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Utilization of Chrome Shavings in the Production of Industrial Gelatin

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Abstract

The leather industry is one of the most polluting sectors in the world, producing a large quantity of solid and liquid waste. So the treatment of solid waste is considered an essential object to reduce environmental pollution which can occur with low cost. So the objective of this study is to find a method to obtain industrial gelatin from chrome shavings with more cost-effective methods in terms of time, chemicals, and wastes generation which reduce environmental pollution. Different factors (pH, swelling time, temperature, and extraction time) were investigated to see how they affected the extraction of gelatin. The extracted gelatin was characterized by bloom, viscosity, FT-IR, Amino acid analysis, and TGA. The optimum conditions of extraction of gelatin from chrome shavings by potassium carbonate are: 5 hour extractions, pH 9.5, 80°C and one swelling day. The result show the extracted gelatin has 23% yield, 219 bloom, 11.4 % nitrogen content which have good thermal stability.

Keywords: Leather waste, recycle, gelatin, alkaline hydrolysis

1. Introduction

The leather industry creates massive volumes of liquid and solid waste for landfilling, as well as emitting unpleasant odors due to the breakdown of skin proteinous material, which releases gases such as NH_3 and H_2S. One tonne of raw hides yields around 250 kg of leather and up to 600 kg of solid wastes. The incineration of these wastes causes significant issues for humans, animals, and plants because it transforms Cr (III) to the toxigenic Cr(VI)[1-3]. As environmental concerns grow, the necessity of clean technology and recycling processes becomes more necessary.

Chrome shavings are regarded as a sustainable raw material source for collagenous protein and chromium. It might be treated to provide gelatin and chromium III resources [4, 5]. This animal hide and skin waste materials may yield desirable and valuable end products [6].

Gelatin is a flavorless solid polymer that is translucent, natural, non-toxic, colorless, and brittle [7]. Collagen from skin, white connective tissue and animal bones is partially hydrolyzed to produce gelatin. in a huge scale, Gelatin is produced from the meat by-products leather industries, and Fish by-products [8-11]. To extract the dried gelatin, various curing, acid, and alkali methods are used to prepare the raw materials.

Gelatin is a significant biopolymer that is widely used in the food and pharmaceutical sectors. Gelatin is used in gelatin desserts, ham coating, fruit toppings, instant sauces, soups, marshmallows, and gummi sweets as a gelling agent, stabilizer, thickening, and texturizer [12-15].

It is also employed as a binding and glazing agent in meats and aspics, as well as a beverage fining agent, vegetable juice, and fruit. Gelatin is used in the pharmaceutical health sector to manufacture the shells of hard and soft capsules for medications,

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capsules (a vitamin supplement), dietary/health supplements, syrups, and other products [16-18].

Alkaline hydrolysis of these leather wastes followed by extraction using sodium carbonate would result in added values to tanners [19].

The traditional method of producing gelatin is a two-step procedure (swelling and extraction). The first stage, swelling in alkali pre-treatment, is designed to weaken the collagen structure by eliminating non-collagenic proteins, hydrolyzing a portion of the peptide linkages, and maintaining the uniformity of the collagen fibers[20, 21]. The second stage includes extracting the polypeptides using hot water, which hydrolyzes portions of the collagen polymer chains. The process of converting collagen to the soluble protein of animal glue (gelatin) entails breaking intramolecular and intermolecular hydrogen bonds, as well as certain peptide connections [22, 23].

The objective of this study is to find a new method to obtain industrial gelatin from chrome shavings, with more cost-effective in terms of time, chemicals, and waste generation. Many variables (temperature, swelling time, extraction time, and pH) were carried to evaluate extracted gelatin from chrome shavings.

2. Experimental

2.1 Materials and chemicals

2.1.1 Materials

Chrome shavings were supplied from Ahmad Amine tannery, Miser El-Kadima region, Cairo, Egypt.

2.1.2 Chemicals

K_2CO_3 was provided by ADWIC, Petroleum ether 60-80 ˚C, HCl was obtained from ADWIC.

2.2 Analyses of chrome shavings

Chrome shavings were obtained from a commercial tannery, kept at room temperature, and analyzed for pH, moisture, ash, fat, total Kjeldahl Nitrogen (TKN) by the semi-micro Kjeldahl method, Chromium and other element were determined by XRF analysis.

2.3 Extraction of gelatin

2.3.1 Extraction of gelatin from chrome shavings at its initial pH

100 g. of chrome shavings with initial pH (3.7-3.3), respectively, were added to 500 ml water for a 1-day swelling time and extraction time 5 hrs. at 80 ºC. The produced gelatin solution was filtrated and let to dry at room temperature for 3 days until constant weight, grinded to powder then subjected to further tests.

