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Film Opacity, Water Vapor Permeability, Water Solubility and Fourier Transform Infrared Radiation (FTIR) 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 film opacity, water vapor permeability, water solubility and fourier transform infrared radiation (FTIR). 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 spectra of the films prepared from blends of duck feet gelatin and PVA (Sample B to D) were similar to those from the pure duck feet gelatin films (Sample F), indicating that there were no major changes in the functional groups of the gelatin produced by interactions between the PVA and the duck feet gelatin. The blended film with the ratio of duck feet gelatin to PVA (sample C=40:60) showed good properties for use as a biodegradable film compared to blended films with other ratios.

Keywords- Duck feet, biodegradable film, film opacity, water vapor permeability, water solubility.

INTRODUCTION

Raw materials such as biopolymers from renewable sources such as gelatin can be used to create biodegradable films (Tharanathan, 2003). Biopolymer-based films are generally sensitive to the relative humidity of environment since they are normally hygroscopic and have limited mechanical resistance compared with synthetic films. One possible method to improve the mechanical characteristics of protein-based films could be mixing these biopolymers with synthetic polymers such as polyvinyl alcohol (PVA), which is also hydrophilic and biodegradable (Carvalho et al., 2009). Additionally, PVA is a highly biocompatible, nontoxic synthetic material and is a common commercial product because it is easily cross linked, has high water permeability, and has excellent film forming properties (Ignatova et al., 2006).

Poultry by-products, mainly the feet and head, have received the most attention as an alternative source of collagen or gelatin. A previous study by Rahman and Shariffah (2012) reported the possibility of extracting gelatin from chicken feet. Other studies have shown that products with a high cartilage content can produce high yields of gelatin, and this relationship applies to the extraction of gelatin from duck feet. According to Ministry of Agriculture and Agro-based Industry, between 2009 and 2014, duck meat production increased from 144,240 to 164,508 metric tons. The large amount of production of duck in Malaysia, especially in Perak, means more by-products are likely to be produced. In Malaysia, the use of duck feet as food is limited because it contains many small bones and cartilage with no muscle. Thus, this limited use provides a readily available source of raw material (duck feet) to produce duck feet gelatin. Moreover, waste from duck meat production would be reduced by maximizing byproduct use for various applications.

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 (film opacity, water vapor permeability, water solubility and fourier transform infrared radiation) 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).

Film Opacity

The opacity of gelatin films was determined using a spectrophotometer (Cary 300 Bio, UV-Vis Spectrophotometer, Varian Instruments, CA, USA) set at a wavelength of 500 nm. Three film specimens were taken from each film sample and cut into rectangular pieces (4.5 × 1.0 cm). The samples were placed on inside transparent plastic 10 mm cuvettes and the absorbance values were measured. The opacities of films were calculated with following equation, according to the method described by (Montero, 2009):

Opacity = Absorbance at 500 nm / Film thickness

Water Vapor Permeability

Water vapor permeability (WVP) of the gelatin films was measured according to the WVP correction method described by McHugh et al., (1996), which is a modification of the American Society for Testing and Materials (ASTM E-96) for determining the WVP of synthetic packaging materials. Glass circular WVP cups with a 4.6 cm diameter were used for this analysis. Distilled water (10 ml) was added to each test cup and film samples were placed over the circular opening, tightened, and secured in place using silicon grease. The cups were maintained under controlled humidity and temperature conditions (50% ± 5% RH and 23 ± 2 °C). The weight loss from each of the 5 replicate samples was monitored for 10-hour period with weights recorded in 1-hour intervals. Water vapor permeability was calculated using the formula:

$$\text{WVP} = \frac{\text{Water vapor transmission rate (WVTR)}}{S\,(R1 - R2)} \times d$$

Where,

S = saturated water vapor pressure at test temperature (Pa)

R1 = RVP in desiccator

R2 = RVP in permeation cup

d = thickness of film (mm)

Water Solubility

Film solubility was determined according to the method described by Gontard and Guilbert (1992). Films were trimmed into small strips (2 x 3 cm) and dried in an oven (Memmert) at 100 °C for 24 hours until they reached a constant weight. Each dried film was then immersed in 100 ml distilled water for 24 hours. Films were then removed from distilled water and re – dried at 100 °C for 24 hours. Final weight was recorded, and water solubility was calculated as:

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

Fourier Transform Infrared Radiation (FTIR)

Any interaction or changes to the structure would occur when different ratios of the duck feet gelatin and PVA were blended together for biodegradable film production. Three replicates of each sample were cut into 3 cm x 3 cm strips to ensure satisfactory results of the peak absorbance reading. The films were clipped to a magnetic sample holder that had a round hole in the center. Then, the spectrum for each film was taken using a FTIR spectrometer equipped with attenuated total reflectance (ATR) and recorded for analysis (Silva et al., 2008).

