STUDY ON OBTAINING KERATIN EXTRACTS FROM LEATHER INDUSTRY BY-PRODUCTS

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Keratin is a biopolymer with numerous functional properties for the production of biomaterials with applications such as: additives for cosmetics, biostimulator for growth and nutrition in agriculture, ecological treatments in reconstruction and protection of leather and furs, as alternative to pollutant chemical compounds. In most of the applications, in either large or niche industries, keratin is used as gels, films, nano- or micro-particles. This study presents the possibilities of using thermal and enzymatic processes of leather industry by-products to obtain keratin extracts. Keratin extracts were characterized by chemical and instrumental analyses: gravimetric, volumetric, potentiometry, Polyacrylamide Gel Electrophoresis, Infrared Spectroscopy, High-Performance Liquid Chromatography, Dynamic Light Scattering. Analytical investigation has shown that the keratin extracts obtained as polydispersions with 5% dry matter have 14% total nitrogen and contain 50% small and medium sized particles (10-500 nm range), such as free amino acids and oligopeptides and 50% larger sized particles (500-5560 nm ranges) such as polypeptides. The IR spectrum of keratin extract is similar to the IR spectrum of collagen from leather.

Keywords: keratin, molecular weight, amino acids

INTRODUCTION

Obtaining keratin hydrolysates from by-products/waste from the leather industry contributes to the recovery of these natural resources, to the reduction of the amount of stored waste and to environmental pollution prevention. The hydrolysates may be used to develop new biomaterials with multiple applications, as well as to design environmentally-friendly treatments for leather and fur with various functionalities.

Wool is a keratinous material with specific structure, mechanical behaviour and physical-chemical properties (Wang *et al.*, 2016). A clean wool fibre contains approximately 82% keratinous protein with high concentration of cysteine, approximately 17%, a protein material with a low cysteine content called “non-keratinous material” mainly localized in the complex of the cell membrane and approximately 1% of the non-protein material is made up of waxy lipids, plus a small amount of polysaccharide material (Lewis and Rippon, 2013).

Keratins are the most abundant structural proteins in epithelial cells and, together with collagen, it forms the most important biopolymer in the organic matter that constitutes animal tissue (Coulombe and Omary, 2002; McKittrick *et al.*, 2012). Keratin is among the most rigid biological materials, with high hardness and elastic modulus, although is is made up only of polymer compounds and rarely contains minerals (Wegst and Ashby, 2004; Szewciw *et al.*, 2010).

Keratinous materials are high in cysteine, which differentiates them from other biopolymers, are usually durable, rigid and non-reactive with the natural environment. They provide mechanical support and various protection functions in the adaptation of vertebrates to the external environment (Schweizer *et al.*, 2006).

Extraction of keratin from wool can be achieved in various ways, all of them involving the presence of reduction or distortion agents to break disulfide bonds (Aldemar *et al.*, 2005; Liu *et al.*, 2004; Aluigi *et al.*, 2007).

Keratin extracts are obtained using acid hydrolysis (Khosa and Ullah, 2014), hydrolysis in alkaline medium (Hill *et al.*, 2010; Staron *et al.*, 2014), enzymatic hydrolysis (Krejci *et al.*, 2011) and ionic liquid extraction (Ji *et al.*, 2014). The various methods of obtaining keratin extracts lead to variations in their composition and properties. These differences allow the use of keratin hydrolysates in the production of materials with multiple industrial applications, in agriculture and niche applications.

This study proposes to transform a low economic value material (sheep wool scraps) into a product with potential for exploitation in various fields: leather processing, cosmetics, agriculture, as wool is a source of organic nitrogen as macronutrient but also sulfur as essential mesonutrient for plant nutrition (for example, in leguminous plants sulfur deficiency leads to reduction of nodules on the roots, increase of soluble nitrogen and slow formation of protein substances).

EXPERIMENTAL

Materials

Raw materials: wool by-products from the leather industry with the following characteristics: dry substance, max. 87%; ash, max. 13%; total nitrogen, min. 13%.

Auxiliary materials: ammonia, 25% solution, CAS 1336-21-6; detergent; anhydrous sodium carbonate, CAS 497-19-8; distilled water; hydrated lime, p.a.; rotulis sodium hydroxide; potassium hydroxide, p.a.

Procedures

In order to obtain keratin extracts, raw wool (LB) was degreased in a FAVE vessel system using a solution of 1 g/L of 25% ammonia, detergent and sodium carbonate anhydrous, under stirring for 12 hours at 35°C.

After degreasing, the wool was dried in a ventilated open space and then fragmented.

Degreased wool (LD) was subjected to alkaline hydrolysis: (a) with 10% hydrated lime (b) with 5% sodium hydroxide, (c) with 5% potassium hydroxide and keratin hydrolysates were obtained according to the technological scheme of Fig. 1.

