BIO-ORGANIC PROTECTIVE EFFECTS OF KAFFIR LIME ESSENTIAL OIL ON GROWTH INHIBITION OF *Colletotrichum gloeosporioides* AND QUALITY CONTROL OF MANGO cv. NAM DOK MAI SRI THONG DURING STORAGE

LOETCHAI CHIT-AREE

DOCTOR OF PHILOSOPHY IN AGRICULTURAL INTERDISCIPLINARY

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ชื่อเรื่อง ฤทธิ์การป้องกันเชื้อราจากน้ำมันหอมระเหยที่สกัดได้จากมะกรูดอินทรีย์ต่อการยับยั้งการเจริญของ *Colletotrichum gloeosporioides* และการควบคุมคุณภาพของมะม่วงพันธุ์น้ำดอกไม้สีทองระหว่างการเก็บรักษา

ชื่อผู้เขียน นายเลิศชัย จิตร์อารี

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บทคัดย่อ

ในการวิจัยครั้งนี้เป็นการศึกษาเกี่ยวกับการใช้ประโยชน์จากน้ำมันหอมระเหยที่สกัดได้จากเปลือกมะกรูดอินทรีย์ซึ่งเป็นวัสดุเหลือใช้ ทั้งนี้เพื่อประยุกต์ใช้ในการป้องกันผลมะม่วงน้ำดอกไม้สีทองจากเชื้อรา โดยในน้ำมันหอมระเหยจากเปลือกมะกรูดสุกและดิบมี สารประกอบทางเคมีที่สำคัญได้แก่ beta-pinene limonene และ beta-citronellol นอกจากนี้ยังพบสารประกอบฟีนอลลิกและสารต้านอนุมูลอิสระด้วยเช่นกัน น้ำมันหอมระเหยจากมะกรูดมีฤทธิ์ต้านทานเชื้อรา *Colletotrichum gloeosporioides* โดยฤทธิ์การต้านทานมีสูงขึ้นตามความเข้มข้นของน้ำมันหอมระเหยที่เพิ่มขึ้น น้ำมันหอมระเหยจากมะกรูดดิบที่ความเข้มข้น 1,500 ppm สามารถลดการพัฒนาของเชื้อโรคได้อย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มควบคุม รวมทั้งมีฤทธิ์มากกว่าน้ำมันหอมระเหยจากมะกรูดสุกที่ความเข้มข้นเดียวกัน จากผลการทดลองสามารถสรุปได้ว่าน้ำมันหอมระเหยจากมะกรูดดิบที่ความเข้มข้น 0.15 เปอร์เซ็นต์ มีศักยภาพที่เหมาะสมสำหรับการเคลือบผลมะม่วงเพื่อควบคุมโรคภายหลังการเก็บเกี่ยว โดยพบว่ามะม่วงที่เคลือบด้วยน้ำมันหอมระเหยจากมะกรูดดิบสามารถลดระดับการเกิดโรคและยืดระยะเวลาการเก็บรักษาได้อย่างมีนัยสำคัญทางสถิติ และไม่มีผลลบต่อการยอมรับของผู้บริโภคใด ๆ เลย

ผลของการเคลือบไคโตซานต่อการเกิดโรค คุณสมบัติทางเคมีฟิสิกส์ และการยอมรับของผู้บริโภคถูกทดลองเพื่อหาความเข้มข้นของการเคลือบไคโตซานที่เหมาะสมกับมะม่วง จากการทดลองพบว่า ภายหลังการเก็บรักษามะม่วง 11 วัน ผลมะม่วงที่เคลือบด้วยไคโตซาน ความเข้มข้น 0.5, 0.75 และ 1 เปอร์เซ็นต์ มีระดับการเกิดโรค ปริมาณของแข็งที่ละลายได้ทั้งหมด การเปลี่ยนแปลงค่าสี a และ b และการสูญเสียน้ำหนักน้อยกว่ากลุ่มทดลองอื่นๆ นอกจากนี้ผลการทดสองทางประสาทสัมผัสยังพบว่า คะแนนความชอบด้านสี รสชาติ และลักษณะปรากฏของผลมะม่วงที่เคลือบด้วยไคโตซานที่ความเข้มข้น 0.5, 0.75 และ 1 เปอร์เซ็นต์ มีค่าน้อยกว่ากลุ่มทดลองอื่น ๆ ทั้งนี้สามารถสรุปได้ว่าไคโตซานที่ความเข้มข้น 0.5, 0.75 และ 1 เปอร์เซ็นต์สามารถคงคุณภาพของผลมะม่วงภายหลังการเก็บ

เกี่ยวได้ 11 วัน ด้วยเหตุนี้ไคโตซานที่ความเข้มข้น 0.5 เปอร์เซ็นต์ จึงถูกเลือกเพื่อใช้ในการทดลองถัดไป

ผลของการเคลือบน้ำมันหอมระเหยจากมะกรูดและไคต่อซานต่อการเกิดโรค คุณสมบัติทางเคมีฟิสิกส์ และการยอมรับของผู้บริโภคถูกศึกษาในการทดลองนี้ โดยผลการทดลองพบว่า การเคลือบผลมะม่วงด้วยน้ำมันหอมระเหยจากมะกรูดดิบที่ความเข้มข้น 1,500 ppm ไคโตซานความเข้มข้น 0.5 เปอร์เซ็นต์ และการเคลือบด้วยน้ำมันหอมระเหยจากมะกรูดดิบที่ความเข้มข้น 1500 ppm ร่วมกับไคโตซานความเข้มข้น 0.5 เปอร์เซ็นต์ ช่วยลดระดับการเกิดโรคและช่วยชะลอการสุกได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม

ผลการทดลองในครั้งนี้สามารถสรุปได้ว่า การพัฒนาการเคลือบผลมะม่วงที่มีส่วนผสมของน้ำมันหอมระเหยจากมะกรูดอาจจะสามารถช่วยปกป้องผู้บริโภคจากการบริโภคอาหารที่อาจปนเปื้อนเชื้อราได้

คำสำคัญ : มะม่วง, ฤทธิ์การยับยั้งเชื้อรา, มะกรูด, น้ำมันหอมระเหย, การปนเปื้อนเชื้อรา

Title	BIO-ORGANIC PROTECTIVE EFFECTS OF KAFFIR LIME ESSENTIAL OIL ON GROWTH INHIBITION OF *Colletotrichum gloeosporioides* AND QUALITY CONTROL OF MANGO cv. NAM DOK MAI SRI THONG DURING STORAGE
Author	Mr. Loetchai Chit-Aree
Degree	Doctor of Philosophy in Agricultural Interdisciplinary
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ABSTRACT

This research studied the utilization of essential oil extracted from the peel organic kaffir lime, an agricultural residual for protecting mangoes cv. Nam Dok Mai Sri Tong from fungal contamination. There are important chemical compounds in the essential oils from ripe and unripe kaffir lime peel, such as beta-pinene, limonene, and beta-citronellol. Bioactive substances, including the total phenolic and antioxidant content of unripe and ripe kaffir lime essential oil, were also found in considerable concentrations.*Colletotrichum gloeosporioides* is resistant to the essential oil of kaffir lime. A more significant concentration of essential oils inhibited fungal growth more effectively. Unripe kaffir lime essential oil at 1,500 ppm can significantly reduce disease development compared to the control and has higher activity than ripe kaffir lime essential oil at the same concentration. This result indicates that the potential of unripe kaffir lime essential oil at 0.15% concentration is suitable for incorporating in fruit coating formulation to control post-harvest fungal contamination. The coated unripe kaffir lime essential oil mangoes can significantly reduce the disease severity index and extend the consumer's storage and acceptance.

The effect of chitosan coating on disease severity index, physicochemical

characteristics, and consumer acceptance of mango fruit was then investigated in order to find the optimal chitosan concentration for mango fruit. The results showed that the disease incidence, total soluble solids, color (a and b) change, weight loss of mango fruits coated with 0.5%, 0.75% and 1% chitosan were lower than that of the other treatments after storage for 11 days. In addition, the sensory evaluation results found that the preference score of color, flavor and appearance was low compared to the other treatments. Therefore, the results indicated that 0.5%, 0.75% and 1% chitosan could preserve the mango cv. Nam Dok Mai Sri Tong for 11 days of storage. Finally, chitosan 0.5% was chosen for the next experiment.

The effect of kaffir lime essential oil or chitosan alone and the combination on the disease severity index, physicochemical properties and consumer acceptability of mango fruit was studied in this experiment. The results showed that the unripe kaffir lime essential oil 1,500 ppm, chitosan 0.50 %, and unripe kaffir lime essential oil 1,500 ppm + chitosan 0.50 % could significantly decrease the disease severity index and delay the fruit ripen in comparison with the control.

Therefore, this work may lead to the development of a mango fruit coating that incorporates kaffir lime essential oil, which may help protect the consumer from fungal contaminating organic food.

Keywords : Mango, Antifungal activity, Kaffir lime, Essential oil, Fungal contamination

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Loetchai Chit-Aree

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List of Abbreviations

UKLO	Unripe kaffir essential oil
RKLO	Ripe kaffir essential oil
C. gloeosporioides	*Colletotrichum gloeosporioides*
DMSO	Dimethyl sulfoxide
FT	First techniques
ST	Second technique
DACP	Diacipentadiene
GAP	Good agricultural practices
CRD	Completely randomized design
LPS	Lipopolysaccharide
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
SOD	Superoxide dismutase
CAT	Catalase
GSH	Glutathione
GPx	Glutathione peroxidase
AHAs	Alpha hydroxy acids
ORAC	Oxygen radical absorbance capacity
FRAP	Ferric reducing antioxidant power assay
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
GAEs	Gallic acid equivalent
DMBA	Dimethylbenz[a]anthracene
MMC	Methylmercury chloride
DD	Degree of deacetylation
DOAC	Direct oral anticoagulant
PDA	Potato dextrose agar
GC-MS	Gas chromatography-mass spectrometry
ANOVA	Analysis of variance

CHAPTER 1
INTRODUCTION

Background and rationale

Mango (*Mangifera indica* Linn.) is an economical fruit growing enormous plantation and production in Thailand. Mango production trends increase every year. Mango production in Thailand was 3,141,950 and 3,308,230 tons in 2010 and 2011 (Office of Agricultural Economics, 2017). Mangos from Thailand were exported to several countries every year. However, mango exportation faced the usual appearance problem because of the poor-quality standard of marketable products such as freshness, chemical residues, cultivation techniques, insects, and diseases after harvesting. This tropical fruit was popular in Thailand because of its delicious taste, beautiful color, and pleasant smell, especially Nam Dok Mai Sri Tong variety, suitable for ripening eating. However, the rotting of fruits during postharvest, storage, transportation and on-shelf affected the quality, quantity combined with an economic decrease to export range quality products. Losses from postharvest fruit diseases range from 1 to 20 percent in Thailand, depending on the product. The crucial postharvest disease of mango was anthracnose caused by *Colletotrichum gloeosporioides*. The disease protection was controllable using systemic fungicides such as benomyl, carbendazim, azoxystrobin, thiophanate methyl, Prochloraz, etc. And the contact fungicide such as copper oxychloride, mencozeb, propineb, and captan. However, these fungicides had a high risk of remaining and harming the end consumer, environment, and farmer. Presently, human being are seriously considering their health and environment. Therefore, organic practices, such as the essential oil from kaffir lime (*Citrus hystrix*) and chitosan, which are the natural organic compounds, are more popular among farmers to avoid fungus attacks.

Kaffir lime is a portion of food and herb. Nowadays, human beings are concerned with food safety, reducing and avoiding the chemicals used in food production. Kaffir lime showed the disease protection property in the report of the

efficacy of kaffir lime 10-gram fresh weight per 100 ml of water could inhibit the infection of *Collectotrichum sp* (Noengpha et al., 2004). Chitosan has been used in various post-harvest fruit quality control, such as Lychee, banana (Krajayklang and Somwan, 2008) and mango (Luimnark, 1998).

Chitosan is a natural biopolymer derived from crustaceous shells such as crabs and shrimp. This compound forms a semipermeable film that regulates the gas exchange, reduces transpiration loss, and slows fruit ripening. Chitosan is applied as a coating; generally, respiration rate and water loss are reduced (Bautista-Baños et al., 2006). This effect has been studied for litchi (Lin et al., 2011), pitaya (Chutichude and Chutichude, 2011), cucumber (El Ghaouth et al., 1991), guava (Hong et al., 2012) and mango (Zhu et al., 2008). Additionally, chitosan has been proven to display toxicity and inhibit fungal growth and development, which causes postharvest diseases in various fruits and vegetables (El Hadrami et al., 2010). According to these positive properties, chitosan has been successfully used as an edible coating to preserve the quality of fruits and vegetables after postharvest.

Therefore, the essential oil of kaffir lime produced from the organic system was used in this study to inhibit *C. gloeosporioides* that cause the rot in mangoes. The experiment was conducted in the laboratory and tested with the mango fruit to extend the shelf life by using only the essential oil of kaffir lime and combined with chitosan for comparison.

Objectives of research

1. To study the chemical composition of kaffir lime essential oil.
2. To study the biological activity of kaffir lime essential oil for inhibiting Anthracnose in the laboratory
3. To study the biological activity of kaffir lime essential oil combined with chitosan for controlling anthracnose in mango cv. Nam Dok Mai Sri Tong

Scope of study

1. Study the effects of kaffir lime essential oil from the organic system for inhibiting *C. gloeosporioides*.

2. Study the utilization of only kaffir lime essential oil and kaffir lime essential oil combined with chitosan on storage quality of mango cv. Nam Dok Mai Sri Tong.

Expected benefits

1. Knowledge of the chemical composition of kaffir lime essential oil.

2. Obtain efficient essential oil of kaffir lime for inhibiting Anthracnose in the laboratory

3. Knowledge of the organic system's extension storage method of mango.

4. Chemical reducing solution in mango production for farmers and customers.

5. Cost reducing solution for safety plant production

CHAPTER 2

LITERATURE REVIEW

Mango

Common Name: Mango

Scientific name: *Mangifera indica* L.

Family Name: Anacardiaceae

Mango is an important tropical fruit in Thailand that there are more than 100 cultivars. It can adapt well to the climate of Thailand. Mango can be grown in almost any area of the country. Therefore, it is one of the most popular fruit for planting and eating in Thailand. However, the proper selection of planting area has to be considered as the followings:

Suitable area for mango cultivation

The area should be 10-300 meters above sea level.

