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Optimisation of process parameters for natural coloured edible film production

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Abstract

Starch is a hydrocolloid group which can produce good and economical edible films. However, starch based edible films have some drawbacks derived from their great sensitivity to water and retrogradation phenomenon which affects the film's mechanical properties. Incorporation of gelatine into starch is expected to produce firmer films with greater resistance to rupture, enhanced extensibility and durability. Therefore, the purpose of this study was to optimise the amount of gelatine and glycerol in natural coloured edible film forming solution to improve its mechanical properties, thus making the edible films more stable toward the environmental stress and applicable for food packaging. The experimental design was performed using Response Surface Methodology (Box Behnken Design) with two variable factors namely gelatine and glycerol. Formulation with the optimal tensile strength of 4.87 MPa and Young's Modulus of 92.29 MPa were obtained with the additional of 40 g gelatine and 18 g glycerol into edible film formulation and the model verification was validated by the prediction accuracy of 97.13% and 99.19, respectively. This study has confirmed that the validated models are fit for the optimal formulation of natural coloured edible film for food packaging applications.

Keywords: *edible film, glycerol, gelatine, Young's modulus, tensile strength*

Introduction

In recent years, edible films and coatings from natural sources have received great interest due to their benefits. These films are totally made of renewable and edible elements, making them more easily degraded than polymers. Numerous studies on the development of edible films for packaging with polysaccharides have been conducted (Marvizadeh et al., 2017; Chatli & Town 2018; Thakur et al. 2018; Ulyarti et al., 2020; Susmitha et al., 2021). The starch isolated from a variety of botanical sources such as cereals (corn, rice, oat, etc.) and tubers (cassava, tapioca, yam, potato, etc) were known to have the capability to build a continuous matrix and available in abundant in nature at a low cost. Although starch is the most relevant polysaccharide polymer used for the development of biodegradable films, it has several disadvantages when compared to multiple synthetic polymers such as strong hydrophilic characteristics and low mechanical properties, which make it unsatisfactory for packaging purposes.

Studies on physical and chemical modifications have been carried out to improve the qualities of starch-based films (Afshar & Baniasadi, 2018; Soo & Sarbon, 2018). Among the strategy is to mix polysaccharides (chitosan, carrageenan and starch) with protein based sources such as gelatine and casein that improved polymers' structure and therefore form a continuous network with better edible film characteristics. Various research on edible and biodegradable films with better qualities have been conducted with the benefits of using two or more ingredients (Marvizadeh et al. 2017; Karim and Bhat, 2011; Silva-Weiss et al. 2013). Before casting and drying processes, plasticisers are generally added to film forming solutions as they effectively extend the film structure, improve flexibility and reduces cohesion within the film network by entering between polymeric molecular chains (Suderman et al. 2018).

Gelatine is classified as GRAS (Generally Recognize as Safe) material, and it is one of the most commonly used biopolymers approved by the Food and Drug Administration (FDA). On top of that, the European Commission, the United Nations Food and Agriculture Organization, the World Health Organisation (WHO) and China National Standards GB 6783-2013 generally recognise gelatine safe for human consumption. Moreover, these regulations strictly control the safety of raw materials and production of gelatine, amend the

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requirements for gelatine with the society development and increase the awareness of health concerns. In edible film production, addition of gelatine give rise to firmer films with greater resistance to rupture, enhanced extensibility and durability. Gelatine is a typical by-product from fish and animal processing (Ahammed et al. 2020), which is commonly used in the food industry (Nazmi et al. 2017; Suderman et al. 2018; Nilsuwan et al. 2019). It is frequently used as biomaterials for food packaging development due to its high contents of proline, glycine and hydroxyproline, which endow the gelatine with excellent film forming ability and help in the formation of a flexible film (Staroszczyk et al. 2014; Nor et al. 2017; Soo & Sarbon, 2018; Wang et al. 2021). Other characteristics including edibility, biodegradability, abundancy, good barrier capacity and transparency, further confirm gelatine as a suitable biopolymer for the production of biodegradable packaging films (Gómez-Estaca et al. 2011) compound gelatine-chitosan films were prepared. The effect of the gelatine origin (commercial bovine hide gelatine and laboratory made tuna skin gelatine; (Haghighi et al. 2019; Rawdkuen et al. 2020). In order to increase the strength and flexibility of edible films, various plasticisers, usually polyols have been widely used. Glycerol is one of the most preferred polyols in edible film studies (Díaz et al. 2011; Jouki et al. 2013; Walfathiyyah et al. 2016). Glycerol is a hydrophilic plasticiser, generally used to improve the mechanical properties of edible films. When glycerol added at the correct level with respect to the biopolymer content, it assists to reduce intermolecular forces and increase the mobility of polymer chains (Hazrati et al. 2021).

