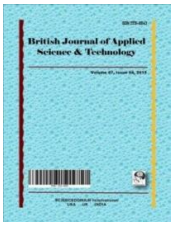


British Journal of Applied Science & Technology
8(3): 254-266, 2015, Article no.BJAST.2015.204
ISSN: 2231-0843

SCIENCEDOMAIN *international*
www.sciencedomain.org

Detanning of Chrome-ladden Collagenous Matrix for Protein Recovery from Tannery Solid Waste

Fernandus Durai Manase Manavalan[1], Muralidharan Chellappa[1] and Rose Chellan[1*]

[1]Department of Biotechnology, CSIR-Central Leather Research Institute, Adyar, Chennai, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author FDMM designed the study, wrote the protocol, managed the analyses of the study, compiled the results and performed the statistical analysis and literature searches. Author MC provided the equipments and managed some of the analysis along with author FDMM. Author RC designed the study along with the first author, wrote the manuscript and interpreted the results. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2015/17107
Editor(s):
(1) Ya-mei Gao, College of Life Science and Technology, Heilongjiang Bayi Agariculture University, Daqing, Heilongjiang, China.
Reviewers:
(1) Rajinder Tiwari, Electrical & Electronics Engineering, Amity University, Lucknow, India.
(2) Anonymous, Tunisia.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1071&id=5&aid=8726

Original Research Article

Received 26th February 2015
Accepted 23rd March 2015
Published 9th April 2015

ABSTRACT

Aim: To optimize conditions for maximum recovery of protein from wet blue as large quantities of chromium containing biological solid wastes are generated in the tanning industry which poses environmental hazard. The chromium recovery from this acidic (pH ≃ 3.5) solid waste is necessary for environmental protection and economic reasons. This study therefore focuses on the conditions that facilitate the optimum recovery of protein from the chrome-tanned biological matrix of tannery with minimum protein loss using H_2SO_4.
Methodology: The Chrome-tanned skin (wet blue) specimen before and after different concentrations of acid treatment were characterized for their mechanical property and thermal stability with a view to observe chemical and morphological characteristics changes by Fourier Transform Infra-Red spectroscopy and Scanning Electron Microscopy respectively. The modification in functional group and chemical composition of the samples is correlated with that of

**Corresponding author: E-mail: chellanrose@yahoo.co.uk;*

the tensile property and thermal decomposition pattern (by Thermo Gravimetric Analysis) and thermal energy (by Differential Scanning Colorimeter) as well.
Results: A gradual change in the mechanical properties of the wet blue was observed with respect to increase in acid concentration. As the acid concentration is increased, the loss of Cr is also increased. The total Cr was estimated in the acid treated wet blue samples and a sudden fall in the Cr content of the specimen after 3% acid treatment was observed. 35% of protein loss was observed in 3% acid treated sample and the % of protein loss increased with acid concentration. FTIR results showed that the acid doesn't aid the conversion of Cr (III) into Cr (VI).
Conclusion: The removal of chromium from chrome-tanned skin could be achieved without transforming Cr (III) into highly toxic Cr (VI), by simple acid treatment.

Keywords: Chrome shavings; wet blue; dechroming; SEM; FTIR; thermal properties.

1. INTRODUCTION

The tanning industry generates substantial quantities of chromium containing proteinous waste in the form of shavings and trimmings. Traditionally much of these wastes have been used as land fill, which in course of time pollute the soil and water through chromium leaching [1]. Extensive research work has been carried out our transformation of chrome shavings into fertilizers and recovery of protein products through hydrolysis. With such products, however, chromium hydroxide sludge was left behind which was eventually discharged on the land. Chrome shavings are also used at limited levels to produce leather board [2] has outlined several methods of detanning chrome shavings and the drawbacks there of. Out of various detanning methods discussed, the acid treatment method has been observed to be less expensive next to the oxidative detanning and decomposition using hydrogen peroxide or chlorine.

A two stage protein recovery process of [3] resulted in higher yield but with generation of increased quantity of chemical sludge. Prior to this, [4] have reported the detrimental effects of alkalinity inducing hydroxides/carbonates on the bloom strength of the protein products from chrome shavings. Therefore, in this study, an attempt has been made to use acid as detanning agent. The wet blue specimen after detanning using different concentrations of acid were characterized for physical, chemical, thermal and morphological properties. The results obtained are discussed in the direction of optimum pre-treatment condition required for the hydrolysis of chrome shavings and recovery of co-products the protein and chromium salt from chrome shavings. These two products have a variety of possible end uses: (i) chromium being a tanning chemical, could be used in tanning operation of leather making there by providing a commercial potential; (ii) the organic part of the chrome shavings being proteinous in nature, it has potential application in food/feed industry as an additive protein or as a coat in pharma products, as a support in graft polymerization [5,6] drug delivery system and wound treatment material in the field of medicine and surgery [7,8,9]. This apart, the protein recovered could also be used in microbiology as a source of nitrogen in the nutrient medium. The tannery solid waste – the chrome shavings, with availability about 6,00,000-8,00,000 tons per annum worldwide offers possibility to be used as resource material for various application. Hence the study on the standardization of optimum conditions for the increased hydrolysis in the subsequent digestion process and product yield assume significance with an objective of value realization from waste. In this study, chrome-tanned skin samples of relatively uniform thickness were used to examine detanning by acid.

2. MATERIALS AND METHODS

2.1 Materials

Specimens of chrome-tanned skin were cut into 10 X 3 cm size and used for acid treatment studies. The samples were immersed in different concentration solutions viz., 2%, 3%, 4% and 5% (V/V) of sulfuric acid for 24 h with occasional shaking. After acid treatment, the detanned samples were dried in shade and subjected to various physical, chemical, thermal, microscopical and spectroscopical analyses. The respective extracts (leachate) were also analyzed for their chemical composition.

