ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)

Vol 10, Issue 5, May 2023

Enzymatic Hydrolysis of Chromed Leather Wastes and Application of Hydrolysate

[1] Karolis Kubilius, [2] Renata Biškauskaitė, [3] Virgilijus Valeika

[1] [2] [3] Department of Polymer Chemistry and Technology, Kaunas University of Technology, Kaunas, Lithuania
Corresponding Author Email: [1] karolis.kubilius@ktu.lt, [2] pawanmartha02@gmail.com, [3] virgilijus.valeika@ktu.lt

Abstract*— The leather industry employs waste from the food industry to produce diverse products, such as garments, footwear, upholstery, etc. However, leather processing use a lot of chemicals and water; this causes huge amounts of solid and liquid waste and has a negative effect on the environment. Chrome shavings are pose a threat of pollution. An alkali-enzymatic hydrolysis method was utilized to obtain collagen hydrolysate as a possible constituent for leather finishing formulations. The new proteolytic enzyme preparation Vilzim PRO Conc was applied for the hydrolysis of chromed shaving and the dependence of collagen hydrolysate properties on conditions of hydrolysis was explored. The application of the collagen hydrolysate for the finishing compositions was assessed as well. It was established that addition of 5% collagen hydrolysate to the finishing compositions increases tensile strength and relative elongation of films obtained from the compositions.

***Index Terms*—chromed shavings, enzyme, hydrolysis, leather finishing.**

I. INTRODUCTION

Chrome shavings are formed during shaving, which is performed to give the leather the desired thickness. Chrome shavings, one of the major proteinous solid wastes of leather industry are posing a pollution threat [1]. Nearly $0.8x10^6$t of chrome shavings are produced from leather industries annually [2].

The occurrence of chromium poses a major problem. Fortunately, there are methods for treating tannery waste containing this element. The storage and incineration of tannery wastes does not eliminate the risk of secondary pollution of the environment with chromium compounds [3]. On the other hand, chromed leather waste can also be easily enough recycled, as such waste consists of proteins and inorganic salts (chromium, etc.). The main problem is the removal of chromium from protein (collagen) in such a case [4].

The most common method of disposal of chromed leather shavings is depositing them in a landfill [5]. Unfortunately, there is a risk that trivalent chromium in landfills can oxidize to a more dangerous form.

The next often proposed technological solution to the problem of waste shavings utilization is the production of secondary or artificial leathers designed for footwear elements, fancy goods or non-woven fabrics as substrates for leather-like materials [6]. Unfortunately, such propositions postpone the problem of end-product recovery to the future and make it even more complicated, as the composition of the product to be recovered becomes even more complex than that of the leather waste used to produce it.

Another trend of utilization consists in detanning to recover chromium (III) compounds and processing the recovered collagen into gelatine, adhesives or protein hydrolysate (fodders, modified polymers, film-forming agents) [6]. The residues of chromium are less important when products are used as fillers for rubber or are reused for leather processing.

Usually, removal of chromium from chromed shavings takes place after detanning of the waste. Depending on the kind of the detanning agent applied, three fundamental means of chromium removal are known chemical and enzymatic methods [7]. Usually, chemical and enzymatic methods are combined. Firstly, the removal of chromium by chemical materials is carried out and, after that, the enzymatic hydrolysis of treated shavings is executed [8]. The two step alkali-enzyme hydrolysis methods are commonly utilized for improved protein recovery efficiency. Sasia *et al*. [4] studied a treatment method that involves first-step denaturation and degradation with alkali followed by inoculation with bating enzyme. Accordingly, hydrolysis using conventional bating enzyme could offer a low-cost alternative for the reuse of chrome-tanned shaving solid waste. Pati *et al*. [9] investigated protein extraction by protease mixed with a-amylase and found that there was a significant change in protein extraction by protease in the presence of a-amylase.

Collagen hydrolysate recovered from chrome-tanned leather through chemical treatments has also been used as feed for anaerobic digesters to produce biogas [10].

Usually, the application of the hydrolysates of chromed leather waste is limited due to the presence of chromium and other materials used for leather processing. While it is possible to separate collagen [11] from chromium by hydrolysis of the finished leather, the separation of collagen hydrolysate from the other aforementioned materials would be prohibitively cumbersome and not economically viable.

Probably, the best decision is to apply the hydrolysates for the processing of leather again. Zarlok *et al*. [12] demonstrated that CH produced from chromium-tanned hide waste by acid hydrolysis in the finishing mixes improved the hygienic properties of finished leather. Pahlawan *et al*. [13]

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)

Vol 10, Issue 5, May 2023

hydrolysed the scraps with NaOH at 90°C and the hydrolysis resulted in two forms of substance, liquid and solid. The liquid substance, as a protein binder has the potential to replace the common binder. It is important to know the quality of leather finished using the protein binder.

