



**Fabrication of Polylactic Acid / Chitosan / Cellulose
Nanocrystal Incorporated with Essential Oil for
Harumanis Mango Postharvest Application**

by

Raja Hasnida Bte Raja Hashim

(1931113122)

A thesis submitted in fulfillment of the requirements for the degree of
Master of Science

**Faculty of Chemical Engineering & Technology
UNIVERSITI MALAYSIA PERLIS**

2023

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful.

All praises to Allah and His blessing for the completion of this thesis. I thank God for all the opportunities, trials and strength that have been showered on me to finish writing the thesis. I experienced so much during this process, not only from the academic aspect but also from the aspect of personality. My humblest gratitude to the holy Prophet Muhammad (Peace be upon him) whose way of life has been a continuous guidance for me.

First and foremost, I would like to sincerely thank my supervisor Assoc. Prof. Ts. Dr. Ahmad Anas Bin Nagoor Gunny, for his guidance, understanding, patience and most importantly, he has provided positive encouragement and a warm spirit to finish this thesis. It has been a great pleasure and honour to have him as my supervisor. I am grateful towards my co-supervisor, Assoc. Prof. Dr. Sam Sung Ting for his knowledge sharing regarding to my research.

My deepest gratitude goes to all of my family members. It would not be possible to write this thesis without the support from them. I would like to thank my dearest husband, Hamzah Bin Abdul Hamid, my parents Raja Hashim Raja Salleh and Farida Bt Ali and also my beloved daughter, Hasya Humaira for their prayers and help.

I offer my special thanks to all my colleagues; Raudzah, Siti Erma, Atikah, Mawaddah, Pn. Nur Zatul Iffah and Zulhilmi ishak for their motivation, prayers and their sincere help during my studies. I also want to extend my thanks to Staff in the Faculty of Chemical Engineering Technology, Unimap for their help and support.

I thank them wholeheartedly. May God shower the above cited personalities with success and honour in their life.

TABLE OF CONTENTS

	PAGE
DECLARATION OF THESIS	i
ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	xii
LIST OF SYMBOLS	xiii
ABSTRAK	xiv
ABSTRACT	xv
CHAPTER 1 : INTRODUCTION	1
1.1 Research Background	1
1.2 Problem statement	4
1.3 Research Questions	6
1.4 Research Objectives	6
1.5 Research Scope	7
CHAPTER 2 : LITERATURE REVIEW	8
2.1 Mango	8
2.2 Postharvest issue	10
2.2.1 Control strategy	11
2.2.2 Chemical fungicides to control postharvest issues.	12

2.2.3	Heat treatment issues	12
2.2.4	Petroleum base plastic packaging	14
2.3	Fruit packaging	15
2.4	Biopolymer	15
2.4.2	Chitosan	16
2.4.3	Polylactic Acid	18
2.5	Cellulose nanocrystal	20
2.5.1	Preparation cellulose nanocrystal	22
2.5.2	Cellulose nanocrystal as packaging materials	23
2.6	Essential oil (EOs) in films as packaging	24
2.6.1	Effects of essential oils on structural effect	25
2.6.2	Effects of essential oils on antioxidant activity	26
2.6.3	Effects of essential oils on Antifungal Activity	27
2.6.4	Effects of essential oils on Water Vapor Permeability and Gas Permeability	29
2.7	<i>Coleus Aromaticus</i>	31
2.7.1	Benefits of <i>Coleus aromaticus</i>	32
2.7.2	<i>Coleus aromaticus</i> essential oil as antioxidant agent	32
2.8	Fruit quality traits	34
2.8.1	Weight loss	34
2.8.2	Solid Soluble Content (SSC)	35
CHAPTER 3 : METHODODOLOGY		37
3.1	Research Framework	37
3.2	Materials	38
3.3	Preparation of CNC through sulphuric acid hydrolysis	38
3.4	Fabrication of biopolymer film based on Polylactic Acid (PLA), Chitosan (CS) and Cellulose nanocrystal (CNC)	38

