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LEATHER AND WOOL BYPRODUCTS PROCESSING FOR BIOACTIVE ADDITIVES

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Abstract: *The leather industry byproducts and coarse wool are valuable bioresources, rich in bioactive proteins, but which are not fully exploited and represent a source of environmental pollution. The biomaterials based on native collagen and keratin are made using sophisticated methods of extraction, synthetic polymers, and organic solvents with toxic potential. The paper presents two methods for collagen gelatin extraction from rabbit skin and bovine pelt, and for keratin solubilization and enzymatic refinery, which were proved to be suitable for the fabrication of new collagen nanofibers and porous keratin powders compatible with wound healing formulations in dressing, creams or gel forms. The neutral and chemical-enzymatic-based technologies for bioactive protein extraction and refinery are presented as alternatives to more expensive and sophisticated methods for native collagen and keratin hydrogels used in making wound healing biomaterials. Bioactive collagen nanofibers and keratin powders for new wound-healing formulations and dressings were successfully obtained. The collagen extracts were found to have good properties for nanofiber fabrication, rabbit collagen showing exceptional characteristics and a high Bloom value. The use of collagen extracts, soluble in ecological solvents with spinnable properties from leather industry byproducts, as well as the preparation of keratin hydrolysate powders from wool waste represents an efficient approach for the capitalization of biomass and the development of new biomaterials with low toxic potential.*

Key words: *collagen, keratin, bioactive proteins, nanofibers, keratin powder, biomaterials.*

1. INTRODUCTION

Protein-based byproducts are rich and not fully exploited resources with high potential to replace synthetic polymers in manufacturing biomaterials for medical applications [1,2].

The paper presents the potential for extraction and refinery of collagen gelatins and keratin hydrolysates from leather industry byproducts or from coarse wool generated in zootechnics.

The neutral and chemical-enzymatic-based technologies for bioactive protein extraction and refinery are presented as alternatives to more expensive and sophisticated methods for native collagen and keratin hydrogels used in making wound healing biomaterials [3]. Bioactive collagen nanofibers and keratin powders for new wound-healing formulations and dressings were successfully obtained. The use of collagen extracts, soluble in ecological solvents with spinnable properties from leather industry byproducts as well as the preparation of keratin hydrolysate powders from wool waste represents an efficient approach for the capitalization of biomass and the development of new biomaterials with low toxic potential.

2. EXPERIMENTAL

2.1 Materials and methods

2.1.1 Materials

The raw materials were purchased from rabbit and sheep breeders. Chemical reagents like sodium carbonate, ammonium solution, sodium hydroxide, and acetic acid were analytical grade and other chemical materials were technical grade: sodium chloride, detergents, sulphuric acid, or formic acid. Valkerase, a keratinase with optimal activity at pH=5.5 and 55ºC, was supplied by BioResource International.

2.1.2 Methods

The methods for spinnable collagen and bioactive keratin extraction and refinery are presented in Figures 1 and 2. Rabbit skins or bovine hides were pre-processed by soaking, unhairing and liming, washing deliming, bating, and degreasing. Rabbit skin in pelt or pickled state was processed following the scheme presented in Figure 1 a. Bovine pelt was processed following the same scheme. The scheme for keratin hydrolysate solubilization from sheep wool and refinery for bioactive wound-healing formulations is presented in Figure 1 b.

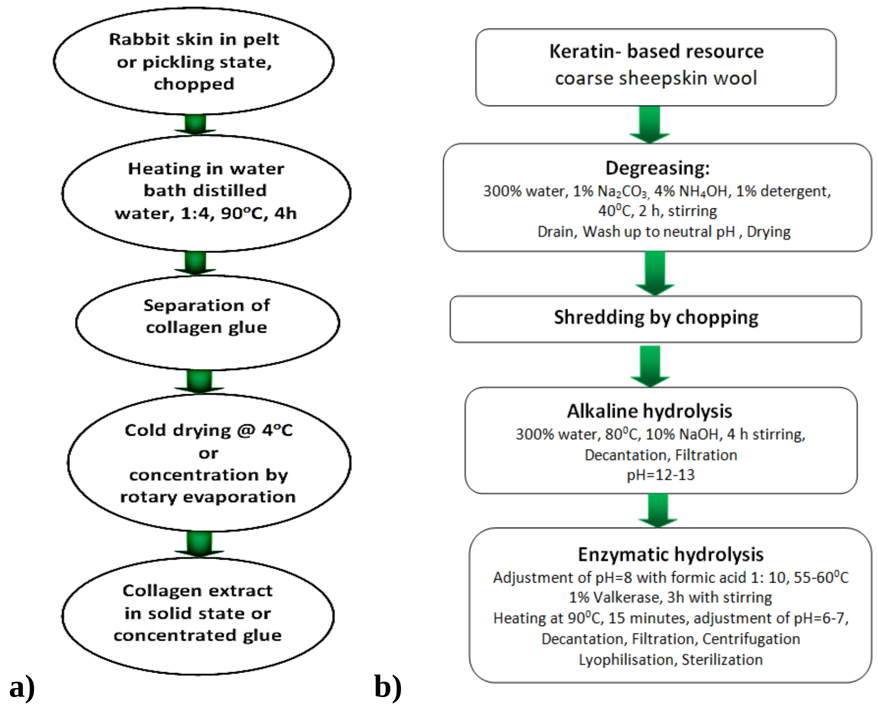

Fig.1: *Technology flowcharts of a) collagen and b) keratin extraction and refinery from leather byproducts or coarse wool*

