



Research article

Keratin hydrolysate as a chrome exhaust aid and keratin filler in leather processing: A cleaner technology approach for tannery solid waste management and leather manufacturing

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ABSTRACT

Hair burning unhairing and dampening of tannery wastes during the hair-saving unhairing process are becoming significant problems in the tanning industry. Therefore, this research article focuses on the extraction of keratin hydrolysate (KH) and its application as a chrome exhaust aid and keratin filler in leather manufacturing process. The structure, morphology and functional groups of the extract were examined using X-Ray Diffractometer (XRD), scanning electron microscopy (SEM) and Fourier transform infrared spectrometer (FTIR), respectively. To study and contrast the degree of improvement in chromium uptake, the KH solution was applied both before tanning on the pickled pelt and after tanning during basification. The thermal stability, physical strength characteristics and organoleptic properties of the leathers obtained were characterized. Furthermore, the environmental impact of the tanning system was assessed through a comparative analysis of the spent liquors. Finally, experimental retanning process was conducted to replace the commercial protein filler (Celatan F: 50, 75, and 100 %) with KH solution, with concurrent processing of control leather using conventional chrome tanning agent at 6 % dosage of chromium. The FTIR analysis of the extract confirmed the presence of alkyl side chains of amino acids as well as carboxylic, amide, carboxyl group and aldehyde functional groups at 1400-1700 cm^{-1} , 3,303.46 cm^{-1} , 3270 cm^{-1} and 2752 cm^{-1} , respectively. XRD spectrum showed two diffraction peaks at 2 theta values of 9.36° and 21.16°, respectively. Leathers with improved mechanical strength, organoleptic properties and thermal stability were obtained with 100 % substitution of Celatan F at pH 6 and 10 % chromium dosage. It was also discovered that the shrinkage temperature of the experimental leather was enhanced to more than 105 °C. Environmental impact evaluation on the spent liquor showed that the complete replacement of Celatan F with KH solution brought about a notable decrease in COD and TDS in the spent liquor. The extraction and application of tannery hair waste-based keratin hydrolysate as an efficient,

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environmentally friendly chrome exhaust aid and keratin filler has been attempted and established in this research article.

1. Introduction

Tanning is the process that turns raw hides and skins into leather, the non-putrescible final product, by stabilizing the triple helical structure of collagen matrix. This chemical modification of the collagen structure is supported by the functional groups on the side chains of the three polypeptide chains that form the spiral helical structure of the collagen protein [1]. Over the course of producing these leathers, the tanning industry generates a large amount of wastes and byproducts throughout the leather-making process, which, if not properly handled, may have detrimental effects on the ecological environment [2]. The environmental impact of the disposed solid wastes such as trimmings, fleshings, chrome shaving wastes, buffing wastes, and keratin wastes is becoming a major concern for tanners around the globe. Therefore, adding value to these solid wastes and converting them into useable end products is an inevitable practice both from the environmental protection and cyclic resource utilization point of view [1,3]. Prior to carrying out other significant tanning processes, hides and skins must pass through the hair removal processes such as hair burning unhairing, paint unhairing, and enzymatic unhairing. During these processes, a substantial amount of solid hair waste is generated and disposed of in landfills, and on the other hand, solid hair waste burned with chemicals contributes to the high values of chemical oxygen demand (COD) and total dissolved solid (TDS) in tannery effluents. Severe environmental pollution issues could result from the uncontrolled discharge of such tannery solid wastes and wastewater without sufficient or effective treatment [4]. For instance, uncontained discharge of hair waste and subsequent open air burning of it results in greater environmental release of greenhouse gases [5]. The other phenomenon observed in this circumstance involving the discharge of tannery effluents into water bodies is eutrophication, in which the growth of aquatic plants is encouraged by an increase in nutrients such as phosphorus and nitrogen from waste discharge to water bodies, resulting in the depletion of dissolved oxygen [6]. Approximately 100 kg of solid hair waste is generated when one tone of hide or skin is converted into leather [5,7]. Therefore, managing the handling of hair waste and discovering appropriate ways to add value are crucial.

Tanning and retanning processes are important steps in the production of leather, where the vital component of the skin structure (collagen) gradually changes its original properties and attains several typical properties, such as exceptional resistance to putrefaction, resistance to wet heat (hydrothermal stability), good mechanical properties, resistance to chemical degradation, improved leather homogeneity, increased durability, cutting value and other physical and organoleptic properties of the final leather product. Mineral tanning materials (chromium, aluminum, iron, and zinc), vegetable tans (mimosa, acacia, and quebracho), aldehyde tans (glutaldehyde and formaldehyde), and oil tans are used to convert putrescible hide/skin to nonputrescible intermediate leather. Likewise, retanning is conducted using inorganic mineral substances (chrome, aluminum, zirconium salts) or organic substances (aldehydes, vegetable tannins, and resins) [8] to fill the interfibrillar void space created due to the removal of unwanted protein materials during beam house leather processing operations [9]. This makes it an important step that is carried out to achieve certain fullness features with the selective filling of the structure and to provide a tight and uniform grain surface for final leather articles [10].

Traditional tanning and retanning processes have their own shortcomings. For instance, the use of chromium in tanning has a limited exhaustion rate that does not surpass 50–70 %, making chromium the major constituent of tannery effluent [11]. Discharge containing a considerable amount of chromium has adverse effects on the environment, is toxic to aquatic life, and pollutes groundwater [12,13]. In addition, trivalent chromium has a high probability of being converted to a hexavalent state in acidic soil, which is carcinogenic and toxic to aquatic life and plant growth at high concentrations [14–16]. Despite these factors, chrome tanning salts are needed in leather processing to produce high-quality leather. Different tanning systems, such as titanium, silicon, and aluminum tannings, have been developed over the last 15 years to avoid toxic chromium compounds; however, since these leathers contain organic salts, they have their own set of drawbacks [17]. On the other hand, the currently used retanning agents are mainly petroleum byproduct-derived, formaldehyde-based fillers, and other synthetic retanning agents [8]. The use of such retanning agents and their presence in leather even at 50 ppm are becoming major causes of concern for leather manufacturers [5,18]. Therefore, it is important to enhance chromium uptake, reduce the amount of chromium discharged into wastewater to below the allowable limit and replace aldehyde-based retanning chemicals with locally produced and environmentally acceptable materials.

Keratin is a group of fibrous structural proteins known as scleroproteins that are mostly found in mammalian body parts and tissue configurations, such as horns, hooves, hair, fur, nails, skins, wool, and bird beaks and feathers [19]. Along with collagen, it is the most significant biopolymer found in mammals and has a higher cysteine content than other fibrous proteins, such as elastin, collagen, and myofibrillar proteins [20]. The epithelial tissues of higher vertebrates are known to contain a greater amount of keratin, which has an important role in protecting the body from chemicals and external mechanical conditions [21]. There are two types of keratins based on its fiber structure and amount of sulfur-containing amino acids. Low-disulfide soft keratin bonds are found in the epidermis and other cellular tissues. Its lower disulfide bond composition compared to that of hard keratin makes it susceptible to hydrolysis by acids and alkalis. However, hard keratins are highly fibrotic and are found in the epidermis of humans and other animals and are characterized by high stiffness due to the presence of a considerable number of disulfide bonds [21].

Many attempts have been made to extract and use different types proteins from tannery solid wastes [22–26]. Similarly, a variety of techniques have been used to extract keratin protein from keratin biomass, but the most important ones include chemical hydrolysis, enzymatic and microbiological treatment, dissolution in ionic liquids, microwave technique, steam explosion technique, and thermal hydrolysis [19]. The extracted keratin has been used for a wide range of applications, such as for fertilizer industries [27]; for

industries producing animal feeds [28,29]; as a biomaterial for example, for the production of keratin-based bioplastic films from chicken feathers [21,30,31]; as a biomedical material using sodium sulfide-based extraction [32]; as an industrial application of tannery wastewater treatment to adsorb chromium [12,33]; and as a replacement for salt in textile processing to enhance dyestuff uptake [26,34]. Therefore, many keratin sources, including waste chicken feathers, wool waste from the textile and wool processing industries, hog hair, horn meal, hoofs, nails and tannery hair waste, have been used for the extraction of keratin hydrolysate [27, 34–41]. It is slowly becoming more common to produce biobased retanning and chrome exhaust aids, as well as use them in the production of leather. There have been numerous attempts to create protein-based chrome exhaust aids and retanning agents from solid tannery and slaughterhouse wastes such as raw trimmings, chrome shavings, fleshing wastes, and cow horn meal waste [42–44]. However, only a small number of studies have been conducted on the use of tannery hair waste and its application in the leather-processing industry. The existing research has primarily focused on the application of keratin hydrolysate (KH) as a chrome exhaust aid on pickled pelts. However, these studies have not adequately addressed the issue of filling looser sections, such as the belly and flank regions of low-grade wet blue leather, due to the negligible impact on chromium uptake observed in these limited works [12, 39,45].