3.4.1	Poly(lactic Acid (PLA), Chitosan (Cs) and Cellulose Nanocrystal (CNC) films preparation	39
3.5	<i>Coleus aromaticus</i> essential oil	39
3.5.1	Gas chromatography/ mass spectrometry (GC/MS) of <i>Coleus aromaticus</i> EO	40
3.6	Addition of <i>Coleus aromaticus</i> essential oil into biopolymer film	40
3.6.1	PLA/Cs/CNC with <i>Coleus aromaticus</i> essential oil films preparation	40
3.7	Characterization of the biopolymer films with additional of essential oil	41
3.7.1	Film thickness	42
3.7.2	Determination of antioxidant activity of a biopolymer films	42
3.7.3	Determination of water absorption of biopolymer films	43
3.7.4	Mechanical properties	43
3.7.5	Fourier transform infrared spectroscopy (FTIR)	44
3.7.6	Scanning Electron Microscopy (SEM)	44
3.7.7	Transmission Electron Microscopy (TEM)	44
3.7.8	Water Vapour Permeability (WVP)	44
3.7.9	Gas Permeability	45
3.8	Isolation of fungi from infected leaves	46
3.9	<i>In-vitro</i> antifungal test	47
3.9.1	Determination of antifungal activity of biopolymer films	47
3.10	<i>In-vivo</i> antifungal test	48
3.10.1	Effect of antifungal activity on artificially wounded and inoculated mangoes	48
3.10.2	Effect of antifungal activity of naturally infected development on unwounded mangoes	49
3.11	Fruit quality test	49
3.11.1	Weight loss	49
3.11.2	Soluble solid content (SSC)	50

3.12	Statistical analysis	50
CHAPTER 4 : RESULTS & DISCUSSION		51
4.1	Fabrication of biopolymer film based on Polylactic Acid (PLA), Chitosan (Cs) and Cellulose nanocrystal (CNC)	51
4.2	Characterization of PLA/Chitosan films with different amount of CNC	52
4.2.1	Scanning Electron Microscopy (SEM)	52
4.2.2	Tensile strength properties of biopolymer films	53
4.2.3	Water Absorption test	56
4.2.4	Gas chromatography/ mass spectrometry (GC/MS) of <i>C. aromaticus</i> EO	57
4.3	PLA/Cs/CNC films with different amount of <i>C. aromaticus</i> essential oil	59
4.3.1	Antioxidant activity of PLA/Cs/CNC embedded with <i>C. aromaticus</i> EO	59
4.3.2	Mechanical properties	60
4.4	Isolation and identification of isolated fungi from infected mango's leaves	62
4.4.1	<i>In vitro</i> antifungal activity of PLA/Chitosan film incorporate with CNC and CAEO	64
4.4.2	<i>In vivo</i> antimicrobial activity of PLA/Chitosan film incorporate with CNC and CAEO (Effect of antifungal activity on artificially wounded and inoculated mangoes)	65
4.4.3	Fourier transform infrared spectroscopy (FTIR)	67
4.4.4	Scanning Electron Microscopy (SEM)	69
4.4.5	Transmission Electron Microscopy (TEM)	71
4.5	Water and gas permeability	72
4.5.1	Water Vapor Permeability	72
4.5.2	Gas Permeability analysis of films	73
4.6	Effect of antifungal activity of naturally infected development on unwounded mangoes	74
CHAPTER 5 : CONCLUSION		77

5.1	Summary	77
5.2	Recommendations	78
	REFERENCES	79
	APPENDIX A	92
	APPENDIX B	94
	APPENDIX C	95
	LIST OF PUBLICATIONS	98
	LIST OF AWARDS	98

©This item is protected by original copyright

LIST OF TABLES

	PAGE
Table 2-1 : Summary of several type of additives that embedded into chitosan films as packaging from previous studies	18
Table 2-2 : Summary of several type of additives that embedded into PLA films as packaging from previous studies	20
Table 2-3 : Main advantages and disadvantages of PLA	20
Table 2-4 : Summary of several type of essential oil that embedded into films as packaging from previous studies.	28
Table 4-1 : The water absorption test of films containing different amount of CNCs.	57
Table 4-2 : Effect of distilled water, ethanol, film with different concentrations of EO on the antifungal activity	65
Table 4-3 : Effect of control, Films without EO, Films with 1.2 % essential oil of <i>C. aromaticus</i> and plastic wrap on weight loss (%) and SSC (%)	76

