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INFLUENCE OF REACTION CONDITIONS ON THE ALKALINE HYDROLYSIS OF CHAMOIS LEATHER WASTE

BĂLĂU MÎNDRU Tudorel[1], BĂLĂU MÎNDRU Iulia[1], PRUNEANU Melinda[1]

[1] "Gheorghe Asachi" Technical University of Iaşi, Romania, the Department of Chemical Engineering in Textile – Leather, Faculty of Textile Leather and Industrial Management, 53 Dimitrie Mangeron Avenue, postal code 700500, Iaşi, Romania

Corresponding author: Bălău Mîndru Iulia; E-mail: ibalau@tex.tuiasi.ro

Abstract: *The leather processing industry is known as an important source of environmental pollution, due to the large amount of waste generated. In this respect there are efforts concerning recovering and capitalizing of these wastes in order to subsequently use them in different applications. This work aimed to obtain collagen forms with convenient features starting from Chamois leather waste resulting from the buffing operation, by alkaline hydrolysis at different temperatures (60°C, 90°C, and 120°C) and reaction time (4, 6 and 8 hours), in a solution of 30% NH_4OH with a pH=11. The effects of the reaction conditions (temperature and reaction duration) on the hydrolysis yield and molecular weight of the extracted polypeptides were investigated. The working version no. 8 (temperature = 120^0C, and reaction time = 6h) was chosen as optimal for obtaining a protein product suitable from the point of view of hydrolysis yield and molecular weight, respectively. For this sample no. 8, tests for checking physical-chemical characteristics were carried out, and also the influence of the hydrolysis upon certain structural changes caused by the reaction conditions was investigated by IR analysis. The reaction conditions led to obtaining polypeptide mixtures with different molecular weights and polydispersity that can be used as auxiliaries to obtain composite materials or as additives for new building materials.*

Key words: *hydrolysis yield, polipeptide mixtures, structural characteristics, IR analyses.*

1. INTRODUCTION

The large amount of solid waste provided by the leather processing industry is a major concern of the researchers in the field. In this respect there are efforts concerning recovering and capitalizing of these wastes to be used as: soil fertilizers [1,2], auxiliaries for leather treatment [2-5], adsorbents for dyes and heavy metals [6,7], adjuvants for preparation of cosmetic products [8,9], biomaterials with applications in medicine [10,11], biosensors [12,13] auxiliaries for building materials [14-18], biodegradable plastic materials [19-21], biofuels [22], composite materials [23].

The hydrolysis of the collagen material as the main component of leather and/or leather waste is based on the general principle of the substitution reactions, according to which chemical function to be modified must be in a non-ionized state. This requires that the protein solutions to be brought at a pH different from the isoelectric point; in this case the consumption of acidic or basic functions of these solutions will take place by conducting the reaction in an acid or alkaline medium, respectively. Depending on hydrolysis conditions, the process leads to a change of the leather

substrate reactivity, a loss of proteinaceous material, associated with a specific molecular weight distribution of polypeptides and a corresponding hydrolysis yield [24].

This work aimed to obtain collagen forms with convenient features by alkaline hydrolysis of Chamois leather waste resulting from the buffing operation, and investigated the effects of the reaction conditions (temperature and reaction duration) on the hydrolysis yield and molecular weight of the extracted polypeptides. The structural changes caused by hydrolysis were also highlighted through IR analysis.

2. MATERIALS AND APPARATUS

The experiments were conducted using the following materials: Chamois leather waste with the following composition: dry matter: 87.98%; protein matter: 63.64%; total nitrogen (TKN): 10.13% (relative to dry matter); Chemical reagents and chemical auxiliaries as: trichloroethylene, NH_4OH, NaOH, nonionic surfactant Boron SE type; acetone, ethyl alcohol; distilled water.

Equipment used: VELP Scientifica UDK 132 with semi-automatic Distillation unit for total nitrogen determination; Krebs Viscometer; Digital balance KERN 474; Laboratory Oven MLW WS100 (Hungary); Laboratory centrifuge, DIGILAB – SCIMITAR Series FTS 2000 spectrometer with ZnSe crystal, 750 - 4000 cm^{-1} range, 4 cm^{-1} resolution.

3. EXPERIMENTAL

Scleroproteins from wastes generated in leather processing are present in a more or less distorted chemical form. Chamois leather waste (dust from buffing operation) is a strong stabilized protein structure as a result of two technological operations: a pre-tanning operation consisting of crosslinking of collagen matrix with glutaraldehyde, followed by the actual tanning using fish (cod) oils as tanning agent. Bringing scleroproteins to a usable form that can be subject to chemical modification requires their release from supramolecular matrix, and liquid phase transition. There are thus obtained the so-called processable protein shapes.

