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Development and Characterization of Halal Gelatin/Curcumin Composite Films for Food Packaging Applications

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Abstract: The Halal status of development of plastic packaging made from animal gelatin is often questionable. Therefore, the alternative source of halal gelatin is required in order to replace the use of pork gelatin with gelatin obtained from marine sources. Gelatin from the warm-blooded fish has similar properties to mammalian sources. The scales of fish are known to be high in collagen that can be a good source of gelatin. In this study, gelatin was extracted from the tilapia scales by heating the scales in distilled water at 70°C. The halal gelatin/curcumin composite films were prepared by using glycerol as plasticizer and sodium dodecyl sulfate (SDS) as an emulsifier. The neat gelatin and gelatin/curcumin composite films were characterized by using field emission scanning electron microscopy (FE-SEM) and Fourier transform infrared spectroscopy (FTIR). The FE-SEM results showed that the low concentration of curcumin was dispersed evenly in the matrix of gelatin but not well dispersed at high concentration of curcumin. The FE-SEM results also showed that the addition of curcumin was able to reduce the visible cracks on the composite films. The FTIR analysis of neat gelatin and gelatin/curcumin composite films showed no significant changes in the functional group of the composite films even after the addition of SDS and curcumin. All the film samples have a significant difference on the L* a* and b* values ($p < 0.05$). This study showed the potential of tilapia scales as a gelatin source and also the addition of curcumin affected the physical and chemical properties of gelatin films.

Keywords: Food Packaging, Curcumin, Tilapia Scales, Gelatin, Composite Films.

1. Introduction

Gelatin is a high molecular weight and a hydrocolloid polypeptide that has gained widespread popularity due to its versatile nature and its ability to be used in a variety of food products [1]. Bovine

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and porcine gelatins are widely used sources of gelatin around the world due to their inexpensive cost and wide availability [2]. However, there are restrictions on the uses of bovine and porcine gelatin due to religious or ethical reasons. However, the uses of gelatin from porcine create a great concern especially for Muslim community since it is not halal in Islam religion, which is mean it is forbidden in Islam. Therefore, due to this issue, the gelatin derived from halal sources as an alternative of porcine gelatin has become an essential thing recently. According to this direction, gelatin from fish has become the best candidate as an alternative source of porcine gelatin. Tilapia fish scales seem to have the potential to produce gelatin with good physicochemical properties. In this study, gelatin was extracted from the tilapia scales by heating the scales in distilled water at 70℃.

There are many new approaches for the use of gelatin as eco-friendly composite films with excellent packaging properties due to its properties. Gelatin has a high potential for the commercial uses in food packaging films because of its unique characteristics such as inexpensive, broad sources, good biocompatibility and biodegradability [3]. However, according to a previous study [4], it shows that the shorter gelatin molecules generated by hydrolysis resulted in a film with a lower junction zone or shorter strands due to the weak bonding during formation of film. As a result, the resultant films had lower mechanical properties and thermal stability. In this case, curcumin is the best option to be used in order to enhance both of the physical and biological properties of gelatin.

Curcumin is the natural yellow pigment in the turmeric roots and the major functional component (2−5%) of turmeric [5]. In addition, curcumin has strong antioxidant properties and has recently received a lot of interest due to its important bioactive potential [6]. Curcumin was applied to natural and synthetic polymer films to give them antioxidant and antibacterial characteristics. There are a lot of studies that have been focusing on enhancing the functional properties of biodegradable films by incorporating various compounds with antioxidant or antimicrobial properties with the biodegradable films in order to produce a biodegradable active packaging material. According to a previous study [7], the gelatin-based film incorporated with curcumin showed significant antibacterial activity while the neat gelatin film, which was not incorporated with curcumin, did not show any antibacterial activity. Therefore, this study would focus in depth of the preparation, characterization in terms of surface morphology, physical, chemical and antibacterial properties, and also the properties of gelatin-based films from tilapia scales incorporated with curcumin for active food packaging application. The effect of different concentrations of curcumin, which are 0.5, 1.0 and 1.5 wt percent based on the gelatin, will be tested on gelatin-based composite films in order to study the physical and biological properties of films.