LIST OF FIGURES

	PAGE
Figure 2-1 : Harumanis mango	9
Figure 2-2 : Decay of Mangoes at retail level	11
Figure 2-3: Chemical structure of chitosan and its derivatives	17
Figure 2-4: Enantiomeric form of Lactic Acid	19
Figure 2-5 : The leaves of <i>Coleus aromaticus</i>	32
Figure 2-6: Chemical structure of Thymol and Carvacrol	33
Figure 3-1: Experimental flowchart	37
Figure 3-2: Schematic diagram of a fabricating films process	41
Figure 4-1: Films with (A) PLA/Cs/CNC 0%, (B) PLA/Cs/CNC 1% (C) PLA/Cs/CNC 2%, (D) PLA/Cs/CNC 3%, (E) PLA/Cs/CNC 4%, (F) PLA/Cs/CNC 5%	51
Figure 4-2 : SEM image of the surface of (A) PLA/Cs film, (B) PLA/Cs/CNC 1% film (C) PLA/Cs/CNC 2% film, (D) PLA/Cs/CNC 3% film, (E) PLA/Cs/CNC 4% film	53
Figure 4-3 : Effect of CNC load on tensile strength of the film. Values in the figure were significantly different ($p < 0.05$)	54
Figure 4-4: Effect of CNC load on elongation break of the film. Values in the figure were significantly different ($p < 0.05$)	55
Figure 4-5: Effect of CNC load on young modulus of the film. Values in the figure were significantly different ($p < 0.05$)	56

Figure 4-6: Water absorption test on Films with different concentration of CNC Values in the figure were significantly different ($p < 0.05$)	57
Figure 4-7 : Chromatogram of essential oil extracted from <i>C. aromaticus</i>	58
Figure 4-8: DPPH scavenging activities of the film. Values in the figure were significantly different ($p < 0.05$)	60
Figure 4-9: Effect of concentration of essential oil on tensile strength of the film. Values in the figure were significantly different ($p < 0.05$)	61
Figure 4-10: Effect of concentration of essential oil on Elongation of the film Values in the figure were significantly different ($p < 0.05$)	62
Figure 4-11: (A) Infected leaves (B) Morphology of for 7 days old colonies growing PDA (C) Microscopic picture of a under 100x magnification (D) Molecular Identification of <i>Curvularia</i> sp.	64
Figure 4-12 : Effect of different types of treatment with 1.2 wt% films on disease incidence of mangoes after 10 days Values in the figure were significantly different ($p < 0.05$)	66
Figure 4-13: Development of fungi at the wound site of the inoculated mangoes after 10 days. (A) Mango treated without film (control), (B) Film without EO, (C) Film with 1.2 wt% EO	67
Figure 4-14: Fourier-transform infrared spectroscopy analysis PLA/Cs//CNC film embedded with and without <i>Coleus aromaticus</i> essential oil. Scanning range from 400 to 4000 cm^{-1} is shown. The presence of chemical groups at the specific wavenumber is shown	69
Figure 4-15 : A (films without essential oil) & B (films with 1.2 % essential oil)	70

Figure 4-16: TEM micrographs of: A: PLA/Cs/CNC film and B: PLA/Cs/CNC film incorporated with *C. aromaticus* essential oil. 71

Figure 4-17 : Water Vapor Permeability analysis of of PLA/Cs A: CNC 0 % , B : CNC 1 % , C : CNC 2% , D: CNC 3% , E : CNC 4% , F: EO 0.4 % , G : EO 1.2 % . Values in the figure were significantly different ($p < 0.05$) 73

Figure 4-18: Gas permeability of PLA/CHI A: CNC 0 % , B : CNC 1 % , C : CNC 2% , D: CNC 3% , E : CNC 4% , F: EO 0.4 % , G : EO 1.2 % 74

©This item is protected by original copyright

LIST OF ABBREVIATIONS

ASTM	American Society Testing Material
CAEO	<i>Coleus Aromaticus</i> Essential oil
CNC	Cellulose Nanocrystal
Cs	Chitosan
DPPH	Diphenyl-Picrylhydrazyl
EO	Essential oil
FTIR	Fourier Transform Infrared Spectroscopy
MCC	Micro Crystalline Cellulose
GCMS	Gas Chromatography /Mass Spectrometry
PDA	Potato Dextrose Agar
PLA	Polylactic Acid
PVA	Poly (Vinyl alcohol) PVA
PP	Polypropylene
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TS	Tensile strength
WVP	Water Vapor Permeability

