OBTAINING OF PROTEIN SUBSTANCES FROM LEATHER INDUSTRY SOLID WASTES

Prof. Dr. Bahri BAŞARAN[1], Tutku Dilara AKKAYA[1], Aykut Sancaklı[2], Demetra SIMION[3], Mıhaela Dına NICULESCU[3], MADALINA IGNAT[3]

[1] Ege University, Faculty of Engineering Department of Leather Engineering, 35100 Bornova –Izmir, Turkey

[2] Etkin Consultancy Co., Istanbul, Turkey

[3]National Research And Development Institute For Textiles And Leather (INCDTP) – Division Leather And Footwear Institute (ICPI), Street: No. 93 Minulescu Ion, box office 031215, Bucharest, Romania

ABSTRACT

Proteins are valuable food source used by all living things such as humans, animals, plants and microorganisms to perform their cellular and vital activities. However, an estimated one billion people worldwide suffer from many diseases due to protein deficiency. This problem is mainly due to the fact that 30% of children in Central Africa and South Asia consume small amounts of protein. Due to protein deficiency, various diseases such as growth delay, edema, liver failure, skin, hair, nail and hair problems occur in humans, and plants and animals are also unhealthy and inefficient. For this reason, it is necessary to prevent these problems by taking supplemental proteins. However, this process is limited due to factors such as shortage of protein source and uneconomical production. The leather industry is an industrial area that is known for its pollution due to the wastes generated as a result of production and tries to continue its production despite criticism. However, 3 types of leather waste, such as tanned, untanned leather and wool wastes, are recyclable due to they have a high protein content. It is possible to obtain very valuable animal-based proteins called collagen from tanned, untanned leather and keratin, from wool wastes. In this study, in addition to reducing the environmental burden by using leather waste as a raw material in order to eliminate the aforementioned protein deficiencies, it will also focus on cheap and effective protein production for a scarce protein source in terms of circular economy. The study from this paper was focused on gelatin extraction from untanned bovine hides waste and hydrolysates extraction from chrome leather waste by using different procedures.

INTRODUCTION

Leather industry generates huge amount of liquid and solid wastes and there are concerns that leather- making activity can have adverse impact on the environment. Out of 1000 kg of raw hide, nearly 850 kg is generated as solid wastes in leather processing. The tannery solid waste are as follows: fleshing, 50-60%; chrome shaving, chrome splits and buffing dust, 35-40%; raw skin trimmings, 5-7%; and hair, 2-5% [1]. Accumulation of these wastes lead to sludge problem and choking of treatment pipes and finally results in reduction in efficiency of treatment plant. Treatment of solid wastes is not cost effective, posing economic burden to the tanners [2]. The main component of solid leather waste consists of protein. Among several classes of proteins (collagen, elastin, keratin, glycoproteins, albumins and globulins), collagen is present in the largest amount and is responsible for the formation of leather by combination with tanning agents. If these protein and other chemicals, which are present in the chemical treated protein, are not utilized properly, it will pose hazardous pollution problem to the environment.

Collagen derivatives extracted from solid organic wastes and by-products are utilized for several industrial applications such as preparation of technical-grade gelatin, protein hydrolysates, collagen peptides and subunits [3-5]. Among many methods, the most commonly one used for collagen extraction is hydrolysis. Hydrolysis can be performed in acid media, alkaline media or by enzymes. When performed in alkaline media, hydrolysis produces protein (collagen hydrolysate or gelatin) in aqueous solution and a solid cake rich in chromium. Although collagen hydrolysate and gelatin extraction from leather solid waste has been already studied [6,7], its later possible applications must be more explored, since new alternatives for its use may be found.

The following diagram shows how solid wastes can be hydrolyzed and used as useful byproduct in many ways

The study from this paper was focused on gelatin extraction from untanned bovine hides waste and hydrolysates extraction from chrome leather waste by using different procedures. By the characterization of soluble and gellable protein fractions from leather industry solid wastes, which state problems for the environment, the obtained data can be used for new management and evaluation methods.

MATERIAL & METHODS

Materials: untanned bovine hides wastes and chrome leather wastes (shavings), formic acid (80%) and tartaric acid (≥99.5%), calcium hydroxide ((≥96%) from SC Cristal R Chim SRL and Alcalase 2.4L (Proteinase from *Bacillus licheniformis*, Subtilisin A) with specific activity ≥2.4 U/g,

Instrumentation: magnetic heater stirrer (AREC.T, Velp), vacuum pump filter system, UV/VIS spectrophotometer (V-550, JASCO), FT/IR-4200 spectrometer, texture analyzer, Zetasizer Nano-ZS.

Methods

The gelatins and collagen hydrolysates were analyzed by gravimetric methods, dry substance (SR EN ISO 4684:2006) and total ash (SR EN ISO 4047:2002), by volumetric methods, in terms of aminic nitrogen (ICPI protocol) and protein substance (SR ISO 5397:1996) and by potentiometric method for pH measurement (SR EN ISO 4045:2008).

The structural characterizations were made by FT/IR-ATR spectroscopy. Mechanical properties of gelatins were determined by texture analyzes. Particle size distribution and Zeta potential for collagen hydrolysates dispersions were determined by Dynamic Light Scattering (DLS) technique.

