Chemical, Physical, and Barrier Properties of Edible Film from Flaxseed Mucilage

Yee Bond Tee,[a,*] Leh Tian Tee,[a] Wichittra Daengprok,[b] and Rosnita A. Talib [c,d,*]

Chemical, physical, and barrier properties of flaxseed mucilage-based films were investigated to determine the appropriate applications for coating or packaging of food or bioproducts. The film samples were formed *via* casting with the addition of glycerol as a plasticizer up to a maximum of 5 wt.%. Fourier transform infrared (FTIR) spectra showed an increase of intensity in the –OH stretching vibration region and the appearance of a new peak at 2883.1 cm^{-1} in the plasticized film samples. These changes may indicate possible interactions between the mucilage and glycerol. With increasing glycerol concentration, the water activity decreased, while the moisture content and water solubility increased. With regard to barrier properties, the water vapour permeability (WVP) and oxygen permeability (OP) notably increased with increasing glycerol content. With these high WVP and OP values, the films can be potentially extended for coating or packaging fresh produce.

Contact information: a: Department of Food Science and Nutrition, Faculty of Applied Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia; b: Faculty of Engineering and Agro Industry, Maejo University, Sansai, Chiang Mai 50290 Thailand; c: Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia; d: Laboratory of Halal Science Research, Institute of Halal Products Research, Universiti Putra Malaysia (UPM), Putra Infoport, 43400 Serdang, Selangor, Malaysia;
* Corresponding authors: teeyb@ucsiuniversity.edu.my; rosnita@upm.edu.my

INTRODUCTION

Packaging plays an important and intermediate role in protecting the quality of food products and extending their shelf life from being deteriorated by the external environment. Synthetic polymers such as polyethylene, polypropylene, polystyrene, and polyvinyl chloride are widely used in food packaging due to their unique functionality, light weight, low cost, and ease in processing (Arora and Padua 2010). However, these non-degradable polymers have been causing serious environmental impact. For instance, plastic bags and waste thrown into the ocean kill millions of sea creatures annually (Chitkara and Goel 2014). To overcome these negative effects, a great deal of research is geared toward exploring natural resources to produce biodegradable films.

Biodegradable packaging such as edible films and coatings are defined as primary packaging and are made from edible components. The packaging consists of a thin layer of edible material that can be coated onto a food product or be developed into a film and used as a packaging material without affecting the packed ingredients. Moreover, the incorporation of nutrients such as vitamins and minerals within the film matrix can enhance the nutritional content of the food product (Pascall and Lin 2013).

Edible films have been developed from flaxseed gel, also known as mucilage (Tee *et al.* 2016). To overcome the brittleness of the film, glycerol acts as a plasticizer and is

added within the range of 1 wt.% to 6 wt.% based on the weight of the mucilage. The addition of 6 wt.% or more glycerol prevents the formation of a solid film.

The present work further characterized the developed flaxseed mucilage-based edible films. The chemical, physical, and barrier properties were investigated, as these are some of the major properties of interest in food packaging and coating materials. Physical determinations such as water activity, moisture content, and water solubility of the films were studied as these properties may affect the shelf life of food products. Fourier transform infrared spectroscopy (FTIR) was used to investigate the chemical components and their interactions in the films. Water vapour and oxygen permeability were studied as the barrier properties because they are needed to understand how to best preserve certain food products.

EXPERIMENTAL

Materials

Brown flaxseeds (*Linum usitatissimum* L.) commercially produced in Mongolia were purchased from Wide Tropism Trading Sdn. Bhd., Selangor, Malaysia. The seeds were packed in vacuum-sealed plastic bags and kept at room temperature. Reagent-grade glycerol, sodium hydroxide (NaOH) pellets, sodium chloride (NaCl) (crystalline), and anhydrous calcium chloride (CaCl$_2$) pellets were purchased from Fisher Chemicals Sdn. Bhd., Selangor, Malaysia.

Mucilage Extraction

A detailed methodology for mucilage extraction was reported previously (Tee *et al.* 2016). Flaxseeds were soaked and mechanically stirred with distilled water (1:30) at an elevated temperature (80 °C to 100 °C) and then cooled to room temperature. The extracted mucilage was separated *via* centrifugation and filtration.