2.2 Tensile Testing

The tensile properties of the dumbbell shaped detanned wet blue specimens were evaluated using INSTRON 3369 (fully computerized) by ISO 3376:2002/IUP6/TM43 at 20˚C with a

relative humidity of 65%. Statistical analysis by One - way ANOVA was carried out using SPSS software and considered significant when p value is lesser than 0.01.

2.3 Estimation of Total Chromium

The Cr content of the detanned wet blue skin as well as its leachate was determined spectrophotometrically [10]. In all the readings the absorbance at 372 nm was corrected against the blank. In this method the trivalent chromium in the samples was converted in to hexavalent chromium through digestion with an oxidizing mixture containing perchloric acid and sulphuric acid which was then analyzed read spectrophotometrically after making up to a known volume.

2.4 Estimation of Total Nitrogen

Acid treated wet blue sample (after dehydration) or its acid extract was digested with conc.H_2SO_4 in the presence of $CuSO_4$ and K_2SO_4 prior to distillation with 40% NaOH. The ammonia liberated by alkali digestion was collected in a boric acid solution and titrated against N/70 N HCl, according to the AOAC (1970) method [11].

2.5 FTIR Spectrum Analysis

The FTIR spectrum of detanned wet blue samples was examined for structural modification, if any in collagen fibers resulting out of detanning. Briefly the specimens were made into powder and then mechanically blended with KBr to obtain a disc which was then analyzed in a NICOLET impact 400 FTIR spectroscope, after being desiccated.

2.6 Thermogravimetric Analysis

The thermal decomposition profile of the detanned skin samples was measured in a thermogravimetric analyser (NETZSCH Instruments, Burlington, MA USA) over a temperature range of 37 – 800ºC at a heating rate of 20ºC per minute under the atmosphere of nitrogen.

2.7 Differential Scanning Calorimetric Analysis

The melting temperature and the respective enthalpies of all the acid treated wet blue samples were determined by using a differential scanning calorimeter [Q 200 – 1420, Thermal Analyser Q 250, Version 23 [12]. A 2 mg sample was heated from 37º – 300ºC at a heating rate of 20ºC/ min in an atmosphere of nitrogen. The stability of the base line was checked before each experiment.

2.8 Scanning Electron Microscopic Analysis

All the detanned samples were first washed in water and then subjected to dehydration by using acetone. Then the specimens were coated with gold using a Hitachi E 1010 ion sputter. Hitachi S 3400N Scanning Electron Microscope was used for the analysis. The micrographs for the cross section / grain surface were obtained by operating SEM at an accelerating voltage of 10 KVA with different magnification levels.

3. RESULTS AND DISCUSSION

3.1 Tensile Strength

The mechanical properties of the sulphuric acid solution treated wet blue samples are illustrated in Fig. 1. Samples treated with H_2SO_4 solution of increasing concentration viz 2,3,4, and 5% (V/V) showed decreasing trend in elastic property (Fig. 1a) and increasing trend for tensile property (Fig. 1b), except in the case of 4% concentration for which need to be explored further. Sudden drop in the elongation parameter at 2% acid treatment, compared to control may be due to the initialization of protein swelling and simultaneous weakening or break down of chromium cross links.

Depending upon the concentration of acid, the chrome laden collagen fibres undergo changes, which in turn could alter the water holding capacity, protein solubilization and chromium leaching. Thus modified micro/macro structure upon drying have the tendency of becoming hard and tough due to the loss of inter fibrillar interactions and hence exhibited increased tensile value, with increasing acid concentration. This gradual change in the mechanical properties with respect to increasing acid concentration may also be correlated with the increasing loss of Cr during incubation, which will be discussed elsewhere in the manuscript.

3.2 Total Chromium

Chromium was determined as total chromium by converting the Cr III present in the sample into Cr VI by oxidation method. The results of total chromium content of the acid treated wet blue sample and the respective leachate are presented in Table 1. The overall chromium content in the acid treated specimens is lower

than that of the untreated specimen (control). The level of Cr (III) which was estimated as Cr (VI) decreases gradually in the treated samples with respect to increase in acid concentration, from 2% to 5% solution. Though 2% acid solution did not leach out much chromium, there is a sudden fall in the Cr content of the specimen after 3% acid treatment.

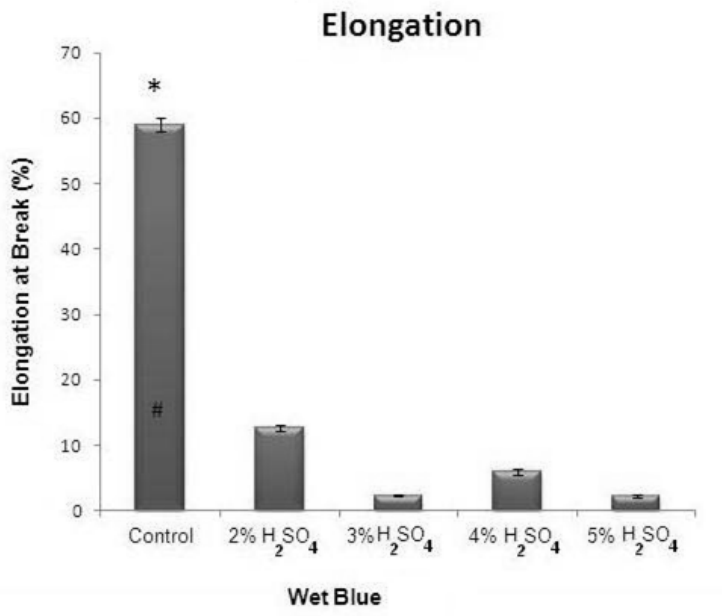


Fig. 1(a)

