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Keratin Isolation Methods From Waste Goose Feather: An Effective Comparison

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Abstract: Conversion of biowaste into value-added material has attracted great interest lately. One of these materials is keratin, which is found in different structures such as nails, hair, beak, wool, feathers, claws and horns. Until now, keratin isolation has been carried out from waste wool, feather, hoof and hair. However, the development of effective techniques to obtain keratin without any damage to the secondary structure of the protein remains a challenging task. Herein, two distinct keratin isolation methods (sodium sulfide and ethylenediamine tetraacetic acid) were compared for the first time from Domestic Goose (*Anser domesticus*) waste feathers. The Kjeldahl method was used for the determination of crude protein by two methods from the obtained keratin powders and their antioxidant activities were conducted. According to our findings, keratin obtained from goose feathers using sodium sulfide showed higher yield (86.34%). On the other hand, the antioxidant activity of keratin obtained from the method prepared using ethylenediamine tetraacetic acid was found to be approximately three times higher than the other method, and our results proved that waste goose down could be considered as a potential source of keratin for further studies.

Atık Kaz Tüyünden Keratin İzolasyon Yöntemleri: Etkili Bir Karşılaştırma

Anahtar Kelimeler
Biyokütle, EDTA, Na_2S, Çözünme

Öz: Biyoatıkların katma değerli malzemeye dönüştürülmesi son zamanlarda büyük ilgi görmektedir. Bu maddelerden biri de tırnak, saç, gaga, yün, tüy, pençe ve boynuz gibi farklı yapılarda bulunan keratindir. Şimdiye kadar atık yün, tüy, toynak ve saçtan keratin izolasyonu yapılmıştır. Bununla birlikte, proteinin ikincil yapısına herhangi bir zarar vermeden keratin elde etmek için etkili tekniklerin geliştirilmesi zorlu bir süreç olmaya devam etmektedir. Burada, Yerli Kaz (*Anser domesticus*) atık tüylerinden ilk kez iki farklı keratin izolasyon yöntemi (sodyum sülfür ve etilendiamin tetraasetik asit) karşılaştırılmıştır. Elde edilen keratin tozlarından ham protein tayini için Kjeldahl yöntemi kullanılmış ve antioksidan aktiviteleri belirlenmiştir. Bulgularımıza göre kaz tüyünden sodyum sülfür kullanılarak elde edilen keratin daha yüksek verim (%86,34) göstermiştir. Öte yandan, etilendiamin tetraasetik asit kullanılarak hazırlanan yöntemden elde edilen keratinin antioksidan aktivitesinin diğer yönteme göre yaklaşık üç kat daha yüksek olduğu tespit edilmiş ve sonuçlarımız atık kaz tüyünün ileriki çalışmalar için potansiyel bir keratin kaynağı olarak kabul edilebileceğini kanıtlamıştır.

1. INTRODUCTION

As one of the most abundant but underutilized protein sources, keratin is the main component of hair, nails, hooves, wool, horns and feathers [1, 2]. However, there are difficulties associated with the disposal and management of these valuable materials. Especially, feathers contain the largest amount of all keratinous waste and is produced largely from poultry processing [3]. Keratin-containing poultry feathers are a very irritating and troublesome waste product of the poultry industry due to their ultimate disposal which could be either parried by incineration or burying; thus, both of these have negative effects on the environment [4].

Keratin is a fibrous, structural protein that is formed by the juxtaposition of many types of amino acids [5]. However, keratin differs from other fibrous proteins in its higher cystine content. These cysteine amino acids have a

stronger structure due to their disulfide bonds [6]. It has a three-dimensional structure thanks to the disulfide bridges caused by cysteine molecules. Thanks to this cross-linked structure and its highly hydrophobic feature, keratin is insoluble in water, nor in nonpolar solvents. Namely, highly stable SS bonds could be denatured just by acid and base [7]. Keratin has a very active chemical structure, it can be easily reduced, oxidized and hydrolyzed thanks to the cystine molecule [5, 8]. Thus, the natural state of the protein is lost in strong inorganic acids or bases. Keratin also reacts with acids and bases, losing its original shape by hydrolysis [7].

It is critically important to develop effective techniques to isolate keratin without damaging this structure of proteins. The fundamental keratin isolation methods used for this purpose in the literature are physicochemical methods [9], enzymatic reactions by hydrolysis of the novel keratinase [10], alkali/acid handling [11], oxidation [12], reduction hydrolysis [13] and processing in ionic solutions [14]. Often used methods such as steam blasting used for the destruction of hard biomass or ionic liquids are high-cost, relatively energy-consuming and difficult to recycle [10]. 2-mercaptoethanol and sodium dodecyl sulfate are the most commonly used chemicals in keratin production [15]. Moreover, using them together with reducing agents such as urea and sodium bisulfite provides high efficiency keratin production in a short time [16]. However, 2-mercaptoethanol is not preferred due to its high cost. Conversely, the use of Na_2S for keratin extraction with high yield is both more economical and commercially available [17]. In the meantime, EDTA acts as an emulsifier and helps the feather stay in dispersion at the peak level, a stable distribution was achieved [18].

