UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF BIODEGRADABLE PROTEIN-BASED FILMS INCORPORATED WITH MANGO KERNEL EXTRACT FOR ACTIVE PACKAGING OF MAYONNAISE

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By

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Chairperson : Badlishah Sham bin Baharin
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Active packaging is a novel idea in which active substance such as antioxidant is incorporated into the packaging to prolong the shelf-life and maintain the quality of food product. This research was focused on developing active packaging by using food industries' by-products. In the first objective, soy protein isolate (SPI) and fish gelatin (FG) were used as the source of biopolymers and different concentration (1, 3 and 5%) of mango kernel extracts (MKE) were added as natural antioxidants. The addition of MKE produced thicker and more opaque films ($p < 0.05$), increased the tensile strength ($p < 0.05$) and antioxidant activity ($p < 0.05$) but decreased the water solubility ($p < 0.05$). The overall observations revealed that SPI outperformed FG as active packaging films. In the second objective, the storage stability of antioxidant films was studied at 25°C, 4°C and -18°C for 90 days. The temperature and time did not have significant effect ($p > 0.05$) on thickness and colour. All the films showed an increase in tensile strength, decrease in elongation and increase in Young's modulus ($p < 0.05$). The highest decrease in antioxidant activity was only 4% throughout the storage time. This shows that the antioxidant activity of the films is stable for the 90 days of storage. In the third objective, the mayonnaise packaged in control and SPI+MKE films turn significantly ($p < 0.05$) darker and the pH values significantly ($p < 0.05$) increased after 6 weeks of storage. The SPI+MKE films were able to slow down the lipid oxidation by 30% in peroxide value analysis, 44% in TBARS analysis, 38% in anisidine value analysis and 65% in total oxidation analysis. Therefore, the usage of the SPI+MKE films could maintain the quality of mayonnaise for a longer time.

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

PEMBANGUNAN FILEM BIODEGRADASI BERASASKAN PROTEIN DITAMBAHKAN DENGAN EKSTRAK BIJI MANGGA SEBAGAI PEMBUNGKUS AKTIF MAYONIS

Oleh

MARYAM 'ADILAH BINTI ZAINAL ARIFIN

Februari 2018

Pengerusi : Badlishah Sham bin Baharin
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Pembungkusan aktif ialah satu teknologi pembungkusan berasaskan penambahan bahan aktif seperti antioksida dalam pembungkus makananan untuk mengekalkan kualiti dan memanjangkan jangka hayat produk. Projek ini bertujuan untuk membangunkan pembungkus makanan berasaskan sisa dan produk sampingan industri. Dalam objektif pertama, *soy protein isolate* (SPI) dan gelatin ikan (FG) telah digunakan sebagai biopolimer dan 1 hingga 5% ekstrak biji mangga telah ditambahkan sebagai antioksida semulajadi. Penambahan ekstrak biji mangga membuatkan filem lebih tebal dan legap ($p < 0.05$), lebih kuat ($p < 0.05$) dan meningkatkan aktiviti antioksida ($p < 0.05$) tetapi mengurangkan kelarutan dalam air ($p < 0.05$). Keseluruhannya, filem SPI menunjukkan ciri-ciri yang lebih bagus sebagai filem berbanding FG. Dalam objektif kedua, kestabilan penyimpanan filem antioksida pada suhu 25°C, 4°C dan -18°C telah dikaji untuk 90 hari. Suhu dan masa penyimpanan tidak memberikan kesan signifikan ($p > 0.05$) kepada ketebalan dan warna filem. Semua filem menunjukkan peningkatan kekuatan, pengurangan kekenyalan dan peningkatan *Young's modulus* ($p < 0.05$). Penurunan antioksida tertinggi yang direkodkan sangat rendah iaitu hanyalah 4% sepanjang tempoh penyimpanan. Hal ini menunjukkan aktiviti antioksida filem ialah stabil untuk 90 hari. Dalam objektif ketiga, mayonis menjadi lebih gelap ($p < 0.05$) dan menunjukkan peningkatan pH selepas 6 minggu penyimpanan. Filem SPI+MKE berjaya memperlahankan pengoksidaan lemak sebanyak 30% dalam analisis nilai peroksida, 44% dalam analisis TBARS, 38% nilai anisidin dan 65% nilai pengoksidaan. Oleh itu, penggunaan filem SPI+MKE sebagai pembungkus mayonis boleh mengekalkan kualiti mayonis untuk jangka masa yang lebih lama.