** denotes control is significantly difference with all the acid treated groups*
 # denotes that control is significantly difference within the acid treated groups

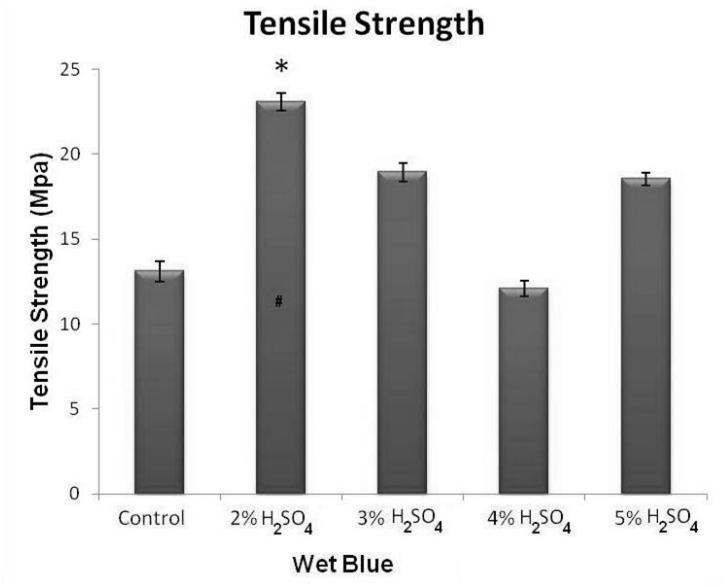


Fig. 1(b)

** denotes 2% acid treated wet blue is significantly difference with all the acid group*
 # denotes that 2% acid treated wet blue is significantly difference within the acid groups

Fig. 1. Mechanical property of H_2SO_4 treated wet blue samples (a) Elongation (b) Tensile Strength. Data expressed as mean ± SD and the results were highly significant i.e. P<0.01

3.3 Total Nitrogen Content

Loss of protein in terms of total nitrogen was observed in acid treated samples. This trend is similar to that of the chromium loss. In this study, the addition of acid to wet blue samples at varying concentrations and drastic decrease of pH resulted in the disintegration of collagen molecule and therefore its release in the acid leacheate (Table 2). Since the collagen macrostructure is stabilized by Cr cross linking, the destabilization of protein by any means would adversely affect the Cr-protein linkage. Therefore the sulphuric acid mediated destruction of protein-Cr linkage result in the release of both the organic and inorganic constituents. It is also understood from this study that the protein integrity is very much essential for holding Cr in it. At an acid concentration of 2% (v/v), the protein solubilization was observed to be only about 14% as against its untreated one, but in the case of 3% acid about 35% protein loss was seen. Though the Cr result of 3% acid treatment is encouraging in view of dechroming, as observed in mechanical properties, the process is also associated with protein leaching to an extent of 35% and causing concern.

3.4 Fourier Transform Infra-red Spectrum

Chrome-tanned collagen matrix contains large amounts of more easily oxidisable organic substances than chromium itself [13]. Hence this study focuses on the standardization of H_2SO_4 concentration for optimum de-chroming with minimal protein loss. At the same time, it would be interesting to find out the conversion of Cr III to Cr VI, if any, in the presence of different concentration of H_2SO_4. Therefore the specimens of acid treated samples were characterized by FTIR technique and the results are presented in Fig. 2(a-e). The FTIR spectrum of the wet blue samples showed absorbance bands at around 3330, 2918, 2851 cm^{-1} indicating the presence of –NH stretching coupled with hydrogen bonding of –OH, $–CH_2$ asymmetrical stretch, and –CH stretching respectively. Similar observations were also made by [14,15], in their FTIR analysis.

The characteristic band at around 1650, 1545 and 1240 cm^{-1} represent the amide–I, amide–II and amide–III respectively as reported by Jackson et al. (1995). In addition to these protein specific bands, some other bands are also present at 866 cm^{-1} and in the absorption range of 466 – 666 cm^{-1}. It has been reported that Cr III displays characteristic absorptions of chromic oxide at <800 cm^{-1}, while Cr VI displays at 850 – 950 cm^{-1} region [16,12].

Based on the results of this study, it is understood that the collagen of the skin matrix is in complexation with chromium through the tanning process, carried out by using Cr III compound, which shows a small absorption band

Table 1. The effect of H_2SO_4 concentration on detanning of wet blue samples. 1) Untreated wet blue sample (control); 2) 2% H_2SO_4 treated wet blue sample; 3) 3% H_2SO_4 treated wet blue sample; 4) 4% H_2SO_4 treated wet blue sample; 5) 5% H_2SO_4 treated wet blue sample

Sl. no	Treatment	Total chromium	
		ATH (mg/100 mg)	Leacheate (mg/100 ml)
1	Wet blue control	2.976	---
2	2% H_2SO_4	2.536	0.440
3	3% H_2SO_4	2.227	0.749
4	4% H_2SO_4	2.108	0.868
5	5% H_2SO_4	1.171	1.805

Table 2. Total nitrogen content in the acid treated wet blue (ATW) samples and their respective leachate

S. no	Treatment	Sample Wt (gm)	N_2 content in ATW (%)	Leachate N_2 (mg/10ml)	N_2 Loss (%)
1	Wet Blue (no treatment)	4.6568	15.40	------	0
2	Wet Blue+2% H_2SO_4	3.7768	15.05	1.05	2.27
3	Wet Blue+3% H_2SO_4	3.7252	14.52	1.75	5.71
4	Wet Blue+4% H_2SO_4	3.9928	14.00	2.00	9.09
5	Wet Blue+5% H_2SO_4	3.6012	13.47	2.45	12.53

- *Sample weight was 100 mg in the case of solid and 10 ml in the case of leachate.*
- *Total volume of leachate in each case was 300 ml*
- *Treatment time 24 hrs*

< 800 cm^{-1} in the IR spectrum. This low intensity peak exhibited in the spectrum of control (tanned pelt as such) as well as in the acid treated sample, clearly reveal that the H_2SO_4 does not promote the conversion of Cr III into Cr VI.

