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Characterizations of keratin for biodegradable films production

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***Abstract*— Keratin is versatile, bioactive polymers, which become very attractive for advanced application, from agriculture, cosmetic, biomaterials and bio-plastics. In this paper, we explored a facile method to prepare biodegradable plastic. The solution keratin was synthesized by alkaline hydrolysis of the wool. Then the solution was deposited on glass by drop casting. The change of structure and chemical compositions of the keratin polymers before and after the treatment was characterized by using by SEM, FTIR. The resulting keratin film was smooth and insoluble in the water. The developed films intended to use as the alternative of synthetic non-biodegradable packaging film.**

***Keywords*— keratin, wool, biopolymer, biodegradable plastic.**

I. INTRODUCTION

The use of natural- based polymers films depends on several features including cost, availability, fictional attributes, optical quality, mechanical properties and resistance to water. These characteristics are interesting for fabrication bio-plastic films as alternative of petroleum-based plastics [1]. Several proteins have been manufactured into bio-plastics such as a proteins from plants (wheat gluten, soy, sunflower, and corn) and animals (gelatin, keratin, casein and chitosan). Of these, keratin –based materials have shown promise for revolutionizing the bioplastic and biomaterial world due to their intrinsic biocompatibility, biodegradability, non-burning and natural abundance [2]. Keratin is a durable, fibrous protein which is mainly present in higher vertebrates (wool, hair, feather, nails and horns) and humans epithelial [3]. Keratin a unique chemical structure due to the great amount of the amino acid cysteine (7-20% the total amino acid residues) in comparison with other proteins (fibroin or collagen). The disulphide covalent bonds confer to keratin a high resistance to chemical and enzymatic and give high stability to the protein structure in terms of strength and stiffness [4]. Keratin is biopolymer with a high degree of chemical functionality and exhibits many properties such as moisture sorption and thermal conductivity [5] keratin has been extracted from wool by the cleavage of the disulfide cystine bonds via reduction (thioglycolic acid) or oxidation treatments (agents peracetic and performic acid)[6] and regenerated into various forms for biotechnological and biomedical application such as sponges, films, hydrogels, and powder[7, 8, 9]. These various from of keratin have been used for biomedical such as tissue engineering. Wool contains up to 95% by weight of pure keratin [10], the wool fiber is made up of three major morphological components, namely the cuticle, the cortex and the cell membrane complex. The thin outer surface of the fiber, the cuticle (is about 400 to 500 nm thick), is a scaly tubular layer which is cystine- rich and highly cross-linked (to from a hydrophobic barrier). The major body of the wool fiber is the cortex (85%), which is composed of elongated cortical cells oriented parallel to the fiber axis, within the cortex of the wool fiber contains micro-fibrils of low sulfur, alpha helix crystalline proteins, fixed within an amorphous matrix of high-sulfur [11]. The cell membrane complex referred to as intercellular cement, performs the function of cementing cortical and cuticle cells together.

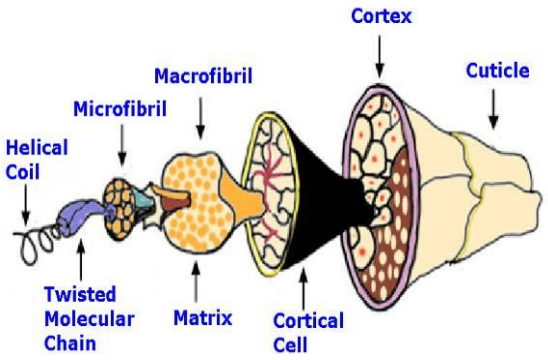

Figure1: structure of the wool fiber

There are large quantities of keratin protein rich biomass is inder-utilized and so it is environmentally and economically important to transform wool into value added products. Recently several methods have been proposed for extraction of the keratin, chemical, mechanical and enzymatic method. Alkalin hydrolysis is one of the most widely used methods for the extraction of keratins, it involves dissolution of wool in an alkali such as NaOH or KOH. In this paper, we focus on the extraction of the keratin solution by alkaline hydrolysis and the films are prepared using a simple drop-casting method. The proprieties of the wool fiber and films are also examined.

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II. MATERIAL AND METHODS

A. *Preparation of keratin solutions and film*

Wool of the sheep was washed with water and detergent. Then, the wool was withdrawn from a combed sliver to eliminate all impurities including and immersed into ethanol to remove fatty matter, washed with distilled water after drying in the atmosphere the four grams of cleaned wool fibers were cut into snippets some millimeters long. Four grams of the wool fibers are immersed in 400 ml 0.5N M NaOH for 3 h at 60°C 5 (figure. 2). after the required time, the resulting solution was appearance of yellow color indicated that the NaOH solution attacked the wool fiber and the color comes from the existing fatty amino acids on the surface of the cuticle. After hydrolysis, the gelatinous keratin was centrifuged for 10 min at 12.000 rpm. The solid precipitate was rinsed with distilled water until neutral pH on a filtrate paper. The solution is finally deposited by drop casting on glass substrates and dried at 50°C to evaporate the water. The resulting films are visually opaque and homogeneous, as show in figure. 3

