Development of Edible Film from Flaxseed Mucilage

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A flaxseed mucilage-based edible film was developed with the addition of glycerol as a plasticizer. Various concentrations of glycerol were blended into the extracted mucilage, and the developed films were studied in terms of physical, mechanical, and morphological properties. As the glycerol concentration was increased from 1 to 5 wt%, the elongation at break of the films prominently increased, whereas the tensile strength and Young's modulus decreased. The film failed to form at 6 wt% glycerol inclusion. The developed film was slightly reddish and yellowish in color, with enhanced transparency as the glycerol concentration in the film increased. Overall, this work demonstrated that with the addition of glycerol as a plasticizer up to 5 wt%, a flaxseed mucilage-based edible film could be developed as a sustainable alternative for food and bioproduct coating or packaging.

Keywords: Flaxseed; Mucilage; Edible film; Mechanical properties; Glycerol; Plasticizer

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INTRODUCTION

Large disposals of synthetic polymers such as polyethylene, polypropylene, polystyrene, and polyvinyl chloride have been causing serious environmental impact. To reduce the volume of disposal, biodegradable polymers have been developed to partly or totally replace synthetic polymers (Janjarasskul and Krochta 2010). In the last few years the use of biodegradable polymers such as starch-based bioplastics, polyester-based bottles, and poly(lactic acid) (PLA) packaging has gradually increased and will continue to increase in the future.

Biodegradable polymer packaging is a type of packaging typically made of proteins, polysaccharides, and lipids, or their combination (Tanada-Palmu and Grosso 2003). Their blends can potentially be developed into edible film. Work in developing these films has experienced large growth in the food and bioproduct industry, as they can potentially improve the products' quality and shelf life (Kolhe *et al.* 2014). For instance, many studies have used polysaccharide-based edible film as a stable material that is feasible for packaging purposes (Dick *et al.* 2016).

In the present work, the development of an edible film from flaxseed mucilage was investigated, with the aim that it serves as an alternative to other edible films and synthetic polymer films. Flaxseed mucilage is the 'gel-like' layer that forms when the seed is soaked in water. The polysaccharide at the epidermal cell layer of the seed coat then becomes viscous with water (Emaga *et al.* 2012). The mucilage consists of

rhamnogalacturonan I and arabinoxylan, with novel side group substitutions that form large aggregates in solution and contribute to its gel-like character (Naran *et al.* 2008).

Nonetheless, one possible drawback of such film is its low elasticity. Recent studies on basil seed and chia seed mucilage-based film showed that the addition of plasticizer aided the formation of film with a notable reduction in the films' brittleness (Khazaei *et al.* 2014; Dick *et al* 2015). Acknowledging such limitation, glycerol, which has been generally regarded as safe (GRAS) by the Food and Drug Administration (FDA, USA), was tested as a potential plasticizer for the edible film development. Given its high workability, efficiency, and flexibility, glycerol is one of the most widely used hydrophilic plasticizers in various polymer manufacturing (Santana and Kieckbusch 2013). The present work studied the effect of adding various concentrations of glycerol as a plasticizer, with characterizations in terms of physical, mechanical, and morphological properties of the developed film.

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 and sodium hydroxide (NaOH) were purchased from Fisher Chemicals Sdn. Bhd., Selangor, Malaysia.

Mucilage Extraction

At a ratio of 1:30, flaxseeds (30 g) were mixed with 900 mL of distilled water. The soaked flaxseeds were stirred at 1000 rpm and 80 to 100 °C for at least 3 h with a hotplate magnetic stirrer (LMS Co. Ltd., HTS-1003, Tokyo, Japan) to produce an optimal mucilage solution. Then, the mucilage solution was cooled to room temperature at 25 °C. To separate the mucilage solution from the flaxseeds, the mixture was poured into 50-mL centrifuge tubes and centrifuged at 3900 rpm for 15 min using a centrifuge machine (Hettich Lab Technology, Universal 320R, USA). To acquire the remaining mucilage bound to the flaxseed coat, the flaxseeds were further filtered with a cheese cloth.

Film Formation

First, 0.1 M NaOH was added dropwise to the mucilage solution until the solution was adjusted to pH 9. The solution was then stirred at 120 rpm and 80 °C for 30 min with a circular-top LED digital hotplate stirrer (Dragon Laboratory Instruments Limited, MS-H280-Pro, Beijing, China). The stirring enabled optimum hydration capacity of the seed's mucilage (Muñoz Hernández 2012). For flaxseed mucilage/glycerol mixtures, glycerol was added to each batch of solution at loadings of 1 to 6 wt%, followed by 5 min of stirring at the same speed and temperature. The solution was then further stirred for 30 min without heating to produce a homogenous solution.