Thus, the purpose of the current study was to evaluate two different methods for keratin extraction in terms of effectiveness and antioxidant activities for future product development. So, the feather hydrolysis using the Na_2S and EDTA methods was preferred due to its low cost and simple hydrolysis herein.

2. MATERIALS AND METHODS

2.1. Sample Collection

Goose feathers were provided by a slaughterhouse in the province of Aksaray, Turkey.

2.2. Pre-treatment of the Feathers

Waste goose feathers collected from goose factories were washed three times with water, cleaned of blood and other dirty and left to dry in daylight. The fat compounds in the goose feather were annihilated by reflux with an organic solvent chloroform for 6 hours. After removing the goose feathers were separated to small pieces.

2.3. Extraction of the Feather's Keratin: *Na_2S Method*

Degreased goose feathers (10 g) were stirred with 100 ml of distilled water, 3.0 N NaOH and 0.2 N Na_2S. The reaction was carried out in a water bath at 25 °C for 1.5 hours. All substances were dissolved in basic medium with the help of magnetic stirrer. Then, the pH of the keratin solution was adjusted to 4.2 with the help of dilute HCl solution to precipitate the keratin. These keratins were washed with acetone and filtered using a vacuum strainer. The resulting keratin was then dried in an oven at 50 °C for a night.

2.4. Extraction of the Feather's Keratin: *EDTA Method*

Dewaxed goose feathers (10 g) were blended with 100 ml of distilled water and 10 g of NaOH. The base was thoroughly dissolved by shaking and mixing by hand. After adding 0.15 g of EDTA to the solution, the keratin solution was poured onto it. This solution was then placed in a 40 °C bath where a stirrer was rotated for 2 hours. After the reaction was complete, neutralization was performed with HCl to pH 4.2. Afterwards, the precipitated keratin was firstly centrifuged, then washed with acetone and finally dried in an oven at 50 °C for 24 hours. At the end, the material was powdered and kept in a vacuum desiccator to be kept dry.

2.5. Kjeldahl Method

The Kjeldahl method was used to evaluate the nitrogen content of the dried hydrogel (1 g) by following method [19]. After determining the total nitrogen of the samples, the crude protein amount was calculated by a conversion factor of 6.25 to convert % nitrogen to % crude protein [20]. Each sample was analyzed in duplicate.

2.6. Antioxidant Activity

The antioxidant activities of keratin powders were determined by making minor modifications from Kaya et al. [21]. First, 10 mg of the keratin samples were weighed and placed in test tubes. Then, 1.0 mL of DPPH solution at 6 x 10^{-5} M concentration was added to each tube. The samples were incubated for 30 minutes at 25 °C in the dark. At the end of the incubation, the entire keratin solution was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Analysis was performed in triplicate for each sample.

DPPH scavenging activities of keratin powders were calculated using the following equation:

$$Inhibition\ (\%) = ((Acontrol - Asample) / Acontrol) \times 100 \quad (1)$$

Acontrol is absorbance of the control, Asample absorbance of keratin powders+ DPPH.

2.7. Statistical Information

Three replicates were prepared for all of the antioxidant activity tests. The results of these analyses were given as means and their standard deviations (means±SD).

3. RESULTS AND DISCUSSION

Within the scope of this study, keratin extraction was targeted using 2 different methods from waste goose feathers, the protein yield of keratin particles calculated and the antioxidant activities of powdered keratins were compared. The stereomicroscopy images of extracted keratin particles and powders were shown in Figure 1.

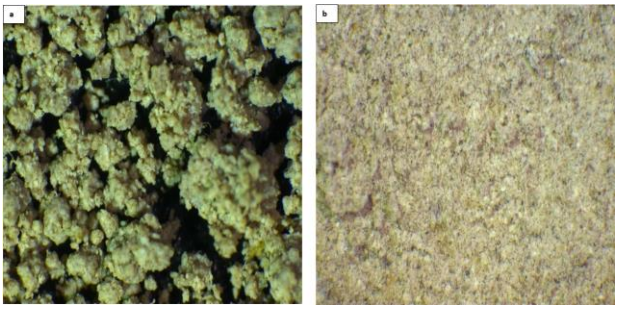

Figure 1. Stereomicroscopy images of keratin particles for Kjeldahl method (a) and powders for DPPH scavenging activity (b) extracted using goose feathers