2. Materials and Methods

2.1 Materials

Tilapia scales were purchased from the Tilapia Fish Supplier in Vietnam. Curcumin Extract Powder ≥95% (solvent soluble) was purchased from supplier Innophos in Spain. Sodium hydroxide pellets (AR) were purchased from Asia Chemie (Thailand) Co., LTD. Copper (II) sulfate anhydrous (AR) was purchased from Friendemann Schmidt. Glycerol anhydrous (AR) was purchased from R&M Chemicals. Mueller Hinton agar (MHA) and brain heart infusion broth (BHI) were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India).

2.2 Extraction of Gelatin from Tilapia Scales

The extraction of gelatin from tilapia scales procedure was performed by using Zakaria and Abu Bakar's technique [8], with a slight modification. 100g of tilapia scales were washed with tap water for an hour in order to remove the superfluous material. Next, the scales were soaked in 500mL of 0.4 (w/v) sodium hydroxide (NaOH) for four hours in order to remove the non-collagenous protein. The scales treated with NaOH were then washed with tap water for an hour. After that, the scales were soaked in 500mL of 0.4 (v/v) of hydrochloric acid (HCl) for four hours. In order to neutralize the pH of tilapia scales after the pre-treatment, the scales were washed again by using tap water. After the pre-treatment, the swollen scales were soaked with distilled water at 1:1 ratio (g:mL) at 70°C for one hour and 30 minutes in order to extract the gelatin. Then, the gelatin extracted was filtered by using two layers of cheese clothes in order to separate the gelatin solution and scales. In order to remove the water content, the solution was evaporated by using a rotary evaporator (Rotary Evaporator N-1300E V EYELA) for 30 minutes at 30rpm with temperature between 40 to 50°C. The filtrate obtained was dried in a hot air oven for 18 hours at 50°C. The gelatin produced was then pulverized into powder and the powder was stored in desiccators for further use.

The extracted gelatin was analyzed by reacting it with NaOH and copper (ii) sulfate ($CuSO_4$) in order to make sure the gelatin powder is positive gelatin. Firstly, 0.1g gelatin powder was dissolved in 5mL distilled water in a test tube. Then, 1mL of NaOH and 2mL of $CuSO_4$ were added. If the solution turns to purple in color, then it is a positive solution of gelatin.

2.3 Characterization of Extracted Gelatin

2.3.1 FTIR Analysis

The FTIR spectroscopy analysis of gelatin powder was analyzed by using the FTIR spectrophotometer. 50mg of gelatin powder was placed directly on the beam exposure stage for measurements. Then, the tip of the instrument was touched directly on the sample. At room temperature, measurements were taken at 4000–500 cm^{-1}, with automatic signals gathered in 32 scans at a resolution of 4 cm^{-1}.

2.4 Film Preparation

Gelatin incorporated with curcumin composite films was prepared by using a casting method as described by Shankar et al [9] with a slight modification. Different concentrations of curcumin, which are 0.5, 1.0 and 1.5 wt percent based on gelatin were mixed in 100 mL of distilled water that contain 1 wt percent (based on gelatin) of sodium dodecyl sulfate (SDS) which is an emulsifier. They were mixed by using a magnetic stirrer and sonicated for 10 minutes in a water bath. Then, 1g of gelatin powder and 30 wt percent of glycerol (based on gelatin) were slowly added into the solution with continuous stirring. The solution was heated at 80°C for 30 minutes. The film-forming solution was casted on a petri dish and dried in a hot air oven at 50°C for two days. The dry film was removed from the plate and stored in the desiccator for two days. The clean gelatin film that is not incorporated with curcumin and SDS were prepared with the same method as control. All samples were prepared in triplicate.

2.5 Characterization of Gelatin and Gelatin/Curcumin Composite Films

2.5.1 Color Analysis

The color of neat gelatin and gelatin composite films were determined by measuring the lightness, redness and yellowness values (L*, a* and b*) by using the Hunterlab Miniscan EZ spectrophotometer.

2.5.2 Surface Morphology

The microstructure of the film samples were analyzed by using field emission scanning electron microscopy (FE-SEM) operated at an acceleration voltage of 5.0 kV. A small portion of each film sample was placed on the FE-SEM specimen holder and sputter-coated with gold for 30 seconds before measurement.

2.5.3 FTIR

The FTIR spectroscopy analysis was carried out in order to identify the presence of organic and inorganic compounds in the film samples prepared. A total reflectance – FTIR spectrophotometer was used to determine the FTIR spectra of the neat gelatin and gelatin composite films. All film samples were cut into pieces and placed directly on the beam exposure stage for measurements.

