Evaluation of biodegradable activity of film from chicken feather keratin

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Abstract. The disposal of plastic caused serious environmental pollution to both land and sea. Chicken feather wastes also contributed to pollution problem and its value has not been fully utilized. This study aimed to produce biodegradable film by utilizing keratin extracted from chicken feathers. Initially, the keratin was extracted from chicken feather prior to the optimization of the keratin biofilm towards the tensile strength. The involved parameters were ratio of keratin to glycerol, ratio of keratin to PVA and drying temperature. The biofilm was proceeded to morphological, chemical structure and colour characterization analysis using SEM, FTIR and chromameter, respectively. The keratin obtained was 0.1296mg/ml. The obtained keratin was then used in the formulation of biofilm. The biofilm with 40:50:10 (volume ratio of keratin: PVA:glycerol) dried at 60°C showed the optimum tensile strength. The FTIR showed the presence of amide and hydroxyl groups. From biodegradability test, it was proven that blend of keratin, PVA and glycerol films can be degraded by microorganisms in soil in which films with higher concentration of glycerol degrading faster compared to those with lesser glycerol concentration. Thus, it can be concluded that the chicken feather keratin offers an alternative as biofilm for biodegradable plastic.

1. Introduction

Plastic bags are widely used in daily life since its advent in late 1970s [1]. They were made by conventional petrochemical method and plays an important role as carriers or for packaging of materials due to easy availability at low cost, have good heat resistance and high tensile strength [2]. However, the synthetic polymer take a long time to be degraded by itself, hence it leads to worsening of environmental pollution.

In order to replace the use of plastics with other material that is more eco-friendly, researches focused on the renewable sources such as polysaccharides [2-3], proteins [4] and lipids [5]. Some examples of materials used in the research as an alternative to plastic include cellulose, starch, pectin, waxes and acylglycerols. However, feathers recently were discovered to have significant potential to manufacture biodegradable materials [2]. The great demand of chicken in food industries caused the production of chicken feather increase dramatically and caused accumulation of waste in ecosystem [6]. Therefore, the investigation on the chicken feather as a keratin source in bioplastic films is crucial as it can replace the daily plastic use and minimize the environmental pollution caused by the chicken feather waste.
2. Methodology

2.1 Preparation of chicken feather keratin

The chicken feathers were first washed with distilled water and dried under the sun for 2 days. Then, they were degreased with hexane in a beaker which act as washing solvent to remove oil contents [7]. The ratio of feathers and hexane by w/v ratio used was 1:5. The degreasing process was repeated for 2 times. The hexane was later evaporated by leaving the beaker overnight in the fume hood. The feathers were sterilized by autoclaving at 121°C for 30 minutes and the weight of defatted wet feathers was weighed [8]. All used chemicals were of analytical grades.

2.2 Extraction and protein determination

The feathers were blended into wool-like structure with a blender and the feathers were soaked in 7M urea solution at 50°C for 1 hour in a mechanical water bath shaker to disrupt the hydrogen bonding in feathers to release keratin [9-10] before immersing in sodium hydroxide solution [11] for 30 minutes under continuous mechanical stirring at 50°C. The amount of sodium hydroxide used were 2ml/g of feathers. The purpose of NaOH was to extract the keratin content from chicken feathers and maintain the keratin in aqueous form.

The prepared mixtures were then filtered using Whatman filter paper before centrifuging at 4000rpm for 10 minutes to separate keratin rich solution with undissolved feathers [9,12]. The presence of protein in the solution obtained (30 µl) were tested using 1.5 ml Bradford solution and left at room condition for 10 minutes. The colour changes were observed and analysed at 590nm with bovine serum albumin as control.