Next, 30 g of the solution was cast onto a Petri dish with a diameter of 95 mm (also expressed as 0.42 g cm^{-2}). A film was formed upon evaporation of the cast mixture in a convection oven at 35 °C for 16 to 20 h. A spatula and forceps were used to remove the films from the Petri dish, as shown in Fig. 1. The films were kept in a desiccator at 25 °C and 52% relative humidity for at least two days prior to subsequent tests. The flaxseed

mucilage-based films were labeled as 'FM-', followed by digits '0' to '6', which denoted the loading (wt%) of glycerol added.

Film Thickness

The thickness of the film was measured using a digital Vernier caliper (NL Scientific, NL 7025 X / 006, Selangor, Malaysia) with a precision of ± 0.01 mm. Measurements were done at five random positions on each film to obtain the mean thickness and were reported with standard deviation.

Tensile Test

Tensile tests were conducted with a material testing machine (Lloyd Instruments, LF Plus, USA) in reference to ASTM D882-12 (2012). Prior to the tests, the samples were cut into dimensions of 70 mm (length) and 20 mm (width). Each sample was clamped between grips, and force and deformation were recorded during extension at 20 mm min^{-1}, with an initial distance between the grips of 60 mm. Tensile strength (TS), elongation at break (EB), and Young's modulus (YM) were measured in five replicates for each sample. The average values of five repetitions of each sample were reported with standard deviation.

Morphology Characterization

The morphologies of tensile fracture surface of sample were observed using a variable pressure scanning electron microscope (LEO Electron Microscopy Inc., LEO 1455VPSEM, USA) at an accelerating voltage of 20 kV. Before scanning, the specimens were sputter-coated with gold (Leica Microsystems Inc., Leica EM ACE200, USA).

Film Color

A colorimeter (Hunter Associates Laboratory, ColorFlex, USA) was used to determine the color of the flaxseed mucilage-based films. The degree of lightness (L) and chromaticity parameters at red-green (α) and yellow-blue (b) were measured. Samples were placed on a white standard plate surface with color coordinates of L = 93.81, α = -1.20, and b = 1.60. Measurements were repeated at five random positions of each film. The total color difference (ΔE) was calculated using Eq. 1. Values were reported as the mean of five readings measured on different positions of each film with standard deviation.

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (1)$$

Light Transmission and Transparency

A UV-Vis spectrophotometer (SI Analytics, UviLine 9400, Mainz, Germany) was used to measure the light transmittance and absorbance of films at the UV range from 200 to 800 nm, as suggested by Shiku *et al.* (2004). The samples were cut into rectangles with an approximate area of 80 mm x 35 mm and inserted into cuvettes. The light transmittance and absorbance were determined against air as a standard. Each obtained absorbance was used to calculate the transparency value of the samples using the equation transparency value = $A600/x$, where $A600$ is the absorbance at 600 nm and x is the thickness (mm) of film (Han and Floros 1997). Greater transparency values represent lower film transparency.

RESULTS AND DISCUSSION

Physical Observation

As shown in Fig. 1a, the flaxseed mucilage film without plasticizer (FM-0) easily tore and appeared brittle, thus requiring extra care during the removal from the casting of the Petri dish. This fragility could be due to the rigidity and ordered format of polysaccharide chains in the mucilage (Guo *et al.* 2009). Therefore, a plasticizer was added to the films to increase their flexibility. The formation of films was successful upon the addition of 1, 2, 3, 4, and 5 wt% of glycerol into the mucilage solutions. When 6 wt% of glycerol was used, the films (FM-6) appeared too oily and sticky, which made them difficult to handle. Thus, FM-6 was not subjected to the tensile test or to the determination of other properties because of its low workability as packaging or coating material. Overall, it was conclusive that the effective glycerol concentration for flaxseed mucilage-based film formation was within the range of 1 to 5 wt%. Sample FM-5 is shown in Fig. 1b.

