HIGH BLOOM GELATIN STRENGTH FROM WHITE LEATHER SHAVINGS

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ABSTRACT. Leather making process is recognized as one of the highly polluting extensive industries which generate huge amounts of solids and liquid tannery wastes. These wastes effect in severely way on the surrounding environment and human health if these polluting not well treated. The aim of this article is to obtain high bloom gelatin from white leather shavings (untanned hide shavings, WLS) via alkaline hydrolysis for improved utilization of leather waste. This research introduces a system of reusing technologies for WLS wastes including preparing gelatin, isolating collagen protein by extracting method then characterization of extracted gelatin. This article recognized to determine efficiency of the thermal and chemical treatments on the white leather waste in recovering the largest amount of gelatin with high bloom strength and a smallest amount of residue. Chemical treatments of white solid wastes by partial hydrolysis of wastes using different factors affecting on hydrolysis such as alkalis concentration, temperature and contact time were discussed. The method verified that the leather wastes can be successfully processed to the powder like leather gelatin products. It was found that the optimum conditions to obtain high bloom gelatin strength from white shaving leather wastes around 150 bloom are 4 % w/v NaOH, 4 hours contact time, 250 rpm at 50 °C.
KEY WORDS: alkaline hydrolysis, gelatin, high bloom strength, SEM, white leather shavings

OBŢINEREA GELATINEI CU PUTERE GELIFIANTĂ MARE DIN DEŞEURI DE PIELE NETĂBĂCITĂ

REZUMAT. Industria de pielărie este recunoscută ca fiind una dintre industriile care poluează foarte mult, care generează cantităţi uriaşe de deşeuri solide şi lichide. Aceste deşeuri au efecte adverse asupra mediului înconjurător şi asupra sănătăţii umane dacă nu sunt tratate cum trebuie. Scopul acestui articol este de a obţine gelatină cu putere gelifiantă mare din deşeuri de piele netăbăcită prin hidroliză alcalină pentru o utilizare mai bună a deşeurilor de piele. Această cercetare introduce un sistem al tehnologiilor de reutilizare a deşeurilor de piele, inclusiv prepararea gelatinei, izolarea proteinei de colagen prin metoda de extracţie, apoi caracterizarea gelatinei extrase. Acest articol a avut scopul de a determina eficienţa tratamentelor termice şi chimice asupra deşeurilor de piele netăbăcită în vederea recuperării unei cantităţi mari de gelatină cu o putere gelifiantă mare şi cu o cantitate mică de reziduuri. S-au discutat tratamente chimice ale deşeurilor solide de piele netăbăcită prin hidroliza parţială a deşeurilor utilizând diferiţi factori care afectează hidroliza, cum ar fi concentraţia de baze, temperatura şi timpul de contact. Metoda a verificat faptul că deşeurile de piele pot fi prelucrate cu succes sub formă de produse din piele gelatină pulbere. Condiţiile optime pentru a obţine gelatină din deşeuri de piele netăbăcită cu putere gelifiantă mare, de 150 grade Bloom, au fost: 4% NaOH w/v, timp de contact 4 ore, 250 rpm la 50°C.
CUVINTE CHEIE: hidroliză alcalină, gelatină, putere gelifiantă mare, SEM, deşeuri de piele netăbăcită

OBTENTION D'UNE GÉLATINE À HAUTE RÉSISTANCE À L'ENFONCEMENT À PARTIR DE DÉCHETS DE PEAU NON TANNÉE

RÉSUMÉ. L'industrie du cuir est reconnue comme l'une des vastes industries qui polluent beaucoup, générant d'énormes quantités de déchets solides et liquides. Ces déchets ont des effets néfastes sur l'environnement et la santé humaine s'ils ne sont pas traités correctement. Le but de cet article est d'obtenir une gélatine à haute résistance à l'enfoncement à partir de déchets de peau non tannée par l'hydrolyse alcaline pour une meilleure utilisation des déchets de cuir. Cette recherche introduit un système de réutilisation des technologies de traitement des déchets de cuir, notamment la préparation de gélatine, l'isolement de protéines de collagène par une méthode d'extraction, et puis la caractérisation de la gélatine extraite. Cet article a eu le but de déterminer l'efficacité des traitements thermiques et chimiques sur les déchets de peau non tannée afin de récupérer une grande quantité de gélatine à haute résistance à l'enfoncement et une faible quantité de résidus. Les traitements chimiques des déchets solides de peau non tannée ont été discutés par l'hydrolyse partielle des déchets en utilisant divers facteurs qui influent sur l'hydrolyse, tels que la concentration des alcalis, la température et le temps de contact. La méthode a permis de vérifier que les déchets de peau pouvaient être traités avec succès sous forme de produits à base de poudre de peau en tripe. Les conditions optimales pour obtenir de la gélatine à 150 degrés Bloom à partir de déchets de peau non tannée ont été: 4% NaOH poids/volume, temps de contact 4 heures, 250 tr/min à 50°C.
MOTS CLÉS : hydrolyse alcaline, gélatine, haute résistance à l'enfoncement, MEB, déchets de peau non tannée

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INTRODUCTION

Leather industry generates a huge amount of solid and liquid tannery wastes during the hide tanning process that produces leather as a supply for shoes, automotive and furniture markets. Environmental risk challenges arise from the nature and the wastes discharged from leather processing. The main part of leather wastes is the hide collagen structure. The leather making process generates large quantities of solid and liquid wastes, one metric ton of rawhide produces on an average 200-400 kg of tanned leather and a range of 600-800 kg of solid wastes and an average consumption of 45-50 m3 of waste liquors. In these process stages, solid wastes generated from leather industry are classified as 1st wastes from untanned hides or skins (white shavings, fleshing wastes) 80% of solid wastes (500-600 kg), 2nd wastes from tanned leather (chrome shaving, buffing dust) 150 kg, 3rd wastes from finished and dyed leather (trimmings) 20-30 kg [1, 2].