The major goal of this study was to produce a biobased liquid KH from tannery hair waste that may be used as a chrome exhaust aid and keratin filler to retan leather. The end-of-waste concept, which is the subject of this study, allows tannery waste products to be treated as secondary raw materials rather than disposed of in landfills. Hair waste produced during the production of leather was specifically treated with alkali hydrolysis, in which different hydrolysis conditions were optimized. The results of this work not only assist in resolving the issue of hair waste in the leather industry but also offer a commercially viable method for the creation of an eco-friendly, biobased liquid chrome exhaust aid and keratin filler. To the best of our knowledge, no studies have been conducted on the production of keratin-based chrome exhaust aid to be applied after tanning during basification and retanning agents from mixed Ethiopian sheep hair waste.

2. Materials and methods

2.1. Materials

Fresh sheepskins were purchased from the Addis Ababa Abattoir, which is located in capital city of Ethiopia, Addis Ababa. Laboratory chemicals and reagents used for analysis are of analytical grade and includes sodium hydroxide (NaOH), 99.0 %; hydrochloric acid (HCl), 37 % w/w; sulfuric acid (H₂SO₄), 98 %; nitric acid (HNO₃), 65 %; perchloric acid, 70 %; sodium chloride (NaCl), 99.5 %; boric acid, 99.5 %; and basic chrome sulfate (33 % basicity). All the laboratory chemicals and reagents including process chemicals for tannery beam houses and retanning, were provided by the Leather and Leather Products Industry Research and Development Cent. The main laboratory instruments and equipment used in this study includes round bottom flasks, a water bath, a digital electronic balance, an analytical weighing balance, a hot air oven, a pilot scale reactor, test equipment such as a Kjeldahl system, an FT-IR Spectrometer (PerkinElmer), an X-Ray Diffractometer (XRD-X-ray tube cu40kv, 44 mA, Rigaku, Ultima IV), and tensile testing machine (testometric/M350) was used to study the mechanical strength characteristics of the crust leather.

2.2. Methods

2.2.1. Raw material collection and preparation

Ethiopian tanneries (Ethiopia Tannery around Mojo and Elico in Addis Ababa) that use the hair-saving method of hair removal provided 6 kg of sheep hair waste for extraction purpose. The composite method of sampling was used to obtain samples of sheep hair waste. Additionally, 75 pieces of fresh sheep skin from the Addis Ababa Abattoir were purchased, and they were unhairing using a hair-saving technique to determine the percentage composition of sheep hair based on the weight of the skin when it was at the wet salted stage. The Leather and Leather Products Industry Research and Development Center's laboratory and model tannery served as the location for the laboratory- and pilot-scale extraction process as well as the paint unhairing process. The Center is situated in Akaki-Kality Sub city, Addis Ababa, Ethiopia. However, some laboratory analyses were conducted at the Central Laboratories of Adama Science and Technology University (ASTU) and Addis Ababa Science and Technology University (AASTU).

To eliminate the salt sodium chloride (NaCl), sodium sulfide (Na₂S), and hydrated lime Ca(OH)₂ used during preservation and paint unhairing, the raw sheep hair sample was washed many times with enough warm water. The sample of washed sheep hair was dried outside in the sun. To partially remove the fat on the surface of the hair, the hair was degreased using a solution of sodium bicarbonate (1 g/L), ammonia (1 g/L), and an anionic degreasing agent (1 g/L). The treated hair sample was subsequently cut into smaller pieces to increase the surface area in contact with the hydrolyzing agent (NaOH) and, consequently, the effectiveness of the extraction.

2.2.2. Laboratory-scale extraction and characterization

Based on the designed experiment employing the response surface methodology (RSM) combined with the Box–Behnken experimental design, the important parameters affecting the extraction process (NaOH concentration, temperature and time) were used to carry out the experiment. 20 g of the samples were soaked in 0.25 L of 0.4 N, 0.70 N, or 1 N NaOH solutions for 1.5, 2.5, or 3.5 h, at 65, 80, and 95 °C, respectively. The samples and solution of the hydrolyzing agent were placed in conical flasks, which were subsequently placed inside an autoclave, high-pressure steam sterilizer, ES-215, for extraction. By using vacuum filtration, the extracted keratin hydrolysate (KH) was further purified, concentrated by evaporation in a water bath and then centrifuged at 10,000 rpm for 5 min. To

prepare for dialysis, the KH solution was concentrated once more in a water bath. The samples were then subjected to membrane dialysis for approximately 72 h. The dialysis product was further concentrated in a water bath and the pH of the concentrated KH solution was subsequently adjusted to the necessary pH of 4.2 using a 2 M HCl (37 %) solution. The precipitated KH solution was then centrifuged at 10,000 rpm for 5 min to produce keratin powder. Table 1 shows the experimental variables considered and protein yield as a response variable.

The total ash content of the keratin powder sample was determined according to the Society of Leather Technologists and Chemists, SLC6 (IUC 7; BS 1309:6) [46,47]. 5 g of the KH sample were weighed and placed in a pre-weighed, clean, and dry crucible dish. It was then heated on a heating mantle inside a cupboard hood until smoking ceased. Then, the dishes were placed in a muffle furnace preheated to 750 °C and heated at that temperature for 3 h. The total ash content of the hair samples was calculated using Equation (1). Furthermore, the structure, morphology, and functional groups of the keratin powder were analyzed using X-Ray diffractometer (XRD) with an X-ray tube cu40kv, 44 mA, Rigaku Ultima IV, FTIR spectrometer with a SHIMADZU IR Affinity-1S, and scanning electron microscopy (SEM) with model JSM840A, respectively.

$$\%Ash = \frac{\text{weight of crucible and ash} - \text{weight of crucible}}{\text{weight of hair sample}} \times 100 \quad (1)$$

2.2.3. Pilot-scale extraction of KH

The optimum conditions from the laboratory-scale extraction optimization reaction (0.4 N NaOH, 80 °C, 3.5 h) were used directly for the pilot-scale extraction; however, the amount and concentration of the solution, including the sample of sheep hair, were scaled up to the pilot scale, in which 4 kg of the treated sheep hair (50 L*0.02 kg)/(0.25 L) = 4 kg was soaked in 0.4 N of 50 L of NaOH solution. After that, the extract was filtered, and its pH was adjusted to 4.5, 5.0, 6.0, 7.0, and 8.0 pH values.

KH started to precipitate at pH 4.5; in particular, clear precipitation was observed at a pH less than or equal to 4.2, and since loosening affects the leather at an extremely high pH above pH 8, the KH extract was adjusted to five different pH values, 4.5, 5, 6, 7, and 8. These pH values were used to investigate the pH rendering relatively higher exhaustion of chromium and spent liquor with minimum values of COD (mg O₂/L), TDS (g/L), and % Cr₂O₃ (g/L). The stainless-steel pilot reactor employed for the extraction process, the raw sheep hair used, the extracted product KH at pilot scale and sample 25 mL kH solution were as shown in Fig. 1a,b, 1c and 1d, respectively.

2.2.4. Chrome tanning process application

Raw sheep skins were conventionally processed until the pickled pelt, after which the pH of the pickle float was adjusted to 2.8 to 3.2 before the application of keratin hydrolysate according to the methods in Tables 2 and 3. The tanning process was applied to both the experimental and control samples, using 6 % basic chromium sulfate (33 % basicity). The percentage of the chromium's primary valence bonds in solution that have an OH- group is known as the basicity of the basic chrome sulfate solution. To explore the relatively high exhaustion of chromium and spent liquor with relatively low COD (mg O₂/L), TDS (g/L), and Cr₂O₃ (g/L) concentrations, the same proportion (20 %) of pH-adjusted KH extract solutions was applied one at a time. The temperature, drum revolution per minute in rpm, float duration, and percentage dose of basic chrome sulfate, all of which affect chrome exhaustion, were all maintained within the ranges of 20–35 °C, 6–8, 40–100 %, and 6 %, respectively. The seventy-five pieces of fresh sheep skin purchased from the Addis Ababa Abattoir were typically processed until they were pickled. One of the five pH values evaluated (4.5, 5.0, 6.0, 7.0, and 8.0) required the

Table 1
Experimental conditions and responses of the predicted values.

