Characterization of dehydrated keratin protein extracted from chicken feather

IOP Conf. Series: Materials Science and Engineering **702** (2019) 012033 doi:10.1088/1757-899X/702/1/012033

Characterization of dehydrated keratin protein extracted from chicken feather

B Y Alashwal, A Gupta* and M S B Husain

Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

*Email: arungupta10@gmail.com

Abstract. Keratin is one of the most important biomaterials due to its relatively good bio-compatibility, high physicochemical with excellent mechanical properties and good film coordination ability. Chicken feathers are a major waste in poultry industries however, 90% of pure keratin can be obtained from the total featherweight. Chicken feathers are abundant of keratin protein which can be considered as a suitable protein source and used as a component in the cosmetic and pharmaceutical industry. The aim of this study is to extract keratin protein from chicken feather source. The chemical structure of the keratin protein was examined by Fourier transform infrared spectroscopy (FTIR). The results showed the presence of, Amide I-III bands, which gave critical information on the protein conformation and alteration in backbone structure of the keratin., Surface morphology analysis was described by Scanning electron microscopy (SEM). The extracted keratin was found to present aspheric shaped, small microspheres with smooth surface. Meanwhile the X-Ray Diffraction results showed the crystalline form of the chicken feather dehydrated keratin. The diffraction peak was indexed for the β-sheet crystalline structure of keratin. In conclusion, the characterization of keratin extracted from chicken feathers was successfully performed using the abovementioned analyses.

1. Introduction

Nowadays, the increase in poultry products consumption led to an increase in waste about by 8.5 billion tons of feather, that is produced from 24 billion from the consumption of chicken annually. The disposal of feather waste has become a global environmental problem due to the traditional and costly feathers disposal strategies [1]. On the other hand, feathers are cheap, eco-friendly as an alternative available abundantly as a natural source of protein (keratin) that it is utilized in many applications such as cosmetic, biomedical and others [2-4].

Keratin from feathers of chicken has included some feature compared to other keratin like the feather keratin of fibrous can extend almost to 6 % and get breaking, but hair keratin can extend to double of length [5-6]. It is famous that feathers are essentially made of a structural keratin protein (>90%), rich in cysteine, and hydrophobic residues that enhances crosslinking by disulfide bonds and includes a variety of predominantly cystine, amino acids, lysine, serine, and proline [7-8].

Extraction of keratin from sheep wool has also been a source of extraction of keratin protein to be used in various cosmetics and biomedical products. According to Fan et al. [9] provided convincing results with up to 72% solubility in the keratin extraction method of wool using L-cysteine. The previous studies have shown that the extract keratin from chicken feathers proved that they produce a total protein mass of up to 53% [4-5]. Several studies reported that increasing protein concentration can contribute to reducing protein degradation during the drying process net gain in kidney protein content [10]. The

reduction of keratin is a multi-step, long-lasting method in that sulphitolysis is a key reaction. The disulfide bond are disrupted by sulfite to ($\mathrm{Keratin-Cys-S-}$) cysteine thiol (reduced keratin) and ($\mathrm{Keratin-Cys-SSO_3^-}$) cysteine-S-sulphonate residue (Bunte salt) [11]:

$$\mathrm{Keratin-Cys-S-S-Cys-Keratin} + \mathrm{SO_3^{2-}} \rightarrow \mathrm{Keratin-Cys-S^-} + \mathrm{Keratin-Cys-SSO_3^-}$$

Keratins are classed into two types of Alpha (α) keratins and beta (β) keratins [12]. (α) keratin proteins are found in all vertebrates, while (β) keratin proteins are found exclusively in feathers of birds and reptiles [13]. In this study, feather keratin was extracted from chicken and convert to powder and the drying under vacuum conditions was first developed for keratin protein biomedical, pharmaceutical and cosmetics applications.

