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EFFECT OF REACTION CONDITIONS ON THE HYDROLYSIS OF WOOL KERATINS

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

[1] Technical University " Gheorghe Asachi of Iaşi, România, Department of Chemical Engineering in Textile – Leather, Faculty of Textile Leather and Industrial Management, B-dul Dimitrie Mangeron, no. 53, postal code 700500, Iaşi, România, E-mail: ibalau@tex.tuiasi.ro

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

Abstract: *Every year millions of tons of keratinous wastes are generated worldwide, therefore from an economic and environmental point of view, it is worthwhile developing processes to use and reuse these resources. Many different procedures and methods can be applied to obtain keratin hydrolysates. Microwave heating is based upon the ability of a particular substance to absorb microwave energy and convert the electromagnetic energy to heat (kinetic energy). An extension of this is application in the field of proteomics (the study of proteins and in particular their structure and function). Large complex proteins can be broken into smaller parts by using microwave heating. This work aimed to perform microwave-assisted hydrolysis of wool keratin wastes in different experimental conditions, and compare it with a conventional method of alkaline hydrolysis, in order to study the efficiency of the treatment induced by this field on the reaction products, subsequently analyzed by specific methods. It was studied the influence of the reaction conditions (reaction time and working recipe) on the keratin hydrolysis yield, and also were analysed the structural changes induced by hydrolysis. A significant reduction of reaction time concurrent with a higher hydrolysis yield was obtained by microwave hydrolysis regardless of the alkali mixtures used; better results were obtained for 60 min treatment. IR analysis performed highlight the structural modifications induced by hydrolysis conditions, the process carried out by means of microwaves leading to a lower degradation of hydrolysis products.*

Keywords: *alkaline hydrolysis, microwave heating, keratin hydrolysates, IR analysis.*

1. INTRODUCTION

Keratin proteins are the major components of hair, feathers, wool and horns and represent an important source of renewable raw materials for many applications. Generally, the organic wastes produced from protein materials, can be processed by different physical or physical chemical methods for obtaining products with various utilizations: nutrients for agriculture [1-2], cosmetics (salves and creams based on keratin) [3], geotextiles, thermal and phonic insulating construction materials [4], nanofibrous materials with various applications for coatings, batteries, sensors, tissue engineering, medical textiles [5], etc. There are also studies for using protein residues of wool, feather and hair in mixtures for obtaining fiber-cement plates [6,7]. One group of promising candidates is the wool keratin wastes, which have the biodegradability and biocompatibility to support cell growth [8].

Every year millions of tons of keratinous wastes are generated worldwide, therefore from an economic and environmental point of view, it is worthwhile developing processes to use and reuse these resources. Wool contains up to 95% by weight of pure keratin, but this has high stability, distinctive physical properties and resistance to chemical attacks due to the presence of inter and intra-molecular disulfide bonds of cysteine amino acids in the wool structure. The cross-linking of the protein chains in wool due to disulfide bonds and inter and intra molecular hydrogen bonding results into the higher stability and lower solubility of keratin. Due to its non-reactive character and strong resilience, keratin can be processed only with great difficulty; for this reason it has to be partly hydrolyzed.

Many different procedures and methods can be applied to obtain keratin hydrolysates. Hydrolysis can be carried out in different process conditions, with different chemical agents. The traditional degradation is usually achieved by thermal hydrolysis in dilute acid or base, or by enzymatic hydrolysis. The decomposition products are almost entirely α-amino acids. [9] Keratin can be extracted by cleavage of the cystine disulfide bonds with reducing agents, such as thiols, to form cysteine, or by sulfitolysis with sodium sulfite to form cysteine and cysteine-S sulfonate or using oxidizing agents, such as peracetic acid, to form sulfonic acid [10]. However, the reductive or oxidative agents used for S-S cleavage, namely sulfites, thiols or peroxides, are harmful, often toxic and difficult to handle. Besides, the treatments result in severe degradation of the protein structure of keratin with reduction of the molecular weight and loss of mechanical properties. More recently, green hydrolysis processes, such as treatments with superheated water and by steam explosion, have been proposed with the aim of avoiding the use of harmful, often toxic, agents. Wool fibers were submitted to green hydrolysis with superheated water in a microwave reactor, in view of potential valorization of keratin-based wastes [11-13].

