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ORIGINAL PAPER

An efficient conversion of waste feather keratin into ecofriendly bioplastic film

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Abstract

Feathers biomass from poultry industry is considered as an important waste product, which creates serious environmental problems. In this study, keratin was extracted from waste chicken feathers using sodium sulfide as a reducing agent under optimized conditions. The extracted keratin particles were used to develop a bioploymeric film by adding microcrystalline cellulose as nano-additive agent. The calculated yield of 80.2% was obtained for keratin from feathers dry weight 25 g (w/w). The extracted keratin was characterized using Fourier transform infrared spectroscopy, scanning electron microscopy (SEM), thermogravimetric analysis, differential scanning calorimetry, wide-angle X-ray diffraction. The physiochemical characteristics of the feathers were compared with the keratin powder. The regenerated keratin particles preserved their chemical composition, thermal strength and stability after chemical extraction. The extracted keratin particles showed 10–20-µm spongy porous microparticles in SEM analysis. The keratin powder was used to synthesize a bioplastic film using glycerol (3.5%) and microcrystalline cellulose (0.2%) in NaOH for 48 h at 60 °C. The calculated thickness of bioplastic film was 1.12×10^{-4} mm with tensile strength of 3.62 ± 0.6 MPa. The Young's modulus and break elongation for synthesized bioplastic film were 1.52 ± 0.34 MPa and 15.8 ± 2.2%, respectively. The feather and keratin showed maximum similarity index of 64.74% (L-alanyl, L-alanyl, L-alanine, *p*-nitroanilide) and 64.32% with D-pantethine, respectively, using OMNIC Specta software. Overall, the study presented a highly efficient method to convert the waste feather biomass into a bioplastic film which can be used in biopolymer, biomedical and pharmaceutical industries.

Keywords Poultry waste · Feather · Keratin · Reducing agent · Characterization · Film synthesis

Introduction

The poultry industry is providing large amounts of protein supplements in the human diet, concomitantly with the production of huge waste biomass. The major fraction of waste biomass generated is in the form of feathers, blood, bone residues, fats, soft matter of meat. These resources are currently converted into meat and bone meal, feather meal, blood meal and fats/oils by rendering process (Lasekan et al. 2013). Feathers are one major waste constituent from the chicken industry. Approximately, five million tons of feathers are produced per year in the world (Poole et al. 2008; Tesfaye et al. 2017), but most of them are dumped to landfills or a smaller amount is used in low-value animal feedstock (Bertsch and Coello 2005; Reddy and Yang 2007). As well, large dumping area is required and they produce higher portion of heavy metals, chemicals and pathogens which have detrimental effects on groundwater and environment (Cavello et al. 2012; Sharma and Gupta 2016). Conversely, exploitation of this waste fraction as feed, fertilizer is reported in recent studies but some challenges of high energy consumption during disposal or conversion remain unsolved (Brandelli et al. 2015). Thus, utilization of feathers biomass becomes more essential to protect the environment and to generate the valuable products. It is necessary to

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utilize feathers in environmentally more sustainable manner for the synthesis of products of commercial use.

Feathers are composed of 90% keratin which is an insoluble, fibrous and structural protein. It is among the most abundant form of hard protein present in the nature (Onifade et al. 1998; Reddy and Yang 2007) and rich in cysteine, arginine, threonine and hydrophobic amino acids, with high nutrient potential (Tiwary and Gupta 2010). Feather keratin has also been studied to develop various valuable bio-based materials (Barone 2009; Barone and Schmidt 2005; Poole and Church 2015; Reddy and Yang 2007; Yin et al. 2013). It contains β-sheet crystallites and is highly cross-linked by cysteine 7 mol% (ARAI et al. 1983). One of the interesting uses of feather keratin is in eco-composites and bioplastics (Pillai 2010). The development of efficient techniques to extract the keratin with minimal deformation of secondary structure of protein remains challenging issue. Various chemical methods like reduction or oxidation (Moritz and Latshaw 2001; Schrooyen et al. 2001b; Yin et al. 2013), enzymatic (Eslahi et al. 2013; Mokrejs et al. 2010; Onifade et al. 1998) and reactions in ionic liquids were reported to dissolve the hard keratin (Ji et al. 2014; Wang and Cao 2012). In the previous study, (Schrooyen et al. 2000, 2001a, b) 2-mercaptoethanol was used in combination with urea and EDTA, to achieve a yield up to 75% in short time period. Urea interrupts the hydrogen bonding and swells the protein chains and gives increased reaction rate. Sodium dodecyl sulfate (SDS) was added to the extracted keratin to prevent the re-cross-linking which makes keratin–SDS complexes having almost similar size to the keratin primary chain (Schrooyen et al. 2001a). Some researchers added SDS during dissolving feather keratin (Martelli et al. 2006a, b; Yin et al. 2013). Although, 2-mercaptoethanol is good for the extraction undamaged keratin, but due to its high cost its commercial use is not viable. The use of Na_2S for the extraction of keratin with good yield is more economical and exploited commercially (Jones and Mecham 1943). Conversely, Na_2S generates strong reducing conditions which have ability to destroy the protein backbone (Happey and Wormell 1949). The feather hydrolysis using Na_2S method could show the industrial utility due to low cost and simple hydrolysis with enough yield can be obtained without damage to the protein primary chain.

