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Development of gelatine-based bio-film from chicken feet incorporated with sugarcane bagasse

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Introduction

Plastics are used worldwide in everyday lives and in different forms such as food packaging, spoons, bottles, pens, shopping plastic bags, chairs and containers.

Recycling rates for most plastic packaging are low although recyclable packaging materials has increased (Hopewell *et al.*, 2009). Plastics take a long period of time for complete degradation as they do not degrade naturally to a large degree when released into the environment due to the many polymers that are exceptionally stable and durable (Webb *et al.*, 2012). In order to substitute these plastics, biodegradable plastics have been developed with the same function that are comparable to traditional petrochemical-based plastics for packaging applications (Song *et al.*, 2009). Biodegradable plastics are plastics in which the degradation mechanism is characterized by the full breakdown of the organic chemical compound by micro-organisms into water, carbon dioxide, methane, biomass and inorganic compounds under aerobic or anaerobic conditions and the action of living organisms (Deconinck and Wilde, 2013). The objectives in the development of biodegradable plastics are to utilize renewable and sustainable sources of raw materials by using crops instead of crude oil and to approach integrated waste management to reduce landfill (Davis and Song, 2006).

In the case of food packaging, edible film from natural polymer is important as an alternative to replace synthetic polymer as it can help to enhance food quality by acting as moisture, gas, aroma and lipid barriers as well as acting as a protection to a food product after the primary package is opened (Rattaya *et al.*, 2009). Generally, edible films are thin, continuous layer of edible material which is renewable such as proteins, lipids and carbohydrates (Jongjareonrak *et al.*, 2006). Examples of edible protein films had been developed from whey (Ramos *et al.*, 2012, 2013), soy (Otoni et al., 2016) and sesame (Sharma and Singh, 2016), gelatin films from skin of cuttlefish (Jridi *et al.*, 2013) and fish (Kaewruang *et al.*, 2013; Nikoo *et al.*, 2014; Weng *et al.*, 2014) while other materials focused on polysaccharides such as carrageenan (Soni et al., 2016), cassava starch (Bergo et al., 2008) and methylcellulose (Rubilar et al., 2015).

Although protein-based films have good gas barrier characteristics compared to synthetic films, they have poor mechanical properties and high water vapor permeability which are the main drawbacks of protein films acting as a packaging material (Hoque *et al*., 2011). Moreover, the main sources of commercial gelatin production are from skin and bones of swine and cattle but the usage of swine skin and bone is considered *haram* (unlawful) for Muslim and Judaism and beef gelatin is only acceptable if prepared according to religious requirements (Badii and Howell, 2006). There is also risk of contamination with bovine spongiform encephalopathy (BSE) if infected cattle skin and bones were used (Grommuang *et al*., 2006). Properties of fish gelatine from skins of Nile Tilapia *Oreochromis niloticus*) and channel catfish (*Ictalurus punctatus*) (Zhang *et al*., 2016), tilapia (*Tilapia zillii*) scales (Weng *et al*., 2014), unicorn leatherjacket (*Aluterus monoceros*) (Kaewruang *et al*., 2013) and Amur sturgeon (*Acipenser schrenckii*) (Nikoo *et al*., 2014) had been carried out as the demand for non-mammalian gelatine increases. Fish gelatine is acceptable for Islam but persisting residual odour in fish gelatin can cause problems if the film is intended for use in mildly flavoured products (Rafieian *et al*., 2015; Sae-Leaw and Benjakul, 2015). Chicken by-products such as chicken deboner residue (CDR) (Rafieian *et al*., 2015), chicken feet to replace cowhides for jokpyun (traditional Korean gel-type food) (Jun *et al*., 2000), chicken bones (Lim *et al*., 2010), chicken skin (Sarbon *et al*., 2013)

Sugarcane bagasse is available abundantly in sugar production and beverage industry and 1 tonne of sugarcane produces 280 kg of bagasse (Cerqueira *et al*., 2007). Although once considered a low value agricultural residue, sugarcane bagasse can be potentially utilized for its cellulose which contributes to stiffness (Afra *et al*., 2013), reinforcing potential (Abraham *et al*., 2011) and biodegradability (Chen *et al*., 2011). Sugarcane produces maximum surplus residue (Hiloidhari *et al*., 2014) and provides 40-50% cellulose content (Sun *et al*., 2004). To our knowledge, this is the first study that incorporates hydrolyzed sugarcane bagasse to study the potential mechanical benefits in protein based bio-film. The aim of this work was to analyse the effect of hydrolyzed sugarcane bagasse incorporation on mechanical and water vapor barrier properties of bio-film derived from chicken feet extract to utilize agricultural by-products as potential food packaging materials.

Materials and methods

Chemicals

Phosphoric acid and hydrogen peroxide were purchased from Fisher Scientific (Loughborough, UK) while sodium hydroxide and sulfuric acid were purchased from RCI Labscan Limited (Bangkok, Thailand) and glycerol from Quality Reagent Chemical ($QReC^{TM}$) (New Zealand). All chemicals were of analytical grade.

Raw materials

Chicken feet produced by Sahafarm Co., LTD were purchased at Tesco Lotus, Hat Yai, Thailand. Sugarcane bagasse was obtained from the wet market in Songkla, Thailand.