The microwave region of the electromagnetic spectrum is classified as that between 0.3 and 300 gigahertz (GHz). Microwaves, like all electromagnetic radiation, comprise oscillating electric and magnetic fields. Microwave heating is based upon the ability of a particular substance to absorb microwave energy and convert the electromagnetic energy to heat (kinetic energy). An extension of this is application in the field of proteomics (the study of proteins and in particular their structure and function). Large complex proteins can be broken into smaller parts by using microwave heating. By knowing the constitution of these smaller parts, it is possible to piece together the sequence of the original protein [14].

This work aimed to perform microwave-assisted hydrolysis of wool keratin wastes in different experimental conditions, and compare it with a conventional method of alkaline hydrolysis [16], in order to study the efficiency of the treatment induced by this field on the reaction products, subsequently analyzed by specific methods. It was studied the influence of the reaction conditions (reaction time and working recipe) on the keratin hydrolysis yield, and also the structural changes induced by hydrolysis were highlighted by IR analysis.

2. EXPERIMENTAL

In order to conduct the laboratory experiments, the following substances have been used: HCl (Merck), NaOH (grains), CH_3COOH, Na_2CO_3, NH_4OH, trichloroethylene, nonionic surfactant, isopropyl alcohol, ethyl alcohol, acetone, distilled water, pH indicator paper, and coarse wool waste.

The apparatuses used consisted of: thermostatic controlled stove, 750 W microwave oven, reaction vessels, centrifuge, IR-ATR spectroscopy using a DIGILAB–SCIMITAR Series FTS 2000 spectrometer with ZnSe crystal, 750÷4000 cm^{-1} range, 4 cm^{-1} resolution. For wool degreasing a classic Soxhlet installation was used.

In the first stage, wool wastes were subjected to dirt cleaning, then the fibers were cleaned and degreased in a solution of 3% Na_2CO_3 followed by an additional treatment with 0.1% nonionic surfactant solution. Then, the material thus treated was subjected to a final scouring with trichloroethylene in a Soxhlet apparatus for 8 hours; the resulting fibers were dried in a thermostatic controlled stove at 30ºC, then washed with a mixture of water: ethanol (1:1, v/v) and dried again at 25ºC for 24 hours. Subsequently, wool fibers were cut to 1÷2 mm in length in order to subject them to alkaline hydrolysis in different alkaline mixtures. Then wool samples with a weight of 5 g each have been held for 2 hours for prior swelling in 200 ml of the treatment mixture. In these experiments two hydrolysis solutions were used: a) 0.5N NaOH: isopropyl alcohol (3:1, v/v); b) 0.5N NH_4OH: isopropyl alcohol (3:1, v/v). The use of isopropyl alcohol is meant to facilitate a more rapid swelling of the protein matrix of wool.

In a first experimental variant, alkaline hydrolysis was performed with the two solutions in mild conditions (at 60ºC for 3 hours, according [15]); in the second, alkaline hydrolysis was conducted in a microwave oven (fig. 1). Thus, a glass bowl with 5 g of sample in the treatment mixture was put in a crystallizer with 300 ml of distilled water, for heat absorption purposes. The samples was treated under the following conditions: working cycles of 1 min with 30" break, total time 30 and 60 min, respectively, power 750 Watts, frequency 2,45 GHz. After the completion of the alkali hydrolysis, the samples were cooled to 25ºC, and then subjected to decantation/filtration for solide residue removal. The liquide phase was treated with a solution of HCl: distilled water (1:1, v/v) in order to precipitate the protein mixture, finding a wool pH_{iz} = 5. After the separation of the precipitates by centrifuging for 20 minutes with a rotational speed of 8000 rpm, the resulting supernatants were washed three times with a mixture of acetone: ethanol (1:1, v/v) followed by drying in a stove at 25ºC for 24 hours. The sequence of operations for obtaining keratin hidrolysates is shown in fig. 2. Hydrolyzed powder resulted from the liquid phase is shown in fig. 3.

3. RESULTS AND DISCUSSIONS

The extraction yield was determined by the following equation:

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

where Y is the extraction yield (%), W_h is the weight (mg) of the dried hydrolyzed powder and W_i is the weight (mg) of the initial sample in the dry state. The results obtained for the extraction yield are shown in fig. 4.

