

## Development of breadfruit flour (*Artocarpus altilis*) with the addition of gelatine as a potential edible film

<sup>1,2</sup>Khairol Nadia, A.H., <sup>2</sup>Russly, A.R. and <sup>2,3,\*</sup>Nur Hanani, Z.A.

<sup>1</sup>Food Science and Technology Research Centre, Malaysian Agriculture Research and Development Institute, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

<sup>2</sup>Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

<sup>3</sup>Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

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### Abstract

The potential application of biodegradable films for food applications has gained interest as an alternative to synthetic plastic packaging. Breadfruit is a common starchy fruit in nature and due to its unique and wide availability, it can be good a source for developing biopolymers. This study aimed to develop packaging films made from unripe green breadfruit (*Artocarpus altilis*) flour with different concentrations of bovine gelatine. The ratios of gelatine and breadfruit flour (G:BF) were 0:5 (G0), 1:5 (G2), 2:5 (G4), 3: 5(G6), 4:5 (G8) and 5:5 (G10). The physical, mechanical and optical properties of these films were investigated. The findings of this study showed that the addition of bovine gelatine in flour solutions had significantly ( $P<0.05$ ) improved the film thickness, tensile strength (TS), optical properties and water vapour permeability (WVP). Breadfruit film with no gelatine (control) showed the lowest WVP ( $P<0.05$ ). Based on the Fourier transform infrared spectroscopy (FTIR) spectra, the chemical interaction of hydroxyl group from gelatine and the carboxyl group of breadfruit flour occurred. Therefore, gelatine helped to improve the water barrier and mechanical properties of the BF films as supported by the scanning electron microscopy (SEM) images. Further study to enhance film properties is necessary to apply the material to food products.

## 1. Introduction

Breadfruit is a tropical fruit and is native to Malaysia, the Southern Pacific and the Caribbean (Carrington *et al.*, 2011). It is from the family of *Moraceae* and is carbohydrate-rich food and a staple diet in some developing countries. There are sixty species of *Moraceae* family tree with three major species over the world namely the breadfruit (*Artocarpus altilis*), the breadnut (*Artocarpus camansi*) and the jackfruit (*Artocarpus heterophyllus*). *Artocarpus altilis* is appropriately known as the seedless breadfruit type that is now widely distributed throughout the tropical world (Carrington *et al.*, 2011). Breadfruit is an energy-rich food, high in complex carbohydrates, low in fat, and a good source of fibre and minerals.

The main disadvantage of breadfruit is the high post-harvest respiration rate, which causes the fruit to ripen in 2–3 days (Roberts-Nkrumah, 2007). Due to the rapid ripening rate, there is an overabundance of breadfruits on the market, particularly during its season. Farmers and

wholesalers are significantly affected. Therefore, breadfruit is normally converted to flour to provide a more stable storage form as well as enhance the versatility of the fruit. Breadfruit flour is more practical and easier to distribute, increases usability, yield and use value and is more easily processed into products with high economic value. Furthermore, breadfruit flour is suitable for use as an ingredient in making patisserie products. This breadfruit flour can also be used as a substitute for wheat flour. The high carbohydrates contained in breadfruit flour are used in bakery products such as buns and bread, and confectioneries such as bars (Nochera and Ragone, 2016), cookies (Barber *et al.*, 2016), and biscuits (Olaoye *et al.*, 2007). It is also used to make pasta (Nochera and Ragone, 2019), and instant noodles (Oke *et al.*, 2018). Gao *et al.* (2019) developed a probiotic beverage using breadfruit flour as a substrate. Breadfruit starch is also commercially used in non-food applications as adhesives in packaging and in the textile and pharmaceutical industries (Graham and De Bravo, 1981). Besides of high content of starch, it is reported

\*Corresponding author.

Email: [hanani@upm.edu.my](mailto:hanani@upm.edu.my)

that breadfruit also has a high content of amylose (22.52%) and amylopectin (77.48%) (Akanbi *et al.*, 2011). These amylose and amylopectin contents in breadfruit flour can also be used to make edible films. Therefore, converting the breadfruit into flour and then making edible films is a wise alternative to avoid dumping and wasting the breadfruit.

However, starch-based edible films have some drawbacks derived from their great sensitivity to water and retrogradation phenomena, which affect the film's mechanical properties. Proteins are generally superior to polysaccharides in their ability to form films with high mechanical and barrier properties besides providing higher nutritional value. Therefore, additional protein in starch will improve the physical and mechanical properties of the starch-based edible films. Gelatine is a protein-based biopolymer obtained from the skin and bones of animals (i.e. bovine, porcine, poultry and fish) and widely used for the formulation of edible packaging films in the food industry (Nur Hanani *et al.*, 2014). It is an ideal material for food packaging due to its versatile advantages such as low price, polymerization, biodegradability, and antioxidant properties. Gelatine addition improves the strength of films with a higher resistance to break and greater extensibility and durability (Acosta *et al.*, 2015; Weng *et al.*, 2015; Luo *et al.*, 2022). Therefore, the objective of this study was to formulate breadfruit flour edible films incorporated with bovine gelatine and characterize the physicochemical properties.