There are several effective methods for the conversion of different biomass like human hair, wool and production of value-aided biomaterial for application in different sectors like tissue engineering (Lee et al. 2015). In some previous studies, keratin from different sources was used to manipulate the properties of different products such as fabrication of chitosan membrane to increase the wettability and tensile strength for biomedical applications (Ma et al. 2017). Similarly, hydrolyzed keratin was used to modify the soy protein film with improved physicochemical properties (Garrido et al. 2018). Furthermore, in a recent study, blend modification of feather keratin-based films using sodium alginate was investigated which expand the application in biomedical industries (He et al. 2017).

This study presented the extraction of keratin from chicken feather waste biomass using alkaline hydrolysis and optimization of extraction conditions. The extracted keratin has been characterized to study its physical and chemical properties. Finally, the keratin particles were used to develop a bioplastic film using microcrystalline cellulose as a nano-additive. The properties of the regenerated film were evaluated to validate the industrial potential of keratin powder.

Material and method

Materials

White colored **c**hicken feathers were collected from a chicken processing plant at Jaya Gading (local market), Kuantan, Malaysia. Sodium sulfide, HCl, NaOH, petroleum ether and cetrimonium bromide (CTAB were purchased from Sigma Aldrich (Kuala Lumpur, Malaysia). Milli-Q water was used to make solutions and washing. Glycerol and microcrystalline cellulose (MCC) were procured from Sigma Aldrich St. Loius MO USA. All chemicals were of analytical grade and used as received.

Pre-treatment of feathers

Wet feathers were cleaned and dried in a ventilated oven at 40 °C for 72 h as described previously ASTM (1997). The feathers were degreased using petroleum ether for overnight, and then, washed with distilled water. The washed feathers were conditioned at 20 °C and relative humidity (RH) 65% for 24 h. After first cleaning, the feathers were treated with CTAB (1 g/L) for 3 h to remove microorganism. Cleaned defatted feather were then chopped into small pieces (2–25 cm) and dried under sunlight for 48 h and stored at 4 °C for further usage.

Keratin extraction

A total 25 g of the chopped feathers were immersed into different concentrations (100, 300, 500 mM) of sodium sulfide (1 L) separately (Kamarudin et al. 2017; Sharma et al. 2016) and then digested at different temperatures (30–65 °C) using mechanical stirrer for 1–6 h. The optimized concentration and temperature for hydrolysis was used for further extraction. The prepared hydrolysate was filtered twice and centrifuged at 10,000 rpm to separate the supernatant. The pH of the solution was adjusted to 3.5 and standardized as reported in previous study (Sharma et al. 2016; 2017a, b).

The keratin sediment was collected, washed, freeze-dried to obtain microporous keratin particles. The total yield of extracted keratin powder was 80.2%.

Protein extraction yield

The extraction yield was calculated using total dry weight of the extracted keratin and initial weight of the processed feather as given in Eq. (1).

$$\text{Extraction yield}\,(\%) = W'/W \tag{1}$$

where W' is the weight of the freeze-dried sample and W is the initial weight of the feather.

The weight difference was calculated. The concentration of the extracted protein was determined with the Bradford protein assay method, using bovine serum albumin (BSA) as a standard. All the experiments were done in triplicates.

Measurements

Surface morphology

The surface morphology of feathers and freeze-dried keratin obtained was analyzed by Ziess EVO 50 scanning electron microscope (SEM). In the preparation step for both, the samples were adhered directly onto an aluminum stub with a thin conductive tape. EDX in the SEM was used to do the elemental analysis of keratin extracted.

Attenuated total reflection–Fourier transform infrared (ATR–FTIR) spectroscopy

Chemical characterization of feathers and keratin extracted were done by using Fourier transform infrared (FTIR) spectroscopy with a Thermo scientific Nicolet iS50 with attenuated total reflection (ATR). It will help to detect the changes in chemical composition of peptides (Mohanty et al. 2005). FTIR was used for chemical characterization in-between the 4000 and 500 cm^{-1} wave number range with transmission mode. The analysis was performed in triplicate. OMNIC Specta software in FTIR was used to verify the composition of the sample by automatically comparing the collected spectrum with the spectra in quality control library. The software compares the sample spectra with the reference spectra in the appropriate category in the library and then tells how much and to which the sample matches in the library spectrum. The software searches the library and then displays a list of the library spectra that best-matched the sample.

Thermal behavior

Thermal behavior measurements of feathers and keratin extracted were investigated by using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). A DSC Q1000 v9.9 Build 303 thermal analyzer was used to determine the melting temperature and to obtain the thermograms. The samples were prepared using standard pan, and measurement was conducted by heating the samples from 35 to 300 °C for feather and keratin extracted with heating rate of 10 °C/min under a nitrogen atmosphere. TGA was performed to determine degradation temperature using TGA Q 500 thermogravimetric analyzer under nitrogen atmosphere, in a temperature range between 10 and 900 °C at ramping time of 10 °C/min. The samples were vacuum-dried at 40 °C. Samples with mass 3 mg were put in aluminum crucible, and thus, the data were analyzed. The change in weight differential difference with temperature was observed.

Wide-angle X-ray diffraction (WAXD)

The difference in different crystal structures of feathers and keratin extracted was compared by Rigaku (brand), MiniFlex II (model) X-ray diffractometer. The patterns were obtained with Cu Kα radiation source. The 2α Bragg angles were scanned for a range of 5–80° using 0.02° step size and 1.0 s per step scan speed.

