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MECHANICAL AND PHYSICAL PROPERTIES OF BIO-NANOCOMPOSITE FILMS BASED ON CHICKEN SKIN GELATIN WITH DIFFERENT CONCENTRATION OF CHITOSAN NANOPARTICLES

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Abstract: Plastics packaging is non-biodegradable and risks to human health and environmental pollution. In contrast, gelatin-based film lack of desired mechanical, thermal and water vapour barrier properties. Thus, this study aimed to investigate the mechanical and physical properties of bio-nanocomposite films based on chicken skin gelatin with different concentration of chitosan nanoparticles (CSNPs). Gelatin/CSNPs film solutions with different CSNPs concentration (0-8%, w/w) were stirred at 45°C for 30 min and oven-dried at 45°C. Film characterization determination includes tensile strength (TS), elongation at break (EAB), Young's modulus (YM), water solubility, water vapour permeability (WVP), film morphology and melting temperature (T_m). Results of the study indicated that incorporation of CSNPs significantly influenced film properties. The addition of CSNPs increased the TS and YM value, which lead to stronger films than the pure chicken skin gelatin films. However the addition of CSNPs decreased the EAB value. Furthermore, WVP and water solubility significantly decreased ($p < 0.05$) by the addition of 6% CSNPs. Morphology images showed that increased CSNPs reduced the film's amorphous character, especially in high level, in which higher CSNPs (8%) resulted in the aggregation of particles in the composites. The nano-reinforcement films showed higher thermal stability as compared to pure chicken skin gelatin films. In conclusion, the film with 6% CSNPs showed the best formulation, as it demonstrated high in TS, YM and T_m value, while low in EAB, water solubility and WVP value compared to other films. The results presented in this study showed the feasibility of using bio-nanocomposite technology to improve the properties of biopolymer films based on chicken skin gelatin.

Keywords*:* Bio-nanocomposite film, gelatine film, chicken skin gelatine, chitosan nanoparticles, film characterization

Introduction

Food packaging plays a vital role in preventing food from directly contaminated or contacted with contaminants, deterioration and thus lengthen the shelf-life of food (Langowski & Wani, 2016; Ramos *et al.,* 2016). The recyclable food packaging materials have been widely introduced into the packaging industry. Nevertheless, petroleum-based plastic is more commonly used than other food packaging material and it is now the second most used material for packaging (Shin & Selke, 2014). This is due to its versatility, cost-effective, require less energy to produce and can be manufactured to have many different properties (North & Halden, 2014). However, plastics bring a lot of convenience to society, but recently, public focus on plastics has centred mostly on human health and environmental concerns, including long-term pollution. Therefore, the use of biodegradable based materials for packaging may contribute to the reduction of total solid waste to certain extends (Kumar *et al.,* 2010). Biodegradable packaging such as bio-nanocomposite film has become significantly concerned by the researcher in recent years due to the claims as environmentally friendly.

The bio-nanocomposite film is defined as a multiphase material comprising of two or more constituents which are continuous phase or matrix particularly biopolymer and discontinuous nano dimensional phase or nanofiller (Othman, 2014). Bio-nanocomposite films are one of the biodegradable plastics and biopolymers. It derived from naturally renewable resources such as polysaccharides, protein, and lipid. Besides, it occupies dominantly in the current market compared with traditional petroleum-based synthetic polymers due to its environmentally sustainable and eco-efficient (Ghuttora, 2016). This is because biodegradable food packaging can be disposed into the bio-waste collection for further natural degradation into organic by-products such as carbon dioxide and water. The increasing interest in biopolymer-based packaging has resulted in the development of protein-based films. Such as soy protein, whey protein, casein, collagen, corn zein, gelatine and wheat gluten, have been investigated for their film properties (Wittaya, 2012). Among all protein sources, gelatine has also been extensively studied in recent years because of their advantages, including its film-forming capacity and its unique characteristics such as transparency, biodegradability and excellent barrier properties against oxygen and carbon dioxide (Chuaynukul *et al.,* 2015).

