UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF TORCH GINGER (Etlingera elatior JACK) INFLORESCENCE ESSENTIAL OIL IN STARCH-BASED EDIBLE FILM FOR CHICKEN MEAT PACKAGING

ANIS ASYILA BINTI MARZLAN

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By

ANIS ASYILA BINTI MARZLAN

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the degree of Master of Science

March 2021

DEDICATION

This thesis is dedicated to

My beloved parents,
Marzlan bin Idris and Khalidah binti Mansor,
My siblings and relatives and not to forget,
To all my friends.

For their unconditional love, prayers and support through this journey.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ASSESSMENT OF ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF TORCH GINGER (*Etlingera elatior* JACK) INFLORESCENCE ESSENTIAL OIL IN STARCH-BASED EDIBLE FILM FOR CHICKEN MEAT PACKAGING

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Essential oils (EOs) are used extensively in wide applications to alternative synthetic compounds. Supercritical carbon dioxide (SC-CO_2) extraction is a sustainable green technology and achieves high purity with better retention of aromatic compounds. The application of torch ginger (*Etlingera eliator* Jack) inflorescence essential oil (TGIEO) in the active packaging system could offer new opportunities to develop a novel food packaging system as functional constituents in order to prolong the shelf-life of food products and ensuring their quality and safety. The poultry processing sector, such as chicken meat, provides a sustainable animal protein source, but it is very perishable and prone to oxidation and microbial growth. Incorporating EOs in active packaging could reduce environmental impacts as reduced chemical waste and offer protection to enhance safety and extend poultry meat's shelf life. Thus, the study's objectives were to optimize the pressure and temperature of SC-CO_2 extraction for TGIEO, evaluate the antioxidant and antibacterial activities of TGIEO incorporation into a starch-based edible film as active packaging, and determine the physicochemical, coliform count, rancidity and sensory analysis of chicken meat packed with TGIEO incorporated into the starch-based edible film during chilled storage. Response surface methodology (RSM) combined with the central composite (CCD) design was employed to optimize the SC-CO_2 extraction conditions which the optimized TGIEO was further incorporated into starch-based active packaging by drying the film-forming solution (FFS) via the casting method. The functional properties of this film in food system were observed by packing the chicken meat samples with TGIEO incorporated into the starch-based edible film (TF). The optimized SC-CO_2 extraction conditions were pressure 286.4 bar and temperature 57.3°C. The TGIEO incorporated into starch-based edible film showed positive antibacterial activities against six pathogenic bacteria (*Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, Salmonella Typhi,* and *Escherichia coli*) and showed inhibition zone (6.0 ± 0.01 - 23.0 ± 0.12 mm). The phytochemical activities of this active packaging were DPPH (67.36 ± 2.48%) and $ABTS^+$ (84.78 ±

0.57%), respectively. At the end of the chilled storage period (3°C ± 1), the chicken sample TF showed the lowest coliform count (4.98 ± 0.07 CFU/g) and the lowest TBARS value (0.212 ± 0.31 mg MDA/kg). There is no significant difference ($p<0.05$) for all the chicken samples for sensory analysis and concluded that the TGIEO does not affect the organoleptic properties. Thus, the optimized SC-CO_2 extracted TGIEO incorporated into starch-based edible film showed significant antibacterial and antioxidant film properties that potentially extends the quality of chicken meat during the chilled storage period.

Keywords: torch ginger, essential oil, active packaging, chicken meat, shelf life

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN SIFAT ANTIBAKTERIA DAN ANTIOKSIDA MINYAK PATI BUNGA KANTAN (*Etlingera elatior* Jack) DALAM FILEM BOLEH MAKAN DARIPADA KANJI UNTUK MENINGKATKAN KUALITI DAN JANGKA HAYAT DAGING AYAM

