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KERATIN HYDROLYSATES OBTAINED FROM SHEEP WOOL FROM THE LEATHER INDUSTRY

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This work presents experiments in making biopolymer-based materials with smart functionalities from renewable resources in the leather industry, applicable in various areas. Keratin hydrolysates were obtained by alkaline hydrolysis in the presence of NaOH and CaO. The physico-chemical characterization, DLS and FT-IR analysis of keratin hydrolysates highlighted the rich content in protein and total nitrogen. The recovery of wool by-products from the leather industry leads to less waste and helps prevent environmental pollution.

INTRODUCTION

Keratin recovered from wool waste is a promising natural material for tissue engineering, cosmetics, pharmaceuticals, applications in leather industry and agriculture. Keratin is an insoluble fibrous protein characterized by stability due to disulfide bonds in the structure [1-4], with a high degree of chemical functionality, and high potential for niche applications (bio-sponges, films, matrices for retention and delivery of substances) [5-8]. The functionalities and the end uses are determined by the hydrolysis conditions for breaking the disulfide bond. The reactivity is given by the amide, carboxyl, sulfide and thiosulfide groups. Wool hydrolyzed by high temperature alkaline hydrolysis in various modified systems has led to the recovery of keratin in the form of intermediate filaments and microfibrillary constituent proteins and matrices [7, 8]. The experiments consist of making biomaterials with controlled characteristics from renewable resources in the leather industry, in order to characterize smart functionalities, applicable in industrial fields, agriculture or niche areas.

METHODS OF INVESTIGATION

The wool recovered from leather processing was degreased in water 1:20 at 35°C, 8h with 1% ammonia solution, 1% sodium carbonate and 1% Borron SE and

hydrolyzed by the alkali method in water 1:20 and 10% calcium oxide or 5% sodium hydroxide at 80°C, 3h, under mechanical agitation to obtain keratin hydrolysates with macromolecular protein chains with high degree of cleavage. The keratin hydrolysates obtained were filtered on black tape filter paper and concentrated to increase the percentage of total nitrogen / protein substance. The concentration process was carried out in Heidolph rotary evaporator, at a temperature of 70°C, with 150 rot/min and at a pressure of 250mbar, for 90 min. Keratin hydrolysates obtained in the presence of calcium oxide were filtered [KerCa1, KerCa3] and concentrated [KerCa2 (4: 1), KerCa4 (3: 1)]. Those obtained in the presence of sodium hydroxide are KerNa1 (unfiltered), KerNa2 (filtered), KerNa3 (refiltered) and concentrated KerNa4 (3:1).

The keratin hydrolysates obtained were analyzed physically and chemically (Table 1), measuring the particle size by DLS method and by FTIR spectroscopy.

Table 1 - Physico-chemical characteristics for keratin hydrolysates obtained in the presence of calcium oxide

No.	Characteristics	KerCa1	KerCa2	KerCa3	KerCa4
1	Dry matter, %	2	10.49	11.87	3.18
2	Ash, %	15.50	13.73	2.53	14.46
3	Total nitrogen, %	13.50	12.96	13.14	13.52
4	Protein substance, %	76	72.83	73.88	76.10
5	pH, pH units	9.39	8.96	8.41	8.79
6	Aminic nitrogen, %	0.66	0.79	0,46	0.41
7	Calcium oxide, %	13	9.63	2.36	12.89

The values for items 3,4,7 are related to the dry substance. The values for item 6 are related to the protein substance.

The ash content shows that KerCa keratin hydrolysates are rich in mineral substances, such as 15.50% KerCa1 or 14.46% KerCa4, but also calcium oxide - 13% in KerCa1 or 12.89% in KerCa4 due to hydrolysis.

The physico-chemical analyses performed for the keratin hydrolysates obtained in the presence of sodium hydroxide highlight important values for total nitrogen, protein and amine nitrogen which enhance the obtained products and underline the high degree of hydrolysis achieved (Table 2).

Table 2 - Physico-chemical characteristics of keratin hydrolysates obtained in the presence of sodium hydroxide

No.	Characteristics	KerNa1	KerNa2	KerNa3	KerNa4
1	Dry substance, %	5.29	5.14	5.14	15.72
2	Ash, %	10.02	9.34	0.53	9.73
3	Total nitrogen, %	13.99	14.40	14.01	13.93
4	Protein substance, %	78.64	80.93	78.79	78.31
5	pH, pH units	10.08	10.03	9.81	10.10
6	Aminic nitrogen, %	0.48	0.48	0.49	0.49
7	Calcium oxide, %	2.08	2.33	undetectable	0.70

The values for items 3,4,7 are related to the dry substance. The values for item 6 are related to the protein substance.

