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EXTRACTION OF KERATIN FROM CHICKEN FEATHER AND ITS ANTIBACTERIAL ACTIVITY

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Abstract

Enormous quantities of chicken are consumed every year that produce an enormous number of feathers as waste material, which is considered an environmental problem. The high content of keratin in feathers makes it a suitable source of protein. In this study, white chicken feathers 50g were collected, washed, dried, solubilized in sodium hydroxide, and protein precipitated with hydrochloric acid to extract crude keratin. The extracted protein was lyophilized, and its protein nature was screened by infrared spectroscopy technique. Results of this research revealed different transmission bands near 1653 cm^{-1}, 1541 cm^{-1}, 1508 cm^{-1}, 1458 cm^{-1}, 1130 cm^{-1}, 1038 cm^{-1}, 1011 cm^{-1}, 472 cm^{-1}, 418 cm^{-1}. These transmission bands confirmed the protein nature of extracted material, band at 1653 cm^{-1} attributed to C=O stretching amide I that occurred in the 1700- 1600 cm^{-1} while band near 1541 cm^{-1} attributed to amid II which occur in the 1580- 1480 cm^{-1} rang, weak bands between 1130 cm^{-1} and 1011 cm^{-1} is associated with amid III. In addition, the results of this research showed that extracted keratin had antibacterial effects against some gram-positive and gram-negative bacterial isolates such as *Enterobacter sakazaki*, *Bacillus cereus, Staphylococcus aureus*, while there was no effect upon *Listeria monocytogenes*, *Micrococcus*. These results indicate that extracted keratin had an antimicrobial property and may be utilized for other purposes, besides big quantities of harmful feathers had been converted to a benefit substance that had many industrial uses.

INTRODUCTION

Chicken waste feathers form a considerable environmental problem because it is not biodegradable material and produced in enormous quantities. The feathers have been destroyed using traditional methods by burning and burying them, which may pollute the air and the ground [1]. Therefore, it is very important to recycle these big amounts of feathers to eco-friendly material. Feathers are among the most inexpensive abundant substances that are considered as renewable protein source due to its high content of protein like keratin that is hydrophilic and biodegradable in which it can be applied in various ways via-chemical processing [2, 3].

The chicken feathers are composed of approximately 90% keratin, it is an important ingredient for cosmetic, shampoo and hair treatment creams [3]. Extracted keratin is eco-friendly, nonabrasive biodegradable insoluble in organic solvents and has good mechanical properties, low density and finally cheap [4]. Beside keratin is insoluble in water, weak acids and bases characterized by high cysteine content and the high strength of keratin due to the di-sulfide bonds between cysteine molecules [5].

This research aims to extract keratin from chicken feathers, which is considered as an environmental pollutant that can be utilized for several purposes and different industrial uses.

MATERIALS AND METHODS

Feather collection and pretreatment

White chicken feathers were collected from the chicken processing plants in Mosul, soaked in concentrated ethyl ether for 24h to remove stains, oil and grease, washed with soap, boiling water and then dried under sunlight over 48h. Then dried feathers were blended and kept carefully in a sterile container.

Extraction of Keratin

Keratin was extracted according to [5] with some modifications. Sodium hydroxide 0.5M was utilized instead of sodium sulfide as follows: Blended feathers (50g) was added into 2L of 0.5M sodium hydroxide and continuously stirred at 40 ^{o}C for over 6h. The keratin solution was filtered to discard the insoluble particles of feathers and centrifuged at 10000 rpm for 10min. The supernatant was then filtered using a 0.45-micron filter paper.

The filtrate was next submitted to dialysis accomplished by utilizing 76×49 cellulose dialysis sleeve at room temperature for 48h. After dialysis, 10 ml of hydrochloric acid 2N was added dropwise to the solution to precipitate keratin at pH 4.2. Then the mixture was centrifuged at 3000 rpm for 10 min and washed the precipitation several times with distilled water to obtain natural pH. The precipitated keratin was lyophilized and kept in a sterile container at room temperature until use.