The problems, solid protein wastes, have admittedly received more attention however; these have been mostly disposed until now to landfill or glue production. Many cleaner processing approaches aimed at reduction of liquid and solid wastes proved to be economically and environmentally beneficial [3-6]. Leather wastes divided into leather waste from tanneries, trimmings from leather goods factories and trimmings from the old leather products. The leather wastes from tanneries are divided into white leather wastes, chrome-containing leather wastes and dyestuff-containing leather wastes. The major part of leather wastes is protein. If protein and other unspent chemicals not utilized or treated correctly they cause severe environmental problems threatening the sustainability of leather making. Various useful products (glue, gelatin, artificial fibrous leathers, and collagen hydrolysate) can be extracted from leather wastes. The best way for their removal is to recover soluble proteins that may have a commercial use. A number of authors have reported about chemical and enzymatic treatment of leather waste [7-9].

Approximately 80 % of dry matter of the hide or skin is made up of complex nitrogenous organic compound known as proteins. Collagen is the ultimate plentiful insoluble protein of animal origin, comprising about 29 % of total protein. It is the major protein of skin, tendon, cartilage, bone, and white fibrous connective tissue in mammals. Collagen extracted from the previous materials is used for clarifying beverages, in cosmetics, in casing for meat products and in a host of biomedical applications. Medical applications of collagen include use in drug delivery systems, sponges for burns and wound and in tissue engineering [10].

Gelatin is a high molecular weight polypeptide derived from collagen; it is obtained by denaturation and solubilization of collagen [11, 12] which is derived from pigskins and bovine hides, bones and fish skin. To produce gelatin, collagen is heated in either acidic or basic solutions to break the covalent bonds between the rods and solubilize the protein. The treatment also separates the polypeptides and hydrolyzes some of the polymer chains. Gelatin quality is measured by Bloom units [13, 14].

There are three methods of gelatin production: acid, alkali, enzymatic processes due to soaking and autoclaving processes. The most widespread methods are acid and alkaline processing followed by heating in water. Acid and base processes produce gelatins commonly point as Type A and Type B, respectively. Following these extraction processes [15-18], gelatin is dried to form coarse granules, fine powders, or thin sheets, which are odorless, tasteless, and yellowish in color. Over many years, gelatin has been an important biopolymer used widespread in food pharmaceutical industries. Commonly, it is used as transparent dessert jelly, but it is widely used in pastry, dessert, factories of meat products and dairy products [19-22].

The best way for their removal is to recover soluble proteins that may have commercial use. This paper deals with one of the major environmental problems of the huge manufacture in Egypt (leather industry). Approximately 80 % of leather solid wastes is generated during pretanning processes in beam

house operations. Untanned trimmings and white leather wastes have negative effects on soil and water resources of the surroundings due to the bad smell produced during their putrefaction and their dangerous chemical contents. In Egypt, production of glue is the only use for white leather shavings. In this research article, production of gelatin with high bloom strength from white leather shavings by different alkalis is our target. Alkali treatment is suitable for more complex collagen as in bovine hides, the purpose of the alkali treatment is destroying chemical crosslinks fiber bundle present in collagen.

Alkali chemical treatments of WLS by partial hydrolysis of wastes using different alkalis (NaOH, KOH, LiOH) and different factors affecting hydrolysis such as alkalis concentration (2-10 % w/v), contact time (1-6 h) and temperature (40-90 °C) were studied.

EXPERIMENTAL

Materials and Methods

Materials

White leather wastes were supplied by tannery from Misr-El-Kadima, Cairo, Egypt. Sodium hydroxide, lithium hydroxide and potassium hydroxide are used as received. All reagents were analytically pure according to the grade of national reagent purity standards.

Methods

The purpose of this research is to recover gelatin from the white leather waste to be used in many applications [21-26]. The study also aimed at evaluation of the different parameters affecting on white leather shavings hydrolysis. White shavings with a pH of 3.8 after basification were obtained from a commercial tannery. The shavings were soaked in 5x their weight of water for cleaning.

Factors Affecting on the Hydrolysis of White Leather Shavings (WLS)

There are different factors affecting on hydrolysis process of WLS by different alkalis (NaOH, KOH, LiOH) have been studied systematically such as concentration of different alkalis, contact time and temperature.

Effect of Different Concentration of Alkalis (Optimum Condition of Alkalis)

The effect of concentration of different alkalis on the hydrolysis of WLS studied as follows: White shavings (20 g) were put into a 250 mL flask and 80 mL of water was added, together with 2-10 % w/v of alkalis (LiOH, NaOH, KOH) equivalent to (1-2.5 mol/L). Then, the flask was put into a water bath at 70 °C and 200 µL surfactant, 250 rpm, for 6 h, the solution was then left to cool down.

Effect of Time on the Hydrolysis (Optimum Condition of Time)

The effect of time on the hydrolysis of WLS studied as follows: white shavings (20 g) were put into a 250 mL flask and 80 mL of water was added, together with optimum condition of concentration of alkalis. Then, the flask was put into a water bath at 70 °C and 200 µL surfactant, 250 rpm, different shaking time from 3 h up to 8 h, the solution was then left to cool down.

Effect of Temperature on the Hydrolysis (Optimum Condition of Temperature)

The effect of temperature on the hydrolysis of WLS studied as follows: white shavings (20 g) were put into a 250 mL flask and 80 mL of water was added, together with optimum condition of concentration of alkalis. Then, the flask was put into a water bath at different temperature from 50-90 °C and 200 µL surfactant, 250 rpm, optimum condition of time, the solution was then left to cool down.

Separation of Gelatin

After hydrolysis of white leather shavings in each of parameters used (conc. of alkali, hydrolysis time, temperature), the extracted gelatin poured into sintered glass filter grade 4 for filtration. The residual solid white cake was washed twice with water for adjusting pH at 7 and then the cake was put into a Teflon mold in a drying oven for complete dryness. The dried

gelatin was ground for obtaining gelatin powder for further characterization.

Determination the Properties of the White Leather Wastes and Extracted Gelatin

Determination of Moisture

Osborne punch was used to cut samples for moisture. The samples were weighed into dry, tared porcelain dishes. The samples were dried for 17 h at 105 °C. The samples were cooled in a desiccator, weighed and the percent moisture determined. 5 g of sample were accurately weighted in a tared dish, and then heated at 105 °C for three hours in air-oven at which the temperature was as uniform as possible; the dish was allowed to cool in a desiccator, and then weighted. The process of heating, cooling and weighting was repeated till constant weight, the moisture content is defined as the percentage loss in weight of the sample.

$$\% \text{ moisture} = (W1 - W2) \times 100/W1 \quad (1)$$

Where: W1 = weight of the sample before drying.