## 2. Materials and methods

### 2.1 Materials and reagents

The raw seedless green unripe breadfruits (*Artocarpus atilis*) also known as 'sukun' were purchased from Pasar Borong Selangor, Malaysia. The fresh and immature green colour of breadfruits were selected at fully grown size 12-16 weeks age. Bovine gelatine powder was purchased from Halalgel Manufacturing (M) Sdn. Bhd. with bloom strength between 151-160. Food-grade glycerol was purchased from Sigma Aldrich. The ethanol 95% was purchased from Fisher Scientific (Leicestershire, United Kingdom). Lemongrass essential oil was purchased from Essential Oil Technology Incubator, MARDI Kuala Linggi Melaka, Malaysia. The 4 mL of lemongrass oil was dissolved with 100 mL ethanol and kept in a chill condition to maintain the shelf life of the lemongrass essential oil.

### 2.2 Production of breadfruit flour

The fruits were peeled, washed and cut into smaller sizes (2 cm × 1 cm) before sliced using a food slicer

(Waring Pro FS150, USA). The sliced breadfruits were dried in an oven drier, Memert UF75, (Schwabach, Germany) at 50°C overnight and reached 3% of moisture content and water activity ( $a_w$ ) at below 0.20%. The dried sliced breadfruits then were ground using a hammer mill pulveriser machine. The flour produced was sieved by using a shaker sieve at size 106 mm. The flour was kept in an airtight container at ambient temperature until further use.

### 2.3 Film preparation

Breadfruit films were prepared by using the solution casting method as reported by Sandra *et al.* (2015) with slight modifications. The breadfruit flour (BF) and bovine gelatine (G) dispersions were prepared separately using different ratios of BF and G. The proportions of G:BF were 0:5 (G0), 1:5 (G2), 2:5 (G4), 3:5 (G6), 4:5 (G8) and 5:5 (G10). BF aqueous dispersions, 50 mL were maintained at 65°C for 10 mins until it reached the gelatinisation temperature. Meanwhile, gelatine was dissolved in 50 mL distilled water at 40°C. Then, glycerol (30%, w/w total solid) as a plasticizer and 1 mL of 4% lemongrass essential oil solution were added to the gelatine dispersions which were then mixed with BF dispersions by magnetic stirring for homogenisation. This process was carried out by using rotor-stator homogeniser (Ultraturrax T25, Malaysia) at 15,000 rpm for 5 mins. The breadfruit flour and gelatine (BFG) solutions were cast into rimmed plastic plates (25.0 cm × 25.0 cm × 2.0 cm). The quantity of each film-forming solution poured onto a plate was 40 mL, then dried overnight at 25°C. The films produced were peeled and stored at a temperature of 25±2°C with relative humidity (RH) 50±5% before further analysis.

### 2.4 Determination of film properties

#### 2.4.1 Film thickness

The thickness of BFG films was determined using a handheld micrometre Dial Thickness Gage (No.7301, Mitutoyo Company, Toyo, Japan). The measurements were taken at 15 random positions around each film to the nearest 0.001 mm and the average values were used.

#### 2.4.2 Water vapour permeability

The Water vapour permeability (WVP) was measured using the updated ASTM E-96 model method (ASTM, 1990) according to Nur Hanani *et al.* (2012). The test cup was filled with 6 mL of purified water and

$$WVP = \frac{(\text{amount of permeant (g)}/\text{time (s)}) \times \text{film thickness (mm)}}{\text{film area (m}^2\text{)} \times \text{pressure difference (kPa)}}$$

the film sample was securely secured to the test cup opening with a rubber gasket. The RH and the temperature of the cups were monitored at 50±5% and

28±2°C, respectively. The weight of test cups was recorded at a time interval of 1 hr for 9 hrs. The WVP was calculated using equation below:

#### 2.4.3 Solubility

The films were cut into 2.0 cm × 2.0 cm strips and the solubility was measured according to Tongdeesontorn *et al.* (2012) with slight modifications. Three pieces of each film were weighed (±0.0001 g), and then dried in an air-circulating oven at 70°C for 24 hrs to obtain the initial dry-mass content. For determination of their initial dry weight ( $W_i$ ), the films were re-weighed and recovered (±0.0001 g). Film discs were immersed with 20 mL of distilled water at 25°C and shaken at room temperature for 24 hrs. The remaining undissolved films were removed and dried at 105°C for 24 hrs and weighed ( $W_f$ ). Film solubility (FS) was calculated using the following equation:

$$FS (\%) = \frac{W_i - W_f}{W_i} \times 100$$

#### 2.4.4 Determination of mechanical properties

The tensile strength (TS), Young's modulus (YM) and elongation at break (EAB) of BFG films were measured according to ASTM-D638-77 using INSTRON 4302 Machine Series iX with 100 N load cell, a velocity of 25 mm/min, with the distance between clamp was 60 mm. Test samples were cut into dumbbell shapes, 50 mm long and 10 mm wide, flaring to 20 mm × 10 mm grip areas on both ends. The data for tensile strength and young modulus were auto-calculated by the INSTRON software. While the data for elongation at break (EAB %) was calculated as follows:

$$EAB (\%) = (l_{max}/l_0) \times 100$$

where  $l_{max}$  is the film elongation (mm) at the moment of rupture and  $l_0$  is the initial grip length (mm) of the sample.

Young's Modulus (YM) was calculated as follows:

$$\text{Young's Modulus (MPa)} = \text{Stress (MPa)} / \text{Strain}$$

where stress is load (N) divided by area (mm<sup>2</sup>) and strain is the change in length (mm) divided by original length (m).

#### 2.4.5 Colour properties

Minolta Chromameter (CR-401, Minolta Camera Co., Osaka, Japan), was used to measure the colour (L, a, b) values. Films were cut into 10 cm × 5 cm and 10 readings at different locations were taken on each film. Five replications were conducted for each treatment and five film samples were used for each replication.

#### 2.4.6 Film opacity

The opacity of films was conducted as defined by Susmitha *et al.* (2021a) and carried out by calculating the absorbance of the film at 600 nm using UV Spectrophotometer (Shimadzu UV-vis 1601, Japan). The film was cut into rectangle pieces and placed directly in a spectrophotometer test cell. An empty test cell had been used as a reference. The opacity of films was calculated using the following equation:

$$T = \frac{Abs600}{x}$$

Where T is the transparency of films, Abs 600 is the value of absorbance at 600 nm and  $x$  is the thickness of films. According to this equation, the high values of T indicate lower transparency and a higher degree of opacity.

#### 2.4.7 Fourier transform infrared analysis

The FTIR spectrometry was conducted to analyse the structural interactions of the BF and G in the films. The IRTracer-100 (Shimadzu Corp., Kyoto, Japan) FTIR spectrometer with an attenuated total reflection (ATR) accessory was used. The chemical structure analysis of edible films was obtained from an average of 45 scans per min at a resolution of 4 cm<sup>-1</sup> over a spectral range of 400 cm<sup>-1</sup> and 4000 cm<sup>-1</sup>. Data analysis of each edible film was done with the Origin 8.5 program.

#### 2.4.8 Scanning electron microscopy analysis

The morphology of the surface and cross-section of films were visualised using scanning electron microscopy (SEM) (JEOL JSM 6400, Tokyo, Japan). The samples were mounted on bronze stubs by means of double-sided tape and were sputtered with gold (Sputter Coater BAL-TEC SCD 005). The photographs were taken at an acceleration of 500 microns.

#### 2.5 Statistical analysis

The Minitab version 16 (Minitab Inc. USA) was used for data analysis. The statistical analysis of the data was carried out by a one-way (unstacked) analysis of the variance (ANOVA) and the differences between the means were evaluated by a Turkey multiple comparison test with a significant range (P<0.05). Results were analysed using triplicate reading.

### 3. Results and discussion

#### 3.1 Thickness

The thickness of the breadfruit films is presented in Table 1. It can be observed that there were no significant changes in the thickness of films with the ratio of starch

and gelatine 0:5 (G0), 1:5 (G2), 2:5 (G4), 3: 5(G6) and 4:5 (G8). However, the thickness of the G10 (5:5) film had significantly ( $P<0.05$ ) increased. The thickness of films may be governed by molecular organisation in the film network, which depends on the types of components, the interaction of components in the film matrix as well as the blend ratio of components used for film preparation. In general, film thickness depends on the composition of the film-forming solution and the nature of its components. The combination of protein from gelatine with amylose from flour after being heated and homogenised resulted in cross-linking of protein hydrolysed and starch gelatinization (Susmitha *et al.*, 2021b). According to Mali *et al.* (2005), thicker films caused the polymer matrix to become denser and higher in inter and intra-molecular interactions and subsequently more resistant to rupture. The thickness of the films is important because it influences other food packaging features, such as water vapour and permeability of gas, dissemination of active compounds and insect protection as well as microbial attack (Carvalho *et al.*, 2014; Murmu and Mishra, 2017).

Table 1. Thickness, water vapour permeability (WVP) and solubility of breadfruit flour and gelatine (BFG) films.

