DEVELOPMENT AND CHARACTERIZATION OF POLYPROPYLENE-BASED ANTIMICROBIAL ACTIVE PACKAGING INCORPORATED WITH TRANS-CINNAMALDEHYDE

by

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Sekian, terima kasih.

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This dissertation is composed of my original work and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

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LIST OF ABBREVIATIONS

Abbreviation	Caption
ASTM	American Society for Testing Materials
GC-FID	Gas chromatography-flame ionization detector
cm	Centimeter
CAS	Chemical Abstracts Service
CFU	Colony forming unit
χ	Crystallinity
T_c	Crystallization temperature
DSC	Differential Scanning Calorimeter
D	Diffusion coefficient
ΔH_c	Enthalpy of crystallization
ΔH_m	Enthalpy of melting
EOC	Essential oil compounds
FTIR-ATR	Fourier Transform Infrared-Attenuated Total Reflectance
g	Gram
h	Hour
J	Joule
kg	Kilogram
LDPE	Low density polypropylene
MJ	Megajoule
MPa	Megapascal
T_m	Melting temperature
m	Meter

µg	Microgram
µL	Microliter
mg	Milligram
mL	Millimeter
mm	Millimeter
min	Minutes
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
Pa	Pascal
PP	Polypropylene
RH	Relative Humidity
rpm	Revolutions per minute
s	Seconds
TGA	Thermogravimetric analysis
CIN	Trans-cinnamaldehyde
WVP	Water vapor permeability
w/w	Weight per weight

LIST OF SYMBOLS

Symbol	Caption
°C	Degree Celsius
=	Equal
<	Less than
L*	Lightness
%	Percentage
±	Plus-minus sign
a*	Redness/greenness
b*	Yellowness/blueness

PEMBANGUNAN DAN PENCIRIAN PAKEJ AKTIF ANTIMIKROBIAL BERASASKAN POLIPROPILENA DIGABUNGKAN DENGAN TRANS-CINNAMALDEHYDE

ABSTRAK

Pembungkusan aktif antimikrobial menyediakan penyelesaian yang ideal untuk industri makanan untuk menjaga kualiti dan keselamatan makanan yang kurang aditif makanan dan makanan yang diproses secara minimum. Dalam kajian ini, pembungkusan filem aktif antimikrobial dihasilkan dengan menambahkan 5% trans-cinnamaldehyde (CIN) 5% w / w dalam matriks polimer polipropilena (PP) menggunakan penyemperitan skru kembar dan penyemperitan filem tiup. Pencirian filem yang dihasilkan dilakukan pada sifat mekanikal, terma dan penghalang wap air. Analisis spektroskopi Fourier transform infrared mengesahkan bahawa CIN berjaya dimasukkan dalam filem ini. Filem ini mengekalkan 44.05% daripada jumlah CIN yang ditambahkan kerana pemprosesan terma. Penambahan CIN meningkatkan pemanjangan pada takat putus filem kerana CIN daya antara molekul rantai polimer dikurangkan. Analisis thermogravimetric menunjukkan filem PP stabil secara termal kerana suhu penurunan PP tidak dipengaruhi oleh penambahan CIN. Differential scanning calorimeter menunjukkan CIN mengurangkan kristalinitas filem, selain mengurangkan entalpi lebur dan suhu penghabluran. Kebolehtelapan wap air dari filem PP-CIN dan PP tidak mempunyai perbezaan yang signifikan. Warna coklat diperhatikan dalam filem PP-CIN kerana ciri CIN yang wujud. Keberkesanan filem aktif dianalisis dengan menggunakan gas-chromatography-flame ionization detector. Hasil kajian menunjukkan bahawa pelepasan CIN mencapai keseimbangan setelah 12 jam dengan pelepasan cepat dalam 6 jam pertama. Pekali penyebaran untuk CIN dari filem adalah 6.31 x 10^{-12} m^2/s. Kaedah agar disk diffusion digunakan untuk

menentukan aktiviti antimikrobial filem yang dihasilkan. CIN meningkatkan sifat antimikrobial filem dengan keberkesanannya menghalang pertumbuhan *Staphylococcus aureus* (bakteria Gram positif). Tidak ada aktiviti antimikrobial yang diperhatikan terhadap *Escherichia coli* (bakteria Gram negatif). Filem PP-CIN dapat berfungsi sebagai pembungkus aktif antimikrobial dan berpotensi memanjangkan jangka hayat makanan.