Oleh

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Minyak pati (EOs) digunakan secara meluas dalam aplikasi yang pelbagai sebagai alternatif kepada sebatian sintetik. Pengekstrakan superkritikal karbon dioksida (SC-CO_2) adalah teknologi hijau yang mampan dan mencapai keaslian yang tinggi dengan pengekalan sebatian aromatik yang lebih baik. Penggunaan minyak pati bunga kantan (*Etlingera eliator* Jack) (TGIEO) dalam sistem pembungkusan aktif dapat memberi potensi baru agar sistem pembungkusan makanan baharu diperkembangkan. Sektor pemprosesan unggas seperti daging ayam menyediakan sumber protein haiwan yang berterusan, tetapi sangat mudah rosak akibat pengoksidaan dan pertumbuhan mikrob. Penambahan EOs dalam pembungkusan aktif dapat mengurangkan kesan persekitaran seperti pembuangan sisa kimia dan memberi peningkatan keselamatan makanan dan memanjangkan jangka hayat daging unggas. Oleh itu, objektif kajian ini adalah untuk mengoptimumkan kondisi pengekstrakan SC-CO_2 terhadap TGIEO, menilai aktiviti antioksida dan antibakteria terhadap penambahan TGIEO ke dalam filem boleh dimakan berasaskan pati sebagai pembungkusan aktif, dan menilai sifat fisikokimia, kiraan koliform, ujian tengik dan penilaian deria daging ayam yang dibungkus dengan filem berasaskan kanji yang ditambahkan TGIEO (TF) semasa penyimpanan sejuk. Kaedah respon permukaan (RSM) yang digabungkan dengan reka bentuk komposit pusat (CCD) digunakan untuk mengoptimumkan tekanan dan suhu pengekstrakan SC-CO_2 dimana TGIEO yang optima selanjutnya ditambah ke dalam filem boleh dimakan berasaskan pati sebagai pembungkusan aktif dengan mengeringkan larutan penghasilan filem (FFS) melalui kaedah *"casting"*. Sifat fungsi filem ini dalam sistem makanan diperhatikan melalui pembungkusan sampel daging ayam dengan filem berasaskan kanji yang ditambahkan TGIEO (TF). Tekanan 286.4 bar dan suhu 57.3°C merupakan kondisi pengekstrakan SC-CO_2 yang dioptimumkan. Penambahan TGIEO ke dalam filem boleh dimakan menunjukkan aktiviti antimikrob yang positif terhadap enam bakteria bawaan (*Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, Salmonella Typhi,* dan *Escherichia coli*) dan menunjukkan zon perencatan (6.0 ± 0.01 -

23.0 ± 0.12 mm). Aktiviti fitokimia filem ini adalah DPPH (67.36 ± 2.48%) dan $ABTS^{+}$ (84.78 ± 0.57%). Pada akhir tempoh penyimpanan sejuk (3°C ± 1), sampel ayam TF menunjukkan kiraan koliform terendah (4.98 ± 0.07 CFU/g) dan nilai TBARS terendah (0.212 ± 0.31 mg MDA/kg). Tidak ada perbezaan yang signifikan ($p < 0.05$) untuk semua sampel ayam bagi penilaian deria dan dapat disimpulkan bahawa TGIEO tidak mempengaruhi sifat organoleptik sampel ayam. Oleh itu, TGIEO yang diekstrak secara optimum melalui pengekstraktan SC-CO_2 dimasukkan ke dalam filem boleh dimakan menunjukkan sifat antimikrob dan antioksidan yang signifikan dan berpotensi meningkatkan kualiti daging ayam dalam tempoh penyimpanan sejuk.

Kata kunci: bunga kantan, minyak pati, pembungkusan aktif, daging ayam, jangka hayat

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LIST OF ABBREVIATIONS

a^*	Degree of redness or greenness
$ABTS^+$	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
b^*	Degree of yellowness or blueness
CCD	Central composite design
CF	Control film
CFU	Colony forming unit
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSC	Differential scanning calorimetry
EAB	Elongation at a break
EOs	Essential oils
FFS	Film-forming solution
GAE	Gallic acid equivalent
GC–MS	Gas chromatography–mass spectrometry
GRAS	Generally regarded as safe
IC_{50}	Inhibition concentration by 50%
L^*	Degree of lightness
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentrations
NF	Not packed with any film
PF	Packed chicken meat
QE	Quercetin equivalent
RSM	Response surface methodology