Film Formation

Film formation *via* a solution casting method was performed as described by Tee *et al.* (2016). The film samples were prepared with flaxseed mucilage/glycerol mixtures at loadings of 1 wt.% to 5 wt.% because a solid film was unable to form at glycerol loading of 6 wt.% and above.

A minimum film diameter of 11 cm was required for the oxygen permeability test. Thus, adhering to the similar expression of 0.42 g/cm^2 from the former methodology, 74.22 g of the mixture was cast into a Petri dish with a diameter of 15 cm. All samples were kept in a desiccator at 25 °C and 52% relative humidity for at least two days prior to testing. They were labeled as 'FM-', followed by digits '0' to '5', which denoted the loading (wt.%) of glycerol added.

Fourier Transform Infrared Spectroscopy (FTIR)

The chemical compositions of glycerol, flaxseed mucilage, and film samples were examined using FTIR (Thermo Fisher Scientific, Nicolet iS5 FT-IR spectrometer, Waltham, MA, USA). Each spectrum was the averaged value of 20 scans in transmittance mode from 4000 cm^{-1} to 600 cm^{-1} at a resolution of 4 cm^{-1}. Results were reported in normalized transmittance mode.

Water Activity (a_w)

The water activity of film samples was determined using a water activity meter (AquaLab, Series 3, Pullman, WA, USA) after calibration with distilled water. Samples with dimensions of 2 cm × 2 cm were loaded into the cap of the equipment for a_w reading. The average values of three repetitions of each sample were reported with standard error.

Moisture Content (MC)

Aluminum containers used to contain the film samples were dried in a convection oven at 105 °C for 3 h and then stored in a desiccator at room temperature. The 2 cm x 2 cm strips from a sample were placed into the container and weighed prior to oven drying at 105 °C for 24 h. They were cooled to room temperature in the desiccator and weighed again. The *MC* was calculated using Eq. 1,

$$MC = \frac{m_1 - m_2}{m_1} \times 100\%$$ (1)

where *MC* is the moisture content of sample (%); m_1 is the weight of sample before drying (g); and m_2 is the weight of sample after drying (g). The average values of three repetitions of each sample were reported with standard error.

Water Solubility (WS)

The dried film samples from the moisture content test were weighed together with a pre-desiccated filter paper No. 4 (Whatman, 100490, UK). The samples were then immersed into 30 mL of distilled water for 24 h at 25 °C with a stirring speed at about 300 rpm with a magnetic hotplate (Lab Mart, model HTS-1003, South Korea). The solution was then filtered with the same filter paper. The remaining pieces of the film samples on the filter paper were dried together in a convection oven at 105 °C for 24 h. After being cooled to room temperature in a desiccator, the filter paper was weighed again. The *WS* of the samples was calculated using Eq. 2,

$$WS = \frac{m_1 - m_2}{m_1} \times 100\%$$ (2)

where *WS* is the water solubility of sample (%); m_1 is the weight of sample on filter paper before water immersion (g); and m_2 is the weight of sample on filter paper after water immersion and drying (g). The average values of three repetitions of each sample were reported with standard error.

Water Vapour Permeability (WVP)

The water vapour permeability (WVP) test was done according to ASTM E96 (2016). Saturated NaCl solution was poured in the bottom of a desiccator to create a relative humidity of 75%. Circular film samples with diameters of 7 cm were prepared and stored in a desiccator at 25 °C and 52% of relative humidity for 48 h.

For the setup of water vapour test cup (Yasuda, No. 318, Tokyo, Japan), the cup was filled with 3 g of anhydrous $CaCl_2$. A film sample was placed on the opening of the cup and enclosed with a ring. The edge around the ring was sealed with a melted mixture of 80 wt.% paraffin wax and 20 wt.% beeswax. The sealed test cup was weighed and placed into the desiccator with the saturated NaCl solution at 20 ± 0.5 °C. The test cup was weighed at 1-h intervals for 8 h. The difference in relative humidity corresponds to a driving force of 1783.13 Pa. The WVP of the sample was calculated using Eq. 3,

$$WVP = \frac{w}{A} \times \frac{x}{P_W(RH_1 - RH_2)} \qquad (3)$$

where WVP is the water vapour permeability (g s^{-1} m^{-1} Pa^{-1}); w is the rate of weight gain of the test cup (g/s); A is the area of the exposed film sample (m^2); x is the film thickness (m); P_w is the water vapour pressure at 20 °C; and RH_1-RH_2 is the relative humidity gradient at 75%. The average values of three repetitions of each sample were reported with standard error.