Therefore H_2SO_4 could be an option for detanning of tanned hide/skin in order to separate the organic protein and the inorganic chromium from the chrome shavings, a solid proteinous waste generated from tanning industry. However, this needs confirmation by analyzing the detan extract, as the Cr VI is highly soluble compare to Cr III.

3.5 Thermogravimetry

Fig. 3 (a-e) shows the thermal decomposition pattern of tanned skin specimens before and after acid treatment. Generally, treatment of collagenous matrix with acid solution results in swelling and in the case of chrome-tanned collagenous pelt, the sample undergoes detanning, when the acid concentration is excess. In thermogravimetry, the tanned specimen (control) showed a dramatic weight loss of 12.5% at a temperature of 125ºC, as against 9% loss for 4 and 5% acid treated samples. This shows that at the initial stage of heating (up to 125ºC), 3% acid treated sample showed high order of resistance with a water loss of only 3.5% and exhibits an increased thermal stability. This trend continues up to 280ºC with a total weight loss of 14% which may be accounted to the loss of both absorbed and bound water. Beyond this temperature, the rate of weight loss increases with increase in acid concentration up to 450ºC. Whereas at 700ºC, 2% acid treated sample showed a minimum of 62% total weight loss compared to other samples. This may be attributed to the presence of more amount of intact chromium.

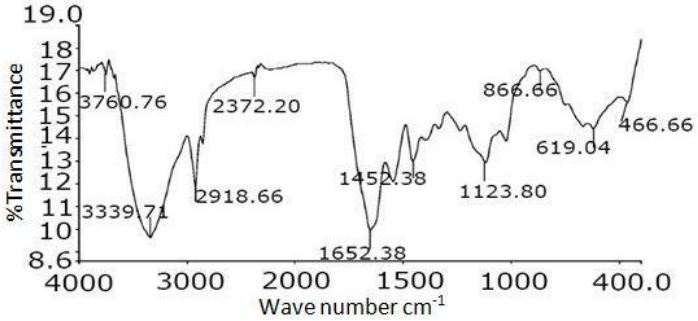
It is obvious from the weight loss data that at 3% H_2SO_4 concentration, the water structure in the tanned collagen matrix is more stable compare to that of higher concentrations. But once the water structure (both absorbed and bound) is disturbed (at about 280ºC) it becomes more labile to pyrolysis. Therefore, the water holding capacity of the specimen of different concentration of acid treatment loses water at different stage of heating. At the same time the loss of protein during detanning by acid solution is also a matter of concern. The objective of this work being the standardization of the concentration of H_2SO_4 for optimum dechroming with minimum protein loss in the liquor, it is suggested based on the TGA data that 2-3% H_2SO_4 solution could be an ideal concentration for de-chroming of chrome-tanned proteinous wastes and for subsequent gelatin extraction.

3.6 Differential Scanning Calorimeter

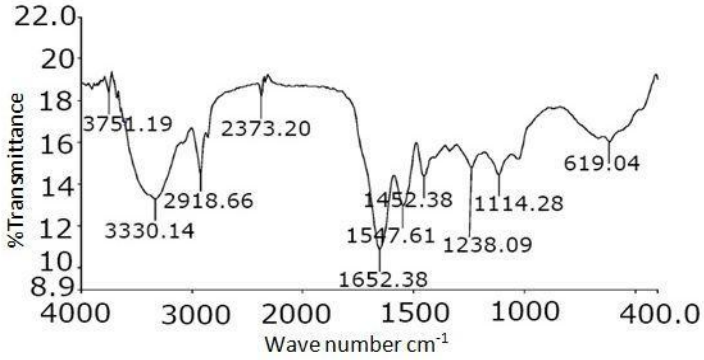
Increase in H_2SO_4 concentration favours increased denaturation, followed by protein loss in the wet blue samples. This may be further explained as the breakdown of H–bond involved in the intrafibrillar and inter-molecular linkages as well as cleavage of protein chain with respect to increasing acid concentration. The thermal characteristics of chrome-tanned skin specimens (wet blue) derived from DSC data (Figs. 4 a-e). The midpoint of first peak of the DSC curve is considered as shrinkage temp (Ts) during which the water is evaporated. The second peak which occurs later is considered as the destruction of microstructure of tanned collagen. In the first event, the Ts of collagen fibril – the macrostructure is destabilized as the inter fibrillar H – bond interactions are broken down. Subsequent heating resulted in the destruction of micro units–the molecules which is indicated by the second peak. Such changes have been reported to be responsible for protein unfolding [17].

The control specimen which was exempted from H_2SO_4 treatment displayed a first endothermic peak at 73ºC in a thermal denaturation process between 35 – 150ºC, with a peak width of 121ºC followed by a minor peak at 120ºC (T_d) whereas the 2% acid treated samples showed a decreased T_s value compare to the control. But the T_d value of the later samples was in the higher side as the time taken for denaturation was more than that required for control sample. However the 4% acid treatment exceptionally showed a T_s value higher than that of control suggesting some kind of stability contributed by unknown factors, in spite of the leaching of Cr as well as protein from the sample. The residual sample showing higher value of Ts, must have been involved in high order of cross linking with chromium, involving some unusual chemical interactions in addition to the well-established coordination bond formation between chromium and protein carboxylic group [18].

