min and cooled immediately before EC measurement as E_1. The EC value was evaluated using Eq:

$$EC (\%) = (E_0/E_1) \times 100$$

3.2.3.10 %DPPH inhibition

%DPPH inhibition was evaluated by following the method of Choi et al. (2006) as previously described in 3.2.1.14.

3.2.3.11 Histological analysis

Histological analysis of banana pulp was observed using transmission electron microscopy (TEM), and the banana tissue was prepared following the method described by Umroong (2018). Banana pulp was fixed in 2.5% glutaraldehyde (in 0.1M sodium phosphate buffer, pH 7.2) at 4 °C for 12 h and rinsed with 0.1M sodium phosphate buffer (pH 7.2) three times. The samples were then fixed with 2% osmium tetroxide in distilled water at room temperature for 2 h. After rinsing with distilled water three times (10 min per time), the samples were dehydrated by gradient acetone concentrations (30%, 50%, 70%, 90%, and 100%) for 15 min each. The samples were infiltrated with acetone: Spurr's resin (2:1, 1:1, 1:2 respectively) for 4 h at room temperature and infiltrated again with pure Spurr's resin overnight. The samples were then placed in capsules contained embedding medium and heated at 80 °C for 7 h. The specimens were cut as ultrathin sections (100 nm) and examined using a TEM (model HT7700, Hitachi, Japan).

3.2.3.12 Statistical analysis

Data of each parameter in each experiment were 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.

3.2.4 Determination of the chitosan coating containing the various concentration of

CTS-MMT nanocomposites as the free-standing film

3.2.4.1 Preparation of free-standing film

Chitosan with a molecular weight of 540 kDa and degree acetylation of 90% (1%

w/v) was dissolved in 0.5% (v/v) acetic acid and shaking at 150 rpm overnight. After that,

0.1% tween 80$^®$was added following CTS/MMT powder in various concentration (0%, 1%,

and 2% w/v) with stirring for 1 h. After bubbles were removed, material solution (100 mL)

was cast using a film block (21 x 29 x 0.5 cm). After drying at room temperature, the

obtained film was neutralized with 1mol/L NaOH solution for 20 min and rinsed with

distilled water before drying at room temperature.

3.2.4.2 Water contact angle (CA)

CA of the film was measured using a contact angle tester (Kruss/ DSA 10 MK2).

The film with a 4 x 4 cm size was placed on the plate tester, a drop of 5 µL distilled water

was placed on the film surface. The CA value was analyzed automatically using the

software.

3.2.4.3 Water absorption and water solubility

The fully dried film was cut into 5 x 5 cm size, and its initial weight was recorded

(W_0); then, it was immersed in 20 mL of distilled water for 24 h. For water absorption

measurement, the soaking film was then absorbed the excess water by blotting with 2

pieces of filter paper for 5 seconds, and its final weight was recorded (W_1). The water

absorption (%) was calculated using the formula as following:

$$\text{Water absorption (\%)} \quad = \quad [(W_1-W_0)/W_0] \times 100$$

For water solubility measurement, the soaking film was dried at 100 °C for 24 h.

The dried film was then left in a desiccator for 30 min and weighed (W_2). The water

solubility (%) was calculated following the formula as followed:

$$\text{Water solubility (\%)} \quad = \quad [(W_0-W_2)/W_0] \times 100$$

3.2.4.4 Water vapor transmission rate (WVTR)

WVTR values of the film were determined according to the method based on ASTM

E 96/E6M-16 (ASTM D 3985, 2002) using a water vapor permeation tester (Labthink model

W3/031) as previously described in 3.2.1.2.

3.2.4.5 Oxygen gas transmission rate (OTR)

OTR values of the film were measured according to the method based on ASTM

D 3985-17 using an oxygen permeation tester (Illinois model 8001L) as previously

described in 3.2.1.3.

3.2.4.6 Statistical analysis

The experiments were analyzed using SPSS software. The means were compared

using Duncan's multiple range tests at a significance level of 0.05.

3.2.5 Determination of the efficiency of chitosan coating containing chitosan-montmorillonite (CTS-MMT) nanocomposites on development of anthracnose disease in banana

3.2.5.1 Isolation of fungi

The fungal *Colletotrichum musae* was freshly isolated from infected banana fruit. Pieces of anthracnose lesion tissue were surface-disinfected with 1% sodium hypochlorite for 3 min, then rinsed with sterilized distilled water 3 times. The samples were placed on potato dextrose agar (PDA) and 100 µg/mL of streptomycin. After incubation for 7 days, growing mycelial were trans cultured on a new PDA medium. The isolation was then identified based on their morphological and molecular technique by DNA sequencing. The pure culture was maintained on a PDA medium.

3.2.5.2 Inoculation on banana fruit

For the inoculation, banana fruit was washed under running tap water and allowed to air dry at room temperature. Fruit was surface sterilized by immersion in 70% ethanol for 10 seconds. After drying at room temperature, banana fruit was wounded by inflicting one 1-mm-deep wound in the three regions of each fruit with a sterile needle. Each wound was inoculated with *C. musae* conidial suspension at 10^6 conidia/mL (10 µL/each wound). After inoculation, the banana was incubated overnight before dipping in the solution treatments.

3.2.5.3 Preparation of coating solution and treatment

The appropriate coating solution from 3.2.4.1 was selected for the experiment (2% CTS-MMT treatment). A chitosan (CTS) solution (1% w/v) was prepared by dissolved in 0.5% (v/v) acetic acid and shaker at 150 rpm overnight for complete dissolving. Then, tween[®] 80 was added at 0.1% (v/v) for improving wettability. The preparation of 2% CTS-MMT, after 1% CTS solution was prepared overnight, CTS-MMT nanocomposites powder was added at a concentration of 2% (w/v) and stirred for 1 h. Then, air bubbles were removed by standing at room temperature for 2 h.

Coating treatment, the banana fruit was dipped for 1 min in each treatment as described below:

Treatment 1: inoculation with distilled water (D.W.)

Treatment 2: inoculation with *C. musae* + dipping in distilled water

Treatment 3: inoculation with *C. musae* + dipping in 0.5% (v/v) acetic

acid solution

Treatment 4: inoculation with *C. musae* + dipping in CTS solution

Treatment 5: inoculation with *C. musae* + dipping in 2% CTS-MMT

solution

After treatment and air dry at room temperature, fruit were stored at 25 ± 2 °C (80% RH) for 9 days. The experiment was performed with a completely randomized design (CRD) with 4 replications, and each replication consisted of at least 2 samples. The samples were analyzed every 3 days.

3.2.5.4 Disease incidence

Disease incidence of banana means the percentage of banana infection measured by counting the number of disease-infected fruit in each time point. The percentage of disease incidence was calculated by using the following formula (Sivakumar et al., 2002).

Disease incidence (%) = [(Number of banana infected)/(Total number of banana)] × 100

3.2.5.5 Disease severity

Disease severity was measured followed the method described by Vilaplana et al. (2018). The diameter of the lesion area was measured, and disease severity was expressed as mm of the lesion area.

3.2.5.6 Hydrogen peroxide (H_2O_2) content

The H_2O_2 content was determined following the method described by Junglee et al. (2014) as the method previously described in 3.2.1.10.

3.2.5.7 %DPPH inhibition

DPPH inhibition analysis followed the method of Choi et al. (2006) as previously described in 3.2.1.11.

3.2.5.8 Total phenolic content (TPC)

Banana pulp was ground into a powder, and then 1 g of the powder was homogenized in cooled 70% ethanol. The samples were centrifuged at 4,750 × g, 4 °C for 15 min, and the supernatants were collected for further analysis. For analysis of the total phenolic contents, the samples were measured using a modified Folin-Ciiocalteu method (Musa et al., 2011). The reaction mixtures consist of 250 μL of the extracted samples and 250 μL of diluted Folin-Ciocalteu reagent. The mixtures were left for 5 min before 500 μL of 7.5% sodium carbonate was added. They were then incubated in the dark condition at room temperature for 90 min. After centrifuge at 5,000 × g, 25 °C for 10 min, absorbance was measured at 765 nm using a microplate reader (SpectraMax® M3, Molecular Devices, LLC., USA). A 70% ethanol addition instead of the extraction sample served as a blank. The total phenolic content was calculated using gallic acid (GA) as the standard. The result was expressed as mg GA/g FW.

3.2.5.9 Ascorbic acid (AsA) content

The ascorbic acid content in banana pulp was determined using the dinitrophenylhydrazine (DNPH) method of Shin et al. (2007). For extraction, 0.3 g of banana powder was homogenized with 1.2 mL of 5% Metaphosphoric acid (in 2M acetic acid). The homogenate was centrifuged at 17,600 × g, 4 °C for 15 min, and the supernatant was collected for further analysis. For analysis, 600 µL of the extracted sample was mixed with 10 µL of 2% 2,6-Dichlorophenolindophenol (DCIP) and incubated at room temperature in a dark condition for 60 min. After incubation, 200 µL of 2% thiourea (in 5% metaphosphoric acid) and 100 µL of 2% dinitrophenylhydrazine (DNPH) (in 4.5M sulfuric acid) were added to the mixtures and then incubated at 60 °C for 2 h. The reaction was stopped by placing the tubes in an ice bath for 5 min, then slowly adding 300 µL of cooled 85% sulfuric acid. Absorbance was measured at 540 nm using a microplate reader (SpectraMax® M3, Molecular Devices, LLC., USA). Adding 5% metaphosphoric acid instead of the extracted sample was used as blank. The total AsA content was calculated using a standard curve of ascorbic acid. The result was expressed as mg/g FW.

3.2.5.10 Glutathione (GSH) content

The glutathione content was measured according to the method described by Chotikakham et al. (2020) with slight modifications. Banana pulp powder (0.3 g) was homogenized with 800 µL of 5% of 5-sulfosalicylic acid at 4 °C, and the homogenate was then centrifuged at 11,000 × g, 4 °C for 20 min. The supernatant was collected for measuring GSH content. The reaction mixture containing 10 µL of the extracted samples, 54.5mM sodium phosphate buffer (pH 7.0), and 0.14% of 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB). The reaction mixtures were incubated at room temperature for 5 min. The absorbance at 412 nm was measured, and the GSH was calculated using a standard curve of GSH.

3.2.5.11 Antioxidant enzyme activities

The ascorbate peroxidase (APX) enzyme was extracted following the method of Hodges and Forney (2000) with slight modifications. Banana pulp was ground to a powder, and 0.3 g of them was homogenized with 1.3 mL of extraction buffer containing 0.1M potassium phosphate buffer (pH 7.0), 1mM ascorbic acid (AsA), 1mM ethylenediaminetetraacetic acid (EDTA), and 2.5% of polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 10,000 × g, 4 °C for 15 min, and the supernatant was collected for APX activity analysis.

The activity of APX enzyme was determined using 200 µL of reaction mixture consisting of 30 µL of the extracted samples, 0.1M potassium phosphate buffer (pH 7.0), 1mM AsA, 0.5mM EDTA, and 1mM H_2O_2. The decrease in AsA was observed at the absorbance of 290 nm. The reaction mixture without AsA was set as blank (molar extinction coefficient = 2.8 $mM^{-1}cm^{-1}$). The APX activity was expressed as U/mg protein and calculated as follow:

$$APX \text{ activity (U/mg Protein)} = \frac{(\Delta A290/min)(\text{Reaction mixure volume})}{(2.8)(\text{Extraction volume})(\text{mg Protein/Extraction volume})}$$

3.2.5.12 Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GR), and Dehydroascorbate reductase (DHAR) enzyme activities

The extraction was performed following the method described by Beers and Sizer (1952). Banana pulp was ground to a powder under liquid nitrogen, and 0.3 g of the powder was homogenized with 1.3 mL of extraction buffer containing 1 M potassium phosphate buffer (pH 7.0), 2.5% PVPP, 4mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride (PMSF). After centrifuge at 10000 × g, 4 °C for 15 min, the supernatant was collected for further analysis.

Superoxide dismutase (SOD) activity was determined following the method described by Deawati et al. (2017) with some modifications based on nitroblue tetrazolium (NBT) reduction. The SOD activity was determined in 200 µL containing 50 µL of the extracted samples, 83 µL of 0.1M potassium phosphate buffer (pH 7.0), 26.4 µL of 5 mM EDTA, 2 µL of 1mM riboflavin, 26 µL of 0.1M methionine, 12.6 µL of 1mM NBT in microplate wells. The microplate was irradiated under fluorescent light at 4,000 lx, room temperature for 15 min. The colorimetric activity was assayed by a microplate reader with an absorbance of 560 nm. The reaction without riboflavin was set as blank, and the reaction without extraction sample was set as control. The inhibition (%) of sample and SOD activity was calculated by using the formula follow:

$$Inhibition\ (\%) = \frac{(Abs.\ control - Abs.\ sample)}{Abs.\ control} \times 100$$

$$SOD\ activity\ (U/mg\ Protein) = \frac{1/50}{mg\ Protein} \times Inhibition\ (\%)$$

Catalase (CAT) activity was assayed according to Nakano and Asada (1987). The activity was analyzed in a 200 µL reaction mixture consist of 40 µL of the extracted samples, 0.1M potassium phosphate buffer (pH 7.0), and 1mM H_2O_2. The decrease in H_2O_2 was observed at the absorbance of 240 nm. The reaction without H_2O_2 was used as blank (molar extinction coefficient = 43.6 mM^{-1}cm^{-1}). The CAT activity was expressed as U/mg protein and calculated as follows:

$$CAT\ activity\ (U/mg\ Protein) = \frac{(\Delta\ A240/min)(Reaction\ mixure\ volume)}{(43.6)(Extraction\ volume)(mg\ Protein/Extraction\ volume)}$$

Glutathione reductase (GR) activity was assayed using the method described by Chotikakham et al. (2020). The activity was determined using 200 µL containing 30 µL of the extracted samples, 0.1M potassium phosphate buffer (pH 7.0), 1mM EDTA, 10mM glutathione disulfide (GSSG), and 5mM β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (β-NADPH) (dissolved in 1% $NaHCO_3$). The NADPH decrease was observed at 340 nm (molar extinction coefficient = 6.2 $mM^{-1}cm^{-1}$), and the reaction without NADPH was used as blank. The GR activity was expressed as U/mg protein and calculated as follows:

$$\text{GR activity (U/mg Protein)} = \frac{(\Delta\,A340/min)(\text{Reaction mixure volume})}{(6.2)(\text{Extraction volume})(\text{mg Protein/Extraction volume})}$$

Dehydroascorbate reductase (DHAR) activity was determined following the method described by Chotikakham et al. (2020). The activity was assayed using 200 µL of a mixture containing 20 µL of the extracted samples, 0.1M potassium phosphate buffer (pH 7.0), 1M EDTA, 2mM Dehydroascorbic acid (DHA), 25 mM reduced glutathione (GSH). The increase in the formation of ascorbate was observed at the absorbance of 265 nm (molar extinction coefficient = 14.7 $mM^{-1}cm^{-1}$), and the reaction without GSH was set as blank. The DHAR activity was expressed as U/mg protein and calculated as follows:

$$\text{DHAR activity (U/mg Protein)} = \frac{(\Delta\,A265/min)(\text{Reaction mixure volume})}{(14.7)(\text{Extraction volume})(\text{mg Protein/Extraction volume})}$$

3.2.5.13 Statistical analysis

The experiments were performed using a completely randomized design (CRD),

and data were analyzed using SPSS software. The means were compared using Duncan's

multiple range tests at a significance level of 0.05.

CHAPTER IV

RESULTS

4.1 Effects of different molecular weights of chitosan coating on physiological responses of banana fruit during storage at 25 ± 2 °C

4.1.1 Chitosan properties

Chitosan used for this study was varied in molecular weights; high molecular weight chitosan (HMW-CTS: 540 kDa), medium molecular weight chitosan (MMW-CTS: 265 kDa), and low molecular weight chitosan (LMW-CTS: 65 kDa). Chitosan characteristics which were shown in Table 1 reveal that percentages of moisture contents of chitosan powder were between 6-7% (LMW-CTS = 6.635 ± 0.01%, MMW-CTS = 7.907 ± 0.02%, HMW-CTS = 7.798 ± 0.25%) with degree of deacetylation (%DD) at 90%. All chitosan powder could dissolve in 0.5% acetic acid more than 97%. HMW-CTS solution had the highest viscosity, followed by MMW-CTS and LMW-CTS solution (85.6, 26.65, and 7.49 cps, respectively).

Table 1. Characteristics of chitosan used in this study.

Chitosan	Molecular weight (kDa)	Deacetylation (%)	Moisture (%)	Solubility (%) (In 0.5% (v/v) acetic acid)	Viscosity (cps)
LMW-CTS	65	90	$6.635 \pm .01^b$	$97.25 \pm .15^a$	$7.497 \pm .06^c$
MMW-CTS	265	90	$7.907 \pm .02^a$	$97.55 \pm .05^a$	$26.65 \pm .13^b$
HMW-CTS	540	90	$7.798 \pm .25^a$	$97.8 \pm .10^a$	85.6 ± 1.01^a

*Values denoted by the same letter in the same column were not significantly different according to Duncan's Multiple

Range Test ($P < 0.05$).

4.1.2 Characterization of derived film

Chitosan coating solution was cast as a free-standing film for their characterization, and the results were shown in Table 2. LMW-CTS obtained the highest WVTR and OTR values (3384 ± 82.6 g/m^2 day and 1532.7 ± 32.0 mL/m^2 day, respectively) compared to those of MMW-CTS (WVTR = 2560 ± 14.4 g/m^2 day; OTR = 2.5 ± 0.1mL/m^2 day, respectively) and HMW-CTS film (2666 ± 0.0 g/m^2 day; 3.9 ± 0.3 mL/m^2 day, respectively). From these results, it was promising that MMW-CTS and HMW-CTS solutions could form a thin film layer which caused the reduction of water and oxygen gas transmission rates.

Table 2. Characteristics of derived films.

Chitosan	WVTR (g/m^2 day)	OTR (mL/m^2 day)
LMW-CTS	3384.0 ± 82.6[a]	1532.7 ± 32.0[a]
MMW-CTS	2560.0 ± 14.4[b]	2.5 ± 0.1[b]
HMW-CTS	2666.0 ± 0.0[b]	3.9 ± 0.3[b]

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

4.1.3 Anatomical characteristics of the chitosan-coated surface of banana peel

On the initial day of treatment, the coating layer on banana peel was visualized under a SEM microscope, and the cross-section images were shown in (Figure 9). Banana peels of fruit coated with D.W. and acetic acid were seen without film coating, while all chitosan-coated fruit expressed thin-film covered on the surface of banana peel (Figures 9A-B). A barely noticeable and thin film was observed on LMW-CTS coated fruit while MMW-CTS coated fruit showed a thicker film and HMW-CTS coated fruit had the thickest film, as indicated by the white arrow (Figures 9C-E).

The surface images of banana peel were presented in Figure 10, and the arrows indicate the stomatal aperture (Figures 10A-B). The stomata obviously appeared on the banana peels of D.W. and acetic acid-treated fruit, whereas the surface of chitosan-coated banana peel expressed a homogenous coating. LMW-CTS coated peel had a thin film layer on the surface, and the film was not sealed thoroughly on its peel (Figure 10C). The film exhibited on LMW-CTS coated fruit did not differ from D.W. and acetic acid-coated fruit (Figures 10A-B). On the other hand, MMW-CTS and HM-CTS coating completely covered the stomata of the fruit (Figures 10D-E).
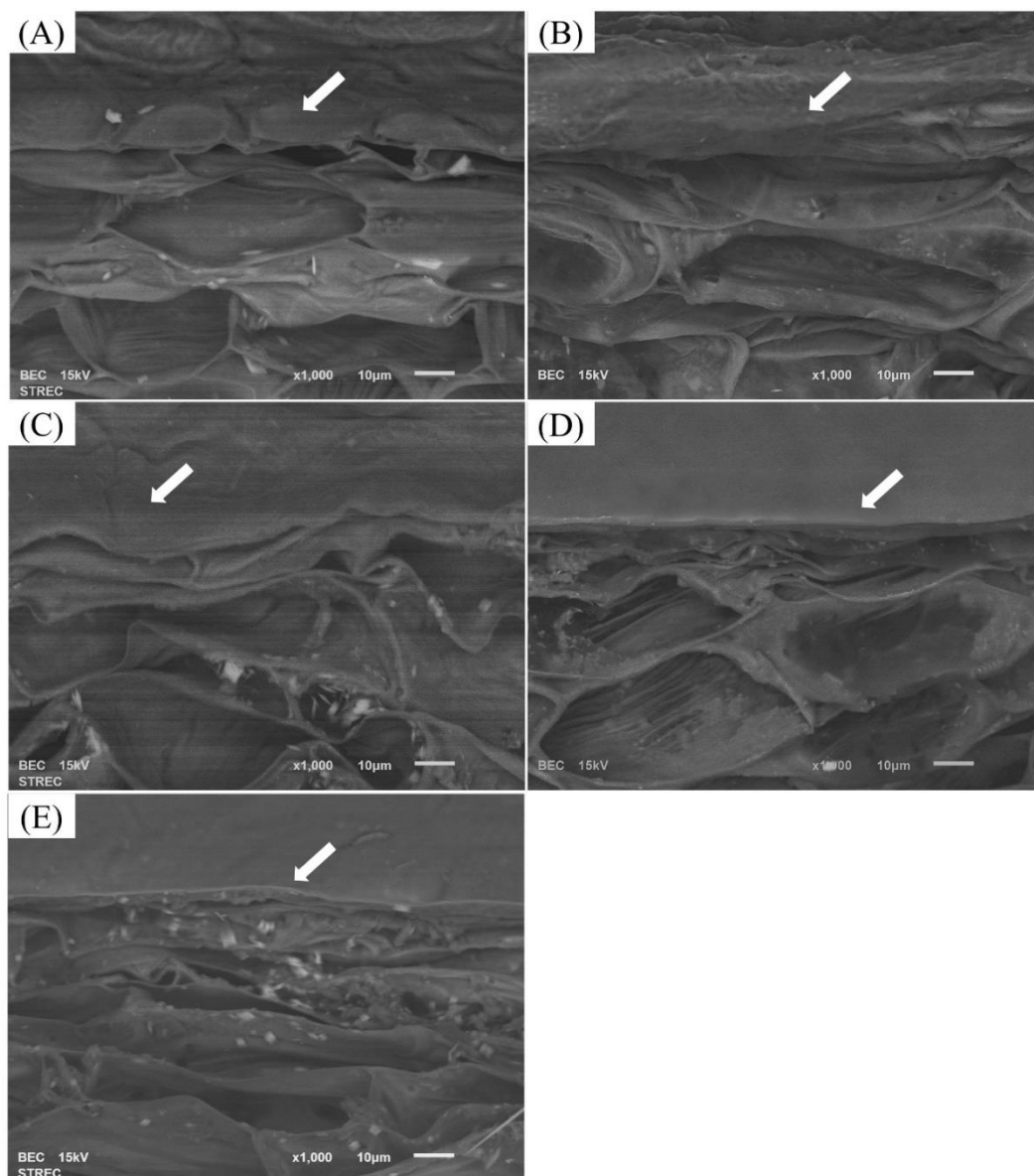
Figure 9. SEM cross-section images of banana peel treated with various molecular weights of chitosan coatings; (A) distilled water, (B) 0.5% (v/v) acetic acid, (C) LMW-CTS, (D) MMW-CTS, and (E) HMW-CTS. The white arrows indicate chitosan film on banana peel after coating on day 0.
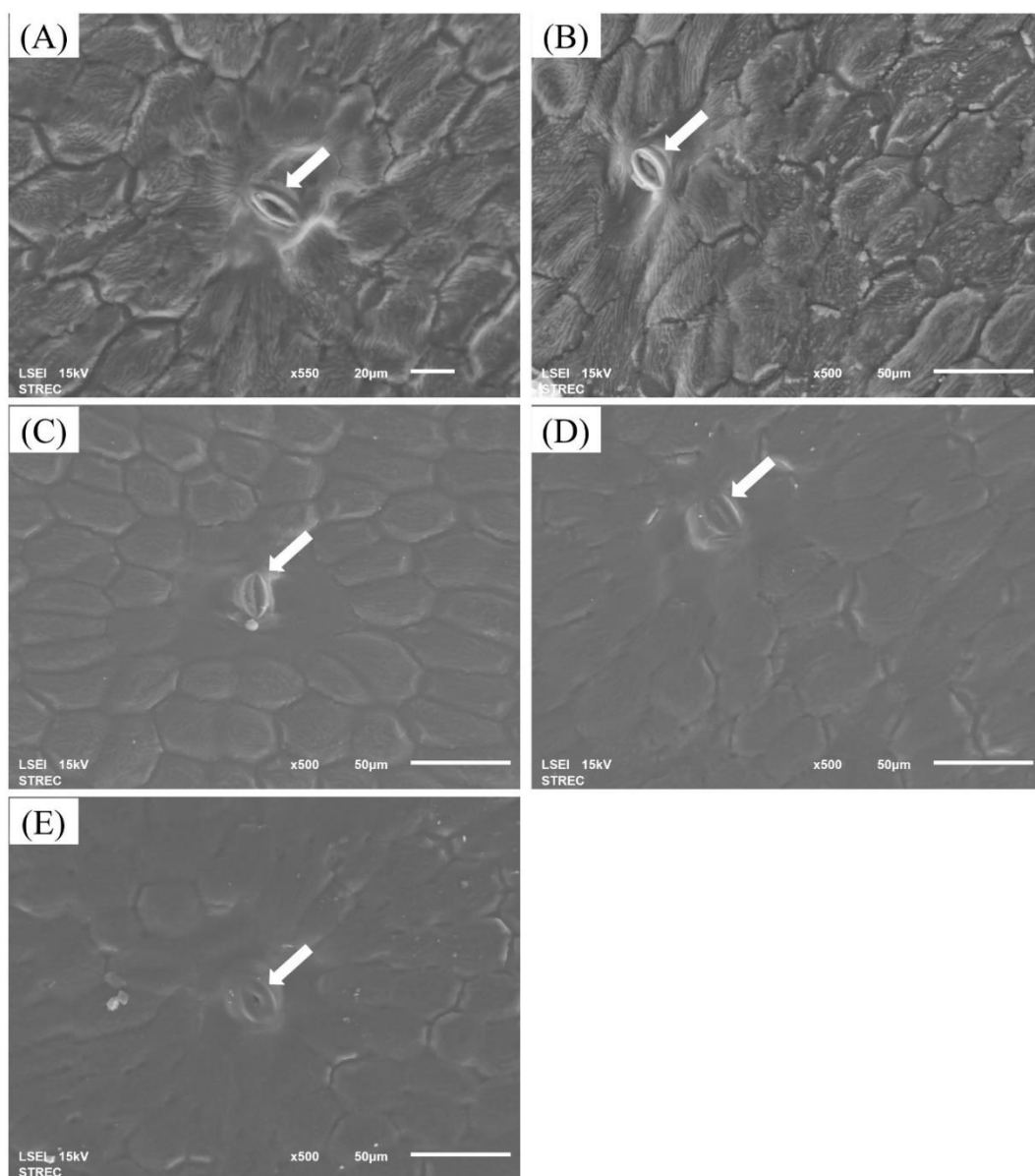
Figure 10. SEM surface images of banana peel treated with various molecular weights of chitosan coatings; (A) distilled water, (B) 0.5% (v/v) acetic acid, (C) LMW-CTS, (D) MMW-CTS, and (E) HMW-CTS. The white arrows indicate chitosan film on banana peel after coating on day 0.

