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Dissolution and characterization of biofunctional keratin particles extracted from chicken feathers

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Abstract. In the present study chicken feathers were hydrolyzed in alkaline environment. The pH value of feather hydrolyzed solution was adjusted according to the principle of isoelectric precipitation. Three kinds of precipitates of keratin polypeptide were collected at pH of 3.5, 5.5 and 7.5 respectively. The keratin solution were freeze dried and denoted as FKP1, FKP2, FKP3 respectively. All keratin particles possessed smooth, uniform and round surface by scanning electron microscope (SEM). FKP1, FKP2 and FKP3 had higher glass transition temperature examined by thermogravimetry (TG). Fourier transform infrared spectroscopy (FTIR) revealed that the extracted keratin retained the most of protein backbone, with the breakage of disulfide cross-links and hydrogen bonds.

1. Introduction

Keratin is present in all mammals having highly conserved amino acid sequence [1]. Keratin is reported for the impact on cell architecture and cell proliferation. Keratin is insoluble, fibrous and structural protein in feathers, which can be applied in many industrial applications. Keratin are three dimensional polymer interlinked by intermolecular bonding of disulfide amino acid and inter and intra molecular bonding of nonpolar and polar amino acids which are the reason for their stability and distinctive physical properties [2].

In food industry, near about $4X10^6$ ton per year are waste of chicken feathers found worldwide [3], some of them are pretreated and used as animal feed [4] and rest disposed in the landfills which cause serious environmental problem. There are mainly three methods of extraction of keratin [5] from the biomass: enzymatic hydrolysis has advantage like less species alteration which is very slow and cannot be used commercially. In the process of acidic hydrolysis the process provides very harsh conditions and can destroy some amino acids. Because of the high hydrolysis mainly alkaline hydrolysis is used for the keratin extraction from chicken feathers. During hydrolysis, chemicals break both types of peptide and disulfide bonds in proteins and as a result the structure of keratin hydrolysate is changed [1]. Reduction hydrolysis can cleave disulfide bonds of protein fibers without any major peptide bond cleavage, thus the microstructure of keratin remain intact [6]. Keratin is one of the most abundant proteins [7, 8, 9, 10]

The main aim of the study is to investigate the transforming of feather waste into bio functional peptide. In the present study three kinds of precipitates of keratin polypeptide were collected at pH of 3.5, 5.5 and 7.5 respectively. The keratin solution was freeze dried and marked as FKP1, FKP2, FKP3 respectively. Chicken feathers were hydrolyzed in sodium sulfide solution and after that by adjusting the pH value of hydrolyzed solution three kind of polypeptide were collected and characterized. The

results showed the feasibility of extraction of keratin with different compositions of the polypeptide with applications.

2. Methods and experiments

2.1. Materials
Chicken feathers were collected from chicken processing plant at Jaya Gading, Kuantan, Malaysia. All chemicals were of analytical grade and were purchased from Sigma Aldrich (Selangore, Malaysia). Milli-Q water was used to make solutions and washing. All chemicals were used without any further processing after receipt.

2.2. Hydrolysis and characterization of polypeptide particles

2.2.1. Alkaline hydrolysis of feathers. 25g of feather was immersed into 0.5M of sodium sufide (1L) solution and then digested under 50°C using mechanical stirrer until feather fibers were dissolved thoroughly. The prepared hydrolysis solution was filtered twice for further process.

2.2.2. Isoelectric-point precipitation process of keratin polypeptides. Isoelectric point (pI) is defined as the pH value at which a particular molecule or surface carries zero net electric charge. It is a process in which proteins or amino acids are precipitated at pH value close to their isoelectric points [11,12]. In this study, the pH value of the prepared feather hydrolysis solution was adjusted to pH 7.5 with 2N HCl, a thick layer of precipitates was settled on the bottom after 24 h. The precipitates were collected and marked as Feather keratin polypeptide (FKP3). The rest feather hydrolysis solution was incubated at pH 5.5 and the precipitates after 24h also collected and marked as Feather keratin polypeptide (FKP2). Lastly the remaining solution is precipitated at pH 3.5 and the precipitates were collected after 24h and denoted as Feather keratin polypeptide (FKP1). In order to wash the salts away and any other impurities FKP1, FKP2, FKP3 were dispersed in water and centrifuged 3 times for 10 mins at 10000rpm. Finally the Feather keratin polypeptides (FKP's) were freeze dried to obtain keratin polypeptide powders.

