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FULL PAPER

Effect of glycerol concentrations on the mechanical and physical properties of chicken skin gelatin-tapioca starch composite films

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Abstract

This study aimed to elucidate the characteristics of chicken skin gelatin-tapioca starch composite films plasticized with different concentrations of glycerol. Gelatin-tapioca starch film solutions with different glycerol concentrations, namely A (0%), B (10%), C (20%), D (30%) and E (40%), were stirred at 45°C for 30 mins and oven-dried at 45°C. The results obtained showed that the tensile strength and Young's modulus of the films had a negative linear correlation with glycerol concentrations, and positively correlated to the elongation at break ($p<0.05$). Water vapour permeability and water solubility of the films were increased with glycerol concentration increased. The plasticized films showed lower thermal stability as compared to unplasticized films. In conclusion, Film D (30% glycerol concentration) offers the most promising potential for industrial food packaging applications.

1. Introduction

Packaging materials widely vary, from plastics, glass, metals, paper and board, biomaterials, or a composite of packaging materials depending on the type of food and function intended (Lechevalier, 2016). With the exception of paper-based products, most packaging on the market is mainly based on nonrenewable materials. In this regard, the use of raw materials from renewable sources, particularly biologically derived products, seems to be the approach toward the manufacture of more sustainable food packaging. This includes edible films, sheets, coatings and pouches (Robertson, 2013). Edible films can be made from proteins, lipids, polysaccharides and composites. Each type offers distinct functional properties. Among those, gelatin is available as by-products or waste from the food processing industry, showing strong potential as a raw material (Sarbon *et al*., 2013).

Gelatin is obtained by partial hydrolysis of collagen, the major constituent of fibrous protein of skin, tendons, cartilage, and bones, and is commonly treated as waste or by-products during animal slaughtering (Sarbon *et al.,* 2013; Rosli and Sarbon, 2015). Some commonly used types of gelatins for film formulation include bovine skin gelatin (Jahit *et al*., 2016; Suderman *et al.,* 2016), fish skin gelatin (Al-Hassan and Norziah, 2012), pigskin gelatin (Pena-Rodriguez *et al.,* 2015), and chicken skin gelatin (Soo and Sarbon, 2018; Suderman and Sarbon, 2019). Despite the good mechanical properties exhibited by chicken skin gelatin films, the high water vapour permeability and high-water solubility of the films tends to limit their application potential (Nor *et al.,* 2017). One of the strategies proposed to address this issue is the blending with other biopolymers, such as polysaccharides, which exhibit film-forming and cross-linking properties.

Due to their abundance in nature, a variety of polysaccharides and their derivatives have been evaluated for potential use as edible packaging, as they are low cost, easy to handle and offer good film-forming properties (Robertson, 2013). At the same time, the cross -linking properties of polysaccharides also make it an ideal composite material to blend with protein in order to take advantage of complementary functional properties and to overcome the flaws in protein-based films. Commonly used polysaccharides in film formulations include tapioca starch (Loo and Sarbon, 2020), corn starch (Aydin and Ilberg, 2016), rice starch (Cheng and Sarbon, 2020), and potato starch (Alias and Sarbon, 2019). Despite their good gas barrier properties, films made from starches are brittle. The functional properties of the film can thus be improved with the addition of chemical additives such as plasticisers.

In film packaging, a plasticiser is a substance added to materials to impart flexibility, workability, and

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elongation (Wypych, 2017). Polyols such as glycerol, sorbitol, xylitol, and mannitol have been used to improve the properties of starch-based edible films (Sanyang *et al.,* 2016; Basiak *et al*., 2017, Bakry *et al.,* 2017). Glycerol is the most widely used plasticiser for improving the mechanical properties and transparency of edible films (Suderman *et al*., 2018). An appropriate amount of plasticising agent added to edible films will act as a spacer between polymer chains to decrease the intermolecular forces between adjacent polymeric chains, thus reducing the brittleness and improving the flexibility and extensibility of a film (Maran *et al.,* 2013). However, it has been reported that the increased plasticiser concentration results in an increase in film thickness, moisture content and solubility, as well as a decrease in the density and water absorption of plasticized films (Sanyang *et al.,* 2016). The concentration of plasticiser used in the film formulation will affect the functional properties of the film produced, meaning that adjustment of the plasticiser concentration in the film formulation is required to achieve the desired functional properties (Suderman *et al.,* 2018).

