

Preparation and Characterization of Water Hyacinth Cellulose/Keratose Composite Films

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Abstract - This research aimed to extract cellulose from water hyacinth for the preparation of composite films with different ratios of hair keratose. The keratose film has more transparency than other films. All films were then investigated their morphology, secondary structure, and thermal properties using scanning electron microscope (SEM), Fourier transform infrared (FTIR) spectrophotometer, and Thermogravimetric analyzer (TGA), respectively. The results found that the native keratose film has smooth surfaces without phase separation, while the cellulose film has rough and some fibers appeared on its surface. The cellulose/keratose composite films have specific absorption peaks of each polymer functional groups. The secondary structure of the keratose film has a β - pleated sheet resulting in the fragile texture and brittle. Adding cellulose increased flexibility and thermal properties of the keratose film. This was due to the keratose and cellulose formed interaction via functional groups. The result suggested that cellulose and keratose are well compatible together. The finding results might be advantaged to use as basic information to prepare the cellulose/keratose composite films obtaining desire properties for applications.

Keywords: Film; Cellulose; Keratose; Secondary structure; Thermal property

1. Introduction

The plastics from petroleum sources are gradually caused by environmental pollution. The people around the world starting to protect this problem in many ways including plastics reducing use (Pico and Barceló, 2019). Development and discovery of biodegradable polymers have been a pervasive interest to replace the plastic from petroleum sources (Liu *et al.*, 2018). Biodegradable polymers are plastics in which could be degraded by the action of living organisms in the environment, such as fungus, bacteria, or various processes in living organisms or environments. In recent year, user - friendly and eco - friendly biopolymer - based materials have been widely proposed, especially for agricultural and marine originated sources of raw materials for biopolymers (Rajabinejad *et al.*, 2018; Brodin *et al.*, 2017; Bertolino *et al.*, 2016; Sagnelli *et al.*, 2016; Kai *et al.*, 2016).

Keratose is a structural protein in which the form of keratin obtained by oxidizing extraction (Wang *et al.*, 2017; Rajabinejad *et al.*, 2018). It is water - soluble. The extracted keratoses degrade relatively fast *in vivo* and have a higher molecular weight in comparison to other types of keratin. The keratoses are highly promising candidates for encapsulation of chemicals for cosmetic, pharmaceutical and biomedical applications due to their non - toxicity, biocompatibility, biodegradability, and non - immunogenicity (Rajabinejad *et al.*, 2018).

Cellulose is one type of carbohydrate. It is a homopolymer of glucose linked together via β - 1,4 - glycosidic bonds (Ul - Islam, Khan and Park, 2012). The structure

of cellulose is a mixture of hemicellulose (20 - 30%) and lignin (15 - 30%) to form the complexation structure (Lee *et al.*, 2008). Recently, the cellulose is widely applied in various industries such as food (Klemm *et al.*, 2005), pharmacy (Shokri and Adibkia, 2013), pulp and paper (Singh *et al.*, 2016), water pollution treatment (Peng *et al.*, 2019), or wine and beer (Chakraborty *et al.*, 2016). The important source of cellulose is the cell wall of the plant (Bledzki and Gassan, 1999). In this work, the cellulose was extracted from water hyacinth, a fresh plant that rapidly grown and widely spread around the world (Wissel, Mayr and Lücke, 2008). The water hyacinth is a virulent cause of water pollution (Sundari and Ramesh, 2012 ; Mochochoko *et al.*, 2013; Juárez - Luna *et al.*, 2019). However, it composed of a high content of cellulose (Zhu *et al.*, 2009). The goal of this work is to extract keratose from human hair and cellulose from the water hyacinth for the preparation of composite films. The films were then characterized and discussed to assess the possible way for the application of both biodegradable materials such as bio - packaging, drug carrier for controlled - release or tissue engineering.

2. Materials and Methods

2.1 Preparation of water cellulose

The water hyacinth samples were collected in the Mahasarakham University then washed with tap water before cutting into small pieces. The samples were dried in an oven for 24h and then crushed into powder. The cellulose was extracted according to previously reported (Wissel, Mayr and Lücke, 2008) with some modifications. The

10g of water hyacinth powder was digested by 100 mL 5% NaOH (w/v) with stirring and warming for 3h. The mixture was then washed with distilled water until neutral. After dried at 90 °C for 24h, the dried sample was then bleached by 5% NaOCl (w/v) at room temperature for 24h. The bleached samples were washed to neutral and dried at 90 °C for 24h. Finally, the samples were hydrolyzed by 5% H₂SO₄ at 60 °C for 8h to obtain the cellulose solution. The cellulose solution was then stirred, washed with distilled water, and filtered before use.

