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Extraction of polypeptide solution from Tannery solid waste (chrome shavings) and its application as Poultry feed

Ariful Hai Quadery[1*], Md. Tushar Uddin[1], Murshid Jaman Chowdhury[2*], Amal Kanti Deb[3].

[1]*Leather Research Institute, BCSIR, Nayarhat, Savar, Dhaka-1350, Bangladesh*
[2]*BCSIR Laboratories Dhaka, BCSIR, Bangladesh.*
[3]*Institute of Leather Engineering & Technology, University of Dhaka, 44-50 Hazaribugh, Dhaka-1209, Bangladesh*

Abstract: *Chrome shavings are obtained as solid waste materials when chrome-tanned leather undergoes the process of shaving operation. The high concentration of trivalent chromium along with organic and inorganic compounds in chrome shaving causes severe ground water contamination on land disposal as well as chronic air pollution during cooling by inhabitant. Chrome shaving also contains rich amount of collagen protein. The aim of this research work was to extract protein or collagen hydrolysate from this polluting solid waste (chrome shavings) by bio-chemical hyrolysis process and its application as poultry feed. FTIR analysis was done to characterize the collagen hydrolysate or protein. AAS analysis of the final product showed that, the prepared collagen hydrolysate is almost free of chromium (1.47ppm).*

Keywords: *Chrome shavings, Bio-chemical Hydrolysis, Solid content of hydrolysate, Kjeldhal Nitrogen, FT-IR, AAS.*

I. Introduction

The leather industry produces a significant amount of chromium bearing hazardous wastes. Contamination of chromium is happened into the surface water, ground water, soil, plant and aquatic lives [1]. Some small businessmen collect this chrome shavings and use for the production of poultry feed ingredient as a protein supplement in an unhygienic and unscientific way which not only creates severe environmental pollution but also the product is very dangerous for the poultry feed as it contains high concentration of chromium. These wastes are converted to protein-concentrate to be used into poultry feed, fish feed, and in production of bio-fertilizers without any appropriate treatment [2].

Research reveals that meat, liver, bones of poultry chicken feed with this unhygienic protein product, contain very high content of chromium in these organs [3]. Therefore poultry chicken meat is very dangerous for human body as the chromium accumulate into human body through food chain.

The main objective of this research work is to process this solid waste i.e. chrome shavings generated by the leather industry and to transform them into new product i.e. protein concentrate or protein hydrolysate that could be used as a protein supplement for the poultry feed industries [4], [5]. The other objectives of the research work are to treat eco-friendly and utilize discarded solid wastes of leather industries, to find, optimize and establish an effective process of extraction of Collagen Hydrolysate from Chrome shaving dust; to estimate the amount of protein content and available amino acids; level of remaining chromium, other heavy metals and to reduce to the minimum the ash content; checking the presence of impurities, and other associated elements present in the extracted material [6].

II. Material and methods

Raw material

The raw material for the preparation of collagen hydrolystae is full chrome shaving dust which was collected from a export oriented tannery and kept at room temperature.

Reagents

Reagents were used for this research work were laboratory or analytical grade.

Analysis of Chrome Shaving Dust

The collected full chrome shaving dust was characterized by both physical and chemical analysis using standard procedures [7]. The moisture content of chrome shaving dust was determined according to SLC-12, the pH according to SLC-13, Nitrogen content according to SLC-7, IUC/10, the amount of total ash, according to IUC/7, chromic oxide content according to SLC-8, oils & fat content according to SLC-4. The analytical measurements were obtained based on the dry weight of chrome shaving dust and triplicate measurements were done for each of the parameter.