The functional properties of a film are important to produce good quality films for food packaging. Functional properties can be divided into mechanical and physical properties. Tensile strength (TS), elongation at break (EAB), and Young's modulus are the most commonly reported attributes used to describe the mechanical properties of edible films, as they are important for application, product performance and consumer acceptance (Suderman *et al.,* 2016). Physical properties that have been studied include water vapour permeability (WVP), transparency and light transmittance, thermal properties, film morphology, water solubility and moisture content (Soo and Sarbon, 2018; Loo and Sarbon, 2020).

Therefore, the objectives of the present study were to formulate chicken skin gelatin-tapioca starch composite film incorporated with different glycerol concentrations, and characterize the mechanical and physical properties of the chicken skin gelatin-tapioca starch composite films produced.

2. Materials and methods

2.1 Materials

Fresh chicken skins were obtained from a local supplier in Kuala Nerus, Terengganu. The chicken skin was transported to Universiti Malaysia Terengganu in chilled condition. Native tapioca starch was purchased from a local market in Kuala Terengganu, Terengganu. Glycerol, the plasticiser used, was purchased from R & M Marketing, Essex, U.K. All chemicals used for analysis were of analytical grade.

2.2 Chicken skin preparation

Chicken skins preparation was conducted following Wan Omar and Sarbon (2016). Then, the chicken skins were washed and weighed before storage in the freezer at a temperature of -18°C for further experiments. The frozen chicken skins were thawed in a chiller (4-5°C) overnight. Visible fat and remaining feathers on the chicken skins were removed before rinsing in excessive water to remove impurities. The chicken skins were cut into small pieces and layered in aluminium trays before being dried in a cabinet drier at 40°C. Each dried skin sample was cut with a scissor into smaller pieces before being ground and defatted using the Soxtec method (AOAC, 2006).

2.3 Chicken skin gelatin extraction

The chicken skin gelatin was extracted following Rasli and Sarbon (2015). To extract the gelatin, 15 g of defatted dried chicken skin was treated with 200 mL sodium hydroxide (0.15%, w/v). The mixture was shaken and stirred slowly for 30 mins at room temperature before being centrifuged at 3500×*g* for 10 mins. To remove non-collagenous protein and pigments, the process was repeated three times. The supernatant was removed and the alkaline solution was changed for each cycle. The alkaline-treated pellets were rinsed with distilled water. The resulting pellets were treated with 200 mL 0.15% (v/v) sulfuric acid for 30 mins and centrifuged at 3500×*g* for 10 mins. The step was repeated with 200 mL of 0.7% (w/v) citric acid solution. The mixture was shaken and stirred slowly for 30 mins at room temperature before centrifugation at 3500×*g* for 10 mins. Each acid treatment was repeated three times and the acid solution was changed for every cycle in order to denature the collagen in the chicken skin matrix. The pellets were washed with distilled water and centrifuged again at 3500×*g* for 15 mins. Then, the final extraction was carried out with distilled water at a controlled temperature of 45°C overnight without stirring. The mixture was filtered in a Büchner funnel with a Whatman filter paper (No. 4) before reducing the volume to 1/10 by a rotary evapourator and freeze-dried. The resultant matter 'gelatin powder' was ground, weighed and stored for further use. The gelatin yield was calculated with the following equation:

$$\text{Yield } \% = \frac{\text{Weight of gelatin powder}}{\text{Defatted weight (g)}} \times 100$$

2.4 Film preparation

The preparation of the film was conducted based on the method proposed by Soo and Sarbon (2018) with some modifications. The film-forming solutions were

prepared by mixing 0.1 g of tapioca starch and 1.0 g of chicken skin gelatin with added plasticisers (glycerol) at different concentrations (0%, 10%, 20%, 30%, and 40%). A total of 5 formulations were developed and designated as 'Formulations A–E'. The tapioca starch solution and gelatin solution were prepared separately. Tapioca starch (0.1 g) was dissolved in distilled water (40 mL) and heated with magnetic stirring in a water bath at 85°C for 30 mins until completely gelatinized. The chicken skin gelatin powder (1.0 g) was dissolved in distilled water (60 mL) at a 45°C water bath for 30 mins until a clear solution was obtained. The chicken skin gelatin solution was added to gelatinized tapioca starch at 45°C and stirring was continued for 30 mins, followed by the addition of plasticiser at different concentrations with constant stirring for another 30 mins until complete dissolution. Then, the solution was cooled to room temperature before being cast into Petri dishes (90 mm in diameter) and dried in a ventilated oven at 45°C for 64-68 hrs. Approximately 25 g of film-forming solution was allocated for each Petri dish to obtain uniform thickness. The films were then removed from the dish and stored in a desiccator containing silica gel at room temperature before the determination of their mechanical and physical properties. The film with 0% glycerol concentration was the control film of the study. Each film formulation was prepared in triplicate.