S-CO_2	Supercritical carbon dioxide
TBARS	Thiobarbituric acid reactive substances
TF	TGIEO incorporated into starch-based film
TFC	The total flavonoid content
TGI	Torch ginger inflorescence
TGIEO	Torch ginger inflorescence essential oil
TPC	The total phenolic content
TS	Tensile strength
WVP	Water vapour permeability
YM	The total phenolic content

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Bioactive compound in essential oil becomes attention as an alternative to synthetic compounds in food production. Essential oil is used extensively in nutraceutical, cosmetic, packaging, and food industries to alternate synthetic compounds. Various food-grade essential oil extensively uses in food products, such as rosemary in salad dressing, while peppermint, lemon, and orange essential oil in dessert and confectionery. Besides, as a flavoring, the essential oil can potentially protect the food from spoilage. Numerous essential oils are recognized as "generally regarded as safe" (GRAS); thus, they can incorporate in edible coatings and films to boost antimicrobial and antioxidant activities (Oriani et al., 2014).

Torch ginger (*Etlingera eliator* Jack) has demonstrated strong antibacterial, antifungal, and antioxidant activity due to the presence of several bioactive compounds (Vairappan et al., 2012). Most of the identified compounds in torch ginger are secondary metabolites with high phenolic content (Nurain et al., 2013; Abdelwahab et al., 2010). Polyphenols tend to substitute them with naturally available antioxidants and inhibit oxidation (Anesini et al., 2008). Various studies have focused on the extraction and utilization of each part of the torch ginger plant (bark, flower, leaves, rhizomes, roots, and stem) as a natural antioxidant and antibacterial agent in food and nutraceutical applications (Bhattacharya et al., 2014; Tao & Liu, 2012). The antibacterial activities of the essential oils of gingers are closely associated with the presence of oxygenated monoterpenes and other bioactive secondary metabolites (Vairappan et al., 2012). The GC-MS data showed the main components found in the torch ginger inflorescence essential oil (TGIEO) were identified as 1- decanol (16.27 %), dodecanal (16.87 %), n-dodecyl acetate (16.40 %), cis-9-tetradecen-1-ol (16.29 %) and 1-hexadecanol (16.34 %) (Susanti et al., 2013). Furthermore, previous studies also showed that 1-dodecanol (13.82%), dodecanal (12.10%), 17-pentatriacontene (10.52%) (Maimulyanti and Prihadi, 2015), á-pinene, decanal, and 1-dodecanol are the most abundant compounds in torch ginger included (Anzian et al., 2017, Meor Hussin et al., 2018).

Current food packaging technologies show that incorporating the bioactive compound to enhance functional and food-protective properties in bio-based films is crucial (Hanani et al., 2014), such EOs as active agents in food packaging (Vilela et al., 2018). Incorporating essential oils into polymeric matrices can improve water vapor resistance by increasing the film's hydrophobic fraction (S´anchez-Gonz´alez et al., 2011). Food packaging is critical for storage, transportation, and consistency preservation during the shelf life of foods. Conventional petroleum-based packaging; plastic is used widely in the food industry as it is durable, has good mechanical and barrier properties, easy processing, low cost, is less permeable to water vapor, and is compatible with various

food products (Assis et al., 2017). Natural compounds incorporated in packaging films can prevent or reduce food decay due to oxidation (López-de-Dicastillo et al., 2012).

Biodegradable polymers can be made from various materials, including polysaccharides, protein, and lipid derivatives. (Peelman et al., 2013; Debiagi et al., 2014). The demand for biodegradable films made from natural sources has risen in recent years as consumers have become more concerned about the environmental impacts of these products and have demanded better quality and appearance (Moradi et al., 2012). Polysaccharides are used to form films and coatings as food packaging because it is abundant and can be produced and obtained at a low cost on a large scale (Bonilla et al., 2013; Oriani et al., 2014). The global export and import of starch worth 9 576 000 tonnes and 9 577 000 tonnes reflected that starch is the sustainable source (FAO, 2018). Starch may be used because of its ability to form films (Bergo et al., 2008; Henrique et al., 2008) and produce odorless, colourless, low-oxygen-permeability polymer matrices (Jiménez et al., 2012; Cano et al., 2014). The current demand is for the production of functional packaging with active compounds that can improve the quality of the packed food products, which further contributes to the food's nutritional value, becomes the current demand (Medina-Jaramilloa et al., 2017).

