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Tensile Strength, Elongation at Breaking Point and Surface Color of a Biodegradable Film Based on a Duck Feet Gelatin and Polyvinyl Alcohol Blend

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Abstract- A biodegradable film was prepared from a blend of duck feet gelatin and polyvinyl alcohol (PVA) and was plasticized by glycerol. The effects of using a different ratio of duck feet gelatin to PVA (A=0:100, B=20:80, C=40:60, D=60:40, E=20:80, and F=100:0) on the quality properties were examined, including an analysis of thickness, tensile strength, elongation at breaking point and surface color. The gelatin extracted from duck feet had a bloom value of 306.9 g. The tensile strength of the films increased as the concentration of the duck feet gelatin increased. However, the percentage of elongation at breaking point decreased, as tensile strength and elongation at breaking point have an inversely proportional relationship. The film made from pure gelatin had a low lightness value (95.13) compared to a sample without gelatin that had a lightness value of 96.91. The blended film with the ratio of duck feet gelatin to PVA (40:60) showed good properties for use as a biodegradable film compared to blended films with other ratios.

Keywords- Duck feet, biodegradable film, bloom value, tensile strength and surface color.

INTRODUCTION

The use of synthetic plastic films for packaging has grown extensively in recent years due to their excellent functional properties. However, these films are usually non-biodegradable, thus leading to environmental pollution that presents serious ecological concerns. Inert and non-biodegradable plastic materials represent approximately 30% of municipal solid waste. Therefore, using biodegradable films could save energy and take an important step toward environmental protection (Moraes et al., 2009). Biodegradable films could partly substitute for commercial packaging materials, which are currently made from non-biodegradable petroleum-based polymeric materials that are major contributor to global warming (Kowalczyk and Baraniak, 2011). Thus, biodegradability is not only a functional requirement but also an important environmental attribute. The concept of using biodegradable films has both user-friendly and eco-friendly attributes (Tharanathan, 2003).

Previous studies have shown that protein-based films can provide desirable mechanical, gas barrier, and transparency properties (Cuq et al., 1995). The mechanical properties of protein-based films are better than those of polysaccharide- and lipid-based films because the proteins have a unique structure (based on 20 different monomers) that provide a wider range of functional properties including a high intermolecular binding potential, which can form bonds at different positions (Bourtoom, 2009). In this study, a biodegradable film was made from blends of PVA/duck feet gelatin and plasticized by glycerol.

The objective of this research was to develop a biodegradable film based on different ratios of duck feet gelatin and PVA and determine the quality properties (thickness, tensile strength, elongation at breaking point and surface color) of the biodegradable films, as well as the optimal ratio of duck feet gelatin to PVA, to achieve the best mechanical strength in the film.

MATERIALS AND METHODS

Materials

Duck feet were purchased from a local duck food industry, Perak Duck Food Industries Sdn. Bhd., which is located in the northern part of Peninsular Malaysia. Butanol, Citric acid, Polyvinyl alcohol (PVA) and Glycerol were purchased from a Local Sigma Aldrich supplier.

Duck Feet Gelatin Extraction

Frozen duck feet (stored at -20 °C) were thawed in a 7 °C chiller for 24 hours. The process of extracting gelatin from the duck feet is explained in Figure 3.2. Duck feet gelatin was extracted using a modified method reported by Kim et al., (2014) based on acid swelling and hot water extraction. After the claws were removed, the feet were then cut into smaller pieces and ground using meat grinder. Ground duck feet were mixed in a 10% butanol solution by w/v (duck feet/solution = 1/20) and continuously stirred for 12 hours by magnetic stirrer at room temperature to

complete the defatting process. Then, is the duck feet were washed with tap water for 5 minutes to remove any remaining butanol. The defatted duck feet were soaked in a 0.1 M citric acid solution by w/v (duck feet/solution = 1/10) at 4 – 7 °C for 24 hours. The swelled duck feet were neutralized with flowing tap water until they reached a pH of 5.5. For the hot water extraction, the product was placed into a beaker with distilled water at a 1:2 ratio of water to swelled duck feet and cooked in a 75 °C water bath for 2 hours. The duck feet extracts were filtered using Whatman filter paper number 4 and cooled at room temperature. The duck feet gelatin was frozen at -18 °C and was freeze-dried using a Millrock Technology LD53 freeze dryer at -50 °C for 3 days. Then, the dried duck feet gelatin was ground using a dry blender to turn it into a powder. The product placed in a tightly sealed container and stored in cool, dry place for later use.