Preparation of keratin bioplastic film

The keratin powder was used to synthesize a bioplastic film using glycerol (3.5%) and microcrystalline cellulose (0.2%) in NaOH for 48 h at 60 °C. To prepare the bioplastic film, 250 mg of dried keratin was dissolved in 5 ml of NaOH (2 N) under 300 rpm vigorous agitation at 45 °C. The mixture was poured on petriplate having 10 cm of diameter greased with greasing agent and dried in oven at 60 °C for 48 h. The film was separated from petriplates and stored for the further analysis. The thickness of the bioplastic film can be varied with amount of keratin solution used. Film thickness was measured with the micrometer. Keratin bioplastic film formed cut into strips (8.5 cm × 2 cm) was conditioned under standard testing environment of 21 °C and 65% relative humidity for 48 h using humidity chamber (Memmert HCP108) before testing the tensile strength. Tensile strength of keratin bioplastic film was performed on XQ-1 fiber tensile tester with crosshead speed of 10 mm/min with gauge length of 50 mm at room temperature according to ASTM Standard D882. Three samples were analyzed, and the average with standard deviations (± SD) was recorded.

Results and discussion

Extraction of keratin from chicken feathers

Feather keratin is insoluble in water, weak acids, weak bases and most of the non-polar solvents. The solubilization of keratin can be initiated by hydrolytic activation, oxidation or reduction of cysteine in its peptide chain (Endo et al. 2008). The hydrolysis of peptide bond in cysteine residue decreases the strength of the peptide chain in keratin particles and forms new hydrogen bonds (Schrooyen et al. 2000). A simple and economical method was reported to break disulfide and hydrogen bonds in alkaline conditions with minor modification as described by (Poole et al. 2008). Generally, physical conditions like temperature, pH value, time and amount and concentration of reducing agent had a considerable influence on the dry weight and final yield of extracted keratin. Thus, the effect of these factors on the keratin productions from chicken feathers was studied.

Factors affecting solubilization of keratin

In this study, Na_2S under highly alkaline conditions (pH 13) was used for the solubilization extraction. The chopped feathers were dissolved in Na_2S at different concentrations 100, 300 and 500 mM. The feathers fractions showed maximum solubility with 500 mM Na_2S after 6 h and showed no change after 6 h. At lower concentration 100 and 300 mM, the feathers biomass was partially dissolved with rachis and most of the undissolved feathers portion left in the beaker. The solubility of feathers was attributed to breakage of disulfide bonds hydroxyl ions in basic medium (Dahl and McKinley-McKee 1981). The dissolved samples of feathers were precipitated using NaOH. The effect of different amount of NaOH used on the precipitation of keratin is shown in Table 1.

The incubation of feathers biomass for shorter time period causes incomplete digestion and poor yield of product. Incubation of feathers in reducing agents for longer time periods causes unspecific cleavage of peptide bonds. Thus, an adequate incubation of feathers with reducing agents was necessary to confer the better yield (Fang et al. 2010). In this study, the maximum yield of 80.2% was obtained in 6 h, and with further incubation it remains unchanged (Fig. 1a). The protein concentration of the treated sample was calculated using Bovine serum albumin as standard. The total protein 1.6 mg/ml was present in the tested sample. Similarly, the dry weight of extracted keratin was determined after each hour and yield was found to be increased concomitantly with time (Fig. 1b).

The effect of temperature on the extraction of keratin from 30 to 65 °C was studied under optimized conditions of incubation time and pH (Fig. 1c). The maximum yield of keratin was obtained at 50 °C, while further increase in the reaction temperature resulted in a decrease in the total yield (Ji et al. 2014; Zhang et al. 2015). The effect of pH in the range 3–13 was studied, and maximum yield was observed at pH 13 (Fig. 1d). Thus, maximum keratin yield was obtained with 500 mM in 6 h at 50 °C.

The effect of different precipitants including HCl, ethanol, and acetone was studied to precipitate the proteins. Among these, the 2 N HCl was found to precipitate the protein quickly from dissolved solution. The excess of sulfur was removed from the precipitated solution using dialysis through 0.2-µm membrane tube. The dialyzed sample was filtered by Whatmann filter paper, and the microparticles were collected. In a previous study, 2-mercaptoethanol and urea were used to dissolve the chicken feathers at pH 13 (Yin et al. 2013). In previous studies, urea and SDS were found to show the maximum keratin yield (Yamauchi et al. 1996). The present yield (80.2%) obtained was higher than previously reported study using Na_2S which was 62% after 24 h of incubation (Poole et al. 2011). In contrast to this, 93% of keratin yield was reported by Yin et al. (2013) using Na_2S. Also in another study, 50 and 30% yield from wool biomass and 3 and 10% keratin yield were recorded from horn–hoof, respectively, using Na_2S (Zoccola et al. 2009). Steam flow explosion method of extraction of feather gave 42% of protein yield (Zhang et al. 2015). The keratin powder with yield of 60% was obtained and used to make pure keratin membrane (Ma et al. 2016).

