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Synthesis and Characterization of Keratin Hydrolisate-Carrageenan Biofilm

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Abstract. Bioplastics became important as oil-based plastic accumulated around the world and polluted the environment. keratin is existed in chicken feathers around 90%. Keratin is an important biomaterial and the most inexpensive abundant biomaterial. Previous study showed that solid-state fermentation of keratinase by bacillus sp. MD24 using chicken feathers a sole carbon and nitrogen source produced soluble keratin hydrolysate. The keratin hydrolysate can be transformed into keratin biofilm which ultimately might be used to make bioplastic. However keratin biofilm has poor mechanical properties. Blending keratin with other biomaterial such as carrageenan might improve keratin biofilm properties. This research aimed to make keratin-carrageenan polyblend and characterize the properties of the polyblend. Keratin hydrolysate was collected from keratinase solid-state fermentation liquid by-product. After dyalisis, soluble keratin was mixed with carrageenan solution at various mass ratio. Prior to mix with carrageenan, Particle size analyzer (PSA) is used to examined the size of soluble keratin. The polybend film was then analyzed using FTIR, SEM, and DMA. SEM photograph showed morphology of film surface which smooth and homogen. FTIR spectra showed the presence of both carrageenan-keratin in the film. DMA shows better mechanical properties of polyblend film.

INTRODUCTION

Recently, the development of petroleum-based plastic materials turn into a common element in daily [1]. This is because of their flexibility and stability but it formed from non renewable materials [2]. Most of the plastics derived from petroleum oil fossil resources and it's hard to reduce the materials. It makes accumulated around the world and polluted the environment with the gas emission during burning the plastics [3].

In order to reduce the impact of petroleum-based plastic materials, one of the solution is to make a renewable resources, environmetally friendly and non toxic materials such as natural polymers [4]. The most abundant natural polymers in daily life is chicken feather in poultry industry [5]. Most of them disposed in around the world and makes too much pollutions. Protein keratin is eisted in chicken feathers. Keratin is a protein that insoluble in water and organic solvent, which makes it difficult to recycle [6]. It is a biopolymer built by α-helix (α-keratin) and β-sheet (β-keratin) structure, which stabilized by hydrogen bonds, peptide bonds, disulfide cross-linkages, salt bridges, and other intramolecular force [7].

The keratin from chicken feathers extracted using Solid State Fermentation of keratinase by *Bacillus sp.* MD 24 a sole carbon and nitrogen source produced soluble keratin hydrolysate. The keratin hydrolysate can be used into a different forms of biotechnological application such as a biofilm for biomedical application [8][9]. The main disadvantage of keratin hydrolysate to be candidate of the film is it shows poor mechanical properties and to improve the mechanical properties of the material by blending the keratin with another materials which can be suitably prepared [10][11]. On the other hand, carrageenan has been widely invetigated as a natural biomaterial for many applications especially in medical materials [12][13][14]. Its due to their biocompatibility, mechanical stability and antimicrobial properties so it can be increase the mechanical properties to developed the film based keratin hydrolysate-carrageenan using glycerin as a plasticizer [15].

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The blending between the components are investigated by Fourier transform infrared spectroscopy (FTIR), Dynamic mechanical analysis (DMA), and Scanning Electron Microscopy (SEM). The data may help to identify the interaction and the stability blending of polypeptide (keratin) and polysaccharides (carrageenan) in different ratios. Blending of keratin hydrolysate-carrageenan could be applicated in wound dressing.

MATERIALS AND METHODS

This is experimental research to blend keratin with other biomaterial such as carrageenan. Keratin waste were collected from the meat industry (slaughterhouse) in the form of chicken feathers at Blimbing market, Malang, Indonesia and kappa carrageenan. Potassium chloride (KCl), Magnesium sulphate ($MgSO_4$), Mono ammonium phosphate ($(NH_4)H_2PO_4$, Calcium carbonate ($CaCO_3$), Potassium dihydrogen phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4) used to prepare *Solid State Fermentation*. Snake Skin Dialysis membranes having average flat width of 33 mm were purchased from Sigma Aldrich.