2.6 Statistical Analysis

The values from the analysis were expressed as mean ± standard deviation of triplicate values. Analysis of Variance (ANOVA) was applied to determine whether there are significant differences between results obtained. The values are considered significant different if the value is $p < 0.05$.

3. Results and Discussion

3.1 Characterization of Extracted Gelatin

3.1.1 Yield of Gelatin

Total yield of gelatin extracted from the tilapia scales is 2.18%. It is found to be lower compared to the other extraction yield that had been reported previously. The yield of gelatin extracted from the black tilapia scales was 16% [8]. The lower yield might be caused by the loss of extracted collagen due to leaching during the series of washing processes or caused by the partial hydrolysis of collagen [10]. Furthermore, the low extraction yield might be due to the low concentration of acid used during the pre-treatment before the process of gelatin extraction. In addition, the extraction yield of gelatin increases when the concentration of acid used during the pre-treatment is increased [11].

3.1.2 FTIR

Figure 1 shows FTIR spectra for gelatin powder extracted from the tilapia scales. There are five different bands that can be identified. Amide A band was recorded at the wave number 3280.29 cm^{-1}, which indicates the gelatin extracted from tilapia scales showed a characteristic of N–H stretching. Generally, a free N–H stretching bond is found at the wave number between 3400 to 3440 cm^{-1} [12]. The position of this band will shift to lower frequencies when the N–H group of a peptide is involved in a hydrogen bond [13]. Furthermore, from this FTIR spectra, Amide I can be found at the wave number 1633.78 cm^{-1}, which indicates the stretching of the carboxyl groups (C=O). Amide II which at the wave number 1531.82 cm^{-1} and 1446.90 cm^{-1} indicated the N–H bending and CH_2 bending, respectively. The peak at 1238.57 cm^{-1} corresponded to N–H stretching of gelatin which is due to the amide III of protein [7].

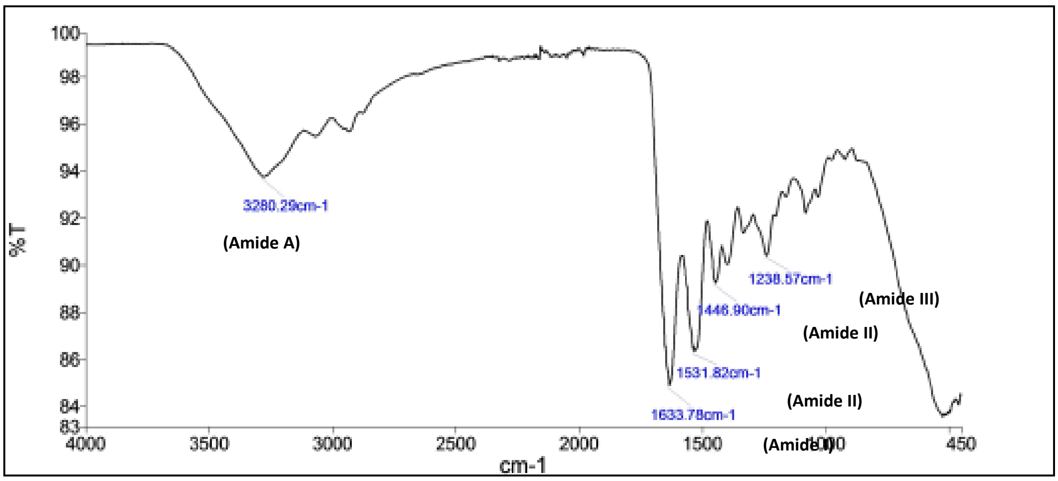

Figure 1: FTIR spectra of gelatin powder extracted from tilapia scales.

3.2 Characterization of Gelatin and Gelatin/Curcumin Composite Films

3.2.1 Color

The analysis of surface color and light transmittance of film are important in its application since it may affect the stability of packaged food as well as the senses of consumers [14]. The neat gelatin which without addition curcumin and SDS was clear and transparent while gelatin/curcumin composite films were yellow in color and the color differed according to the concentration of curcumin added into the gelatin films. Table 1 shows the result for color analysis of gelatin films. From the result obtained, the neat gelatin film had the highest L* value (lightness) and a* value (redness/greenness) but had the lowest b* value (yellowness). The composite films with the concentration of curcumin 1.5 wt percent had the lowest L* value and a* value but had the highest b* value. The yellow pigment of curcumin was primarily responsible for the formation of yellow color of gelatin/curcumin composite films [7].