2.2 Preparation of solution

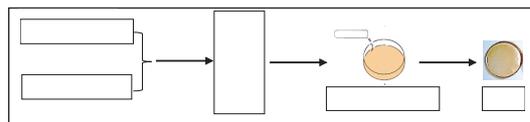
Human hair was collected from volunteer aged between 21 and 22 years, Maha Sarakham Province, Thailand. The hair was warmed to 40 °C before immersing in n - hexane for 12h to remove some lipids. Hexane, NaOH, peracetic acid, and Tris(hydroxymethyl)aminomethane used for the oxidizing extraction were purchased from Sigma - Aldrich®.

Keratose was obtained from human hair by oxidizing extraction followed the method previously reported (Rajabinejad *et al.*, 2018) with some modifications. The hair samples were extracted with peracetic acid with the ratio of hair: oxidizing solution of 1:30 (w/v). The mixture of hair and oxidizing reagent was left in a thermostatic water bath for 24 h at 25 °C. The hair was washed to remove unreacted reagent, with distilled water and dried at 90 °C for 1h. The hair was then vigorously shaken in TRIS 1 M at 37 °C for 2 h. The resulting solution of keratases was filtered with a dialysis membrane (MW cut off = 3 kDa), neutralized with sodium hydroxide, and dialyzed for 3 days against distilled water.

At the end of the dialysis, the solution was centrifuged to remove insoluble hair. The concentration of extracted keratose was checked by the evaporation technique and adjusted to 1.5% (w/v) with distilled water.

2.3 Preparation of composite films

The cellulose (CE) and keratose (KE) solutions with different ratios (4:0, 3:1, 1:1, 1:3, and 0:4) were prepared and stirred homogeneously for 30 min. The small chains of CE can dissolve in the KE solution via H - bond interaction between hydroxyl groups and water. The mixture was then cast onto a 4.5 cm diameter petri dish followed by solvent evaporation at room temperature for 24 h. The films were peeled off. The obtained films were placed in a desiccator until investigation. The schematic for preparation of the films as followed;



Scheme 1. A schematic illustration showing the preparation of CE/KE composite films.

2.4 Morphological observation

All of the films were dehydrated and cut into ~1cm length before observing their morphology using a scanning electron microscope (SEM) (JEOL, JSM - 6460LV, Tokyo, Japan). The film fractures were coated with gold (Au) to enhance conductivity before scanning.

2.5 Secondary structure investigation

The secondary structure of films was analyzed by Fourier transform infrared (FTIR) (Perkin Elmer - Spectrum Gx, USA) in the spectral region of $4000 - 400 \text{ cm}^{-1}$ at 4 cm^{-1} spectral resolution and 32 scans with air as reference.

2.6 Thermal property analysis

A thermogravimetric analyzer (TGA) (SDTQ600, TA - Instrument Co. Ltd., New Castle, DE, USA) was used for thermal investigation of the native and composite films. Simply, 3 - 5 mg microparticle was heated from $50 - 1000 \text{ }^\circ\text{C}$ with $20 \text{ }^\circ\text{C}/\text{min}$ of heating rate under nitrogen atmosphere.

3. Results

3.1 Transparency of films

(Fig. 1) shows the transparency of all films. The results indicated that the cellulose (EC) (Figure 1a) had white, dense, and lower transparency than brown keratose (KE) film (Figure 1b). The transparency and color of CE/KE composite films varied following the ratio of native polymers. All composite films have higher transparency than CE, but in lower than KE film.

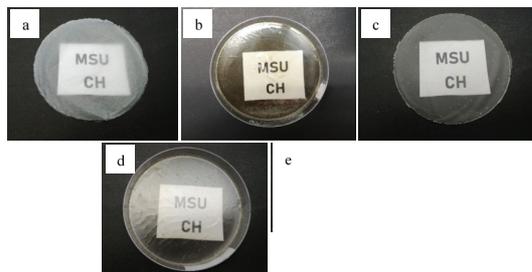


Figure 1. Transparency of films; CE (a), KE (b), CE/KE composite at 3:1 (c), 1:1 (d) and 1:3 (e) ratios.