©This item is protected by original copyright

LIST OF SYMBOLS

<i>h</i>	Hour
<i>A_m</i>	Area of films (cm ²),
<i>p_h</i>	The pressure of feed (mmHg)
<i>p_l</i>	The pressure permeates (mmHg)
<i>g</i>	gram
wt %	Weight percent
v/v	Volume/volume
<i>μl</i>	Micro litre
<i>W_d</i>	Weight of dry film
<i>W_w</i>	Weight of films at equilibrium

©This item is protected by original copyright

Pembikinan Polilaktik /Kitosan/ Selulosa Nanokristalin Biopolimer Digandingkan Dengan Minyak Pati untuk Aplikasi Lepas Tuai Mangga Harumanis

ABSTRAK

Mangga 'Harumanis' ialah kultivar komersial terkemuka di utara Semenanjung Malaysia, yang juga merujuk sebagai "raja mangga" di Malaysia dan mempunyai permintaan pengguna maksimum. Cabaran utama yang dihadapi oleh petani mangga Harumanis ialah penyakit lepas tuai mangga. Penggunaan asid Polilaktik dan Kitosan sebagai pembungkusan semasa lepas tuai boleh memberikan alternatif mesra alam kepada pendekatan konvensional. Namun, disebabkan kos yang lebih tinggi, kekuatan mekanik yang lemah, penahan air dan gas yang lemah dan aktiviti antimikrob antioksidan yang lemah bagi filem, pembikinan biopolimer masih agak terhad. Penambahan (selulosa nanokristalin) dan minyak pati kepada filem adalah untuk meningkatkan sifat filem. Lapisan yang membolehkan kawalan penahan kimia dan berfungsi sebagai penahan tambahan untuk meningkatkan kualiti keseluruhan dan kestabilan buah dicipta oleh keupayaan minyak pati untuk mengekalkan dan bahan tambahan untuk mengurangkan pemindahan air dan gas antara produk dan alam sekitar. Tujuan penyelidikan ini adalah untuk menggabungkan selulosa nanokristalin (CNC) dan minyak pati *Coleus aromaticus* (CAEO) dan konstituennya ke dalam bahan pembungkusan baharu asid polilaktik (PLA)/Kitosan (Cs) dan untuk mengkaji kesan CNC dan CAEO tentang sifat filem PLA/Cs sebagai pembungkusan buah. Filem itu dibuat melalui teknik tuangan pelarut. Sifat antimikrob, antioksidan, mikrostruktur dan kekuatan mekanik filem yang diperolehi telah dikaji. Sifat mekanik menunjukkan nilai kekuatan tegang (KT) yang lebih tinggi dengan penggabungan 2% CNC. Walau bagaimanapun, filem PLA/Kitosan-CNC pada 3% dan 4% CNC mempamerkan nilai KT yang menurun. Filem PLA/Cs telah ditambah baik dengan penambahan sejumlah kecil CNC. Tetapi, apabila CAEO ditambah, ia menambah baik sifat kebolehlenturannya. Analisis SEM menunjukkan bahawa permukaan filem PLA/Cs lebih licin apabila 1% dan 2% daripada CNC dan mula menghasilkan buih dan retak pada 3% dan 4%. Permukaan filem menjadi lebih kasar apabila CAEO dimasukkan ke dalam filem. Aktiviti antioksidan filem biopolimer meningkat dengan ketara ($p < 0.05$) dengan peningkatan CAEO. Ujian in-vitro dan in-vivo digunakan untuk menilai kesan rencatan filem terhadap kulat terasing daripada mangga (*Curvularia sp.*) dengan membandingkan filem dengan kepekatan minyak pati yang berbeza dengan rawatan kawalan. Resapan cakera digunakan untuk menilai perencatan pertumbuhan miselia, yang mana filem yang digandingkan dengan 1.2% CAEO menunjukkan hasil terbaik. Untuk in-vivo, kejadian penyakit berjaya dikurangkan oleh filem yang digandingkan dengan 1.2% CAEO. Semua spektrum FTIR campuran PLA/Cs/CNC dan CAEO memaparkan jalur ciri bahan berasaskan PLA. TEM menunjukkan kehadiran CAEO, dan kehomogenan titisan minyak dikesan dalam matriks PLA/Cs/CNC. Keputusan menunjukkan filem biopolimer yang digabungkan dengan CAEO boleh digunakan sebagai pembungkusan aktif dalam industri buah-buahan dan menjadi alternatif yang menjanjikan kepada rawatan kawalan semasa yang digunakan untuk mengawal penyakit lepas tuai pada mangga Harumanis.

