International Journal of Environment, Agriculture and Biotechnology
Vol-8, Issue-6; Nov-Dec, 2023
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Extracting chromium-free protein hydrolysate from leather tanning wastes

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Received: 07 Nov 2023; Received in revised form: 09 Dec 2023; Accepted: 18 Dec 2023; Available online: 24 Dec 2023

Abstract— *Leather tanning produces a variety of solid wastes, the most common of which is chrome shaving waste (CSW), which accounts for about one-third of the total solid waste produced from leather tanning. The biggest problem with this waste is that it contains chromium, which is a pollutant to the environment if the waste is disposed of by traditional methods such as landfilling or incineration. Therefore, the study aims to convert CSW into a proteinaceous material that does not contain chromium, so that it can be used in various applications with added value. In this study, chromium was removed from CSW using either an acid method or an alkaline oxidative method. In the acid method, sulfuric acid (1 molar) was used, while the other method was performed using a mixing ratio of 5:0.2:0.5:1 of CSW: potassium bicarbonate: hydrogen peroxide: water, respectively. The resulting protein was then hydrolyzed using acetic acid (1.5 molar). A chemical analysis of the CSW was performed to determine the percentage of chromium removed from both methods. The percentage of protein hydrolysis was also determined, as well as chromatographic analysis and amino acid analysis of the resulting proteins. The removed chromium was also reused in the tanning of sheepskin samples. The results showed that the alkaline oxidative method for chromium removal was better than using sulfuric acid, with a chromium removal percentage of 94.8% compared to 70.5%. The chromium removed was used in the tanning of leather without any differences in the properties of the resulting leather compared to traditional chrome tanning. The resulting hydrolyzed proteins were found to be collagenous proteins with an amino acid composition that can be used in various applications such as plant fertilizers. Therefore, the treatment and use of CSW achieves economic and environmental benefits for the leather tanning industry, thus achieving sustainability.*

Keywords— ***chrome removal, collagen, leather tanning, sustainability***

I. INTRODUCTION

Chrome tanning is a widely employed method for leather tanning worldwide because it offers cost-effective production of high-quality leather. However, it is also recognized as the most environmentally harmful method due to the generation of solid and liquid waste containing significant levels of trivalent chromium (Huffer and Taeger, 2004; Kolomaznik *et al*., 2008).

Among the various pollutants resulting from leather tanning, chrome shaving waste (CSW) is a prominent contributor, accounting for approximately one-third of the total solid waste produced. The disposal of CSW through incineration or landfilling poses risks as it introduces toxic chromium salts into the soil. These contaminants can permeate the groundwater or be directly absorbed by plants and animals, thereby posing ecological concerns. Additionally, incineration emits harmful gases, and the conversion of trivalent chromium to its carcinogenic hexavalent form can occur (Beltrán-Prieto, *et al*., 2012).

Consequently, the conversion of CSW into environmentally friendly materials becomes imperative to harness its potential for alternative beneficial applications. Previous research has explored various treatment methods and systems to utilize chromium leather waste, such as energy and protein hydrolysate production, panel manufacturing, and the generation of value-added products (Pati *et al*., 2014).

Recent studies have specifically focused on the production of proteinaceous materials from leather waste, which holds promise as a valuable product for different purposes (Rajabimashhadi *et al*., 2023; Maistrenko *et al*., 2022; Parisi *et al*., 2021). However, the challenge lies in the extraction of chromium salts from the waste. Chemical treatments have been predominantly employed to achieve this objective, involving the dissolution of chromium salts from leather waste or extracted ash. Although alkaline hydrolysis is more popular than acid hydrolysis using mineral acids for chrome shaving dust, the latter is commonly utilized due to its ease of use and cost-effectiveness (Rahaman *et al*., 2017; Pantazopoulou and Zouboulis, 2020; Kokkinos, *et al*., 2021). The extracted chromium can then be reused within the tanning industry (Nasr *et al*., 2022; Sharaf *et al*., 2013; Rao *et al*. 2002).

This study aligns with Egypt's 2030 strategy to mitigate environmental pollution and maximize waste utilization by transforming it into value-added materials. The primary objectives of this study are to produce chromium-free protein suitable for various applications and to facilitate the recycling of chromium extracted from CSW for reuse in chrome tanning.