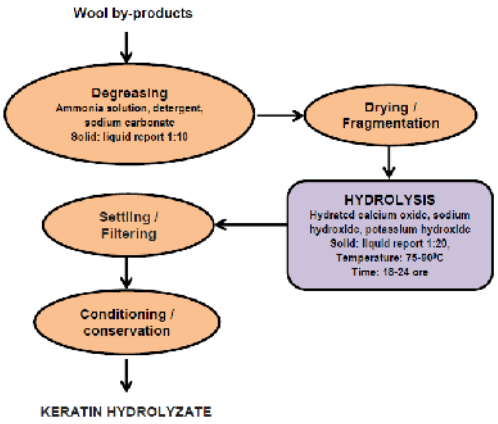


Figure 1. Technological scheme for obtaining keratin hydrolysates

Analytical Methods

The keratin extracts were analysed in terms of dry substance and total ash by gravimetric methods, total nitrogen and protein substance, aminic nitrogen by volumetric methods, pH by potentiometric method, molecular weight by SDS-PAGE electrophoresis. Particle size and distribution was determined by ZetaSizer device Nano ZS (Malvern, UK), IR spectral analysis by FT/IR-4200 (Jasco) with ATR device equipped. Static contact angle of keratine hydrolysate was evaluated with contact angle analysis equipment, VGA Optima XE system, AST Products SUA.

RESULTS AND DISCUSSIONS

Keratin hydrolysates with distinct colors were obtained depending on the alkaline hydrolysis medium: (a) clear orange (KHA1), (b) brown (KHA2), (c) tan (KHA3). The unsolubilized wool residue has a different appearance and consistency: a) RL1, from the hydrolysis with calcium oxide has a brittle consistency, b) RL2 from the hydrolysis with sodium hydroxide is compact and has a more rigid consistency, c) RL3 from hydrolysis with potassium hydroxide is voluminous and thick.

Keratin hydrolysates obtained by alkaline hydrolysis were analyzed in terms of chemical and physical-chemical properties and specific properties were identified, using different instrumental techniques: electrophoresis, DLS, IR spectroscopy.

Physico-chemical analyses showed significant and close values for total nitrogen, which reveals extraction of large amounts of proteins (Fig. 2). Proteinaceous material is between 65.29% (KHA3) and 79.02% (KHA1), and the amino nitrogen up to 3.21% (KHA1). The high values of ash range between 12.28% (KHA1) amd 25.62% (KHA3), showing that the keratin hydrolysates are rich in minerals used in hydrolysis (Ca, Na, K).

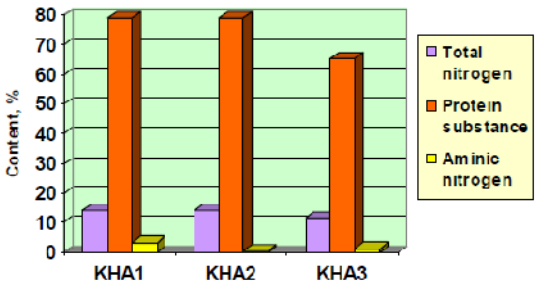


Figure 2. Protein composition of keratin hydrolysates

Determining Distribution of Molecular Weights in Keratin Hydrolysates by SDS - PAGE Electrophoresis

The electrophoretic pattern of keratin is associated with two main groups of protein specific to keratin, intermediate filamentous proteins and matrix proteins.

Figure 3 shows the distribution of molecular masses in keratin hydrolysates experimentally obtained by alkaline hydrolysis from wool by-products.

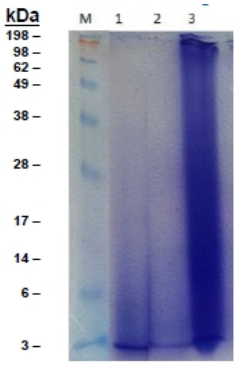
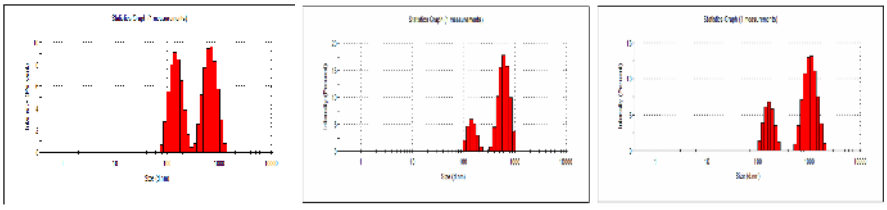


Figure 3. Distribution of molecural weights in keratin hydrolysates: M-marker;1-KHA1; 2-KHA2; 3-KHA3

Two high molecular weight bands are visible (45-60 kDa) attributed to filamentous proteins with a low sulfur content and more low molecular weight bands attributed to proteins with a high content of sulfur (20-10 kDa) and a high content of glycine/tyrosine (6-9 kDa) (Vasconcelos *et al.*, 2008). These considerations highlight the presence of proteins with a high content of sulfur (20-10 kDa) but rich in glycine/tyrosine (6-9 kDa) with low molecular weight, mainly in keratin hydrolysate (KHA1), but also in keratin hydrolysate (KHA3). Proteins with a low sulfur content and high molecular weight assigned to filamentous proteins are more evident in keratin hydrolysate (KHA3).

