

Chicken gelatin films: rheological properties of film forming solutions and film characterisation as influenced by starch incorporation

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Abstract

The aim of the present work was to develop chicken skin gelatin films incorporated with different concentrations of rice starch prepared by casting techniques. Six film-forming solutions were prepared separately with different blend ratios of chicken skin gelatin to rice starch: A (0/100), B (5/100), C (10/100), D (15/100), E (20/100), and F (25/100). The rheological properties of the film-forming solutions (FFS) were determined using frequency sweep. The mechanical and physical properties of the respective films were also evaluated. With the increase in rice starch concentration, the storage (G') modulus of FFS increased dramatically with loss (G'') modulus as the oscillatory frequency rising to contribute to gel behaviour ($G' > G''$). As rice starch concentration increased, the chicken skin gelatin films also demonstrated higher tensile strength, elongation at break, and water vapour permeability, but reduced the solubility of gelatin films in water. Additionally, elevation in melting point values indicated that the thermal stability of the composite films was enhanced with the increment of rice starch concentration. Film F (with 25% rice starch) yielded the optimal film formulation, as it had the highest tensile strength and a high elongation at break value. Thus, film F shows the best potential as a film for food packaging.

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Keywords

chicken skin gelatin,
film forming solution,
rice starch,
rheological properties

Introduction

Food packaging is an essential element of the food manufacturing process and food industry. The food packaging market has introduced recycled materials such as aluminium, glass, and paper. Nevertheless, these materials have been progressively substituted over the last half century by plastic, which are synthetic polymers from petroleum resources, due to its availability in large quantities at low cost and favourable functionality characteristics (Lopez-Gil *et al.*, 2014). However, plastic is non-biodegradable and difficult to recycle, leading to its accumulation in land and water (Al-Hassan and Norziah, 2012). Therefore, biodegradable packaging in films and coatings has become the focus of worldwide attention in recent years due to a looming loss of petroleum resources and for environmental reasons.

Biodegradable films are thin, transparent, and flexible layers prepared from natural biopolymers such as proteins, polysaccharides, lipids, or a combination of these components (Bergo and Sobral, 2007). Gelatin is the most preferable protein derivative as the base material for formulating biodegradable films due to its natural abundance, renewability, low cost, film-forming ability, and excellent functional properties (Arvanitoyanni, 2002). A study by Sarbon

et al. (2013) found that chicken skin, which serves as an alternative to mammalian gelatin, shows similar chemical composition to bovine gelatin, and exhibits better physicochemical properties. Unfortunately, gelatin-based films suffer from major limitations due to their high moisture sensitivity, high brittleness, and poor thermal stability (Nur Hazirah *et al.*, 2016). Therefore, current researchers are highly interested in the overall effects of adding other biopolymers, cross linking agents, plasticisers, and strengthening agents with antimicrobial or antioxidant properties on the functional properties of gelatin-based film (Bakry *et al.*, 2017).

The use of starch in biodegradable films is based on its linear polysaccharide amylose and highly branched polysaccharide amylopectin (Woggum *et al.*, 2014). Rice (*Oryza sativa* L.) starch is popular as an attractive material for biodegradable films due to its high amylose content (Wittaya, 2012). However, applications of starch film alone are limited by its mechanical properties and lack of an efficient barrier against low polarity compounds (Bourtoom and Chinnan, 2008). Thus, a study on the blending of starch and gelatin at different ratios to decrease the solubility and water vapour permeability of films, and increase their tensile strength has been conducted (Soo and Sarbon, 2018).

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Rheological analysis of film-forming solutions is necessary for optimising processing design, since the rheological properties of biopolymer solutions can influence the spreadability, thickness, and uniformity of liquid coating layer and film performance (Silva-Weiss *et al.*, 2013). The rheological properties of film-forming solutions can be tested via both stationary shear flow and dynamic oscillatory tests to understand the structure-property relations between biopolymers (Alias and Sarbon, 2019). The mechanical and physical properties of protein films can be modified with the addition of polysaccharides, causing a decrease of elongation at break but increasing the tensile strength and elastic modulus.

Thus, the objectives of the present work were (a) to formulate biodegradable chicken skin gelatin films incorporated with rice starch, and (b) to characterise the rheological properties of film-forming solutions, and their physical and mechanical properties.

Materials and methods

Materials

Fresh chicken skins were obtained from Kuala Nerus, Terengganu, Malaysia. Chicken skins were transported to the laboratory in chilled conditions. Upon arrival, visible fat and flesh were mechanically removed using a knife. The skins were thoroughly washed and weighed for wet weight, before stored in a freezer at -18°C until further use. Native rice starch was purchased from Sigma-Aldrich (M) Sdn. Bhd. All chemicals used for gelatin extraction were of analytical grade.

Methods

Chicken skin preparation

The chicken skins were prepared following the methods of Rasli and Sarbon (2015). Frozen chicken skins were thawed in a chiller ($4 - 5^{\circ}\text{C}$) overnight. The skins were then cut into pieces (2 - 3 cm) after thoroughly rinsed in excess water to remove impurities, and then dried in a cabinet drier (FSD-380, Protech, Malaysia) at 45°C overnight. The dried chicken skins were ground with a blender (Panasonic, Malaysia) before being defatted using the Soxhlet method (Soxtec® Avanti System 2055, Foss, Sweden) (AOAC, 2006).

