BIODEGRADABLE RETANNING MATERIAL FROM TANNERY TRIMMING WASTE: EXTRACTION, PREPARATION AND APPLICATION

Md. Farhad ALI[1*], Papia HAUQUE[2], Md. ABDULLA-AL-MAMUN[1], Tamanna JAMAN[1]

[1]Institute of Leather Engineering and Technology, University of Dhaka, Bangladesh

[2]Dept. of Applied Chemistry and Chemical Engineering, University of Dhaka, Bangladesh

Received: 18.09.2023 Accepted: 04.12.2023 https://doi.org/10.24264/lfj.23.4.7

BIODEGRADABLE RETANNING MATERIAL FROM TANNERY TRIMMING WASTE: EXTRACTION, PREPARATION AND APPLICATION

ABSTRACT. The leather industry has long grappled with environmental challenges associated with waste disposal. One ton of wet-salted hides or skins generates approximately 650 kg of solid waste. The majority of trims and hair waste remain underutilized, with hair often discarded or used inefficiently. We initiated the creation of a retanning agent devoid of formaldehyde by utilizing discarded tannery trimmings, with the goal of promoting a circular economy approach in the leather processing industry in our sustainability efforts. Optimal hydrolysis conditions were determined, involving an alkaline (3.75% w/w NaOH) pre-treatment followed by thermal hydrolysis at 100 °C for 5 hours, effectively maximizing the use of trimmings. Similar treatments were applied to hair waste, particularly red sheep hair, yielding successful hydrolysis. Keratin hydrolysate-g-methacrylic acid (KH-g-MA) copolymers were synthesized through in situ polymerization, employing hydrolysates in a redox system. Characterization was carried out using dynamic light scattering and Fourier transform infrared spectroscopy. These newly developed copolymers were applied as retanning agents in the leather industry, enhancing leather qualities such as fullness, grain tightness, and color brightness. Moreover, they improved leather mechanical strength and reduced the need for post-tanning chemicals. This innovative approach not only addresses solid waste issues but also contributes to greener leather processing, thereby fostering a more sustainable environmental landscape.

KEY WORDS: retanning material, trimming waste, retanning application

MATERIAL DE RETĂBĂCIRE BIODEGRADABIL DIN ȘTUȚUITURĂ DE LA TĂBĂCIREA PIEILOR: EXTRACȚIE, PREGĂTIRE ȘI APLICARE

REZUMAT. Industria de pielărie se confruntă de mult timp cu provocările de mediu asociate cu eliminarea deșeurilor. O tonă de piei sărate umede generează aproximativ 650 kg de deșeuri solide. Majoritatea deșeurilor cum ar fi ștuțuitura și părul rămân neutilizate, părul fiind adesea aruncat sau folosit ineficient. S-a realizat un agent de retăbăcire fără formaldehidă utilizând ștuțuitură, cu scopul de a promova o abordare a economiei circulare în industria de prelucrare a pieilor în vederea dezvoltării sustenabilității. S-au determinat condițiile optime de hidroliză, ceea ce a presupus o pretratare alcalină (3,75% g/g NaOH) urmată de hidroliză termică la 100°C timp de 5 ore, maximizând în mod eficient utilizarea ștuțuiturii. S-au aplicat tratamente similare deșeurilor de păr, în special blana de oaie roșie, realizând cu succes procesul de hidroliză. S-au sintetizat copolimeri de tip hidrolizat de cheratină-g-acid metacrilic (KH-g-MA) prin polimerizare in situ, folosind hidrolizatele într-un sistem redox. Caracterizarea a fost efectuată utilizând difuzia dinamică a luminii și spectroscopia în infraroșu cu transformată Fourier. Copolimerii dezvoltați au fost aplicați ca agenți de retăbăcire în industria de pielărie, îmbunătățind calitățile pielii, cum ar fi plinătate, compactizare și strălucirea culorii. Mai mult, au îmbunătățit rezistența mecanică a pielii și au redus necesarul de substanțe chimice post-tăbăcire. Această abordare inovatoare nu numai că rezolvă problemele legate de deșeurile solide, ci contribuie și la o prelucrare mai ecologică a pielii, încurajând astfel un peisaj ecologic mai durabil.

CUVINTE CHEIE: material de retăbăcire, ștuțuitură, aplicare la retăbăcire

MATÉRIAU DE RETANNAGE BIODÉGRADABLE ISSU DES ROGNURES DE TANNERIE : EXTRACTION, PRÉPARATION ET APPLICATION

RESUME. L'industrie du cuir est depuis longtemps confrontée aux défis environnementaux liés à l'élimination des déchets. Une tonne de peaux salés humides génère environ 650 kg de déchets solides. La majorité des rognures et des poils restent sous-utilisées, les poils étant souvent jetés ou utilisés de manière inefficace. Nous avons lancé la création d'un agent de retannage sans formaldéhyde en utilisant des rognures de tannerie, dans le but de promouvoir une approche d'économie circulaire dans l'industrie du cuir dans nos efforts vers le développement durable. Les conditions optimales d'hydrolyse ont été déterminées, impliquant un prétraitement alcalin (3,75 % p/p de NaOH) suivi d'une hydrolyse thermique à 100 °C pendant 5 heures, maximisant efficacement l'utilisation des rognures. Des traitements similaires ont été appliqués aux poils, en particulier aux poils de mouton roux, réalisant avec succès le processus d'hydrolyse. Des copolymères d'hydrolysat de kératine-g-acide méthacrylique (KH-g-MA) ont été synthétisés par polymérisation in situ, en utilisant des hydrolysats dans un système redox. La caractérisation a été réalisée par diffusion dynamique de la lumière et spectroscopie infrarouge à transformée de Fourier. Ces copolymères nouvellement développés ont été utilisés comme agents de retannage dans l'industrie du cuir, améliorant les qualités du cuir telles que la fermeté, l'adhérence de fleur et la luminosité des couleurs. De plus, ils ont amélioré la résistance mécanique du cuir et réduit le besoin de produits chimiques de post-tannage. Cette approche innovante résout non seulement les problèmes de déchets solides, mais contribue également à un traitement du cuir plus écologique, favorisant ainsi un paysage environnemental plus durable.

MOTS CLÉS : matériau de retannage, rognures, application de retannage

[*] *Correspondence to: Md. Farhad ALI, Associate Professor, Institute of Leather Engineering and Technology, University of Dhaka, Bangladesh, email: farhad.ilet@du.ac.bd*

INTRODUCTION

The leather industry plays a pivotal role in the modern economy, employing various chemical and mechanical processes to transform animal hides and skins into a chemically and physically stable material. This transformation facilitates the production of a wide range of goods that cater to diverse human needs. Typically, the primary raw materials for the leather industry are hides and skins, which are byproducts of the meat and meat product industry. It is worth noting that the leather sector could be recognized as environmentally friendly to some extent due to its utilization of waste products derived from the cattle processing business [1].

Traditional leather production methods are notorious for causing significant pollution, characterized by noxious odors, organic waste discharge, and excessive water consumption [2]. These methods generate waste products comprising solids and release offensive odors due to the degradation of the proteinaceous material found in hides and skins. Additionally, the process emits gases such as NH3, H2S, and CO2. The conversion of raw animal skins and hides into finished leather results in the creation of various waste materials, some of which pose environmental hazards [3].

