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# Functional and antioxidant properties of protein-based films incorporated with mango kernel extract for active packaging

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ABSTRACT

This research is focused on developing active packaging by using food industries' by-products. Soy protein isolate (SPI) and fish gelatin (FG) were used as the sources of biopolymers and different concentrations of mango kernel extracts (MKE) from 1 to 5% were added as natural antioxidants. Thicker and more translucent films ($p < 0.05$) were produced when a greater concentration of MKE was incorporated in both films. The mechanical test revealed that the addition of MKE increased the tensile strength of both films ($p < 0.05$), with higher tensile strength recorded in FG films than in SPI films. The incorporation of MKE significantly ($p < 0.05$) decreased the water solubility up to 22 and 33%, in FG and SPI films, respectively. The water vapor permeability (WVP) of SPI with the incorporation of MKE improved up to 10%. In contrast, FG films incorporated with MKE showed higher WVP in comparison with the control. The antioxidant activity increased with a greater concentration of MKE incorporated in both antioxidant films ($p < 0.05$) with more impact in SPI films compared to FG film in DPPH, FRAP and ABTS analysis. DPPH analysis on SPI films revealed the highest antioxidant activity (89%) with the inclusion of 5% MKE extract. Though both films were found to have the potential to be developed as antioxidant films, yet the overall observations revealed that SPI outperformed FG as active packaging films.



## 1. Introduction

Statistically, one-third of the food produced (approximately 1.3 billion tons) in the world for human consumption is wasted per year (FAO, 2011). The elimination of the food waste and food by-products poses a serious threat to the environment due to the high amount of nitrogen and phosphorous content (Dorta, Lobo, & González, 2012). To make it worse, there are very limited options for the waste treatment. However, the waste and by-products also contain some valuable products such as proteins and phenolic compounds that can be reused to lessen the environmental pollution. These products have the potential to be developed as active packaging due to their film-forming and antioxidant properties. Active packaging is a smart system that involves the interaction between packaging and the packaged food; either by migration to the headspace or onto the food-contact surface to ensure fresh product (Ozdemir & Floros, 2004). Bio-based active packaging is suitable to substitute conventional plastic packaging as it is not exposed to issues such as phthalate leaching (Weng & Zheng, 2015). The recovery of these high-value compounds from waste and by-products could provide a cheaper source for active packaging production and a new function for the by-products (Baiano, 2014; Wang, Hu, Ma, & Wang, 2016).

Polysaccharides, proteins, and lipids are the common basic materials to form biodegradable active packaging (Dutta, Tripathi, Mehrotra, & Dutta, 2009; Liu, Meng, Liu, Kan, & Jin, 2017). They can act as the carriers for active substances such as antioxidant, antimicrobial and oxygen scavenger. However, protein is preferable among other materials due to its better gas barrier and mechanical properties than polysaccharide and lipid films (Nur Hanani, Roos, & Kerry, 2014b). Polysaccharide films have lower water and gas barrier due to higher hydrophilicity while lipid films have lower tensile strength as compared to protein (Bourtoom, 2008).

Soy protein isolate (SPI) is widely used in the edible film formation due to its abundance, low cost, biodegradability and good film-forming ability (Galus, Mathieu, Lenart, & Debeaufort, 2012; Qiu et al., 2015). SPI is naturally derived from defatted soybean which is the waste product of soybean oil production (Kumar,

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Choudhary, Mishra, Varma, & Mattiason, 2002). SPI is enriched in protein such that the minimum protein content is 90%. It can be used in numerous applications such as in hydrogels, adhesives, plastics, films, coatings and emulsifiers (Koshy, Mary, Thomas, & Pothan, 2015; Zhao, Yao, Fei, Shao, & Chen, 2013).

Meanwhile, gelatin is a heteropolymer that is extracted from collagen through controlled hydrolysis (Martucci, Gende, Neira, & Ruseckaite, 2015; Maryam Adilah & Nur Hanani, 2016). Gelatin has the potential to be used as biopolymer for edible films due to its ability to form mechanically strong film network, abundance, and biodegradability (Wu et al., 2015). Fish gelatin (FG) is derived from fish industry by-products such as scales and skins and is a preferable gelatin source as it is free from religious issues and bovine spongiform encephalopathy disease or known as mad cow disease (Benbettaïeb, Karbowiak, Brachais, & Debeaufort, 2016).

Mango (*Mangifera indica* L.) belonging to the family of Anacardiaceae and to date, around 1000 mango varieties were identified (Jahurul et al., 2015). Generally, mango contains about 20–60% seed of the whole fruit and normally more than half (45–75%) of the seed was made up of the kernel (Asif et al., 2016; Maisuthisakul & Gordon, 2009). Mango was used in a vast amount to produce products such as puree, juice and nectar (Jyotshna, Srivastava, Killadi, & Shanker, 2015). However, the mango industry eliminates more than one million tons of mango seeds annually without any further commercial applications (Abdalla, Darwish, Ayad, & El-Hamahmy, 2007a). Mango seed kernel has the highest antioxidant activity, followed by the seeds of tamarind, longan, avocado, and jackfruit (Matsusaka & Kawabata, 2010). Mango kernel extract (MKE) is a rich source of gallic acid, ellagic acid, ferulic acid, cinnamic acids, tannins, vanillin, coumarin, and mangiferin that exhibits antioxidant properties (Soong & Barlow, 2004). Therefore, mango kernel extract (MKE) is a promising good source of natural antioxidant for active packaging.

