KERATIN HYDROLYSATE FROM WOOL BY-PRODUCTS AS AN ADDITIVE FOR DYEING BOVINE LEATHERS

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ABSTRACT. In this research, the aim was to obtain keratin hydrolysate from wool waste from sheep breeders and use it in technologies for dyeing bovine hides. The keratin hydrolysate (KerNa), obtained by alkaline hydrolysis with sodium hydroxide, was physico-chemically analyzed, determining the protein substance in the amount of 80.65%, highlighting the bands specific to peptides and compounds with sulfur by FTIR spectroscopy and particle size by DLS technique, obtaining majority populations at 161 nm and 615 nm. Bovine hides were treated with keratin hydrolysate, in different stages of the dyeing process, and an increase in the dyeing resistance to wet and dry rubbing and the dyeing resistance to water drops was obtained, as well as the improvement of the specific color parameters. Leathers dyed with the use of the KerNa additive showed an increase in the friction resistance of the dyeing (grade 5/5) and brighter colors according to ISO Brightness. Treatments based on protein-rich keratin hydrolysate, applied in various stages of the dyeing process, interact with the leather's collagen or tanning agents, giving the finished leathers improved properties of shade, shine and softness. The good results obtained in the applications of keratin hydrolysate in the leather industry show that the keratin extract can be the basis for obtaining new biomaterials with various applications. The utilization of wool waste from animal husbandry leads to a decrease in the amount of waste and the prevention of environmental pollution.

KEY WORDS: keratin hydrolysate, dyeing resistance, wool by-products

HIDROLIZATUL DE CHERATINĂ OBȚINUT DIN SUBPRODUSE DE LÂNĂ UTILIZAT CA ADITIV ÎN VOPSIREA PIEILOR DE BOVINE

REZUMAT. În această cercetare s-a urmărit obținerea unui hidrolizat de cheratină din deșeuri de lână de la crescătorii de ovine și folosirea în tehnologii de vopsire a pieilor de bovină. Hidrolizatul de cheratină (KerNa), obținut prin hidroliză alcalină cu hidroxid de sodiu, a fost analizat fizico-chimic, determinându-se substanța proteică în valoare de 80,65%, punându-se în evidență benzile specifice peptidelor și compușilor cu sulf, prin spectroscopie FTIR și dimensiunea particulelor prin tehnica DLS, obținându-se populații majoritare la 161 nm și la 615 nm. S-au tratat piei bovine cu hidrolizat de cheratină, în diferite etape ale procesului de vopsire și s-a obținut o creștere a rezistenței vopsirii la frecare umedă și uscată și a rezistenței vopsirii la picătura de apă, precum și îmbunătățirea parametrilor specifici de culoare. Pieile vopsite cu utilizarea aditivului KerNa au prezentat o creștere a rezistenței la frecare a vopsirii (nota 5/5) și culori mai strălucitoare conform ISO Brightness. Tratamentele pe bază de hidrolizat de cheratină bogat în proteine, aplicate în diverse etape ale procesului de vopsire interacționează cu colagenul pielii sau agenții tananți conferind pieilor finite proprietăți îmbunătățite de nuanță, strălucire și moliciune. Rezultatele bune obținute în aplicațiile hidrolizatului de cheratină în industria de pielărie arată că extractul de cheratină poate sta la baza obținerii de biomateriale noi cu aplicații diverse. Valorificarea deșeurilor de lână din zootehnie conduce la scăderea cantității deșeurilor și prevenirea poluării mediului înconjurător.

CUVINTE CHEIE: hidrolizat de cheratină, rezistența vopsirii, subproduse de lână

L'HYDROLYSAT DE KÉRATINE OBTENU À PARTIR DE SOUS-PRODUITS DE LAINE UTILISÉ COMME ADDITIF DANS LA TEINTURE DES PEAUX DE BOVINS

