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Short Communication

Effect of Extraction Parameters on Some Properties of Keratin obtained From Waste Chicken Feathers

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ABSTRACT

Extraction parameters are important in determining the physicochemical properties of extracts. This work aimed to investigate the effect of extraction parameters on the physicochemical properties of keratin powder obtained from chicken feather wastes. Keratin was extracted using alkaline hydrolysis under different experimental conditions viz: concentration of sodium hydroxide, retention time and temperature. Extracted keratin powder was subjected to physicochemical, bulk powder and microbiological investigations. The powder was also subjected to high-resolution analyses such as scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). An optimal yield of 62% keratin was obtained from 3.0 M NaOH solution at 37°C and 24 hours' reaction time. Extracted keratin powder was odourless, light-brown and insoluble in neutral and acidic solutions but soluble in alkaline medium. It exhibited a moisture sorption capacity of 98% with fair to good flowability. SEM revealed a rough surface powder particle with a mean diameter of 100 µm. FTIR spectroscopy revealed the presence of amide groups while DSC showed a melting point of 110°C. Extraction conditions impacted yield, physicochemical and powder properties of keratin from chicken feathers. The powder may be useful as an excipient in colon-targeted drug delivery due to its pH-dependent solubility.

***Keywords*:** Keratin, alkaline hydrolysis, chicken feather, waste material, natural polymer.

Introduction

The use of polymers in drug delivery is critical in improving the performance of drug molecules and accommodating the demand in pharmaceutical production. The application of synthetic and semi-synthetic polymers raises various concerns such as high cost and toxicity.[1] There is therefore, a continuous search for natural polymers as excipients intended for use in drug delivery. Natural polymers sourced from animals, plants, and microorganisms as well as agricultural wastes are abundant, inexpensive and compatible with biological systems.[2] Some of these extracted natural polymers include gelatin, cellulose, starch, and alginate. Poultry farms are sources of agricultural wastes and the feathers generated from processing animals become environmental pollutants if not properly managed and they could be hazardous to health.[3] Feathers as poultry wastes are regularly thrown away and often litter the environment with little or no use in developing countries.

Studies have shown that feathers are rich in keratinous biomaterial/biomass composed of nearly 91% keratin.[4] Structurally, keratin is a fibrous protein consisting of various amino acids linked by peptide bonds.

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The utilization of keratin as an industrial raw material helps to reduce the use of harmful petrochemicals. It has been used as a source of amino acids in animal feeds, bio-composites, cosmetics, biomedicals, and drug delivery.[5] Formulations such as gels, films, beads and nano/micro-particles have been developed with keratin as a constituent.[6] Knowledge of extraction parameters, and physicochemical and powder flow properties is vital in improving the excipient potentials of keratin in dosage formulation.

Several methods have been employed for the extraction of keratin. They include; reduction, hydrolysis, oxidation, ionic liquid, microbial, enzymatic, microwave irradiation, and steam explosion methods.[7] The hydrolysis method is extremely productive and has a lower loss of amino acids.[8] Keratin is difficult to extract because of its high level of cross-linked disulfide bonds.[9] The first required step is to disrupt the intra-molecular crosslink disulfide bond to remove the embedded keratin moiety without damaging the amino acid chain.[10] The reduction of the disulfide bond of cysteine facilitates solubilization of keratin in the extraction medium and aids keratin extraction.[9,11] Some researchers have reported keratin extraction and yield from chicken feathers using the hydrolysis extraction method under various processing conditions.[12-14] The uniqueness of this study resides in refurbishing chicken feather waste and developing a need for its utilization which could be environmentally useful and economically profitable. Therefore, this work aims to investigate the effect of some extraction parameters on the yield of keratin from chicken feathers and evaluate the physicochemical, powder and microbiological properties of the extracted keratin to determine its suitability as an excipient in drug delivery.

Materials and Methods

Materials

Hydrochloric acid (Sigma-Aldrich, Germany), sodium hydroxide (Loba Chemie, India). Nutrient and Sabouraud dextrose agar (Titan

Biotech Ltd, India). Clean fresh chicken feathers were selected individually from the feather dump of the chicken processing unit of a poultry farm in Benin City (Latitude - 6.33815 and Longitude - 5.62575), Nigeria in the month of April 2021. Other chemicals used were of analytical grade and were used without further purification.