Annually, the leather industry discards approximately 850 kg of raw hides, a stark contrast to the 150 kg of hides needed to produce one tonne of leather. Within the tanning industry, common operations contribute to a solid waste output ranging from 2% to 5%. These operations include shaving (50%-60%), chrome shaving (35%-40%), chrome splits, and buffing dust (35%-40%), as well as skin trimming (7%). Notably, eighty percent of this waste originates from the beam house, nineteen percent from the tanning process, and the remaining one percent from the finishing process [4].

Within the tannery industry, only 25% to 55% of the produced materials are deemed usable, leaving a substantial amount as waste [5]. The consumption of disposable goods plays a significant role in contributing to pollution. To address this issue, organic and fermentative compounds have been explored for the hydrolysis of protein-containing waste, resulting in the production of bioactive peptides [6]. The chemical composition of goatskins primarily comprises water (60-70%), proteins (25-30%), fat (2%), and miscellaneous elements (1%) [7]. Notably, collagen protein, a vital component derived from this solid waste, constitutes a substantial portion, accounting for 25–30% of animal protein [8].

Slaughterhouses, meat processing plants, poultry processing plants, and the wool textile industry generate substantial amounts of waste materials containing fibrous proteins such as collagen, elastin, and keratin. These wastes encompass viscera, skin remnants, meat trimmings, bones, blood, bristle, horns, hooves, animal hair, feathers, and low-quality raw wool unsuitable for spinning [9, 10]. The degradation processes of keratin-rich waste materials from diverse industries stand to gain significant advantages from the incorporation of keratinolytic enzymes [11].

Alpha- and beta-keratins, characterized by dominant motifs of alpha helices and beta sheets, respectively, exhibit dense packing and stability owing to a high degree of inter- and intra-molecular disulfide bonds, non-covalent hydrogen bonds, and hydrophobic interactions [12]. Alpha-keratins, with molecular weights ranging from 60 to 80 kDa and low sulfur content, constitute 50–60% of wool fiber, primarily located in the cortex. Meanwhile, beta-keratins, which predominantly compose the protective cuticle, are present in smaller quantities. Gamma-keratins, making up 20–30% of wool fiber and having molecular weights ranging from 11 to 28 kDa, boast a high sulfur concentration, facilitated by their abundant cysteine and tyrosine content, serving as disulfide crosslinkers and aiding in maintaining cortical superstructures [13].

Keratin structures are water-insoluble, enzyme-resistant (pepsin, trypsin, and papain), and mechanically stable [14]. However, waste materials rich in keratin do not accumulate in nature due to the presence of keratinolytic bacteria capable of breaking down keratin. In environments where keratinous materials accumulate, keratinolytic bacteria are frequently identified [15].

Notably, a defining feature distinguishing keratin from other structural proteins like elastin and collagen is its high cysteine concentration at the protein's N- and C-terminal regions. Based on cysteine content and, consequently, disulfide linkages, keratins can be categorized into soft and hard keratins. Epidermal keratins, classified as soft keratins, possess a cysteine concentration of less than 2%. On the other hand, hard keratins include feathers (with a cysteine concentration of 4–8%) and hair and wool (with a cysteine level of 10–17%) [16].

MATERIALS AND METHODS

Materials

Wet-salted goat raw trimmings and sheep hair waste were collected from the Tipara tannery in Hemayetpur, Savar, Dhaka. Various chemicals, including sulfuric acid, sodium hydroxide, hydrogen peroxide, methacrylic acid (MAA), potassium persulfate ($K_2S_2O_8$), and sodium metabisulfite ($Na_2S_2O_5$), were purchased from Hatkhola scientific store in Dhaka. The leather preparation process exclusively utilized industrial-grade chemicals. To determine the required chemical proportions for the leather-making process, wet blue leather from a goat was first weighed after being shaved.

Pretreatment of Wastes

To remove any salts or other contaminants that had settled on the surface of the hair and trimmings, we conducted a thorough washing with distilled water. Subsequently, we air-dried the hair, and the purified trimmings were soaked in water until they reached a moisture content of approximately 65% by weight. These two raw materials were then utilized to create a retanning agent.

Optimizing the Hydrolysis of Raw Trimmings

After rehydration, the initial step involved subjecting the raw trimmings to a pre-treatment procedure, which included the application of either acid, alkali, or alkali-hydrogen peroxide, followed by thermal hydrolysis. To carry out this process, the rehydrated trimmings, maintaining a dry weight to water ratio of 1:10, were broken down into smaller segments and immersed in water along with one of the designated pre-treatment agents for a period of 6 hours. Trimmings that had not been pretreated were hydrolyzed at 100°C for five hours. In Fig. 1 we see the entire experimental procedure carried out. The hydrolysis process required a quantity of chemicals equal to the dry weight of the raw trimmings.

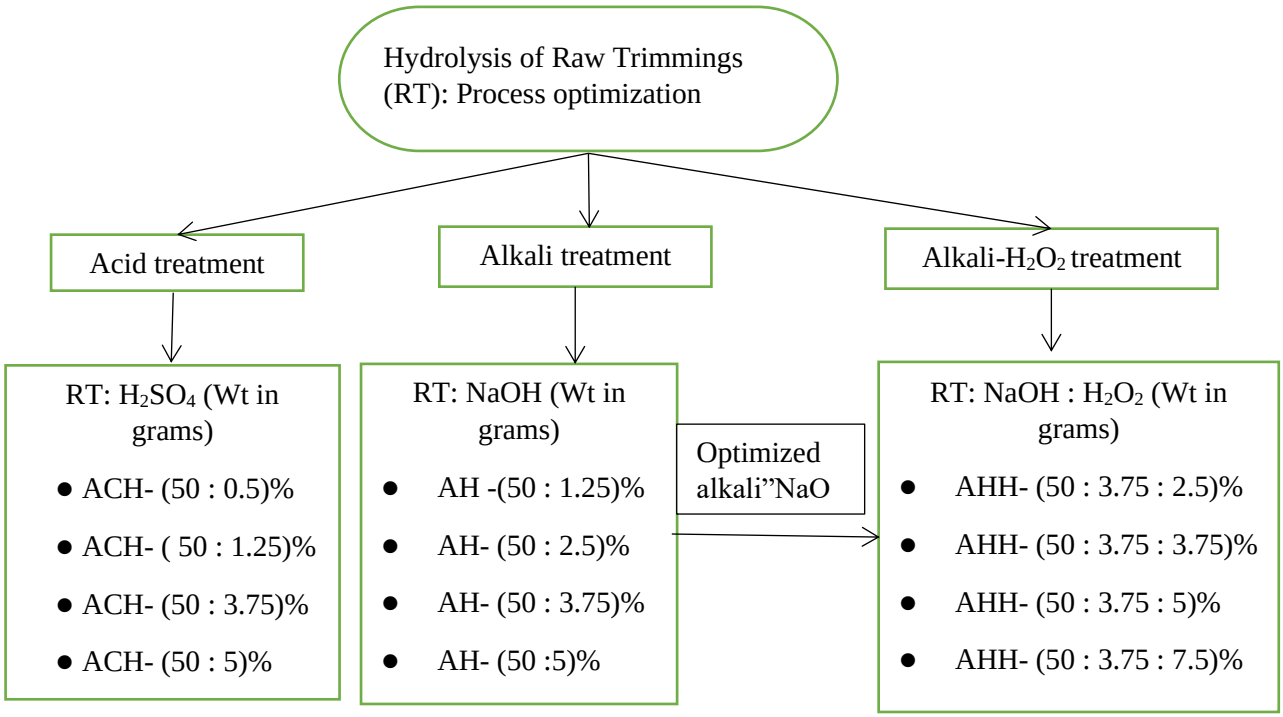

Figure 1. Complete experimental procedure for optimizing hydrolysis of trimmings

We used Eq. (1) to determine the hydrolysis efficiency, often known as the degree of hydrolysis.

$$DH\ (\%) = \frac{W_{RT} - W_{IT}}{W_{RT}} \times 100 \quad (1)$$

In this context, DH represents the degree of hydrolysis, while W_{RT} stands for the initial weight of unprocessed trimmings, and W_{IT} signifies the weight of trimmings that remain insoluble after the hydrolysis process.