DEVELOPMENT AND CHARACTERIZATION OF POLYPROPYLENE-BASED ANTIMICROBIAL ACTIVE PACKAGING INCORPORATED WITH TRANS-CINNAMALDEHYDE

ABSTRACT

Antimicrobial active packaging provides an ideal solution for food industry to preserve the quality and safety of reduced additives and minimally processed food. In this study, antimicrobial active film packaging was produced by incorporating 5% w/w trans-cinnamaldehyde (CIN) in the polypropylene (PP) polymer matrix using twin-screw extrusion and blow film extrusion. Characterization of the films produced were conducted on mechanical, thermal and water vapor barrier properties. The Fourier transform infrared spectroscopy analysis confirmed that the CIN was successfully incorporated in the film. The film retained 44.05% of total CIN added due to thermal processing. The addition of CIN increased elongation at break of the film as the intermolecular forces between polymer chains were reduced. Thermogravimetric analysis showed the PP film was thermally stable as the degradation temperature of PP was not affected by the addition of CIN. Differential scanning calorimeter showed CIN reduced the crystallinity of the film, aside from reducing the enthalpy of melting and crystallization temperature. Water vapor permeability of the PP-CIN, and PP films had no significant difference. A brownish color was observed in PP-CIN film due to inherent characteristic of CIN. The efficacy of the active film was analyzed by using gas chromatography-flame ionization detector. The results demonstrated that the release of CIN achieved equilibrium after 12 hours with rapid release in the first 6 hours. Agar disk diffusion method was used to determine the antimicrobial activity of produced films. CIN improved the antimicrobial properties of the film by effectively inhibited the growth of *Staphylococcus aureus* (Gram-positive bacteria). No

antimicrobial activity was observed against *Escherichia coli (*Gram-negative bacteria). The PP-CIN film was able to function as antimicrobial active packaging and potentially extend the shelf life of food.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Packaging plays an important role in food supply chain. Plastic packaging is the most used packaging material due to its benefits over other packaging materials such as metal, paper and glass. The preference of plastic packaging material by food manufacturing industry is because plastics are lightweight, easy to process, flexible, durable, ergonomics and can be designed easily. Among all the types of plastics, polypropylene (PP) is one of the widely used materials. Generally, PP possess good resistance to chemicals, excellent clarity and is a considerably stiff material. Packaging materials protects the packaged food by maintaining the quality and safety of food before reaching to the consumers. The packaging materials are characterized by their mechanical properties, thermal properties, and barrier properties towards water vapor and oxygen diffusion. Food packaging is under continuous development in order to maximize the functions of food packaging to provide better protection on the food quality and safety.

The development of packaging in food industries is driven by the consumers demand for healthier, minimally processed and preservative-free products (López et al., 2007). Active packaging, which has been the focus in food industries, involves the interaction of environment, package and product intended to prolong the shelf life, enhance safety and improve the sensory properties of the product (Ribeiro-Santos et al., 2017). The quality and safety of food can be preserved by absorbing undesirable compound, such as ethylene, oxygen and moisture, by active agent from food or by releasing active agent into the food to add desired compounds, such as antimicrobial substance, antioxidant substance and flavor, into the packaging.

The trend of antimicrobial packaging development is due to the aim of producing preservative-free products and to lower the levels of preservatives added directly to the food when the preservative agents are incorporated to the packaging materials (López *et al.*, 2007). Antimicrobial active packaging can be achieved by incorporating antimicrobial agent in the packaging in different forms. Direct blending of antimicrobial agent into the polymer matrix could limit or prevent the growth of microorganisms and control the release of active agent. However, it could affect the original properties of the polymer. Preservative agents which are from natural sources are popular choices of food industry to use as active agents due to the consumers demand on natural additives.

Essential oils which are natural extracts have shown a significant antioxidant ability and strong antimicrobial agent against many types of foodborne pathogens and food spoilage bacteria (Diniz do Nascimento *et al.*, 2020; Mith *et al.*, 2014). Trans-cinnamaldehyde (CIN) is one of the essential oil compounds which is found as a main active constituent in cassia and cinnamon essential oil. CIN has yellow color and is in the form of liquid. It is recognized as Generally Recognized as Safe (GRAS) to be use in food application. The outstanding antimicrobial properties of CIN has been investigated in many studies against bacteria and fungi (Cetin-Karaca & Newman, 2018; Du *et al.*, 2021; Firmino *et al.*, 2018; Kot *et al.*, 2020; Lee *et al.*, 2020; Purkait *et al.*, 2020; Wieczyńska *et al.*, 2016). The antimicrobial activity of a compound depends on its molecular structure. The efficacy of antimicrobial agent against Gram-positive and Gram-negative bacteria should be studied as they possess different cell membrane structure which responsible in providing resistance towards antimicrobial activity.

Several authors studied the incorporation of CIN and cinnamon extracts into different polymers. Srisa & Harnkarnsujarit (2020) incorporated CIN in poly(lactic acid)/poly(butylene adipate-co-terephthalate) films at 2, 5 and 10%. de Souza *et al.* (2014) and Villegas *et al.* (2019) incorporated cinnamon extracts in cassava starch biocomposite films, and PLA/organoclay, respectively. Jia *et al.* (2021) added 5 % cinnamon extract in gliadin films whereas Qin *et al.* (2015b) incorporated in poly(lactic acid)/poly(trimethylene carbonate) at 3, 6, 9 and 12 %. Yang *et al.* (2020) also fabricated bionanocomposites polysaccharide composite nanofiber film loaded with cinnamon extract. Research is lacking regarding investigation of PP films incorporated with CIN.