Table 1: Color Analysis of Gelatin Films

Films	L*	a*	b*
Gelatin	81.62 ± 0.96	0.92 ± 0.04	-1.98 ± 0.13
Gel/Cur$^{0.5}$	79.62 ± 1.31	-6.02 ± 0.10	24.30 ± 1.26
Gel/Cur$^{1.0}$	79.42 ± 0.51	-5.96 ± 0.11	33.20 ± 1.65
Gel/Cur$^{1.5}$	75.42 ± 0.40	-6.53 ± 0.23	61.08 ± 0.72

3.2.2 Surface Morphology

Figure 2 shows the FE-SEM micrograph of film samples. From the FE-SEM micrograph, it can be seen that there are cracks all over the surface of the films. The cracks in thin films are mainly caused by incompatibility in the thermal expansion coefficients of the substrate and thin film [15]. The cracks also might be due to the films made from fish gelatin. The films made from fish gelatin have the potential to become brittle due to the existence of disulfide bonds, hydrogen bonds, electrostatic forces and hydrophobic molecules [16]. According to a previous study [17], the films from fish gelatin also have lower water vapor permeability than the films made from mammalian gelatin due to the differences in the composition of amino acids.

However, the cracks were reduced after the addition of curcumin into the gelatin films. From the FE-SEM micrograph, at the highest concentration of curcumin which is at 1.5 wt percent, the cracks of the film were less visible compared to the other film. This proved that curcumin was able to improve the physical properties of gelatin films. The addition of curcumin which is up to 1 wt% to the gelatin-based food packaging will greatly improve its mechanical properties and also the water vapor barrier properties [7]. Furthermore, due to the surfactant, SDS, curcumin was dispersed uniformly in the gelatin/curcumin composite [7]. However, the surface morphology of the gelatin/curcumin composite films differed depending on the curcumin concentration. At lowest concentration of curcumin which is at 0.5 wt percent, curcumin was dispersed evenly in the matrix of gelatin while at the highest concentration of curcumin which is at 1.5 wt percent, the particles can be clearly seen in the FE-SEM micrograph. Therefore, it proved that the curcumin was dispersed evenly in the matrix of gelatin, mainly owing to the application of SDS, which indicates the high compatibility, intermolecular interaction and affinity between curcumin and polymer matrix [7].

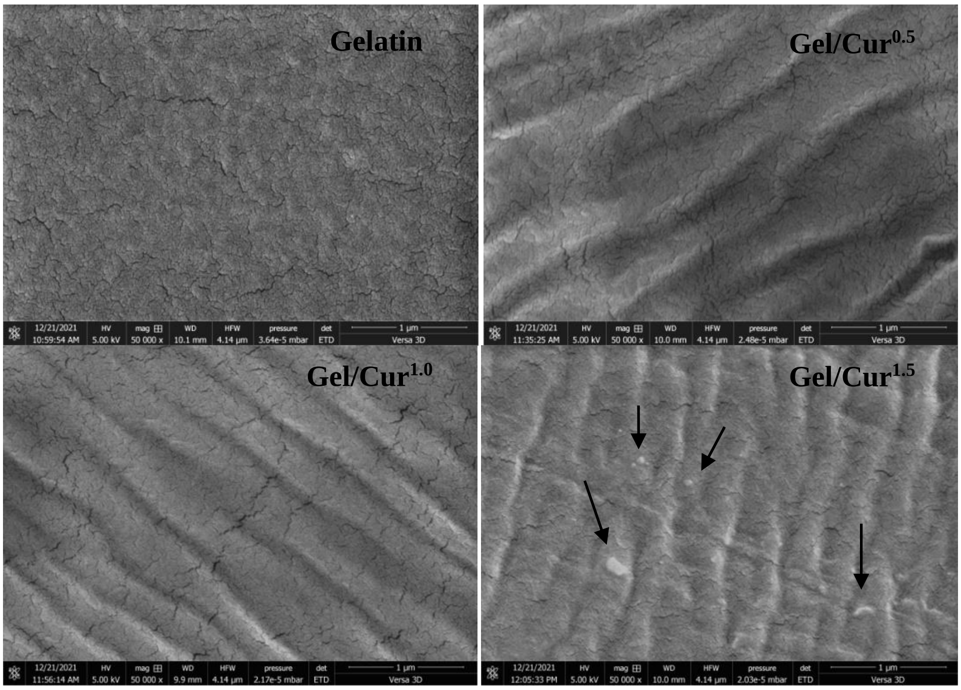

Figure 2: FE-SEM micrograph of neat gelatin and gelatin/curcumin composite films.