Table 1: Efficiency of the Process Optimization for Hydrolyzing Raw Trimmings

Acid hydrolysis		Alkali hydrolysis		Optimized alkali-H_2O_2 hydrolysis	
Sample ID	DH (%)	Sample ID	DH (%)	Sample ID	DH (%)
$ACH_{0.5}$	47	$AH_{1.25}$	71	$AHH_{2.5}$	84
$ACH_{1.25}$	54	$AH_{2.5}$	74	$AHH_{3.75}$	89
$ACH_{3.75}$	61	$AH_{3.75}$	76	AHH_{5}	93
ACH_{5}	69	AH_{5}	81	$AHH_{7.5}$	93

* Note: Acid hydrolysis (ACH), alkali hydrolysis (AH), and alkali hydrogen peroxide hydrolysis (AHH), respectively.

Production of Retanning Agent from Unprocessed Trimmings

50 grams of goat raw trimmings were rehydrated (moisture content: 65% w/w) in 250 milliliters of water that contained 2.5 milliliters of hydrogen peroxide and 1.9 grams of sodium hydroxide. Following that, the mixture was mechanically stirred for a total of 6 hours. The trimmings turned sticky and swelled after being treated with hydrogen peroxide and alkali. The swelled scraps were subjected to hydrolysis at 100 °C for 5 hours. Hydrolysis was followed by cooling and centrifugation to remove the insoluble components. Just before the freeze-drying process, the pH of the supernatant was adjusted with acetic acid to a more controllable 8.5. The freeze-dried material served as a

retanning agent in the leather production process (Fig. 3).

Preparation of Keratin Hydrolysate: Process Optimization

The dried sample was crushed using a crushing machine. After adding 20 g of NaOH to 500 ml of water, 100 g of crushed hair were included in the mixture (weight of hair: volume of NaOH solution ratio 1:5). The substance was heated to 90 °C and stirred magnetically for 4 hours. After hydrolysate the hair was cooled at room temperature and it was centrifuged for 5 minutes at 5000 rpm to separate out the insoluble components. The solution was then performed on keratin hydrolysate.

The following formula was used to calculate the degree of hydrolysis of hair:

$$\text{Degree of hydrolysis \%} = \frac{W_{RH} - W_{UH}}{W_{RH}} \times 100 \quad (2)$$

where, W_{RH} = Raw hair weight, W_{UH} = Undigested hair weight.

Table 2: Enhancing the Hair Hydrolysis Process and Alkali Percentage Optimization

Sample ID	DH%
AH (10: 1)	50%
AH (10: 1.25)	62.5%
AH (10: 1.5)	82%
AH (10: 2)	91%

*Note: Alkali hydrolysis (AH), Degree of hydrolysis (DH). Percentage of alkali = 1, 1.25, 1.5, 2. Weight of hair = 10 gm)

Development of Retanning Agent from Keratin (Graft Polymerization Process)

In a 100 ml reactor vessel equipped with three necks, an extra funnel, a water reflux condenser, a thermometer, and a stirrer were installed. Subsequently, 10 ml of keratin hydrolysate was introduced into the reactor. The reactor should be filled with 10 ml of the redox initiators $K_2S_2O_8$ (18.49 x 10^{-3} moles/liter) and stirred for 30 minutes at room temperature. Simultaneously, the monomer MAA was gradually introduced at a concentration of 1.3887 mol/L into the designated inlet, with continuous stirring maintained for a duration of 30 minutes at a speed of 350 rpm. Following this initial 30-minute period, the mixture underwent an additional hour of stirring at a temperature of 85°C. The reaction was then continued for 1 hour after the addition of 5 ml of the reducing agent $Na_2S_2O_5$ (0.00350 mol/L). To get the pH of the finished product to 5.0, 40 percent of sodium hydroxide was then added. The viscous polymer was formed, the reactor's temperature was lowered to room temperature, and it was transferred to a plastic container and kept there. Keratin hydrolysate and the copolymer formed by grafting keratin hydrolysate with g-methacrylic acid, referred to as KH and KRA respectively, are both designated as "KH" and "KRA" (grafted keratin retanning agent). KRA displayed complete water solubility. To assess the grafting percentage, methanol was introduced into the graft copolymer solution to separate the loosely attached (non-covalently bound) free homopolymer. After a period of a few hours, a precipitate formed and was separated from the solution. Subsequently, the sample was weighed, measured, and placed in an evaporating dish. Employing established procedures involving weight variations, the grafting percentage and grafting efficiency were determined.

Physical and Chemical Testing Analysis

Moisture and Ash Content Analysis

Hydrolysate powder, samples of the powder were first weighed and put in an oven at 105°C for 30 min to determine the moisture content of the protein. After 30 minutes, the sample was weighed and recorded.

Ash content of protein hydrolysate powder: The oven-dried sample was taken in a silica crucible, weighing about 1 g. On the hot plate, the sample-containing crucible was fired and burned until it was scorched. The crucible and sample were then held in a muffle furnace and heated for four hours at 80°C. The ash that developed after it had been allowed to cool

was weighed. The percent of ash was found out using the following formula:

$$\% \text{ Ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample taken (g)}} \text{ x } 100\ \% \quad (3)$$

Analysis of Particle Size

The particle size distribution of hydrolyzed protein powder (HPP) and KRA was assessed utilizing the Dynamic Light Scattering (DLS) zeta sizer 3000HS at a temperature of 25°C.

FTIR Analysis

FTIR (Fourier-Transform Infrared Spectroscopy) was employed to capture the spectra of the substances in the 4000-400 cm^{-1} range. This was accomplished using potassium bromide (KBr) pellets that had been compressed before analysis.

Thermal Gravimetric Analysis (TGA)

A thermo-gravimetric analyzer (TGA 8000) was employed for this test. Temperature Program: In a nitrogen atmosphere, heat at a rate of 10 °C per minute from 50 to 600 °C while purging at a rate of 20 ml per minute.

X-Ray Diffraction Analysis (XRD)

For the XRD analysis, a Japan Science 2200PC X-ray diffractometer was employed. Diffractograms were recorded at 2 using a Cu-K beam (=0.1543 nm) mono chromated by a nickel filter, and the wavelength range was 20–100. The final form of the product was examined using the H-600 transmission electron microscope under settings that replicated its initial manufacture, such as a 20mA X-ray tube, a scanning speed of 10/min, and a current rating of 40KV.