Mango can grow in both upland and lowland areas where does not flood.

The slope of the area should not be more than 15 percent.

The transportation and communication in the area should be convenient.

Suitable properties of soil for mango cultivation

Suitable soil should be a sandy loam with good drainage.

The pH of the soil should be medium to slightly acidic, about 5.5-7.5. Mangoes can generally grow and produce good yields in a relatively wide pH of soils.

Suitable climate for mango cultivation

The whole year average suitable temperature is about 20-34 ºC.

Mango needs a dry period before flowering for 2 months and a low temperature of about 15-20 ºC continuously for 2 weeks, depending on the variety.

Some mango varieties do not require low temperatures to stimulate flower buds, such as off-season flowering variety.

The optimal rainfall should be between 700-1500 mm per year, spreading evenly (Department of Agriculture, 2016).

Increasing quantity and improving quality of production

Flower bud development

Mango will stop the growth for a while and after that, it will initiate the inflorescence. At this stage, water should be given a small amount and then gradually increased to promote inflorescence growth.

Increase fruit setting

After mangos start to set the fruit, water should be increased by 7-10 days after fruiting and continuously increase the water supply until it reaches the fully required mango level.

Promoting the development of fruit

By continuously watering mango throughout and stop watering before harvest for 10-15 days. Apply the fertilizers according to the fruit development stage.

Prevention of damage production

Fruits were wrapped when the fruit was 45-60 days. It will promote good quality of mango fruit such as beautiful skin, reducing the loss of fruit, reducing or preventing the destruction of some diseases and insects, etc. (Department of Agriculture, 2016).

Fertilizer applies for mango cultivation

Permanently remove weeds in the canopy before applying fertilizers.

When mango tree is 1-2 years, apply 15-15-15 fertilizer at the rate of 1-2 kg per plant per year, divided into 2 times equally at the beginning and end of the rainy season. Spread fertilizer on the soil around the tree and disk the soil to cover the fertilizer. If mango has already provided the fruit or more than 3 years, it is periodically fertilized according to the following development stage.

Vegetative growth period After harvesting and pruning, apply 15-15-15 or 20-10-10 or 30-10-10 fertilizer at a rate 1-2 kg per plant per time. Also, apply organic fertilizers at the rate of 10-20 kg per plant per time by adding fertilizer in the canopy then disk the soil to cover the fertilizer. Apply fertilize again when the mango starts to initiate leaves set 2 using the same formula and rate of fertilizer.

Flower bud formation period 2-3 months before flower bloom, add 12-24-12 or 8-24-24 fertilizer at rate of 1-2 kg per plant for 2-4 years trees, rate of 2-4 kilograms per plant for 5-7 years plant and 4-6 kg per plant from 8 years trees and more.

Fruit-producing period After flowering 1 month, apply 15-15-15 fertilize at rate of 1-2 kg per plant.

Fruit quality improvement period Before harvesting for 1 month, apply fertilizer 13-13-21 at the rate of 1-2 kg per plant (Department of Agriculture, 2016).

Irrigation for mango cultivation

Irrigation method

Mini sprinkler system This system is convenient, reducing labor and plants absorb water evenly.

Hose or small canal system The system cost is lower than the sprinkler system, but the controls of water quantity for the plant are complex, uneven, and use more water, labor, and time than the sprinkler system.

Water quantity

Tree nourishing period The mango water requirement is approximately 0.5 times the climate evaporation rate. Therefore, if the water evaporation of climate was a 5 mm per day (1 mm evaporation is 1 liter of water per square meter), 3 m diameter canopy of mango tree will require about 22.5 liters of water per tree per day (time).

After fruiting, it is critical that the water requirement of mangoes was highest, about 0.7-0.8 times the evaporation rate. Therefore, if the water evaporation of climate was 5 mm per day, 5 m diameter canopy of mango tree will require approximately 87.5-100 liters of water per plant per day (time).

Frequency of watering

The frequency of watering depends on the soil texture and the weather. Sandy soil should be water 2-3 days per time. Clay soil should be water 4-5 times a day. However, observing soil moisture and mango leaf could support a watering plan. From the above example (Tree nourishing period), mango trees require 22.5 liters per tree per day and farmers want to water 4 days per time, so the total amount of water must be 90 liters per time (Department of Agriculture, 2016).

Harvesting

Harvesting time

Harvesting time could affect mango quality, shelf life period, and consumer acceptance. The harvesting time for fresh edible mango was mature fruit but not ripened yet. Namely, the physical development of this mango is enough to ripen. It can be detected from various characters such as creamy skin, shape, fruit color, and pulp color.

Data of day number after fruiting or inflorescence initiation until harvesting could obtain from the estimation from last year. However, the weather conditions have contributed to the harvest date discrepancies. Thus, the farmer could test by dipping mango fruit in water. The mature fruit has more specific gravity than water so that it will sink in water.

Harvesting method

Use carefully practical methods during harvesting. Must not cause mango to wound, scratch, crack or bruise.

In the case of processing mango, if mango trees were shaken, mango must not be dropped onto the ground and must have a canvas or support material to reduce the falling and dirty with soil.

They were harvesting mango fruit by maintaining a long stalk. Prevent the flowing latex from fruit. Use a container for convenient transportation of mangos. Containers should add support material to protect the bumping during transportation, such as fruiting plastic baskets that can be stacked without pressing on mangos in lower baskets.

Transport quickly harvested mangos in the shade and cool area during waiting to complete the harvest. Transport them quickly to the packing area for the post-harvest process (Department of Agriculture, 2016).

Postharvest Technology

Fresh mango preservation

The quality deterioration's physical and biological delay can be a preservative or extended shelf-life of mango fruit. Thus, when mango arrives, the packing house should do the following

The defective fruit such as wounded fruit or anomalous fruit caused by diseases such as anthracnose and stalk rotting or damaged fruit by insects such as thrips, aphids, black mold, etc. were excluded to prevent the germ spreading source that causes rot later. The Mango stalk was cut, and the stalk length was not more than 1 cm to flow out the latex from the fruit.

Wait for the remaining latex to slowly drain from the fruit until fruit dry by inverting the fruit onto the grid. The inverted head part was laid down on not sharp material or did not cause a wound or bruise. The latex flows through the drain and collecting tubes until it is completely dry—clean mango in clean water. Water that has been cleaned should be flowing or changing often. Cleaned water may add with some detergent for fruit to be more hygiene. The detergents should be recognized and do not cause any negative effects on hygiene and are safe for consumers, such as chlorine 7 5 mg/kg. Desiccate water on mango skin until dry. Sort fruit size and quality level, then load mango into a container or conduct the next step for storage, transportation or selling.

Mango life extension

Extending mango life while waiting for sale or transport may be used one method or more of the following methods

Fruit wrapping

To reduce dehydration, crash, abrasion and to potentially prevent disease, may use wrapping material with packaging before selling as follows

Use mesh foam to reduce the bumping.

Use wrapping paper to reduce friction.

Use small pores plastic to reduce dehydration and adjust the wrapping condition to be moderate humidity without condensation.

Temperature reduction and relative humidity increase

Slowing down respiration and dehydration could extend the freshness of mango for a more extended time. The optimum storage condition is 13-15 ºC of temperature and 85-95% relative humidity. Avoid the use of below 13ºC temperature for storage. Because it may cause dark skin color or changed color shin, soften and juicy fruit. In severe cases, mango fruit will not ripen due to chilling.

Produce cold resistance to mango fruit by gradually reducing the temperature periodically to mango fruit for adapting to low-temperature conditions.

Skin coating

To maintain the shiny skin, reduce dehydration, and extend the shelf life.

The coating material may contain carnauba wax or fat from vegetables or animals.

The coating material must be safe for the consumer and will not have any negative effect on mango quality, such as the effect on gas exchange quantity until occur the abnormal respiration then cause an unpleasant smell and taste. Therefore, the coating is not a popular method in Asian countries and Australia to reduce risk, as described above.

Use of ethylene synthesis inhibitors

To extend the storage time, ethylene synthesis inhibitor may be used in the future to delay ripening, such as diacipentadiene (DACP)

Ripening

Mango could ripen regularly and be ready for sale or consumption and reduce the risk of rotting.

Ripening method

1. Fumigation in a closed room with ethylene gas. The concentration of ethylene gas is 0.01 microliters per liter at 20-25 ºC. The relative humidity is 90-95 percent for 24 hours. This method could delay the maturation for 3-7 days.

2. Incubation by acetylene gas or gas charcoal wrapped in newspaper at the rate of 50 grams per 15 kg of mango. Must be careful, and mango could not touch with gas charcoal. Cover with canvas for 1-2 nights, then open the canvas and mango will ripen.

3. Dipping in 750 ml per liter of ethephon solution containing 2-chloroethyl phosphonic acid as an active ingredient for 2-3 minutes and then dried for ripening. Covered with canvas for 1 night, then open the cover and mango will start to ripen

***Note**: In the case of Nam Dok Mai Sri Tong mango, after incubation for 2 nights with gas charcoal, the taste of mango is still sour. Therefore, mango will continue in the incubator for 3 days to mature; then, mangoes are ripe and very sweet (Department of Agriculture, 2016).*

Standard criteria of mango

Error criteria

Error criteria of mango quality and size allowed in each container for mango fruit that quality and size are not the same as the specification are as follows.

1) Quality error criteria

1.1) Extra class

The discrepancy is not more than 5% by number or weight of quality mangoes either not following the requirements of the special class (Section 2) but satisfying the quality of class I (Section 2.2) or the quality is still within the error criteria of first-class quality (Section 1.2)

1.2) Class I (Class I)

The discrepancy is not more than 10% by the number or weight of quality mangoes that do not follow the requirements of Class I (Section 2), but the quality could accept by the second class (Section 2.3), or the quality is still within the error criteria the second-class quality (Section 1.3)

1.3) Class II (Class II)

The discrepancy is not more than 10% by number or weight of quality mangoes that do not follow the requirements of Class II (Section 2.3) or the quality

could not enter the minimum requirements (Section 2.1), but mango fruit must not be bruised, spoiled or appearance that is not suitable for consumption

2) Size error criteria

Each mango code could mix with larger or smaller mango in the next layer, which is not more than 10% by number or weight of mango. The size difference in each container must not be greater than the criteria in Table 1 (National Bureau of Agricultural Commodity and Food Standards, 2017).

Table 1 Size error criteria

Size code	Standard size (g)	Smaller or bigger than standard size (g)	Size difference in each container (g)
1	>450	350 ->550	150
2	>350 - 450	300-500	75
3	>250 - 350	200-400	75
4	>150 - 250	125-300	50
5	100 - 150	75-200	25

Packaging

1) Uniformity

Each container of mangos must be consistent in terms of variety, quality, size and color. In the mango that can see from outside of the container, this mango must represent all production.

2) Containers

The mangos must be packed so that mango can be stored well. The material was used inside the container must be clean and of good quality. The material should be able to prevent damage affecting the mango quality. If the used materials are paper or commercial information stamps, non-toxic inks or adhesives are required. The containers must be good quality, hygienic and strong for shipping, can preserve mango, no smells and no stranger.

Food additives

The type and amount of food additives used in mangos shall follow the relevant laws and regulations.

Contaminants

The type and amount of contaminants in mangos shall follow the relevant laws and regulations.

Toxic residue

Type and content of toxic residues in mangos shall be under the relevant laws and TAS 9002 Agricultural Standard on Toxic Residues: Maximum Toxic Residues and TAS 9003 Agricultural Standard on Toxic Residues: Maximum residue content contaminated from unavoidable causes.

Hygiene

Mangoes must be processed hygienically by following Good Agricultural Practices (GAP) standards and TAS 9035 Agricultural Standards on Good Practices for Packing House of fresh fruits and vegetables or other equivalent standards (National Bureau of Agricultural Commodity and Food Standards, 2017).

Anthracnose

The cause of the disease was *Colletotrichum gloesporioides* Penz.

Symptoms This disease damage had both the quantity and quality of the mangoes. It can destroy almost all mango parts, whether a seedling, young shoots, young leaves, inflorescences, flowers, young fruit to mature fruit and harvested fruit. Causes symptoms, at least as the wound spots remain on the leaves, branches and fruit. If the disease infection is severe, it will cause dry leaves, distorted and fallen leaves, dry inflorescences, not fruiting, rotten fruit and then fall as well as rotten fruit after harvest. This will damage the transportation of mangoes to foreign countries.

Symptoms in the seedling stage Symptom will find both in the leaves and stems. If diseased seedlings are weak or die, they cannot be used as stock. It will significantly damage commercial graft production. Leave symptom initiated from a small spot on the young leaves. Leaf-blade on sports looks clearer than other areas around this spot. The disease spot will expand into rings of different sizes depending

on the humidity and maturity of the leaves. The wound edges are visible in dark brown color. In high humidity conditions, the lesions on very young leaves are large and expand quickly, and then many lesions combine on leave in a large area. When leaves are old, the entire leave will dry or distort because the disease destroys some parts of the leaves. If temperature and humidity conditions are not suitable, the lesions on leaves were small dots and scattered. The middle of the lesion was the brown color, a lighter brown than the edge of the lesion. The middle of the lesion was thinner than the leaf blade. It may tear and fall off when exposed to water and the wound look like a hole.

Symptoms on the young trunk is a relatively black wound. The oval-shaped lesion is along the length of the trunk. If disease symptoms were severe, the wound would expand rapidly until around the trunk, and the tree will dry and dead. However, the lesion may not spread well if the seedlings are diseased when the tissues mature. The lesion will be black oval-shaped and shrink slightly. There will be some black or orange beads set in a band inside the wound in the middle. If the disease occurs on young shoots, they will dry out, and the shoot color will be black-brown. Finally, the whole tree may die as well.

Inflorescence symptoms are sporadic dark brown spots on the peduncle and flower stalk, causing the flowers to wither and fall off. If it is not too severe, it will cause less fruiting. However, if it is a lot, it will not produce. Occasionally, symptoms of peduncle burns are found; this will eventually dry out. Pathogens may destroy young fruits. The color of young fruit will turn brown, black and the fruit will fall off. Big fruits that have not yet been old can also suffer from the disease. The environment is suitable: high humidity and the optimal temperature (24-32 ° C).

