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Title: Towards zero solid waste: utilising tannery waste as a protein source for poultry feed

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Example citation: Paul, H., Antunes, A. P. M., Covington, A. D., Evans, P. and Phillips, P. S. (2013) Towards zero solid waste: utilising tannery waste as a protein source for poultry feed. Paper presented to: *28th International Conference on Solid Waste Technology and Management, Philadelphia, PA, USA, 10-13 March 2013*. Philadelphia USA: The Journal of Solid Waste Technology and Management. ISSN 1091-8043.

Version: Presented version

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Towards zero solid waste: Utilising tannery waste as a protein source for poultry feed.

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Abstract: Zero waste is now a strongly emerging issue for sustainable industrial development where minimisation and utilisation of waste are a priority in the leather industry. In a tannery hides and skins converted in to leather through various processes. Approximately 20% (w/w) of the chrome containing tannery solid waste (TSW) is generated from one tonne of raw hides and skins. However, tannery solid waste may also be a resource if it is managed expertly as we move towards zero waste.

This research illustrates the potential of tannery solid waste as a poultry feed additive. An oxidation method was used to achieve 95% of dechroming rate of chrome tanned waste followed with thermal and enzymatic treatment to produce gelatin solution and collagen concentrates. The thermal stability and fibre structure of samples were analysed by Differential Scanning Calorimeter (DSC) and Scanning Electron Microscope (SEM). Protein content and fourteen amino acid concentrations were determined using amino acid analysis. 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.

The nutrient requirements for poultry feed vary according to the purpose for which they have been developed. The high content of arginine, leucine, threonine, serine and methionine in the extract were of a sufficient level for poultry feed. Hexavalent chromium test was performed and showed that levels of the metal were low enough to be used in feed additives. In addition, the extracted product showed 75% digestibility (*in vitro*) and appears that treated TSW may be utilised in poultry feed, this demonstrates a clear example of waste utilisation. In Bangladesh plans are being formed to use the extract in poultry feed production.

Keywords: zero waste, leather solid waste, dechroming, hydrolysis, protein concentrate, amino acid, poultry feed.

1. Introduction:

1.1 Zero waste

Zero waste is now a strongly emerging issue for sustainable industry development where reduction, minimization, and utilization of waste are simultaneously realized (Phillips *et al*., 2011).

The zero waste concept encompasses a broad range of strategies including volume minimization, reduced consumption, design for recycling and reduced toxicity. In addition changes can be made in product design, manufacturing processes, consumer behaviour or material flow logistics, reduction and minimization remain a central component of the zero waste concept (Braungrat, 2007).

There is a requirement to keep in mind the need for sustainability and at the same time optimizing the product life cycle and minimizing 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). Zero waste and sustainable development have obvious potential for a preventive approach (Greyson, 2007). Environmental pressures will continue dominating tannery process development until tanneries approach zero waste discharge to the environment. It is important to use any waste because then they become co-products (Wang *et al*., 2009).

The waste management hierarchy is an ordered set of preferred practices that can be used to reduce the amount of waste being disposed. 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. Therefore, the higher levels of the hierarchy are more environmentally benign than the lower level in most cases; with landfilling waste in the ground as the least desirable approach to waste management (Bain *et al*., 2010).

Integrated waste management includes seeking management methods to reduce waste at its source before it even enters the waste stream. Disposal is not a sustainable solid waste management. More especially, 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, nothing left` (Ngoc *et al*., 2009).

It is an important function of leather science to provide technologists with sufficient understanding of the principles of the chemistry and biochemistry to make sustainable changes in their operations. Developments in this direction should ideally be economically attractive at the same time, for waste streams, by adding value to the byproducts. Profit is a big incentive for change (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. Water is seen as one of the chemicals needed for the process and not as a commodity that is of limited availability.

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 (Catalina *et al*., 2007). 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. 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.

Table 1: Solid waste generated (kg) during processing of 1 tonne hides and skins (Alexander *et al*.,1991 and Buljan *et al*., 2000)

Solid Waste	**Alexander**	**Buljan**	**Bangladesh**	**Quantity generated tonnes per annum**
Untanned Waste				
Raw Trimmings	120	100	100	8,500
Fleshings	70-230	300	250	21,250
Tanned Waste				
Split	115	107	100	8,500
Shavings	100	99	100	8,500
Crust/ Finished Cutting	32	25	30	2,550

1.3 Chrome Tanned waste

Nowadays chrome tanning is the predominant method in leather manufacture, which results in a large amount of chrome-containing solid waste. It is an important environmental problem of tanning production for 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 (Reich, 2007) in Table 2. Usually chrome content of fully tanned leather is 4 % Cr_2O_3 (Covington, 2009).