Table 1 Effect of NaOH quantity on the dissolution of the keratin extracted

S. no.	Keratin powder (mg)	NaOH (2 N) added (ml)	Consistency
1.	500	5	Very thick
2.	500	10	Thick
3.	500	15	Diluted
4.	500	20	Very diluted

Characterization of extracted keratin from feather and film formed

Surface morphology

The surface morphology of washed feathers and extracted keratin particles was explored by SEM analysis. The SEM images for the feather and extracted keratin are shown in Fig. 2a, b. SEM images revealed that the keratin powder (Fig. 2c, d) consists of spongy microporous particles

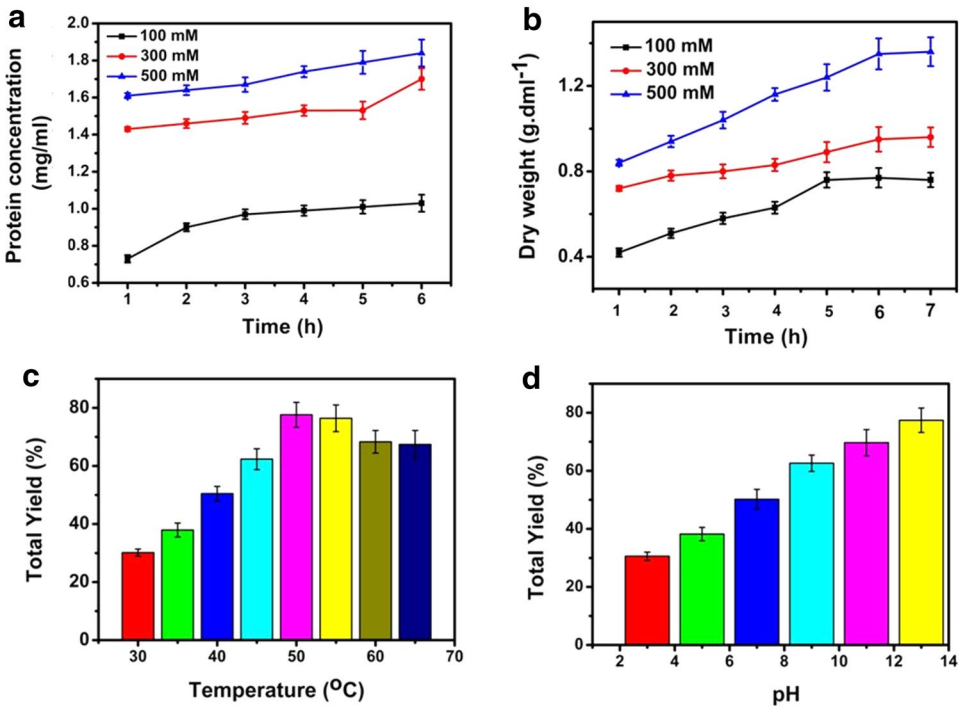

Fig. 1 **a** Effect of incubation time on the protein concentration dissolved in 100, 300, 500 mM of sodium sulfide. **b** Comparison of dry weight of the protein obtained after each 1 h in different concentrations of sodium sulfide 100, 300, 500 mM (v/v), **c** effect of extraction temperature, **d** effect of pH on extraction. *All the experiments were carried out in triplicates ($n = 3$) for each sample, and mean average values with standard error (± SD) were calculated and represented in the error bars

which are randomly arranged porous microstructures. This showed a microsphere with diameter of 1–2 µm. The similar morphology was observed in previous reports for feather and wool keratin particles (Rad et al. 2012; Yin et al. 2013; Zhang et al. 2013). The extracted dried keratin has large number of applications in water treatment, feed ingredients and surface modifications (Bertsch and Coello 2005; Guo et al. 2014; Touaibia and Benayada 2005).

The elemental composition of feather and keratin was studied with EDX spectra Fig. 3a, b. Both feather and keratin composed of O, Na, Al, S, Ca and Cu in addition to carbon. Chlorine fraction was observed in spectra of keratin particles which attributed to the precipitation with HCl. The weight proportion (% w/w) associated with S was 36.2 and 15.43 and that with Na was 0.582 and 6.57 in the feathers and extracted keratin, respectively.