Gelatin extraction

Chicken skins gelatin were extracted following the methods of Sarbon *et al.* (2013). The defatted dried chicken skins were pre-treated

sequentially in 0.15% (w/v) sodium hydroxide (NaOH), 0.15% (v/v) sulphuric acid (H_2SO_4), and 0.7% (w/v) citric acid, with a skin to acid/alkali ratio of 1:14. The mixture was mixed well and gently stirred using a magnetic stirrer for 30 min at room temperature, before centrifuging (CR 22N, Hitachi, Japan) at 3500 g for 10 min. This step was repeated three times for each pre-treatment. The alkaline solution was changed each time to remove non-collagenous proteins and pigments, while the acid solution was changed every 30 min to denature the collagen in the chicken skin matrix. After each pre-treatment, the pellets were thoroughly rinsed with distilled water to remove any residual salts, and centrifuged at 3500 g for 10 min. The final extraction was carried out with distilled water using a water bath shaker (WNE14, Memmert, Germany) at a controlled temperature (45°C) overnight. The extract was filtered in a Büchner funnel with Whatman No.4 filter paper. The filtered gelatin solution was evaporated under vacuum to reduce its volume to 1/10 by using a rotary evaporator (Rotavapor R25, Buchi, Switzerland) at 45°C . The concentrated gelatin solution was kept in the freezer overnight prior to freeze-drying. The dry matter was ground with a blender (7011HS, Waring, Japan) to obtain the gelatin powder.

Preparation of film forming solution

Film-forming solutions (FFS) were prepared based on the method described by Soo and Sarbon (2018) with slight modification. Six formulations of film were developed with different percentages of rice starch, based on total weight basis of chicken skin gelatin (4 g) in 100 mL distilled water: A (0/100), B (5/100), C (10/100), D (15/100), E (20/100), and F (25/100). A total of 30% (w/w) glycerol was added as plasticiser for all formulations.

Rice starch and chicken skin gelatin solutions were prepared separately. Rice starch was dissolved in distilled water and heated in a water bath shaker at 90°C for 30 min until completely gelatinised. The chicken skin gelatin powder was dissolved in distilled water at 45°C for 30 min using a hot plate magnetic stirrer until a clear solution was obtained. Chicken skin gelatin solution was added to gelatinised rice starch at 45°C , and continued stirring for 30 min, followed by the addition of glycerol with constant stirring for another 30 min. The FFS were then cooled to room temperature. Control films were prepared in the same way but without the addition of rice starch. Each film formulation was prepared in triplicate.

Preparation of chicken skin gelatin-rice starch composite film

Composite film samples were prepared using the casting technique described by Jahit *et al.* (2016). To obtain uniform thickness, approximately 25 g of each FFS was casted onto flat Petri dish with an internal diameter of approximately 8.5 cm. The film-forming solutions were oven-dried (UNB500, Memmert, Germany) at 45°C for 48 h. Then, all dried composite films were detached from the Petri dish. Prior to film property testing, all films were conditioned in a desiccator containing silica gel (0% RH) for at least 24 h, then stored in airtight plastic containers until subjected to further analyses (Nazmi *et al.*, 2017; Nor *et al.*, 2017).

Rheological properties of film-forming solutions

Frequency sweep

Dynamic oscillatory measurement of FFS was conducted following the procedures described by Alias and Sarbon (2019). The dynamic viscoelastic behaviour of FFS was determined by using a rheometer (AR2000 Advanced Rheometer; TA Instruments, New Castle, DE, USA) equipped with a cone and plate geometry (cone angle 4°, 60 mm diameter). Temperature was controlled using a Peltier system.

To begin, FFS (1 mL) of each formulation was carefully poured on the surface of the lower plate. Each solution was held for 5 min at room temperature to allow stress relaxation and temperature equilibration. Then, the linear viscoelastic region (LVR) was determined by performing small amplitude oscillatory strain (SAOS) sweep tests on all film-forming solutions, with strain values between 0.01 to 20.00% and 1 Hz. The critical strain was defined as the limit strain of the linear viscoelastic region (LVR) that marks the end of the linear stress-strain relation. From the results of these tests, a strain amplitude of 1% within the linear viscoelastic domain in FFS was chosen to perform the dynamic oscillatory tests. A dynamic frequency sweep was conducted over a range of 0.01 to 50 rad/s at 25°C within the identified LVR for each FFS. The results were analysed using the software Rheology Advantage Data Analysis V.5.3.1 (TA Instruments). Viscoelastic parameters including storage or elastic modulus (G') and the loss or viscous modulus (G'') were then determined.

Mechanical properties of composite films

Tensile strength determination

The tensile strength (TS) of the films was determined using a texture analyser (TA-XT2i, Stable Micro System, Surrey, UK) based on the ASTM D882 method modified by Nur Hazirah *et al.* (2016). Film specimens were cut into rectangular

strips of 10 mm wide and 70 mm long. The film thickness was measured at five different positions using a micrometre (Coolant Proof 293-331-30, Mitutoyo, Japan), and averaged in order to determine the elastic behaviour of films as a function of the thickness. Film strip was placed onto texture analyser attached with grip pairs of AT/G probe and 5 kg load cell. Initial grip length between the top and bottom parts of the grip was set to 50 mm. The films strips were stretched by moving the upper grip at crosshead speed of 120 mm/min until broken. All measurements were performed at room temperature and 50% relative humidity. For each formulation, triplicate measurements were conducted to obtain the mean. The TS of each film was calculated by dividing the maximum load for breaking the film by its cross-sectional area (m^2) using Eq. 1:

$$TS = \frac{F_{\max}}{(b \times d)} \quad (\text{Eq. 1})$$

where, TS = tensile strength of film (MPa), F_{\max} = maximum load needed to pull the film sample apart (N), b = width of film (mm), and d = average thickness of film (mm).