W2 = weight of the sample after drying.

Determination of Ash

The dried samples were ashed at 600 °C for two hours then cooled in a desiccator and weighed to determine ash content and percent volatile substance calculated on a moisture-free basis. In a burnt platinum crucible about 5 g of sample was accurately weighted, the sample was carefully ignited in a muffle furnace at about 600 °C for about 2 hours. Finally the crucible with its contents was cooled in a desiccator and weighted. The ignition, cooling and weighting were repeated till constant weight.

$$\% \text{ ash} = \text{Wt. of residue} \times 100/ \text{ Wt. of original fat} \quad (2)$$

Determination of Total Kjeldahl Nitrogen (TKN)

TKN was determined by the semi-micro Kjeldahl method. Solid samples weighed to the nearest 50 mg and liquid samples measured to 1 mL and transferred to a 30 mL digestion flask. Digestion catalyst (1.2 g), a few boiling chips and sulfuric acid (2 mL) added. The samples were digested for two hours. The samples were carefully transferred to the filling funnel and NaOH solution (10 mL) will be added. The mix was distilled to a 125 mL Erlenmeyer flask containing boric acid saturated solution (10 mL). The samples were titrated with standardized HCl to the gray endpoint.

Determination of Fat

For fat determination, samples were weighed into appropriate flasks and 6N HCl (75 mL) added. The samples were hydrolyzed for 2 h. The hydrolysate was transferred to a separating funnel and the fat was extracted with chloroform or petroleum ether. The chloroform layer was put in dry, tared crystallizing dishes, the chloroform evaporated and the samples were held at 60 °C for 16 h. The samples were cooled in a desiccator and then weighed.

pH Measurement of Leather Waste

The tests for the determination of the initial pH value were accomplished as follows: 5 g of white shavings sample was placed in 100 mL of distilled water at room temperature during two hours with agitation. After decantation without filtration of soluble matter, proceed to the determination of the pH of the prepared liquor using the pH-meter.

pH Measurement of Gelatin

The British Standard was adapted and one gram of gelatin sample was dissolved in 100 mL warm distilled water. The solution was cooled to 25 °C and the pH was measured with a standard pH meter.

Yield of Gelatin

The yield of extracted gelatin obtained was determined as follows:

$$\text{Yield (\%)} = (\text{dry weight of the gelatin/dry weight of white leather shavings}) \times 100\ \% \quad (3)$$

Characterization

Determination of Gel Strength

The gel strength (Bloom) was determined according to British Standard 757:1975 method (BSI, 1975), by using a texture analyzer (CT3 Brookfield, USA). A solution containing 6.67 % (w/v) gelatin was prepared by mixing 7.50 g of gelatin and 105 mL of distilled water in a Bloom bottle with stopper. The mixture was swirled and left to stand at room temperature for 3 h, allowing the gelatin to absorb water and swell. The Bloom bottles were then transferred to a water bath maintained at 65 °C and held for 25 min with occasional swirling to dissolve the gelatin. The bottles were taken out of the water bath, allowed to cool for 15 min at room temperature and then placed in a cold-water bath (Brookfield gelatin bath system, model TC-550MX refrigerated bath) maintained at 10 °C and held at this temperature for 18 h before the determination of the gel strength. The Bloom bottle was placed centrally under the plunger (Delrin probe, which is clear acrylic AOAC and GMA cylinder with sharp edge; TA10, 12.7 mm diameter) of the instrument. The Bloom strength was determined with a load cell of 10 kg and crosshead speed of 0.5 mm/s. The maximum force (g) was determined when the probe penetrated to a depth of 4 mm into the gel.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were taken with a Nexus 670 FTIR spectroscopy (Nicolet, United States) over the range of 400–4000 cm-1 with a resolution of 4 cm-1; the KBr disk technique was applied.

Dynamic Light Scattering (DLS)

The particle size investigation of the aqueous dispersions was carried out with Zetasizer Nano S (Malvern Instr., UK) equipped with a monochromatic He-Ne laser lamp (λ = 633 nm) as light source (ALV GmbH, Germany) at an angle of 173°. All measurements of dispersed samples were carried out in disposable cuvettes.

X-Ray Fluorescence (XRF)

It used to identify and determine the concentrations of elements in solid, powdered and liquid samples. XRF is capable of measuring all elements from Beryllium to Uranium and beyond levels often below one part per million. X-ray fluorescence was carried out using Axios, WD-XRF sequential spectrometer. Sample was crushed then ground in Herzog mill to powder, then sieved through 0.063 mm sieve. The samples were prepared as pressed disks, through mixing 7 g of fine powder of each sample with 1.6 g of binding wax in small mill, at 380 rpm, for one minute. Sample was kept in standard aluminum cup, pressed in automatic machine less than 130 KN, the yield disk spacemen was used in qualitative and quantitative analysis of elements.

High Performance Liquid Chromatography, HPLC Amino Acid Analyzer

HPLC consists of pump for pumping of mobile phase (liquid), Octocatyl silica column (C18-column). It provided with photodiode UV detector. It is used for determination of bulky organic compounds. Liquid chromatography 300, amino acid analyzer - Eppendorf, Germany, and flow rate: 0.2mL\min, pressure of buffer from 0-50 bar, pressure of reagent to 0-150 bar, reaction temp 123 °C, was used for amino acid analysis.

Preparation of sample to amino acids analysis:

1 - 1g of gelatin was weighed in a hydrolysis tube, then 1 mL of 6 N HCL.

2 - The solution was frozen and evacuated from the tube with vacuum pump.

3 - The hydrolysis tube was closed by melting the glass with a suitable gas-burner.

4 - Depending on original material, hydrolysis was carried out in an oven with a uniform temperature distribution of 110 °C for 24 hours, then the tube was cooled down in an ice-bath after hydrolysis. Afterwards, the solution was centrifuged in order to precipitate insoluble components.