Gelatine is a natural water-soluble protein and it is dominantly used as a major component of biodegradable films as food packaging all over the world due to its low cost, renewable and excellent film-forming properties (Jorge *et al.,* 2015). However, gelatin films do have some limitation such as low tensile strength and poor barrier against water vapour due to its hydrophilic nature. These properties are the main drawbacks of gelatin films for the application as a packaging material (Mu *et al.,* 2012). A study by Nur Hanani *et al.* (2013) revealed that limitations of a single gelatin film can be solved by combining selected biopolymers to form composite films with better properties. Nevertheless, a study by Nazmi *et al.* (2017) revealed that a composite chicken skin gelatin films still have limitation such as poor water barrier properties. Single and composite chicken skin gelatin film has been reported to exhibit high potential as biodegradable film packaging (Nor *et al.,* 2017; Nazmi *et al.,* 2017; Rasid *et al.,* 2018).

In response to the problem, the nanoparticle is blended into gelatine based films due to its significant improvements to the polymer matrix in terms of mechanical and physical properties at lower concentration (Hosseini *et al.,* 2014). Nanoparticles have better interfacial adhesion with the polymer matrices in comparison to the respective micro or macroscopic reinforcements due to their small size and high surface to volume ratio. Incorporation of nanoparticles into conformal thin films leads it to have a large interfacial area in biopolymer matrix (Müller *et al.,* 2017). Besides, nanocomposite could exhibit dramatic modification in their molecular mobility, improve the relaxation behaviour and consequently improve the thermal and mechanical properties of the film (Mohanty *et al.,* 2015). Nanocomposite technology using nanoparticles such as organic nanoparticles (Sahraee *et al.,* 2017) and inorganic nanoparticles (Rouhi *et al.,* 2013) incorporated to the films have proved to be an effective way to enhance the mechanical and physical properties of biopolymers. A study by Hosseini *et al.* (2014) showed that the incorporation of chitosan nanoparticles into various biopolymer matrices noticeably improved the film's characteristics.

The characteristics of bio-nanocomposite films can be classified into physical and mechanical properties. Performance and functionalities as a protection layer on a food product are found to significantly affect by the physical and mechanical properties of bio-nanocomposite films. Mechanical characteristics of bio-nanocomposite films can be determined by measurement of the tensile strength (TS), elongation at break (EAB) and Young's modulus (YM) (Rouhi *et al.,* 2013). In addition, physical properties measurements of films are including water solubility, water vapour permeability, thermal properties, crystallinity properties and morphology of films (Sahraee *et al.,* 2017). Recently, several studies have investigated bio-nanocomposite films as food packaging materials. The research introduces nanoparticles as a promising option in improving the mechanical and physical properties of biopolymer-based packaging materials (Rouhi *et al.,* 2013; Sadegh-Hassani & Mohammadi Nafchi, 2014). Thus, bio-nanocomposite materials can be an alternative source for packaging products due to their biodegradability and environmentally friendly.

Hence, bio-nanocomposite films based on chicken skin gelatine and chitosan nanoparticles have been considered as a promising option for improving the properties of biopolymer-based food packaging materials to the extent the shelf life of foods and products. Chitosan nanoparticles (CSNPs) is commonly used as an antimicrobial agent and blended with other polymer films to produce antimicrobial films such as cellulose/chitosan, starch/chitosan, starch/chitosan/lauric acid, guar gum/chitosan, polyethylene oxide (PEO)/chitosan, and glucomannan/chitosan/nisin (Romainor *et al.,* 2014). This is due to their thermal stability tended to increase following CSNPs content (Hosseini *et al.,* 2016). Not only that, but it also shows improvement of thermal and mechanical properties in films (De Moura *et al.,* 2011). Therefore, this study is attempted to investigate the effect of chitosan nanoparticles as nanofiller on mechanical and physical properties of chicken skin gelatine based composite films as a material for food packaging.

Materials and Methods

Raw Materials

The fresh chicken skin was obtained from Jang Maju Enterprise, Kuala Nerus, Terengganu. The chicken skin was kept in icebox during transportation to Universiti Malaysia Terengganu. Then, the chicken skin was washed and weighted (cleaned weight). The chicken skin was stored in a freezer at a temperature of -18 ^{o}C for further experiments. Chitosan nanoparticle was purchased from Sisco Research Laboratories Pvt. Ltd., India (SRL). All chemicals used for analysis were of analytical grade.