As known, feathers contain 91% insoluble keratin and 1% oil; the rest is water [22]. For this purpose, firstly, the keratin oil was removed and then isolated from the insoluble goose down S-S cross-links that were broken in basic medium. An aqueous solution containing NaOH was used to break the keratin, disulfide bonds and provide solubility. Keratin was then precipitated with hydrochloric acid. Sodium sulfide (Na_2S) was chosen as the emulsifier in the first place because positive results were obtained with this chemical in many previous studies [23, 24, 25]. In general, physical conditions such as temperature, time, and concentration of reducing agent had a significant effect on the final yield of the isolated keratin [26]. Gül Çelik et al. found the best protein yield in Na_2S (93.3%) compared to the extraction using only NaOH as a result of keratin extraction from chicken feathers [26]. Similarly, Pourjavaheri et al. found the yield as 88% in the extraction with sodium sulfide, but 66% with L-cysteine method [24]. Sharma et al. obtained the yield of keratin extracted with Na_2S to be 80.2% under highly alkaline conditions [23]. Sinkiewicz et al. obtained 84% and 82% efficiency, respectively, by using mercaptoethanol and sodium bisulfite [27]; however, in our study, similar keratin yield was obtained at a lower cost. Gül Çelik et al. also proved that the yield increased as the temperature and time increased, and they observed the best yield at 60 °C [26]. Further, 25 °C obtained 87.6% efficiency [26] as in our study. Similarly, the previous study determined the optimum yield for keratin production within 2.5 hours at 23°C [5]. In Gül Çelik et al.'s study, the efficiency reached 90% as it was kept for 6 hours [26], however, in our study, 86% efficiency was achieved almost in one sixth of the time, which is a significant reduction in labor savings. Although, it has been shown in previous studies that alkaline hydrolysis takes longer to occur than acidic ones [25], but this study refuted this thesis.

Zeydanlı showed that the yield of powdered keratin obtained from feathers by EDTA method depends on the amount of feather, temperature, NaOH quantity, EDTA and hydrolysis reaction time [7]. Since the previous studies have shown that keratin yield is inversely proportional with increasing temperature [7, 23], reactions were carried out at 25 degrees and 40 degrees in the current study. Because excessive amount of EDTA concentration would be harmful to the product, it was used only 0.15 g. Similarly, conducted study has shown that by reducing the EDTA concentration, the efficacy is increased by half [7].

Looking at their antioxidant activities, keratin powders obtained using EDTA showed higher inhibition (65.21%) (Table 1, Figure 2). However, Alahyaribeik and Ullah (2020) obtained the best antioxidant activity from keratin by using sodium sulfite rather than 2-mercaptoethanol and mixture of sodium sulfite and SDS [25]. Mostly, the differences in inhibition activities of various keratin extraction methods are related to amino acid residues, various molecular weight of keratin, and production procedures [25]. Antioxidants possess an increasing interest due to their protecting functions in food and pharmaceutical products towards oxidative injuries and in the body and oxidative damage-intermediated pathological processes. Screening of antioxidant properties of plants and plant-derived agents necessitate suitable methodologies that address the machineries of anti-oxidant activities and emphasis on the kinetics of the reactions including the antioxidants. Several studies evaluating the antioxidant activity of various samples of research interest using different methods in food and human health have been carried out [28, 29, 30, 31, 32]. In our study we evaluated antioxidant activities of keratin by DPPH assay.

Table 1. DPPH radical scavenging activity of keratin powders obtained by two different extraction methods

Methods	Inhibition (%)
Na_2S	24.14±5.79
EDTA	65.21±0.13

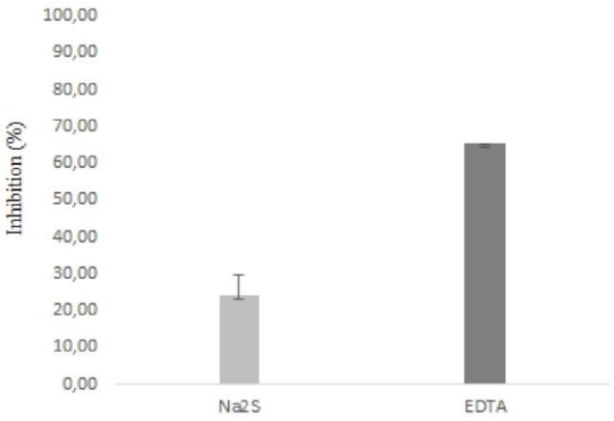

Figure 2. Antioxidant activities of keratins extracted by goose feathers determined by DPPH method

4. CONCLUSION

Na_2S and EDTA were used during keratin extraction in this manuscript. The yield obtained from goose feathers extracted with Na_2S and EDTA were 86.34% and 80.94%, respectively. The antioxidant activities of the powdered keratins obtained as a result of two extractions were

examined and detected as 24.14% and 65.21% for Na_2S and EDTA, respectively. As a result, keratin extraction efficiencies were compared by using Na_2S and EDTA chemicals separately and the best yield was obtained with Na_2S. It has been proven that the use of keratin can be increased with this study, which has high antioxidant activities especially for EDTA method. Considering the high protein structure of keratin, the addition of antioxidant activity strengthens the possibilities for further use in future for medical, tissue engineering and bioengineering studies.

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