2.3.2 Extraction of gelatin by alkaline hydrolysis K_2CO_3

a. 100 g. of chrome shavings were added to 500 ml water adjusting pH (9, 9.5, 10, and 10.5); by adding required amounts of potassium carbonates.
b. Different factors were changed as the swelling time before extraction process (1, 3, 5, 7 days), extraction time (3, 4, 5, 6 hrs.), and temperature of extraction processes (60- 70- 80- 90˚C).
c. The produced gelatin solution was filtrated and let to dry at room temperature for 3 days until constant weight, grinded to powder then subjected to further tests [19].

2.4 Analyses of extracted gelatin

The extracted gelatin was analyzed for pH, ash, moisture, protein as total Kjeldahl Nitrogen (TKN) by the semi-micro Kjeldahl method, fats were determined according to the soxhlet extraction method, turbidity, chromium, and other elements by XRF measurement, FT-IR, amino acid composition by the amino acid analyzer and thermal stability by TGA.

2.4.1 pH Measurement of chrome shavings

Initial pH values of samples were accomplished as the following way: 5 g. of samples were placed in 100 ml. distilled water at room temperature for two hours with agitation. After decantation without filtration of soluble matter (aqueous fraction), we proceed to determine the pH of the filtrated solution using the pH meter.

2.4.2 Determination of moisture content

10 g. of the sample were accurately weighted in a tared dish, then heated at 105 ºC for three hours in the oven at which the temperature was as uniform as possible; the dish was allowed to cool in a desiccator and then weighed. 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 samples.

$$\text{Moisture \%} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where:

W_1 = weight of the sample before dryness.

W_2 = weight of the sample after dryness

2.4.3 Determination of ash content

In a burnt platinum crucible about 10 g. of the sample was accurately weighed, the sample was carefully ignited in a muffle furnace at about 600 °C for about 2 hrs. Finally, the crucible with its contents was cooled in a desiccator and weighed. The ignition,

cooling, and weighting were repeated till constant weight.

$$\text{Ash \%} = \frac{W_1 \text{ of residue}}{W_2 \text{ of the original sample}} \times 100$$

2.4.4 Determination of Nitrogen content

TKN (Total Kjeldahl Nitrogen) was determined by the semi-micro Kjeldahl method, to calculate Total Nitrogen (TN).

2.4.5 Determination of fat content

a. Empty flask was firstly placed in the muffle furnace at 105 °C for two hours to ensure its stable weight.
b. Sample 1 g. was adjusted to soxhlet and petroleum ether was added to fill about 250 ml.
c. The soxhlet apparatus was connected and turned to water cool then switched the heating mantle.
d. The sample was heated for about 10 hrs.
e. The extract solvent was evaporated and dried in an oven furnace at 80 ºC and kept in desiccators.
f. Weight of the flask with extracted fat; to determine the amount of fat in the sample [24].

$$\text{Fat \%} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

2.4.6 X-Ray fluorescence analysis

X-ray fluorescence spectrometry (XRF) is a method of elemental analysis that assesses the presence and concentration of various elements by measurement of secondary X-radiation from the sample that has been excited by an X-ray source. Classically elements from the heaviest down to atomic number 9 (F) can be determined at levels of few mg/kg (ppm). Newer developments with a wavelength-dispersive spectrometer (WD-XRF) allow determining some of the ultra-low atomic number elements including (O). XRF was carried by AXIOS, WD-XRF Sequential spectrometer, Panalytical, 2005.

2.4.7 Amino acids analysis

Amino acid analysis was carried out by liquid chromatography 300, amino acid analyzer - Eppendorf - Germany, at a flow rate of: 0.2 ml/min, the pressure of buffer from 0-50 bar, the pressure of reagent to 0-150 bar, reaction temp 123 ºC, was used for amino acid analysis.

2.4.8 FT-IR Spectral analysis

Infrared spectra were recorded with a JASCO FT-IR, Nicolet, Model 670. The samples were measured as thin films using the diffuse reflectance mode of IR spectroscopy. The contribution of CO_2 in air, moisture, and oxygen was eliminated by measuring the background spectra before every sample. Bands were recorded in the region from 400 to 4000 cm^{-1} with Deuterated triglycine Sulfate (DTGS) detector at National Research Centre.

2.4.9 Turbidity of gelatin solution

Turbidity of gelatin solution was measured by a turbid meter (Lovi bond - Germany) at the National turbidity unit (NTU) in National Research Centre.