RÉSUMÉ. Dans cette recherche, l'objectif a été d'obtenir un hydrolysat de kératine à partir de déchets de laine d'éleveurs ovins et de l'utiliser dans les technologies de teinture des peaux de bovins. L'hydrolysat de kératine (KerNa), obtenu par hydrolyse alcaline à la soude, a été analysé physico-chimiquement, pour déterminer la substance protéique à hauteur de 80,65%, mettant en évidence les bandes spécifiques aux peptides et composés soufrés, par spectroscopie FTIR et la taille des particules par DLS, obtenant de populations majoritaires à 161 nm et 615 nm. Les peaux de bovins ont été traitées avec de l'hydrolysat de kératine, à différentes étapes du processus de teinture, et une augmentation de la résistance de la teinture au frottement humide et sec et de la résistance de la teinture aux gouttes d'eau a été obtenue, ainsi qu'une amélioration des paramètres de couleur spécifiques. Les cuirs teints avec l'utilisation de l'additif KerNa ont montré une augmentation de la résistance au frottement de la teinture (grade 5/5) et des couleurs plus vives selon la norme ISO Brightness. Les traitements à base d'hydrolysat de kératine riche en protéines, appliqués à différentes étapes du processus de teinture, interagissent avec le collagène de la peau ou les agents de tannage, conférant aux peaux finies des propriétés améliorées d'ombre, de brillance et de douceur. Les bons résultats obtenus dans les applications de l'hydrolysat de kératine dans l'industrie du cuir montrent que l'extrait de kératine peut être à la base de l'obtention de nouveaux biomatériaux aux applications variées. L'utilisation des déchets de laine issus de l'élevage entraîne une diminution de la quantité de déchets et la prévention de la pollution de l'environnement.

MOTS CLÉS : hydrolysat de kératine, résistance à la teinture, sous-produits de la laine

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INTRODUCTION

Keratin is used in various applications based on natural biomaterials. In addition to the potential of assembling into organized structures, keratin is also composed of bioactive elements suitable for the initiation of biocompatible interactions with various substrates [1]. Keratin-rich waste pollutes the environment and is released in increasing quantities as by-products from agro-industrial or leather processing operations [2-4]. Keratin from renewable sources from the leather industry is abundant, available and easily harvested. Extracted keratin hydrolysates can be processed into various products using established techniques specific to a wide range of fields [5, 6]. Keratinous materials have a high protein content consisting of at least 17 amino acids that can be used in animal feed or agricultural fertilizers. Degradation of keratin waste can therefore provide an inexpensive source of protein and amino acids [7]. Keratinous materials, formed by specifically organized keratinized cells, filled with proteins, mainly fibrous keratins, are natural polymeric composites that present a complex hierarchical structure that varies from nano to centimeter: polypeptide chain structure, filamentous matrix structure, lamellar structure, structure in sandwich-type layers. These fibrous keratins are the most abundant and have an average molecular mass in the range of 40-60 kDa [8-10]. On the other hand, keratinous materials have a high cysteine content that differentiates them from other biopolymers, they are usually durable, hard and non-reactive with the natural environment. They provide mechanical support and various protective functions in the adaptation of vertebrates to the external environment [11]. Matrix proteins have a high content of cysteine, glycine and tyrosine amino acids. Those fractions with a high content of cysteine have a molecular weight in the range of 11-26 kDa, and those fractions with a high content of glycine and tyrosine residues have a molecular weight between 6 and 9 kDa [10, 12]. Keratins and keratinized materials are often discussed in terms of α- and β-keratins. Keratins are classified into α-keratins (amorphous model) from keratinized tissues and β-keratins, which are found in bird feathers [13, 14]. Since ordered structures (α- or β-keratins) predominate, keratinized materials are conveniently distinguished by these components. In addition, the two usual secondary structures, α-helix and β-sheet, are the two major internal support structures in proteins [15]; therefore, they are usually used to classify keratins [13].

The preparation of keratin hydrolysates from the waste wool generated by sheep breeding contributes to the design of new biomaterials and to the prevention of environmental pollution. The obtained keratin hydrolysates can be used for the creation of new biomaterials with multiple applications, as well as for the design of new ecological treatments for hides and furs with different functionalities, which will lead to a reduction in the consumption of petroleum origin auxiliaries and synthethic dyes.

EXPERIMENTAL

Materials and Methods

Wool waste was purchased from seep breeders from Constanța County, ammonium and sodium carbonate was supplied by SC Cristal RChim SRL, sodium hydroxide was supplied by Lachner. The bovine wet-blue leathers were prepared in Leather Research Department by standard technology. Keratin hydrolysate was obtained from wool waste by alkaline hydrolysis in the presence of sodium hydroxide. All reagents used were analytical grade.