Preparation of protein solution from chrome shavings by treating with alkali and enzyme

In the first step of hydrolysis, 50 gm of full chrome shaving dust was measured and taken into 1000 ml beaker. 150% of distilled water and 0.1% of non ionic surfactant were added to this and heated at 70-72 ºC for 15-20 minutes [8]. After complete soaking of chrome shaving dust, 3% of MgO was added and heated at 70-72 ºC for 30 minutes. Continuous stirring was maintained during the whole hydrolysis process. The pH was adjusted at 8.5-9.5 using MgO [9]. After complete maceration, the temperature was reduced to 45-55 ºC and then added to this 0.1% of trypsin enzyme [10]. Hydrolysis was continued for 2 hours with frequent agitation of 120 rpm. Water soluble collagen hydrolysate was extracted from the chrome shaving slurry during hydrolysis. After completion of hydrolysis, the temperature was raised at 85 ºC and heated for 10 to 15 minutes to completely deactivate the enzyme. After the deactivation of enzyme, the slurry was cooled down to 40-45 ºC and the collagen hydrolysate was separated from the slurry through vacuum filtration without disturbing the sludge. The prepared collagen hydrolysate was collected and preserved at 4 ºC in an incubator.

The chrome cake after vacuum filtration was further hydrolyzed at the second and third steps in the same procedure. The collagen hydrolysate were separated, collected and preserved in the same way as first step of hydrolysis. Then the protein hydrolysate was analyzed.

Solid Content of Prepared Collagen Hydrolysate

The solid content of prepared collagen hydrolysate was determined by SLC-114.

pH of Hydrolysate

The pH of prepared collagen hydrolysate samples was determined using the standard method, SLC-13.

Nitrogen and Protein Content

The nitrogen and protein contents were determined by the standard procedure using BUCHI Digest BUCHI AUTO Kjeldhal Unit K-370.

Chromium Content

Total chromium content of the prepared collagen hydrolysates was determined by a Perkin Elmer A Analyst-200 model Atomic Absorption Spectrophotometer.

Calcium and Magnesium Content

Ca and Mg content of the prepared collagen hydrolysates were determined by a Perkin Elmer A Analyst-200 model Atomic Absorption Spectrophotometer.

Ash Content

The ash content of the prepared collagen hydrolysate samples was determined by SLC-7.

FTIR Analysis

The FTIR analysis of the collagen hydrolysate was carried out by a Shimadzu FTIR analyzer.

Result and Discussion

Analysis of Chrome Shaving Dust

Physical and Chemical properties

The physical and chemical parameters of chrome shavings shown in table 1.

SL.No.	Parameters	Values
01.	Color	Bluish
02.	Moisture Content (%)	21.96
03.	Apparent Density (g/ml)	0.89
04.	Chrome Content (%)	3.42
05.	Ash Content (%)	5.18
06.	Oils & fats content (%)	0.88
07.	pH (10% aqueous solution)	3.72
08.	Nitrogen Content (%)	14.53
09.	Protein Content (%)	90.81

Table 1: Characteristics of Full Chrome Shaving Dust.

Shaving wastes are generated during leather processing steps called beam house process, in which leather are processed in watery environments, water content of solid wastes diffuse to a great extent in accordance with how and where they are collected, storage condition and climate. According to the results of water content determination in shaving dust, water content was found to 21.96 %.The chrome shavings were analyzed for pH, ash, fat, protein and chromium oxide. The results are presented in Table -1 as can be seen the analysis indicates that this waste has important percentage of proteins 90.81% and chromium oxide 3.42%. The analytical data obtained is nearly same as reported in the literature [11]. The chemical composition of these wastes is very interesting and allows scope for recovery of proteins.

Analysis of Collagen Hydrolysate

Physical Properties

Physical properties of Collagen Hydrolysate are shown in table 2.

Parameters	Result
General appearance	Viscous gel and sticky
Color	Light yellow or Creamy
Odor	Odorless
pH	8.03
Solid content (%)	70.72

Tab-2: Physical properties of Collagen Hydrolysate

Physical analysis of prepared collagen hydrolysate shows that (Table-2) it is a light yellow or creamy color, viscous gel, odorless and sticky type liquid. The pH is 8.03, which is complied with the reference value [12].

Chemical Properties

The Chemical properties of prepared collagen hydrolysate are shown in Table 3.