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I certify that a Thesis Examination Committee has met on 19 February 2018 to conduct the final examination of Maryam 'adilah binti Zainal Arifin on her thesis entitled "Development of Biodegradable Protein- Based Films Incorporated with Mango Kernel Extract for Active Packaging of Mayonnaise" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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Declaration by graduate student

I hereby confirm that:

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LIST OF ABBREVIATIONS

ABTS	[2,2-azinobis(3-ethylbenzothiazoline-6-sulphonate)]
Ala	Alanine
AOAC	Association of official analysis chemists
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
AV	Anisidine value
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
Cys	Cysteine
DPPH	2,2-diphenyl-1-picrylhydrazyl
EAB	Elongation at break
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FG	Fish gelatin
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
GRAS	Generally recognized as safe
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine

MKE	Mango kernel extract
PE	Polyethylene
PET	Polyethylene terephthalate
Phe	Phenylalanine
PP	Polypropylene
Pro	Proline
PV	Peroxide value
PVC	Polyvinyl chloride
RH	Relative humidity
RSA	Radical scavenging activity
SEM	Scanning electron microscopy
Ser	Serine
SPI	Soy protein isolate
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
Thr	Threonine
TOTOX	Total oxidation
TPC	Total phenolic content
TPTZ	[2,4,6-Tris(2-pyridyl)-s-triazine]
Trp	Tryptophan
TS	Tensile strength
Tyr	Tyrosine
Val	Valine
WVP	Water vapour permeability
YM	Young's modulus

CHAPTER 1

INTRODUCTION

Food industry is one of the most important industries in the world. However, the huge amount of waste and by-products produced by this industry imposed a danger to the environment. According to Machado, Grosso, Nouws, Albergaria and Delerue-Matos (2014), there are three different methods that were used to manage the food industry waste and by-products. Firstly is by reducing the waste by improving the processing technique, secondly is by recycling or recovering and thirdly is by waste treatment. However, recycling and recovering of waste is the better option as it is more cost-effective and development of new value-added products are possible (Goula & Lazarides, 2015; Machado et al., 2014). The waste and by-products of food industries also have been reported to contain numerous beneficial components such as starch, fibre, minerals, lipid, phytochemicals and bioactive compounds like antioxidants and antimicrobials (Martins, Pinho, & Ferreira, 2017). Recovery of these compounds from by-products and waste will help to reduce the waste management problem and they can be a source of cheaper food additives or nutraceutical products (Kowalska, Czajkowska, Cichowska, & Lenart, 2017). Therefore, the application of these waste and by-products as antioxidant film could be an alternative to reduce the waste management problem.

Antioxidant film is a type of active packaging obtained by incorporating antioxidant in the polymer; either synthetic polymers such as polyethylene and polypropylene or biodegradable polymers such as polysaccharide, protein and lipid. However, biodegradable polymers are favoured as they are environmentally-friendly. Among the biodegradable polymers, protein has the most potential to be developed into antioxidant film. This is because biodegradable film made from protein has better mechanical properties, gas barrier and functional groups as compared to polysaccharide and lipid films (Arvanitoyannis & Dionisopoulou, 2010). In this research, antioxidant film will be developed by using waste and by-products of food industries which are soy protein isolate, fish gelatin and mango kernel. The storage stability and the application of this antioxidant film on mayonnaise to reduce lipid oxidation will be determined in the later section of the research.