Elongation at break determination

Elongation at break (EAB) of films was simultaneously determined with TS. Elongation at break was expressed as a percentage of the change in initial gauge length of a sample at the point of sample failure by the following equation (Nur Hazirah *et al.*, 2016) using Eq. 2:

$$EAB = \frac{l_{\max}}{l_0} \times 100 \% \quad (\text{Eq. 2})$$

where, EAB = elongation at break of film (%), l_{\max} = film elongation at the moment of rupture (mm), and l_0 = initial grip length of film (mm).

Physical properties of composite films

Water vapour permeability determination

The water vapour permeability (WVP) of the films was measured using the gravimetric modified cup method based on ASTM E96 standard method as described by Nazmi *et al.* (2017). Film pieces of 30 × 30 mm were individually sealed in each glass permeation bottle containing 10 g of silica gel (the desiccant) to produce 0% relative humidity below the film. The films were held in place with silicone vacuum grease. The surface area of the film covering the bottle end was calculated, and the initial weight of test bottle together with film was measured to the

nearest 0.0001 g using an analytical balance (AX224 M-Pact, Sartorius, Germany). Next, all covered bottles were placed in a desiccator containing distilled water ($50 \pm 2\%$ RH) at room temperature. The weights of each test bottle with film (± 0.0001 g) were measured hourly for 7 h. To measure the WVP as a function of the thickness, the thickness average of each film strip at five different positions was measured using a micrometre (Coolant Proof 293-331-30, Mitutoyo, Japan). Measurements were performed in triplicate for each film formulation to obtain the average mean. The WVP of the film was calculated using Eq. 3:

$$\text{WVP} = \frac{w \times x}{A \times t \times (P_2 - P_1)} \quad (\text{Eq. 3})$$

where, WVP = water vapour permeability of film ($\text{g mm/m}^2 \text{ h Pa}$), w = weight gained by the cup (g), x = average thickness of film (mm), A = permeation area of film (m^2), t = time gained (h), and $P_2 - P_1$ = difference in partial pressure of atmosphere with silica desiccant and pure water.

Film solubility determination

Film solubility was determined following the method modified from Nor *et al.* (2017). Film pieces of 20×20 mm were dried at 70°C in an oven for 24 h, and then weighed to the nearest 0.0001 g for its initial dry weight by using an analytical balance (AX224 M-Pact, Sartorius, Germany). Dried films were separately immersed in 20 mL of distilled water in plastic containers. The containers were capped and shaken by an orbital shaker for 24 h at room temperature. Then, both the solution and insoluble film pieces were poured onto No.1 Whatman filter paper previously dried at 105°C for 24 h, and weighed before using. After that, filter papers containing insoluble film pieces were oven-dried (UNB500, Memmert, Germany) at 105°C for 24 h to determine the final dry weight of film (± 0.0001 g). The experiment was done in triplicate for each film formulation. The film solubility in water was calculated using Eq. 4:

$$\text{Film solubility (\%)} = \frac{\text{Initial dry film weight} - \text{Final dry film weight}}{\text{Initial dry film weight}} \times 100\% \quad (\text{Eq. 4})$$

Thermal properties determination

The thermal analysis of the films was conducted following the method described by Nazmi *et al.* (2017) with slight modification, using a differential scanning calorimeter (DSC) (Q2000,

Modulated TA Instrument, USA) equipped with a cooling device (Intercooler II). Temperature calibration was performed using an Indium thermogram ($T_m = 156.6^\circ\text{C}$, and enthalpy $\Delta H = 28.3$ J/g). Approximately 5 ± 0.5 mg of film was weighed using the analytical balance (AX224 M-Pact, Sartorius, Germany) into aluminium pans, and hermetically sealed. An empty aluminium pan was used as a reference and for suitable heat capacity. Both sample pan and reference pan were scanned together over a temperature range of 10 to 150°C with a heating rate of $10^\circ\text{C}/\text{min}$. Nitrogen gas was used to flush the DSC cell at a flow rate of 20 ml/min to maintain an inert environment. The maximum transition or melting temperature (T_m) was identified as the temperature where the endothermic peak occurred. This thermal property was calculated with aid of the Pyris Thermal Analysing System ver. 5.00.03 software.

Film morphology determination

Film morphology was determined based on the method used by Nilswan *et al.* (2016). Morphology of film surface and cross-section was visualised using a scanning electron microscopy (SEM) (JSM-6360LA, JEOL, Japan). For cross section, film samples of 2×2 cm were fractured by immersion in liquid nitrogen for 30 s prior to visualisation. The samples were then mounted on bronze stubs perpendicularly to their surface using a double-sided adhesive tape and sputter coated with gold in order to make the samples conductive. Images were captured at an accelerating voltage of 10 kV with a magnification range of 300 to $1500\times$.

