J Food Sci Technol (September 2021) 58(9):3338–3345
https://doi.org/10.1007/s13197-020-04893-6

ORIGINAL ARTICLE

Effect of pH on functional, gas sensing and antimicrobial properties of bio-nanocomposite gelatin film for food packaging application

N.N. Azizun[1] · Wan M. Khairul[2] · Adibah Izzati Daud[3] · N.M. Sarbon[1]

Revised: 3 October 2020 / Accepted: 6 November 2020 / Published online: 21 November 2020

Abstract A bio-nanocomposite film is a polymer blend with nanofiller dispersed in a biopolymer matrix. The aim of this study is to investigate the functional, gas sensing and antimicrobial properties of bio-nanocomposite films incorporated with chicken skin gelatin/ tapioca starch/zinc oxide at different pH levels (pH 4, 6, 7 and 8). Bio-nanocomposite films were prepared using a casting technique followed by the characterization of their functional, gas sensing and antimicrobial properties. Film formulations with pH at different levels showed increased thickness, colour and water vapour permeability (WVP) ($p < 0.05$). In addition, the increase of pH in films in chicken skin gelatin bio-nanocomposite films increased the tensile strength (TS), while decreasing the elongation at break (EAB). The highest response for ammonia gas in chicken skin gelatin bio-nanocomposite films was obtained at pH 7, with quick response time (τ_{res}) within 10 s. The inhibition zone of *Staphylococcus aureus* in chicken skin gelatin bio-nanocomposite films increased with increasing pH levels. Overall, chicken skin gelatin bio-nanocomposite films with a pH level of 8 were found to have the optimal formulation, with the highest values in thickness, and TS, with the lowest values for WVP and EAB. In conclusion, bio-nanocomposite chicken skin gelatin films with an alkaline pH are a superior packaging material.

Keywords Food packaging · Bio-nanocomposite films · Zinc oxide nanoparticles · pH of films · Chicken skin gelatin · Gas sensing

✉ N.M. Sarbon
norizah@umt.edu.my

[1] Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[2] Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[3] Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Malaysia Perlis, 02100 Padang Besar, Perlis, Malaysia

Introduction

Packaging is defined as a coordinated system of ensuring high-quality, safe food products delivery to satisfy consumers and which provides containment, security, storage, communication, utility and performance (Robertson 2013). Food packaging is typically made from non-biodegradable materials such as polylactic acid (PLA) and polystyrene (PS) which can cause environmental problems and pollution (Othman 2014). Nowadays, interest has increased in using eco-friendly materials from bio-polymers in biodegradable packaging to enhance food quality, health and prolong shelf life (Nazmi et al. 2017; Ahmad et al. 2012). Unfortunately, bio-composite films still have drawbacks in terms of mechanical, thermal and barrier properties (Othman 2014). Therefore, researchers aim to develop friendlier packaging with the incorporation of bio-composite films with nano-fillers to produce bio-nanocomposite films with superior properties (Shapi'i and Othman 2016).

A bio-nanocomposite film is a polymer mixture with a nano-filler in which polymer matrices are in a continuous phase and nano-filers (nanoscale 1–100 nm) are in a discontinuous phase to create a stable multiphase material framework (Shankar et al. 2016). Bio-nanocomposite films have improved physical properties compared to composite films, such as water vapour permeability, thermal

properties, and film thickness. The use of nano-filler in bio-nanocomposite films has attracted interest because it can provide antimicrobial properties for food packaging (Lee et al. 2020). Nano-fillers that have been used in film production include cellulose, chitin, chitosan, clay, metal (silver, copper) and metal oxides (zinc oxide).