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

Fil Opacity

This property is important if the film is to be used to coat foods and/or for packaging (Gontard and Guilbert 1992). Generally, the transparency of film is an indicator of miscibility of polymer blends (Li et al., 2006). Film opacity values should be low relative to values of transparent films. The opacity value of the films from Sample A to F are presented in Figure 1.

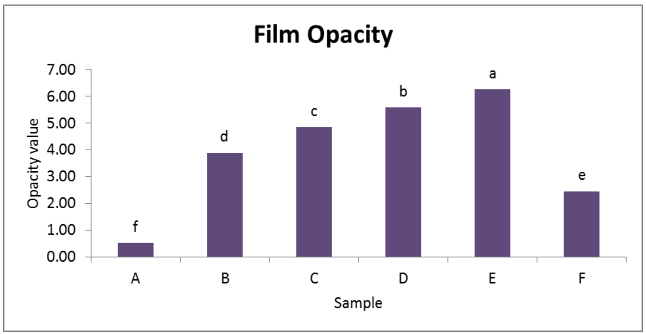

Figure 5: Opacity of films with different ratios of duck feet gelatin and PVA
* Mean values with different letters indicate significant differences at (P < 0.05)

It was remarkable that the films containing only the pure polymers (Sample A and F) had the two lowest values for opacity, namely, 0.52 ± 0.04 and 2.45 ± 0.16, respectively, and were thus more transparent than mixtures of the two products. Low opacity values indicate that the absorbance value measured by the UV-vis spectrometer was low, as more light could pass through the film. There were statistically significant variations in the observed opacities of the films. The opacity values increased when duck feet gelatin was incorporated into PVA solution. The film opacities of the blended films were 3.89 ± 0.03, 4.86 ± 0.18, 5.59 ± 0.26, and 6.26 ± 0.19 for Samples B, C, D, and E respectively. Transmission of light to films was affected by the protein concentration, increasing as the duck feet gelatin concentration increased. Films with high protein contents and with greater thickness tended to transmit less light. Thus, films with greater protein contents and thickness would absorb light more effectively than those with lower protein contents and thickness. Hence, the opacity values of films with higher protein contents and thicknesses were high (Jeongranak et al., 2006). Film transparency (low opacity) is related to the internal structure that is defined by the component rearrangement in the film matrix that occurs during the drying process (Chambi & Grosso, 2005).

Water Vapor Permeability

Biodegradable films act as a barrier for protecting foods from the surrounding environment, especially the transfer of moisture, which is the main factor examined in this analysis. Therefore, the water vapor permeability value needs to be as low as possible (Martins et al., 2012). Figure 2 shows that the film from sample A (PVA only) had the lowest water vapor permeability value (0.2997 g.mm/m2.h.kPa), which means that it exhibits good barrier properties in terms of preventing moisture absorption and emission. However, in some cases, such as the packaging of fruits or vegetables, plastic films with higher water vapor permeability values are needed to enhance the release of vapor produced from respiration. This release will prolong the shelf life and the freshness of fruits or vegetables.

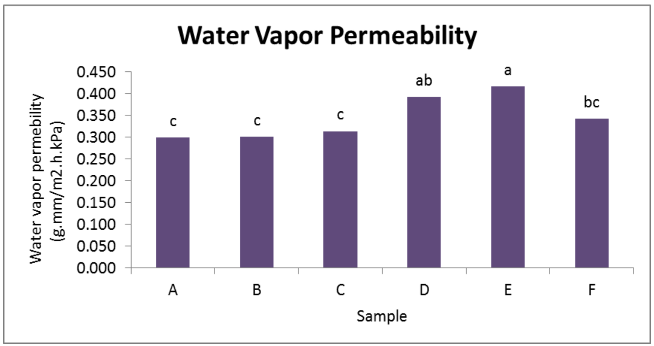

Figure 2: Water vapor permeability of film with different ratios of duck feet gelatin and PVA
* Mean values with different letters indicate significant differences at (P < 0.05).