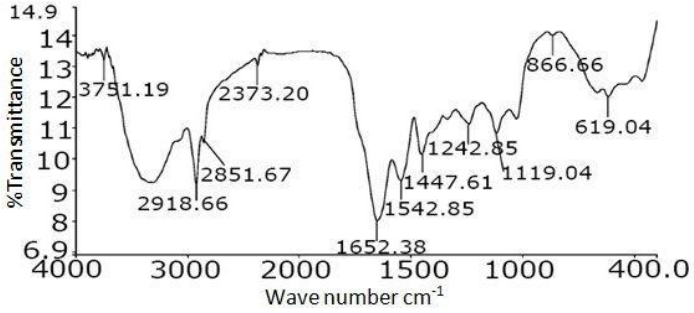
(a)


%Transmittance
Wave number cm-1
3760.76
2372.20
2918.66
3339.71
1652.38
1452.38
1123.80
866.66
619.04
466.66

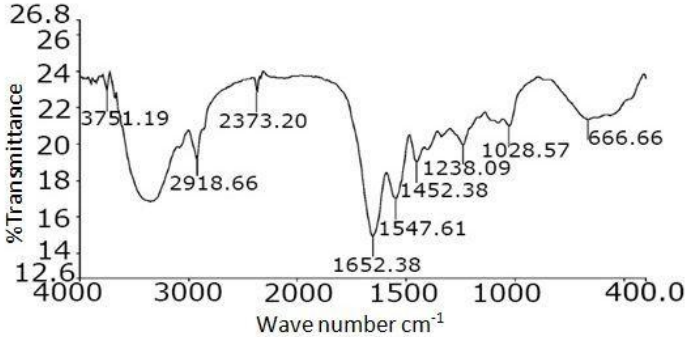
(b)


%Transmittance
Wave number cm-1
3751.19
2373.20
2918.66
3330.14
1652.38
1547.61
1452.38
1238.09
1114.28
619.04

(c)


%Transmittance
Wave number cm-1
3751.19
2373.20
2851.67
2918.66
1652.38
1542.85
1447.61
1242.85
1119.04
866.66
619.04

(d)


%Transmittance
Wave number cm-1
3751.19
2373.20
2918.66
1652.38
1547.61
1452.38
1238.09
1028.57
666.66

(e)

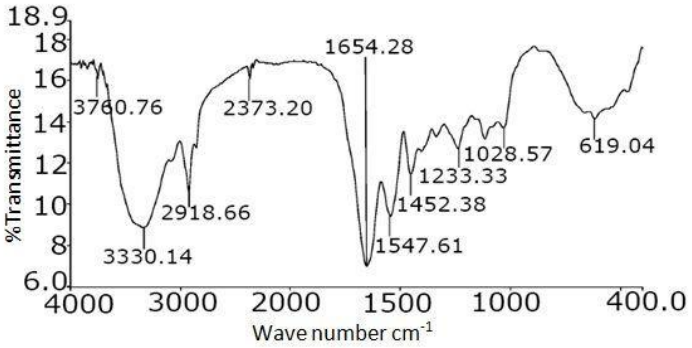
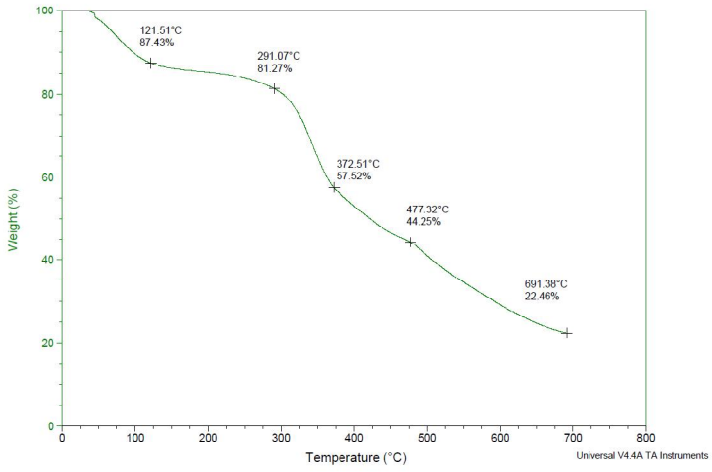
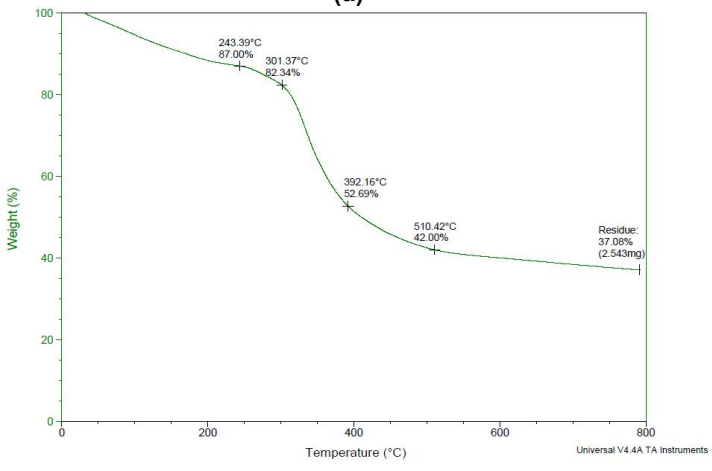