Zinc oxide is metallic nanofiller mostly used for its excellent ability to interface and disperse particles in a protein matrix. It offers high surface energy and a large volume ration region (Shankar et al. 2016). Zinc oxide blended with bio-composite film has been generally recognized as safe (GRAS) by the Food and Drug Administration (Espitia et al. 2013). In addition, zinc oxide inhibits the growth of Gram-positive and Gram-negative bacteria and also spores that can otherwise survive under high thermal conditions (Lee et al. 2020). Specific processing conditions such as pH treatments have been used to enhance zinc oxide nanoparticle (NP) dispersion in the polymer matrix and enhance the properties of nanocomposite films (Nagarajan et al. 2015). Increasing interest in eco-friendly packaging has led to the creation of starch, protein and lipid-based bio-nanocomposite films. Protein-based films such as gelatin have also attracted attention for the improvement of film characteristics (Said and Sarbon 2019; Alias and Sarbon 2019). To date, to the best of our knowledge, no studies have been conducted on the effects of pH on gelatin-based films.

Gelatin can be obtained from the skin, tendon and connective tissues. It has a positive charge when its pH is lower than the isoelectric point, and if the pH is higher than the isoelectric point it will have a negative charge (Ma et al. 2018). Electrostatic repulsion between gelatin molecules occurs beyond the isoelectric point (pI) of pH values; thus, pH modification can also be used as an alternative method for enhancing physical and mechanical properties. There have been many studies carried out on gelatin used in bio-nanocomposite film, including chicken skin gelatine/zinc oxide NP (Lee et al. 2020), bovine gelatin (Martucci and Ruseckaite 2010) and fish skin gelatine/zinc oxide NP (Arfat et al. 2016). Chicken skin gelatin has been reported to have strong potential as an alternative to other commercial gelatins due to its high bloom value (355 g) (Sarbon et al. 2013). However, single chicken gelatin film has a drawback in terms of its water vapour barrier properties. The pH value of the composite films is one important factor that needs to be concerned in order to control the physical and mechanical properties of films (Nazmi et al. 2017).

Therefore, the main purpose of this work was to improve single chicken skin gelatin films with incorporation of tapioca starch and zinc oxide nanoparticles at different pH levels. The physical, mechanical, gas sensing and antimicrobial properties of the bio-nanocomposite films as affected by different pH levels were also investigated.

Materials and methods

Fresh chicken skins and tapioca starch were obtained from a local market in Kuala Terengganu, Malaysia. The skins were kept on ice during transport to the laboratory and stored at −80 °C after being washed and weighed. Zinc oxide nanoparticles were purchased from Sisco Research Laboratories, India. All reagents, including solvents, chemicals, and materials, were purchased from different commercial suppliers and were used without further purification.

Preparation of chicken skin

The frozen chicken skins were thawed overnight in a chiller (4–5 °C). The thawed chicken skins were washed thoroughly to remove impurities, cut into 2–3 cm pieces, and dried in a cabinet dryer overnight at 55 °C. The completely dried skins were then ground up using a Waring blender (PANA MK-5087M, Panasonic, Shah Alam, Malaysia) before being defatted using the Soxhlet method (AOAC 2006). The defatted chicken skins were measured to obtain the dry weight.

Gelatin extraction

Gelatin was extracted from the chicken skins according to the method used by Lee et al. (2020). To extract the gelatin, 15 g of defatted dried chicken skin was added with 200 ml sodium hydroxide (0.15%, *w/v*). The mixture was blended well at room temperature for 30 min before centrifugation at 3500 × *g* for 10 min. Then, the resultant pellets were mixed with 200 ml of 0.15% (w/v) sulphuric acid, followed by 200 ml of 0.7% (w/v) citric acid. Each treatment was repeated three times. The pellets were then washed with distilled water for 10 min. Final extraction was carried out in distilled water in controlled temperature (45 °C) for overnight shaking. The resultant mixture was filtered with Whatman filter paper (no. 4). The extracted gelatin was concentrated using a rotary evaporator until its volume was reduced to 1/10 of original volume at 45 °C and then freeze dried. The freeze-dried gelatin powder was grounded, weighed and stored for further used. The yield of gelatin was calculated based on dry weight of chicken skin as shown in Eq. (1):

$$\text{Yield of gelatin } (\%) = \frac{\text{Weight of gelatin powder (g)x 100}}{\text{Weight of defatted dried skin (g)}} \quad (1)$$

