THE INFLUENCE OF ALKALINE HYDROLYSIS OF WOOL BY-PRODUCTS ON THE CHARACTERISTICS OF KERATIN HYDROLYSATES

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Wool by-products represent an important bioresource for designing new biodegradable materials, alternative to synthetic products for regenerative medicine, cosmetic formulations, food package, fertilizers etc. Keratin is the main structural protein of wool, with highest concentration of cysteine as compared to other proteins. Research has been carried out on the physico-chemical properties of keratin hydrolysates obtained from wool by-products by using different concentrations of sodium hydroxide. The influence of sodium hydroxide concentrations from 8% to 25% on keratin hydrolysate characteristics was shown by chemical analyses, SDS-PAGE electrophoresis, particle size and Zeta potential measurements. It was concluded that the alkaline hydrolyse allows efficient solubilisation of wool by-products and producing of keratin hydrolysates with different molecular weights and cysteine concentrations. The use of 10% sodium hydroxide allows to obtain keratin hydrolysates with high molecular weight and higher concentration of cysteine meanwhile 15% sodium hydroxide represents the threshold for low molecular hydrolysates and low cysteine concentrations. The experiments showed the thresholds for sodium hydroxide concentration related to different properties of keratin hydrolysates.

Keywords: wool by-products, keratin hydrolyzate, alkaline hydrolysis.

INTRODUCTION

At present, there is a particular interest in research on biodegradable materials obtained from renewable sources such as proteins, polysaccharides and lipids. The use of biodegradable materials is the most effective solution to solve environmental pollution problems generated by synthetic polymers from petroleum origin (Dou *et al.,* 2016; Moore *et al.,* 2006; Mauri and Anon, 2006).

Keratin is a group of insoluble, filamentous proteins, with high-sulfur content forming the bulk of epidermal appendages such as wool, claws, horns, beaks, and feathers (Wang *et al.,*2016). Keratin extracted from wool is a biopolymer with excellent properties that synthetic polymers cannot achieve (Cardamone, 2010).

The study of biological materials opens the way for discovering new materials by providing principles and mechanisms obtained from the micro and macro multifunctional natural design (Meyers *et al*., 2008; Meyers *et al*., 2012).

The research of the biochemistry, structure, physical and chemical properties of keratin and keratinised materials is important for the development of new advanced ecological materials and models based on keratin (Holkar *et al.,* 2018). Keratin based products showed bioactive properties in many pharmaceutical, medicine, cosmetic and agriculture products (Sundar *et al*., 2010). Bhavsar and coworkers obtained nitrogen rich product from raw wool hydrolyzed with superheated water in a semi-industrial reactor (Bhavsar *el al.,* 2016).

Keratin can be extracted using strong acid, alkali, high concentration of salt solutions or expensive enzymes. Recent methods use ionic liquids for keratin extraction from wool, feathers and hair with improved yield (Ji *et al*., 2016).

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The paper presents the influence of sodium hydroxide concentration on keratin hydrolysate properties in view of designing different applications with improved ecological impact.

MATERIALS AND METHODS

Materials

The raw wool was purchased from sheep breeders, NaOH rotulis was purchased from Lach-Ner, Czech Republic, NH_3 (25% solution), Na_2CO_3 and the detergent were purchased from Chimreactiv, Romania.

Wool Degreasing and Hydrolysis

The raw wool was degreased in water in ratio 1:10 with 1% Na_2CO_3, 1% NH_3 and 1% detergent by shaking for 8 hours at 35°C in a FAVE drum. The alkaline hydrolysis of the degreased wool was made in a solution containing water in ratio 1:20 and NaOH at 80°C, by stirring for 3 hours, followed by decantation, filtration and separation (Fig.1). Five concentrations of NaOH: 8%, 10%, 15%, 20% and 25% were used and five keratin hydrolysates were obtained: KerNa8, KerNa10, KerNa15, KerNa20 and KerNa25. The solubilisation of wool was complete for alkaline hydrolysis processes with 10%, 15%, 20% and 25% NaOH and no waste wool was generated.