Table 2: Minimum tanning material quantities required for leather formation.

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/by-products problem that deal with tanners, minimizing the quantity of waste generated and maximizing the return on 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 utilization (Cabeza *et al.*, 1998).

1.4 Poultry feed Nutrition and toxicity:

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. For example turkey poults and broiler chickens have high amino acid requirements to meet the needs for rapid growth. The mature rooster has lower amino acid requirements than does the laying hen, even though its body size is greater and its feed consumption is similar. Minerals are required for the formation of the skeleton, as components of various compounds with particular functions within the body, as cofactors of enzymes, and for the maintenance of osmotic balance within the body of the bird. Calcium and phosphorus are essential for the formation and maintenance of the skeleton. 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. It has been shown to have antioxidative properties in vivo and it is integral in activating enzymes and maintaining the stability of proteins and nucleic acid. Its primary metabolic role, however, is to potentiate the action of insulin through its presence in an organometallic molecule called the glucose tolerance factor (GTF).

Almost all of the sources of chromium in the earth's crust are in the trivalent state (Cr^{3+}), and naturally occurring chromium compounds in the hexavalent oxidation state are rare. Hexavalent chromium (Cr^{6+}) compounds are thus man-made products. 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. 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 utilization by livers of chicks and poults in vivo and in vitro (NAP, 1997).

2.Experimental

2.1 Materials and Method:

Chrome containing leather shavings and splits were collected from the tannery of the Institute for Creative Leather Technologies (ICLT), Northampton, UK. The samples were cut into 1 cm square and frozen 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 :Characteristics of the tanned leather waste

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 Step:

In order to achieve maximum elimination of chromium from the sample, without affecting the molecular structure of collagen fibres, grinding of the material is applied to increase surface area. For optimum dechroming, the material must be fragmented into long fibres (Cot *et al*., 1999).The chrome tanned splits 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.

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

2.2.1 The Dechroming rate: The chromium content (calculated as Cr_2O_3) in splits, shavings and various liquor 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 or dechroming of the material was analysed using differential scanning calorimetry (DSC) in moist conditions using peak temperature as a reflection of hydrothermal stability and the dechrome of the chrome tanned material.

2.2.3 Scanning Electron Microscopy and EDXA:

The dechromed materials fibre orientation were investigated using SEM (ISO 17131:2012) and elemental analysis with EDXA systems. 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 250ml and 500 ml water with 3% MgO (w/v), 3% $NaHCO_3$ (w/v)to increase the alkalinity to pH 8-9 . Dechromed shavings and splittingss, alkaline water, were tumbled at the following experimental conditions. In general 3-5 extractions are obtained in the temperature range 55-95^oC (Babel *et al.*,1998). DCS and DCT were studied by changing three parameters: time, water to materials mass ratio and temperature. 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 5.

Table 5 :Operating conditions for DCS and DCT						
Water Quantity (ml)	250			500		
Temperature (^oC)	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 of DCS/DCT. By filtration the water soluble protein (gelatin) was obtained, concentrated and dried at 105 ^oC which resulted in a gelatine powder product.

2.4 Isolation of protein concentrates using enzyme treatment:

The insoluble residue was collected and the materials suspended in water with liquid proteases (from *Bacillus licheniformis*, Sigma-Aldrich, UK). Salts were added (1% $CaCO_3$ w/v, 2% $NaHCO_3$ w/v) to maintain the pH level to 9-10 into following operating condition (Table 6).

Table 6: 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 at supernatant, concentrated and dried at 105^{o}C . Shavings (W_s), splittings (W_t) and protein concentrates (W_p) were dried at -40^{o}C in a freeze dryer and weighted. The % yield was calculated as **(W_p/ W_s x 100)**.

2.5 Protein and amino acid analyses:

Hydroxyproline and collagen content 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.

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) are generated each year. Table 3 shows that there is a need for accurate characterisation to optimise the subsequent process using standard methods.