Preparation of bio-nanocomposite film

Chicken skin gelatin/tapioca starch/zinc oxide NP (ZnONP) composite films were prepared using the casting method following Lee et al. (2020). ZnONP stock solution was prepared by dissolving 0.12 g of ZnONP for 1 h in 100 ml boiling distilled water. Then, 4 g of chicken skin gelatin powder, 0.8 g of tapioca starch and 30% (*w*/w) of glycerol were measured. Chicken skin gelatin powder and tapioca starch were separately dissolved in distilled water at 45 and 80 °C, respectively. The chicken skin gelatin solution was added into the tapioca starch solution and stirred continuously, followed by the addition of ZnONP solution. After that, glycerol was added at 45 °C for 30 min under continuous stirring. The pH levels were adjusted to pH 4.0 (Formulation A), pH 6.0 (Formulation B), pH 7.0 (Formulation C) and pH 8.0 (Formulation D) with 1 N HCL and each blend was maintained at 80 °C with constant stirring for 30 min. Afterward, 25 ml of bio-nanocomposite solution was then poured into a petri dish. The films were dried in oven dryer until completely dried. The physical, mechanical and antimicrobial properties of the chicken skin gelatin/tapioca starch/ZnONP composite films were then investigated.

Physical properties of bio-nanocomposite films

Water vapour permeability

Water vapour permeability was determined using the method described by Soo and Sarbon (2018). Firstly, 10 g of silica gel (0% RH) was weighed and placed in a glass bottle. Then, a 22 mm diameter glass vial containing silica gel was sealed and weighed along with the film to measure the initial weight before being put in a desiccator filled with distilled water. The weight of the glass bottle was measured at 1 h intervals over an 8 h period. The water vapour permeability of film was calculated as in Eq. (2):

$$\text{Water vapour permeability } \left(\text{gmm}/\text{m}^2\text{sPa}\right) = \frac{\text{W} \times \text{X}}{\text{A} \times \text{t} \times \Delta\text{P}} \quad (2)$$

where W is the weight gained by the cup (g), X is the average of film thickness (mm), A is the region of permeation (m^2), t is the time gained (s), and ΔP is the difference of partial pressure (Pa).

Thermal properties

The thermal properties of the bio-nanocomposite films were determined using a differential scanning calorimeter (DSC Q2000, TA Instrument, Pittsburgh, PA USA) following Lee et al. (2020). Approximately 5 mg (±0.001) of film was weighed using a micro balance (MYA 5.4Y, Radwag, Radom, Poland) and placed in a hermetically sealed aluminium sample pan. The reference was an empty pan, sealed with a lid to provide a sufficient heat power. The samples were measured at a heating rate of 10 °C/min ranging from 0 to 175 °C. The temperature of the denatured gelatin film was taken at which one half as the top of the peak. The total energy required to denature the film (the enthalpy change, ΔH) was obtained by measuring the area under the peak. The endothermic peak was selected as the melting temperature for gelatin film. Each reading was carried out in triplicate and the average recorded.

Gas sensing evaluation of ammonia (NH_3)

The responses of bio-nanocomposite films based on chicken skin gelatin/tapioca starch/ZnONP (A-D) with an optical gas sensor in five different time intervals (0 s, 10 s, 1 min, 15 min, 30 min) were investigated by direct exposure of area 4 cm^2 films to ammonia gas (NH_3) in a close vessel. The *in-situ* measurements were performed using UV-visible spectrophotometer (Shimadzu 1601 series) following Khairul et al. (2017). The absorbance values obtained were used to calculate the responses of A-D films towards NH_3 using Eq. (3):

$$\text{R } (\%) = \frac{\text{A}_0\text{--A} \times 100}{\text{A}} \quad (3)$$

where A_0 represents the absorbance films after exposure to NH_3, while *A* represents the baseline absorbance of A-D films (in air).