Preparation of Chicken Skins

The frozen chicken skin was thawed in a chiller for overnight. Visible fat and feathers were removed before rinsing in excessive water to remove impurities. It was then dried in cabinet drier at 45^{o}C for overnight. The dried chicken skin was ground before defatted using Soxtec method (AOAC, 2006) and was kept in an airtight container for further use.

Gelatin Extraction

The extraction of chicken skin gelatin was conducted following the method used by Sarbon *et al.* (2013) with some modifications. A defatted dried chicken skin (15g) was soaked with 200 ml sodium hydroxide (0.15%, w/v), 0.15% (v/v) sulphuric acid and 0.7% (w/v) citric acid solutions sequentially. Each soaking treatment was repeated three times. After, each soaking treatment, the solution mixture was centrifuged at 3500 × *g* for 10 min to obtain the pellets. The pellets were rinsed with distilled water followed by centrifugation at 3500 × *g* for 10 min before final extraction. The final extraction was carried out with distilled water using a water bath shaker at a controlled temperature (45 ^{o}C) for overnight. The extract was filtered in a Büchner funnel using Whatman No. 4 filter paper. The filtered gelatin solution was evaporated under vacuum to reduce its volume to 1/10 using a rotary evaporator at 45 ^{o}C before freezing dried. The dry matter was ground to obtain gelatine powder and stored for further use.

Preparation of Bio-Nanocomposite Film

The film-forming solution was prepared based on the method described by Soo and Sarbon (2018) with some modification. The film-forming solutions were prepared by mixing 4g of chicken skin gelatine and 30% (w/w) of glycerol with added chitosan nanoparticles at 5 different concentrations (0, 2, 4, 6 and 8%, based on dried chicken skin gelatine). Chitosan nanoparticle at different concentrations was first dispersed into 50ml of 1% (v/v) acetic acid until completely dissolved. Meanwhile, chicken skin gelatine powder (4g) was dissolved in distilled water (50ml) at 45^{o}C for 30 min until a clear solution was obtained before transferred into chitosan solution and then continued to stir for another 30 min. This step was followed by the addition of glycerol as a plasticizer for continuous stirring for 30 min until complete dissolutions and then sonicated for 10 min. Then, the solution was cooled to room temperature before cast into a flat petri dish with the amount of 25g each, followed by drying in a ventilated oven at 45°C until dry. The dried films were carefully peeled off then stored in a desiccator containing silica gel before subjected to further analysis. Each film formulation was prepared in triplicates.

Tensile Strength (TS), Elongation at Break (EAB) and Young's Modulus (YM) Determination

Tensile strength (TS), Elongation at break (EAB) and Young's Modulus (YM) of films was determined using a texture analyser (TA. XT2. Plus, Stable Micro System, UK) following Nur Hazirah *et al.* (2016). The film was cut into rectangular strips of 1 × 7 cm. The film strip was placed onto texture analyser that attached with grip pairs of AT/G probe and 30 kg load cell. Initial grip length between up and down parts of the grip was set to 50 mm. The films strip was stretched by moving upper grip at a crosshead speed of 120 mm/min until broken. The tensile strength (TS) was calculated as follows:

$$\text{Tensile strength (MPa)} = \frac{\text{Maximum load (N)}}{\text{Film width (mm)x Film thickness (mm)}}$$

The elongation at break (EAB) was calculated as follows:

$$\text{EAB} = \frac{\text{Final elongation (mm)at the moment of rupture}}{\text{Initial grip length (mm)}} \text{ x 100}$$

The Young's modulus was calculated by the following equation:

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

Water Solubility Determination

The film solubility in water was determined as the following method applied by Nur Hazirah *et al.* (2016). Film pieces of 2 × 2 cm were dried in the oven at 105°C for 24 hrs and weighted to obtain initial dry weight. The dried film was immersed separately with 30 ml of distilled water in plastic containers. The container was capped and shaken gently (70 rpm) by an orbital shaker (IKA-KS501, Germany) for 24 hrs at room temperature. The remaining pieces or insoluble films that did not dissolve was filtered and rinsed with distilled water and then dried at 105°C for 24 hrs to determine the final dry weight of the film. The percentage of solubility was calculated as the following equation:

$$\text{Solubility (\%)} = \frac{\text{Initial dry film weight} - \text{Final dry film weight}}{\text{Initial dry film weight}} \text{ x 100}$$