Symptoms on the fruit are black spots, round shapes or holes that size ranges from small as a pin head to a large diameter of 2 - 4 cm, depending on the severity. In the wound area, cracks and small black grains line inside the wound will be found. When mango fruit matures, the wound will spread out and cause the whole fruit to rot during ripening incubation or transportation. Rotted black spots on this fruit damage almost all varieties of mango. Anthracnose could attach to the fruit without any symptoms. However, the disease will show many damage symptoms when

suitable later, such as ripe fruit or high humidity during storage or packaged for transportation.

Anthracnose can be prevented and eliminated by many types of chemicals. This is the only way to quickly and timely mitigate the damage from this disease. The application must be used according to the timing of the pathogen's infection to reduce wastage and chemicals are more effective in the case of exported mango. The disease prevention must be done regularly, primarily during mango produces young leaves, flowering and fruiting periods when mangos are susceptible to infection. The chemical spraying in regular anthracnose spreading sites could reduce the damage from leaf disease, which will affect the leaf fertility and then affect the good flowering and fruiting. Pruning and destroying diseased branches and young branches that occur at the base of big branches in the canopy, which is a source of disease accumulation, is another way to reduce the number of pathogens. Many plant protection substances such as benomyl, mancozeb, captan, copper oxychloride, etc., can be used to prevent and eliminate anthracnose effectively. The selection of any substance depends on the severity of the disease in each environment. Before the mango starts, the inflorescence should be sprayed with chemicals to prevent and eliminate insects and plant diseases once. To reduce the number of insects and diseases that will disturb the new inflorescences that have started to blossom. After that, spray every 10-15 days until the mango has young fruit. Absorbent chemicals, such as benomyl, may be more effective for spraying during heavy rains or near harvest because they will affect the product quality. They also help to reduce the damage from rot as well. For the flowering and fruiting of mangoes, other chemicals should be sprayed alternately as appropriate, such as the flowering stage may be used mancozeb, the young fruiting stage may be used captan or copper fungicide, big fruit stage may be used benomyl, etc. Harvested mango should be immersed in a solution of thiabendazole (Pronto 40) mixed with water at 50 ° C for 5 - 10 minutes and then dried to eliminate any bacteria (Department of Agriculture, 2017). According to the experimental report of Nuanrat and Boonroj (2011), it was found that the chemical that can inhibit the growth of *C.*

gloeosporioides by 100% compared with the control is Prochloraz at a concentration of 2,000 ppm.

Kaffir lime

Common Name: Kaffir lime, Mauritius papeda, Leech lime.

Scientific name: *Citrus hystrix* DC.

Family Name: RUTACEAE

General characteristics: Kaffir lime is a small tree with hardwood and light brown smooth bark. The trunk has many branches from the lower part of the trunk; therefore, the appearance was bush. Trunk and branch have long, sharp spines. Kaffir lime has a compound leaf that petiole could extend into the fin to look like a leaf blade. Kaffir lime leaves are thick, smooth, glossy, green and dark green with age. Leaves are deflated in the middle of the leaf. Therefore, it seems leaves split into 2 or 2 leaves attach each other. Leaf width is about 2.5-5 cm and length is 5-12 cm.

The leaves are very fragrant because of the oil glands. Flowers are perfect sex flowers. The flowers are a bouquet of white flowers that initiate at the top or axillary area of the bush. Each bouquet has about 1-5 flowers; the flowers are white cream with 5 petals and have hairs covered. Inside, the flowers have yellow stamens. The flowers are slightly fragrant and they will fall easily when they it old. Kaffir lime fruit is quite round with 5-7 cm diameter. Kaffir lime fruit is like a tingling orange and the fruit is slightly larger than the lemon fruit. The fruit shape varies depending on the species. The fruit peel is thick, dark green, rough, undulating or nodular. Inside the peel, there are many essential oil glands. There are corks at the head and end of the fruit. When fruit is ripe, it turns yellow. The inside of the fruit consists of juicy meat. There were 5-10 seeds inserted in the middle of the fruit. The fruit had a slightly sour taste.

Kaffir lime propagation

Kaffir lime propagation can be done in many methods such as layering, grafting, budding and seeding. However, the popular methods are layering, splitting and seeding. After the seedlings are ready to be planted, dig a hole for the width x length x depth of approximately 50 x 50 x 50 cm. Add cow dung mixed with soil at the bottom of the hole, cut the black bag out, and bring the seedlings to the plant. Add the soil into the hole and water, then cover with the straw. Place a stake in the hole to prevent swaying when the wind blows. In general, kaffir lime was grown with 2 x 2 meters of distance between plant and row. Therefore, 1 rai will have 400 kaffir lime plants. If planted at 1.5 x 1.5 meters, 1 rai will be 1067 trees. This planting pattern was used when farmers wanted to sell kaffir lime leaves. Since the leaves are cut for sale every 3 - 4 months, the kaffir lime bush will not be very close together. If you want to grow for selling a kaffir lime fruit. Growers may be planted at a distance of 4 x 4 meters, 1 rai will have 200 trees or 5 x 5 meters 1 rai will have 65 trees, etc. Kaffir lime is grown well in all types of soil. The cultivation can be planted at various patterns depending on the purpose and area of the growers.

Chemical composition

Kaffir lime essential oil consists of two main substances: terpenes and non-terpene or oxygenated compounds. For example, kaffir lime skin contains 4% of quickly evaporating volatile oil. The main constituents were 30% beta-pinene, 29% limonene, beta-phellandrene, citronellal. Linalool, borneol, camphor was also found, sabinene, germacrene D, aviprin.

The coumarin group was umbelliferon, bergamottin, oxypeucedanin, psoralen, N- (iminoethyl) -L-ornithine (L-NIO) water. Citric acid was found in fruit water. When distilled with steam, the kaffir lime leaves will have approximately 0.08% volatile oil with the main component about 65% of L-citronellal, citronellol, citronellol acetate. It was also found that sabinene, alpha-pinene, beta-pinene, alpha–phellandrene, limonene, terpinene, cymene, linalool and other substances found are indole alkaloids, rutin, hesperidin, diosmin, alpha-tocopherol. The nutritional value of kaffir lime can be separated as follows.

Nutritional value of kaffir lime leaves (100 g)

171 kcal energy

6.8 g of protein

3.1 g fat

29.0 g carbohydrate

8.2 g fiber

1672 ml calcium

20 mg phosphorus

3.8 mg of iron

303 μg vitamin A

0.20 mg thiamine

0.35 mg riboflavin

1.0 mg niacin

20 mg vitamin C

4.0 g of ash

Nutritional value of the kaffir lime skin (100 g)

21.3 g of carbohydrates

2.8 g protein

1.1 g of fat

3.4 g dietary fiber

322 ml calcium

62 mg phosphorus

1.7 mg of iron

0 mg vitamin B1

0.13 mg vitamin B2

115 mg vitamin C

Nutritional value of kaffir lime juice (100 g)

10.8 g of carbohydrates

0.6 g protein

0 g fat

0 g of dietary fiber

20 ml calcium

20 mg phosphorus

0.6 mg iron

0.02 mg vitamin B1

58 mg vitamin B2

55 mg vitamin C

Pharmacological studies

Anti-inflammatory activity Two types of coumarins obtained from kaffir lime were bergamottin and N- (iminoethyl) -L-ornithine (L-NIO), inhibiting the secretion of nitric oxide (NO) in vitro. Nitric oxide causes Inflammation secreted from the macrophages of stimulated mice by lipopolysaccharide (LPS) and interferon-g (IFN- g) with IC_{50} values of 14.0 μM and 7.9 μM, respectively. Three coumarins, bergamottin, oxypeucedanin and psoralen, were able to inhibit nitric oxide formation when tested in RAW 264.7 macrophage cells of mice stimulated with lipopolysaccharide (LPS) and interferon.

Liver-protective activity Study of the liver-protecting effect of kaffir lime leaves in rats by using the extract of 80% methanol from 200 mg/kg kaffir lime leaves was given 7 days before adding 2 g / kg paracetamol for 5 days to induce hepatotoxicity in rat liver on Day 5. Using silymarin at a dose of 1 0 0 mg/kg as standard. On Day 7, there was an assessment of liver function, including liver enzyme levels (ALT, AST, ALP), total bilirubin, total protein, blood serums and hepatic antioxidants (SOD, CAT, GSH and GPx). The results show that kaffir lime leaf extract helps to restore the liver. It caused liver enzyme levels and antioxidant enzymes to return to normal levels and were statistically significant ($p < 0.001$). This study concluded that kaffir lime leaf extract could protect the liver against paracetamol poisoning.

Effect on hair conditioning Kaffir lime essential oils such as b-pinene (30.6%), limonene (29.2%), sabinene (22.6%), citronellal (4.2%) and fruit juices which contains various vitamins and citric acid (2.56%) when mixed into hair conditioner products compared to chemical alpha hydroxy acids (AHAs), it was found to improve hair condition.

Antioxidant activity The antioxidant activity of the water extract and acetone of the 3 types of kaffir lime leaves (fresh, boiled and fried leaves) was used by three test methods: ORAC, FRAP and DPPH radical scavenging activity. Fried leaves showed the highest antioxidant activity and followed by fresh kaffir lime leaves and boiled kaffir lime leaves, respectively. The chemical composition analysis of the leaves found nine flavonoids, such as theobromine, cyanidin, myricetin, peonidin, quercetin, luteolin, hesperetin, apigenin, and isorhamnetin. Total flavonoids were 1110 ± 74.1, 556 ± 29.7 and 1235 ± 102.5 mg per 100 g of dried leaves, fresh leaves, and boiled leaves. It showed that the highest content was hesperetin. The polyphenols content was 2.0 (fresh), 1.8 (boiled) and 1.9 (fried) g. GAEs / 100 g fresh weight.

Anti-chromosome fracture effect Test in the pedal rat using a micronucleus test in red blood cells to see the inhibition effect of chromosome fracture. Feeding fresh kaffir lime leaves, size 0.2 and 0.4 g. dried weight/1kg/day for 14 days and then add carcinogenicity DMBA (40 mg / kg 1 kg) or MMC (1 mg11kg). After that, the blood was checked at 0, 24 and 48 hrs. The results showed that fresh kaffir lime leaves at 0.2 g / 1 kg trended to reduce similarly chromosomes fractures caused by MMC and DMBA at 24 hrs and 48 hrs, respectively. It was concluded that the cooking process, especially boiling, reduced the content of polyphenol and flavonoids and reduced the antioxidant capacity in kaffir lime leaves. Kaffir lime leaves at the concentration of 0.2 g/1 kg had reduced micronucleus. This is due to the induction of a direct carcinogen, MMC, and a small amount of DMBA-activate liver enzyme carcinogen.

Antibacterial activity Kaffir lime skin extract with alcohol and water and kaffir lime skin extract with ethanol (80%) at a concentration of 5 mg/sheet by Disc diffusion method was found to inhibit bacteria *Staphylococcus aureus*. While the essential oil from the kaffir lime skin at a concentration of 0.5 mg/sheet could not inhibit *S. aureus.*

Antifungal activity Juice extract of kaffir lime leaves at the concentrations 1:10, 1:50, 1: 500 were tested by disc agar diffusion method. The result showed that juice extract inhibited Trichophyton mentagrophytes at 38, 26 and 11%. The juice extract at the ratio 1:50 and 1: 500 was slightly antifungal *Microsporum gypseum*, 25 and 17% respectively.

Kaffir lime essential oil

Kaffir lime can be cooked in many dishes and as a medicinal plant. Two parts are commonly used, such as leaves and fruit. Not only used for such benefits, kaffir lime can also be extracted for the essential oil. The kaffir lime is grown in general appear 3 varieties as follow

1. Large leaf variety. The 7 -8 leaf position from the top has an average length of 10.84 cm and a leaf width of 4.21 cm. The canopy shape was sparse, with large bunches of fruit and large fruit.

2. Generally grown variety. The leaves and fruit are smaller than the first variety. The leaf length was 10.31 cm and the leaf width was 3.79 cm.

3. Small leaves variety. The leaf length was 7.41 cm and the leaf width was 2.92 cm. The tree node was short; the leaves were dark green.

After extracting the essential oil from all 3 varieties, it was found that the essential oils were not statistically different (Boonsiri et al., 2009). Scientists worldwide, including Thailand, have focused on herbal extracts to inhibit the growth of microbial spores. They tried to study the benefits and possibilities of chemical substitutes Because it is a resource that is abundant in Thailand and is safer than chemicals, reduces toxic residue, reduces the resistance of some pests. The research studies on kaffir lime using a solution obtained from 10 g fresh weight of kaffir lime per 100 ml of water can inhibit *C. gloeosporioides*. Because kaffir lime contains B-pinene, citronellal and citric acid as an important compound that inhibits the growth of microorganisms (Noengpa et al., 2004). The research on comparing amount extraction of 3 types of citrus, namely lemon, grapefruit and kaffir lime, by distillation and solvent extraction methods using alcohol and petroleum ether had the highest % yield at 2.188, 2.563 and 2.429 W/W, respectively. Kaffir lime extracted by this

method was able to inhibit *Staphylococus aureus* well. Kaffir lime extract by distillation inhibited the infection 35 mm better than that of lemons and grapefruit (Hiran et al., 2009).

Chitosan

Chitosan is a chain of D-glucosamine polymer. It is chemically named poly-β (1,4) -2-amino-2-deoxy-D-glucose or poly-β (1,4) -2-amino-2-deoxy-D-glucan. Chitosan is sometimes called deacetylated chitin because chitosan is a derivative of chitin caused by chitin deacetylation with alkaline. Chitin removes acetyl ($COCH_3$) from the chitin structure, or hydrolyze group $–NHCOCH_3$, which is eliminated to amino (NH_2) at the second carbon by chitosan formation. The amount of chitosan depended on deacetylation by measuring the degree of deacetylation in percentage of deacetylation (% DD). It is the reduction of the acetyl group in chitin, the result is an increase of the amine group, which increases the polycationic activity on the polymer, increasing the chitinization state. It could be soluble at below pH 6. After chitosan has an amino group in a structure, resulting in relatively good solubility. pH and ionic strength influence chitosan production. The high hydrophilic group or polar properties can be soluble in water and absorbed by hydrogen bonds to form a viscous substance. Therefore, it can be used as a coating for industrial benefit (Nicolas et al., 2010).