2.5 Determination of tensile strength, elongation at break and Young's modulus

The tensile strength (TS), elongation at break (EAB) and Young's modulus of each film were determined following Nur Hazirah *et al.* (2016) using a Texture Analyzer (Stable Micro Systems Model TA-XT plus, UK) with some modifications. The film was cut into a size of 10×70 mm. In order to measure the elastic behaviour of the films as a function of the thickness, a manual micrometre (Mitutoyo, Japan) with an accuracy of 0.001 mm was used. The thicknesses of each film were measured at five different positions to obtain the average and standard deviation. Tensile strength and EAB were performed as a tension test at a strain of 300% with the initial grip separation set at 40 mm and film width of 1 cm. The films were subjected to an applied force with a load cell of 5 kg and a cross-head speed of 120 mm/min. Then, the film strip was mounted between grip pairs of AT/G probes and stretched by moving the upper grid until the film broke. The TS, EAB and Young's modulus were calculated using the following equations:

$$\text{Tensile Strength (MPa)} = \frac{\text{Maximum load (N)}}{[\text{Sample width (mm)} \times \text{Film thickness (mm)}]}$$

$$\text{EAB (\%)} = \frac{\text{Final length at breakage (mm)} - \text{Initial length of sample (mm)}}{\text{Initial length of the sample (mm)}} \times 100$$

$$\text{Young's modulus} = \frac{\text{Strength of drag force (MPa)}}{\text{Elongated film (\%)}}$$

All final determinations were recorded as the mean of three measurements.

2.6 Determination of water vapour permeability

The water vapour permeability (WVP) of the film was measured using the method described by Jahit *et al.* (2016) with slight modification. The film was sealed over the area of clean and dry cylinder glass bottles (20×20 mm) with 10 g of silica gel (0% RH) in the bottles by using silicon vacuum grease. Together with the film, each bottle was weighed to obtain the initial weight. The bottles were placed in a desiccator containing distilled water at room temperature. The weight of each bottle was recorded daily for a week and the WVP was calculated using the following equation:

$$\text{WVP}\left(\frac{\text{gmm}}{\text{cm}^2\text{hPa}}\right) = \frac{\Delta w \times x}{A \times t \times \Delta P}$$

Where Δw is the weight difference (g), x is the average film thickness (mm), A is the area of the film surface exposed to the per meant (cm^2), t is the time of gain (h) and ΔP (Pa) is the difference of partial pressure of atmosphere with silica gel and single water (3159 Pa at 25°C).

2.7 Determination of thermal properties

The thermal properties of the film were measured based on the method proposed by Sarbon *et al.* (2015) using a differential scanning calorimeter (DSC) (DSC Q2000, Canada) with some modifications. Each 5 (±0.01) mg sample was placed on an aluminium pan and an empty pan was used as a reference. Then, the pan was hermetically sealed before heating over a temperature range of 10 to 150°C at a scanning rate of 10°C/min. Nitrogen gas was used to flush the DSC cell at a flow rate of 20 mL/min to maintain an inert environment. The glass transition temperature (T_g) was calculated as the temperature where the endothermic peak occurs. All determinations were recorded as the mean of two measurements.

2.8 Determination of water solubility

Film water solubility was investigated based on the method described by Saberi *et al.* (2016). The films were cut into 10×30 mm samples and dried at 110°C for 24 hrs in an oven before being weighed to obtain the initial dry weight ($W_{Initial}$). Then, each sample was immersed in 20 mL of distilled water in a clean plastic container, capped and shaken gently (70 rpm) for 24 hrs at room temperature using an orbital shaker (IKA-KS501, Germany). The remaining pieces of films that did not dissolve were filtered and rinsed with distilled water and

then dried at 110°C for 24 hrs to constant final dry weight (W_{Final}). All determinations were recorded as the mean of three measurements. The percentage solubility was calculated using the following equation:

$$\text{Solubility (\%)} = \frac{W_{initial} - W_{Final}}{W_{initial}} \times 100$$

2.9 Statistical analysis

All analyses were performed in triplicate and the data was presented by mean±standard deviation. Then, the data obtained were analyzed by using the one-way Analysis of Variance (ANOVA) of Minitab-14.0 software. The comparison of means was performed by Fisher's test with a confidence level of $\alpha<0.05$.

