Dechroming Optimisation of Chrome Tanned Leather Waste As Potential Poultry Feed Additives: A Waste to Resources Approach

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Abstract

Moving towards zero waste required that the industry adopts a circular economy. There is a need to drive a circular economy approach where all resources are reclaimed and reused if possible; waste is kept to an absolute minimum. Resource recovery for the leather industry waste will give financial and economic benefits. This research explores the potential utilisation of tannery solid waste as poultry feed additive. Dechroming rate can be controlled to produce a final product with a low level of chromium satisfying the requirements for poultry feed. Enzymatic treatment was used to obtain protein concentrates after thermal treatment. The hydrothermal stability and fibre structure of samples were analysed by differential scanning calorimeter (DSC) and scanning electron microscope (SEM). Energy dispersive X-ray analysis (EDXA) peaks revealed the chromium content and relative content of each element. High performance liquid chromatography (HPLC) was used to compare the amino acid composition with wheat and soya bean meal that is conventionally used in poultry feed.

Proximate analyses and other essential inorganic elements were determined by inductively coupled plasma optical emission spectrometers (ICP-OES) and showed that levels of the metal were within the limit to be used in feed additives. The molecular weight distribution of the protein concentrates by sodium dodecyl-polyacryamide gel electrophoresis (SDS-PAGE) indicated <10kDa.In addition, the extracted product showed 75% digestibility (*in vitro*).This demonstrates a clear example of waste utilisation.

Keywords: Dechromed waste, hydrolysis, protein concentrate, amino acid content, poultry feed.

1. Introduction:

1.1 Circular economy

Circular economy focuses on resource-productivity and eco-efficiency improvement in a comprehensive way. In a circular economy, as opposed to a traditional linear economy, products are intended to be more sustainable, as their design is based around reusable parts, allowing for a simpler end of life recovery process (Jing Hu *et al.*, 2011). Zero waste is now a strongly emerging issue for sustainable industry development where reduction, minimisation, and utilisation of waste are simultaneously realized (Phillips *et al.*, 2011).The zero waste concept comprehends a broad range of approaches including volume minimization, reduced consumption, design for recycling and reduced toxicity. (Braungrat *et al.,* 2007).

There is a requirement to keep in mind the need for sustainability, at the same time optimising the product life cycle and minimising pollution and waste. Life Cycle Engineering can address the eco-efficiency such as: the product, the production, the process and the discharge (Hauschild *et al*., 2005). Old products would become new resources for the economy or for nature. Resource scarcity would be replaced by resource abundance, with benefits for technological, economic, environmental and social progress. (Greyson, 2007). Environmental pressures will continue dominating tannery process development until tanneries approach zero waste discharge to the environment (Wang *et al*., 2009).

The waste hierarchy ranks waste management options in terms of sustainability. Waste disposal at the bottom of the hierarchy, has the greatest impact on the environment and is typically the least cost effective waste management solution. The hierarchy has five components, generally ordered in decreasing preference as follows: (i) waste minimisation, (ii) reuse, (iii) material recycling, (iv) energy recovery and (v) waste disposal (Bain *et al*., 2010).

Integrated waste management includes seeking management methods to reduce waste at its source before it even enters the waste stream. Sustainable solid waste management aims to offer a chance to prevent waste through designs based on the full life cycle of the product, similar to natural cycles, which function without producing waste. By this way, waste should, like any residue, be thought of as potential inputs for starting new processes. Waste materials that are generated must be recovered for reuse and recycling to reach the goal of using everything with nothing left (Ngoc *et al*., 2009).

Progression towards zero environmental impact from BETNEEC (best available technology, not entailing excessive cost) to CATNAP (currently applied technology, narrowly avoiding prosecution) should ideally be economically attractive at the time, including using waste streams, by adding value to the byproducts. (Covington, 2009).

1.2 Leather Waste

Environmental matters cannot be taken in isolation from leather making, as every facet of pollution or residual material is a direct function of manufacture (Richard, 2004). Converting raw hides to finished product involves significant chemical and water usage and generates a substantial amount of waste (BLC, 2000). Waste includes all items that people or companies no longer have any use for, which they either intend to get rid of or have already discarded. However, waste can also be a resource if managed correctly (Ngoc *et al*., 2009). Processing industries can cause adverse changes in the immediate environment (Mbuligwe *et al*., 2006).