Oxygen Permeability (OP)

The oxygen transmission rate of a film sample was acquired in accordance with ASTM D3985-05 (2010) using an Oxygen Permeability Analyser (MOCON Inc., OpTech®-O2 Platinum, Minneapolis, MN, USA) at 25 °C. Circular film samples with diameters of 11 cm were prepared and stored in a desiccator at 25 °C and 52% of relative humidity for 2 h prior to the test.

Before testing, the samples were sealed with a silicone ring, which was coated with vacuum grease within the diffusion chamber. Pure nitrogen was purged at a flow rate of 10 mL/min until 0% O$_2$ was recorded. Oxygen was then purged continuously in the chamber at 760 ± 5 mmHg. The test was stopped until at least three consecutive transmission rate readings were obtained. The OP of the sample was calculated using Eq. 4,

$$OP = \frac{OTR\,x}{\Delta P_{O2}} \qquad (4)$$

where OP is the oxygen permeability (m^3 mm^{-2} s^{-1} Pa^{-1}); OTR is the oxygen transmission rate of flaxseed mucilage-based film (m^3 m^{-2} day^{-1}); x is the film thickness (m); and ΔP_{O2} is the oxygen pressure difference across the film (Pa). The average values of three repetitions of each sample were reported with standard error.

RESULTS AND DISCUSSION

FTIR Spectra

The FTIR spectra of glycerol and film samples of FM-0 to FM-5 are shown in Fig. 1. In the FTIR spectrum of glycerol, the peak at 3285.3 cm^{-1} corresponded to the O-H stretching of alcohol and also denoted the hydroxyl functional group. The two peaks at 2931.8 cm^{-1} and 2879.4 cm^{-1} were indicative of –C-H stretching (Rani *et al.* 2016).

Comparing the spectrum between the neat flaxseed mucilage sample (FM-0) and that of the glycerol plasticized film samples, the intensity of the –OH stretching vibration at 3500 cm^{-1} to 3200 cm^{-1} region had notably increased. A new peak at 2883.1 cm^{-1} appeared upon the addition of glycerol, which represented the vibration band of –C-H stretching (Alix *et al.* 2008; Kandekar *et al.* 2015). Moreover, there were new peaks and gradual increases in band intensities between 1100 cm^{-1} and 920 cm^{-1} as the concentration of glycerol in the films increased (FM-1 to FM-5) compared with neat mucilage (FM-0). These results could be indicative of interactions between the flaxseed mucilage and glycerol, feasibly as hydrogen bonds, as glycerol is a polyol plasticizer.
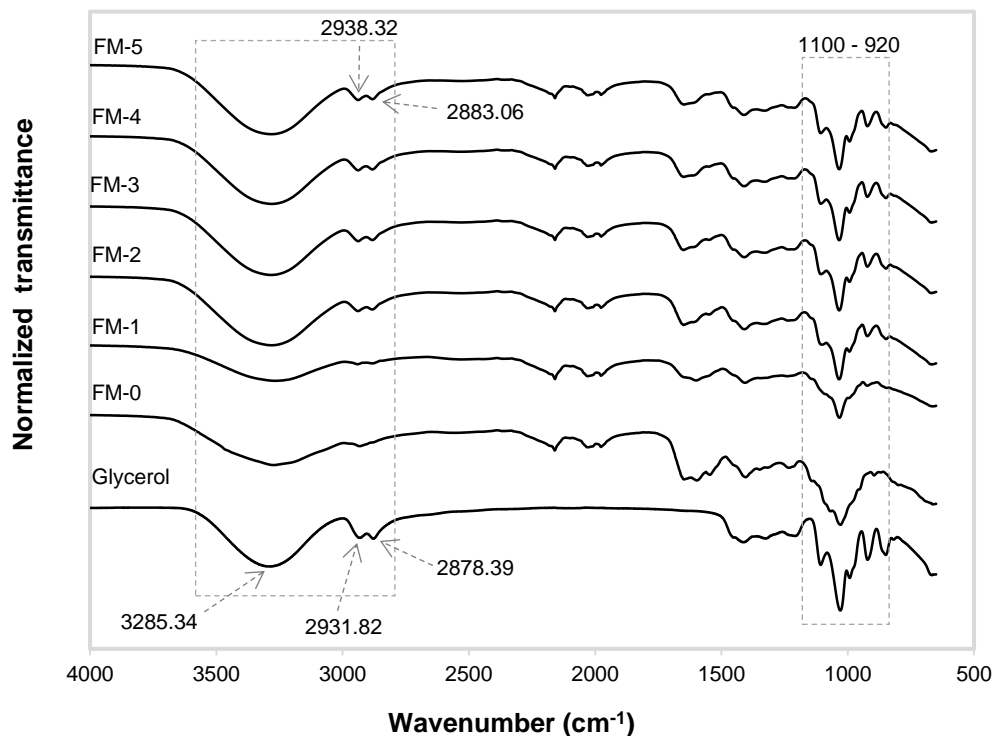
Fig. 1. Normalized transmittance as a function of wavenumber (cm^{-1}) for glycerol and film samples at various glycerol concentrations in the FTIR region from 600 to 4000 cm^{-1}