Methods

Collection and pre-treatment of chicken feathers

Collected chicken feathers were inspected to ensure no adulteration with other feathers. The feathers were subjected to no further identification since the site of collection was a chicken-only poultry. The collected and inspected mass of feathers were pre-treated by washing them with soapy water to remove blood, grease and dirt. The feathers were thereafter defatted by soaking in acetone to remove oils or lipid content and then sun-dried. Dried feather samples were then blended using a domestic electric blender (Qasa, Qlink group, China) and kept in a sealed plastic bag until further use.

Extraction by alkaline hydrolysis

Keratin was extracted from the pulverized powder using the hydrolysis method described by Alashwal[14] and Nagai and Nishikawa[15] with some modifications. Ten (10) grams of milled feather was dissolved in different molar concentrations (0.1, 1.0, 2.0, 3.0, and 6.0 M) of sodium hydroxide (NaOH) at room temperature. The content was stirred and allowed to stand for 24 hours. Then, the mixture was centrifuged (SF-400, Yongkang Hengtu Technology Co., Ltd, China) at 5000 rpm for 10 minutes. The supernatant was decanted and collected while the insoluble filtrate/undissolved feather was discarded.

Keratin neutralization and precipitation

The supernatant solution collected was placed in a 500 mL conical flask and an equimolar concentration of HCl was added dropwise to precipitate the keratin. The mixture was centrifuged at 5000 rpm for 10 minutes to separate the precipitates and the supernatant. The precipitate or insoluble residue was collected, washed with distilled water and air-dried to a constant weight. The percentage yield of the keratin extract was determined.

Effect of retention time on extraction yield

Ten grams of the feathers were also dissolved in an optimized concentration of sodium hydroxide at different solubilization/retention times of 1, 3, 6, 12, and 24 hours. The optimum time of feather solubilization was noted.

Effect of varying temperature on extraction yield

Ten (10) grams of feathers were dissolved in an optimized concentration of sodium hydroxide for an ideal solubilization time at different temperatures including refrigerator temperature (8°C), room/ambient temperature (28°C), and incubator (60°C). Keratin extraction yield was noted for each temperature of extraction.

Characterization of extracted keratin

Physicochemical characterizations

The following physicochemical characterizations were carried out on the extracted keratin.

Organoleptic properties

The texture, colour, taste and odour of keratin were tested by five different individuals and the matched impressions of not less than any three individuals (constituting a simple majority) were recorded.

Chemical identification tests

The following chemical tests were carried out on the extracted keratin using standard procedure.[16]

a. Test for proteins

(i) Biuret test: This test for keratin was performed by transferring 5.0 mg of the powder into a test tube and adding 1.0 mL of 1.0 % potassium hydroxide solution. Drops of 1.0 % copper sulphate solution were added to the mixture and shaken with each drop until colour changes in the solution were observed under an electronic microscope and recorded.

(ii) Xanthoproteic reaction: A few drops of concentrated nitric acid were added into a test tube containing a 2 mL dispersion of keratin powder. The content was allowed to cool and ammonia solution was added in few drop and the precipitate colour computed.

b. Test for carbohydrates

(i) Fehling's test: In a test tube containing 1.0 mL of a 1.0 %w/v aqueous dispersion of keratin, freshly prepared Fehling's solutions A and B were added and heated for 5 min in a water bath. The occurring change in colour was noted.

(ii) Molisch's test: In a test tube containing 1.0 mL of 1.0 %w/v aqueous dispersion of keratin, two drops of alpha-naphthol solution were added and 1.0 mL of concentrated sulphuric acid were carefully poured down the side of the test tube. The resulting colour change was noted.

c. Test for fixed oils

A 5.0 mg of keratin powder was placed on a filter paper and a drop of 10.0 %w/v acetone was added to it. The filter paper was air-dried and the drop spot observed for translucency.

Solubility profile

The solubility profile of the keratin powder was carried out with a previously described method.[17] The following solvents were used: water, 0.1 M HCl, 0.1 M NaOH and 0.1 M phosphate buffer (pH 6.4) and at different temperatures of 30, 45 and 60°C in a thermostated water bath. A 1.0 g keratin powder was introduced into 10 mL of the test liquid, the powder dispersion was stirred and filtered in a pre-weighed filter paper. The un-dissolved powder was air-dried and weighed with the filter paper (Kerro BL3002, England) and the difference in weight was used as a measure of solubility of the keratin powder.