Mechanical properties of bio-nanocomposite film

Tensile strength (TS) and elongation at break (EAB)

Tensile strength (TS) and elongation at break (EAB) were measured following Nur Hazirah et al. (2016). The TS and EAB values were measured using a Texture analyser (Stable Microsystem, TA.XT Plus, Godalming, Surrey, United Kingdom). Each film was cut into 10 × 70 mm rectangles. The film thickness was measured using a micrometre (Digital Micrometer 406-350, Mitutoyo Corp., Kanagawa, Japan) at five different locations to obtain the average thickness to determine the elastic behaviour of films. Each film strip was mounted with a 50 N load cell between the grip pairs and 50 mm away from the film strips. The speed of crosshead was set at 1 mm/s. Then, the upper grip was moved until the film broke through the load cell. Tensile strength (TS) was calculated using the formula given in Eq. (4):

$$\text{Tensile strength } (\text{MPa}) = \text{F}_{\text{max}}/\text{A} \quad (4)$$

where F_{max} is max load (N) needed to pull the sample apart and A is cross sectional area (mm^2) of film sample.

Elongation at break (EAB) was calculated according to the following formula as in Eq. (5):

$$\text{Elongation at break } (\%) = (l_{max}/l_0) \times 100 \quad (5)$$

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

Antimicrobial properties

Disc diffusion method

The antimicrobial activity of film samples was measured using the disc diffusion according to Lee et al. (2020). The growth of pathogenic bacteria, Gram-positive bacteria (*Staphylococcus aureus*) were observed. Both bacteria were cultured on Tryptone Soya Agar (TSA) for 18 h at 37 °C. Mueller-Hinton agar was poured into petri dish and the agar was allowed to solidify. A single colony of bacteria was inoculated and spread evenly on each Petri dish using a sterilized swab. The dishes containing the bacteria cultures were pre-incubated at 37 °C for 10 min. The films were cut into a circular shape with a diameter of 4.5 mm. Then, the films were placed onto the agar containing bacteria culture using sterilized forceps. The plates then underwent 24 h of incubation at 37 °C. The inhibition zone was measured using a ruler in mm. Each reading was carried out in triplicate and the average recorded.

Statistical analysis

Statistical tests were performed using Minitab version to analyse the data obtained from all analysis. One Way Analysis of Variance (ANOVA) values were calculated and the significant differences among the value of each film properties were determined using Turkey's test with a significance level of $p < 0.05$.

Results and discussion

Physical properties of the bio-nanocomposite films

Water vapour permeability

The effects of pH on the water vapour permeability (WVP) of chicken skin gelatine/tapioca starch/ZnONP composite films are shown in Table 1. There were no significant differences in WVP values between all formulations ($p > 0.05$).

The highest WVP values in this study were obtained by the films in acidic (pH 4) and neutral condition (pH 7). The WVP values were also influenced by the thickness of films (Hamaguchi et al. 2007). The thickness value obtained in the current study, showed that films at pH 4 (0.176 mm) and pH 7 (0.189 mm) had the lowest thicknesses as compared to other formulation. The lowest thicknesses will develop fewer hydrogen bonds between the chicken skin gelatin, tapioca starch and ZnONP, leading to a less compact structure with larger inter-chain spaces in the film matrix (Lee et al.2020). This will increase the transmission of water vapour through films (Chinabhark et al. 2007). However, the highest WVP value obtained in acidic condition may also be due to less intermolecular crosslinking, thus resulting in films with a lower strength and a less dense structure. Moreover, addition of tapioca starch in gelatin film will influence the WVP values due to formation of strong cohesive bond between the polymer in film formulation, thus resulting in higher thickness as well as lower WVP values (Loo and Sarbon 2020).

