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Original Research Article

Investigation of the effect of oxidation on keratin obtained from chicken feathers

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Abstract

Purpose: *To investigate excipient properties of oxidized extracted keratin (OEK) compared to extracted keratin (EK) obtained from chicken feathers.*
Methods: *Keratin from whole chicken (Gallus gallus domesticus) feathers was extracted under conditions of optimum yield. Oxidation was carried out with hydrogen peroxide (H_2O_2) for 15 mins, washed, filtered, dried and stored in airtight containers. Organoleptic properties, chemical tests, pH, true density, hydration capacity, swelling capacity, flow properties and chemical functionalities using UV/visible spectroscopy, Fourier transform-infrared spectroscopy (FT-IR) and High-performance liquid chromatography (HPLC) were investigated.*
Results: *The yield of EK was 28 % while that of OEK was 89.2 % (obtained from EK). The EK was dark brown, fine and powdery with a characteristic odour. However, OEK was pale brown, smooth texture and odourless. Furthermore, OEK had significantly lower hydration capacity, and true density as well as significantly higher pH, swelling capacity, and improved flow compared to EK ($p < 0.05$). UV and FTIR spectra were superimposable. The wavelength of maximum absorption was 244.50 to 306.50 nm (EK) and 269 to 335.50 nm (OEK). The transmission band was attributed to CH3, C=O, N-H, C-H, C-S, and N-H stretching. Furthermore, HPLC analysis revealed varying amounts of amino acids and total amino acid content was higher in EK compared to OEK.*
Conclusion: *Oxidation of EK from chicken feathers enhances appearance, and flow, and reduces protein content with a significant reduction in cysteine residues. Drug release modulation properties of OEK would need to be investigated in further studies.*

Keywords: *Keratin, Oxidized, Extracted, Excipient, Properties*

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INTRODUCTION

Chicken (*Gallus gallus domesticus*) feathers constitute a significant portion of waste, and international regulations prohibit disposing of these biodegradable materials with high energy potential in landfills. Instead, chicken waste feathers are being recognized as a valuable resource, leveraging on their unique properties to produce various high-value byproducts, such as

keratin, fibers, hydrogels, and more, aiming to maximize their economic potential and minimize waste [1].

Feathers are predominantly made up of beta-keratin, a type of fibrous protein that confers exceptional strength and flexibility. Chicken feather waste encompasses the feathers that are shed, removed, or collected from various chicken sources, such as chickens, turkeys, ducks, and geese, throughout the entire chicken farming and processing cycle. These feathers are generally regarded as a waste product of the chicken industry and are often discarded or disposed as a byproduct of processing operations [2]. While chicken waste accounts for a relatively small portion of total animal waste, managing feather waste requires special attention and necessitates the development of efficient methods for reusing and recycling chicken feathers. Finding effective ways to reuse and recycle waste chicken feathers is crucial for sustainable waste management [3]. Innovating and improving existing excipients, formulations and facilities has been the core focus of recent research in pharmaceutical technology [4]. This is due to the chemical modification of pharmaceutical excipients which has resulted in the development of other excipients with improved functionalities [5]. However, they may also be obtained by combining existing materials [6]. Oxidized keratin extracts also referred to as keratose lack free thiols, and therefore its supra-structural network assembly only depends on non-covalent interactions [7]. As a result, bulk degradation of keratose is faster compared to keratin constructs [7]. Both keratin and keratose have been incorporated in films, gels, and scaffolds through supra-structural assembly of closely-packed protein subunits. These have been used as carriers to deliver drugs and growth factors. The desired therapeutic effect depends on the release kinetics associated with the keratin constructs. This is further influenced by the surface and bulk interaction of the drugs with keratin and keratin degradation behaviour. As a result, understanding the intermolecular interactions between keratin and the payload compounds will help in the design of more efficient keratin-based implantable constructs, delivery systems, and medical devices [7,8].

Oxidative treatment of keratin fibers promotes urea-dependent morphological change by changing films from opaque to transparent [9]. Oxidative treatment with hydrogen peroxide (H_2O_2) promotes yield, and results in high molecular weights, compared to other conventional methods that are more deleterious. This extraction method influences the yields, molecular structure, self-assembly, and aqueous behaviour. Proteins extracted with H_2O_2 demonstrate excellent film-forming capability, higher aqueous solubility, and no S-S reformation. This enables the development of biodegradable protein films and more efficient delivery systems [10]. This study therefore investigated the excipient properties of OEK compared to EK obtained from chicken feathers.

