UNIVERSITI PUTRA MALAYSIA

POTENTIAL OF GELATIN FILMS WITH SILVER-KAOLIN AND CINNAMON BARK OIL AS PRIMARY PACKAGING FOR PACKED SALAD

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By

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Chair: Associate Professor Nur Hanani binti Zainal Abedin, PhD
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The interest in renewable packaging film has increased since the rose of synthetic-based plastics disposal has caused serious issues towards environmental pollution. Active packaging is an alternative solution in improving the shelf life while sustaining the nutrients and quality of food products during storage. Gelatin is a protein that extracted from animals' skins and bones and has great potential as the renewable sources for packaging films. This research focused on how the physical, mechanical, and water barrier retention of the gelatin film were enhanced as active substances were incorporated into the packaging film for food products. In the first objective, this study discovered the potential application of kaolin (Kln) and silver/kaolin (Ag/Kln) in fish gelatin (FG)-composite films as active packaging material. Three different types of kaolins; raw Kln, Ag/Kln (1:2) mix and Ag/Kln (1:1) mix with various concentrations (15%, 30% and 45%) were prepared by solution casting. For the water barrier properties, the wettability test indicated that the addition of kaolin in gelatin films produced hydrophobic films and lower ($p<0.05$) water vapour permeability, regardless of the kaolin type and concentration. Scanning electron microscopy images portrayed that higher inclusions of Ag/Kln compounds are able to develop smoother surface and homogenous cross-section. In addition, by incorporating these two materials, films with great antimicrobial effect ($p<0.05$) towards both Gram-positive and Gram-negative bacteria were produced. Elevation of Ag/Kln concentration also proved to lower ($p<0.05$) the transmission of ultraviolet-vis light through the films. In the second objective, an active packaging film composed of silver/kaolin-gelatin (FG_Ag/Kln) incorporated with cinnamon bark essential oil (CBEO) of different concentrations (0% as control film, 3%, 6%, and 9%) was established. Increased in CBEO improved the mechanical properties of the films by increasing the Young's modulus (376.20 to 1637.40 MPa) and tensile strength (3.51 to 32.47 MPa). Additionally, decreases in the water vapour permeability (1.02 to 0.77%), water solubility (8.80 to 7.20%), and moisture content (0.46 to 0.32%) of the films were observed, while the water contact angle for all the films were higher than 100° (116.38 – 123.00°). These findings indicated good wettability, improved hydrophobic properties, and enhanced water retention property of the films as CBEO concentration increased. As CBEO concentration increased, both the Gram-positive and Gram-negative bacteria

were also effectively inhibited. These findings indicated the potential of 3-9% CBEO in enhancing the functional properties of FG_Ag/Kln composite film. In the third objective, the application of FG film reinforced with Ag/Kln (1:1) and CBEO as packaging materials was observed and compared. The potential of the packaging film to protect and maintain the quality of ready-to-eat salad (Romaine lettuce) was investigated for 13 days. The overall observations revealed that films contained the highest CBEO incorporation had improved and extended ($p<0.05$) the shelf life of ready-to-eat salad. Therefore, these findings suggested that FG film with Ag/Kln with CBEO has high potential and beneficial for food packaging applications.