Water Vapour Permeability (WVP) Determination

The film's water vapour permeability (WVP) was measured using a modified method by Nor *et al.* (2017). Film pieces of 2 cm × 2cm was mounted onto a clean and dry cylinder glass bottles containing 10 g silica gel (0% RH) in the bottles and sealed by using parafilm. Together with film, each bottle was weighted to obtain initial weight. The bottles were stored in a desiccator with distilled water at room temperature. The weight of bottles was recorded daily for a week and the WVP was calculated by using the following equation:

$$\text{WVP}(\text{g mm h}^{-1}\text{m}^{-2}\text{kPa}^{-1}) = \frac{\Delta\text{W (g)x} \times \text{(mm)}}{\text{t (h)x A (m}^2\text{)x }\Delta\text{P (kPa)}}$$

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

Film morphology determination

Film morphology was observed based on the method proposed by Nazmi *et al.* (2017). Morphology of film surface and cross-section was visualized by using a scanning electron microscope (SEM) (JEOL, JSM-6360LA, Japan). The film was fractured by immersion in liquid nitrogen before visualisation.

Then, the film was mounted on copper stubs perpendicularly to its surface using a double-sided adhesive tape and sputtered with gold to make the sample conductive. Images were captured at an acceleration voltage of 10 kV with magnification range 300 to 1500X.

Thermal Properties Determination

Thermal properties of the film were measured based on the method proposed by Sarbon *et al.* (2015) using differential scanning calorimeter (DSC) (DSC Q2000, USA). Approximately 5 mg ± 0.01 mg of the sample was fixed on an aluminium pan and the empty pan was used as a reference. The pan was hermetically sealed before heating over the temperature range of 25 to 200^{o}C at a scanning rate of 10 ^{o}C/min. To maintain the inert environment, nitrogen gas was used to flush the DSC cell at a flow rate of 20 ml/min. The melting temperature (T_m) was obtained from the thermograms as the temperature where the endothermic peak occurs. The measurement was conducted in duplicate for each formulation.

Statistical Analysis

All the analysis was performed in triplicate and the data was presented by mean ± standard deviation. Then, the data obtained were analysed using one-way Analysis of Variance (ANOVA) of MINITAB-16 statistical software. The significant difference between the means was determined by Fisher's test with a confidence level as $p< 0.05$.

Results and Discussion

Tensile Strength (TS)

The effect of chitosan nanoparticles concentration on TS of chicken skin gelatine/chitosan nanoparticles films is shown in Table 1. The TS values of films showed an increasing trend from 2.29 to 4.22 MPa for 0-6% CSNPs concentration added. However, the increase of CSNPs concentration up to 8% decreased the TS value of the film. The TS value of films with 2, 4, 6 and 8% was significantly higher ($p < 0.05$) than film without CSNPs.

The increase in chitosan nanoparticles concentration from 2 to 6% in the bio-nanocomposite film formulation causes an increase in the tensile strength of the film. The improvement of TS properties of bio-nanocomposite films can be attributed to different mechanisms such as the favourable chitosan nanoparticles filling of empty spaces in amorphous regions and favour a good interface between the matrix and the nanoparticles. This creates a strong network by enhancing hydrogen bonds and increasing of crystallinity in the matrix (Sahraee *et al.,* 2017). High mechanical strength of the bio-nanocomposite films may be due to efficient load transfer to the nanocrystal network, leading to more uniform stress distribution and minimize the stress concentration area (Khan *et al., 2*012). The TS value of film with 2, 4 and 6% CSNPs were significantly higher ($p < 0.05$) than control film (0% CSNPs). This is due to the electrostatic interaction between the positive charges of nanoparticles and negative charges of the biopolymer matrix. This promoted more extensive biopolymer helical and formation of the strong film (Oun & Rhim, 2017). Increasing the concentration of chitosan nanoparticles up to 8%, TS of bio-nanocomposite film were not in the increasing trend. This was due to nanoparticles did not increase the TS of bio nanocomposite films further. This is due to the lack of interaction between the nanoparticles and matrix polymer.