Pre-treatment of feathers

The feathers were soaked in detergent for 2 hours and dried approximately 48 hours. After dry the feathers, It cut around 5 cm and stored in closed containers

Degradation of chicken feathers under Solid State Fermentation

Chicken feathers in Erlenmeyer flask was added around five grams to 100 mL. The solution contained 0.2 g/L of KCl; 0.2 of $MgSO_4.7H_2O$; 1 g/L of $(NH_4)H_2PO_4$; 2 g/L of $CaCO_3$; 0.5 g/L of KH_2PO_4; and 0.5 g/L of K_2HPO_4 (pH 8). *Bacillus sp*. MD24 was added as a pre-culture in the mixture. The mixture was incubated for a month. Liquid by-product from Solid-State Fermentation (hydrolysate) was separated by centrifugation at 5000 rpm. Hydrolysate keratin was dialysed for 8 hours. Then the keratin size in hydrolysate checked by Particle Size Analyzer (PSA).

Preparation Keratin Carrageenan biofilm

20 ml soluble keratin after dialysis mixed with different concentration of carrageeWnan, as shown in Table 1. Then, the mixture mixed with magnetic stirrer for an hour with adding two drops of glycerin as plasticizer. The keratin-carrageenan solutions were poured into an petri dish. The mixture placed in oven at 60°C for 24 h. Biofilm was then labeled and stored for analysis.

TABLE 1. Design of keratin and carrageenan composite films

Sample	$V_{Keratin}$ [1]	$m_{Keratin}$	$V_{Carrageenan}$ [2]	$m_{Carrageenan}$	(Keratin : Carrageenan) Weight Ratio
KK_0	-	-	20 ml	14,32 mg	0:1
KK_1	20 ml	15,56 mg	10 ml	7,16 mg	3,17 : 1,46
KK_2	20 ml	15,56 mg	20 ml	14,32 mg	2,08 : 1,92
KK_3	20 m	15,56 mg	30 ml	21,48 mg	1,72 : 2,38

[1] 0.778 mg ml^{-1}, [2] 0.716 mg ml^{-1}

Characterization

Scanning Electron Microscopy (SEM)

The surface morphology of keratin-carrageenan films were studied under FEI's electron microsope Inspect-S50 at 1000x magnification. SEM is used to identify the molecular structure of the sample to check if the mixtures are well-mixed.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of keratin-carrageenan film were analyzed using series FTIR intrument. The spectra collected from 4000 cm^{-1} to 500 cm^{-1} frequency. The data were identify using th FTIR Spectrum Software.

Dynamic Mechanical Analysis

The mechanical analysis in extension with the samples of approximately 5 mm x 0.19 mm. The DMA was operated at a fixed frequency of 1 Hz with 10^{-3}N initial force in 37°C. The tensile strength, Modulus young, and Elongation break were evaluated from the force displacement data.

RESULTS AND DISCUSSIONS

After incubation for 1 month at 37°C, the degradation of chicken feathers using Solid- produce brownish yellow soluble keratin (Fig.1). Salt that still exists in keratin is dialysis uses a dialysis membrane. The salt content is Na_2SO_4. $BaCl_2$ solution is used to determine wether the salt content of Na_2SO_4 has come out of the soluble keratin. The loss of the entire Na_2SO_4 salt content in the soluble keratin is characterized by the absence of $BaSO_4$ when the $BaCl_2$ solution is dripped in solution. The salt content is very disturbing in the process of film formation. The average size of soluble keratin was 728.9 ± 22 nm.(Fig.2)

(a) (b)

FIGURE 1. Degradation of chicken feathers (a) Solid State Fermentation (SSF) result on 2 weeks and (b). Hydrolysate keratin after a month

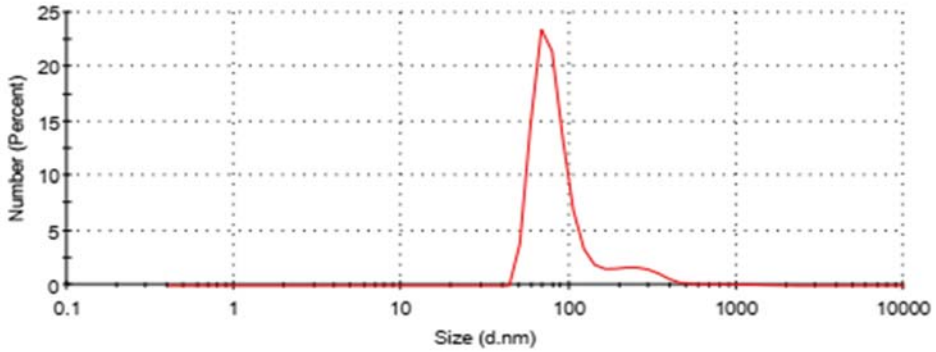


FIGURE 2. Result particle size of hydrolysate keratin using Particle Size Analyzer (PSA)

Biofilms are made by mixing keratin solution with carrageenan solution. Variation in the amount of carrageenan volume is intended to find out how well carrageenan can combine with keratin to form biofilms. Biofilms with

keratin mixture has a yellowish brown color while the biofilm without keratin has a transparent color. The thickness of the biofilm is also increased in proportion to the amount of carrageenan were added.