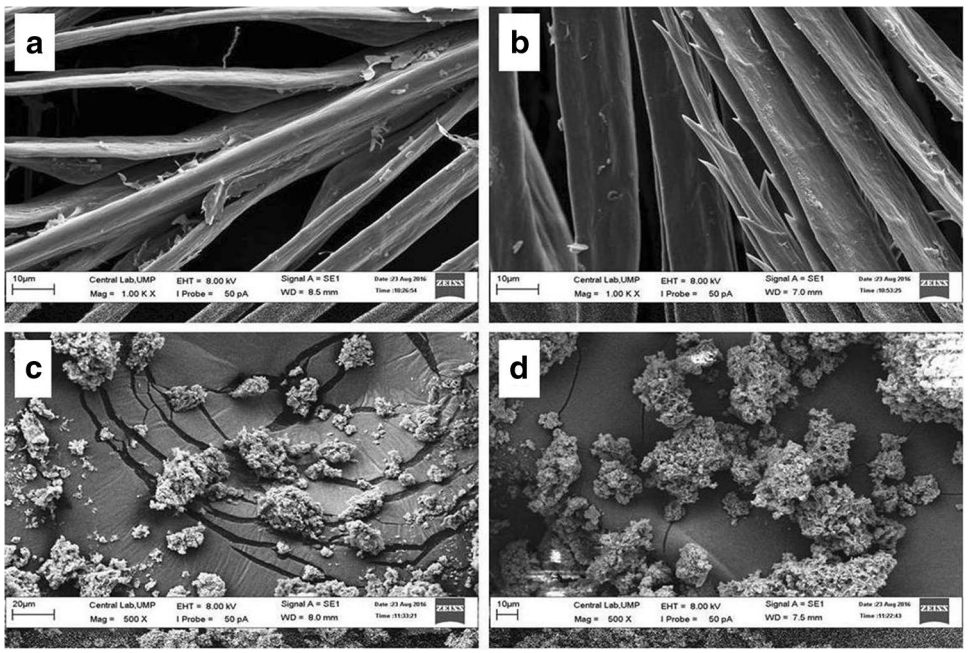

Fig. 2 SEM images of the **a**, **b** feather and **c**, **d** extracted keratin

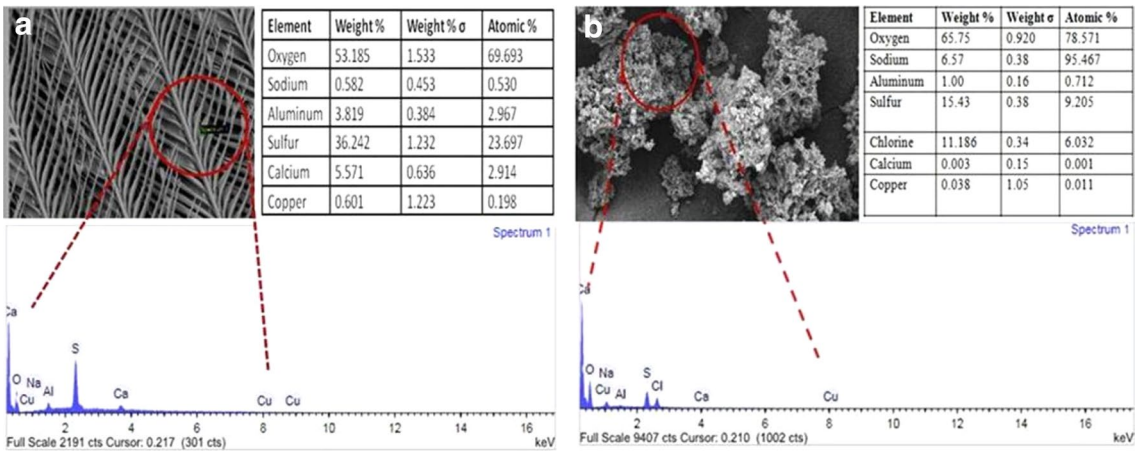

Fig. 3 Elemental analysis of feather and keratin particle using EDX spectra

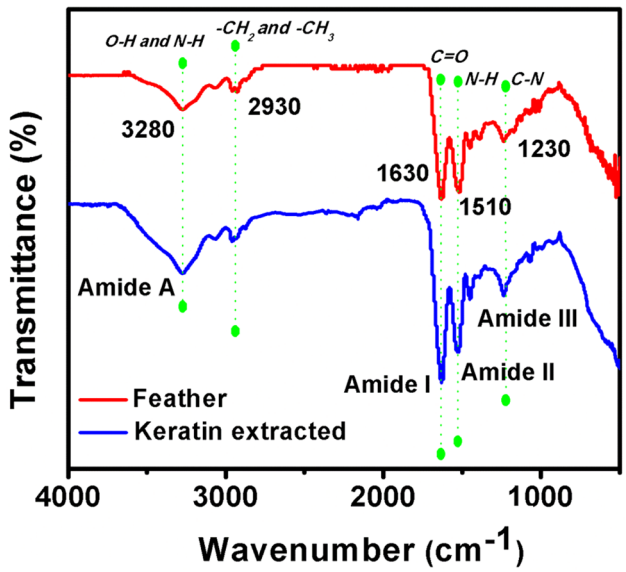

Fig. 4 FTIR spectra of feather and extracted keratin

ATR–FTIR analysis

The conformational changes in the polypeptide chains of keratins were analyzed from ATR-FTIR spectra (Fig. 4). In the ATR-FTIR spectra, an absorption band was scrutinized in the range of 3275–3295 cm^{-1}. The transmission bands appeared in the range 3000–2800 cm^{-1} were related to symmetrical CH_3 stretching vibration (Edwards et al. 1998; Eslahi et al. 2013). The strong transmission band was attributed to C=O stretching (amide I) which occurred in the range of 1700–1600 cm^{-1} (Aluigi et al. 2007; Mohanty et al. 2005). The transmission band for amide II appeared in the range of 1580–1540 cm^{-1} was attributed to N–H bending and C-H stretching (Eslahi et al. 2013). The weak band between 1300 and 1220 cm^{-1} indicated the amide III band which is derived from C–N stretching and N–H bending (Vasconcelos et al. 2008; Wojciechowska et al. 1999) and signals from C=O bending and C–C stretching vibration (Idris et al. 2013; Zhang et al. 2013). The peak at 990 and 580 cm^{-1} was associated with C–S and S–S bonds (Vasconcelos et al. 2008). Amide I–III bands give critical information on the protein confirmation and alteration in backbone structure of proteins (Ma et al. 2016). The transmission band in-between 750 and 600 cm^{-1} is related to N–H out-of-plane bending (Pavia et al. 2008).