This study was consistent with the studies conducted by Rouhi *et al.* (2013) and Alboofetileh *et al.* (2013) on the effect of nanoparticles in both gelatin-based film and polysaccharides-based film. They found that TS of bio-nanocomposite film was increased with the increased of nanoparticles concentration. However, above a certain concentration, TS value of film might be decreased. Hosseini *et al.* (2014) claimed that increased in TS value of bio-nanocomposite film remarks the enhanced stiffness of the film because of the nanoparticles reinforcement effect. This was due to effective interactions between homogeneously dispersed nanoparticles and biopolymer matrix. Thus, it created strong intermolecular forces between nanoparticles and biopolymer matrix and increased the crystallinity of film (Sahraee *et al.,* 2017). Contrary, the reduction of TS of bio-nanocomposite films could be attributed by the agglomeration of nanoparticles in the film (Abdollahi *et al.,* 2013a). The agglomeration of nanoparticles could reduce the interaction of nanoparticles in the polymer matrix, thus lead to lower TS value of bio-nanocomposite film when too many nanoparticles were added.

Elongation at Break (EAB)

The effect of chitosan nanoparticles concentration on EAB value of chicken skin gelatin/chitosan nanoparticles composite films is shown in Table 1. The EAB values of the film were showed a decreasing trend from 270.92 to 190.40% for the film with 0, 2, 4 and 6% CSNPs. However, the EAB value increased as nanoparticles increased to 8%. The EAB value of film with 6 and 8 % CSNPs was significantly decreased ($p < 0.05$) compared with the film without CSNPs added. The decreased in EAB could be due to the rigid nature of nanoparticles that restricts the motion of biopolymer matrix in terms of increase in intermolecular attractive forces, making the polymer network denser and thus less permeable (Alboofetileh *et al.,* 2013; Khan *et al.,* 2012). Besides, strong interactions and bonding formed between polymer chains limit the mobility of polymer chains and therefore leading to low extensibility of the films. However, increasing of nanoparticles is an indication of an increase in the brittleness of the film. Therefore, the increased of EAB value at 8% CSNPs caused the agglomeration of nanoparticles in the matrix polymer which lead to lower interface interaction.

The result obtained in this study agreed with the studies conducted by Müller *et al.* (2017) and Khan *et al.* (2012) on the effect of nanoparticles in both protein and polysaccharide-based film. They found that EAB of bio-nanocomposite film decreased with the increased of nanoparticles concentration. At 6% CSNPs concentration, the EAB value in the current study was 190.4%, which was comparably higher than the fish gelatine/chitosan composite film (44.7%) (Hosseini *et al.,* 2014). The superior film elongation properties of the chicken skin gelatine films could be due to high viscosity and elasticity of chicken skin gelatine. The higher viscosity in chicken skin gelatine/chitosan nanoparticle film, the solution will lead to higher retention of water molecule during the drying of the film (Daud *et al.,* 2012). Thus, decreasing intermolecular attraction and increasing polymer mobility which facilitates film elongation.

Young's Modulus (YM)

The effect of chitosan nanoparticles concentration on the YM of chicken skin gelatine/chitosan nanoparticles films is shown in Table 1. The YM of the film had a similar increasing trend with TS properties (as previously discussed) for the film with 0, 2, 4, 6% CSNPs, however, the value decreased for the film with 8% CSNPs. The YM value of chicken skin gelatine film increased significantly with the increased of CSNPs concentration from 2 to 6%. The increased of YM can be related to good interfacial interaction between nanoparticles and the biopolymer matrix. Besides favourable of CSNPs in filling of empty spaces in gelatine polymer, it also creates a strong network by enhancing hydrogen bonds and increasing crystallinity in the matrix (Sahraee *et al.,* 2017). Another study reported that electrostatic interaction between the positive charges of CSNPs and negative charges of chicken skin gelatine polymers potentially promote more extensive biopolymer helical and formation of the strong film (Oun & Rhim, 2017). Similarly, to the TS value, the decreased of YM value for the film with 8% CSNPs was due to nanoparticles did not interact well with the matrix polymer which causes lower interface interaction.