2. Materials and Methods

2.1. Materials

Chicken feathers were taken from the chicken was obtained from Balok Poultry Farm Sdn. Bhd. Malaysia, Malaysia. Sodium hydroxide and hydrochloric acid were obtained from Sigma-Aldrich.

2.2. Extraction of keratin protein from chicken feather

Preparation of the feather keratin was based on the previously studied techniques which put 50 g feather with 1 L sodium hydroxide into the conical flask. The mixture was kept at 50 °C and continuously stirred for 5 hours. Then, the mixture was filtered by filter paper and the biomass waste was removed by centrifugation at 10,000 rpm for 10 min [14, 16-17].

2.3. Protein neutralization

The filtrate solution collected earlier was placed in a beaker and stirred. 2 N hydrochloric acid was added drop wise to the solution for making neutral pH. The solution was again centrifuged at 10,000 rpm for 5 minutes and filtered using cellulose filter paper with Grade 1 from Whatman to ensure it is particle free then stored for the further analysis.

2.4. Total mass concentration of Keratin

The content of the keratin protein from the 50 g feather per letter was determined by dehydrating 5 ml of the solution at 104 °C for 24 h in the oven. The powder keratin was 0.24 g and the total mass concentration of keratin was calculated from equation (1):

$$Mass\ concenteration\ of\ keratin = \frac{m}{V} \times 100\% \quad (1)$$

where m is the weight of the solute after evaporation and V is the weight of the initial mixture.

The mass concentration of keratin as powder was $4.8\mathrm{g/mL}$ from 1L keratin.

2.5. Dehydration of keratin

Under vacuum condition, a 50 mL of keratin solution was poured in Pyrex glass dish and dried in the vacuum oven for 20 hours, at 60 °C and 20-inch Hg.

2.6. Characterization of keratin protein

Characterization of keratin was performed by FTIR, SEM and, XRD analyses as shown below:

2.6.1 Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of keratin were read from 400 to 500 $\mathrm{cm^{-1}}$ utilizing a Perkin-Elmer Model 1000 Series FTIR spectrophotometer. All data obtained for keratin was analysed using the software of FTIR [16].

2.6.2 Scanning electron microscopy (SEM)
The surface images of the keratin protein powder sample were investigated by SEM using Hitachi TM3030Plus [17].

2.6.3 X-ray diffraction (XRD)
The keratin protein in crystal structures was examined by X-ray diffractometer a RIGAKU, (Miniflex II, Japan. The models were collected with a radiation source of the Cu Kα. The crystallinity index (Crl) of the samples was described by equation (2) [18]:

$$Crl(\%) = \left(\frac{A_{Crystal}}{A_{Total}}\right) * 100 \quad (2)$$

Where, ($A_{Crystal}$) is the sum of the areas below the crystalline diffraction peaks and (A_{Total}) describes the total area below the diffraction curve 2θ ranging from 5 to 50°.

3. Results and Discussion

3.1. Fourier transform infrared spectroscopy (FTIR)
The FTIR measurement was utilized to identify the chemical structure of the keratin as shown in figure 1. In FTIR spectra an absorption band was scrutinized in the range of 3274-3287 cm^{-1}. The result indicates that the band at 3274.15 cm^{-1} correspond to the peptide bonds (–CO–NH–). The wavenumbers at 1633, 1559 and 1392 cm^{-1} are the waves of the peptide bonds identified as amides I, II and III which provide important information on the keratin protein structure and alteration in backbone composition of the keratin protein. thus, Amide I, is the combination of α-helix and β-sheet and amide III can be attributed to β-sheet structure [19]. As shown, the Amide I region can be deconvoluted to provide more information about the structure presents. at wavenumber 1126 cm^{-1} was described (-OH) the groups of carboxylic acids and the (N-H) band of transmission at 750 to 500cm^{-1}. Hence, FTIR proved the appearance of amino acids like and threonine, cysteine, and glutamine in the keratin sample [17]. Therefore, it can be concluded that the extracted keratin possesses more β-sheet structures as compared to α-helices.