II. MATERIAL AND METHODS

- Chrome shaving wastes:

The chrome shaving wastes used in this study were supplied by Elshafei Sons' Tannery, El-Max region, Alexandria, Egypt. CSW was dried at 25±3°C for five days in an open and shaded place without the use of any of thermal drying methods. Characterization of CSW was determined. Volatile matter, ash, fat, total Kjeldahl nitrogen, chrome contents, total energy and pH values of CSW were determined according to (ASTM, 2014).

- Removing chrome from CSW:

Chrome removal from chrome shaving waste (CSW) was accomplished using two primary methods: alkaline oxidative hydrolysis or acid hydrolysis.

- Alkaline oxidative hydrolysis

CSW was mechanically treated with leaching solution using occasional stirring for 45 min at ambient temperature. According to Nasr (2023), leaching solution was prepared using CSW, potassium carbonate, hydrogen peroxide, and water in a ratio (w/w) of 1:0.5:0.2:5 (w/w), respectively. After treating with leaching solution, CSW was filtered and rinsed twice with water. De-chromed shaving wastes were obtained after rinsing by pressing, whereas the filtrate, containing chromium salts were collected for subsequent chromium sulfate preparation.

- Acid hydrolysis

According to Nasr *et al*. (2022), 10 grams of CSW were precisely weighed and transferred to a beaker. Subsequently, 100 ml of sulfuric acid (1M) solution was added to the beaker. The beaker was then placed on a hot plate and maintained at 50°C for three hours, with continuous stirring at 500 rpm. This hydrolysis step facilitated the precipitation of chromium present in the CSW as Cr(OH)3. The Cr(OH)3 precipitate was separated from the solution by filtration using Whatman filter paper. The filtered Cr(OH)3 was then dried and weighed. The remaining supernatant solution was centrifuged multiple times at 15,000 rpm to remove any residual impurities and subsequently stored.

- Preparation of protein hydrolysates

The protein hydrolysates were prepared from de-chromed shaving wastes that prepared previously by alkaline oxidative and acid hydrolysis. The process was carried out according to Selvaraj *et al*. (2019), using 2.5 g of the sample, which was separately dissolved in 100 mL of 1.5 M acetic acid solution. The solutions were then stirred at 80°C for 6 hours. Afterward, the solutions were centrifuged at 10,000 rpm for 30 minutes. The supernatant was collected, and the residue was dried at 80°C to remove moisture content. Figure 1 shows the protein hydrolysates from CSW after removing chromium by acid and alkaline oxidative methods.

Hydrolysis percentage, FTIR and amino acid analysis were determined for protein hydrolysate to evaluate its properties for using in varied purposes. Hydrolysis percentage was calculated according to Selvaraj *et al*. (2019) based on the residual weight of the chrome shaving waste, using the following formula.

$$\text{Hydrolysis percentage} = \frac{\text{Initial weight taken} - \text{Residual weight}}{\text{Initial weight taken}} \times 100$$

The spectrophotometer (Bruker Varian 70 transform infrared using Platinium ATR unit) was used to analyze the chemical structure and the fingerprint of CSW or protein hydrolysates. Small quantity of the sample was used and scanned directly on the instrument stage.

The polypeptides acquired from CSW and protein hydrolysates were analyzed for amino acid composition. The amounts of different amino acids present were also estimated after hydrolysis using Sykam amino acid analyzer SW.

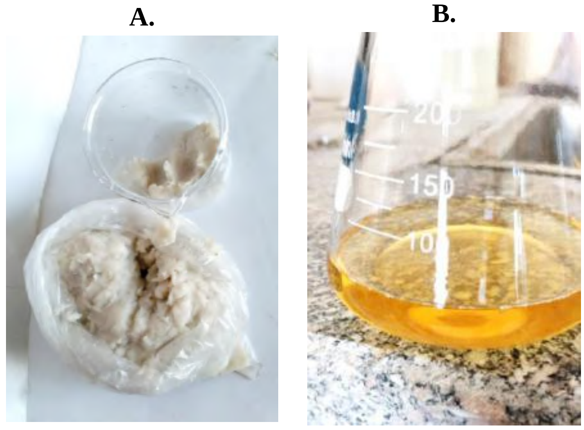

Fig. 1: Protein hydrolysate after removing chromium (A. oxidative alkaline, B. Acid hydrolysis)

- Re-using extracted chromium in leather tanning.