Fabrication of Polylactic Acid / Chitosan / Cellulose Nanocrystal Incorporated with Essential Oil for Harumanis Mango Postharvest Application

ABSTRACT

'Harumanis' mango is the leading commercial cultivar on north Peninsular Malaysia, which also refer as "king of mangoes" in Malaysia and have the maximum consumer demand. The major challenges face by Harumanis mango's farmer is mango postharvest diseases. The application Polylactic acid and Chitosan biopolymer as packaging during postharvest could provide an eco-friendly alternative to those conventional approaches. However, due to higher costs, poor mechanical strength, poor water and gas barrier and poor antioxidant antimicrobial activity of the films, biopolymer packaging is still relatively limited. The purpose of adding nanofillers (cellulose nano crystals) and essential oils to films is to improve their properties. The ability of essential oils to retain and additives to prevent water and gas transfer between product and environment produce a layer that allows for the control of chemical barriers and serves as an additional barrier to improve the overall quality and stability of fruit. Hence, the aim of this research is to incorporate Cellulose nanocrystal (CNC) and *Coleus aromaticus* essential oil (CAEO) and their constituents into a new packing material of poly-lactic acid (PLA)/ Chitosan (Cs) and to examine the effect of CNC and CAEO on the properties of PLA/Cs films as fruit packaging. The films are fabricated via solvent casting technique. The obtained films' antimicrobial, antioxidant, microstructural, and mechanical properties were studied. Mechanical properties studies showed the incorporation of CNC indicated higher Tensile strength (TS) value by incorporation of 2% of CNC. However, the PLA/Chitosan-CNC films at 3% and 4% CNC concentration exhibited a decreased TS value. PLA/Cs films were improved with the addition of a small amount of CNC. On the other hand, when the CAEO was added, it improves the elongation breaks which reflect on its flexibility properties. Scanning electron microscopy (SEM) analysis revealed that SEM analysis showed that the surface of PLA/Cs films smoother when 1% and 2 % of CNC and start to produce bubbles and crack on 3 % and 4 %. The surface of films became rougher when CAEO was incorporated into the film. Antioxidant activity of biopolymer film significantly increased ($p < 0.05$) with increasing CAEO. *In-vitro* and *in-vivo* tests were used to evaluate the inhibitory effect of the film against isolated fungi from mango (*Curvularia* sp.) by comparing the film with different concentration of essential oil with the treatment of control. Disk diffusion was used to evaluate mycelial growth inhibition, which the film incorporated with 1.2 wt% CAEO showed the best result. For *in-vivo* with wounded mango, the disease incidence was successfully reduced by the film incorporated with 1.2 wt% CAEO. All FTIR spectra of PLA/Cs/CNC and CAEO blends displayed the characteristic bands of PLA-based materials. TEM analysis shows the presence of CAEO, and homogeneity of oil droplets was detected in the PLA/Cs/CNC matrix. The results indicate the biopolymer film incorporated with CAEO could be used as active packaging in fruit industry and be a promising alternative to current control treatment that used for controlling postharvest disease on Harumanis mango.