Statistical analysis

All data obtained were presented as mean \pm standard deviation. Statistical analysis was performed using One-Way Analysis of Variance (ANOVA) of Minitab ver. 14.0 software for Windows (Minitab Inc., USA). The significant differences between the means were determined by Fisher's test with a confidence level of $p < 0.05$.

Results and discussion

Rheological properties of film-forming solutions Dynamic viscoelastic

The dynamic viscoelastic properties of FFS can be evaluated through frequency sweep to assess the effect of starch on changes in microstructure and stability of the gelatin FFS during processing, storage, and transportation. By using small amplitude oscillatory strain (SAOS) sweep tests at 25°C , all

chicken skin gelatin-rice starch FFS showed a 0.1 - 10% strain range, where G' and G'' moduli remained constant, thus indicating a linear viscoelastic domain. Critical strain at 1% was defined before frequency sweep as the limit strain of the linear viscoelastic region (LVR) marking the end of the linear stress-strain relation.

Figure 1 shows the storage or elastic modulus (G') and the loss or viscous modulus (G'') of chicken skin gelatin-rice starch FFS. All FFS showed similar dynamic viscoelastic behaviour, with a typical liquid-like response at low frequencies from 0.1 to 22.5 rad/s, where the loss modulus was greater than the storage modulus. Both moduli increased as oscillatory frequency rose from 22.5 to 50.00 rad/s, but G' showed greater increments, especially for FFS with high rice starch content, which provides a more interacting starch chains for the formation of network structure (Peressini *et al.*, 2003). The G'' was also frequency dependence, suggesting that a high oscillatory shear might weaken the original gel structure, and turned it into a looser structure (Anvari and Chung, 2016). As a consequence, G' of all film formulations overcame G'' at higher frequencies ranging from 42.5 to 50 rad/s, thus contributing a gel behaviour ($G' > G''$) to FFS.

These results are consistent with a study by Jătăriu *et al.* (2013) which showed solid-like characteristic for gelatin-chitosan hydrogel with $G' > G''$ over a large range of frequencies between 0.01 and 100 s^{-1} . Similar observations were shown for starch-methylcellulose-based FFS with viscous behaviour ($G'' > G'$) during low frequency range, and then behaved like a gel ($G' > G''$) during high frequency range. In addition, all G' (around 4 to 400 Pa) of single rice starch FFS prevailed over the G'' (around 1 to 60 Pa) along the frequency range between 0.01 and 100 rad/s (Lawal *et al.*, 2011). Sarbon *et al.* (2013) also revealed that G' values of chicken skin gelatin solution were higher than G'' for all concentrations of 3, 5, 7, and 10% (w/v) at 10°C in the frequency ranges of 0.01 and 100 Hz, due to the network formation after reaching the gelling point. Higher frequency dependence of G'' for fish gelatin-gum Arabic coacervated gels was observed by Anvari and Chung (2016), with a larger degree increment around 6 to 200 Pa as the oscillatory frequency increased from 0.1 to 10 Hz.

Mechanical properties of chicken skin gelatin-rice starch films

Tensile strength

Tensile strength (TS) is the measurement of film strength. Higher TS leads to stronger film with

stronger intermolecular force between the polymer chains (Al-Hassan and Norziah, 2012). The influence of rice starch concentration on the TS of chicken skin gelatin-based composite films is presented in Table 1. The addition of rice starch ranging from 0 to 25% in the film formulation led to a significant increase ($p < 0.05$) in TS from 2.26 to 3.53 MPa. Film formulation F showed the highest TS value, which was significantly different from film formulations A, B, and C. However, there were no significant differences on TS between film formulations C, D, and E.

Table 1. Tensile strength and elongation at break of chicken skin gelatin-rice starch films of different formulations.

Film Formulation	Tensile strength (MPa)	Elongation at break (%)
A	2.26 ± 0.30 ^c	194.27 ± 18.39 ^{ab}
B	2.39 ± 0.36 ^c	210.37 ± 35.43 ^{ab}
C	2.73 ± 0.33 ^{bc}	227.58 ± 45.58 ^a
D	3.15 ± 0.60 ^{ab}	198.89 ± 27.47 ^{ab}
E	3.21 ± 0.32 ^{ab}	163.47 ± 29.69 ^b
F	3.54 ± 0.41 ^a	189.77 ± 25.27 ^{ab}

A = film with 0% rice starch; B = film with 5% rice starch; C = film with 10% rice starch; D = film with 15% rice starch; E = film with 20% rice starch; and F = film with 25% rice starch. Values are means ± standard deviations. Different superscript lowercase letter within similar column indicate significant difference ($p < 0.05$) based on Fisher's test.

The increasing trend in TS of chicken skin gelatin composite films may be attributed to the addition of rice starch that could be easily inserted and crosslinked with polymer chains of chicken skin gelatin (Ahmad *et al.*, 2015). The formation of strong hydrogen bonding between chicken skin gelatin and rice starch molecules lowers the molecular mobility and enhanced chain entanglement (Soo and Sarbon, 2018). Therefore, films with high starch concentration showed tighter and more compact film matrix structures, leading to thicker and stronger films (Fakhouri *et al.*, 2015; Loo and Sarbon, 2020).