Obtaining Keratin Hydrolysate

The wool was degreased, chopped and hydrolyzed by the alkaline method in the presence of sodium hydroxide, at 80°C, for 4

hours, in a steel reaction vessel with automated control of stirring and temperature to obtain soluble keratin hydrolysates, according to the scheme presented in Figure 1.

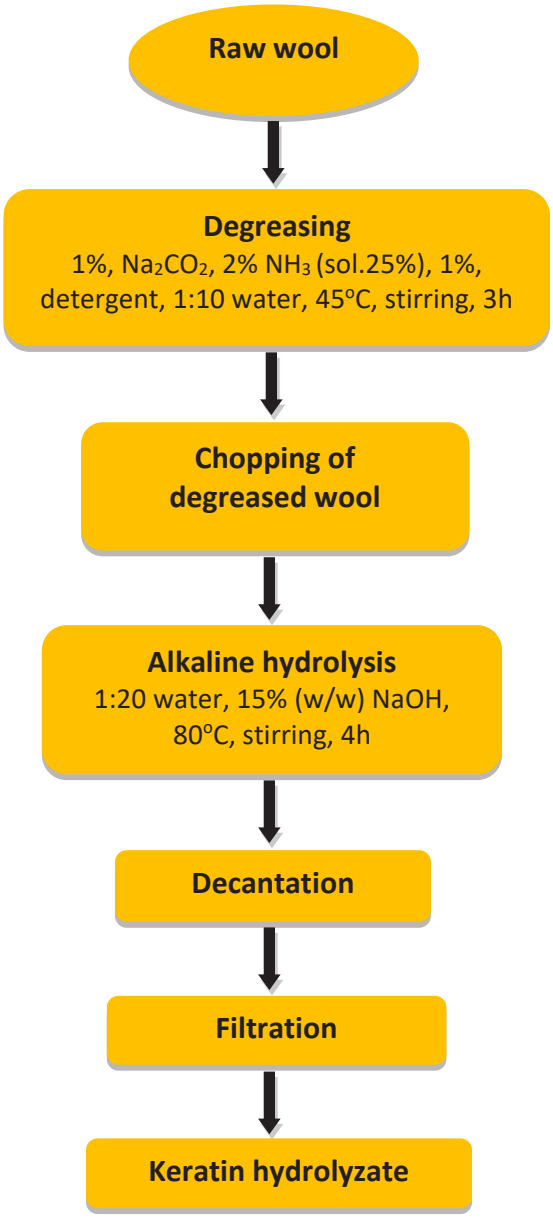


Figure 1. Technological scheme for obtaining keratin hydrolysate by alkaline hydrolysis

Characterization of Keratin Hydrolysate

The keratin hydrolysate obtained by alkaline hydrolysis was characterized by physico-chemical analyses regarding the content in dry substance (SR EN ISO 4684:2006), total ash (SR EN ISO 4047:2002), total nitrogen and protein substance (SR ISO 5397:1996), pH (STAS 8619/3:1990) and amino nitrogen (ICPI Method).

Determination of Particle Size by DLS Technique

The keratin hydrolysate obtained was analyzed using Malvern's Zetasizer Nano ZS equipment. Three measurements were made to determine the size of the particles and the Zeta potential.

Characterization of Keratin Hydrolysate by FTIR

Fourier Transform Infrared Spectroscopy (FTIR) spectra of samples were obtained using Nicolet iS50 FTIR spectrophotometer in the wave number ranging from 400 cm^{-1} to 4000 cm^{-1}, using attenuated total reflection (ATR).

Experiments on Leather Dyeing with Keratin Hydrolysate

The keratin hydrolysate was tested as additive for leather dyeing following the dosing stages presented in Table 2.

Analyses of Dyed Leathers Using Keratin Hydrolysate as Additive

The leather characteristics were assessed for colour fastness to cycles of to-and-fro rubbing (SR EN ISO 11640:2013), resistance to water drop (STAS 8259/3-68) and softness (SR EN ISO 17235:2002). The color characteristics were measured by using the Datacolor CHECK II portable spectrophotometer provided with a color analysis software.

RESULTS AND DISCUSSIONS

Physico-chemical Analysis of Keratin Hydrolysate

The physico-chemical characteristics of keratin hydrolysate obtained by alkaline hydrolysis with sodium hydroxide is rich in total nitrogen content (13.93%) and protein substance (80.65%), which can influence the results obtained in the treatment of bovine leathers in dyeing processes (Table 1).