The aim of the current research was to explore the hydrolysis of chromed leather using new enzyme preparation; to apply the collagen hydrolysate obtained from chromed shavings for leather finishing and to establish the properties of the finished leather.

II. EXPERIMENTAL

A. Materials

All chemical materials used for the experiments were of analytical grade. Proteolytic enzyme preparation (EP) Vilzim PRO Conc "Baltijos enzymai" (Lithuania) with proteolytic activity at pH 11 and temperature 50°C – 1400 units/g. The chromed shavings from calf leather obtained from tannery "Kedainiu oda" (Lithuania) contained 3.7% Cr_2O_3 were used for hydrolysis. Acrylic resin RA-2312 ("Stahl", Netherlands); polyurethane acrylate mix polimerisate PU-Binder 5954 ("DLH LEDERTECHNIC", Austria) and polyurethane mix Filler 150 ("EstCo", Slovakia) were used for coating films formation.

B. Procedure

The hydrolysis of the shavings was carried out according to the method described by Cantera *et al*. (1994): 10g of shavings; 2% (% here and further from the mass of shavings) $Ca(OH)_2$; distilled water (1200%) and mixing for 30 minutes; NaOH 10% and mixed 2 hours; EP Vilzim PRO Conc and mixed 2, 4, 6 or 8 hours. Treatment temperature 50°C. After treatment, the liquid hydrolysate was filtrated from the solid part.

C. Analysis and testing

The enzymatic activity of EP was determined according to the Anson method [14]. The content of chromium in shavings and collagen hydrolysate (CH) was determined according to the ISO standard [15]. The method prescribes oxidation of the chromium presented in the solution into a hexavalent state using hydrogen peroxide, and an analysis of the solution by iodometric titration.

Nitrogen determination was carried out according to the Kjeldahl procedure [16]. The dynamic viscosity of CH was determined using Ubbelohde viscometer. The tensile strength and elongation of films were determined using dynamometer Zwick/Roell BDO-FBO.5TH ("ZwickRoell GmbH&Co" Germany).

D. Statistical analysis

All data were expressed as the average value of triplicate measurements. One sample was used for one measurement. The standard deviations did not exceed 5% for the values obtained.

III. RESULTS AND DISCUSSION

A. Determination of the activity of enzyme preparation

In order to determine the suitability of the new enzyme preparation Vilzim PRO Conc, the dependence of its proteolytic activity on the medium conditions was determined.

Fig. 1 and 2 show the dependence of the enzyme activity on the pH of medium and temperature. It is seen that both parameters significantly influence the activity of EP. The maximum activity of the EP is observed at pH 11 and 50°C – 1410 units/g, respectively, suggesting that this EP has a high proteolytic activity.

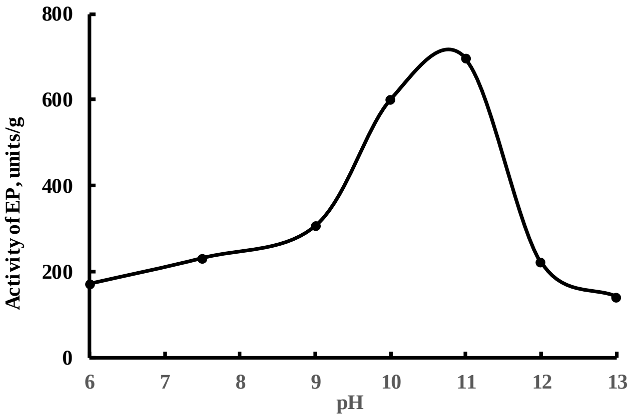

Figure 1. Dependence of the activity of enzyme preparation Vilzim Pro Conc on pH of medium at 30°C

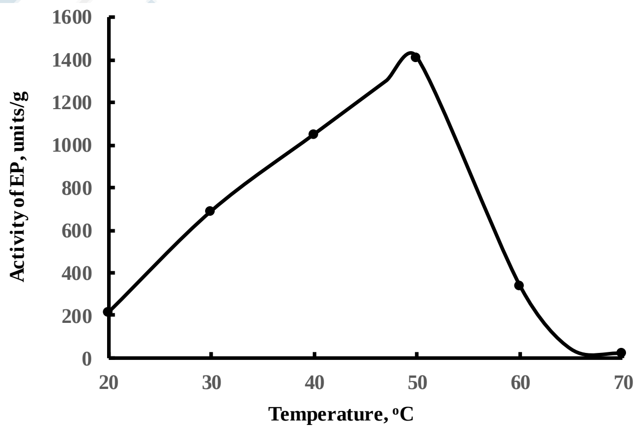

Figure 2. Dependence of the activity of enzyme preparation Vilzim Pro Conc on temperature at pH 11

B. Assessment of the influence of hydrolysis parameters on the properties of hydrolysis products

The next step was to establish the influence of hydrolysis conditions on the properties of CH. The influence of the amount of EP for enzymatic stage, temperature and enzymatic stage duration were investigated. To establish effect of enzyme preparation, various amounts of EP were used in the enzymatic stage of the hydrolysis process: 0 (control), 2, 4 and 6%. (Table I); temperature 50°C; duration 4 hours.