The objectives of this research were to develop antioxidant active packaging from by-products (SPI, FG, and MKE) and also to determine the effect of different MKE concentration on the physical, mechanical and antioxidant properties of SPI and FG films.

## 2. Materials and methods

### 2.1. Chemicals

Fish gelatin (FG) derived from fish scales in the range of 240–260 bloom was supplied from Custom Collagen (Addison, Illinois, USA). Soy protein isolate (SPI) with 92% protein content was purchased from MP Biomedicals (Solon, Ohio, USA). ABTS [2,2-azinobis(3-ethylbenzothiazoline-6-sulphonate)], Folin Ciocalteu reagent and hydrochloric acid were all supplied by Merck and Co. (Darmstadt, Germany). Potassium persulphate, sodium carbonate, gallic acid and acetic acid were purchased from R&M Chemicals (Selangor, Malaysia) whereas anhydrous glycerol (99.5% purity), sodium acetate trihydrate and ferric chloride ($FeCl_3$) were supplied by Systerm (Karlsruhe, Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl) and TPTZ [2,4,6-Tris(2-pyridyl)-*s*-triazine] were purchased from Tokyo Chemical Industry (Tokyo, Japan). Absolute ethanol (99.9% purity) and soy lecithin were purchased from John Kollin Corporation (Midlothian, UK) and Modernist Pantry (Eliot, Maine USA).

### 2.2. Extraction of mango kernel

The mango seeds were cleaned and the mango kernels were separated manually. The kernels were then cut into smaller pieces and dried at 50 °C for 24 h (Abdalla, Darwish, Ayad, & El-Hamahmy, 2007b; Augustin & Ling, 1987). The dried kernel was powdered using a grinder (Tefal, Rumilly, France). The mango kernel was extracted by adding ethanol in 5:1 (v/w) ratio. The mixture was left in dark for 24 h with regular shaking. The residue was removed by using Whatmann no. 4 filter paper and the supernatant was evaporated at 40 °C. The mango kernel extract (MKE) was used for the film preparation.

### 2.3. Proximate composition of MKE

The protein content was determined using kjeldahl method (Foss Analytical AB, 2003). The fat, ash and moisture content was analysed according to AOAC (1995). The carbohydrate content was determined using arithmetic difference.

### 2.4. Film preparation

The film was prepared according to Tongnuanchan, Benjakul, and Prodpran (2013). Distilled water was heated to 70 °C and 3.5% gelatin was added and mixed for 30 min. Glycerol (30% w/w based on FG content), MKE (1, 3 and 5% w/w based on FG content) and soy lecithin were added as emulsifier (25% w/w based on MKE content) and stirred at 50 °C for another 30 min. Soy lecithin is used as emulsifier because it can promote homogeneous distribution of extract in the protein film solution better than other types of emulsifiers such as Tween 20 and Tween 80 (Tongnuanchan, Benjakul, & Prodpran, 2014). The mixture was then homogenized at 5000 rpm for 3 min using a homogenizer (Heidolph Instruments GmbH & Co., Schwabach, Germany). The film solution was spread on polystyrene petri dish plate (14 × 14 $cm^2$) with the initial thickness around 0.050 ± 0.005 mm and dried at 25 °C and 50% relative humidity (RH) for 24 h. The film solution without the addition of MKE was used to prepare the control film sample. The films were conditioned at 50± 5% RH and at a temperature of 23 ± 2 °C for at least 48 h in a dry cabinet (Che Scientific Co., Kwai Chung, Hong Kong) prior to testing. The same method was used to prepare SPI films.

### 2.5. Physical properties of films

#### 2.5.1. Film thickness

The film thickness was determined with a digital micrometer (Mitutoyo Absolute, Tester Sangyo Co. Ltd., Japan). The thickness was measured in ten randomly selected locations on each film and then an average value was calculated.

#### 2.5.2. Water solubility

The water solubility of the films was determined in accordance with the procedures of Kavoosi, Rahmatollahi, Mohammad Mahdi Dadfar, and Mohammadi Purfard (2014) with slight modifications. To determine the initial dry mass, the film samples were cut into square strips (2 × 2 $cm^2$) and dried at 100 ± 5 °C for 24 h. It was then submerged in 50 ml distilled water for 24 h at 23 ± 2 °C. The soaked sample was then re-dried at 100 ± 5 °C for 24 h to get the final dry mass. The water solubility of the film was calculated using equation (1):

$$\text{Solubility } (\%) = \frac{(\text{Initial dry mass} - \text{Final dry mass}) \times 100}{\text{Initial dry mass}} \quad (1)$$

#### 2.5.3. Water vapor permeability (WVP)

The WVP was performed according to Nur Hanani, O'Mahony, Roos, Oliveira and Kerry, (2014a). Distilled water (6 ml) was first