The findings in the present work are supported by Fakhouri *et al.* (2012) who demonstrated the significantly increasing TS of manioc starch-gelatin film by adding more starch. For chicken skin gelatin-based films, film formulation with 10% tapioca starch and 20% rice flour gave the highest TS values of 3.27 and 2.91 MPa, respectively (Soo and Sarbon, 2018; Loo and Sarbon, 2020). However, these two highest TS values were lower than the highest TS value obtained by film

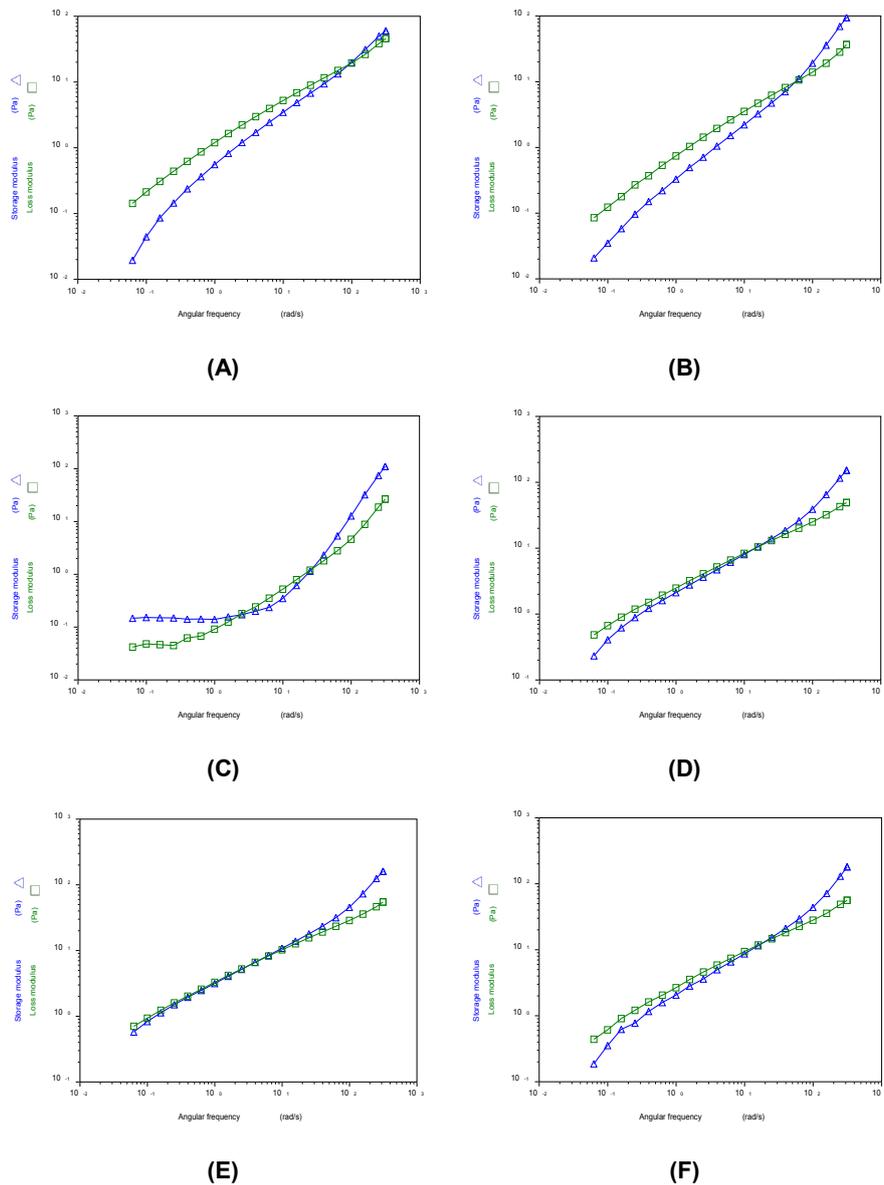


Figure 1. Frequency sweep curves of the elastic modulus (G') and viscous modulus (G'') of chicken skin gelatin-rice starch film forming solution of different formulations. A = film with 0% rice starch; B = film with 5% rice starch; C = film with 10% rice starch; D = film with 15% rice starch; E = film with 20% rice starch; and F = film with 25% rice starch.

formulation F containing 25% rice starch in the present work. This might be due to the higher amylose content up to 30.4% in rice starch, making it able to form stronger hydrogen bonding with chicken skin gelatin, thus contributing to film strength with a higher TS value (Woggum *et al.*, 2014). There are some studies showing that higher gelatin content gave higher TS values of composite films, such as cassava starch-bovine gelatin film (Tongdeesontorn *et al.*, 2012) and corn starch-gelatin film (Fakhouri *et al.*, 2015).

Elongation at break

Elongation at break (EAB) determines the flexibility of the film in terms of the film's ability to

stretch, which is determined at the point at which the film breaks under tensile testing. Higher EAB reflects more flexible and stretchable film. Table 1 shows the EAB of chicken skin gelatin-based composite films with various rice starch concentrations added. The EAB values were significantly increased ($p < 0.05$) from 194.27 to 227.58% for film formulations A to C, while decreased to 163.47% for film formulation E. Interestingly, EAB increased again to 189.77% for film formulation F. There was a significant difference ($p < 0.05$) in EAB between film formulations C and E. Nevertheless, there were no significant differences found for EAB among film formulations A, B, D, and F.