Film Preparation

The film forming solutions were prepared from a mixture of gelatin (solution A), PVA (solution B), and glycerol as the plasticizing solution. The process of preparing the film-forming solution is explained in Figure 3.3. To prepare solution A, gelatin was hydrated for 30 minutes at room temperature and then dissolved in a 55°C water bath. For solution B, the PVA was slowly added to distilled water and stirred with magnetic stirrer. It was homogenized until it was fully dispersed and was then heated to the temperature at which it was solubilized - between 90 to 98°C (Chiellini et al., 2001). Mixing continued at this temperature until the PVA was fully dispersed. These solutions were then mixed and homogenized for 15 minutes at room temperature to produce film-forming solutions with 2, 4, 6 and 8 g of PVA/10 g of macromolecules (gelatin + PVA). The ratio of gelatin/PVA was on a dry weight basis (10 g), and the amount of water added was 200 ml. Additionally, films made only from gelatin (0 g PVA/10 g of macromolecules) or PVA (10 g PVA/10 g of macromolecules) were also produced for use as comparisons for the films prepared with blends of gelatin and PVA. Next, 3 g of glycerol were added to the mixture and stirred for 15 minutes at 55 °C. Finally, the film-forming solutions were cast on polyacrylic glass plates (27.5 x 13.0 cm) and dried at room temperature for 24 hours. Before carrying out the analyses, the films were conditioned inside a desiccator containing sodium bromide solution (50 ± 5% relative humidity) at 23 ± 2 °C for 2 days (Montero, 2009).

Yield of Gelatin

The ratio of duck feet gelatin powder weight to the total weight of the ground duck feet was used as the gelatin yield. The yield of extracted gelatin was calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight of gelatin (g)}}{\text{Weight of ground duck feet (g)}} \times 100$$

Gel Strength Analysis

Gel strength of the gelatin gels was determined according to British standards (BSI, 1975). Gelatin gel was prepared by dissolving 6.67% (w/v) gelatin powder with distilled water in a bloom jar at room temperature and hydrating it for 30 minutes. It was then heated in a water bath at 60 °C for approximately 15 minutes until the gelatin was completely dissolved. The gelatin solution cooled at room temperature before being left to mature in a chiller at 10 °C for 16 to 18 hours prior to analysis. A TA. XT2 Texture Analyzer (Stable Microsystem, Surrey, UK) was used to determine the gel strength with a 5 kg load cell and a standard radius cylinder (P/0.5) probe. The maximum force (g) was obtained when the probe penetrated the gel to a depth of 4 mm. The maximum force (g) was defined as the resistance to penetration and was then translated into bloom strength (g).

Film Thickness

The thickness of the films was measured by averaging these thicknesses at five different positions, using a handheld micrometer (Mitutoyo, Tokyo, Japan) with a precision of 0.01 mm. This micrometer was used to calculate the moisture permeability of the films.

Tensile Strength and Elongation at Break (EAB)

The mechanical properties of the films were determined by conducting a tensile strength test according to American Society for Testing and Materials (ASTM Standard, 2003), with some modifications. Each film was cut into 3 replicate samples measuring 8 cm x 1.5 cm. The texture analyzer was calibrated with a 5 kg load before being used for tensile strength analyses (Stable Microsystem, Surrey, UK). Both ends of film strips were marked with a 2 cm line. The initial separation distance and the velocity were fixed at 40 mm and 0.40 mm/s, respectively. The trigger force used was automatically set at a 300 mm/min pre-test speed and a 600 mm/min post-test speed. The cell load capacity of the texture analyzer was 30 kg, and the return distance was 190 mm. Tensile strength and elongation at breaking point were determined directly from the stress-strain curves using Texture Exponent 32 software V.4.0.5.0. (SMS).

$$TS\ (Pa) = (F / A)$$

where

TS = tensile strength, Pa (Nm-2)
F = maximum force at break
A = cross sectional area of film (m2)

$$EAB\ (\%) = \frac{l - lo}{lo} \times 100$$

where

l = length extended at breaking point of sample
lo = initial length of sample

Surface Color

The surface color of films was measured using a Gunter Colorimeter (Minolta Spectrophotomer CM-3500d, Minolta Co. Ltd., Osaka, Japan). The colorimeter was first calibrated to standard black and white tiles. Three replicates of film samples were used to measure the film surface color with the help of a computerized system (Spectra Magic software, version 2.11 Minolta Co. Ltd,

Osaka, Japan). The Hunter Lab color scale was used, where the color coordinates ranged from L* = 0 (black) to L* = 100 (white), −a* (greeness) to +a* (redness), and –b* (blueness) to +b* (yellowness).