2.4.10 Gelatin yield

The gelatin extraction yield was calculated by the following equation [25]:

$$\text{Gelatin yield \%} = \frac{\text{Wt. of extracted gelatin}}{\text{Wt. of leather waste}} \times 100$$

2.4.11 Determination of bloom strength

The analytical measurement of gelling power is the Bloom value. Measurement of the bloom (gel) strength is still the most important quality parameter for gelatin. The Bloom value is the weight in grams that is required for a specified plunger to depress the surface of a standard, thermostatted gel to a defined depth under standard conditions. Bloom strength of gelatin was defined as the force in grams required for pressing a 12.5 mm diameter plunger 4 mm into 112 g. of a standard 62 % w/v gelatin gel at 10 ^{o}C and was carried by using Brooke field Lfra Texture analyzer (Lfra 1000 made in the USA).

2.4.12 Determination of viscosity

The viscosity of the gelatin solution is determined at 60 ºC by measuring the flow time of 100 ml of the solution and was carried by using brooke field dv-e viscometer model number (LvDve 230).

2.4.13 Thermogravimetric analysis

The thermal properties were determined using TGA Perkin -Elmer thermal analysis controller AC7-DX TGA7, using a heating rate of 10 °C/min. in a nitrogen atmosphere, temperatures range from room temperature up to 500°C at National Research Centre.

3. Results and Discussion

3.1Characterization of chrome shavings

3.1.1 Physical and simple chemical analysis

The basic chemical composition and physical characteristics of chrome shavings were analyzed, and the results are shown in table (1):

Table (1): Chemical composition of chrome shavings

Color	Faint green - blue
pH	3.76±0.075
Ash content	6.81±0.026 %
Moisture content	52.5±0.069 %
Nitrogen content	10.62±0.036 %
Fat content	0.70±0.052 %

According to table (1), chrome shavings contain 52.5 % moisture, 10.62 % nitrogen, 6.81 % ash, and 0.70 % fat

3.1.2 Elemental analysis

The elemental analysis of chrome shavings was presented in Table (2).

Table (2): Elemental analysis of chrome shavings leather wastes

Element	Amount %
Carbon (C)	32.277±0.078
Hydrogen (H)	4.862±0.039
Nitrogen (N)	10.893±0.027
Sulfur (S)	2.140±0.041

As shown from table (2), the major components of chrome shavings were C and N which represent more than 43% due to the presence of amino acids

3.1.3 XRF analysis of chrome shavings

X-ray fluorescence spectrometric was conducted to identify the trace element components in chrome shavings, XRF data of chrome shavings are shown in table (3). As shown in table (3), Cr (5.349 %) is the largest amount in chrome shavings owing to the tanning process, which uses $Cr_2(SO_4)_3$ as the major tanning ingredient [26]. Cl, S, and Ca are the next: 0.594, 0.399, and 0.400, respectively. The final three elements are hair removal and preservation. Other elements are backed to the natural structure of the skin account for small amounts.

Table (3): XRF analysis of the chrome shavings

Main constituents	Wt. %
Silicon (Si)	0.024
Aluminum (Al)	0.018
Iron (Fe)	0.108
Chromium (Cr)	5.349
Phosphoure (P)	0.011
Sulfur (S)	0.399
Calicum (Ca)	0.207
Mganisum (Mg)	0.004
Potassium (K)	0.014
Sodium (Na)	0.081
Chloro (Cl)	0.594
LOI	93.19

LOI: lowest ignition percentage

3.1.4 FT-IR analysis of chrome shavings leather wastes

FT-IR spectrum was used to provide preliminary chemical structure by identifying the functional groups contained in chrome shavings and leather wastes, as shown in Fig. (1). Bands at 3000-3500 cm^{-1} correspond to -OH overlaps with -NH stretching vibration. Another band at 2956, 2930, 2750 cm^{-1} is caused by the stretching vibration of aliphatic -CH,-CH_2,-CH_3 and one strong band at 1543 cm^{-1} was assigned to the vibration of -NH amide bending, and another band at 1632 cm^{-1} relates to the vibration of -C=O stretching, as well as weak bands in the area 1000 - 1250 cm^{-1} caused by the -CN and -CO groups of chrome leather wastes [27].