Volatile compounds are present in EOs and are responsible for their biological activities, which potent to minimize or even eliminate the presence of microorganisms and/ or reduce the phenomenon of lipid oxidation (Ribeiro-Santos et al., 2017). The quality of chicken meat is altered by cross-contamination and can be caused by the oxidation of lipids in the presence of O_2. Active packaging contains an active substance such as an antioxidant that can interact with the headspace and packaged product to prolong its shelf-life and quality (Martucci et al., 2015). The innovation of bio-based active packaging containing antimicrobial compounds could minimize environmental impacts and offer protection for food microbiological contamination (Qin et al., 2017). Applying essential oils to food and food packaging extends food stability during storage and can perform an antimicrobial, antioxidant or flavoring function. EO minimizes or even eliminates microorganisms and/ or reduces the phenomenon of lipid oxidation (Ribeiro-Santos et al., 2017). Therefore, active packaging containing antibacterial and antioxidant compounds was suggested as a functional strategy to enhance chicken meat's safety and shelf life (Medina-Jaramillo et al., 2017).

1.2 Problem statements

Synthetic antioxidants obtained from chemical processes are commonly incorporated into food to inhibit or slow the oxidation of food during exposure to environmental factors. The increasing evidence of chronic diseases correlated with the limitation of synthetic food additives as they may exert harmful effects on human health (Farahmandfar et al., 2018). Natural preservatives obtained from plant sources have been actively studied as an alternative to synthetic materials. The concerns about the safety of some chemical preservatives have prompted rising interest in natural alternatives. Therefore, there is a need to find safe and effective replacements for chemical preservatives and treatments. Various extraction methods to extract torch ginger essential

oils such as hydrodistillation (Vairappan et al., 2012) and (Wijekoon et al., 2013); steam distillation (Susanti et al., 2013) and (Wijekoon et al., 2013); solvent extraction (Maimulyanti & Prihadi, 2015) and (Anzian et al., 2017); subcritical CO_2 extraction (Anzian et al., 2020 were reported.

For instance, the adverse effect of previous extraction methods, such as applying high heat in the steam distillation process, could alter the quality of the essential oils. The wide range of parameters level of the extraction makes the selected extraction method's optimization crucial. Supercritical CO_2 extraction is suitable for recovering various constituents, especially for non-polar organic compounds, because mild extraction conditions were achieved as CO_2 has a low critical temperature and pressure, making this process viable for broad applications (Mahdi et al., 2014). Advanced methods are considered the most promising extraction techniques due to less extraction time, low energy consumption, low solvent used, and less carbon dioxide emissions (Abdul Aziz et al., 2018). Thus, supercritical CO_2 extraction is suitable for recovering various non-polar organic compounds as conventional extraction has low yield efficiency and adverse effects on the bioactive compounds (Molino et al., 2020).

The increase in food demand is mounting plastic usage, which the accumulation and inappropriate disposal of these packaging contributed to the negative environmental impact since it takes a very long time to decompose (Debiagi et al., 2014). These driving research efforts to develop biodegradable films as a replacement for or reduction in the use of non-biodegradable packaging. Due to the pollution issues associated with plastic food packaging, sustainable packaging made from renewable materials has been extensively researched to replace synthetic polymers (Medina-Jaramilloa et al., 2017). Starch is an excellent example of polysaccharides that can be utilized due to its excellent performance to form odorless and colourless film structures with low oxygen permeability (Cano et al., 2014; Jiménez et al., 2012; Bergo et al., 2008). The incorporation of essential oils as active packaging becomes attention as it could reduce environmental impacts and offer protection against chemical contaminations. A previous study showed the efficacy of oregano and garlic EOs use within a structural matrix controlled the microbial action, which ensured that antimicrobials were distributed homogeneously and remained ineffective doses during the storage period (Fernandez-Pan et al., 2014). On the other hand, torch ginger inflorescence essential oil (TGIEO) possesses bioactivity actives, and to the extent, the application of TGIEO into food matrices is still limited in the literature. Thus, in this present study, TGIEO incorporated as a natural constituent into starch-based films.