3. Results and discussion

3.1 Tensile strength

The effects of glycerol concentration on the tensile strength of chicken skin gelatin-tapioca starch composite films are shown in Table 1. There was a significant difference ($p<0.05$) between the control film (Formulation A) and the rest of the formulations (Formulations B, C, D, and E). However, there were no significant differences observed between Formulations B, C, D, and E, indicating that these films had similar tensile strength, regardless of the percentage of glycerol added in the formulation.

Table 1. The tensile strength (TS), elongation at break (EAB) and Young's modulus of film formulations at different glycerol concentrations

Formulation	Tensile strength (MPa)	Elongation at break (%)	Young's Modulus (MPa)
A	22.42±4.75^{a}	1.71±0.27^{d}	1305.2±75.6^{a}
B	4.52±0.46^{b}	71.64±9.05^{c}	6.4±1.5^{b}
C	2.95±0.44^{b}	130.82±1.75^{b}	2.3±0.3^{b}
D	2.46±0.31^{b}	188.69±6.49^{a}	1.3±0.2^{b}
E	1.35±0.01^{b}	193.76±1.71^{a}	0.7±0.0^{b}

A = Film with 0% of glycerol, B = Film with 10% of glycerol, C = Film with 20% of glycerol, D = Film with 30% of glycerol, E = Film with 40% of glycerol. Values are presented as mean±SD. Values with different superscripts within the same column are significantly different.

Generally, an increase in glycerol concentration in a film formulation causes a decrease in tensile strength. This could be related to the arrangement of different film components in the matrix, the addition of glycerol affects the cohesion forces in the matrix. The incorporation of gelatin with tapioca starch induces crosslinking reactions between the two components, increasing the mechanical resistance of the material. The addition of glycerol acts to decrease the crosslinking density in the starch-gelatin film. The mechanical properties of the control formulation (0% glycerol) are generally brittle and rigid with the highest TS value. This may be due to the stronger interactions between the polymer chains in the absence of a plasticiser. The lower TS value (1.348 MPa) in the highly plasticized films (40% glycerol) could be due to the interruption of gelatin-starch polymer matrix continuity by glycerol. These discontinuities cause possible weakening of the interchain forces in the presence of glycerol compounds, therefore reducing the cohesion forces of the matrix and its mechanical resistance (Acosta *et al.,* 2015).

A similar trend of decreasing TS values with increasing glycerol concentrations has been reported by Nor *et al.* (2017) on chicken skin gelatin-based films. When compared at similar glycerol concentrations added (0-20% glycerol), the TS values (22.42 to 2.95 MPa) from the current study were found to be higher than those of single chicken skin gelatin films (33.66 to 1.75 MPa) (Nor *et al.,* 2017) and single tapioca starch films (3.82 to 2.21 MPa) (Adamu *et al.,* 2017). This may be due to the cross-links between the tapioca starch and chicken skin gelatin molecules increasing the mechanical resistance of the material as compared to the single gelatin and single starch films. At 30% glycerol concentration, the TS value (2.46±0.31 MPa) was comparably higher than fish gelatin-sago starch composite film (1.28±0.25 MPa) (Al-Hassan and Norziah, 2012). This could be due to the higher hydroxyproline content in the chicken skin gelatin compared to fish gelatin, which results in higher gel strength in the former gelatin, thus producing a film with better mechanical resistance (Sarbon *et al.,* 2013).

3.2 Elongation at break

The EAB values significantly increased ($p<0.05$) from 1.71 to 193.76% for Formulations A to E (Table 1). There were significant differences ($p<0.05$) among all formulations, except between Formulations D and E. The addition of glycerol increased the EAB values of the films. This was significant when the glycerol ratio increased up to 30%. The low EAB of Formulation A (0% glycerol) could be related to the strong interactions and bonding formed between polymer chains, which limits the mobility of polymer chains, leading to lower extensibility of the films. Meanwhile, the increase in film extensibility for protein-starch matrix by glycerol addition is attributed to the plasticizing effect due to the orientation of the glycerol functional groups in the matrix (Acosta *et al.,* 2015). The polar groups (-OH) in the glycerol's chain favour the interaction of the plasticiser with gelatin-starch polymers and weaken the polymer chain attraction forces. As a result, the presence of a high amount of glycerol molecules in films with

higher glycerol concentration (40%) tends to act as a "lubricant" to reduce the cohesion and intermolecular forces, and along with film-forming polymer chains, soften the polymer network and facilitate the slippage of the molecular chains during film stretching, thus increasing the EAB value of the films (193.76%) (Acosta *et al.,* 2015; Wypych, 2017).