Soy protein is a by-product of soybean oil production. Soy protein isolate (SPI) with a 90% minimum protein content is preferable as the raw material for biodegradable film due to its high protein content, high nutrition content, good film-forming ability, inexpensive and availability (Zhao, Xu, Mu, Xu, & Yang, 2016; Li et al., 2016). The high protein content in SPI contributes to its exceptional film-forming properties. It has numerous amino groups, contributed by the high content of amino acids such as arginine and lysine (Wang, Li, Yan, Huang, & Dong, 2016). Numerous functional groups such as hydroxyl, carboxyl and sulphide groups allow the interaction of SPI film with other compounds such as antioxidant (Zhang et al., 2016).

Fish gelatin is a hydrocolloid extracted by the hydrolysis of collagen from the residual of fish such as skin and scales (Hazaveh, Mohammadi Nafchi, & Abbaspour, 2015). Even though the commercial usage of fish gelatin is fairly limited as compared to mammalian gelatin, the application of fish gelatin as biodegradable film is gaining more interest. This is because it has low oxygen permeability, good film-forming ability and emulsifying properties (Byun, Bae, & Whiteside, 2012). The films produced from fish gelatin also have good gas barrier and good mechanical properties (Benbettaïeb, Karbowiak, Brachais, & Debeaufort, 2016). Fish gelatin is also relatively cheaper as it is produced from the by-products of fishery industry. Fish gelatin extracted from warm water fish is more suitable as biodegradable film as it contains higher proline and hydroxyproline than cold water fish gelatin. Higher proline and hydroxyproline is related to higher gelling and melting temperature (Santos et al., 2014).

Mango processing generates a high amount of waste (40-60%), mainly in the form of peels and kernels. Generally, 15-20% of the waste is contributed by the kernels (Nawab, Alam, Haq, & Hasnain, 2016). Statistically, more than 1 million tonnes of mango seeds are wasted annually (Torres-León et al., 2016). Mango kernel is a rich source of phenolic acids, antioxidants, gallotannins and polyphenols (Maisuthisakul & Gordon, 2012). According to Soong and Barlow (2006), the mango kernel extract has approximately 87% more gallic acid than the longan seed extract (Soong & Barlow, 2006). Mango kernel extract has the highest antioxidant based on the wet and dry basis as compared to tamarind, longan, avocado and jackfruit seeds (Soong & Barlow, 2004). Despite the huge potential of mango kernel as natural antioxidant that has been reported, there is no commercial application of mango kernel that has been published yet. Therefore, the usage of mango kernel as the source of natural antioxidant in antioxidant film provides a new alternative for the mango kernel waste management.
The main function of antioxidant film is to reduce the lipid oxidation in food product, especially high-fat product. Mayonnaise is an oil-in-water emulsion that is made up of 70 to 80% fat (vegetable oil), acidifying ingredients (vinegar) and egg yolk. Other additives such as salt, flavours, sweetening and food seasonings are optional in the production of mayonnaise (Chivero, Gohtani, Yoshii, & Nakamura, 2016). Mayonnaise is commercially used in dressing and sauces as well (Håkansson, Chaudhry, & Innings, 2016). The high-fat content and low pH makes it invulnerable to microbial growth (Ghorbani Gorji, Smyth, Sharma, & Fitzgerald, 2016). Therefore, the quality indication of mayonnaise is normally determined from its lipid oxidation rather than microbial spoilage. Lipid oxidation happens when volatile aldehydes, ketones, alcohols, furans, hydrocarbons or acids are formed. The development of these volatile substances and their mixture produced the rancid aroma (Sainsbury, Grypa, Ellingworth, Duodu, & Kock, 2016).

In this study, active packaging is developed using the industries' by-products. There are 3 objectives in this study;

1. To prepare and analyze the functional and antioxidant properties of protein-based films incorporated with mango kernel extract for active packaging.
2. To evaluate the storage stability of soy protein isolate films incorporated with mango kernel extract at different temperature.
3. To investigate the storage stability of mayonnaise packaged in soy protein isolate film incorporated with mango kernel extract.

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