The result in this study was consistent with the study conducted by Hosseini *et al.* (2014) and

Sadegh-Hassani & Mohammadi Nafchi (2014) on the effect of nanoparticles in both protein and polysaccharide-based film. They found that YM of the bio-nanocomposite film increased with the increased of nanoparticles concentration. However, above a certain concentration, YM value of the film might decrease. Khan *et al.* (2012) claimed that nanofiller reinforced films usually tend to become more brittle as the concentration of the reinforcing particles increased and thus increased the stiffness of the film. Moreover, incorporation of nanoparticles into polymer matrix resulted in strong interactions between nanofiller and matrix, which restricted the movement of the matrix and hence increased the YM value of the film.

Water Solubility

The effect of CSNPs concentration on the water solubility of chicken skin gelatine/chitosan nanoparticles films is shown in Table 1. The CSNPs concentration on the water solubility of the film showed a decreasing trend (from 83.61 to 52.42%). The water solubility of the film with CSNPs concentration from 2 to 6% was significantly decreased ($p < 0.05$) compared to film with CSNPs concentration 8%. The water solubility of bio-nanocomposite films was decreased as the CSNPs concentration increased. This was attributable to the consequence of enhancing interaction of chitosan nanoparticles and gelatine matrix which lead to more compressed structure and suppressed the diffusion of water into the structure (Sahraee *et al.,* 2017). The strong hydrogen bond between the nanoparticles and the film matrix was reported to cause significant reduction in water solubility of films due to water molecules unable to break the hydrogen bonds sufficiently (Sahraee *et al.,* 2017). The water solubility of 6% film was the lowest value due to high crystallinity of nanoparticles and the strong hydrogen-bonded networks with the polymer matrix. It was mentioned that incorporation of nanoparticles improved the cohesiveness of biopolymer matrix while decreasing water sensitivity (Abdollahi *et al.,* 2013b). The decreased water solubility value at 8% CSNPs could be due to the agglomeration of nanoparticles occurs in the matrix polymer.

The result in this study was inconsistent with the studies conducted by Hosseini *et al.* (2014) and Abdollahi *et al.* (2013b) on the effect of nanoparticles in both protein and polysaccharide-based film. They found that water solubility of bio-nanocomposite film decreased with the increased of nanoparticles up to a certain extend. The water solubility of chicken skin gelatine/chitosan nanoparticles film obtained was comparably lower (52.42%) than the fish gelatine/chitosan composite film (85.64%) (Jridi *et al.,* 2014). This was due to the formation of the strong interaction between small particles size fillers and biopolymer matrix which lead to more compressed structure and suppressed the diffusion of water into the structure (Sahraee *et al.,* 2017). Thus, incorporation of nano-sized particles enhanced the water solubility of films as compared to micro-sized particles.

Water Vapor Permeability (WVP)

The effect of CSNPs concentration on WVP of chicken skin gelatine/chitosan nanoparticles films is shown in Table 1. The WVP of the film shown a decreasing trend for the film with CSNPs concentration from 2 to 6%, however, WVP increased for the film with CSNPs concentration of 8%. WVP value for film with CSNPs concentration from 2 to 8% were significantly decreased ($p < 0.05$) compared to film with CSNPs concentration of 0%.

The present findings showed that water vapour permeability of bio-nanocomposite films decreased when the CSNPs concentration was increased. This was due to the impermeable nature of the CSNPs, forcing gas molecules wiggle around them instead of taking a straight path perpendicular to the surface of the film. This would increase the effective path length for the diffusion of gas molecules through the film and reduce WVP of the film (Müller *et al.,* 2017). According to Duncan (2011), a nanoparticle has been attributed to the creation of a tortuous pathway for water vapour to diffuse in or out of the bio-nanocomposite matrix. Decreasing WVP of the film also due to the formation of hydrogen bonds between CSNPs and chicken skin gelatine matrix, which lead to the decrease in the number of free hydroxyl groups that give the hydrophilic character to biopolymers (El Miri *et al.,* 2015).