1.2 Problem Statement

Sustainability in food waste management is important to minimize the impact of food waste on environment. Food waste is generated throughout the food system, which include production, distribution and consumption. About 1.6 billion metrics of food is wasted per year globally (FAO, 2013). Managing the food waste through landfills releases methane as food decay. This greenhouse gas results in global warming which leads to climate change. The economically impacts of food waste should not be underestimated as food waste management is costly. The trend of consumers preferring reduced additives and minimally processed food offers additional challenges to food industry to maintain the quality and safety of food. This is because reduced additives and minimally processed food are prone to microbial deterioration which would increase food waste. The growth of foodborne spoilage bacteria can cause undesirable physical changes on food, such as color, texture, smell and flavor, while pathogenic bacteria may cause disease or illness to consumer.

Therefore, the development of antimicrobial active packaging by blending CIN with PP provides an alternative to extend the shelf life of food while meeting the consumers demand.

1.3 Objectives

The main objective of this research was to develop and characterize antimicrobial active packaging based on PP containing trans-cinnamaldehyde.

In order to achieve the main objective, the specific objectives are listed below

1. To produce PP and CIN-containing PP films using twin-screw extrusion and blow film extrusion
2. To analyze the effect of CIN on mechanical, thermal and water vapor barrier properties of film
3. To investigate the efficacy of the produced films to act as active packaging via release test and antimicrobial analysis

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to active packaging

Traditional food packaging serves four basic functions, namely containment, convenience, communication and protection (Otles & Sahyar, 2020). Firstly, containment allows easy handling and transportation of food in food supply chain. Food packaging brings convenience to consumers as it consists of various shapes and sizes with different consumption methods, allowing consumers to enjoy the food to the fullest. Packaging communicates with consumers regarding product information, nutritional content, and preparation instruction through labelling. Protection of packaging is basically passive by keeping the food separated from external condition, which could prevent loss of food volume and food contamination from outer environment. Shelf life of a food is significantly reduced due to deterioration attributed to oxidation, enzymatic activity, moisture transfer and microbial growth (Petruzzi *et al.*, 2017). Over the last decades, additive-free, minimally processed and safe food are leading the trend in food industry due to the increasing consumer demand (Yildirim & Röcker, 2018). To promise an acceptable shelf life of these high-demand food, the protective function of packaging can be designed further using innovative packaging technologies such as active packaging (Brockgreitens & Abbas, 2016).

Active packaging interacts with food by "deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food" (European Commission, 2009). Active packaging system can be categorized into active releasing systems and active scavenging systems. Active scavenging system performs its role as absorber by removing undesired substances, such as carbon dioxide, oxygen, moisture, ethylene and odor, present in

the packaging; whereas packaging in active releasing system acts as emitters through the addition of desired compounds, such as antimicrobial agent, antioxidant, flavors and ethylene, into the packaging, either the headspace or onto food surface (Ahvenainen, 2003). Active compounds can be incorporated in different formats such as sachets, labels, coating on the surface of food or directly incorporate into the packaging material's polymer matrix. Different active packaging technologies are applied in food applications according to the desirable benefits provided by the packaging system and the types of food.

2.2 Antimicrobial active packaging

Antimicrobial active packaging system could inhibit the growth of microorganisms in packaged food by incorporating active microbial substances in the packaging system. It is designed to replace the traditional method of direct addition of antimicrobial substances to food, which would limit the activity of the substances due to the interactions with the complex food matrix (Yildirim & Röcker, 2018). Antimicrobial active packaging system can be divided into three types according to the types of antimicrobial agents incorporated. First, systems with non-volatile antimicrobial agents, such as organic acids and their salts, metals and enzymes, would require direct food contact so that they can be released into the food by diffusion. Besides, volatile antimicrobial agents, such as inorganic gases, spices and herbs extract essential oils and their pure compound, can be released from the packaging into the headspace of the packaging without direct food contact. The third type is nonmigratory antimicrobial packaging, where food contact is required as the antimicrobial agents are attached at the polymer backbone (Yildirim & Röcker, 2018). This could be achieved

by surface modification of the packaging with antimicrobial agents or using polymer packaging with inherent antimicrobial properties (Yildirim, 2011).

2.3 Essential Oil Compounds (EOC)

Natural antimicrobial compounds such as essential oils are trending due to increasing awareness of consumers towards safety of chemical additives and the effectiveness on antimicrobial activity (Kapetanakou & Skandamis, 2016). Puškárová *et al.* (2017) demonstrated the antimicrobial activity of six essential oils against *Escherichia coli, Salmonella typhimurium, Yersinia enterocolitica, Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecalis, Bacillus cereus, Arthrobacter protophormiae, Pseudomonas fragi, Chaetomium globosum, Penicillium chrysogenum, Cladosporium cladosporoides, Alternaria alternata,* and *Aspergillus fumigatus*. Of all six essential oils, four of them showed very strong antibacterial activity against all tested strains at both full strength and reduced concentrations. In addition, Pattnaik *et al.* (1996) tested ten essential oils against 22 bacteria strains. The results showed that all essential oils inhibited 15 bacteria strains, and four essential oils were effective against all tested bacteria strains.