Run No.	Actual factors			Absorbance at 540 nm	Protein concentration (mg/mL)	% protein yield
	Temperature (°C)	Concentration (N)	Time (h)			
1	80	0.75	2.5	0.7914	66.58	82.70
2	65	0.4	1.5	0.7455	62.645	80.12
3	80	1	3.5	0.7437	62.494	78.94
4	95	1	2.5	0.6816	57.177	74.33
5	65	0.4	3.5	0.8029	68.773	82.89
6	65	1	2.5	0.6956	58.374	74.35
7	95	0.75	2.5	0.7308	61.386	77.54
8	80	0.4	3.5	0.8210	69.111	86.57
9	95	0.4	2.5	0.7613	64.002	79.16
10	80	0.75	1.5	0.779	65.526	82.08
11	80	0.75	2.5	0.787	66.1675	83.58
12	80	0.5	1.5	0.786	67.350	83.301
13	65	0.75	2.5	0.748	62.893	78.12
14	95	0.75	3.5	0.719	60.398	78.20
15	95	1	1.5	0.676	56.717	74.03
16	95	0.75	1.5	0.714	59.935	77.60
17	65	0.75	1.5	0.761	63.945	78.07
18	95	0.4	2.5	0.758	63.681	81.78
19	80	1	1.5	0.742	62.352	78.76
20	65	1	3.5	0.712	59.753	73.59

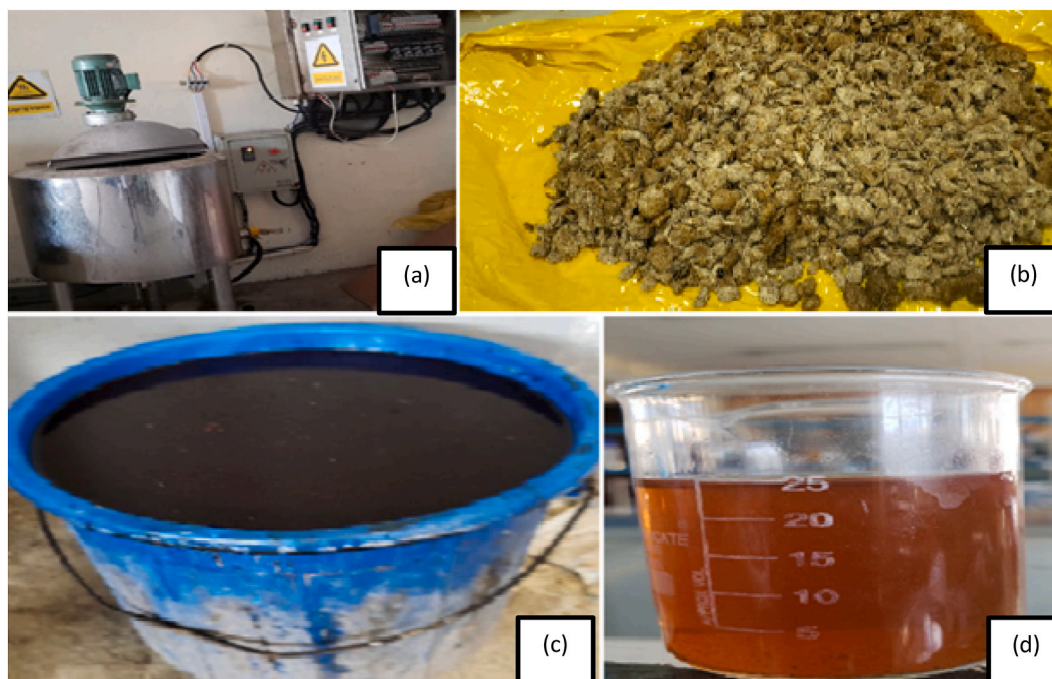


Fig. 1. Pilot scale extraction: (a) stainless steel reactor (b) Raw sheep hair (c) Pilot scale extraction product (d) KH display using 25 mL beaker.

Table 2

Process methods soaking, unhairing and liming of raw sheep skin.

Process	%	Chemicals	Temp. (°C)	Time (min.)	pH, °Be etc.
Wash 3× Drain	300	Water	25	10	°Be < 1.0
Soaking	200	Water	25		
	1	Sodium carbonate			
	0.25	Biocide (Cortymol BAC)			
	0.15	Cletapon BAC			
	0.2	Wetting Agent (Selastol EC)		20	
		Adjust drum automatic and run 5 min every hour			pH 10
Next day	0.3	Agglutan PR		90	Total soaking time 22 h
Drain/Rinse/Unload and pile					
Paint solution	100–150 g/L	Sodium Sulphide			°Be = 14-16
	400–600 g/L	Lime			°Be = 28-30
To the empty drum add	300	Water	25		
	0.1	Hydrated Lime		5	
Add unhaird skins	0.1	Wetting Agent (Eusapon OD)		20	
		Adjust drum automatic and run 10 min every hour			pH 12
					Total Liming time 22 h
(Wash/Drain) 3×					

Note: °Be is degree baume, 3X = three times.

use of nine sheep skins at once, six of which were divided in half along the backbone to be used as materials with the same properties for both the experimental and control runs. Three identical-sized pilot-scale stainless steel drums were set up to conduct three separate runs for one pH at a time (drum-1 KH solution applied prior to tanning, drum-2 KH solution applied following tanning during basification, and the third drum served as a control with no KH solution application). To investigate the pH rendering relatively high exhaustion and the exact point of application using a total of 45 pieces of sheep skin, five such operations were conducted for each pH. Based on the weight of fleshed pelt, a constant KH dose of 20 % in solution or 1.43 % in powder form based on the KH solution solid matter of 0.0721 g/mL was utilized. For both the experimental and control processes, the same amount of chromium was used (6 % based on the weight of the fleshed pelt). To test the chromic oxide concentration of the spent liquor, TDS, and COD chrome tanning spent liquors from both the control and experimental operations were collected.

After aging for at least 48 h, wet blue samples were taken from both the experimental and control process drums for the shrinkage test and chromic oxide content estimation to investigate the increase in chromium uptake due to the application of the KH extract.

Table 3
Process methods for the delimiting, bating, pickling and tanning of sheep skin pelts.

Process	%	Chemicals	Weight	Time (min.)	pH, °Be etc.
Wash 2×	150	Water, 30		10	
Drain					
Delimiting	200	Water		20	Drain
	0.3	Decal N			
	50	Water, 30			
	1.2	Decaltal N			
	0.3	Ammonium sulfate		40	pH 7.5–8.0
Drain					
Bating	50	Water, 33			
	0.5	Bating enzyme (Codymac 1.0 M)		30	
	0.5	Degreasing Agent (Silastol 380)			
	0.5	Tergolix DA 511		60	
Drain					
Wash 2×					
Drain					
Pickling		No float			
	8	Common salt		10	°Be 7-8
	0.3	Formic acid		15	
	0.3	Formic acid (2 portions) 1:10		30	
	0.2	Sulfuric acid 98 1:10		15	
	0.2	Sulfuric acid 98 1:10		90	pH 2.8–3.0
Tanning	3	BCS		30	
	4	BCS		90	
	50	Water, 40		30	
	2	Tanning auxiliary (Implanal DC)		30	
	0.4	Basifying agent (Neutrigan MOS)		5 h	pH 3.8–4.0
	0.2	Fungicide (Cortymol FUN)			

Finally, chrome liquors from the control and experimental processes drums were collected for chromic oxide, COD, and TDS analysis. Determined the optimum pH and point at which the KH solution should be applied (before or after tanning), the next step was to make an investigation on how different dosages affect the product quality and spent liquor characteristics.

Since no previous research has been performed, it was thought vital to undertake a new investigation on the optimal dosage of KH, which should be closer to the dose of commercially available chemicals for chrome exhaustion (1.5 % of MgO) [48]. Therefore, 30 sheepskin samples were employed for these experiments, considering various concentrations of 35, 30, 25, 15, 10, and 5 % KH in solution and 2.5 %, 2.2 %, 1.8 %, 1.08 %, 0.7 %, and 0.4 % in powder form, respectively based on the solid matter (0.0721 g/mL) of the KH solution. Six sheepskins were used for each experimental trial to optimize the percentage of KH dosage. However, the optimum pH and point of application (before or after tanning) were taken from the previously determined optimum pH value and point of application with optimum chromium uptake and lower TDS, chromic oxide, and COD in the spent liquor.

2.2.4.1. Determination of chromic oxide content in the wet blue leather. Determination of the chromium content (chromic oxide) was conducted for each of the experimental and control samples using colorimetric method used by Ref. [49], where 0.5 g of wet blue samples from the butt portion of both the experimental and control samples were taken in a 250 mL Erlenmeyer flask and digested using the perchloric acid digestion method (SLTC 1965) [50], where oxidation of the wet blue was carried out using an acid mixture (sulfuric acid, nitric acid, and perchloric acid) on a heating mantle in a hood. The technique of titration was subsequently used to estimate the content of the chromic oxide. To further complete the digestion for 10 min, 10 mL of distilled water was added. Then, following the completion of acid digestion, the samples were cooled to room temperature, transferred to a 100 mL measuring flask, mixed with distilled water, and then titrated. Finally, the percentages of chromic oxide by mass and chromic liquor by mass were determined using Equations (2) and (3), respectively.

$$\%Cr_2O_3 = \frac{T * 0.00253 * 100 * C_f}{M} \quad (2)$$

where T is the volume of consumed sodium thiosulfate during titration. M is the weight of the sample (wet blue) and C_f is correction coefficient and 0.00253 g is the amount of Cr_2O_3 consumed by 1 mL of 0.1 N sodium thiosulphate.

$$\text{Chromic liquor percentage by mass} = \frac{T * 0.00253 * 100 * C_f}{V} \quad (3)$$

where V is the volume of the sample liquor.