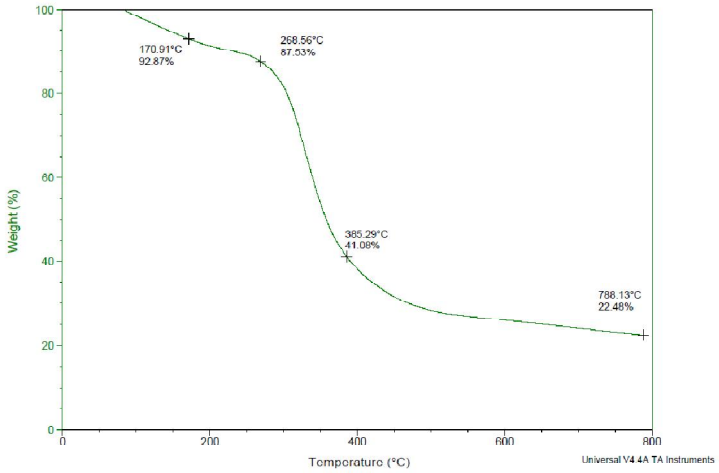
Fig. 2 a) FT-IR spectra of the untreated wet blue hide (control) before chromium extraction b) 2% acid treated wet blue c) 3% acid treated wet blue d) 4% Acid Treated wet blue e) 5% acid treated wet blue



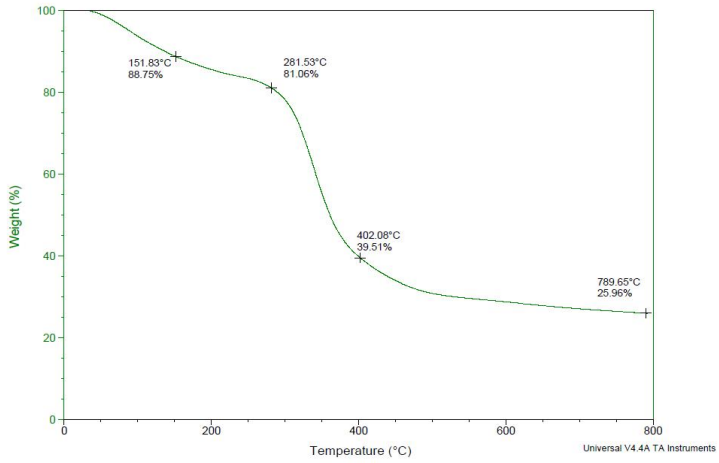
(a)



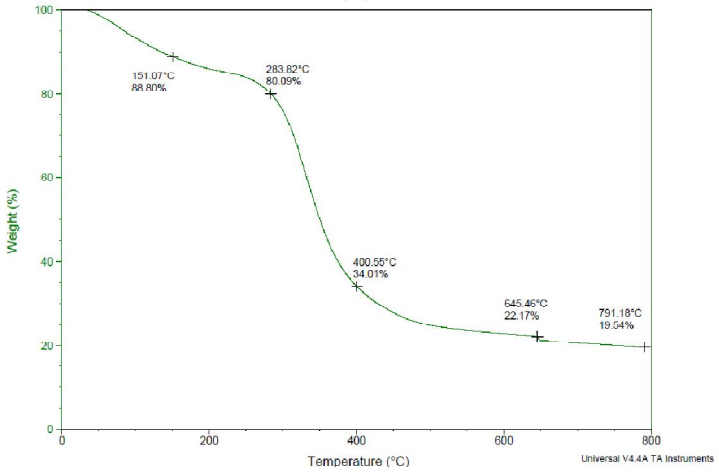
(b)



(c)



(d)



(e)

Fig. 3. TGA Analysis of H_2SO_4 treated wet blue hide. TGA curve of a untreated wet blue sample heated from room temperature up to 700ºC. a) Untreated (Control) b) 2% H_2SO_4 treated c) 3% H_2SO_4 treated d) 4% H_2SO_4 treated e) 5% H_2SO_4 treated

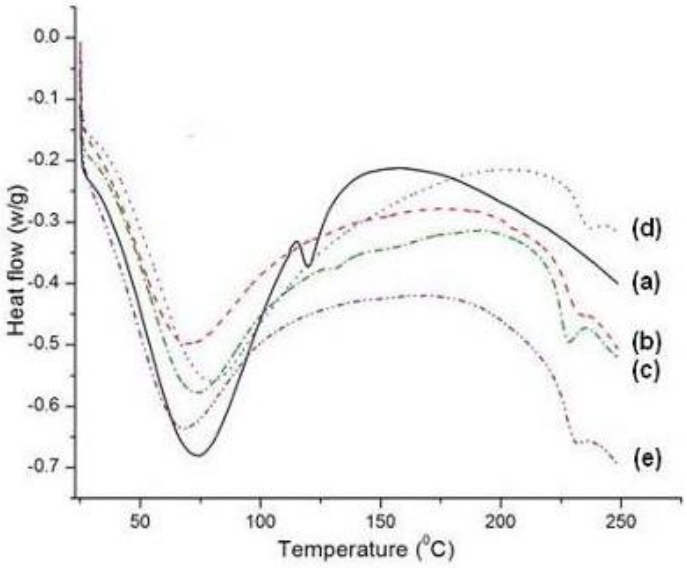


Fig. 4. DSC Analysis of H_2SO_4 treated wet blue sample. a). Untreated – wet blue (control), b). 2% H_2SO_4 treated wet blue, c). 3% H_2SO_4 treated wet blue, d). 4% H_2SO_4 treated wet blue, e). 5% H_2SO_4 treated wet blue

The loss of Cr and protein in the acid treated wet blue and appearance of these components in the acid leacheate was depending upon the concentration of acid used. This confirms that the addition of H_2SO_4 facilitates the destabilization of collagen–Cr interaction and promotes the solubilization of the matrix to some extent at the given ambient temperature. This process of solubilization must have been preceded by swelling, through water uptake. The increased water holding capacity could be one of the factors that contribute to stabilization against heat denaturation. This property was also observed by relatively decreased structural destabilization which is revealed by a broader endotherm as in the case of 4% acid treated sample.