EXPERIMENTAL

Materials

Sodium hydroxide (NaOH, Molychem, Mumbai, India), chicken feathers were obtained from a local poultry farm in Delta State, Nigeria. Sodium lauryl sulphate, hydrochloric acid (HCl, Unicon instruments, Ambala Cantt. India), hydrogen peroxide (Arkema, France), methanol (Merck, USA), phenylisothiocyanate (CDH Fine Chemical, India), sodium phosphate (Kemin Industries, Inc., USA), acetonitrile (INEOS, Germany), sodium acetate (Hemadri chemicals, India), triethylamine (Sigma-Aldrich, USA).

Extraction of keratin

Keratin from chicken feathers was extracted under conditions of optimum yield reported by Meko *et al* [11]. Feather samples were soaked in sodium lauryl sulfate solution for 24 h to remove stains, oil, and grease. Thereafter, the feathers were washed, dried under sunlight for 72 h, pulverized and stored in bags. Dried (100 g) chicken feathers were dissolved in 3.0 M NaOH solution at 24 ^oC, stirred and allowed to stand for 24 h. The mixture was centrifuged (REMI R-24) at 5000 rpm for 10 min. The supernatant was collected and the insoluble feathers were discarded. The supernatant solution collected was kept in a 500 mL conical flask and an equimolar concentration of HCl was added dropwise to precipitate keratin. The samples were filtered, washed with distilled water and air-dried to a constant weight.

Oxidation of keratin

Keratin was oxidized following the method of Meyer and Seuss-Baum [12]. Dry keratin (30 g) was dissolved in 10 mL distilled water to create a thin paste. Thereafter, 15 mL of undiluted hydrogen peroxide was added to the paste and stirred for 10 to 15 min. The mixture was allowed to oxidize for 1 h while stirring at intervals. A small amount of distilled water was added to stop the reaction. A large filter paper was used to remove the excess liquid. The sample was

washed with water several times, air-dried and stored in a closed container.

Evaluation of parameters/indices

Yield (Y)

Yield of the extracted and oxidized samples was calculated using Eq 1.

$Y = (W_1/W_2)100$ …… (1)

where W_1 is the weight of whole feathers, W_2 is the weight of extracted or oxidized samples

Organoleptic evaluation

Organoleptic properties (colour, odour and texture) of extracted keratin and oxidized samples were evaluated for their taste, odour, colour and texture by three independent observers and the outcome was recorded.

Chemical tests

Test for proteins (biuret test)

Keratin (5 mg) was transferred to a test tube and 1 mL of 1 % potassium hydroxide (KOH) solution was added. Thereafter, drops of 1 % copper sulphate solution were added and shaken following each addition and observed for colour change under an electronic microscope.

Test for carbohydrates (Fehling's test)

Aqueous dispersion of 1 mL 1 %w/v keratin was prepared in a test tube and freshly made Fehling's solution A and B was added and heated for 5 min in a water bath. The colour change was recorded.

Test for fixed oils

Keratin (5 mg) was placed on a filter paper and a drop of 10 %w/v acetone was added. The filter paper was air-dried and the drop spot was observed for translucency.

Powder characterization

Determination of pH

The pH of 2 % w/v aqueous dispersion of samples was determined using a pH meter (Hanna pH meter model H12211)

True density

Particle density was evaluated by solvent displacement method using liquid paraffin as the non-solvent. An empty 25 mL pycnometer was weighed (W). The pycnometer was filled with n-hexane and weighed (W_1). Difference between W_1 and W was calculated as W_2. Thereafter, the sample (1 g) (W3) was carefully transferred into a pycnometer. Excess fluid was wiped off and the resultant weight was taken (W_4). Determinations were done in triplicate and true density was calculated using Eq 2.

$Pt\ (g/mL) = (W_2/V)*(W_3/W_3–W_4+W_2+W)$ ….. (2)

where V = 25 mL

Hydration capacity (HC)

Keratin (1 g) was placed in a 15 mL plastic centrifuge tube and 10 mL water was added. The tube was shaken intermittently over a 2 h period and left to stand for 30 min. This was centrifuged for 10 min at 3000 rpm. The supernatant was decanted and powder weight after water uptake and centrifugation was determined. Hydration capacity was calculated using Eq 3.

$HC = x/y$ …… (3)

where x is the weight of moist powder after centrifugation and y is the weight of dry powder.