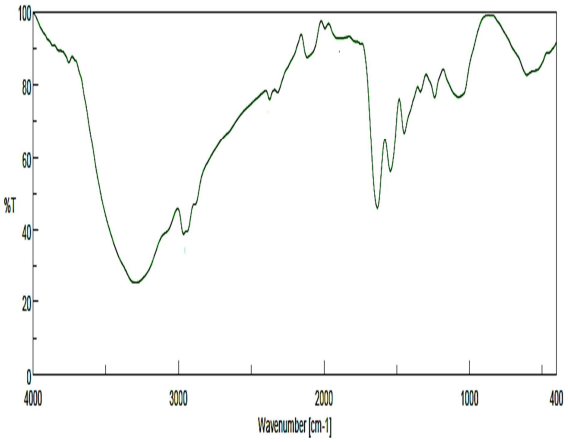


Figure (1): FT-IR analysis of chrome shavings

3.1.5 Amino acids analysis

The amino acid composition of chrome shavings was shown in Figs. (2, 3); Figs (2,3) reveal that chrome shavings had comparatively high amounts of the amino acids proline (Pro), glycine (Gly), glutamic (Glu), and aspartic acids (Asp). Other important amino acids include alanine (Ala), arginine (Arg), threonine, and trace amounts of valine, methionine, isoleuicne and tyrosine[28] .

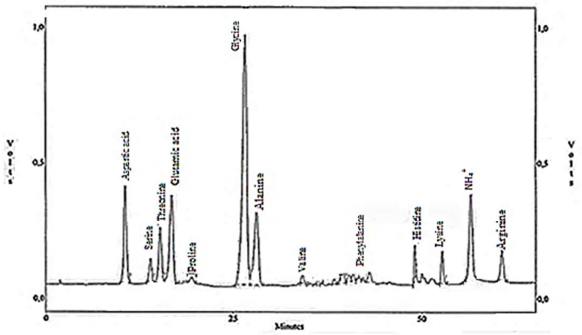


Figure (2): Amino acids of chrome shavings channel (A)

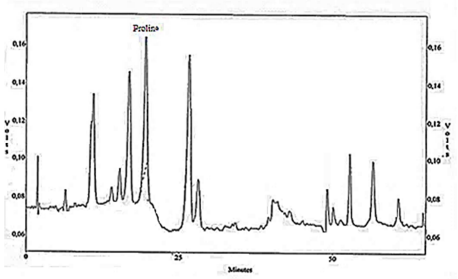


Figure (3): Amino acids of chrome shavings channel (B)

3.2 Gelatin extraction from chrome shavings

Gelatin may be produced from different collagen sources via acidic, alkaline, or enzymatic hydrolysis to disrupt the H-bonds and covalent amide bonds between the rods [29] as shown in Fig.(4).

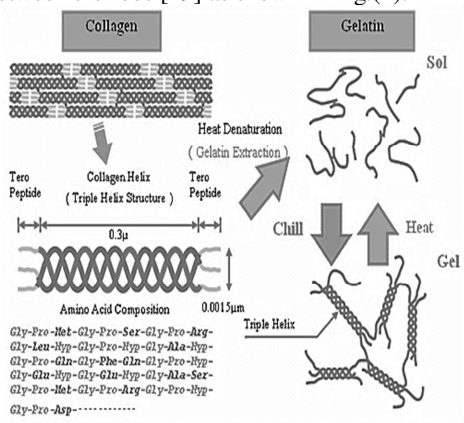


Figure (4): Gelatin production mechanism

3.2.1 Extraction of gelatin from chrome shavings at its initial pH.

Extraction of gelatin from chrome shavings at its initial pH is considered as acidic hydrolysis because of its acidic nature. The result of gelatin extraction is negative because these chrome shavings are tanned by chromium sulfate which stabilizes the hide collagen structure through chrome crosslinking [30]. Thus increasing its resistance against chemicals and hot water and reduce its ability to swell as shown in Fig. (5).

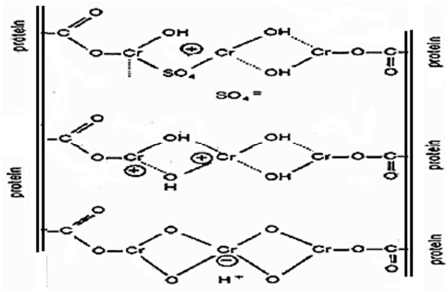


Figure (5): Crosslinking chrome with collagen (tanning process)

3.2.2. Extraction of gelatin by alkaline hydrolysis of chrome shavings

Technical gelatins have been extracted successfully from chrome shavings by using potassium carbonates. Their physical properties (bloom and viscosity) allow its use as industrial type.

The main reason for gelatin extraction by K_2CO_3 is that they are basic salts for weak acid with midly pKa 10.6.

$$K_2CO_3 + HOH <--------> 2K^+ + HCO_3^- + OH^-$$

3.3 Factors affecting gelatin extraction from chrome shavings

3.3.1 Effect of pH

The effect of pH on gelatin extraction process was evaluated through bloom and viscosity determination of the extracted gelatin at different pHs, by changing the amount of potassium carbonates at constant extraction time (6 hrs.), and swelling (1 day), extraction temperature (80 ^oC), data are presented in table (4).