4.1.4 Postharvest quality of banana during storage

    4.1.4.1 Ethylene production and respiration rate

    The respiration rate and ethylene production of banana fruit during storage at 25 ± 2 °C were observed for 15 days and shown in Figure 11. As a result, the respiration rate of fruit coated with D.W., 0.5% acetic acid, and LMW-CTS treatments reached the climacteric peak on day 9 of storage. In contrast, fruit coated with HMW-CTS and MMW-CTS had a delayed climacteric peak on day 12 of the storage period. HMW-CTS coating group had the lowest respiration rate on days 9 and 15 of storage (Figure 11A). The ethylene production showed a similar trend with respiration rate, fruit coated with MMW-CTS and HMW-CTS treatments had a retarding of ethylene production, which reached the peak on day 15 of storage. While fruit coated with D.W., 0.5% acetic acid and LMW-CTS treatments reached the ethylene peak on day 12 of storage (Figure 11B).

(A)

(B)
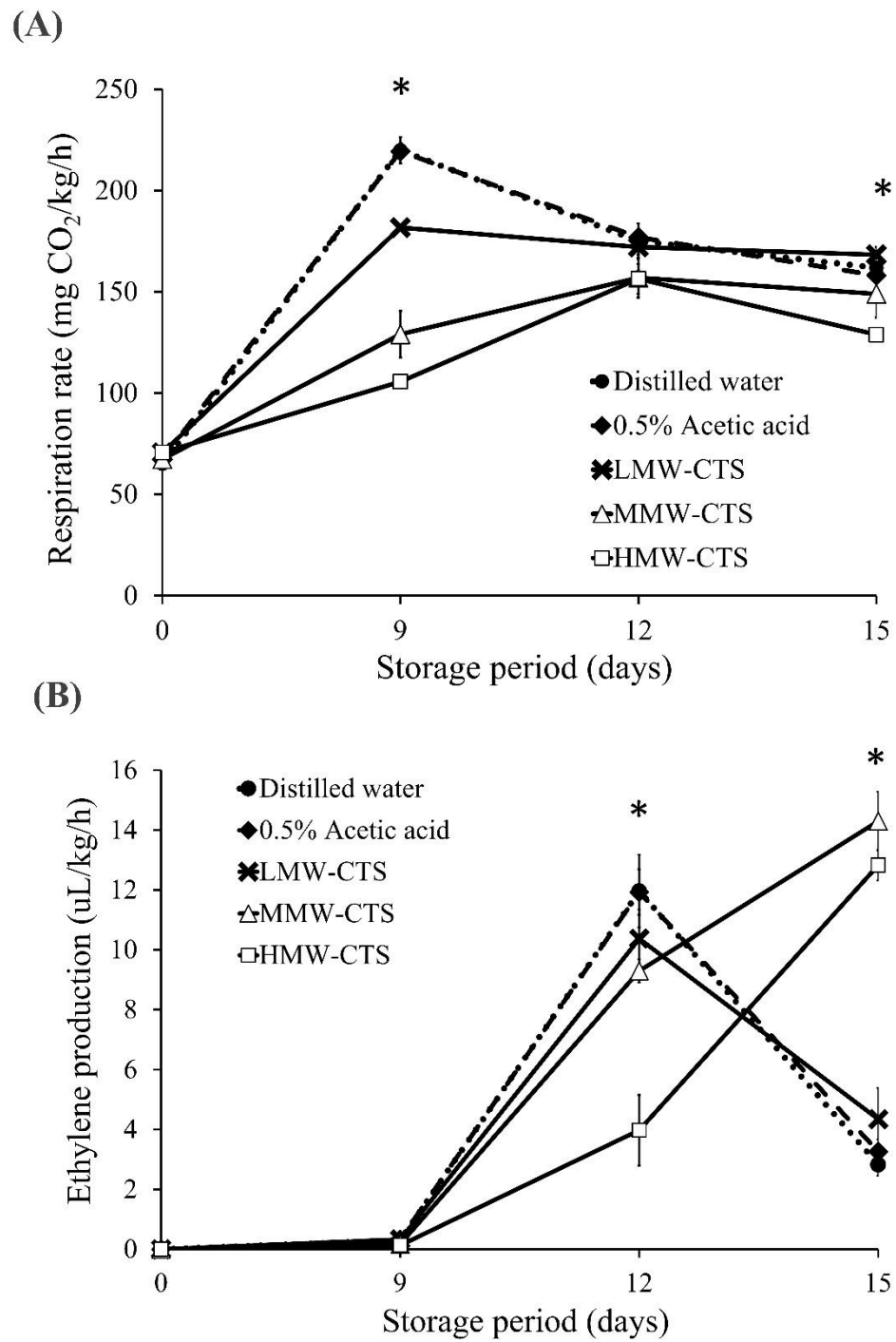
Figure 11. Effects of chitosan coating on respiration rate (A) and ethylene production

(B) of banana fruit during storage at 25 ± 2 °C for 15 days.

4.1.4.2 Weight loss and total soluble solids (TSS)

Weight loss of banana fruit was exhibited in Figure 12A. During ripening, fruit weight loss increased gradually throughout the storage period. There was no significant difference among treatments during the first 9 days of storage. HM-CTS coated fruit presented the lowest percentage of weight loss compared to other treatments.

Total soluble solids (TSS) was presented in Figure 12B. TSS content continued to increase with ripening development from approximately 6 to 23 °Brix for banana coated with D.W., acetic acid, LMW-CTS, and MMW-CTS solutions and from 6 to 21 °Brix for fruit coated with HMW-CTS solution. Fruit coated with D.W. and acetic acid treatments exhibited a sharp increase of TSS and peak at day 9 of storage. While LMW-CTS, MMW-CTS, and HMW-CTS treatments showed a delay of TSS peak on day 12 of storage. The results suggested that fruit coated with HMW-CTS showed the lowest TSS value throughout the storage period.

(A)

(B)
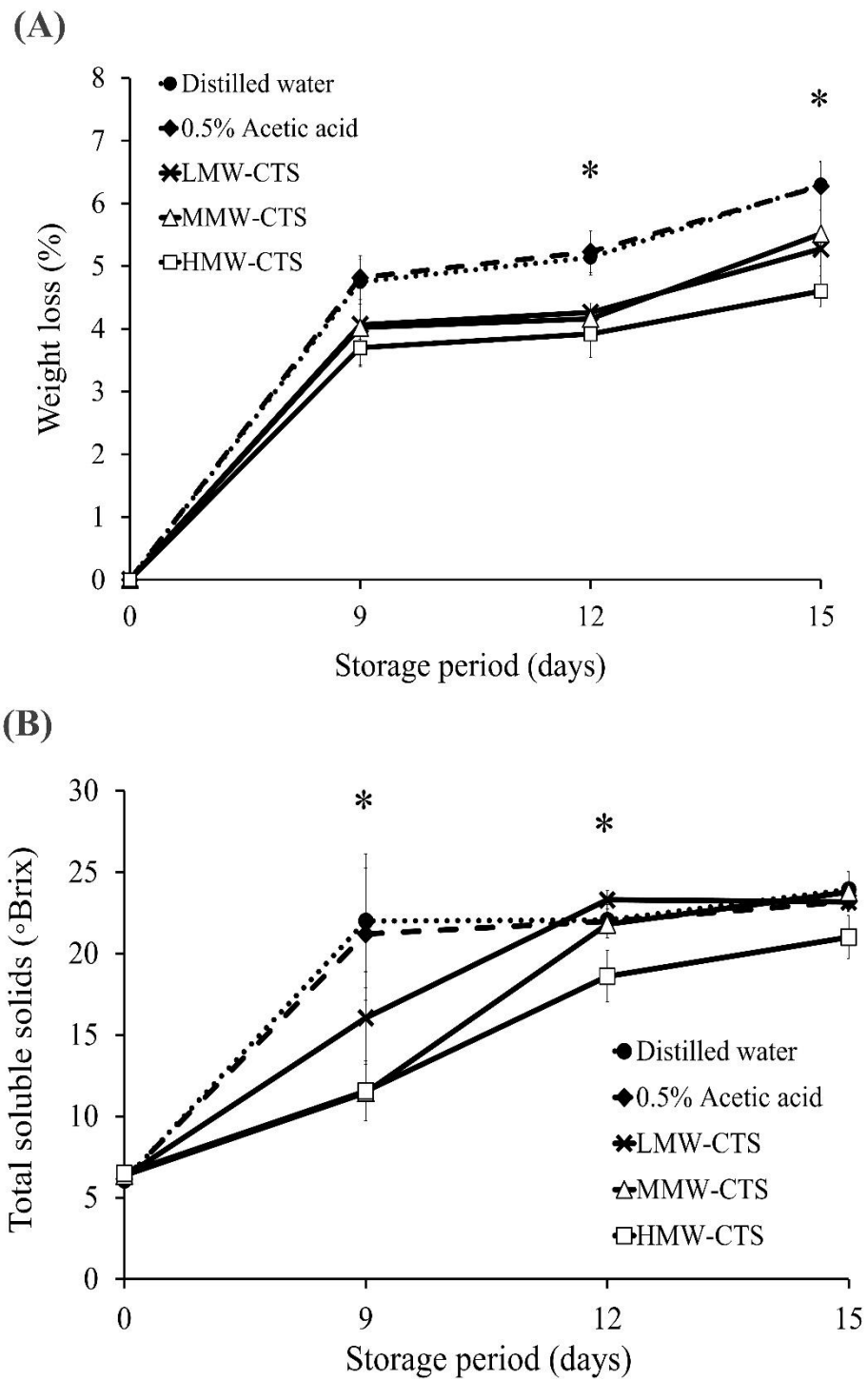
Figure 12. Effects of chitosan coating on weight loss (A) and TSS (B) of banana fruit

during storage at 25 ± 2 °C for 15 days.

4.1.4.3 Peel color change

The peel color of the banana was expressed in Figure 13 and Figure 14 as L and hue value. Fruit coated with D.W. and acetic acid treatments started changing from green to yellow on day 9 of storage. While MMW-CTS and HMW-CTS coating could delay peel color change which showed yellow peel on day 12 of storage (Figure 13). L value showed a correlation with color changes which L value increased sharply and peaked on day 9 of storage for fruit coated with D.W., acetic acid, and LMW-CTS treatments. MMW-CTS and HMW-CTS coated fruit showed a peak of L value on day 12 of storage. The Hue angle values for MMW-CTS and HM-CTS coated fruit were also higher, corresponding to the peel color (Figures 14A-B).
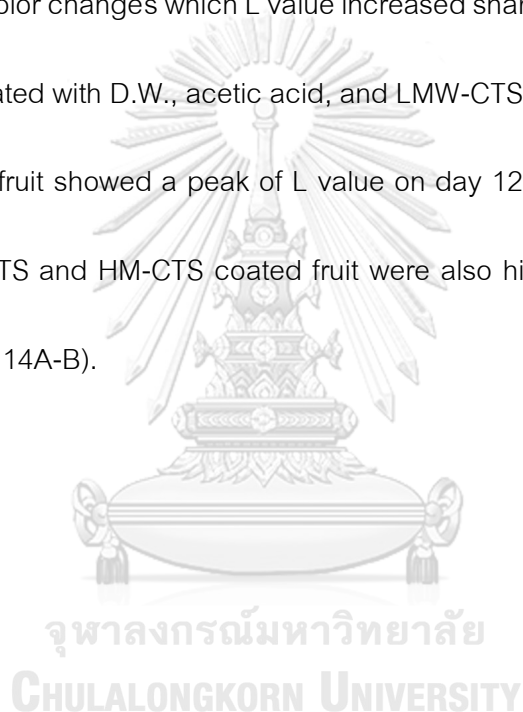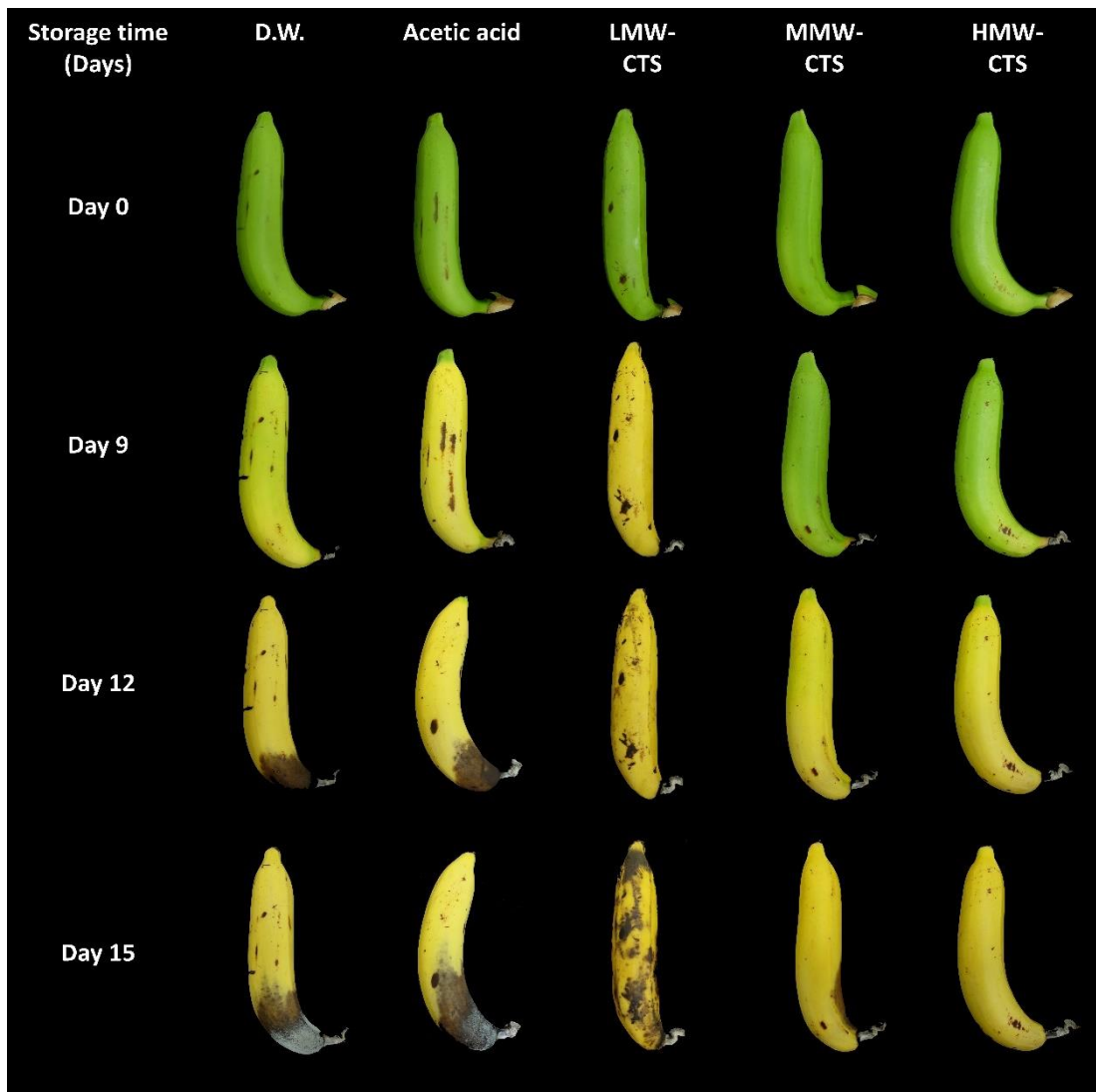
Figure 13. Peel color changes of banana in control and chitosan-coated fruit during

storage at 25 ± 2 °C for 15 days.

(A)

(B)
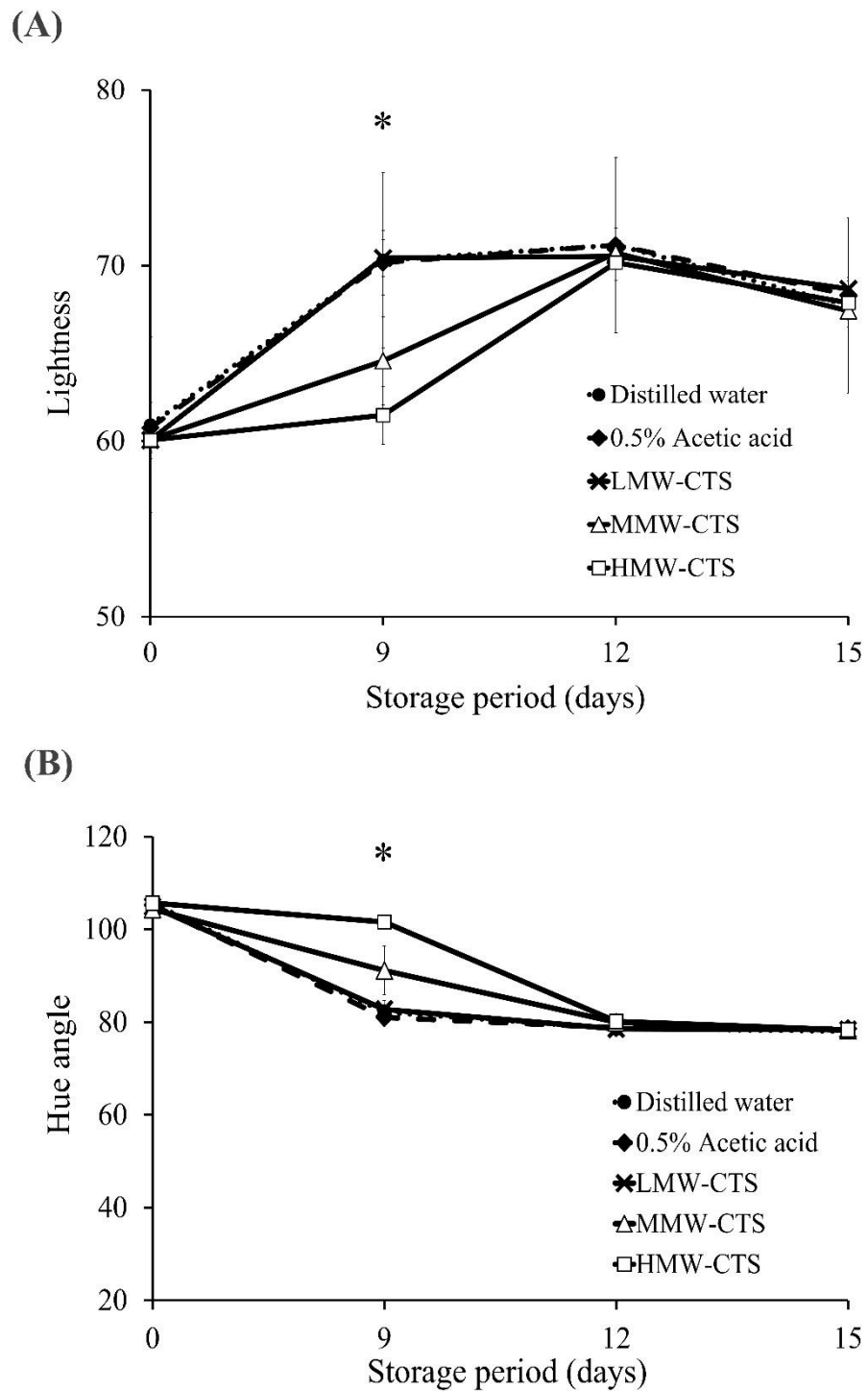
Figure 14. Effects of chitosan coating on L value (A) and hue angle (B) of banana fruit

during storage at 25 ± 2 °C for 15 days.

4.1.5 Banana fruit softening during storage

    4.1.5.1 Fruit firmness

    During ripening, banana fruit firmness declined continuously until the end of the

storage period. All chitosan treatments could retard firmness loss on day 12 of storage.

MMW-CTS and HMW-CTS treatments could maintain their firmness throughout the storage

period showing significantly lower firmness losses on days 12 and 15 of storage (Figure

15).
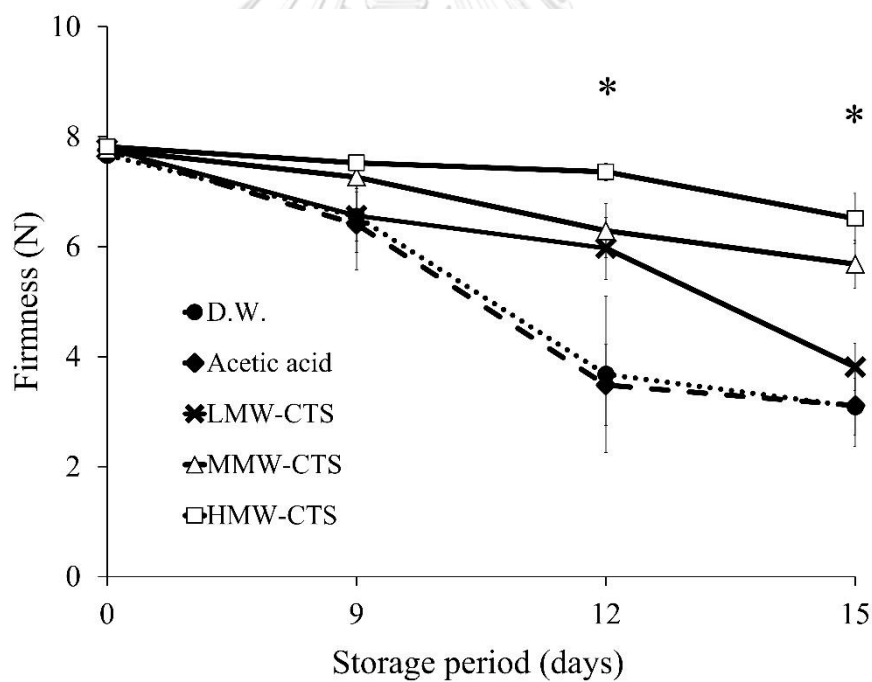
Figure 15. Changes in fruit firmness of banana coated with HMW-CTS, MMW-CTS, and

LMW-CTS during the storage of fruit at 25 ± 2 °C for 15 days.

4.1.5.2 Water-soluble pectin

Water-soluble pectin contents of banana peel were shown in Figure 16. The results revealed that water-soluble pectin content increased during the storage period. Fruit coated with MMW-CTS and HMW-CTS treatments showed significant increases in water-soluble pectin compared to fruit coated with D.W., acetic acid, and LMW-CTS treatments on days 9, 12, and 15 of storage.
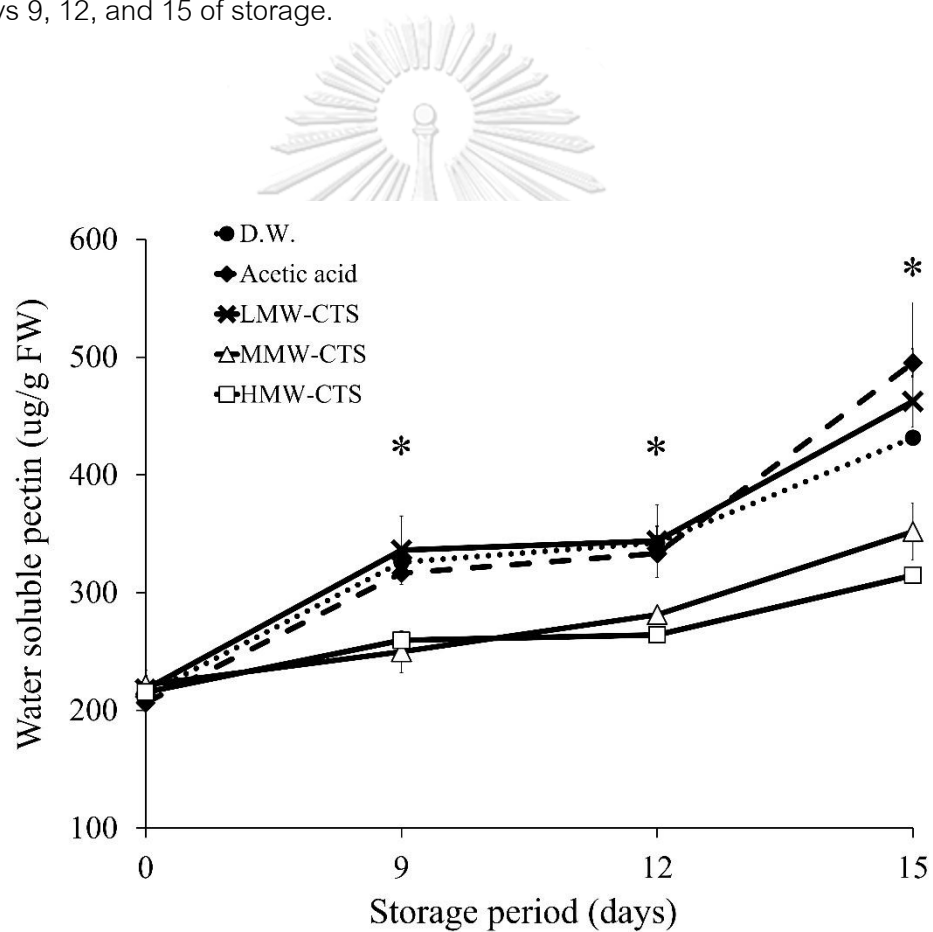
Figure   16. Changes in water-soluble pectin of banana pulp coated with HMW-CTS, MMW-CTS, and LMW-CTS during the storage of fruit at 25 ± 2 °C for 15 days.

4.1.5.3 Cell wall degrading enzymes

The Polygalacturonase (PG) activity of banana peel was shown in Figure 17A. The PG activity was found to increase continuously during ripening. Fruit coated with D.W., acetic acid, and LMW-CTS treatments demonstrated higher PG activities throughout the study. While the activities of PG in MMW-CTS and HMW-CTS coated fruit increased slowly compared to other treatments and showed a significant difference from other treatments on day 12 of storage.

Pectate lyase (PL) activity of banana peel expressed the same trend as PG activity, showing an increase with ripening. MMW-CTS and HMW-CTS treatments could retard PL activity significantly throughout the storage period compared to the other treatments (Figure 17B).