Chitosan has been utilized and has researched numerous experiments. Chitosan at 0 .8 % was the best to delay the germination of *Fusarium solani*, *Sclerotium rolfsi* and *Pythium aphanidermatum* (soilborne pathogen) and *Macrophomena phaseolina* (seedbone pathogen) spores

Chitosan coatings with and without lemon essential oil affected volatile strawberry data, affecting the perception of aroma and fruit flavors. The coating affects the metabolic processes of the fruit. Especially chitosan only supports the formation of ester and 2,5-dimethyl-4-methoxy-3 (2H) furanone in a short time after coating, which may improve the perception of a smell. However, adding lemon

essential oil to the coating adds terpenes from lemon essential oil to the fruit's volatile compounds; it also promotes cellular physiological changes, increasing the fermentation process to modify the volatile composition of general fruit. The chitosan coating effect was not a tactile response. This may be because of the difference in the range of consumer acceptance. The changes caused by lemon essential oil were recognized and prevented fungal spoilage (Perdones et al., 2016)

CHAPTER 3

RESEARCH METHODOLOGY

Materials

Mango fruit cv. Nam Dok Mai Sri Tong

Acitic acid 0.5 % Rankem Thailand

Gas Chromatography-Mass Spectrometry (GC-MS)

Chitosan Sigma USA

Weighing machine CP3202S Sartorius company Germany

Colorimeter CR-410 KONIKA MINOLTA Japan

pH meter pH/lon S220 MERTRO USA

Refractometer MASTER Series ATAGO USA

Penetrometer FT-011 PENETROMETER Italy

Stirrer SM-10 MAGNETIC STIRRER Germany

Thermometer

Incubator

Distilled water

Lactophenol cotton blue

Compound microscope

Stereo microscope

Rotary evaporator

Micropipette

Fume hood

Laminar flow cabinet

Hot air oven

Autoclave

Hemocytometer

Chromatographic tank

Measuring cylinder

Airbrush

Capillary tube

Tissue bottle

Brown glass bottle

Conical flask

Beaker

Camera

Stirring rod

Methods

1. Fungal culture

The *C. gloeosporioides* DOAC 2361 isolate originated from mango (*Mangifera indica* Linn.) was provided by the Plant Protection Research Development office, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. For further experiments, the fungus was cultured using potato dextrose agar (PDA, Difco) media and maintained at 25 °C for further experiments.

2. Plant materials and essential oil extraction

Unripe and ripen fruits of kaffir lime were collected from the local garden at Chantaburi province, Thailand. Both the fruits were washed with distilled water and subjected to essential oil extraction. Two thousand grams of fresh peels were removed from unripe and ripened fruits. Each fruit peel was immersed in a 5 L round bottom flask of distilled water and hydro-distilled using Clevenger apparatus set for 3h. Water and essential oil were recovered in a decant bowl, and anhydrous magnesium sulfate was used to dry water traces. Unripe and ripen kaffir lime essential oil was stored at 4 °C in a brown vial prior to being used for the experiment.

3. Bioactive compound analyses by GC-MS

A modified method was used to analyze the chemical components of essential oils using a gas chromatograph equipped with mass spectrometry (GC-MS) (Thirabunyanon and Hongwittayakorn, 2013). The sample preparation was done using 6 µl of each UKLO or RKLO mixed with 1 ml of dichloromethane (J. T. Baker). This chemical component analysis was performed using a GC-MS of an Agilent 6890 series GC (Agilent Technologies, Palo, Alto, CA, USA) coupled to an Agilent 5973 series MS and operated in electron impact mode (70 eV). An HP-5MS capillary column of 30 m x 0.25 mm x 0.25 µm film thickness (Agilent J&W, USA) was used. Each essential oil sample of 1 µl was injected with an injector operating in a split mode ratio of 20:1. The gas chromatograph's oven temperature was programmed as follows: at 60 °C for 2 min, increased at 15 °C per/min to 220 °C and then held constant for 15 min. Helium was used as carrier gas with a flow velocity of 1 ml/min^{-1}. Mass selective detector scanning ions of 15 – 300 atomic mass units were used. Temperatures of the ion source and quadrupole mass analyzer were kept at 230 and 150 °C, respectively. Analyzing the time for each sample was 60 min. The chemical compounds were identified by MSD Chem station software (Agilent Technologies) and matched with the commercial library (NIST98.L and WILEY275.L).

4. Total phenolic compound analyses

The bioactive content as total phenolic compounds from UKLO and RKLO were determined using Folin-Ciocalteu reagent according to modified previously described methods (Abu Bakar et al., 2009; Bajalan et al., 2017). Briefly, 1 ml of each URKO or RKLO sample was added with 5 ml of the Folin–Ciocalteu reagent (Merck) and then mixed thoroughly. After that, 4 ml of 7.5% sodium carbonate (Merck) was added to each sample and mixed thoroughly. The samples were left in the darkroom for 120 min at room temperature. Finally, the samples were determined using a spectrophotometer at 765 nm in comparison to the prepared blank. The total phenolic compounds were expressed as mg gallic acid equivalent per gram (mg GAE/g) of dry weight. This experiment was analyzed in triplicate.

5. Antioxidant activity analyses

The bioactive content as antioxidant activity from UKLO or RKLO was determined using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH, Sigma-Aldrich). The DPPH radical scavenging solution was prepared in ethanol (0.2 mM). The UKLO or RKLO at 0.5 mL was added with 1 mL of DPPH solution and mixed thoroughly. Then, the sample was incubated in the dark for 30 min at room temperature and, after that, read at 517 nm using a spectrophotometer against a blank. As a reference, positive controls of gallic acid (Merck) and **α**-tocopherol (Sigma) were compared. The sample's concentration was provided as 50% inhibition (IC_{50}), which was calculated by plotting inhibition percentages against the sample concentrations (Bajalan et al., 2017; Sepahvand et al., 2014). This evaluation was analyzed in triplicate.

$$\% \text{ DPPH free radical} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test sample.

6. Antifungal activity of kaffir lime essential oils on mycelial growth in vitro conditions

The effects of essential oil extracted from kaffir lime peel with different concentrations on mycelia growth were tested in vitro using the poison food technique (Bussaman et al., 2012). The essential oil concentrations of UKLO and RKLO were used in concentrations such as 750, 1,500, 3,125, 6,250, 12,500, 25,000 and 50,000 ppm. Kaffir lime essential oil of each concentration was disseminated individually as an emulsion in sterilized water containing 5% dimethyl sulfoxide (DMSO). The emulsion solution was added to warm PDA media and poured into the sterile Petri dishes. PDA dishes mixed with Prochloraz (commercial fungicide) and 5% of DMSO were served as positive and negative controls, respectively. The experiments were performed in 10 replicates for each treatment. The 5 mm diameter circular hole was cut with a sterile cork borer from the margin of 7-day-old cultures on the PDA and placed in the center of each Petri dish containing the DMSO, essential oil and Prochloraz treatments. The inoculated dishes were incubated at 25

°C and the diameter of fungal colonies was determined every day for 7 days. Mycelial growth inhibition was calculated as the following formula (Kowsari et al., 2014).

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100,$$

A = diameter of a fungal colony grown on the negative control plate, B = diameter of a fungal colony grown on the plate containing kaffir lime essential oil at different concentrations and Prochloraz.

7. Effect of the kaffir lime essential oil on disease development in vivo conditions

Mature yellow mango fruits (*Mangifera indica* L. cv. Nam Dok Mai Sri Tong) were provided by Thamai Agriculture Cooperative Limited, Chanthaburi province, Thailand. Mango fruit was washed with sterile distilled water and dried at air room temperature. Prochloraz and 5% DMSO were used as a positive and negative control, respectively. All experimental solutions (kaffir lime essential oil, Prochloraz and 5% DMSO) were treated to mango fruits with two different techniques (Estrada et al., 2000; Lima et al., 2014; Lima Oliveira et al., 2018). First techniques (FT), five mango fruits were soaked in each treatment solution for 5 s and dried with air at room temperature. Then mango fruits were pin-wounded in 2 mm depth and then a 5-mm diameter circular disk of *C. gloeosporioides* was placed on the wound for the inoculation. The mango fruits were put in a square plastic basket. The basket was covered with a plastic bag. The sterile distilled water was sprayed into the plastic bag and then the plastic bag was sealed to keep the high moisture. The plastic bags were incubated at 25°C until the fruits rotted at 9 days.

The second technique (ST), mango fruits, was wounded and continued the process described above. However, the mango fruits were incubated at 25 °C for 24 hours. After that, the plastic bag, basket and *C. gloeosporioides* disk were removed from mango fruits. Five mango fruits were soaked in each treatment solution for 5 s

and dried with air at room temperature. The treated fruits have continued incubation at 25 ºC until the fruits rotted at 9 days. In both techniques, lesion development diameter was measured, and which also was calculated the percentage of lesion development inhibition as the following formula.

$$\% \text{ lesion development inhibition} = \frac{A - B}{A} \times 100,$$

A = diameter of a fungal colony grown on the negative control mangoes, B = diameter of a fungal colony grown on the mango containing kaffir lime essential oil and Prochloraz.

8. Effect of kaffir lime essential oil coating on the disease severity index, physicochemical properties, consumer acceptability and shelf-life extension

One hundred and five days after flower blooming, mango fruits were harvested and used as samples in this experiment. The experimental design was a Completely Randomized Design (CRD) with 4 replications. Three treatments were 5% DMSO, 1,500 ppm UKLO and 250 ppm Prochloraz. First, the Mango fruit was washed with sterile distilled water and dried at air room temperature. Then the fruits were soaked in each solution of the treatments and dried at air room temperature. The mango fruits were stored in an incubator at 25 ºC, and the disease severity index was monitored daily until the fruits rotted at 9 days. Disease severity index in treated and control fruits was daily recorded after coating by estimating the surface lesions on a scale from 1 to 6, where 1 = no lesions, 2 = >0–5%, 3 = >5–25%, 4 = >25–50%, 5 = >50–75%, 6 = >75–100% of the total area on mango fruit (Corkidi et al., 2006; de Oliveira KÁ et al., 2017; Lima Oliveira et al., 2018; Sefu et al., 2015).

On 9th day after coating, the mango fruit of three treatments (5% DMSO, 1,500 ppm UKLO and 250 ppm Prochloraz) were determined the weight loss, firmness, soluble solids and titratable acidity. Fruit firmness and soluble solids were checked by using a fruit penetrometer with an 11 mm diameter tip (Effegi, Alphonsine, Italy) and a refractometer (ATAGO, model N1), respectively. The titratable acidity was

determined by titration with 0.1 M NaOH. The consumer acceptability of mango fruit was evaluated on the 9th day after coating. Samples of mango fruits in three treatments (5% DMSO, 1,500 ppm UKLO and 250 ppm Prochloraz) were presented in random order to 40 trained panelists for sensory evaluations. They were rated on a nine-point hedonic scale ranging from 1 (“dislike extremely”) to 9 (“like extremely”) (Estrada et al., 2000; Sharma and Kulshrestha, 2015). Each panelist was evaluated for visual appearance, color, smell, flavor, texture and overall acceptability.

A shelf life extension of coated UKLO mangoes was assessed in this study. The mangoes were randomized divided into three treatments: 5% DMSO, 1,500 ppm UKLO and 250 ppm Prochloraz. Sixteen mangoes per treatment were designed. After coating and storing in an incubator at 25 ºC, the mangoes' shelf life extension was observed every day until it spoiled and then recorded (Naeem et al., 2018; Sefu et al., 2015).

9. Effect of chitosan coating on the disease severity index, physicochemical properties and consumer acceptability.

One hundred and five days after flower blooming, mango fruits were harvested and used as samples in this experiment. The experimental design was a Completely Randomized Design (CRD) with 4 replications. Six treatments were water, acetic acid, 0.25%, 0.50%, 0.75% and 1% chitosan, respectively. Mango fruit was washed with sterile distilled water and dried at air room temperature. Then the fruits were soaked in each solution of the treatments and dried at air room temperature. The mango fruits were stored in an incubator at 25 ºC, and the disease severity index was monitored daily until the fruits rotted at 11 days. Disease severity index in treated and control fruits was daily recorded after coating by estimating the surface lesions on a scale from 1 to 6, where 1 = no lesions, 2 = >0–5%, 3 = >5–25%, 4 = >25–50%, 5 = >50–75%, 6 = >75–100% of the total area on mango fruit (Corkidi et al., 2006; de Oliveira et al., 2017; Oliveira et al., 2018; Sefu et al., 2015).

On 11th day after coating, the mango fruit of six treatments (water, acetic acid, 0.25%, 0.50%, 0.75% and 1% chitosan) were determined the weight loss, color, soluble solids and titratable acidity. The color was determined by using a spectrophotometer (Minolta). Total soluble solids were checked using a refractometer (ATAGO, model N1). Finally, the titratable acidity was determined by titration with 0.1 M NaOH.

The consumer acceptability of mango fruit was evaluated on the 11th day after coating. Samples of mango fruits in six treatments were presented randomly to 20 trained panelists for sensory evaluations. They were rated on a nine-point hedonic scale ranging from 1 (“dislike extremely”) to 9 (“like extremely”) (Estrada et al., 2000; Shrama and Kulshrestha, 2015). Each panelist was evaluated for visual appearance, color, smell, flavor, texture and overall acceptability.

10. Effect of kaffir lime essential oil and chitosan coating on the disease severity index, physicochemical properties and consumer acceptability.

One hundred and five days after flower blooming, mango fruits were harvested and used as samples in this experiment. The experimental design was a Completely Randomized Design (CRD) with 4 replications. Six treatments were water, 1,500 ppm UKLO, 1,500 ppm RKLO, 1500 ppm UKLO + 0.5% chitosan, 1500 ppm RKLO + 0.5% chitosan and 0.5% chitosan, respectively. Mango fruit was washed with sterile distilled water and dried at air room temperature. Then the fruits were soaked in each solution of the treatments and dried at air room temperature. The mango fruits were stored in an incubator at 25 ºC, and the disease severity index was monitored daily until the fruits rotted at 11 days. Disease severity index in treated and control fruits was daily recorded after coating by estimating the surface lesions on a scale from 1 to 6, where 1 = no lesions, 2 = >0–5%, 3 = >5–25%, 4 = >25–50%, 5 = >50–75%, 6 = >75–100% of the total area on mango fruit (Corkidi et al., 2006; de Oliveira et al., 2017; Oliveira et al., 2018; Sefu et al., 2015).