Only 255 kg of finished leather (grain and embossed split) is obtained for every 1000 kg wet salted hides processed, i.e. just 25.5% of the raw material becomes finished leather (Aquim *et a*l., 2010). Large volumes of wastewater contaminated with the chemicals and organic matters pose greater challenge than the treatment of waste water. The cost of setting up and operating an effluent treatment plant is also directly related to water consumption (Saravanan *et al*., 2010). To process 1000 kg of wet salted hides, around 40 m^3 of water (process and technical water) is required and out of 452 kg of process chemicals used only 72 kg are retained in and on leather and 380 kg are wasted and discharged in various forms.The total quantity of sludge (including biological treatment) dewatered to approximately 30% of dry substance will be approximately 420 kg for one tonne of wet salted hides. Only 53% of corium collagen and 15% of the chemicals purchased are retained in the finished leather (Buljan *et al*., 2000). The annual production of raw material (hides and skins) processed in Bangladesh is about 85,000 tonnes. An estimated amount of solid (tanned and untanned) waste during the processing of one tonne of salted hides/skins according to various authors and Bangladeshi leather industries is shown in Table 1 (Paul *et al*., 2013).

Table 1: Solid waste generated (kg) during processing of 1 tonne hides and skins in Bangladesh (Paul *et al*., 2013).

Solid Waste	**Bangladesh**	**Quantity generated tonnes per annum**
Untanned Waste		
Raw Trimmings	100	8,500
Fleshings	250	21,250
Tanned Waste		
Split	100	8,500
Shavings	100	8,500
Crust/Finished cutting	30	2,550

1.3 Chrome tanned waste

Approximately 200 kg of the chrome containing tannery solid waste (TSW) is generated from 1000 kg of raw hides and skins. Currently chrome tanning is the predominant method in leather making, which results in a large amount of chrome-containing solid waste. It is an environmental problem of tanning production due to its relatively high content of environmentally damaging Cr(III) salts. Stather and Pauligk has addressed the question regarding the minimum tanning material quantities required for conversion of hide to leather in Table 2 (Reich, 2007). However Gustavson (1956) has mentioned that an amount of 1% Cr_2O_3 fixed by collagen is sufficient to produce the maximum degree of stabilisation of collagen measured by the heat of activation and the entropy. Usually chrome content of fully tanned leather is 4 % (w/w) Cr_2O_3 (Covington, 2009).

Table 2: Minimum tanning material quantities required for leather formation (Reich, 2007).

Tanning material type	**Minimum tanning material quantity, in terms of hide substance**
Basic Chromium sulphate	0.9-1.25% Cr_2O_3
Formaldehyde	0.7-0.8% CH_2O
Various syntans and vegetable tanning agents	Dependent upon type : 6-12%; in all cases<20%

Chrome split and shavings are two vital waste streams that need to be handled expertly. There are two approaches to the waste/byproducts problem that tanners deal with, minimising the quantity of waste generated and maximising the return through by-products. Landfill has been widely practiced for disposal of chrome-containing tannery wastes. This is rather expensive as because of fewer landfill sites and transportation cost increases and environmentally in appropriate way of handling a waste material that has the potentiality for utilisation (Cabeza *et al.*, 1998).

1.4 Nutrition and toxicity of poultry feed:

Poultry farming is one of the fastest growing and most promising industries in Bangladesh. Its steady growth influences the economic growth of the country and contributes to the improvement in human nutritional status through consumption of meat and eggs. In poultry production system depends solely on compound feed, the cost of which represents 65-70% of the total production costs. It is important to search for unconventional feed ingredients of poultry feed as a solution to sky rocketing prices of novel feed ingredients like corn and soya. There is a short supply of these ingredients in different parts of the world and their use for poultry feed is in competition with their uses in human food and bioenergy (Jayatillake, 2011).