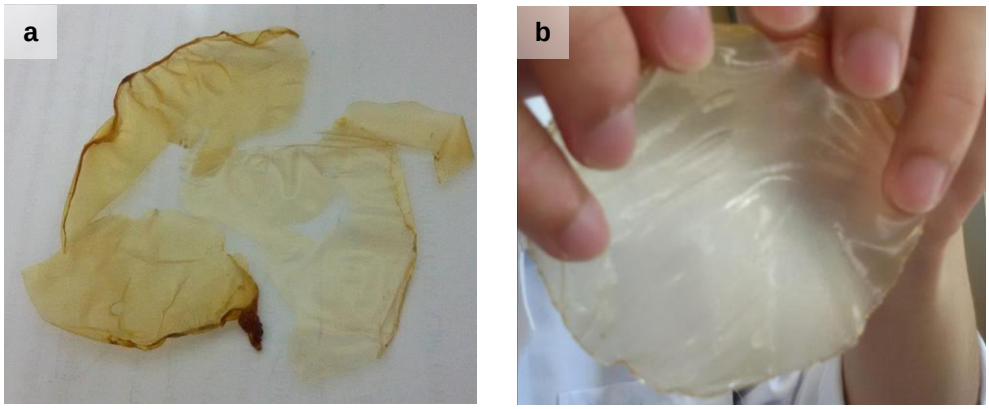

Fig. 1. Flaxseed mucilage-based films with formulation of (a) FM-0 and (b) FM-05

Film Thickness

Table 1 shows the effect of various concentrations of glycerol on the thickness of flaxseed mucilage-based films. The samples with glycerol concentrations up to 5 wt% gradually increased in thickness, from 0.05 mm (FM-0) to 0.22 mm (FM-5). The low standard deviation values in the film thickness may indicate even spreading of the samples during casting on the Petri dishes (de Moraes *et al.* 2013).

Table 1. Thickness of FM Films with Various Concentrations of Glycerol

Sample	Thickness (mm)
FM-0	0.05 ± 0.01
FM-1	0.09 ± 0.01
FM-2	0.11 ± 0.01
FM-3	0.20 ± 0.01
FM-4	0.21 ± 0.01
FM-5	0.22 ± 0.01

Values are given as average (± standard deviation).

Similarly reported, thicker mucilage or gum-based edible films were produced upon the addition of glycerol as a plasticizer (Ahmadi *et al*. 2012; Jouki *et al.* 2013; Khazaei *et al*. 2014; Dick *et al.* 2015). This increase in thickness was from the swelling process, in which the attractive force between the plasticized mucilage polymer and water increased (Jouki *et al.* 2013; Khazaei *et al.* 2014). Films with higher glycerol concentration absorbed more moisture to some extent compared with films with lower glycerol concentration.

Tensile Properties

The tensile properties of all FM film samples are tabulated in Table 2. The TS of the samples significantly decreased, from 16.61 MPa (FM-0) to 0.24 MPa (FM-5), with a gradual increase in glycerol addition to the films. Concurrently, the YM also significantly decreased, ranging from 174.77 MPa (FM-0) to 0.08 MPa (FM-5). Conversely, the EB of FM films significantly increased, even with the addition of only 1 wt% of glycerol.

Table 2. Tensile Properties of FM Films with Various Concentrations of Glycerol

Sample	Tensile Strength (MPa)	Elongation at Break (%)	Young's Modulus (MPa)
FM-0	16.61 ± 3.04	12.24 ± 6.50	174.77 ± 33.06
FM-1	0.82 ± 0.21	213.36 ± 29.80	0.40 ± 0.04
FM-2	0.73 ± 0.71	234.54 ± 44.58	0.34 ± 0.03
FM-3	0.51 ± 0.13	237.98 ± 67.15	0.28 ± 0.02
FM-4	0.33 ± 0.12	238.36 ± 33.40	0.17 ± 0.07
FM-5	0.24 ± 0.09	279.98 ± 27.55	0.08 ± 0.04

Values are given as average (± standard deviation).

From the tensile test, it was concluded that the film's flexibility and extensibility was notably enhanced with the addition of glycerol as a plasticizer, adequate even at 1 wt%. The small glycerol molecules could have penetrated between the mucilage polymer matrix, thereby causing a "cross-linker" effect in the matrix (Ahmadi *et al.* 2012; Jouki *et al.* 2013). The GLY-FM (glycerol and flaxseed mucilage) hydrogen bonds may have replaced some of the FM-FM (flaxseed mucilage and flaxseed mucilage) hydrogen bonds (Khazaei *et al.* 2014). This led to the reduction of inter-chain interaction and an increase in the polymer chains' mobility, which improved the film's extensibility (Ahmadi *et al.* 2012).