Melting point

Keratin powder was filled into a capillary tube and inserted into the heating chamber of the melting point apparatus (Gallenkamp, England). The temperature of heating chamber was slowly raised from room temperature at 0.5°C per minute until the sample melted as observed through a magnifying lens and the melting temperature was recorded. This was done in triplicate and the average melting temperature was noted.

pH determination

A 1.0 g quantity of extracted keratin was mixed with 100 mL of phosphate buffer and centrifuged for 5.0 minutes. Thereafter, the pH of supernatant layer was noted, in triplicate using a digital pH meter (Hanna Instruments, USA).

Percentage moisture content

About 1.0 g of keratin powder was put in a petri dish and then dried in a desiccator containing activated silica gel. The powder was weighed at different time intervals over 48 hours until no further loss in weight was observed. The moisture content was then calculated as a difference between the initial and final weight of keratin after drying and expressed as a percentage.

Moisture uptake/capacity

About 1.0 g of keratin powder was weighed into a petri dish and kept in a desiccator containing distilled water (100 % relative humidity). The powder was reweighed and the new weight was recorded after 48 hours. Moisture uptake was calculated as a difference between initial and final weight and expressed as a percentage.

Physico-technical characterization of extracted keratin

Bulk properties

Extracted keratin (20 g) was transferred into a 100 mL measuring cylinder and volume occupied by the powders (bulk volume) was recorded. The measuring cylinder was then tapped 100 times on a horizontal base using a locally fabricated tapping machine and the volume occupied after tapping (tapped volume) was recorded. The

average of triplicate values was determined and bulk and tapped densities were calculated using Equations 1 and 2.

$$\text{Bulk density} = \frac{\text{mass of powder}}{\text{bulk volume}} \quad \ldots\ldots (1)$$

$$\text{Tapped density} = \frac{\text{mass of powder}}{\text{tapped volume}} \quad \ldots\ldots (2)$$

Carr's compressibility index[18] and Hausner ratio[19] for keratin powder were calculated using the Equations 3 and 4.

$$\text{Carr's index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times \frac{100}{1} \quad \ldots\ldots (3)$$

$$\text{Hausner's ratio} = \frac{\text{tapped density}}{\text{bulk density}} \quad \ldots\ldots (4)$$

Flow rate and angle of repose

The funnel method was used in these evaluations; a retort stand was place on a flat surface and a glass funnel hanging about 5.0 cm from the surface was clipped to it. Keratin powder (20 g) was poured into the funnel, the stopper was detached from the funnel's tip and time taken for keratin to entirely flow through the funnel orifice was noted. Furthermore, the heap height formed by granules and diameter of the heap base were computed.[20] This procedure was performed in triplicate and the average determination noted. Flow rate and angle of repose (θ) were calculated using Equations 5 and 6.

$$\text{Flow rate} = \frac{\text{mass of powder}}{\text{time of flow}} \quad \ldots\ldots (5)$$

$$\theta = \tan^{-1}\frac{h}{r} \quad \ldots\ldots (6)$$

Where; h = height of the conical powder heap, r = radius of the circular base.

True (Particle) density

True density of keratin was determined using a density bottle. A 25 mL density bottle was weighed as (w1). The 25 mL specific gravity bottle was filled with water, wiped of any excess water and weighed as (w2). The water was poured out, the bottle was rinsed with acetone and dried. Then 10 g of keratin was put into the bottle (w3) and then filled with water. It was weighed (w4) after wiping off any excess water from the bottle. The various weights were noted and used to determine the true density of keratin powder using Equation 7.

$$\rho = \frac{w3-w1}{(w2-w1)-(w4-w3)} \times D \quad \ldots\ldots (7)$$

Where ρ is the particle density of the powder and D is the density of water.

Powder porosity

The porosity of keratin powder was estimated by subtracting the ratio of the bulk density to the true density using Equation 8.

$$\text{Powder porosity} = 1 - \frac{\text{Bulk density}}{\text{True density}} \quad \ldots\ldots (8)$$

Microbial load characterization of keratin powder

Total viable aerobic plate count (Bacterial and fungal counting)