Table (4): Effect of pH on gelatin extraction

pH	Bloom g.	Viscosity ml.poise	Yield %
9	68	42	12
9.5	78	52	29
10	53	39	25
10.5	36	27	27

Table (4) clearly shows that pH 9.5 is the optimal pH for gelatin extraction, as it provided appropriate viscosity 52 ml.poise and the highest bloom values 78 g. . At pH 9, gelatin's poor solubility produces low yield, bloom, and viscosity.

Over pH 9.5, the extracted gelatins have low bloom and viscosity owing to collagen fiber breakdown, resulting in a combination of free amino acids and gelatin. High alkaline concentrations improved gelatin production while lowering gel strength [3]. Potassium carbonate concentrations have a powerful effect on gelatin production, gel strength, and viscosity.

3.3.2 Effect of extraction time

The effect of extraction time on gelatin extraction processes was evaluated through bloom and viscosity determination of the extracted gelatin at different extraction time at constant; pH (9.5), swelling time (1 day) and extraction temperature (80 °C), the data are presented in the Fig. (7) and table (5):

Table (5): effect of extraction time on the gelatin extraction

Extraction time (hrs.)	Bloom g.	Viscosity ml.poise	Yield %
3	65	115	11
4	110	62	29
5	145	64	41.5
6	123	54	42.5

Table (5) shows that 5 hours extraction time was optimal for the highest bloom values 145 g. But extraction times of 3 and 4 hours were insufficient to provide adequate bloom and viscosity, whereas extraction times of more than 5 hours resulted in weak bloom and viscosity. Longer extraction times led to a greater yield, but much worse gel strength and viscosity due to excessive collagen fraction degradation from heating and extraction of other free amino acids [3].

3.3.3 Effect of the swelling time

The effect of swelling time on gelatin extraction processes was evaluated for gelatin derived from chrome shavings through determination of its bloom and viscosity at constant other parameters, swelling pH (9.5), extraction time (5 hrs.) and extraction temperature (80 °C), the data are presented in table (6).

Table (6): Effect of swelling time on the gelatin extraction

Swelling time (days)	Bloom g.	Viscosity ml.poise	Yield %
1	210	112	37.63
3	195	98	41.12
5	170	67	39
7	153	57	32

According to table (6), one day swelling is the ideal swelling period for gelatin extraction since it results in moderate viscosity 112 ml.poise and the high bloom values 210 g. Due to increased cleavage of collagen fibers, the extracted gelatin exhibits poor bloom and viscosity after 1 swelling day.

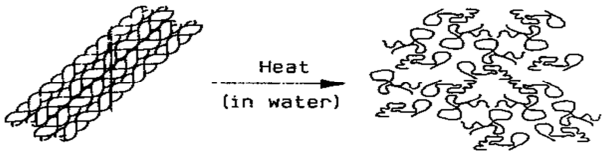
3.3.4 Effect of extraction temperature

The effect of extraction temperature on bloom and viscosity of gelatin extraction processes from chrome shavings was studied at other constant parameters; swelling pH (9.5), extraction time (5 hrs.) and swelling time (1 day), the data are presented in table (7).

Table (7): Effect of extraction temperature on the gelatin extraction

Extraction temparture °C	Bloom g.	Viscosity ml.poise	Yield %
60°C	74	33	44
70°C	190	93	37.36
80°C	219	153	23
90°C	138	54	15

According to Table (7), the optimum extraction temperature is 80 °C, which results in moderate viscosity 153 ml.poise and high bloom values 219 g. Because the solubility of gelatin reduces at temperatures lower than 80 °C, the yield was reduced. However, at 90 °C, the extracted gelatin was influenced by increasing temperature and hydrolyzed to free amino acids, resulting in a decrease in bloom, viscosity, and yield. Temperatures from 60 ^{o}C - 80 ^{o}C can promote intramolecular bond formation between chains and consequently gelatin with stronger gelling ability can be obtained [31].



3.4 Characterization of extracted gelatin

3.4.1 General properties of extracted gelatin sample

General properties of extracted gelatin were evaluated and presented in table (8):

Table (8): General properties of extracted gelatin

Color	pH	Moisture content %	Ash content %	Nitrogen content %	Fat content %	Turbidity
Faint yellow	9.10	17.8	4.68	11.40	0.40	45

The clarity of gelatin solution depends on the conditions during and after extraction, The extracted gelatin by K_2CO_3 showed good transparency [32].

The extracted gelatin exhibited amino acids including glycine, proline, alanine [12, 33], glutamic acid, arginine, aspartic acid [34], and others which are presented in Fig.(6,7). Glycine represents the main constituent followed by proline, alanine, arginine, glutamic and aspartic acids respectively. Other amino acids were present in low yield.