Table 1. Physico-chemical characteristics of keratin hydrolysate

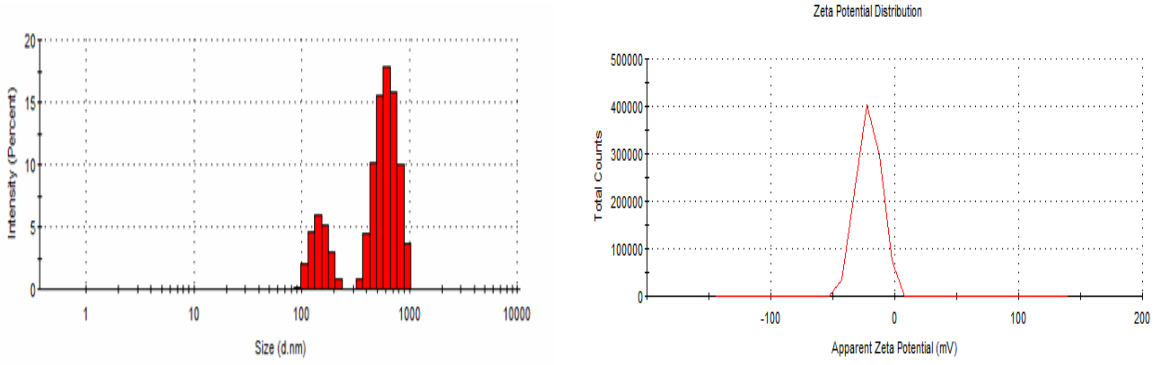
Characteristics	KerNa
Dry substance, %	15.72
Ash, %	9.73
Total nitrogen*, %	13.93
Protein substance*, %	80.65
pH, pH units	10.10
Aminic nitrogen**, %	0.49

* reported to the dry substance.

** reported to the protein substance.

Determination of Particle Size by DLS Technique

Two major populations were highlighted at 164nm and 615 nm (Figure 2 (a)), with an average particle size of 776 nm and a polydispersity of 0.707. The measured Zeta potential has a value of -20.9mV (Figure 2 (b)).



(a) (b)

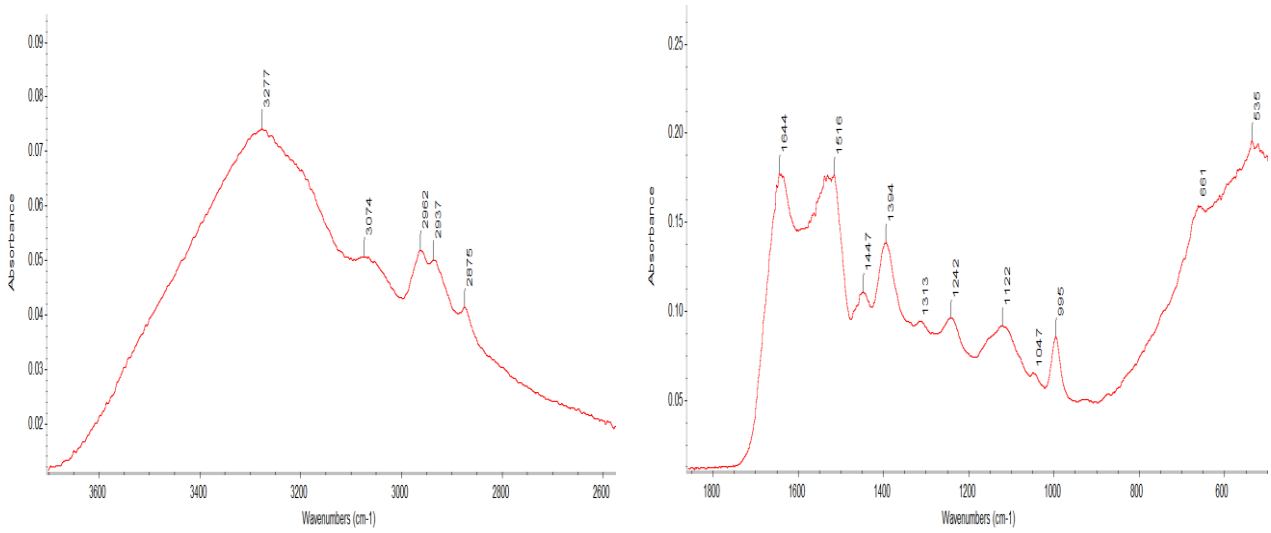
Figure 2. Histogram of particle sizes distribution (a), and Zeta potential (b) of keratin hydrolysate (KerNa)