Total viable aerobic plate count was carried out using the pour plate method described by the International Standards.[21] A stock solution of 1.0 L, (0.1 M) phosphate buffer was made with distilled water and keratin solution was prepared by dissolving some powder with some quantity of stock solution. Thereafter, five (5)-fold dilution of keratin solution was prepared by diluting 1 ml of the keratin solution into 4.0 mL buffer serially into different test tube. A 1.0 mL of each sample (A (distilled water), B (buffer solution), and C (keratin solution)) was aseptically pipetted into a sterile 9.0 cm petri plate respectively and 20 mL of sterilized molten agar medium (that has been kept below 45°C) was added to the plate. The petri dish was carefully swirled to ensure proper mixing of the medium with inoculum. For bacterial count, nutrient agar was used while sabouraud dextrose agar was used for fungal enumeration. After agar solidification, plates were incubated in upturn position at 35-37°C (for bacterial counting) and 25 - 27°C (for fungal counting) for 1 - 3 days. Plates were daily observed for countable colonies presence.

Morphological characterization of extracted keratin powder

Sieves of different aperture sizes were arranged in decreasing order of sizes with a receiver placed at the base. The nest of sieves was clamped on a standard mechanical sieve shaker (Endicott's Ltd, UK). A 35 g amount of keratin powder was placed on the topmost sieve and a mechanical shaker was operated for 30 minutes. Thereafter, the particles obtained in the different sieves were weighed and the average diameter was calculated using Equation 9 as described by Ansel[22] and a distribution curve was plotted.

$$\text{Average diameter} = \frac{\sum(\text{weight retained}) \times (\text{mean aprture})}{\sum \text{weight retained}} \quad \ldots\ldots (9)$$

Scanning electron microscope (SEM)

The shape and surface analysis of the keratin was investigated by scanning electron microscopy. The keratin powder was mounted on a stub with adhesive carbon and coated in 20 nm carbon with a quorum Q150R ES mini sputter coater, and the pictures of the sample were then analyzed by scanning with an SEM equipment (Phenom PRO-X SEM, Netherlands) equipped with an oxford XMax 50 silicon Drift energy dispersive x-ray detector at 15KV under high vacuum.

Infrared spectroscopy

Keratin powder sample was analyzed using about 1 - 2 mg of keratin powder blended with 200 mg potassium bromide (KBr) powder and compressed into a pellet using a tablet press. The pellet was placed in the sample compartment. Spectra were recorded on a BX, Perkin-Elmer FT-IR spectrometer at an IR scan range of 4000 - 600 cm^{-1} (Waltham, Massachusetts, USA).

Differential Scanning calorimetry (DSC) characterization

A 5.0 mg keratin powder was weighed in an aluminium pan and sealed in an inside compartment. The pan was placed in the heating compartment of the DSC and heating of the sample was carried out at 10°C/minute from 30 to 500°C under nitrogen as the purge gas, at a flow rate of 70 mL/minute using the Tryte instrument, China.

Statistical analysis

Statistical analysis was performed using Microsoft Excel Office 15 and IBM SPSS Version 23. All experiments were carried out in triplicates and the mean values were expressed with their standard deviations (SD). The difference between means was determined using one-way analysis of variance (ANOVA) at a significant value of $p < 0.05$.

Results and Discussion

Yield of keratin

Percentage yield at different concentrations of sodium hydroxide

The result of the percentage yield of keratin from chicken feathers at different concentrations of sodium hydroxide is shown in Figure 1. The yield varied from 3.0 ± 0.057 to 62 ± 0.577%. There was significant difference ($p < 0.05$) in the effect of concentration of sodium hydroxide (NaOH) on the yield of keratin. The 3.0 M concentration gave the highest yield of 62 ± 0.577%, followed by the 6.0 M concentration which gave a yield of 38 ± 0.577% while 0.1 M concentration gave the least yield of 3.0 ± 0.057%.

Percentage yields showed that 6.0 M concentration did not produce the best yield because the high concentration of sodium hydroxide may have caused damage and straightened the double helix peptide bond of keratin, hence, denaturing a majority of the amino acids. Furthermore, adverse or extreme pH change induces unfolding of keratin double helix.[23] Therefore, a high extraction concentration may not always mean a high yield, but an optimum concentration is required for further processing. The 3.0 M concentration gave the highest keratin yield of 62%. Although some studies have reported keratin yield of 29.3 to 93.7% by varying the concentrations of sodium hydroxide,[13,14] they further reported that the differences in yields may also be due to different experimental conditions and processes. The processes explored in this study are viable, easy, and cost-effective.