The presence of high amino acid content, tend to exhibit better gelling properties than those with low levels of amino acids. Gelatin is not a nutritionally complete protein. It contains no tryptophan and is deficient in isoleucine and methionine. The other sulfur-containing amino acid, cysteine is absent.

Ammonia evolved during liming due to the hydrolysis process of the terminal amide group of the collagen molecule [35].

Hydrolysis of the amide group

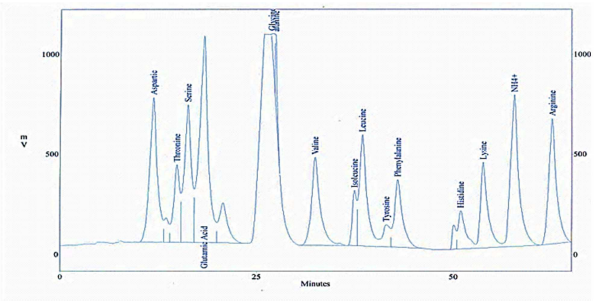


Figure (6): Amino acids of extracted gelatin channel (A)

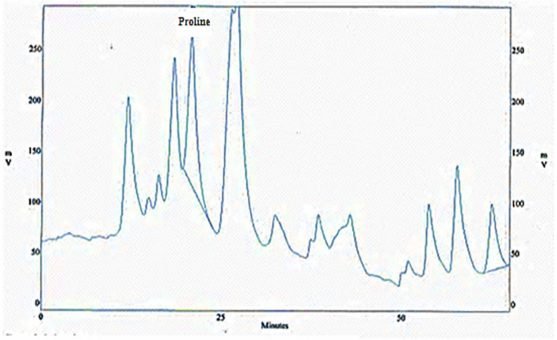


Figure (7): Amino acids of extracted gelatin

4.4.3 FT-IR analysis of extracted gelatin

FT-IR spectra were carried out to give preliminary chemical structure by identifying the functional groups present in extracted gelatin. Gelatin spectra, show absorption bands situated in the amide region, the results are shown in Fig. (8). A broad band can be seen between (3250-3420) cm^{-1} is corresponding to the stretching vibration of -NH overlaps with -OH group, Another band at 2930 cm^{-1} resulted from the stretching vibration of -CH aliphatic. Two intense bands at (1661, 1650) cm^{-1} for gelatin, were assigned to -NH bending vibration of amide bands of gelatin (amide I and amide II modes, respectively), amide I represent -C=O stretching/ hydrogen bonding coupled with $-COO^-$ and amide II represents -NH bending coupled with -CN stretching. Amide III (1406 cm^{-1}) represents -NH bending vibration. Weak bands in region 1000 - 1250 cm^{-1} resulting from -CN and -CO groups of leather amino acids (T. Aewsiri et al, 2009; Z. Khiari et al, 2011).

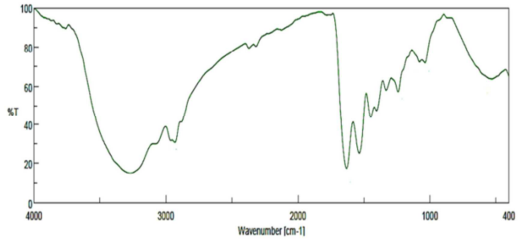


Figure (8): FT-IR analysis of extracted gelatin at optimum conditions by different extractions (K_2CO_3)

3.4.4. XRF analysis of extracted gelatin.

X-Ray fluorescence spectrometric study was carried out to determine the elemental constituents of the extracted gelatin. The data are presented in table (9), from the results; it is found that the percentage of chromium ions.

From table (9), it is clear that, the wt. percentage of sulfur0.96 for which represents the highest elements value present in gelatin. This is due to the unhairing process which causes cleavage of sulfur - sulfur bond. The wt. percentage of Cr is 0.20; other elements represent a very small fraction of the total. According to the previous results, when gelatin was extracted by K_2CO_3, the proportion of chromium ion dropped compared to leather waste percentage. While the carbonates provided the necessary alkaline medium, due to the absence of hydroxyl anion inhibits the formation of the undesired chromium hydroxide.