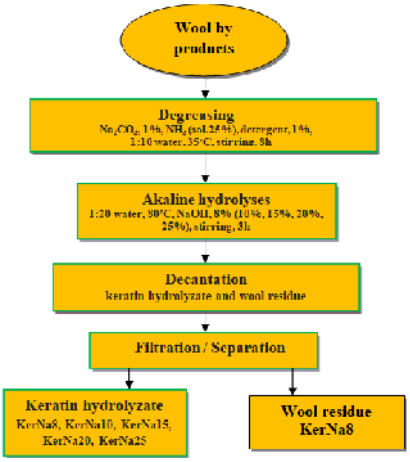

Figure 1. The flow chart for obtaining keratin hydrolysates

Keratin Hydrolysate Characterization

The obtained keratin hydrolysates (KerNa8, KerNa10, KerNa15, KerNa20 and KerNa25) were physically and chemically analyzed for: dry matter (SR EN ISO 4684:2006), ash (SR EN ISO 4047:2002), total nitrogen and protein (SR ISO 5397:1996), pH (STAS 8619/3:1990), aminic nitrogen (ICPI Method), cysteine and cystine sulfur (SR 13206:1994) content.

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Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed for molecular weight evaluation according to Laemmli's method (Laemmli, 1970) with a Vertical Dual-Gel Units (VWR Austria).

Particle size and Zeta potential of keratin hydrolysates were measured by using a Zetasizer Nano ZS (Malvern).

RESULTS AND DISCUSSION

The physical-chemical characteristics of keratin hydrolysates are presented in Table 1. The keratin hydrolysate obtained in the presence of 8% NaOH (KerNa8) is characterized by a significant percentage of protein content (79.86%), total nitrogen (14.18%) and highest concentration of cysteine (6.67%) and cystine sulfur (1.78).

It can be seen that the use of 15% sodium hydroxide substantially increase the aminic nitrogen concentration in correlation with keratin molecule breaking and cysteine and cystine sulphur decreasing. Table 1 shows that the wool hydrolyse with 10% sodium hydroxide can be a threshold for lower aminic nitrogen concentration (lower molecular weight) and higher cysteine and cystine sulphur concentration. The threshold for obtaining keratin hydrolysate with low molecular weight and low concentration of cysteine and cystine sulphur is represented by the wool hydrolysate with 15% sodium hydroxide.

Table 1. Physical-chemical characteristics of the keratin hydrolysates

No.	Characteristics, UM	KerNa8	KerNa10	KerNa15	KerNa20	KerNa25
1	Dry matter, %	4.16	4.31	5.16	4.68	5.22
2	Ash*, %	12.50	12.30	17.25	19.87	24.14
3	Total nitrogen*, %	14.18	14.62	13.18	13.03	11.30
4	Protein*, %	79.56	82.13	74.03	73.29	63.60
5	pH, units of pH	11.40	12.04	12.52	12.62	12.90
6	Aminic nitrogen**, %	0.60	0.93	2.09	2.04	1.81
7	Cysteine, %	6.67	5.74	2.38	1.03	-
8	Cystine Sulphur, %	1.78	1.53	0.63	0.27	-

*values reported on a dry matter basis, **value reported on protein basis

The physical-chemical analyses results are in agreement with SDS-PAGE electrophoresis. The bands for KerNa8 and KerNa10 are more prominent than the KerNa15, KerNa20 and KerNa25 bands (Fig.2) suggesting higher molecular weights, in agreement with lower values for aminic nitrogen (Table 1).

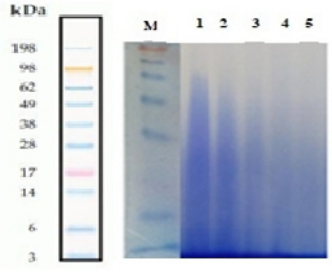

Figure 2. SDS-PAGE electrophoresis for keratin hydrolysate: M - marker, 1- KerNa8, 2 – KerNa10, 3 – NaKer15, 4 – KerNa20, 5 – KerNa25

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The molecular weights of keratin hydrolysates range between 3 and 62 kDa for KerNa8 and KerNa10, between 3 and 38 kDa for KerNa15 and KerNa20 and between 3 and 28 kDa for KerNa25.