Swelling capacity (SC)

The tapped volume occupied by 5 g of the powder V_X, was recorded. Thereafter, the powder was dispersed in 85 mL of water, made up to 100 mL, and left standing for 24 h. Sediment volume, V_V, was measured. Determination was done in triplicate and swelling capacity was computed using Eq 4 [13].

$SC = V_v/V_x$ …… (4)

Density

Samples (10 g) were placed inside the measuring cylinder and bulk volume (V_1) was recorded. The samples were subjected to 100 taps and tapped volume (V_2), was recorded. Bulk density (BD), tapped density (TD, Hausner's ratio (HR) and Carr's index (CI) were calculated using Eqs 5 – 8 where W represents the weight of the sample.

$BD = W/V_1$…….. (5)

$TD = W/V_2$ …….. (6)

$HR = TD/BD$ …… (7)

$$CI = \{(TD–BD)/TD\}100 \text{ ……..} (8)$$

Flow rate (FR) and angle of repose (θ)

A clean glass funnel was clamped to a retort stand such that the distance from the tip of the funnel to the horizontal base was 5 cm. The funnel orifice was opened and the flow rate was determined in triplicate using Eq 9 and Eq 10.

$$FR = W/t \text{ ……} (9)$$

$$\theta = \tan^{-1} 2(h/d) \text{ ……} (10)$$

where h is the height of the powder heap, d is the powder heap diameter, W is the weight of the sample (g), and t is the flow time (s).

Amino acid analysis

High-performance liquid chromatography (HPLC) analysis

The HPLC equipment used was a spectra physics (San Jose, CA) HPLC apparatus comprising of an 8700 XR ternary pump, a 20-µL Rheodyne (Cotati, CA) injection loop, an SP8792 column heater, 8440 XR UV-vis detector and a 4290-integrator linked via Labnet to a computer running WINner 8086 software (operating system, MS.DOS version 3.2). For separation, a 250- × 4.6-mm column packed with 5-µm Spherisorb C_{18} (Sugelabor, Madrid, Spain) was used.

Preparation of samples and standards

Before derivatization, sample proteins were hydrolyzed. A lyophilized sample (0.1 g) was transferred into a 16- × 125-mm screw-cap Pyrex (Barcelona, Spain) tube and 15 mL of 6 N hydrochloric acid was added. The tube was thoroughly flushed with nitrogen gas, quickly capped, and placed in an oven at 110 °C for 24 h [14]. The tubes were filtered under vacuum (Whatman #541, Maidstone, England) to remove solids after hydrolysis. The filtrate was made up to 25 mL with water, and an aliquot was filtered through a 0.50-µm pore-size membrane (Millipore, Madrid, Spain). A standard solution containing 1.25 µmol/mL of each amino acid in 0.1 N hydrochloric acid was created.

Derivatization procedure

Derivatization was done following the method of Elkin *et al* [15] with some modifications. A standard solution (5, 10, 15, or 20 µL) or 50 µL of sample solution was pipetted into a 10 × 5 mm tube and dried under vacuum at 65 °C. Thereafter, 30 µL of methanol-water-phenyl isothiocyanate (2:2:1 v/v) was added to the residue and then removed under vacuum at 65 °C. A derivatizing reagent (30 µL) comprising methanol-water-phenyl isothiocyanate (7:1:1 v/v) was added, and the tube was agitated and left to stand at room temperature for 20 min. The solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4 °C prior to analysis. Before injection, 150 µL of diluent consisting of 5mM sodium phosphate with 5 % acetonitrile was added to each tube.

Chromatographic procedure

Chromatography was carried out at a constant temperature of 30 °C using gradient elution. Eluant A was an aqueous buffer prepared by adding 0.5 mL/L Triethylamine to 0.14 M sodium acetate and titrating it to pH 6.20 with glacial acetic acid. Eluant B was acetonitrile : water (60:40 v/v). The gradient program is shown in Table 1.

Table 1: Gradient program employed for the separation of phenylthiocarbamyl (PTC)-amino acids

Time (min)	Flow rate (mL/min)	Eluent A (%)	Eluent B (%)
0	1.0	90	10
12.0	1.0	70	30
20.0	1.0	52	48
22.0	1.0	0	100
24.0	1.0	0	100
30.0	1.5	0	100
37.0	1.0	90	10

Statistical analysis

Data was analyzed using Statistical Packages for Social Sciences (SPSS, version 22.0; (IBM, Armonk, NY, USA). Measurement data were presented in mean ± standard deviation (SD) and compared using the student t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Yield

The yield of extracted keratin was 28 %. The oxidized sample was 89.2 % (obtained from the extracted keratin).