In this study, the development of natural coloured edible film from rice flour with the addition of glycerol, halal gelatine (bovine) and natural dyes from *Bunga Telang (Clitoria ternatea)* has been carried out. The film produced was blue coloured, stretchable, more resistance toward shear and tear, also has basic stable properties as a film for food packaging materials. This study was designed to optimise two variables gelatine and glycerol to the natural colour edible film forming solution in order to enhance mechanical characteristics, to produce more environmentally stable edible films and appropriate for food packaging application.

Materials and method

Materials

Rice flour (brand Erawan) was purchased from local distributor PJ Grocer (M) Sdn Bhd, (Petaling Jaya, Selangor). Bovine gelatine powder was purchased from Halalgel Manufacturing (M) Sdn. Bhd. (Wangsa Maju, Kuala Lumpur) with bloom strength between 151–160 g and dried *Bunga Telang* were obtained from Bee Nursery (M) Sdn. Bhd. (Shah Alam, Selangor). Glycerol was obtained from Sigma-Aldrich (Steinheim, Germany).

Film preparation

The natural colour was extracted by mixing 6 g of dried *Bunga Telang* (*Clitoria ternatea*) in 800 mL of distilled water while stirring on a heating stove at 80 °C. The solution was then filtered through a muslin cloth. Gelatine (0 – 40 g) was weighed and added into the extracted coloured solution at 45 °C until the gelatine was fully dissolved. Rice flour (40 g) was added into the gelatine solution, followed by addition of 12 – 24 g glycerol as a plasticiser. The mixture was stirred for 30 mins at 85 °C until the starch solution fully gelatinised. A total of 250 mL film forming solution was uniformly poured on a Teflon coated mould and then dried in a cabinet drier (Floor Model Laboratory Dryers FDD-720) at 55 °C for 12 H. The films were then peeled off and were conditioned for 48 H in a humidity test chamber with relative humidity set at 50% and temperature of 25 °C.

Film thickness

Thickness of the films was measured by digital micro meter (Mitutoyo 293 251, Tokyo, Japan) with an accuracy and precision of ± 0.001. Measurements were taken randomly from 15 different points for individual film samples and their average value was calculated.

Moisture and solubility

Film moisture and solubility was determined according to Tongdeesoontorn et al. (2011) with slight modification. Three pieces of each film (2.0 cm × 2.0 cm) were weighed (±0.0001 g) and then dried in an air circulating oven at 70 °C for 24 H to obtain the initial dry mass content. For determination of their initial dry weight (W_i), the films were re-weighed. Film discs were immersed and soaking with 20 mL of distilled water at room temperature for 24 H. The remaining undissolved films were removed and dried at 105 °C for 24 H and their weight were recorded (W_f). The percentage of film solubility (% FS) was then calculated using the following equation:

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

where:
W_i= initial dry film weight and W_f = final dry film weight.

Tensile properties of films

Tensile strength (TS), Young's modulus (YM) and elongation at break (EAB) of films were measured according to ASTM-D638-77 method. It is a specific method for testing the tensile strength of plastics and other resin materials as well as calculating their mechanical properties, and outlines accuracy requirements for the test frames and accessories used. This method was performed using INSTRON (model 4302 Machine Series iX, England) with 100N load cell, a velocity of 25 mm/min,

with the distance between clamp of 60 mm. Test samples were cut into dumb bell shaped with 50 mm long and 30 mm width, flaring to 20 x 10 mm^2 grip areas on both ends. The data for tensile strength (TS), elongation at break (EAB) and Young's modulus (YM) was auto calculated by the INSTRON software.