Similarly, a study by Nagarajan et al. (2015) on tilapia skin gelatin/Cloisite Na^+ also obtained decreased WVP values from 2.3 to 1.96×10^{-11} gmm/m^2s^1Pa as pH levels increased. This is because more intermolecular protein crosslinking occurred under alkaline conditions due to repulsive forces developed in the gelatin film formulation. This will result in a dense film structure, reducing the WVP value (Chinabhark et al. 2007). The findings in the current study are also in agreement with a study by Lee et al. (2020) on chicken skin gelatin, tapioca starch and ZnONP composite films, in which the WVP values obtained ranged from 1.52 to 1.24×10^{-8} gmm/m^2s^1Pa. The similarly decreased WVP values obtained were due to the inclusion of ZnONP in film formulation. The reinforcement of ZnONP in film formulation will improve the WVP value, as more compact structures are formed which reduce the water vapour migration rate through the film (Arfat et al. 2016).

Thermal properties of films

The effects of pH on melting temperature (T_m) of chicken skin gelatine/tapioca starch/ ZnONP composite film are presented in Table 1. The current results showed that the T_m values of chicken skin gelatine/tapioca starch/ZnONP composite film were not influenced by the pH level. All films exhibited a single endothermic peak in range of 48.12 to 51.18 °C. The single endothermic peak in all film formulations indicated the homogeneity of the films under acidic and alkaline conditions (Tongdeesoontorn et al. 2011). The presence of the endothermic peak was due to the breakage of the hydrogen bonds, accompanied by the convergence of various processes such as water evaporation, melting and recrystallization. The imperfections in gelatin crystallites and the interaction of glass transition of

Table 1 Water vapour permeability and melting temperature of chicken skin gelatine/tapioca starch/ZnONP composite films at different pH level

Formulation	Water vapour permeability (gmm/m^{2}sPa)	Melting temperature (°C)
A	1.76 ± 0.32^a	49.14 ± 2.02^a
B	1.55 ± 0.11^b	51.18 ± 5.21^a
C	1.75 ± 0.39^a	48.12 ± 1.60^a
D	1.47 ± 0.11^b	50.80 ± 0.57^a

Water vapour permeability and melting temperature of film formulation at different pH levels. A = film with pH 4; B = film with pH 6; C = film with pH 7; D = pH 8. The different superscript letters ($^{a, b}$) within column indicate a significant difference ($p > 0.05$). Data reported are mean values ± standard deviation

α-amino acid also block the polypeptide chains (Soo and Sarbon 2018). The increment of T_m from pH 4–6 was mainly due to the formation of intramolecular forces such as hydrogen bonds between chicken skin gelatin, tapioca starch and ZnONP in films (Sahraee et al. 2017). Therefore, a compact and homogenous structure was formed between the components, requiring more heat energy to break the bonds in the film and increasing the melting point (Lee et al. 2020).

The findings in this study were in contrast with the study by Lee et al. (2020), who studied chicken skin gelatin/tapioca starch incorporated with ZnONP in which no pH treatment was done on the film (T_m 61.86–93.42 °C). The current study obtained lower T_m values due to the pH treatment of the film. The pH level in film matrix leads to decrease the degree of electrostatic repulsion and ionic interaction in the chicken skin gelatin. The faster denaturation of protein in acidic and alkaline conditions can evolve and modify its properties by disrupting three-dimensional structures and the hydrogen bonds in the film formulation (Shankar and Rhim 2019). Therefore, less heat is required to break the structure and bonds of the film matrix.

Sensing evaluation of the sensor towards NH_3 for application in detecting fish spoilage

The sensing response towards ammonia (NH_3) gas of the bio-nanocomposite films based on chicken skin gelatin incorporated with tapioca starch and zinc oxides was observed via the differences in UV-visible spectra before and after the films were exposed to NH_3 gas. Figure 1 shows the sensing response of the optical A-D films with various time intervals (0 s, 10 s, 1 min, 15 min, and 30 min) of exposure towards NH_3 to define the responsiveness of the optimum film condition for the detection of NH_3 gas.