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

POTENSI FILEM GELATIN DENGAN PERAK-KAOLIN DAN MINYAK PATI KULIT KAYU MANIS SEBAGAI BAHAN PEMBUNGKUSAN UTAMA UNTUK SALAD

Oleh

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Permintaan terhadap bahan pembungkusan yang boleh diperbaharui telah meningkat sejak peningkatan masalah pencemaran alam sekitar yang disebabkan oleh pelupusan plastik yang berasaskan sintetik. Pembungkusan aktif adalah penyelesaian alternatif dalam meningkatkan jangka hayat di samping mengekalkan nutrien dan kualiti produk makanan semasa penyimpanan. Gelatin adalah protein yang diekstrak dari kulit dan tulang haiwan serta mempunyai potensi besar sebagai bahan pembungkusan makanan. Penyelidikan ini memfokuskan terhadap penambahbaikan yang boleh dilakukan kepada sifat fizikal, mekanikal, dan kadar ketelapan air filem gelatin dengan menambahkan bahan-bahan aktif. Dalam objektif pertama, kajian ini telah menemukan potensi penggunaan kaolin (Kln) dan perak-kaolin (Ag/Kln) dalam filem komposit gelatin sebagai bahan pembungkusan aktif. Tiga jenis kaolin yang berbeza; kaolin, campuran Ag/Kln (1:2) dan campuran Ag/Kln (1:1) dengan pelbagai kepekatan (15%, 30%, dan 45%) telah disediakan. Ujian kadar ketelapan air telah menunjukkan bahawa penambahan kaolin dalam filem gelatin telah menghasilkan filem yang hidrofobik dan kebolehtelapan wap air yang lebih rendah ($p<0.05$), tanpa mengira jenis dan kepekatan kaolin. Imbasan mikroskopi elektron menggambarkan bahawa penambahan Ag/Kln yang lebih tinggi mampu menghasilkan permukaan yang sekata dan keratan rentas yang homogen. Di samping itu, dengan campuran dua bahan aktif ini, filem dengan kesan antimikrobial yang signifikan terhadap bakteria Gram-positif dan Gram-negatif dapat dihasilkan. Peningkatan kepekatan Ag/Kln dalam filem gelatin ikan juga terbukti dapat menurunkan ($p<0.05$) kadar transmisi sinar ultra ungu. Dalam objektif kedua pula, filem pembungkusan aktif yang terdiri daripada perak/kaolin-gelatin (FG_Ag/Kln) telah digabungkan dengan minyak pati kulit kayu manis (CBEO) dengan kepekatan yang berbeza (0% sebagai filem kawalan, 3%, 6%, dan 9%). Pemerhatian secara menyeluruh menunjukkan peningkatan CBEO telah meningkatkan daya tahan (376.20 hingga 1637.40 MPa) dan kekuatan ketegangan (3.51 hingga 32.47) filem. Selain itu, penurunan kadar kebolehtelapan wap air (1.02 hingga 0.77%), kadar kebolehlarutan (8.80 hingga 7.20%), dan kandungan kelembapan filem (0.46% hingga 0.32%) juga turut dikuasai oleh filem FG_Ag/Kln, manakala, sifat penghalang air untuk semua jenis filem

mempunyai nilai yang tinggi dari 100° (116.38° – 123.00°). Penemuan ini menunjukkan sifat hidrofobik yang meningkat seiring dengan penambahan kepekatan CBEO. Bakteria Gram-positif dan Gram-negatif juga dapat dihalang dengan berkesan berdasarkan peningkatan kepekatan CBEO. Penemuan ini menunjukkan potensi 3-9% CBEO dalam memperbaiki sifat kefungsian filem komposit FG_Ag/Kln. Seterusnya, dalam objektif ketiga, pengaplikasian filem gelatin ikan yang ditambah dengan Ag/Kln (1:1) dan CBEO sebagai bahan pembungkusan makanan telah dikaji dan dibandingkan. Potensi filem pembungkusan untuk melindungi dan mengekalkan kualiti salad yang sedia dimakan (salad Romaine) disiasat selama 13 hari. Pengamatan secara menyeluruh menunjukkan bahawa filem yang mengandungi gabungan Ag/Kln dan kepekatan CBEO yang tertinggi telah berjaya menambahbaik dan memanjangkan ($p<0.05$) jangka hayat salad. Oleh itu, penemuan ini menunjukkan bahawa filem gelatin ikan dengan gabungan Ag/Kln dan CBEO berpotensi tinggi dan mempunyai keistimewaan sendiri sebagai bahan pembungkusan makanan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree Master of Science. The members of the Supervisory Committee were as follows:

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I hereby confirm that:

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This is to confirm that:

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LIST OF ABBREVIATIONS

%	Percentage
℃	Degree Celsius
◦	Degree
µl	Microlitre
µm	Micrometre
σ	Sigma
Abs	Absorption
AFM	Atomic force microscopy
Ag/Kln	Silver/Kaolin
ANOVA	Analysis of variance
ATCC	American type culture collection
CA	chlorhexidine acetate
CA	Contact angle
CBEO	Cinnamon bark essential oil
CFU	Colony forming units
cm	Centimetre
CPB	Cetylpyridinium bromide
Da	Dalton
DDM	Disc diffusion method
EAB	Elongation at break
EFSA	European Food Safety Authority
EO	Essential oil
FDA	Food and drugs administration
FFS	Film forming solution
FG	Fish gelatin
g	Gram
GPA	Gigapascals
GRAS	Generally recognized as safe
h	Hour
HDPE	High-density polyethylene
IZD	Inhibition zone diameter
kDa	Kilodaltons (molecular weight)
kg	Kilogram
kHz	Kilohertz
Kln	Kaolin
kN	Kilonewton
kV	Kilovoltage
LDPE	Low-density polyethylene
m	Mass
MAP	Modified atmosphere packaging
MC	Moisture content
mg	Milligram
min	minutes
ml	Millilitre
mm	Millimetre
MPa	Millipascal
N	Newton
n	Sample size