Dietary requirements for protein are actually requirements for the amino acids contained in the dietary protein. Protein and amino acid requirements vary considerably according to the productive state of the bird, that is, the rate of growth or egg production. Minerals such as calcium and phosphorus are required for the formation of the skeleton, as components of various compounds with particular functions within the body, as cofactors of enzymes. Sodium, potassium, magnesium and chloride function with phosphates and bicarbonate to maintain homeostasis of osmotic relationships and pH throughout the body (NAP, 1994).

Chromium (Cr) has been considered an essential nutrient for humans and animals. Chromium is absorbed primarily in the small intestine. Chromium toxicity is primarily associated with exposure to hexavalent chromium compounds. Trivalent and hexavalent chromium compounds behave differently in the body. However most of the Cr^{6+} is believed to be reduced to Cr^{3+} by extracellular fluids before reaching sites of absorption in the small intestine. Information is meagre on chromium toxicity for poultry feed. Dietary concentrations of chromium ranging from 3 to 1,000 $CrCl_3$ mg/kg caused effects on growing chicks. Research with poultry has shown that supplemental dietary chromium can be used to alleviate some of the toxic effects of vanadium in growing chicks and laying hens. Evidence also has been obtained that supplemental chromium at 20 mg/kg of diet as $CrCl_3$ increases the rate of glucose utilisation by livers of chicks and poults *in vivo* and *in vitro* (NAP, 1997

2.Experimental

2.1 Materials and Method:

Chrome containing leather shavings (CS) and splits (CT) were collected from the tannery of Institute for Creative Leather Technologies (ICLT), Northampton, UK by using conventional tanning process with 33% basic chromium sulfate followed by post tanning mechanical operation. CT were cut into 1 cm square and stored until required. All chemicals used in the dechroming process: sodium sulfate, sodium carbonate, calcium hydroxide, 1N sodium hydroxide, 30% hydrogen peroxide, sodium chloride, sulphuric acid were supplied by Fisher Scientific, UK Ltd. Analyses were carried out according to methods shown in Table 3.

Sample Digestion: The sample was digested by microwave-assisted digestion (MARS6, CEM Corporation, USA). Acid mixture (7 ml HNO_3 and 3 ml HCl) was added to 1 gm of sample (EN 14602).

Metal content was determined by Inductively Coupled argon Plasma Optical Emission Spectrometers (ICP-OES; iCAP 6000, Thermo Fisher Scientific, UK).

Table 3 : Properties of tanned leather waste with standard methods

Parameter	**Method**	**Tanned waste**	
		Cr-Splits	Cr-Shavings
pH [a]	BS EN ISO,4045:2008	4.77	4.10
Moisture (%)[a]	SLC-113	38.89	36.09
Sulphated total ash (%)[a b]	BS EN ISO,4047:1998	10.67	9.82
Total Kjeldahl Nitrogen (TKN) (%)[a b]	IUC-10	15.28	14.50
Hide substance (%)[a b]	IUC-10	85.87	81.51
Hydroxyproline(%) [a b]	IUC-17	10.08	9.78
Collagen content (%) [a b]	IUC-17	80.65	78.24
Cr_2O_3(%) [a]	BS EN ISO,5398-4:2007	4.12	3.72
Fat(%) [a]	BS EN ISO,4048:1998	0.97	1.10

[a] N=3-5,where N=number of replicates for each sample, [b] Moisture free basis.

2.2 Dechroming Procedure of CS and CT:

In order to achieve maximum elimination of chromium from the CS and CT, without affecting the molecular structure of collagen fibres, the material has grounded to increase surface area. For optimum dechroming, the material must be fragmented into long fibres (Cot *et al.*, 1999).The CT were ground to a consistent size of 2-5 mm and the chrome shavings were used directly for dechroming. Both materials were moisture free.