CHAPTER 1 : INTRODUCTION

1.1 Research Background

Nowadays, fruits and vegetables are highly demanded in the market because of its nutritional value. Fruits and vegetables account for 40-50% of food losses, bringing the total to a yearly loss of US\$750 billion (FAO, 2013). Because postharvest fruits and vegetables are highly perishable, their preservation is an ongoing challenge for the food industry. Fruits and vegetables are also sensitive to microbial attack damage. Microbial spoilage are mostly caused by fungi, bacteria, yeast and moulds (Mardiyya, 2019). Fungi might be one of the cause massive yield losses due to their potential to cause severe crop-damaging diseases). As the fruit ripen, the fungal pathogens will turn into aggressive growth. The fungi are necrotrophs during the aggressive stage which kill the host cell and obtain nutrients from the host which causes the rot of fruits (Abd-Alla & Haggag, 2013)

Mango is one of the major economically fruit crops accounted for more than half of total global major tropical fruit production in recent years (Mitra, 2016). The global production of mangoes was 2141000 tonnes in 2020 and Asian as second world exporter of mangoes produces 42 % of total global export of mangoes (Actimage, 2021). Mangoes are also among the important and popular fruit in Malaysia and mostly found in northern regions such as Perlis, Kedah and Perak. Mango cultivars grown in Malaysia such as 'Maha 64', 'Harumanis', 'Sala' and 'Telor'. 'Harumanis' is the leading commercial cultivar on north Peninsular Malaysia, which also refer as "king of mangoes" in Malaysia and have the maximum consumer demand not only in Malaysia but also in the international market (Talib et al., 2020). Best quality of 'Harumanis' mangoes can only be grown in Perlis and

well known as one of the uniqueness of Perlis among Malaysian cultivars (Uda et al., 2020). 'Harumanis' mangoes possess significant losses in the range of 10-30% prior reaching to the consumers due to the pathogenic fungus growth during the postharvest storage (Yusuf et al., 2018). The major challenges faces by Harumanis mango's farmer is mango postharvest diseases. Conventional approach to control the outbreaks of disease by use of chemical fungicides such as benomyl, carbendazim or propiconazole (Uda et al., 2020), the use of plastic packaging (Ayhan, 2011)(Abejón et al., 2020) and physical treatment such as Hot Water treatment (HWT) (Bambalele et al., 2021).

However, those conventional approaches during postharvest can have a number of negative consequences. The drawbacks when using chemical fungicides is the possibility of residue on the produce. If the fungicide is not properly applied or the produce is not thoroughly washed before consumption, the residue can be harmful to human health. Furthermore, many fungicides are toxic to beneficial insects and microorganisms, disrupting the natural ecosystem and leading to the development of resistant pathogens (Perumal et al., 2022). On the other hand, the continuous production of petroleum based plastics causes disposal issues, exacerbating the problem of plastic pollution and its rise in the environment (Pires et al., 2021). Plastic packaging materials are not biodegradable, and degrading in the environment can take hundreds of years (Cvek et al., 2022). This can result in the accumulation of plastic waste in landfills and oceans, endangering wildlife and marine life (Abejón et al., 2020)(Basumatary et al., 2020). Another drawback from conventional approaches is when application of Hot Water treatment. Hot water treatment (HWT) is a method of controlling postharvest decay that relies on the use of water at temperatures above 40 degrees Celsius. They may also have some limitations, such as low persistence, the risk of adverse effects on produce quality, or technological

issues for commercial application (Usall et al., 2016). Consequently, more efficient and environmentally approaches to control postharvest issues are deemed necessary.

The use of Polylactic Acid and Chitosan biopolymer as packaging has increased over the past year (Cvek et al., 2022)(dos Santos et al., 2017)(Siracusa et al., 2017). The main benefits of bio-based packaging films include their environmental friendliness, biodegradability, and the nutritional value of their food products. However, the application of PLA/Chitosan biopolymer as food packaging have some disadvantages including low water and gas permeability ,poor mechanical properties (Kusmono et al., 2021)(Chouwatat et al., 2010), low antioxidant and antimicrobial properties (Özge et al., 2013).