DLS analysis presented in Table 2 shows the tendency of increasing of low size population of particles with increase concentration of sodium hydroxide used for wool solubilisation. The third particle size population with lowest dimension appears in the case of the most hydrolysed product, KerNa25. The association tendency of protein polydispersions can explain higher particles sizes in the case of the most hydrolysed keratin (KerNa25). The stability of keratin hydrolysates ranges between -22.8 mV and -27 mV, values which are not very far from the stability value of ± 30 mV.

Table 2. Particle sizes and Zeta potential for keratin hydrolysate

No	Keratin hydrolysate	Particle populations (%) and size (nm)						Pdl	Zeta potential, mV
		Majority population 3		Majority population 2		Majority population 1			
		Size	%	Size	%	Size	%		
1	KerNa8	-	-	110.9	17.3	648.6	82.7	0.699	-25.2
2	KerNa10	-	-	146.6	24.5	631.1	75.5	0.872	-25.5
3	KerNa15	-	-	131.8	27.1	1027	72.9	1	-27.0
4	KerNa20	-	-	110.4	24.0	539.9	76.0	0.754	-26.4
5	KerNa25	51.80	7.7	189.0	31.6	714.5	60.7	0.675	-22.8

Five keratin hydrolysates were obtained using different concentrations of NaOH, following the technological steps shown in Fig 1. The five keratin hydrolysates are individualized by their physico-chemical characteristics. The total nitrogen values are similar for KerNa8 and KerNa10. From KerNa15 it was observed a small decrease in content of total nitrogen (Figure 3) in correlation with ash content increase. The chemical action of the alkaline medium is manifested on wool keratin by the unbinding of the electrovalences, the breaking of the cystine bridge and the hydrolysis of the peptide bonds. These changes are largely influenced by the pH value of the reaction medium, the working time and temperature (Ifrim, 1979).

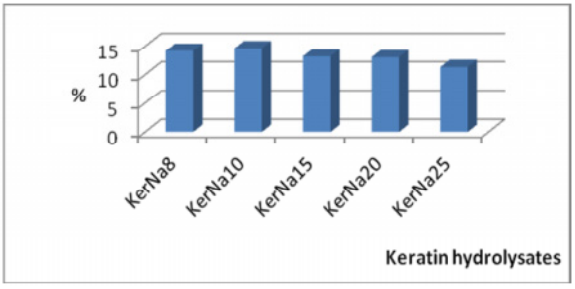

Figure 3. The influence of sodium hydroxide on the total nitrogen content of keratin hydrolysates

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Research result has highlighted that alkaline-solubilized keratins are poorer in sulfur with increasing NaOH concentration, by decreasing cystine sulphur content from 1.78% in KerNa8 to 0.27% in KerNa20. Aminic nitrogen values (Fig. 4) increase from 0.6% for KerNa8 to 2.09% for KerNa15, showing that alkaline hydrolysis has been enhanced the keratin molecule breaking.

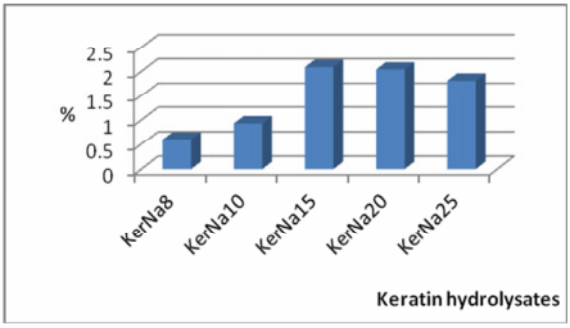

Figure 4. The influence of sodium hydroxide on aminic nitrogen content in keratin hydrolysates

Particle size measurement for the five obtained keratin hydrolysates confirms the chemical-physical analyses and SDS-PAGE electrophoresis results. In Figure 5 it can be seen the tendency of decreasing the share of high particle size and increasing of particle populations with low particle size.