(A)

(B)
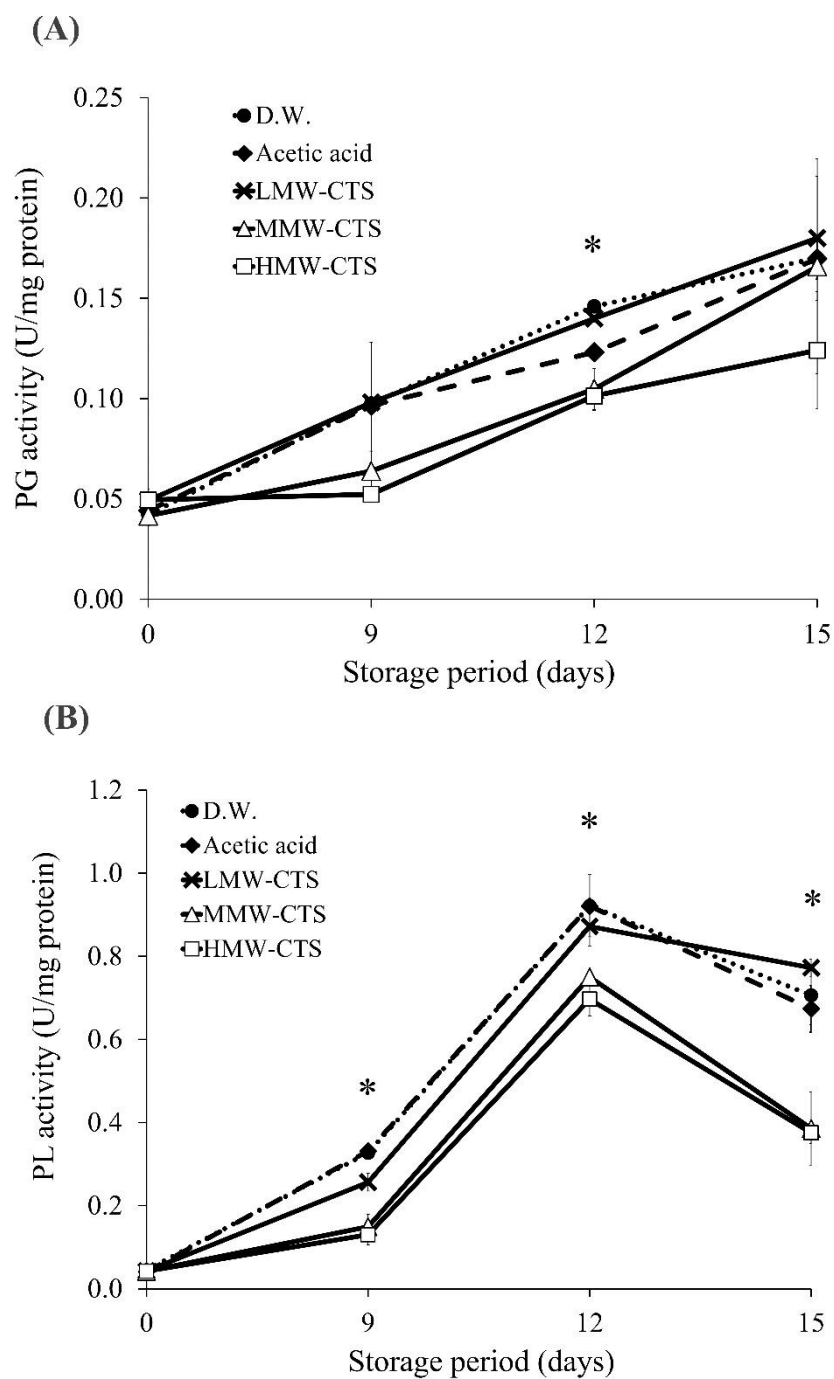
Figure 17. Changes in activities of polygalacturonase (PG) (A) and pectate lyase (PL)

(B) of banana pulp coated with HMW-CTS, MMW-CTS, and LMW-CTS during the storage

of fruit at 25 ± 2 °C for 15 days.

4.1.6 Physiological responses of banana fruit to chitosan coating during storage

4.1.6.1 H_2O_2 content and DPPH inhibition

H_2O_2 content decreased slightly on the first 9 days of storage and increased thereafter. Fruit coated with all chitosan treatments found no significant difference compared to fruit coated with D.W. and acetic acid throughout the storage period (Figure 18A).

DPPH inhibition of banana pulp increased on the first 9 days of storage then decreased continuously until the end of the storage period. Fruit coated with D.W., acetic acid, LMW-CTS, and MMW-CTS showed no significant difference among treatments. In comparison, fruit coated with HMW-CTS treatment showed the highest value of DPPH inhibition throughout the storage period significantly on day 12 of storage (Figure 18B).

(A)

(B)
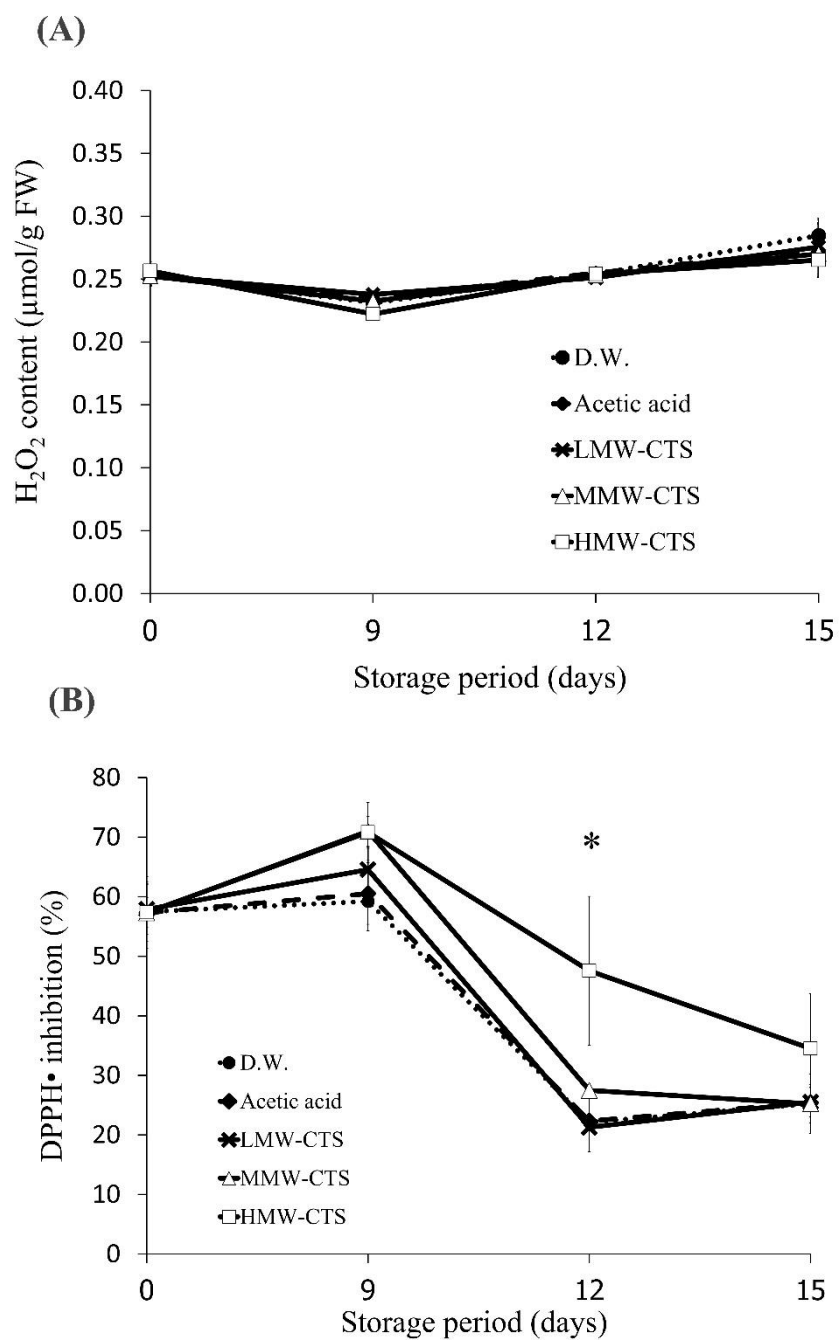
Figure 18. Changes in H_2O_2 content (A) and (%) DPPH inhibition (B) of banana peel coated with HMW-CTS, MMW-CTS, and LMW-CTS during the storage of fruit at 25 ± 2 °C for 15 days.

4.1.6.2 Malondialdehyde (MDA) content

As shown in Figure 19, MDA content in banana pulp increased continuously during storage. Banana treated with MMW-CTS treatment showed lower MDA content, while HMW-CTS coated fruit had the lowest MDA value on day 12 of storage compared to other treatments.
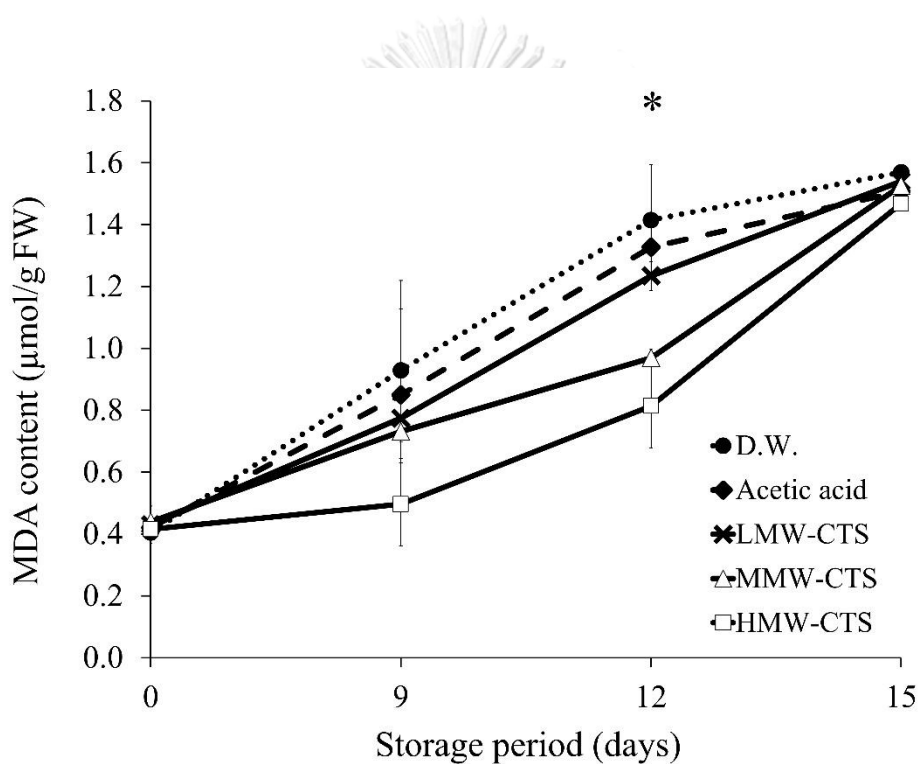
Figure 19. Changes in MDA content of banana peel coated with HMW-CTS, MMW-CTS, and LMW-CTS during the storage of fruit at 25 ± 2 °C for 15 days.

4.1.6.3 Disease severity

The effect of chitosan coating on the disease severity of banana fruit was presented in Figure 20. The symptoms of anthracnose disease started to appear and were observed on day 9. Then the severity increased sharply until the last day of storage. Fruit coated with D.W. and acetic acid treatments showed the highest disease severity value at the end of the storage period. While banana coated with MMW-CTS and HM-CTS treatments decreased disease severity markedly and showed a significant difference on day 15 of storage.
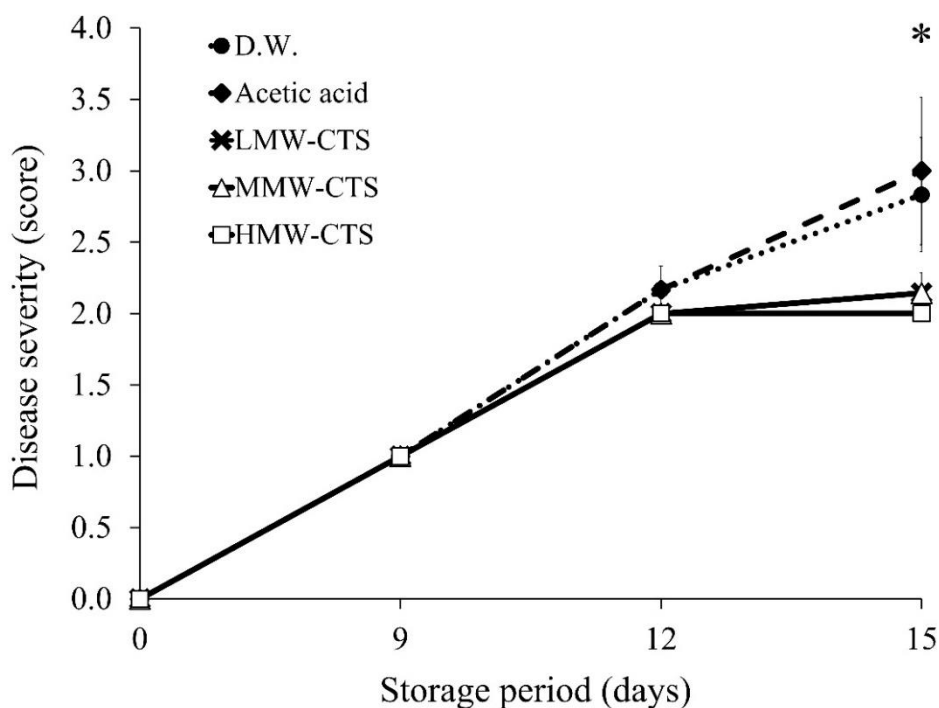
Figure 20. The disease severity of the banana coated with HMW-CTS, MMW-CTS, and LMW-CTS during the storage of fruit at 25 ± 2 °C for 15 days.

4.2 Determination of the appropriate chitosan coating containing chitosan-

montmorillonite (CTS-MMT) nanocomposites to prolong shelf life of banana during

storage at 25 ± 2 °C

4.2.1 SEM analysis of coated thin film

After coating, banana peel was observed using SEM analysis, and the SEM

images were shown in Figure 21. The stomata appeared visibly on the surface of fruit

coated with D.W. (Figure 21A). All chitosan-coated fruit showed completely sealed

stomata compared to D.W. coated fruit (Figures 21B-E). The surface of the film on CTS

coated fruit was smooth, while the CTS supplemented with CTS-MMT-coated fruit

presented a rougher surface compared to those neat CTS coated fruit. At 1% and 2%

CTS-MMT nanocomposites addition, there was no or little accumulation of nanoparticles

(Figures 21C-D). However, when CTS-MMT was increased to 4%, multiple large

agglomerations of nanoparticles could be observed by naked eyes (Figure 21E).
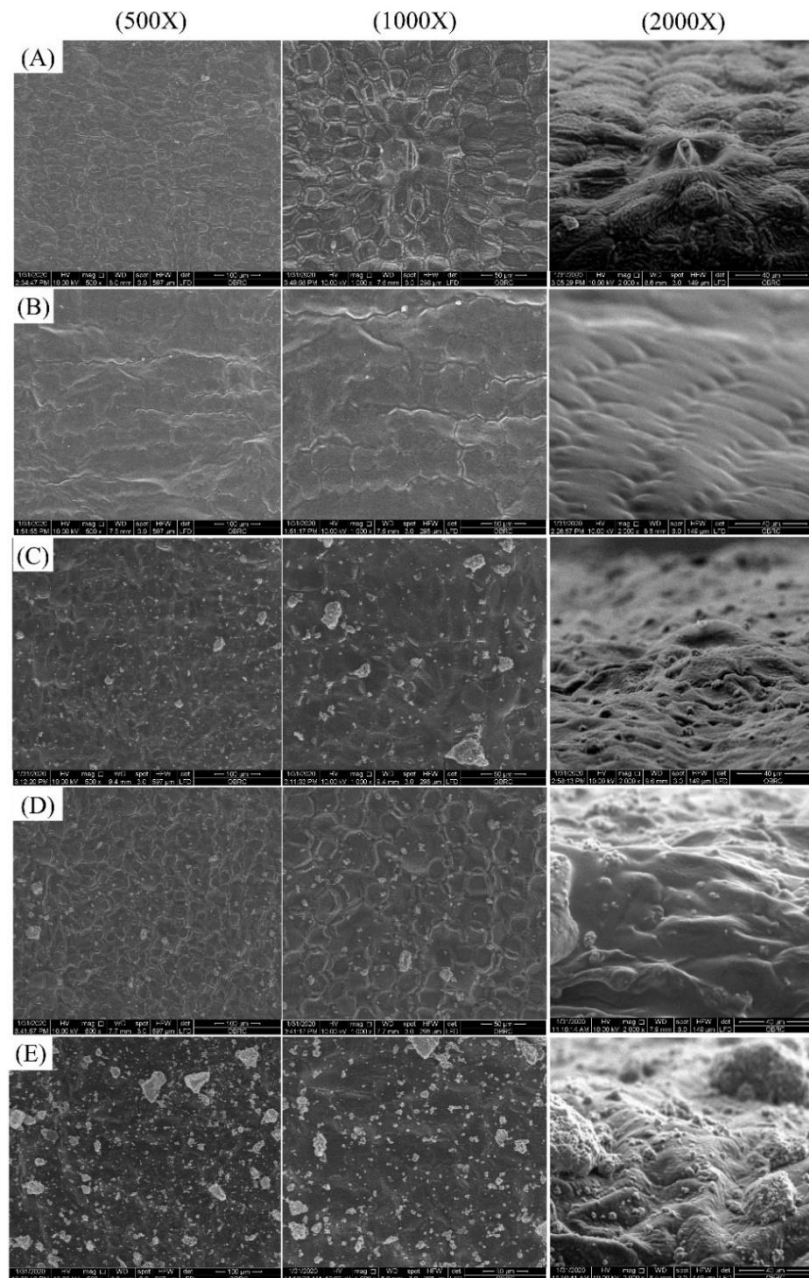
Figure 21. The surface area of banana peel after coating with various treatments: (A) D.W. coated fruit; (B) CTS coated fruit; (C) 1% CTS-MMT coated fruit; (D) 2% CTS-MMT coated fruit; (E) 4% CTS-MMT coated fruit investigated on the initial day by scanning electron microscope.

4.2.2 The effects of the coating on banana postharvest qualities

4.2.2.1 Weight loss and firmness

The results of weight loss and firmness were shown in Figures 22A-B. During ripening,

the weight loss of banana fruit increased continuously. Banana fruit coated with all

treatments showed no significant difference among groups throughout the storage

period. However, fruit treated with 2% CTS-MMT showed the highest fruit firmness after

storage for 15 days, followed by CTS and 1% CTS-MMT treatments. While D.W. coated

fruit had the lowest firmness significantly on days 12 and 15 of storage.

(A)

(B)
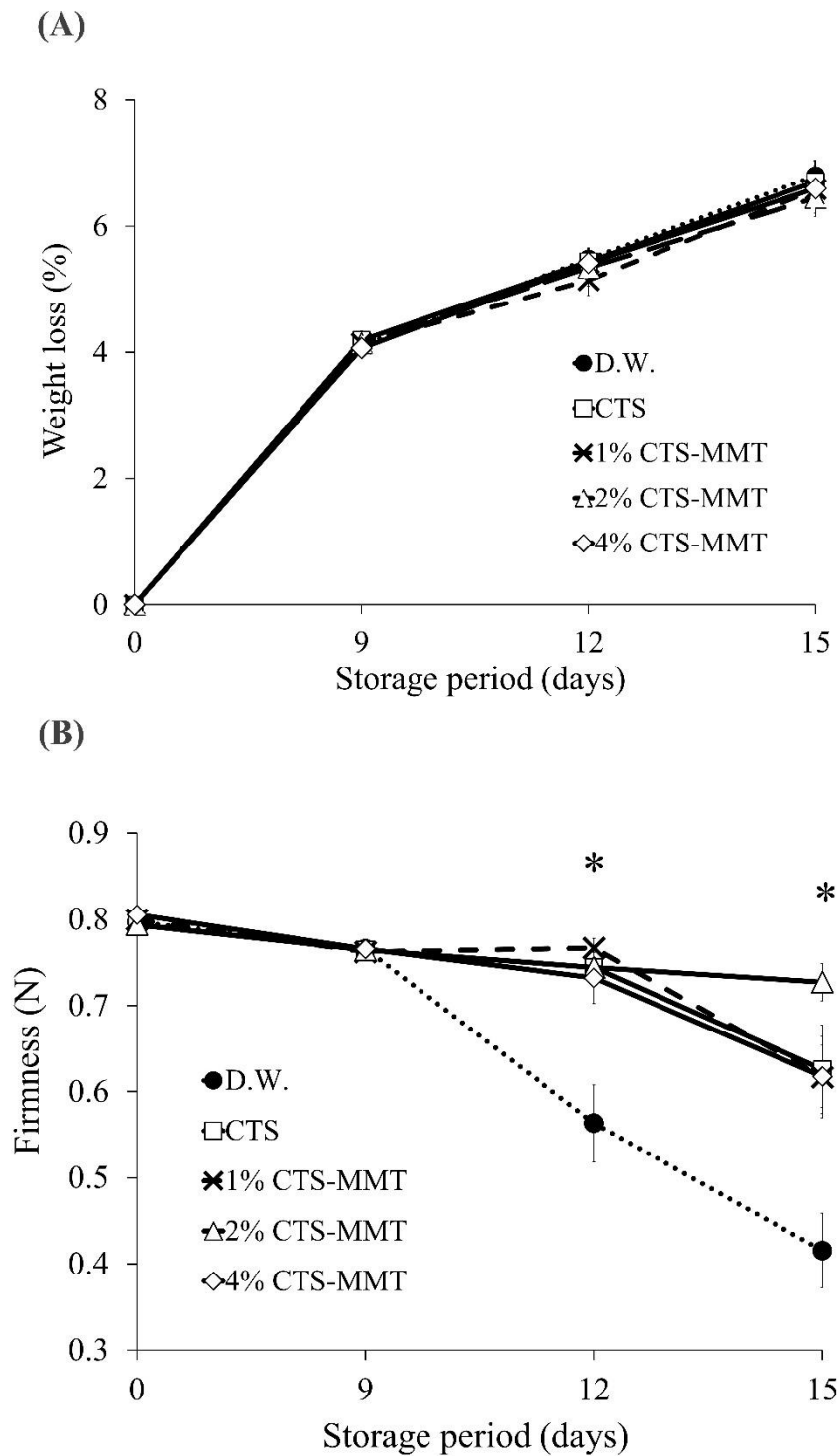
Figure 22. Weight loss (A) and firmness (B) of banana fruit coated with various treatments

and stored at 25 ± 2 °C. for 15 days. Vertical bars indicate ± SE.

4.2.2.2 TSS content

The results in Figure 23 showed that the TSS content of banana pulp increased gradually during ripening. Fruit coated with D.W. had the highest TSS content value significantly throughout the storage period due to ripening. For CTS and 1% CTS-MMT coated group, TSS content was not different among treatments. However, TSS content was lower when fruit coated with 2% CTS-MMT treatment, which showed the lowest content after storage for 15 days compared to other treatments.
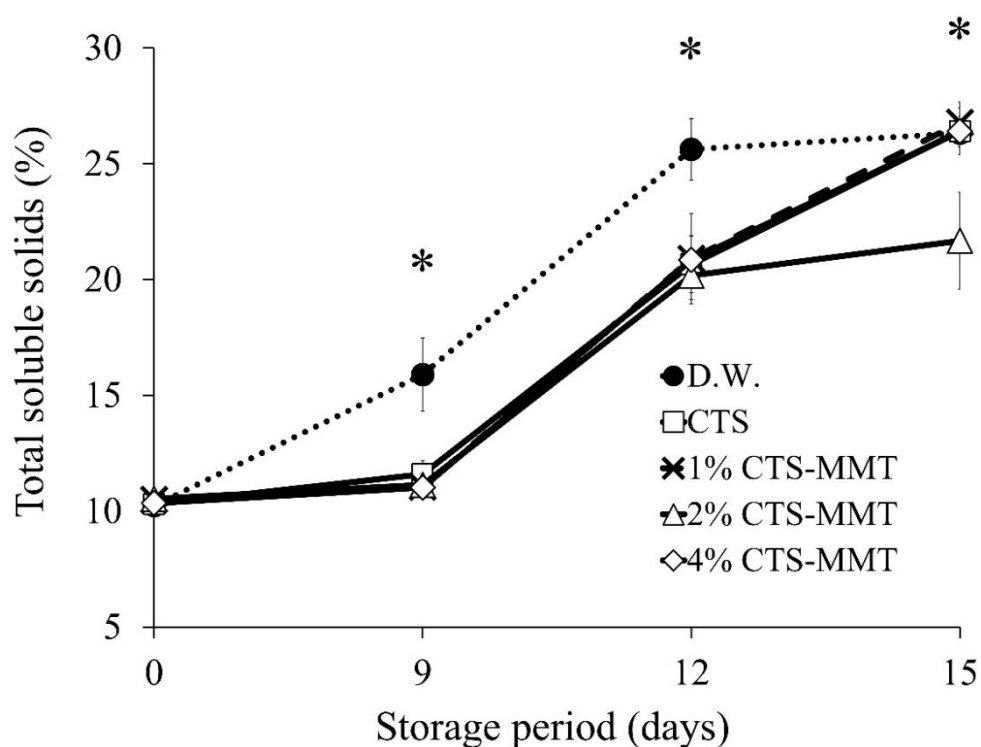
Figure 23. TSS content of banana fruit coated with various treatments and stored at 25 ± 2 °C. for 15 days. Vertical bars indicate ± SE.