2.2.4.2. Hydrothermal stability test. A Thiess shrinkage tester was used to examine the hydrothermal stability of the wet blue leather for each of the experimental and control process samples. According to the official method of sampling and testing followed by Refs. [1, 51]. A rectangular test piece of wet blue leather measuring 50 mm by 3 mm was cut from both the experimental and control samples

using an oil dynamic micro clicking press machine. The test piece was then suspended inside the apparatus containing distilled water for measuring the shrinkage temperature, and the water was heated at a rate of 2 °C with heat from a Bunsen burner until the leather specimen reached the desired shrinkage temperature. The device was equipped with a reference metal that displays a variation in the length of the wet blue line. The temperature that caused the length of the wet blue to change was ultimately recorded for all the trials of the experimental and control runs based on readings on the inserted thermometer as T_s (°C), where, T_s is shrinkage temperature.

2.2.5. Retanning process application of KH

After the completion of the tanning process, the wet blue leathers were piled for at least 48 h so that cross-linkage of chrome tannage by oxalation was allowed to undergo for the better strength and stability of wet blue leather. Oxalation and Oxalation are two of the three stages in the crosslinking of chrome tannages. In the first stage, chromium complexes react with the carboxyl groups in proteins. As the tannage's pH rises, particularly during basification, the hydroxyl replaces the sulfate associated with chromium, and chromium atoms share these hydroxyl groups through olation. The tannage becomes more stable when the complex gives up hydrogen ions and oxalation results upon further drying during piling. The samples were prepared for the next process by conducting preparational operations, such as through feed samming, shaving the wet blue to the required thickness of the final leather product, 0.9 mm for the sheep upper leather. The mechanically adjusted wet blue leathers were used for the subsequent operation of the retanning process based on the designed methods, as shown in Table 4A number of retanning processes were conducted at different pH values of 5, 6, 7, and 8, substituting the commercial protein filler (Celatan F) with the produced keratin filler (50 %, 75 % and 100 %). This approach made things easier to determine the product's optimum pH for retanning application. After the crust leather was produced, it

Table 4
Retanning, dyeing and fat liquoring for sheep upper.

Process	%	Chemicals	Weight	Time (min.)	pH, °Be etc.	
Add	150	Water, 40 °C				
	0.5	Silastol R687				
	0.3	Silastol 380		30		
					Drain/Wash/Drain	
Rechroming	150	Water, 40 °C				
	1	Pelllan KFL		20		
	0.3	Formic Acid		10	pH = 3.2/3.3	
	3	Novaltlan PF		30		
	5	BCS		40		
	1	Sodium Formate		40	pH = 4.0	
L/O/N, Drain Wash/Drain						
Neutralization	150	Water, 35 °C				
	2	Magnopal RHN		30		
	2	Clarimol AK		40	pH = 5.5 Drain/Wash/Drain	
Retanning	50	Water, 40 °C				
	4	Magnopal RHN		20		
	3	Taningan OS				
	3	Mimosa				
	2	Qubracho				
	5	Retanal MD 80		30		
Dyeing	4	Dye		40	Check penetration	
Fat liquoring	100	Water, 50 °C				
	3	Lipsol J622				
	1	Perfectol WR				
	2	Lipsol MSG		45		
	2	Taningan OS		30		
	Fixation	1	Formic Acid		15	
		1	Formic Acid		15	
2		Formic Acid		30	pH = 3.4/3.6 Drain	
150		Water				
0.4		Formic acid		10		
0.375	Black dye					
0.125	Blue dye		15			
0.5	Formic acid		15			
0.425	Black dye					
0.075	Blue dye		15			
0.5	Formic acid		15			
					Drain/Wash/Drain	
		Adjust Water, 50				
	0.75	Formic acid		30		
	0.5	Formic		30		

L/O/N = Leave overnight.

was determined that it was crucial to assess the quality of the crust leathers produced as well as to check whether the leather would ultimately serve its intended purpose in various environmental settings.

2.2.5.1. Estimation of nitrogen content. Following the end of the tanning and retanning processes, the experimental and control samples were taken for the purpose of estimating the nitrogen content of the leather using the Kjeldahl method after its decomposition [45,52]. Where, 1 g of treated leather sample was taken in triplicate, digested using the prepared acid mix to produce ammonium. The ammonium was then treated with an alkaline solution (50 % sodium hydroxide) to convert it to ammonia, and the mixture was distilled using steam at a high temperature. Boric acid was used to condense and trap the ammonia that had escaped from steam distillation. The amount of boric acid was measured by titration, and the average was computed using the dry weight of the leather sample.

2.2.5.2. Tensile strength and percentage elongation of crust leather. Test specimens of both the experimental and control types of leather were prepared and conditioned as per IUP -1 and IUP- 3 [2,53]. The specimens were subsequently tested using tensile testing machine (testometric/M350) as per IUP 6 (ISO 3376:2011) [54] under the same constant rate of loading during all the measurements. A dumbbell-shaped sample is punched out using a standard steel die with dimensions of 110 mm total length and 25 mm diameter, but the machine used for measuring the tensile strength test is only 50 mm between the two grips. This particular shape aids in concentrating the stresses in the narrow portion, forcing the sample to break there; otherwise, the sample may break near the jaw of the machine, which will give a wrong value.

The thicknesses of each test specimen were measured to the nearest 0.01 mm using a thickness gauge at three locations, the average of which was calculated and recorded. The width of each specimen was measured using a graduated scale and used to calculate the cross-sectional area. The distance between the grips of the tensile tester was set to 50 mm for the standard test specimen, and the test specimens were inserted between the grips; thereafter, the grips were tightened with sufficient pressure. Finally, the testing machine was operated at a speed of 100 mm/min to the maximum force until the sample broke; then, the machine was immediately stopped, and the distance between the two grips was measured. The tensile strength and elongation at break of the sample leathers were calculated using Equation (4).

$$\text{Tensile strength} \left(\frac{\text{N}}{\text{mm}^2} \right) = \frac{\text{Breaking load (N)}}{(\text{thickness} \times \text{width}) \text{mm}^2} \quad (4)$$

Since the percentage elongation of the leather is also a useful index of leather stretching quality, it was measured simultaneously with the tensile strength; two reference marks were made in the narrow portion of the specimens before the start of testing, and the distance between these marks was measured. Finally, the extension due to the applied load was expressed as the percentage of elongation at that load, as calculated from Equation (5).

$$\% \text{ Elongation} = \frac{\text{Change in length between the jaws at the instant of break (m)}}{(\text{initial length between the jaws (m)})} \times 100 \quad (5)$$

2.2.5.3. The double-edge tear strength of crust leather. The double-edge tear strength (Baumann tear strength) was measured for the crust leather samples from both the control and experimental processes. The crust leather samples were cut to a size of 70 mm × 40 mm with the central cut to the longer edge of 50 mm in length. The tear strength was calculated by taking the ratio of the maximum load (N) exerted to the maximum upthrust distance the grain moved at the instant of grain burst as shown by equation (6). Test specimens of the leathers were prepared and conditioned as per IUP 1 and 3, and the specimens were tested using tensile testing machine (testometric/M350) as per ISO 3377: 2016/IUP -8 used by Refs. [47,55]. Test specimens were cut using the cutting knife 50 mm × 25 mm with a central slot and conditioned for 48 h; thereafter, the thickness of each specimen was measured. The specimens were subsequently inserted through the slit into sample holder clamps that were fixed to the tensile tester. The test was conducted by operating the tester at a rate of 100 mm/min until the test specimen was torn apart, where the maximum force was recorded, and the tear strength of the measured samples was calculated using equation (6).

$$\text{Tear strength} \left(\frac{\text{N}}{\text{mm}} \right) = \frac{\text{maximum tear load (N)}}{\text{thickness (mm)}} \quad (6)$$

2.2.5.4. Distension and strength of grains of crust leather. Test specimens were prepared and conditioned as per IUP 1 & 3. For each sample, triplicate specimens were cut using an oil dynamic micro clicking press machine. The specimens were tested for distension and strength of grain ball at burst using tensile testing machine (testometric/M350) as per the ISO 3379:2015/IUP 9 used by Refs. [46,47]. The average load (N) and distension (mm) at burst for the specimens were measured and recorded.

2.2.5.5. Crust leather organoleptic properties. Organoleptic properties such as softness, fullness, roundness, grain smoothness, grain tightness, color uniformity, and general appearance were assessed by hand and visual examination for all the experimental and control dyed crust leathers, where the leathers were rated on a scale of 0–10 points for each functional property.