A similar trend of increasing EAB value with the increasing glycerol concentration was reported by Nor *et al.* (2017) on chicken skin gelatin-based films. When compared to a similar glycerol concentration range (0-20% glycerol), the EAB values in the current study were lower than those of single chicken skin gelatin films (3.87 to 148.3%) (Nor *et al.,* 2017) and single tapioca starch films (3.93 to 536%) (Adamu *et al.,* 2017). This could be attributed to the cross-linking reaction of chicken skin gelatin with tapioca starch molecules, which limited the chain movement and strengthen polymer chains, therefore producing more rigid films compared to single gelatin or single starch films. Moreover, the greater extensibility of single tapioca starch films compared to chicken skin gelatin-tapioca starch films could be due to the higher content of low amylose content starch, which has a better ability to form helical complexes with glycerol (Acosta *et al.,* 2015). Additionally, the increasing glycerol concentrations showed a typical pattern of decreasing TS and increased EAB which has also been reported by several authors for starch-based and gelatin-based films (Nor *et al.,* 2017). The opposite interaction of TS and EAB could be regarded as the interference of the polymer chain by the added glycerol, which reduces the intermolecular forces between gelatin-starch polymers, reducing the rigidity of the matrix and improving film flexibility.

3.3 Young's modulus

The Young's modulus values were significantly decreased ($p<0.05$) from 1305.2 to 0.7 MPa for Formulations A to E (Table 1). There was a significant difference ($p<0.05$) between the control (Formulation A) and the rest of the formulations (Formulations B, C, D, and E). However, there were no significant differences ($p>0.05$) between Formulations B, C, D, and E, indicating that these films performed similarly to Young's modulus regardless of the percentage of glycerol added in the formulation.

Generally, Young's modulus value decreases with increasing glycerol concentration, indicating that the stiffness decreases as glycerol (0-40%) are added to the film formulation. This behaviour could be related to the structural modification of the gelatin-starch network when a plasticiser is incorporated. The glycerol acts by reducing the cross-link density in the matrix and facilitating the mobility of the polymer chains, consequently improving the film flexibility under stress (Farahnaky *et al.,* 2013). The films of Formulation A did not have any additional plasticiser and were thus comprised of a very dense gelatin-starch network that leads to high film stiffness and Young's modulus value. In the other formulations with a plasticiser (Formulations B to E), the added glycerol tends to come in between the lateral branches of the gelatin-starch polymer chain structure and weaken the bonds, lowering the viscosity of the more crystalline starch molecule chains, therefore reducing the film rigidity and stiffness.

A similar trend of decreasing Young's modulus value with increasing glycerol concentration was reported by Farahnaky *et al.* (2013) (wheat starch-based films) and Villacres *et al.* (2014) (tapioca starch-based films). When compared to similar glycerol concentrations (20-40% glycerol), the values from the current study (2.3 to 0.7 MPa) were lower than those of single tapioca starch films (163 to 0.13 MPa) (Villacres *et al.,* 2014) and single wheat starch films (6280 to 288 MPa) (Farahnaky *et al.,* 2013). This might be due to the increased protein concentration in the film formulation increasing the triple-helix content and causing the reduction of the degree of swelling. This increase in the energy stored elastically in the gel network with the increase of intermolecular cross-links (Sarbon *et al.,* 2013). At 30% glycerol concentration, Young's modulus value (1.3 MPa) was comparably lower than fish gelatin-sago starch films with a value of 1.2×10^{6} MPa (Al-Hassan and Norziah, 2012). Chicken skin gelatin-tapioca starch composite is less stiff compared to the fish gelatin -sago starch films with the same glycerol ratio, probably due to the higher bloom value of chicken skin gelatin compared to fish skin gelatin, which contributes to the higher gel strength and gel-forming quality of the chicken skin gelatin (Sarbon *et al.,* 2013).