Chrome shavings (CS-10 g/100ml) and Chrome splits (CT-10 g/100ml) were placed in sodium sulphate (5% w/w) and sodium carbonate (4% w/w) solution for 30 minutes followed by calcium hydroxide (3% w/w) for 1 hour. Sodium hydroxide solution (0.1% v/v) was then added.

$$Cr(OH)SO_4 + Ca(OH)_2 = Cr(OH)_3 + CaSO_4$$
$$Cr(OH)SO_4 + NaOH = Cr(OH)_3 + Na_2SO_4$$

Hydrogen peroxide (10% v/v) was added to the solution and stirred for 2 days, the oxidation effect was investigated for 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 hours of treatment.

$$2Cr(OH)_3 + 3\ H_2O_2 = H_2Cr_2O_7 + 5H_2O$$

Water was removed by filtration. The materials were washed three times with sodium sulphate solution (10% w/v) and filtered. The materials were soaked with sodium chloride solution (6% w/v) and sulphuric acid solution(1% v/v) for acid steeping for 1 hour and filtered. The materials were washed twice with sodium sulphate solution (10% w/v) and sodium chloride (6% w/v) and filtered. The dechromed CS and CT were then allowed to air dry at room temperature.

$$2H_2Cr_2O_7 + 6\ H_2SO_4 = 2Cr_2(SO_4)_3 + 7\ H_2O$$

2.2.1 The Dechroming rate: The chromium content (calculated as Cr_2O_3) in splits, shavings and liquors from the dechroming steps were determined by ICP-OES with the following equation:

$$\textbf{Dechroming rate (\%)} = 100[Cr_{shav} - Cr_{dec})/Cr_{shav}]$$

2.2.2 Thermal stability of the dechromed material was analysed using Differential Scanning Calorimetry (Mettler Toledo DSC-822) in moist conditions using the peak temperature as a reflection of hydrothermal stability.

2.2.3 Scanning Electron Microscopy and Energy Dispersive X-ray Analysis (EDXA):

The dechromed materials fibre orientation were investigated using SEM (ISO 17131:2012, Hitachi S-3000N, Japan) and elemental analysis with EDXA systems ; accelerating voltage 20kv, collecting time 300 sec. The results obtained were compared against a control, untanned materials e.g. hide powder.

2.3 Isolation of gelatin using thermal treatment:

Dechromed shavings (DCS) and dechromed splits (DCT) obtained from the above experiments were suspended in 250 ml and 500 ml water with 3% w/w MgO, 3% w/w $NaHCO_3$ to increase the alkalinity to pH 8-9 . In general 3-5 extractions are obtained in the temperature range 55-95^{o}C (Babel *et al.,*1998). Samples (50 gm) were placed in a flask on water bath and stirred at 50 rpm and at the desired operation temperature for the intended extraction time. The water quantity, temperature and contact time used for each experiment are shown in Table 4.

Table 4 :Operating conditions for DCS and DCT

Water Quantity (ml)	250			500		
Temperature (^{o}C)	60-70	70-85	85-95	60-70	70-85	85-95
Time (h)	2	2	2	2	2	2
Extract	1	2	3	4	5	6

The extract obtained by the use of above methods described contains liquid protein and an insoluble residue from the DCS/DCT. By using filtration the water soluble protein (gelatin) was obtained, concentrated and lyophilised which resulted in a gelatin powder product.

2.4 Isolation of protein concentrate using enzyme treatment:

The insoluble residue was collected and the materials suspended in water with liquid proteases (*Bacillus licheniformis*, Sigma-Aldrich, UK). Salts were added (1% w/v $CaCO_3$, 2% w/v $NaHCO_3$) to maintain the pH level to 9-10 with the following operating conditions (Table 5).

Table 5: Operating conditions for enzyme treatment								
Enzyme Activity	2U/g		3U/g		4U/g		5U/g	
Temperature (^oC)	70		70		70		70	
Time (h)	1	2	1	2	1	2	1	2
Experiment	1	2	3	4	5	6	7	8

The solution obtained from the above experiment were centrifuged at 5000 rpm (Megafuge 16 R, Thermo scienfic, UK) . The protein solution was separated, concentrated and freeze dried at -40^{o}C.Shavings (W_s), splittings (W_t) and protein concentrates (W_p) were lyophilised and weighted. The % yield was calculated as **(W_p/ W_s x 100)**.