2.2.3. Surface Morphology. The surface morphology of FKP1, FKP2 and FKP3 was analyzed by scanning electron microscope. It is performed by using Hitachi TM3030Plus. In the preparation step, the samples were adhered directly onto an aluminium stub with a thin self adherent carbon film.

2.2.4. TGA analysis. Thermo gravimetric measurements of precipitates were performed on Research Instruments TGA Q 500 under nitrogen atmosphere, in a temperature range between 10°C and 900°C at ramping time of 10°C/min. The samples were vacuum dried at 40°C. Samples with mass 3mg were put in aluminium crucible and thus, the data was analyzed.

2.2.5. FT-IR analysis. Chemical characterization of precipitates of different pH was done by using Fourier transform infrared (FT-IR) spectroscopy. It will help to detect the changes in chemical composition of peptides [13]. Nicolet iS5 from Thermo scientific FT-IR was used for chemical characterization hers in between the 4000cm^{-1} and 500cm^{-1} wave number range.

3. Results and discussion

3.1. Alkaline hydrolysis of feather and isoelectric precipitation of keratin polypeptide
In the alkaline process of the hydrolysis the disulfide bond (S-S) and partial peptide bonds were broke. Bradford test were used to test the protein content. The hydrolysis was done under the following operating conditions: 0.5M/L sodium sulfide, 50°C and 6 hr. when time was increased to 24 hr and 48 hr, there was not much significant increase in hydrolysis degree of feather keratin. Thus, it can be concluded that prolonged hydrolysis time couldn't increase the degree of hydrolysis at the same temperature and alkali conditions. The purpose of the study was to confirm the possibility of making

biofunctional polypeptides from feather keratin using isoelectric precipitation process. The different isoelectric precipitates were collected at different pH range. It has been verify that three types of polypeptides with distinctive compositions were collected successfully. Furthermore, both chemical and physical properties of the polypeptides will be scrutinized through a series of characterization after freeze dry.

3.2. Surface morphology of keratin polypeptide powders

The morphology of the surface layer was studied by SEM analysis. In Figure 1 the regenerated keratin presents small microsphere, this is in accordance with the study on wool and feather keratin in journals [14, 15]. Fig 1 displayed the SEM images of FKP1, FKP2 and FKP3 respectively. The dried keratin is consisted of spherical, tightly packed micro-particles and random arranged porous microstructures.

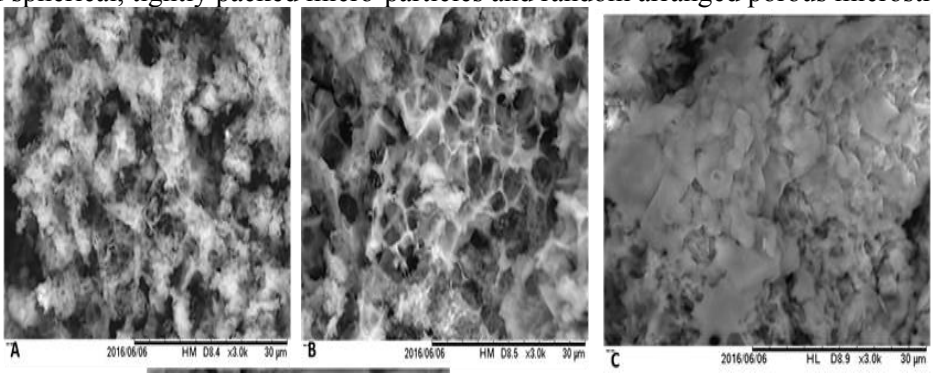

Figure 1. SEM images of (A)Feather keratin polypeptide at pH 3.5 (FKP1) (B) Feather keratin polypeptide at pH 5.5 (FKP2) (C) Feather keratin polypeptide at pH 7.5 (FKP3)