3.4 Water vapour permeability

The effects of glycerol concentration on water vapour permeability of chicken skin gelatin-tapioca starch composite films are shown in Table 2. The WVP values were significantly increased ($p<0.05$) from 5.718×10^{-8} to 1.325×10^{-7} g mm cm^{-2} h^{-1} Pa^{-1} for Formulations A to C, while WVP values decreased slightly from 1.325×10^{-7} to 1.268×10^{-7} g mm cm^{-2} h^{-1} Pa^{-1} for Formulations C to E. There were significant differences ($p<0.05$) between all formulations, except between Formulations D and E.

The WVP of the composite films produced was found to be affected by the glycerol concentration incorporated in the film formulations. Initially, the addition of glycerol concentration led to a significant

increase in their WVP values. For the unplasticized films (0% glycerol), the low permeability could be due to the gelatin-starch chain aggregation progress and the increase in the matrix compactness of the films (Acosta *et al.,* 2015). The increasing glycerol addition to the film composition for Formulations A to C (0-30% glycerol), generally increased the WVP of the studied films due to its more hydrophilic character. This fact could be attributed to increased hydrophilic groups in the final mass of the sample as the glycerol concentration increases, thus allowing the easier permeation of water found to be decreased slightly. This could further be attributed to the interaction of water vapour and glycerol with the protein matrix reaching a maximum. The decrease in WVP could have resulted from glycerol becoming less effective in transferring water vapour beyond a certain threshold in the mixtures, due to the interactions between glycerol molecules, which would reduce the number of OH groups binding to water/water vapour.

Table 2. The water vapour permeability (WVP), water solubility and glass transition temperature (T_g) of film formulations at different glycerol concentration.

Formulation	WVP ($\times 10^{-7}$ $\frac{gmm}{cm^2 hPa}$)	Water solubility (%)	Glass transition temperature T_g (°C)
A	0.571±0.05^{c}	45.887±4.60^{b}	79.34±0.59^{a}
B	1.182±0.09^{b}	54.84±6.68^{ab}	64.93±2.45^{b}
C	1.325±0.03^{a}	58.03±4.39^{a}	53.62±2.05^{c}
D	1.276±0.05^{bc}	62.88±6.57^{a}	50.27±1.63^{cd}
E	1.268±0.10^{bc}	63.98±1.86^{a}	48.05±0.18^{d}

A = Film with 0% of glycerol, B = Film with 10% of glycerol, C = Film with 20% of glycerol, D = Film with 30% of glycerol, E = Film with 40% of glycerol. Values are presented as mean±SD. Values with different superscripts within the same column are significantly different.

When compared to similar glycerol concentrations (0 -20% glycerol), the WVP values of the present study (5.718×10^{-8} to 1.325 g mm cm^{-2} h^{-1} Pa^{-1}) were lower than the WVP values of single chicken skin gelatin film (1.5×10^{-6} to 2.4×10^{-6} g mm cm^{-2} h^{-1} Pa^{-1}) (Nor *et al.,* 2017). This was due to the cross-linkage between the OH groups and NH groups of gelatin molecules with the OH groups of starch molecules which improve the interaction of polymers in the matrix, which consequently improved the biopolymer cohesiveness and reduced the WVP. This is also an indication of successful cross-linking treatment with the incorporation of tapioca starch in the present study, reducing the moisture sensitivity of the films. At 30% glycerol concentration, the WVP value (1.276×10^{-7} g mm cm^{-2} h^{-1} Pa^{-1}) is comparable with that reported by Nur Hazirah *et al.* (2016) on gelatin blended films with carboxymethyl cellulose, wherewith a WVP value of 1.01×10^{-7} g mm cm^{-2} h^{-1} Pa^{-1}. This shows that the composition of each matrix manages to exhibit similar interaction reactions between the respective components in the protein-polysaccharide composite films. Therefore, the WVP of the film was significantly increased with the higher glycerol concentration, yet, this phenomenon ceases when a certain interaction threshold is achieved.

3.5 Water solubility

The effects of glycerol concentration on the water solubility of chicken skin gelatin-tapioca starch composite films are shown in Table 2. The water solubility values were significantly increased ($p<0.05$) from Formulations A to E. There was a significant difference ($p<0.05$) between all formulations, except between Formulations C, D and E.