In comparing Samples A and F, the water vapor permeability of Sample F (0.3426 ± 0.04 g.mm/m2.h.kPa) was higher than that of Sample A (0.2997 ± 0.01 g.mm/m2.h.kPa). Proteins containing voluminous chains will be more loosely packed (Chambi & Grosso, 2005). Therefore, films which contain only duck feet gelatin (Sample F) had greater permeability. Moreover, gelatin chains undergo a conformational disorder-order transition and partly regenerate the collagen triple helix structure during the gelation process. This process formed thermoreversible networks by associating helices in junction zones stabilized by hydrogen bonds (Bigi et al., 2004). Interstitial water molecules may act as hydrogen-bond bridges, thus contributing to stability of the helix (Jo et al, 2005).

However, the water vapor permeability values of blend films, Sample B (0.3013 ± 0.04 g.mm/m2.h.kPa), C (0.3141 ± 0.04 g.mm/m2.h.kPa), D (0.3928 ± 0.04 g.mm/m2.h.kPa), and E (0.4167 ± 0.01 g.mm/m2.h.kPa) gradually increased as the concentration of duck feet gelatin increased. According to Souza et al. (2010), the water vapor permeability of biodegradable films is dependent on many factors. Water permeability of a films should be affected by factors including the plasticizer used, surrounding temperature, hydrophilic-hydrophobic ratio, relative humidity of the integrity of the film, the mobility of the polymeric chains, and the crystalline-amorphous zone ratio.

As the concentration of duck feet gelatin increased, the water vapor permeability value of the blended films increased. The water vapor permeability of Sample E had a higher value than Sample B because Sample E contained lower amounts of hydrophobic amino acids and higher amounts of hydroxyproline. For the plasticizer, glycerol is well recognized as having a higher plasticizing effect than sorbitol but reduced resistance toward water vapor permeability (Gennadios et al., 1996). Thus, the type and number of molecules of plasticizers in the film affected water vapor permeability in Sample B. This result agrees with those reported by Nurhanani et al. (2011) who found that the water barrier properties of blended films had higher water vapor permeability values compared with film made from gelatin only. Overall, the water vapor permeability values observed in this study were similar to the values observed by Silva et al. (2008), who worked with films made from gelatin-PVA blends. However, these same authors observed that films made from pure PVA were less hygroscopic than gelatin films.

Water Solubility

According to Turhan and Sahbaz (2004), the dissolution of hydrophilic polymers involves the penetration of water to the polymer bulk, swelling, and subsequent disruption of intermolecular forces such as hydrogen and Van der Waal forces between polymer chains. A low dissolution rate indicates high cohesion of the polymer matrix via numerous interactions such as hydrogen bonds between polymer chains. The films from Sample A had highest percentage of water solubility at 87.37 ± 0.10%, while Sample F had lowest percentage of water solubility at 18.13 ± 0.72% (Figure 3). As the concentration of gelatin increased, the water solubility percentage of films decreased significantly. A low percentage of water solubility indicates that the film can protect or enhance product integrity, hence considered to be a good film for water resistance.

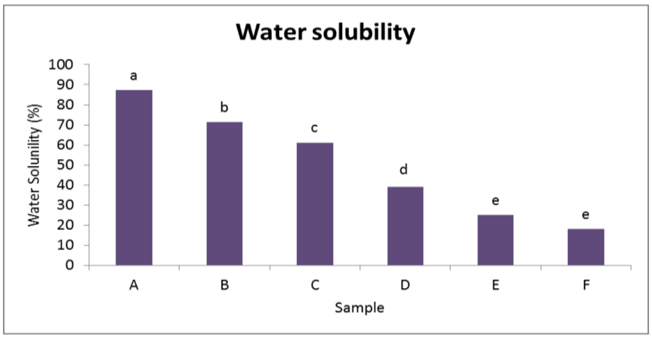

Figure 3: Water solubility of films made with different ratios of duck feet gelatin and PVA

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

These results agree with those reported by Chiellini et al., (2011), who observed that increasing the gelatin concentration decreases the solubility of films made from PVA blended with gelatin. Chambi and Grosso (2006) reported that gelatin was able to renature during gelling and the film-forming process and re-acquires part of the triple helix structure of collagen, which is a protein demonstrating a high degree of organization. Therefore, by increasing the amount of duck feet gelatin in the blends, the films produced can form more organized networks compared to the PVA network alone. These organized networks may refer to a high interaction density in films, which is supported by the finding that film solubility decreased and the structures were not easily disintegrated.