RESULTS AND DISSCUSION

1. Physico-chemical characterisation

The total nitrogen determined for KerCa keratin hydrolysates varies between 12.96% (KerCa2) and 13.52% (KerCa4), the concentrated hydrolysate having the highest value. The protein substance determined from 76.10% at KerCa4, 76% at KerCa1 up to 72.83% at KerCa2 shows a high degree of hydrolysis in which the peptide bonds of the protein were broken down to amino acids. Amine nitrogen values show the cleavage of macromolecular keratin chains. In the case of concentrated keratin hydrolysates, there were increases in the total nitrogen up to 13.52%, the protein substance up to 76.10% and the calcium oxide up to 12.89% in the case of KerCa4 and of amine nitrogen up to 0.79% for KerCa2.

Keratin hydrolysates obtained in the presence of sodium hydroxide have an important percentage of total nitrogen of 14.40% and protein substance of 80.93% in the case of KerNa2 sample and 0.49% amine nitrogen, showing splitting of keratin macromolecular chains.

2. Determination of particle size by DLS technique

The KerCa2 sample has smaller particle sizes highlighted by the majority population size of 821nm (Fig 1) compared to 1032 nm for the KerCa1 sample.

In the case of KerNa hydrolysates, KerNa3 sample has three major populations at 5560 nm, 687 nm and 118 nm and an average particle size at 934 nm (Fig. 1) and the KerNa4 sample has two majority populations, at 337 nm and 12 nm and an average of 4008 nm. The particle size distribution for KerNa3 highlights the three major

populations and the Zeta potential is -19.5nV. The KerNa4 keratin hydrolysate sample shows a larger average particle size at 4008 nm, smaller populations and a Zeta potential of -12.4 mV.

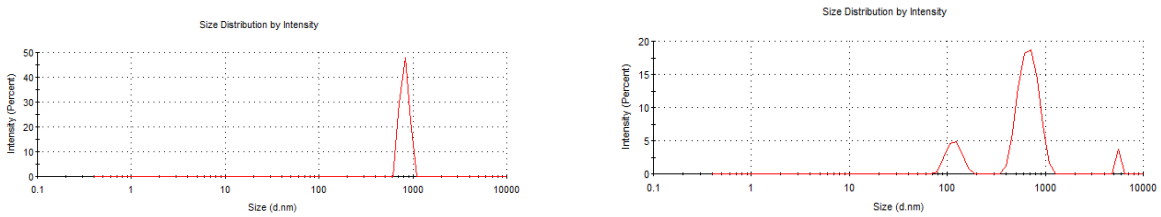

Fig. 1 *- Particle size distribution (nm) for KerCa2 (left) and KerNa3 (right)*

3. FTIR characterization of keratin hydrolysates

Keratin hydrolyzates analyzed by FTIR have spectral bands attributed to amide A from 3312.14cm^{-1}-3275.5cm^{-1} and the specific band of amide I from 1636.3cm^{-1} and 1637.27cm^{-1} are present in all analyzed samples.

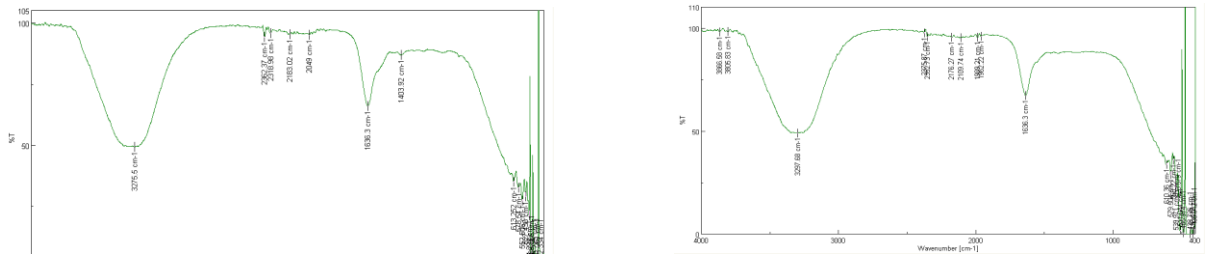

Fig.2 *- FTIR spectra of keratin hydrolysates KerCa2 (left) and KerNa3 (right)*

Infrared spectra of keratin hydrolysates have characteristic bands at 3300-3200 cm^{-1}, the broadband that accumulates the OH, NH, NH_2 bands predominantly derived from protein compounds, at 2370-2340 cm^{-1}, corresponding to the aliphatic CH bond, at 1650-1600 cm^{-1} corresponding to the bands characteristic of proteins, at 600-650 cm^{-1} and 400-550 cm^{-1} corresponding to the sulfur compounds and specific to the stretching vibration $\nu_{C\text{-}S}$.

CONCLUSIONS

Keratin hydrolysates obtained are valuable through protein and total nitrogen content, highlighted by physico-chemical analyses. Keratin hydrolysates can be used to make new biomaterials with various applications (pharmaceuticals, medical products, cosmetics, agriculture and leather industry). Recovery of wool by-products leads to less waste and prevents environmental pollution.

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