3. Results and discussion

3.1. Extraction optimization and characterization results of the extracted KH

Biuret test method of analysis for the extract revealed that the optimum parameters of temperature (80 °C), concentration (0.4 N), extraction time (3.5 h), and protein yield (88.6 %) were attained. The treated samples were taken in triplicate to determine the ash content, and the results are depicted in Table 5. As the table shows, the average ash content from the analysis data was 12.73 % of the sample weight, which is in consistent with the value reported by Ref. [27], slightly higher than the one reported by Ref. [18], 10 % and almost in agreement with that of [27], which is $12.16 \pm 1.42\%$ whereas ash content of most commercial re-tanning agents sometimes falls in the range of 30–40 % w/w [18]. This indicates that the experimental extract has relatively less hazardous inorganic oxides and salinity compared with most commercial retanning agents and the higher ash percentage indicates an excessive amount of the hydrolyzing agent employed. The findings suggests that the lesser ash contents are more likely to be green because ash content is a key feature of any retanning agents manufactured and employed in the leather manufacturing [40]. Furthermore, one of the biggest issues facing the tanning industry is the treatment of wastewater containing a high salt load. In general, while characterizing KH to be used as a retaining agent, the higher proportion of ash content indicates less amount of organic active component and a higher production of effluent load because it produces more unreacted oxides and other salts [40]. Additionally, it is important to take the ash content of the retanning agent into account during the characterization of the produced retanning agent. Currently, leather chemical manufacturers are under pressure to develop retanning agents with low salt contents.

The Fourier transform infrared analysis result was as shown in Fig. 2. In the spectrum the hydrogen-bonded -N-H and -O-H stretching motions of the amide functional group and absorbed water account for the relatively broad peak in the range of 3250–3300 cm^{-1} . The stretching motions of the -C-H and -N-H groups cause considerably less intense peaks in the range of 2900–3100 cm^{-1} . The carbonyl group of the amide moiety corresponds to the peak that can be observed in the range of 1400–1700 cm^{-1} .

On the other hand, at a wavelength of 1237.03 cm^{-1} , the stretching vibrations of the C–N and C–C functional groups and the bending vibrations of the N–H functional group in -CNH were observed. A less pronounced peak at 1541.11 cm^{-1} corresponds to the bending vibration of the -C-N-H group, whereas the peak at 1400.52 cm^{-1} was caused by the bending vibration of the functional group -CH₂. The -C-N- group stretching vibration is responsible for the less intense sharp peaks between 1200 and 910 cm^{-1} . Furthermore, the presence of characteristic peaks in this FT-IR pattern, such as amide -N-H, -C=O, -C-N-, and -CNH, confirms the existence of the amino acid building blocks that constitute peptide groups of keratin protein [40]. The characteristic structure and distinctive mobility of significant functional groups in its molecular structures, such as -CO-NH-, -NH₂, -CNH, and -C-H, are likely to result in peak signals in the FTIR pattern of keratin extract in transmittance mode [7]¹, [18]¹. Fig. 3a and (b) shows the morphologies of the KH powder extracted from tannery sheep hair waste using SEM at various resolutions of $\times 170$ and $\times 600$, respectively. The morphological view resembles that extracted at 80 °C and reported by Refs. [56–58]; however, it is more porous, deeper in color, and has more hollow spaces.

The XRD analysis result of the sheep hair keratin powder is as shown in Fig. 4. The XRD analysis result showed two diffraction peaks at the diffraction angles of (2θ) at 9.36° and 21.6° which were attributed to the presence α - helix and β - sheet structures, respectively. It was also observed from the XRD spectrum that the two places in the keratin structure giving crystalline nature for the keratin had diffraction peaks of varying intensity, with the more intense and less broad peak α - helix displayed at 2 theta (9.36°) and on the other hand, the one observed at 21.6° had short and broader peak.

3.2. Application of KH as a chrome exhaust aid and keratin filler

3.2.1. Optimization of the product pH, percentage dose and points of application

The effects of five pH values of KH solution (4.5, 5, 6, 7, and 8), the percentage of the applied KH solution (5 %, 10 %, 15 %, 20 %, 25 %, 30 %, and 35 %), and the points of application (before or after tanning) were considered in the optimization study. Several runs with KH solution at different pH values ranging from 4.5 to 8 and percentage dosages ranging from 5 % (0.4 % in powder form based on the determined solid matter of 0.0721 g/mL) to 35 % (2.5 % in powder form) were performed to determine the pH and percentage dose at which KH performs best. The results of the investigation revealed that the content of chromic oxide in wet blue leather to be better when the KH solution was applied at pH (5) than at pH (4.5) as depicted in Table 6, which is due to precipitation of KH at pH (4.5) that hinders its penetration. Similarly, the chromic oxide content was observed to be lower at higher pH values of 7 and 8 compared to pH value of pH 5 and pH 6, as shown in Table 6. On the other hand, it was demonstrated in Table 9 that chromic oxide in the spent liquor observed to be higher at pH values above pH (6), possibly because of the reverse breaking of bonds formed between chromium and the collagen structure and the replacement of the active site with KH molecules at higher pH values. Additionally, pH has an important role in regulating surface charge and the isoelectric point (pI) of leather, which has an important effect on leather processing because it influences how chemicals penetrate, distribute, and fix in the leather matrix [59]. This indicates that a pH of 6 is the optimum pH for

Table 5
Ash content of KH extract from sheep hair waste.

Trial No.	1	2	3	Mean \pm SD
Ash content	12.8	12.72	12.68	12.73 \pm 0.10

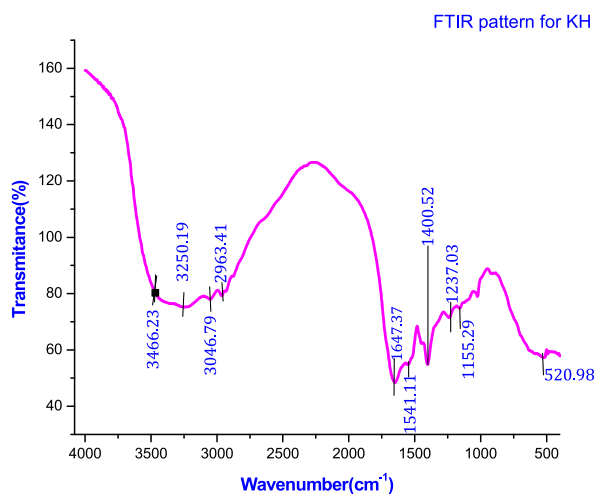


Fig. 2. FTIR pattern of the extracted KH.

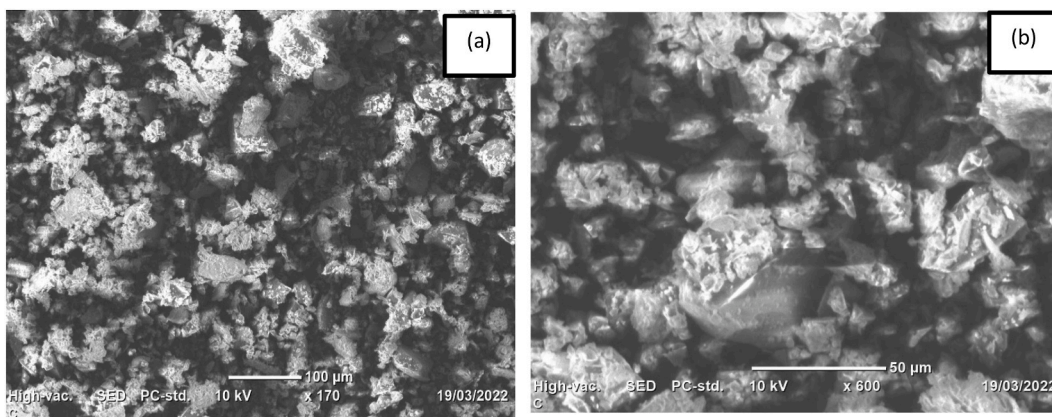


Fig. 3. SEM images of the KH product powder (a) $\times 170$ resolution and (b) $\times 600$ resolution.

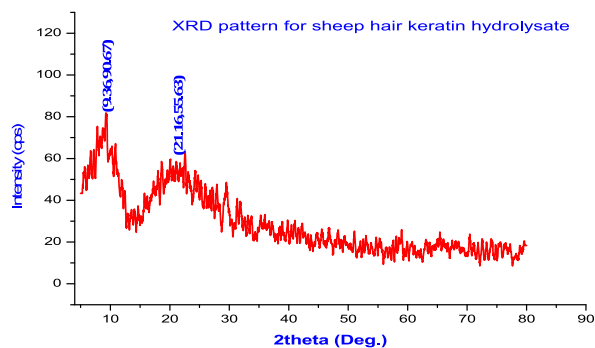


Fig. 4. XRD spectrum for sheep keratin powder.

mass production [40].