According to Sharaf *et al*. (2013), the chromium sulfate (33% basicity) was prepared from exhausted solution by adding concentrated sulfuric acid as an acid catalyst agent and table sugar as a reducing agent in the reaction. 25 g of chrome shaving ash were mixed with 62.5 ml water in a beaker; 23 g of concentrated sulfuric acid (Sp. Gr. 1.84) were added carefully and stirred well. The beaker was under cooling with continuous stirring while 6.25 g of table sugar were added slowly in 10 lots over three hours. During the reaction the color of the liquor changed gradually from orange to green and finally to bluish green indicating the completion of the reduction. The prepared chromium sulfate was used in tanning pieces of pickled sheep samples (20 cm × 20 cm) and compared with other samples tanned with normally commercial 33% basicity of chromium sulfate.

Tanned leathers' samples were assessed physically and chemically for thickness, tensile strength, elongation, split tear strength, water absorption, permeability to water vapor, pH, and contents of ash, chrome and moisture were analyzed according to (ASTM, 2014).

- Statistical analysis:

Data were analyzed using GLM procedure of SAS (2008) to evaluate the differences among different treatments. The fixed model that used in the analysis of removing chrome.

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where Yij is the observation taken (k), μ is an overall mean, Ai is a fixed effect of the (i) de-chroming method (alkaline oxidative or acid hydrolysis), eij is a random error assumed to be normally distributed with mean=0 and variance=σ2e.

III. RESULTS AND DISCUSSION

Chrome shaving wastes (CSW) characteristics:

Results for CSW analysis are presented in Table (1). High concentration of nitrogen (12.28%) was found in CSW as a protein substance consisting mainly of collagen fibers. Low fat content (0.31%), low pH (3.92 ml mol/L) and high chromium content (3.68%) were found in CSW due to tanning processing steps, in which the fat is removed by the action of alkalis in unhairing step to facilitate the access of tanning material into collagen fibers, which usually react to the chromium sulfate at low value of pH. The total ash content was 10.28% due to the different elements in collagen fibers such as carbon, nitrogen and sulfur, in addition to other elements comes from different chemical used in tanning especially chromium salts. These obtained values of CSW properties were in accordance with previous investigations (Pati *et al*., 2014; Scopel *et al*., 2018).

Protein hydrolysates characteristics.

Table 2 demonstrates the influence of the chromium removal method on chrome shaving waste (CSW) and the subsequent hydrolysis rate of the resulting product. The results reveal a significant impact of the chromium removal method on both the residual chromium content ($P<0.05$) in the final waste and the recovered chromium content ($P<0.01$).

Table 1: Chemical properties of chrome shaving waste (CSW).

Parameter	CSW	ASTM
Volatile matter (%)	23.8	D-6403
Fat (%)	0.31	D-3495
Total Kjeldahl nitrogen (TKN)	12.28	D-2868
Total ash (%)	10.28	D-2617
Cr (%)	3.68	D-6714
pH (ml mol/L)	3.92	D-2810

The alkaline oxidative method proved superior to the acid method in removing chromium. The recovered chromium content reached 94.84% compared to 70.56% for the alkaline oxidative and acid methods, respectively. Similarly, the residual chromium content in the waste after chromium removal was significantly lower for the alkaline oxidative method (0.19%) compared to the acid method (1.08%).

Comparing the findings of this study with those of previous studies, Nasr *et al*. (2022) and Rahaman *et al*. (2017) demonstrated a positive correlation between the concentration of sulfuric acid and the amount of chromium removed. In Nasr *et al*. (2022) study, chromium removal reached 74% at a sulfuric acid concentration of 1 molar, closely aligning with the results of this study. However, Rahaman *et al*. (2017) study reported a lower chromium removal rate (55.5%) at a sulfuric acid concentration of 5 molar compared to the value obtained in this study.

Table 2: Effect of hydrolysis method on removing chrome from chrome shaving waste and percentage of hydrolysis.

Hydrolysate group	**Cr in residual (%)**	**Cr recovery (%)**	**Percentage of hydrolysis (%)**
Acid hydrolysis	1.08[a]	70.56[b]	59.33[b]
Alkaline oxidative hydrolysis	0.19[b]	94.84[a]	81.61[a]
Over all of means	0.64	82.70	70.47
SEM	0.21	5.61	4.99
Significance	**	**	**

Significance: ** P<0.01.

Means in the same column having different superscripts are significantly different (P<0.05).

Despite limited utilization in previous studies, the alkaline oxidative method employed in this study yielded chromium removal rates comparable to those reported in previous studies of Nasr (2023) and Siska (1993), which were 95.43% and 98%, respectively.