The result obtained was consistent with the studies conducted by Sahraee *et al.* (2017) and Khan *et al.* (2012) on the effect of nanoparticles in both protein and polysaccharide-based film. They found that WVP of the bio-nanocomposite film decreased with the increased of nanoparticles concentration. However, above a certain concentration, WVP of the film could decrease. The WVP of chicken skin gelatine/chitosan nanoparticles bio-nanocomposite film that obtained in the current study (1.31 g mm/h m^2 kPa) was comparably lower than the fish gelatine/chitosan composite film (2.872 g mm/h m^2 kPa) (Jridi *et al.,* 2014). This was because of nanoscale fillers have a much higher surface area to volume ratio and much higher interfacial volume as compared to micro-sized fillers that blended into films (Müller *et al.,* 2017). The good interface between nanofillers and biopolymer matrix, creating a strong network and increases the effective path length for the diffusion of gas molecules through the film. Incorporation of nano-sized particles improved the gas barrier properties of films as compared to micro-sized particles.

Thermal Properties

The melting temperature (T_m) of chicken skin gelatine/chitosan nanoparticles films with different CSNPs concentration are presented in Table 1. The T_m of the film shown an increasing trend for the film with CSNPs concentration from 0 to 8%. The T_m value for film with CSNPs concentration from 2 to 8% were significantly increased ($p < 0.05$) compared to film with CSNPs concentration of 0%.

The increase of T_m of chicken skin gelatine film with the increased of CSNPs concentration can attribute to the 2 important mechanisms (Frone *et al.,* 2013). The first mechanisms related to the effect of heterogeneous nucleation mechanism of nanoparticles which induces a decrease of the free energy barrier and fasten the crystallization of film. The second mechanisms are nanoparticles modifies the orientation of polymer chain, causing to more regular and compact chain arrangement which turn larger size and more perfect crystals. The T_m of the film was mainly affected by the strength of intermolecular forces, as well as by the degree of the flexibility and length of the chain. According to Balakrishnan *et al.* (2017), the high melting temperature of the film was due to the polar side such as the hydroxyl group that enhance the strength of the intermolecular bonds.

The finding was in an agreement with the studies conducted by Sahraee *et al.* (2017) and Chen *et al.* (2001) who found that T_m of bio-nanocomposite film increased with the increased of nanoparticles concentration. Choo *et al.* (2016) claimed that the formation of a hydrogen bond between nanoparticles and biopolymer matrix restricts the movement of molecular chains because of cross-linking and hence improved the thermal properties of bio-nanocomposite films. Contrary, T_m of bio-nanocomposite films reduced due to the degree of crystallinity and crystallization rate can be affected by crystallization in narrow spaces (Choo *et al.,* 2016). If the space is so narrow, the spherulitic growth is restricted, primary nuclei are not available for heterogeneous crystallization. Consequently, homogeneous nucleation appears. This can lead to a low crystallization rate, degree of crystallinity and melting point of the film (Müller *et al.,* 2017).

Table 1: Tensile strength (TS), elongation at break (EAB), Young's modulus (YM), water solubility, water vapour permeability (WVP) and melting temperature (T_m) of chicken skin gelatin-based nanocomposite films with different CSNPs

CSNPs concentration (%)	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)	Water solubility (%)	Water vapour permeability (g mm/ h m^2 kPa)	Melting point (^{o}C)
0	2.29 ± 0.41^{c}	270.92 ± 17.02^{a}	0.85 ± 0.14^{c}	83.61 ± 3.41^{a}	2.72 ± 0.52^{a}	149.27 ± 0.99^{b}
2	3.76 ± 0.03^{ab}	245.32 ± 26.28^{a}	1.54 ± 0.17^{b}	68.14 ± 1.49^{b}	2.04 ± 0.41^{b}	155.16 ± 2.69^{a}
4	4.11 ± 0.15^{a}	239.64 ± 6.67^{ab}	1.72 ± 0.11^{b}	66.15 ± 0.80^{b}	1.75 ± 0.25^{bc}	155.90 ± 1.20^{a}
6	4.22 ± 0.41^{a}	190.40 ± 7.52^{c}	2.22 ± 0.25^{a}	52.42 ± 1.21^{c}	1.31 ± 0.09^{c}	157.37 ± 1.64^{a}
8	3.27 ± 0.35^{b}	204.57 ± 34.78^{bc}	1.65 ± 0.47^{b}	53.15 ± 1.12^{c}	1.97 ± 0.39^{bc}	157.80 ± 0.01^{a}