2.3.1 Properties of EOC

Essential oils are a complex mixture of 20 to 60 low molecular weight organic compounds at different concentrations, with terpenes, terpenoids and phenylpropenes as the dominant constituents (Burčul *et al.*, 2020; Chouhan *et al.*, 2017). These phytochemicals are volatile organic compounds of low molecular weight below 300 g/mol, which are synthesized and secreted in cytoplasm of plant cells (Dhifi *et al.*, 2016). Essential oil compounds can have different flavors, colors and odors that yield

distinct characteristics according to species, plant age, plant part and environment (Suppakul, 2016).

Terpenes composed of mainly monoterpenes and sesquiterpenes, which differ in the number of isoprene units, but longer chains like diterpenes and triterpenes also present (Chouhan *et al.*, 2017). Terpenoids are derived when oxygen molecules are added and methyl groups are moved or removed from terpenes (Chouhan *et al.*, 2017). Phenylpropanoids are non-terpene compounds with C6-C3 carbon skeleton. They are biosynthesized through phenylpropanoid pathway, where amino acid phenylalanine is converted to cinnamic acid followed by subsequent reduction to aldehyde (Mousavi Khaneghah *et al.*, 2018). Examples of phenylpropanoids are cinnamaldehyde, eugenol and safrole. Essential oil constituents can have different chemical functionalities, which include alcohol (geraniol, linalool, terpineol, and menthol), phenol (carvacrol and thymol), aldehyde (citral and citronellal), ketone (carvone and camphor and ether (eucalyptol) (Guimarães *et al.*, 2019). The antimicrobial action of terpenoids is determined by their functional group where the modification of terpenes takes place (Griffin *et al.*, 1999).

2.3.2 Antimicrobial Efficacy of EOC

The antimicrobial activity of terpenes is investigated by Guimarães *et al.* (2019), which revealed 16 out of 33 terpenes and terpenoids commonly found in essential oils have shown antimicrobial activity when tested against *Bacillus cereus*, *Salmonella* Typhimurium, *Escherichia coli* and *Staphylococcus aureus*. According to Davidson *et al.* (2001) and Dorman & Deans (2000), aldehydes or phenols, such as cinnamaldehyde, carvacrol, eugenol, citral and thymol have the highest antimicrobial activity followed by terpene alcohols and ketones or esters whereas terpene

hydrocarbons are mostly inactive. The antimicrobial properties are further confirmed by the mode of action proposed. The mechanism is affected by the chemical structure of antimicrobial compounds and the strain microorganism used (Dorman & Deans, 2000).

Compounds with hydroxyl groups, such as thymol, carvacrol and eugenol are found to be highly reactive and effective as they rupture cell membrane by inactivating the enzymes through hydrogen bond with active sites of enzymes, thus causing membrane dysfunction and content depletion (Chauhan & Kang, 2014; Guimarães *et al.*, 2019). Apart from disrupting cytoplasmic membrane, phenolic compounds also affects the driving force of protons, active transport, electron flow, and coagulation of cell contents (Dorman & Deans, 2000; Knobloch *et al.*, 1986; Lambert *et al.*, 2001). The combination therapy of an antimicrobial compounds with terpenes which are low molecular weight have shown inhibitory effect by acting on the fungal and bacterial biofilm production (Zacchino *et al.*, 2017).

Although most individual components have been recognized as antimicrobial agents, they show highly divergent values of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) due to different efficacy of compounds attributed by functional groups and the types of bacteria strain (García-Salinas *et al.*, 2018). The effect of antimicrobial compounds on Gram-positive and Gram-negative microbes differs due to the difference in cell membrane structure, which provide resistivity to the cell. Gram-negative bacteria possess thin peptidoglycan and outer membrane rich in lipopolysaccharides, which limit the entry of hydrophobic substances (Chouhan *et al.*, 2017). In addition, the outer membrane of Gram-negative bacteria composed of porins, hydrophilic channels for transportation

of low molecular weight substances (Lopez-Romero *et al.*, 2015; Nikaido, 2001). In contrast, Lopez-Romero *et al.* (2015) & Magiatis *et al.* (2002) proposed that Gram-positive bacteria greater resistance towards action of antimicrobial compounds may be due to rigidity contributed by thicker layer of peptidoglycan, making the antimicrobial agents difficult to penetrate. However, several studies have shown that Gram-positive bacteria have lower antimicrobial activity, which is due to the thickness of peptidoglycan wall that is not dense enough to act as a barrier to small antimicrobial molecules (Chouhan *et al.*, 2017; Zinoviadou *et al.*, 2009).