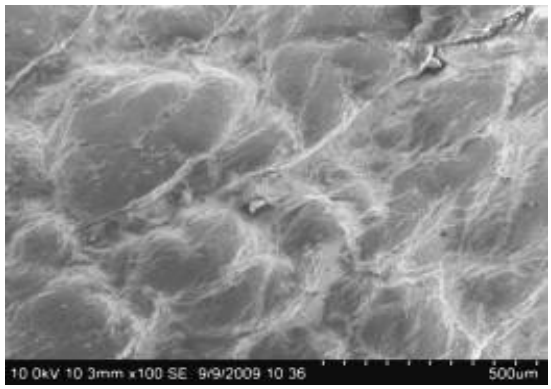
3.7 Scanning Electron Microscopic Analysis

The scanning electron microscopic examination of the specimens clearly exhibits the differences in the surface morphology between the samples treated with different concentration of H_2SO_4 (Fig. 5a). For the convenience of comparison of the data, a 100X magnification of all the micrographs are presented. As could be seen from the results, the increasing concentration of H_2SO_4 treatment, imparted a specific change in the matrix morphology.

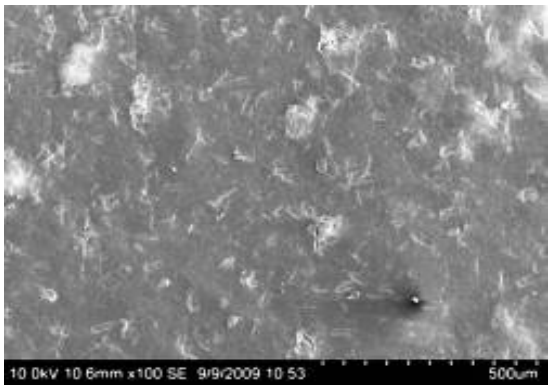
The surface layers of all the treatments were continuous and intact however, there was a loss in the smoothness of the grain surface. For instance, the 2% acid treated sample showed a smooth grain surface with salt deposition at the hair holes (Fig. 5b). This smoothness may be attributed to the swelling of tanned matrix where the hair pores are also closed. At 3% acid concentration, the sample started developing wavy folding as a result of onset of dehydration or/ and deproteinization from inside layers of the tanned matrix (Fig. 5c). Whereas, the 4% acid treatment more clearly indicate protein dissolution underneath the grain surface, causing a high order of wrinkles (Fig. 5d), probably by breaking the acid labile chemical interactions and leaving behind the acid stable fibrillar material at this concentration. The 5% acid treatment showed a maximum wrinkles on the surface, in proportion to the dissolution of the grain layer with the exposure of inner fibrillar bundles of tanned matrix (Fig. 5e).

These observations are also supported by the protein content of the acid extract of the individual samples. Similar to the effect of acid concentrations, the temperature effect on the tanned skin has also been studied by [19]. They have reported the loss of fibrous character of the material with increase of temperature. Therefore, it is obvious that both temperature and the abnormal acidity affect the structural organization and integrity of the tanned matrix. This phenomenon could be advantageously adapted for the extraction of proteinous product, if

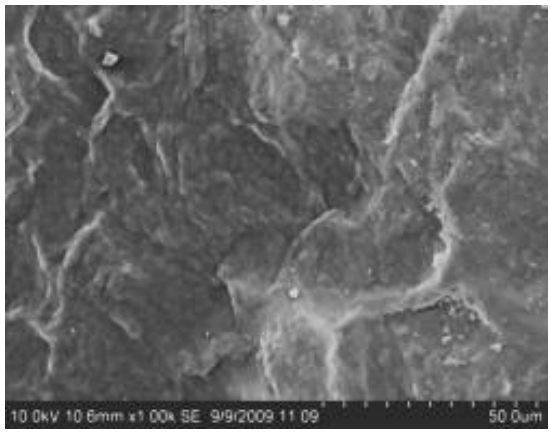
intended, from the scrap of chrome-tanned proteinous waste materials such as chrome shavings, which are generated in large quantity in tannery and pose disposal problem.



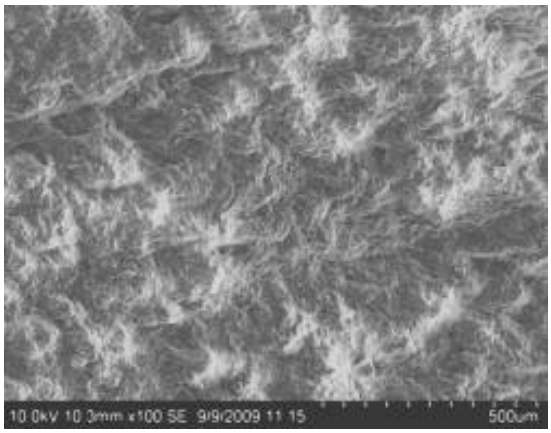
(a)



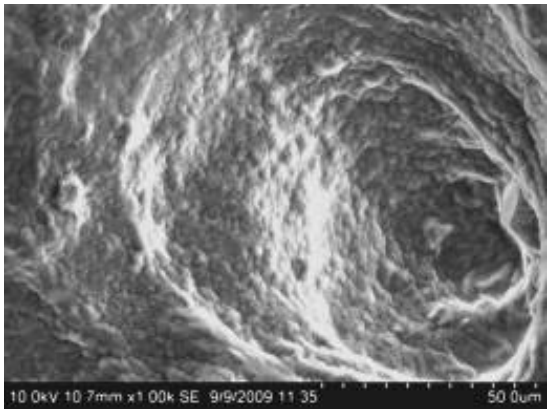
(b)