Table 1. Water Activity (a_w), Moisture Content (MC), and Water Solubility (WS) of FM Films with Various Concentration of Glycerol

Sample	Water Activity	Moisture Content (%)	Water Solubility (%)
FM-0	0.665 ± 0.004	19.41 ± 1.54	12.16 ± 0.98
FM-1	0.590 ± 0.003	27.08 ± 2.08	21.44 ± 0.07
FM-2	0.558 ± 0.004	34.93 ± 2.41	30.67 ± 0.98
FM-3	0.515 ± 0.003	48.44 ± 1.95	43.64 ± 0.91
FM-4	0.504 ± 0.002	69.51 ± 2.01	65.88 ± 1.53
FM-5	0.485 ± 0.004	77.82 ± 1.62	72.18 ± 0.72

Data are reported as means ± standard deviation.

Physical Properties

Table 1 shows the physical properties of film samples, including a_w, MC, and WS. The a_w gradually decreased from 0.67 (FM-0) to 0.49 (FM-5) as the glycerol concentration increased. This could be due to the water-binding ability of glycerol. Glycerol is a polyhydric alcohol humectant used to adjust or decrease water activity by binding with water in food products (Edwards 2000; Sikorski 2007; Belitz *et al.* 2009; Acton 2013).

The a_w value of FM-0 was above the minimum water activity range (0.65 to 0.61) that is susceptible for osmophilic yeasts (including *Saccharomyces rouxii*) and molds (such as *Aspergillus echinulatus* and *Monascus biporus*). The a_w for FM-1 to FM-5 were below 0.61, which may prevent microbial proliferation due to unfavorable conditions for their growth (Barbosa-Cánovas *et al*. 2007).

The MC of film samples exponentially increased from 19.41% to 77.82% with

increasing glycerol concentration. This could be due the hygroscopic nature of glycerol. Hydrogen bonds develop between flaxseed mucilage and hydroxyl groups along glycerol chains, which replace the interactions between mucilage molecules (Yang and Paulson 2000; Dick *et al.* 2015). Thus, the change in moisture content reflected the formation of the glycerol-mucilage and glycerol-water interactions. As the glycerol concentration increased, more water molecules were able to bind within the films, making them more hydrophilic (Arvanitoyannis and Biliaderis 1999; Zhang and Han 2006; Seyedi *et al.* 2014).