More so, taking cognizance of the small amount of starting material, yield obtained is higher in comparison to other reported yield from chicken feathers.[13,14]

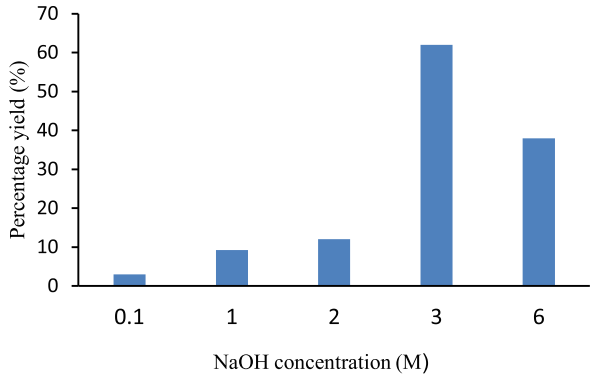

Figure 1: Percentage yield of keratin powder at different concentrations of sodium hydroxide

Effect of retention time on the extraction of keratin

Retention time refers to the time taken to solubilize feathers without causing damage to the peptide bonds in the amino acid chains. The optimum concentration of 3 M sodium hydroxide which produced the highest yield of keratin was used to determine the effect of retention or solubilization time on the quantity of feathers solubilized and the keratin yield. The effects of different retention times on the percentages of solubilized feather and keratin yield are shown in Table 1. It is represented as the percentages of keratin yield and solubilized feathers that is estimated from the un-dissolved feather at the different retention times. The result showed a high percentage of feathers solubilized at various retention times of 3, 6 and 12 hours to be 90, 93, and 96%, respectively. But these high percentages of feathers solubilized produced low yields of keratin while the retention time of 24 hours which had a high percentage of 99% solubilized feathers also had a high keratin yield. This observed result means that, firstly, a high percentage of solubilized feathers does not equate to a high keratin yield. This finding is in line with an earlier report by Panwad and Pairote[12] which revealed that up to 94% of the poultry feathers were soluble in sodium hydroxide solution at 70°C for 75 minutes and keratin yield was 34%. Secondly, the yield of keratin is directly affected by the retention or solubilization time. Hence, the quantity of feathers solubilized or dissolved is a function of retention time. Thus, an increase in retention time, does not only results in an increased percentage of dissolved feathers but also an increased percentage yield. Additionally, the variation in the solubilized feather produce at the different time which were significantly different ($p < 0.05$). Similar results have been reported by a study on the solubilization of feathers as a function of time, giving a keratin yield of 62% at 24 hours at 30°C using sodium sulphide.[24] Therefore, obtaining a maximum keratin yield, an optimum retention or solubilization time is required to aid the complete feather solubilization and breakdown of the crosslinked disulfide bonds.

Table 1: Effect of retention time on percentages of solubilized feathers and keratin yield

Time (hour)	Feather solubilized/dissolved (%)	Yield (%)
1	10 ± 1.154	5.9 ± 0.0577
3	90 ± 0.577	26.0 ± 0.000
6	93 ± 0.577	35.7 ± 0.577
12	96 ± 0.001	38.1 ± 0.0577
24	99 ± 0.577	62.0 ± 0.577

Effect of temperature

The percentage yields of keratin at different extraction temperatures are shown in Figure 2. Result of keratin yields at the various temperatures ranged from 25 ± 1.527 to 58 ± 1.000%. Extraction at room temperature (28°C) gave the highest yield followed by the refrigerator temperature (8°C) while the water bath temperature (60°C) had the least yield.

Temperature is an important factor in keratin extraction. The low yield observed at an elevated temperature in the incubator may be the result of denaturation of keratin caused by the increased thermal energy.[25,26] Also, keratin undergoes hydrogen bonding or interaction which is responsible for protein structure and function. This weak hydrogen bond is easily broken at elevated temperatures resulting in an unfolding or loss of structural integrity. Thus, a dysfunctional keratin chain becomes available for precipitation and yields less keratin.