To bring biopolymer-based food packaging from concept to reality, it is critical to strengthen their composition while also modifying and improving their properties. As a result, another solution under consideration is the use of organic or inorganic nanoparticles such as such as zinc oxide (ZnO), silver (Ag), titanium dioxide (TiO₂), silicon dioxide (SiO₂), and iron (III) oxide (Fe₃O₄) (Anaya-Esparza et al., 2021) to improve the properties of the films, as demonstrated in the study by (Wei et al., 2016). Incorporating homogeneously dispersed nanoparticles with a high aspect ratio and surface area into the biopolymer matrix is regarded as a promising innovation. Previous researchers have conducted studies on biopolymer films using various nanofillers such as cellulose nanocrystals (W. Yang et al., 2016)(Pires et al., 2021). Because of its sustainability, abundance, large surface area to volume ratio, lightness, and high mechanical strength (Basumatary et al., 2020), Cellulose nanocrystal has drawn the attention of researchers as a potential nano-reinforcement for biopolymer films

(Szymańska-Chargot et al., 2020). The incorporation of antioxidant and antimicrobial compounds into the polymeric matrix, such as essential oils (EOs) extracted from various plant compartments, can boost the antioxidant and antimicrobial activity of the packaging films (Bonilla, J., Talón, E., Atarés, L., Vargas, 2013)(Shemesh et al., 2015)

Thus, this research puts forward the hypothesis that the PLA/Chitosan films that combines with nanofillers such as cellulose nanocrystal and essential oils can be improve in term of the mechanical strength, structural, antioxidant capacity, antimicrobial capacity as well as the barrier to apply as fruit packaging.

1.2 Problem statement

Harumanis mango is a worldwide commercial fruit and it is a seasonal fruit. When it's come to the season, the major challenges in commercializing the fruits in the industry was maintaining its quality (Kore et al., 2016) (Firdaus & Mohd, 2014). There were several pathogenic fungus causes the rotting of fruit for example *Colletotrichum spp.* (Abdullah et al., 2016), *Aspergillus spp* (Krishnapillai & Wijeratnam, 2013), *Alternaria spp.*, *Botrytis spp.* And *Curvularia spp* (Lydia I. Rivera-Vargas³, Manuel Pérez-Cuevas⁴ & Asencio⁵, 2020) which infect the fruit cultivation, packaging process and during distribution (Abd-Alla & Haggag, 2013).

In recent years, conventional approach such as chemical synthetic fungicides, petroleum-based plastic packaging and hot water treatment are thought to be an effective way to effectively control pathogenic fungus during postharvest. All the approaches were applied to inhibit the growth of fungi and to prolong the shelf life of the fruit. However,

using chemical synthetic fungicides on fruit for a long time is not an effective way to combat pathogenic fungus growth on fruit since chemical synthetic fungicides are very toxic, thus lead to environmental pollution and serious health problems for human (Xing et al., 2014). Petroleum-based packaging has numerous negative effects on the environment, wildlife, and human health, so it is critical to consider alternative, sustainable packaging materials. On the hand, application of hot water treatment (HWT) during postharvest requires a significant amount of energy, which can be costly and increases the carbon footprint of the postharvest process and the high temperatures used in HWT can cause thermal damage to the produce, lowering its quality, appearance, and shelf life (Huan et al., 2017).

The application Polylactic acid and Chitosan biopolymer as packaging during postharvest could provide an eco-friendly alternative to those conventional approaches such as petroleum-based packaging materials. However, the use of biopolymer packaging is still relatively limited and having issues on mechanical properties, gas barrier, antioxidant capacity and antimicrobial capacity.

There are several research that use the biopolymer films containing Cellulose nanocrystal (Fortunati et al., 2014; Sullivan et al., 2015) as filler and essential oils (Antunes et al., 2012; Sessa et al., 2015). The main objective of incorporating the nanofillers, essential oils and their constituents into films is to improve the films. Essential oil has the ability to retain and additives to reduce water and gas transfer between product and environment, this layer allow control of chemical barriers and also act as an additional barrier to improve the overall quality and stability of fruit (Nur et al., 2017)

Thereby, the main objective of this research is to blend Cellulose nanocrystal (CNC) to improves the mechanical properties of biopolymer packaging and incorporate *Coleus aromaticus* EO (CAEO) as well as their constituent's into polylactic acid (PLA)/ Chitosan (Cs) biopolymer film and to use their antioxidant and antimicrobial capacity to improve the films as mango packaging. To the best of the researcher knowledge, the combination of these types of biopolymers and essential oil has never been identified.

