

## Extraction of Amino Acids and Proteins from Chrome Leather Waste

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### Abstract

Hides are the by-product of the meat business, which are converted into leather through tanning processes. Solid wastes of leather industry are raw trimmings, flashings, chrome shavings, buffing dusts and keratin wastes. Chromium salts are used in processing of leather, which creates difficulties in its throw out. The leather industry waste has a large amount of nitrogen content which is amino acids, i.e. protein, which can be used for different purposes, after extraction through different methods. The objective of the present work is to isolate protein products from chrome leather waste and to evaluate acid and alkali for efficiency to convert chrome leather waste into useful products, as the most practiced methods are acid and alkali methods, for extraction of protein. Chromium is precipitated by raising its pH through an alkali, in to  $\text{Cr}(\text{OH})_3$  while the insoluble protein is collected by lowering the temperature. Atomic absorption spectrophotometer is used for chromium determination, for protein determination Biuret and Kjeldhal's method was used.

The highest amount of protein was extracted by using 40 ml and 80 ml 10% sulfuric acid for the shaking time 60 and 30 minutes at 40°C and 25°C respectively. In alkali method 7 g of NaOH at 50°C and 10 g of MgO at 50°C for 6 hours give the best results. While the high amount of temperature and time along with high quantity of acid denature the protein.

The results of chromium and protein determination was analyzed by using SPSS one-way ANOVA factorial design.

**Keywords:** Protein extraction; Alkali method; Acid method

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### Introduction

Hides go to the tanner as a by-result of the meat business. The tanning process, and creates much more prominent amounts of by-products and waste than leather. Land application for the transfer of chromium-containing tannery and other by-products has been generally worked on among the huge majority of the twentieth century; however less landfill sites can be found each day and the expense of transportation and transfer increments.

Leather manufacturing is a conventional and critical worldwide mechanical area, which gives different completed leather qualities and its items [1-4]. With an increase in global demand for leather goods, leather industry achieves a rapid growth and causes an increase in investment. Genuine leather cannot be

obtained from any artificial method and must be converted from raw hides of animals [5].

The byproduct of leather industry is a major issue of pollution in countries related to the production of leather like China, India, Brazil, Pakistan and others as, one ton of wet salted hides yields only 200 kg of leather but over 600 kg of solid waste, or by-product [6]. This waste should be recycled or refuse to overcome the pollution issues which would ultimately cause health problems for human and other living beings.

As formation of leather from slaughter cattle, sheep, goats, buffalo, goats etc. hides is not a simple process, but they are changed through physical, chemical and mechanical processes, in which chromium is used in large amount. The chrome shavings

are the one of byproduct of the industry, which is being used in the present study.

According to study data year 2014 of Food and Agriculture Organization (FAO) of the United Nation, in Pakistan bovine animals are 74,094, number of sheep and lambs is 29,050 and goats and kids are 65,464 thousand heads and heavy and light leather produced from bovine animals 1.9 and 221.9, 99.4 million square feet respectively and 3.6 million square feet. Therefore, Pakistan is also suffering from the pollution issue with regard to this industry.

The leather industry waste has a large amount of nitrogen content which is amino acids, i.e. protein, which after extraction can also be used for different purposes in agriculture, poultry feed, etc. For the extraction of protein part along with chromium different technologies are being used and new or modified every day.

The most practiced are alkali or acid-based hydrolysis have been found. Alkali hydrolysis products obtained showed no gel strength and low molecular weight fragments.

As all other heavy metals chromium is also precipitated at high pH means in alkali conditions. It precipitates in the form of  $\text{Cr}(\text{OH})_3$  and collected as residue of filter on the filter paper. The protein content is left infiltrate the insoluble protein is collected by lowering the temperature up to  $4^\circ\text{C}$ , while the soluble contents are in less amount that is negligible or degraded amino acids which are of no worth. By comparative analysis of acid and base technique most appropriate method can be analyzed and used for the processing of chrome leather waste which will also help us to minimize the pollutant content, a step to healthier and safer environment.

Because of the measures of chromium in the leachate coming about because of hide draining tests, chromium sulfate tanned leather squanders are all the time considered dangerous squanders. To conquer this issue, one alternative could recuperate the chromium and, thusly, bringing down its substance in the cowhide scrap. With this goal, chromium leather scrap was drained with sulfuric corrosive arrangements at low temperature likewise going for amplifying chromium evacuation with least assault of the hide lattice. The impacts of leather scrap measurement, sulfuric corrosive and sodium sulfate fixation in the arrangements, and also extraction time and temperature on chromium recuperation were considered, and, moreover, natural lattice corruption was assessed.

## Materials and Methods

The sample of chrome leather waste (CLW) was collected from a local garment and shoes industry in Lahore. All the work was conducted in lab, in the Department of Environmental Sciences, University of Veterinary and Animal Sciences Lahore. While the sample was shared by using the shredder in the lab of College of Earth and Environmental Sciences (CEES), University of the Punjab, Lahore.

Two methods were used for the extraction of amino acids and proteins from the chrome leather waste that were;

(1) Alkali method

(2) Acid method.