(a) (b) (c) (d)

FIGURE 3. Keratin hydrolysate-carrageenan biofilm (a) KK_0 (b) KK_1 (c) KK_2 (d) KK_3

In Figure 4, Characteristic peaks of kappa carrageenan were observed at 3505 1/cm shows the O-H stretch, the bands at at 1223 1/cm shows the O=S=O stretch, the bands at 941 1/cm shows the C-O-C in 3,6-anhydro-D-galactose, and the bands at 858 1/cm represent C_4-O-S stretch vibration.

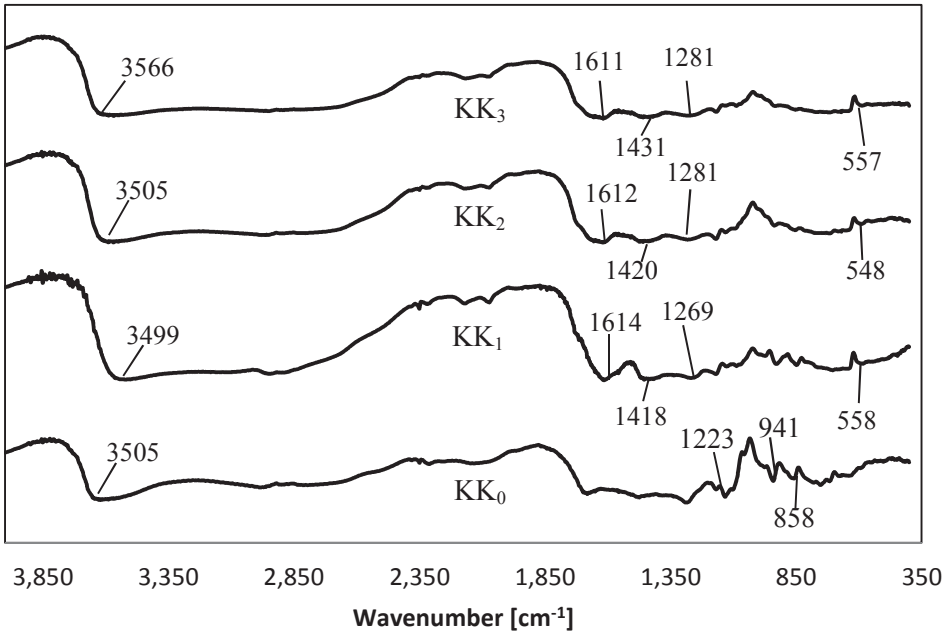
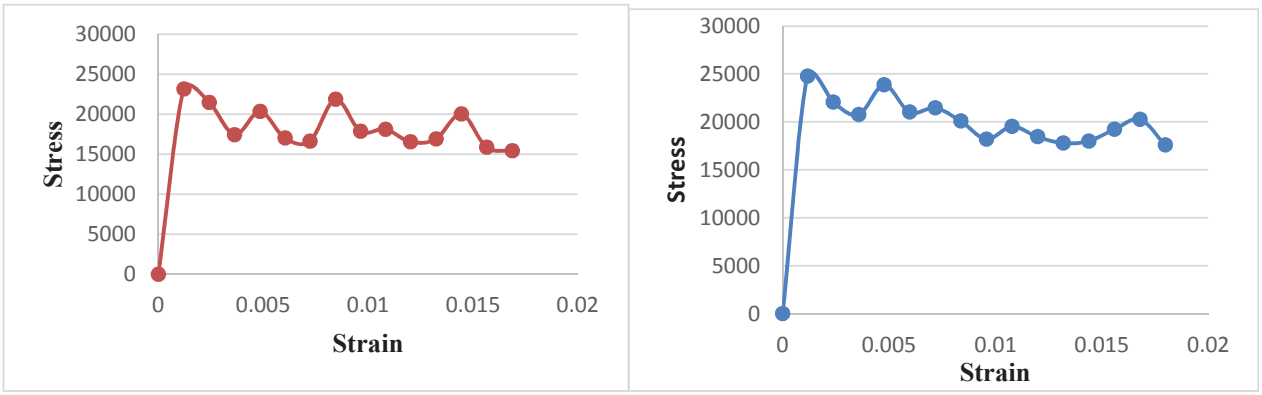