5 - Centrifuged solution was evaporated at approximately 40 °C in a rotary evaporator.

6 - The sample was dissolved with 1-2 mL of sample diluting buffer; then the sample was ready for analysis.

Thermal Gravimetric Analysis (TGA)

Thermal analysis was studied with a Perkin Elmer thermogravimetric analyzer (rate=10 °C/min) from room temperature to 600 °C at the National Research Center of Egypt.

Differential Scanning Calorimetry (DSC)

DSC was carried out in a TA Instruments Q100 to determine glass transitions (Tg), crystallization temperatures (Tc) and melting points (Tm). The thermal history was erased during the first run at a high heating rate up to 200 °C, followed by cooling cycle to -50 °C. After that, the heating rate was modulated ± 1.0 °C min-1 with heating rate 5.0 °C min-1 to 200 °C. According to ASTM D 3418, Tg was calculated as the midpoint temperature.

Scanning Electron Microscopy (SEM)

SEM is thus a useful technique for evaluating the effects of various treatments on the leather surface and it can be used to assess the penetration of the copolymer through leather and onto the hierarchy of the structure. Leather samples (1cm2) were subjected to sputter coating with gold ions which acted as a conducting medium during scanning, and observed using a Philips Quanta 250 electron microscope.

RESULTS AND DISCUSSION

Characterization of White Leather Shaving (WLS)

It is very important to characterize the WLS before hydrolysis for obtaining extracted content was in the range 0.39-0.5 %. All the percentage represented in a dry basis except for moisture.

Table 1: Properties of white leather shaving

Characteristic	Mean Value*
pH	4 ± 0.3
Ash content	2.8 ± 0.2 %
Moisture content	11 ± 2 %
Nitrogen content	14 ± 1 %
Fat content	0.55 ± 0.2 %

Mean Value* ± SD triplicate analysis

Chemical Hydrolysis of White Leather Shavings (WLS)

Effect of Different Concentration of Alkalis

The effect of concentration of different alkalis on the hydrolysis of WLS was studied, for obtaining optimum conditions of conc. of alkalis which is a step growth for further parameters. Table 2 showed the effect of different concentrations on the gelatin extraction from WLS. The experimental results proved that as the percent of conc. of alkalis increases, the percent of the hydrolysable protein increases, where at 2 % w/v conc. of alkalis the WLS were not completely hydrolyzed and at 4 % w/v, 6 % w/v conc. of alkalis is adequate and enough for obtaining gelatin with good bloom strength but at 8 % w/v and 10 % w/v conc. of alkalis waste is completely hydrolyzed to hydrolysable protein.

Table 2: The effect of different concentrations on the gelatin extraction from white leather shavings (WLS)

	NaOH			KOH			LiOH		
Conc. %	Bloom G	Viscosity Poise	Yield weight %	Bloom G	Viscosity Poise	Yield weight %	Bloom G	Viscosity Poise	Yield weight %
2	-----	-----	-----	-----	-----	-----	-----	-----	-----
4	160 ±5	120 ±4	80 ±2	140 ±5	163 ±4	79 ±3	135 ±4	160 ±2	79 ±3
6	100 ±4	80 ±5	70 ±3	110 ±6	150 ±6	70 ±2	111 ±4	120 ±3	71 ±2
8	80 ±6	65 ±3	50 ±2	70 ±3	90 ±8	52 ±4	76 ±6	60 ±1	55 ±4
10	40 ±7	55 ±6	20 ±4	30 ±7	50 ±4	27 ±6	35 ±8	40 ±4	25 ±5

-----Not enough hydrolysis
Mean Value ± SD triplicate analysis

From Table 2, it is obvious that, 4 % w/v conc. of alkalis is the best concentration for gelatin extraction and it gives good viscosity and bloom values. At 2 % w/v conc. of alkalis there is not enough hydrolysis to the shavings. Over 6 % w/v conc. of alkalis there is a decrease in the bloom and viscosity values, due to the hydrolysis of the collagen fibers, yielding mixture of free amino acids and hydrolysable protein. High concentration of alkaline materials decreased the gelatin yield and gel strength [27, 28]. In conclusion, 4 % w/v conc. of all alkalis is the best concentration for gelatin extraction because it gives good viscosity and high bloom values which is very important in case of application in the further part of the research especially in biodegradable packaging.

Effect of Extraction Time on the Hydrolysis of White Leather Shavings (WLS)

The time of extraction is a very important parameter for studying the hydrolysis of WLS for saving money for industrial application. The data was recorded in Table 3. The experimental results proved that as time increased the gel strength increased up to 4 h after that it tends to decrease.

From Table 3 it is obvious that, 4 h extraction time is the optimum time required for gelatin extraction, besides high viscosity and good bloom values. At 3 h extraction time is not enough time to yield acceptable bloom and viscosity. However, over 5 h the extracted gelatin was hydrolyzed resulting in weak bloom and viscosity, longer extraction times give very low yield. Extracted gelatin has low gel strength and viscosity due to excessive damage and breaking down the collagen fractures with longer heating and possibly extraction of hydrolysable proteins. In conclusion, 4 h is the best time for gelatin extraction because it gives good viscosity and bloom values.

Table 3: Effect of time on the hydrolysis of white leather shavings (WLS)

Extraction time Hours	NaOH			KOH			LiOH		
	Bloom G	Viscosity Poise	Yield weight %	Bloom G	Viscosity Poise	Yield weight %	Bloom G	Viscosity Poise	Yield weight %
3	-----	-----	-----	-----	-----	----	-----	-----	-----
4	155 ±6	140 ±7	78 ±6	160 ±3	135 ±4	77 ±4	140 ±6	150 ±6	78 ±4
5	125 ±5	100 ±5	77 ±5	130 ±5	100 ±8	65 ±4	120 ±5	89 ±7	66 ±3
6	80 ±6	90 ±4	34 ±4	90 ±7	80 ±6	26 ±3	70 ±7	65 ±4	27 ±4
7	40 ±8	30 ±8	13 ±7	30 ±6	40 ±4	10 ±6	30 ±6	20 ±7	15 ±5

--- = Not enough hydrolysis
Mean Value ± SD triplicate analysis

Effect of Temperature on the Hydrolysis of White Leather Shavings (WLS)

The thermal effect is very efficient parameter in case of studying the hydrolysis of WLS. Table 4 show the effect of temperature on the hydrolysis of white leather shavings.