The positions of the bands indicated the protein structure as reported in previous studies (Ha et al. 2005), and amide I is the combination of α-helix and β-sheet (Martinez-Hernandez et al. 2005; Senoz and Wool 2010), and amide III can be attributed to β-sheet structure (Fu et al. 1999). As shown in the figure, the amide I region can be deconvoluted to provide more information about the structure presents (Fig. 5a, b). The FTIR spectrum obtained was deconvoluted using OMNIC Specta software into three Gaussian peaks. The three maxima positions were observed at 1636, 1647, 1659 cm^{-1}, for feathers, while for extracted keratin three peak maxima were observed at 1632, 1645, 1664 cm^{-1}. Therefore, it can be concluded that the feather and extracted keratin possess more β-sheet structures as compared to α-helices. Table 2 indicated that the contents of α-helix were decreased after dissolution and regeneration process, and it is in agreement with previous study (Idris et al. 2014).

The similarity index between the single-component search result and the spectrum of feather and keratin is striking, even though four components have been found with similarity from 64 to 55% in each case (Table 3). In single-component search result using the OMNIC Specta software, the algorithm has identified four components which showed the correlation between four components of actual and synthetic spectra (Fig. 5c, d). This similarity between the single-component search results showed

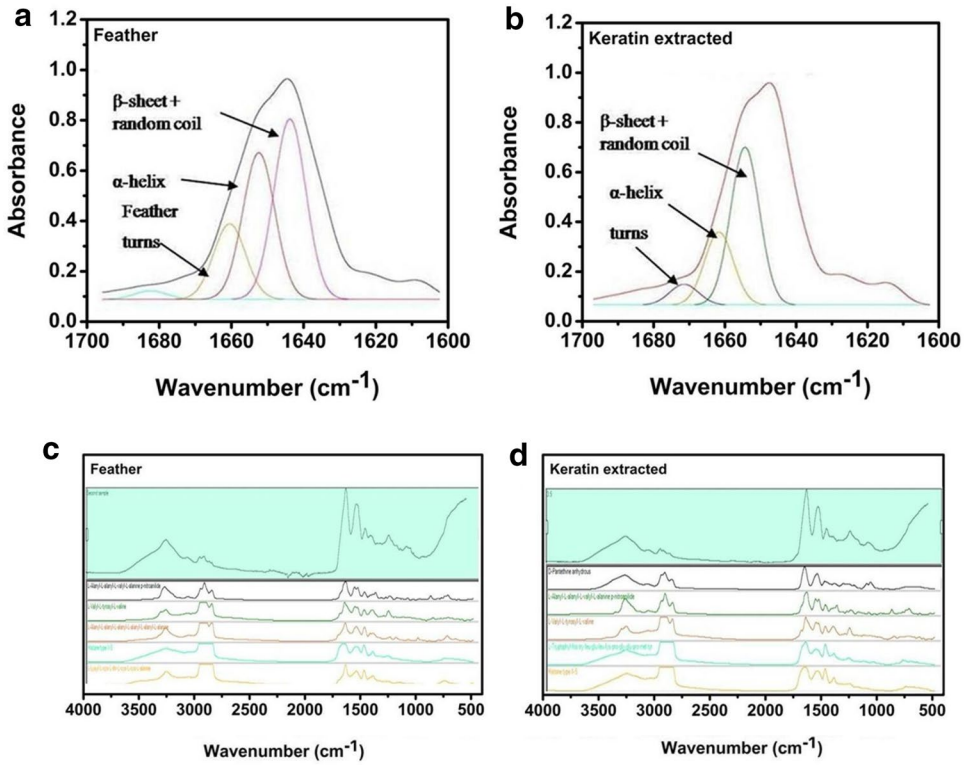

Fig. 5 **a**, **b** Deconvolution spectra of amide I region in feather and extracted keratin which were fitted using Gaussian fitting function; **c**, **d** Showing the results of similarity between the single-component spectra of library with sample of feather and keratin extracted

Table 2 Percentage fraction of β-sheet + random coil and α-helix of feather and extracted keratin, which were presumed after Fourier self-deconvolution

S. no	Material	Wave number cm^{-1}	Assignment	% Fraction
1.	Feather	1636	β-sheet + random coil	61
		1647	α-helix	46
		1659	Turns	18
2.	Keratin extracted	1632	β-sheet + random coil	58
		1645	α-helix	36
		1664	Turns	25

Table 3 Composition similarity with the OMNIC Specta software

S. no.	Feather		Keratin	
	Match	Name	Match	Name
1.	64.74	L-Alanyl-L-alanyl-L-alanine *p*-nitroanilide	64.32	D-Pantethine anhydrous
2.	56.30	L-Valyl-L-tyrosyl-L-valine	64.26	L-Alanyl-L-alanyl-L-alanine *p*-nitroanilide
3.	55.51	L-Alanyl-L-alanyl-L-alanyl-L-alanyl-L-alanyl-L-alanine	57.00	L-Valyl-L-tyrosyl-L-valine
4.	55.44	Histone type II-S	56.47	L-Tryptophyl-his-try-leu-lys-pro-gly-glu-pro-met-tyr

that the four components were present in fitting percentages (Table 3). The feather and keratin extracted showed maximum similarity index of 64.74% (L-alanyl, L-alanyl, L-alanine, *p*-nitroanilide) and 64.32% with D-pantethine, respectively. The algorithm has identified four components, and they showed the correlation between the actual spectrum and the synthetic spectrum consisting of the different components in fitting percentages.