Evaluation of the Post-tanning Phase, Involving a Comparison of the Effectiveness of KRA against a Commercially Available Retanning Agent

We conducted four leather retanning experiments to assess the effectiveness of the newly developed retanning agent in the post-tanning process, comparing the outcomes with those achieved using a commercial retanning agent. The raw material for this study was four wet blue goat skins that had been shaved down to a diameter of 1.1 mm. The skins were then split down the middle along the neck, with the left halves serving as the control (IL) and the right halves serving as the experimental (EE) samples (IR, 2R, 3R, 4R, 5R, 6R). Three right sides were processed with 2%, 4%, and 8% protein hydrolysate powder, while three left sides were processed with 4%, 8%, and 12% KRA. The left half was treated with a technical polypeptide-based retanning agent at a concentration of 6%. The compactness and density of the fibers would be different for each species. Everything else about the retanning process, including the chemicals, coloring material, and fat liquor, was the same for both the standard and observational runs.

Conducting Assessments of Leather's Physical Properties

Employing the INSTRON universal testing machine following the IUP methods to measure various physical properties of leather, including tensile strength, percentage of elongation at break (STM 566), stitch tear strength (DIN 53331), grain crack load (SATRA STM 463), and distention at the grain crack. These measurements were taken from matched pairs of leather samples extracted from symmetrical locations for both the control and experimental groups. The leather sample was chosen for additional research due to its exceptional mechanical properties [17].

Post-tanning Liquor Assessment

Liquor that was not used during the observational and standard procedures (4% KRA, protein-based and traditional retanning agent) was gathered and analyzed for impurity metrics like chemical oxygen demand (COD), total solids (TS), biochemical oxygen demand (BOD), total dissolved solids (TDS), and total suspended solids (TSS) [18].

RESULTS AND DISCUSSION

Raw Trimmings Hydrolysis: Process Optimization

Type-1 collagen makes for 26% of the dry weight of rehydrated goat trimmings (moisture: 65% w/w), while keratin (hair) accounts for 8% of the dry weight [19]. Collagen and keratin differ greatly in their structural makeup and amino acid composition. With a molecular weight of 300 kDa, type-1 collagen is a protein made up of three left-handed polypeptide chains coiled-coil-helical in the right direction. However, keratin has a molecular weight of between 44 and 66 kDa [20] and an α-helix structure. While keratin is maintained via disulfide bond/hydrophobic contact and colored via melanin, collagen relies mostly on non-covalent interactions and is conjugated with polysaccharide. Therefore, collagen hydrolysis conditions may vary from keratin hydrolysis conditions. Three alternative pre-treatment techniques were used to optimize the hydrolysis conditions, and their impact on the effectiveness of the hydrolysis process was researched. Table 1 displays the results of several tests assessing the efficiency of hydrolysis. Table 1 makes it clear that only around 69% of the raw trimmings are hydrolyzed by the acid treatment, which is ineffective for achieving complete hydrolysis. Perhaps the extensive compound of keratin and the presence of a slippery surface account for the protein's exceptional resistance to acid treatment. When sodium hydroxide was used in place of sulfuric acid, hydrolysis efficiency increased significantly; the highest efficiency was 81% when sodium hydroxide was used at a concentration of 5%. Table 1 further shows that 7.5% sodium hydroxide ($AH_{3.75}$) has a comparable hydrolysis efficiency to that of 5% sodium hydroxide (AH_5). As a result, the hydrogen peroxide in subsequent experiments ranged from 2.5 to 7.5% w/w while the sodium hydroxide concentration was maintained at 3.75%. Table 1 shows that the hydrolysis efficiency has increased with the addition of hydrogen peroxide, reaching a high of 93% for 5% hydrogen peroxide. When it was raised by more than 5%, no improvement was seen. As a result, the best way for efficient hydrolysis is to pre-treat raw trimmings with 3.75% NaOH and 5% H_2O_2.

Retanning Chemical Production from Raw Trimmings

Preliminary hydrolysis data shows that the raw trimmings can be fully hydrolyzed with an effective pre-treatment with alkali and hydrogen peroxide. In the pre-treatment phase, hydrogen peroxide oxidizes the keratinocytes already present in the hair's microfibrils structure, leaving the hair more susceptible to thermal damage [21]. This process, depicted in (Fig. 3), oxidizes cystine amino acids to cysteic acids [16] under alkaline conditions, yielding a product abundant in sulfonic functional groups.

Collagen enlarges as a consequence of heightened osmotic pressure within the matrix, leading to the separation of fibre bundles. The increased surface area of broken fibres in collagen makes it more reactive to water. Thus, we treated collagen and keratin with alkaline hydrogen peroxide, which destabilized them both. Subsequently, heat treatment was utilized to cleave the polypeptide chains. The hydrolyzed segments notably display clearly identifiable functional groups, including amino, carboxyl, and sulfonic acid groups, as visualized in (Fig. 2). Using hydrogen peroxide as a pretreatment has many advantages. That is because (a) we toned down the original color of the results, making them suitable for a wider spectrum of pastel-hued products, and (b) we reduced their intensity. To ensure chemical linkages between the substance and the leather matrix, a sulfonic group is produced (More details about how this occurred are given below). (c) Oxygen and water were produced as hydrogen peroxide broke down. The hydrolyzed product was chilled and then separated to get rid of the fat and other insoluble parts. The liquid solution's pH was decreased to 8.0–8.5 before it was freeze dried. The grinding was used as a retanning agent throughout the leather-making process.

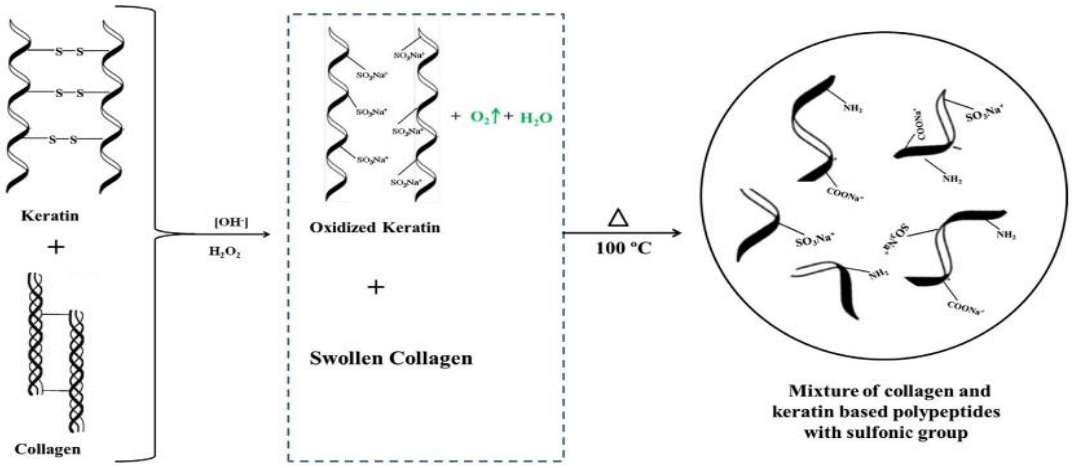

Figure 2. Fragment decomposition in an alkaline-hydrogen peroxide environment

(a) (b) (c) (d)