4.2.2.3 Peel color

From Figure 24, fruit coated with D.W., CTS, and 1% CTS-MMT treatments started changing color from green to yellow on day 9 of storage. While fruit coated with 2% and 4% CTS-MMT treatments started changing color on day 15 of the storage period. The results corresponding with L and hue angle value, which L value of fruit coated with D.W., CTS, and 1% CTS-MMT treatments increased sharply on the first 9 days of storage and hue angle decreased continuously during storage. For fruit coated with 2% and 4% CTS-MMT treatments, the L value showed the highest value on day 15 of storage and had the lowest value throughout the storage period (Figures 25A-B). It indicated that 2% and 4% coating solutions could delay color changes of banana peel during storage.
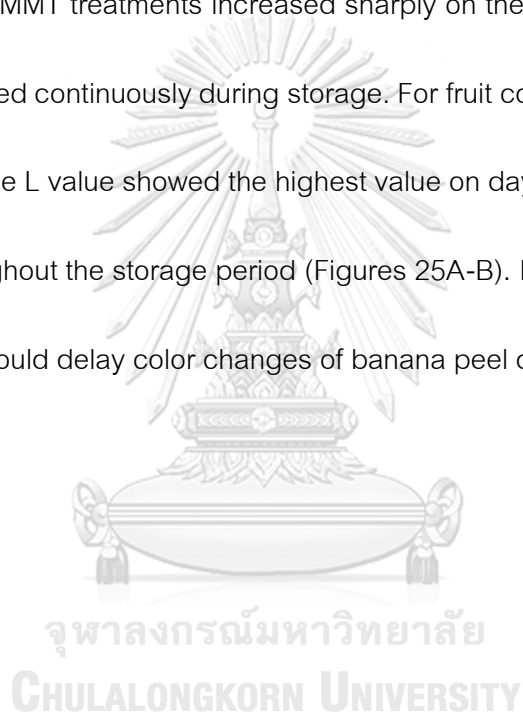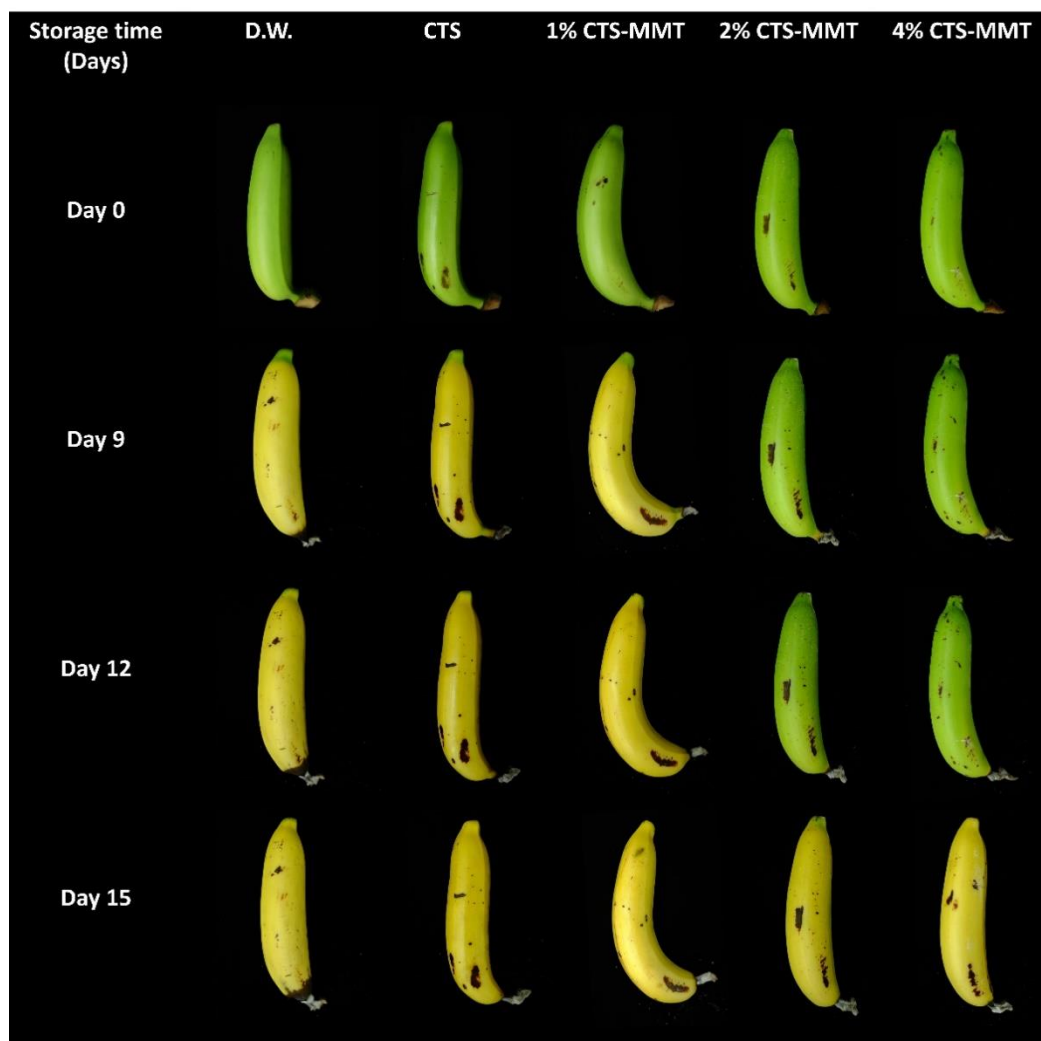
Figure 24. Peel color change of bananas-coated with various treatments and stored at
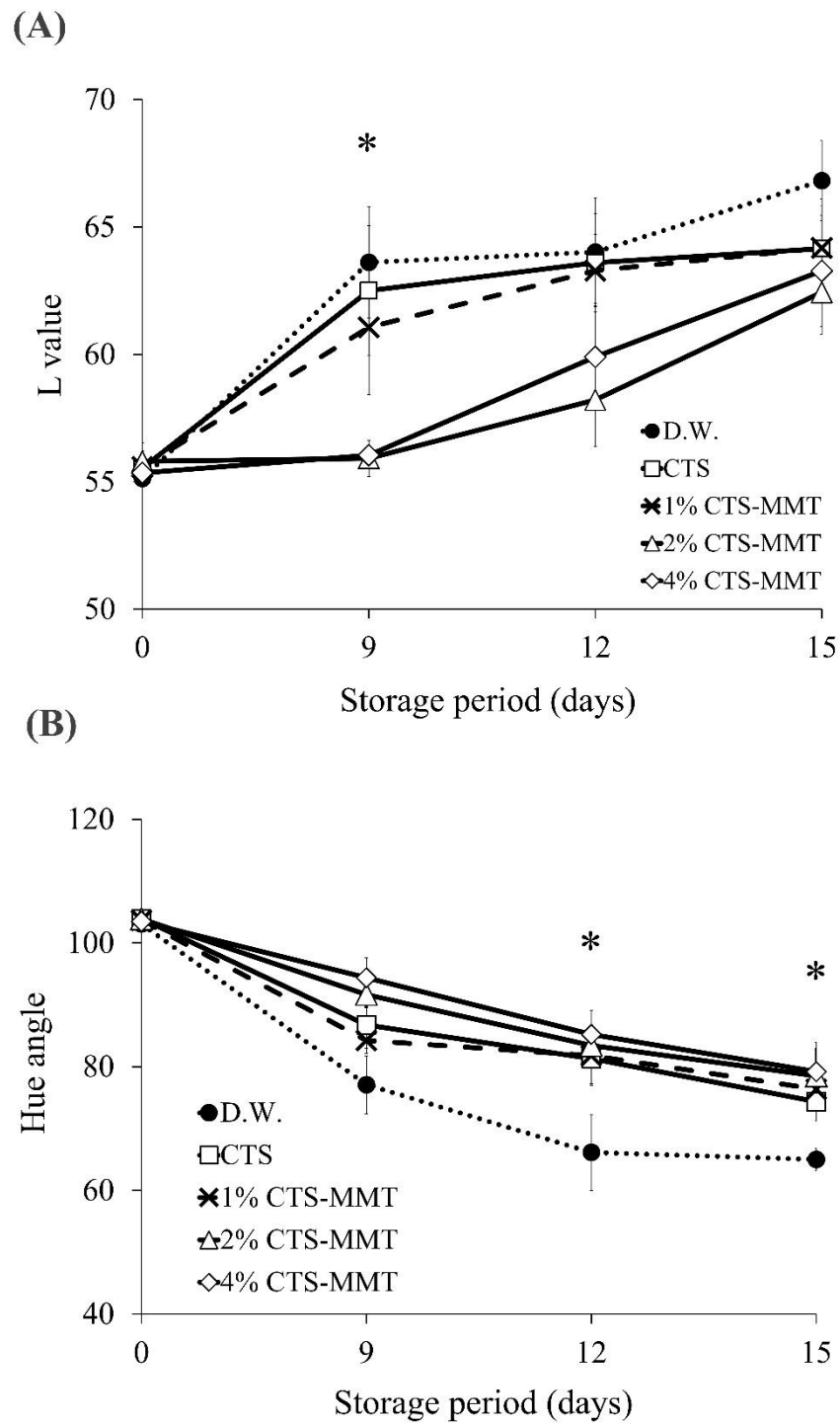
25 ± 2 °C for 15 days.

Figure 25. L value (A) and hue angle (B) of banana fruit coated with various treatments

and stored at 25 ± 2 °C. for 15 days. Vertical bars indicate ± SE.

4.2.2.4 Respiration rate and ethylene production

During storage, changes in the respiration rate and the ethylene production of banana fruit were investigated and presented in Figures 26A-B. The respiration rate of fruit coated with D.W. has reached its peak on day 9 of storage. For all chitosan coating groups except the 2% CTS-MMT group, the fruit showed respiration peak on day 12 of storage while 2% CTS-MMT coating fruit showed a climacteric peak on day 15 of storage.

The ethylene production presented a similar pattern with respiration rate which all chitosan coating groups except 2% CTS-MMT group showed ethylene production peak on day 12 of storage while 2% CTS-MMT coating fruit showed the peak on day 15 of storage. However, 2%-CTS-MMT coated fruit presented the lowest rate of ethylene production significantly on day 12 compared to other treatments. These results suggested that 2% CTS-MMT treatment could delay fruit respiration rate and reduce ethylene production of banana fruit during storage.

(A)

(B)
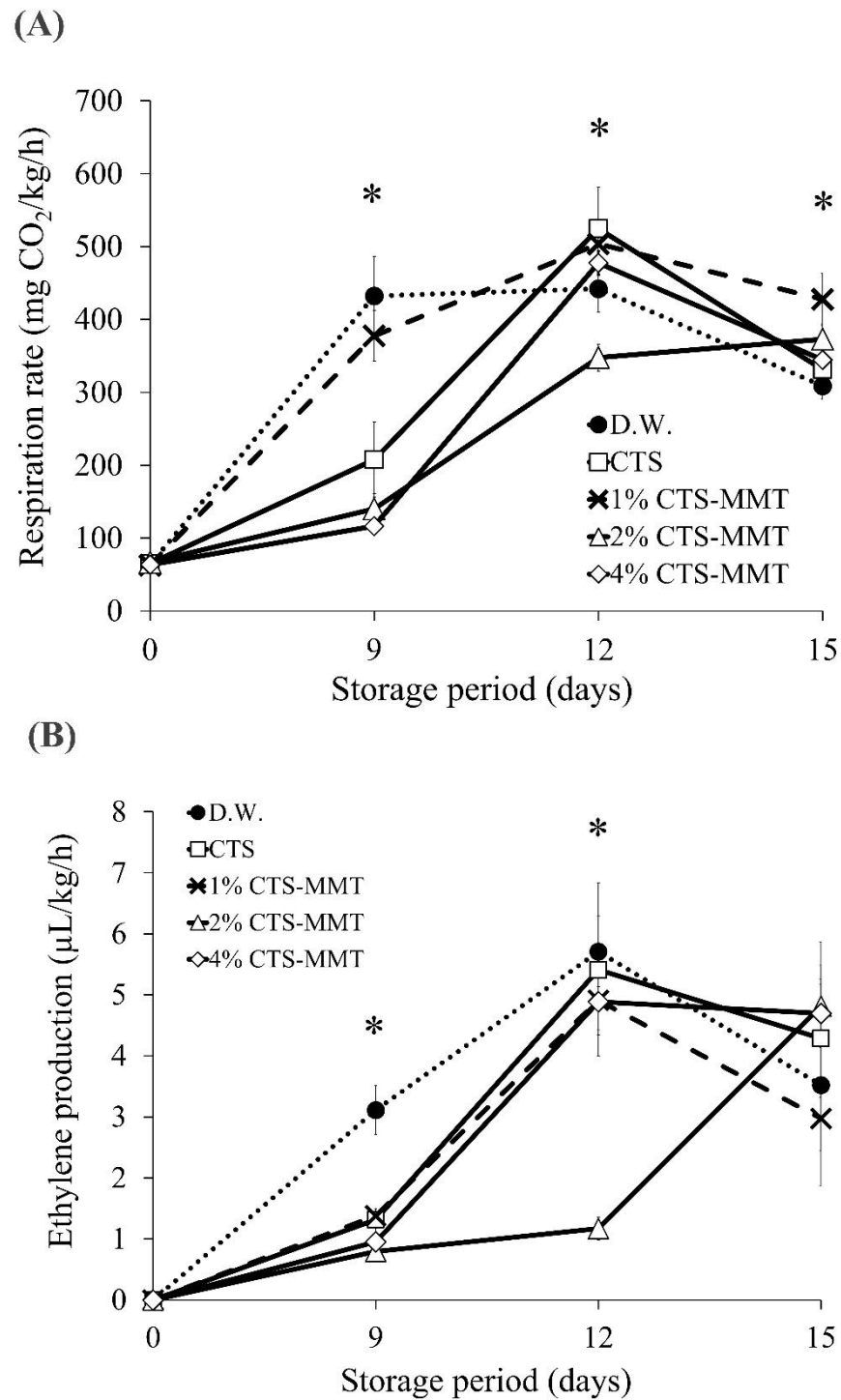
Figure 26. Respiration rate (A) and ethylene production (B) of banana fruit coated with

various treatments and stored at 25 ± 2 °C for 15 days. Vertical bars indicate ± SE.

4.2.2.5 MDA content and electrolyte leakage

MDA content and electrolyte leakage were shown in Figures 23A-B. MDA content of banana fruit increased with storage time increase. Fruit coated with D.W. showed the highest value after storage for 9 days compared to other groups. For CTS and 1% CTS-MMT coating groups, MDA content increased sharply on day 12 of storage while 2% and 4% coated fruit had significantly lowest value on days 9 and 12 of storage time.

Electrolyte leakage showed the result patterns the same as MDA content which EC value increased continuously with storage time increase. Fruit coated with 2% CTS-MMT presented the lowest EC value significantly on days 9, 12, and 15 of storage. It indicated that the supplementation coating of 2% CTS-MMT could retard the increase of MDA level and electrolyte leakage value of banana fruit during storage.

(A)

(B)
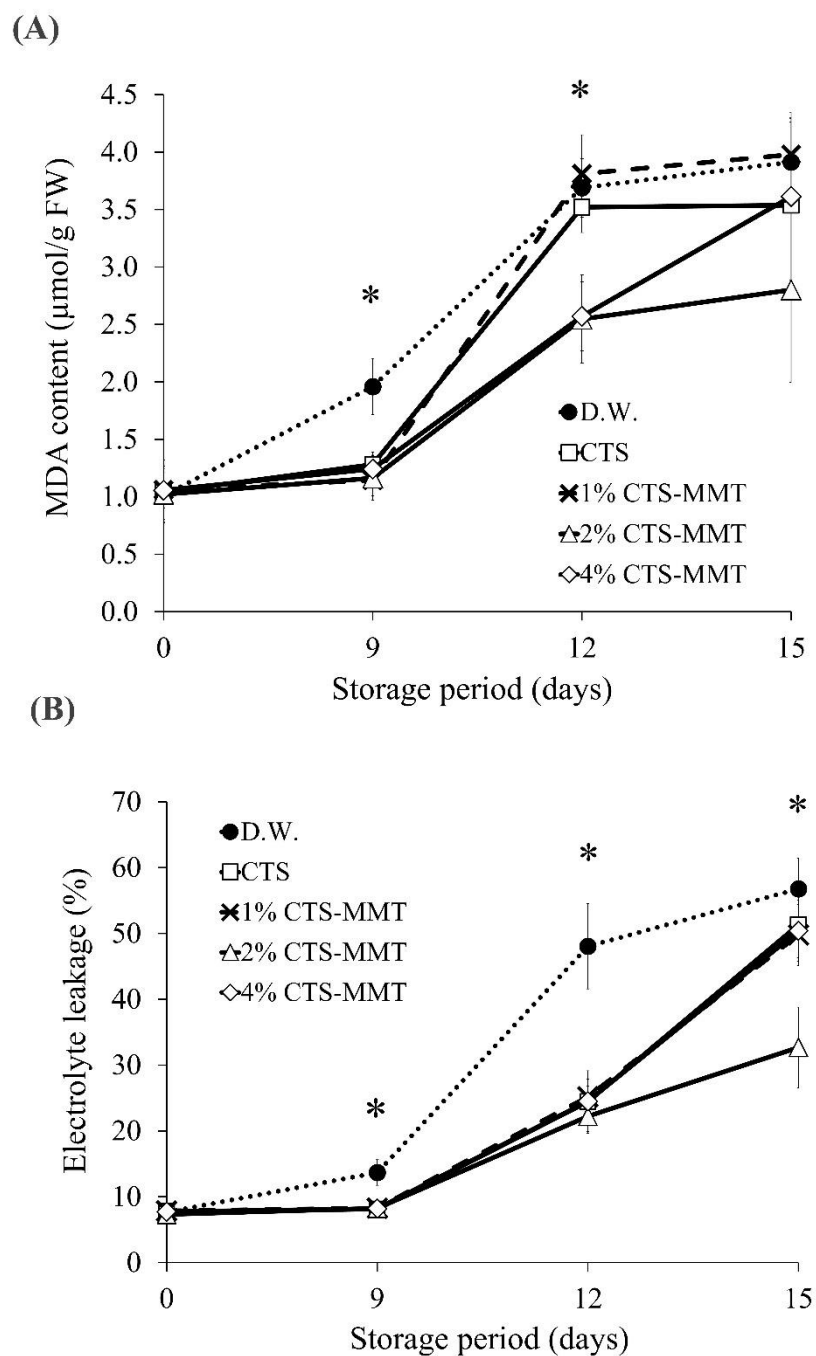
Figure 27. MDA content (A) and electrolyte leakage (B) of banana fruit coated with

various treatments and stored at 25 ± 2 °C for 15 days. Vertical bars indicate ± SE.

4.2.2.6 %DPPH inhibition assay

Figure 28 presents antioxidant capacity in terms of DPPH inhibition and FRAP assay. As a result, the level of DPPH inhibition decreased during storage for 12 days and then increased slightly on day 15 of storage. Fruit coated with D.W., CTS, and 1% CTS-MMT treatments presented a lower value than 2% and 4% CTS-MMT coated fruit. However, fruit coated with 2% CTS-MMT had the highest level of DPPH inhibition throughout the storage period significantly on day 12 of storage. As a result, it indicated that 2% CTS-MMT treatment could maintain antioxidant capacity during ripening.
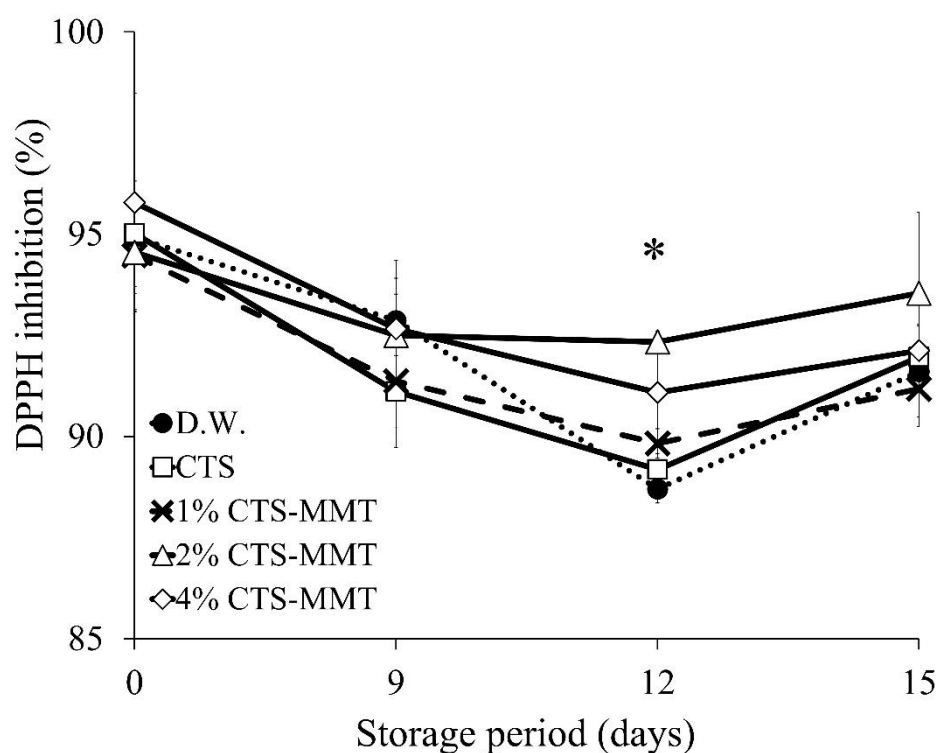
Figure 28. DPPH inhibition of banana fruit coated with various treatments and stored at 25 ± 2 °C for 15 days. Vertical bars indicate ± SE.

4.2.2.7 Histological analysis

Banana fruit coated with various treatments and stored for 15 days were observed for the changes of the microstructure of banana pulp by using a TEM microscope compared to banana fruit on the initial day. The results were displayed in Figure 29. As the results from the day 0 image (Figure 29A), the plasma membrane was intact, and its cell wall remained close to the cell wall. After storage for 15 days (Figures 29B-F), the plasma membrane separated from its cell wall, and the cell wall became thinner than day 0 fruit. While fruit coated with CTS and 1% CTS-MMT were not much different in microstructure from D.W. coated fruit. However, fruit coated with 2% and 4% CTS-MMT treatments showed that their plasma membrane remained close to its cell wall. Therefore, 2% and 4% CTS-MMT treatments could maintain the integrity of banana pulp during storage.
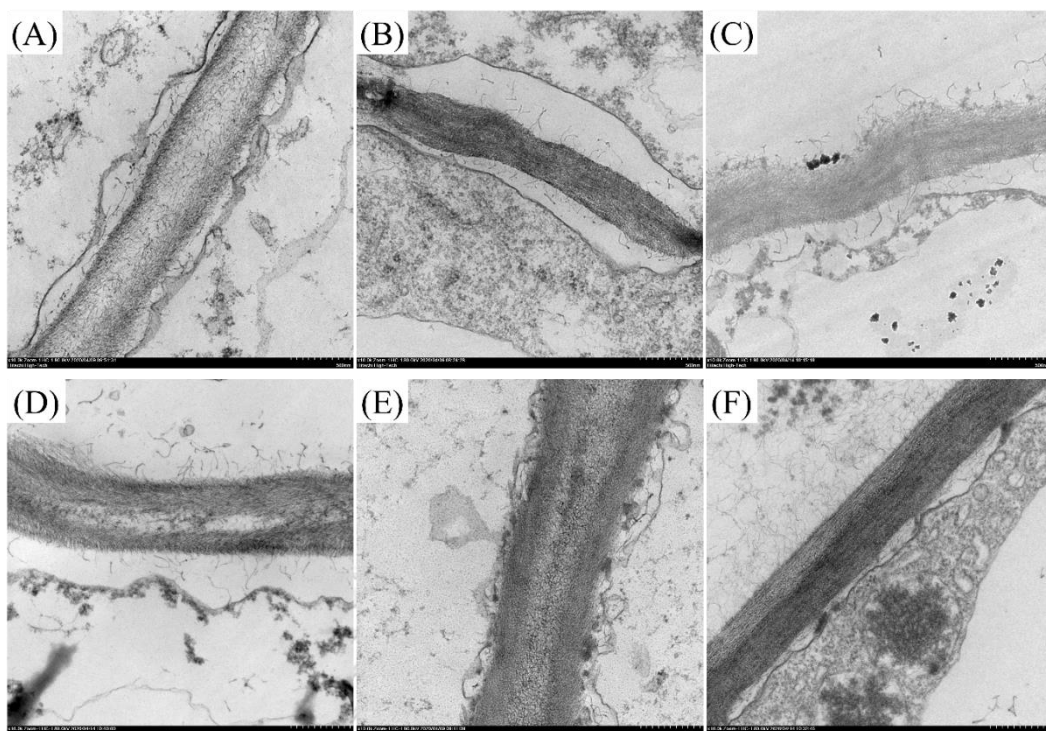
Figure 29. Transmission electron micrographs at 10,000 × magnification of banana pulp:

(A) Control fruit at harvest (day 0); (B) D.W.-coated fruit at day 15 of the storage; (C) CTS

coated fruit at day 15 of the storage; (D) 1% CTS-MMT-coated fruit at day 15 of the storage

(E) 2% CTS-MMT-coated fruit at day 15 of the storage; (F) 4% CTS-MMT-coated fruit at

day 15 of the storage.

4.2.3 Characteristics of CTS-MMT nanocomposites film

4.2.3.1 Water contact angle, water absorption, and water solubility

In Table 3, the neat CTS film showed the hydrophilic nature by expressing the water contact angle at 52.7°. For CTS supplement with CTS-MMT nanocomposites at 1% and 2% (w/v), the value increased up to 63.3° and 77.6°, respectively, which indicated more hydrophobic when compared to neat CTS film.

For water absorption analysis, neat CTS film and the film supplement with CTS-MMT at 1% were not significantly different (184 ± 7% and 193 ± 15%, respectively). However, after the addition of 2% MMT to CTS film, the water solubility reduced significantly (119 ± 7%).

The water solubility of neat CTS film was 9.1 ± 0.4% whereas, after the addition of CTS-MMT nanocomposites at 1% and 2% to CTS film, it became more hydrophobic by showing the water solubility lower than those CTS film (6.7 ± 0.7% and 6.3 ± 0.5% respectively).

Table 3. Properties of chitosan film supplemented with CTS-MMT nanocomposites.

Film type	Water Contact Angle (degree)	Water absorption (%)	Water solubility (%)
CTS	52.7 ± 5.5^b	184 ± 7^a	9.1 ± 0.4^a
1% CTS-MMT	63.3 ± 2.0^b	193 ± 15^a	6.7 ± 0.7^b
2% CTS-MMT	77.6 ± 3.1^a	119 ± 7^b	6.3 ± 0.5^b

* Values indicated by the same letter in the same column were not significantly difference using Duncan's Multiple Range Test

$(P < 0.05)$.

4.2.3.2 Water Vapor Transmission rate (WVTR) and Oxygen Transmission

Rate (OTR)

The value of WVTR and OTR were shown in Figures 30A-B. The value of WVTR of

neat CTS film was $29.6 \times 10^2 \pm 3.4$ g/m^2 day. After 1% and 2%, CTS-MMT nanocomposites

were added, WVTR values were not significantly different compared to neat CTS film (30.1

± 2.2 g/m^2 day for1% CTS-MMT and 28.0 ± 1.9 g/m^2 day for 2% CTS-MMT). However, the

OTR of the film was significantly reduced after addition with 2% of CTS-MMT

nanocomposites (10.9 ± 2.4 mL/m^2 day) compared to neat CTS film (22.6 ± 2.1 mL/m^2

day). These results indicated that CTS film supplemented with CTS-MMT nanocomposites

at 2% could increase the oxygen barrier property.