Extraction of gelatine from chicken feet sample

Preparation of chicken feet sample was carried out according to Grommuang *et al*. (2006). The chicken feet were ground with meat grinder (4 mm mesh size) and washed several times with cold water. The ground chicken feet were then centrifuged at room temperature for 5 minutes and stored at -20°C for further use. Extraction of gelatine from chicken feet sample was done by pre-swelling the ground chicken feet first with 2.14% phosphoric acid at 20°C for 48 hours as described by Grommuang *et al*. (2006). It was then washed thoroughly with tap water until the pH reached 6 - 7. Extraction was done with distilled water for 5 hours at 70°C in water bath. The extract was concentrated at 70°C with vacuum evaporator, chilled to set gel, ground and air dried overnight at 40°C before further grinding if necessary. Kjeldhal method (AOAC, 2000) was used to determine the protein content in the extracted chicken feet gelatine. The protein content of the extracted chicken feet gelatine was carried out in triplicate and the average value was calculated.

Purification of cellulose from SCB

Purification of cellulose from SCB was carried out as described by Teixeira *et al*. (2011) with slight modification. Oven dried SCB was blended to pass through 40 mesh screen. Five grams of dried SCB was then digested with 6% NaOH solution for 4 hours in 60°C water bath. It was then stirred with magnetic stirrer while 100 mL hydrogen peroxide solution (11% v/v) was added slowly to the flask and stirred vigorously for 90 mins. The SCB was filtered and washed with distilled water until neutral pH.

Preparation of hydrolyzed SCB

Hydrolyzed SCB was prepared according to Teixeira *et al*. (2011) with slight modification. SCB was dispersed in 100 mL of 6M H_2SO_4 at 50°C. It was stirred vigorously for 2 hrs 500 ml cold distilled water (4°C) was added to stop the reaction. The pH of the solution was adjusted to pH 6 - 7 through dialysis in tap water with cellulose membrane before storing the suspension in refrigerator. Moisture content of the hydrolyzed SCB suspension was carried out in triplicates (AOAC, 2000).

Preparation of gelatine film with different percentage of glycerol

Film forming solution (FFS) was prepared as described by Tongnuanchan *et al*. (2012, 2013) Gelatine powder was mixed with distilled water to obtain the protein concentration of 3.5% (w/v). The mixture was heated at 70°C until completely dissolved. . Glycerol which acts as plasticizer was added at concentrations of 25% and 35% (w/w) of protein content. The film was then prepared by casting 4.0 g FFS onto a rimmed silicone resin plate (50 x 50 mm^2) and air-blown for 12 hrs at 25°C. The film was further dried at 25°C and 50±5% relative humidity for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany) (Prodpran et al., 2007). The resulting films were peeled off manually and subjected to analyses.

Preparation of gelatine film incorporated with different weight percentage of hydrolyzed SCB

To incorporate the hydrolyzed SCB, modification of methods by Nagarajan *et al*. (2014) and Gilfillan *et al*. (2014) were applied. Gelatine powder was mixed with distilled water to obtain the protein concentration of 3.5% (w/v). The mixture was heated at 70°C until completely dissolved. Then, glycerol was added at concentrations of 35% (w/w) of protein content as a plasticizer. Hydrolyzed SCB suspension of 0.00, 0.131, 0.262, 0.393 and 0.524 g (dry basis) to produce 0, 2.5, 5, 7.5 and 10% (w/w, on dry protein basis) were prepared by homogenizing for 20 secs at 11,000 rpm (IKA Labortechnik homogenizer, Selangor, Malaysia). The hydrolyzed SCB suspensions were added to the film forming solution slowly and the mixtures were homogenized for another 1 min at 11,000 rpm. The final volume of the film forming suspensions were made up to 150 ml and were sonificated for 30 mins using sonicating bath (Elmasonic S 30 H, Singen, Germany) and stirred gently for 30 mins at room temperature in order to obtain a homogeneous suspension. Before casting the film forming suspensions, they were degassed for 10 mins using sonicating bath. The film was then prepared by casting 4.0 g film forming suspension onto a rimmed silicone resin plate (50 x 50 mm^2) and air-blown for 12 hrs at room temperature before drying in an environmental chamber (WTB Binder, Tuttlingen, Germany) for 24 hrs at 25°C and 50 ± 5% RH. The resulting films were peeled off manually and subjected to analyses. Gelatine film without SCB (control) is named SCB 0 and those incorporated with 2.5, 5, 7.5 and 10% SCB were named SCB 2.5, SCB 5.0, SCB 7.5 and SCB 10.0 respectively. Prior to testing, film samples were conditioned for 48 h at 25°C and 50 ± 5% RH (Ahmad et al., 2012).

Determination of film properties

Film thickness

The thickness of films were measured using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan) as described by Fazilah and Maizura (2010). Measurements were taken at fifteen random positions around each film of 10 film samples and average value was calculated.

Mechanical properties

Tensile strength (TS) and elongation at break (EAB) of the films were determined as described by Iwata *et al*. (2000) using Universal Testing Machine (Lloyd Instruments, Hamsphire, UK). Five film samples (2 x 5 cm^2) were first conditioned for 48 hrs at 25°C and 50 ± 5% RH before testing. The film samples were clamped under tensile loading using a 100 N load cell with initial grip length of 3 cm and cross-head speed at 30 mm/min. Tensile strength (MPa) was calculated by dividing the maximum load (N) needed to pull the sample film apart by the cross-sectional area of the sample. Percentage of elongation at break was calculated by the film elongation at the moment of rupture divided with the initial grip length of samples multiplied by 100%.