Statistical Analysis

Statistical analysis were carried out using SPSS 22.0 for Windows (SPSS Inc. Chicago, IL). After all data and analyses results were collected, One-way Analysis of Variance (ANOVA) tests were performed. Film samples with different ratio of duck feet gelatin to PVA were compared using Duncan's multiple range test with a 5% significant difference level.

RESULTS AND DISCUSSIONS

Gelatin Yield

A total of 78.15 g of duck feet gelatin powder was obtained from 2.4 kg of ground duck feet, which is equivalent to a yield of 3.3%. The percentage of gelatin powder derived from chicken feet was reported to be 4.1% by Rahman & Shariffah (2012). According to research by Liu et al. (2012), the type of acid used in the acid swelling extraction process can influence the percent yield of gelatin. As reported by Ashar and Herickson (1992), the swelling percentage of samples with citric acid is higher than that of the samples soaked with hydrochloric acid. The percent yield of gelatin from citric acid swelling was 4% higher than that obtained from hydrochloric acid swelling (Liu et al., 2012). The yield probably depends greatly on the proportion of fractions of different proteins in the duck feet. Adjusting the pH by either lowering it to pH 4.0 or increasing it to pH 10.0 could weaken the binding ability between the molecular structures of the gelatin and could result in swelling of the gelatin (Li, 1993). Gudmundsson (2002). This finding suggests that the H+ concentrations that are either too high or too low can result in a low yield and a poor gel quality. Low pH is favorable for achieving a maximum extraction rate but is detrimental to the physical properties of the gel as it produces more degradation and proliferation of lower-molecular weight peptides (Johnston-Banks, 1990).

Gel Strength

Gel strength of duck feet gelatin was tested and compared to commercial bovine gelatin. Based on the test carried out, gel strength of duck feet gelatin was 306.9 g, while the gel strength of commercial bovine gelatin was 216.8 g. The gel strength of commercial bovine gelatin was tested again to ensure the value stated by manufacturer, which was 225 g. The reduced value of gel strength measured for the commercial bovine gelatin may have been due to the long storage period. According to Johnston-Banks (1983), there are three levels of gelatin quality: low quality (150 g), medium (150 - 220 g), and high (220 g and above). Thus, the duck feet gelatin in this study had a high-quality level. The present study found that the gel contained 17 amino acids, and histidine and cysteine were not detected. Glysine was the dominant amino acid found in duck feet collagen (20.46%). Hydroxyproline is an amino acid obtained from proline. The amino acids (hydroxyproline and proline) in the duck feet collagen were at 17.97%, which is in agreement with the amino acid content of interstitial collagen (approximately 17%) reported by Gomez-Guillen et al. (2002). Both amino acids and glycine play important roles in gel strength. Low amino acid content also indicates poor gelling power (Wangtueai and Noomhorm, 2009). Gelatin that contains high amounts of the amino acids glycine, proline, and hydroxylproline have been shown to have high gel strength compared to gelatin with low amino acid content (Muyonga et al., 2004).

Film Formation and Thickness

Figure 1 shows the average thickness of the duck feet gelatin and PVA blend films. All films produced were visibly homogenous and transparent with an excellent appearance typical of gelatin-based films. The thickness of all films was found to be uniform when the film-forming solutions for each formulation were replicated.

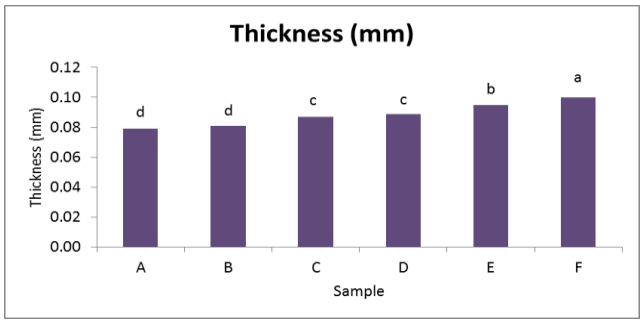

Figure 1: Thickness of blended films with different ratios of duck feet gelatin to PVA
* Mean values with different letters show differences in significance at ($P < 0.05$)

The films were removed easily from the casting plates and had a smooth surface. Additionally, the viscosity (based on visual observation) of the film-forming solutions increased as the amount of PVA (g/10 g of macromolecules) increased. The level of irregularities in the film-forming solutions depends mainly on their rheological properties (Peressini et al., 2003). PVA solutions generally shear thinly and may exhibit significant thixotropy. The viscosity of the film-forming solutions increased with increased molecular weights and concentrations of the solutions. According to Cuq et al. (1995), casting a high viscosity film-forming solution is very difficult and requires a dispersing machine (spreader). Nevertheless, the film-forming solution studied in this experiment had low to medium viscosity, which readily allowed for casting these solutions by free flow (Carvalho et al., 2009).