(c)



(d)



(e)

Fig. 5. Scanning electron microscope analysis of a) untreated wet blue (control) b) 2% H_2SO_4 treated wet blue. c) 3% H_2SO_4 treated wet blue d) 4% H_2SO_4 treated wet blue. e) 5% H_2SO_4 treated wet blue

4. CONCLUSION

In conclusion, H_2SO_4 treatment of chromium tanned collagen matrix leads to weakening or breakdown of Cr-protein linkage, resulting in dechroming and deproteinisation depending upon the concentration of acid used. This could be controlled by decreasing the incubation time as well. Thermal energy is another phenomenon which can also be used wisely for enhanced cleavage of protein–chromium linkage. This pH and temperature mediated destabilization of tanned matrix can be successfully exploited in the utilization of chrome shavings for the recovery of value added gelatin / protein product and chromium. More importantly, the acid detanning method of this study does not promote the toxic Cr (VI) formation; hence the Cr (III) recovered in this process could be safely reused. The protein recovered is a valuable bio-material that could find hide application, when utilized in an appropriate manner.

This study, therefore, provide a lead that facilitate the removal of chromium from chrome-tanned skin by simple treatment without transforming Cr III and Cr VI. Chrome shavings – the scrap generated by subjecting the chrome-tanned skin to levelling by mechanical operation, are posing pollution threat due to Chromium content in it. The quantum of chrome shavings generated being very huge, it is imperative to provide a vast open land for the disposal this waste. Again, this waste when used as land fill would release the Chromium III or its toxic species into the soil and then to ground water. Hence this study has high relevance the environmental pollution.

ACKNOWLEDGEMENTS

M. Fernandus Durai gratefully acknowledges the Management of Sacred Heart College for granting permission to pursue the Ph.D.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Brown EM, Thomson CJ, Taylor MM. Molecular size and conformation of protein recovered from chrome shavings. JALCA. 1994;89:215–20.
2. Heideman E. Disposal and Recycling of chrome-tanned material. JALCA. 1991;86;331-33.
3. Brown EM, Taylor M, Marmer WM. Production and potential uses of co-products from solid tannery waste. JALCA. 1996;91:270-76.
4. Taylor MM, Diefenderf EJ, Marmer WN, Brown EM. Effect of various alkalinity inducing agents on chemical and physical properties of protein products isolated from chromium containing leather waste. JALCA. 1994;89:221-28.
5. Harada O, Sugita M, Yamamoto T, Sangen O. Structure of the solubilised collagen – graft – poly (methyl methacrate) films obtained by initiator free photo graft polymerization. Kobunshi Ronbunshu. 1994;51:710-16.
6. George A, Radhakrishnan G. Grafting of ethyl acrylate onto gelatine in water isopropanol medium. Macromol. Chem. Phys. 1984;128:173-79.
7. Chatterji PM. Gelatinwithhydrophilic / hydrophobicgraft and glutaraldehyde cross links. J Appl Polym Sci. 1989;37:2203-12.
8. Jeyanthi R, Panduranga Rao K. *In vivo* biocompatibility of collagen-poly (hydroxyethyl methacrylate) hydrogels. Biomaterials. 1990;11:283-43.
9. Santin M, Huang SJ, Iannace S, Ambrosia L, Nicolaris L, Peluso S. Synthesis and characterization of a new interpenetrated poly (2- hydtroxyethylmethacrylate) - gelatine composite polymer. Biomaterials. 1996;17:1459-67.
10. Monteiro MIC, Fraga ICS, Yallouz AV, de Oliveira NMM, Ribeiro SH. Determination of total chromium traces in tannery effluents by electrothermal atomic absorption spectrometry, flame atomic absorption spectrometry and UV – visible spectophotometric methods. Talanta. 2002;58:629-33.
11. Horwitz Ed, W. Association of Official Analytical Chemists. Official Methods of Analysis. 11th ed. Washington, U.S.A; 1970.
12. Hoang ME, Hughes AE, Mathews JF, Prattz KC. Surface chemistry of supported chromium oxide on lanthanum carbonate. J Catal. 1997;171:313-19.
13. Erdem M. Chromium recovery from chrome shaving generated in tanning process. J Hazard Mater. 2006;129:143-46.
14. Onyancha D, Mavura W, Ngila C, Ongoma P, Chacha J. Studies of chromium removal from tannery wastewaters by algae biosorbents, *Spirogyra condensata* and *Rhizoclonium*

hieroglyphicum. J Hazard Mater. 2008; 158:605-14.
15. Palpandi C. Extraction of chitin and chitosan from shell and operculum of mangrove gastropod Nertia (Postia) crepidularia Lamarck. Int J Med Med Sci. 2009;1:198-05.
16. Abu – Zied BM. Structural and catalytic studies of silver/ chromia catalyst. Appl Catal A: Gen. 2000;98:139-53.
17. Del Angel SS. Study of denaturation of corn protein during storage using differential scanning calorimetry. J Food Chem. 2003;83:531-40.
18. Nakamoto K. Infrared and Raman spectra of Inorganic and Coordination Compound, John Wiley and Sons, Inc; 1978.
19. Tahiri S. Thermal behavior of chrome shavings and of sludge recovered after digestion of tanned solid wastes with calcium hydroxide. Waste Manage. 2007;27:89-95.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1071&id=5&aid=8726