The time-dependent response mechanism of the gas sensor towards NH_3 gas was operated at room temperature (T = 28 ± 1 °C). All of the experimental work-ups were carried out for 30 min in the ambient environment of standard relative humidity between 80 and 85%. The

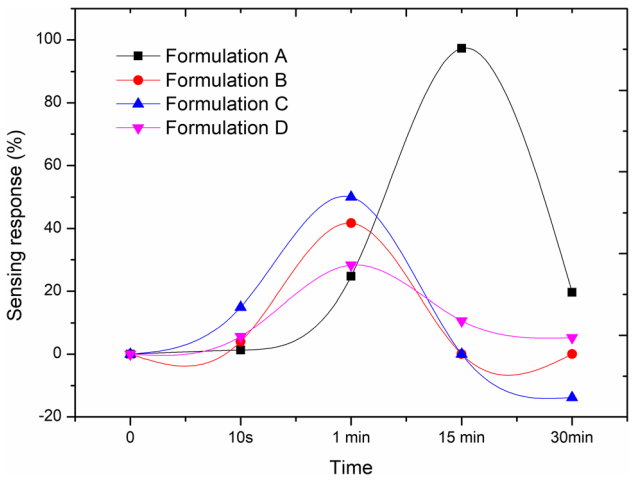

Fig. 1 Sensing response of film formulation at different pH level. A = film with pH 4; B = film with pH 6; C = film with pH 7; D = pH 8

results revealed that bio-nanocomposite films based on chicken skin gelatin/tapioca starch/ZnONP composite at pH 7 (Formulation C) exhibited the highest sensing response of 15% to ammonia gas with the shortest response time of within 10 s. Differences in UV-visible spectra are associated with the formation of phenolic oxygen anions in tapioca starch from functional moieties of amylose and amylopectin. The ammonia (NH_3) volatile gas is supposed to diffuse into the film to form hydrated ammonia (NH_3.-H_2O) and then hydrolyzed to produce hydroxyl ions (OH) and ammonium ions (NH_4^+) in the following process:

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$$

The increases and decreases of absorbance values are affected by the condition of the film at different pH values. The sensing response of the shortest time within 10 s decrease to the lowest values of 1.3%, 4.0% and 5.6% for Formulation A, Formulation B, and Formulation D respectively, indicated that the interaction between bio-nanocomposite films is at the weakest point in acidic and alkaline condition (pH 4, 6 and 8). Additionally, when prolonging the time of exposure between films and NH_3 gas to 15 min, the sensing response of both neutral and alkaline conditions started to decrease drastically. This is

because of the participation of NH_3 gas embedded in the films, which due to the alkalinity nature of NH_3 gas, can increase the pH values of the films. A prolonged exposure time between film and NH_3, except for Formulation A, increased the sensing response to 97.3%. This is because the initial pH value (pH 4) of Formulation A increased with the addition of NH_3 to the neutral point, allowing the film to have excellent interaction with NH_3 at a neutral condition. However, all types of film formulations (A-D), decreased to the point of saturation when they reached 30 min of exposure time with NH_3, due to the change of film condition from neutral to alkaline, revealed the weak sensing response towards ammonia gas due to its compact structure; the excess O-H group in the alkaline condition of the film unable to bind with the compound present in gelatin films. This finding was in accordance with a study by Korposh et al. (2006), who reported that only neutral condition of nano-assembled thin film gas sensors could accelerate the response between bio-nanocomposite films and ammonia gas. The quick and strong sensing response of the indicator films is an expected and essential characteristic for intelligent food packaging applications. In that sense, the bio-nanocomposite based on chicken skin gelatine/tapioca starch/ZnONP composite may be considered for use as an NH_3 gas sensor for detecting fish spoilage.