The easiest way to bring scleroproteins in liquid phase is by alkaline hydrolysis; in this respect the laboratory experiments consisted in nine working variants involving autoclaving at different temperatures (60°C, 90°C, and 120°C) and reaction time (4, 6 and 8 hours), in a solution of 30% NH_4OH with a pH=11. Thus, each sample consisting of 250 g of Chamois powder waste was subjected to a wetting/swelling and degreasing process with a non-ionic surfactant/trichlorethylene (1:5 wt/wt) mixture at 20ºC, for 24 hours, and then was dried in a thermo-regulated oven at 50ºC, followed by cooling to 20ºC. Subsequently, the degreased product was placed in a batch reactor (Fig.1) equipped with a stirring system, a vapor condenser and pressure automatic control at 2 atm, and subjected to hydrolysis process under the above-mentioned conditions.

The resulting mixture was cooled to 20ºC, and then separated by centrifugation at 6000 rpm for 30 min. The obtained liquid component was then subjected to the operations of extraction, separation, purification, and drying by lyophilization resulting thus a fine powder mixture. The process flow for obtaining the hydrolyzed polypeptide mixture is shown in Fig. 2.

4. RESULTS AND DISCUSSIONS

The hydrolysis yield of the lyophilized samples resulting from experiments was determined by the following equation:

$$Y = \frac{W_h}{W_i} 100 \quad \textbf{(1)}$$

where Y is the hydrolysis yield (%), W_h is the weight (g) of the dried hydrolysate powder and W_i is the weight (g) of the initial sample in the dry state.

The results obtained for the nine experimental variants are presented in Fig. 3. In order to determine the average molecular weight of the lyophilized protein mixture Sörensen method (AOAC 1995) [25] was used and the results are presented in Fig. 4.

Fig. 1: *Batch reactor for carrying out alkaline hydrolysis (autoclave)*

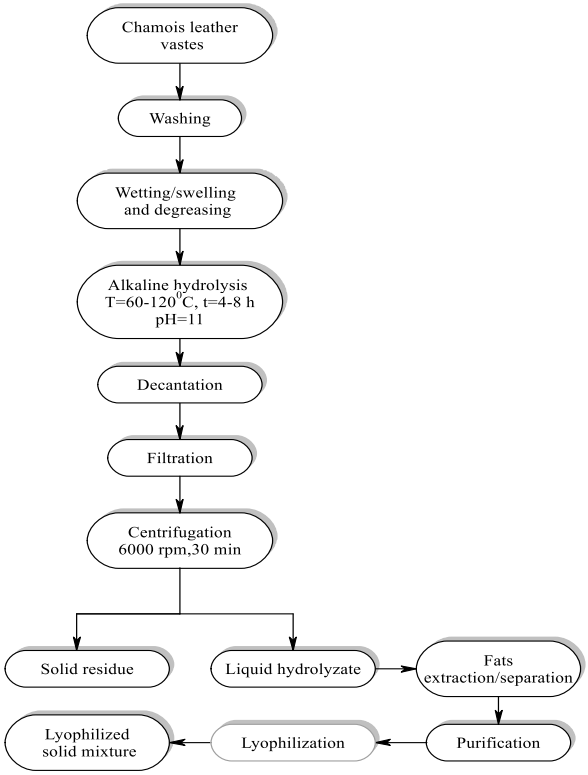

Fig. 2: *The sequence of operations for obtaining polypeptide hydrolysates by alkaline hydrolysis*

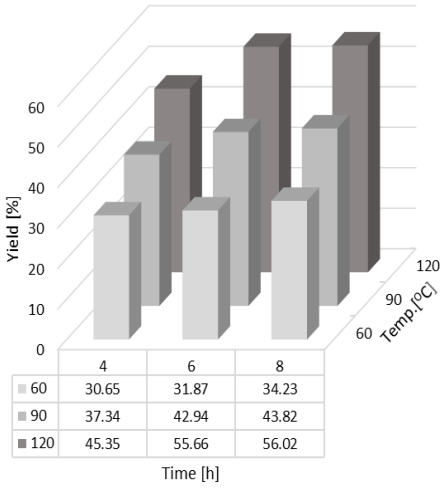

Fig. 3: *Dependence of hydrolysis yield on temperature and time*

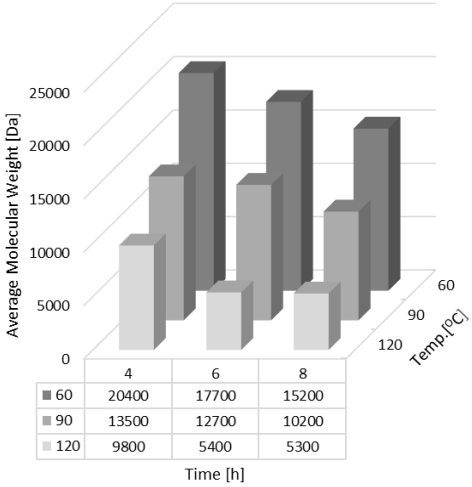

Fig. 4: *Dependence of average molecular weight on temperature and time*

Analyzing data in Figure 3 and 4 it can be seen a noticeable increase in the yield of protein hydrolysis with the increasing of reaction time and temperature; at the same time occurs a most advanced fragmentation of polypeptide chains demonstrated by molecular weight reduction. The composition of the amino nitrogen (according Sörensen method) of the extracted hydrolysates indicates the presence of polypeptides with average molecular weight of 5300 - 20400 Da.