2.5 Protein and amino acid analyses:

Hydroxyproline and collagen content (IUC-17) were determined by using UV- Visible spectrophotometric analysis. For amino acid analysis the sample was transferred into a 250 ml round bottom flask and placed in a heating mantle at 110^{o}C for 24 hour with 6N HCl. The solution obtained was kept in an evaporating dish to evaporate HCl on a water bath. It was then filtered into a 25 ml volumetric flask through a Whatman no.1 filter paper and injected with 0.1NHCl. The solution was run through an amino acid analyser (Schimadzu, Japan). The analyser showed the standard curve for standard solution and another curve for sample solution. By comparing the two curves and integration (retention time, area), the amount of amino acids (%) was calculated.

2.6 Crude protein and the hexavalent chromium was analysed with AOAC official method 988.05 and CEN/TS 144495:2003 respectively in the final product.

2.7 SDS-PAGE: SDS-PAGE was performed using a Mini-PROTEAN 3 system (Bio-Rad, USA). Each individual gel (80X73mm) was composed of a 20mm staking gel (5% acrylamide/bis; T30%,C3.3%) and a 53 mm separating gel (10% acrylamide/bis ; T30%, C3.3%).5µl of protein concentrates sample (1mg/ml) was mixed with 1:2 sample buffer (0.5 M Tris-HCl at pH 6.8, 25% Glycerol,10% (w/v) SDS,0.5% (w/v) bromophenol blue:0.2 ml, 50µl β-Mercaptoethanol) and heat 95^{0}C at 5 minutes. 10 µl sample was loaded with a pipette using gel loading tips. The wide range of protein standard from 6,500 Da to 200,000 Da was included. Then, the electrophoresis was performed at 25mA/gel. When the dye reached the bottom of the gel, the current was turned off. The gel was stained with 0.1% Coomassie brilliant blue (R250) in 50% methanol,10% glacial acetic acid and overnight destained by a mixed solution of 10% methanol, 7% acetic acid.

3.Results and Discussion

Table 1 shows that the solid waste problem from Bangladeshi tanneries is a large with substantial amount of chrome containing waste (about 17,000 tonnes) generated each year.

3.1 Dechroming: It is anticipated the danger and deleterious effect is exacerbated if the pH change is rapid. In the case of alkali swelling, the mechanisms operating are charge effects and lyotropy. The effect of alkali on collagen is to break the natural salt links, to make the protein anionic (Covington, 2009):

$$^{-}COO \ \ldots\bar{} \ H_3N^+ - \ OH^- \longleftrightarrow {}^{-}COO \ + H_2N-$$

During oxidation (hydrogen peroxide) in alkaline condition, all of the combined or deposited chrome (Cr^{3+}) oxidizes to (Cr^{6+}) by generating some peroxochromates (yellow orange colour *in situ*) followed by reduced again Cr^{3+}.

Due to the bleaching and activating effect of hydrogen peroxide, a final clear product was obtained. Once the chromium was oxidized, the complexing capacity of chrome-fibre interaction was lost and eliminated through several washes. In the dechroming stages 94.62% and 93.44% of dechroming rate were achieved for CS and CT respectively after 48 hours. Within 2 hours 54.30% and 50.48% chromium (as a $\%Cr_2O_3$) removed from the materials. After 6 hours Cr_2O_3 content (%) in CS and CT were 0.83% and 1% that indicates chromium present in the materials is less than the required level of tanning materials.

3.2 Thermal stability: In Figure 1 it has revealed that after 2 hours oxidation the hydrothermal stability of dechromed materials is below $80^{O}C$ in wet condition.

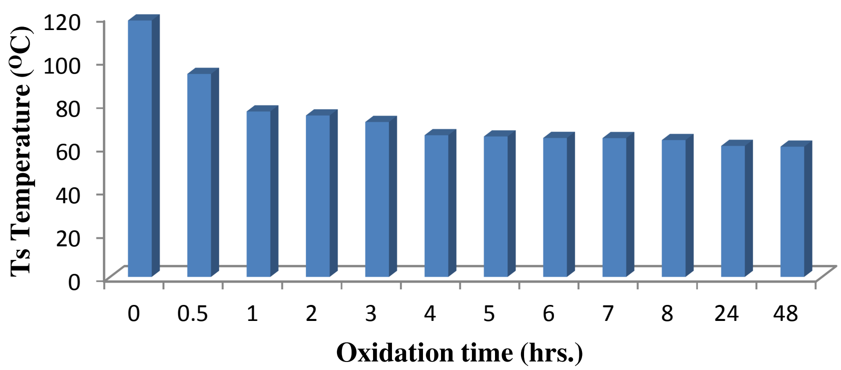


Figure 1: Different oxidation time (hr.) with Shrinkage temperature (Ts)