Water Vapor Permeability (WVP)

WVP of the films were determined using American Society for Testing and Materials (ASTM) method (ASTM, 2004) as described by Rattaya *et al*. (2009). The film was sealed on an aluminum permeation cup containing dried silica gel (0% RH) with silicone vacuum grease and a rubber gasket was used to hold the film in place. The cups were placed in a desiccator containing distilled water at 30°C. The aluminum permeation cups were weighed at every 1 hr intervals for 8 hrs period. WVP of film was calculated as follows:

$$\text{WVP} \ (\text{gm}^{-1}\,\text{s}^{-1}\,\text{Pa}^{-1}) = wxA^{-1}\,\text{t}^{-1}\,(P_2 - P_1)^{-1};$$

where, w = weight gain of the cup (g); x = film thickness (m); A = area of exposed film (m^2); t = time of gain (s), and(P_2 - P_1) = vapor pressure difference across the film (Pa).

Color measurement

Color of each different film was determined using a CIE colorimeter (Hunter Associates Laboratory Inc., USA). Color of the film is expressed as $L*$ - (lightness/brightness), $a*$ - (redness/greenness) and $b*$ - (yellowness/blueness) values. The total difference in color ($\Delta E*$) was calculated according to the equation of Gennadios *et al.* (1996) as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where, ΔL^*, Δa^*, and Δb^* are the differences between the color parameter of the film samples and the color parameter of the white standard,

($L^* = 93.63$, $a^* = -0.88$, and $b^* = 0.33$) when test done on films with different glycerol

percentage, and

($L^* = 93.59$, $a^* = -0.95$, and $b^* = 0.44$) when test done on films incorporated with hydrolyzed sugarcane bagasse.

Light transmittance and transparency value

Light transmittance of the films was measured in ultraviolet (UV) and visible range from 200 nm to 800 nm using a UV-Visible spectrophotometer (model UV-1800, Shimadzu, Kyoto, Japan) (Shiku *et al*., 2004). The transparency value of film sample was calculated based on the equation of Han and Floros (1997) as shown below :

$$\text{Transparency value} = \frac{(-\log T_{600})}{x}$$

where, T_{600} = the fractional transmittance at 600 nm, and x = the film thickness (mm).The higher the transparency value indicates the lower transparency of film.

Scanning Electron Microscopy (SEM)

Microstructure of surface and cross-section of film samples were determined as described by Tongnuanchan *et al*. (2013) using scanning electron microscopy (SEM) (Quanta 400, FEI, Eindhoven, the Netherlands). Film samples were fractured under liquid nitrogen before visualization for cross-section. The film samples were mounted on bronze stub and sputtered with gold using Sputter coater (SPI-Module, West-Chester, PA, USA) in order to make the sample conductive. Photographs were taken at an acceleration voltage of 15 kV.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test. For pair comparison, T-test was used (Steel and Torrie, 1980). Results are presented as mean ± standard deviation and the probability value of $p < 0.05$ is considered as significant. Where relevant, an asterisk (*) is used to indicate which values are presented as mean ± standard deviation. Statistical analysis was performed using the Statistical Package for Social Sciences version 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, NY).

Note:

Films from gelatine with different percentage of glycerol were first produced, tested and analyzed in order to determine which film is suitable to proceed to form films incorporated with different weight percentage of SCB.

Results and discussion

Protein content in extracted chicken feet gelatine and moisture content of hydrolyzed SCB suspension

The chicken feet gelatine contains about 74.22 % of protein. The moisture content of the hydrolyzed SCB suspension was 98.63%. The dry basis of the SCB was calculated by subtracting 98.63% with 100 % which resulted in 1.37 g. This means that there was 1.37 g of SCB for every 100 ml of the hydrolyzed SCB suspension.

Properties of gelatine film with different percentage of glycerol

Thickness

The thickness of films with different percentage of glycerol is as shown in Table 1. It is not significantly different) between the films containing 25% (0.058 mm) and 35% glycerol (0.060 mm). The glycerol did not affect the film thickness as glycerol was dissolved with the gelatine during preparation of FFS. Negligible differences in thickness of gelatine-based films with different levels of glycerol were also mentioned by Vanin *et al.*(2005), Kokoszka *et al.* (2010), Tongnuanchan *et al.*(2012) and Chamnanvatckatit *et al.* (2014).

Table 1. Properties of films from chicken feet gelatine with different percentage of glycerol

Mechanical Properties

TS and EAB of the film with different percentage of glycerol are as shown in Table 1. There is significant difference ($p < 0.05$) for both TS and EAB. It can be seen that TS of the film decreased from 44.86 MPa to 34.20 MPa when the glycerol percentage increased 10%. As for the EAB of the film, the value increased about two-fold; from 15.99% to

33.30%. Glycerol concentration affects the film properties by improving the film extensibility and reducing its resistance as reported by Jouki *et al*. (2013). Glycerol improves the flexibility of gelatine-based film but decreases its stiffness.