Table 4: Effect of temperature on the hydrolysis of white leather shavings (WLS)

Extraction temperature °C	NaOH			KOH			LiOH		
	Bloom G	Viscosity Poise	Yield weight %	Bloom G	Viscosity Poise	Yield weight %	Bloom G	Viscosity Poise	Yield weight %
40	----	----	----	----	----	----	----	----	----
50	160 ±4	120 ±4	79 ±3	140 ±4	163 ±7	78 ±2	130 ±6	160 ±5	76 ±2
60	130 ±3	100 ±5	61 ±4	120 ±6	117 ±5	60 ±3	99 ±3	120 ±4	84 ±3
70	100 ±6	80 ±3	40 ±5	100 ±8	83 ±5	43 ±4	74 ±4	90 ±3	63 ±4
80	50 ±7	56 ±5	23 ±7	80 ±5	62 ±8	24 ±6	60 ±4	66 ±7	46 ±2
90	40 ±7	34 ±7	13 ±8	50 ±7	30 ±6	12 ±8	40 ±4	36 ±8	20 ±8

---- Not completely hydrolyzed
Mean Value ± SD triplicate analysis

The experimental results proved that as temperature increased there will be an extensive increase in the hydrolysable protein up to 90 °C. From Table 4, it is obvious that 50 °C is the optimum extraction temperature required as it gave high viscosity and good bloom values. At temperature lower than 50 °C solubility of gelatin decreases, so the yield was decreased. However over 60 °C the extracted gelatin was affected by increasing temperature and hydrolyzed to free amino acids. Different temperatures are used in extraction of gelatin but most of them are in the range from 50 °C to 60 °C. Temperatures from 45 °C to 60 °C can enhance bond formation within molecules between strands and therefore gelatin with stronger crystallization ability can be obtained. However, temperatures above 60 °C lead to broken chains within the particles giving weaker gelatin ability. Lower extraction temperatures, on the other hand, lead to low yields. The higher yield percent of the gelatin is obtained at moderate temperature.

In conclusion, the optimum condition for obtaining higher bloom strength around 150 bloom with an average molecular weight of 20000 - 25000 g mol^{-1} or 20-25 kilo Daltons was 4 % w/v NaOH, 4 hours contact time, 250 rpm at 50 °C. After that, characterization of the extracted gelatin was investigated chemically and instrumentally to check suitability for industrial or biomedical application.

Characterization of Extracted Gelatin

There is increasing attention in the extraction process of gelatin and its derivatives due to the rising tendency to use gelatin to replace synthetic agents in various industrial processes, which results in a greater approval of the by-products from animal slaughter. Gelatin's characteristics depend on the raw material and the extraction conditions, which subsequently determine its application. Gelatin has a wide range of applications in the food, pharmaceutical, cosmetic and photographic industries, among others; also gelatin quality is highly affected by physico-chemical characteristics, not only by species and tissue extract, but also by processing methods [27].

The properties of extracted gelatin samples from different alkalis are showed in Table 5.

Table 5: The properties of extracted gelatin

Extraction Material	Color	Moisture Content %	Ash Content %	Nitrogen Content %	Fat Content %	Turbidity
NaOH	Brown	5.5 ±0.5	4.5 ±0.6	13.5 ±0.8	0.45 ±0.03	72
KOH	Brown	5.1 ±0.4	5.4 ±0.4	11.6 ±0.5	0.40 ±0.02	45
LiOH	Brown	4.7 ±0.6	5.3 ±0.5	10.9 ±0.7	0.42 ±0.03	101

Mean Value ± SD triplicate analysis, All the percentage represented in a dry basis except for moisture.

From Table 5 it is clear that the moisture content of all extracted gelatin from three alkalis was from 4 to 5.5 % with the highest value for NaOH and lower value with LiOH. The ash content was in the range from 4-6 %. However the nitrogen content has a high value 14 % in case of NaOH and lower value in case of 11 % in case of LiOH. The gelatin extracted from the LiOH is more turbid than the others extracted gelatin from NaOH and KOH and the fat content from all the extracted gelatin was in range 0.39 -0.45 %.

FTIR Analysis of Extracted Gelatin

Figure 1 and Table 6 show a typical FTIR spectrum for extracted gelatin at 50 °C using 4 % w/v of different alkalis which completely resemble each other, taking NaOH and KOH solution with bloom 150 g as example. It displays three major peak regions noticeable as 1st region (3600-2700 cm^{-1}), 2nd region (1900-900 cm^{-1}), and 3rd region (400-900 cm^{-1}). The regions are specifying to the bonds as amide A and B; also amide I, II and III; and amide IV, V and VI.

The FTIR spectrum shows characteristic transmittance peaks of the chemical functional groups of the gelatin in Figure 1 and Table 6. The gelatin fibril found to be broadening and a slight shift to lower wave number of the amide A peak, so associated with increased inter-molecular interactions of gelatin. The absorbance bands are identified as follows: a strong and broad overlapping band in the range of 3600-3300 cm^{-1} was assigned to NH bond in

the peptide group of collagen and protein and for the OH of carboxylic group in the protein, the bands at 2938, 2877 cm^{-1} are from -CH3 and -CH2 stretching vibrations for the gelatin. The strong band at 1646,1652 cm^{-1} is due to C=O overlapping between carbonyl groups of gelatin, the bands at 1335 cm^{-1} and 1433 cm^{-1} are due to -CH3 and -CH2 bending vibration. The band at 1225 cm^{-1} was assigned to NH bending vibration of the amide group and the band at 1174, 1051 cm^{-1} is attributed to the strong C-O-C of amide bond in gelatin stretching vibrations, the bands at 987 cm^{-1}, 969 cm^{-1}, 695 cm^{-1} are the skeletal stretch C-C- and CH out off plane. For the region from 750 to 500 cm^{-1} especially for extracted gelatin by KOH, the C-S stretching mode is generally was observed related to cysteine and cystine [29]. Also, another characteristic skeletal deformation is due to the C-S-H and O=C-S out of plane bending appears at 400-500 cm^{-1}.