Meanwhile, samples with 6 wt% of glycerol could not be continued in the tensile test because of their extreme stickiness. The glycerol concentrations (1 to 5 wt%) tested in the present study were distinctly different from those in several related studies, as glycerol was added at loadings between 25 to 75 wt% to seed mucilage-based films (Ahmadi *et al.* 2012; Jouki *et al.* 2013; Khazaei *et al.* 2014; Dick *et al*. 2015). The differences in glycerol concentrations could be due to the differences in the film-forming solution formulations and the film development methods.

Morphology Characterization

Figure 2 shows micrographs of the tensile fracture surfaces of samples FM-0, FM-3, and FM-5. The unplasticized film showed a clear cut surface, with some clearly visible brittle tensile fracture. Upon addition of glycerol, the fracture surfaces seemed smoother, with a more homogeneous surface. As the glycerol content in the films increased, the fracture surfaces became smoother. The glycerol particles seemed well

dispersed and embedded in the biopolymer matrix. Because of the close molecular structures of the mixtures, there was good compatibility, miscibility, and interfacial adhesion between the glycerol and mucilage polymer, which thereby produced tougher films (Santana and Kieckbusch 2013; Petchwattana and Covavisaruch 2014). These observations were supported by the increase in EB and decrease in YM of plasticized films in the present study.

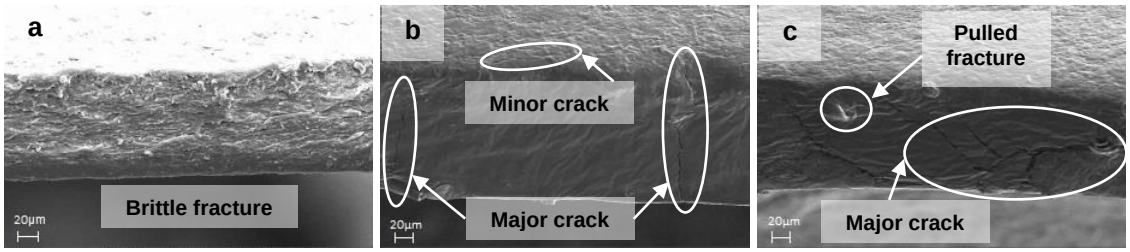

Fig. 2. VPSEM micrographs at 250x magnification of cross-sectional tensile fracture surface of (a) FM-0; (b) FM-3; and (c) FM-5

The general fracturing behavior of the matrix, such as pulled fracture and cracks, was also observed. Upon increasing the glycerol concentration in the FM films, the cracks on the tensile fracture surface increased and became more prominent (Figs. 2b and 2c). The large cracks could have been generated from the phase separation of glycerol particles and mucilage matrix. As the glycerol concentration in the FM films increased, the phase separation between the plasticizer and mucilage matrix increased, thereby causing cracks when energy was applied during the tensile test (Ma and Jing 2012).

As reported by Lu *et al.* (2007), film samples with more major cracks had higher EB values compared with films without any cracks on the tensile fracture surfaces. Similarly observed in the present work, FM-5, which had many major cracks compared with the rest of the samples, had the highest EB. Its polymeric matrix was deformed and stretched furthest prior to tensile failure.

Film Color

The color parameters of all FM film samples are tabulated in Table 3. The addition of glycerol concentration up to 5 wt% in the FM films caused the *L* values (lightness) to decrease significantly, whereas *a* values (green-red) increased significantly. Although the *b* values (blue-yellow) increased, the changes were comparatively minor, and the films' color remained in the yellowish hue (Fig. 1).

Table 3. Color Measurements of FM Films with Various Concentrations of Glycerol

Sample	Color			Δ*E*
	L	*a*	*b*	
FM-0	31.87 ± 1.88	-0.596 ± 0.36	6.45 ± 1.13	86.92 ± 13.22
FM-1	22.72 ± 2.55	0.36 ± 0.24	6.80 ± 0.06	100.63 ± 1.60
FM-2	22.72 ± 5.11	0.54 ± 0.55	7.68 ± 0.89	111.91 ± 14.63
FM-3	22.47 ± 0.57	0.57 ± 0.03	7.90 ± 1.04	115.01 ± 12.09
FM-4	20.47 ± 0.46	0.98 ± 0.60	8.22 ± 0.04	120.26 ± 2.76
FM-5	20.46 ± 2.32	1.17 ± 0.01	8.25 ± 2.20	127.11 ± 26.54

Values are given as average (± standard deviation).