FIGURE 4. The measuremenets of Films using Fourier transform infrared spectroscopy (FTIR) in different ratios

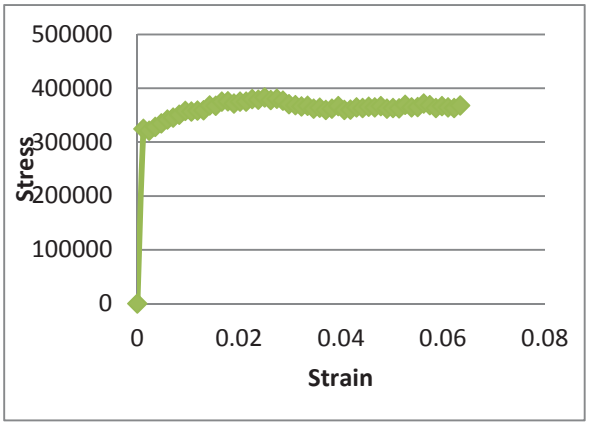
Spectra of FTIR keratin in region 350-3850 1/cm are given in Figure 4. The amide A band was showed stretching vibration of N-H bonds at 3566 1/cm, 3505 1/cm, and 3499 1/cm. The amide I band showed the C=O stretching vibration at 1611 1/cm, 1612 1/cm, and 1614 1/cm. The amide II was showed to N-H bending and C-H stretching vibration at 1431 1/cm, 1420 1/cm, and 1418 1/cm. Less absorption band was showed at amide III, it is because the C-N and C-O stretching, N-H and O=C-N bending vibration at 1281 1/cm, 1281 1/cm, and 1269 1/cm. The FTIR spectra indicated the exist of protein keratin.

The keratin hydrolysate-carrageenan biofilm from different ratios was investigate the interactions on mechanical properties of the membranes using Dynamic Mechanical Analysis (DMA). The tensile strength, Young's Modulus and Elongation break of keratin hydrolysate-carrageenan with different ratios obtained from stress-strain curves are diplayed in Figure 5.

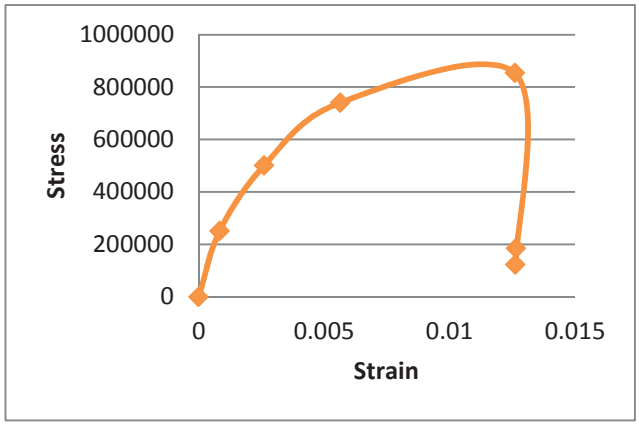


(a) KK_0

(b) KK_1



(c) KK_2



(d) KK_3

FIGURE 5. Mechanical properties of biofilm with different keratin hydrolysate-carrageenan ratios as stress-strain diagrams