The main characteristics of the protein extracts prepared from leather and wool byproducts were assessed in view of identifying the main properties of spinnable products and bioactive keratins for new biomaterial formulations. In this sense, the following characteristics were determined: dry substance (SR EN ISO 4684:2007), ash (SR EN ISO 4047:2002), total nitrogen and protein content (SR ISO 5397:1996), pH (STAS 8619/3:1990), viscosity (DV2T™, Brookfield), average particle size, polydispersity, conductivity and Zeta potential of 0.3% filtered solutions through 0.45µm filter (Zetasizer Nano ZS), Bloom test (TEX'AN TOUCH 50N texture analyzer). In order to test new protein extracts for biomaterials formulations, electrospinning mats were fabricated using acetic acid as a solvent for collagen extracts and alginate-polyacrylate gel base for keratin powder integration.

3. RESULTS

The collagen extracts were found to have good properties for nanofiber fabrication, rabbit collagen showing exceptional characteristics due to the known composition of C-terminal region with longer α1 chains due to alanine and arginine amino acids [4] and preservation of subunits like ß (dimer of α-chain), and Υ (trimer of α-chain) in gelatin state [5]. In Table 1 can be seen that the Bloom test is exceptionally high [6] for rabbit collagen as compared to bovine collagen and this made rabbit collagen more suitable for nanofiber spinning as compared to bovine collagen with a lower Bloom test value. The other characteristics like particle size, stability, polydispersity or conductivity showed to be less important as compared to bloom test value for nanofiber preparation by using only nontoxic solvents like acetic acid. Keratin powder with porous morphology was prepared and showed good compatibility for wound dressing formulations in gel forms.

Table 1: *Physical-chemical characteristics of collagen and keratin extracts for biomedical applications*

Characterization	Rabbit collagen	Bovine collagen	Keratin powder
Dry substance, %	6.21±0.35	7.69±0.35	95.54±0.35
Ash, %	0.81±0.24	7.67±0.24	15.07±0.24
Total nitrogen, %	15.78±0.34	12.87±0.34	11.07±0.34
Protein content, %	98.63±0.34	72.35±0.34	69.99±0.34
pH, pH units	6.1±0.10	6.3±0.10	6.85±0.10
Conductivity, mS/cm	0.128	2.05	0.969
Viscosity, cP	64,080	54,500	-
Particle size, average nm	321.9	284.9	394.9
Zeta potential, mV	-12.40	-9.77	-23.5
Polydispersity	0.851	0.411	0.604
Bloom test, g	657.4	215.3	-

Collagen gelatins processed from rabbit skin or bovine hide byproducts according to the scheme presented in Fig. 1a were tested for nanofibers manufacture by electrospinning (Fig. 2 a,c), confirming that the chosen extraction method was appropriate. Collagen nanofibers were successfully prepared by electrospinning in order to manufacture new wound healing patches as can be seen in Fig.1 d.

Keratin hydrolysate was solubilized from coarse sheep wool preliminary degreased, chopped and alkaline hydrolyzed, followed by the enzymatic refinery, centrifugation lyophilization (Fig.2b) and sterilization, according to the scheme presented in Fig. 1b. Keratin hydrolysate in lyophilized powder was compatible with different components used for designing new wound healing cream and gel formulations (Fig.1 e,f) proving that the extraction and refinery methods were suitable for the research aim.

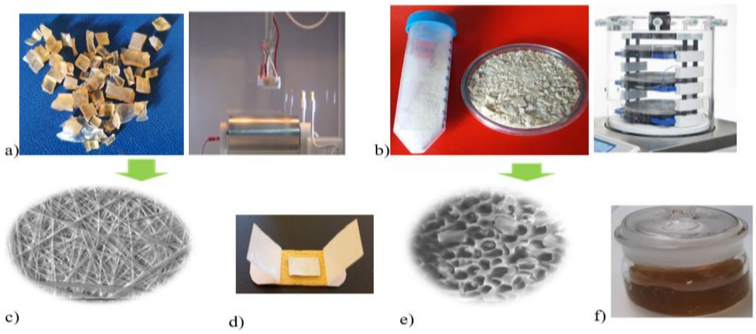

Fig.1 Images of a, c,d) collagen gelatin from rabbit skin processed by electrospinning in view of wound healing patches manufacturing and b,e,f) lyophilized keratin processed as a porous powder in view of new wound-healing creams or gels fabrication.

4. CONCLUSIONS

Collagen gelatins were extracted from rabbit skin and bovine pelts and showed spinnable properties in the acetic acid solvent which represents a safer alternative for organic solvents and more expensive native collagen use. Keratin hydrolysate was solubilized by alkaline hydrolyses and refined by enzymatic molecule tailoring and conditioned by lyophilization in porous powder form which showed good compatibility in wound-healing gel formulations. The research is in progress for in vitro and in vivo testing of new bioactive additives in new wound-healing dressings.

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