2.3 Preparation and characterization of bioplastic film

Polyvinyl alcohol (PVA) solution was first prepared by dissolving PVA crystal (1g:1ml) in distilled water at 80°C. The extracted keratin solution, PVA and glycerol with volume ratio of 30:50:20 were mixed on a hot plate at 60°C for 2 hours [11]. Then, 15ml of the solution was pipetted into a petri dish and dried in an oven at 60 °C for 72 hours. Table 1 shows the films that obtained with different parameters.

| Parameter                          | Values         |
|-----------------------------------|----------------|
| Ratio of keratin:glycerol         | 40:10          |
|                                   | 35:15          |
|                                   | 30:20          |
| Ratio of keratin:PVA              | 35:55          |
|                                   | 40:50          |
|                                   | 45:45          |
| Temperature of drying (°C)        | 40 °C          |
|                                   | 60 °C          |
|                                   | 80 °C          |

2.3.1 Tensile strength analysis. Texture Analyser (TA.XT plusC) was used to measure the tensile strength of the film produced. The film strips made with different concentration of glycerol, PVA and dried at different temperature will be cut into size of 60mm x 10mm and stretched for approximately 100mm at speed of 1mm/s [13-14]. The tensile strength of each films were later calculated using Equation 1.

\[
Tensile\ strength = \frac{F_x}{A}
\]  

Where

F: Force (g)

x: Distance of top grip from original position (m)
t: Time to break (s)
A: Cross sectional area of films (m²)

2.3.2 Fourier Transform Infrared Spectroscopy (FTIR). The prepared biofilms were first cut into sizes of 10 x 10 mm prior to analysis using FTIR (Spectrum 100, Perkin-Elmer, Fremont, CA, USA). The frequency used ranged between 400 cm⁻¹ to 4000 cm⁻¹ and the data collected were analysed using FTIR Spectrum Software [10,13,15].

2.3.3 Scanning Electron Microscope (SEM). The morphology of the optimized and control biofilms were cut into dimensions of 10mmx10mm and coated with platinum before studying under the scanning electron microscope, JSM-6460LA (JEOL, Japan) at voltage of 20kV and magnification of x2000[9-11].

2.3.4 Colorimeter. The colour of the optimized biofilms was analysed using Minolta Chroma Meter (CR-400). The meter was first calibrated using a standard white calibration plate before proceeding with tests. The films were directly placed under the observer of the chromameter for measurements [14]. Hunterlab color scale was used to evaluate the results, with lightness=0 to 100 (black and white) and chromaticity parameters of +a in red direction, -a in green direction, +b in yellow direction and –b in blue direction.

2.3.5 Biodegradability test. Films of different concentration of keratin and glycerol were first cut into size of 20 x 20 mm. Then they were buried under gardening soil used for planting. Appearances and weight loss of films were noted every 2 days for a period of 7 days until the films were degraded. During the period of experiment, some water was added into the soil to keep the soil sufficiently moist. When the samples were taken out from soil for readings, they were first lightly cleaned off unwanted soil particles using a moist cloth and dried before weighing [10,16]. The weight loss calculation was based on Equation 2.

\[
\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}}
\] (2)

3. Results and Discussion

3.1 Tensile strength analysis

3.1.1. Effect of ratio of keratin:glycerol. Figure 1 shows the graph of tensile strength against the type of film with different concentration of glycerol. The tensile strength of films decreases when the concentration of glycerol increases, from an initial value of 28.13 Pa for 0% glycerol film to 11.07 Pa for 20% glycerol film. Although the 20% glycerol film shows slightly higher tensile strength than that for 15% glycerol film, the slope is generally going downwards. This is because more glycerol content increases free volume or voids in film which causes an increase in mobility and less resistance to stretching. This is in line with previous theories which showed similar decrease in tensile strength with increasing glycerol content in films [17-18].
3.1.2 Effect of ratio of keratin:PVA. Figure 2 showed the graph of tensile strength against the type of film with different concentration of PVA. Based on figure 2, the tensile strength of films decreases when the concentration of PVA increase, from an initial value of 15.23Pa for 45% PVA film to 3.10Pa for 90% PVA control film without keratin. According to previous, tensile strength showed linear reduction when PVA concentration increased in contrast to other studies which was attributed to typical behaviour of homogenous and thermodynamically miscible system [20].