Chamnanvatckatit *et al.* (2014) stated that glycerol gives plasticizing effect because it decrease the inter- and intra molecular attractive forces resulting TS to decrease and EAB to increase with the increasing of glycerol concentration. Plasticizer can be easily inserted between polymer chains to produce a "cross-linker" effect that decreases the free volume of the polymer and at the same time improves the extensibility of the films and diminishes mechanical strength (Jouki *et al*., 2013). Other studies showed similar result concerning the effect of glycerol as plasticizer on protein-based films which include muscle proteins of Thai tilapia (Sobral *et al*., 2005), whey protein (Ramos *et al*., 2013) and bovine gelatine (Chamnanvatckatit *et al*., 2014).

Water Vapor Permeability (WVP)

WVP of the film prepared from chicken feet gelatine with 25% and 35% glycerol are shown in Table 1. There is no significant difference between the gelatine film with 25% and 35% glycerol. The WVP for 25% glycerol gelatine film is 2.04 x $10^{-11}gm^{-1}s^{-1}Pa^{-1}$ and 2.14 x $10^{-11}gm^{-1}s^{-1}Pa^{-1}$ for 35% glycerol gelatine film. WVP increases as the glycerol percentage increases. This is due to lower water barrier in higher content of glycerol.

Glycerol enhances the water vapor permeability as it modifies the molecular organization of the protein network and increases the free volume leading to lesser dense network hence, films are permeable to water as it ease the water diffusion (Al-Hassan and Norziah, 2012). Arvanitoyannis *et al*. (1998) stated that the water vapor transfer rate increases proportionally with the increasing of the total plasticizer content (water and polyols) in the polymer matrix.

Chamnanvatckatit *et al*. (2014) with similar results also stated that glycerol is hydrophilic in nature which led to the hygroscopic characteristics of the films thus increases the moisture content of the film as well as the WVP of the film.

Color measurement

Differences in color between the gelatine film with 25% and 35% glycerol are presented in Table 2. As mentioned, $L*$ is the lightness/brightness and $a*$ is redness/greenness whereas $b*$ is the yellowness/blueness values. The values of $L*, a*$ and ΔE^* have significant difference ($p < 0.05$) between the films from gelatine with 25% and 35% glycerol. However, the b^*values showed no significant difference between the two types of films. Based on the study carried out by Chamnanvatckatit *et al*. (2014), addition of different concentrations of glycerol to bovine protein films does not impact the color of the resulting films. However, Jouki *et al*. (2013) reported otherwise, when different glycerol concentration were added to cress seed gum films. All of the color parameters except a-value of the films were significantly changed when glycerol concentration increased.

Table 2. Film colors made from chicken feet gelatine with different percentage of glycerol

Light transmittance and transparency value

The light transmission in the UV range (200-280 nm) for film with 25% glycerol is from 0.02% to 21.54% while film with 35% glycerol is from 0.03% to 19.25%. As for visible range (350-800 nm), the light transmittance ranges from 72.48% to 87.58% and 66.75% to 85.62% for 25% and 35% glycerol gelatine-based film respectively (Table 3). This conveys that there is a slight decrease in light transmission with the increase of percentage of glycerol.

Table 3. Light transmittance and transparency values of films from chicken feet gelatine with different percentage of glycerol

In addition, the increased in percentage of glycerol had no significant differences on the transparency value between the two types of film. The transparency value differs by 0.02 indicating the increased in glycerol percentage do not affect the transparency value of the films. The resulting gelatine films were transparent and also clear which is suitable for use as see-through packaging. Gelatine has low content of tyrosine and phenylalanine; aromatic amino acids that are sensitive to chromophores which absorb light at wavelength below 300 nm (Li *et al*., 2006). The aromatic amino acids are important as an UV barrier property of protein films as gelatine film without glycerol has higher barrier for light transmission and UV range compared to film added with glycerol.

Analysis

Based on the results for thickness, mechanical properties, water vapor permeability, color and light transmittance as well as transparency value tests of the films from chicken feet gelatine with different percentage of glycerol, film with 35% glycerol was chosen to be incorporated with different weight percentage of hydrolyzed SCB. Film with 35% of glycerol has lower TS but higher EAB. By incorporating hydrolyzed SCB, it was hoped that the TS increases and WVP of the film can further be lowered.

Properties of films from chicken feet gelatine incorporated with different percentage of dry weight SCB

Thickness

Thickness of the film incorporated with different percentage of dry weight SCB is shown in Table 3. Generally, thickness of a film increases as the amount of weight percentage of SCB increases ($p < 0.05$). The hydrolyzed SCB is likely distributed on the gelatine film and increase the thickness of the film. However, the thickness of the film is the same between the control film with 0% and 2.5% of dry weight SCB. There is no effect on the thickness of the film as the amount of SCB is not significant.

Table 4. Properties of films from chicken feet gelatine incorporated with different percentage of dry weight SCB

Mechanical Properties

The mechanical properties of films incorporated with different percentage of dry weight SCB are presented in Table 4. Incorporating SCB in the gelatine film is supposed to increase the TS of the film. However, as shown in Table 4, the TS increased slightly from

22.50 MPa to 23.07 MPa for the film with 0 wt % and 5.0 wt % SCB respectively. The TS then decreased to 20.88 MPa and 19.76 MPa with 7.5 wt % and 10.0 wt % SCB incorporated respectively. This is in agreement with Gilfillan *et al*. (2012) and Prachayawarakorn *et al*. (2010) where fiber overloading resulted in decreasing tensile strength.