Obtaining mean polypeptides with average molecular weight exceeding 10,000 Da, in conjunction with lower hydrolysis yields at a reaction temperature of 60^0C-90^0C, can be attributed to the so-called effects of "superficial erosion" due to possible the existence of cross-linking bridges resulting from the tanning of Chamois leather with cod oil. This range of molecular weights will be studied further in order to obtain products with potential applications in composite materials.

By increasing the hydrolysis temperature to 120^0C together with the reaction time (up to 8 hours) occur so-called "erosion in volume" processes that determine obtaining polypeptides with low molecular weight and low polydispersity. At this temperature, no significant differences were observed for the yield values and the average molecular weights obtained for a reaction time of 6 and 8 h, respectively. This aspect can be valorized to obtain new value-added products based on Chamois leather waste, with potential applications in building materials.

Given the above results, the working version no. 8 (temperature = 120^0C, and reaction time = 6h) was chosen as optimal for obtaining a protein product suitable from the point of view of hydrolysis yield and molecular weight, respectively. For sample no.8 tests for checking physical-chemical characteristics were carried out and the results are shown in Table 1.

Table 1. *Physical and chemical characteristics of hydrolyzed product no.8*

Property	**Value**
Average Molecular Weight , Da	3400
Density (related to dried protein form), g/cm^3	0.42
Components (relative to dry matter) %	
- total dry matter	34.62
- water soluble components	9.20
- total fats	19.39
- protein matter	36.79
Total nitrogen (Kjeldahl Method), %	14.62
Total ash, %	4.74
Hydrolysis yield **(**related to total protein), %	55.66

In order to study the the structural changes caused by the hydrolysis in an alkaline medium, IR analysis of the hydrolyzed sample no.8 along with Chamois leather powder as control sample was performed (fig.5).

Spectra from figure 5 show typical bands of collagen such as: the amide A band associated with the free N-H stretching vibration, and free water in the range of 3400 to 3440 cm^{-1}; amide B related to the stretch of CH_2, was found at ~ 2900 cm^{-1}; amide I band with its characteristic frequencies 1600-1700 cm^{-1}, mainly associated with the stretching vibrations of the C=O group along the polypeptide backbone, is a sensitive marker of the peptide secondary structure; amide II at 1500–1550 cm^{-1} corresponding to N-H bending vibrations; and amide III at 1200–1300 cm^{-1} related to C-H stretching. Normally, the amide I band is strong, the amide II band is weak and the amide III band is moderate. Area and location of individual peak is changed according to the changes in the structure of collagen. Fibril formation, which increases intermolecular interactions in collagen, is associated with broadening and slight shift to lower wavenumber of the amide A (at ~ 3300 cm^{-1}), as we can see from the spectrum of the control sample, suggesting that more NH groups were involved

in the hydrogen bonding, in contrast to the hydrolyzed samples where this band is more intense and occurs at ~ 3400 cm^{-1}; this could be also associated with an increase of free –OH groups content. On the other hand, the amide II and III adsorption show a lower signal for hydrolyzed sample that can be attributed to the conformational changes in the secondary structure of collagen, with the increasing of random-coil structures.

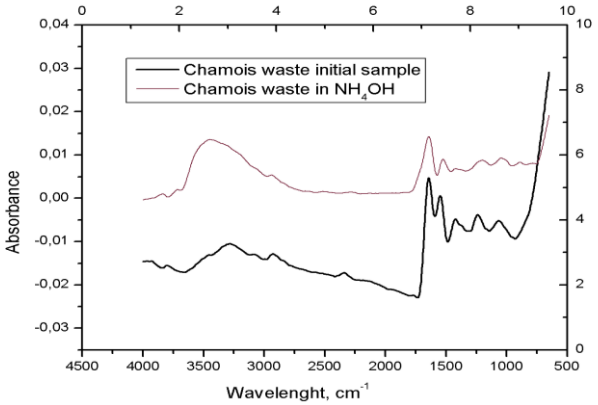

Fig. 5: *IR spectra of Chamois powder and his hydrolysate in NH_4OH (120^0C, 6 h reaction time)*

5. CONCLUSIONS

1. Starting from waste resulting from buffing operation of chamois leather, were obtained polydisperse colloidal solutions containing protein fractions with an average molecular weight of 5300 - 20400 Da.
2. A noticeable increase in the hydrolysis yield with the increasing of reaction time and temperature occurred, at the same time with a most advanced fragmentation of polypeptide chains, demonstrated by molecular weight reduction. The structural changes were also revealed by infrared analysis performed.
3. Polypeptides with low molecular weight and low polydispersity resulting at high temperature can be valorized to obtain new value-added products based on Chamois leather waste, with potential applications in building materials.

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