Organoleptic and qualitative analysis

Extracted keratin was dark brown in colour, fine and powdery with a characteristic odour.

However, the oxidized sample was pale brown, smooth texture and odourless (Table 1).

Table 1: Organoleptic and qualitative analysis

Property/test	Extracted keratin	Oxidized extracted keratin
Organoleptic		
Colour	Dark-brown	Light-brown
Texture	Fine and powdery	Fine and powdery
Odour	Mild characteristic odour	Odourless
Chemical test		
Test for protein	Positive	Positive
Test for carbohydrate	Negative	Negative
Tet for lipids	Negative	Negative

Table 2: Physicochemical properties of keratin (mean ± SD)

Property	Extracted keratin	Oxidized extracted keratin
pH	6.14±0.00	6.23±0.00**
Swelling capacity (cm^3)	1.15±0.02	1.43±0.04**
Hydration capacity (g)	0.60±0.10	1.10±0.28**
True density (g/mL)	4.18±0.18	1.17±0.16*

*P < 0.05 significantly lower, **p < 0.05 significantly higher vs extracted keratin

Physicochemical of keratin

Oxidized extracted keratin (OEK) had significantly lower hydration capacity, and true density compared to extracted keratin (EK) ($p < 0.05$). Furthermore, pH, and swelling capacity were significantly higher in OEK compared to EK samples ($p < 0.05$; Table 2).

Flow properties

There was no significant difference in Hausner's ratio, Carr's index, and angle of repose between the two samples ($p > 0.05$). However, oxidation resulted in lower Hausner's ratio, Carr's index, higher flow rate and angle of repose (Table 3).

Chemical characterization

The UV spectrum of the extracted and oxidized samples showed similar chemical bands. The wavelength of maximum absorption was 244.50 to 306.50 nm (extracted sample) and 269 to 335.50 nm (oxidized sample; Figure 3). Conformational changes in the polypeptide chains were analyzed and the absorption band was scanned from 500 to 4000 cm^{-1}. A transmission band appeared in the range of 3000 – 2800 cm^{-1} which is related to symmetrical CH_3 stretching. The transmission band at 1700 – 1600 cm^{-1} is attributed to C=O stretching (amide I). The transmission band for amide II appears at 1580 – 1540 cm^{-1} attributed to N-H bending and C-H stretching as in the extracted keratin sample. The peak at 993.37 cm^{-1} was associated with C-S bonds. Transmission band related to N-H out-of-plane bending occurred between 750 and 600 cm^{-1} (Figure 4).

Protein profiles

Protein profiling indicated the presence of glycine, alanine, serine, proline, valine, threonine, isoleucine, leucine, aspartate, lysine, methionine, glutamate, phenylalanine, histidine, arginine, tyrosine, tryptophan, and cystine in both samples, however at varying amounts. Extracted keratin from chicken feathers had a total protein content of 80.35622 mg/100 g while the oxidized sample had a total protein content of 79.64146 mg/100 g. There was no significant difference in protein concentration in both samples ($p > 0.05$), although present at varying concentrations (Figure 4).

DISCUSSION

The abundance of chicken feather waste is a direct result of the massive global chicken production industry, driven by the escalating demand for chicken products to satisfy the dietary needs of a growing human population. The large-scale industrial farming of chicken has led to a significant generation of feathers as waste material from farms, slaughterhouses, and processing facilities, substantially contributing to the overall volume of agricultural waste produced by the chicken industry [16]. Feather waste's high protein content and robust makeup (90% resilient) make it a promising resource for development and valorization [17].