Table 1. Properties of collagen hydrolysate dependent on the amount of EP Vilzim PRO Conc used for hydrolysis.

Enzyme preparation amount, %	Collagen hydrolysate qualitative indexes		
	Cr_2O_3 concentration, mg/l	Nitrogen content, g	Dynamic viscosity, Pa·s
0	9.3	0.74	-
2	5.4	0.75	2.09
4	4.8	0.79	1.61
6	8.8	0.69	1.42

Assessment of the obtained data (Table 1) allows conclusion that 4% EP is appropriate amount to obtain qualitative CH.

Afterwards, the influence of the duration of the enzymatic stage on the properties of CH was determined (other conditions: 4% EP Vilzim PRO Conc; temperature 50ºC).

Table 2. Influence of enzymatic treatment duration on properties of collagen hydrolysate

Enzymatic stage duration, hours	Collagen hydrolysate qualitative indexes		
	Cr_2O_3 concentration, mg/l	Nitrogen content, g	Dynamic viscosity, Pa·s
2	5.8	0.76	1.51
4	4.9	0.78	1.62
6	3.0	0.81	1.70
8	2.8	0.96	1.71

Temperature was the last parameter, which influence on the CH properties was checked (Table 3). Other conditions: 4% EP; duration 4 hours.

Table 3. Influence of enzymatic stage temperature on properties of collagen hydrolysate

Enzymatic stage temperature, ºC	Collagen hydrolysate qualitative indexes		
	Cr_2O_3 concentration, mg/l	Nitrogen content, g	Dynamic viscosity, Pa·s
30	17.8	0.72	1.12
50	4.9	0.78	1.30
60	46.0	0.83	1.58

After hydrolysis, two products are produced: collagen hydrolysate and solid product containing mainly non-soluble compounds of chromium and calcium. The constitution of solid product is presented in Table 4. Conditions of enzymatic stage of hydrolysis: 2, 4 or 6% EP; temperature 50ºC; duration 4 hours.

The obtained results have shown that EP is characterized as an efficient agent leading to qualitative hydrolysis of collagenous wastes. The optimal conditions for the enzymatic stage of hydrolysis are: 4% EP Vilzim PRO Conc, temperature 50ºC; duration 4 hours.

Table 4. Influence of enzymatic treatment parameters on constitution of solid product of hydrolysis

Enzyme preparation amount, %	Cr_2O_3 amount, %*	Nitrogen amount, %*
2	82.5	24.2
4	93.3	27.2
6	94.8	32.3

* the percentages are calculated on the basis of the amount of the relevant material present in the waste before treatment

C. Properties of leather finishing films produced using collagen hydrolysate

The properties of the films obtained using compositions of conventional leather finishing materials and collagen hydrolysate obtained under the above hydrolysis conditions were further investigated.

To reach this aim, the CH obtained under optimal conditions of the hydrolysis was mixed with other film forming materials used in leather industry in various proportions; films from mixtures produced and mechanical properties of the films evaluated. The results are presented in Table 5 and Table 6.

The addition of 5% CH to the first composition (Table 5) improves the tensile strength of the film. Further increase of the CH amount in the composition leads to a decrease of relative elongation of the film.

The investigation of the properties of films obtained using the second composition (Table 6) shows similar results: the addition of 5% CH to the composition improves mechanical properties of the film.

Table 5. Properties of films formed from first composition mixtures.

Composition, %				Mechanical properties	
Collagen hydrolysate	RA-2312	PU-Binder 5954	Water	Tensile strength, N/mm^2	Relative elongation, %
0	12	23	65	6.36	463
5	12	23	60	9.15	482
10	12	23	50	9.6	444
21	12	23	44	10.6	385

Table 6. Properties of films formed from second composition mixtures.