Film	Thickness (mm)	WVP ( $10^{-8}$ g mm kPa <sup>-1</sup> s <sup>-1</sup> m <sup>-2</sup> )	Solubility (%)
G0	0.16±0.01 <sup>c</sup>	1.69±0.20 <sup>d</sup>	72.86±1.97 <sup>a</sup>
G2	0.16±0.00 <sup>c</sup>	1.88±0.10 <sup>cd</sup>	68.86±1.31 <sup>a</sup>
G4	0.17±0.04 <sup>c</sup>	2.09±0.08 <sup>ab</sup>	66.25±0.48 <sup>a</sup>
G6	0.17±0.00 <sup>c</sup>	1.94±0.11 <sup>bc</sup>	67.01±1.36 <sup>a</sup>
G8	0.20±0.04 <sup>b</sup>	2.18±0.05 <sup>a</sup>	67.17±2.35 <sup>a</sup>
G10	0.24±0.00 <sup>a</sup>	2.12±0.00 <sup>ab</sup>	67.19±2.02 <sup>a</sup>

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ( $P<0.05$ ). G0: Control, G2: 2 g gelatine, G4: 4 g gelatine: G6: 6 g gelatine, G8: 8 g gelatine and G10: 10 g gelatine.

### 3.2 Water vapour permeability

The WVP of the films had shown an increment trend when the concentration of gelatine was increased (Table 1). This was due to the stronger interactions between amylose and amylopectin chains in the starch of breadfruit flour with the molecule of protein from gelatine. When gelatine was added, it installed itself between these chains and increased its free volume, allowing for more water diffusion. The increase in WVP was related to an increase in biofilm polarity due to the presence of more gelatine (Davanço *et al.*, 2007; Soo *et al.*, 2018). Norfarahin *et al.* (2018) reported that the WVP of the films increased ( $P\leq 0.05$ ) when the thickness increased. This fact can be attributed to the increased content of hydrophilic groups in the final mass of the

sample. As the film thickness increases, the film provides higher resistance to mass transfer across it, therefore the equilibrium water vapour partial pressure at the inner surface increases (Longares *et al.*, 2004).

### 3.3 Film solubility

In Table 1, the increment of gelatine contents into the films had decreased the solubility of the films. However, the changes were not significant. The films possibly have a high-stable starch-protein polymer network since they did not break apart after being immersed in water for 24 hrs (Norfarahin *et al.*, 2018). Increased thickness of composite films by gelatine addition has also contributed to a decrease in solubility. The result suggested that the thicker the film, the stronger the interaction between starch and protein, therefore reducing the solubility. Some studies have also shown that it may be due to the formation of hydrogen bonds between the OH groups of gelatine and the functional groups of starch in breadfruit flour. These intermolecular interactions improved the water resistance and stability of films (Hoque *et al.*, 2010; Limpan *et al.*, 2010). Furthermore, physical interference may occur in the gelatin polypeptide chains, which leads to a significant blockage of gelatin's ability to interact with water molecules, thus reducing the solubility of films (Hosseini, 2013). A lower film solubility is required for storage, while a higher solubility of film is useful when edible films are needed to coat food products for cooking (Maizura *et al.*, 2007).

### 3.4 Mechanical properties of breadfruit flour films

#### 3.4.1 Tensile strength and elongation at break

Tensile strength (TS) of edible breadfruit starch film at different formulations of bovine gelatine are summarised in Table 2. The G0 possessed the highest tensile, at 0.88 MPa. The addition of gelatine in G2 decreased 28.41% of tensile value. Following the addition of gelatine in G4, the tensile strength of the film Table 2. Mechanical properties of the breadfruit flour and gelatine (BFG) films.

Film	Tensile Strength (TS) (MPa)	Elongation at break (EAB) (%)	Young modulus (YM) (MPa)
G0	0.88±0.21 <sup>a</sup>	11.58±2.98 <sup>c</sup>	56.00±24.24 <sup>a</sup>
G2	0.63±0.06 <sup>b</sup>	12.21±2.39 <sup>c</sup>	36.73±8.74 <sup>b</sup>
G4	0.46±0.06 <sup>c</sup>	13.37±3.61 <sup>bc</sup>	26.82±8.28 <sup>b</sup>
G6	0.41±0.09 <sup>c</sup>	13.83±2.35 <sup>bc</sup>	22.22±7.98 <sup>b</sup>
G8	0.43±0.10 <sup>c</sup>	16.32±1.57 <sup>ab</sup>	22.28±11.51 <sup>b</sup>
G10	0.49±0.10 <sup>bc</sup>	17.85±1.47 <sup>a</sup>	27.62±11.77 <sup>b</sup>

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ( $P<0.05$ ). G0: Control, G2: 2 g gelatine, G4: 4 g gelatine: G6: 6 g gelatine, G8: 8 g gelatine and G10: 10 g gelatine.

decreased significantly ( $P < 0.05$ ) to 26.98%. However, a significant increase ( $P < 0.05$ ) in EAB was observed in films of G6, G8 and G10. It was found that the EAB ranged between 11.58% to 17.85%. The film without gelatine had the lowest EAB values. It appeared that the increase of gelatine had significantly increased at ( $P < 0.05$ ) 5.16% EAB values. This showed that the film became more flexible and the polymer chains can slide each other more readily during tensile deformation. The desired amount of gelatine added could be easily inserted in breadfruit flour polymer chains, producing a ‘crosslinker’ effect (Ahmad *et al.*, 2015).