Table 3: Flow properties of the samples (mean ± SD)

Sample	Bulk density (g/mL)	Tapped density (g/mL)	Hausner's ratio (HR)	Carr's index (CI; %)	Flow rate (g/sec)	Angle repose (º)
EK	0.55±0.02	0.58±0.02	1.06±0.05	5.65±4.92	1.77±0.03	18.61±1.49
OEK	0.96±0.04	1.01±0.04	1.05±0.00	4.92±0.19	3.15±0.24*	18.68±3.38

*P < 0.05 vs EK, EK: extracted keratin, OEK: oxidized extracted keratin

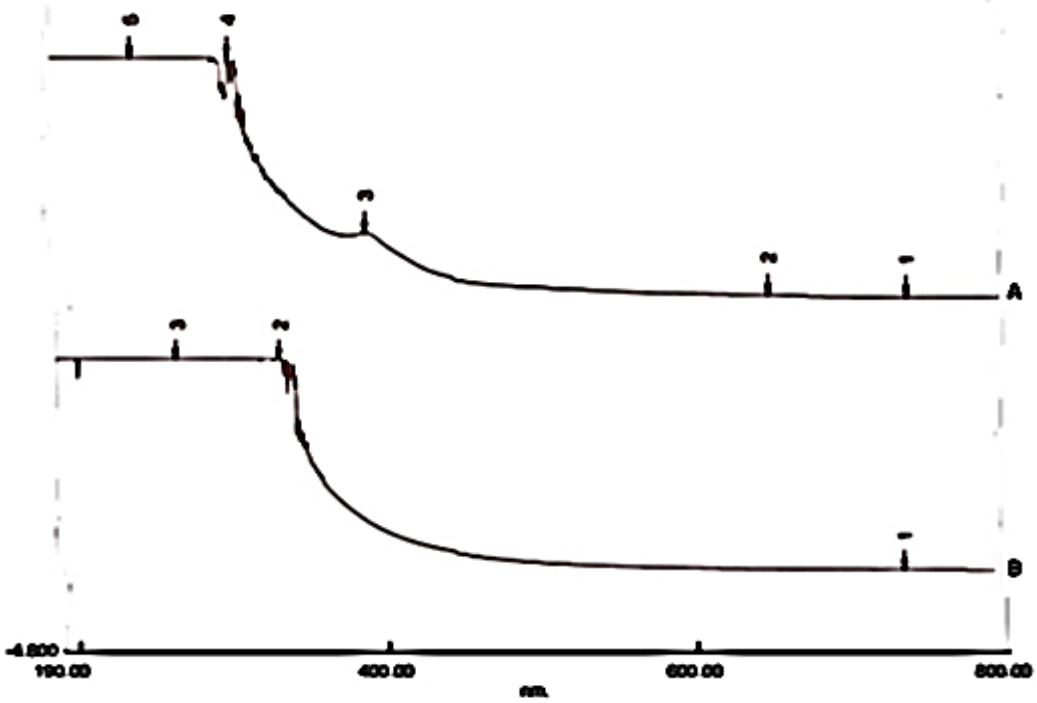

Figure 3: Ultraviolet spectrum of keratin. A: Extracted keratin, B: Oxidized extracted keratin

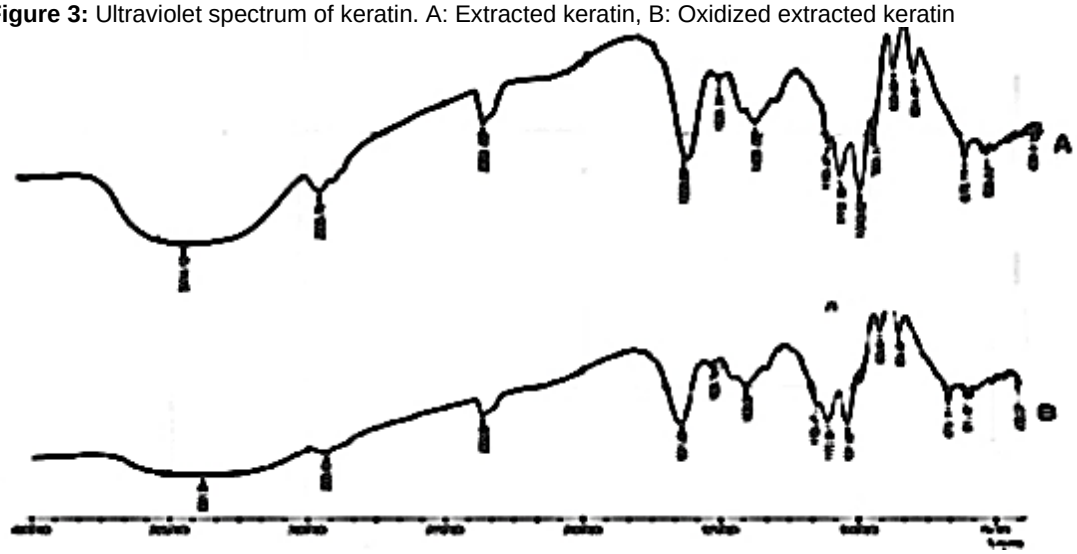

Figure 3: Fourier transform infrared spectroscopy (FT-IR) of keratin. A: Extracted keratin, B: Oxidized extracted keratin