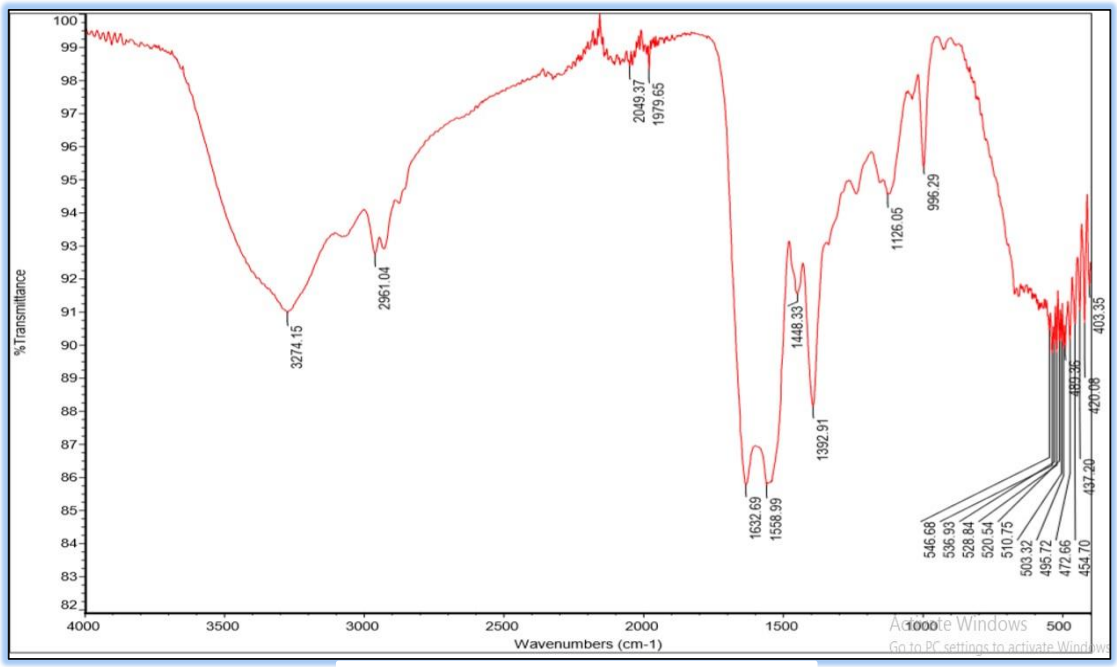

Figure 1. The FTIR spectra of keratin

3.2. Scanning electron microscopy (SEM)

The morphological characteristic of the keratin displays the highly porous structure in the surface with multitude features, like the specimen explained a horny layer, and very thick. The SEM image in figure 2 confirmed that keratin seemed as small particles in aggregates form. The extracted keratin presents aspheric shaped, small microspheres with smooth surface, The surface morphology of these microparticles is similar to the keratin particles synthesized from wool and feather keratin in previous studies [20]. The dried keratin consisted of globular, tightly packed microparticles and randomly arranged microstructures.