The films made of only PVA (Sample A) or duck feet gelatin (Sample F) had the lowest (0.079 ± 0.001 mm) and highest (0.100 ± 0.000 mm) thickness values, respectively. There was a continuous increase in the thickness value as the concentration of duck feet gelatin increased; thus, a specific pattern was observed in the sample thickness. The thickness values for Sample B, C, D and E were 0.081 ± 0.001, 0.0087 ± 0.001, 0.089 ± 0.002 and 0.095 ± 0.003

mm, respectively. Films with increasing concentrations of duck feet gelatin displayed significantly different ($P<0.05$) thicknesses. Generally, film thickness also affects film properties such as mechanical properties, water vapor permeability, light transmission, and film transparency (Kaewprachu & Rawdkuen, 2014).

Tensile Strength and Elongation at Break

To use biodegradable films as packaging materials, it is important to develop films that possess favorable mechanical properties. Mechanical properties are largely associated with the distribution and density of intermolecular and intramolecular interactions in the network. These interactions are dependent on the arrangement and orientation of polymers chains in the film (Chambi and Grosso, 2006). Therefore, as the concentration of duck feet gelatin increased, the tensile strength also increased, as shown in Figure 2.

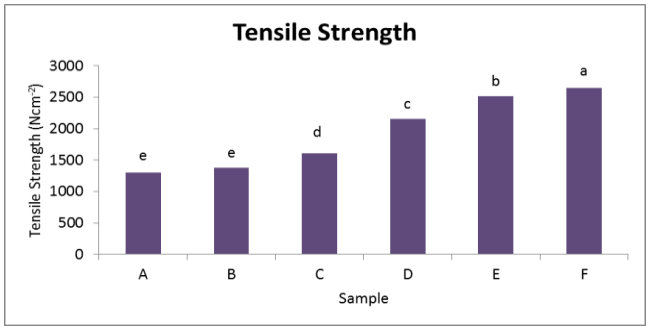

Figure 2: Tensile strength of films with different ratios of duck feet gelatin and PVA.
*Mean values with different letters indicate significant differences at ($P < 0.05$)

In Figure 2, Sample F displayed the highest tensile strength (2654.18 ± 21.59 Ncm-2), whereas Sample A had the lowest value (1306.25 ± 6.12 Ncm-2). The tensile strength of the blended films increased significantly as the concentration of duck feet gelatin increased. Sample B had a tensile strength of 1377.64 ± 58.60 Ncm-2, while Samples C, D, and E had tensile strengths of 1609.82 ± 55.79 Ncm-2, 2151.44 ± 42.60 Ncm-2, and 2518.44 ± 51.81 Ncm-2, respectively. Higher concentrations of the high molecular weight fraction of duck feet gelatin were related to a higher breaking force (tensile strength) and a noticeably lower elongation of the film. A study by Thomazine et al. (2005) examined two different types of gelatin-based films from higher molecular weight gelatins. Using differential scanning calorimetry (DSC), the gels with higher molecular weight gelatins were significantly more resistant, more rigid, and less elastic than films from low molecular weight gelatins. High tensile strengths are generally necessary for films to withstand the normal stresses encountered during their application, subsequent shipping, and food handling. In general, protein films are brittle and susceptible to cracking because of the strong cohesive energy density of the polymer (Arvanitoyannis et al., 1998).

Miya et al. (1984) studied similar blends and observed that PVA molecules in the blend tend to disrupt the crystallinity of proteins. In this study, PVA molecules added to duck feet gelatin also tended to disrupt gelatin crystallinity. However, in contrast, an increase in the tensile strength of the blended films has been reported (Park et al., 2001). The lower strength of pure PVA films may thus be due to its low degree of polymerization.