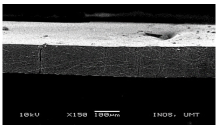
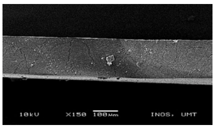
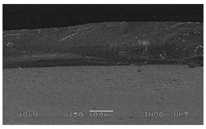
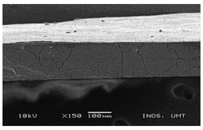
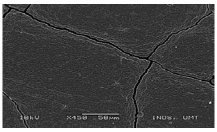
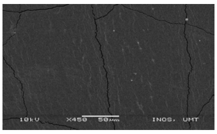
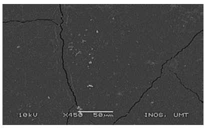
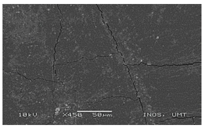
All data reported are mean values ± standard deviations. The different superscript letter $^{(a-c)}$ indicate a significant difference ($p < 0.05$) within the same column.

Film Morphology

Figure 1 shows the micrographs corresponding to the surface section and cross-section of chicken skin gelatine/chitosan nanoparticles films of the different formulation. The microstructure of films highly depends on the interaction between the film components and drying conditions, which affects the final physical properties of the films (Acosta *et al.,* 2015). The morphology surface section of the films with CSNPs 0 and 2% was smoother, more homogeneous, and fewer cracks than the film with CSNPs 6 and 8%. This was due to the proper interaction occur between film matrix and nanoparticles caused to have good compatibility (Sahraee *et al.,* 2017). This finding was consistent with the study conducted by Khan *et al.* (2012), who revealed that the addition of nanoparticles may cause changes in the film surface section, since the non-reinforced films exhibited a smooth surface with few cracks, as expected for a homogeneous material. However, morphology surface section of the films with 6 and 8% CSNPs showed rough surface, with increasing density of crack deflection sites that resulted in an increasing number of ripples and ridges as nanoparticles concentration increased. This was due to poor distribution and most of the CSNPs were agglomerated by hydrogen-bonded free hydroxyl groups (Azizi & Mohamad, 2018). The similar finding also demonstrated in Abdollahi *et al.* (2013a) that the dispersion of nanoparticles at the higher loading levels of cellulose nanoparticles (5%) was not uniform and showed some agglomeration.

The morphology cross-section for films with CSNPs concentration of 2, 6 and 8% was denser and compact structure compared to film with 0% CSNPs. This is explained by the improved mechanical and barrier properties of the nanocomposite films due to good bonding strength between nanoparticles and polymer (Nagarajan *et al.,* 2015). Anionic and cationic nature of matrix and nanoparticles can cause strong interaction between film and filler molecules (Jridi *et al.,* 2014). Hosseini *et al.* (2014), revealed that the morphology cross-section of the fish gelatine films incorporated with 2, 6 and 8% CSNPs was relatively denser structure compared to the pure fish gelatine film.

Table 2: Cross-section and surface section morphology of bio-nanocomposite films formulations at different chitosan nanoparticles concentrations

	Formulations			
	0%	**2%**	**6%**	**8%**
Cross-section				
Surface section				

Conclusion

Incorporation of different concentration of chitosan nanoparticles in the chicken skin gelatine-based film formulation affects the mechanical and physical properties of bio-nanocomposite films. The increased of CSNPs concentration increased the tensile strength, Young's modulus and melting temperature of the films, but decreased the elongation at break, water solubility and water vapour permeability of the films. The film with higher CSNPs concentration possessed a compact and denser biopolymer structure, leading to a good bonding strength between nanoparticles and biopolymer as shown in morphologies studies. The film with 6% CSNPs appeared as the best formulation attributed to the significantly improved the mechanical and physical properties of bio-nanocomposite films. Hence, the film with 6% CSNPs holds high potential for further food packaging application studies.

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