Characterization of Keratin Hydrolysate by FTIR

The infrared absorption spectrum of keratin hydrolysate (KerNa) shows characteristic absorption bands attributed to peptides (-CONH), known as amide I, amide II, and amide III as well as sulfur compounds [16-18]. The broad band appearance indicates hydrogen bonding at 3277 cm^{-1}. Absorption bands at 3074 cm^{-1}, 2962 cm^{-1}, 2937 cm^{-1} can be attributed to the differences between O–H, N–H and stretching modes C -H [19-21].

Amides present in the IR spectrum absorption bands located at 1644 cm^{-1} and 1516 cm^{-1} due to the coupling between the valence vibration of the C=O bond and the deformation vibration of the N-H bond; the two bands are usually described as the amide I band (due mainly to the valence vibration of the C=O bond) and the amide II band (due to a vibrational coupling between an N-H bond deformation frequency and a C-N bond elongation frequency) [22-24].

The band at 1242 cm^{-1} corresponds to C-N stretching vibrations and C=O bending which are identified as amide III [17, 25, 26]. The fingerprint area (1400-400 cm^{-1}) of the IR spectrum contains numerous absorption bands that characterize the molecular structure as a whole (skeleton vibrations: deformation, combination, harmonics) [27, 28].



(a) (b)

Figure 3. FTIR spectrum of the keratin hydrolysate obtained with sodium hydroxide (KerNa), (a) 3600 cm^{-1} - 2600 cm^{-1}, and (b) 1800 cm^{-1} - 500 cm^{-1}

Thiols (R-SH) present in the IR spectrum a characteristic absorption band determined by the valence vibration of the S-H bond located at 2675 cm^{-1}. This band is of weaker intensity and less influenced by the association through hydrogen bonds. In thioethers (R-S-R), the absorption band determined by the valence vibration of the C-S bond appears at 661 cm^{-1} and 535 cm^{-1}, corresponding to the cross-linking disulfide group [17].

The valence vibration of the S=O bond (stretching vibration) produces a band located at 995 cm^{-1} characteristic of sulfoxides (R_2SO) and two bands at 1242 cm^{-1} and 1122 cm^{-1}, and 1313 cm^{-1} and 1394 cm^{-1}, respectively, characteristic of sulfones (R_2SO_2) [17, 29].

The valence vibration of the >C=S bond produces an absorption band at 1047 cm^{-1} [17,

29]. The band at 2675 cm^{-1} is in the area where the absorption bands appear due to the vibration of the -S-H bond (elongation vibration) in the structure of thiols [17].

Testing and Evaluating the Functionality of Keratin Hydrolysate with Applications in Bovine Leather Dyeing

Treatments with keratin hydrolysate obtained by hydrolysis with sodium hydroxide were applied in various stages of the dyeing operation on samples of bovine hides. Three samples of bovine leather were dyed in the presence of keratin hydrolysate in different stages of the dyeing operation compared to a control sample (Table 2). The experiments regarding the use of keratin hydrolysate as a dyeing additive were carried out according to the scheme and notations described in Table 2.

Table 2: Bovine leather samples obtained and application of the keratin hydrolysate in the technological process

No.	Samples obtained, notations	Description
1	V_0	Control sample (without keratin hydrolysate)
2	V_N	Treatment with keratin hydrolysate in the neutralization stage
3	V_1	Keratin hydrolysate treatment in the dyeing stage
4	$V_{1(5.2)}$	Treatment with keratin hydrolysate at pH=5.2, before fixing the dye

The dyed bovine leathers obtained (Figure 4) were analyzed for determining the fastness of the dyeing to dry and wet rubbing, measuring the softness and determining the resistance of the dyeing to water drop (Table 3).