EAB of films decreases as the amount of percentage of dry weight SCB increases as shown in Table 4. The EAB of film decreased steadily from 59.97% to 24.82% for the film with 0 wt % and 10.0 wt % of SCB respectively. Slavutsky and Bertuzzi (2014) reinforced starch films with cellulose nanocrystals obtained from sugarcane bagasse and stated high value of TS as the sugarcane bagasse was dispersed properly in the matrix structure. In addition, EAB value decreases due to the rigid nature of the sugarcane bagasse. Another similar study was conducted by Gilfillan *et al*. (2014) where starch was incorporated with sugarcane bagasse nanofibres. The TS doubled but started to decrease at fibre loadings above 10 wt % while EAB decreased by up to 70% compared to film with no nanofibres.

Water Vapor Permeability (WVP)

WVP of films from chicken feet gelatine incorporated with different weight percentage of SCB showed significant differences ($p < 0.05$) between the films (Table 4). WVP of films decreased with the increasing levels of weight percentage of SCB incorporated in the film. The WVP of the films decreased from $2.18 \times 10^{-11} gm^{-1}s^{-1}Pa^{-1}$ to $1.56 \times 10^{-11} gm^{-1}s^{-1}Pa^{-1}$ which is the SCB 0 (control) to SCB 10.0 (10 wt % SCB), which are the highest and lowest WVP of the films respectively. However, there is no significant difference between SCB 7.5 and SCB 10.0. This may due to the uneven dispersion of SCB on the film samples for 7.5 wt % and 10.0 wt %.

A high WVP of film is not desirable due to its usage and performance (Pereda *et al*., 2011). From the results in this experiment, addition of hydrolyzed SCB improved the water vapor barrier properties of the film slightly. Rawdkuen *et al*. (2012) reported similar results by adding catechin-lysozyme combination (CLC) in fish gelatine film. The barrier properties improved as the moisture transfer between the food and the surrounding atmosphere is lowered when the film was applied to heterogeneous food product. Ahmad *et al*. (2012) stated that the water vapor transfer process in films depends on the hydrophilic-hydrophobic ratio of the film constituents. In addition, film thickness also influences the water vapor permeability as thicker film can absorb more water from the environment (Rawdkuen *et al*., 2010). In order to utilise the gelatine based bio film incorporated with SCB as a potential food packaging film, resistance of the film to water is desirable if the film is to be used for the preservation of intermediate or high moisture foods (Ozdemi and Floros, 2008). Films with good solubility had been proposed as packaging material for instant noodle seasoning bags and instant beverages or as casing for sausages, biscuits and candy (Wan *et al*., 2015). The water solubility and swelling of the bio film should be determined in future studies.

Color measurement

The color properties, $L*$ (lightness/brightness), $a*$ (redness/greenness) and $b*$ (yellowness/blueness) values of the films from chicken feet gelatine incorporated with different levels of weight percentage of SCB are shown in Table 5. It can be concluded that all the color parameters were affected by the amount of weight percentage of SCB being incorporated in the film. The value increases proportionally with the weight percentage of SCB and there is significant difference ($p <0.05$) for the three parameters. The total color differences (ΔE^*) also showed significant difference ($p < 0.05$). Control (SCB 0) showed the lowest value while the highest weight percentage (SCB 10.0) showed the highest value with 3.48 and 3.61 respectively.

Table 5. Film colors of chicken feet gelatine incorporated with different percentage of dry weight SCB

Light transmittance and transparency value

Generally, films often exhibit lower light transmission in the UV range than in the visible range (Rawdkuen *et al*., 2012). Transmission of UV light of the film from control film to incorporation of sugarcane bagasse (SCB 10.0) in chicken feet gelatine film at 280 nm decreased from 22.20 to 9.95%. Hence, the films are successful in preventing the UV light and possibly retard lipid oxidation induced by the UV light. The light transmittance of the films at different wavelengths decreases as the weight percentage of the SCB incorporated in the film increases (Table 6).

Table 6. Light transmittance and transparency of films from chicken feet gelatine incorporated with different percentage of dry weight SCB

There is significant difference ($p < 0.05$) on the transparency among all the films with different weight percentage of SCB. The transparency value increases as the amount of weight percentage of SCB incorporated increases. Transparency value increased from 0.99 (SCB 0) to 2.37 (SCB 10.0)with higher transparency value indicating that the films have lower transparency. The increase of transparency value is most probably due to the hydrolyzed SCB incorporated as the hydrolyzed SCB is solid and not transparent which made the film not entirely clear.

Scanning Electron Microscopy (SEM)

SEM micrographs of the surface and cross-section of films from chicken feet gelatine incorporated with different levels of weight percentage of hydrolyzed SCB are illustrated in Figure 1.

Figure 1. Scanning Electron Microscopy micrographs of surface (magnification: 500x) and cross section (magnification: 1800x) of films from chicken feet gelatine incorporated with different levels of weight percentage of SCB. The SCB 0 which is the control film

showed smooth and homogeneous surface. The cross-section of the control film also showed smooth surface. As the weight percentage of hydrolyzed SCB increases, the surface of the films showed increment in white spots. The white spots are believed to be the hydrolyzed SCB.