(A)

(B)
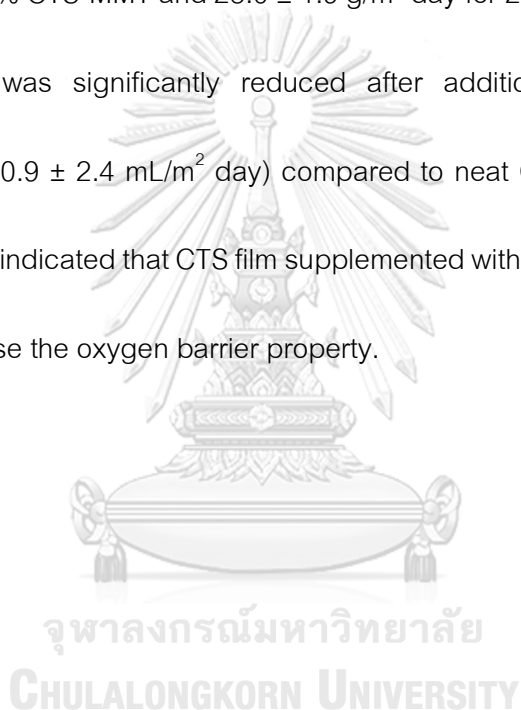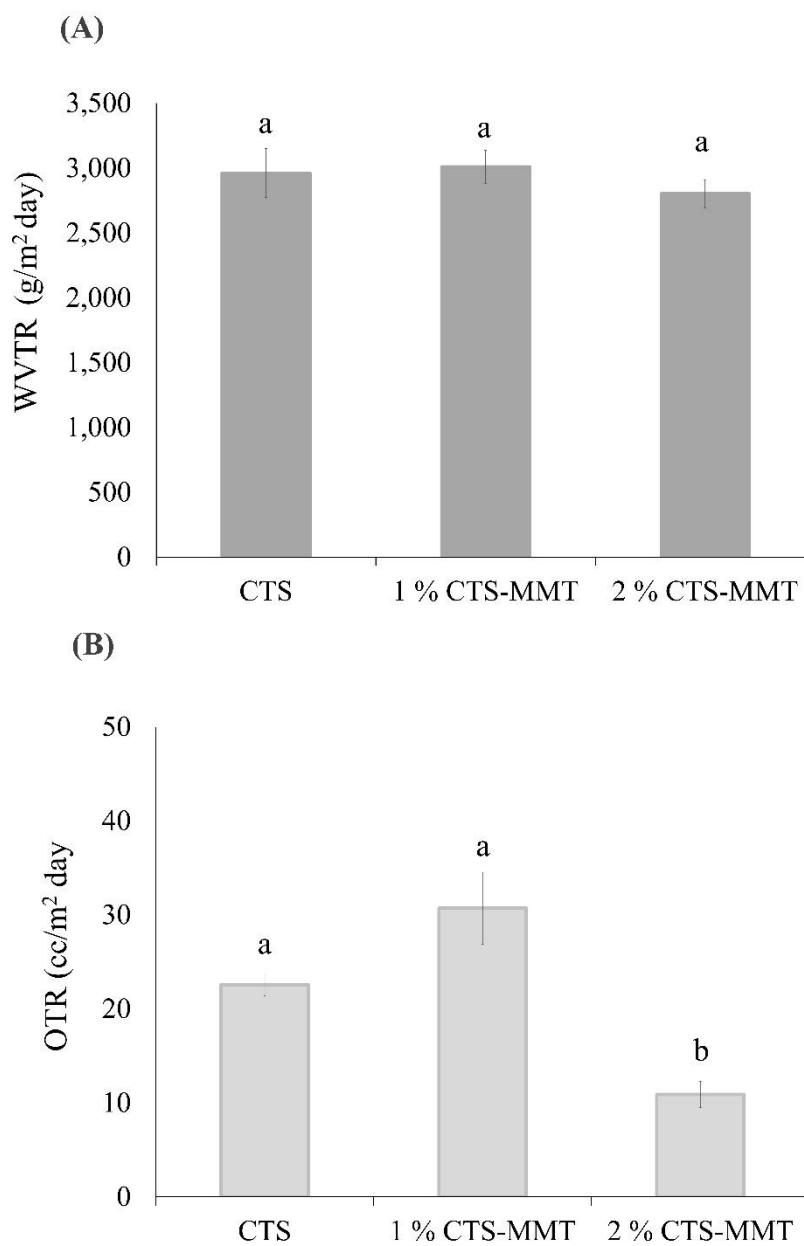
Figure 30. Water vapor transmission rate (WVTR) (A) and oxygen transmission rate (OTR) (B) of CTS film supplemented with CTS-MMT nanocomposites in various concentrations.

4.3 Determination of the efficiency of chitosan coating containing chitosan-montmorillonite (CTS-MMT) nanocomposites on development of anthracnose disease in banana

4.3.1 Disease incidence and severity

The development of anthracnose disease of banana fruit after inoculation and coating with various treatments was displayed in Figure 31 and Figure 32. The inoculated fruit with sterile distilled water was used as control fruit which did not present any disease symptom throughout the storage period. While inoculated fruit followed by dipping with distilled water (D.W.) and acetic acid showed disease incidence more than 80%, and it showed disease lesion area more than 1 mm after inoculation for 3 days. While fruit coated with CTS and 2% CTS-MMT appeared obvious infection after inoculation on day 6 (about 1.8 mm). However, there was no significant difference in anthracnose disease development observed in CTS, and 2% CTS-MMT coated fruit during the storage period. As a result, coating banana fruit with CTS and 2% CTS-MMT could reduce the severity of anthracnose disease (3.3 mm and 3.1 mm at day 9) than fruit coated with D.W. and acetic acid treatments (3.9 mm and 4.1 mm at day 9 of storage). Therefore, anthracnose infection in banana fruit was delayed in fungal development when fruit was treated with CTS and 2% CTS-MMT treatments.
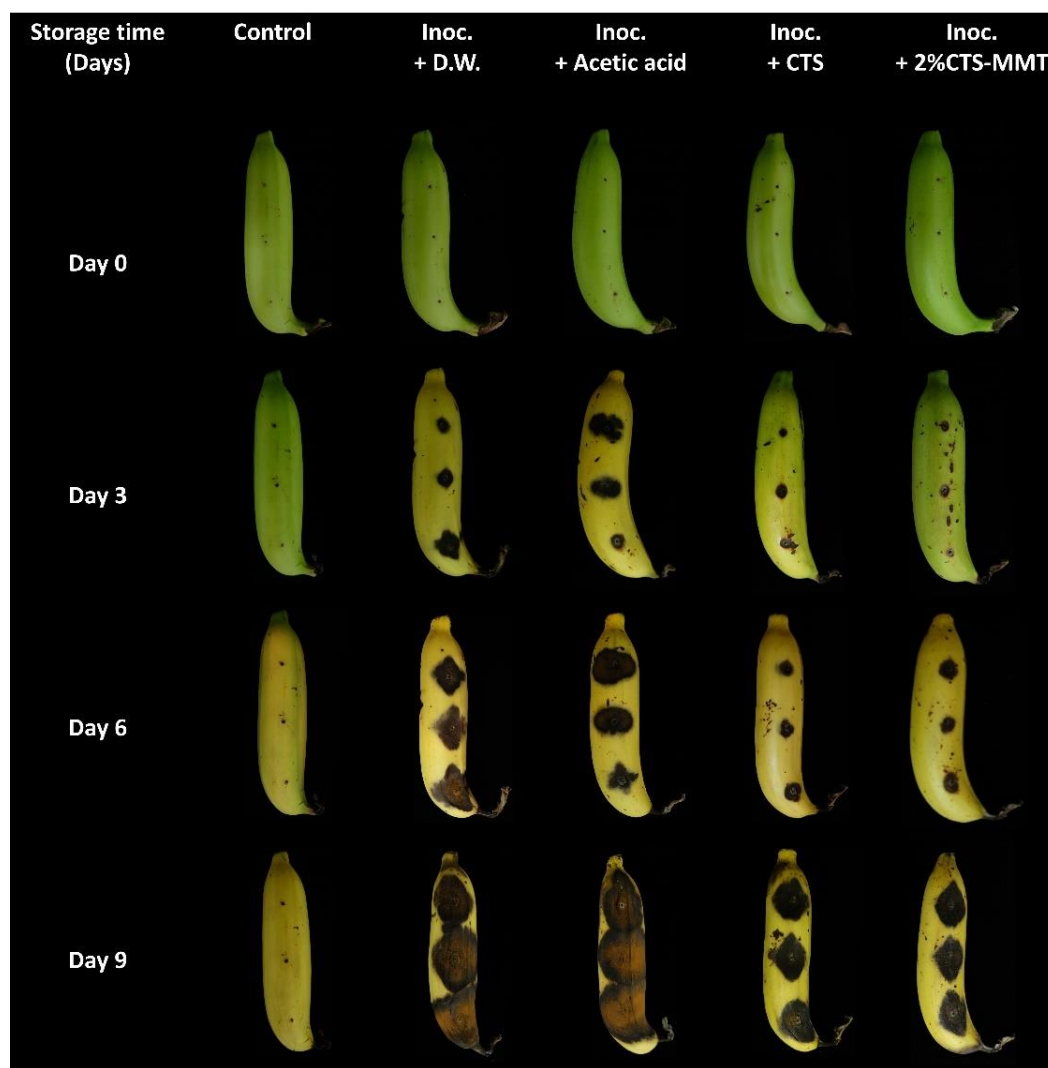
Figure 31. Disease development of inoculated fruit after coating during storage at 25 ± 2

°C for 9 days.

(A)

(B)
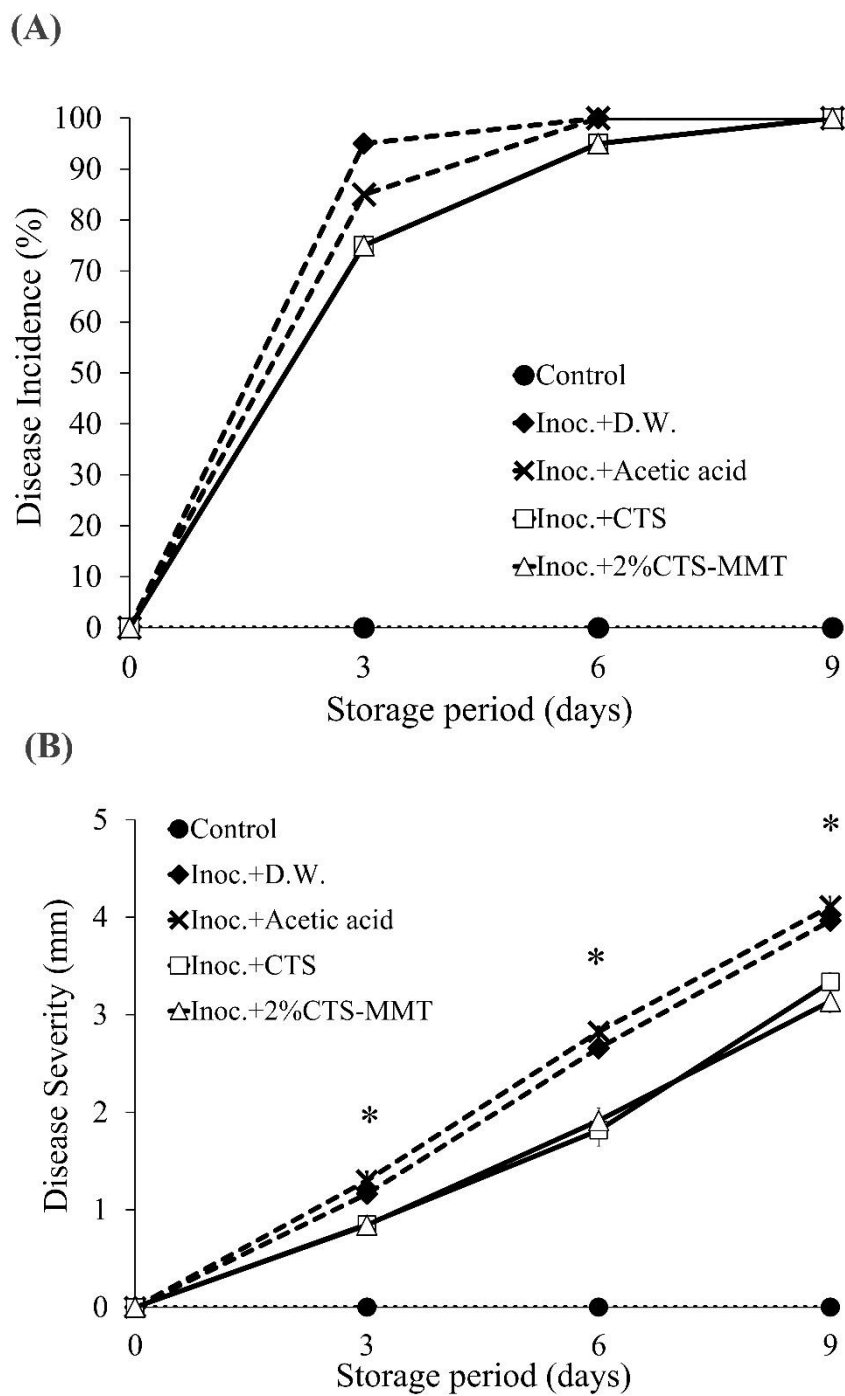
Figure 32. Disease incidence (A) and disease severity (B) of control fruit and coated fruit

during storage at 25 ± 2 °C. for 9 days. Vertical bars indicate ± SE.

4.3.2 H_2O_2 content, DPPH inhibition

The content of H_2O_2 in banana pulp was shown in Figure 33A. The results found that H_2O_2 content in control fruit increases gradually during ripening. For all inoculation groups, a high level of H_2O_2 content increased after inoculation for 3 days and then declined thereafter. Treatment of 2% CTS-MMT coating caused a significant reduction of H_2O_2 content after inoculation for 3 days compared to the other inoculated groups. Therefore, our results indicated that banana fruit coated with 2% CTS-MMT treatment was the most effective in inhibiting H_2O_2 production during ripening.

The antioxidant capacity was measured by using DPPH inhibition assay and shown in Figure 33B. As a result, the control fruit showed an increase and then decreased until the end of storage. In contrast, inoculation groups showed a decrease continuously during the ripening process. However, fruit coated with CTS and 2% CTS-MMT coated groups showed the highest DPPH inhibition during storage compared to other infected groups.

(A)

(B)
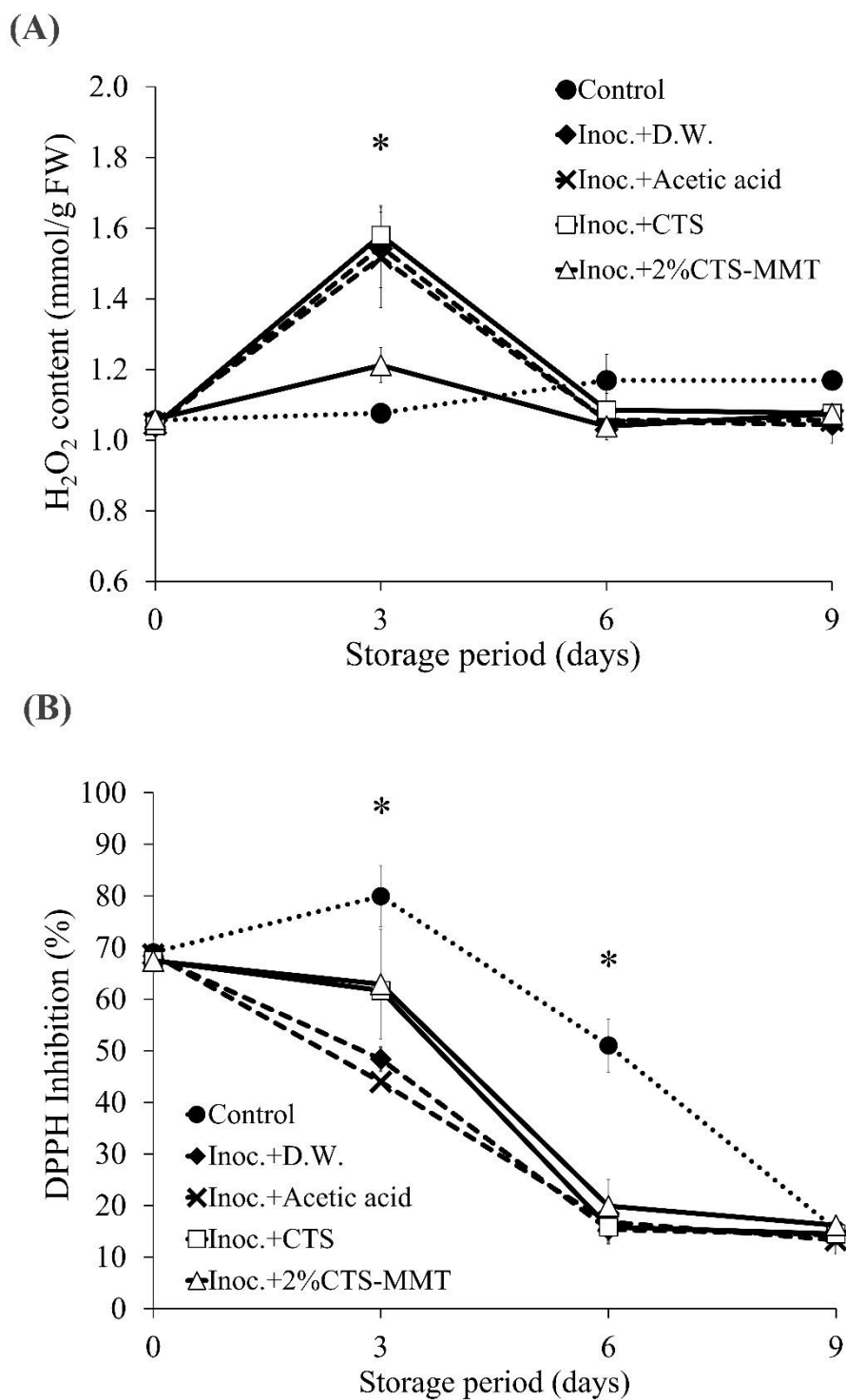
Figure 33. H_2O_2 content (A) and DPPH inhibition (B) of control fruit and coated fruit during

storage at 25 ± 2 °C. for 9 days. Vertical bars indicate ± SE.

4.3.3 Antioxidant contents

4.3.3.1 Total phenolic content (TPC)

Total phenolic content was displayed in Figure 34. As a result, TPC in all groups increased in the first 3 days of storage and then decreased until the end of the storage period. After inoculation for 3 days, the inoculation fruit coated with CTS treatment showed higher content of TPC in the banana pulp. However, 2% CTS-MMT treatment showed higher TPC content than CTS treatment showing a significant difference on day 3 after inoculation.
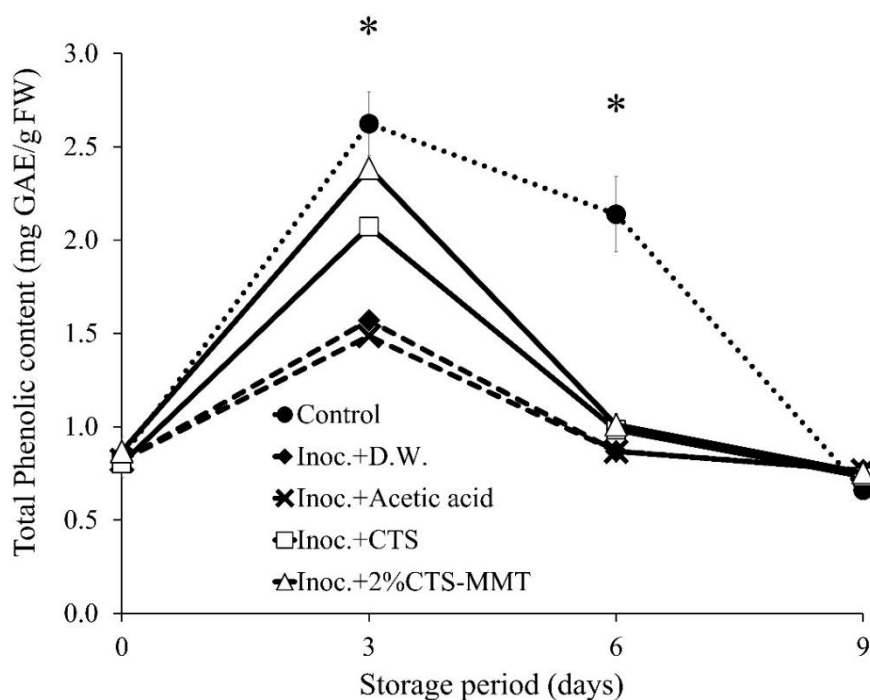
Figure 34. Total phenolic content of control fruit and coated fruit during storage at 25 ± 2 °C. for 9 days. Vertical bars indicate ± SE.

4.3.3.2 Ascorbic acid (AsA) content

Figure 35 displayed the ascorbic acid level in the banana pulp. The AsA content of the control group decreased on day 3 and then continuously increase until the end of the storage period. For inoculated groups, it increased sharply until day 6 and then decreased on day 9 of storage. After inoculation for 3 days, fruit coated with 2% CTS-MMT had the lowest AsA content compared to among inoculation group. However, it showed the highest value on day 6 and day 9 of storage. As a result, 2% CTS-MMT coating could enhance AsA content during the ripening process.
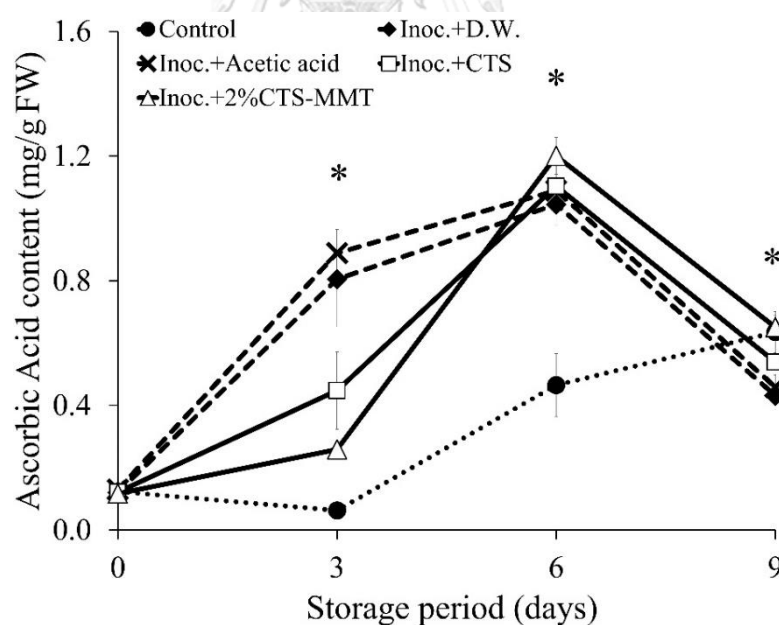
Figure 35. The ascorbic acid content of control fruit and coated fruit during storage at 25 ± 2 °C. for 9 days. Vertical bars indicate ± SE.

4.3.3.3 Glutathione (GSH) content

The GSH content in banana pulp decreased slightly after storage for 3 days for all groups. All infected groups showed continuous decreasing until day 6, then increased slightly on day 15 of storage. While control fruit showed a different pattern, the GSH content increased sharply on day 6 then decreased on day 15 of storage. Among treatments, 2% CTS-MMT treated fruit had the highest GSH content than infected fruit treated with the other treatments. The results were significantly different on days 3 and 6 of storage (Figure 36).
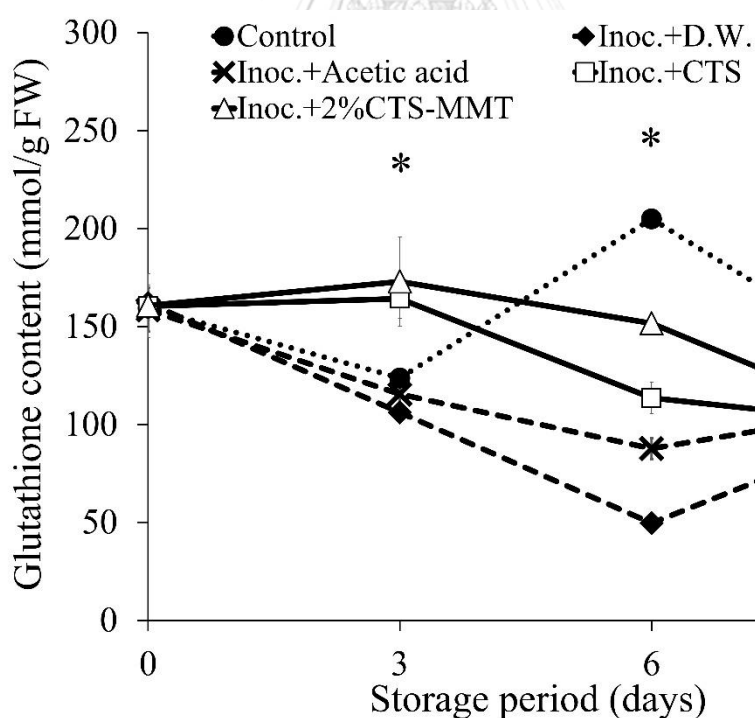
Figure 36. Glutathione content of control fruit and coated fruit during storage at 25 ± 2 °C. for 9 days. Vertical bars indicate ± SE.

4.3.4 Antioxidant enzyme activities

4.3.4.1 Superoxide dismutase (SOD) activity

SOD activity of banana pulp was displayed in Figure 37. The results revealed that SOD activity in all groups increased during ripening and reached the peak on day 6, then decreased until the end of the storage period. However, the activity of SOD showed no difference among groups throughout the storage period.
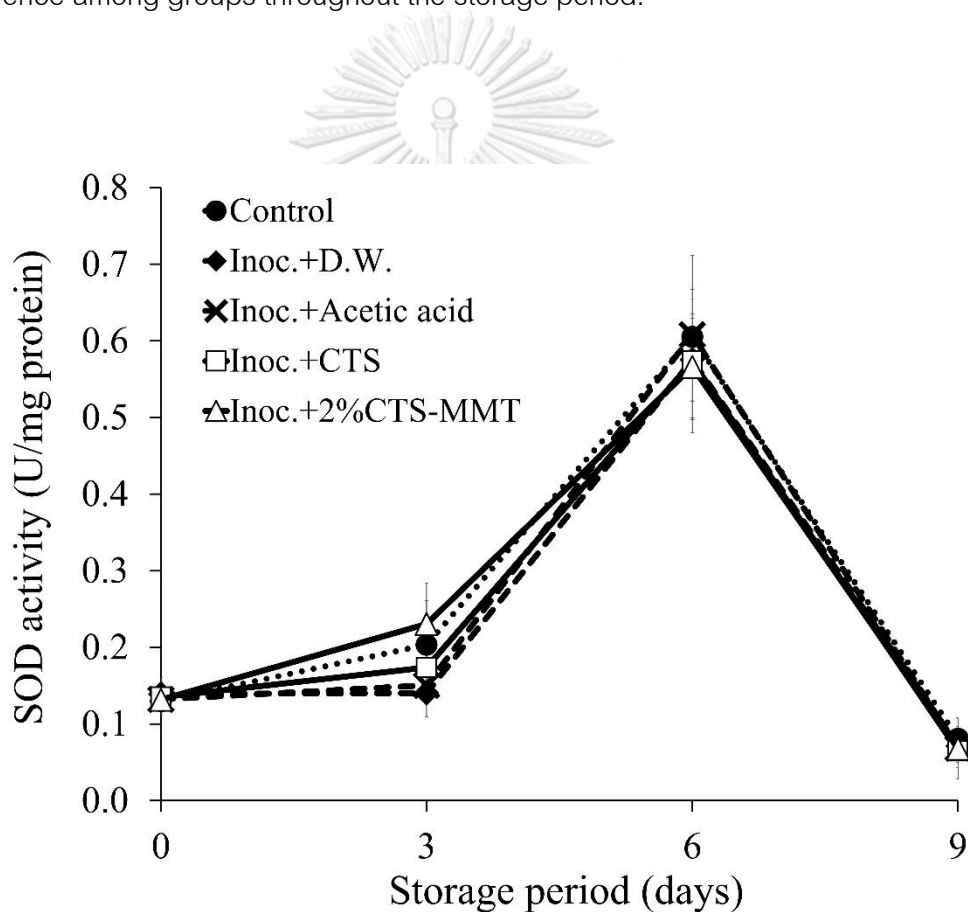
Figure 37. Superoxide dismutase (SOD) activity of control fruit and coated fruit during storage at 25 ± 2 °C. for 9 days. Vertical bars indicate ± SE.