According to Tong *et al.* (2008), generally, the increased TS precedes a decrease in EAB whereby TS and EAB values usually show an inverse rate. In this result, TS values showed some decrement while the EAB value had some increment. An edible film used for packaging food products typically requires adequate mechanical strength and extensibility to resist external stress and maintain its integrity and barrier properties (Rao *et al.*, 2010). The decreased TS values and increased EAB values in this study were reflected by the amount of gelatine added into the starch polymer chain of breadfruit flour and reacted with available hydroxyl groups in gelatine, thus the formation of strong hydrogen bonds lowering the molecular mobility and resulting in higher chain entanglement as well as higher molecular slippage upon tensile deformation (Arfat *et al.*, 2014; Ahmad *et al.*, 2015). Besides, the excess levels of bovine gelatine added into films interfered with the interaction with breadfruit flour starch molecules in the film matrix. Starch’s intra-molecular hydrogen bonds are formed, resulting in a phase separation between the two main components and the non-uniform network, thus decreasing TS and increasing EAB value in films (Xu *et al.*, 2005).

The findings of our study are supported by Ahmad *et al.* (2015) who reported that the incorporation of rice flour with gelatine films caused a significant decrease in TS from 25.33 to 13.26 MPa and EAB values from 14.72 to 6.46%. Therefore, the addition of gelatine influenced the TS and EAB of breadfruit flour film.

### 3.4.2 Young modulus measurement

Young's modulus suggests film resistance to elastic deformation, representing the degree of stiffness and surface strength. Table 2 shows the Young’s Modulus (YM) values for BFG films. The addition of gelatine concentrations into breadfruit flour films had a significant effect on YM measurement. Film without the addition of gelatine (G0) had the highest value of YM, 56.00 MPa which was parallel with the results of tensile strength (TS) in this study. The Young's modulus values

were clearly reduced when gelatine was added and this decrease led the films to lose their stiffness and become more elastic. This behaviour was due to the influence of gelatine on the chemical composition of the films. Protein chains tend to aggregate through helical structures, while amylopectin and amylose chains progressively aggregate through the formation of hydrogen bonds (Cano *et al.*, 2014). Films with higher values of YM resulted as less flexible and more rigid compared to those with lower values of YM. This condition occurs because the linear chains of amylose in starch are able to interact via hydrogen bonds to a higher extent and be more easily entangled than the branched amylopectin chains, which act as self-reinforcement (Wittaya, 2012).

## 3.5 Colour and opacity of the films

### 3.5.1 Colour measurement

Colour analysis was conducted to determine the effect of gelatine addition into breadfruit flour films as shown in Table 3. The addition of bovine gelatine concentration significantly ( $P < 0.05$ ) reduced the lightness of the films. This is because the yellowish colour contributed by bovine gelatine and the changes in the thickness of the films led to more opaque. However, further increasing the concentration did not cause any significant changes in the rest of the edible films.

Table 3. Colour properties of breadfruit flour and gelatine (BFG) films.

Film	L*	a*	b*	Opacity
G0	89.39±0.37 <sup>a</sup>	-0.69±0.046 <sup>c</sup>	15.80±0.68 <sup>c</sup>	4.81±0.036 <sup>c</sup>
G2	87.77±0.363 <sup>b</sup>	-0.44±0.08 <sup>b</sup>	19.12±0.49 <sup>b</sup>	5.88±0.19 <sup>c</sup>
G4	86.76±0.69 <sup>c</sup>	-0.26±0.06 <sup>a</sup>	20.53±0.82 <sup>a</sup>	5.25±0.17 <sup>de</sup>
G6	85.86±0.58 <sup>d</sup>	-0.17±0.07 <sup>a</sup>	21.35±0.76 <sup>a</sup>	5.48±0.41 <sup>cd</sup>
G8	86.16±0.36 <sup>cd</sup>	-0.42±0.04 <sup>b</sup>	21.10±0.38 <sup>a</sup>	6.58±0.07 <sup>b</sup>
G10	86.45±0.68 <sup>cd</sup>	-0.51±0.12 <sup>b</sup>	20.61±0.83 <sup>a</sup>	7.58±0.16 <sup>a</sup>

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ( $P < 0.05$ ). G0: Control, G2: 2 g gelatine, G4: 4 g gelatine: G6: 6 g gelatine, G8: 8 g gelatine and G10: 10 g gelatine.

