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Recovery of Collagen Hydrolysate from Chrome Leather Shaving Tannery Waste through Two-Step Hydrolysis using Magnesium Oxide and Bating Enzyme

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Abstract

Chrome-tanned solid waste emanating from leather industry is usually disposed of to the environment through landfill which not only pollutes the environment but also wastes the protein resource contained in it. Protein recovery for re-use in secondary industrial processes presents the best strategy for its re-utilisation. Dechroming by hydrolysis is the most practiced method of protein and chromium recovery from tanned solid waste. The alkali-enzyme two step hydrolysis methods are commonly utilised for improved protein recovery efficiency. However, enzyme cost and temperature dependence of the heat stable alkali enzyme has made the process economics difficult and therefore unattractive. The objective of the present study was to explore a relatively inexpensive method of recovering collagen hydrolysate through a two-step hydrolysis incorporating conventional bating enzyme. The method of treatment involved a first-step denaturation and degradation with alkali followed by inoculation with bating enzyme. The ash content, total kjeldahl nitrogen, dry matter and chromium content of the collagen hydrolysates obtained are reported. Protein recovery at 58.20% and 50.76% efficiency were obtained for the separate alkali and enzyme hydrolysis respectively. A combined protein recovery rate of 79.45% efficiency was obtained for the two-step process. The results of this study indicate that hydrolysis dechroming employing the use of conventional bating enzyme could offer a low-cost alternative for the effective treatment and reuse of chrome-tanned shaving solid waste.

INTRODUCTION

The leather industry makes use of raw hides and skins which are by products of meat industry. The hides and skins are composed of protein collagen which is the basis for chemical modification by the tanner to produce leather.[1] Currently, over 90% of the world leather production involves the use of chromium salt to convert the hides and skins into non-putrescible material called wet-blue.[2]

It has been reported that processing one ton of raw salted hide leads to a 20% conversion to leather while 60% is channelled out as both tanned and un-tanned solid waste.[1,2] During the mechanical process of shaving, approximately, 30% of the leather substance is given out as shaving waste in the form of scraps which contain majorly protein waste and about 3-5% chromium content.[3,4] Based on recent unpublished research work by KIRDI (Kenya Industrial Research and Development Institute), it was revealed that tanneries produce an average of 13,000 tons of chrome shavings weekly in Kenya.[5] Globally, an annual production of about 0.8 million tons of leather shavings has been reported.[6] The disposal of these shavings is done mostly by on site open burning and land filling.[5,7]

Leather shavings are seldom utilised because of their shapes and cross link features.[4] The most common way of managing the leather shavings is disposing them into landfill.[8] Land filling is expensive and environmentally inappropriate way for handling tannery solid waste.[3] The other major concern that relates to disposal of chromium containing waste on land is the possible oxidation of trivalent chromium to the more harmful hexavalent form.[4] Hexavalent chromium is not only carcinogenic and mutagenic to living organisms but also leads to liver damage, pulmonary congestion and causes irritation resulting in ulcer formation.[10]

The ever increasingly stringent restrictions concerning the disposal of chromium containing solid waste by tanneries have been the motivation driving research aimed at its re-utilisation.[6] Among the strategies involved is the creation of new avenues for effective utilisation to create wealth from waste. Chrome shavings are now considered a potential source for marketable protein.[10] However, the presence of the chromium in this kind of waste has made the endeavour more challenging. Moreover, the increase in dumping costs, the difficulty of finding new land fill sites and the rise in the protein price have increased focus in the re-utilisation of chrome shavings.[9]

Dechroming by hydrolysis is the most practiced method of recovery of collagen and chromium from tanned solid waste. It is based on dissociation of the functionalised groups in the chrome shavings.[3] The method of treatment in hydrolysis can be acidic, alkaline or enzymatic. Protein hydrolysates obtained in the hydrolysis contain valuable peptides and amino

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acids resource that has been value added to make fertilisers, bio-degradable polymers and additives for both leather and cosmetic industries.[11]

The limitations in protein recovery efficiencies have led to combination hydrolysis, in which case a combination of two of the methods above is used.[3] Commonly referred to as two-step process, alkali followed by enzyme hydrolysis has been the most commonly used hydrolysis dechroming method.[12-16] Alkalis such as sodium hydroxide, calcium oxide, lime and magnesium oxide have been employed in the first step. The remaining residue is subjected to enzyme hydrolysis to degrade the protein and increase deproteination efficiencies. However, the high cost and high temperature dependence of heat stable alkali proteases has made the process uneconomical.