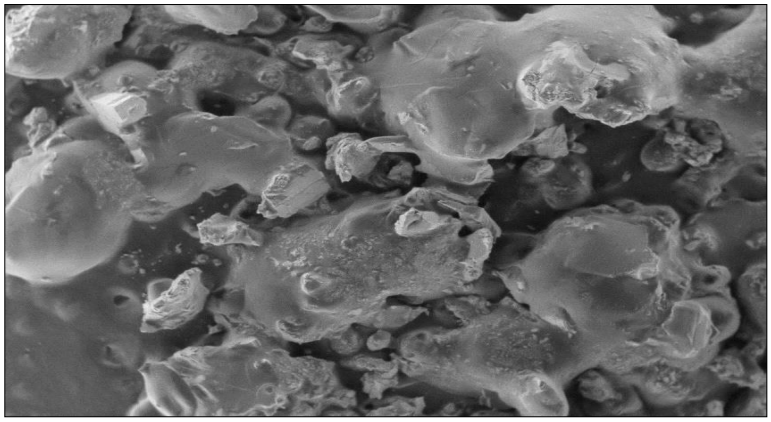

Figure 2. The SEM of keratin

3.3. X-ray diffraction (XRD)

The XRD results determined that the extracted keratin was mainly presented in a semi-crystalline form so, that its aqueous state maintained the crystal. XRD analysis of keratin molecules in figure3 showed a strong peak at 32.75 degrees, which determined the presence of keratin nanoparticles [21]. The dominant peaks at 2θ = 9-10.76° and at 19-32.75° were allocated to α-helix and β -sheet, respectively [22]. The keratin nanoparticles show the diffraction properties of α-helix that appear at 2θ = 10.76 ° and β-sheet at 2θ = 20.50 ° [23].The peak ranges from 5 degrees to 46 degrees, with the width of an amorphous structure [24]. From the above results, it can be concluded that keratin possesses two types of crystal structure α and β. However, keratin contains a greater amount of β-sheet. Although keratin molecules are also slightly amorphous, the peaks have not yet changed the formation of nanoparticles that have shown that the crystal form of keratin is retained in the formulation of nanoparticles which showed in a previous study that after the formation of nanoparticles keratin remained unchanged [6].