The color of FM films, which became slightly reddish (*a*+) and yellowish (*b*+) from the addition of glycerol plasticizer, can also be more wholly described using Δ*E*, which shows the degree of total color difference from the standard color plate. The Δ*E* values increased with increasing glycerol content. A similar trend of decreasing *L* values and increasing *a* and *b* values with increasing plasticizer concentration has been reported (Salamanca *et al*. 2011; Dick *et al*. 2015).

Light Transmission and Transparency

In the food and bioproduct industry, a film's UV light absorption capability is important, as it may determine its potential application in the food and bioproduct packaging or coating. The film can prolong the shelf life of fat-based food products by absorbing UV light and thus suppress oxidative degradation (López and García 2012). The light transmittance percentage at selected wavelengths (from 200 to 800 nm) and the transparency values for FM films are tabulated in Table 4. Lower transparency values denote higher transparency of a film material. Based on Table 4, as the glycerol concentration in the FM films increased, the transparency value of the films decreased, with FM-5 as the most transparent film. The improvement in transparency between an unplasticized sample (FM-0) and plasticized sample (FM-5) can be observed in Fig. 3.

Table 4. Light Transmittance (%) and Transparency Value (A_{600}/mm) of FM Films

Sample	Light transmittance (%) at various wavelengths (nm)								Transparency value
	200	280	350	400	500	600	700	800	
FM0	0.02	0.03	1.82	2.37	7.33	9.04	10.60	12.03	36.84 ± 23.09
FM1	0.02	0.04	1.92	3.58	11.60	14.80	16.60	18.70	18.81 ± 13.33
FM2	0.01	0.03	3.17	4.76	15.80	20.20	22.50	25.20	15.40 ± 13.33
FM3	0.01	0.03	4.46	7.11	27.00	37.60	43.50	47.50	7.46 ± 7.61
FM4	0.02	0.03	3.84	6.03	21.10	29.40	34.70	38.70	7.19 ± 6.61
FM5	0.77	2.30	7.65	12.40	22.80	27.40	29.90	32.10	4.54 ± 2.71

Values are given as average (± standard deviation).

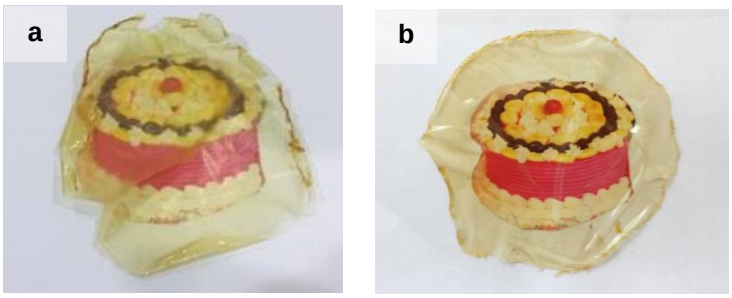

Fig. 3. The observation of transparency of flaxseed mucilage films against a coloured illustration: (a) unplasticized flaxseed mucilage film; (b) plasticized flaxseed mucilage film with 5 wt% glycerol

The light transmittance percentage of FM-0, FM-1, FM-2, FM-3, and FM-4 were low (ranging from 0.01% to 0.04%) between 200 and 280 nm. These low percentages may indicate the excellent UV barrier capability of the films, which is important for food and bioproduct protection. From the comparison of the light transmittance percentage with other studies, the barrier against the UV radiation of these FM films was reportedly higher than that of the synthetic polymer films. For example, low-density polyethylene

(LDPE) and oriented polypropylene (OPP) range from 4.6% to 67.5% in the same wavelength spectrum, in addition to the rest of the tested wavelengths (Shiku *et al. 2*004). With these data obtained, it is anticipated that the developed flaxseed mucilage-based film could serve as an alternative protective layer to food and bioproducts if used as a coating or packaging material.

CONCLUSIONS

1. A maximum of 5 wt% glycerol can be added as a plasticizer in flaxseed-extracted mucilage for the development of edible films intended as sustainable alternatives to food and bioproduct coating or packaging.
2. From the physical, mechanical, and morphological studies, the addition of 1 wt% glycerol as a plasticizer in the flaxseed mucilage-based film was sufficient to form a physically stable film. This addition was sufficient because FM-1 had already achieved desirable ductility in addition to its sufficient transparency and color, which suggested protection of the product against UV radiation, if used as packaging or coating.

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