It was also observed that raising the pH to 7 or 8 resulted in softer crust leather and is suggested for softy upper leather, but doing so is not environmentally recommended because it increases the concentration of Cr_2O_3 , TDS, and COD in the spent liquor, hence, in tannery wastewater. This is because as the pH of the float increased from 6.0 to 8.0 during the application of KH at higher pH values, the electro positivity of the leather surface rapidly decreased, demonstrating a phenomenon in which the subsequent exhaust agents, specifically KH, tend to penetrate into the neutralized leather matrix rather than bind to the leather surface; on the other hand, the

Table 6Effects of varying pH and points of application on the %Cr₂O₃ content of wet blue leather.

pH	Dosage of KH solution (%)	Points of application	% Cr ₂ O ₃ in the wet blue leather (Mean ±SD)
4.5	5	After tanning (Experimental)	2.62 ± 0.15
5	5	After tanning (Experimental)	3.70 ± 0.24
5	20	Before tanning (Experimental)	2.88 ± 0.53
		After tanning (Experimental)	3.75 ± 0.17
		Control	2.31 ± 0.10
6	20	Before tanning (Experimental)	3.45 ± 0.24
		After tanning (Experimental)	3.81 ± 0.16
		Control	2.59 ± 0.15
7	20	Before tanning (Experimental)	2.56 ± 0.34
		After tanning (Experimental)	2.60 ± 0.71
		Control	3.22 ± 0.37
8	20	Before tanning (Experimental)	2.14 ± 0.18
		After tanning (Experimental)	2.50 ± 0.06
		Control	2.64 ± 0.47

Note: before tanning: on the pickled pelt, after tanning: after completion of tanning process during basification.

Table 7

Nitrogen content of the sheep skin at the raw, wet blue and crust leather stages.

Sample Number	Dosage of KH (%)	Nitrogen content for raw skin (%)	Nitrogen content of wet blue (%)	Nitrogen content of crust leather (%)
1.	5	12.0 ± 0.4	14.1 ± 0.3	14.8 ± 0.2
2.	10	12.7 ± 0.1	15.4 ± 0.2	16.2 ± 0.2
3.	15	13.8 ± 0.2	17.1 ± 0.1	18.6 ± 0.3
4.	20	13.3 ± 0.4	16.5 ± 0.1	18.0 ± 0.2
5.	25	13.7 ± 0.1	17.1 ± 0.3	18.7 ± 0.2
6.	30	13.4 ± 0.2	16.7 ± 0.2	18.2 ± 0.2
7.	35	13.8 ± 0.3	17.2 ± 0.3	18.8 ± 0.1

Table 8

Hydrothermal stability test results.

S/No	Percentage dosage (%)	Shrinkage temperature (°C)			mean ±SD
		Trial-1	Trial-2	Trial-3	
1	5	>105	>105	>105	>105
2	10	>107	>107	>107	>107
3	20	>107	>107	>107	>107
4	25	>105	>105	>105	>105
5	30	>105	>105	>105	>105
6	35	104	105	103	104 ±1
7	Control	105	101	103	103 ±2

penetration of the remaining chromium in the float will be restricted. This leads to a reduction in the proportion of chromic oxide in a wet blue leather test sample [60]. The nitrogen content of the same material at any pH and dosage of KH was lower when KH was applied after tanning during basification, and it was discovered that when KH was added after tanning at the basification stage, the percentage of Cr₂O₃ in the wet blue leather was better. On the other hand, the percentage of chromic oxide in the experimental wet blue leather was lower than that in the control wet blue leather when the sample was treated with KH applied at the pickled pelt stage. This is because when deciding which chemical should be applied first, molecular weight and particle size differences must be taken into account; in this particular case, chrome has a higher molecular weight (size); Consequently, the chromium cannot penetrate the collagen matrix if the collagen matrix has already been filled with smaller KH particles [60]. In this case, increasing chrome astringency and creating new active sites by adding KH after the tanning process is complete permits the remaining chromium in the float to be exhausted [61]. It was therefore demonstrated that the best sequence was to favor the one with the higher molecular weight.

3.2.2. Analysis result of the experimental and control wet blue leather

3.2.2.1. Chromic oxide content analysis results for the experimental and control leathers. The experiments were conducted in a triplicate, and the average of the values was used for data analysis. The analysis results showed a better percentage of chromic oxide (Cr₂O₃) in the experimental wet blue leather than in the conventionally processed control wet blue leather around the optimum pH value of pH (6). Furthermore, there was an improvement in the leather mechanical strength and organoleptic properties. Table 6 demonstrate that for the experimental wet blue leather samples produced, the percentage of Cr₂O₃ was observed to be always greater than that of in the

Table 9
TDS and Cr₂O₃ analysis results for tanning spent liquors at a 20 % KH dosage.

pH	Points of KH application	TDS (g/L)	Cr ₂ O ₃ (g/L)
4.5	After tanning at (5 %) KH dosage	67.42 ±0.85	9.62 ±0.15
5	Before tanning	66.05 ±6.95	9.045 ±1.45
	After tanning	49.33 ±0.71	6.164 ±1.21
	Control	66.17 ±6.34	6.231 ±0.44
6	Before tanning	48.07 ±2.86	7.437 ± 0.36
	After tanning	42.53 ± 4.31	4.69 0± 0.81
	Control	49.55 ± 5.03	6.164 ± 0.80
7	Before tanning	64.20 ±3.30	6.7 00± 2.13
	After tanning	61.70 ±4.40	6.7 00± 1.65
	Control	49.55 ±5.03	6.164 ±2.76
8	Before tanning	107.37 ±8.41	7.638 ±1.43
	After tanning	89.93 ±6.52	7.434 ±0.65
	Control	97.57 ±8.80	6.365 ±1.2

control wet blue leather when the KH solution was applied at pH 5 and 6. However, some variations were observed when the KH solutions were applied at pH 4.5, 7 and 8. So, both pH 5 and 6 were observed to be always better than their corresponding control process, indicating that the use of KH solution in the tanning process around these pH values improves chromium uptake to a greater extent.

As it was observed from Table 6 the percentage chromic oxide (% Cr₂O₃) content in the wet blue leather increased from 2.62 % to 3.7 % when the pH value of the applied KH solution raised from 4.5 to 5 at 5 % dosage, which implies that KH at pH 5 improved chromic oxide uptake better than it did at pH 4.5 due to the fact that at pH 4.5, the KH solution is about to start precipitation, and clear precipitation was observed just below this pH, especially at pH 4.2. On the contrary, the percentage chromic oxide in the experimental wet blue leather was clearly seen to drop from 3.81 % to 2.6 % and 2.5 % as the pH value of the KH solution applied raised to pH (7) and (8), respectively. That means beyond pH 6, there was a noticeable decrease in the percentage chromic oxide content of the wet blue leather, which may have been caused by KH replacing the already bonded wet blue in the collagen matrix due to a decrease in the astringency of chromium at those pH values [11,59,61].

As shown in the tabulated data, the percentage of chromic oxide content in the KH-treated wet blue leather was consistently greater when applied after tanning than those in which KH is applied before tanning, indicating an enhancement in chromium uptake. However, for some pH values e.g. pH (7) and (8), when KH was applied on the pickled pelt prior to tanning and after tanning, the chromium uptake was even lower than that in the control leathers. The reason for this is because KH applied before tanning to the pickled pelt fills up the interfibrillar gaps and spaces, making it difficult for chromium to penetrate [62]. However, by producing more active sites for chromium to form bonds, adding KH after tanning during basification aids in the uptake of the remaining chromium in the float. On the other hand, it was observed that the % Cr₂O₃ in the spent liquor of the experimental process was observed to decrease with an increasing of pH values from pH (5) to (6), as shown in Table 9, after which subsequent increases end up with an increase of chromic oxide content of the spent liquor. In addition, some characteristics of the spent liquor wet blue leather sample processed at higher pH values, specifically, TDS and % Cr₂O₃ (g/L), seen to be greater than even those of the control spent liquor as depicted by Table 9. This is because as the size of the void spaces in the collagen matrix decreases, the rate of KH penetration will initially exceed that of chromium, and as the void space decreases further, the rate of KH penetration also decreases, resulting in more keratin protein in the spent liquor. Higher COD and TDS values indicates greater keratin protein accumulation, which is not advisable from an environmental and economic point of view.

The percentages of Cr₂O₃ in wet blue leather at various pH values both for the experimental and control processes are as shown in Fig. 5. The figure shows that KH performed best at pH 6, outperformed both the control and all other experimental pH values. As the pH

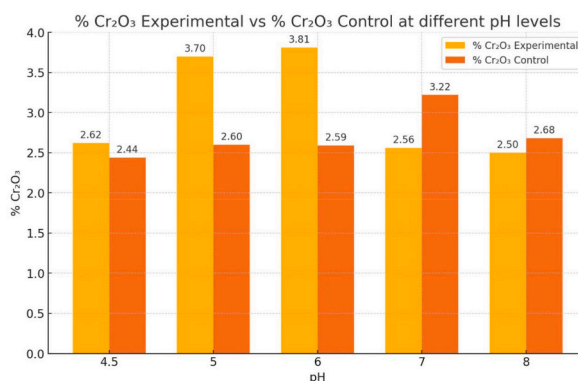


Fig. 5. Diagram showing %Cr₂O₃ in wet blue leather at different pH values of experimental processes.

of KH solution adjusted to more than 6, the values of percentage chromic oxide content ($(\%Cr_2O_3)$) were even lower than those in the control leather samples.

