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Extraction and Characterization of Keratin from Poultry Feathers

P. E. Dim, M. O. Edoga

Abstract – *The objective of this study was to extract and characterize-feather keratin. Poultry feathers were first pretreated and ground to 1.00 mm particle size. Subsequently, the keratin was extracted from the feather powder with an aqueous solution of sodium sulphide at 40^0C for 3 hrs. This allowed the extraction of 78 % of the keratin present in the dry feather. The characterization of the keratin sample was carried out to determine its nitrogen content, sulphur content, ash content, specific gravity, and moisture content, respectively. Infrared and ultraviolet analyses of keratin were also carried out. The results obtained from this work confirmed the sample to be poultry feather-keratin.* ***Copyright © 2011 Praise Worthy Prize S.r.l. - All rights reserved.***

Keywords*: Keratin, Characterization, Extraction, Poultry Feather, Solubilization*

E-PRINT

I. Introduction

Poultry feathers are approximately 91% protein (keratin), 1% lipid, and 8 % water, [1]. The amino acid sequence of a poultry feather is very similar to that of other feathers and also has a great deal in common with reptilian keratins from claws [2]. This sequence is largely composed of cysteine, glycine, proline, and serine and contains almost no histidine, lysine or methionine [3]. Keratin is a fibrous structural protein component of hair, wool, nail, claws, horn, hoofs, and quill of feathers. These proteins generally contain large quantities of the sulphur-containing amino acids particularly cysteine. A quarter of the amino-acids in keratin are cysteine whose ability to form strong bridging (disulphide) bonds with other cysteine unit accounts for keratin's great stability. This is due to extensive cross linking and strong covalent bonding within its structure. Keratin shows good durability and resistance to hydrolytic and thermal degradation. Efforts to extract keratin proteins from feathers illustrate this point. Extraction is difficult because it can only be achieved if the disulphide and hydrogen bonds are broken. Schrooyen [4] found keratin to be insoluble in polar solvents, such as water, as well as in non-polar solvents. The most common method for dissolving feather keratins is solubilization with concormitant peptide bond scission via acid and alkali hydrolysis, reduction of disulphide bonds with alkaline sodium sulphide solution, or combination of enzymatic and chemical treatment. Although these techniques are effective for extracting 75% yield of keratin [5], efforts should be intensifying extracting higher percentage yield of keratin from poultry feathers. This work therefore has become imperative because the opportunity to use this interesting protein-rich-keratin from agricultural byproduct has risen which invariably will augment the stability-durability property of some hydrophilic petrochemical if properly incorporated.

Take for instance, a typical petrochemical adhesive, urea-formaldehyde adhesive, which traditionally is hydrophilic, can become hydrophobic if the feather-keratin is properly incorporated into its structure. It has also been used as component of various kinds of composites [6]; as a component of biodegradable non-woven [7]; and in biotechnology.

II. Materials and Method

Sodium hydroxide, sodium sulphide, ethylene alcohol (BDH Chemical Ltd, England), distilled water, ariel (Procter and Gamble Nig. Ltd), Heating mantle (Electrothermal Britian), bench top density meter model RS232 (Fisher scientific UK) and Cellulose dialysis sieve (Sigma and Aldrich). The specific gravity of keratin was determined using an instrument called Bench top density meter with model RS 232.The nitrogen content was determined by the Kjejdahl method, whereas the sulphur content was determined by the sheniger standard method [8].

II.1. Collection and Pre-Treatment of Poultry Feathers

The poultry feather for obtaining keratin, were collected from various poultry farms in Minna, Nigeria. The feather were soaked and washed with hot water and detergent for six consecutive times. The poultry feathers were filtered and dried under the sun and atmospheric condition.

Subsequently, the feathers were washed with ethylene alcohol and dried under the sun and atmospheric condition.

After drying, they were ground to reduce their size as well as create a better surface area for contact with solvent.

Manuscript received and revised December 2010, accepted January 2011

II.2. Solubilization of Keratin in Aqueous Solution of Sodium Sulphide

Twenty five grams of sodium sulphide was weighed and dissolved in distilled water. The solution was poured into a round bottom flask and subsequently 50 grams of poultry feather was added to the solution. The content of the flask was heated at a temperature of 30 ^{0}C for 3hrs. At the end of heating the keratin solution was filtered in order to separate the insoluble parts of the poultry feather from the soluble part. Dialysis was carried out using a cellulose dialysis sleeve. The dialysis was performed at environmental temperature for over three days. After dialysis operation, the keratin was recovered from the solution by spatter-drying. Drying was carried out over some seconds at temperature of 147^{0}C at the inlet and 85^{0}C at the outlet [9]. The keratin was weighed and recorded. This procedure was repeated at constant poultry feather weight, sodium sulphide concentration and time but at different temperatures.