The first increasing trend in EAB of chicken skin gelatin-rice starch films was attributed to the suitable amount of dispersed rice starch molecules to produce the apparent plasticising effect by weakening the chain-chain interactions and promoting chain mobility. Therefore, the polymer matrix became more flexible, since polymer chains could slide past each other more readily during tensile deformation (Ahmad *et al.*, 2015). However, EAB was then decreased, perhaps due to the formation of intermolecular interactions between rice starch and gelatin molecules, contributing to the reduction of film flexibility and then reducing the EAB of film (Nazmi *et al.*, 2017). When adding an inappropriate level of rice starch into chicken skin gelatin films, a heterogeneous film matrix was formed with discontinuities along the entire film network, thus rendering chicken skin gelatin-rice starch films less resistant to breakage (Ahmad *et al.*, 2015).

The findings in the present work are consistent with studies by Soo and Sarbon (2018) and Loo and Sarbon (2020) which showed the highest EAB values of chicken skin gelatin films when incorporating with 20% rice flour (79.31%) and 10% tapioca starch (95.34%), respectively. In comparison, rice starch-chicken skin gelatin films have a double EAB values, perhaps due to the presence of higher amylose content up to 30.4% in rice starch responsible to form stronger and flexible films with higher resistance to elongation (Woggum *et al.*, 2014). A decreasing trend was noted in a study by Ahmad *et al.* (2015) which showed the EAB of rice flour-fish gelatin films decreased from 14.72 to 6.46% with increasing rice flour content. Fakhouri *et al.* (2015) also revealed that EAB of composite film was decreased from 7.53 to 5.15% with the increment of 3% (w/w) corn starch in Type A gelatin film. In contrast, Fakhouri *et al.* (2012) observed that the increments of both manioc starch and type A gelatin concentrations enhanced the EAB of composite film from 4.28 to 6.53%.

Physical properties of chicken skin gelatin-rice starch films

Water vapour permeability

Water vapour permeability (WVP) is an important property that greatly influences the utility of a film in food systems (Ahmad *et al.*, 2015). Lower WVP of film reflects a good protective barrier towards moisture migration between food and the surrounding atmosphere. WVP of chicken skin gelatin-rice starch films prepared at different film formulations is illustrated in Table 2. The results

obtained in the present work showed that an increasing trend in WVP ranged from 1.67×10^{-3} to 2.13×10^{-3} g mm/h m² Pa with the increased concentration of rice starch from 0 to 25% in chicken skin gelatin based films. WVP of film formulation A was the lowest and showed a significant difference ($p < 0.05$) with film formulation F. Conversely, there was no significant difference found for WVP between film formulations B, C, D, and E.

Table 2. Water vapour permeability, film solubility, and thermal properties of chicken skin gelatin-rice starch films of different formulations.

Film Formulation	Water vapour permeability ($\times 10^{-3}$ g mm/h m ² Pa)	Film solubility (%)	Melting point, T _m (°C)
A	1.67 ± 0.37 ^b	94.48 ± 0.40 ^a	56.09 ± 2.08 ^c
B	1.69 ± 0.13 ^{ab}	92.64 ± 0.30 ^{ab}	56.89 ± 3.61 ^{bc}
C	1.80 ± 0.21 ^{ab}	89.20 ± 1.24 ^{bc}	59.93 ± 1.01 ^{abc}
D	1.90 ± 0.20 ^{ab}	85.31 ± 3.45 ^c	61.02 ± 0.42 ^{ab}
E	1.97 ± 0.31 ^{ab}	74.44 ± 3.49 ^d	61.25 ± 0.32 ^{ab}
F	2.13 ± 0.24 ^a	72.47 ± 1.69 ^d	61.55 ± 0.71 ^a

A = film with 0% rice starch; B = film with 5% rice starch; C = film with 10% rice starch; D = film with 15% rice starch; E = film with 20% rice starch; and F = film with 25% rice starch. Values are means ± standard deviations. Different superscript lowercase letter within similar column indicate significant difference ($p < 0.05$) based on Fisher's test.

Film formulation A (control) showed the lowest WVP due to the presence of hydrophobic amino acids (glycine, 33.70%; proline, 13.42%; alanine, 10.08%; and leucine, 2.63%) in single chicken gelatin film, which led to lower free volume and compact film structure, thus attracting less water molecules from the environment (Sarbon *et al.*, 2013; Ahmad *et al.*, 2015). Increasing the WVP of chicken skin gelatin-based blended films upon increasing rice starch concentration might be due to the presence of hydrophilic hydroxyl groups in amylose macromolecules of rice starch, thus leading to greater water affinity that facilitates the migration of water vapour molecules through the film (Ahmad *et al.*, 2015).