3.2 Morphology of films

(Fig. 2 and 3) show morphology both surface (upper row) and cross - section (lower row) of films. At low magnification (Fig. 2), the native CE film has a rough surface according to the random woven of fiber with clearly observed at high magnification (Figure 3). However, the CE film formed dense texture without separation as revealing by cross - section (lower row). The KE film has dense in texture without cracks. At high magnification, film still has a dense texture with many pores in small sizes. Considering the CE/KE composite films, the morphology of each ratio varied by the content of the polymer. At high content of cellulose (3:1), the film has a rough surface and appears fibers woven together. Increasing magnification, the texture found various sizes of fibers at the edge of film. At the equal ratio (1:1), the composite film has a smooth surface but in lower than the KE film. Moreover, the texture of the film has a small size of particles embedded in its surface. At high content of keratose (1:3), the morphology of film was similar but smoother than the 1:1 ratio. However, the film has a crack in texture at high magnification (Fig. 3).

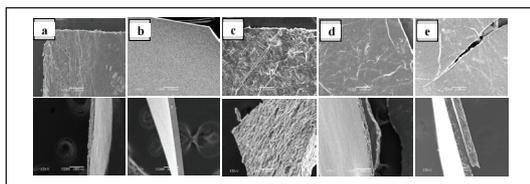


Figure 2. SEM micrographs of surface (upper row) and cross - section (lower row) of films; CE (a), KE (b), CE/KE composite at 3:1 (c), 1:1 (d) and 1:3 (e) using 200X magnification.

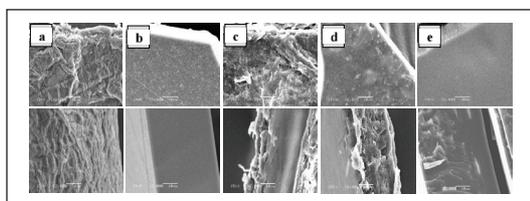


Figure 3. SEM micrographs of surface (upper row) and cross - section (lower row) of films; CE (a), KE (b), CE/KE composite at 3:1 (c), 1:1 (d) and 1:3 (e) using 2000X magnification.

3.3 Secondary structure of films

(Fig. 4) shows FTIR spectra of the films. All the main peaks can be assigned following Pocasap *et al.* (2020). The CE film indicated the absorption peaks at 3339 (assigned to H - CO vibration), 2899 (assigned to CH_2 vibration and C - H stretching), 1680 (assigned as $\text{HC}=\text{O}$ stretching), 1519 (C - H in aldehyde ring) and 1057 cm^{-1} (C - O - H) (Figure 4a) while the absorption peaks of KE film appeared at 3205 (assigned to CH_2 vibration and H - C=O amide A), 1678 (assigned as C=O stretching, amide I), 1519 (O=C - NH, amide II), and 1042 cm^{-1} (O=C - SH stretching) (Figure 4e). The CE/KE composite films (Figure 4b,c,d) showed variable absorption peaks depending on the content of cellulose or keratose.

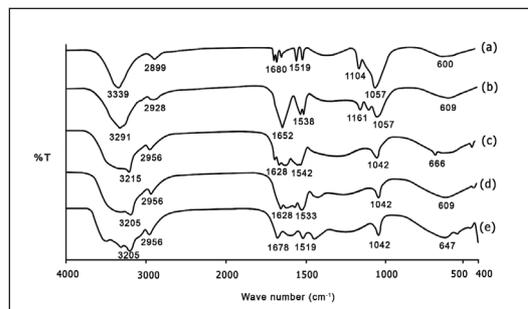


Figure 4. FTIR spectra of films; CE (a), KE (e), CE/KE composite at 3:1 (b), 1:1 (c) and 1:3 (d).

3.4 Thermal property of films

(Fig. 5) shows the TG curves of films. All prepared films have decomposition peaks of more than 3 points. The first point is less than $100\text{ }^\circ\text{C}$, second is a range $150 - 300\text{ }^\circ\text{C}$ and the third is a range of $300 - 400\text{ }^\circ\text{C}$. The maximum temperature of decomposition rate ($T_{d,max}$) of films was clearly observed by DTG curves as shown in (Fig. 6).

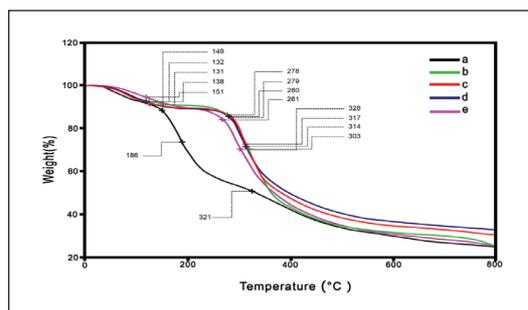


Figure 5. TG curves of films; CE (a), KE (e), CE/KE composite at 3:1 (b), 1:1 (c) and 1:3 (d).