3.1 Dechroming: Before oxidation one must first treat the tanned leathers with alkali (pH range between 6 to 8) so that the alkali may reach the deepest layers of the leather, creating a sufficient concentration and potentiality of (OH^-) between the fibres. It is assumed 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):

$$^{-}COO^{-} \ldots. \; H_3N^{+} - \; \underset{\longleftarrow}{OH^-} \; \longrightarrow \; -COO^{-} + H_2N-$$

Oxidation is pH dependent and it is therefore important to maintain the pH between 10 and 10.5 during oxidation time. During oxidation with peroxides (hydrogen peroxide) in alkaline condition, all of the combined or deposited chrome (Cr^{3+}) oxidizes to (Cr^{6+}) by generating some peroxochromates of a yellow orange colour *in situ*. Hydrogen peroxide was selected because of its price, simplicity and hazardous-free final products of redox reaction. In the dechroming stages 94.62% of dechroming rate is achieved (Table 7).

Table:7 Effect of oxidation time on chrome splits and shavings

hr	Chrome Splits (%Cr_2O_3)	Dechroming rate(%)	Chrome Shavings (%Cr_2O_3)	Dechroming rate (%)
0	4.12		3.72	
0.5	2.44	40.77	1.85	50.26
1	2.36	42.71	1.80	51.61
2	2.04	50.48	1.70	54.30
3	1.91	53.64	1.68	54.83
4	1.73	58.00	1.61	56.72
5	1.39	66.26	1.20	67.74
6	1.00	75.72	0.83	77.68
7	0.71	82.76	0.56	84.94
8	0.41	90.04	0.35	90.59
24	0.34	91.74	0.28	92.47
48	0.27	93.44	0.20	94.62

3.2 Thermal stability: Due to the bleaching and activating effect of hydrogen peroxide, a final clear product was obtained. Once the whole chromium has been oxidized, it has lost all its complexing capacity and it is eliminated through several washes, thus remaining the collagenic fibre 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). In Figure 1 it is shown that after 5 hour dechroming the materials peak temperature at about 60^{o}C.

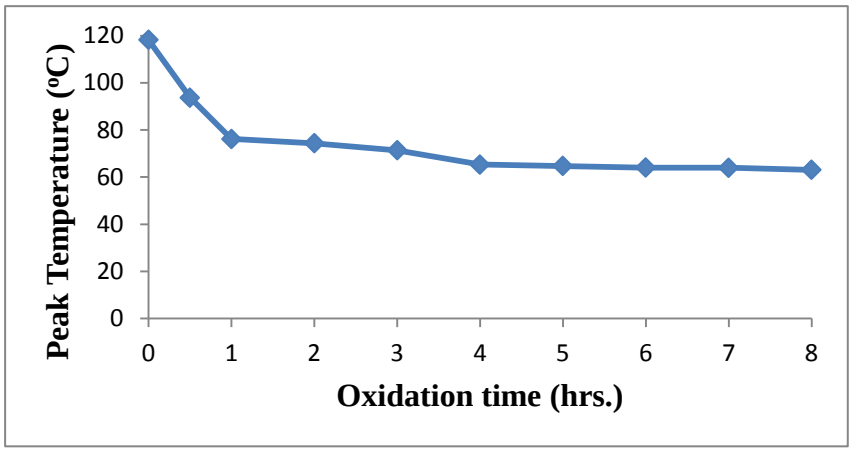


Figure 1: Different oxidation time (hr.) with peak temperature

3.3 Yield: Dechromed shavings (DCS) has given higher yield than dechromed splits (DCT) with enzyme solution. However both materials yield is above 90% shown in the Figure 2.