II.3. Determination of Ash Content

A crucible was placed in a muffle furnace for about 15 minutes at 350^{0}C. The crucible was removed and cooled to room temperature and was weighed. Feather keratin sample was added into the crucible and it was weighed with the content. The crucible and the content were placed inside the muffle furnace and slowly the temperature was increased from 200^{0}C - 450^{0}C. This sample was ashed until it became whitish in colour. It was removed from the furnace to a dessicator and allowed to cool to room temperature after which the crucible and the content was reweighed and recorded. This was used to determine the ash content.

II.4. Determination of Protein Content of Feather Sample

About 2g of wet feather sample was weighed into a 50 ml kjeldahl flask. 20 ml of concentrated sulphuric acid and one kjeldahl catalyst tablet was also added into the flask. About 0.5g of dry feather sample was weighed into a 50 ml kjeldahl flask. 5 ml of concentrated sulphuric and a half kjeldahl catalyst was also added into the flask. Subsequently, the content of the flask was heated with a heater, starting with a low heat for 15 minutes, and was increased to medium heat for about 30 minutes again and finally was heated until the digestion was completed. During the heating process, the flask was rotated at a given interval until the digestion was over. The flask and its content was allowed to cool, and the sample residue was filtered and washed. Subsequently, 5 ml of 2 % boric acid was added into 100 ml conical flask (as receiving flask), 3 drops of mix indicator was added to the flask content. The receiving flask was placed so that the tip of the condenser tube was below the surface of the boric acid, 10 ml of 40% sodium hydroxide was added, and the joints were tightened and about 50 ml was distilled into the receiving flask. The distillate was titrated with a standard mineral acid (0.01 M HCl) and the results obtained were used to calculate the protein content of the feather sample.

II.5. Infrared and Ultraviolet Analyses

Infra-red analyses were carried out on the keratin using Perkin Elmer 1310 IR (FTIR) spectrophotometer and Nujol as solvent. Ultraviolet analyses were also carried out.

III. Results and Discussion

From Table I the result shows that as temperature increased, the yield also increased, but after 40^{0}C, the yield decreased while the temperature was still increasing. As shown in Table I, increase in yield was recorded between 30^{0}C and 40^{0}C, while decrease in yield was recorded between 40^{0}C and 50^{0}C, respectively. These suggest that between 30^{0}C and 40^{0}C, percentage yield varied directly with temperature, while between 40^{0}C and 50^{0}C percentage yield varied indirectly with temperature. On the basis of the results obtained, the best temperature is 40^{0}C and the maximum yield is 78% [10]. Thus, the percentage yield value of extracted keratin from poultry feather was found to be between the ranges of 16% to 78% as presented in Table II. The keratin sample is characterized by nitrogen content of 14.46 - 15.23%, sulphur content of 2.1- 2.37% and ash content of 0.85-1.1%. From Table III, it can be seen that the average protein content of the three feather samples is 97.43%.

TABLE I
EFFECT OF TEMPERATURE ON THE YIELD OF KERATIN

Temperature (^{0}C)	Extracted Keratin (g)	Percent Yield (%)
30	8.00	16.00
35	12.00	24.00
40	39.00	78.00
45	30.00	60.00
50	22.00	44.00

TABLE II
PHYSICOCHEMICAL PROPERTIES OF KERATIN SAMPLES

Keratin sample	Nitrogen content (%)	Sulphur content (%)	Ash content	Colour	Specific gravity
A	15.20	2.30	0.90	Brown	0.81
B	14.80	2.10	1.10	Brown	0.80
C	15.23	2.37	0.80	Brown	0.85
D	14.46	2.17	0.99	Brown	0.90
E	14.90	2.10	0.93	Brown	0.92

TABLE III
PROTEIN CONTENT OF FEATHER SAMPLES

Feather samples	1	2	3
Protein content (%)	97.43	97.30	97.56
Average protein content (%)	97.43	97.43	97.43

Figs. 1-5 show the ultraviolet spectra of the keratin samples.

The spectra of the samples showed a strong and sharp absorption at the peak which corresponds to 195 nm, 208 nm and 349 nm.

But it is observed that the prominent appearance of peak at 208 nm shows the presence of amino groups and primary amide hydrogen, as well as amino acids.

While the diminished peak at 195 nm and 349 nm may be due to the presence of some trace amount of secondary amide.

The prominent peak at 208 nm therefore shows a clear evidence of the presence of amino acids which is the building block of keratin substance.

Generally, the spectra show a similar range of resonance for all the samples.