3.2.3 FTIR

FTIR was conducted in order to distinguish the intermolecular interaction between the gelatin film matrices with the compounds that present in curcumin. Figure 3 shows the FTIR spectra of neat gelatin and gelatin/curcumin composite films. The spectrum of gelatin showed a number of different peaks ranging from 3285 cm^{-1} to 1037 cm^{-1}. The broad peaks were observed in all film samples at ~3287 cm^{-1} which indicates the O–H stretching vibration of gelatin. The intense peak at ~1633 cm^{-1} corresponded to the carbonyl (C=O) stretching/hydrogen bonding coupled with COO [18]. The peaks appearing between 1547 to 1543 cm^{-1} in all of the film samples indicates the bending vibration of the N–H group of amines. In addition, all the film samples showed peaks from 1452 to 1239 cm^{-1} corresponding to the vibrations of C–N and N–H stretching [19]. Furthermore, the peak at ~1037 cm^{-1} that appeared in all film samples could be due to the interactions between the O–H group of glycerol which acts as plasticizer and the gelatin films [20].

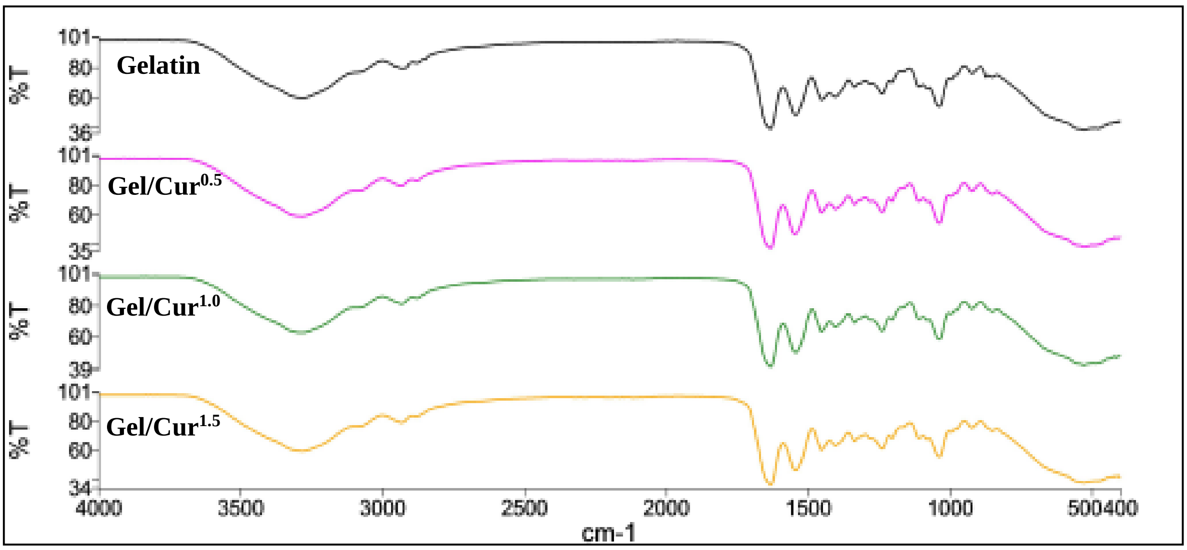

Figure 3: FE-SEM micrograph of neat gelatin and gelatin/curcumin composite films.

4. Conclusion

The environmentally friendly of halal gelatin/curcumin composite films from tilapia scales were prepared by a casting method using glycerol as the plasticizer. This study also proved that the tilapia scales has potential to be a good source of halal gelatin which is able to replace the mammalian gelatin. The addition of curcumin into gelatin films affected the physical and chemical properties of gelatin films. The color of gelatin film changed from colorless to yellow in color after the incorporation of curcumin into film depending on the concentration of curcumin used. The FTIR analysis of neat gelatin and gelatin/curcumin composite films showed no significant changes in the functional group of the composite films, showing that there are no structural changes in gelatin after combining with SDS and curcumin.

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