Test	Result
Nitrogen Content (%)	10.34
Protein Content (%)	64.63
Chromium Content (ppm)	1.47
Magnesium Content (ppm)	1030
Calcium Content (ppm)	Traces
Ash Content (%)	2.10

Table 3: Chemical properties of prepared Collagen Hydrolysate

From the table -3, it is shown that the percentage of nitrogen & protein content is reasonable. The chromium content of the prepared sample is only 1.47 ppm (Chrome content of full chrome shaving dust was 3.42%), which is much less than the reference value (< 4.5 ppm). So the collagen hydrolysate prepared by this method is completely safe & potential [13]. The Magnesium content of the prepared sample is 1030 ppm (due to 3% of MgO was used in the hydrolysis process), which is complied with the reference value [14]. The ash content of prepared sample is 2.10%, which is much less than that of the untreated full chrome shaving dusts (5.18%). Science the prepared hydrolysate contains trace amount of chromium, the ash content of the prepared sample reduced to a large extent. The ash content of collagen hydrolysate was not found to be very low due to high content of Magnesium present in the sample.

FT-IR Analysis

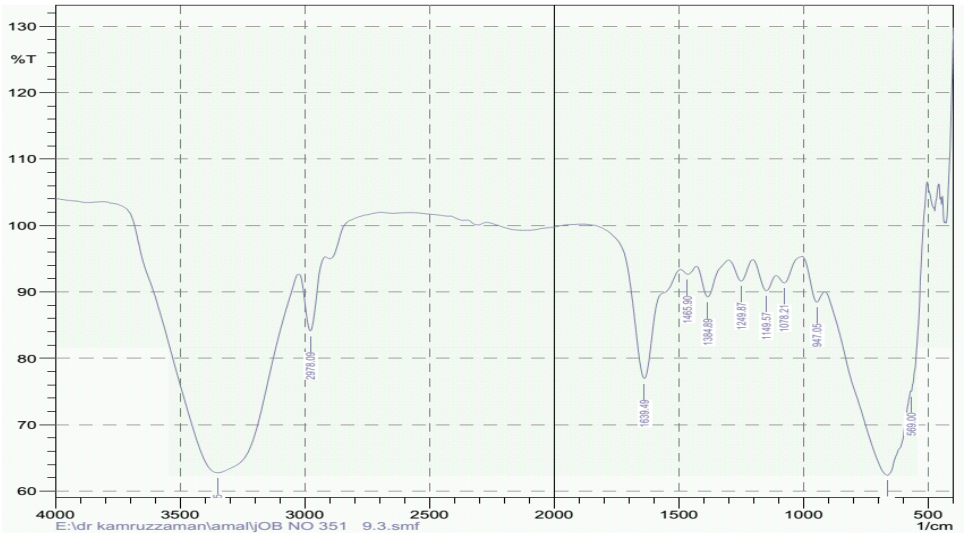

Figure 1: IR Spectra of Protein hydrolysate

In the above figure, the collagen hydrolysate (CH) is characterized by amide A (3329 cm^{-1} – 3360 cm^{-1}), amide B (2978 cm^{-1} – 3285 cm^{-1}) bands, associated with NH stretching modes. Amide I (1633 cm^{-1} – 1640 cm^{-1}), amide II (1384 cm^{-1} – 1550 cm^{-1}) and amide III (1149 cm^{-1} – 1260 cm^{-1}) are characteristic for collagen in random coil protein [15], [16] which proved that the collagen was in hydrolysate form.

III. Conclusion

From the above data it can be concluded that, a safe and scientific method has been developed for the preparation of collagen hydrolysate from the tannery solid wastes (i.e. chrome shavings). This method is eco-friendly and can be referred as biochemical method. The prepared collagen hydrolysate is almost free of chromium (1.47 ppm). The prepared collagen hydrolysate also contained appreciable amount of magnesium (1030 ppm) which is essential minerals for poultry feed. Thus, the product will be very potential for use as poultry feed. The ash content of the prepared collagen hydrolysate was found to be minimum and it was much less than the reference value (4.9%). The protein content of the prepared collagen hydrolysate was also reasonable and it was found 64.63%.So the chrome containing leather waste is not ordinary because it has a treasure of valuable material.

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