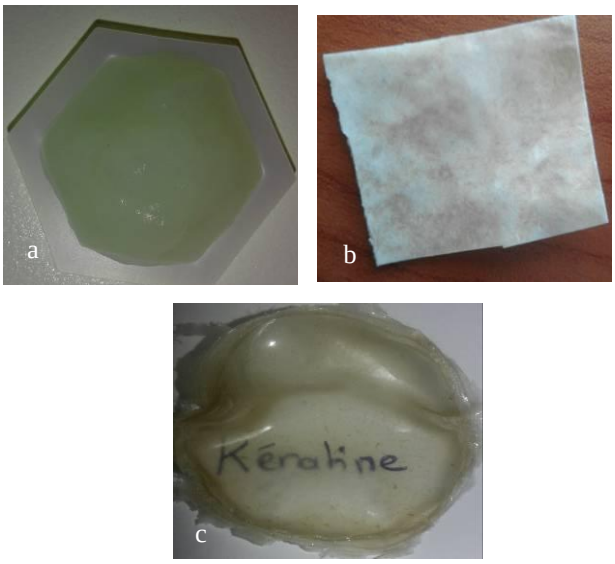

Figure.3. Preparation of keratin solution by alkaline hydrolysis, a) the gelatinous keratin, b) Visual appearance of keratin film obtained by swollen wool fibers, c) film keratin transparent

B. *Characterization*

The morphologies and structures of the wool fabrics and films were observed using Scanning electron microscopy (SEM) type PHILIPS XL 30 ESEM,and fluorescence microscopy**.** The surface functional groups of the films and original wool were qualitatively detected by a fourier transforms infracted. The wool original cut into powder were pretreated by mixing with Kbr in from of disks, the spectra were acquired for 100 Scans, from 4000 to 400 cm^{-1} with a resolution of $4cm^{-1}$. The dielectric properties was measured using a impedance meter (HP 4284A) at room temperature as a function of frequency from 1KHz to 1MHz, a thick keratin film 0.64mm placed between two metal electrodes, this structure creates a plane capacitor which capacity , resistance (R) and dielectric losses are measured by a impedance meter. The polarization of the structure is done by an alternating electric field with oscillation amplitude of 1V .

III RESULTS AND DISCUSSION

A. Influence of water on keratin film

The water vapor permeability is influenced by the hydrophilicity of the material, it well know that the water molecules have the high plasticizing effect in the protein films, under the plasticization of the water, the increase in the free volume of the keratin wool film system results in a protein network with less density, which consequently increases the water diffusion in the protein matrix []. Fig.3 shows, when the keratin film is exposed to water vapor; it causes swelling and deformation of the film. Immersing these films I the water for several days allow the film to swell quickly without dissolving

Fig. 3: keratin film, a) before exposure to water, b) after exposure to water

B. *Morphological analysis*

The application of fluorescence microscope can be a valuable supplement to other methods used to evaluate the homogeneity and level of dispersion of a keratin solution on a glass substrate. Fig.4 presents microscopic images of keratin film obtained using fluorescence microscope. It indicates that surface is roughness .

The morphological structure of wool fiber before and after treatments is shown in fig. By observing, we can see that the surface of natural wool has a wrinkled structure and scales having the sharp edges protrude outward from the fiber on the cuticle (fig. 5). After hydrolyze, it was clear that the surface of keratin film were quite different morphology than wool fiber. The keratin films are obtained by flattening the swollen wool fibers and burst after alkaline treatment. Figure. 5 showed that the surface plat and the presence of cracks were visible in many places on the surface. This is likely due to the length of wool fiber

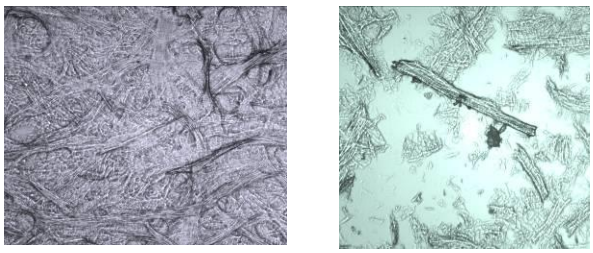

Figure. 4: microscopic of keratin film

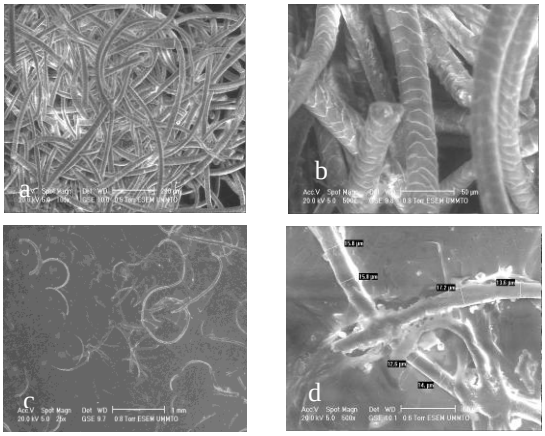

Figure 5: Scanning electron micrographs of untreated wool and keratin preparation from alkaline hydrolysis, a, b) SEM of wool fiber, c, d) SEM of film keratin made by drop casting.