2.3.3 EOC: trans-cinnamaldehyde

Cinnamaldehyde or 3-phenyl-2-propenal, is a main active constituent of cassia and cinnamon oil. Cinnamaldehyde is a hydrophobic aromatic aldehyde and naturally exists as trans isomer (Figure 2.1). Trans-cinnamaldehyde is a liquid characterized by its yellowish color, spicy aroma and strongly reminiscent of cinnamon (Bauer *et al.*, 2001). The physical properties of trans-cinnamaldehyde are displayed in Table 2.1 referring to National Center for Biotechnology Information (2021). According to FDA's CFR (2021), trans-cinnamaldehyde is classified as Generally Recognized as Safe (GRAS) molecule and is approved for use in foods. It is well known for its antibacterial and antifungal effects.

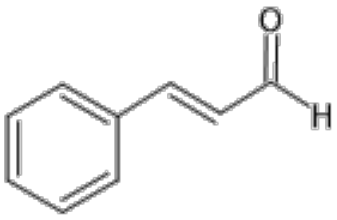

Figure 2.1: Chemical structure of trans-cinnamaldehyde.

Table 2.1: Physical properties of trans-cinnamaldehyde.

Properties	Description
CAS number	14371-10-9
Molecular Formula	C_9H_8O
Molecular Weight	132.16 g/mol
Density	1.048 g/cm^3 at 20 °C
Boiling Point	246–253 °C
Melting Point	-7.5 °C
Flash Point	71.11 °C

Xing *et al.* (2014) reported that the antimicrobial effect of cinnamon oil increases with increasing cinnamaldehyde concentration in cinnamon oil, thus confirming the role of cinnamaldehyde in microbial inhibition. Previous studies have proven the broad-spectrum antimicrobial activity of trans-cinnamaldehyde against *Pseudomonas fluorescence, Pectobacterium carotovorum, Agrobacterium tumefaciens, Salmonella enterica, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa, Bacillus cereus, Listeria monocytogenes* and *Salmonella typhimurium* (Cetin-Karaca & Newman, 2018; Du *et al.*, 2021; Firmino *et al.*, 2018; Kot *et al.*, 2020; Lee *et al.*, 2020; Purkait *et al.*, 2020; Wieczyńska *et al.*, 2016).

The high electrophilic characteristics of carbonyl group adjacent to the double bond activates trans-cinnamaldehyde to react with nucleophiles such as amino groups and protein sulfhydryl of the microbial cell (Neri *et al.*, 2006). The minimum inhibitory concentrations (MICs) of cinnamaldehyde against *S. aureus*, and *E. coli* have been reported to be 195, and 98 μg/mL, respectively (Tian *et al.*, 2016). Xing *et al.* (2014) proposed that the antimicrobial properties are attributed to irreversible cell modifications induced by interference of cinnamaldehyde on enzymatic reactions of cell wall synthesis, for example, plasma membrane destruction, loss of cell wall integrity and rigidity, cytoplasmic contents depletion, mitochondrial breakdown and

cell folding. Trans-cinnamaldehyde also has high antioxidant activity as it is a free radical scavenger and an enzyme inhibitor (Carvalho *et al.*, 2016; Davaatseren *et al.*, 2017; López-Mata *et al.*, 2018).

2.4 Packaging Strategy for an Efficient Antimicrobial Action

Antimicrobial packaging system can be achieved by several methods. Antimicrobial-containing sachets can be placed inside the package to gradually release the compounds over time (Suhag *et al.*, 2020). According to Ju *et al.* (2020), antimicrobial sachets contribute to shelf life extension of food. Coating method coats a layer of antimicrobial matrix on the packaging surface. This method promises the concentration of antimicrobial agents with minimum loss but its limitation is similar to direct addition of antimicrobial substances to food, which would reduce the antimicrobial efficiency due to excessive neutralization of antimicrobial substances by food complex (Quintavalla & Vicini, 2002). The antimicrobial agents can also be immobilized in the polymer matrix through covalent or ionic bonding. The release of antimicrobial agents depends on the types of interaction between the functional groups of antimicrobial compound and the polymer (Chawla *et al.*, 2021).

2.4.1 Direct blending

Film with antimicrobial effect can be produced by direct incorporation of bioactive agents in a polymer matrix, whereby this method would require understanding on the shearing and thermal resistance properties of the antimicrobial substances (Chawla *et al.*, 2021). This is because the processing operations during film fabrication such as extrusion, lamination, drying and printing involves high temperature that may cause compounds degradation or evaporation, resulting in loss of antimicrobial activity (Suppakul *et al.*, 2003). Direct blending the compounds into

the packaging is able to masked its strong flavor characteristic partially (Ruiz-Navajas *et al.*, 2013).

2.5 Effect of incorporation of EOC on food packaging materials

To fabricate antimicrobial active packaging, antimicrobial agents can be added into the food packaging materials. Antimicrobial packaging can improve shelf life of food, while also assuring food quality and food safety (Bastarrachea *et al.*, 2011). Spoilage and pathogenic microorganisms mostly grow on food surfaces. The foodborne microorganisms are inhibited by antimicrobial compounds carried by polymers interacting with the food surface. In addition, constant and adequate presence of antimicrobial agents on food surface is ensured by controlling the diffusion rate of antimicrobial agents via the polymer (Gharsallaoui *et al.*, 2016). The efficacy of this active packaging system strongly depends on the interactions of antimicrobial-package, package-food and the environmental conditions (Corrales *et al.*, 2014).