### Alkali Method

One hundred grams of ground chrome leather waste were taken and it was shaken in one liter of water, for 24 hours. At room temperature and at  $50^\circ\text{C}$ . Three alkalies NaOH, MgO and CaO were used. NaOH was used in three quantities 5 g, 7 g and 18 g for 3 hours, 3 hours and 1 hour respectively, 5 g, 10 g and 20 g of MgO and CaO were used, all the treatments of MgO were done for the time period of 6 hours while the time for first two treatments of CaO was 12 hours as shown in **Table 1**, while the third treatment of CaO was proceed for 6 hours. At  $98^\circ\text{C}$  see the sample was filtered Buchner porcelain funnel with Whatman filter paper was used. Protein was separated at  $4^\circ\text{C}$  which was insoluble and chrome sludge was digested. The chrome analysis was done by Atomic Absorption Spectrophotometer.

### Acid Method

One hundred grams of ground chrome leather waste was taken and it was shaken in one liter of water, for 24 hours. At room temperature and  $50^\circ\text{C}$ . Then it was filtered, the filtrate solution was treated to raise its pH up to 10, with 6 M NaOH which precipitate the chromium in to  $\text{Cr}(\text{OH})_3$ . It was filtered again and the residue was placed in the oven at  $600^\circ\text{C}$  for 2 hours, after acidic digestion the chromium determination by using atomic absorption spectrophotometer was done in the following **Table 2**. Filtrate from the solution was also left at this step. At the other hand the residue were treated with 40, 50, 60 and 80 ml of 10% sulfuric acid at the temperature 25, 40 and  $60^\circ\text{C}$  with the shaking time 30, 60 and 90 minutes for each treatment of 10% sulfuric acid respectively than after filtration the residue was again treated as above and filtrate was also left. After all the observation was taken, the results of chromium and protein determination was analyzed by using SPSS one-way ANOVA factorial design.

## Results and Discussion

Strong cowhide squander contains a rich measure of collagen proteins. Changing the asset and understanding its high usage are the necessities of ecological security, practical improvement, and monetary development. Collagen filaments can be effectively harmed while removing and preparing. In this way, response conditions are not to be excessively solid. This causes a not high extraction rate of collagen protein. In the meantime, there are issues, for example, contamination and cost in the extraction of collagen protein. Collagen protein has different applications in nourishment supplement, makeup, biomedical materials, creature nourishes, and so forth we trust that the potential improvement of the market for separating collagen protein from calfskin is tremendous.

### Alkali Method

For alkali method three alkalis, NaOH, MgO and CaO were used in different quantities 5 g, 7 g and 18 g for 3 hours, 3 hours and 1 hour respectively, 5 g, 10 g and 20 g of MgO and CaO were used,

**Table 1:** List of CLW dissolved in the alkali medium.

Alkali	Quantity	Temperature	Time	CLW Dissolved	CLW Left	Protein	Cr
NaOH	5	25	3	30.91	19.09	2.73	2.64
	7	25	3	44	6	2.03	2.72
	18	25	1	46.8	3.2	0	4.52
	5	50	3	46	4	1.64	2.61
	7	50	3	37.8	12.2	3.14	2.84
	18	50	1	47	3	0	4.92
MgO	5	25	6	10.1	39.9	1.02	0.94
	10	25	6	18.8	31.2	2.7	2.28
	20	25	6	23.6	26.4	1.02	3.26
	5	50	6	14	36	2.31	1.2
	10	50	6	20.3	29.7	3.23	2.1
	20	50	6	26.5	23.5	0.73	3.61
CaO	5	25	12	10.9	39.1	1.34	1.53
	10	25	12	21.1	28.9	3.12	2.01
	20	25	6	26.6	23.4	1.24	4.2
	5	50	12	14.3	35.7	2.41	1.26
	10	50	12	24.7	25.6	2.31	2.18
	20	50	6	27.4	22.6	1.01	4.35

**Table 2:** List of CLW left in the acid.

10%Sulfuric	Temperature	Shaking time	CLW Dissolved	CLW Left	Protein	Cr
40	25	30	0.75	42.5	0	1.62
40	40	60	41.5	8.5	2.2	3.41
40	60	90	24.49	25.71	1.87	2.37
50	25	30	6.98	43.02	0	3.65
50	40	60	10.3	39.7	0.35	1.6
50	60	90	16.96	33.04	0.72	2.84
60	25	30	21.86	28.14	1.01	1.73
60	40	60	32.21	17.7	1.73	3.21
60	60	90	42	8	0	3.5
80	25	30	28.82	21.72	2.07	0
80	40	60	45	5	0	4.01
80	60	90	42.98	7.05	0	3.79

treatments of MgO were done for the time period of 6 hours while the time for first two treatments of CaO was 12 hours while the third treatment of CaO was proceed for 6 hours. The highest amount of CLW was dissolved in NaOH, while the lowest one in CaO. The raise of temperature increases the dissolved amount of CLW. Wenwei Liang et al. utilized NaOH 9% to hydrolyze chrome shavings under temperature of 120°C for 6 hours.