4.3.4.2 Ascorbate peroxidase (APX) activity

APX activity of control fruit increased sharply on the first 6 days of storage and decreased thereafter. Inoculated fruit coated with CTS and 2% CTS-MMT showed the same activity patterns with control fruit which reached the activity peak on day 6 of storage but showed significantly enhanced APX activity among the infected group. While inoculation fruit coated with D.W. and acetic acid, the activity decreased continuously with storage time increase (Figure 38). The results suggested that coating fruit with CTS and 2% CTS-MMT treatments could induce APX activity during ripening.
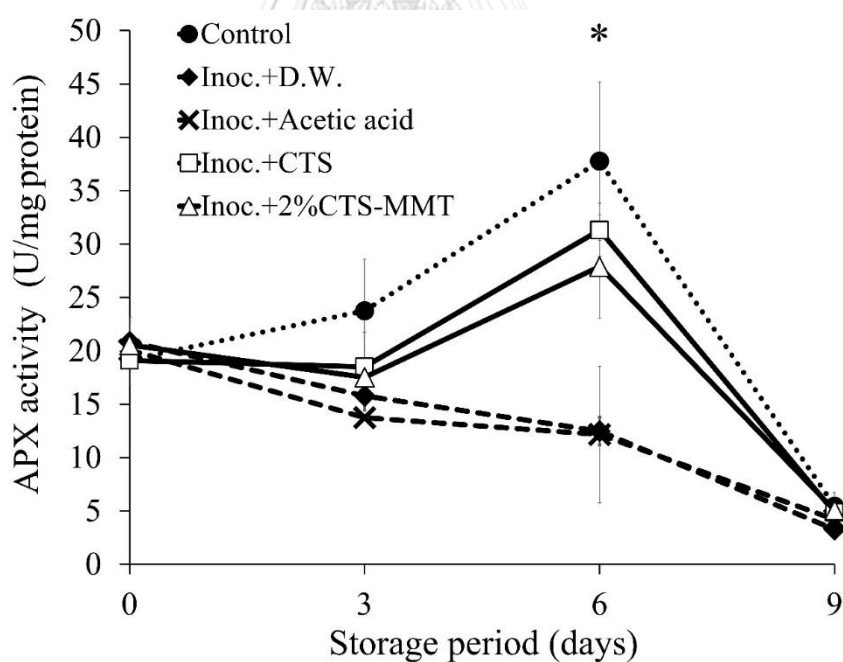
Figure 38. Ascorbate peroxidase (APX) activity of control fruit and coated fruit during storage at 25 ± 2 °C. for 9 days. Vertical bars indicate ± SE.

4.3.4.3 Catalase (CAT) activity

During storage, CAT activities of banana fruit had a similar pattern as APX activities, which showed a peak on day 6 and then decreased. Among the inoculation group, fruit coated with CTS had the highest value of CAT activity followed by fruit coated with 2% CTS/MMT compared to another infected group. The CAT activity showed a significant difference on day 6 of storage (Figure 39).
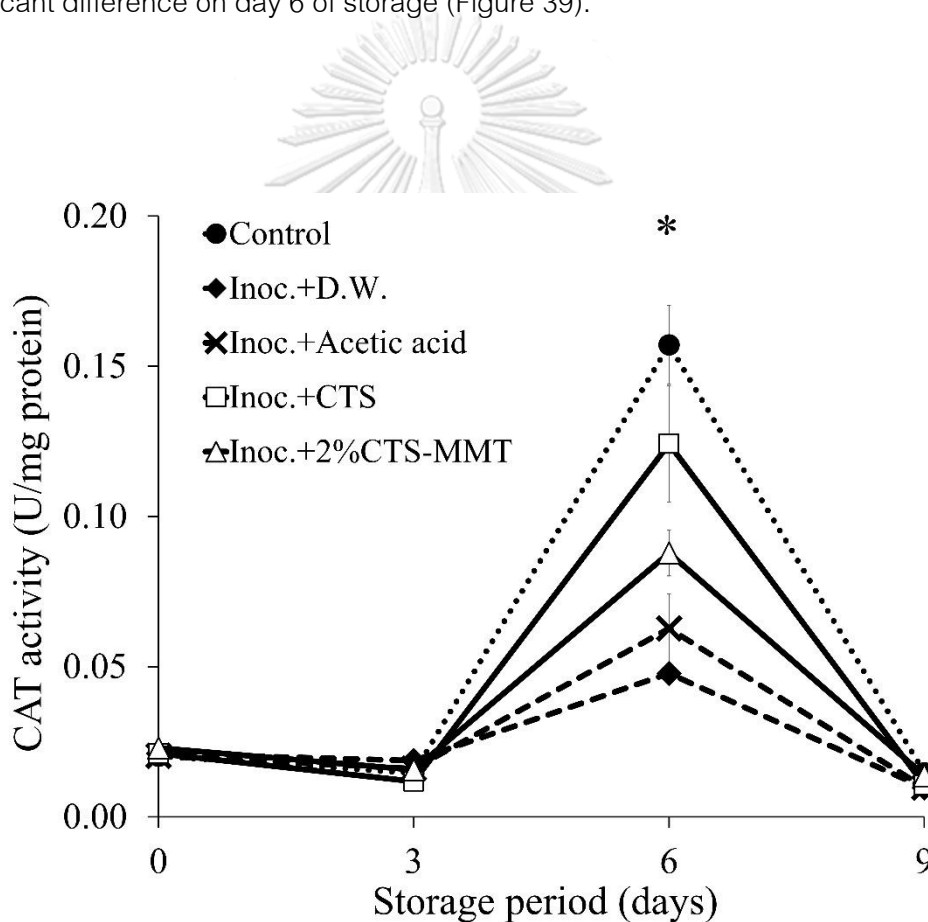
Figure 39. Catalase (CAT) activity of control fruit and coated fruit during storage at 25 ± 2 °C. for 9 days. Vertical bars indicate ± SE.

4.3.4.4 Glutathione reductase (GR) activity

GR activity in the banana pulp of control fruit decreased till day 6 and increased on day 9 of storage. However, the inoculation group showed different patterns, fruit coated with D.W. and acetic acid showed that GR activity decreased with storage time. While fruit coated with 2% CTS-MMT and CTS increased sharply on day 6 after inoculation and decreased on day 9 of storage. Fruit coated with 2% CTS-MMT coating had the highest GR activity significantly on days 3 and 6 compared within the inoculation group followed by the CTS coating group (Figure 40).
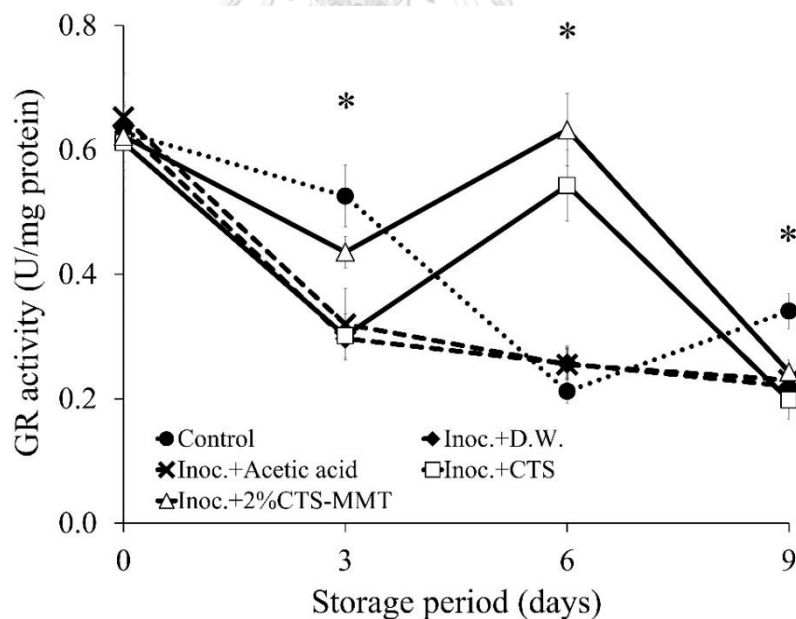
Figure 40. Glutathione reductase (GR) activity of control fruit and coated fruit during storage at 25 ± 2 °C. for 9 days. Vertical bars indicate $\pm$ SE.

4.3.4.5 Dehydroascorbate reductase (DHAR) activity

DHAR activities in all treatments expressed the same patterns, and the activity decreased on the first 3 days, then reached the highest activity on day 6 and decreased sharply on day 9 of storage. After inoculation for 3 days, fruit coated with D.W. and acetic acid had the highest DHAR activity. However, after inoculation for 6 days, fruit coated with 2% CTS-MMT had the highest activity followed by CTS treatment. As a result, coating banana fruit with 2% CTS-MMT after infection could stimulate antioxidant activities in the banana pulp during ripening (Figure 41).
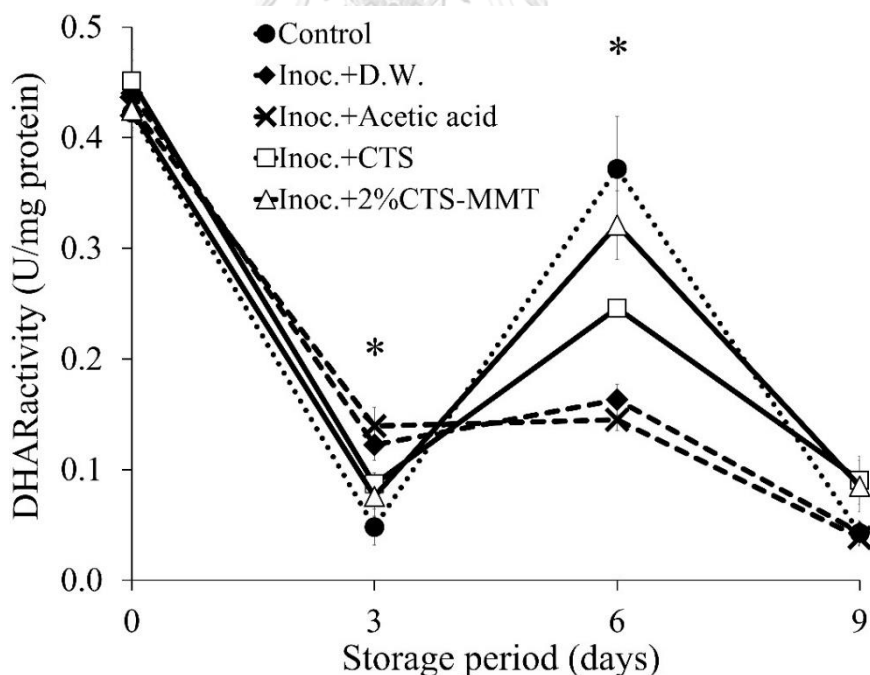
Figure 41. Dehydroascorbate reductase (DHAR) activity of control fruit and coated fruit during storage at 25 ± 2 °C. for 9 days. Vertical bars indicate ± SE.

CHAPTER V

DISCUSSION

5.1 Effects of different molecular weights of chitosan coating on physiological responses of banana fruit during storage at 25 ± 2 °C

5.1.1 Chitosan properties, chitosan thin film, and anatomical characteristics of chitosan coating on banana peel

The chitosan specific properties depend on its type, degree of deacetylation of chitin (%DD), and molecular weight (Nakatsuka and Andrady, 1992). Our study was focusing on the effects of its molecular weights on their property. The chitosan used in the experiment was demonstrated in Table 1; HMW-CTS showed the highest viscosity followed by MMW-CTS and LMW-CTS, respectively. The viscosity of chitosan solution directly affected the thickness of chitosan film. Table 2 exhibited that HMW-CTS and MMW-CTS solution could form a thin film layer that reduced water and oxygen gas transmission rates. The same result was also reported in the previous study (Yan et al., 2001).

The anatomical characteristics of the chitosan-coated surface of banana peel were studied using a scanning electron microscope and presented in Figures 14 and 15. From the cross-section images, HMW-CTS coated fruit showed the highest thickness film (as indicated by white arrow) followed by MMW-CTS, while LMW-CTS showed a very thin

film and could not be investigated in the Figure 9. The peel surface observation in Figure 10 reviewed that all chitosan treatments showed the homogenous coating on a banana peel. However, HMW-CTS coating showed a complete seal on the stomata compared to other coatings. It indicated that the HMW-CTS solution had the highest ability to form a thin film and cover smoothly on a banana peel. Confirm by Yan et al. (2001) study where expressed film coating prepared from low molecular weight (130 kDa) appeared less permeable to water 55% compared to high molecular weight (1,000 kDa) chitosan.

5.1.2 Effects of coating on postharvest quality of banana during storage

Since chitosan properties depend on its molecular weight, therefore, a various molecular weight of chitosan was applied as banana fruit coating in this study. Our results found that banana fruit coated with HMW-CTS and MMW-CTS showed a delay in the climacteric peak and ethylene production peak compared to other treatments. These results indicated that HMW-CTS and MMW-CTS coatings could reduce the respiration rate and ethylene production of the banana fruit during storage. It was in accordance with the OTR result where HMW-CTS and MMW-CTS films had the highest ability in O_2 barrier property. Thus, the coating led to a retard in respiration and ethylene production during storage compared to other treatments. The result was also supported by previous studies which reviewed that chitosan could act as a barrier film so, it creates a modified internal atmosphere leading to a reduced respiration rate and retard fruit ripening (Xu et al., 2018).

Furthermore, similar results have been reported in peach (Jiao et al., 2019) and mango (Xing et al., 2020) fruit when coated with the chitosan-based solution.

The loss of fruit weight mainly reflects the water status of plant tissue, which resulted from the increase in transpiration and respiration processes (Zhu et al., 2008). HMW-CTS coating could delay banana weight loss due to its barrier property of thin film covered on banana peel led to a restriction in water evaporation between fruit and surrounding air resulting in retarding fruit weight loss. It was supported by SEM images which HMW-CTS showed the thickness film and covered tightly on the stomata. Previous studies also found that chitosan coating could decrease mango weight loss (Zhu et al., 2008) and papaya (Ali et al., 2011) during storage.

HMW-CTS coating also reduced the total soluble solid value representing the amounts of solids dissolved in a solution mostly refer to sugar content (Jones and Scott, 1983). The TSS value was linked with the respiration process relate to the carbohydrate conversion to sugars (Macrae et al., 1992). The retarding of TSS increase may attribute to the chitosan thin film covered on banana peel led causing the low internal O_2 level. Furthermore, its barrier reduced the O_2 influx, which is needed in the final step of ethylene biosynthesis in converting ACC to ethylene by ACC oxidase (Rudus et al., 2013). The study of Li and Yu (2001) also reported a similar result where peach fruit coated with

chitosan solution had a reduction in TSS value together with respiration rate and ethylene production during storage.

Banana peel was changed from green to yellow because of chlorophyll degradation and carotenoid production (Thomas and Janave, 1992). It started changing from day 9 of storage, while banana fruit coated with HMW-CTS and MMW-CTS treatments delayed changing on day 12 of storage. Retarding in fruit color changes were also attributed to chitosan thin film reducing O_2 and ethylene exposure directly affecting chlorophyll degradation in guava and sweet pepper (Hong et al., 2012; Xing et al., 2011).

5.1.3 Effects of coating on banana fruit softening during storage

Fruit firmness is a key parameter that reflects the softening which influences consumer acceptability of fruit. The results were consistent with the weight loss, TSS, and peel color change, where HMW-CTS coating showed the best in maintaining their firmness by delaying the decrease of firmness throughout the storage period. The retardation of fruit softening with chitosan coating agrees with previous research of Petriccione et al. (2015). The firmness of strawberry was maintained when coated with chitosan solution, which showed firmness reduction values of only 7.84% on day 9 of storage time compared to uncoated fruit increased the value up to 28.52%.

Cell wall components including cellulose, hemicellulose, and pectin were found to degrade during fruit ripening resulting in softening (Seymour et al., 1990). Pectin was

an essential component of the primary and middle lamella, mainly reported decreasing via pectin solubilization during banana ripening (Amnuaysin et al., 2012). Our research found that MMW-CTS and HMW-CTS coatings could delay pectin solubilization as reflected in lower water-soluble pectin value at the end of storage. These results were in concordance with the study of Liu et al. (2016), which found a delay in pectin solubilization of cherimoya fruit coated with chitosan solution.

Generally, fruit softening is related to cell wall degrading enzymes. Polygalacturonase (PG) and pectate lyase (PL) were two critical enzymes in pectin degradation. PG plays a role in catalyzing the hydrolytic cleavage of α-(1-4) galacturonan linkages (Fischer and Bennett, 1991). PG activity in banana peel continuously increased with storage time increase. Fruit coated with HMW-CTS and MMW-CTS solution slowly increased compared to other treatments. PL is the pectin depolymerizing enzyme that cleaves α-(1,4) galacturonosidic linkages of PGA (polygalacturonic acid) via a β-elimination reaction (Fischer and Bennett, 1991). PL activity expressed the same trend with PG activity, which increased gradually with storage time. HMW-CTS and MMW-CTS treatments maintained lower PL activity during the first 12 days of storage. Our results clearly demonstrated that HMW-CTS and MMW-CTS treatments could reduce the PG and PL activities during storage. The reduction of these two enzyme activities could effectively retarding pectin degradation led to the maintenance of banana fruit firmness. Therefore,

HMW-CTS and MMW-CTS coating applications could maintain banana firmness by reduced PG and PL activities. Similar results were reported in fresh-cut papaya (Gonzalez‑Aguilar et al., 2009) and cherimoya (Liu et al., 2016) where the activities of PME and PG were lower than control. Furthermore, Gonzalez‑Aguilar et al. (2009) reported that cherimoya coated with chitosan plus citric acid could retard or suppress of *AcXETs* (xyloglucan endotransglycosylases) and *AcPE* (Pectinesterase) genes expression, which encodes cell wall hydrolase enzyme. Based on our results, HMW-CTS and MMW-CTS coatings retarded or suppressed the cell wall hydrolase enzymes and positively affected fruit firmness.

5.1.4 Effects of coating on DPPH inhibition, malondialdehyde contents, and disease severity of banana

Chitosan coating has been reported that it could promote antioxidant activity in many plants. However, their antioxidant activity depends on their molecular weight and the degree of deacetylation. (Kim and Thomas, 2007; Samar et al., 2013). Our results suggested that the application of HMW-CTS treatment showed the highest level of effectiveness in maintaining antioxidant capacity by expressing the highest %DPPH inhibition throughout the storage period compared to other treatments, which may be due to the oxygen penetration was suppressed. Ghasemnezhad et al. (2013) and Kou et al. (2014) also reported that strawberry and pear coated with chitosan solution expressed the highest antioxidant capacity. Based on the results, it can be concluded that HMW-

CTS coating was appropriate to increase antioxidant capacity in 'Hom Thong' banana fruit during storage.

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids in response to postharvest oxidative stress during storage (Ayala et al., 2014). Generally, during ripening, MDA content showed an increase. Our results demonstrated that fruit coated with HMW-CTS and MMW-CTS treatments had a lower MDA content than other treatments. The treatments could reduce oxidative stress that occurred in membrane lipid, which led to retard fruit senescence. It might be caused by a film barrier of chitosan thin film causing oxygen reduction, which is the primary reactant in the lipid peroxidation process (Johnson and Decker, 2015). Petriccione et al. (2015) reported a similar result, which found that strawberry coated with 1% and 2% chitosan expressed a delay in increasing MDA content.

The result of disease severity showed that HMW-CTS and MMW-CTS treatments had the potential to control banana disease. This could be due to the coating causes a reduction in oxygen influx resulting in a decline of fruit respiration rate and a slow ripening process, thus increasing its disease resistance. The previous studies reviewed that chitosan had an antioxidant activity against many microbials including *Colletotrichum musae* (Maqbool et al., 2010), *Pseudomonas fragi*, *Shewanella putrefaciens* and *Aeromonas hydrophila* (Fernandez-Pan et al., 2015), and *Colletotrichum gloeosporioides*

(Jitareerat et al., 2007; Jongsri et al., 2017). Chitosan effectively affected microbial

membrane disturbing by disrupting the monolayers of microorganisms (Krajewska et al.,

2011). It also induced the chitinase and B-1,3-glucanase activities in mango (Jitareerat et

al., 2007; Jongsri et al., 2017). Our result was consistent with previous research of Hossain

and Iqbal (2016) where the disease severity score of banana fruit was lower than uncoated

fruit. The same results were also reported in longan (Jiang and Li, 2001), citrus (Chien et

al., 2007), and mango (Jongsri et al., 2017) fruit when coated with chitosan solution.

5.2 Determination of the appropriate chitosan coating containing chitosan-

montmorillonite (CTS-MMT) nanocomposites to prolong shelf life of banana during

storage at 25 ± 2 °C

5.2.1 SEM analysis of coated fruit

The micromorphology of coating materials on banana peel was observed under a

scanning electron microscope (SEM). CTS coated fruit appeared completely sealing the

stomata, and the surface of the film was smooth. The CTS-MMT supplemented film

presented a rougher surface and showed a good dispersion of CTS-MMT in the film

matrix. The increasing surface roughness when added MMT was also reported by Neji et

al. (2020). However, when the addition of CTS-MMT was high (4% w/v), it caused many

large agglomerations of nanoparticles which were visibly observed. The SEM images

confirm that CTS-MMT nanocomposites at 1% and 2% combination resulted in a good

dispersion in the CTS matrix and covered banana peel surface completely. This effect

may have further contributed to a reduction of respiration rate and water loss during

banana storage.

5.2.2 Effects of the coating on banana postharvest qualities

The postharvest qualities of banana fruit were observed in various treatments.

Weight loss, fruit firmness, and TSS content are major parameters that indicate fruit

ripening (Valero et al., 2007). Banana fruit treated with 2% CTS-MMT treatment showed

the most beneficial effect on maintaining fruit firmness and showed the lowest TSS value.

It means that 2% CTS-MMT coating could retard the hydrolysis process of starch to sucrose in banana fruit during development to ripen. The increasing barrier property of O_2 uptake was reported to slow down softening process via metabolic activity (Maleki et al., 2018). The results were strongly supported by OTR measurement which 2% CTS-MMT film had the lowest OTR value compared to the other films. In addition, the surface layer of film that affected the reduction of oxygen and ethylene permeation also affected the delay in fruit color changes, which indicated that the ripening process was delayed. L and H angle values could represent banana color changes when fruit reached the ripening stage, L value increased, and H angle decreased (Chen and Ramaswamy, 2002).

5.2.3 Effects of the coating on respiration rate and ethylene production

Since the respiration rate of fruit can typically be used as an indicator of storage potential (Saquet and Streif, 2017), ethylene can also trigger fruit ripening and senescence, leading to reduced storage life. Our results expressed that the respiration rate of 2% CTS-MMT coated group had a delay in climacteric peak compared to the other groups. Ethylene production rate showed a similar trend with respiration rate where 2% CTS-MMT coated fruit expressed the lowest ethylene production. Since the layer of CTS-MMT clay addition created a tortuous diffusion (Cui et al., 2015; Vilarinho et al., 2020), 2% CTS-MMT coating had the greatest ability to create O_2 and CO_2 barriers. Therefore, it might cause CO_2 accumulation in banana fruit, leading to reduced banana respiration rate and ethylene production during fruit ripening. Xu et al. (2018) also reported that CTS thin

film created a barrier result in CO_2 accumulation occur in the fruit, and the respiration rate was reduced.

5.2.4 Effects of the coating on MDA content, electrolyte leakage, and antioxidant capacity

MDA content and electrolyte leakage values are used as indicators of membrane injury, causing the lipid peroxidation process (Wang et al., 2015). As shown in the result, fruit in all groups increased these two parameters with storage time increase. The combination coating treatment at 2% CTS-MMT found retarding in the increasing MDA level (reduced up to 32%) and EC value, and it was maintained at lower levels throughout storage time. It means that 2% CTS-MMT combination coating had the most effective in maintaining membrane integrity compared to other treatments.

This study presented the changes in antioxidant capacity of banana fruit after coating in terms of DPPH assays. The highest value of these two parameters was recorded in 2% CTS-MMT combination coating fruit followed by 4% CTS-MMT combination coating sample. In contrast, the lowest values were recorded in samples of D.W. and CTS treatments. From these results, CTS coating could not improve antioxidant capacity in terms of DPPH assay but, when combined with CTS-MMT at 2%, antioxidant capacity was improved. Previous reports also found the enhancing antioxidant activity when montmorillonite was added into the coating material (Guimaraes et al., 2016) the most

effective in increasing antioxidant capacity by expressing the highest %DPPH inhibition compared to the other treatments.

5.2.5 Effects of the coating on histological analysis

The microstructure images of banana pulp after coating for 15 days were observed under TEM compared to the initial day (day 0). In this study, 2% and 4% CTS-MMT treatments could retard the structure destroy of the plasma membrane and cell wall of banana pulp cell, its cell wall were still intact and plasma membrane still retained integrity and close to its cell wall after storage for 15 days. Goulao and Oliveira (2008) found that the loss of firmness during ripening relation to plant cell wall component degradation and membrane integrity loss. The barrier property of thin film affected in delaying pectin dissolution and reducing cell wall hydrolase enzyme activity (Zhao et al., 2018), and therefore it maintained its integrity. The result indicated that CTS-MMT combination with CTS solution at 2% and 4% effectively reduced loss of membrane integrity and cell wall degradation resulting in maintaining fruit firmness and delaying fruit ripening.

5.2.6 Properties of free-standing film supplemented with CTS-MMT nanocomposites

Water contact angle measurement could demonstrate the degree of hydrophilicity or hydrophobicity of film. The value of more than 90° is considered to have hydrophobic properties (Niu et al., 2019). Our results suggested that the addition of CTS-MMT into the chitosan matrix caused the decrease in hydrophilicity of film by showing the higher value of CA, which means that the surface of chitosan film became more hydrophobic. Since chitosan functional groups can form more hydrogen bonds with hydroxyl domains of CTS-MMT clay, it results in more rigidity of combination film (Shahbazi et al., 2017). Thus, the incorporated treatment could enhance the resistance of film against water. These results were related to the solubility value where the combination film could reduce the water solubility of film. It was clear that chitosan film had poor resistance to water. Still, when CTS-MMT nanocomposites were added to the chitosan matrix, it could enhance the ability of water-resistant chitosan-based film.