Figure 3. Progression from raw goat trimmings to the final retanning agent: (a) Rehydration of the raw trimmings, (b) Treatment of trimmings with Alkali-H_2O_2, (c) Protein hydrolysate, and (d) Development of the ultimate retanning agent

Hydrolysis of Sheep Hair: Process Optimization

The majority of the keratin found in red sheep hair is of the α-type and has α-helix structure [22]. To form a left-handed coiled-coil structure in the form of a dimer, two right-handed α-helices are linked together through disulfide bonds. Keratin's mechanical resilience is a result of a blend of factors, including hydrophobic interactions within the polypeptide chains, the existence of disulfide connections formed by cysteine amino acids, and the keratin's restricted ability to dissolve [23]. It takes a lot of time and expense to hydrolyze substances using enzymes. In addition to producing very modest amounts of hair wastage, acid hydrolysis is ineffective for entirely hydrolyzing hair. Alkali is a highly efficient method for hydrolyzing keratinous waste since it speeds up the process and reduces the amount of time needed [24]. Therefore, it is recommended to hydrolyze hair using the alkali approach. The alkali concentration, extraction duration, and temperature all affect how effectively hair is hydrolyzed. In a high-pH alkaline setting, surpassing a pH level of 9.2, sodium hydroxide disrupts the α-helix configuration of cystine and accelerates the breakdown of sturdy disulfide bonds and other covalent linkages.

This process leads to the creation of small, easily soluble peptides (Fig. 4). With increasing sodium hydroxide concentration, the effectiveness of hydrolyzing red sheep's hair gradually increases (Table 2). After two hours in contact with 2% NaOH at 90°C, the resulting hydrolysate shows no signs of significant hair filaments. Hence, it is safe to state that the process of dissolution is complete. Hair was hydrolyzed to a maximum of 91% with a cheap chemical, sodium hydroxide, at roughly 2% of its cost (Table 2). The fast hydrolysis time and simple process

used in the research can be applied on a larger scale in industry.

Development of Keratin Retanning Agent: Graft Polymerization

According to tests on hair hydrolysis, 2% sodium hydroxide proved successful for completely hydrolyzing hair. In the presence of the redox initiators $K_2S_2O_8/Na_2S_2O_5$, graft copolymerization of MAA monomers was carried out at 85 °C for 3 hours on the keratin hydrolysate backbone. The results showed that 53% of the grafts were successful and 89.7% of the grafts were efficient. The drafting success rate and drafting % were determined by analyzing the weight variation between the donor and recipient. Sulfate anion radicals were created when potassium persulfate was thermally induced to breakdown at 85 °C [25]. The radical produced triggers the initiation of active sites, leading to the generation of larger radicals responsible for kickstarting the graft copolymerization process of MAA onto the structure of KH. This ultimately leads to the development of a dense and thick substance known as KRA (Fig 6). To achieve this, a hydrogen radical is removed from one of the keratin hydrolysate's structural groups (-COOH, -SH, -OH, and -NH2) [26] (Fig. 5).

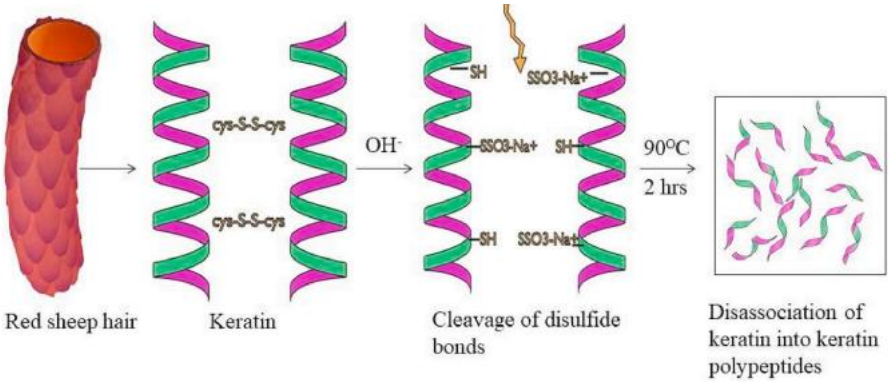

Figure 4. Hydrothermal and alkaline treatment of keratin, depicted schematically

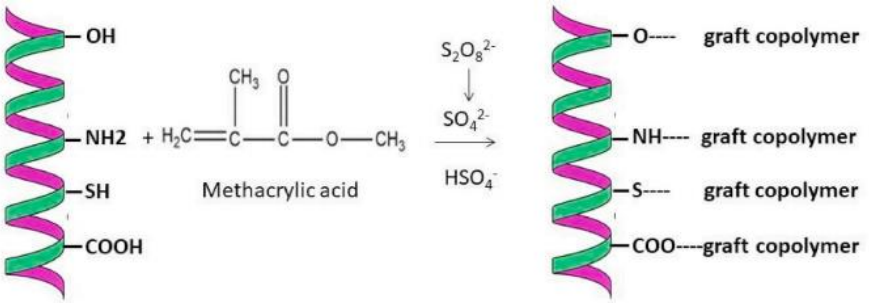

Figure 5. Schematic depiction of pendant functional groups in keratin hydrolysate, indicating potential grafting sites

(a) (b) (c) (d)

(e)

(f)

Figure 6. Enhancement of retanning agent derived from tannery waste hair, specifically addressing: (a) Hair waste, (b) Crushed hair, (c) Alkali hydrolysis, (d) Keratin hydrolysate (KH), (e) Graft polymerization process, (f) Developed retanning (KRA)

Developed Product Characterization

Moisture and Ash Content

The developed product (PHP) is a free-flowing powder with an approximate 7% w/w moisture content. Any retanning agent's ash content, which also affects how environment friendly a product is, as a crucial component. Wastewater treatment is a major problem for the leather industry because of the high salt load it contains; this load is exacerbated by the presence of retanning agents. Comparatively, the ash percentage in the newly formed product is approximately 15% (w/w), but it is between 30 and 40% (w/w) in technical retanning agents. The result revealed that produced product is hazardous substance-free and rich in organic content.