Table 6: FTIR spectra and assignments of extracted gelatin

Region	Extracted gelatin	
	Peak wave number cm^{-1}	Assignment
Amide A	3500	NH stretch
	3422	OH stretch
	2936	CH2 asymmetrical stretch
	2850	CH2 symmetrical stretch
Amide I	1647	C=O stretch coupled with COO-
Amide II	1524	NH bend coupled C-N stretch
	1455	CH2 bend
	1331	CH3 bend, CH2 wagging of proline
Amide III	1246	NH bend
	1047	C-O stretch
Amide IV	863	-C-C-, Skeletal stretch
Amide V	687	CH out off plane, skeletal stretch
Amide VI	611	CH out off plane, skeletal stretch

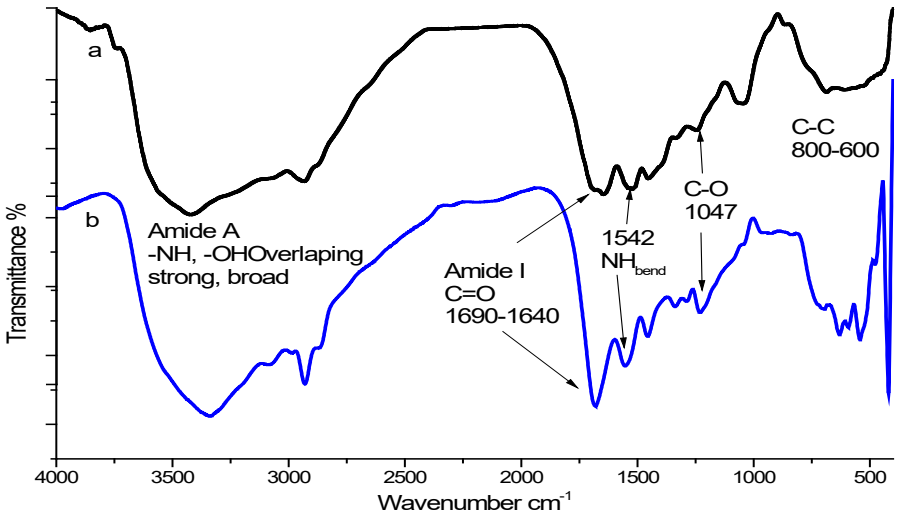


Figure 1. FTIR spectra of extracted gelatin with different alkalis: (a) NaOH, (b) KOH

All the previous detected peaks confirmed the structure of the extracted gelatin. The results obtained are in similarity in the literature [30].

Dynamic Light Scattering (DLS)

DLS can be considered a main tool to understand and verify models pertaining to the dynamics of biopolymers (gelatin) in dilute solution. It allows determining the size and hydrodynamic radius of biopolymers molecule in solution. As shown in Figure 2, the particle size distribution of gelatin which dispersed in aqueous medium presence relative particle diameter measurement distribution over narrow range from 0.45-1.6 µm with percentage more than 90 % of particles by volume distribution. Low polydispersity, narrow distribution of particle size (870 nm) of gelatin indicated to the ability to form homogenized distribution of blended film gelatin, which gives gelatin film a range of application from few µm as packaging films.

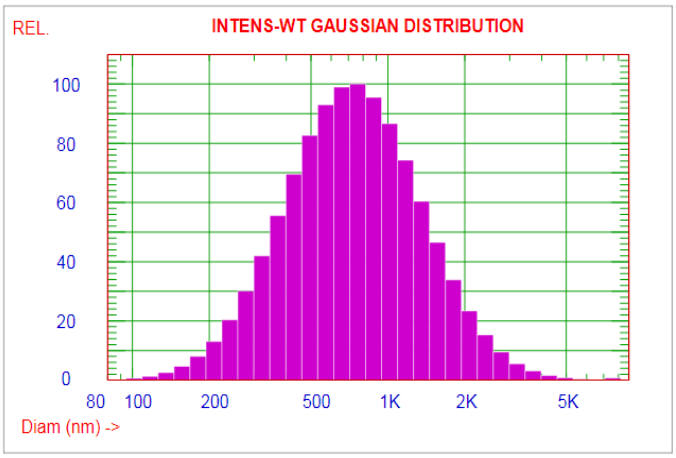


Figure 2. DLS image of particle size distribution of extracted gelatin with different ratios

Amino Acids of Extracted Gelatin at Optimum Condition

The amino acid composition of the gelatin extracted from white leather shavings (WLS) were presented in Table 7. The experimental results showed that under the conventional acidic hydrolysis conditions, complete hydrolysis of the white leather shavings (WLS) was proceeded with yield of 16 amino acids as revealed in Table 7. It was observed that, all of asparagines and glutamine are completely hydrolyzed to aspartic and glutamic acids, respectively. It is difficult to

determine cysteine by acid hydrolysis and also tryptophan is completely damaged. Glutamic acid reaches the maximum amount of 20% while, aspartic acid and lysine reach to 10%. Tyrosine is partially hydrolyzed and trace amounts are present in the hydrolysate, low amount of serine and threonine due to partial hydrolysis of about 3 and 4 %, respectively. The results obtained match with the literature [31].

Table 7: Amino acids composition (%) of the extracted gelatin

Amino Acids	%	Amino Acids	%
Aspartic Acid	10.35	Leucine	3.54
Threonine	3.93	Isoleucine	6.57
Serine	3.72	Phenylalanine	2.57
Glutamic Acid	19.78	Tyrosine	4.04
Glycine	3.86	Histidine	3.78
Alanine	3.76	Lysine	10.56
Valine	4.63	Arginine	4.66
Methionine	2.82	Proline	9.06
NH_4^+	2.16		

X-Ray Fluorescence Spectrometric Analysis of Extracted Gelatin

XRF study was carried out to determine the elemental constituents of extracted gelatin from white leather shavings by 4 % w/v of different alkalis.