Table (9): XRF analysis of extracted gelatin at optimum conditions

Main constituents	**Wt. % (K_2CO_3)**
Silicon (Si)	0.13
Aluminium (Al)	0.0.4
Iron (Fe)	0.25
Chromium (Cr)	0.20
Phousphours (P)	0.009
Sulfur (S)	0.96
Calicum (Ca)	0.40
Maganisum (Mg)	0.01
Bromine (Br)	0.030
Potaissum (K)	0.18
Sodium (Na)	0.44
Chloro (Cl)	2.12
Coblat (Co)	0.11
LOI	**95.14**

LOI: lowest ignition percentage

3.4.5 Thermal gravimetric analysis (TGA) of gelatin extracted by K_2CO_3

Gelatin sample extracted by K_2CO_3 from chrome shavings exhibited an initial weight loss of about 18 % at a temperature between 50-190°C because of the release of water included in gelatin structure which is

presented in fig. (9). The loss reached 44 % at a temperature between 190-420°C, which resulted from the burning of the hydrocarbon chain of the gelatin chain (**-**CO-NH-CH-CH_2). The third inflection at 420-520 °C included the degradation of the rest chain with loss in weight reached to 20 %. At temperature after 470 °C, the residual ash formation was about 9 % of the initial weight.

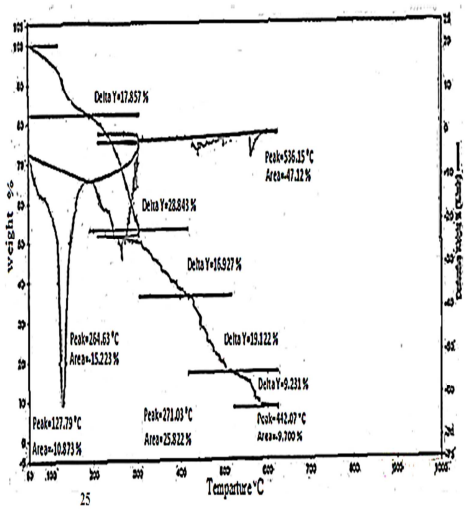


Figure (9): TGA of gelatin extracted by K_2CO_3

4. Conclusion

Recycling chrome shavings becomes necessary which causes big environmental contamination. So, Industrial gelatin has been extracted from chrome shavings without precipitation of chromium. The extraction yield is 23% with bloom 219, nitrogen content 11.40 %, low percentage of chromium content 0.20 %, and good thermal stability, which have a great economic for tanners to utilize From chrome shavings to gelatin cleaner production. optimum conditions of extraction of gelatin from chrome shavings by potassium carbonate are 5-hour extractions, pH 9.5, 80°C, and one swelling day.

5. Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

6. Acknowledgments

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7. Reference

1. Kanagaraj, J., et al., Solid Wastes Generation in the Leather Industry and Its Utilization for Cleaner Environment. Cheminform, 2006. **37**.
2. Sundar, V., et al., Recovery and Utilization of Proteinous Wastes of Leather Making: A Review. Reviews in Environmental Science and Biotechnology, 2011. **10**: p. 151-163.
3. Gudmundsson, M. and H. Hafsteinsson, Gelatin from cod skins as affected by chemical treatments. Journal of Food Science, 2006. **62**: p. 37-39.
4. Cabeza, L.F., et al., Processing of leather waste : Pilot scale studies on chrome shavings. Isolation of potentially valuable protein products and chromium. Waste Management, 1998. **18**: p. 211-218.
5. Kumaraguru, S., T.P. Sastry, and C. Rose, Hydrolysis of Tannery Fleshings Using Pancreatic Enzymes: A Biotechnological Tool for Solid Waste Management. Journal of The American Leather Chemists Association, 1998. **93**: p. 32-39.
6. Cot, J., et al., Minimisation of Industrial wastes: Adding value to collagen materials. J Soc Leather Technol Chem, 2003. **87**: p. 9799.-
7. Lalani, J. and A. Misra, 4 - Gene Delivery Using Chemical Methods, in Challenges in Delivery of Therapeutic Genomics and Proteomics, A. Misra, Editor. 2011, Elsevier: London. p. 127-206.
8. Kariduraganavar, M.Y., A.A. Kittur, and R.R. Kamble, Chapter 1 - Polymer Synthesis and Processing, in Natural and Synthetic Biomedical Polymers, S.G. Kumbar, C.T. Laurencin, and M. Deng, Editors. 2014, Elsevier: Oxford. p. 1-31.
9. Winkler, A.M., Chapter 38 - Albumin and Related Products, in Transfusion Medicine and Hemostasis (Third Edition), B.H. Shaz, C.D. Hillyer, and M. Reyes Gil, Editors. 2019, Elsevier. p. 229-233.
10. Elsayed, h. and S. Ibrahim, Biodegradable package films of poly(L-Lactic) acid/extracted gelatin blend from white leather fibers. Egyptian Journal of Chemistry, 2020. **63**(8): p. 3059-3074.
11. Elsayed, H., et al., High Bloom Gelatin Strength from White Leather Shavings. Leather and Footwear Journal, 2018. **18**: p. 259-274.
12. Saha, D. and S. Bhattacharya, Hydrocolloids as thickening and gelling agents in food: a critical review. Journal of food science and technology, 2010. **47**(6): p. 587-597.
13. Sultan, M., et al., Active packaging gelatin films based on chitosan/Arabic gum/coconut oil Pickering nano emulsions. Journal of Applied Polymer Science, 2021. **n/a**(n/a): p. 51442.
14. Ibrahim, H.M., et al., Production of Antibacterial Cotton Fabrics via Green Treatment with Nontoxic Natural Biopolymer Gelatin. Egyptian Journal of Chemistry, 2019. **62**(Special Issue (Part 2) Innovation in Chemistry): p669.-655 .
15. Ibrahim, S., H. Elsayed, and M. Hasanin, Biodegradable, Antimicrobial and Antioxidant Biofilm for Active Packaging Based on Extracted Gelatin and Lignocelluloses Biowastes. Journal of Polymers and the Environment, 2021. **29**(2): p. 472-482.
16. Ramendra, P., et al., Gelatin Biopolymer: A Journey from Micro to Nano. Journal of Pharmacy Research, 2014. **8**: p. 1387-1397.