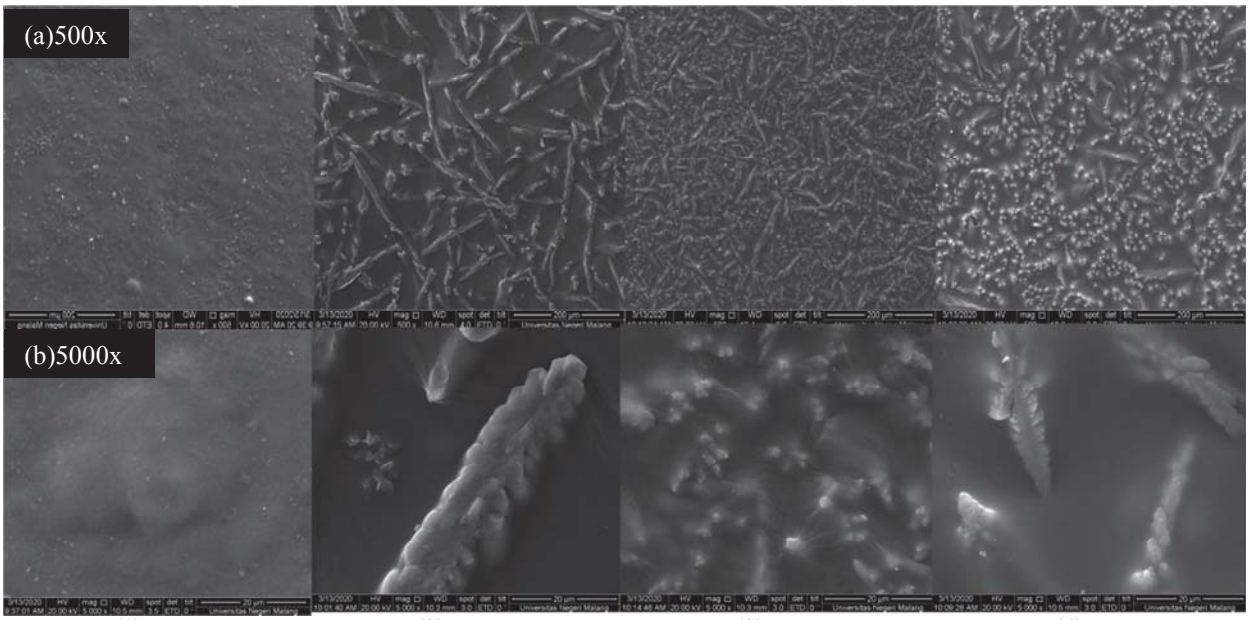
Tensile strength, young's modulus, and elongation break are shows in Table 2. These graphics shows that the mechanical properties change in different ratios. The polyblend was prepared with glycerin at level concentration 0.05 g.

TABLE 2. Mechanical properties of biofilm with different keratin hydrolysate-carrageenan ratios

Sample	Tensile Strength (N/m^2)	Young's Modulus (MPa)	Elongation Break (%)
KK_0	23152.1	18.96	1.7
KK_1	24757.8	21.27	12
KK_2	324060.0	26.77	12.1
KK_3	853427.5	67.74	1.3

The films tensile strength increased with the addition of carrageenan. It makes the polyblend more flexible and less brittle. The increases in Young's modulus in Table 2 showed that the polyblend more rigid with the increasing addition of keratin hydrolysate. The interactions between polysaccharides and polypeptides influence the mechanical properties of the polyblend. The mechanical properties of the polyblend materials can be controlled by keratin hydrolysate.

The morphological structures of obtained keratin hydrolysate-carrageenan film surfaces in various ratios was explored by Scanning Electron Microscopy (SEM) analysis. The SEM image in Figure 6. The structure of keratin hydrolysate-carrageenan interacted by the chemical of the films and the the texture of keratin hydrolysate-carrageenan polyblends were depended by it ratios.



(1) KK_0 (2) KK_1 (3) KK_2 (4) KK_3

FIGURE 6. The morphological image at (a)500x magnification and (b)5kx magnification of (1) KK_0 (2) KK_1 (3) KK_2 (4) KK_3 biofilm

The SEM result reveal that KK_0 as a control of carrageenan had a compact structure even at higher magnifications. Nevertheless, compared with KK_1, KK_2 and KK_3 with addition of keratin hydrolysate makes the film more rigid and compact as we know keratin has a strong and stabil structure.

CONCLUSIONS

The result of material evaluation, this blending keratin hydrolysate-carrageenan biofilm in different ratios was successfully produced. The increasing of carrageenan content, flexibility, solubility and moisture content of the films increase. Keratin hydrolysate blending makes the film more rigid showed by the Scanning Electron Microscopy (SEM) analysis. These biofilm are expected to combine both of the protein and polysaccharides. FTIR analysis shows that the absorption peaks carrageenan shows the amide bands of the polypeptide (keratin). The mechanical properties using Dynamic Mechanical Analysis (DMA), the films tensile strength increased. It makes the polyblend more flexible and less brittle. The increases of Young's modulus shows that the polyblend more rigid with increasing addition of keratin hydrolysate. It makes the intercation of polysaccharides and polypeptides well combine the mechanical properties.

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