The optical characteristics of the film including colour and opacity are key factors which determine the film’s appearance as food packaging materials. It is an important factor in their marketability and applicability. The lightness (L\*), redness/greenness (a\*) and yellowness/ blueness (b\*) values of breadfruit films incorporated with different levels of gelatine are shown in Table 3. The breadfruit flour films had higher L\* values but low a\* and b\* values due to the colour of the original breadfruit flour which was slightly yellowish and creamy. The degree of brightness (L\* value) of material was the ability of a material to reflect light that

hits its surface. The control BF film was the lightest (89.39) among all samples. The  $L^*$ ,  $a^*$ , and  $b^*$  values decreased with the increase in gelatine concentration, indicating reduced lightness with decreased redness and increased yellowness of the films. Edible films produced from BF with the highest concentration of gelatine were the most yellowish in colour compared to the control film. This was probably due to the gelatine's natural colour which is yellowish. On the other hand, the breadfruit flour also had a creamy and slightly yellowish colour causing the film to have low transparency and become more yellowish (Arifin *et al.*, 2020).

The increase in the  $b^*$  values was parallel to the decrease in the  $L^*$  values, which showed that the films turned yellowish and thus became darker. Gelatine concentrations in the breadfruit flour may have caused the films to absorb more low-wavelength light than the film without gelatine addition, resulting in a further increase in the  $b^*$  value. The incorporation of gelatine concentration increased the opacity values of all the breadfruit flour films. In general, film G10 had a significant ( $P < 0.05$ ) difference in opacity values with films G0, G2, G4, G6 and G8 which indicates that the higher the gelatine concentration, the more opaque films produced. Opaque films can be used when protection against incident light is required, in particular for products that are sensitive to degradation reactions catalysed by light. According to Fakhri *et al.* (2015), the opacity of films differed depending on the content of amylose, the molecules of a linear nature and seem to favour strong hydrogen bonds between hydroxyl groups of adjacent chains. The increase in film opaqueness may also prevent oxidative deterioration, avoiding discolouration and off-flavours in food products, as the exposure to visible and ultraviolet light was decreased (Yuan *et al.*, 2015). However, customer acceptability can also be affected by the colour of the films used to wrap food products (Ojagh *et al.*, 2010).

### 3.6 Fourier transform infrared spectroscopy analysis

The Fourier transform infrared spectroscopy (FTIR) technique was used to identify the interaction of the film produced as the result of different molecule interactions between breadfruit flour and bovine gelatine. Figure 1 presents FTIR spectra of breadfruit flour and bovine gelatine blended film, in which Amide A, Amide I, Amide II and Amide III bands from protein derivatives were observed. Amide A at 3300 wavenumbers represents N-H stretching vibration (Wu *et al.*, 2013). The FTIR spectra pattern for breadfruit flour G0 (control) film shows a broad peak located around  $3265.69 \text{ cm}^{-1}$ . This was assigned to O-H stretching which was affected by the inter-molecular or intra-molecular

hydrogen bonds. With the addition of bovine gelatine, the peak of O-H stretching shifted toward higher wavelength from  $3265.69 \text{ cm}^{-1}$  (G0) to  $3268.38 \text{ cm}^{-1}$  (G2),  $3273.16 \text{ cm}^{-1}$  (G4),  $3267.53 \text{ cm}^{-1}$  (G6),  $3271.01 \text{ cm}^{-1}$  (G8) and  $3271.88 \text{ cm}^{-1}$  (G10). The shifted wavenumbers into a higher number, indicating that the hydrogen bonds acting on the -OH groups for the blends. This was due to the intermolecular interaction between the hydroxyl group from gelatine and the carboxyl group from breadfruit flour, thus reducing the amount of hydrogen bonds that can act on the free hydroxyl group.

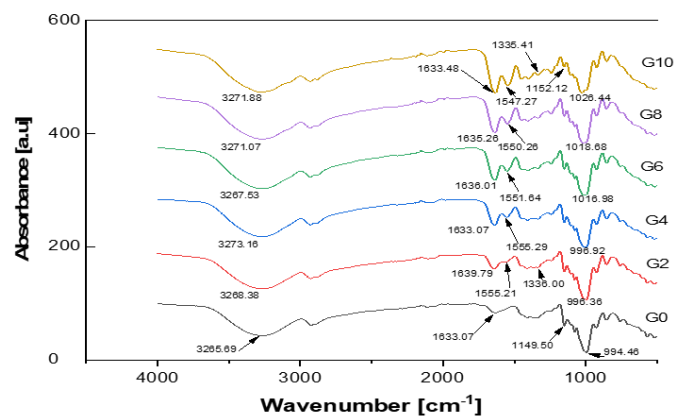


Figure 1. Fourier transform infrared spectroscopy (FTIR) analysis of intermolecular interactions of breadfruit flour edible films.