Scanning electron microscopy

The films external surfaces and cross sections were observed with a JSM-5610LV Scanning Electron Microscopy (SEM) (JEOL, Tokyo, Japan), following the description by Maran et al. (2013). Prior to the observation, the surfaces were sputter-coated with a gold layer to avoid charging under the electron beam. The films were cut into specific size and fractured on the double sided adhesive tape immediately. The fracture surfaces were sputtered with gold and photographed. The photographs were taken at an acceleration of 800 micron.

Experimental design and optimisation

The experimental design using Response Surface Methodology (RSM) Box Behnken Design was performed a commercial statistical package, Design Software Expert® version 6.0.10 Stat-Ease, Incorporation, Minneapolis, Minnesota with two variable factors namely gelatine and glycerol. A total of 13 film formulations have been created with five centre points, two axial points and six replications. The full quadratic effect of gelatine and glycerol were significant at (p <0.05) on the responses, namely solubility (SOL), thickness (T), tensile strength (TS), elongation at break (EAB), young modulus (YM), and colour (a*) value. The independent variables were gelatine and glycerol concentration (*Table 1*). For the adjustment of the responses as function of independent variables, following second order polynomial equation (1) was used:

$$Y = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 12X1\,X2 + \beta 11X12 + \beta 22X22 + \varepsilon \quad (1)$$

where Y = response variable namely tensile strength (MPa); and young's modulus (%); independent coded variables include concentrations of X1 gelatine (%), X2 = glycerol (%), X12 = gelatine * glycerol, X22 = glycerol* glycerol. The test of statistical significance was performed on the total error criteria, with a confidence level of 95%. Analysis of variance (ANOVA) was used to determine the significant effects of variables on the responses.

Table 1. Coded and uncoded values of variables and their levels

Independent variables	Coded levels	–1	0	1
Gelatine (g)	A	0.0	20.0	40.0
Glycerol (g)	B	12	18.0	24.0

Statistical analysis

Design Software Expert® version 6.0.10 Stat-Ease, Incorporation, Minneapolis, Minnesota, USA) was used to design the experimental plan for RSM. This software also used for the regression analysis of the data obtained, estimation of the coefficients of the regression equation and to perform the analysis of variance (ANOVA).

Results and discussion

Model fitting

In RSM, the relationship between the variables, which were concentration of gelatine (A) and glycerol (B) were expressed mathematically using polynomial model. The independent and dependent variables were fitted to the second-order polynomial model equation except for L value (fitted for mean model) and examined for their goodness of fit. The ANOVA was performed to test the adequacy of the model. In order to obtain the fitted models to predict the responses, the parameters must be significant at the probability level, p value of less than 0.05 (p <0.05). In this study, the responses, namely solubility, moisture, tensile strength (TS), Young's modulus (YM) and elongation at break were significant at p <0.05. However, thickness and elongation at break (EAB) value obtained insignificant values (p >0.05) for the model response. Thus, this response was not adequate and did not apply for the optimisation process as summarised in *Table 2*.

The final equations developed in terms of coded factors are given below:

$$Y1 = 40.46 – 0.5063A - 3.743B + 0.00639A2 + 0.087B2 + 0.0188AB \quad (2)$$

$$Y2 = 2887 + 0.05A – 278.1B + 0.1066A2 + 6.644B2 – 0.152AB \quad (3)$$

where A is starch gelatine and B is glycerol concentration.

The quadratic models in term of coded variables are shown in Equation (2) and (3) where (Y1) represents tensile strength and (Y2) represents Young's modulus as a function of concentration of gelatine and glycerol.

Thickness of the films

Thickness of starch based edible films is an integral property which affects its possible applications. Some food products may require thick films to enhance their shelf life and moisture resistance. Thickness of the film will also affect its solubility in water due to the bonding of the hydrogen. The increasing hydrogen bond causes the structure of the mutual starch molecules to bind and form a compact network, thus reducing the solubility of the film (Arifin et al. 2020). The thickness values of starch based natural coloured edible films varied from 0.215 to 0.326 mm (*Table 3*). Films with higher concentrations