2.5.1 Mechanical, physical and chemical properties

The incorporation of essential oil compounds may alter the characteristics of packaging material including mechanical and barrier properties. The change in tensile properties is due to change in crystallinity of polymer disrupted by antimicrobial molecules. According to Han & Floros (1997), the mechanical properties of polymer is not influenced, if the antimicrobial molecules have a lower molecular weight than polymeric material and the antimicrobial molecules are able to fill in the void volume of amorphous region of polymer without altering the polymeric structure.

The thermal properties of polymer are found mostly not affected by antimicrobials, while the incorporation of antimicrobial compounds may improve

water vapor and gas barrier properties of polymer (Bastarrachea *et al.*, 2011). This is due to the hydrophobicity of polymer is increased, leading to lower water vapor permeability (Suppakul *et al.*, 2006). The barrier properties are also affected by the solubility change and pinholes formation due to the structural change (Bastarrachea *et al.*, 2011). In terms of physical properties, the surface morphology of polymer added with antimicrobials may show holes or pores created in the polymer matrix, which in turn will influence the tensile and barrier properties of the polymer (Bastarrachea *et al.*, 2011). According Suppakul *et al.* (2006), the addition of antimicrobials may reduce the transparency of the polymer. The effect of the antimicrobial incorporation on the appearance of packaging is important as it affects the application of packaging and the consumer acceptance.

2.5.2 Functional properties

The antimicrobial agents incorporated in the polymer will be released into the food packaging to act on the microbes. The release rate is determined by the diffusion of the antimicrobial substances through the polymer and their solubility in food (Bastarrachea *et al.*, 2011). According to Bastarrachea *et al.* (2011) & Corrales *et al.* (2014), antimicrobials having lower diffusion coefficients in packaging than in food matrices result in smaller amount being released to the food. The antimicrobial properties of packaging is also affected by the interaction of antimicrobial agent with packaging through hydrogen bonds, hydrophobic and electrostatic interactions (Corrales *et al.*, 2014). Various types of antimicrobial agent and packaging materials have been investigated to increase the antimicrobial efficacy of the active packaging system. Among the individual compounds, carvacrol, cinnamaldehyde, citral, eugenol and thymol are some of the most popular antimicrobial agents. The recent findings on

antimicrobial activity of packaging incorporated with essential oil compounds are summarized in Table 2.2.

Table 2.2: Summary of findings of antimicrobial effect of food packaging incorporated with carvacrol, cinnamaldehyde, citral, eugenol or thymol.

EOC	Film Matrix	Tested microorganisms	Findings	Food Product	References
Carvacrol	Fish gelatin	*Escherichia coli O157:H7* *Staphylococcus aureus*	Slightly bigger inhibition zone of *E. coli* than *S. aureus* Antimicrobial effect against both bacteria increase with increased concentration of carvacrol from 0.2 to 0.6 % w/v No antimicrobial effect was shown against both bacteria after 15 days of storage	–	Neira *et al.* (2019)
	Whey protein isolates nanofibers	*Listeria monocytogenes* *Staphylococcus aureus* *Salmonella enteritidis* *Escherichia coli*	Showed nearly 2.0-fold higher antimicrobial activity against all microbes	–	Wang *et al.* (2019)
	Ethylene vinyl alcohol (EVOH)	*Aspergillus flavus* *Aspergillus parasiticus*	Reduced growth rate of both microbes with increasing concentration from 0 to 4 mg/plate	–	Mateo *et al.* (2017)
	Chitosan, guar gum, and whey protein isolate	*Bacillus cereus* *Escherichia coli*	Reduced *E. coli* count by 36% with 0.75% w/w carvacrol Reduced *B. cereus* count by 29% with 0.75% w/w carvacrol	–	Dhumal *et al.* (2019b)

Table 2.2: Continued.

EOC	Film Matrix	Tested microorganisms	Findings	Food Product	References
Carvacrol	Sago starch and guar gum	*Bacillus cereus* *Escherichia coli*	Sensitive (inhibition diameter 18 to 23 mm) against *B. cereus* and *E. coli* with 0.75% w/w carvacrol	–	Dhumal *et al.* (2019a)
	Flaxseed gum-sodium alginate	*Vibrio Parahemolyticus* *Shewanella putrefaciens* *Staphylococcus aureus* *Pseudomonas fluorescens*	Exhibited good antimicrobial activity against all tested bacteria Inhibition ratio increased with increasing concentration of carvacrol from 0 to 2.0 mL/L *V. parahemolyticus* was the most resistant strain whereas *S. aureus* was the most sensitive strain	–	Fang *et al.* (2019)
	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)	*Listeria innocua* *Escherichia coli*	Greater antimicrobial effect against *E. coli* than *L. innocua*	cheese, chicken breast, fresh-cut pumpkin and melon	Requena *et al.* (2019)
Cinnamaldehyde	Ethylene vinyl alcohol (EVOH)	*Aspergillus flavus* *Aspergillus parasiticus*	Stopped growth rate of both microbes on low concentration	–	Mateo *et al.* (2017)

Table 2.2: Continued.