The SCB 0 which is the control film showed smooth and homogeneous surface. As the weight percentage of hydrolyzed SCB increases, the surface of the films showed increment in white spots. The white spots are believed to be the hydrolyzed SCB. The cross-section of control film also showed smooth surface. The surface became rougher with the increase of weight percentage of SCB. However, through the micrographs, it can be deduced that the hydrolyzed SCB did not form a strong matrix with the protein matrix of gelatine. There is a weak bond between the SCB and the gelatine film and the hydrolyzed SCB merely formed a layer on top of the gelatine film. It can be seen that the hydrolyzed SCB did not disperse homogeneously on the gelatine film but agglomerate instead. Hence, further treatment of the SCB should be applied for a better dispersion of the SCB on the gelatine-based film.

Gilfillan *et al*. (2014) reported that the sugarcane nanofibres are well attached to the starch matrix based on the SEM micrographs of the composite from starch with SCB nanofibres. The SEM micrograph of starch film reinforced with cellulose nanocrystals obtained from SCB showed that the dispersion of the cellulose nanocrystals are homogeneous within the polymer matrix (Slavutsky and Bertuzzi, 2014). In this study, the SCB used were chemically hydrolyzed. The SCB particle size is still noticeably large (Figure 1). This may have affected the dispersion of the SCB on the gelatine film. The structure of cellulose fibers can be damaged by excessive hydrolysis (Gilfillan *et al*., 2014). It is suggested that further treatments be carried out on hydrolyzed SCB. Pre-treatment (Salehudin *et al*., 2013) and combination of mechanical refining and enzymatic treatment were found to produce homogenous nanofibrils from sugarcane bagasse (Santucci *et al*., 2016).

Conclusion

A higher percentage of glycerol used in the gelatine-based film, resulted in lower TS and higher EAB. Film containing 35% glycerol in gelatine extracted from chicken feet were further incorporated with different weight percentage (0, 2.5, 5.0, 7.5 and 10.0 wt %) of SCB. Although the mechanical strength of the film could not be improved by incorporating SCB, there was only slight improvement in the WVP barrier properties. As the weight percentage of SCB increases, the WVP of the film decreases. In addition, film from gelatine extracted from chicken feet incorporated with 5.0 wt % of SCB has the best properties when all the tests were taken into consideration. The thickness, color and transparency value of the film with 5.0 wt % of SCB were similar to the control film. However, the TS of SCB 5.0 film is increased and the WVP is lowered slightly. This limits the application of the film as biomaterial and further research to treat the hydrolysed SCB is recommended. The bio-film developed in this study incorporates sugarcane bagasse into the film derived from chicken feet and demonstrated an increment in tensile strength and reduction of water vapor permeability. This study is of value to food practitioners looking into utilising agricultural wastes (e.g. animal by-product and sugarcane bagasse).

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Table 1 Properties of films from chicken feet gelatine with different percentage of glycerol

Glycerol (%)	**Thickness (mm)**	**TS (MPa)**	**EAB (%)**	**WVP ($x10^{-11} gm^{-1} s^{-1} Pa^{-1}$)**
25%	0.058 ± 0.003^a	44.86 ± 1.66^a	15.99 ± 6.24^a	2.04 ± 0.29^a
35%	0.060 ± 0.003^a	34.20 ± 0.97^b	33.30 ± 6.79^b	2.14 ± 0.11^a

Results are presented as mean ± sd. Different superscript letters in the same column indicate significant difference by independent samples T-test ($p < 0.05$).
TS - Tensile strength
EAB - Elongation at break
WVP - Water vapor permeability

Table 2 Film colors made from chicken feet gelatine with different percentage of glycerol

Glycerol (%)	L^*	a^*	b^*	ΔE^*
25%	90.77 ± 0.06^a	-1.30 ± 0.04^a	3.01 ± 0.27^a	3.94 ± 0.14^a
35%	91.29 ± 0.10^b	-1.40 ± 0.03^b	3.18 ± 0.07^a	3.73 ± 0.08^b

Results are presented as mean ± sd. Different superscript letters in the same column indicate significant difference by independent samples T-test ($p < 0.05$)

Table 3 Light transmittance and transparency values of films from chicken feet gelatine with different percentage of glycerol.

Glycerol (%)	**Light transmittance (%) at different wavelength (nm)**								**Transparency value***
	200	**280**	**350**	**400**	**500**	**600**	**700**	**800**	
25 %	0.02	21.54	72.48	79.94	84.30	85.96	86.89	87.58	1.08 ± 0.05^a
35 %	0.03	19.25	66.75	74.95	80.54	83.02	84.53	85.62	1.10 ± 0.14^a

*Mean ± SD

Different superscript letters in the same column indicate significant difference by independent samples T-test ($p < 0.05$).