N/m	Newton per metre
nm	Nanometre
Pa	Pascal
PCA	Plate Count Agar
PE	polyethylene
PET	Polyethylene terephthalate
PG	Peptidoglycans
PP	Polypropylene
ppm	Part per million
PPO	Polyphenol oxidase
PS	Polystyrene
PVC	polyvinyl chloride
RH	Relative humidity
rpm	Revolutions per minute
Rq	Root mean square roughness
RTE	Ready-to-eat
sec	Seconds
SEM	Scanning electron microscopy
sp.	Species
SPC	Standard plate count
spp.	Species plural
TPC	Total plate count
TS	Tensile strength
UV	Ultra-violet
v/v	volume per volume
w/v	Weight per volume
w/w	Weight per weight
WCA	Water contact angle
WS	Water solubility
WVP	Water vapour permeability
YM	Young's modulus

CHAPTER 1

INTRODUCTION

1.1 Background

Food packaging is a concept to protect food products from external influences such as microorganism, moisture, and gases, as well as to maintain the quality and safety of the food product, hence, prolong its shelf-life. Out of numerous materials of packaging, plastic has always been the major packaging material in the food production area due to the lightweight, convenient, and cheaper to manufacture.

Plastic can be used to replace metal and possess the same ability in storing food products. Polyethylene terephthalate (PET), for example, has become a huge success story. Introduced in 1978 as a lightweight, shatterproof replacement for glass, this thermoplastic container started out with one major drawback: the decomposability (Prashanth and Tharanathan, 2007). Issues regarding the high impact of petroleum-based synthetic polymers such as polyethylene (PE), polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), and polystyrene (PS) on the environment occurred long ago. Unreasonably, these plastics had caused many toxic pollutions towards the environment by being passed through the plug holes and eventually ending up in the oceans, lakes, and seas. Researchers also found that plastic ingested by animals such as turtles, fish, and birds had resulted in blocking their intestines. The toxicity might have been passed to us, as consumers, due to the natural food chain. Therefore, bio-based food packaging has been investigated as an alternative for synthetic polymers.

To overcome this problem, packaging material with natural active ingredients can be an alternative solution (de Carvalho and Junior, 2020). They can be decomposed and have other excellent characteristics along with the advanced technology emerging these days. Over the decades, food packaging industry has evolved to provide better food protection as well as able to lengthen the shelf-life of certain food products (Trinetta, 2018). In fact, by having an active food packaging material that also has other functional properties such as antibacterial or antioxidant effect, it could increase its value.

Active packaging improves the functionality of traditional packaging with new elements (Wyrwa and Barska, 2017). Referring to the active packaging term, it means that it could provide better functions (Soroka, 2008) compared to conventional petroleum-plastic packaging. For example, oxygen scavengers, antimicrobials, and antioxidant properties to prevent food spoilage and deterioration while promotes longer shelf-life. Examples of active compounds including from essential oils and extracts from plants, fruits, and vegetables that contain active materials which are high sources of antioxidant and antimicrobial properties (Chang et al., 2016; Gavril et al., 2019). Usually, these involve the modification of the packaging atmosphere by absorbing or scavenging the unwanted external influences. Natural preservatives are added into the packaging material to scavenge the excessive water, flavour, or odour to replace scavenging sachets and ensuring the safety of food products. Antimicrobial packaging for instant, is used to inhibit the pathogenic bacteria growth and prolong the food products' shelf-life.

Since the request for naturally preserved and minimal processed foods is soaring up, food packaging industry has gained more interest in developing active packaging material. Different elections of active natural compounds would result in different applications depending on the active compounds incorporated. Nevertheless, the objective is still the same, which is to allow longer delivery and storage time and to prevent food waste. Consequently, lead to fewer environmental effects and ecological problems.

Natural-based polymers from polysaccharides (starch, carrageenan, alginate, agar, pectin, chitosan, cellulose, and pullulan) (Bonilla et al., 2013; Dang and Yoksan, 2016), proteins (gelatin, casein, gluten, and whey protein) (Arfat et al., 2014; Tongnuanchan et al., 2014), or lipids (beeswax, vegetable oil, essential oil, and fatty acids) have been the main interest in developing biodegradable films or coatings. These biopolymers could provide extra protection for food products from excessive water, gas, and microorganisms, as well as sustaining the quality of the food products depending on the combinations of the polymers with additional active compounds.