Bating enzyme is an industrial preparation of a mixture of proteases. Conventionally, bating enzyme is employed in leather manufacture to dissolve scud and remove inter-fibrillary proteins for the opening up of fibre structure for subsequent tanning operation. It's readily available and its cost is friendly. Additionally, it is packed with a diluent – ammonium salt, which buffers the enzyme at pH8.3-8.5 for optimal activity. The temperature for optimal activity is 33-37°C which is favourable for classical tannery operations. Enzymes are very specific in their mode of action and collagen has a highly defined structure. According to Covington, its breakdown can only be achieved through specific collagenases.[1] To induce collagen breakdown using proteases, a pretreatment with alkali is mandatory. This pre-treatment increases the vulnerability of collagen to proteolytic attack. This study looked at the possibility of exploiting the use of conventional bating enzyme in the two-step hydrolysis of chrome shavings.

EXPERIMENTAL PROCEDURES

Materials

The chrome shavings tannery waste employed in this research were acquired from KIRDI leather processing pilot plant and stored at room temperature. Analytical grade magnesium oxide and sodium carbonate were bought from Brackett Supplies (K) Limited while Microbates (1600 LVU) bating enzyme was acquired from Priyann (K) Enterprises Limited.

Method

Characteristics of chrome shavings

The collected chrome shavings were analysed for fat, ash, protein as total kjeldahl nitrogen (TKN), chromium, moisture and pH according to the protocols described in the official methods SLC 4, 6, 7, 8, 113 and 120 of 1996 respectively.

Alkaline hydrolysis of chrome shavings

A series of 25g of chrome shavings were weighed into conical flasks and subjected to alkaline hydrolysis in a 600% w/w float water with 6% w/w magnesium oxide at 70°C for 48 hours in an incubated shaker model IST-4075. 1.0% w/w sodium carbonate was gradually added at intervals to raise the pH to an optimum of 9-10 for the alkali hydrolysis. The sample was centrifuged and filtered through Whatman filter paper No 1. The filtrate and chrome residue was stored at 4°C. The filtrate was analysed for ash, protein, chromium and dry matter.

Optimisation of enzyme assay parameters

Optimisation of enzyme concentration

The chrome residue from the alkaline treatment was warmed to room temperature. Its pH was adjusted to an optimal range of 8.3-8.5 using a few drops of 0.5N sulphuric acid. Bate enzyme hydrolysis experiments were carried out with varying enzyme concentrations. A range of 0.25-1.0% w/w enzyme concentration was chosen. Protein in the resulting hydrolysate was measured as TKN and studied as a function of enzyme concentration.

Effect of time on the enzymatic degradation of chrome shavings

To determine the effective time for hydrolysis of chrome shavings, bate enzyme concentration of 1.0% w/w was studied for 36 hours with a 6 hour time interval. The chrome residue was warmed and the pH adjusted to the optimal range prior to inoculation of the bate enzyme. Protein in the resulting hydrolysate was measured as TKN and studied as a function of time.

Bate enzyme hydrolysis of chrome shavings

The chrome sludge from the first step was warmed to room temperature, 200% w/w float and 0.1% non-ionic surfactant was added and shaken at 35-37°C for one and a half hours. The pH was adjusted to the optimal range (8.3-8.5) with few drops of 0.5N sulphuric acid. 1.0% w/w of the bate enzyme was added and the samples shaken further for 30 hours at 35°C. The solution was centrifuged and stored at 4°C. The hydrolysates was analysed for ash, protein, chromium and dry matter.

RESULTS AND DISCUSSION

Characteristics of chrome shavings

Table I summarises the results of the proximate analysis of the chrome shavings.

TABLE I
Chemical characteristics of chrome shavings

	[a] Mean ± SD
% Fat	1.32 ± 0.04
% Inorganic ash	13.29 ± 0.62
% TKN	16.30 ± 0.14
% Chromium	3.04 ± 0.06
% Moisture	19.62 ± 0.41
pH (10% Aqueous solution)	3.45 ± 0.05

[a] Triplicate measurements expressed on a dried in basis

The values are typical of chrome shavings and consistent with other determinations from the literature.[16-18] Chrome shavings vary in chemical composition based on the chemical offer, variation in the processing recipe, storage time and processing technique employed in the manufacture of the leather in the tannery.

Alkaline hydrolysis of chrome shavings

Chrome shavings are chemically depicted as collagen-chromium complex. Hydrolysis of this waste involves the breakdown of bonds responsible for its stability.[19] Two such types exist: native cross links responsible for collagen stability and insolubility as well as the collagen-chromium bond.[1] The latter is as a result of the covalent linkage between the complex chromium ion and the ionized carboxyl groups of aspartic and glutamic acid on collagen.[1,20]

The chrome shavings were firstly subjected to the action of alkali for denaturation and degrading the protein fraction. The optimal conditions needed for this work were temperature of 68-72°C and pH of 9-10. The temperature provided requisite energy for the disruption of the bonds in collagen-chromium complex. The alkaline condition was achieved by use of magnesium oxide and sodium carbonate. The percent offer of the alkalinising agent will depend on prevailing pH of the chrome shavings and the degree of hydrolysis sought. Lower pH values may necessitate higher quantities to reach pH high enough for the hydrolysis of the sample.[19] The collagen was broken down to large molecular weight peptides into the aqueous solution while the chromium was converted to an insoluble state at the prevailing alkaline conditions.[12,17,21] The degradation was achieved by the nucleophilic attack of the hydroxyl anion on covalent and non-covalent cross links in the chrome shavings. The chemical characteristics of the hydrolysate resulting from this treatment are presented in Table II.