Similar to the trend in MC, the WS of film samples also exponentially increased with increases in glycerol content from 1 wt.% to 5 wt.%. This result also could be due to the hygroscopic nature of glycerol and its plasticization effect. The smaller molecules of glycerol, which are plasticizers within the mucilage matrix, were dispersed between the polymer chains and interrupted the interactions between the mucilage-mucilage interactions (Sánchez-González *et al.* 2010; Ghasemlou *et al.* 2011; Ahmadi *et al.* 2012). Subsequently, more water molecules were attracted into the polymer matrix and formed higher mobility regions with greater interchain distances. This in turn increased the WS of the film samples (Jouki *et al.* 2013; Jouki *et al.* 2014; Khazaei *et al.* 2014; Dick *et al.* 2015).

Barrier Properties

Table 2 lists the WVP and OP of flaxseed-mucilage based films plasticized with various concentrations of glycerol. The flaxseed film without glycerol (FM-0) was brittle and easily cracked (Tee *et al.* 2016). Thus, FM-0 could not be subjected to the barrier tests. In addition, the OP for FM-5 could not be determined due to its viscidity. It adhered to the testing platform and tore when flushed with gas.

Table 2. WVP and OP of FM Films with Various Concentration of Glycerol

Sample	WVP ($\times 10^{-13}$ kg m m^{-2} s^{-1} Pa^{-1})	OP ($\times 10^{-18}$ m^3 m m^{-2} s^{-1} Pa^{-1})
FM-0	N.A.	N.A.
FM-1	2.83 ± 0.07	3.03 ± 0.41
FM-2	4.34 ± 0.11	553.43 ± 106.76
FM-3	10.93 ± 0.24	30967.89 ± 469.71
FM-4	16.43 ± 0.46	52416.43 ± 1289.42
FM-5	19.50 ± 0.58	N.A.

Data are reported as means ± standard deviation. N.A., not available

The WVP and OP of the plasticized film samples (FM-1 to FM-5) notably increased with the increase of glycerol content. This could be due to the hydrophilic nature of glycerol, which favored the adsorption of water and increased the WVP of the plasticized films (Gontard *et al.* 1994; Jouki *et al.* 2013). Moreover, the molecular dispersion of glycerol increased (or introduced) free volumes within the mucilage matrix. Hence, the water vapour and gas molecules diffused more easily across the polysaccharide networks (Sothornvit and Krochta 2001; Ahmadi *et al.* 2012; Jouki *et al.* 2014; Khazaei *et al.* 2014; Seyedi *et al.* 2014).

Compared with some plastic materials, the present samples reported higher WVP and OP. For instance, those made from polyvinylidene chloride (PVDC) film reportedly had a WVP of 2.22×10^{-16} kg s^{-1} m^{-1} Pa^{-1} and an OP of 5.90×10^{-20} m^3 m m^{-2} s^{-1} Pa^{-1}; the high density polyethylene (HDPE) film with a WVP of 2.31×10^{-16} kg s^{-1} m^{-1} Pa^{-1} and an

OP of 4.94 x 10^{-18} m^3 m m^{-2} s^{-1} Pa^{-1}; and the low density polyethylene (LDPE) film with a WVP of 9.14 x 10^{-16} kg s^{-1} m^{-1} Pa^{-1} and an OP of 2.16 x 10^{-17} m^3 m m^{-2} s^{-1} Pa^{-1} (McHugh *et al.* 1996; Miller and Krochta 1997; Shit and Shah 2014). For the packing of fresh produce such as fruits and vegetables, LDPE packaging is typically microperforated for sufficient gas and water vapour transfer (Allan-Wojtas *et al*. 2008). Because of the higher WVP and OP values, the present flaxseed mucilage-based seed film could be further developed as an alternative to packing fresh produce with less microperforation.

CONCLUSIONS

1. There were interactions, feasibly hydrogen bonds, between the flaxseed mucilage and the glycerol, which contributed to the improved properties of the developed films.

2. Starting at FM-1, all glycerol-plasticized flaxseed mucilage films had an a_w value of 0.59 or less, which could sufficiently curb microbial proliferation due to unfavorable conditions for microbial growth.

3. With sufficiently high WVP and OP values in FM-1, the flaxseed mucilage-base has the potential to be developed as a biodegradable packaging or an edible coating for fresh produce.

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