Table 8: XRF analysis of extracted gelatin at 4% w/v of different alkalis

Main constituents	(NaOH), Wt.%	(KOH), Wt. %	(LiOH), Wt. %
Si	0.154	0.139	0.186
Al	0.123	0.134	0.322
Fe	0.234	0.314	0.386
P	0.118	0.089	0.127
S	1.35	0.98	1.168
Ca	1.166	1.120	1.230
Mg	0.048	0.050	0.039
Br	--	0.030	0.003
Cu	--	----	----
K	0.175	0.248	0.230
Na	0.256	0.129	0.316
Sr	-	---	---
Cl	1.333	1.230	1.654
Li	--	----	0.123
Co	--	0.001	----
Loss On Ignition, LOI	93.88	95.14	94.21

From Table 8, it is obvious that, the percent of sulfur are (1.35, 0.98 and 1.168) for different alkalis NaOH, KOH, LiOH, respectively. It is one of the highest values of element percent in gelatin. This is due to the cleavage of S-S bonds in cystine and cysteine by the different alkalis. Other elements have few contributions with minor percentages. The higher value of Na in case of using LiOH is not logical but it may be related to apparatus analysis error. The higher value of Loss On Ignition related

to the organic part of the extracted gelatin (N, C, O, H) with percent 94% - 96%.

Thermal Gravimetric Analysis (TGA) of Extracted Gelatin

TGA is a thermoanalytical technique that follows the change in weight of gelatin as a function of temperature. The response to thermal treatment depends on the structure and morphology of gelatin at each step. Thermal behaviours for gelatin extracted by NaOH, KOH and LiOH from white shaving leather waste completely resemble and are presented in Figure 3.

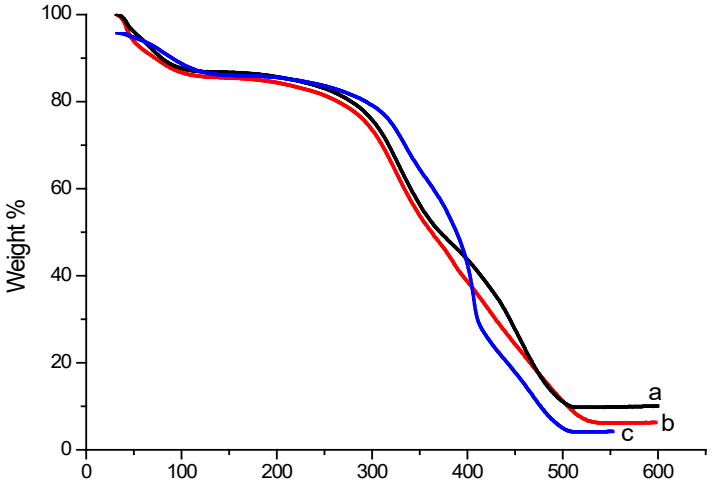


Figure 3. TGA of gelatin extracted by different alkalis (a) NaOH, (b) KOH (c) LiOH

It is clear that there are three main degradation stages: 1st stage represents dehydration volatilization of low molecular weight substances and moisture. The 2nd stage is the main degradation stage and the third stage is the carbonization stage. The gelatin sample extracted by different alkalis from WLS has an initial weight loss of about 12 % at temperature between 50-127 °C due to the evaporation of water included in gelatin. There is a gradually decreased in the weight loss of the gelatin which reaches to 60 % at temperature between 130-440 °C due to burning of hydrocarbon chain of the gelatin chain. The 3rd peak at 440-600 °C included the degradation of the rest of gelatin with a weight loss 19 %. After 600 °C, the ash formed with about 9 % of the initial weight. The results obtained are comparable and similar with the literature [32, 33].

Differential Scanning Calorimetry (DSC)

DSC analysis gives information about the glass transition behavior, T_g of the extracted gelatin. Commonly, If T_g of gelatin lies below room temperature, the gelatin films be more flexible and good elastomers. If T_g of gelatin above room temperature the gelatin films takes rigid and brittle behavior. Thus, known information of T_g is important in the selection of materials for various applications.

The slow traditional DSC scan at 10 °C/min gives broadening endothermic peak that is observed from almost -100 °C up to 200 °C in extracted gelatin samples (Figure 4). For regularity, the three thermal transitions of gelatin experimental in increasing order of temperature and will be referred to as T_g (glass transition) \ T_m (melting) \T_i (isomerization), T_g in the range of 40–50 °C and a melting peak (T_m) in the range of 133–144 °C and melting enthalpy (normalized per unit mass) of 33 J/g. The results obtained match with the literature [34].