Infra- red spectrophotometer analysis

The dry mass of the keratin sample was measured by pouring the lyophilized keratin in a petri-plate. The petri-plate was weight before and after pouring the lyophilized keratin and then analyzed in IR-instrument in chemistry department model= Waf-510 resolution=4 Scan time= 32.

Bacterial strains sensitivity test

Several positive and negative bacterial isolates were utilized in this research such as *Enterobacter Sakazaki, Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, and Micrococcus* that obtained from the Microbial bank of Biology Department, College of Science, Mosul University. The bacteria sensitivity test was carried out using the Kirby-Bauer method [6]. The isolates were grown in tryptic soy broth, incubated for 24h at 37℃. After that, bacterial culture was spread on Muller- Hinton agar plate using sterile swabs with sterilized filter paper disk that saturated with extracted keratin 10 mg/ml as follows: 1mg of lyophilized were keratin dissolved in 1 ml of Dimethyl Sulfoxide (DMSO). Then, 100 disk filter papers were immersed and saturated with this solution, then incubated at 37℃ for 48h. Inhibition zones were measured and recorded in millimeters. Results were interpreted according to a standard inhibition zones claimed [7].

RESULTS AND DISCUSSION

Keratin (2g) was extracted from 50g of Chicken feathers utilizing sodium hydroxide like previous studies such as [8], which found in their results that the alkaline hydrolysis of feathers is necessary for the disulfide bond S-S and partial peptide hydrolysis. While Dhayanithi [9] concluded that the alkaline treatment of feathers is cheap and economically viable, therefore it could serve as a basis in the development of the ecologically safe complex. The feathers fiber treated of sodium hydroxide was better than of the untreated fiber, which indicates that sodium hydroxide treatment was responsible for the removal of a greater amount of amorphous content from the fiber. Kamarudin, et al. also concluded that among the reducing agents, sodium hydroxide had been utilized for keratin extraction from chicken feathers that gave 43.8% of keratin. Sharma [10] confirmed that the dissolving rate of feathers will be high only if the solution is highly alkaline with pH range from 10-13.

This is because in the alkaline state the proton will be removed from the amino-group and the ionic bond formed by electrostatic attraction of the NH^{3+} group of the diamine and the COO- group of dicarboxylic acid can be broken. Guevarra [8] confirmed that sodium hydroxide plays an important role in dissolving feathers because without alkaline state protons can't be removed thus can't break the ionic bonds. While utilization of hydrochloric acid for protein precipitation was accepted [10], which further confirmed that hydrochloric acid was the best protein precipitant among ethanol and acetone.

Infra-red technique was utilized in this research for understanding the chemical structure of extracted material as shown in (Fig 1) that indicated the presence of C-N , N-H , and C=O bonds, which revealed amino acids presence in the extracted substance and its protein natures. Extracted protein revealed the characteristic transmission bands near 1653, 1541, 1508, 1458, 1130, 1038, 1011, 631, 472, 418cm^{-1}. Bands analyzing interpreted that transmission bands on 1653 cm^{-1} attributed to C=O stretching amid I while band near 1541 cm^{-1} attributed to amid II which occur in the rang 1580- 1480 cm^{-1} for N-H bending and C-H stretching as [11] confirmed. The weak band between 1130 and 1011 is associated with amide II band that is derived from C-N stretching and N-H bending as [12] assure. These results were in line with other researchers such as [13] that demonstrated the same absorption bands appearance, which mainly assigned to the vibration of peptide bonds amide (I, II and III). And [14] that obtained peaks at 1672, 1598, 1276 cm^{-1} indicating vibration known as amid I,II and III which is

the result of β sheet of protein structure. [12] revealed at their results that the peaks at 990 cm^{-1} and 580 cm^{-1} were the characteristic absorption of C-S and S-S bonds. Many researchers also revealed that many bands indicated protein conformation in 1650cm^{-1} and 1630 cm^{-1} for amid I, 1540 cm^{-1} , 1520 cm^{-1} for amid II and 1230 cm^{-1} 1270 cm^{-1} for amid III such as [15,16].