Determining Particle Sizes Using Dynamic Light Scattering (DLS)

Measurements of the intensity of reflected light indicate a narrow spectrum of particle sizes for the three types of keratin hydrolysate, Figure 4, including mostly medium-sized particles in the range of 100-1000 nm, specific to oligopeptides and larger peptides.



(a) (b) (c)

Figure 4. Particle size distribution in keratin hydrolysates: (a) KHA1, obtained with hydrated lime; (b) KHA2, obtained with sodium hydroxide; (c) KHA3, obtained with potassium hydroxide

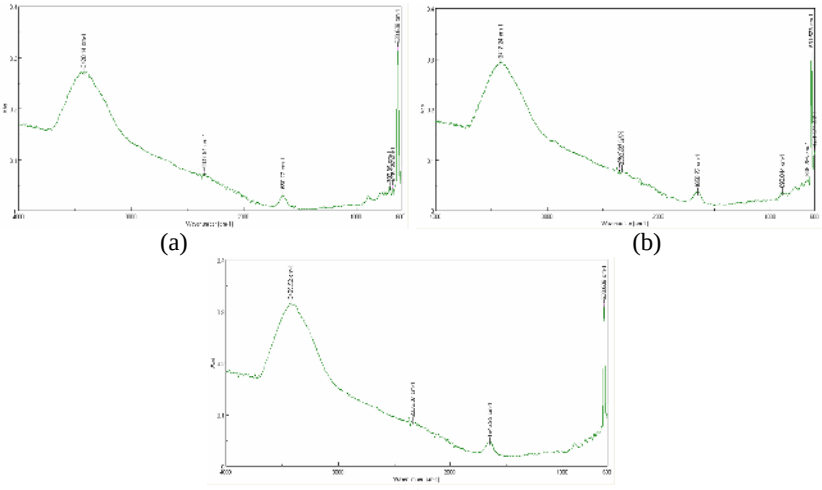
However, the KHA1 hydrolysate also contains smaller particles in the higher area of the 10-100 nm range, specific to amino acids and small oligopeptides, but also a small percentage of particles greater than 1000 nm, namely polypeptides. Hydrolysates KHA2 and KHA3 do not have particles smaller than 100 nm, however, hydrolysate KHA3

shows a higher percentage of particles of over 1000 nm. These data are consistent with results of electrophoresis which reveal the presence of compounds with molecular weights lower than 20 KDa, and some compounds with molecular weight higher than 45KDa.

Structural Analysis of Keratin Hydrolysates Using FT-IR Spectroscopy

Infrared absorption spectra of keratin hydrolysates (KHA1, KHA2, KHA3) show characteristic bands attributed both to peptides (-CONH) of amide I, amide II, and amide III types, and to sulfur compounds.

Among these, there are bands specific to primary amides at 3400 cm^{-1} and secondary amides at 3440 cm^{-1} attributable to the stretching vibration ν_{N-H} and the band specific to tertiary amides at 1650 cm^{-1} attributable to the stretching vibration $\nu_{C=O}$, present in the three spectra of keratin hydrolysates (Fig. 5). The bands at 600-700 cm^{-1} attributed to stretching vibration ν_{C-S} and at 800-900 cm^{-1} attributed to rocking vibration δ_{S-H} are specific to sulfur compounds (Khosa and Ullah, 2014; Hill *et al.*, 2010; Staron *et al.*, 2014).



(a) (b) (c)

Figure 5. FT-IR spectral analysis of keratin hydrolysates: (a) KHA1; (b) KHA2; (c) KHA3

Determination of Contact Angle and Surface Tension of Keratin Hydrolysates

Determining the contact angle value of keratin hydrolysates, in relation to glass as inert material indicates a strongly hydrophilic behavior, recording very low values: 9.25° for KHA1, 7.75° for KHA2, 6.32° for KHA3. These hydrophilic contact angles are the premises for good adhesion of keratin hydrolysates to non-hydrophobic porous surfaces, for example, unfinished tanned leather or plant seeds.

Surface tension values of keratin hydrolysates: 40.77 mJ/m^2 for KHA1, 46.49 mJ/m^2 for KHA2, 46.18 mJ/m^2 for KHA3, confirm the presence of low intermolecular forces that cause a high capacity for wetting surfaces.

CONCLUSIONS

Keratin extracts were obtained from wool by-products from the leather and fur industry, through alkaline hydrolysis.

Extracting keratin from by-products contributes to decreasing the amount of by-products stored and prevents pollution, while recovering and reusing residual protein as eco-friendly products for applications in industry and in bio-economy.

Using keratin extracts in agriculture has the advantage of providing organic nitrogen as macronutrient and sulfur as mesonutrient.

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