Mechanical properties of bio-nanocomposite Film

Tensile strength (TS)

The effects of pH on tensile strength (TS) of chicken skin gelatin bio-nanocomposite films are presented in Table 2. The TS values were increased (5.08–8.48 MPa) with the increase in pH (pH 4–8). There were significant differences among all the formulations ($p < 0.05$). The lowest TS values were obtained at acidic conditioned (pH 4) due to negative or positive gelatin charges, resulting in the electrostatic repulsion and unfolding of the triple-helix chains. Thereby the chains become less rigid and lowering the strength of the film network (Gioffrè et al. 2012). The increase in TS value was due to the increase of film thickness, because the addition of solute concentration will provide more dissolve solids to occupy voids in the film matrix (Arham et al. 2016). This leads to a more crystalline film with a greater strength. The reinforcement of zinc oxide nanoparticles in the film formulation will lead to the formation of stronger intermolecular forces between the polymer chains (Al-Hassan and Norziah 2012).

The current findings are in contrast with the study by Gioffrè et al. (2012) who studied the effect of pH on pig skin gelatin film where the tensile strength ranged from 0.26–1.7 MPa. The highest TS value in this current study was due to the higher amino acid levels (13.42% proline and 12.13% hydroxyproline) in chicken skin gelatin compared to pig skin gelatin (13.10% proline and 9.8% hydroxyproline), encouraging the formation of triple helix structure between the polymer chains (Sarbon et al. 2013). However, a study by Lee et al. (2020) on chicken skin gelatin, tapioca starch and ZnONP showed contrasts in TS values (22.96–50.43 MPa) as compared with the current findings. The lowest TS levels in current study were due to pH levels in the film ranging from pH 4 to 8. With an acidic pH, the repulsive force between the charged of gelatin molecule would hinder the interaction between the chain by reducing the strength of the film network. While at alkaline pH the stronger network was formed through the interaction of the proteins through hydrogen bonds, hydrophobic and ionic interaction (Weng et al. 2014).

Elongation at break (EAB)

The effects of pH on elongation at break (EAB) of bio-nanocomposite films are presented in Table 2. The EAB values decreased (98.67–49.73%) with decreasing pH level (pH 4–8). There were significant differences between all formulations ($p < 0.05$). The highest EAB value under acidic conditions (pH 4) was due to the presence of weaker bonds in the film matrix (Arfat et al. 2016). This finding was confirmed by Tongnuanchan et al. (2011), who

Table 2 Tensile strength, elongation at break and inhibition zone of *Staphylococcus aureus* in chicken skin gelatin/tapioca starch/ZnONP

Formulation	Tensile strength (MPa)	Elongation at break (%)	Inhibition zone of *Staphylococcus aureus* (mm)
A	5.08 ± 0.21^{c}	98.67 ± 2.53^{a}	3.10 ± 5.37^{b}
B	7.72 ± 0.68^{ab}	58.31 ± 5.42^{c}	13.97 ± 1.53^{a}
C	7.00 ± 0.36^{b}	81.71 ± 0.67^{b}	15.67 ± 1.29^{a}
D	8.48 ± 0.04^{a}	8.48 ± 0.04^{d}	15.00 ± 0.70^{a}

Tensile strength, elongation at break and the inhibition zone of film formulation at different pH level. A = film with pH 4; B = film with pH 6; C = film with pH 7; D = pH 8. The superscript letters ($^{a–d}$) within column indicate a significant difference ($p < 0.05$). Data reported are mean values ± standard deviation

claimed that the unfolded or stretched protein molecules would develop molecular weakness in film forming solution at acidic condition. Therefore, allowing more molecular slippage of protein in film matrix resulted in increasing EAB values. Kumar et al. (2010) reported that the reduction in EAB values of film was caused by the inclusion of ZnONP in films, which restricts the movement of polymers and lessens the flexibility of the films.