Gelatin has obtained high attention in producing natural biopolymers because of its special attributes, including the natural colloidal features, abundance, having excellent biocompatibility, and non-toxicity (Extabide et al., 2017). Gelatin is also suitable and fit to serve as a base in combining with various active ingredients other than having the desired physical properties (Nilsuwan et al., 2018). It was discovered that gelatin possesses great heat-sealability compared to other biopolymers owing to its inherent chemical structure (Abedinia et al., 2018). Besides, gelatin is also a sustainable resource and could be derived from numerous sources, particularly, from animals' skins and bones. Issues regarding the gelatin outsource from pigs' intestines, skins, bones, and Bovine Spongiform Encephalopathy (CBE) have led the researchers to come out with new relevant alternative (fish gelatin), simultaneously fulfil the Halal and Kosher food markets.

Gelatin from fish is a denatured polypeptide extracted through thermal hydrolysis from retreated collagen in fish skin (Kim and Mendis, 2006). It is high in molecular weight protein chains (α-chain), approximately 300 kDa, which also contains three distinguish polypeptide α-chain curling and forming a triple-helical structure (Boran and Regenstein, 2010). Its amino acid (hydroxyproline and hydroxylysine) composition and molecular weight contribution determine the fish gelatin quality. The length of the protein chains is also one of the characterizations in stipulating the quality of fish gelatin (Benjakul et al., 2012).

Gelatin's capability in producing edible film and having an excellent film-forming ability were the reasons for the high exploitation in developing biodegradable film (Etxabide et al., 2016). Massive attention has been received due to its transparency, decomposability, and aroma barrier properties which are the desired food packaging material characteristics (Nilsuwan et al., 2018).

Generally, in the food industry, gelatin is mostly used as a gelling agent and stabilizer in food, while some use it as a vitamin capsules coating (Mariod and Fadul, 2013). It is an irreversible hydrolysis form of collagen, which the process has alternate the protein fibrils to peptides (300, 000 Da). When gelatin is mixed with hot water, it is easily dissolved, and gel-like when cooled. However, gelatin does not dissolve well in cold water, even it is soluble in polar solvents.

Some studies on gelatin have explored the potential of gelatin as an active packaging material blended with another type of protein (Kchaou et al., 2020) or in combination with other natural biopolymers (Jridi et al., 2020; Zhao et al., 2020). Some research focus mainly on gelatin with the incorporation of active compounds; essential oil, and plant extracts (Wang et al., 2020; Staroszczyk et al., 2020) aiming for the enhancement of physicochemical properties. More detailed research towards the application of developed active gelatin films on food products is necessary to determine the potential usage in food industry.

The combination of gelatin with other natural active ingredients not only produce an environmentally friendly packaging material, but also an active packaging that is safe and feasible for packed food products. Kaolin clay (Kln) is an active substance that can be added into the gelatin film to enhance its functional properties. With the incorporation of Kln, the physical, water barrier, and optical properties are showing an increase of performance towards the film as the kaolin clay acts as a filler to improve the hygroscopicity of the film (Malek and Ramli, 2015). This means Kln has proved its ability in developing new alternative food packaging material in shifting the trend of using petroleum-based plastics.

The potential of silver (Ag^+) has also been recognized as active compound in packaging (Qin et al., 2019; Roy et al., 2019). The Ag^+ could inhibit the pathogenic bacteria growth and prolong the shelf-life of the food products (Prabhu and Devaraju, 2018; Vieira et al., 2020). However, due to the limitation of Ag^+ migration towards the packed food product, the amount of Ag^+ to be included in food packaging needs to be in a controlled amount. Interestingly, the research regarding silver/kaolin (Ag/Kln) reinforced in gelatin film as an active packaging is still limited. To elevate the antimicrobial activity of the film, cinnamon bark essential oil (CBEO) can be introduced. There is also a major concern towards ready-to-eat (RTE) salad including Romaine lettuce regarding its shelf-life and the browning effect (Zhan et al., 2012; Yu et al., 2018). Therefore, active packaging film based on gelatin with Ag/Kln and CBEO has been proposed to prolong the shelf-life of this vegetable.

In this study, the potential of gelatin from fish skin as a packaging film had been investigated with three objectives. The first objective was to determine the effects of different types and concentrations of silver/kaolin on the properties of the gelatin film. Secondly, to explore the potential of cinnamon bark essential oil (CBEO) on the gelatin film with silver/kaolin as active packaging. Lastly, for the third objective, an application of gelatin films reinforced with silver/kaolin and CBEO for prolonging the shelf-life of packed salad (Romaine lettuce) was conducted.

1.2 Objectives

i. To determine the effects of different concentration and type of silver/kaolin on the properties of gelatin film.
ii. To investigate the effect of cinnamon bark essential oil on the gelatin film with silver/kaolin as active packaging.
iii. To develop active packaging material for prolonging the shelf life of packed salad.

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