Table 4 Properties of films from chicken feet gelatine incorporated with different percentage of dry weight SCB

Film sample	**Thickness (mm)**	**TS (MPa)**	**EAB (%)**	**WVP ($\times10^{-11} gm^{-1}s^{-1}Pa^{-1}$)**
SCB 0	0.066 ± 0.002^{a}	22.50 ± 1.97^{bc}	59.97 ± 5.83^{c}	2.18 ± 0.08^{d}
SCB 2.5	0.066 ± 0.003^{a}	22.68 ± 1.14^{c}	41.67 ± 4.95^{b}	2.06 ± 0.04^{c}
SCB 5.0	0.073 ± 0.004^{b}	23.07 ± 0.67^{c}	35.75 ± 3.59^{b}	1.85 ± 0.08^{b}
SCB 7.5	0.085 ± 0.004^{c}	20.88 ± 1.36^{ab}	27.99 ± 3.46^{a}	1.61 ± 0.07^{a}
SCB 10.0	0.087 ± 0.007^{c}	19.76 ± 0.67^{a}	24.82 ± 4.50^{a}	1.56 ± 0.05^{a}

Results are presented as mean ± sd. Different superscript letters in the same column indicate significant difference by Duncan's multiple range tests ($p < 0.05$).
TS - Tensile strength
EAB - Elongation at break
WVP - Water vapour permeability
SCB: Sugarcane bagasse

Table 5 Film colors of chicken feet gelatine incorporated with different percentage of dry weight SCB

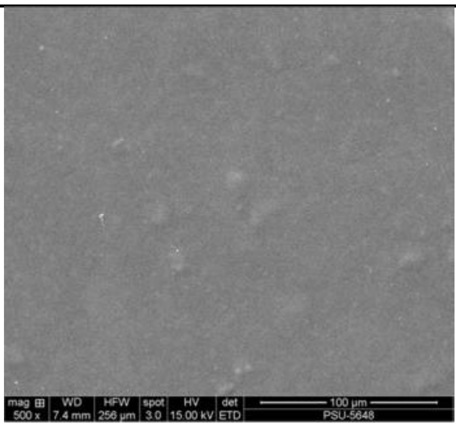
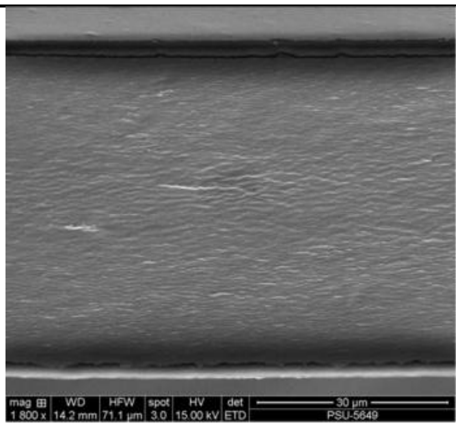
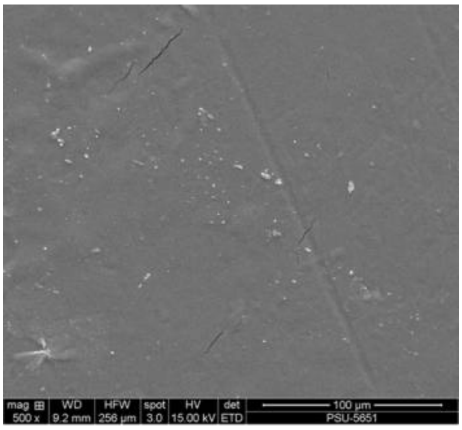
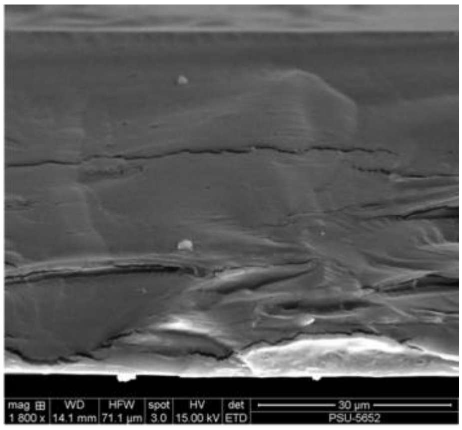
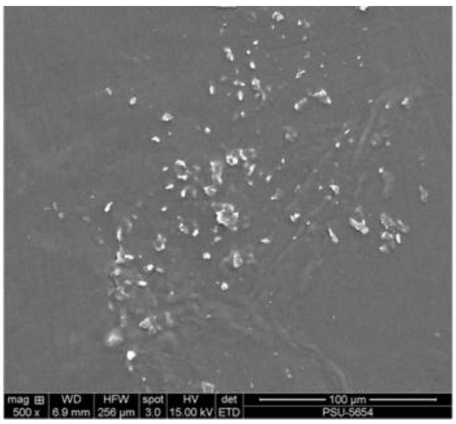
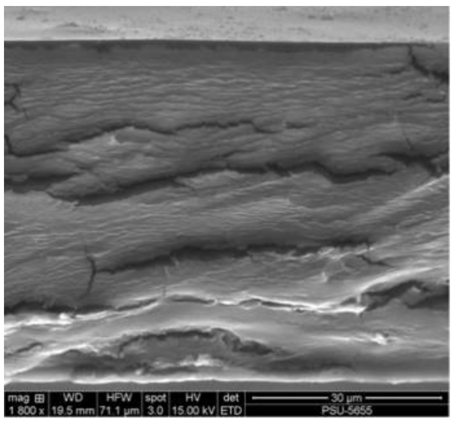
Film sample	$\boldsymbol{L^*}$	$\boldsymbol{a^*}$	$\boldsymbol{b^*}$	$\boldsymbol{\Delta E^*}$
SCB 0	90.85 ± 0.07[a]	-1.30 ± 0.06[a]	2.61 ± 0.21[a]	3.48 ± 0.06[a]
SCB 2.5	90.86 ± 0.09[a]	-1.25 ± 0.04[b]	2.75 ± 0.12[b]	3.57 ± 0.05[b]
SCB 5.0	91.01 ± 0.16[b]	-1.24 ± 0.03[b]	2.90 ± 0.21[c]	3.58± 0.04[b]
SCB 7.5	91.26 ± 0.02[c]	-1.23 ± 0.03[b]	3.22 ± 0.08[d]	3.59± 0.06[b]
SCB 10.0	91.55 ± 0.08[d]	-1.21 ± 0.07[b]	3.40± 0.06[e]	3.61± 0.07[b]