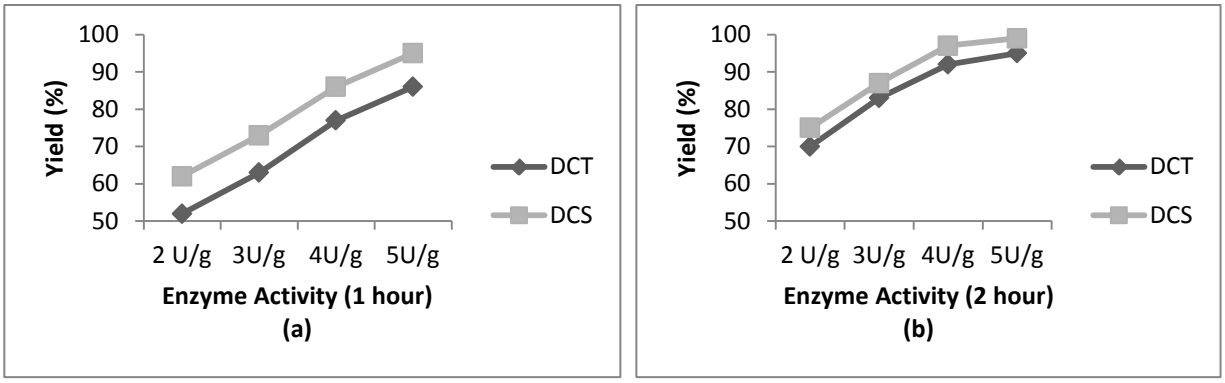
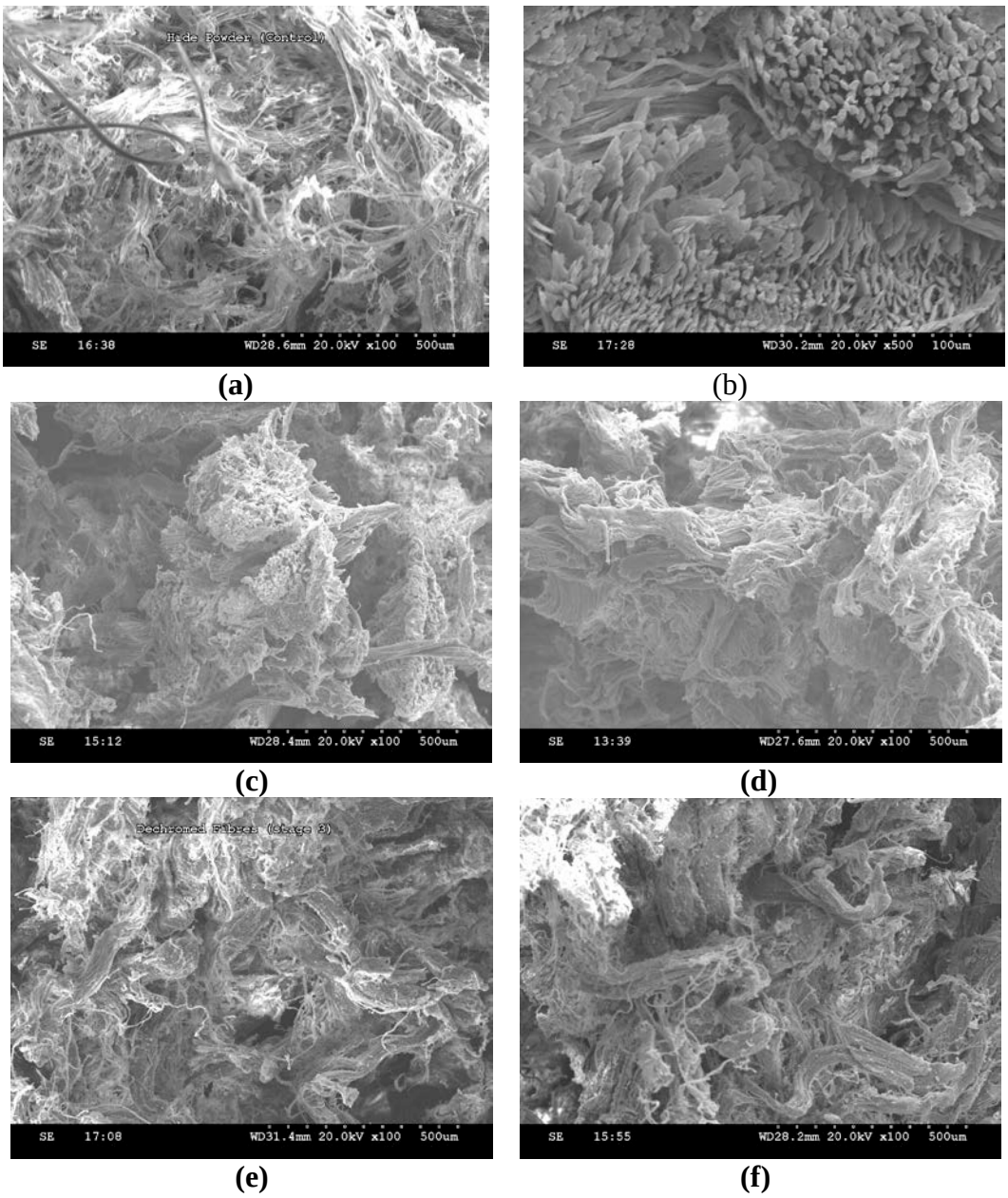


Figure 2: Effect of enzyme treatment with time (a) for 1 hr and (b) for 2 hrs and the yield of protein concentrates.