According to Finch (1983), the water solubility of PVA depends on its crystallinity and on the structure of the amorphous region of the polymer. To explain the behavior of the water solubility of the films, samples of pure gelatin and pure PVA, with no previous treatment, were mixed with equal parts of distilled water and analyzed by differential scanning calorimetry (DSC). The results suggested that gelatin presented a single, highly visible endothermal peak with initial and peak temperatures of 28.3 and 32.3 °C, respectively. PVA, however, presented two peaks. The first peak was very broad with an initial temperature of 46.2 °C and a peak temperature of 72.3 °C ($\Delta T = 26.1$ C). The second peak was very sharp ($\Delta T = 3.4$ C) with initial and peak temperatures of 98.8 and 102.2 °C, respectively. To a certain extent, these results may explain the crystallinity of the PVA films, because the heat treatments for solution B and PVA solution (see Methodology) was done at 95°C, which was not sufficient to dissolve the crystallites observed in the second endothermal peak (102°C). Therefore, during the analysis of water solubility, the PVA remained soluble in water.

Fourier Transform Infrared Radiation

FTIR analysis was conducted to ensure that no interaction occurred between the duck feet gelatin and the PVA when they were incorporated together as biodegradable film. Generally, chemical bonds vibrate at characteristic frequencies; thus, an exposure to infrared radiation will cause them to absorb radiation at frequencies that match the vibration modes. The spectra of the films containing only duck feet gelatin, blends of both polymers (different ratios of duck feet gelatin to PVA), and only PVA are shown in Figure 4. Despite being a qualitative analysis, the positions of the peaks obtained from the spectrum in the FTIR spectroscopy analysis are still sensitive to conformations at the macromolecular or molecular levels. The spectra of the films prepared from blends of duck feet gelatin and PVA (Sample B to D) were similar to those from the pure duck feet gelatin films (Sample F), indicating that there were no major changes in the functional groups of the gelatin produced by interactions between the PVA and the duck feet gelatin.