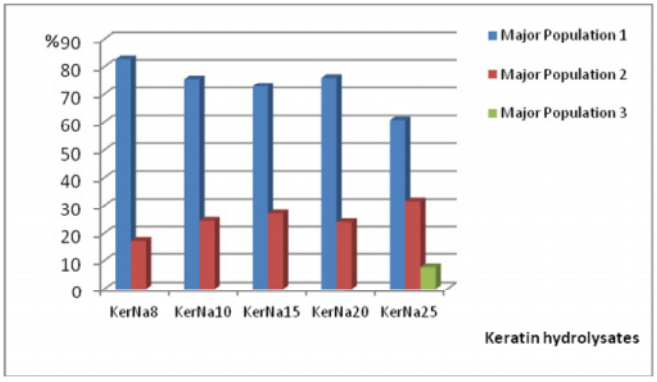

Figure 5. The influence of alkaline hydrolysis on particle size populations of keratin hydrolysates

CONCLUSIONS

The production of keratin hydrolysates from wool by-products represents an ecological route for designing new added values products: biodegradable food packages, organic biostimulants and fertilizers, biodegradable auxiliaries (flame retardants, tensides etc). The research results showed that the complete solubilisation of wool can be achieved by alkaline hydrolyse with sodium hydroxide starting with 10% concetration. Different keratin hydrolysates with high molecular weights and high

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cysteine content can be obtained by using 8% sodium hydroxide in atmospheric conditions of reaction. The hydrolyse of wool with 15% of sodium hydroxide seems to be a threshold for getting low concentration of cysteine and low molecular weight keratin.

REFERENCES

Bhavsar, P. *et al*. (2016), "Superheated Water Hydrolysis of Waste Wool in a Semi-Industrial Reactor to Obtain Nitrogen Fertilizers", *ACS Sustainable Chem Eng*, 4, 6722-6731, https://doi.org/10.1021/acssuschemeng.6b01664.

Cardamone, J.M. (2010), "Investigating the microstructure of keratin extracted from wool: Peptide sequence (MALDI-TOF/TOF) and protein conformation (FTIR)", *Journal of Molecular Structure*, 969, 97–105, https://doi.org/10.1016/j.molstruc.2010.01.048.

Dou, Y. *et al*. (2016), "The Structure, Tensile Properties and Water Resistance of Hydrolyzed Feather Cheratin-Based bioplastics, *Chin J Chem Eng*, 24(3), 415-420, https://doi.org/10.1016/j.cjche.2015.11.007.

Holkar, C.R. *et al*. (2018), "Valorization of keratin based waste", *Process Safety and Environmental Protection*, 115, 85–98, https://doi.org/10.1016/j.psep.2017.08.045.

Ifrim, S. (1979), *Chemistry of Wool* (in Romanian), Editura Tehnica, Bucharest.

Ji, Y. *et al*. (2014), "Extraction of keratin with ionic liquids from poultry feather", *Separation and Purification Technology*, 132, 577–583, https://doi.org/10.1016/j.seppur.2014.05.049.

Laemmli, U.K. (1970), "Cleavage of structural proteins during the assembly of the head of bacteriophage T4", *Nature*, 227, 680-685, https://doi.org/10.1038/227680a0.

Mauri, A.N. and Anon, M.C. (2006), "Effect of solution pH on solubility and some structural properties of soybean protein isolate films", *J Sci Food Agric*, 86(7), 1064-1072, https://doi.org/10.1002/jsfa.2457.

Meyers, M.A. *et al*. (2008), "Biological materials: structure and mechanical properties', *Prog Mater Sci*, 53, 1–206, https://doi.org/10.1016/j.pmatsci.2007.05.002.

Meyers, M.A., McKittrick, J. and Chen, P.Y. (2013), "Structural biological materials: critical mechanics–materials connections", *Science*, 339(6121), 773–9, https://doi.org/10.1126/science.1220854.

Moore, G.R.P. *et al*. (2006), "Influence of the glycerol concentration on some physical properties of feather cheratin films", *Food Hydrocolloid*, 20(7), 975-982, https://doi.org/10.1016/j.foodhyd.2005.11.001.

Sundar, S., Kundu, J. and Kundu, S.C. (2010), "Biopolymeric nanoparticles", *Sci Technol Adv Mater*, 11, 014104, https://doi.org/10.1088/1468-6996/11/1/014104.

Wang, B. *et al*. (2016), "Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration, *Progress in Materials Science*, 76, 229–318, https://doi.org/10.1016/j.pmatsci.2015.06.001.

https://doi.org/10.24264/icams-2018.I.4