Keratin characterization properties

Results from the evaluations carried out on the keratin powder are shown in Table 2. Organoleptic properties of extracted keratin showed that the powder was light shade creamy to brown in appearance, odourless, slightly salty to taste and powdery-fine in texture. The keratin powder dispersions showed a violet and yellow colouration to biuret and xanthoproteic test respectively confirming that keratin is a structural fibrous protein. It also gave negative or no colour change in the test for carbohydrates and no translucency for lipid test. Solubility varied in different solvents; insoluble in water (neutral) and hydrochloric acid (acidic) and soluble in sodium hydroxide, and phosphate buffer (alkaline). An increase in temperature did not affect its solubility in any of these solvents. It had a melting point of 110 to 150°C and a pH of 8.80 ± 0.000. The pH showed that keratin is alkaline in nature. The keratin powder had a moisture content of 2.0 ± 0.010% and a moisture sorption capacity of 98 ± 0.000%. Moisture content connotes the amount of water in a powder and this implied an acceptable amount of moisture. On the other hand, moisture sorption capacity expresses the sensitivity of the keratin powder to absorb moisture and its physical stability when stored in a moist condition. Keratin powder showed an excessively high moisture sorption capacity indicating that it is hygroscopic.

Also, keratin has several active functional groups which are prone to binding or chemical interaction with foreign agents under certain conditions.[27] Most of the functional groups are entrapped in the cross-linked or folded network mainly due to disulphide (–S–S–) link/bond.[8] Chemical treatments might have caused an unfolding in the network and exposed the crosslinked or hidden functional groups creating more reactive sites for interaction with water molecules. Therefore, the hygroscopic nature of keratin indicates that keratin is affected by moisture and should be stored away from moist environment.

Furthermore, powder flow behavior has been reported to affect manufacturing productivity and in turn, the quality of the product such as weight and content uniformity.[28] Thus, details on powder flow properties are necessary for efficiency in manufacturing operations such as powder mixing, compression and filling into capsules. The bulk and tapped densities of the keratin powder showed a reduction in volume, implying that the powder was consolidated or closely packed because of the particle's small diameter and low porosity. This observed result implied that the keratin powder particles diameter may be small and also closely packed with low void space, hence these properties may aid in the efficient packaging and storing of the powder.

The keratin powder exhibited Carr's index and Hausner's ratio of 27.0% and 1.30, respectively. Carr's indices below or equal to 16% indicates good compressibility, while values above or equal to 35 % indicate cohesiveness.[18] 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. The Carr's index for the extracted keratin indicated poor compressibility while the Hausner's ratio showed a fair flow. Carr's compressibility index and Hausner's ratio have been used as indirect method of measuring powder flowability[29].

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The keratin powder had a flow rate of 5.0 g/second and an angle of repose equivalent to 26.0°. Generally, powders having angle of repose below 25° have very good flow properties, whereas, angle values above 50°C correspond to unsatisfactory flow.[30] Also, the keratin powder exhibited a true density value of 1.3 g/mL, making it lighter than the test liquid (water). True density of powder excludes pores or void space in the powder.

Porosity values of the keratin powder was 0.39%, indicating a powder with little pores or void spaces between the particles. Porosity of a powder is caused by voids between particles as well as pores within the particles.[31] Porosity values tells of the extent of perforation in a powder bed, implying that the particles are separated from each other. Results obtained from this study revealed powder of low porosity. This observation could be attributable to their particle sizes which were in the range of 0.05 to 2.0 mm. In summary, the micromeritic results obtained for the keratin powder indicated a fair to good flow powder; properties that may aid in its effective flow into pharmaceutical containers, capsules or hoppers.

Microbial result of keratin powder

Table 3 shows results from the microbial evaluation of the keratin powder in different culture media. The absence of colonies in sample A indicates that distilled water is a sterile solution. There was microbial growth in sample B containing nutrient agar which is a bacterial medium and none in SDA medium, while keratin solution had no noticeable presence of bacteria or fungi. Therefore, only the buffer solution had a slight quantity of bacterial contamination without the presence of fungi. The acceptance criteria for microbiological quality of nonsterile products for pharmaceutical use is 10^3 CFU/mL.[21] The observed 8 x 10^{-5} CFU/mL was in line with acceptable criteria. Therefore, the buffer solution might have been slightly contaminated as a result of poor handling of the buffer salt during weighing.

Morphological parameters of keratin powder

Particle size

The result of particle size distribution of keratin extracted from chicken feathers is shown in Figure 3. From the sieve analysis method, the sieve size ranges from 1000 - 100 mm. The particle size distribution chart revealed that keratin powder particles with size 0.5 ± 0.000 mm were the most prevalent in the powder bed. The average particle size of the keratin powder is 0.5 ± 0.000 mm and falls in a range of a moderate fine sand particle. The poly distribution of keratin showed and asymmetrical curve or negative skew distribution.