Table 3: Test of mechanical properties

Testing parameter / unit		LR+2%	LR+4%	LR+8%	LR+12%
Tensile strength (N/mm^2)	PHP Applied Leather	21.65	16.28	22.23	
	KRA Applied Leather		18.29	24.58	27.41
	Control LR	18.11			
Elongation at Break (%)	PHP Applied Leather	155.28	154.47	159.49	
	KRA Applied Leather		148.68	155.45	123.99
	Control LR	164.32			
Stitch Tear Strength (kg/cm)	PHP Applied Leather	87.5	91.2	78.7	
	KRA Applied Leather		123.6	158.7	168.00
	Control LR	103.8			
Load at Grain Crack (kg)	PHP Applied Leather	20	21	30	
	KRA Applied Leather		19	26	23
	Control LR	21			
Distension at Grain Crack (mm)	PHP Applied Leather	7.7	8.8	9.1	
	KRA Applied Leather		8.5	8.4	7.4
	Control LR	8			
Water Vapor Permeability	PHP Applied Leather	12.73	12.15	12.29	
	KRA Applied Leather		15.45	15.90	16.50
	Control LR	10.79			

*Protein hydrolysate powder (PHP), Keratin retanning agent (KRA), Leather (LR)

Color Rub Fastness Test

Table 4: Observation for leather (Dry condition)

Sample ID	Gray scale rating					
	32(rev)	64(rev)	128(rev)	256(rev)	512(rev)	1024(rev)
Control LR (0%)	5	5	5	5	5	5
LR+2%PHP	5	5	5	5	5	3/4
LR+4%PHP	5	5	5	5	5	4
LR+8%PHP	5	5	5	5	4	4/5
LR+4%PHP	5	5	5	5	4/5	4
LR+8%PHP	5	5	5	5	5	4/5
LR+12%PHP	5	5	5	5	4	3/5

Table 5: Observation for felt (Dry condition)

Sample ID	Gray scale rating					
	32 (rev)	64(rev)	128(rev)	256(rev)	512(rev)	1024(rev)
Control LR (0%)	5	5	5	5	5	2/3
LR+2%PHP	5	5	5	5	5	5
LR+4%PHP	5	5	5	5	5	5
LR+8%PHP	5	5	5	5	5	4/5
LR+4%PHP	5	5	5	5	4/5	4
LR+8%PHP	5	5	5	5	5	4/5
LR+12%PHP	5	5	5	5	4	4/5

Table 6: Perspiration Fastness Test

Sample ID	Gray scale Rating (1024 rev)	
	For Leather	For Multi-fiber
Control LR (0%)	2/3	2/3
LR+2%PHP	2/3	2/3
LR+4%PHP	4/5	3/4
LR+8%PHP	4/5	4
LR+4%PHP	4	4/5
LR+8%PHP	4/5	¾
LR+12%PHP	4/5	4

Analysis of Particle Size

The mean particle size of Protein Hydrolysate Powder (PHP) and KRA was determined Using DLS measurements. To assess whether a retanning agent interacts and penetrates the leather matrix sufficiently, its particle size must be measured. Insufficient penetration of the leather is caused by retanning agents with larger particle sizes, particularly those with a diameter of more than 1000 nm. PHP and KRA have particle diameters of 291 nm (7a) and 717 nm (7b) respectively, which are thought to be sufficient for penetrating the leather matrix.

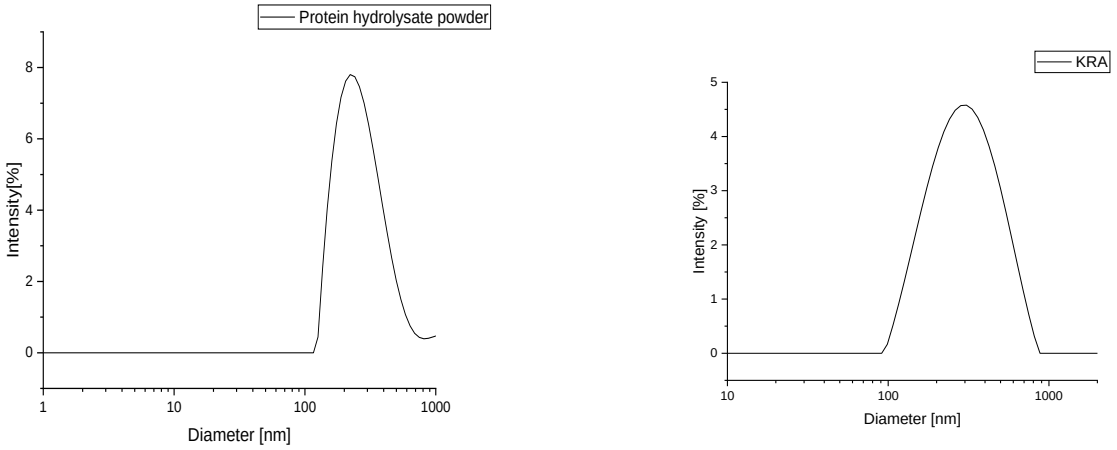

Figure 7. (a) Protein hydrolysate powder (PHP) particle size; (b) Size of the keratin-based retanning agent particles

FTIR Analysis

In Figure 8(a), the produced leather's FTIR spectrum and that of the protein retanning agent are displayed. Significant spectral signals may be found at 3369 cm^{-1} (N-H stretching vibration), 3304 cm^{-1} (CH2 asymmetrical stretching vibration), and 2923 cm^{-1} (N-H stretching vibration) for amides A and B, respectively. Each amide exhibits distinct peaks at 1647 cm^{-1}, 1640 cm^{-1}, 1537 cm^{-1}, 1546 cm^{-1}, 1327 cm^{-1}, and 1238 cm^{-1}, respectively. Specifically, Amide I corresponds to the stretching vibration of the C=O bond in the peptide backbone, while Amide II and Amide III denote the bending of N-H and the stretching vibration of C-N bonds, respectively. A peak at 1043 cm^{-1} and another at 1034 cm^{-1} show that the production process involved the use of cysteic acid, which contains sulfonic acid. These peaks agree with the stretching vibrations of S=O that are observed in cysteic acid, both symmetrically and asymmetrically [27]. The produced retanning agent has a significant amount of amino, carboxyl, and sulfonic functional groups, as shown by the results above.

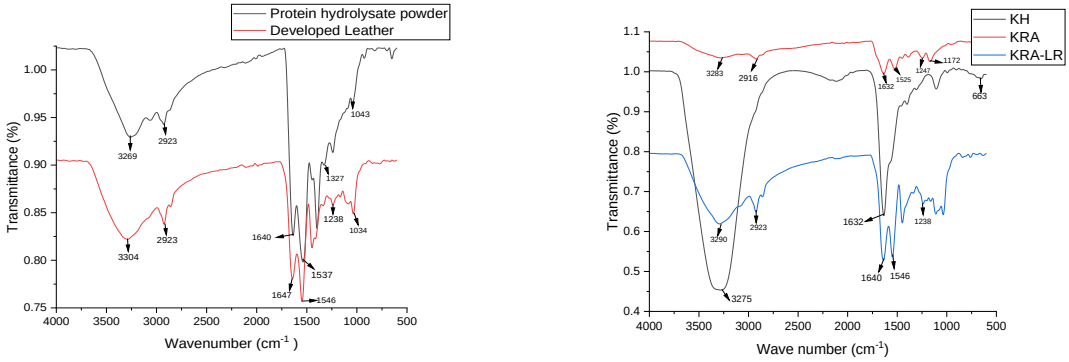

Figure 8. (a) FTIR spectrum of PHP and the leather produced through development, (b) FTIR spectrum of KH, KRA and developed leather (KRA-LR)

KH, KRA, and KRA-LR IR spectra are shown in Figure 8(b). Peptide N-H stretching vibration is responsible for the bands about 3310-3230 cm^{-1} (–CONH–). The bands at 2916 cm^{-1} and 2923 cm^{-1} in KRA and KRA-LR are indicative of the rotational oscillations of CH, CH_2, and CH_3. The frequency range from 1632 to 1640 cm^{-1} is characteristic of amide I. KRA and KRA-LR both showed amide II bands at 1525 cm^{-1} and 1546 cm^{-1}. C-S structural properties have been attributed to the 570-710 cm^{-1} bands (KH and KRA).