EOC	Film Matrix	Tested microorganisms	Findings	Food Product	References
Cinnamaldehyde	Chitosan, poly(vinyl alcohol), fish gelatin	*Listeria monocytogenes* *Staphylococcus aureus* *Salmonella enteritidis* *Escherichia coli*	Inhibition diameters were slightly higher against Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) than Gram-negative (*S. enteritidis* and *E. coli*) Inhibition zone increased when concentration increases from 0 to 1.6 w/v	–	Hosseini *et al.* (2021)
	Polylactic acid (PLA)	*Escherichia coli* *Listeria monocytogenes*	Antibacterial activity of the film against *L. monocytogenes* was higher than *E. coli* Antibacterial activity against both bacteria reached 100% when cinnamaldehyde content is 10%	–	Zhang *et al.* (2020)
	Poly(lactic acid) (PLA) and poly(ε-caprolactone) (PCL)	mesophilic and psychrophilic counts	Growth rate of both mesophilic and psychrophilic bacteria slowed down Film with 9 wt% cinnamaldehyde was more effective in reducing microbial count	button mushrooms	Qin *et al.* (2015a)
	Gliadin	*Staphylococcus aureus* *Escherichia coli*	Inhibited the growth of *E. coli* and *S. aureus*	–	Jia *et al.* (2021)

Table 2.2: Continued.

EOC	Film Matrix	Tested microorganisms	Findings	Food Product	References
Cinnamaldehyde	Polycaprolactone (PCL)	*Staphylococcus aureus* *Escherichia coli*	Completely inactivated the growth of the artificially inoculated *E. coli* and *S. aureus* with 5 wt % cinnamaldehyde	–	Uzunlu & Niranjan (2017)
	Polylactic acid (PLA)	*Staphylococcus aureus* *Escherichia coli*	Effective in antibacterial activity on *S. aureus* and *E. coli* from 20% More effective against *S. aureus* than *E. coli*	–	Akgün *et al.* (2020)
	Low-density polyethylene (LDPE)	*Staphylococcus aureus*	Showed 2.5 ± 0.15 log reduction after 10 days storage	chicken mutton, cheese, and grape foods	Manukumar & Umesha (2017)
	Polypropylene (PP)	*Staphylococcus aureus* *Escherichia coli*	Showed significant antibacterial activity against both strains Diameter of inhibition zone of both strains was similar	–	Kaplan *et al.* (2020)
Citral	Chitosan, guar gum, and whey protein isolate	*Bacillus cereus* *Escherichia coli*	Reduced *E. coli* count by 44% with 1.0% w/w citral Reduced *B. cereus* count by 31% with 1.0% w/w citral	–	Dhumal *et al.* (2019b)

Table 2.2: Continued.

EOC	Film Matrix	Tested microorganisms	Findings	Food Product	References
Citral	Sago starch and guar gum	*Bacillus cereus* *Escherichia coli*	Very sensitive (inhibition diameter 24 to 28 mm) against *B. cereus* with 1.0% w/w citral Extremely sensitive (inhibition diameter > 29 mm) against *E. coli* with 1.0% w/w citral	–	Dhumal *et al.* (2019a)
	Polyethylene terephthalate (PET)	*Escherichia coli* *Staphylococcus aureus*	Bactericidal effects against *E. coli* (2.1 log) and *S. aureus* (4.3 log) at concentrations of 20% dry matter	–	Thielmann *et al.* (2021)
	Alginate	*Escherichia coli*	Percentage of growth inhibition increased with storage time	–	Alarcón-Moyano *et al.* (2017)
	Guar gum, sago starch and whey protein isolate	*Escherichia coli* *Bacillus cereus*	*E. coli* exhibited greater inhibition diameter than *B. cereus*	–	Dhumal *et al.* (2019c)
Eugenol	Chitosan, guar gum, and whey protein isolate	*Bacillus cereus* *Escherichia coli*	Reduced *E. coli* count by 40% with 0.5% w/w eugenol Reduced *B. cereus* count by 23% with 0.5% w/w eugenol	–	Dhumal *et al.* (2019b)

Table 2.2: Continued.

EOC	Film Matrix	Tested microorganisms	Findings	Food Product	References
Eugenol	Chinese yam starch	*Escherichia coli* *Listeria monocytogenes* *Staphylococcus aureus*	Displayed slightly better antibacterial activity on *E. coli* than the other two bacteria Film with 3% eugenol was able to extend the shelf-life of pork	pork	Cheng *et al.* (2019)
	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)	*Listeria innocua* *Escherichia coli*	Greater antimicrobial effect against *E. coli* than *L. innocua*	cheese, chicken breast, fresh-cut pumpkin and melon	Requena *et al.* (2019)
	Chitosan, acorn starch	*Escherichia coli* *Staphylococcus aureus*	Observed better inhibition of the films with higher eugenol concentrations Inhibition zone of eugenol-containing films against *E. coli* were slightly larger than that of *S. aureus*	–	Zheng *et al.* (2019)
Thymol	Gelatin	*E. coli O157:H7* *Bacillus subtilis*	Higher antimicrobial effect against *B. subtilis* than *E. coli O157:H7* Showed prolonged (48 h) inhibition effect against both bacteria	–	Li *et al.* (2020)
	Soybean protein isolate	*Escherichia coli*	Showed profound antimicrobial activity	–	Wu *et al.* (2021)

Table 2.2: Continued.