3.4 SEM and EDXA: SEM images confirm the fibre structure with limited disorientation. There is a decrease of chromium content within the fibre structure; at the end of dechroming it is likely to be untanned hide powder (Figure 3). EDXA is used to confirm qualitatively the decreasing chromium content in the fibre structure (Figure 4).

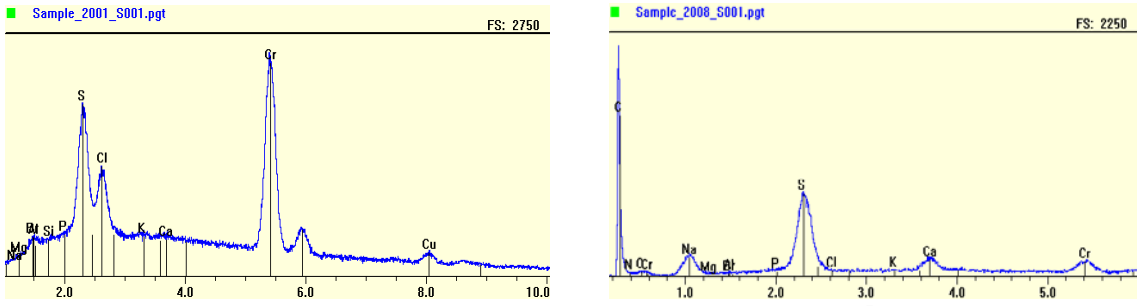


(a) (b)

(c) **(d)**

(e) **(f)**

Figure 3: (**a);** hide powder (control), (**b)**; chrome tanned leather (intact) (**c)**; ground chrome tanned fibre, (**d-f)**; dechromed stages (20kV x100 magnification).



(a) **(b)**

Figure 4: Elemental analysis by EDXA; (**a)**- starting material (ground chrome tanned shavings , (**b)**- dechromed material (after 8hrs oxidation).

3.5 Extraction of protein concentrate: Isolation of protein is carried out by thermal and enzymatic treatment as per operating condition mentioned in Table 5 and Table 6. The insoluble residue is lowered with extraction time and it exits nearly 50 % as shown in the Table 8.

Table 8: Extraction experiments for dechromed shavings (DCS) and Dechromed splits (DCT).

Extract	Water (ml)	T(^{o}C)	Time (h)	Insoluble Residue w/w %)	Gelatin Solution (w/w %)
DCS-1	250	60-70	2	69	31
DCS-2	250	70-85	2	58	42
DCS-3	250	85-95	2	49	51
DCS-4	500	60-70	2	65	35
DCS-5	500	70-85	2	54	46
DCS-6	500	85-95	2	45	55
DCT-1	250	60-70	2	71	29
DCT-2	250	70-85	2	64	36
DCT-3	250	85-95	2	55	45
DCT-4	500	60-70	2	70	30
DCT-5	500	70-85	2	65	35
DCT-6	500	85-95	2	48	52

3.6 Amino acid analysis: The protein concentrate is analysed for crude protein and amino acid composition, the results are shown in Table 9 and compared with wheat and soya bean meal. The final product contains about 83% protein. The Table 9 is also mentioned the requirement of certain amino acid for broilers and laying hens (Evonik, 2012).

Table 9: Essential Amino Acid (% or dry substance) in different commercially feed products with extracted protein concentrate.

Amino Acids (%)	**Isolated 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	-
HydroxyProline	10.64	1.3	2.3	-	-
Glycine	11.66	0.52	2.0	0.3-1.0	-
Alanine	1.63	0.45	2.0	-	-
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

The hexavalent chromium was analysed (Method CEN/TS 144495:2003) in the final product and the levels are below detection limit. Digestibility (*in vitro* method) of the materials was found to be 70% to 75%.

4.Conclusion:

Moving towards zero waste requires that industry adopts a circular economy. In Bangladesh, substantial environmental degradation occurs in the crude disposal of tannery solid waste. 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 very 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. This paper show how a waste can be changed to a valuable product by adopting a sustainable approach. Further research would needs to be undertaken into these by-products with the aim of establishing their value for a wide range of animal feed.

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