The present findings showed that the water solubility increased significantly when the glycerol concentration increased. This is attributable to the high affinity of glycerol to actively react with the –OH groups present in the starch and gelatin to make ether linkage with the available hydroxyl groups (Detduangchan, 2012). With a high concentration of glycerol available in the film formulation, excess glycerol that does not bind with the starch molecules will interact with water and interrupt the network via hydrogen bonds. This will cause a decrease in the matrix cohesiveness and increased water solubility (Farahnaky *et al.,* 2013). This can be observed from the significant increase in the water solubility for every 10% increment of glycerol added from Formulations A to C. At high glycerol concentrations (20 -40%), the polar groups (-OH) along the chains of the glycerol are able to develop the polymer-glycerol hydrogen bonds to replace the polymer-polymer interactions in the polymer matrix, leading to the high affinity toward the polar water molecules, thus increasing the film's water solubility (Nor *et al.,* 2017).

The range of water solubility in the current study (45.887 to 63.981%) is comparable to the films studied by Munoz *et al.* (2012) which were comprised of mucilage of *Salvia hispanica* and whey protein concentrate, with a water solubility ranging from 48.30 to 63.96%. The high solubility of chicken skin gelatin-tapioca starch films can be due to the hydrophilic nature of the starch, as well as the higher solubility exhibited by the gelatin. A similar increasing trend was reported by Nor *et al.* (2017) on the increase of water solubility, with a 0-20% glycerol concentration added in the chicken skin gelatin films, which ranged between 55.60 to 86.75%. This is suggesting a higher water solubility of chicken skin gelatin films as compared to the water solubility of chicken skin gelatin-tapioca starch films in the present

study (45.89 to 58.03%).

3.6 Thermal properties

The glass transition temperature (T_g) of chicken skin gelatin-tapioca starch composite films with different glycerol concentrations is presented in Table 2. There was a significant difference ($p<0.05$) between all formulations on T_g values. As the glycerol concentration in the film formulation increased, the glass transition temperature of the film decreased. Incorporating glycerol (0 to 40%) into the composite films significantly decreased the T_g value of the films.

T_g is the temperature at which the bonding of the polymers of the gelatin-starch matrix starts to relax and undergo a transition in structure from a glassy solid state to a rubbery viscous state, allowing the film to become more flexible (Zhang *et al.,* 2014). The present finding shows that the glass transition temperature of the films decreases as the glycerol concentration increases. The presence of only a single T_g for blended polymers indicates the good compatibility of the component polymers (Al-Hassan and Norziah, 2012). Glycerol in the composite films functions by creating more free volume in the polymer chains with the incorporation of increasing glycerol content; this decreases the overall cohesion and interaction forces between the polymer chains and increases the mobility of polymer chains (Wypych, 2017). As the glycerol concentration incorporated increased from Formulations A to E, the extra free volume in the intermolecular lattices created by the glycerol increased; thus, less thermal energy was required for the movement of the chains, resulting in the lowering of T_g values.

Based on the findings of the current study, the T_g of the chicken skin gelatin-tapioca starch films (79.34°C to 48.05°C) were within the range of the T_g of single bovine gelatin film which decreased from 137.5 to 56.5° C (Rivero *et al*., 2010). The similar thermal stability of the films could be related to the imino acid composition of chicken skin gelatin, which helps to stabilize the triple helix and thus increases the thermal stability of the films when heated (Sarbon *et al.,* 2013). The decrease in T_g also indicated an increase in the interactions of gelatin, glycerol and starch molecules as a result of cross-linking reaction (Podshivalov *et al.,* 2017). Additionally, the T_g of film at 30% glycerol content (Formulation D) was found to be 50.27°C, which is comparable to the gelatin-CMC film reported by Nur Hazirah *et al*. (2016) with film T_g of 56.36°C. The higher thermal stability of gelatin-starch films compared to single starch-based films is attributable to the interaction of the two biopolymers. The formation of intermolecular hydrogen bonds between OH^- of starch and NH_3^+ of gelatin increases the interchain interactions as a result of blending and cross-linking reactions.

4. Conclusion

In conclusion, different glycerol concentrations in the film formulation affected the mechanical and physical properties of the chicken skin gelatin-tapioca starch composite films. An increase in the glycerol concentrations increased elongation at break, water vapour permeability, and water solubility, but decreased the tensile strength, Young's modulus and the thermal stability of the films. Overall, Film D appeared to be the best formulation due to its low tensile strength and high elongation at break (EAB) value. Hence, Film D demonstrates the greatest potential for further food packaging application studies.

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