Typically the native collagenic materials shrinkage temperature (Ts) is at $65\text{-}70^{O}C$ (Covington, 2009). Therefore it is likely to be a untanned hide powder.

3.3 SEM : The remaining collagenic fibres of DCS and DCT is practically unaffected, the chromed collagen fibres are left at a stage previous to the tanning phase, remaining in raw or pickle stage, not stabilised or crosslinked (Cot, 2004).SEM images confirm the fibre structure with limited disorientation.

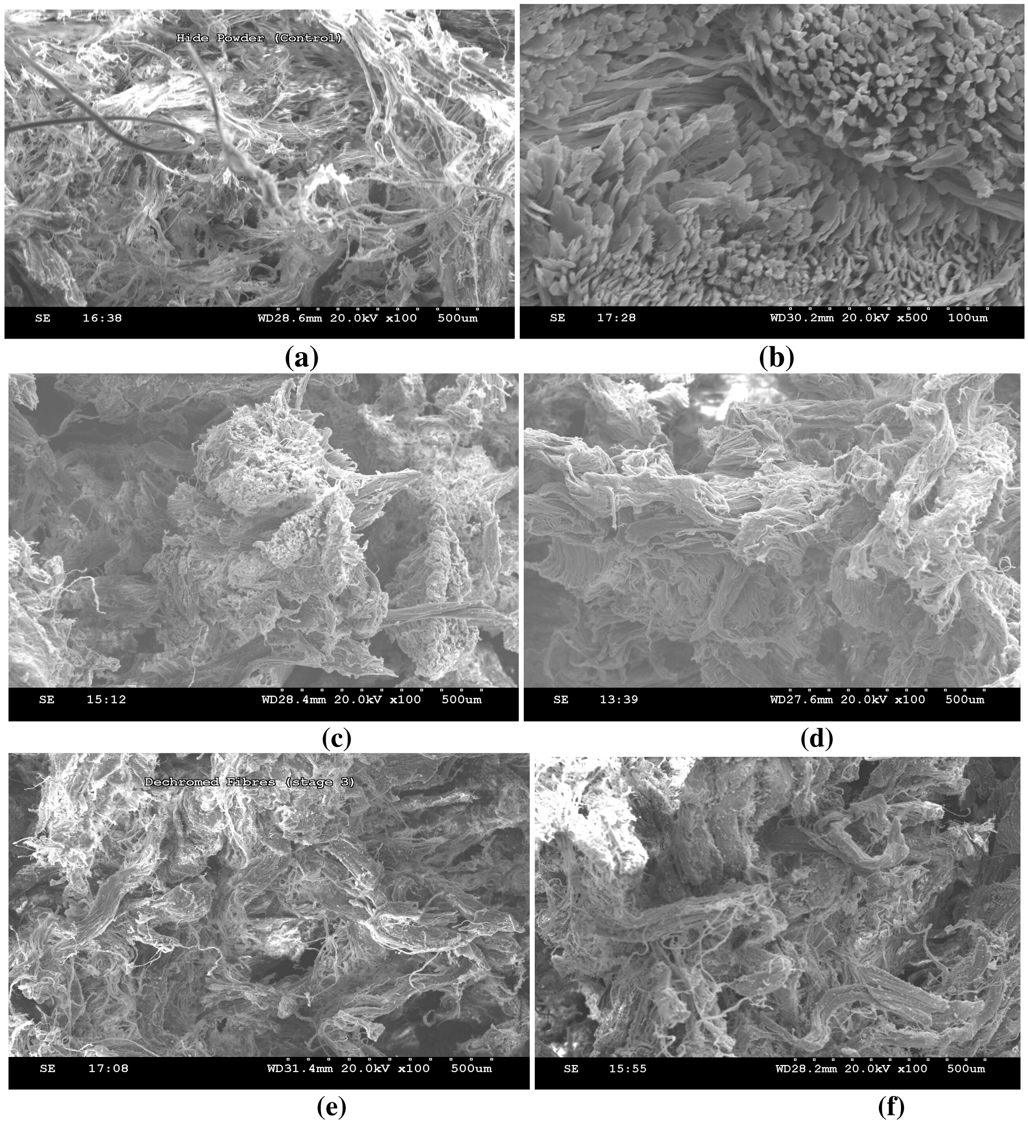
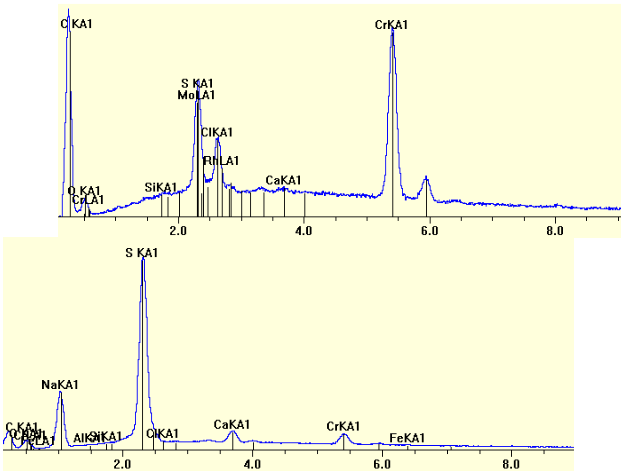