Table 2. Analysis of variance for the response variables

Source	df	Tensile strength			Young modulus			Elongation at break			Thickness		
		Sum of squares	p-value	F-value	Sum of squares	p-value	F-value	Sum of squares	p-value	F-value	Sum of squares	p-value	F-value
Model	5	122.69	0.000	25.94	604657	0.000	173.24	1979.62	0.008	8.04	0.017	0.034	4.70
A	1	18.52	0.003	19.85	6009	0.022	8.61	1107.64	0.002	22.51	0.009	0.009	12.88
B	1	11.05	0.011	11.68	381558	0.000	546.6	84.23	0.232	1.71	0.003	0.069	4.60
AB	1	20.38	0.002	21.54	1329	0.021	1.90	479.70	0.017	0.86	0.000	0.577	0.577
A^2	1	18.04	0.003	19.07	5024	0.031	7.20	42.39	0.384	3.22	0.003	0.087	0.09
B^2	1	27.25	0.001	28.80	157961	0.000	226.29	158.49	0.116	9.75	0.003	0.09	
Error	7	6.62			4886			344.50			0.005		
Lack of fit	3	5.15			1735			334.03			0.005		
Pure error	4	1.48			3151			10.47			0.000		
Total	12	129.32			609544			2324.12			0.022		
R-Squared		0.9488			0.9920			0.8515			0.770		

of gelatine and glycerol had higher thickness due to the increased of compressive strength of edible films (Ulyarti et al. 2020). Similar results regarding increase in film thickness with an increment of gelatine concentrations have been reported earlier by Sarbon et al. (2013) and Acosta et al. (2015). According to Ahmad et al. (2015), thicker films caused the polymer matric became denser, displayed higher in inter and intra molecular interactions and consequently resulting more resistant to rupture.

Tensile strength of films

The tensile properties of starch based edible films are crucial when they are determined to bear external force and preserve their integrity without breaking. This property will affect the application of the product packaged in the films as well as its subsequent handling and transport (Shaikh et al. 2019). Sufficient mechanical strength and flexibility are necessary for packaging film to endure external stress, as well as to maintain its integrity and barrier properties during packaging (Rao et al. 2010). The tensile strength for common commercial polymer (polypropylene) was 3,200 to 5,000 Psi (equal to 22.06 to 34.47 MPa) (Hisham 2016). When high amount of gelatine (40 g) mixed with either high (24 g) or low (12 g) amount of glycerol, it was found that the tensile strength of film was quite good, which were 9.879 and 7.517 MPa, respectively. However, when low gelatine (20 g) mixed with moderate amount of glycerol (18 g), it creates the lowest tensile strength of 0.366 MPa (*Table 3*). *Figure 1(a)* presents the effect of varying gelatine and glycerol concentrations on tensile strength of films. At the optimal of gelatine (40 g) and glycerol (18.64 g) concentration, the tensile strength of 4.87 MPa could be obtained. The tensile strength has been affected by both gelatine and glycerol levels. The concentration of gelatine in film forming solutions affected the self-adhesion of starch polymers and the rate of matrix formation during film preparations. The increasing of both gelatine and glycerol concentrations have increased interactions between these polymers. The addition of glycerol will increase the thickness of edible film as the glycerol molecules has occupied the voids in the film matrix and interact with edible film forming polymer, resulted in increasing the distance between the polymer (Sudaryati et al. 2010). According to Vieira et al. (2011) and Ahmadi et al. (2012) high concentration of glycerol will increase the ability to absorb moisture and cause the swelling process, thus increasing the film thickness. Bourtoom (2008) has found that the plasticiser added to the edible film can bind with starch to form starch plasticiser polymer, whereby the starch-starch bond is replaced by the starch-glycerol-starch bond, subsequently led to improvement of the film thickness. Sun (2013) study on composite films from pea starch/peanut proteins also reported an increment of tensile strength with an increasing in the gelatine concentration. The results are in agreement with those of Acosta et al. (2015) barrier and mechanical's finding. The addition of gelatine gave rise to harder films with a higher resistance to break, also showed