From fig. 4 it can be seen that the best values in terms of hydrolysis efficiency were obtained for the samples treated with the mixture of NaOH: isopropyl alcohol, both for the normal hydrolysis treatment and for the hydrolysis carried out by means of microwaves, comparing to the samples hydrolyzed in the same conditions but in alkaline medium using NH_4OH. This is due to increased swelling capacity of this mixture and also to pronounced disulfide bridges breaking in NaOH medium. NH_4OH has weaker basicity and lower swelling capacity and therefore lower access to the two areas (inner and outer) of the keratin matrix, but even in these conditions the yields obtained are satisfactory. Microwaves enhance hydrolysis processes concurrent with the significant reduction of reaction time regardless of the alkali agent used in mixtures. The best results for microwave-assisted hydrolysis yields were obtained for a reaction time of 60 min.

Fig. 1: *Reaction wessel inside of the microwave oven*

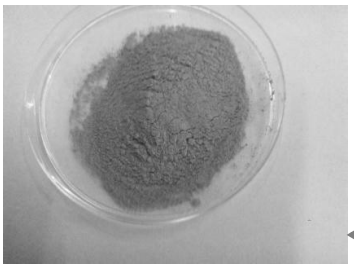


Fig. 3: *Hydrolyzed powder*

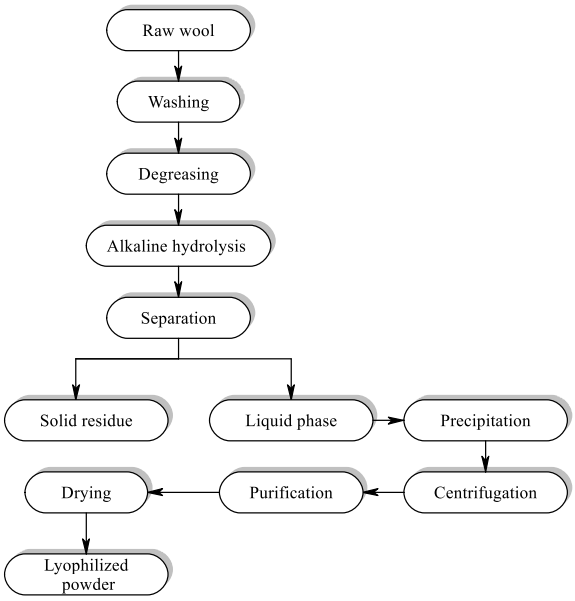
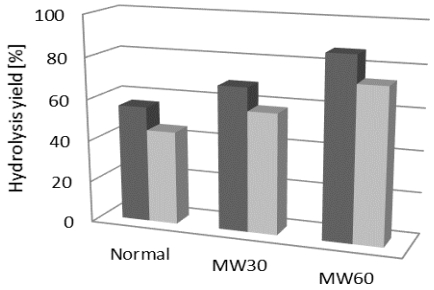


Fig. 2: *The operations flow for obtaining keratin hydrolysates*



	Normal	MW30	MW60
NaOH:isopropyl alcohol	56	69	87
NH4OH:isopropyl alcohol	45	58	74

Fig.4: *Dependence of hydrolysis yield on reaction conditions (Normal: conventional alkaline hydrolysis; MW30 and MW60: microwave-assisted hydrolysis at 30 min and 60 min, respectively).*

Spectral analysis

IR spectra of keratin hydrolysates in different alkaline media along with a control wool sample are shown in the fig. 5 and 6.

In the fig. 5 and 6 the spectra of control sample, show characteristic adsorption bands due mainly to the peptide bonds (-CO-NH-). Amide A and B are found at 3250–3300 cm^{-1}, connected with the N-H stretching vibrations and at ~ 2900 cm^{-1}, respectively, related to stretching modes of the C-H alkyl chains. The region between 1700-1500 cm^{-1} contains the most intense features in the IR spectrum, arising from the amide groups, predominantly from protein structures such as amide I band at ~1650 cm^{-1}, mainly due to the C=0 stretching vibration coupled to the in-plane bending of the N-H and stretching of C-N bonds; amide II band much weaker at ~ 1540 cm^{-1}, due to the coupled N-H in-plane bending and C-N stretching vibrations; and amide III which appears as a weak band at

1240-1260 cm^{-1} resulting from an in-phase combination of C-N stretching and N-H in-plane bending, with some contribution of C-C stretching and C=O bending vibrations.