1.3 Research Questions

- i) How to fabricate and characterize different amount of CNC on physicochemical properties and tensile strength of PLA/Cs film?
- ii) How are *C.Aromaticus* Essential oil affect the physicochemical properties of PLA/Cs/CNC films ?
- iii) What is the best assessment to evaluate the quality of fruit after using PLA/Cs/CNC/CAEO?

1.4 Research Objectives

- i. To fabricate and investigate different amount of CNC on physicochemical and tensile strength of PLA/Cs film
- ii. To evaluate the Physicochemical properties of PLA/Cs/CNC embedded with *C.Aromaticus* Essential oil films
- iii. To evaluate In-vitro and In vivo assessment on the quality of fruit after using PLA/Cs/CNC/CAEO?

1.5 Research Scope

PLA and chitosan were used because chitosan itself has advantages of biodegradability and excellent film forming. The biopolymer films will be prepared with different concentration of PLA/ Cs, which CNC and essential oil is fabricated by using solution casting method. The essential oil from *C. aromaticus* were supplied from IBNU MBBS Enterprise. The tensile strength properties of the produced biopolymer films were analyzed using ASTM D882 method. Specimens were prepared from rectangular film strips (1 cm × 10 cm), dimensions. A universal testing machine, Instron (Model 5569) with 1kN load cell was operated at a crosshead speed of 2 mm/ min. The morphological characterization (SEM) were characterized with 500x, 1 K, 5K and 10 K magnification. For *in-vitro* assessment (antifungal & antioxidant) the sample were varies essential oil concentration (0, 0.4, 0.8 and 1.2 %). For *In Vivo* analysis, the fruit were collected from Perlis State Department of Agriculture, Malaysia was free from injury and infections, and were used in the experimentation of this work. In this study, the fruits maturity was week 8 (Day 1) selected, because 'Harumanis' are typically harvested 60-120 days after flowering, or about 8 weeks after the fruits reach a diameter of 4 cm.

CHAPTER 2 : LITERATURE REVIEW

2.1 Mango

Mango (*Mangifera indica* L.) was considered as one of the most popular fruits among the people in the developed countries and tropical area (Aris, 2015). The demand on the mango fruit is high until today due to its richness in variety, delicious taste, pleasant flavour, high nutritional value, and attractive appearance. Furthermore, mango is a popular fruit in Malaysia, this is because mangoes is a good source of dietary antioxidants for example carotenoids, ascorbic acid and phenolic compounds (Krishnapillai & Wijeratnam, 2013)

There are many types of mangoes cultivar in Malaysia such as Maha 64, Harumanis, Sala and Telor. Among the mango cultivar, 'Harumanis' is the leading commercial cultivar on north Peninsular Malaysia, which have the maximum consumer demand not only in Malaysia but also in the international market. Harumanis mango can only be found in Perlis and well known as one of the uniqueness of Perlis among Malaysian. Moreover, Harumanis also being considered as the "King of Mangoes" because of its excellent taste, deliciousness, aromatic fragrance and has a very nice texture. Figure 2-1 shows Harumanis mango fruit.



Figure 2-1 : Harumanis mango (Firdaus & Mohd, 2014)

Harumanis mango, a variety of the mango fruit *Mangifera Indica L*, is said to be one of the best varieties in the world. However, Harumanis mango countered with many problems like browning or blackening of tissues prior to culture due to oxidation of phenolic compound by polyphenolic oxidase enzyme present in the tissue when excised. As the fruit ripen, the fungus will switch to a pathogenic stage and branch into the fruit's internal tissue. Pathogenic fungal control and management techniques such as chemical fungicide, sanitation, heat treatment and low temperature storage and plastic packaging are manufacturing practices (Abd-Alla & Haggag, 2013). Therefore, one of potential of the most practical practice to overcome these issues is via using biopolymer films as control technology to maintain the quality of the fruits.

The incorporation of filler such as cellulose nanocrystal and additives such as plant essential oils into biopolymer film is an interesting way to improve their industrial applications. Essential oils that extracted from plants and spices exhibit antimicrobial and antioxidant properties (Ng et al., 2014). The addition of filler into biopolymer films enhance the mechanical properties of a biopolymer films (Szymańska-Chargot et al., 2020). For biopolymer films, active compounds such as essential oils may be added to