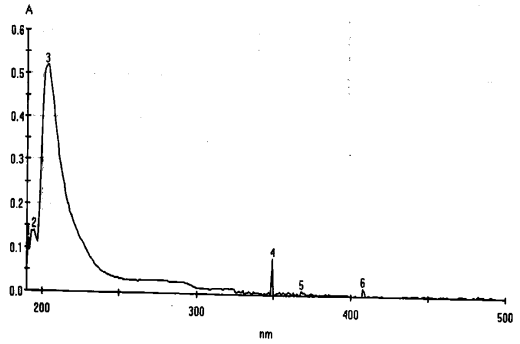

Fig. 1. UV spectra curve of keratin sample A

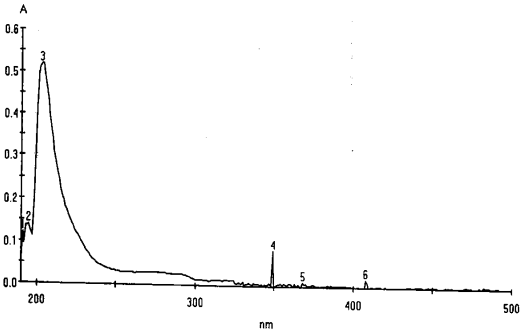

Fig. 2. UV spectra curve of keratin sample B

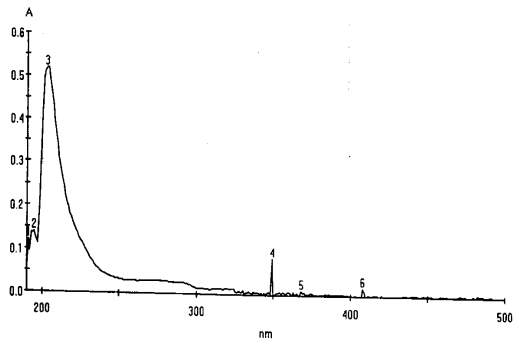

Fig. 3. UV spectra of keratin sample C

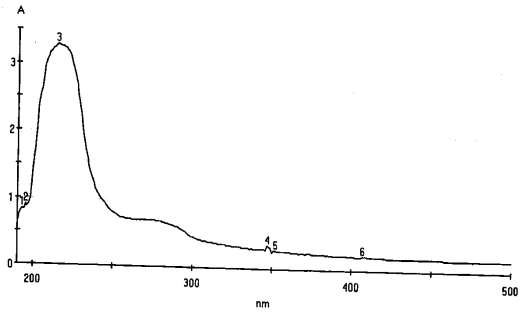

Fig. 4. UV spectra curve of keratin sample D

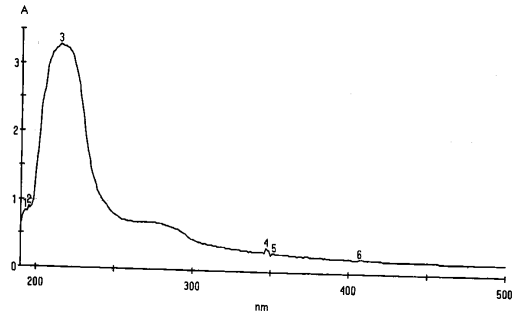

Fig. 5. UV spectra curve of keratin sample E

The infrared spectra of the same keratin samples were recorded as shown in Figs. 6-10.