The chicken industry has long utilized feather waste as a component in animal feed, while it has also found applications in sports and furniture production through small-scale industrial processes. However, scientists are now actively exploring innovative ways to repurpose feather waste, driving rapid growth in related research and development. Efforts to minimize feather waste production are underway, focusing on the creation of eco-friendly products that offer sustainable solutions [17].

The utilization of feather biomass is important for environmental protection and to generate valuable pharmaceutical and cosmetic products. Feathers are made up of 90 % keratin (an insoluble, fibrous and structural protein). It is one of the most abundant forms of hard protein in nature [18] and rich in cysteine, arginine, threonine and hydrophobic amino acids, with high nutrient potential [19]. Studies on feather keratin have been pursued recently to develop valuable bio-based materials [20]. This study investigated the properties of extracted and oxidized keratin (keratin oxidized after extraction) from chicken feathers. The yield of the sample was below that reported by Meko *et al* [11] at the same concentration (58 %).

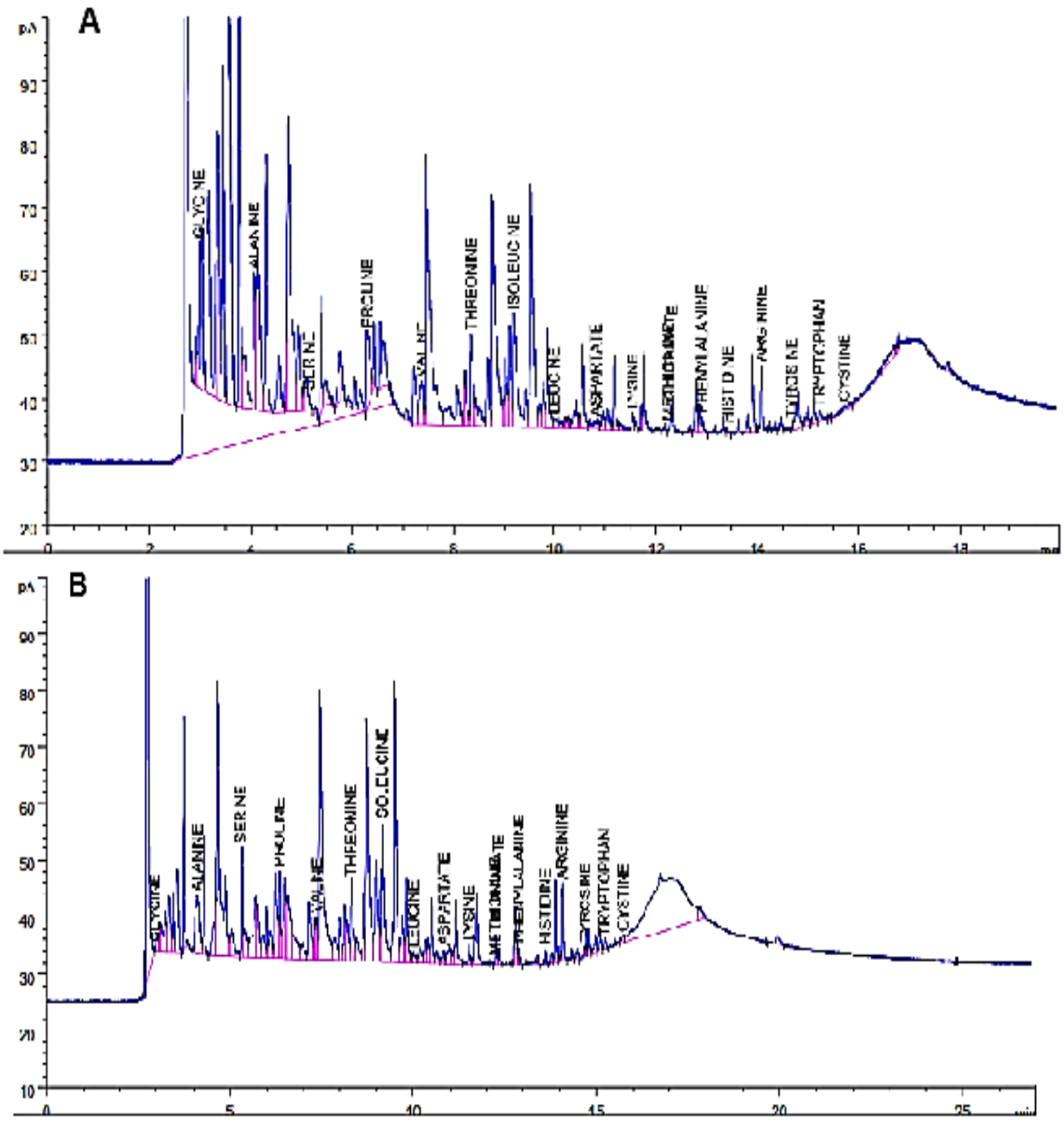

Figure 4: Protein profiles. A: Extracted keratin showed a total protein content of 80.35622 mg/100 g. B: Oxidized extracted keratin showed a total protein content of 79.64146 mg/100 g

The organoleptic properties showed that extracted keratin was dark brown in colour with a mild characteristic odour while the oxidized form was light-brown and odourless. Both samples were fine in texture. Dispersions of keratin showed a violet colouration to the biuret test suggesting that keratin is a fibrous protein. There was no carbohydrate and no translucency for lipid test. These findings agree with the results of Meko *et al* [11]. The pH showed that keratin was slightly neutral (pH between 6 – 7). Hydration capacity was lower in the oxidized sample compared to the extracted sample from the feather wastes.

Moisture sorption capacity refers to the sensitivity of keratin to absorb moisture as well as its physical stability when stored in a moist environment [11]. Result of the moisture sorption capacity was comparable to the findings of Meko *et al* [11] indicating hygroscopicity. Under certain conditions, several active functional groups present in keratin are susceptible to binding or chemical interaction with foreign agents. Majority of these functional groups are entrapped in the cross-linked or folded network mainly due to the disulphide (S-S) link/bond [21]. Chemical treatments may have unfolded the network and exposed the crosslinked or hidden functional groups. This creates more reactive sites for