3.1.3 Effect of temperature of drying oven. Figure 3 shows the graph of tensile strength against the film dried at different temperature in oven. The tensile strength for film dried at 60 °C shows the highest tensile strength of 15.35 Pa, follow by 11.44 Pa for film dried at 40 °C and 8.30 Pa for film dried at 80 °C. Film dried at 80 °C has the lowest tensile strength probably due to fast drying which made the linking between keratin, PVA and glycerol to be less completed, while the linkage for film dried at 40 °C was more completed but some PVA may have solidified during drying. Currently there are no research being done to investigate the effect of drying temperature, but 60 °C had been widely used as drying temperature of films [11,13].
3.2 Characterization of the prepared biofilm

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR). Figure 4 shows FTIR for films at different volume ratio of K:PVA:Gly and pure PVA. By comparing pure PVA film with films mixed with keratin and glycerol, few functional groups can be identified based on their structural formula. S-S, C-N, N-H and C=O bonds came from keratin while O-H and C-O bonds came from PVA and glycerol. Later by comparing films with different concentration of keratin and glycerol pure PVA film, it can be shown that most parts of the spectrums for these three films were almost identically same.

Figure 4. FTIR for films at different volume ratio of K:PVA:Gly and pure PVA.

3.2.2 Scanning Electron Microscope (SEM). The effect of glycerol on the morphology of the prepared biofilm is evaluated by SEM (Figure 5). The left film (control) shows a smooth surface without cavity and holes. This indicates that keratin and PVA bonded well without the interference of plasticizer to cause empty voids to appear. When 10v/v% glycerol is added, empty holes starts. This may be caused by higher degree of dispersion of glycerol as plasticizer into the polymer matrix. This is in line with
studies done which stated that the degree of phase separation is directly proportional to glycerol content [10].

![Figure 5. SEM for biofilm of (a) control and (b) 40:50:10](image)

3.2.3 Chromameter. Table 2 shows the colour readings of plastic biofilms for PVA+Gly and the optimized biofilm. From the table, the value of b* increased and L* decreased with the presence of keratin and small amount of glycerol. Since the colour of keratin extracted from chicken feathers was brownish yellow colour, while the colour of glycerol was milky white, the colour of film became yellower when keratin content increased and glycerol decreased in sample. This result is in line with results shown previous researchers [19].

| Film       | L*   | a*   | b*   |
|------------|------|------|------|
| PVA+Gly    | 81.57| -0.24| 6.00 |
| 40:50:10   | 72.50| 1.35 | 24.71|

3.3 Biodegradability test

The biodegradability test using biofilm of control and 40:50:10 are shown in Figure 5. It displays the condition of biofilm when buried under the soil from day 1 to day 7 and weight loss percentage for each film. Based on Figure 6, the degradation rate for film 40:50:10 was faster as compared to control biofilm. The colour for all biofilms changed at day 3, from an initial brown colour to colourless which may be attributed to the degradation of keratin responsible for the brown colour. The results obtained are in line with previous report [10].
4. Conclusion
Keratin was successfully extracted from chicken feathers with concentration of 0.1296mg/ml and tested positive with Bradford reagent. Parameters like concentration of glycerol, PVA and drying temperature in oven were examined to check their effects on characteristics of films produced by physical characterizations which showed that keratin films generally are yellow in colour, have higher tensile strength at lower concentration of plasticizer and PVA, and have lesser empty voids or holes on films’ surface with lower plasticizer content. Keratin:PVA:glycerol with volume ratio of 40:50:10 dried at 60°C was found to be the best film in terms of tensile strength. The films produced from keratin were proven to be degradable when buried under the soil. The biodegradability of the optimized biofilm is faster than control biofilm which is due to lesser amount of PVA and keratin needed to be degraded. In conclusion, the idea of replacing conventional plastics with biodegradable plastics have been proven to be viable.

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Acknowledgement
The authors greatly acknowledge the Department of Chemical Engineering Technology for providing the facilities to complete the research.