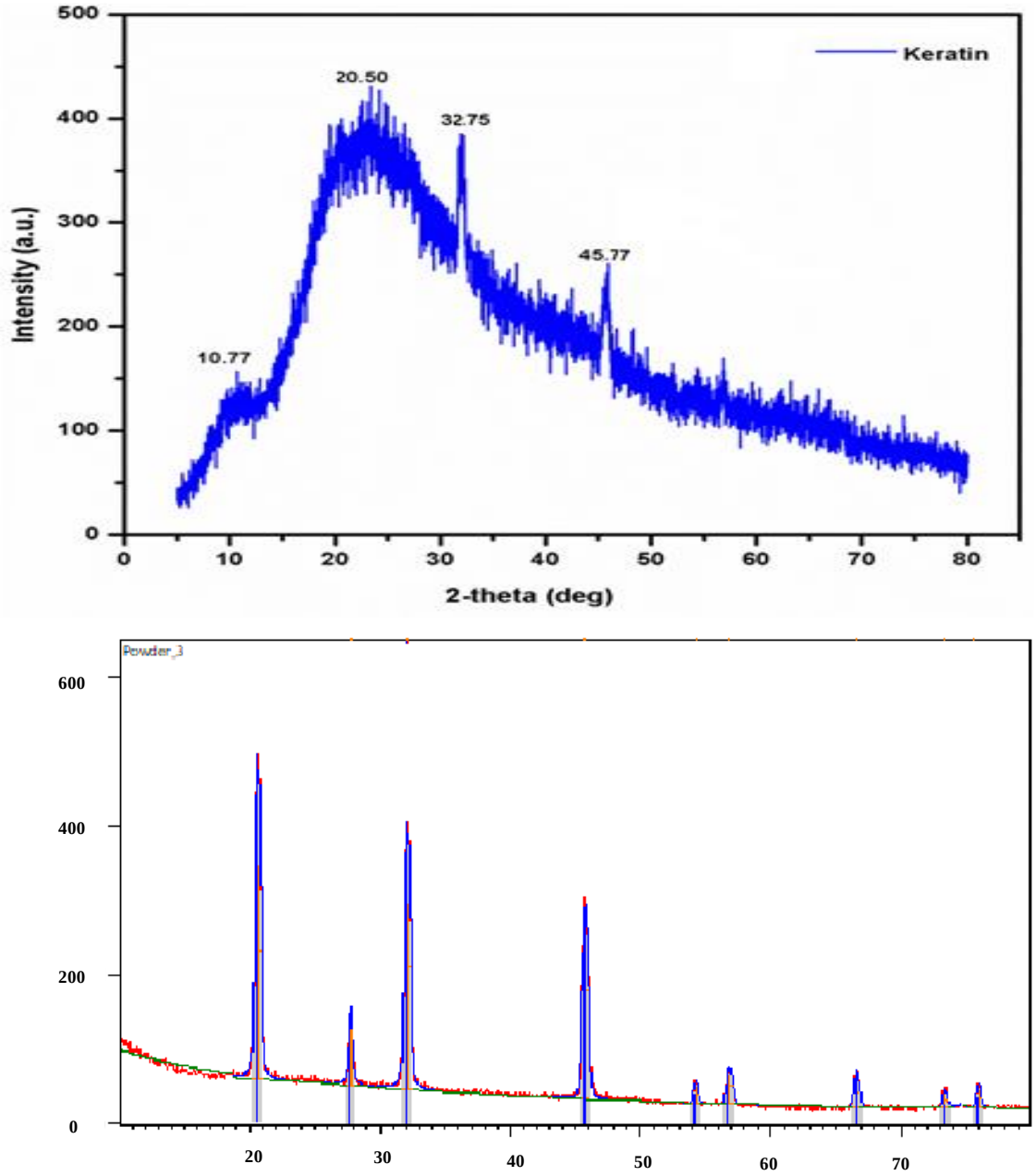

Figure 3. The XRD of keratin

4. Conclusions

In a conclusion, the study aimed to efficiently extract keratin from the biomass waste of chicken feathers and dry the keratin using vacuum oven conditions and obtained success. The fine particles of keratin were studied for morphology and crystalline areas and the appropriate functional groups were analyzed using different assays. Keratin extract, with sequential properties of distinct amino acids, stable properties, and concentration of its mass, has excellent potential for use in future studies and commercial applications.

Acknowledgements
Authors are thankful to University Malaysia Pahang (UMP) for financial support (Financial Aid (PGRS) number: 18033) and providing facilities.