On 11th day after coating, the mango fruit of five treatments (1,500 ppm UKLO, 1,500 ppm RKLO, 1500 ppm UKLO + 0.5% chitosan, 1500 ppm RKLO + 0.5% chitosan and 0.5% chitosan) were determined the weight loss, firmness, soluble solids and titratable acidity. Fruit firmness and soluble solids were checked by using a fruit penetrometer with an 11 mm diameter tip (Effegi, Alphonsine, Italy) and a refractometer (ATAGO, model N1), respectively. The titratable acidity was determined by titration with 0.1 M NaOH.

The consumer acceptability of mango fruit was evaluated on the 11th day after coating. Samples of mango fruits in three treatments (1,500 ppm UKLO, 1,500 ppm RKLO, 1500 ppm UKLO + 0.5% chitosan, 1500 ppm RKLO + 0.5% chitosan and 0.5% chitosan) were presented in random order to 40 trained panelists for sensory evaluations. They were rated on a nine-point hedonic scale ranging from 1 (“dislike extremely”) to 9 (“like extremely”) (Estrada et al., 2000; Shrama and Kulshrestha, 2015). Each panelist was evaluated for visual appearance, color, smell, flavor, texture and overall acceptability.

11. Statistical analysis

All experiments were conducted using a completely randomized design and analysis of variance (ANOVA) using the general linear models. Mean separation by Duncan multiple range tests was carried out for all parameters. The least significant difference between treatment means used the 5% level of significance. All statistical analyses were performed by SPSS version 25.

CHAPTER 4
RESULT AND DISCUSSION

1. Chemical composition of kaffir lime essential oil

As human food is now consumer considering concern about food contamination. These contaminated foods are from several routh of coming. Fungal contamination with human foods is one factor of human disease. In this study, human food safety to consumers, biocontrol, or organic control from the action of kaffir lime essential oil was investigated to protect mango anthracnose caused by C *gloeosporioides* using *in vitro* and *in vivo* evaluation. Bioactive components such as chemical composition, total phenolic compounds, and antioxidant activity were also examined in this prospective kaffir essential oil. Disease severity index, physicochemical characteristics, consumer acceptance, and shelf-life extension were all examined after the essential oil was applied to the mangos.

The major chemical composition of essential oil from unripe and ripened kaffir lime peel and the relative percentage of each compound measured by GC-Mass spectroscopy analysis are shown in Table 2. The major compounds in unripe peel oil were beta-pinene (23.67%), beta-citronellol (13.96%) and 4-terpineol (11.92%), respectively. In ripening peel, the essential oil was primarily composed of limonene (24.62%) followed by lesser quantities of beta-pinene (16.71%) and beta-citronellol (8.0%), respectively. Compared to UKLO and RKLO, several bioactive compounds were obtained only in RKLO, such as cholesterin, squalene and 4-octen-3-one.

This study used two types of kaffir lime peels to extract the essential oil. After GC-MS analysis, the result showed that UKLO contained 17 constituents, of which the major constituents were beta-pinene, beta-citronellol, 4-terpineol, limonene, and sabinene. In the case of RKLO, the results showed that this essential oil contained 25 constituents and the major constituents were limonene, beta-pinene, beta-citronellol, 4-terpineol, and cholesterin. Thus, the analysis results of UKLO and RKLO agreed well with the previous study, which showed high beta-pinene

and limonene contents. However, there was a variation in the chemical composition of kaffir lime oil in this study and the previous reports (Kerdchoechuen et al., 2010; Suwannayod et al., 2018; Warsito et al., 2017). The cause of variation may be plants such as leaf and fruit, the fruit's ripeness, the cultivation, storage process, and extraction procedures (Fanciullino et al., 2006).

Table 2 Chemical composition (relative area %) of essential oils extracted from kaffir lime peels

(Mean ± SD, n = 2)

Compounds	Retention time (min)	Kaffir lime essential oil	
		Unripe	Ripen
alpha-Pinene	5.103	1.81 ± 0.01	1.30 ± 0.11
Sabinene	6.269	10.49 ± 0.01	5.10 ± 0.44
beta-Pinene	6.362	23.67 ± 0.07	16.71 ± 1.42
beta-Myrcene	6.846	0.94 ± 0.03	0.97 ± 0.05
alpha-Terpinene	7.695	0.94 ± 0.01	0.97 ± 0.10
Benzene	8.017	0.46 ± 0.01	0.38 ± 0.02
Limonene	8.17	10.99 ± 0.01	24.62 ± 1.93
1,4-Cyclohexadiene	9.17	1.96 ± 0.01	1.86 ± 0.15
trans-Linalool oxide	9.91	4.34 ± 0.08	4.30 ± 0.32
Terpinolen	10.426	0.48 ± 0.01	0.52 ± 0.04
2-Furanmethanol	10.545	2.33 ± 0.06	2.33 ± 0.20
Cyclohexene	11.104	2.44 ± 0.06	1.50 ± 0.01
iso-Pulegol	12.572	0.75 ± 0.01	0.41 ± 0.15
Citronella	13.131	7.90 ± 0.04	2.86 ± 1.16
4-Terpineol	13.949	11.92 ± 0.27	7.44 ± 4.06
Linalyl propionate	14.564	4.43 ± 0.10	4.56 ± 1.82
beta-Citronellol	15.904	13.96 ± 0.23	8.00 ± 4.32
Citronelly acetate	19.802	-	0.33 ± 0.15
alpha-Copaene	22.625	-	0.47 ± 0.33
delta-Cadinene	34.972	-	0.40 ± 0.04
4-Octen-3-one	43.645	-	0.52 ± 0.04
Cholesta-4,6-dien-3-ol	43.999	-	0.43 ± 0.42
Cholesterilene	45.977	-	6.08 ± 5.14
Squalene	55.59	-	1.23 ± 0.11
Pyrazine	55.864	-	0.59 ± 0.17

2. Total phenolic compounds of kaffir lime essential oil

The total phenolic compounds from unripe and ripe kaffir essential oil by the Folin-Ciocalteu reagent were determined, shown in Table 3. Comparing unripe and ripe kaffir essential oil, the total phenolic compounds from UKLO were found to have a tendency higher than that of RKLO. These total phenolic compounds of UKLO and RKLO were 16.17 and 15.76 GAE/g, respectively.

The bioactive compound was obtained, such as total phenolic compounds from the kiffir essential oil. It is known that these compounds contain the hydroxyl groups, which had action to destroy radicals (Bajalan et al., 2017), indicating that the phenolic compounds are beneficial for health modulation. In this observation, URLO and RKLO have phenolic compounds. Furthermore, similar to our result, the rich phenolic contents from kaffir essential oil were also reported (Mahomoodally et al., 2019). Thus, the kaffir essential oil might have an important role in human food application.

Table 3 Compositions of total phenolic compounds and antioxidant activity from kaffir essential oil

(Mean ± SD, n = 3)

Treatment	Total phenolic compounds (GAE/g)	Antioxidant activity (IC_{50}, mg/ml)
UKLO	16.17 ± 1.81a	36.14 ± 9.11a
RKLO	15.76 ± 1.74a	31.62 ± 2.07a
Gallic acid	-	2.95 ± 0.16c
α-Tocopherol	-	7.03 ± 0.23b

Note: Means followed by the different alphabet are significantly different at 5% level.

3. Antioxidant activity of kaffir lime essential oil

The DPPH scavenging activity of unripe and ripe kaffir essential oil was measured, shown in Table 2. As reference compared to which of the gallic acid and α-tocopherol as known antioxidants. Both UKLO and RKLO have an antioxidant activity less than the reference known antioxidants. However, which antioxidant activity from UKLO and RKLO did not differ between them. As a known antioxidant compared, the antioxidant capacity with the value of IC_{50} in UKLO, RKLO, gallic acid and α-tocopherol was 36.14, 31.62, 2.95 and 7.03 mg/ml, respectively.

Antioxidant activity is a good health index known to be useful for consumers (Ramli et al., 2020). DPPH assay expressed the H-donating capacity of compounds, and antioxidants acting on hydrogen removal protect lipids against oxidation (Djebari et al., 2021). The results obtained in this study as URLO and RKLO had relatively high antioxidant activity as of DPPH assay with the IC_{50} value of 36.14 and 31.62 mg/ml, respectively. As confirmed, this property of kaffir lime essential oil, our results were better than the previous report that exhibited at 0.761 µg/ml using DPPH assay (Mahomoodally et al., 2019). This good antioxidant activity of kaffir lime essential oil indicated that it might be useful for consumer application.

4. Effect of kaffir lime essential oil on in vitro mycelial growth

Effect of unripe and ripen kaffir lime essential oils (UKLO and RKLO) on in vitro mycelial growth of *C. gloeosporioides* was shown in Figure 1 (A; control (5% DMSO), B; 50,000 ppm UKLO, C; 50,000 ppm RKLO, D; 25,000 ppm UKLO, E; 25,000 ppm RKLO, F; 12,500 ppm UKLO, G; 12,500 ppm RKLO, H; 6,250 ppm UKLO, I; 6,250 ppm RKLO, J; 3,125 ppm UKLO, K; 3,125 ppm RKLO, L; 1,500 ppm UKLO, M; 1,500 ppm RKLO, N; 750 ppm UKLO, O; 750 ppm RKLO and P; Prochloraz). After 7 days of inoculation, the results showed a significant difference in colony diameter among treatments. The highest colony diameter was found in PDA containing 5% DMSO (control) and followed with 750 ppm RKLO, 750 ppm UKLO, 1,500 ppm RKLO and 3,125 ppm RKLO, which the diameter was 8.48, 3.17, 1.90, 1.53 and 0.26 cm, respectively. However, there was no mycelial growth in PDA containing more than

1,500 ppm UKLO and 3,125 ppm RKLO (Table 4 and Fig 1). Mycelial inhibition significantly differed among treatments. The inhibition was 100% in PDA containing more than 1,500 ppm UKLO and 3,125 ppm RKLO. The mycelial inhibition of 3,125 ppm RKLO, 1,500 ppm RKLO, 750 ppm UKLO and RKLO was 96.93%, 81.96%, 77.59% and 62.62%, respectively. The lowest mycelial inhibition was found in 5% DMSO treatment, 0.00% (Table 4).

In fact, *in vitro* antifungal kaffir lime oil activities showed that UKLO at a 1,500 to 50,000 ppm concentration exhibited the highest inhibition against C. gloeosporioides mycelial growth (100%) RKLO at 3,125 to 50,000 ppm also could inhibit. It may be due to the antifungal chemical composition activities in UKLO and RKLO, such as limonene and beta-pinene (Hong et al., 2015; Nazzaro et al., 2017). A similar study reported that ethyl acetate extract of kaffir lime showed a broad spectrum of inhibition against all Gram-positive bacteria, yeast and molds *Saccharomyces cerevisiae* and *Aspergillus fumigatus* (Chanthaphon et al., 2008). However, the percentage of mycelial inhibition in this study was higher than that reported (Bussaman et al., 2012). This is due to the different extraction methods of hydro-distillation in this study, but it was a rotary evaporator. This process may affect the chemical constituents in kaffir lime extraction and influence the extraction's antifungal properties.

Table 4 Effect of unripe and ripen kaffir lime essential oils (UKLO and RKLO) on in vitro mycelial growth colony diameter and mycelial growth inhibition of *C. gloeosporioides*

(Mean ± SD, n = 10)

Treatments	Colony diameter (cm)	Mycelial inhibition (%)
Control (5% DMSO)	8.48 ± 0.04[a]	0.00 ± 0.00[e]
750 ppm UKLO	1.90 ± 0.61[c]	77.59 ± 7.16[c]
750 ppm RKLO	3.17 ± 0.41[b]	62.62 ± 4.81[d]
1,500 ppm UKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
1,500 ppm RKLO	1.53 ± 0.61[d]	81.96 ± 7.14[b]
3,125 ppm UKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
3,125 ppm RKLO	0.26 ± 0.02[e]	96.93 ± 4.85[a]
6,250 ppm UKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
6,250 ppm RKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
12,500 ppm UKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
12,500 ppm RKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
25,000 ppm UKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
25,000 ppm RKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
50,000 ppm UKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
50,000 ppm RKLO	0.00 ± 0.00[e]	100.00 ± 0.00[a]
Prochloraz	0.00 ± 0.00[e]	100.00 ± 0.00[a]

Note: Means followed by the different alphabet are significantly different at 5% level.