Amide-I represents C=O stretching vibration coupled with C-N stretch, and C-C-N bending. The Amide-I band is the most sensitive spectral region to the protein's secondary structure. Each peak was in the same wavelength position for both temperatures but with differences in their intensity (Nur Hanani *et al.*, 2011). The graph shows the peak increase for Amide-I in breadfruit flour and bovine gelatine blended film from  $1633.07 \text{ cm}^{-1}$  (G0) to  $1639.79 \text{ cm}^{-1}$  (G2),  $1638.61 \text{ cm}^{-1}$  (G4),  $1636.01 \text{ cm}^{-1}$  (G6),  $1635.26 \text{ cm}^{-1}$  (G8), and  $1633.48 \text{ cm}^{-1}$  (G10) as the increment of gelatine concentrations. Amide-II arises from the wagging vibration of N-H groups, stretching vibrations of C-N groups and also the wagging of C-O groups. From the result, the Amide-II band did not appear in the control breadfruit flour film (G0). However, it appeared in sample G2 as soon as gelatine was added to the film blends showing a wavelength of  $1555.21 \text{ cm}^{-1}$  (G2). Then, the peak was shifted to  $1553.29 \text{ cm}^{-1}$  (G4) to  $1551.64 \text{ cm}^{-1}$  (G6),  $1550.26 \text{ cm}^{-1}$  (G8),  $1547.27 \text{ cm}^{-1}$  (G10) with the increment of gelatine's concentrations into the film. The differences observed were due to the alteration of the secondary structure of gelatine polypeptide chains caused by the addition of gelatine. Amide-III represents in-plane wagging of C-C and N-H groups of bound amide and vibrations of stretching C-N and C-C groups also a vibration of wagging C-O groups. It usually appears in the range of  $1300 \text{ cm}^{-1}$  to  $1200 \text{ cm}^{-1}$ .

From the result, the amide-III band in bovine gelatine film had a prominent shift from  $1336\text{ cm}^{-1}$  (G2) to  $1335\text{ cm}^{-1}$  (G10) when gelatine concentration was increased to 50% (G10) in the breadfruit flour-based films. These results showed that the  $-\text{OH}$  group in breadfruit flour and amino groups in gelatine were ingested during the blending process. This finding was similar to other researchers (Yakimets *et al.*, 2005; Su *et al.*, 2010; Ahmad *et al.*, 2015) who reported similar results for bovine gelatine where Amide-I, Amide-II and Amide-III peaks were found at the wavenumber of  $1633$ ,  $1536$  and  $1240\text{ cm}^{-1}$ , respectively. The peak situated around  $1033\text{ cm}^{-1}$  was related to the interaction anything between the plasticizer (OH group of glycerol) and film structure (Bergo and Sobral, 2007). Generally, similar spectra of all samples were obtained in the range of  $1800\text{-}700\text{ cm}^{-1}$  covering Amide-I, Amide-II and Amide-III. The absorption bands in the IR spectra were assigned to the vibrations of the corresponding functional groups based on published data for gelatine. Staroszczyk *et al.* (2014) and Dong *et al.* (2006) reported that the spectrum of gelatine shows characteristic absorption bands at  $1653$ ,  $1541$ ,  $1238\text{ cm}^{-1}$  attributed, respectively, to  $\nu\text{C}=\text{O}$  and  $\nu\text{N}$  stretching vibration of groups in Amide I, to  $\delta\text{NH}$  and  $\nu\text{CN}$  vibrations of group I Amide II and to  $\nu\text{CN}$  and  $\delta\text{NH}$  in the Amide III band.

The addition of bovine gelatine proportion in the film caused conformational changes in breadfruit flour starch and its polysaccharide chains. The band at  $1148\text{ cm}^{-1}$  was previously speculated to be the polysaccharide (1  $\rightarrow$  4) glycosidic bond stretching vibration (C-O-C) (Wu *et al.*, 2013). The absorption peak at  $1103\text{ cm}^{-1}$  may maybe caused by the vibration of the C-O bond at the C4 position of a glucose residue (Jia *et al.*, 2020). The result from this figure indicated that the addition of gelatine concentrations resulted in an increment in its wavenumbers from  $1149.50\text{ cm}^{-1}$  (G0) to  $1149.84\text{ cm}^{-1}$  (G2),  $1150.20\text{ cm}^{-1}$  (G4),  $1150.48\text{ cm}^{-1}$  (G6),  $1150.84\text{ cm}^{-1}$  (G8), and  $1152.12\text{ cm}^{-1}$  (G10). This also affected the band range  $1103\text{ cm}^{-1}$ , which showing the increment trends of wavenumber from  $1103.38\text{ cm}^{-1}$  (G0),  $1103.46\text{ cm}^{-1}$  (G2),  $1103.62\text{ cm}^{-1}$  (G4),  $1104.22\text{ cm}^{-1}$  (G6)  $1104.74\text{ cm}^{-1}$  (G8), and  $1105.92\text{ cm}^{-1}$  (G10).