Thermal behavior

The phase behavior using DSC thermogram of feather and extracted keratin was studied and depicted in Fig. 6a. The endothermic melting peak for feather and extracted keratin was observed at 60 and 80 °C, respectively, with the release of bound water molecules (Zhang et al. 2015).

Thermal degradation of feather and extracted keratin was studied using TGA (Fig. 6b). The stability of keratin during extraction was well maintained, and all the samples showed a more or less similar pattern. The thermogravimetric analysis showed two decomposition stages for feathers and extracted keratin. A starting degradation temperature of 100 °C was attributed to loss of bound water. The weight loss in the temperature range from 240 to 400 °C is mainly due to breakage of disulfide bond and β-sheet conformation (Idris et al. 2014; Ullah et al. 2011) and skeletal degradation (Martinez-Hernandez et al. 2005). Also, the volatile compounds including SO_2 and H_2S were released due to the cleavage of the disulfide bonds that occurred between 230 and 250 °C (Menefee and Yee 1965). The keratin decomposed to other lighter products and volatile compounds such as HCN, CO_2, H_2O and H_2S (Popescu and Augustin 1999). The results are similar to the previously reported results (Zhang et al. 2015). The total 75% weight loss was observed after heating to 900 °C.

Wide-angle X-ray diffraction

The analysis done was by wide-angle X-ray diffraction (WAXD) to determine the crystal phase of the samples. Figure 7 shows the XRD spectra of feather and extracted keratin. The results of XRD specified that the feather and the extracted keratin mainly existed in semicrystalline form, and even after hydrolysis it retained the crystallinity. There are three types of crystal diffraction peaks: the meridional reflections of 0.51 nm (2θ between 15° and 31°) for α-helix structure, the equatorial reflections of 0.465 nm (2θ between 16° and 31°) for β-sheet structure and the equatorial

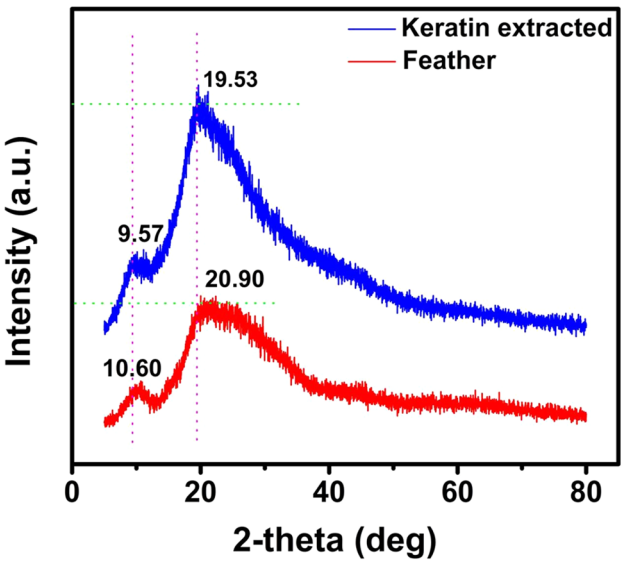

Fig. 7 XRD pattern of feather and extracted keratin

reflections of 0.98 nm ($2\theta = 9°$) for α-helix and β-sheet structure (Feughelman et al. 2002; Xu et al. 2006). The peaks at 8°–9° indicated the diffraction patterns of α-helix configuration (Khosa and Ullah 2014; Poole and Church 2015). The changes occurred in the molecular structure are shown in Fig. 7. The two strong peaks at $2\theta = 9°–10°$ and at 15°–31° were allocated to α-helix and β-sheet, respectively (Cao 2000; Nishikawa et al. 1998). Both feather and extracted keratin show diffraction characteristics of α-helix appearing at $2\theta = 9.7°$ and of β-sheet at $2\theta = 21.2°$ and $2\theta = 8.4°$ and of β-sheet at $2\theta = 19.8°$, respectively (Rao and Gupta 1992). The diffraction peak at $2\theta = 13°$ was allocated for the amorphous region. The study indicated that partial crystallinity of the keratin particles is retained after regeneration process.

The diffraction peak at 19.6° and 21.2° was indexed for the β-sheet crystalline structure of keratin and the peak at 17.8° indexed for α-helix diffraction pattern (Idris et al.