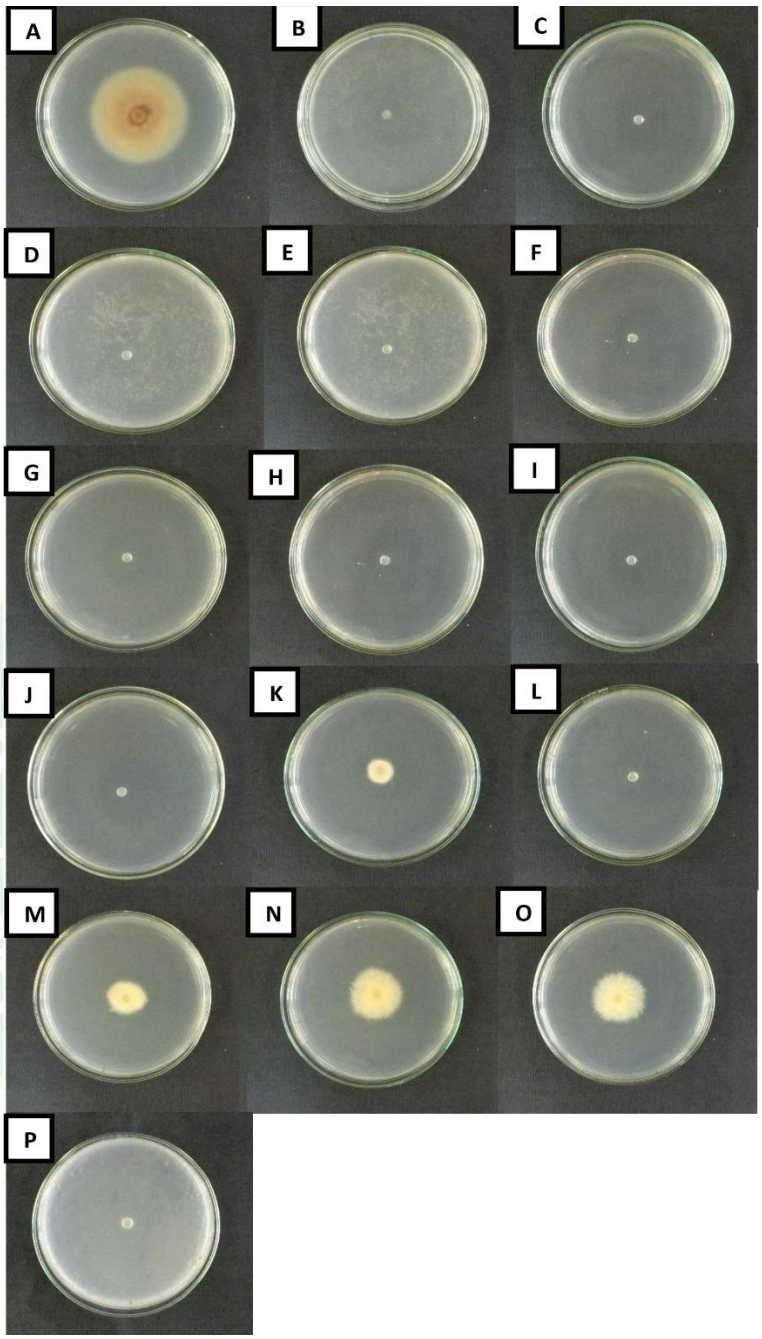

Figure 1 Effect of unripe and ripen kaffir lime essential oils (UKLO and RKLO) on in vitro mycelial growth of *C. gloeosporioides.*

5. Anthracnose suppression in vivo conditions on mango fruits by kaffir lime essential oil

Effect of unripe and ripen kaffir lime essential oils (UKLO and RKLO) on *in vivo* mycelial growth of *C. gloeosporioides* using first technique (FT) was shown in Figure 2 (A; control (5% DMSO) at day 0, B; control at day 5, C; control at day 9, D; UKLO at day 0, E; UKLO at day 5, F; UKLO at day 9, G; RKLO at day 0, H; RKLO at day 5, I; RKLO at day 9, J; Prochloraz at day 0, K; Prochloraz at day 5 and L; Prochloraz at day 9). Effect of unripe and ripen kaffir lime essential oils (UKLO and RKLO) on *in vivo* mycelial growth of *C. gloeosporioides* using the second technique (ST) was shown in Figure 3 (A; control (5% DMSO) at day 0, B; control at day 5, C; control at day 9, D; UKLO at day 0, E; UKLO at day 5, F; UKLO at day 9, G; RKLO at day 0, H; RKLO at day 5, I; RKLO at day 9, J; Prochloraz at day 0, K; Prochloraz at day 5 and L; Prochloraz at day 9). According to the previous experiment results, 1,500 ppm of kaffir lime essential oil was found to have a high level of activity against *C. gloeosporioides* mycelial growth. Therefore, it was selected for further testing to suppress anthracnose on mango fruit. There was no significant difference in lesion diameter between 5 % DMSO of the first and second techniques, 2.63 and 2.58 cm. The lesion diameter in 5 % DMSO of the first and second techniques was highest compared to the other treatments. The lesion diameter of UKLO, RKLO and Prochloraz treatments using the second technique showed a bigger lesion diameter than the UKLO, RKLO and Prochloraz treatments using the first technique. There was no difference in lesion diameter between UKLO, RKLO and prochloraz treatments for the second technique. Only UKLO treatment revealed a similar lesion diameter as Prochloraz treatments in the first technique. Finally, the UKLO and Prochloraz treatments using the first technique expressed the smallest lesion diameter, 1.19 and 1.07 cm, respectively (Table 5, Fig 2 and 3).

The effect of UKLO and RKLO on *in vivo* mycelial inhibition of *C. gloeosporioides* is shown in Table 4. There was a significant difference in lesion inhibition among treatments. The lowest inhibition was found in 5% DMSO of both techniques. The inhibition percentage of UKLO, RKLO and Prochloraz treatments using the first technique were higher than those using the second technique. For the

first technique, only UKLO treatment showed a similar inhibitory level as Prochloraz treatment, while there was no significant difference in lesion inhibition among UKLO, RKLO and Prochloraz treatments using the second technique. The highest inhibition was Prochloraz and UKLO treatments using the first technique: 55.78% and 50.83%, respectively.

The UKLO, RKLO and Prochloraz with the first technique showed higher inhibition than those using the second technique. It indicates that the antifungal activities of UKLO, RKLO and Prochloraz before *C. gloeosporioides* attacking were higher effective than those after *C. gloeosporioides* attacking. Interestingly, the result of lesion inhibition exhibited that UKLO and RKLO could reduce lesion on mango fruit as similar to Prochloraz in both the first and second techniques. These are indicated that essential oil from kaffir lime peel was efficient in inhibiting *C. gloeosporioides.* A previous study reported that it kept anthracnose severity (lesion development) below 5% during much of the 12 days experimental period, while severity on untreated fruit reached 29% (Estrada et al., 2000). Furthermore, Preharvest application of biocontrol agents in fruit crops, viz., apples, grapes, strawberries, and avocados, significantly controlled the postharvest disease symptoms caused by Colletotrichum's species Botrytis and Rhizopus (Nazzaro et al., 2017). These indicated that kaffir essential oil might be applied as an organic protecting agent using pre- and post-fungal contamination.

Table 5 Effect of unripe and ripen kaffir lime essential oils (UKLO and RKLO) on in vivo disease lesion diameter and disease lesion inhibition of *C. gloeosporioides*

(Mean ± SD, n = 16)

Treatments	Lesion diameter (cm)	Lesion inhibition (%)
Control + FT	2.63 ± 0.25a	0.00 ± 0.00
Control + ST	2.58 ± 0.14a	0.00 ± 0.00
UKLO + FT	1.19 ± 0.11cd	50.83 ± 4.71
UKLO + ST	1.92 ± 0.25b	21.63 ± 10.06
RKLO + FT	1.39 ± 0.28c	42.56 ± 11.45
RKLO + ST	2.04 ± 0.24b	16.73 ± 9.72
Prochloraz + FT	1.07 ± 0.16d	55.78 ± 6.79
Prochloraz + ST	2.03 ± 0.23b	17.14 ± 9.20

Note: Means followed by the different alphabet are significantly different at 5% level.

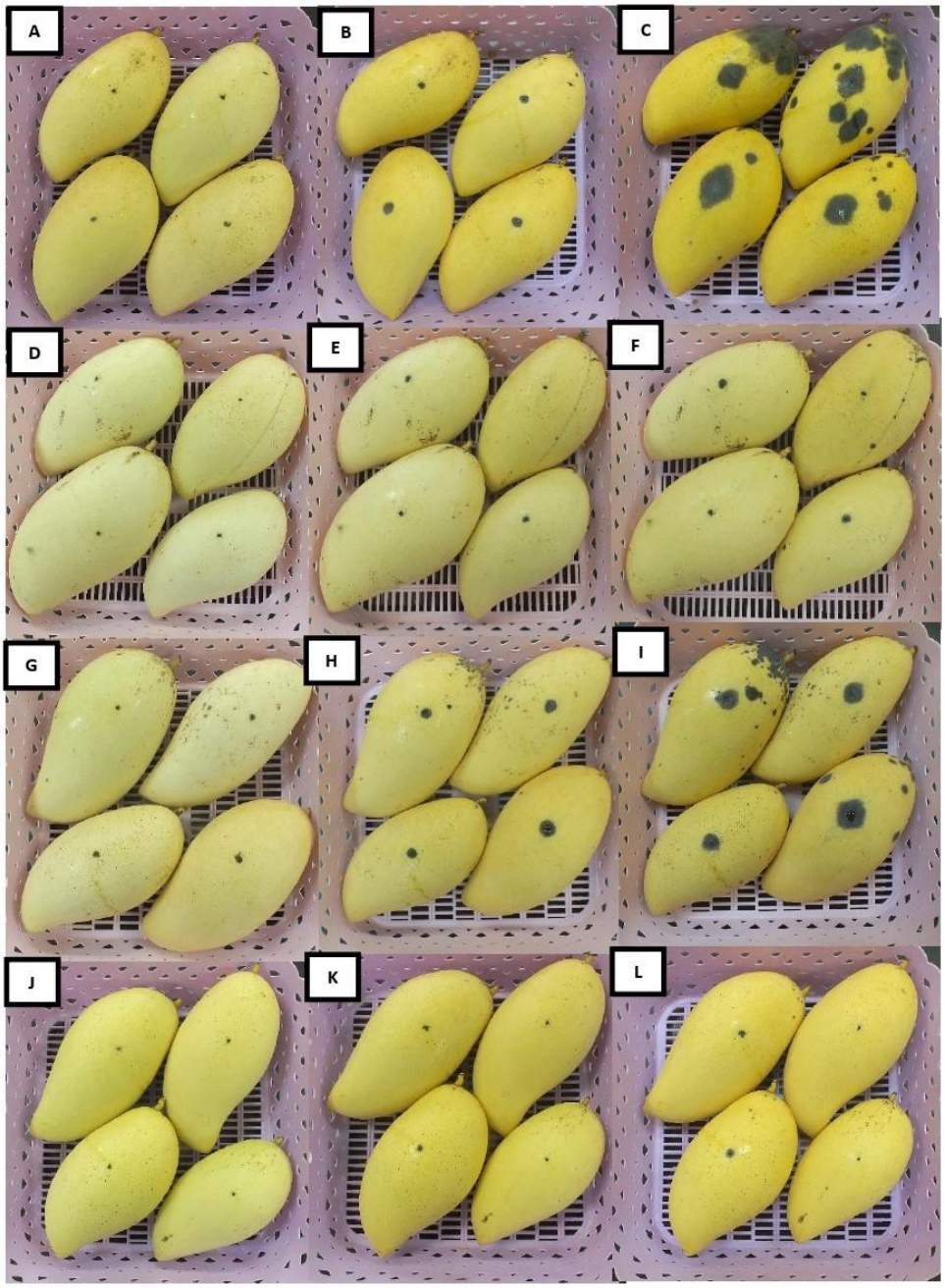

Figure 2 Effect of unripe and ripen kaffir lime essential oils (UKLO and RKLO) on *in vivo* mycelial growth of *C. gloeosporioides* using first technique (FT).

Figure 3 Effect of unripe and ripen kaffir lime essential oils (UKLO and RKLO) on *in vivo* mycelial growth of *C. gloeosporioides* using second technique (ST).

6. Effect of kaffir lime essential oil coating on the disease severity index

The disease severity index in control, UKLO and Prochloraz treatments is shown in Fig 4 and 5. In Figure 5, A; control (5% DMSO) at day 0, B; control at day 3, C; control at day 6, D; control at day 9, E; UKLO at day 0, F; UKLO at day 3, G; UKLO at day 6, H; UKLO at day 9, I; Prochloraz at day 0, J; Prochloraz at day 3, K; Prochloraz at day 6 and L; Prochloraz at day 9. The results showed that the mango coating with UKLO at 1,500 ppm had significantly reduced the disease severity index ($p < 0.05$) as scored 1.00 compared to scored 3.13 of the control group. However, compared to the 1,500 ppm UKLO and Prochloraz, there was no significant difference between the groups.

Commercially applying kaffir lime oil using by coating on mango, resulting in the UKLO coating group could significantly reduce the disease severity index and using by coating on mango, resulting in UKLO coating group could significantly reduce disease severity index and Prochloraz compared to uncoating group of control. Several chemical compounds were obtained from UKLO, such as beta-pinene, beta-citronellol, 4-terpineol, limonene and sabinene. Moreover, bioactive compounds, including total phenolic and antioxidant compounds, were also obtained. These beneficial compounds might be protecting fungal contamination that can contaminate the mango during storage. It is not only to protect against fungal anthracnose but also this UKLO could preserve mango as the exact incidence. Thus, the essential oil from other plants, such as 0.45% ginger and 0.075% cinnamon, was reported to reduce disease incidence and severity in mango anthracnose caused by *C. gloeosporioides* (Sefu et al., 2015).

Furthermore, coating *mango* cv. Tommy Atkins combines chitosan and *Mentha piperita* L. essential oil (de Oliveira et al., 2017) or chitosan and *Cymbopogon citratus* (D.C. ex Nees) Stapf. the essential oil can reduce the anthracnose lesion's severity that contaminated several pathogenic *Collectotrichum* species (Lima Oliveira et al., 2018). These suggest that UKLO might be suitable as an organic protective fungal contaminating compound of human food such as mango.

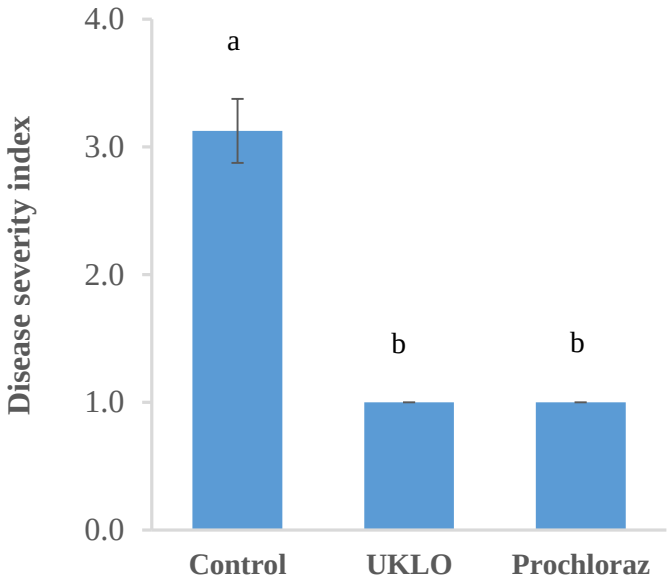

Figure 4 The mango coating with 5% DMSO (control), 1,500 ppm UKLO and Prochloraz on disease severity index

Means followed by the different alphabet are significantly different at 5% level (Mean ± SD, n = 16).