EOC	Film Matrix	Tested microorganisms	Findings	Food Product	References
Thymol	Sodium alginate	*E. coli O157:H7* *Staphylococcus aureus*	Exhibited remarkable inhibition of the growth of *E. coli* and *S. aureus*	–	Chen *et al.* (2021)
	Jackfruit straw and starch, jackfruit skin and starch	*Staphylococcus aureus* *Escherichia coli*	10 wt% thymol exhibited positive inhibitory effect against *E. coli* and *S. aureus*	–	Shukor *et al.* (2021)
	Peanut protein isolate	*Escherichia coli* *Staphylococcus aureus* *Lactobacillus plantarum* *Pseudomonas aeruginosa*	Antimicrobial activity increased significantly by the addition of thymol *S. aureus* and *L. plantarum* were more susceptible than *E. coli* and *P. aeruginosa*	–	Zhong *et al.* (2017)
	Polylactic acid (PLA)	*Escherichia coli* *Staphylococcus aureus*	Higher antimicrobial effect against *S. aureus* than *E. coli*	–	Ramos *et al.* (2020)
	Polylactic acid (PLA) and kenaf	*Escherichia coli*	Reduced *E. coli* inoculated on the surface after 30 days storage at 10°C	Processed sliced chicken	Tawakkal *et al.* (2017)

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Polypropylene PM383 (melt flow rate at 230 °C = 3.0 g/10 min, density = 0.9 g/cm^3) manufactured by Lotte Chemical Titan (Johor, Malaysia) was purchased from Toiling Corporation (Penang, Malaysia). The PP resin was supplied in the form of granules. Trans-cinnamaldehyde (99%) was purchased from Acros Organics (New Jersey, USA).

3.2 Film Preparation

3.2.1 Twin-Screw Extrusion and Palletization

Trans-cinnamaldehyde (CIN) was manually mixed with polypropylene (PP) resins with a concentration of 0 and 5% weight basis in a zip lock bag to form a homogenous mixture. The mixture was fed into the feed hopper and extruded using a Scientific Twin-Screw Extruder Type LTE 20-40 (Lab Tech Engineering Company LTD, Samutprakarn, Thailand). The 10 heating zones of the barrel were set from 145 to 190 °C, motor screw speed of 100 rpm and feeder screw speed of 50 rpm. This process was carried out with lights off to minimize the loss of light-sensitive active agent. The molten extrudate left the die in a string form and was cooled by passing through trough filled with cold water. The extruded strands were cut into pellets by passing through a Strand Pelletizer Type LZ-120 (Lab Tech Engineering Company LTD, Samutprakarn, Thailand) at a speed of 12.5 rpm. The pellets produced were collected in aluminum bags and labelled accordingly. The aluminum bags were then vacuum packed and stored at 4 ± 2 °C.

3.2.2 Blown Film Extrusion

PP film without additive (PP) and PP containing 5% CIN (PP-CIN) were produced using extrusion blown film machine MINI-40 (Queen's Machinery Co., Ltd., Taipei, Taiwan). The barrel temperature of four zones (feed zone, metering zone, compression zone and die zone) were set at 190 to 250 °C. The screw and roller speed were 400 rpm. The film produced were collected in rolls and kept in aluminum bags. The aluminum bags were labelled, vacuum packed and stored at 4 ± 2 °C.

3.3 Film Characterization

3.3.1 Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR) spectroscopy

Films were determined for absorption spectra using Shimadzu IRPrestige-21 FTIR spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Spectra were collected using attenuated total reflectance (ATR) technique at a resolution of 4 cm^{-1}. A total of 32 scans were conducted from wavenumber ranging from 600 to 4000 cm^{-1}. Dried CIN was analyzed to identify and compare the spectra of CIN. Analysis was performed in triplicate for each sample.

3.3.2 Quantification of Antimicrobial Agent

The quantification of antimicrobial agent was carried out according to Iñiguez-Franco *et al.* (2012) with some modifications. Pieces of the film weighing approximately 0.2 g were extracted with methanol in stirring water bath at 40 °C. The extractions were performed several times at 24, 48, 72 and 96 h to ensure complete extraction of trans-cinnamaldehyde. Quantification of trans-cinnamaldehyde incorporated in the film was conducted by using gas chromatography with a flame ionization detector (GC-FID, GC-2010 Plus, Shimadzu, Kyoto, Japan) equipped with