Chicken meat is a highly perishable food due to its high nutrients content and high susceptibility to microbial contamination, which is the primary reason for the quality deterioration of chicken meat. The quality of chicken meat is altered by two main reasons, including microbial contamination and lipid oxidation. Therefore, chicken meat's safety and quality have gained high interest at all supply chain levels. Plant essential oils were proposed as natural alternatives to enhance safety and extend poultry meat's shelf-life. However, this sector's applications are limited due to the essential oil's low stability and rapid oxidation. In addition, the concentration of essential oil possesses odor and taste (Maryam Adilah & Nur Hanani, 2016), while chicken meats are highly

susceptible to lipid oxidation resulting in off-flavor, color, and texture changes, as well as safety concerns (Bostami et al., 2017). So, the effect of the active packaging in this present study on the quality of chicken meat is crucial to be determined.

Therefore, the current research aims to determine the optimized SC-CO_2 extraction's temperature and pressure on yield and quality of torch ginger which will be further used as active packaging constituents for food products. Pressure and temperature were evaluated in this study because a previous study indicated an increase in temperature (40 to 60°C) caused a significant increase in the yield of extraction from *Moringa oleifera* leaves, while a further increase resulted in a slight increase in the yield (Zhao & Zhang 2013). On the other hand, higher pressures are applied to take advantage of the impact of compression on the vegetal matter, which improves mass transfer and the release of essential oil from the plant matrix (Yousefi et al., 2019). Therefore, it can conclude that many bioactive compounds are potentially being extracted at a higher pressure and attributed to the quality of torch ginger essential oil. Therefore, the application of TGIEO in active packaging could offer new opportunities to develop a novel food packaging system.

1.3 Research hypothesis

The supercritical carbon dioxide (SC-CO_2) extraction conditions of torch ginger (*Etlingera eliator* Jack) inflorescence essential oil (TGIEO) on high yield and antioxidant solid activity will be successfully optimized by employing response surface methodology (RSM). The functional properties of TGIEO can be utilized by incorporating into the polymer as a natural antioxidant and antimicrobial constituents in the food packaging system.

1.4 Objectives

The objectives of this study were:

1. To optimize the pressure and temperature of supercritical carbon dioxide extraction for torch ginger inflorescence essential oil.

2. To evaluate the antioxidant and antibacterial activities of torch ginger inflorescence essential oil incorporation into a starch-based edible film as active packaging.

3. To determine the physicochemical, coliform count, and rancidity of chicken meat packed with torch ginger inflorescence essential oil incorporated into the starch-based edible film during chilled storage.

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Anis Asyila Binti Marzlan was born and raised at Kuala Pilah, Negeri Sembilan. She received her early education at Sekolah Rendah Tunku Kurshiah, completed her high school at Mara Junior Science College (MJSC) Serting and preuniversity at Centre of Foundation Studies for Agricultural Science, Universiti Putra Malaysia (UPM)**.** As UPM is her alma mater, she holds a BSc (Food Studies) major in Food Marketing in October 2018, and the same year, she pursued her research study in MSc of Science Food Technology under the supervision of Associate Professor Dr. Anis Shobirin Meor Hussin. As the beginning of her contribution towards the research area, she published several research articles and one of the noted achievements was her article was published in a Top 10% Q1 Journal; Industrial Crops & Products in May 2020. She has also actively engaged as a facilitator for a curricular credited course (Volunteerism Development) at the Co-Curriculum and Student Development Centre, UPM since September 2019.

LIST OF PUBLICATIONS

Publication in Referred Journal:

Marzlan, A. A., Muhialdin, B. J., Abedin, N. H. Z., Manshoor, N., Ranjith, F. H., & Hussin, A. S. M. (2021). Improving the quality and shelf life of chicken meat by incorporating Torch Ginger (*Etlingera elatior* Jack) inflorescence essential oil in starch-based active packaging. *Carbohydrate Polymers.* (Submitted)

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