The similar trend of current study obtained decreased EAB values as compared to the results of a study by Arfat et al. (2016), who studied fish skin gelatin/ZnONP where the EAB value obtained from 64.31% to 43.47%. This was due to the inclusion of ZnONP in gelatin-based film, reducing the availability of water and glycerol to interact with gelatin (Muller et al. 2017). Nanoparticles in film are associated with glycerol, which loses its plasticizing effect that contributes to the flexibility of film and leading to a decrease in EAB values. However, present study was in contrast with a study by Gioffrè et al. (2012) who studied effect of pH on pig skin gelatin where the EAB values were from 260% to 440%. The current finding obtained the lowest EAB values due to the reinforcement of zinc oxide nanoparticles in chicken skin gelatin composite films. The reinforcement will enhance the formation of hydrogen bonds and other interactions between the nanoparticles and component in film, leading to the formation of a compact structure (Sahraee et al. 2017).

Antimicrobial properties

Disc diffusion method

Table 2 shows the inhibition zone of chicken skin gelatin/tapioca starch/ZnONP composite films against gram-positive pathogen *Staphylococcus aureus* (*S. aureus*). The result indicates that the inhibition zone of *S. aureus* is influenced by pH levels with a film diameter of 4.5×4.5 mm^2. The bio-nanocomposite film of Formulation A showed the lowest inhibitory effect on *S. aureus*. In contrast, there is no significant different of antimicrobial activity against *S. aureus* was achieved between Formulation B, C and D.

The inhibitory zone of *S. aureus* in this study increased up to pH 7 (15.67 mm). This finding was supported by Medvedova and Valik (2012), who found that *S. aureus* can grow from pH 4 to 10, while the optimum growth of *S. aureus* is at pH 6–7. This is because the negatively charged alkaline pH condition would result in electrostatic repulsion between bacteria and surface, thereby increasing the inhibition zone (Nostro et al. 2012). In addition, the inhibition zone of bacteria for acidic and alkaline conditioning increases with increasing film solubility, because ZnONP could be distributed evenly with solubilized film matrix to a greater extent (Arfat et al. 2016). The reinforcement of ZnONP into film formulations enhances the inhibitory zone of *S. aureus* (Lee et al. 2020). According to Azeredo et al. (2011), the size of zinc oxide eases penetration through the bacterial cell walls, followed by effective interaction with the interior components of bacteria, improving the antimicrobial properties of films. However, there is lack of findings on the antimicrobial properties of film at varies pH level.

The findings in the current study show a similar trend as a study by Nostro et al. (2012) which studied the effects of pH on antimicrobial properties of biofilm. In that study, the inhibition zone increased from 0.010 to 1.053 mm with increasing pH level. This might be due to the presence of negatively charged of film at alkaline pH lead to the electrostatic repulsion between bacteria and film surface. The current study is in contrast to a study by Arfat et al. (2016) on the effects of pH on antimicrobial activity of fish protein isolate/fish skin gelatin-ZnONP (FPI/FSG-ZnO nanocomposite films) at pH 3 and 11. The inhibition zone of bacteria in films increased for both pH 3 and 11 (13–25 mm) with increased ZnONP. The addition of nano-size ZnO in film will facilitate the penetration of ZnO through the cell membrane of bacteria, followed by effective interaction with the interior component of bacteria.

Conclusion

In conclusion, the physical, mechanical, gas sensing and antimicrobial properties of bio-nanocomposite chicken skin gelatin films were significantly affected by pH treatment at different levels. The pH treatments of the film matrices improved the water vapour permeability (WVP), and thermal properties. In addition, pH treatment of the film matrix also increased tensile strength (TS) while reducing elongation at break (EAB). The pH treatment in film were influenced the gas sensor toward pure NH_3 in film. The pH treatment of the film matrix with ZnONP enhanced the inhibition zone of the bacteria *S. aureus*. Film formulation D (pH 8) was found to be the optimal formulation, since it had the highest thermal properties, TS, and inhibition zone of *S. aureus*, while possessing the lowest WVP and EAB values.

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