3.3. TGA Measurement

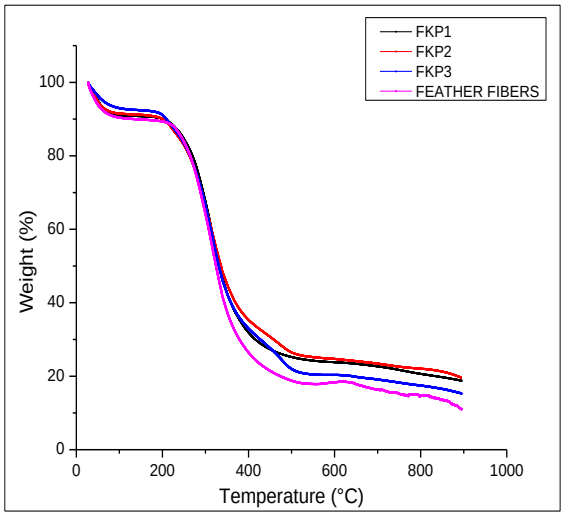

Figure 2. The TG curve of the feather fibers, FKP1, FKP2 and FKP3

The TG curve of the feather keratin polypeptides FKP1, FKP2 and FKP3 as well as feather fibers obtained. Figure 2 displayed the TG curve of feather fiber, FKP1, FKP2 and FKP3. All the samples were quickly decomposed from 250°C to 350°C and produced a significant amount of residue. The total weight loss was about 90%, 82%, 81.4%, 85.7% for feather fiber, FKP1, FKP2 and FKP3 respectively. The thermal decomposition started slowly 150°C with evolution of water and decomposition increased above 220°C showing notable weight loss. The study showed that the feather fiber is more stable as compare of polypeptides.

3.4. FTIR measurement

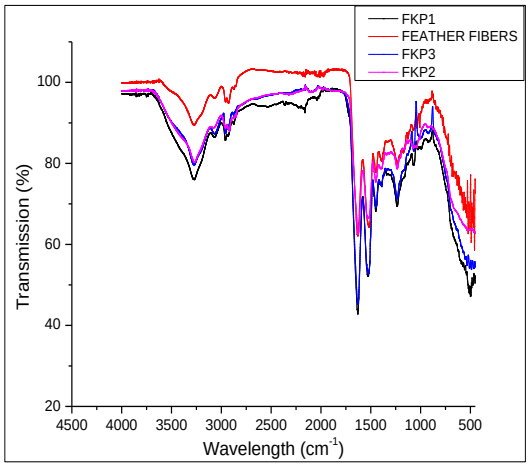

Figure 3. FTIR spectra of the feather fibers, FKP1, FKP2 and FKP3

FTIR measurement was used understand the chemical structure and the results are shown in Figure 3. The obtained results confirm that there are no significant changes in chemical structure of fibers after alkaline treatment. When comparing the absorbance spectra of all samples, their characteristic peaks are similar and are in comparison with other study [16]. On the other hand, the isoelectric precipitation have small effect on the chemical structure of protein, all the treatment showed transmission bands to the peptide bonds (-CONH) and are known as Amide A, Amide I, Amide II, Amide III [17,18]. The broad transmission band region from 3500 cm^{-1} to 3200 cm^{-1} can be attributed to stretching vibration of -O-H and -N-H (Amide A) [19]. The Amide A band which falls at 3282 cm^{-1}, is connected with stretching vibration of N-H bonds. Bands which fall in 3000-2800 cm^{-1} are related to C-H stretching bonds. The strong transmission band is attributed to C=O stretching (Amide I) which occurs in the range of 1700 cm^{-1} to1600 cm^{-1} [13] . The transmission band (Amide II) in the range of 1580 cm^{-1} to 1480 cm^{-1} is for N-H bending and C-H stretching [20]. The weak band between 1300 cm^{-1} to 1220 cm^{-1} is associated with Amide III band which is derived from C-N stretching and N-H bending [21, 22].

4. Conclusion

The proposed procedure for the treatment of feather by using alkali process is cheap and economically viable. Therefore, it could serve as a basis in the development of complex ecologically safe and efficient biotechnology for improved feather wastes utilization applicable in different applications. The aim of the study is the extraction of keratin from chicken feather waste by using reducing agent. Three types of keratin polypeptide precipitations were collected at selected pH value (3.5, 5.5 and 7.5) respectively. The physical and chemical properties of freeze dried FKP1, FKP2 and FKP3 powders were studied through different experiments. SEM image showed spherical and porous microstructures. FTIR spectra showed that the bisulfate bonds were broken and indicted no significant changes in the chemical composition and macromolecular conformation of the hydrolyzed fibers. Though, the feather remainders consisted mostly of β-sheets confirmation. TGA revealed that the polypeptides kept a little lower stable as compare to feather fibers.

In whole, keratin can be extracted by applied method and used in different applications such as coatings, films, packaging, cosmetics and biodegradable composites.

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