interaction with water molecules. Keratin is hygroscopic (affected by moisture) and should therefore be stored away from moist environment.

Powder flow behavior affects manufacturability, and in turn, product quality such as weight and content uniformity [22]. Carr's compressibility index and Hausner's ratio are used as indirect methods of measuring powder flowability [24]. Carr's indices below or equal to 16 % indicate good compressibility, while values above or equal to 35 % indicate cohesiveness [23]. Hausner's ratio shows the inter-particle friction between particles and ratios below or equal to 1.25 indicates good flowability while those above or equal to 1.6 denotes poor flow. There was no significant difference in HR and CI for both samples. However, OEK showed significantly higher flow rate.

Keratin proteins are rich in certain amino acids like glycine, alanine, serine and valine comparable to the findings of this present study. Minor amounts of methionine, lysine, and tryptophan are also present. Cysteine and cystine (containing sulphide and disulphide bonds) are responsible for creating covalent bonds and therefore, account for the physicochemical properties of keratin [25]. Studies have revealed that chemical treatment changes the amino acid composition of various keratin. For example, during hydrolysis, amino acids such as tryptophan are destroyed; cystine, cysteine, methionine and tyrosine undergo incomplete degradation, and asparagine and glutamine are converted into aspartic and glutamic acid, respectively. Cystine disulfide bonds are cleaved during extraction resulting in the production of low molecular weight proteins and peptides as new residues [26]. Oxidized keratin is chemically derived from keratin following oxidation with H_2O_2. This reaction is responsible for the conversion of some sulphur atoms in cysteine and cystine residues in keratin to the corresponding sulfonic acid grouping (cysteic acid) [9]. This accounted for the reduction in cysteine residues following oxidation with hydrogen peroxide in this present study. It has been shown that oxidation of keratin reduces protein solubility (resulting in higher protein precipitation), induces morphological change from opaque to transparent, and promotes the formation of cysteic acid [9].

CONCLUSION

The yield of oxidized keratin obtained from extracted keratin is high. This means that the process of oxidation did not result in significant losses, and the samples were efficiently recovered after drying. Oxidation of EK from chicken feathers enhances appearance and flow, and reduces protein content with a significant reduction in cysteine residues. Therefore, future studies should investigate the possibilities of harnessing the potential of this protein polymer in drug delivery systems.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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