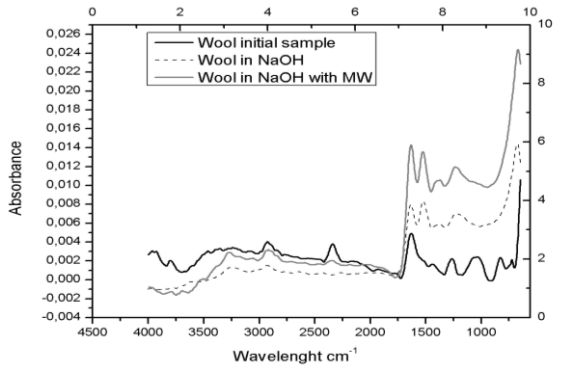


Fig. 5: *IR spectra of keratin hydrolysates in NaOH and in NaOH with microwave, 60 min.*

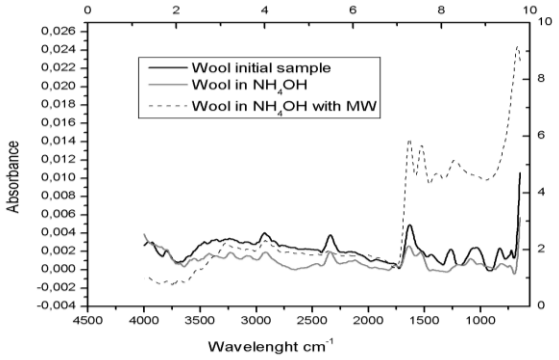


Fig. 6: *IR spectra of keratin hydrolysates treated in NH_4OH and in NH_4OH with microwave, 60 min*

In the spectra of all keratins extracted by hydrolysis, the signal strength in some areas decreases, and wool treated normally in NaOH mixture shows the most attenuated signal, which is due to the degradation of hydrolyzed keratin; the hydrolysis carried out by means of microwaves leads to a lower degradation. The amide I, II and III are the most sensitive probes for the conformational changes in the proteins. The literature [16] suggests that every chemical interference within a keratin fiber leads to a decrease in the share of a-helix as compared with a raw, untreated sample. The amide I adsorption which is known to be sensitive to the secondary structure of polypeptides shows a lower signal intensity for all treated samples, much more attenuated for the normal treated samples; this could be atributted to conformational changes in secondary structure, with a decrease in the α-helix structure, possible accompanied by an increase in the random-coil structure. What is interesting to note is that unlike this attenuation of the vibration of amide I, the signal in the spectral region of the amide II reveals no decrease in hydrolysate samples, which could be determined by a reforming of the α-helix of the amide II after treatment. Amide III remains relatively stable during microwaves hydrolysis. The intense peaks at ~ 1200 cm^{-1}, observed in the infrared spectrum of all keratin treated samples, are related, respectively, to the asymmetric and symmetric S-O stretching vibrations of the cysteine-S-sulfonate residues (bunte salts), formed through the reaction of cystine with sulfites during the hydrolysis of protein from wool. It can also be noted changes in the intensity of the characteristic peaks and of the surfaces in the area of the respective peaks at the wavelength 650 cm^{-1} which is characteristic for C–S bonds. Thus, it can clearly be seen the presence of this peak at the control samples. The alkali: alcohol mixtures treatment strongly affects the disulfide bridges, contributing to their partial dissolution or complete break during the treatment, highlighted on graphic by strong decrease of the peak intensity or even by its disappearance.

4. CONCLUSIONS

1. Hydrolysis yield of wool samples shows that the best results are obtained for the samples hydrolyzed in NaOH, both for the conventional hydrolysis treatment and for the alkaline hydrolysis carried out by means of microwaves, comparing to the samples hydrolyzed in the same conditions but in alkaline medium using NH_4OH.

2. A significant reduction of reaction time concurrent with a higher hydrolysis yield was obtained by microwave hydrolysis regardless of the alkali mixtures used; better results were obtained for 60 min treatment.
3. Even if microwave-assisted hydrolysis using NH_4OH mixtures results in lower reaction yield, it has the advantage that prevents the contamination of the protein hydrolysates, in contrast to hydrolysis in NaOH media that requires additional purification steps.
4. IR analysis highlights the structural modifications induced by hydrolysis conditions, the process carried out by means of microwaves leading to a lower degradation of hydrolysis products.
5. Further studies are considered in order to determine the molecular weights of keratin hydrolysates obtained in different conditions and to identify the possible applications fields.

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