For each type of leather waste, two types of extraction protocols were developed, leading to 4 samples.

Gelatin extraction from untanned bovine hides wastes. The experiment was carried out in three stages with intermediate filtration (Experiment 1) and an experiment was carried out, at a ratio of 1:4 hides scraps/water, at 5.5-6.0 pH adjusted with 1:3 diluted formic acid, at 80˚C, by stirring for 6 hours with pH regulation (adjusted in 2 hours intervals) at every 2 hours, followed by cooling and static settling at 4-6oC for 16-24 hours, detachment of residues from the base and homogeneous gelatin collection (Experiment 2).

Collagen hydrolysate extraction from chrome leather waste (shavings). The experiment was carried out in two stages with intermediate filtration (Experiment 1) and another experiment was carried out non-stop running in single-stage by alkaline-enzymatic hydrolysis (Experiment 2). Both processes started with alkaline hydrolysis at a ratio of 1:8 leather/water, 10% calcium hydroxide, stirring for 5 hours at 80oC. At the end of the first stage, the pH was adjusted around 8.5-9.0 with tartaric acid solution, it was cooled to 60oC and treated with 1.25 mL Alcalase 2.4L. The dispersion was stirred for 2 hours at 60oC and heated under stirring regime, to 85oC-90oC for 10-15 minutes for enzyme deactivation and final vacuum filtrated.

Obtained samples were coded as follows:

GS-1 is the gelatin sample from the experiment in three stages.

GC-2 is the gelatin sample from single stage experiment (continuous process).

CH-1 is the collagen hydrolysate sample from one stage alkaline-enzymatic hydrolysis.

CH-2 is the collagen hydrolysate sample obtained by alkaline-enzymatic hydrolysis in two stages.

LEATHER WASTES ARE RAW MATERIAL, NOT WASTE

CONCLUSIONS

- According to the results obtained, it has been concluded that the gelatin GC-2 obtained by single stage process has higher bloom test value and has higher dry substance content than gelatin GS-1 extracted in three stages and hydrolysate CH-2, obtained by alkaline-enzymatic hydrolysis in two stages contains a higher quantity of small particles attributed to oligopeptides and free amino acid components.
- The gelatins and collagen hydrolysates obtained in this study showed appropriate characteristics of multi-purposes products to use solely or in combination with other ingredients for auxiliary materials formulations for leather processing, cosmetics, tissue engineering and agriculture.

ACKNOWLEDGEMENTS

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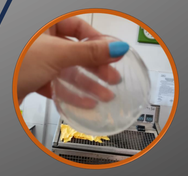

RESULTS

Table 1. Gelatin samples characteristics

Sample	Dry substance, %	Protein content, %	pH value	Ash content, %
GS-1	6.01	98.18	7.37	0.83
GC-2	7.61	79.02	6.09	0.66

It can be observed that the gelatin sample obtained in a three stages experiment (GS-1) has a higher content of protein.

The obtained results for the chemical characteristics of collagen hydrolysates samples are presented in Table 2.

Table 2. Collagen hydrolysates characteristics

Sample	Dry substance, %	Protein content, %	pH value	Ash content, %
CH-1	7.01	87.45	11.1	11.98
CH-2	8.17	88.74	9.3	6.73

The Zeta potential results of the collagen hydrolizates obtained are presented in Table 3:

Table 3. Size distribution and Zeta Potential for collagen hydrolysate samples

Sample	Size, nm (populations, average)				Pdl	Zeta Potential, mV
	Populations, nm/%			Average, nm		
CH-1	744/60	5/20	2/18	906	0.837	-9.1
CH-2	3/70	750/25	3966/3	627	0.359	-5.32

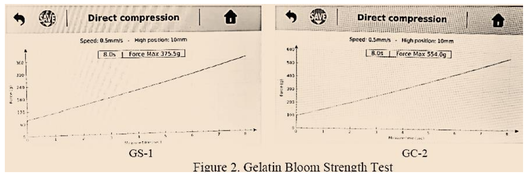

Figure 2. Gelatin Bloom Strength Test

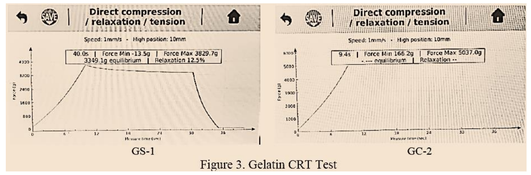

Figure 3. Gelatin CRT Test

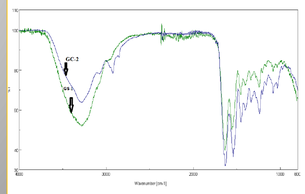

Figure 4. Overlapping FTIR-ATR spectra for gelatin samples: GS-1 and GC-2

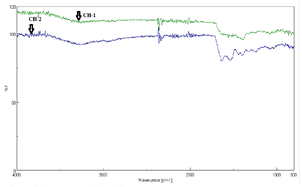

Figure 5. Overlapping FTIR-ATR spectra for collagen hydrolysates: CH-1 and CH-2 hydrolysates

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