Results are presented as mean ± sd. Different superscript letters in the same column indicate significant difference by Duncan's multiple range tests ($p < 0.05$).
SCB: Sugarcane bagasse

Table 6 Light transmittance and transparency of films from chicken feet gelatine incorporated with different percentage of dry weight SCB.

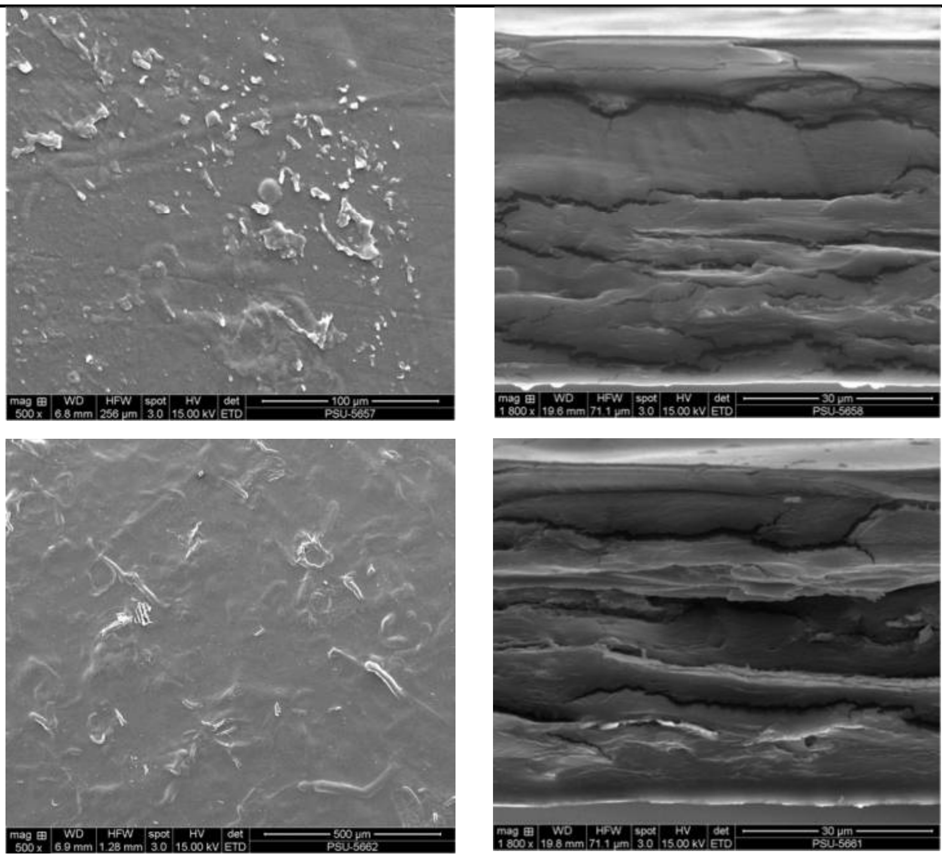
Film sample	Light transmittance (%) at different wavelength (nm)								Transparency value*
	200	280	350	400	500	600	700	800	
SCB 0	0.02	22.20	73.13	80.35	84.35	85.94	86.83	87.43	0.99 $\pm$ 0.09[a]
SCB 2.5	0.02	19.88	68.94	76.91	81.08	82.62	83.54	84.23	1.06 $\pm$ 0.09[a]
SCB 5.0	0.04	17.23	63.44	72.65	77.07	78.66	79.62	80.32	1.48 $\pm$ 0.13[b]
SCB 7.5	0.02	12.57	51.79	61.16	65.37	67.03	68.03	68.81	1.84 $\pm$ 0.19[c]
SCB 10.0	0.02	9.95	47.52	58.21	62.90	64.79	65.92	66.80	2.37 $\pm$ 0.14[d]

*Mean $\pm$ SD
Different superscript letters in the same column indicate significant difference by Duncan's multiple range test ($p < 0.05$).
SCB: Sugarcane bagasse

Weight Percentage of SCB (%)	Surface	Cross-section
SCB 0		
SCB 2.5		
SCB 5.0		

SCB 7.5

SCB 10.0



SCB: Sugarcane bagasse

Figure 1. Scanning Electron Microscopy micrographs of surface (magnification: 500x) and cross section (magnification: 1800x) of films from chicken feet gelatine incorporated with different levels of weight percentage of SCB. The SCB 0 which is the control film showed smooth and homogeneous surface. The cross-section of the control film also showed smooth surface. As the weight percentage of hydrolyzed SCB increases, the surface of the films showed increment in white spots. The white spots are believed to be the hydrolyzed SCB.