Table 3: Fastness characteristics of dyed bovine leathers

Characteristics			U.M.	Samples / Determined values			
				V_0	V_N	V_1	$V_{1(5.2)}$
Colour fastness to cycles of to-and-fro rubbing	dry	20 cycles	marks	5/5		5/5	5/5
		50 cycles		5/4.5	5/4	5/5	5/5
		100 cycles		5/4.5		5/5	5/5
		500 cycles		5/3		5/4	5/4
	wet	20 cycles		4-5/3		5/4-5	5/4
		50 cycles		4/2	4/2-3	4-5/4	4/3-5
		80 cycles		2/1		3-4/4	3-4/3
Softness, ring opening Ø 25mm			-	2.0	3.0	3.9	3.3
				2.2	3.1	3.3	3.4
				2.2	3.2	3.0	3.5
				Average:	Average:	Average:	Average:
				2.1	3.1	3.4	3.4
Resistance to water drop			marks	3	4	4-5	4

The fastness characteristics measured show an improved resistance of the dyeing, compared to the control.

Samples V_0 V_N V_1 $V_{1(5,2)}$

Figure 4. Bovine leathers dyed in the presence of KerNa hydrolysate

Measurement of Leather Color Characteristics with the Datacolor CHECK II Portable Spectrophotometer

With the help of the CIEL*a*b* and CIEL*C*h software systems, specific to the equipment, the chromatic coordinates of the color of each leather sample dyed in the presence of keratin hydrolysate were obtained. The determined color parameters are: L* represents the degree of lightness, the maximum value for L* is 100 (perfect white), and the minimum is 0 (perfect black); a* represents the shade between green (-a*) and red (+a*); the negative value b* is blue while a positive one is yellow; C* (chroma) provides indications regarding the purity (high values) or complexity (low values) of the mixture; h is the hue angle, it reflects the proportion between the chromatic components a* and b*.

The ISO Brightness Index is a parameter calculated from spectrophotometric data that describes the color change of a test sample. This test is most commonly used to evaluate color changes in a material caused by exposure to the open air, real or simulated, but also to determine the degradation produced by light [30].

The measurement of the ISO Brightness index shows an accentuated brightness for the samples obtained with KerNa treatment with a maximum in the case of the sample $V_{1(5,2)}$ (Figure 5).