Conversely, the hydrolysis rate of the resulting proteinaceous material was higher (P<0.05) in the waste obtained from chromium removal using the alkaline oxidative method (81.61%) compared to the acid method (59.33%). The hydrolysis percentages obtained in this study are in the range reported by Selvaraj *et al*. (2019), who used different concentrations of acetic acid from 0.5 molar to 2.5 molar, which gave hydrolysis percentages in the range of 48% to 87.5% with increasing acid concentration.

Therefore, the alkaline oxidative method emerged as the most suitable approach for achieving a proteinaceous material with exceptionally low chromium content.

Fourier-transform infrared (FTIR) spectroscopy was utilized to examine the impact of hydrolysis on changes in functional groups during the hydrolysis process. Figure 2 displays the FTIR spectra of CSW (chrome shaving waste) and its hydrolysates. The spectra demonstrate a reduction in the peak intensity of the amide I band, located at approximately 1610 cm^{-1}, which corresponds to C=O stretching. A prominent peak is observed in the amide II region, around 1540 cm^{-1}, representing the out-of-phase combination of -CN stretching and -NH bending. Additionally, a moderately intense peak is detected in the amide III region, around 1260 cm^{-1}, associated with the in-phase combination of -CN stretching and -NH bending. The specific peak positions are summarized in Table 3.

Interestingly, the amide I peak, found at approximately 1608 cm^{-1}, exhibits minimal variations under different hydrolysis conditions. Since the amide I peak is highly sensitive to the secondary structure of proteins, alterations in hydrolysis methods or waste sources have negligible influence on the conformation of the polypeptide chains present in the protein hydrolysate.

On the other hand, the amide II and III peaks consistently appear at approximately 1542 cm^{-1} and 1260 cm^{-1}, respectively, with relatively little variation observed across different hydrolysis groups.

Table 4 presents the amino acid analysis of CSW and the protein hydrolysates derived from CSW. The three hydrolysates exhibited comparable amino acid profiles, with a high abundance of glycine, proline, alanine, and hydroxyproline. The amino acid composition of the hydrolysates aligns with previous studies (Ammasi *et al*., 2020; Aftab *et al*., 2006), supporting their suitability for diverse applications, including encompassing cosmetics, adhesives, printing, and photography. The release of hydroxyproline indicates that amino acids are also produced during the degradation process, potentially opening up avenues for their purification and utilization in poultry feed.

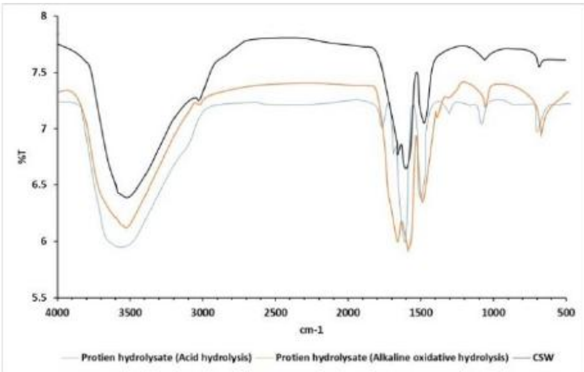

Fig 2: FTIR spectra of CSW and their hydrolysates.

Table 3: Peak positions of protein hydrolysate from chrome shaving and raw trimming wastes.

Group		**Amide I (cm^{-1})**	**Amide II (cm^{-1})**	**Amide III (cm^{-1})**
Protein hydrolysate	Acid hydrolysis	1609	1543	1248
	Alkaline oxidative hydrolysis	1612	1544	1278
Chrome shaving waste (CSW)		1614	1540	1280

The results of FTIR and amino acid analysis showed that the proteinaceous material produced after chromium removal and hydrolysis is a collagenous material, as its results agreed with previous research.

Therefore, the liquid form of the protein that obtained from acid hydrolysis could serve as a plant fertilizer or undergo electrospinning to produce nano-filters for medical applications, such as wound dressings. In its solid form that obtained from alkaline oxidative hydrolysis, the protein can act as an absorbent for dyes, fats, or heavy metals generated by the leather tanning industry, contributing to environmental protection and industry sustainability.

Using extracted chromium in leather tanning

Table 4: Amino acids compositions of chrome shaving wastes (CSW) and their proteins hydrolysates.