Similar phenomena were observed in a previous study by Podshivalov *et al.* (2017) which revealed that the increment of starch content caused a significant increase of WVP in potato starch-gelatin film. Similarly, the addition of 0 to 20% rice flour (Soo and Sarbon, 2018) and 5 to 25% tapioca starch (Loo and Sarbon, 2020) in chicken skin gelatin-based film increased the WVP values by 2.14 to 5.00×10^{-3} g mm/h m² Pa and 1.90 to 5.78×10^{-3} g mm/h m² Pa, respectively. Conversely, a study by Ahmad *et al.* (2015) reported a contrast observation with

decreasing in WVP of rice flour-fish gelatin films from 4.42 to 3.72×10^{-10} g/s m Pa upon increasing the rice flour content. Single rice flour film showed the highest WVP of 7.28×10^{-10} g/s m Pa which was probably because of its more hydrophilic nature as compared to fish gelatin, together with its less compactness and lower ordered network that allowed the penetration of water vapour more effectively.

Film solubility

Table 2 represents the influences of rice starch concentration on the solubility of chicken skin gelatin-based composite films in water. The solubility of chicken skin gelatin-based films in water significantly decreased ($p < 0.05$) from 94.48 to 72.47% with increased levels of rice starch. Film formulation A showed the highest value of film solubility in water, and was significantly difference ($p < 0.05$) as compared to film formulations C, D, E, and F. Yet, there were no significant difference on film solubility between film formulations A and B; between film formulations B and C; between film formulations C and D; as well as between film formulations E and F.

Due to the hydrophilic nature of gelatin, film formulation A (control) containing only gelatin in film matrix had higher affinity with water molecules, thus resulting in higher water solubility. The descending trend in water solubility for chicken skin gelatin films upon adding rice starch might be due to the inter- and intramolecular interactions between polymer chains in the film matrix (Ahmad *et al.*, 2015). During the blending process, both hydroxyl group and amide group of chicken skin gelatin formed strong hydrogen bonds with the hydroxyl groups of rice starch, thus leading to the reduction of available functional groups for binding water molecules (Tongdeesoontorn *et al.*, 2012).

Similar results were obtained in the previous studies for rice flour-fish gelatin films (94.46 to 57.96%) (Ahmad *et al.*, 2015), Type A commercial gelatin-corn starch films (36.53 to 16.52%) (Fakhouri *et al.*, 2012), chicken skin gelatin-tapioca starch films (93.66 to 83.29%) (Loo and Sarbon, 2020), and chicken skin gelatin-rice flour films (93.66 to 82.91%) (Soo and Sarbon, 2018). The chicken skin gelatin-based composite films had higher solubility in water as compared to other composite films, perhaps because the chicken skin gelatin had a higher amount of hydrophilic amino acids than other gelatins, such as positive charges of lysine (4.66%) and polar tyrosine (1.22%), which energetically favour contact with water (Sarbon *et al.*, 2013).

Thermal properties

The thermal stability of film plays important role in optimising the potential application of biodegradable film. The melting transition temperature or melting point (T_m) of chicken skin gelatin-based films both with and without the addition of rice starch is shown in Table 2. The T_m values of chicken skin gelatin-based films significantly increased ($p < 0.05$) with increased concentration of rice starch. There was a significant difference ($p < 0.05$) noted between film formulations A and F, but no significant differences were found between film formulations B, C, D, and E.

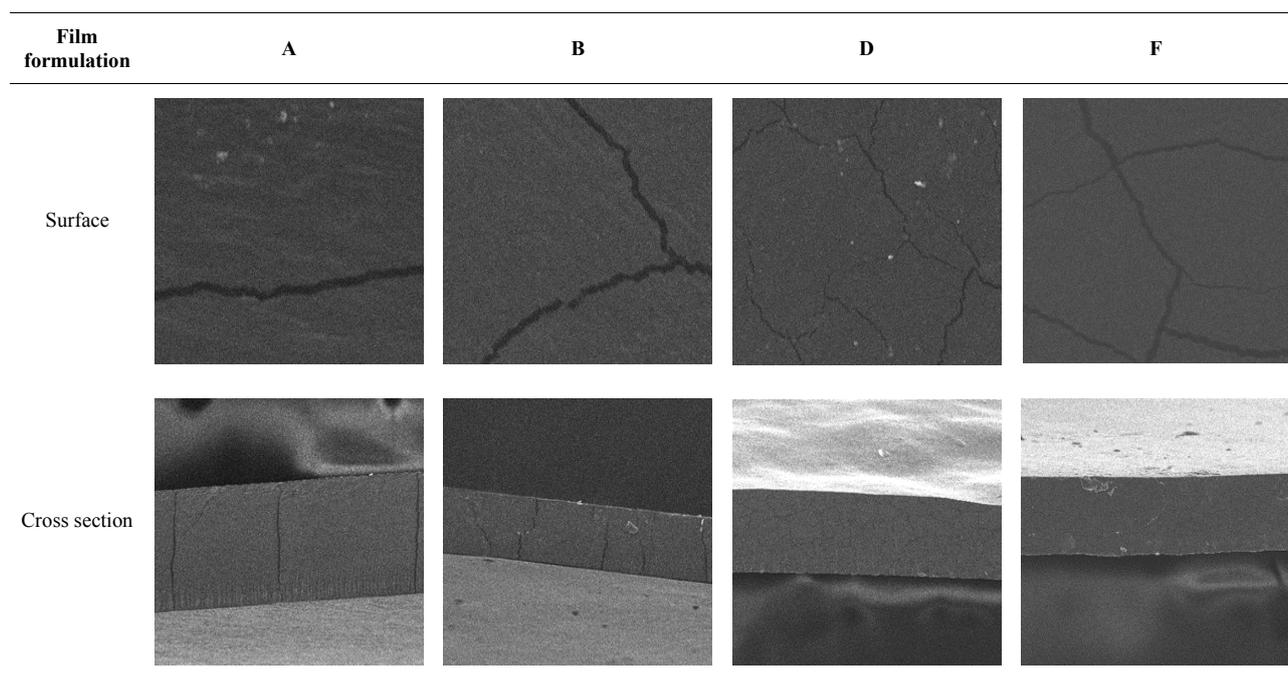
In the present work, the increasing in T_m of chicken skin gelatin-based films was caused by elevation of rice starch concentration as a crosslinker to promote the hydrogen bonding interactions and reduce the mobility of biopolymer chains in the film matrix, thus producing highly heat stable films (Soo and Sarbon, 2018; Loo and Sarbon, 2020). A study by Nur Hazirah *et al.* (2016) also revealed that the incorporation of crosslinkers into gelatin films improved the thermal stability of film by shifting T_m towards a higher value. The appearance of T_m endothermic peak for film formulations A to F might be related to the melting of crystalline domains of chicken skin gelatin film (Hoque *et al.*, 2010) associated with the helix-coil transition and disruption of molecular ordered structure (Ahmad *et al.*, 2015).