Figure 5 The mango coating with control (5% DMSO), 1,500 ppm UKLO and Prochloraz on disease severity caused by fungal contamination

7. Effect of kaffir lime essential oil coating on the physiochemical properties

The physiochemical properties of DMSO, 1,500 ppm UKLO and Prochloraz treatments are shown in Table 6. There was no significant difference in weight loss, firmness, soluble solids, and titratable acidity of mango fruits among treatments. The weight loss of mango fruit in DMSO, UKLO and Prochloraz treatments was 7.19, 7.11 and 7.97, respectively. The firmness of mango fruit ranged from 0.53 to 0.65. The soluble solid was from 12.50 to 13.50 °Brix. The titratable acidity was 0.31, 0.27 and 0.25 in DMSO, UKLO and Prochloraz treatments, respectively.

Physicochemical properties of mango during storage are important points of product quality preservation. After commercially applying a coated UKLO mango for 9 days, the weight loss percentage was not different among groups, but this UKLO tends to protect against weight loss compared to other groups. This reason may correlate with the interval time of storage. Our results, similar to a previously reported chitosan and alginate-based coatings enriched with cinnamon essential oil, can protect against mango weight loss during storage at days 12 and 14, but not affect days 0 – 10 (Yin et al., 2019). The firmness of the coated UKLO mango during storage showed a tendency compared to the control and Prochloraz. Since increasing time of storage is correlates with diseases, the mango firmness. This may be why the pectin in mangoes gradually decomposes into soluble pectin; thus, firmness is a key indicator of mango freshness (Koh et al., 2017). However, it is not obtained in firmness change of coated UKLO mango in our investigation but due to preservation time. As firmness of uncoated mango was gradually significantly decreased beginning at day 10, while cinnamon and ginger essential oils could preserve the firmness during its storage (Sefu et al., 2015).

The change insoluble solid of coated UKLO mango was not significantly discovered in this investigation, but it only tends to be lower than control and Prochloraz groups. This soluble solid is known as an indicator of fruit quality and it's directly proportional to sugar content; also, this content is closely related to metabolism, such the more substances are consumed, reducing soluble solid as the same incidence (Jiang et al., 2013). The coated mango with chitosan and alginate-based enriched with cinnamon essential oil can begin to significantly decrease the

soluble solid during the storage period after day 8 (Yin et al., 2019). This lower soluble solid may due to a long time of mango preservation.

As the titratable acidity of coated UKLO mango did not differ from among control and Prochloraz groups. This factor is known as one of the most important indicators of mango quality. Thus, the titratable acidic content is gradually converted into sugar during mango storage due to later reduction in this titratable acidity (Hong et al., 2012). Significantly, the coated mango's essential oil may reduce the mango's respiration rate, slowing their metabolic reaction processes and maintaining them with a fresh and full appearance (Yin et al, 2019). The low concentration of cinnamon oil at 0.025% of 10 days did not change the percentage of titratable acidity of coated mango, but not in a high concentration of coated mango, which is higher than that of the control group (Sefu et al., 2015). For this reason, our investigated unchanged results may be due to the storage time and UKLO used concentration.

Table 6 Effect of kaffir lime essential oil coating on weight loss, firmness, soluble solid and titratable acidity of mango fruits after storage for 9 days

(Mean ± SD, n = 4)

Treatments	Weight loss (%)	Firmness (Kg/cm^2)	Soluble solid (°Brix)	Titratable acidity (%)
Control	7.19 ± 0.53^a	0.61 ± 0.03^a	13.50 ± 2.52^a	0.31 ± 0.22^a
UKLO	7.11 ± 0.55^a	0.65 ± 0.15^a	12.50 ± 1.73^a	0.27 ± 0.07^a
Prochloraz	7.97 ± 1.13^a	0.53 ± 0.03^a	13.25 ± 1.89^a	0.25 ± 0.07^a

Note: Means followed by the same alphabet are not significantly different at 5% level.

8. Effect of kaffir lime essential oil coating on the consumer acceptability

Effect of kaffir lime essential oil coating on the score of visual appearance, color, smell, flavor, texture, and acceptability of mango fruits after storage for 9 days results were shown in Table 7. The results showed no difference in the score of visual appearance, color, smell, flavor, texture, and overall acceptability of mango fruits coated by DMSO, UKLO, and Prochloraz. The visual appearance and color score ranged from 6.58 to 6.93 and from 6.60 to 6.75. The smell score of mango fruit was 6.33, 6.65 and 6.15 in DMSO, UKLO and Prochloraz treatments, respectively. In flavor and texture, the score was from 5.90 to 6.45 and from 6.03 to 6.45, respectively. Finally, the overall acceptability score of mango fruit in DMSO, UKLO and Prochloraz treatments was 6.35, 6.58 and 6.58, respectively.

Consumer acceptability is one of the main indicators when using UKLO as in commercial applications. Although all parameters, including visual appearance, color, smell, flavor texture and overall acceptability, were not different among groups, all indicators in UKLO tend to be better than that of a control group or some of them also higher than Prochloraz. Our observation, mango smell from UKLO group, had relatively clear better than control and Prochloraz at the scoring rate of 6.65, 5.90 and 6.15 in UKLO, control and Prochloraz, respectively. This good smell may come directly from the UKLO, since kaffir essential oil is good for general people. In fact, kaffir essential oil can also be commercially applied in the food industry, perfumery, aromatherapy, and medicinal usage (Agouillal et al., 2017; Ng et al., 2011; Srifuengfung et al., 2020). As the comparison of UKLO and Prochloraz, the exportation of these mangoes worldwide is normally using Prochloraz to protect against fungal contamination. There is no difference in the consumer acceptability of these two groups, indicating that UKLO may be used as an alternative organic protective compound against fungal contamination in mangoes.

Table 7 The mango coating with control (5% DMSO), 1,500 ppm UKLO and Prochloraz on the score of consumer acceptability

(Mean ± SD, n = 40)

Treatment	Consumer acceptability					
	Visual appearance	Color	Smell	Flavor	Texture	Overall acceptability
Control	6.58a	6.60a	6.33a	5.90a	6.03a	6.35a
UKLO	6.93a	6.75a	6.65a	6.35a	6.15a	6.58a
Prochloraz	6.90a	6.75a	6.15a	6.45a	6.45a	6.58a
F-test	ns	ns	ns	ns	ns	ns

Note: Means followed by the same alphabet are not significantly different at 5% level.

9. Effect of kaffir lime oil coating on shelf-life extension

As shown in Figure 6. the coated UKLO mangoes were significantly extended ($p < 0.05$) shelf-life extension from the day of beginning until it was spoiled during storage at 12.25 days compared to that of the uncoated control group at 9.50 days. However, these coated UKLO mangoes were not different from the Prochloraz group, which prolonged shelf life at 12.50 days. Prolong shelf life is one indicator that is very important when carrying out mango management during transportation and at the time of selling in the markets. The coated UKLO mangoes significantly extended the shelf life during storage; this made it easy in the processing management and might get more earning at the end. The extended days of coated UKLO mangoes were increased by about 2.75 days more than uncoated mangoes. This means that it extends at about 22.44% of storage interval time. This result is similar to cinnamon and ginger essential oil (Sefu et al., 2015) and guar gum coating coupled with essential oils from *Nigella sativa*, *Coriandrum sativum*, *Foeniculum vulgare* and *Laurus nobilis,* which for mango postharvest preservation (Naeem et al., 2018). This

suggested that UKLO may be useful for prolonging the shelf life of mango preservation.

The economic feasibility can be achieved by producing a combination of high-value products such as essential oils, pectin, phenolic compounds and antifungals. Based on the available technology, irregular supply of primary raw material, and considering the market demand, a more generalized biorefinery can be prescribed, focusing on biomaterials and biochemicals that include ethanol, essential oils, phenolic compounds, methane, and syngas. Consequently, this study proposed a new industrial approach for the treatment of kaffir lime peel waste to obtain products (as antifungal products) and biorefinery applications for controlling mango fruit anthracnose by *C. gloeosporioides*.

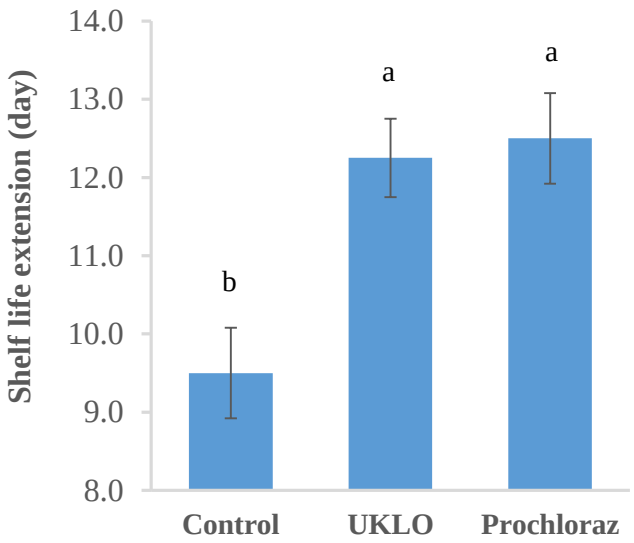

Figure 6 The mango coating with control (5% DMSO), 1,500 ppm UKLO and Prochloraz on the shelf-life extension

Means followed by the different alphabet are significantly different at 5% level (Mean ± SD, n = 16).

10. Effect of chitosan coating on the disease severity index

Chitosan is a natural biopolymer that contains many benefits for humans. One of chitosan utilization is using for coating agricultural production to extend storage time such as chili (Lacap and Photchanachai, 2018), papaya (Kakaew et al., 2008) and orange (Paiboonsombat and Panhasemsuk, 2010). This experiment showed that mango coating with chitosan at 0.50%, 0.75% and 1.00% had significantly reduced the disease severity index ($p < 0.05$) as scored 1.00 compared to 1.75 and 2.00 of the acetic acid and water treatments. However, there was no significant difference in severity index among the chitosan treatments (Table 8 and Fig 7). In Figure 7, Upper row; left = water coating, middle = 0.5% acetic acid, right = 0.25% chitosan. Lower row; left = 0.50% chitosan, middle = 0.75% chitosan, right = 1.00% chitosan. This may be due to the coating of chitosan covering the natural opening hole on fruit surface and resulted in decreased deterioration from postharvest disease microorganism similar to the report of strawberry (Boonyakiat and Sehanam, 2003; Seehanam et al., 2006).

Table 8 Effect of chitosan coating on the disease severity index and weight loss of mango cv. Nam Dok Mai Sri Tong after storage for 11 days.

Treatments	Disease severity index	Weight loss (%)
Water	2.00a	15.40a
Acetic acid 0.5%	1.75ab	15.08a
Chitosan 0.25 %	1.25bc	12.48a
Chitosan 0.50 %	1.00c	9.76b
Chitosan 0.75 %	1.00c	9.59b
Chitosan 1.00 %	1.00c	9.51b
F-test	*	**

Note: Means followed by the different alphabet are significantly different at 5% level.

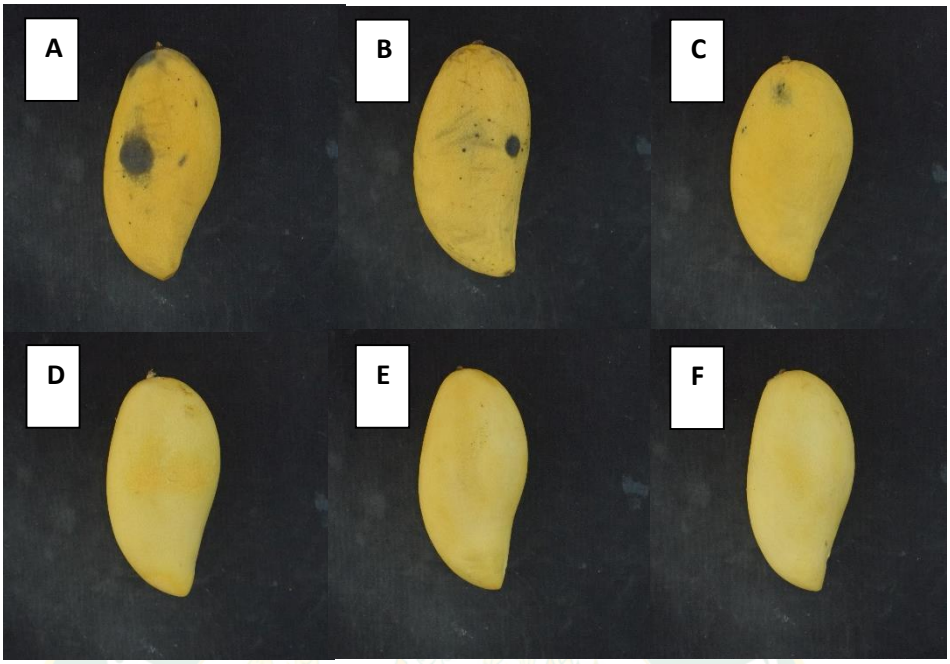

Figure 7 Effect of chitosan coating on the external change and disease severity index of mango cv. Nam Dok Mai Sri Tong after storage for 11 days.

11. Effect of chitosan coating on the physicochemical properties

After coating agricultural production with chitosan, the gas exchange was limited, and the production respiration also was reduced. Finally, the water in production could maintain. Similarly, the results in this experiment also revealed that the weight loss of mango fruit coating with chitosan at 0.50%, 0.75%, and 1.00% was 9.76%, 9.59% and 9.51%, respectively, which were significantly lower than those of mango coating with the other solutions. However, there was no significant difference when compared the weight loss of mango coating with chitosan at 0.50%, 0.75% and 1.00% (Table 9).