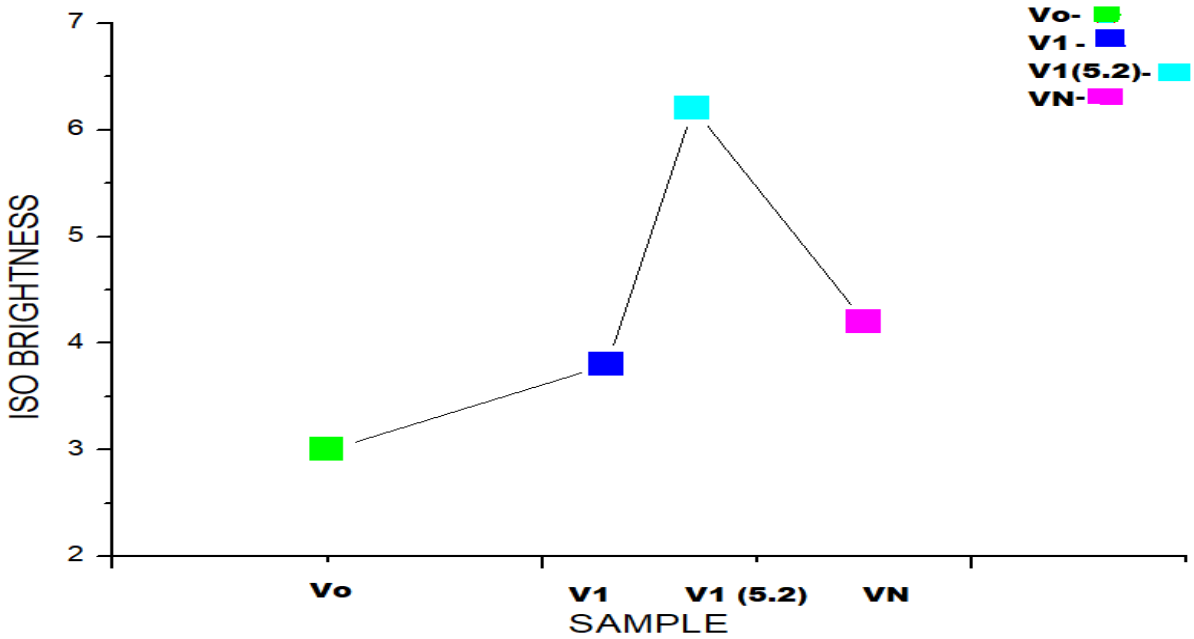


Figure 5. ISO Brightness index for bovine leathers

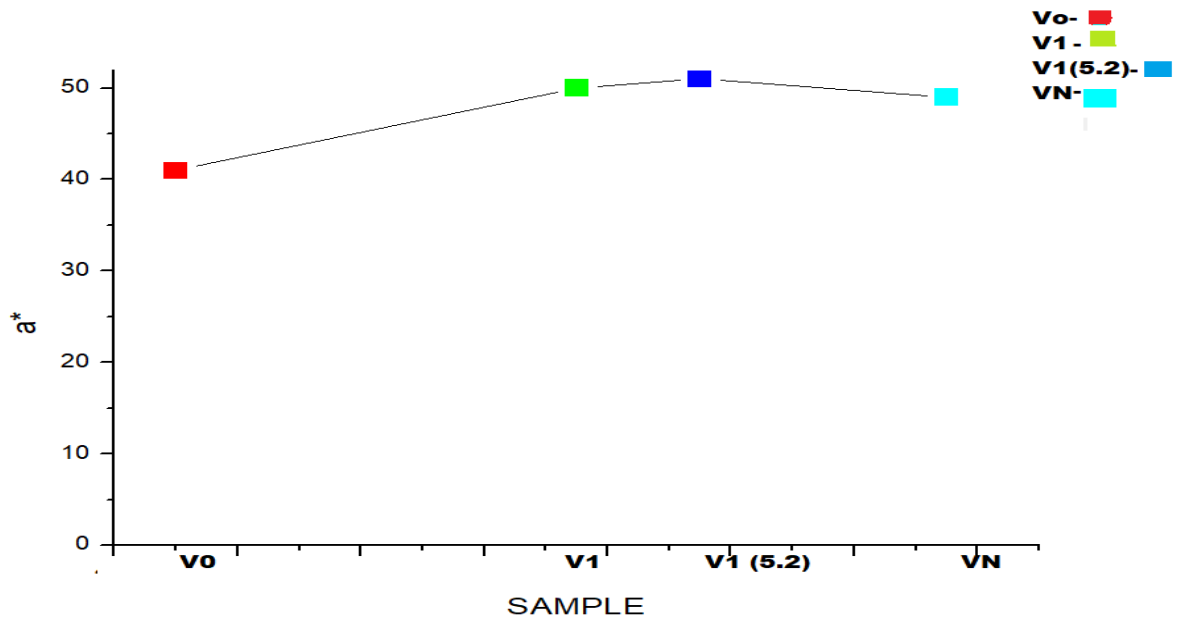


Figure 6. Chromatic component a* for bovine leather

The measurement of the chromatic component a* shows higher values (red component) for the bovine leather samples treated with KerNa compared to the control, V_0 (Figure 6).

By extracting keratin from the wool waste through alkaline hydrolysis in the presence of sodium hydroxide, keratin hydrolysate (KerNa), rich in protein substance, was obtained, which, through specific treatments applied in leather dyeing technology, leads to improved characteristics of finished products. Bovine leathers were treated with keratin hydrolysate, KerNa, in different stages of the dyeing process and an increase in the dyeing fastness to wet and dry rubbing and the dyeing resistance to water drop was obtained, as well as the improvement of the specific color parameters.

CONCLUSIONS

The main conclusions of this research can be summarized as follows:

- Keratin hydrolysate (KerNa), obtained by alkaline hydrolysis with sodium hydroxide from wool by-products, was tested and evaluated in treatments on bovine leathers during the dyeing operations, obtaining an increase in the rubbing resistance of the dyeing (mark 5/ 5) and brighter colors given by ISO Brightness.
- Treatments based on protein-rich keratin hydrolysate, applied in various stages of the leather processing process, interact with the leather's collagen and tanning materials, giving it improved properties of color and softness.
- The good results obtained in the applications of keratin hydrolysate in the leather industry show that keratin extract can be the basis for obtaining new biomaterials with various applications.
- The utilization of wool by-products leads to a decrease in the amount of waste and the prevention of environmental pollution.

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