17. Abdullah, M.S.P., et al., Recent Advances in the Use of Animal-Sourced Gelatine as Natural Polymers for Food, Cosmetics and Pharmaceutical Applications. Sains Malaysiana, 2018. **45**.
18. Baseer, R.A., et al., Modified cotton fabrics with poly (3-(furan-2-carboamido) propionic acid) and poly (3-(furan-2-carboamido) propionic acid)/gelatin hydrogel for UV protection, antibacterial and electrical properties. Arabian Journal of Chemistry, 2020. **13**(6): p. 5614-5626.
19. Mohamed, O., G.M. Taha, and N. El-Sayed, Technical gelatine from chrome tanned shavings. Int. J. of Environment and Waste Management, 2013. **11**: p. 335-349.
20. Schmidt, M.M., et al., Collagen extraction process. 2016. **23**: p. 913-922.
21. Jafari, H., et al., Fish Collagen: Extraction, Characterization, and Applications for Biomaterials Engineering. Polymers, 2020. **12**(10.(
22. Devi, A. Extraction, Characterization and Application of Gelatin fromCarcharhinus amblyrhyncho and Sphyraena barracuda. 2016.
23. Montero, P. and M. Gomez-Guillen, Extracting Conditions for Megrim (Lepidorhombus boscii) Skin Collagen Affect Functional Properties of the Resulting Gelatin. Journal of Food Science, 2000. **65**: p. 434-438.
24. Horwitz, W., Official methods of analysis of AOAC International. 2000, Gaithersburg, Md.: AOAC International.
25. Ahmad, T., et al., Extraction, characterization and molecular structure of bovine skin gelatin extracted with plant enzymes bromelain and zingibain. Journal of Food Science and Technology, 2020. **57**(10): p. 3772-3781.
26. Font, R., et al., Physico-chemical characterization and leaching of tannery wastes. Waste Management & Research, 1998. **16**(2): p. 139-149.
27. Ellis, W.O., et al., Growth of and aflatoxin production by Aspergillus flavus in peanuts stored under modified atmosphere packaging (MAP) conditions. Int J Food Microbiol, 1994. **22**(2-3): p. 173-87.
28. Aftab, N., et al., Biodegradation of Leather Waste by Enzymatic Treatment. Chinese J. Process Eng., 2006. **6**.
29. Lin, L., et al., An overview of gelatin derived from aquatic animals: Properties and modification. Trends in Food Science & Technology, 2017. **68**.
30. Covington, A.D., Modern tanning chemistry. Chemical Society Reviews, 1997. **26**(2): p. 111-126.
31. Djagny, V.B., Z. Wang, and S. Xu, Gelatin: a valuable protein for food and pharmaceutical industries: review. Crit Rev Food Sci Nutr, 2001. **41**(6): p. 481-92.
32. Fernández-Díaz, M., P. Montero ,and M. Gomez-Guillen, Gel properties of collagens from skins of cod (Gadus morhua) and hake (Merluccius merluccius) and their modification by the coenhancers magnesium sulphate, glycerol and transglutaminase. Food Chemistry - FOOD CHEM, 2001. **74**: p. 161167.-
33. von Hippel, P.H., The Macromolecular Chemistry of Gelatin. Journal of the American Chemical Society, 1965. **87**(8): p. 1824-1824.
34. Masuelli, M. and M. Sansone, Hydrodynamic Properties of Gelatin - Studies from Intrinsic Viscosity Measurements2012. .
35. Barbooti, M., S. Raouf, and F. Kamar, Optimization of Production of Food Grade Gelatin from Bovine Hide Wastes. 2008.