The IR spectra obtained from breadfruit flour showed  $994.46\text{ cm}^{-1}$  C-O, C-C stretching with some C-OH contributions from  $994.46$ ,  $996.36$ ,  $996.92$ ,  $1016.98$ ,  $1018.68$ , and  $1026.44\text{ cm}^{-1}$  COH bending and CH<sub>2</sub>-related modes, C-O-C symmetrical stretching and C-H deformation. A band near  $946\text{ cm}^{-1}$  is interpreted as a skeletal mode indicating the  $\alpha$ -1,4 linkage of glucose molecules in starch amylose (Bartošová *et al.*, 2018).

### 3.7 Scanning electron microscopy

The SEM micrographs of the surface and cross-section of the control film and BFG films (G10) are presented in Figure 2. These two samples were chosen to demonstrate the differences in surface and cross-section structure morphology between film without gelatine (G0) and with the highest amount of gelatine (G10).

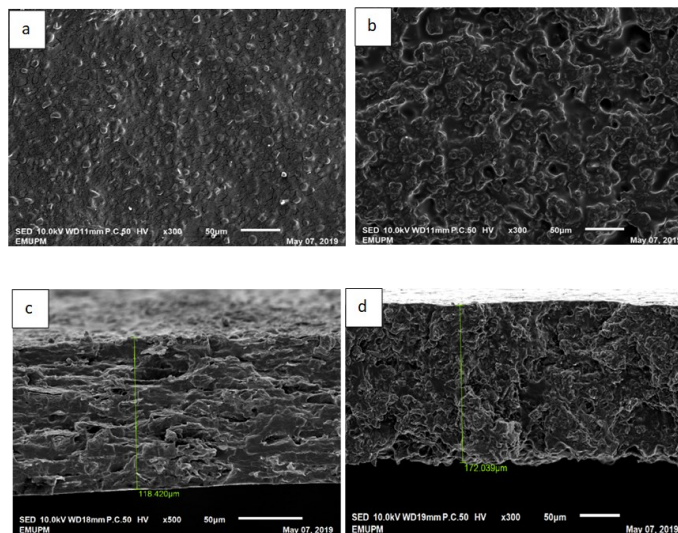


Figure 2. a) Surface morphology on breadfruit flour edible film without gelatine (G0), b) surface morphology on breadfruit flour edible film with an addition of gelatine (G10), c) cross-section on breadfruit flour edible film without gelatine (G0), d) cross-section on breadfruit flour with an addition of gelatine (G10).

The surface of the G0 film was rough, slightly irregular, protruded and bumpy surface Figure 2(a). After adding the bovine gelatine at a ratio 5:5, the film showed a smoother, even and homogenous surface Figure 2(b). This is composed of the intermolecular interaction in the film matrix that was contributed by gelatine, as supported by the FTIR spectra. The entanglement of breadfruit flour and bovine gelatine chains via covalent and non-covalent bonding promoted the smoothness of the surface of the film (Jeya Shakila *et al.*, 2012; Arfat *et al.*, 2014). No distinction separation was observed in the matrix of BFG films. For cross-section, films without gelatine addition (G0) (Figure 2c) and films with the highest gelatine G10 (Figure 2d) clearly showed differences in terms of film thickness as discussed previously. As can be seen, the control films exhibited an uneven surface, porous and rough network. However, a non-porous and smooth cross-section was noticeable in the G10 film, indicating the addition of bovine gelatine promoted the smoother cross-section in breadfruit flour films. This was related to the homogenous network and good compatibility between gelatine and breadfruit flour indicating that an ordered structure was formed without layering phenomenon, which was similarly observed by Ahmad *et al.* (2015)

using rice flour and gelatine. Tongdeesontorn *et al.* (2012) observed the smooth structure of composite films from cassava starch. However, Al-Hassan and Norziah (2012) observed that there were discontinuous zones and cracks randomly distributed along the network in the sago starch and fish gelatine films. Thus, the microstructures of films were governed by molecular organisation in the film network, which depended on the types of components, the interaction of components in the film matrix as well as the blend ratio of components used for film preparation.

#### 4. Conclusion

In summary, this study has demonstrated that the addition of gelatine (G) in breadfruit flour (BF) films improved the physical and mechanical properties. The thickness, tensile strength, solubility and water vapour permeability of the breadfruit flour films showed improvements with the addition of gelatine. Furthermore, the BFG films exhibited strong UV light absorbance and may, therefore, provide good photoprotection. The significant reinforcement of BF is contributed by the well-dispersed gelatine component within the BF matrix and the strong interfacial interactions between this flour and gelatine, as supported by the FTIR spectra and SEM images. Breadfruit flour with an addition of 50% gelatin makes films to have higher resistance to break and greater extensibility therefore the BFG films demonstrated a good potential characteristic as edible film for food packaging applications.

#### Conflict of interest

The authors declare no conflicts of interest.

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