References

[1] Husain M, Gupta A, Alashwal B and Sharma S 2018 Synthesis of PVA/PVP Based Hydrogel for Biomedical Applications: A Review Energy Sources, Part A Recovery, Utilization, and Environmental Effects 2388-93
[2] Husain M S B, Gupta A and Alashwal B Y and Sharma S 2018 Synthesis of Hydrogel Using Keratin Protein from Chicken Feather Proceedings of ICCEIB 2018 **193** p 16
[3] Alashwal B Y, Bala M S, Gupta A, Sharma S and Mishra P 2019 Improved properties of keratin-based bioplastic film blended with microcrystalline cellulose: A comparative analysis *Journal of King Saud University-Science* In Press
[4] Gupta A, Kamarudin N B, Kee C Y G and Yunus R B M 2012 Extraction of keratin protein from chicken feather *Journal of Chemistry and Chemical Engineering* **6** (8) 732
[5] Navone L and Speight R 2018 *Understanding the dynamics of keratin weakening and hydrolysis by proteases* (Public Library of Science) p e0202608
[6] Ma B, Qiao X, Hou X and Yang Y 2016 Pure keratin membrane and fibers from chicken feather *International Journal of Biological Macromolecules* 614–621
[7] Sharma S, Gupta A, Chik S M S, Kee C G, Mistry B M, Kim D H and Sharma G 2017 Characterization of keratin microparticles from feather biomass with potent antioxidant and anticancer activities *International journal of biological macromolecules* 189–196
[8] Fraser R D B and Parry D A D 2017 *Filamentous structure of hard β-keratins in the epidermal appendages of birds and reptiles in Fibrous Proteins: Structures and Mechanisms* (Springer, Cham) 231–252
[9] Fan J, Lei T D, Li J, Zhai P Y, Wang Y H, Cao F Y and Liu Y 2016 High protein content keratin/poly (ethylene oxide) nanofibers crosslinked in oxygen atmosphere and its cell culture *Materials & Design* 60–67
[10] A Shavandi, Bekhit A E-D A, A Carne, and Bekhit A 2017 Evaluation of keratin extraction from wool by chemical methods for bio-polymer application *Journal of bioactive and compatible polymers* 163–177
[11] Sinkiewicz I, Śliwińska A, Staroszczyk H and Kołodziejska I 2017 *Localization of Alpha-Keratin and Beta-Keratin (Corneous Beta Protein) in the Epithelium on the Ventral Surface of the Lingual Apex and Its Lingual Nail in the Domestic Goose (Anser Anser f. domestica) by Using Immunohistochemistry and Raman Microspectros* (The Anatomical Record 300)
[12] Skieresz-Szewczyk K., Jackowiak H, Buchwald T and Szybowicz M 2017 *Localization of Alpha-Keratin and Beta-Keratin (Corneous Beta Protein) in the Epithelium on the Ventral Surface of the Lingual Apex and Its Lingual Nail in the Domestic Goose (Anser Anser f. domestica) by Using Immunohistochemistry and Raman Microspectros* (The Anatomical Record 300)
[13] Fraser R D B and Parry D A D 2017 *Filamentous structure of hard β-keratins in the epidermal appendages of birds and reptiles in Fibrous Proteins: Structures and Mechanisms* (Springer, Cham) 231–252
[14] Bao Y, Ma J and Li N, 2011 Synthesis and swelling behaviors of sodium carboxymethyl cellulose-g-poly (AA-co-AM-co-AMPS)/MMT superabsorbent hydrogel *Carbohydrate Polymers* 76-82
[15] Sharma S, Gupta A, Chik S M S B T, Kee C Y G and Poddar P K, 2017 *Dissolution and characterization of biofunctional keratin particles extracted from chicken feather*s (IOP Publishing) 12013
[16] Ramakrishnan N, Sharma S, Gupta A and Alashwal B Y 2018 Keratin based bioplastic film from chicken feathers and its characterization *International Journal of Biological Macromolecules* **111** 352-58
[17] Kamarudin N B, Sharma S, Gupta A, Kee C G, T Chik S M S B, and Gupta R 2017 *Statistical*

investigation of extraction parameters of keratin from chicken feather using Design-Expert (3 Biotech) 127

[18] Idris A, Vijayaraghavan R, Patti A F, and MacFarlane D R 2014 Distillable protic ionic liquids for keratin dissolution and recovery *ACS Sustainable Chemistry & Engineering* 1888–94

[19] Wang Y-X, and Cao and X.-J 2012 Extracting keratin from chicken feathers by using a hydrophobic ionic liquid *Process Biochemistry* 896–899

[20] Barone J R, Schmidt W F and Gregoire N T 2006 Extrusion of feather keratin *Journal of Applied Polymer Science* 1432–1442

[21] Ayutthaya S I N, Tanpichai S and Wootthikanokkhan J 2015 Keratin extracted from chicken feather waste: extraction, preparation, and structural characterization of the keratin and keratin/biopolymer films and electrospuns *Journal of Polymers and the Environment* 506–516

[22] Song K, Xu H, Xie K, and Yang Y, 2017 Keratin-Based Biocomposites Reinforced and Cross-Linked with Dual-Functional Cellulose Nanocrystals *ACS Sustainable Chemistry & Engineering* 5669–5678

[23] Esparza Y, Ullah A and Wu J 2017 Preparation and characterization of graphite oxide nano-reinforced biocomposites from chicken feather keratin *Journal of Chemical Technology & Biotechnology* 2023–2031

[24] Yin X-C, Li F-Y, He Y-F, Wang Y, and Wang R-M, 2013 Study on effective extraction of chicken feather keratins and their films for controlling drug release *Biomaterials Science* 528–36