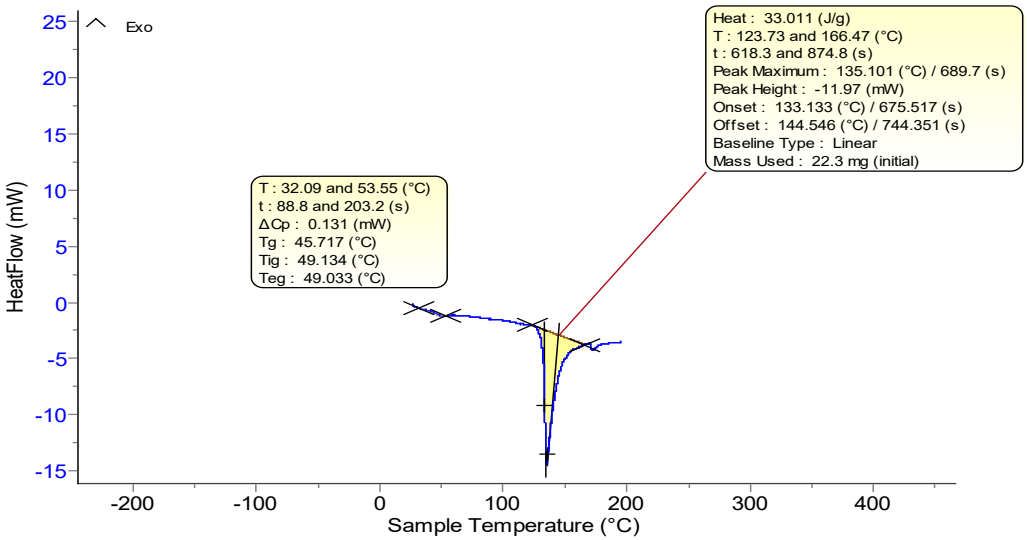
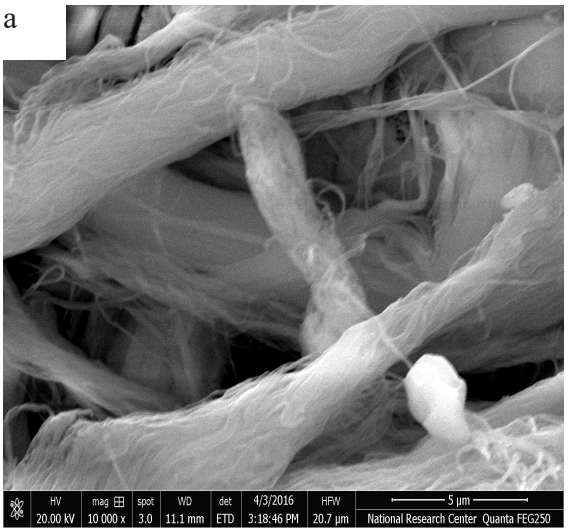
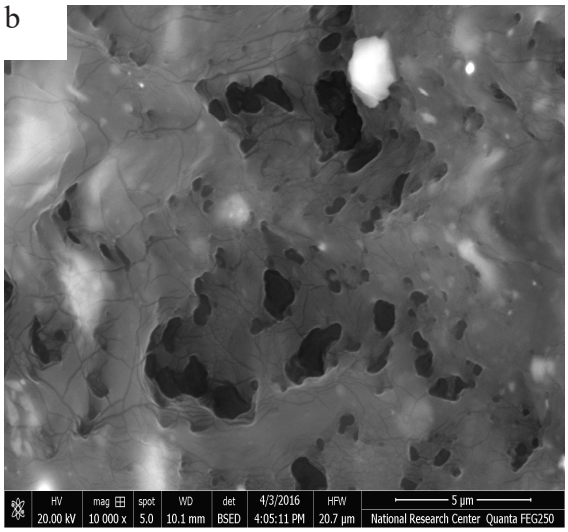


Figure 4. Differential scanning calorimetry (DSC) thermograms of extracted gelatin

Scanning Electron Microscope (SEM) for White Leather Shaving and Extracted Gelatin with Different Alkalis

Surface area morphology and microstructure of gelatin are recognized as vital tools for understanding its industrial, chemical and biomedical applications [35]. Figure 5 shows SEM images of white leather shavings, extracted gelatin with different alkalis at 10000 x magnifications. The white leather shavings had a striped fiber bundle with irregularly filamentous shape Figure 5(a), while the extracted gelatin from different alkalis showed pores with flake shape Figure 5(b-d). The collagen extracted from alkalis had a complex fibril form, related to the higher wet-ability, i.e. the ability to be adsorbed and be soluble in water [36, 37]. Therefore, extracted gelatin is used as a hydrating agent in field of cosmetics. As described by the comparatively well-distributed pore structure might be suitable for biomedical application as mentioned by many researchers [38].





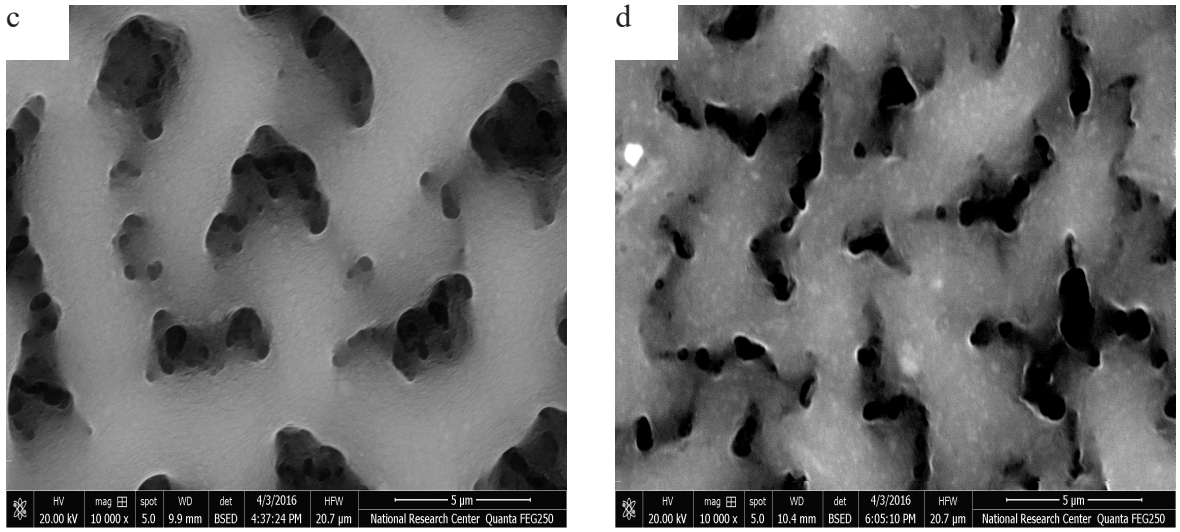


Figure 5. SEM images of a) white leather shavings; and extracted gelatin with different alkalis: b) NaOH; c) KOH; d) LiOH

CONCLUSION

The aim of our article is to obtain high bloom gelatin from white leather shavings via combining the fields of leather technology and waste management technology for enhanced utilization of leather waste. Chemical treatments of white solid wastes by partial hydrolysis of wastes using different alkalis and different factors affecting hydrolysis such as alkalis concentration, contact time and temperature were studied. The system included preparing gelatin from white leather waste, isolating collagen protein from white leather waste by extracting method and characterization of extracted gelatin. The method verified that the leather wastes can be successfully processed to the powder gelatin products. It was found that the optimum conditions to obtain high bloom gelatin strength from white shaving leather wastes around 150 bloom are 4% w/v NaOH, 4 hours contact time, 250 rpm at 50 °C. FTIR spectra show the presence of functional groups of gelatin with different alkalis. TGA of extracted gelatin reaching to higher temperature 440 °C, and glass transition temperature at 45 °C. SEM of the extracted gelatin from different alkalis showed pores with flake shape with the comparatively well-distributed pore structure that might be suitable for biomedical application especially in biodegradable packing materials.

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