Figure 2: (**a);** hide powder (control), (**b**); chrome tanned leather (intact) (**c**); ground chrome tanned fibre, (**d-f**); dechromed stages; (20kV x100 magnification).

3.4 <u>EDXA</u>: EDXA is used to confirm qualitatively and quantitatively for DCS and DCT samples (Figure 3). It is clear the peak for Cr is higher than other inorganic elements before dechroming. On the other hand Cr content is significantly reduced after 8hours dechroming.

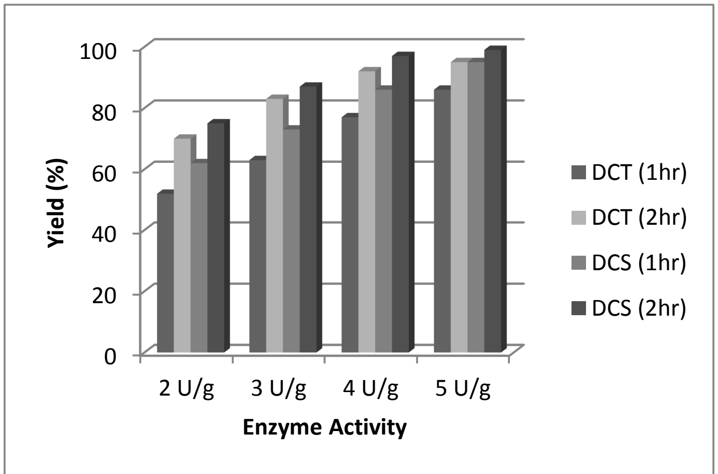


(a) **(b)**

Figure 3: Elemental analysis by EDXA; (**a**)- starting material (ground chrome tanned shavings , (**b**)- dechromed material (after 8hrs oxidation).

3.5 <u>Extraction of protein concentrate</u>: Isolation of protein is carried out by thermal and enzymatic treatment as per operating condition mentioned in Table 4 and Table 5. The insoluble residue is lowered with extraction time . The effect of temperature is significantly affect to extract gelatin solution, however about 50% of insoluble residue is present in solution.

3.6 <u>Yield</u>: In Figure 4 it has shown the effect of enzyme treatment as the yield of protein concentrates for 1 hr and 2 hrs. Dechromed shavings (DCS) has given higher yield than dechromed splits (DCT) with enzyme solution. However both materials yield is above 90%. It is established the maximum amount of protein concentrate recovered with minimum residue by enzyme treatment.