Amino acid	Protein hydrolysate		CSW
	Acid hydrolysis	Alkaline oxidative hydrolysis	
	mg/gm	mg/gm	mg/gm
Glycine	127.12	116.25	123.43
Proline	50.31	46.22	51.43
Alanine	46.09	40.53	45.82
Hydroxyprolin e	36.1	33.06	34.85
Glutamic acid	29.19	26.66	30.19
Arginine	19.97	18.49	20.89
Aspartic	17.67	16.35	17.31
Serine	11.91	11.02	12.72
Leucine	8.45	8.89	10.54
Lysine	9.6	9.95	10.11
Valine	6.53	8.18	8.95
Theronine	6.91	5.69	6.07
Isoleucine	4.22	4.27	4.98
Phenylalanine	4.22	4.62	5.91
Methionine	3.84	2.13	3.03
Histidine	1.15	1.78	1.59
Tyrosine	0.77	1.42	1.17
Total	384.06	355.5	389

Figures 3 and 4 show the results of physical and chemical properties of tanned leather samples produced using traditional vs. recovered chromium sulfate. There are insignificant differences between the two groups. This trend was found in previous studies that done by Nasr *et al*. (2022) and Rao *et al*. (2002). Thus, the usage of recovered chromium did not degrade the properties of produced leather which had similar properties to that tanned with traditional basic chrome sulfate tanning. Therefore, recycling chrome from CSW could be a promised practice to achieve additional environmental and economic benefits.

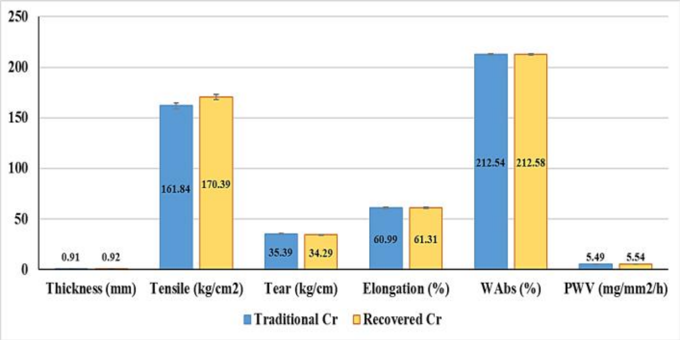

Fig. 3: Physical properties of tanned leather samples produced using traditional vs. recovered chromium sulfate

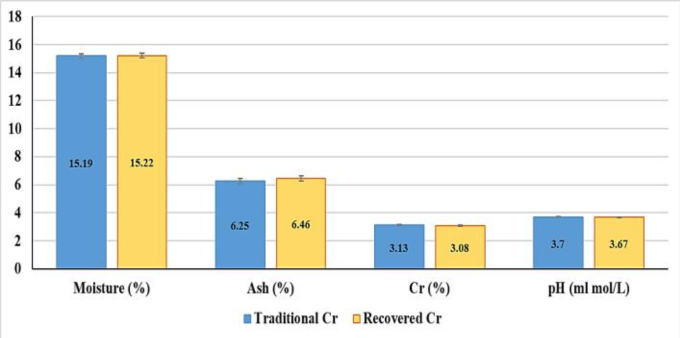

Fig. 4: Physical properties of tanned leather samples produced using traditional vs. recovered chromium sulfate.

IV. CONCLUSION

The study suggests that chrome shaving waste (CSW) from leather tanning is a proteinaceous material that can be reused after chromium removal, and that chromium can be recycled for reuse in leather tanning. Chromium can be removed from CSW using either acid hydrolysis or alkaline oxidative methods. The alkaline oxidative method was found to be the most effective method for chromium removal, with a removal percentage of about 95%. The proteinaceous material produced after chromium removal and hydrolysis is collagenous, and can be used in a variety of other applications, such as plant fertilizers and adhesives, printing, and photography. This approach can help to maximize the value of CSW, reduce environmental pollution from leather tanning, and achieve sustainability.

FUNDING

This research was supported financially by the Academy of Scientific Research and Technology (ASRT), Ministry of Scientific Research (MoSR), Egypt.

ACKNOWLEDGMENT

The present document was achieved in the frame of project titled “Nanotechnological interventions to convert tannery solid waste into value added nanofibrous membrane”, supported by the Academy of Scientific Research and Technology (ASRT), Ministry of Scientific Research (MoSR), Egypt with Desert Research Center, in cooperation with Department of Science & Technology (DST), Ministry of Science and Technology, India.

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