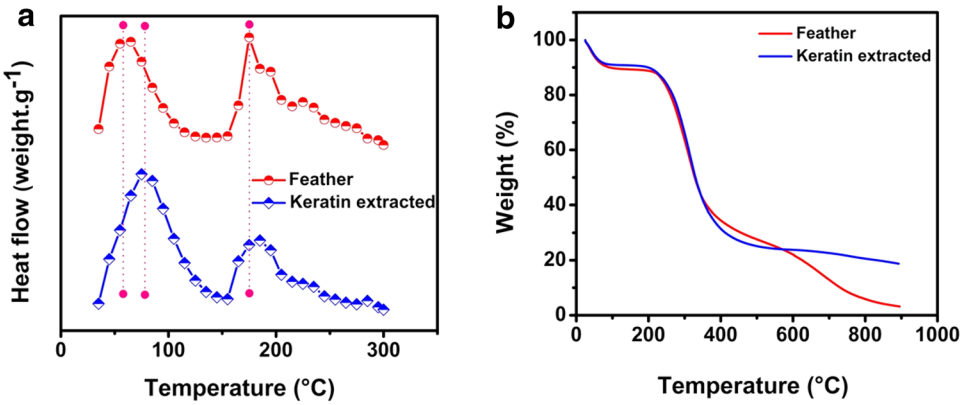

Fig. 6 **a** DSC thermogram and **b** TGA plots of feather and extracted keratin

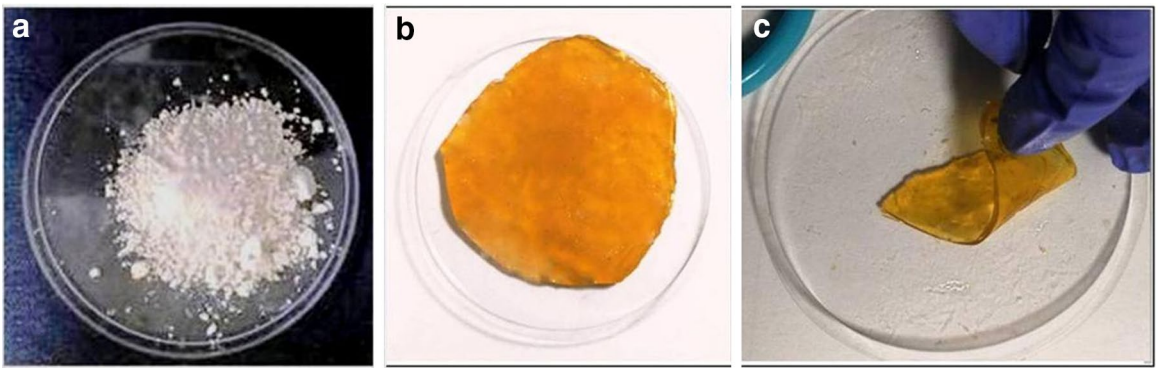

Fig. 8 Development of a bioplastic film using extracted keratin powder **a** extracted keratin powder **b** made bioplastic film

2013). All the samples were found to consist of β-sheets as reported previously. The intensity of peak specifies that the feather and extracted keratin contained large amount of β-sheet conformation and very small amount of α-helix conformation; also the extracted keratin have more content of β-sheet than the chicken feather. It is well identified that the keratin is semicrystalline and is macromolecular, and now it has been confirmed by the XRD analysis. In the extraction process of keratin, the original crystal domain was destroyed by dissolution and regenerated. All the destroyed crystals cannot rebuild and attain their original morphology, which leads to the crystallinity difference in the feather and keratin.

Application of extracted keratin in bioplastic film synthesis

The dissolved feather keratin was casted on the petriplates to prepare bioplastic film. The prepared bioplastic film is shown in Fig. 8. Microcrystalline cellulose was used as a nano-filler. Mechanical properties of biopolymer are very important to determine the industrial utility of the synthesized film. The calculated thickness of keratin-based film was 1.12×10^{-4} mm with tensile strength of 3.62 ± 0.6 MPa. The Young's modulus and break elongation for synthesized bioplastic film were 1.52 ± 0.34 MPa and $15.8 \pm 2.2\%$, respectively.

Some studies showed the fragile nature of film with the pure keratin (Aluigi et al. 2008; Yin et al. 2013). The film prepared with glycerol less than 20 wt% of keratin, was too fragile to measure mechanical properties. 0.3 g glycerol per gram of keratin was flexible with 7.56 and 27.61 MPa tensile strength and Young's modulus, respectively (Yin et al. 2013). The use of glycerol provides flexibility to the film. The results agree with other results on the addition of glycerol (Schrooyen et al. 2001a; Tanabe et al. 2002). Film was prepared by adding glycerol at level concentrations of 0.01, 0.03, 0.05, 0.07 and 0.09 g. The films tensile strength decreased from 16.6 ± 5.5 to 2.0 ± 0.2 MPa, and the elongation at break increased from $1.7 \pm 0.7\%$ to $31.9 \pm 4.5\%$, for samples without plasticizer and prepared with 0.09 g glycerol/g keratin, respectively (Moore et al. 2006). Extracted keratin solution was mixed with different concentrations of glycerol (2–10%) to produce plastic films and then investigated for its characterization (Ramakrishnan et al. 2018). It is important to improve the strength of the film; thus, it will open a new gateway for the research on bioplastics using waste biomass.

Conclusion

The highly porous keratin microparticles were extracted using chemical method and used to synthesize a bioplastic film. Overall, we provided an efficient method to utilize the waste biomass of poultry industry. Here, we successfully synthesized the bioplastic film using extracted keratin from the chicken feathers. These keratin microparticles can also be used to synthesize the various products like hair treatment cream, shampoos, anti-aging cream and wound healing cream of commercial use. In addition to feathers, bones, fats, other by-products are also disposed from the poultry industry. We need to develop more efficient processing systems for the sustainable management such waste products of poultry farms and food industry. Conclusively, in-depth studies are needed to extract the keratin and other useful substances from the waste biomass fractions and transform these into commercial commodities. Thus, this study provides an interface for the academia with industry with developments of products of mankind use.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest among all authors.

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