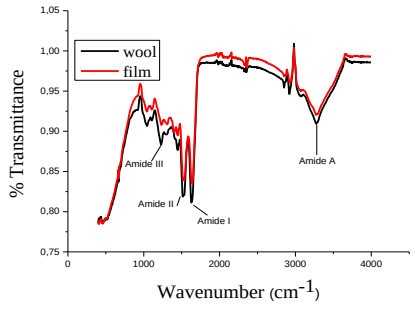

Figure. 6: FTIR spectra of wool and keratin film

c. FTIR analysis

Infrared transmission spectra of pure keratin film and wool fiber are shown in figure 6. It indicates that both pure keratin film and wool fiber have the property of hydrogen bond, amide I, amide II, and amide III [12]. The amide I is considered to be related to stretching vibration of C=O bond , which is located in 1624 cm^{-1} on both the curve of wool fiber and keratin film is related to stretching . The amide II with the band at 1523 cm^{-1} is derived from N-H bending and C-H stretching. The peack of the amide III band of wool fiber wool fiber and film keratin lies in 1220 cm^{-1} and 1244 cm^{-1} respectively, mainly related to the combination effect of N-H stretching vibration and C=O benging vibration. The amide A band, which falls at 3282cm^{-1} is attributed to the stretching vibration of O-H and N-H, The band at 2924 cm^{-1} is attributed to the symmetrical CH_3 stretching vibration. In this study, the characteristic peack at 1624 cm^{-1} was near the characteristic B-sheet. The characteristic peack at 1523 cm^{-1} states the presence of a- helix conformation. To sum up, compared with wool fiber, a- helix structure, B- sheet were existed in keratin film.

c. The dielectric characterization of keratin

to evaluate the dielectric characteristics of the keratin thin film, dielectric constant (έ) and loss dielectric loss (tanδ) was measured at ambient condition (25°C, 25%RH) within the frequency range of 1kHz to 1M Hz under 1 v. Dielectric constant depends on the presence of of amino acid (–CO-CR-NH-) and contain neutral polar and charged side chains in the keratin, they can be considered as palarizable materials. figure .6 shows, the dielectric constants and dielectric loss of the keratin decrease with the frequency increase, The έ value of (έ=29, tanδ= 0.17at 1 Hz,) at higher frequency above 1 MHz, the dielectric constants is 23.5.

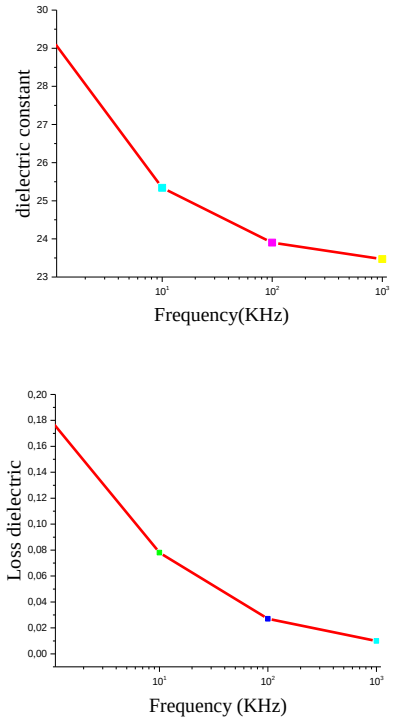

Figure. 7: The dielectric characterization of keratin, a) dielectric constant, b) loss dielectric.

IV. CONCLUSIONS

The present need is to find the green and ecofriendly methods for the extraction of keratin biomass with the minimal usage of harmful acids and chemicals, for this reason, in this study, we have detailed processes for the production of keratin films by alkaline hydrolysis using NaOH. In the first step of the process, The resulting solution was appearance of yellow color indicated that the NaOH solution attacked the cystine disulfide bonds of the external cuticle layer which became soluble on degradation, the color of this solution comes from the existing fatty amino acids on the surface of the cuticle. The films are prepared using a simple drop-casting method and were characterized by SEM and FTIR showed that the surface is roughness and the presence of microfibrils from cortical and cuticle cells. The results showed that there are α -helix structure, β -sheet in the pure wool fiber, as well as in the keratin film. This films are a highly hydrophilic character, immersing these films in the water for several days allow the films to swell quickly without dissolving. The addition with other properties of wool keratin, which is naturally biocompatible, biodegradable and low –cost indicates that keratin films are the appropriate candidate for biotechnology applications and to use as the alternative of synthetic non-biodegradable packaging

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