The stretching of keratin's -OH groups causes the bands in the KRA in the 3200–3600 cm^{-1} range. Absorption is seen in the range from 2550 to 3800 cm^{-1}, where both the carboxylate and alcoholic Maximum utilization bands are present. Moreover, there were supplementary spectral characteristics signifying the creation of graft copolymers, such as a prominent peak at 1172 cm^{-1} (representing the C-O-C component of PMMA) and approximately 1247 cm^{-1} (associated with C—O stretching), both of which were absent in the KH spectrum. The additional signature peak at 1689 cm^{-1} was observed in KRA (Fig. 7b), indicating grafting of the acrylic acid's carboxylic acid (-COOH) group (Fig. 8b) [28]. Alterations in the NH signals serve as evidence of the graft reaction occurring between the NH compounds and the free radicals of MMA, as evidenced by shifts at 3286, 3075, and 1592 cm^{-1}.

TGA Test

To examine the thermal behavior of the prepared leather sample, thermogravimetric analysis (TGA) was done. 50 °C was the initial temperature, and 1500 °C was the end temperature. The control sample and the leather samples numbered 1 through 4 were among the four samples used in the TGA test. Figure 9 shows the TGA graphs that were produced for those samples.

Each sample lost 5% of its weight, according to the TGA thermogram, which is consistent with a melting range of 50–80 °C. Weight loss was seen before the substance reached its melting point, demonstrating its hydrous character. When the temperature rises from 600 to 650 °C, there is a 13% weight loss. The melting peak is followed by the degradation peak, which starts to lose mass at temperatures of approximately 800 °C.

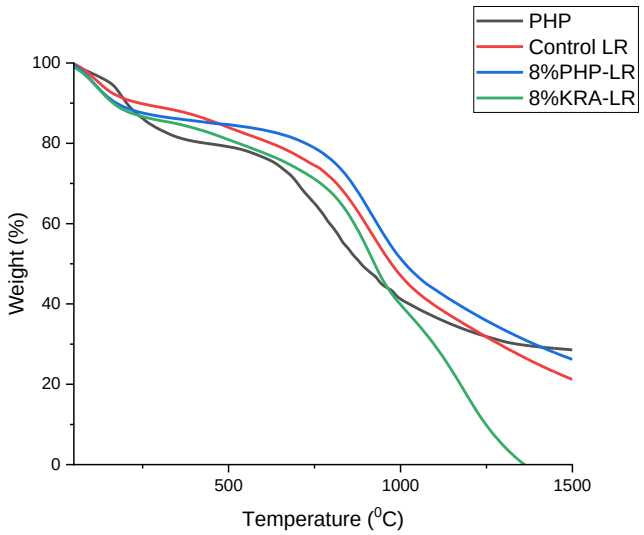

Figure 9. TGA data of different sample

XRD Analysis

The extracted keratin and protein hydrolysate powder (PHP) displayed in Fig. 10 were of high quality, according to the XRD data. In order to preserve the crystal, the extracted keratin was primarily supplied in a semi-crystalline form. Keratin molecules performed XRD examination, which revealed a strong peak at 22.46 degrees that indicated the existence of keratin [29]. The main peaks at 2θ = 9-10.50° and 19-34.8°, respectively, were attributed to the α-helix and β-sheet. The keratin exhibits the α-helix's diffraction characteristics at 2θ = 9.52 ° and the β-sheet's at 2θ = 22.46 °. The peak has an amorphous structure and a range that varies from 5 to 45 degrees. The results discussed above lead to the conclusion that keratin has two different crystal structures: α and β. On the other hand, keratin contains a greater amount of β-sheet. Amorphous keratin molecules are another characteristic. On the contrary, the PHP was obtained from raw trimming, which contains

two types of protein such as collagen and keratin. The XRD results of PHP showed amorphous structure and a strong peak at 2θ = 21.02°, which determined the presence of both proteins.

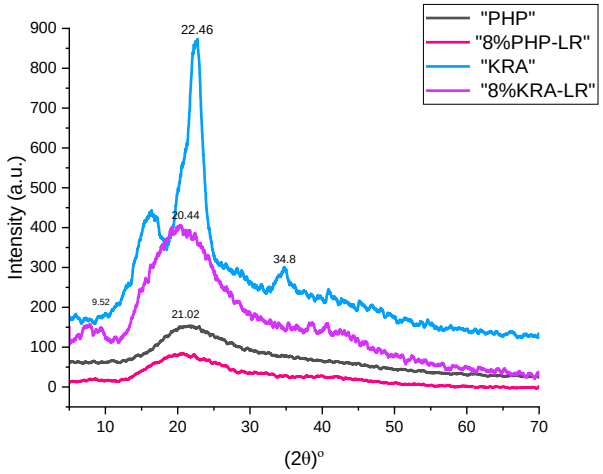

Figure 10. XRD graph of different sample

CONCLUSION

In this current study, we have developed an eco-friendly and biodegradable retanning solution using raw tannery waste trimmings and hair, all without the incorporation of any harmful substances or toxic compounds. Experiment leather was treated with 8% PHP and KRA which enhanced organoleptic and mechanical qualities compared to the control sample. In terms of fullness and roundness, the experimental leather was rated higher (8/10) than the control leather (7/10). The tensile strength, grain fracture resistance and color intensity of the experimental leather revealed higher vales than those of the control leather. Further, when compared to commercial retanning agents, the use of 8% PHP and KRA resulted in a lower emission of COD, TDS, TS and TSS, all of which were indicative of a smaller negative impact in the environment. This innovative approach involves creating an eco-friendly retanning agent using discarded trimmings and waste hair, providing an enhanced alternative to synthetic retanning agents in leather production. This not only exemplifies a sustainable business model but also plays a significant role in revolutionizing the tannery sector.

Acknowledgements

The authors would like to thank the Institute of Leather Engineering and Technology, University of Dhaka for technical and analytical support. The authors acknowledge University Grant Commission Bangladesh for financial support for this research work.

REFERENCES

1. Murali, S., Balaraman, M., Jonnalagadda, R.R., Leather Solid Waste: An Eco-benign Raw Material for Leather Chemical Preparation – A Circular Economy Example, *Waste Manage*, **2019**, 87, 357-367, https://doi.org/10.1016/j.wasman.2019.02.026.
2. Rao, P.S., Palanivel, S., Water Management in Leather Industry, *J Sci Ind Res (India)*, **2001**, 70, 6, 443-450.
3. Kanagaraj, J., Velappan, K.C., Solid Wastes Generation in the Leather Industry and Its Utilization for Cleaner Environment, *Chem Inform*, **2006**, 37, 49, 541-548, https://doi.org/10.1002/chin.200649273.
4. Saha, B., Azam, F.A.B., Probable Ways of Tannery's Solid and Liquid Waste Management in Bangladesh – An Overview, *Text Leather Rev*, **2021**, 4, 2, 76-95, https://doi.org/10.31881/TLR.2020.25.
5. Jadhav, N.C., Jadhav, A., Waste and 3R's in Footwear and Leather Sectors, in: Muthu, S.S. (ed.), *Leather and Footwear Sustainability*, **2020**,