In addition, 2% CTS-MMT film was found to exhibit a lower value of OTR, which implied that it largely improved the O_2 barrier properties of chitosan film. Kasirga et al. (2012) reported that the addition of clay created a tortuous path in chitosan film; thus, O_2 hardly passes through it. O_2 was known to involve in fruit respiration and lipid oxidation (McGlasson et al., 1971); therefore, enhancing the O_2 barrier property influences fruit's shelf life. This development indicated that chitosan film adding 2% CTS-MMT nanocomposites presented a high performance as fruit coating.

5.3 Determination of the efficiency of chitosan coating containing chitosan-

montmorillonite (CTS-MMT) nanocomposites on development of anthracnose disease in

banana

Banana is susceptible to several diseases, especially anthracnose caused by the

fungus, *Colletotrichum musae,* resulting in postharvest losses (Maqbool et al., 2010). The

present study presented that the uncoated infected group showed higher disease

severity. At the same time, the infected group coated with CTS and 2% CTS-MMT

treatments resulted in the inhibition of disease development. Therefore, chitosan film-

forming covered on banana peel could inhibit fungus growth. Since chitosan could

penetrate inside the pathogen nuclei and disruption its cell membrane, causing

intracellular leakage and, finally, cell death (Safari et al., 2021). Furthermore, Liu et al.

(2007) also found that chitosan presented a potent inhibition of fungal germination and

development. This result was the same with the research of Limon et al. (2021) where *C.*

gloeosporioides growth was inhibited when mango fruit was coated with chitosan solution.

ROS plays a significant role as the first line of plant defense response against

pathogens infection (Shetty et al., 2007). Some previous studies demonstrated that an

excess ROS in plant tissues might cause its cell membrane damage, and disease-

resistance was reduced, resulting in disease development in fruit (Lin et al., 2017). Thus,

the lower levels of ROS accumulation prevented cell membrane damage, and the

disease-susceptibility was reduced. Our study found that after infection for 3 days, H_2O_2

accumulation increased rapidly. The treatment of 2% CTS-MMT could keep the H_2O_2

content at a normal level in infected fruit like those control fruit. In addition, DPPH inhibition

which represented antioxidant capacity was also induced by CTS and 2% CTS-MMT

treatments compared with the infected group. This finding agreed with the study of Nair

et al. (2018) where chitosan coating could enhance antioxidant capacity in guava fruit.

Phenolic compound is the secondary plant metabolites which involved in the

interaction between plants and pathogens. It is well known as an antimicrobial compound

and contribution to the resistance of disease (Petkovsek et al., 2009). Our study exhibited

that CTS and 2% CTS-MMT treatments significantly promoted phenolic compound

accumulation of the infected banana group. According to Gatto et al. (2016), phenolic

extracts could promote the disease resistance of sweet cherry fruit. This compound may

react with proteins of pathogens; thus, causing the loss of enzyme function (Schwalb and

Feucht, 1999). In agreement with our study, Liu et al. (2007) also reported that chitosan

coated tomato showed an increase in phenolic content after being treated for 3 days.

Furthermore, AsA and GSH play an essential role in the non-enzymatic

scavenging system. AsA acts as a substrate of APX that converts H_2O_2 into H_2O (Rezayian

et al., 2019). AsA can either be oxidized to monodehydroascorbate (MDHA) or converts

to dehydroascorbate (DHA). DHAR is catalyzed to AsA by the dehydroascorbate

reductase (DHAR) enzyme, which uses glutathione (GSH) as a substrate (Wang et al.,

2013). In this study, the infected fruit expressed the highest AsA content throughout the storage period. CTS and 2% CTS-MMT treatments significantly increased the AsA content after storage for 6 days and increased the GSH content throughout the storage period.

The antioxidant enzymes play a significant role in the oxidative stress defense mechanism. SOD is well known to catalyze superoxide (O_2^-) to H_2O_2 and O_2. Then, H_2O_2 can be further scavenged by APX, CAT, GR, and DHAR enzymes (Sevillano et al., 2009). Our results showed that the activity of SOD showed no significant difference among the group. However, the activities of APX, CAT, GR, and DHAR of infected groups were lower than those of uninfected groups during storage. Since the pathogen infection might induce ROS accumulation in fruit (Baker and Orlandi, 1995), thus it resulted in the inhibition of antioxidant activity. CTS and 2% CTS-MMT coating treatments significantly increased the activities of APX, CAT, GR, and DHAR of infected groups during storage, which increased and peaked at day 6 of the storage and then decreased after that. Zheng et al. (2017) found that chitosan coated kiwi fruit had a lower disease severity. The activities of SOD, CAT, and APX were enhanced, demonstrating that chitosan treatment effectively affected disease resistance kiwi fruit. In addition, Jiang et al. (2018) also reported that litchi fruit coated with chitosan treatment exhibited higher levels of DPPH radical scavenging, activities of antioxidant enzyme, and contents of AsA and GSH compared to control fruit. Based on these results, it could be concluded that chitosan

reduced the severity of anthracnose disease of banana fruit by regulation of the nonenzymatic and enzymatic antioxidant activities. These results demonstrated that applications of CTS and CTS-MMT coating could be effectively employed to extend postharvest longevity and quality of banana fruit. As a best management practice to increase financial returns per unit cost, the production of coating material needs to be scaled up to industrial scale. More study about the stability of coating solution and its retention and their toxicological effects should be considered to improve the quality of coating for the future commercial application.

CHAPTER VI

CONCLUSION

6.1 Effects of different molecular weights of chitosan coating on physiological responses of banana fruit during storage at 25 ± 2 °C

In the present study, the HMW-CTS solution had the highest viscosity solution and presented the highest thickness of coated film followed by MMW-CTS and LMW-CTS. WVTR and OTR results exhibited that MMW-CTS and HMW-CTS treatments effectively act as a barrier of water vapor and oxygen gas transmission compared to LMW-CTS film. The investigation of different MWs of chitosan coated on banana fruit during storage at 25 ± 2 °C, HMW-CTS treatment showed a complete seal on the banana peel. Also, HMW-CTS retarded fruit respiration rate and ethylene production during storage results in delaying fruit softening. In addition, the HMW-CTS coated group expressed the highest ability to decelerate the fruit ripening process by decreasing weight loss, peel color change, and TSS content. The activities of cell wall degrading enzymes, including PG and PL, were retarded and resulted in softening inhibition. Furthermore, HMW-CTS could reduce disease severity and enhance antioxidant capacity by maintaining the highest %DPPH inhibition and reducing cell membrane injury. Therefore, HMW-CTS is appropriate for 'Hom Thong' banana fruit coating as softening retardation and prolonging their shelf life.

6.2 Determination of the appropriate chitosan coating containing chitosan-montmorillonite (CTS-MMT) nanocomposites to prolong shelf life of banana during storage at 25 ± 2 °C

Compared to the various amounts of CTS-MMT nanocomposites combined with chitosan solution, 2% CTS-MMT treatment appeared completely sealing on the stomata, and SEM images showed that CTS-MMT nanocomposites had an excellent dispersion in the CTS matrix. Coating banana fruit with 2% CTS-MMT showed more effectiveness by maintaining postharvest qualities of fruit throughout the storage period. The treatment could maintain fruit firmness, reduce TSS content and retard peel color changes of fruit.

In addition, 2% CTS-MMT coating could delay the ripening process by retarding respiration rate and ethylene production compared to other groups. Furthermore, it could enhance antioxidant capacity leading to reduce cell membrane injury. Histological analysis exhibited that 2% CTS-MMT coating had the most effective in maintaining cell membrane integrity compared to the other groups. The results of the free-standing film showed that 2% CTS-MMT film had a higher ability of water resistance compared to CTS film by increasing the degree of water contact angle, lowering water absorption and water solubility values. In addition, 2% CTS-MMT film had a lower OTR value compared to the other films. Based on the results, the combination of CTS-MMT nanocomposites in CTS solution at 2% was appropriate as a banana coating for extending the 'Hom Thong' banana shelf life.

6.3 Determination of the efficiency of chitosan coating containing chitosan-montmorillonite nanocomposites on development of anthracnose disease in banana

CTS and 2% CTS-MMT treatments on banana fruit effectively reduced anthracnose disease incidence and severity caused by *Colletotrichum musae*. The coating treatment induced disease resistance by enhancing antioxidant capacity. The contents of TPC, AsA, and GSH had a higher accumulation than the infected group, as well as the increased activities of APX, CAT, GR, and DHAR when fruit coated with CTS and 2% CTS-MMT treatments. These results demonstrated that the combination of CTS-MMT nanocomposites with CTS solution at 2% affected the disease resistance of 'Hom Thong' banana fruit against anthracnose.
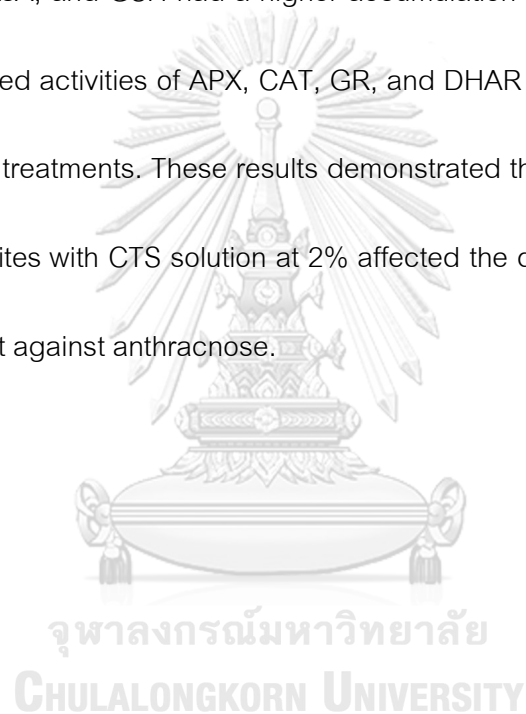
REFERENCES

Adao, R. C., & Gloria, M. B. A. 2005. Bioactive amines and carbohydrate changes during ripening of Prata'banana (*Musa acuminata× M. balbisiana*). Food Chemistry, 90(4), 705-711.

Alboofetileh, M., Rezaei, M., Hosseini, H., & Abdollahi, M. 2013. Effect of montmorillonite clay and biopolymer concentration on the physical and mechanical properties of alginate nanocomposite films. Journal of Food Engineering, 117(1), 26-33.

Ali, A., Muhammad, M. T. M., Sijam, K., & 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(2), 620-626.

Allegra, A., Sortino, G., Inglese, P., Settanni, L., Todaro, A., & Gallotta, A. 2017. The effectiveness of Opuntia ficus-indica mucilage edible coating on post-harvest maintenance of 'Dottato'fig (*Ficus carica* L.) fruit. Food Packaging and Shelf Life, 12, 135-141.

Amarante, C., Banks, N. H., & Ganesh, S. 2001. Characterising ripening behaviour of coated pears in relation to fruit internal atmosphere. Postharvest Biology and Technology, 23, 51-59.

Amnuaysin, N., Jones, M. L., & Seraypheap, K. 2012. Changes in activities and gene expression of enzymes associated with cell wall modification in peels of hot water treated bananas. <u>Scientia Horticulturae</u>, 142, 98-104.

Ampaichaichok, P., Rojsitthisak, P., & Seraypheap, K. 2014. Effects of different molecular weights of chitosan coatings on postharvest qualities of 'Nam Dok Mai' mango. <u>2nd International Conference on Food and Agricultural Sciences</u>, 77, 16-20.

Anthony, K. K., George, D. S., Baldev Singh, H. K., Fung, S. M., Santhirasegaram, V., Razali, Z., & Somasundram, C. 2017. Reactive oxygen species activity and antioxidant properties of *Fusarium* infected bananas. <u>Journal of Phytopathology</u>, 165(4), 213-222.

AOAC International. 2000. <u>Official methods of analysis (17th ed.)</u>. MD: AOAC Int.. Method.

ASTM D 3985. 2002. Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor. In A. International (Ed.), <u>Annual Book of ASTM Standards</u>. United States.

Ayala, A., Muñoz, M. F., & Argüelles, S. 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. <u>Oxidative Medicine and Cellular Longevity</u>, 1-31.

Baker, C. J., & Orlandi, E. W. 1995. Active oxygen in plant pathogenesis. Annual Review of Phytopathology, 33(1), 299-321.

Baldwin, E. A., Nisperos, M. O., Hangenmaier, R. H., & Baker, R. A. 1997. Use of lipids in edible coatings for food products. Food Technology, 51, 56-62.

Bautista-Banos, S., Hernandez-Lopez, M., Bosquez-Molina, E., & Wilson, C. L. 2003. Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit. Crop Protection, 22(9), 1087-1092.

Beers, R. F., & Sizer, I. W. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry, 195(1), 133-140.

Bhardwaj, A., Alam, T., & Talwar, N. 2019. Recent advances in active packaging of agri-food products: a review. Journal of Postharvest Technology, 7(1), 33-62.

Boudhrioua, N., Giampaoli, P., & Bonazzi, C. 2003. Changes in aromatic components of banana during ripening and air-drying. LWT-Food Science and Technology, 36(6), 633-642.

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.

Butler, B. L., Vergano, P. J., Testin, R. F., Bunn, J. M., & Wiles, J. L. 1996. Mechanical and barrier properties of edible chitosan films as affected by composition and storage. Journal of Food Science, 61(5), 953-956.

Cakmak, H., Kumcuoglu, S., & Tavman, S. 2018. Production of edible coatings with twin-nozzle electrospraying equipment and the effects on shelf-life stability of fresh-cut apple slices. Journal of Food Process Engineering, 41(1), e12627.

Chen, C. R., & Ramaswamy, H. S. 2002. Color and texture change kinetics in ripening bananas. LWT-Food Science and Technology, 35(5), 415-419.

Chien, P. J., Sheu, F., & Lin, H. R. 2007. Coating citrus (*Murcott tangor*) fruit with low molecular weight chitosan increases postharvest quality and shelf life. Food Chemistry, 100(3), 1160-1164.

Choi, Y., Lee, S. M., Chun, J., Lee, H. B., & Lee, J. 2006. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. Food Chemistry, 99(2), 381-387.

Chotikakham, S., Faiyue, B., Uthaibutra, J., & Saengnil, K. 2020. Exogenous methyl salicylate alleviates senescent spotting by enhancing the activity of antioxidative ascorbate-glutathione cycle in harvested 'Sucrier' bananas. Scientia Horticulturae, 267.

Costa, C., Conte, A., Buonocore, G. G., & Del Nobile, M. A. 2011. Antimicrobial silver-montmorillonite nanoparticles to prolong the shelf life of fresh fruit salad. International Journal of Food Microbiology, 148(3), 164-167.

Cui, Y., Kumar, S., Kona, B. R., & van Houcke, D. 2015. Gas barrier properties of polymer/clay nanocomposites. Rsc Advances, 5(78), 63669-63690.

Daungban, S., Pumisutapon, P., Topoonyanont, N., & Poonnoy, P. 2017. Effects of explants division by cutting, concentrations of TDZ and number of sub-culture cycles on propagation of 'Kluai Hom Thong'banana in a temporary immersion bioreactor system. Thai Journal of Science and Technology, 6(1), 89-99.

Deawati, Y., Onggo, D., Mulyani, I., Hastiawan, I., & Kurnia, D. 2017. Activity of superoxide dismutase mimic of [Mn (salen) OAc] complex compound non-enzymatically in vitro through riboflavin photoreduction. Molekul, 12(1), 61-69.

Dhindsa, R. S., & Matowe, W. 1981. Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. Journal of Experimental Botany, 32(1), 79-91.

Duran, M., Aday, M. S., Zorba, N. N. D., Temizkan, R., Buyukcan, M. B., & Caner, C. 2016. Potential of antimicrobial active packaging 'containing natamycin, nisin,

pomegranate and grape seed extract in chitosan coating'to extend shelf life of fresh strawberry. Food and Bioproducts Processing, 98, 354-363.

Fernandez-Pan, I., Mate, J. I., Gardrat, C., & Coma, V. 2015. Effect of chitosan molecular weight on the antimicrobial activity and release rate of carvacrol-enriched films. Food Hydrocolloids, 51, 60-68.

Finney Jr, E. E., Ben-Gera, I., & Massie, D. R. 1967. An objective evaluation of changes in firmness of ripening bananas using a sonic technique. Journal of Food Science, 32(6), 642-646.

Fischer, R. L., & Bennett, A. B. 1991. Role of cell wall hydrolases in fruit ripening. Annual Review of Plant Biology, 42(1), 675-703.

Florent, A., Loh, A. M., & Thomas, H. 2015. Nutritive value of three varieties of banana and plantain blossoms from Cameroon. Journal of Agricultural Sciences, 5(2), 52-61.

Gatto, M. A., Sergio, L., Ippolito, A., & Venere, D. D. 2016. Phenolic extracts from wild edible plants to control postharvest diseases of sweet cherry frui. Postharvest Biology and Technology, 120, 180-187.

Ghasemnezhad, M., Zareh, S., Rassa, M., & Sajedi, R. H. 2013. Effect of chitosan coating on maintenance of aril quality, microbial population and PPO activity of pomegranate

(*Punica granatum* L. cv. Tarom) at cold storage temperature. Journal of the Science of Food and Agriculture, 93(2), 368-374.

Gonzalez-Aguilar, G. A., Valenzuela-Soto, E., Lizardi-Mendoza, J., Goycoolea, F., Martínez-Téllez, M. A., Villegas-Ochoa, M. A., et al. 2009. Effect of chitosan coating in preventing deterioration and preserving the quality of fresh-cut papaya 'Maradol'. Journal of the Science of Food and Agriculture, 89(1), 15-23.

Goulao, L. F., & Oliveira, C. M. 2008. Cell wall modifications during fruit ripening: when a fruit is not the fruit. Trends in Food Science & Technology, 19(1), 4-25.

Goulao, L. F., & 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.

Guimaraes, I. C., dos Reis, K. C., Menezes, E. G. T., Borges, P. R. S., Rodrigues, A. C., Leal, R., Hernandes, T., de Carvalho, E.H.N., & Vilas Boas, E. V. D. B. 2016. Combined effect of starch/montmorillonite coating and passive MAP in antioxidant activity, total phenolics, organic acids and volatile of fresh-cut carrots. International Journal of Food Sciences and Nutrition, 67(2), 141-152.

Hirano, S., Yamaguchi, R., Matsuda, N., Miura, O., & Kondo, Y. 1977. Chitosan-aldehyde gel a novel polysaccharide gel produced from chitosan and aldehydes. Agricultural and Biological Chemistry, 41(8), 1547-1548.

Hodges, D. M., & Forney, C. F. 2000. The effects of ethylene, depressed oxygen and elevated carbon dioxide on antioxidant profiles of senescing spinach leaves. Journal of Experimental Botany, 51(344), 645-655.

Hoidy, H. W., Ahmad, M. B., Al-Mulla, E. J., & Ibrahim, N. A. 2010. Modification of montmorillonite by difatty acyl thiourea using cation exchange process. Oriental Journal of Chemistry, 26(2), 409-414.

Hong, K., Xie, J., Zhang, L., Sun, D., & 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.

Hossain, M. S., & Iqbal, A. 2016. Effect of shrimp chitosan coating on postharvest quality of banana (*Musa sapientum* L.) fruits. International Food Research Journal, 23(1), 277-283.

Jiang, X., Lin, H., Lin, M., Chen, Y., Wang, H., Lin, Y., Shi, J., & Lin, Y. 2018. A novel chitosan formulation treatment induces disease resistance of harvested litchi fruit to *Peronophythora litchii* in association with ROS metabolism. Food Chemistry, 266, 299-308.

Jiang, Y., & Li, Y. 2001. Effects of chitosan coating on postharvest life and quality of longan fruit. Food Chemistry, 73, 139-143.

Jiao, W., Shu, C., Li, X., Cao, J., Fan, X., & Jiang, W. 2019. Preparation of a chitosan-chlorogenic acid conjugate and its application as edible coating in postharvest preservation of peach fruit. Postharvest Biology and Technology, 154, 129-136.

Jitareerat, P., Paumchai, S., Kanlayanarat, S., & 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(2), 211-218.

Johnson, D. R., & Decker, E. A. 2015. The role of oxygen in lipid oxidation reactions: a review. Annual Review of Food Science and Technology, 6, 171-190.

Jones, R. A., & Scott, S. J. 1983. Improvement of tomato flavor by genetically increasing sugar and acid contents. Euphytica, 32(3), 845-855.

Jongsri, P., Rojsitthisak, P., Wangsomboondee, T., & Seraypheap, K. 2017. Influence of chitosan coating combined with spermidine on anthracnose disease and qualities of 'Nam Dok Mai' mango after harvest. Scientia Horticulturae, 224, 180-187.

Jongsri, P., Wangsomboondee, T., Rojsitthisak, P., & Seraypheap, K. 2016. Effect of molecular weights of chitosan coating on postharvest quality and physicochemical characteristics of mango fruit. LWT - Food Science and Technology, 73, 28-36.

Joshi, G. V., Kevadiya, B. D., Patel, H. A., Bajaj, H. C., & Jasra, R. V. 2009. Montmorillonite as a drug delivery system: intercalation and in vitro release of timolol maleate. International Journal of Pharmaceutics, 374, 53-57.

Junglee, S., Urban, L., Sallanon, H., & Lopez-Lauri, F. 2014. Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. American Journal of Analytical Chemistry, 5(11), 730-736.

Junqueira-Gonçalves, M. P., Salinas, G. E., Bruna, J. E., & Niranjan, K. 2017. An assessment of lactobiopolymer-montmorillonite composites for dip coating applications on fresh strawberries. Journal of the Science of Food and Agriculture, 97(6), 1846-1853.

Kasirga, Y., Oral, A., & Caner, C. 2012. Preparation and characterization of chitosan/montmorillonite-K10 nanocomposites films for food packaging applications. Polymer Composites, 33(11), 1874-1882.

Ke, Z., & Yongping, B. 2005. Improve the gas barrier property of PET film with montmorillonite by in situ interlayer polymerization. Materials Letters, 59(27), 3348-3351.

Keegstra, K. 2010. Plant cell walls. Plant Physiology, 154(2), 483–486.

Kevers, C., Falkowski, M., Tabart, J., Defraigne, J. O., Dommes, J., & Pincemail, J. 2007. Evolution of antioxidant capacity during storage of selected fruits and vegetables. Journal of Agricultural and Food Chemistry, 55, 8596-8603.

Kim, K. M., Son, J. H., Kim, S. K., Weller, C. L., & Hanna, M. A. 2006. Properties of chitosan films as a function of pH and solvent type. Journal of Food Science, 71(3), E119-E124.

Kim, K. W., & Thomas, R. L. 2007. Antioxidative activity of chitosans with varying molecular weights. Food Chemistry, 101(1), 308-313.

Kojima, K., Sakurai, N., Kuraishi, S., & Kokubo, A. 1994. Changes in firmness and chemical constituents of banana fruits during ripening. Japanese Journal of Tropical Agriculture, 38(4), 293-297.

Kou, X. H., Guo, W. L., Guo, R. Z., Li, X. Y., & Xue, Z. H. 2014. Effects of chitosan, calcium chloride, and pullulan coating treatments on antioxidant activity in pear cv."Huang guan" during storage. Food and Bioprocess Technology, 7(3), 167-681.

Krajewska, B., Wydro, P., & Janczyk, A. 2011. Probing the modes of antibacterial activity of chitosan. Effects of pH and molecular weight on chitosan interactions with membrane lipids in Langmuir films. Biomacromolecules, 12(11), 4144-4152.

Kulikov, S. N., Chirkov, S. N., Il'Ina, A. V., Lopatin, S. A., & Varlamov, V. P. 2006. Effect of the molecular weight of chitosan on its antiviral activity in plants. Applied Biochemistry and Microbiology, 42(2), 200-203.

Kumar, S., Mudai, A., Roy, B., Basumatary, I. B., Mukherjee, A., & Dutta, J. 2020. Biodegradable hybrid nanocomposite of chitosan/gelatin and green synthesized zinc oxide nanoparticles for food packaging. Foods, 9(9), 1143.

Kumar, S., Ye, F., Dobretsov, S., & Dutta, J. 2019. Chitosan nanocomposite coatings for food, paints, and water treatment applications. Applied Sciences, 9(12), 2409.

Kurecic, M., & Smole, M. S. 2012. Polymer nanocomposite hydrogels for water purification. In Nanocomposites-New Trends and Developments (pp. 161-185).

Lertsutthiwong, P., Noomun, K., Khunthon, S., & Limpanart, S. 2012. Influence of chitosan characteristics on the properties of biopolymeric chitosan–montmorillonite. Progress in natural science: Materials International, 22(5), 502-508.

Lertsutthiwong, P., Noomun, K., Khunthon, S., & Limpanart, S. 2012. Influence of chitosan characteristics on the properties of biopolymeric chitosan–montmorillonite. Progress in Natural Science: Materials International, 22, 502–508.

Li, H., & Yu, T. 2001. Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. Journal of the Science of Food and Agriculture, 81(2), 269-274.

Li, K., Jin, S., Li, J., & Chen, H. 2019. Improvement in antibacterial and functional properties of mussel-inspired cellulose nanofibrils/gelatin nanocomposites incorporated with graphene oxide for active packaging. Industrial Crops and Products, 132, 197-212.

Li, M., Slaughter, D. C., & Thompson, J. F. 1997. Optical chlorophyll sensing system for banana ripening. Postharvest Biology and Technology, 12(3), 273-283.

Lim, J. Y., Lim, T. H., & Cha, B. J. 2002. Isolation and Identification of *Colletorichum musae* from Imported Bananas. The Plant Pathology Journal, 18(3), 161-164.

Limon, T., Birke, A., Monribot-Villanueva, J. L., Guerrero-Analco, J. A., Altúzar-Molina, A., Carrion, G., Goycoolea, F. M., Moerschbacher, B. M., & Aluja, M. 2021. Chitosan coatings reduce fruit fly (*Anastrepha obliqua*) infestation and development of the fungus Colletotrichum gloeosporioides in Manila mangoes. Journal of the Science of Food and Agriculture, 101(7), 2756-2766.

Lin, B., Du, Y., Liang, X., Wang, X., Wang, X., & Yang, J. 2011. Effect of chitosan coating on respiratory behavior and quality of stored litchi under ambient temperature. Journal of Food Engineering, 102(1), 94-99.

Lin, Y., Chen, M., Lin, H., Hung, Y. C., Lin, Y., Chen, Y., Wang, H., & Shi, J. 2017. DNP and ATP induced alteration in disease development of *Phomopsis longanae* Chi-inoculated longan fruit by acting on energy status and reactive oxygen species production-scavenging system. Food Chemistry, 228, 497-505.

Liu, Liu, J., Li, H., Yuan, C., Zhong, J., & Chen, Y. 2016. Influence of postharvest citric acid and chitosan coating treatment on ripening attributes and expression of cell wall related genes in cherimoya (*Annona cherimola* Mill.) fruit. Scientia Horticulturae, 198, 1-11.

Liu, J., Tian, S., Meng, X., & Xu, Y. 2007. Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. Postharvest Biology and Technology, 44(3), 300-306.

Lohani, S., Trivedi, P. K., & 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(2), 119-126.