In general, pH values between 5 and 6 were found to be optimal for chromium uptake. In particular, pH (6) was seen to be the ideal pH for pilot-scale extraction, and at this pH, KH can also function as a basifying agent in addition to being used as an exhaust aid, which helps to minimize or avoid the application of sodium bicarbonate.

3.2.2.2. Nitrogen content of the wet blue and crust leathers. The wet blue leather treated with KH showed an increase in nitrogen content due to bond formation of the amino groups in KH with collagen and the chromium structures. When the keratin hydrolysate solution was applied to the pickled pelt before tanning, the sample became saturated with KH. As a result, the percentage of chromic oxide in the 0.5 g sample used for the analysis decreased due to the incorrect sequence of application. [Table 7](#) demonstrates that treating the sample leather with KH during the tanning process and using KH as a keratin filler increases the nitrogen content of the wet blue and crust leather, where a percentage increase in KH uptake with dosage was observed and further increase in dosage has a negligible effect on the nitrogen content.

3.2.2.3. Shrinkage test. As shown in [Table 8](#) samples from all of the processed wet blue leather at a common optimum KH solution pH of 6 and various percentage dosages were taken and subjected to a hydrothermal stability test.

The table shows that the use of KH as an exhaust –aid during the chrome tanning process improved the leather's hydrothermal stability, as evidenced by an increase in the shrinkage temperature well above the minimum recommended shrinkage temperature of 90 °C. Here, it was found that the majority of the wet blue leather samples from the control process shrank at temperatures lower than 105 °C when the conventional amount of chromium (6 %) was utilized. However, the experimental samples experienced shrinkage when the temperature exceeded 107 °C, which is the primary indication of increased chromium uptake.

3.2.3. Environmental assessment of the spent liquors

3.2.3.1. Tanning spent liquor analysis results for chromic oxide, COD, and TDS. [Table 9](#) shows the TDS and Cr_2O_3 analysis results for the spent liquor taken both from the control and experimental process drums at various pH levels and the two points of applications (before tanning on the pickled pelt and after tanning during basification).

Applying KH extract after completion of the tanning process (during basification) resulted in lower TDS and % Cr_2O_3 in the spent liquor, which indicated that most of the chemicals offered were taken up by the sample materials. Furthermore, KH offered at pH 6 performed best, with the lowest values of TDS (g/L) and % Cr_2O_3 compared with those of spent liquors taken from the control process drums and other experimental runs with pH values of 5, 7, and 8 [7]. [Table 10](#) shows the analysis results for TDS (g/L), COD (g O_2/L) and Cr_2O_3 g/L in spent liquor, and it can be seen that as the % KH dose increases from 10 % to 30 %, both TDS and chromic oxide in the experimental spent liquor are less than their values in the spent liquor from the control sample processing drum. However, beyond 10 % dosage the COD values for spent liquors from the experimental process sample exceeds that of those from the control process samples. This is due to the fact that the collagen matrix has only a limited number of active sites and it was shown in [Table 10](#) that even if the chromic oxide content in the wet blue increases with dosage of KH to some extent, the considerable amount of KH was seen to remain in the float contributing for an increase in COD value.

However, a further increase in dosage resulted in a greater effluent load than that of the spent liquor from the control sample drum. However, as shown in [Table 11](#), above a 10 % dose of KH solution, the COD (g O_2/L) of the spent liquor from the experimental process sample drums was greater than that from the control process sample drum, so this makes the COD an important parameter for determining the optimum dosage of KH solution to be 10 %, considering both environmental and economic factors.

Additionally, it can be seen from the information presented in the table that at a constant pH of 6, the tanning spent liquor's COD (g O_2/L) increases with % KH, and at higher pH values, minimal uptake of tanning chemicals occurs.

3.2.3.2. Retanning spent liquor analysis for COD and TDS. To analyze the retanning spent liquors for TDS, nine experimental runs and one control run were carried out with the substitution (100 %, 75 %, and 50 %) of the commercially available protein fillers (Celatan F). [Table 12](#) shows that the leather that underwent 100 % substitution had a lower TDS (g/L) value, indicating that adding KH as a keratin filler between the dyeing and fat liquoring stages of the retanning process improved the uptake of other chemicals, reducing the TDS in the spent liquor. In comparison to the spent liquor from the control process drum and other experimental processes, it was determined that a pH of 6.0 and 100 % substitution produced the best results, with a better outcome of a lower TDS value (19.3 g/L).

3.2.4. Analysis results for experimental and control crust leather

3.2.4.1. Mechanical strength characteristics

3.2.4.1.1. Tensile strength and percentage elongation of crust leather. Using tensile testing machine (testometric/M350), the mechanical strength characteristics of the experimental and control crust leather at different percentage substitutions were evaluated as shown in [Table 13](#). Determining the tensile strength of leather is a requirement in the leather industry because it is the only way for leather to withstand various mechanical operations, such as shaving at the wet blue stage, staking during crusting, and buffing of the intermediate leather product. As it was seen from the tabulated data all the 50 %, 75 % and 100 % substitutions for the Celatan F (Commercial protein filler) with KH resulted in tensile strength values above the minimum requirement (15 N/mm²). In addition, the

Table 10
TDS, % Cr₂O₃, Cr₂O₃ (g/L), and COD for different dosages of KH at pH (6).

S/No	% KH dosage	Cr ₂ O ₃ ($\frac{g}{L}$)	TDS (g/L)	COD (g O ₂ /L)
1	5	3.6	23.3	4.98
2	10	3.9	25.2	5.4
3	20	4.7	47.3	15.6
4	25	4.9	39.3	21.6
5	30	4.9	51.4	32.0
6	35	7.1	68.7	36.7
7	49	7.8	87.7	–
8	Control	6.2	54.2	7.5

Table 11
COD analysis results for spent tanning liquor at pH (6) and (8).

S/No	1	2	3	4	5	6	7	8
pH of KH solution	6	6	6	6	6	6	8	Control
% KH dosage	10	15	20	25	30	35	20	No KH
COD (g O ₂ /L)	5.4	9.7	15.6	21.6	32.0	36.7	36.7	6.7

Table 12
TDS and COD analysis results for the spent liquor retanning process.

S/No	1	2	3	4	5	6	7	8	9	10
pH of KH	8	7	6	8	7	6	8	6	5	Control
Substitution (%)	100	100	100	75	75	75	50	50	50	C
TDS (g/L)	26.25	28.54	19.30	23.19	26.74	23.10	28.65	28.62	23.80	31.00
COD (g O ₂ /L)	57.10	59.45	54.27	62.50	65.47	57.18	62.70	54.30	54.4	54.6

100 % substitution was observed to produce crust leather with better tensile strength compared with the other percentage substitutions and the control process.

3.2.4.1.2. The double edge tear strength of the crust leather. Testing tear strength is an important task for estimating the durability of leather to withstand tearing stresses encountered during the manufacturing of shoes, garments, gloves, and upholstered products. Therefore, it is preferable for customers to perform such tests than for determining tensile strength. Both control and experimental leathers have a mean tear load along or parallel to the backbone higher than that across/perpendicular to the backbone, as presented in [Table 13](#), because fibers are more oriented to the backbone and are highly resistant to tear load, whereas fibers across the backbone are not oriented toward the backbone; rather, they are less compact and far apart from each other.

3.2.4.1.3. Detention and strength of the grains of crushed leather (ball burst). The results of the analysis are presented in [Table 13](#), and the grain layer of leather is prone to cracking on the shoe surface, especially at the toe area, due to its exposure to strain during the shoemaking process and because of the need to fill the leather matrix with too many retanning chemicals. Therefore, leather for shoe preparation passes through a grain crack load and has good dimensional properties.

3.2.4.2. Crust leather assessment and manual evaluation of bulk properties. The bulk properties of the control and experimental crust sheep upper leathers, such as general appearance, grain tightness, smoothness, fullness, softness, roundness, and color consistency, were evaluated via hand and visual inspection by an experienced group of professionals. Experts graded all other crust leathers from 0 to 10 using the best export-grade crust leather as the benchmark. For each functional attribute, the experimental leathers were rated, and the average of those ratings was determined and the results were displayed in [Fig. 6](#), where the best export grade of the sheep upper leather was 10. It was discovered that the organoleptic properties of crust leathers from experimental samples, such as softness, fullness, tightness, color uniformity and general appearance, were better than those of the control leather. The required bulk properties for sheep upper leathers were not met by experimental sample leathers produced at pH values of 7.0 and 8.0 and with only a 50 % substitution of protein filler, which indicates that the developed keratin filler is more effective than the commercially obtained filler.

Table 13
Mechanical strength characteristics of experimental and control leathers at different % KH substitutions.