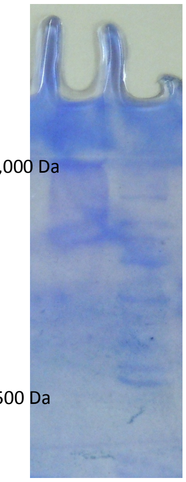


Figure: 4 Figure:5

3.7 Molecular weight distribution (MWD): Results showed the band corresponding approximiately10kDa in figure 5. Low molecular weight distribution may be the indication of higher enzymatic hydrolysis. This result revealed to get higher digestibility to protein concentrates.

3.8 Amino acid analysis: The nutrient requirements for poultry feed vary according to the purpose for which they have been developed. The final product contains 82.81% protein. The high content of arginine, leucine ,threonine,serine and methionine are of a sufficient level for poultry feed. The Table 6 is also mentioned the requirement of certain amino acid for broilers and laying hens (Evonik, 2012).

Table 6: Essential Amino Acid (% or dry substance) in different commercially feed products with extracted protein concentrate.

Amino Acids (%)	**Extracted Protein concentrate (%)**	**Wheat (%)**	**Soya bean Meal (%)**	**Broilers (%)**	**Laying hens (%)**
Threonine	2.67	0.36	1.8	0.49-0.80	0.48-0.73
Serine	4.59	0.59	2.4	0.3-0.8	-
Valine	0.74	0.53	2.1	0.59-1.00	0.61-0.91

Methionine	2.26	0.20	0.64	0.31-0.50	0.35-0.52
Isoleucine	4.10	0.41	2.0	0.53-0.86	0.55-0.83
Leucine	1.12	0.85	3.6	0.75-1.36	0.83-1.25
Tyrosine	2.07	1.00	3.9	0.84-1.47	0.83-1.25
Histidine	1.76	0.32	1.2	0.24-0.42	0.21-0.31
Lysine	2.31	0.34	2.8	0.73-1.27	0.69-1.04
Arginine	7.25	0.59	3.3	0.78-1.30	0.72-1.08

3.9 Proximate analyses and Inorganic elements: Proximate and inorganic elements analyses are carried out for determining the nutritive value of extracted protein compared with traditional poultry starter. CP is much higher in extracted protein. The inorganic elements content are notable (Table 7). Thus there is no negative impact on final product.

Table 7 : Proximate analyses and Inorganic elements

Parameters	**Poultry Starter**	**Extracted Protein**
Crude Protein (CP)%	19-22	82.81
Fat%	2-4.5	0.47
Moisture%	7.5-10	7.5
Ash%	5	8.5
Chromium ($Cr^{3+)}$, ppm	3-1000	880
Calcium, ppm	10,000-20,000	13,000
Magnesium, ppm	400-600	560
Sodium, ppm	1200-1500	1087
Potassium, ppm	2500-4000	1293
Chromium (Cr^{6+})ppm	<3	0
Digestibility,% (*in vitro*)	75-85	75

4.Conclusion:

Zero waste is a strongly emerging issue for sustainable industrial development where prevention and utilisation of waste are a priority in the leather industry. However, tannery solid waste may also be a resource if it is managed correctly as we move towards zero waste.

In Bangladesh, substantial environmental degradation occurs in the crude disposal of tannery solid waste. A true circular economy can not be realised unless there is sustainability. An increase in the utilisation of potential and traditional feed ingredients by processing industries will lead to the development of new feedstuffs. Tannery solid waste is however a potentially vital source of protein once dechromed. Dechroming rate can be controlled to produce a final product with a low level of chromium and satisfies the requirement for poultry feed. The chemicals used in this vital process do not impinge of the final quality of the product. Some (Ca, Na) are in themselves advantageous in the final product. The yellowish final product with no smell contains the required amount of essential inorganic elements. This paper show how a waste can be changed to a valuable product by adopting a sustainable approach.

As disposal, treatment and raw material costs continue to rise and as markets for secondary materials develop, closing the resource loop will help us all to improve productivity and efficiency while reducing environmental impact. Further research would need to be undertaken into these by-products with the aim of establishing their value for a wide range of animal feeds.

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