Normally, the fruits will respirate, change color, increase soluble solids and decrease titratable acidity after postharvest, in the case of mango cv. Nam Dok Mai Sri Tong, the light yellow color on the fruit surface at the unripened stage, will be the dark yellow color at the ripening stage. After 11^{th} storage day, the result of ΔL value ranged from 10.60 to 11.95 and did not differ among the treatments. However, there was a significant difference in Δa and Δb between the control and treatments. Namely, the mango coating with chitosan at 0.50%, 0.75% and 1.00% showed a

significant lower of Δa and Δ b than the water, acetic acid, and chitosan at 0.25%. The significant difference of Δa and Δb between 0.50%, 0.75% and 1.00% of chitosan treatments did not appear. Chitosan at 0.50%, 0.75% and 1.00% treatments significantly reduced the soluble solids compared to the remaining treatments (Table 9). In addition, chitosan at 0.50%, 0.75% and 1.00% treatments also significantly maintained the titratable acidity. However, there was no significant difference in the soluble solids and titratable acidity between 0.50%, 0.75% and 1.00% of chitosan treatments (Table 10). This may indicate that chitosan at 0.50%, 0.75% and 1.00% could postpone the ripen stage of mango fruit. It may be due to the coating of chitosan could slow down the fruit respiration and gas exchange; therefore, the fruit ripens are also delayed. The result was in line with Kwanhong andRatanachinakorn (2014) research, which reported that the chitosan coating could retard the color change and preserve the freshness of rose apple.

Table 9 Effect of chitosan coating on the color change of mango cv. Nam Dok Mai Sri Tong after storage for 11 days.

Treatment	Color change		
	ΔL	Δa	Δb
Water (T1)	11.95a	7.60a	8.26a
Acetic acid 0.5% (T2)	11.23a	7.50a	8.18a
Chitosan 0.25 ppm (T3)	11.41a	5.79a	6.84a
Chitosan 0.50 ppm (T4)	11.22a	2.38b	4.88b
Chitosan 0.75 ppm (T5)	10.60a	2.87b	4.67b
Chitosan 1.00 ppm (T6)	10.84a	2.26b	4.84b
F-test	ns	**	**

Note: Means followed by the different alphabet are significantly different at 5% level.

Table 10 Effect of chitosan coating on the soluble solids and titratable acidity of mango cv. Nam Dok Mai Sri Tong after storage for 11 days.

Treatment	Soluble solids (°Brix)	Titratable acidity (%)
Water	17.47a	0.33b
Acetic acid 0.5%	18.00a	0.27b
Chitosan 0.25 ppm	17.17a	0.34b
Chitosan 0.50 ppm	15.27b	0.93a
Chitosan 0.75 ppm	15.33b	1.06a
Chitosan 1.00 ppm	15.20b	1.10a
F-test	**	**

Note: Means followed by the different alphabet are significantly different at 5% level.

12. Effect of chitosan coating on the consumer acceptability

The sensory evaluation results found that the mango coating with chitosan at 0.50%, 0.75%, and 1.00% had a significantly lower acceptability score in visual appearance, color, and flavor than the other solutions. However, the acceptability score in smell, texture, and overall acceptability did not differ among all treatments. The score of consumer acceptability corresponded to the result of soluble solids and titratable acidity. The score results showed that the consumer accepted the visual appearance, color and flavor of mango coating with water, acetic acid and chitosan at 0.25% more than chitosan at higher concentrations (Table 11). This may be because mango coating with water, acetic acid, and chitosan at 0.25% turned to the ripen stage while the remaining treatments were still unripened. This result could be the evidence that the chitosan at 0.50%, 0.75% and 1.00% could slow down the ripening stage of mango fruit.

Table 11 Effect of chitosan coating on the consumer acceptability of mango cv. Nam Dok Mai Sri Tong after storage for 11 days.

Treatment	Consumer acceptability					
	Visual appearance	Color	Smell	Flavor	Texture	Overall acceptability
Water	8.55a	8.56a	8.50	8.60a	7.46	7.50
Acetic acid 0.5%	8.45a	8.44a	8.38	8.33ab	7.38	7.57
Chitosan 0.25 ppm	8.27a	8.22a	8.13	8.00b	7.15	6.86
Chitosan 0.50 ppm	7.73b	7.83b	8.00	6.87c	6.62	6.21
Chitosan 0.75 ppm	7.82b	7.89b	8.03	6.80c	6.54	6.57
Chitosan 1.00 ppm	7.72b	7.87b	8.06	6.67c	6.69	6.28
F-test	*	*	ns	**	ns	ns

Note: Means followed by the different alphabet are significantly different at 5% and 1% levels.

13. Effect of kaffir lime essential oil and chitosan coating on the disease severity index

After coating with water, kaffir lime essential oil and chitosan for 11 days, the result showed that the coating with kaffir lime essential oil, chitosan and the combination of kaffir lime essential oil and chitosan significantly reduced the disease severity index of mango. This result was following the previous experiments, which showed that kaffir lime essential oil and chitosan could decrease the disease severity index of mango. However, the disease severity index of mango coating with ripe kaffir lime essential oil 1500 ppm was significantly higher than the unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 % and the combination of kaffir lime essential oil and chitosan treatments (Table 12). These results also agreed with the previous experiments, which reported that unripe kaffir lime essential oil expressed the higher inhibition of mycelial growth.

Finally, the mango coating with unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 % and the combination of kaffir lime essential oil and chitosan treatments show a significantly similar level of the disease severity index. This result may indicate that the coating with unripe kaffir lime essential oil 1500 ppm could reduce the disease severity index as same as chitosan 0.50 % and the combination of kaffir lime essential oil and chitosan. This may be because of several chemical compounds such as beta-pinene, beta-citronellol, 4-terpineol, limonene, and sabinene, including total phenolic and antioxidant compounds in unripe kaffir lime essential oil affected on the disease severity index. Thus, the results were the same approach

Table 12 Effect of kaffir lime essential oil and chitosan coating on the disease severity index of mango cv. Nam Dok Mai Sri Tong after storage for 11 days.

Treatments	Disease severity index
Water (T1)	3.60a
Unripe kaffir lime essential oil 1500 ppm (T2)	1.00c
Ripe kaffir lime essential oil 1500 ppm (T3)	1.80b
Chitosan 0.50 % (T4)	1.00c
Unripe kaffir lime essential oil 1500 ppm + Chitosan 0.50 % (T5)	1.00c
Ripe kaffir lime essential oil 1500 ppm + Chitosan 0.50 % (T6)	1.20c
F-test	**

Note: Means followed by the different alphabet are significantly different at 1% level.

Table 13 Effect of kaffir lime essential oil and chitosan coating on the weight loss, soluble solids and titratable acidity of mango cv. Nam Dok Mai Sri Tong after storage for 11 days.

Treatment	Weight loss (%)	Soluble solids (°Brix)	Titratable acidity (%)
Water	14.63a	16.80a	0.34d
Unripe kaffir lime essential oil 1500 ppm	8.90d	13.00c	0.98a
Ripe kaffir lime essential oil 1500 ppm	11.12b	14.20b	0.69c
Chitosan 0.50 %	9.06d	13.10c	0.95a
Unripe kaffir lime essential oil 1500 ppm + Chitosan 0.50 %	8.86d	12.80c	0.99a
Ripe kaffir lime essential oil 1500 ppm + Chitosan 0.50 %	9.68c	13.50bc	0.83b
F-test	**	**	**

Note: Means followed by the different alphabet are significantly different at 1% level.

14. Effect of kaffir lime essential oil and chitosan coating on the physicochemical properties

The mango coating with water showed the significant highest weight loss and followed by the ripe kaffir lime essential oil 1500 ppm, ripe kaffir lime essential oil 1500 ppm + chitosan 0.50 %, chitosan 0.50 %, unripe kaffir lime essential oil 1500 ppm and unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % treatments, respectively. On the other hand, there was no significant difference in the weight loss of unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 % and unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % treatments (Table 13). This result indicated that unripe kaffir lime essential oil could reduce weight loss in the same level as

chitosan and the combination coating between unripe kaffir lime essential oil and chitosan.

There was a significant difference in soluble solids among treatments. The mango coating with water showed the significant highest soluble solid (16.80 ºBrix). The unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 %, unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % and unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % treatments could significantly maintain the soluble solids of mango (Table 13). In the case of titratable acidity, the results showed that the mango coating with unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 %, and unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % treatments could significantly delay the reduction of titratable acidity of mango as compared with the remaining treatments (Table 13). When the mango fruits naturally ripen, the soluble solids will increase and the titratable acidity will decrease. This result may indicate that unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 % and unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % could effectively delay the ripening of mango. It may be because of the decrease in weight loss that is one of the essential reasons that could affect the quality of fruit products. Similar results also found in the experiment of Xing et al. (2015) reported that chitosan, cinnamon oil, and chitosan + cinnamon oil could delay the ripen of Chinese jujube fruit.

The change in color of the fruit is also a ripening indicator. The peel of a mango is a pale yellow tint when it is unripe. After the fruit ripen, the peel will turn a dark yellow tint. In this experiment, the results showed no significant difference in *L color of mango peel among treatments while the *a and *b colors were found the difference. The significant difference in *a and *b color was similar. Namely, the highest *a and *b color was found in the water treatment. The significant low of *a and *b color was found in the unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 % and unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % treatments (Table 14).

Table 14 Effect of kaffir lime essential oil and chitosan coating on the color of mango cv. Nam Dok Mai Sri Tong after storage for 11 days.

Treatment	Color		
	*L	*a	*b
Water	68.02	15.05a	45.45a
Unripe kaffir lime essential oil 1500 ppm	67.56	8.29d	34.82d
Ripe kaffir lime essential oil 1500 ppm	68.21	11.14b	40.80b
Chitosan 0.50 %	67.75	8.56cd	34.54d
Unripe kaffir lime essential oil 1500 ppm + Chitosan 0.50 %	68.38	8.27d	34.46d
Ripe kaffir lime essential oil 1500 ppm + Chitosan 0.50 %	68.13	9.23c	36.23c
F-test	ns	**	**

Note: Means followed by the different alphabet are significantly different at 1% level.

It may indicate the unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 % and unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % could slow down the ripening of mango fruit. This result was according to the results of soluble solids and titratable acidity. Thus, it may reveal that the unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 %, and unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % were suitable to use as the organic coating for the disease protection and delay fruit ripening.

15. Effect of kaffir lime essential oil and chitosan coating on the consumer acceptability

After storage for 11 days, the mango was evaluated the consumer acceptability such as visual appearance, color, smell, flavor, texture and overall acceptability. There was a significant difference in treatments' visual appearance, color, flavor, texture, and overall acceptability. The water coating treatment found the highest scores of visual appearance, color, flavor, texture, and overall acceptability. The lowest score of visual appearance, color, flavor, texture and overall acceptability was found in unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % treatment which did not significantly differ in comparison to the unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 % and ripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % treatments (Table 15). This result may indicate that the mango coating with water already turned to the ripen stage; therefore, the score of visual appearance, color, flavor, texture, and overall acceptability was significantly higher than the other treatments.

Similarly, the mango coating with unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 %, unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % and ripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % was still in the unripe stage. Therefore, the score of visual appearance, color, flavor, texture and overall acceptability was significantly low as compared to the water coating treatment. This result also showed that the ripening stage of mango was postponed by the coating of unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 %, unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % and ripe kaffir lime essential oil 1500 ppm + chitosan 0.50 %.

Table 15 Effect of kaffir lime essential oil and chitosan coating on the consumer acceptability of mango cv. Nam Dok Mai Sri Tong after storage for 11 days.

Treatment	Consumer acceptability					
	Visual appearance	Color	Smell	Flavor	Texture	Overall acceptability
Water	8.20a	8.45a	7.15a	8.40a	8.45a	8.35a
Unripe kaffir lime essential oil 1500 ppm	6.50bc	6.35b	6.90a	6.55c	6.60c	6.50c
Ripe kaffir lime essential oil 1500 ppm	6.80b	6.50b	7.00a	7.15b	7.00b	6.90b
Chitosan 0.50 %	6.45bc	6.45b	6.85a	6.50c	6.55c	6.55bc
Unripe kaffir lime essential oil 1500 ppm + Chitosan 0.50 %	6.40c	6.20b	6.90a	6.40c	6.50c	6.45c
Ripe kaffir lime essential oil 1500 ppm + Chitosan 0.50 %	6.55bc	6.45b	6.95a	6.65c	6.70bc	6.65bc
F-test	**	**	ns	**	**	**

Note: Means followed by the different alphabet are significantly different at 1% level

CHAPTER 5
CONCLUSION

Using kaffir lime oil from peel waste, a new concept was developed in this study. Mango fruit is treated with a low-toxicity, an ecologically friendly process that protects it from fungal infestation. The main composition components of UKLO and RKLO were beta-pinene, limonene, and beta-citronellol, according to the classification of several chemical compounds of UKLO and RKLO. UKLO and RKLO contained significant amounts of bioactive compounds, including total phenolic and antioxidant content. Kaffir lime essential oil is efficient against C. gloeosporioides. A larger concentration of essential oils inhibited fungal growth more effectively. UKLO at 1,500 ppm can significantly reduce disease development compared to the control and has higher activity than RKLO at the same concentration. This result indicates that the potential of UKLO at 0.15% concentration is suitable to be incorporated in fruit coating formulation to control post-harvest fungal contamination. The coated UKLO mangoes can significantly reduce the disease severity index. Physicochemical indicators and consumer acceptability are absent during mango storage for 9 days.

The effect of chitosan coating on disease severity index, physicochemical characteristics, and consumer acceptance of mango fruit was then investigated in order to find the optimal chitosan concentration for mango fruit. The results showed that the disease incidence, total soluble solids, color (a and b) change, weight loss of mango fruits coated with 0.5%, 0.75% and 1% chitosan were lower than that of the other treatments after storage for 11 days. In addition, the sensory evaluation results found that the preference score of color, flavor and appearance was low compared to the other treatments. Therefore, the results indicated that the Nam Dok Mai Sri Tong for 11 days of storage. Therefore, chitosan 0.5% was chosen for the next experiment.

The effect of kaffir lime essential oil or chitosan alone and the combination on the disease severity index, physicochemical properties and consumer acceptability of mango fruit was studied in this experiment. The results showed that

the unripe kaffir lime essential oil 1500 ppm, chitosan 0.50 %, and unripe kaffir lime essential oil 1500 ppm + chitosan 0.50 % could decrease the disease severity index and delay the fruit ripen. Therefore, this work may lead to the development of a mango fruit coating that incorporates kaffir lime essential oil and may be used to protect organic food from fungal contamination.

Suggestion

1. Apply this result in a field experiment to reduce cost and production.
2. Promote kaffir lime production
3. Study the storage method for preserving the bioactive compounds of kaffir lime essential oil.
4. Use kaffir lime essential oil with the other plant production.

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