Composition, %					Mechanical properties	
Collagen hydrolysate	RA-2312	PU-Binder 5954	Filler 150	Water	Tensile strength, N/mm^2	Relative elongation, %
0	11	22	6	61	4.44	582
5	11	22	6	56	5.27	645
9	11	22	6	52	4.17	508
18	11	22	6	43	4.22	529

IV. CONCLUSION

The collagen hydrolysate obtained from chromed shavings after alkali-enzymatic hydrolysis using enzyme preparation Vilzim PRO Conc can be used as a constituent in compositions with conventional leather finishing materials for leather finishing coatings. The addition of 5% collagen hydrolysate to the finishing compositions increases tensile strength and relative elongation of films obtained from the compositions. Further increase of the collagen hydrolysate content in the films leads to their worse mechanical properties

REFERENCES

[1] S. Shakilanishi, N. K. C. Babu, and C. Shanthi, “Exploration of chrome shaving hydrolysate as substrate for production of dehairing protease by Bacillus cereus VITSN04 for use in cleaner leather production”, J. Clean. Prod., vol. 149, pp. 797-804, 2017.

[2] J. R. Rao, P. Thanikaivelan, K. J. Sreeram, and B. U. Nair, “Green route for the utilization of chrome shavings (chromium-containing solid waste) in tanning industry”, Environ. Sci. Technol., vol. 36, pp. 1372-1376, 2002.

[3] K. Chojnacka, D. Skrzypczak, K. Mikula, A. Witek-Krowiak, G. Izydorczyk, K. Kuligowski, P. Bandrów, and M. Kułażyński, **“**Progress in sustainable technologies of leather wastes valorization as solutions for the circular economy,**”** J. Clean. Prod., vol. 313, 127902, 2021.

[4] A.A. Sasia, P. Sang, and A. Onyuka, (2019), “Recovery of Collagen Hydrolysate from Chrome Leather Shaving Tannery Waste through Two-Step Hydrolysis using Magnesium Oxide and Bating Enzyme”, J. Soc. Leath. Tech. Ch., vol. 103, pp. 80-84, 2019.

[5] E. M. Brown, M. M. Taylor, and W. N. Marmer, “Production and potential uses of co-products from solid tannery waste“, J. Am. Leather Chem. Assoc., vol. 91, pp. 270–276, 1996.

[6] A. Prepiorkowska, K. Chronska, and M. Zaborski, “Chrome-tanned leather shavings as a filler of butadiene–acrylonitrile rubber”, J. Hazard. Mater. Vol. 141, pp. 252-257, 2007.

[7] A. Pati, R. Chaudhary, S. and Subramani, “A review on management of chrome-tanned leather shavings: A holistic paradigm to combat the environmental issues”, Environ. Sci. Pollut. Res., vol. 21, 11266-11282, 2014.

[8] D. T. Adeoye, O. A. Adeyi, I. B. Kathir, and A. Ejila, “De-chroming of Chrome Tanned Leather Solid Waste using Modified alkaline Hydrolysis Process.”, Int. J. Eng. Res. Technol., vol. 3, pp. 620-623, 2014.

[9] A. Pati, R. Chaudhary, S. and Subramani, “Biochemical method for extraction and reuse of protein and chromium from chrome leather shavings: A waste to wealth approach”, J. Am. Leather Chem. Assoc., vol. 108, pp. 365-372, 2013.

[10] A. Pati, and R. Chaudhary, “Studies on the Generation of Biogas from Collagen Hydrolysate Obtained from Chrome Shavings by Alkaline Hydrolysis: A Greener Disposal“, Method. Res. J. Recent Sci., vol. 2, pp. 234-240, 2013.

[11] C. S. Cantera, C. A. Greco, M. De Giusti and R. Bereciartua, “Dechroming of shavings. Part 1. Enzymic Alkaline Treatment. Study of Variables”, Das Leder, pp. 265-270, November 1994.

[12] . Zarlok, K. Smiechowski, and M. Kowalska, “Hygienic Properties of Leather Finished with Formulations Containing Collagen Hydrolysate Obtained by Acid Hydrolysis”, J. Soc. Leath. Tech. Ch., vol. 99, pp. 297-301, 2015.

[13] I. F. Pahlawan, S. Sutyasmi, and G. Griyanitasari, “Hydrolysis of leather shavings waste for protein binder”, Book Series: IOP Conference Series-Earth and Environmental Science, vol. 230, Article Number: 012083, 2019.

[14] Standard USSR: GOST 20264-88. Enzyme preparations. Methods of determination of proteolytic activity.

[15] Standard ISO 5398-1:2007. Leather - Chemical determination of chromic oxide content - Part 1: Quantification by titration.

[16] A.A. Golovteeva, D.A. Kutsidi, and L.B. Sankin, Laboratornyj Praktikum po Khimiyi i Tekhnologiyi Kozhy i Mekha; Legkaiya i Pischevaiya Prom: Moscow, Russia, 1982. (In Russian)