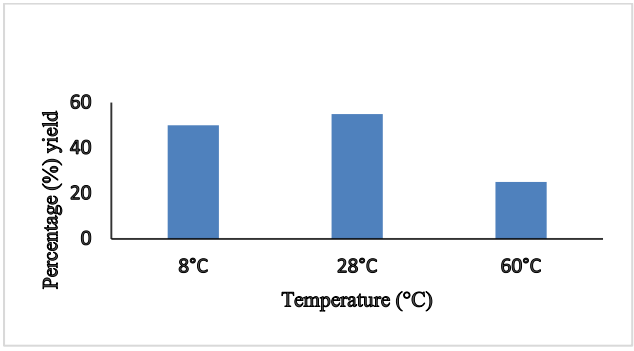

Figure 2: Percentage yield of keratin at different extraction temperatures

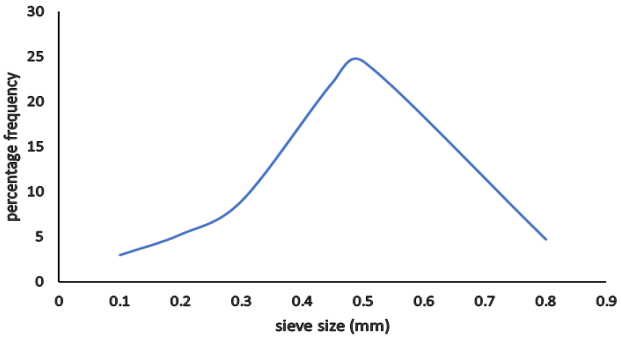

Figure 3: Particle size distribution profile of keratin powder

SEM

Results from electron scanning microscopy are shown in Figure 4. The microgram revealed keratin powder as discrete particles, topography having an unsmooth surface and irregular shapes ranging from oval to cuboids. The SEM also revealed keratin powder particle average size in diameter as 100 µm.

High resolution properties

FTIR

Spectrum from the FTIR analysis of the keratin powder is shown in Figure 5. The spectrum of the extracted keratin revealed the presence of amides functional groups. Peaks around 1625.1 cm^{-1} were assigned to N-H stretching vibration of 1^o amine compound of keratin. Wavelengths around 2102.93 cm^{-1} correspond to COO stretching vibration of carboxylic acid compound. Broad bands around 3060.1 and 3261.4 cm^{-1} were assigned to OH stretching vibration of 1^o and 2^o alcoholic compounds respectively. Though broad OH-stretching band are associated with alcohols, this observation in the keratin spectrum may be due to the water content of the keratin powder resulting from insufficient drying.

Keratin contains functional groups such as amino (-NH_2), carboxylic acid (-COOH), hydroxyl (–OH) and sulfhydryl (SH)[32]. The wavenumber bands from the FTIR verified the presence of these functional groups which are predominant in proteins thus the sample is keratin protein.

DSC

The DSC thermogram of keratin shown in Figure 6 revealed an endothermic trough from 30°C, a sharp endothermic trough at 110°C and a third occurring at 350°. Thermogram of keratin showed an endothermic trough which means that keratin absorbed heat during phase transition from 30 to 90°C indicating loss of water/moisture (that is water of hydration). Transition remained constant until a change in keratin nature from solid to liquid that is sol-gel transition revealing a sharp endothermic peak at 110°C this indicates a melting point of keratin. A transition occurred until an exothermic peak around 350°C which may be an indication of keratin denaturation or degradation.