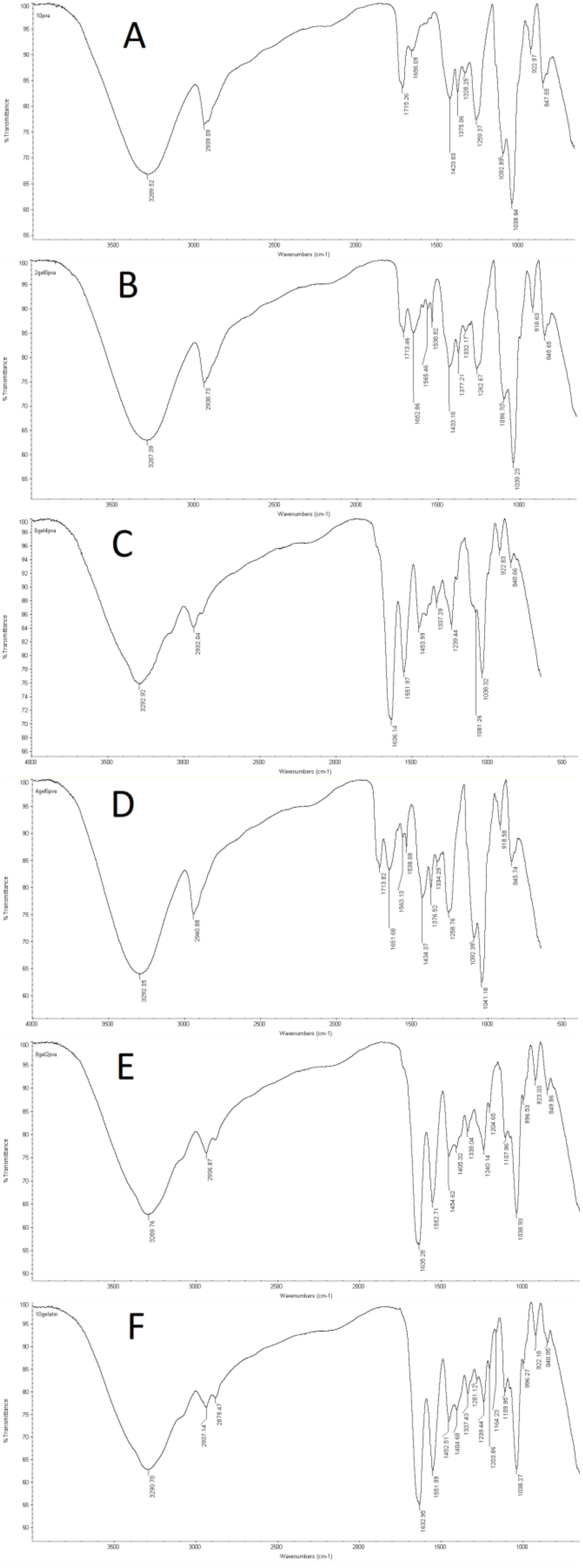

Figure 4. FTIR spectraof the film made with different ratios of duck feet gelatin and PVA.

There are three characteristic signals in the FTIR spectra of proteins: amides I, II and III. The amide I band arises from the stretching of C=O bonds of the proteins. The amide II band is due to vibrations of N–H bonds and vibrations due to stretching of the C–N bonds. The amide III band corresponds to vibrations in the planes of the C–N and N–H of the amide bonds or vibrations of the CH2 groups of glycine, an amino acid abundant in gelatin. The amide II and amide III peaks are less sensitive to the secondary structure of proteins such as gelatin (Yakimets et al., 2007). The FTIR spectra of PVA showed a broad peak approximately 3289.52 cm-1, which indicates stretching of hydroxyl groups. The band for CO stretching and OH bending was observed at 1092.89 cm-1. The FTIR spectrum of gelatin showed that the regions between 3000-3600 cm-1 and 1100-1700 cm-1 contained the most potentially useful information on the structure of the gelatin. An analysis of the amide I band of Sample F showed a peak at 1632.95 cm-1. The peak at 1632.95 cm-1, characteristic of the coiled structure of gelatin, may also be associated with CO and CN stretching and was insensitive to the PVA content (Yakimets et al., 2007). In the amide II band, a peak appeared at 1551.99 cm-1 in the films made from only duck feet gelatin (Sample F).

However, in the films prepared from blends of gelatin and PVA, the peaks remained same, although no peak was observed for Sample A. In the amide III domain, a highly visible peak appeared at 1239.44 cm-1, and a less visible peak was present at 1203.86 cm-1 for the film from Sample F. This peak was insensitive to the concentration of PVA, but the peak at 1239.44 cm-1 shifted to 1258.76 cm-1 for Sample D and to 1262.67 cm-1 for Sample B. A characteristic peak was observed at 1038.34 cm-1 for the film from Sample A, which is sensitive to crystallization. This behavior may indicate that there was some interaction between the gelatin and the PVA that affected the crystallinity of the films. (Silva et al., 2008). Moraes et al., (2009) analyzed films made from blends of gelatin and PVA and plasticized by glycerol using X-ray diffraction and observed patterns typical of partially crystalline materials. The spectrum of the pure gelatin films in this study also revealed a peak at 3290.78 cm-1, which is normally associated with NH stretching. However, the spectra of the films made from blends of gelatin and PVA (Sample B to E) and the Sample A films all showed a peak at 3289.71 cm -1, which is normally associated with OH stretching vibration.

CONCLUSIONS

It was observed in the blended films B to E, the values of water vapor permeability and the percentage of water solubility decreased as the concentration of PVA increased. The addition of duck feet gelatin improved water permeability and the solubility properties of the film. The spectra of the films prepared from blends of duck feet gelatin and PVA (Sample B to D) were similar to those from the pure duck feet gelatin films (Sample F), indicating that there were no major changes in the functional groups of the gelatin produced by interactions between the PVA and the duck feet gelatin. When comparing all of the blended film ratios, the sample C with a 40:60 ratio of duck feet gelatin to PVA had the best properties for use as a biodegradable film, such as good opacity, low water vapor permeability, and a low percentage of water solubility.

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