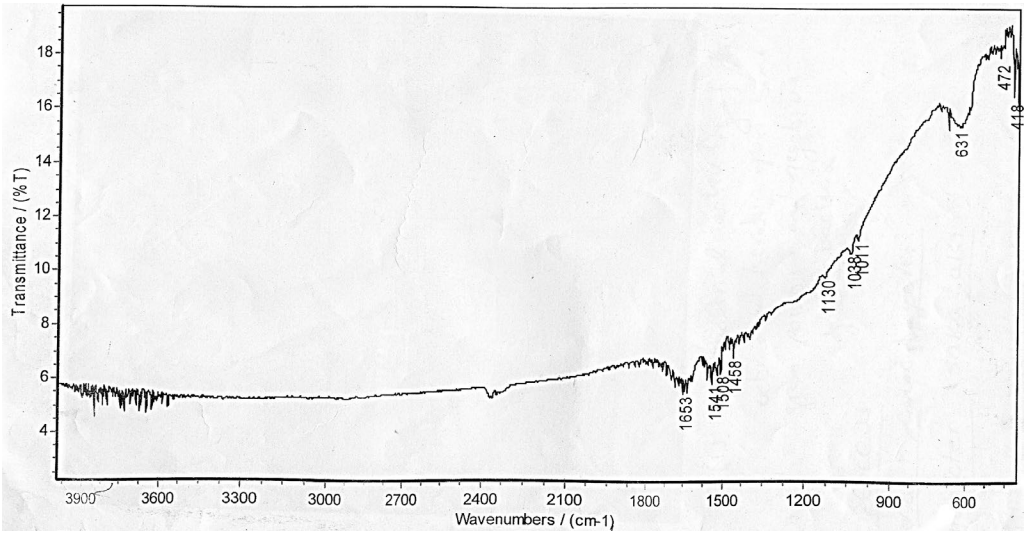

Figure 1. IR-Spectroscopy analysis of extracted keratin

The results of this research also revealed that filter paper disks saturated with keratin solution (1mg/ml) exerted an inhibition zone more than 12 mm. Therefore, extracted keratin considered as antimicrobial agents against some important gram-positive bacteria such as *Staphylococcus aureus*, which resist to antibiotic and are considered as big health problems, and environmental contaminants bacteria like *Bacillus cereus*, and the most common bacterial pathogens *Enterobacter sakazaki*. There were no growth inhibition effect against listeria monocytogenes and micrococcus isolates as shown in (Fig 2 and Fig 3). These results are similar with [17] results, which concluded that keratins contain amino acid sequences with high biological activities as antibacterial agents.

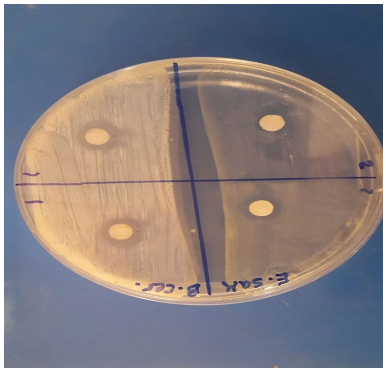

Figure 2. Anti- bacterial activity of extracted keratin against 2 isolates of gram positive *Bacillus cereus* and Gram negative *Enterobcter sakazaki*

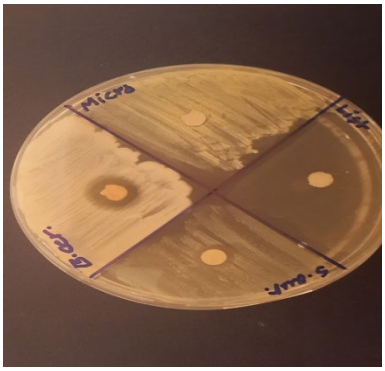

Figure 3. Antibacterial activity of extracted keratin against some bacterial isolates such as *Bacillus cereus*, *Staphylococcus aureus, Listeria*, and *Micrococcus*

CONCLUSION

The present research was successfully converted chicken waste feathers that pose an environmental problem due to its time-consuming decomposition to a benefit substance such as keratin. The extracted material was analyzed by IR infrared spectroscopy to confirm the protein nature of this material. The present research also shows the derived keratin from chicken feathers can be used for several purposes such as antimicrobial agents.

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