Macrae, E., Quick, W. P., Benker, C., & Stitt, M. 1992. Carbohydrate metabolism during postharvest ripening in kiwifruit. Planta, 188(3), 314-323.

Maduwanthi, S. D. T., & Marapana, R. A. U. J. 2017. Biochemical changes during ripening of banana: A review. International Journal of Food Science and Nutrition, 2(5), 166-169.

Maleki, G., Sedaghat, N., Woltering, E. J., Farhoodi, M., & Mohebbi, M. 2018. Chitosan-limonene coating in combination with modified atmosphere packaging preserve postharvest quality of cucumber during storage. Journal of Food Measurement and Characterization, 12(3), 1610-1621.

Malinovsky, F. G., Fangel, J. U., & Willats, W. G. 2014. The role of the cell wall in plant immunity. Frontiers in Plant Science, 5, 178.

Maqbool, M., Ali, A., Ramachandran, S., Smith, D. R., & Alderson, P. G. 2010. Control of postharvest anthracnose of banana using a new edible composite coating. Crop Protection, 29(10), 1136-1141.

Marriott, J., & Palmer, J. K. 1980. Bananas-physiology and biochemistry of storage and ripening for optimum quality. Critical Reviews in Food Science & Nutrition, 13(1), 41-88.

McGlasson, W. B., Palmer, J. K., Vendrell, M., & Brady, C. J. 1971. Metabolic studies with banana fruit slices II. effects of inhibitors on respiration, ethylene production, and ripening. Australian Journal of Biological Sciences, 24(4), 1103-1114.

Meng, X., Yang, L., Kennedy, J. F., & Tian, S. 2010. Effects of chitosan and oligochitosan on growth of two fungal pathogens and physiological properties in pear fruit. Carbohydrate Polymers, 81(1), 70-75.

Meredith, D. S. 1960. Studies on *Gheosporium musarum* Cke. & Massee causing storage rots of Jamaican bananas: I. anthracnose and its chemical. Annals of Applied Biology, 48(2), 279-290.

Moran, F., Nasuno, S., & Starr, M. P. 1968. Extracellular and intracellular polygalacturonic acid trans-eliminases of *Erwinia carotovora*. Archives of Biochemistry and Biophysics, 123(2), 298-306.

Moreno, J. L., Tran, T., Cantero-Tubilla, B., Lopez-López, K., Becerra Lopez Lavalle, L. A., & Dufour, D. 2020. Physicochemical and physiological changes during the ripening of banana (*Musaceae*) fruit grown in Colombia. International Journal of Food Science & Technology, 56(3), 1171-1183.

Musa, K. H., Abdullah, A., Jusoh, K., & Subramaniam, V. 2011. Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): effect of extraction techniques and solvents. Food Analytical Methods, 4(1), 100-107.

Nair, M. S., Saxena, A., & Kaur, C. 2018. Effect of chitosan and alginate based coatings enriched with pomegranate peel extract to extend the postharvest quality of guava (*Psidium guajava* L.). Food Chemistry, 240, 245-252.

Nakano, Y., & Asada, K. 1987. Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. Plant and Cell Physiology, 28(1), 131-140.

Nakatsuka, S., & Andrady, A. L. 1992. Thermogravimetric determination of starch content in starch–polyethylene blend films. Journal of Applied Polymer Science, 45(11), 1881-1887.

Neji, A. B., Jridi, M., Nasri, M., & Sahnoun, R. D. 2020. Preparation, characterization, mechanical and barrier properties investigation of chitosan-kaolinite nanocomposite. Polymer Testing, 84, 1-9.

Nilsen-Nygaard, J., Strand, S. P., Varum, K. M., Draget, K. I., & Nordgard, C. T. 2015. Chitosan: gels and interfacial properties. Polymers, 7(3), 552-579.

Niu, B., Shao, P., Chen, H., & Sun, P. 2019. Structural and physiochemical characterization of novel hydrophobic packaging films based on pullulan derivatives for fruits preservation. Carbohydrate Polymers, 208, 276-284.

Pathak, N., & Sanwal, G. G. 1998. Multiple forms of polygalacturonase from banana fruits. Phytochemistry, 48(2), 249-255.

Paul, T., Halder, S. K., Das, A., Ghosh, K., Mandal, A., Payra, P., Barman, P., Mohapatra, P. K. D., Pati, B. R., & Mondal, K. C. 2015. Production of chitin and bioactive materials from Black tiger shrimp (*Penaeus monodon*) shell waste by the treatment of bacterial protease cocktail. 3 Biotech, 5(4), 483-493.

Pedroso, C., Lage, D. A. C., Henz, G. P., & 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, 219-225.

Petkovsek, M. M., Stampar, F., & Veberic, R. 2009. Accumulation of phenolic compounds in apple in response to infection by the scab pathogen, *Venturia inaequalis*. Physiological and Molecular Plant Pathology, 74(1), 60-67.

Petriccione, M., Mastrobuoni, F., Pasquariello, M. S., Zampella, L., Nobis, E., Capriolo, G., et al. 2015. Effect of chitosan coating on the postharvest quality and antioxidant enzyme system response of strawberry fruit during cold storage. Foods, 4, 501-523.

Petriccione, M., Mastrobuoni, F., Pasquariello, M. S., Zampella, L., Nobis, E., Capriolo, G., et al. 2015. Effect of chitosan coating on the postharvest quality and antioxidant

enzyme system response of strawberry fruit during cold storage. Foods, 4(4), 501-523.

Pino, J. A., & Febles, Y. 2013. Odour-active compounds in banana fruit cv. Giant Cavendish. Food Chemistry, 141(2), 795-801.

Prasanna, V., Prabha, T. N., & Tharanathan, R. N. 2007. Fruit ripening phenomena–an overview. Critical Reviews in Food Science and Nutrition, 47(1), 1-19.

Qi, H., Hu, W., Jiang, A., Tian, M., & Li, Y. 2011. Extending shelf-life of fresh-cut 'Fuji'apples with chitosan-coatings. Innovative Food Science & Emerging Technologies, 12(1), 62-66.

Ranasinghe, L., Jayawardena, B., & Abeywickrama, K. 2003. Use of waste generated from cinnamon bark oil (*Cinnamomum zeylanicum* Blume) extraction as a post harvest treatment for Embul banana. Food, Agriculture and Environment, 1(2), 340-344.

Ren, J., Tong, J., Li, P., Huang, X., Dong, P., & Ren, M. 2021. Chitosan is an effective inhibitor against potato dry rot caused by *Fusarium oxysporum*. Physiological and Molecular Plant Pathology, 113, 101601.

Rezayian, M., Niknam, V., & Ebrahimzadeh, H. 2019. Oxidative damage and antioxidative system in algae. Toxicology Reports, 6, 1309-1313.

Rhim, J. W., Hong, S. I., Park, H. M., & Ng, P. K. 2006. Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. Journal of Agricultural and Food Chemistry, 54(16), 5814-5822.

Robertson, G. L. 1979. The fractional extraction and quantitative determination of pectic substances in grapes and musts. American Journal of Enology and Viticulture, 30(3), 182-186.

Rudus, I., Sasiak, M., & Kepczynski, J. 2013. Regulation of ethylene biosynthesis at the level of 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene. Acta Physiologiae Plantarum, 35(2), 295-307.

Safari, Z. S., Ding, P., Nakasha, J. J., & Yusoff, S. F. 2021. Controlling Fusarium oxysporum tomato fruit rot under tropical condition using both chitosan and vanillin. Coatings, 11(367)

Salama, H. E., & Aziz, M. S. A. 2020. Optimized carboxymethyl cellulose and guanidinylated chitosan enriched with titanium oxide nanoparticles of improved UV-barrier properties for the active packaging of green bell pepper. International Journal of Biological Macromolecules, 165, 1187-1197.

Salcedo, I., Aguzzi, C., Sandri, G., Bonferoni, M. C., Mori, M., Cerezo, P., et al. 2012. In vitro biocompatibility and mucoadhesion of montmorillonite chitosan nanocomposite: A new drug delivery. Applied Clay Science, 55, 131-137.

Samar, M. M., El-Kalyoubi, M. H., Khalaf, M. M., & Abd El-Razik, M. M. 2013. Physicochemical, functional, antioxidant and antibacterial properties of chitosan extracted from shrimp wastes by microwave technique. Annals of Agricultural Sciences, 58(1), 33-41.

Saquet, A. A., & Streif, J. 2017. Respiration rate and ethylene metabolism of 'Jonagold'apple and 'Conference'pear under regular air and controlled atmosphere. Bragantia, 76, 335-344.

Schwalb, P., & Feucht, W. 1999. Changes in the concentration of phenolic substances in the bark during the annual development of the cherry tree (Prunus avium L.). Advances in Horticultural Science, 13(2), 71-75.

Sevillano, L., Sanchez-Ballesta, M. T., Romojaro, F., & Flores, F. B. 2009. Physiological, hormonal and molecular mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. Journal of the Science of Food and Agriculture, 89(4), 555-573.

Seymour, G. B., Colquhoun, I. J., Dupont, M. S., Parsley, K. R., & Selvendran, R. R. 1990. Composition and structural features of cell wall polysaccharides from tomato fruits. Phytochemistry, 29(3), 725-731.

Shahbazi, M., Rajabzadeh, G., & Ahmadi, S. J. 2017. Characterization of nanocomposite film based on chitosan intercalated in clay platelets by electron beam irradiation. Carbohydrate Polymers, 157, 226-235.

Shetty, N. P., Mehrabi, R., Lutken, H., Haldrup, A., Kema, G. H., Collinge, D. B., & Jorgensen, H. J. L. 2007. Role of hydrogen peroxide during the interaction between the hemibiotrophic fungal pathogen Septoria tritici and wheat. New Phytologist, 174(3), 637-647.

Shin, Y., Liu, R. H., Nock, J. F., Holliday, D., & 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(3), 349-357.

Singh, S. S., Devi, S. K., & Ng, T. B. 2014. Banana lectin: a brief review. Molecules, 19(11), 18817-18827.

Sivakumar, D., Hewarathgamagae, N. K., Wijeratnam, R. W., & Wijesundera, R. L. C. 2002. Effect of ammonium carbonate and sodium bicarbonate on anthracnose of papaya. Phytoparasitica, 30(5), 486-492.

Subagio, A., Morita, N., & Sawada, S. 1996. Carotenoids and their fatty-acid esters in banana peel. Journal of Nutritional Science and Vitaminology, 42(6), 553-566.

Thakur, R., Pristijono, P., Bowyer, M., Singh, S. P., Scarlett, C. J., Stathopoulos, C. E., & Vuong, Q. V. 2019. A starch edible surface coating delays banana fruit ripening. LWT - Food Science and Technology, 100, 341-347.

Thomas, P., & Janave, M. T. 1992. Effect of temperature on chlorophyllase activity, chlorophyll degradation and carotenoids of Cavendish bananas during ripening. International Journal of Food Science & Technology, 27(1), 57-63.

Umroong, P. 2018. Leaf anatomy and minimal structure in leaves of *Hydrocotyle umbellata* L., obtained from water stress, were examined under electron microscope and light microscope. Microscopy and Microanalysis Research–The Journal of The Microscopy Society of Thailand, 31(1), 29-33.

Usuki, A., Hasegawa, N., Kato, M., & Kobayashi, S. 2005. Polymer-clay nanocomposites. Inorganic Polymeric Nanocomposites and Membranes, 135-195.

Valero, C., Crisosto, C. H., & Slaughter, D. 2007. Relationship between nondestructive firmness measurements and commercially important ripening fruit stages for peaches, nectarines and plums. Postharvest Biology and Technology, 44(3), 248-253.

Vilaplana, R., Pazmino, L., & Valencia-Chamorro, S. 2018. Control of anthracnose, caused by *Colletotrichum musae*, on postharvest organic banana by thyme oil. Postharvest Biology and Technology, 138, 56-63.

Vilarinho, F., Vaz, M. F., & Silva, A. S. 2020. The use of montmorillonite (MMT) in food nanocomposites: Methods of incorporation, characterization of MMT/polymer nanocomposites and main consequences in the properties. Recent Patents on Food, Nutrition & Agriculture, 11(1), 13-26.

Wang, J., You, Y., Chen, W., Xu, Q., Wang, J., Liu, Y., Song, L., & Wu, J. 2015. Optimal hypobaric treatment delays ripening of honey peach fruit via increasing endogenous energy status and enhancing antioxidant defence systems during storage. Postharvest Biology and Technology, 101, 1-9.

Wang, X., Du, Y., & Luo, J. 2008. Biopolymer/montmorillonite nanocomposite: preparation, drug-controlled release property and cytotoxicity. Nanotechnology, 19(6), 065707.

Wang, Y., Luo, Z., Du, R., Liu, Y., Ying, T., & Mao, L. 2013. Effect of nitric oxide on antioxidative response and proline metabolism in banana during cold storage. Journal of Agricultural and Food Chemistry, 61(37), 8880-8887.

Wannabussapawich, B., & Seraypheap, K. 2018. Effects of putrescine treatment on the quality attributes and antioxidant activities of 'Nam Dok Mai No. 4'mango fruit during storage. Scientia Horticulturae, 233, 22-28.

Williams, C. 1995. Healthy eating: clarifying advice about fruit and vegetables British Medical Journal, 1453-1455.

Win, T. O., Srilaong, V., Kyu, K. L., Poomputsa, K., & Kanlayanarat, S. 2006. Biochemical and physiological changes during chlorophyll degradation in lime (*Citrus aurantifolia* Swingle cv.'Paan'). The Journal of Horticultural Science and Biotechnology, 81(3), 471-477.

Xia, Y., Rubino, M., & Auras, R. 2019. Interaction of nanoclay-reinforced packaging nanocomposites with food simulants and compost environments. Advances in Food and Nutrition Research, 88, 275-298.

Xing, Y., Li, X., Xu, Q., Yun, J., Lu, Y., & Tang, Y. 2011. Effects of chitosan coating enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annuum* L.). Food Chemistry, 124(4), 1443-1450.

Xing, Y., Yang, H., Guo, X., Bi, X., Liu, X., Xu, Q., et al. 2020. Effect of chitosan/Nano-TiO$_2$ composite coatings on the postharvest quality and physicochemical characteristics of mango fruits. Scientia Horticulturae, 263, 1-7.

Xu, D., Qin, H., & Ren, D. 2018. Prolonged preservation of tangerine fruits using chitosan/montmorillonite composite coating. Postharvest Biology and Technology, 143, 50-57.

Yadav, S., Mehrotra, G. K., & Dutta, P. K. 2021. Chitosan based ZnO nanoparticles loaded gallic-acid films for active food packaging. Food Chemistry, 334, 127605.

Yan, X. L., Khor, E., & Lim, L. Y. 2001. Chitosan-alginate films prepared with chitosans of different molecular weights. Journal of Biomedical Materials Research, 58(4), 358-365.

Yang, G., Yue, J., Gong, X., Qian, B., Wang, H., Deng, Y., et al. 2014. Blueberry leaf extracts incorporated chitosan coatings for preserving postharvest quality of fresh blueberries. Postharvest Biology and Technology, 92, 46-53.

Zhao, Y., Deng, L., Zhou, Y., Yao, S., & Zeng, K. 2018. Chitosan and Pichia membranaefaciens control anthracnose by maintaining cell structural integrity of citrus fruit. Biological Control, 124, 92-99.

Zheng, F., Zheng, W., Li, L., Pan, S., Liu, M., Zhang, W., Liu, H., & Zhu, C. 2017. Chitosan controls postharvest decay and elicits defense response in kiwifruit. Food and Bioprocess Technology, 10(11), 1937-1945.

Zhong, Y., Zhuang, C., Gu, W., & Zhao, Y. 2019. Effect of molecular weight on the properties of chitosan films prepared using electrostatic spraying technique. Carbohydrate Polymers, 212, 197-205.

Zhu, X., Wang, Q., Cao, J., & Jiang, W. 2008. Effects of chitosan coating on postharvest quality of mango (Mangifera indica L. cv. Tainong) fruits. Journal of Food Processing and Preservation, 32(5), 770-784.

Zhuang, C., Zhong, Y., & Zhao, Y. 2019. Effect of deacetylation degree on properties of Chitosan films using electrostatic spraying technique. Food Control, 97, 25-31.

จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

APPENDIX

APPENDIX A

1. Results of the characteristic of the produced CTS-MMT nanocomposites powder

1.1 The d-spacing value and size of MMT and CTS-MMT powders

Table A1. d-spacing value and size of MMT and CTS-MMT powders

Powder	d-spacing value (Å)	Size (nm)
MMT	12.6	nd
CTS-MMT	15.5	262.5 ± 15.4

1.2 X-Ray Diffraction (XRD) analysis

After CTS-MMT nanocomposites were produced, the characteristics of nanocomposites were analyzed and presented in Figure A1 and Table A1. The XRD diffraction peak of CTS-MMT nanocomposites was found at $2\theta = 5.7°$ while the original MMT powder diffraction peak appeared at $2\theta = 7.0°$. For d-spacing results, CTS-MMT nanocomposites and original MMT powder had their d-spacing at 15.5 Å and 12.6 Å, respectively.
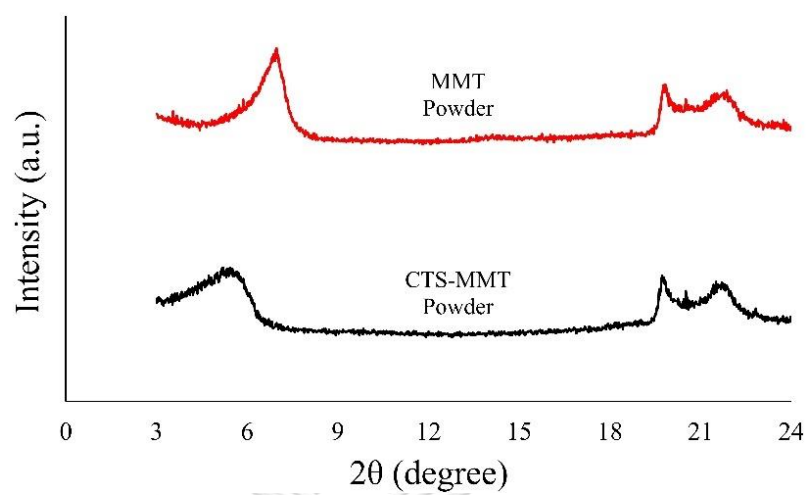
Figure A1. XRD patterns of MMT and CTS-MMT nanocomposites.

1.3 Fourier transform Infrared Spectroscopy (FTIR) analysis

The FTIR spectrum peak of CTS-MMT nanocomposites powder was measured and presented in Figure A2. CTS-MMT nanocomposites demonstrated characteristic bands at 3,435 and 1,634 cm^{-1} (N-H bands) and 2928 cm^{-1} (aliphatic C-H band).
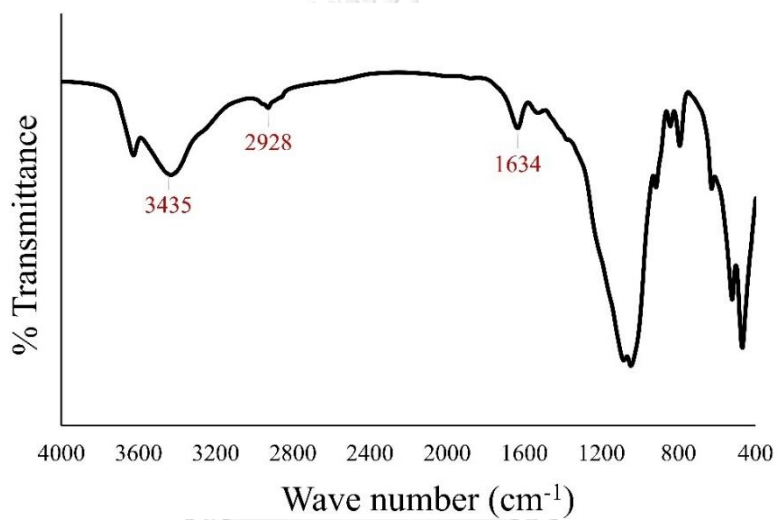

Figure A2. FTIR spectrum peak of CTS-MMT nanocomposites.

2. Results of the properties of free-standing CTS film supplemented with CTS-MMT nanocomposites

2.1 X-Ray Diffraction (XRD) analysis

The XRD patterns of CTS film and CTS supplement with CTS-MMT nanocomposites were measured and presented in Figure A3. As results, the neat CTS film had 4 characteristic crystalline peaks at about 2θ = 5.9°, 11.4°, 15.5°, and 19.9°, respectively. CTS supplemented with CTS-MMT at 1 % and 2 % (w/v), the board characteristic peaks of CTS film were observed, and the board peak of CTS-MMT nanocomposites at 2θ = 5.7° appeared.
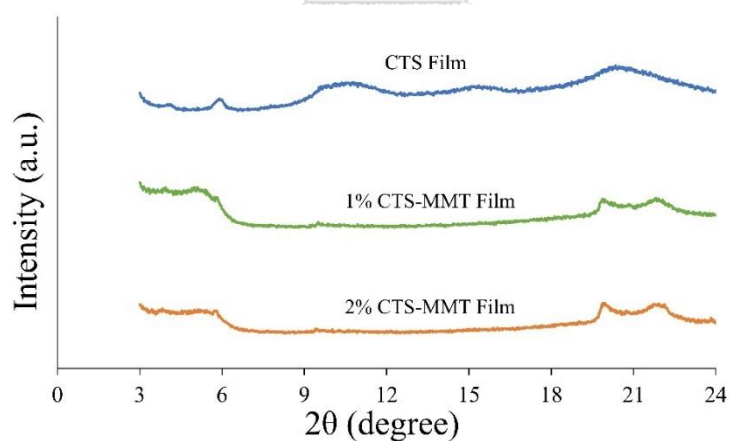

Figure A3. XRD pattern of CTS film supplemented with CTS-MMT nanocomposites in various concentrations.

2.2 Fourier Transform Infrared Spectroscopy (FTIR) analysis

The results of the FTIR analysis were shown in Figure A4. The spectra of neat CTS film demonstrated the broad peak between 3500-3200 cm^{-1} (N-H stretching vibration). The peaks were observed at 2869 cm^{-1} (C-H stretching), 1652 cm^{-1} (C=O stretching; amide I group), 1586 cm^{-1} (N-H blending of primary amine), and 1324 cm^{-1} (CH_3 symmetrical deformation).
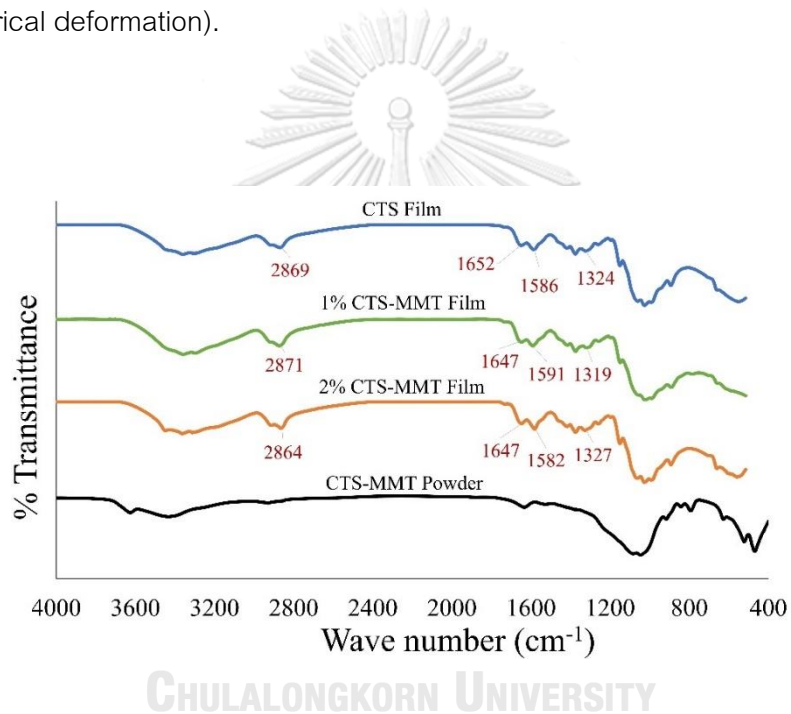

Figure A4. FTIR spectra of CTS film supplemented with CTS-MMT nanocomposites in various concentrations.

APPENDIX B

Methodologies

1. Particle size and surface area

The size of CTS-MMT nanocomposite powders was measured by dynamic light scattering using a Zeta sizer (Nano-ZS, Malvern Instruments Ltd., UK). The surface area was measured by the BET method.

2. X-ray diffraction (XRD)

The XRD measurements of the material were carried out by X-ray diffraction (XRD; model PW3710, 154 Philips, Netherlands) at room temperature with Cu Kα radiation (λ = 1.5418 Å). The XRD scanning was performed with speed at 0.2° 2θ/step, and the detector was set at a voltage of 40 kV and a current of 30 mA.

3. Fourier transforms infrared (FTIR)

FTIR measurements were recorded by a Fourier transforms infrared spectrophotometer (Perkin Elmer, Inc., 160 USA) using the KBr pellet technique. The determination was performed under a transmittance mode at the scan range of 400 - 4000 cm^{-1} and a sample scan of 16 times/sample with a resolution of 4 cm^{-1}.

APPENDIX C

1. Comparison of the isolated fungus and *Colletotrichum musae* sequences

The sequence of isolated fungus

GTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACTGAGTTTACGCTCTG
CAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGGGTCCCCGTGACCC
TCCCGGCCCCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCT
GATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGA
TCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG
CAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGC
GGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCC
TACAGCAGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGC
GTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCC
AATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCA

Results

>MH863549.1 *Colletotrichum musae* strain CBS 125356 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

AGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACT
GAGTTTACGCTCTGCAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAG
GGTCCCCGTGACCCTCCCGGCCCCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGA
GGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAA
ACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT

AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
GCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTT
GGTGTTGGGGCCCTACAGCAGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCG
GAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGC
CGTAAAACCCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTG
AACTTAAGCATATCAATAAG

Score	Expect	Identities	Gaps	Strand
1051 bits(569)	0.0	569/569(100%)	0/569(0%)	Plus/Plus

VITA

NAME	Arisa Wantat
DATE OF BIRTH	23 April 1990
PLACE OF BIRTH	Phitsanulok
INSTITUTIONS ATTENDED	She graduated with the second class honor of the degree of Bachelor of Science, Department of Biology, Faculty of Science, Naresuan University. After that she continued on Master Degree in the Department of Botany, Faculty of Science, Chulalongkorn University from 2013-2015. Then, she continued on PhD Program in Biotechnology, Faculty of Science, Chulalongkorn University since 2015.
HOME ADDRESS	33/1 Moo. 11, Pan sao, Bangrakham, Phitsanulok
PUBLICATION	Wantat, A., Rojsitthisak, P., Seraypheap, K., 2021. Inhibitory effects of high molecular weight chitosan coating on 'Hom Thong' banana fruit softening. Food Packaging and Shelf Life. 29: 100731.