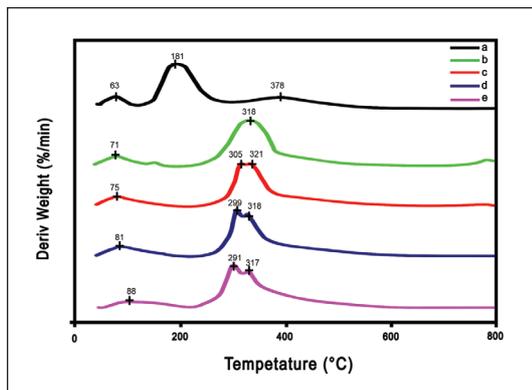


Figure 6. DTG curves of films; CE (a), KE (e), CE/KE composite at 3:1 (b), 1:1 (c) and 1:3 (d).

4. Discussion and Conclusion

Water hyacinth is a water pollutant and caused a big problem with water pollution worldwide. It composed of the high content of cellulose which has been interested in applications. The cellulose showed good properties such as high strength, heat durability, and biodegradability. Moreover, it could be mixed with other polymers to prove some properties including poly (vinylidene fluoride) or PVDF (Zhang, 2012), silk fibroin (Zhou, 2013), and chitosan (Abdul Khalil *et al.*, 2016). SEM images indicated that the texture of cellulose film linked together without phase separation. This morphology was similar to the previous report (Qua *et al.*, 2011). However, the consistency of fiber was lower than the amino acid components in keratose. The homogeneous size of amino acids in keratose supported the transparency and smooth surfaces of the film. Considering the CE/KE composite films, the difference types and ratios used were the main factor on the film morphology.

With FTIR spectra, cellulose film showed peaks of hydroxyl group (- OH) at

3700 - 3000 cm^{-1} , (carbonyl group C=O) at 1700 - 1800 cm^{-1} (Karatzos *et al.*, 2012), and the methyl group (- CH_2) at 2900 - 2800 cm^{-1} (Fan, Dai and Huang, 2012). Furthermore, the peaks of hemicellulose at 1735 cm^{-1} and lignin at 1240 cm^{-1} (Sun *et al.*, 2020) were also observed. The keratose film showed absorption peaks of the amide group (R - COONH - R), which the main bonding of amino acids. Amide I (1700 - 1600 cm^{-1}) is the responsible peak of the carbonyl group (- CO -), amide II (1600 - 1500 cm^{-1}) is responsible peak of amine group (- NH -) and methyl group (- CH -), and amide III (1300 - 1150 cm^{-1}) is a responsible peak of - CN - stretching, plane - NH - , - C - C - and - CO - stretching (Sharma *et al.*, 2017). The absorption peak at 1040 cm^{-1} confirmed cysteic acid group of the oxidized keratin (keratose) (Pakkaner *et al.*, 2019). The absorption peaks from FTIR spectra indicated that the keratose film has a β - sheet structure (1519 cm^{-1} , amide II). This film has rapidly brittle and very hard to manual. The high content of hydroxyl groups in cellulose supported the hydrophilic property of the film. This helped to increase the flexibility of the film. The mixture of cellulose and keratose resulted to change of the absorption peaks of the main functional groups by increasing of the wave number to higher values. However, they still appeared in all of the main groups which varied by the ratio of the polymer. This suggested that cellulose and keratose were good compatibilities via interaction formation such as hydrogen bonds, hydrophobic interaction, and van der valve force. The keratose supported the strength of cellulose film while cellulose helped to decrease the brittle of keratose. This mention was observed from the SEM images and FTIR results. Films

showed decomposition peaks at least 3 points. At lower 100 °C is caused by water evaporation in polymer molecules (Dou *et al.*, 2019). The temperatures in the range of 150 - 300 °C are the decomposition point of hemicellulose and lignin and 300 - 400 °C are the decomposition of cellulose chain. The keratose showed a decomposition point in the range of 250 - 300 °C (Rajabinejad *et al.*, 2018). The composite films showed variable decomposition points depending on the ratio used as like as the thermal property. Cellulose content helped to increase the thermal property of the films. However, the highest $T_{d, max}$ found in the composite film at 1:1 ratio. This result confirmed the compatibility of the cellulose and keratose and 1:1 ratio of CE/KE composite showed the best properties of prepared film.

In conclusion, the properties of films depended on many factors such as type of polymer, the mixed ratio as well as the preparation methods. The results obtained from this work suggested that the cellulose could be extracted from the waste water hyacinth. This cellulose could also be used as a biodegradable polymer for blending with keratose or other materials for value - added and replaced of non - degradable polymer like synthetic plastics.

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6. References

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