Table 3. Box Behnken quadratic design and observed values of response variables

Run	Gelatine (g)	Glycerol (g)	Thickness (mm)	Moisture (%)	Solubility (%)	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)
1	40	12	0.246	34.647	58.113	7.518	621.33	14.093
2	20	18	0.265	17.734	60.654	0.532	9.46	33.723
3	40	24	0.326	21.588	57.353	9.897	58.8	51.928
4	0	24	0.264	17.059	58.974	1.068	21.72	9.124
5	20	18	0.262	17.959	62.526	0.768	13.08	35.280
6	20	18	0.264	21.873	61.143	0.447	7.56	35.746
7	0	12	0.215	17.987	70.784	7.718	511.33	15.093
8	40	18	0.387	14.81	64.372	3.438	87.0	49.039
9	20	18	0.265	21.873	62.398	0.366	6.27	31.655
10	20	24	0.258	12.319	59.753	1.131	31.64	20.830
11	0	18	0.243	11.268	63.409	1.525	44.2	9.321
12	20	18	0.264	20.069	51.617	1.845	71.59	33.541
13	20	12	0.244	18.116	67.407	5.003	492.56	30.215

greater extensibility and stretchability enhancement. The addition of plasticiser (glycerol) in the films assisted the film to be more flexible and reduce the possibility of the films to break. Plasticisers with low volatility are added to polymeric materials to modify the functional properties of the films, increasing their extensibility, flexibility, elasticity, rigidity and mechanical properties by positioning themselves between the polymer chains and disrupting the hydrogen bonding (Nur Hanani et al. 2011). The increase of flexibility in the films plasticised with glycerol were affected by the looser structure of the network which was caused by the reduction of protein-protein interactions due to the presence of glycerol, subsequently led to a greater chain mobility (Mourad et al. 2013). Marcilla (2009) suggested that the addition of glycerol have good interaction with the polymer due to its structure and hydrophilic character. Therefore, it increased the tensile strength of the films

Young's modulus

The Young's modulus of natural coloured edible films ranged between 6.27 to 621.33 MPa (*Table 3*). Young's modulus determined the flexibility of edible film. The higher the values, the more flexible the film is. It was found the highest Young's modulus of 621.33 MPa achieved when high amount of gelatine (40 g) mixed with low amount of glycerol (12 g). However, when the amount of glycerol increased to 24 g, the Young's modulus decreased drastically to 58.8 MPa. On the other hand, when low amount of gelatine (20 g) mixed with 18 g of glycerol, the film produced showed the lowest of Young's modulus, which was 6.27 MPa (*Table 3*). This phenomenon indicated the optimal ratio of glycerol and gelatine plays a critical role to strengthen the flexibility of film produced. *Figure 1*(*b*) presents the effect of varying gelatine and glycerol concentration on Young's modulus and concluded that film plasticised with glycerol has good mechanical strength. It was noted that as gelatine concentration increased, the Young's modulus of film also increased. Su et al (2010) suggested that glycerol with a small molecule plasticiser penetrates between polymer chains, weakening the interaction between polyer materials in polysaccharides and proteins film, thus increasing its flexibility and extensibility YM; tensile strength, TS and % elongation EAB. Sothornvit and Krochta (2001) demonstrated that hygroscopic nature of glycerol contributes more plasticisation effect when compared to any plasticiser, thus increasing the mobility of polymer chains and leading to increased stretchability and flexibility of films.

Model optimisation and validation of processing parameters on natural coloured edible films making

The desirability function was used for simultaneous optimisation of the two responses. The aim was to maximise tensile strength and Young's modulus while minimising thickness. These responses were considered to study the possibility of choosing one formulation to optimise the properties of natural colour edible films. The possible optimal solutions for the formulation and processing parameters for starch gelatine natural coloured edible film are shown in *Table 4*. Based on the optimisation modelling, at the gelatine concentration of 40 g and glycerol of 18 g with the targeted tensile strength and Young's modulus at 5 and 100 MPa, respectively, the predicted value generated from RSM system for tensile strength and Young's modulus value was 4.87 MPa and 92.29 MPa respectively. The experiment value of tensile strength and Young's modulus at 5.01 and 93.04 MPa (n = 3) was obtained by conducting the experiment in triplicate using the optimal processing parameters (*Table 4*). The data obtained verified the tensile strength and Young's modulus with high percentage of prediction accuracy of 97.13% and 99.19% respectively, which validate the optimisation modelling in the edible film formulation. The optimal points were confirmed by the good correlation between the actual and predicted values as reported earlier by (Koh et al. 2010) on the optimisation

and model verification on the synthesis of medium- and long chain triacylglycerols.