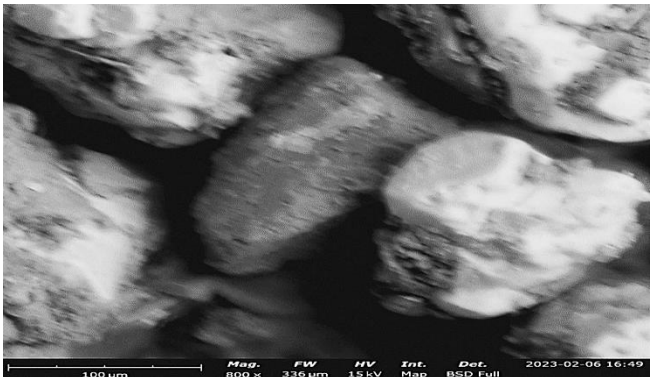

Figure 4: Scanning electron micrograph of keratin powder

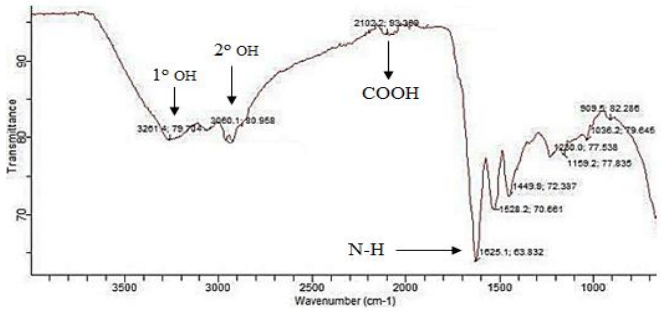

Figure 5: FTIR spectrum of the extracted keratin powder

Table 2: Some characterization properties of the keratin powder studied

Property	Tests	Characteristic
Organoleptic	Appearance	Cream to light brown
	Taste	Slightly salty
	Odour	Odourless
	Texture	Fine and powdery
Chemical/ Physicochemical	Test for proteins	Positive
	Test for carbohydrate	Negative
	Test for lipids	Negative
	Melting point (°C)	110 – 150
	pH	8.80 ± 0.000
	Moisture content (%)	2.0 ± 0.010
	Moisture reuptake (%)	98 ± 0.000
Solubility profile	Water	Insoluble
	0.1 M HCl	Insoluble
	0.1 M NaOH	Soluble
	0.1 M phosphate buffer	Soluble
Powder Parameters	Bulk density (g/mL)	0.80 ± 0.011
	Tapped density (g/mL)	1.10 ± 0.010
	Carr's index (%)	27.0 ± 0.057
	Hausner's ratio	1.30 ± 0.005
	Flow rate (g/sec)	5.00 ± 0.000
	Angle of repose (^{o})	26.0 ± 0.011
	True density (g/mL)	1.30 ± 0.005
	Powder porosity (%)	0.39 ± 0.010

Table 3: Total viable aerobic count from culture media

Test sample + Media	CFU/mL
Sample A	
Distilled water in nutrient agar	-
Distilled water in Sabouraud's dextrose agar (SDA)	-
Sample B	
Buffer solution in nutrient agar	8×10^{-5}
Buffer solution in Sabouraud's dextrose agar (SDA)	-
Sample C	
keratin solution in nutrient agar	9×10^{-5}
Keratin solution in Sabouraud's dextrose agar (SDA)	-

(-) means no colony.

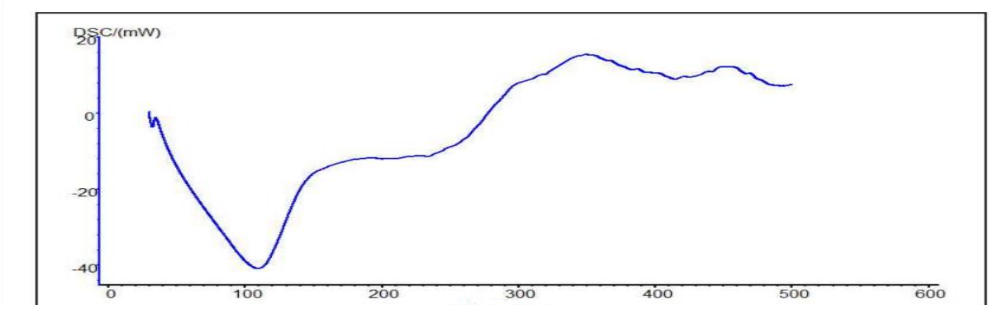

Figure 6: DSC thermogram of keratin powder

Conclusion

The method and processes used in the extraction of keratin in this study was easy, cheap and environmentally friendly. A simple modulation of the extraction parameters resulted in a high yield of keratin from chicken feathers. The highest yield of keratin was extracted with 3.0 M concentration at a maximum retention time of 24 hours and a room temperature of 28°C. The extracted keratin was moisture-sensitive with a high moisture sorption capacity and thus hygroscopic. The keratin powder was naturally alkaline and solubilized in an alkaline medium hence does not break down or solubilize in an acid or water medium. The powder had a fair to good flowability but a poor compressibility. The powder had little or no microbial content as it did not support growth of bacteria or fungi. The powder may find its usefulness in the pharmaceutical industry as an excipient in colon-targeted drug delivery due to its pH-dependent solubility.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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