The left amount of CLW was also measured for all alkali treatments, as the temperature and quantity of the alkali is increased the left amount of CLW decreased, as 18 g of NaOH give the minimum amount of left CLW i.e 3 g. Also calfskin shavings being waste from full chrome tanned cowhides are more hydrophobic than calfskin buffing which is gotten amid post tanning operations. Cowhide shavings squanders have more chromium focus and fiery remains content than the calfskin buffing waste since much part of the cowhide especially the dermis in which chromium is retained is shaved off amid the shaving operation [7]

The main objective of the study was to extract protein from CLW, the maximum protein was extracted from CLW when the

conditions were optimum otherwise the protein denatured, the high amount of temperature or high quantity of alkali denatures the proteins. 7 g of NaOH at 50°C and 10 g of MgO at 50°C for 6 hours give the best results. In chrome shavings were firstly taken care of under gentle extraction conditions (for instance, generally low temperature, low antacid dose and short extraction time), so that collagen items, which were less demanding to break down, were gotten first. At that point extraction conditions were slowly fortified to concentrate collagen items that were generally hard to break up. Rehashing 2-4 times, extraction was finished. This fractionation technique can separate collagen protein that beats items from the general soluble base strategy in each assessment file. For the general salt strategy, collagen extraction part is 25.9%; cinder substance is 10.3%; and chromium substance is 23.2 mg/kg. For the fractionation strategy, these numbers were 43.3%; under 2%; and under 2 mg/kg, individually.

Higher the quantity of alkali more it precipitates the chromium as by using 18 g of NaOH at 50°C 4.92% of total chromium was precipitated. This outcome demonstrates that there is

an expansion in convergence of chromium with increment in centralization of sodium carbonate in both cowhide buffing and shaving squanders. The rate of increment was however seen to diminish at higher centralizations of sodium carbonate. The watched increment in chromium focus with increment in the convergence of antacid could be as a consequence of capacity of sodium carbonate to accelerate the chromium. This demonstrates the higher the grouping of soluble base the higher the convergence of chromium recuperated. This is not amazing in light of the fact that chromium has been accounted for to be accelerated by a few hastening specialists incorporating sodium carbonate in the expulsion of chromium as chromium salts [8].

## Acid Method

For acid method 40, 50, 60 and 80 ml of 10% sulfuric acid at the temperature 25, 40 and 60°C with the shaking time 30, 60 and 90 minutes for each treatment of 10% sulfuric acid was used. Highest dissolved amount 42.98 g was obtain by 80 ml of 10% sulfuric acid at 60°C with 90 min shaking time. Corrosive of low focus can decimate salt bonds amongst particles and Schiff bases, and make collagen strands grow and disintegrate. Hence, acidic corrosive, citrus extract or hydrochloric corrosive of 0.5 mol/L and with pH 2-3 can be utilized to concentrate collagen[9], examined the impacts of citrus extract, acidic corrosive, and hydrochloric corrosive arrangements on the disintegration rate of collagen protein extricated from pig skin. They presumed that citrus extract has the best impacts, and afterward acidic corrosive, and in conclusion hydrochloric corrosive.

Highest left amount of CLW was 43.02 by using 50 ml of 10% sulfuric acid at 25°C with shaking time of 30 minutes. It can be observed from this result that samples treated with higher acid concentration gave higher percentage recovery. However the

rate of increase declined with increase in acid concentration as from the addition of 0.6 M sulphuric acid on leather buffing waste and 0.8 M of the acid on leather shaving waste.

The high amount of protein was extracted by using 40 ml and 80 ml 10% sulfuric acid for the shaking time 60 and 30 minutes at 40°C and 25°C respectively. While the high amount of temperature and time along with high quantity of acid denature the protein. sodium hydroxide, smelling salts and corrosive acidic inferred gelatins contained 1.162, 1.117 and 0.015 ppm of Cr(VI), separately. The acidic corrosive hydrolysis convention created a gelatin with hints of chromium (VI) much lower than the antacid hydrolysed gelatins, as the majority of the chromium was available as Cr (III) because of the corrosive pH.

Chromium is precipitated at the first step in the acid method when CLW is treated with 6 M NaOH. The highest precipitate was collected when 80 ml of 10% sulfuric acid was used at 40°C with the shaking time of 60 minutes. For engineered squander containing starting Cr. conc. of 3000, 4000 and 6000 mg/l, the real NaOH dosage required was 3, 7 and 10% separately, more than stoichiometric measurements and for spent chrome tan liquor (10000 mg/l) it was 20% more. Comes about because of the electroplating business by utilizing enacted carbon as a part of the pH scope of 5.5 to 8.0 demonstrated that the evacuation of chromium declines with increment in pH and a sudden diminishing in expulsion from 88% to around 40%, when the pH increment from 5.5 to 7.0. This was further lessened from 40 to 27%, when the pH was raised from 7.5 to 8.0 [10].

## Conclusion

It is concluded from this research that protein can be extracted from Chrome Leather Waste from both acid and alkali method but the acid method is more effective.

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