261-293, https://doi.org/10.1007/978-981-15-6296-9_10.
6. Duong, H., Grolle, K.C.F., Nga, T.T.V., Zeeman, G., Temmink, H., van Eekert, M., Protein Hydrolysis and Fermentation under Methanogenic and Acidifying Conditions, *Biotechnol Biofuels*, **2019**, 12, 1, 1-10, https://doi.org/10.1186/s13068-019-1592-7.
7. Abdelhedi, O., Mora, L., Jridi, M., Toldra, F., Nasri, M., Proteolysis Coupled with Membrane Separation for the Isolation of Bioactive Peptides from Defatted Smooth Hound Byproduct Proteins, *Waste Biomass Valorization*, **2023**, https://doi.org/10.1007/s12649-023-02303-4.
8. Cao, C., Xiao, Z., Ge, C., Wu, Y., Animal By-products Collagen and Derived Peptide, as Important Components of Innovative Sustainable Food Systems—A Comprehensive Review, *Crit Rev Food Sci Nutr*, **2021**, 62, 1, 1-25, https://doi.org/10.1080/10408398.2021.1931807.
9. Limeneh, D.Y., Tesfaye, T., Ayele, M., Husien, N.M., Ferede, E., Haile, A., Mengie, W., Abuhay, A., Gelebo, G.G., Gibril, M., Kong, F., A Comprehensive Review on Utilization of Slaughterhouse By-Product: Current Status and Prospect, *Sustainability*, **2022**, 14, 1-20, https://doi.org/10.3390/su14116469.
10. Jayathilakan, K., Sultana, K., Radhakrishna, K., Bawa, A.S., Utilization of Byproducts and Waste Materials from Meat, Poultry and Fish Processing Industries: A Review, *J Food Sci Technol*, **2012**, 49, 3, 278–293, https://doi.org/10.1007/s13197-011-0290-7.
11. Shestakova, A., Timorshina, S., Osmolovskiy, A., Biodegradation of Keratin-Rich Husbandry Waste as a Path to Sustainable Agriculture, *Sustainability*, **2021**, 13, 16, 1-11, https://doi.org/10.3390/su13168691.
12. Makwana, K.M., Mahalakshmi, R., Implications of Aromatic–Aromatic Interactions: From Protein Structures to Peptide Models, *Protein Sci*, **2015**, 24, 12, 1920-1933, https://doi.org/10.1002/pro.2814.
13. Bragulla, H.H., Homberger, D.G., Structure and Functions of Keratin Proteins in Simple, Stratified, Keratinized and Cornified Epithelia, *J Anat*, **2009**, 214, 4, 515-559, https://doi.org/10.1111/j.1469-7580.2009.01066.x.
14. Sypka, M., Jodlowska, I., Bialkowska, A.M., Keratinases as Versatile Enzymatic Tools for Sustainable Development, *Biomolecules*, **2021**, 11, 12, 1-30, https://doi.org/10.3390/biom11121900.
15. Herzog, B., Overy, D.P., Haltli, B., Kerr, R.G., Discovery of Keratinases Using Bacteria Isolated from Marine Environments, *Syst Appl Microbiol*, **2016**, 39, 1, 49-57, https://doi.org/10.1016/j.syapm.2015.10.004.
16. Feroz, S., Muhammad, N., Ratnayake, J., Dias, G., Keratin-based Materials for Biomedical Applications, *Bioact Mater*, **2020**, 5, 3, 496–509, https://doi.org/10.1016/j.bioactmat.2020.04.007.
17. Colak, S.M., Kaygusuz, M., Study on the Mechanical Properties of Leathers Reinforced with Supporting Materials, *Annals of the University of Oradea, Fascicle of Textiles, Leatherwork*, **2021**, 113-124, License: CC BY-NC.
18. Di Gregorio, S., Giorgetti, L., Ruffini Castiglione, M., Mariotti, L., Lorenzi, R., Phytoremediation for Improving the Quality of Effluents from a Conventional Tannery Wastewater Treatment Plant, *Int J Environ Sci Technol*, **2014**, 12, 4, https://doi.org/10.1007/s13762-014-0522-2.
19. León-López, A., Morales-Peñaloza, A., Martínez-Juárez, V.M., Vargas-Torres, A., Zeugolis, D.I., Aguirre-Álvarez, G., Hydrolyzed Collagen—Sources and Applications, *Molecules*, **2019**, 24, 22, 4031-4041, https://doi.org/10.3390/molecules24224031.
20. Aluigi, A., Vineis, C., Varesano, A., Mazzuchetti, G., Ferrero, F., Tonin, C., Structure and Properties of Keratin/PEO Blend Nanofibers, *Eur Polym J*, **2008**, 44, 8, 2465-2475, https://doi.org/10.1016/j.eurpolymj.2008.06.004.
21. Dias, G., Reis, M.F., Hair Cosmetics: An Overview, *Int J Trichology*, **2015**, 7, 1, 2–15, https://doi.org/10.4103/0974-7753.153450.
22. Ramya, K.R., Thangam, R., Madhan, R., Comparative Analysis of the Chemical Treatments Used in Keratin Extraction from Red Sheep's Hair and the Cell Viability Evaluations of This Keratin for Tissue Engineering Applications, *Process Biochem*, **2020**, 90, 223-232, https://doi.org/10.1016/j.procbio.2019.11.015.
23. Perța-Crișan, S., Ursachi, C.Ș., Gavrilaș, S., Oancea, F., Munteanu, F.-D., Closing the Loop with Keratin-Rich Fibrous Materials, *Polymers*, **2021**, 13, 11, 1896, https://doi.org/10.3390/polym13111896.
24. Nur-E-Alam, M., Akter, N., Chakma, S., Fatema, K., Azad, A.K., Chowdhury, J.M., Mia, A.S., Alkali Enzymatic Extraction of Keratin Protein from Chicken Feather Waste in Bangladesh, *Iran J Energy Environ*, **2019**, 10, 4, 235-241, https://doi.org/10.5829/IJEE.2019.10.04.02.
25. Ye, Q.F., Wang, C.Y., Wang, D.H., Sun, G., Xu, X.H., Hg^0 Absorption in Potassium Persulfate Solution, *J Zhejiang Univ Sci B*, **2006**, 7, 5, 404–410, https://doi.org/10.1631/jzus.2006.B0404.
26. Alahyaribeik, S., Ullah, A., Methods of Keratin Extraction from Poultry Feathers and Their Effects on Antioxidant Activity of Extracted Keratin, *Int J Biol Macromol*, **2020**, 148, 4, https://doi.org/10.1016/j.ijbiomac.2020.01.144.
27. Parker, S.F., Assignment of the Vibrational Spectrum of L-cysteine, *Chem Phys*, **2013**, 424, 75–79, https://doi.org/10.1016/j.chemphys.2013.04.020.

28. Chhabra, V., Deep, A., Kaur, R., Kumar, R., Functionalization of Graphene Using Carboxylation Process, *International Journal of Science & Emerging Technologies*, **2012**, 4, 1, 13-19.
29. Liu, X., Nie, Y., Meng, X., Zhang, Z., Zhang, X., Zhang, S., DBN-based Ionic Liquids with High Capability for the Dissolution of Wool Keratin, *RSC Adv*, **2017**, 7, 4, 1981-1988, https://doi.org/10.1039/C6RA26057H.