These findings parallel those of studies by Soo and Sarbon (2018) and Loo and Sarbon (2020). Two T_m values obtained in the thermogram could be due to the partial immiscibility of gelatin and starch. The first T_m related to the presence of chicken skin gelatin, and the second T_m contributed by starch were elevated with the increment of incorporated tapioca starch or rice starch concentration in chicken skin gelatin films. However, the results obtained in the present work differed with a previous study by Tongdeesoontorn *et al.* (2012) which showed a decrease in T_m of film from 149.28 to 111.29°C upon increasing starch content in the cassava starch-bovine gelatin composite matrix.

Film morphology

Scanning electron microscopy images of surface and cross section of chicken skin gelatin-rice starch films with different formulations are represented in Table 3. Film formulations A, B, D, and F were chosen to study the surface and internal cross structure morphology of chicken skin gelatin film incorporated with different concentration of rice starch. Film formulation A was important as a control

Table 3. Scanning electron microscopy images of surface and cross section of chicken skin gelatin-rice starch films of different formulations.



A = film with 0% rice starch; B = film with 5% rice starch; D = film with 15% rice starch; and F = film with 25% rice starch.

for single chicken skin gelatin films, while the purpose of choosing film formulations B, D, and F was to compare their differences in morphology in adding the least, the median and the most percentages of rice starch into the composite films.

For the surface section, it was observed that the micrograph of film A (control) exhibited a rough and bumpy surface with wide and longer crack. The findings of de Carvalho and Grosso (2004) stated that gelatin films plasticised with glycerol have discontinuous zones characterised by cracks distributed along the length of the network as a result of the preferential channels that occurred through drying. The increment of rice starch concentration enhanced the surface characteristic since film F showed a more compact, flat, smooth, and homogenous surface than film B. This indicates that an ordered structure was formed without layering phenomenon (Ahmad *et al.*, 2015). However, film D had the tiniest and shortest scattered cracks on the surface. The incorporation of rice starch enhanced the smoothness of surface for chicken skin gelatin films due to the hydrogen bonding between gelatin and rice starch as well as inter- and intramolecular bonding between film components (Nazmi *et al.*, 2017; Soo and Sarbon, 2018). These observations are similar to those of studies by Nazmi *et al.* (2017), Soo and Sarbon (2018), and Loo and Sarbon (2020), in which surface morphology of chicken skin gelatin

films became smoother with the incorporation of rice flour, tapioca starch, and CMC, respectively.

In the case of cross section, heterogeneity with two separation layers was not detected. The morphology of film A showed a slightly rough laminated surface with more long-lined structured cracks as compared to films B, D, and F, which had smoother surfaces with short and scattered cracks. The improvement of internal cross structure upon increasing starch concentration might be due to the homogenous network and good compatibility between chicken skin gelatin and rice starch (Soo and Sarbon, 2018). These findings parallel with observations of chicken skin gelatin films incorporated with rice flour (Soo and Sarbon, 2018), tapioca starch (Loo and Sarbon, 2020), and CMC (Nazmi *et al.*, 2017), respectively. The roughness of cross section morphology in gelatin-based films decreases with the increment of polysaccharide content. Tongdeesoontorn *et al.* (2012) also agreed that blending with cassava starch could improve the internal structure of gelatin films.

Conclusion

In conclusion, the physical and mechanical properties of chicken skin gelatin film were significantly affected by different concentrations of rice starch incorporation. The G' increased

dramatically than G'' as oscillatory frequency rose for FFS with higher rice starch content to contribute a gel behaviour ($G' > G''$). The increased rice starch content significantly increased the tensile strength and elongation at break, water vapour permeability, and thermal properties of gelatin films, while reducing the film solubility as well as providing a smoother surface and cross section morphology. Overall, chicken skin gelatin film with 25% rice starch (film formulation F) was found to be the optimal film formulation, as it had the highest tensile strength and higher elongation at break. Thus, formulation film F has the potential to be considered as an alternative food packaging material over other biodegradable films.

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