Morphological properties

The scanning electron micrographs of films without gelatine have rough, coarser and cracked irregularly surfaces (*Figure 2a*) while films with gelatine have smooth and homogeneous structure 'cement like' surfaces (*Figure 2b*). The addition of gelatine into starch based edible films has improved the surface texture and flexibility of the film as depicted in *Figure 2b*.

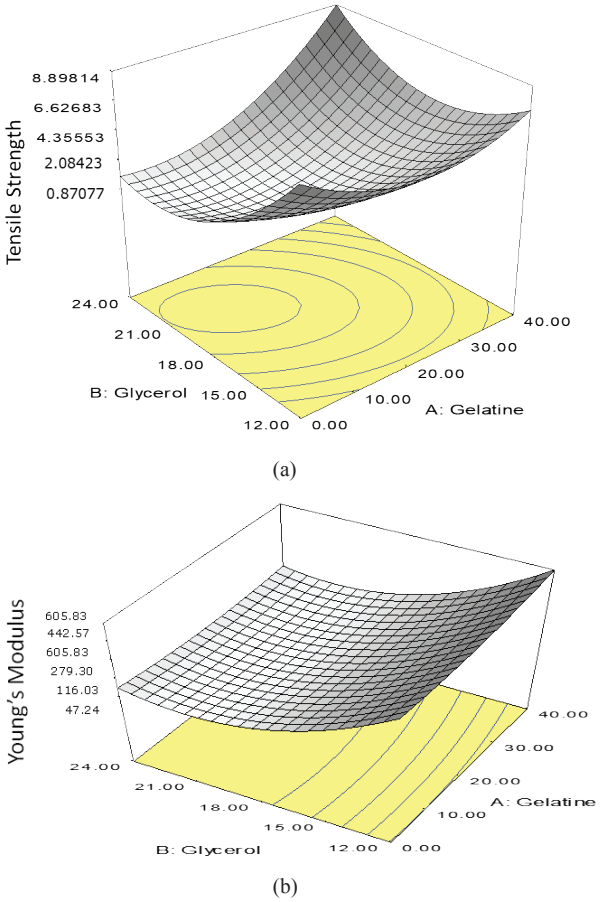

Figure 1. Response surface graphs showing the effect of gelatine and glycerol concentration on (a) tensile strength; (b) Young's modulus of the natural-coloured edible film

For cross section, it showed that films without gelatine have a wider porosity size (*Figure 2c*). A film with additional of gelatine showed more comparatively homogeneous and compact matrix without disruption and pore formation (*Figure 2d*), which confirmed the integrity of the structure with strong strength (Ahmad et al. 2015). This phenomenon might be caused by the continuous phase formation of polymeric matrix and strong molecular interactions among starch and gelatine. Compact structure of natural coloured edible film with more structural solidity might be responsible for its good mechanical properties like tensile strength and elongation at break value, make the films more resistance toward rupture, improve thickness and water vapour permeability values. Therefore, films are more stable at ambient temperature.

Conclusion

Natural coloured edible films were prepared, optimised and the essential characteristics for their potential usage were analysed. The results indicated that the addition of gelatine and glycerol at the optimum concentration resulted in an increment of the tensile strength and Young's modulus of the natural colour edible film. The optimisation of RSM modelling showed the quadratic model was fitted well and the optimal values of tensile strength and Young's modulus were validated within the specified range of independent parameters with the prediction accuracy of 97.13% and 99.19%, respectively. The micrographs of natural coloured edible film without gelatine revealed its rough, coarser and slightly irregular surface. Starch and gelatine blends exhibited good film forming properties, indicating these films can be utilised for packaging or coating applications in food industries.

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Table 4. Optimisation and model verification of mechanical properties on natural coloured edible films

Optimisation model	Mechanical properties (MPa)		Accuracy prediction (%)
Gelatine (g): 40 Glycerol (g): 18.64	Predicted value	Experimental value	
Tensile strength (MPa): 5	4.87	5.01 ± 0.17 (n = 3)	97.13
Young modulus (MPa): 100	92.29	93.04 ± 0.88 (n = 3)	99.19