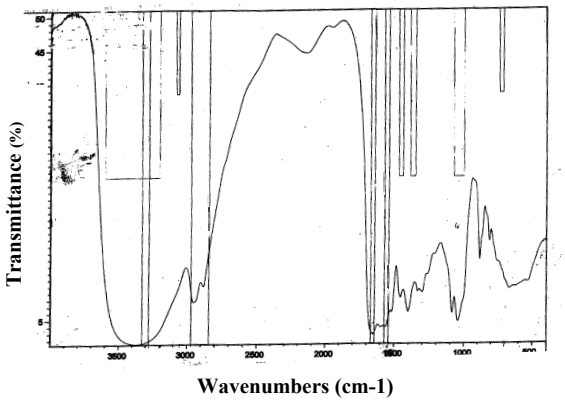

Fig. 6. IR spectra curve of keratin sample A

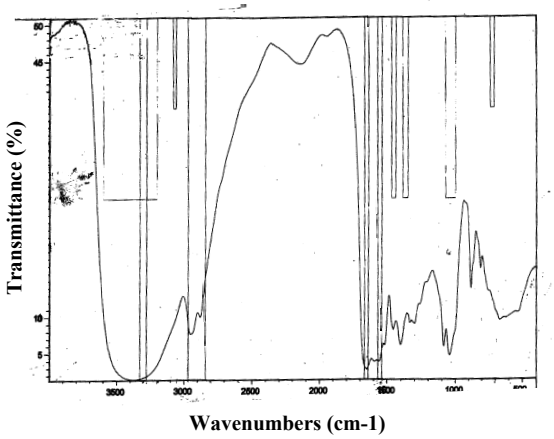

Fig. 7. IR spectra curve of keratin sample B

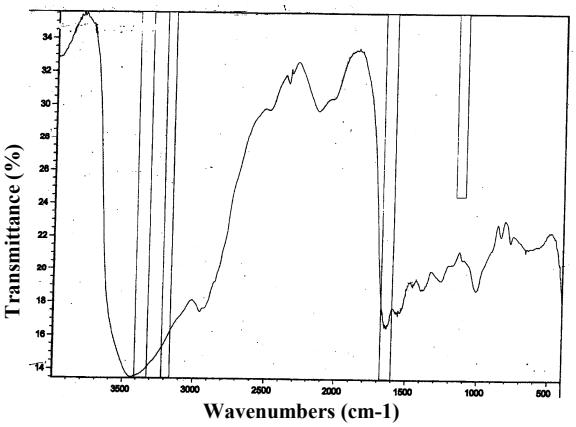

Fig. 8. IR spectra curve of keratin sample C

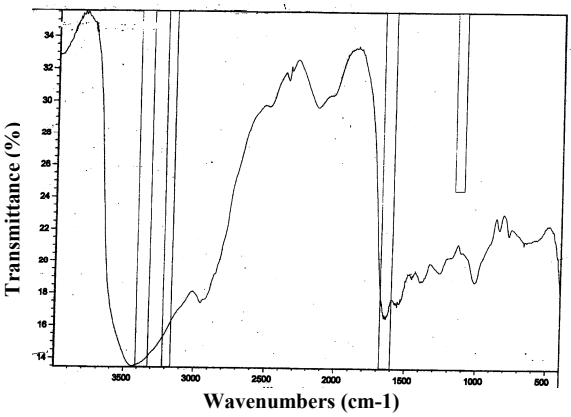

Fig. 9. IR spectra curve of keratin sample D

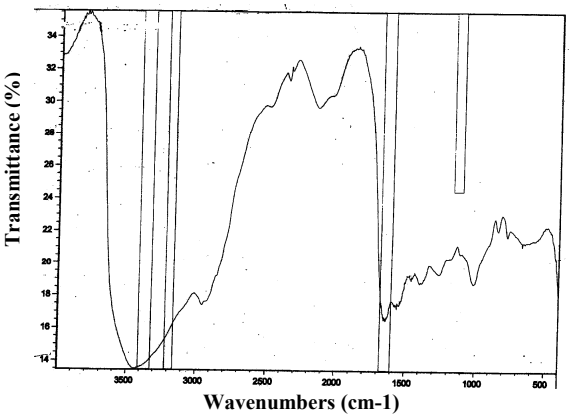

Fig. 10. IR spectra curve of keratin sample E

The spectra of the keratin samples show a strong absorption band at 3410 cm^{-1}.This band is broad and typical of hydrogen bonded N-H and OH. It is also observed that within the region of 3200-3510cm^{-1}, the absorption is strong and broad which is peculiar to hydrogen bonded N-H and OH. At the region of 2850-3000cm^{-1} the intensity was small and sharp, which is an indication of C-H stretching vibration. The strong absorption observed at 1651cm^{-1} which is sharp and small confirms the presence of C = O which is characteristics of carbonyl group. The strong and small absorption bands at 1403cm^{-1}, 1042cm^{-1} and 667cm^{-1} confirms the presence of C-N, C-C, and C-S bond, respectively. Finally, the S-S, stretching vibration is weak and falls within 400-500cm^{-1}. This absorption also confirms the presence of primary and secondary amides. The infrared spectra of the samples also showed a similar range of resonance and are consistent with the UV spectra. Consequently, the infrared absorptions are in agreement with the ultraviolet absorption carried out previously. This observation also confirms the sample to be keratin.

IV. Conclusion

On the basis of the results obtained from the extraction and characterization of keratin from poultry feathers, the following conclusions are made: The best thermodynamic conditions for extracting keratin from poultry feathers to obtain the highest percentage yield of 78% at 40^0C for 3 hours. This study also revealed that the percentage yield of keratin brownish in colour was found to be within the range of 16 – 78% value. The poultry feather sample used for this work was found to contain 97.43% protein content. The results of UV and IR analyses confirmed the presence of keratin an active principle of poultry feather.

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