The relationship between tensile strength and elongation breaking point (Figure 3) was inversely proportional. This outcome means that film samples with highest tensile strengths will have the lowest amounts of elongation at their breaking points because films that are hard to break (require more force) have lower flexibilities. However, flexibility of biodegradable films, measured as elongation at breaking point, should be adjusted according to the applications for which it is intended. The elongation at breaking point of the film from Sample A, 402.17 ± 9.22%, was higher than that of the films prepared with blends of duck feet gelatin and PVA (Sample B, C, D and E), as well as Sample F, 92.63 ± 4.43%. This elongation behavior at break has been reported by Chiellini et al., (2011). Additionally, the addition of glycerol to films improves their flexibility properties (Pranoto et al 2005).

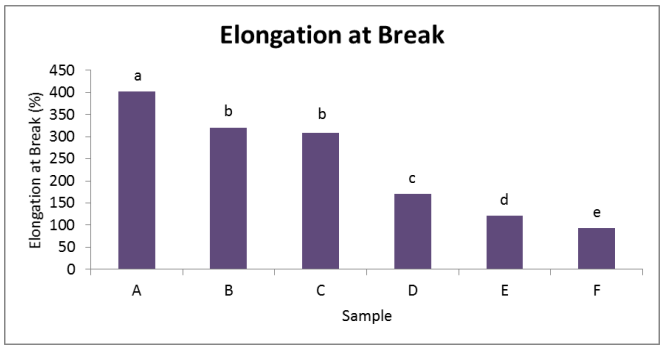

Figure 3: Elongation at breaking point for films with different ratios of duck feet gelatin and PVA
* Mean values with different letters indicate significant differences at ($P < 0.05$).

Surface color

The parameters of the different films' color differences, L*, a*, and b* are presented in Table 1. The film from Sample A showed the highest lightness, or L* value (96.91), and this parameter decreased significantly as the concentration of duck feet gelatin increased. This was due to the color of the PVA solution itself, which is clear and colorless, while the duck feet gelatin solution is cloudy. Even though the film from Sample F had a low lightness value (95.13), it was not far from the target value of standard white plate. The lightness values of Sample C and D dropped suddenly from 95.94 to 95.18 because the amount of duck feet gelatin had surpassed the amount of PVA in Sample D.

Table 1: Color difference parameters, L*, a* and b* of film with different ratios of duck feet gelatin and PVA blend.

Sample	L*	a*	b*

A	96.91±0.00[a]	-0.01±0.01[a]	0.23±0.02[f]
B	96.41±0.01[b]	-0.03±0.01[ab]	1.06±0.01[e]
C	95.94±0.01[c]	-0.07±0.06[bc]	1.49±0.00[d]
D	95.18±0.01[d]	-0.10±0.01[cd]	1.62±0.02[c]
E	95.16±0.01[e]	-0.12±0.03[d]	1.90±0.02[b]
F	95.13±0.01[f]	-0.20±0.01[e]	2.30±0.03[a]

* Mean values with different letters indicate significant difference at ($P < 0.05$).

For the a* values, all films showed negative values, which were visualized as a green tint. The greenness of all of the films increased as the concentration of duck feet gelatin increased, with the film from Sample F having the highest a* value of -0.20. The lowest greenness value was observed in Sample A with -0.01. The greenness values for the blended films gradually increased. In general, the b* value observed increased as the concentration of duck feet gelatin increased. The yellowness of all of the films is primarily a result of the color of duck feet gelatin itself. The gelatin solution was not clear, but rather had a yellowish tint as the concentration of duck feet gelatin increased. Thus, films with high concentration of duck feet gelatin (Sample F) had the highest value of yellowness at 2.30. The film from Sample A had the lowest yellowness value at 0.23, but as the duck feet gelatin was incorporated into film-forming solution, the value gradually increased.

CONCLUSIONS

The mechanical properties of tensile strength and elongation at breaking point of the films prepared with blends of duck feet gelatin and PVA varied across the spectrum of values that existed between films prepared from pure duck feet gelatin and PVA. The tensile strength of the films increased as the concentration of the duck feet gelatin increased. However, the percentage of elongation at breaking point decreased, as tensile strength and elongation at breaking point have an inversely proportional relationship. Incorporation of duck feet gelatin into films decreased lightness as the concentration duck feet gelatin increased due to the cloudy properties of the gelatin. It also increased the yellowness of the films, which is a result of the duck feet gelatin itself. When comparing all of the blended film ratios, the sample with a 4:6 ratio of duck feet gelatin to PVA had the best properties for use as a biodegradable film.

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