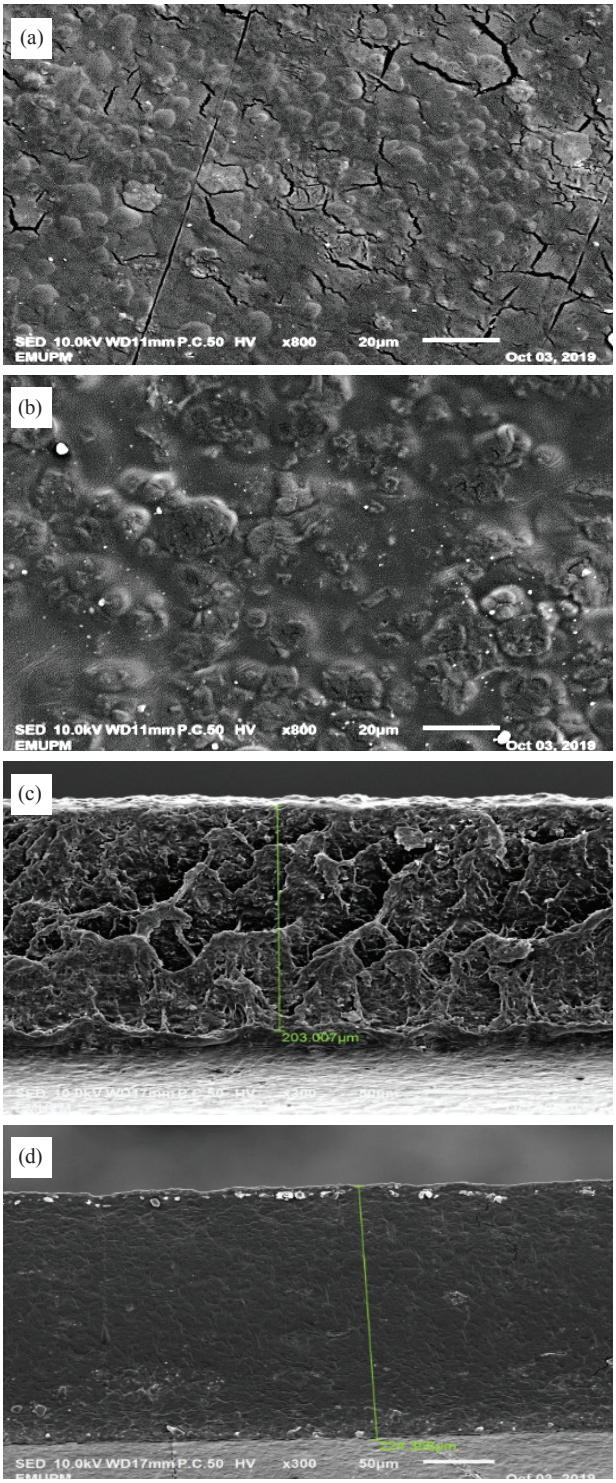

Figure 2. (a) Surface of natural coloured edible film without gelatine; (b) Surface of natural coloured edible film with gelatine; (c) Cross-section of natural-coloured edible film without gelatine; (d) Cross-section of natural-coloured edible film with gelatine

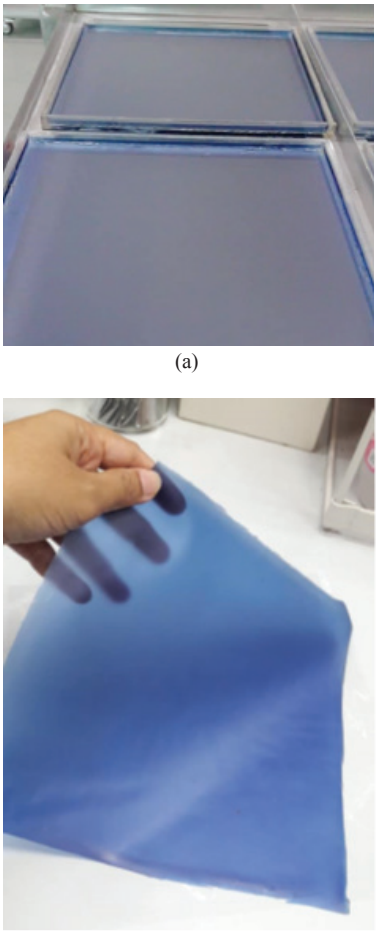

Figure 3. (a) Smooth and homogenous structure of natural-coloured edible film; (b) Improved surface texture and flexibility of the natural-coloured edible films

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