KH substitution (%)	Tensile strength (N/mm ²)	Elongation at break (%)	Tear load (N/mm ²)	Distension at burst (mm)	Load at burst (N)
50	20.48 ±3.67	60.06 ±8.80	26.98 ±3.89	10.3 0 ± 0.66	246 ±55.3
75	25.46 ±2.80	65 .00 ± 0.86	35.45 ±8.8	14.21 ±8.80	480 ±20.8
100	25.71 ±0.85	62 .00 ± 2.8	37 .00 ± 6.8	12.78 ±8.80	490 ±10.2
Control	22.63 ±0.60	55 .00 ± 1.8	32 .00 ± 4.2	12.04 ±0.45	405 ±8.4
Standards	>15 N/mm ²	40–80 %			

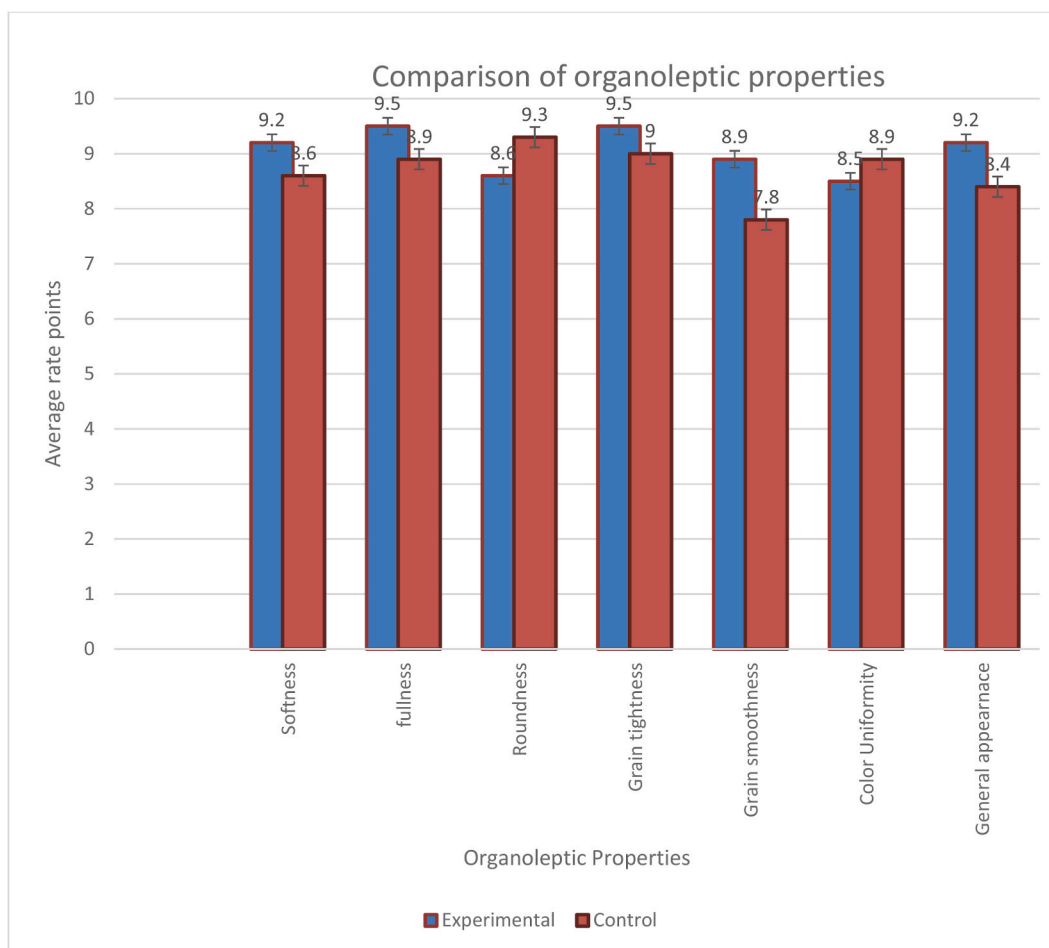


Fig. 6. Organoleptic properties of the control and experimental leathers.

4. Cost benefit analysis

In general, an approximate cost benefit analysis by equally sharing the overall country wise tannery sheep hair waste generation among the 36 tanneries in the country was conducted. Country wise Ethiopian tanneries have an average soaking capacity of 107,850 pcs of sheep skins considering an average wet salted weight of 1.2 kg per pcs and 20–30 % weight by mass of the skin is hair, the total generation per batch of processing (actual current working of 14 days per batch) was calculated to be 25,884 kg as a whole and 719 kg of sheep hair waste is being generated per tannery. Assuming 16 working hours per day implies 224 working hours are required two weeks to extract 599 kg of sheep hair, which is 569 kg after deduction of 5 % weight loss during washing.

In a 50 L pilot scale reactor a batch of extraction 4 kg of sheep skin is employed for 3 h of extraction time, this implies that to extract 569 kg of sheep hair 426.75 h is needed. A reactor can work 24 h per day (224 h per two weeks).

So, number of reactors needed = $426.75/224 = 1.91 \cong 2$ reactors for each tannery are required to handle all their own generated hair waste. Therefore, Cost of two 50 L pilot scale stainless steel reactors with its filter bags system will be $2 \times 350,000$ ETB = 700,000 ETB. ETB = Ethiopian birr. Assuming average area for end leather product from skin and cowhide is 4.6sq.ft and 24 square feet, respectively. So, the total produced area of leathers will be 13,800 sq. ft, 6,587.2 sq. ft and 6,528 sq. ft for sheep, goat and cow, respectively with a total cost of 0.74 ETB per sq.ft. Table 14 shows the overall summary of the cost benefit analysis.

5. Conclusion

In this investigation alkaline hydrolysis was employed to successfully hydrolyze tannery hair waste to produce keratin hydrolysate. It was observed that parameters, including temperature, time, and concentration of the hydrolyzing agent (NaOH solution) have significant impact on the extraction processes, specially, the concentration of the NaOH solution was seen to have a significant effect on the extracted keratin hydrolysate. Solid matter of the KH extract was analyzed to be 0.0721 g/mL. The FTIR analysis of the extract confirmed the presence of alkyl side chains of amino acids as well as carboxylic, amide, carboxyl group and aldehyde functional groups at $1400\text{--}1700\text{ cm}^{-1}$, $3,303.46\text{ cm}^{-1}$, 3270 cm^{-1} and 2752 cm^{-1} , respectively. On the other hand, XRD spectrum showed two diffraction

Table 14
Approximate cost benefit analysis.

S/No	Fixed cost	Ethiopian Birr (ETB)	Operation cost	Ethiopian Birr (ETB/day)
1	Cost of 50 L stainless steel reactor with filter bag system	700,000.00	Cost of collection and handling (labor cost)	429
2	Overall cost for groundwater supply system development such as labor cost, motor and digging and piping system cost	42,260.00	Drying after washing, sun drying,	0
	Total fixed cost	742,260	Cost of electric power	112.516
		Amount per day	Cost of chemicals (NaOH and HCl)	4008.9
			Total operation cost	4,550
1	Money saved from substitution of Celatan F and bicarbonate	1995.26ETB/day	Total KH solution to be produced per day	2678 L/day
2	Cost of remaining KH with 30 % deduction compared to commercial protein filler	4,290.6ETB/day	KH required as a substitution for bicarbonate and Celatan F	65.78 L/day
3	Total income from KH production	6285.89ETB/day	Remaining KH	202.08 L/day
4	Net income after deduction of operation cost	1,735.88ETB/day		
5	Payback period = fixed cost/1,735.88	428 days		
6	Considering 260 working days per year payback period = 428/260 = 1.65 years			
	≅ 2years			

peaks at 2 theta values of 99.36° and 21.16° due to alpha helix and beta-sheet structures. Extraction parameter conditions such as concentration of 0.4 N NaOH solution, extraction temperature of 80 °C, and extraction time of 3.5 h were determined to be the optimum parameters at which maximum protein yield of 86.57 % and 14.64 % nitrogen content were achieved using biuret test method and Kjeldahl method, respectively. The application of KH extract as a chrome exhaust aid and keratin filler provided a promising outcome when the product is applied at pH 6 and percentage dosage of 10 % after the completion of tanning process during basification. 100 % substitution of the commercial protein filler (Celatan F) with keratin solution ends up with leathers with improved mechanical strength characteristics, organoleptic properties and thermal stability. Furthermore, it brought about a notable decrease in COD and TDS in the spent liquor. In general, the application of KH in leather processing was observed to enhance uptake of chromium and other retanning chemicals which resulted in leather with better hydrothermal stability, mechanical strength characteristics and organoleptic properties. In addition, it was observed to have unpredictable benefit for the ecological environment due to its significant reduction of tannery effluent load. Finally, it can be concluded that the study has multi-objective purpose of applying circular economy of waste recycling and reuse and environmental protection through effluent load reduction.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of supporting data

All data sets generated during this study are included in this published article.

CRedit authorship contribution statement

Ashagrie Mengistu: Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Kenatu Angassa:** Writing – review & editing, Supervision, Conceptualization. **Israel Tessema:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Getaneh Andualem:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Bereket Yiheyese:** Writing – original draft, Software, Methodology, Investigation. **Daniel Berhane:** Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Mikiyas Abewaa:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Mulat Kassie:** Supervision, Methodology, Investigation, Data curation, Conceptualization. **Berhanu Telay:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

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