TABLE II
Composition of hydrolysate from alkaline treatment

	[a] Mean ± SD
% Inorganic ash	14.15 ± 0.20
% TKN	14.55 ± 0.54
Chromium (ppm)	2.89 ± 0.05
% Dry matter	16.30 ± 0.02

[a] Triplicate measurements expressed on a dried in basis

The hydrolysate had 14.15 % ash, 14.55% total Kjeldahl nitrogen, 16.30% dry matter and 2.89mg/L chromium. Other than the chromium content, the analyses are in good agreement with literature values.[14,15] The chromium in the hydrolysate is due to solubilisation as a result of pH adjustments in the hydrolysis stage. It could also be, according to other studies, water-soluble chromic compounds and surface unreacted chromium passing together with the collagen hydrolysate into the aqueous solution.[22,23] The quantity of ash consist of solubilised chromium, traces of Mg^{2+} ions from hydrolysis and mineral salts anchored into the leather during the wet operations of leather processing.[13,16] The dry matter content relates to the quantities of peptides passed into the aqueous solution as collagen hydrolysates whose concentration is expressed as % TKN. The hydrolysis yield realised with MgO hydrolysis was 58.20%, which is lower than previously reported. This is primarily due variations in temperature and percent offer of alkali employed in the study.[24]

The data above, make it evident that protein extraction was not complete. Mu *et al.* deduced three possible explanations for the incomplete hydrolysis in alkaline medium namely; resistance of trivalent chromium complexes to alkali oxidation, formation of covalent cross links between alkaline amino acids and hydroxyl amino acids that can only be broken down in acidic medium and presence of a structure inaccessible to water due to hydrophobic interactions between non-polar and alkaline amino acids which become hydrophobic.[24]

Optimisation of enzyme assay parameters

Effect of enzyme concentration on enzymatic degradation of chrome shavings

At low enzyme concentration, as shown in Fig. 1, the degradation of chrome shavings was slow. This is indicative of slow diffusion and penetration of the bate enzyme through into the inner layers of the substrate. Maximum degradation was observed at 0.75% w/w bate concentration which is preceded by a linear increase in the rate.

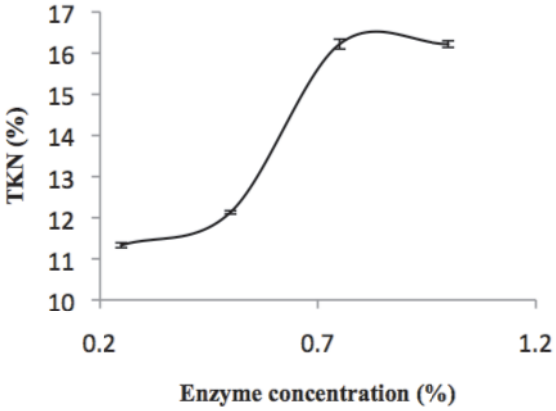

Figure 1. Effect of enzyme concentration on hydrolysis.

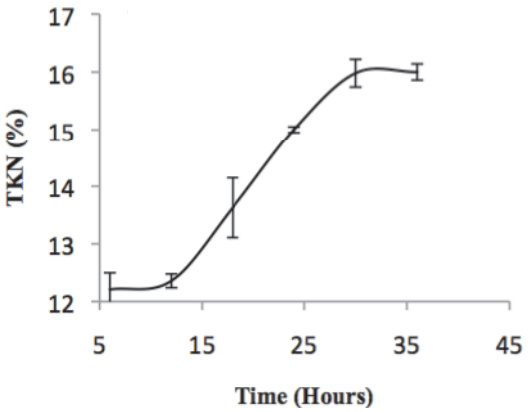

Figure 2. Effect of time on hydrolysis.

Effect of time on the enzymatic degradation of chrome shavings

The bate enzyme degradation of chrome shavings was slow for the first 12 hours of incubation, but increased linearly to 30 hours, where maximum yield was obtained. The slow degradation is indicative of low accessibility of the substrate to the enzyme. Figure 2 is a graphical representation of the effect of time on hydrolysis of chrome shavings.

Bate enzyme hydrolysis of chrome shavings

In this study, the denatured chrome shavings were subjected to a second step hydrolysis employing bate enzyme. The extent of hydrolysis for any enzyme catalyzed reaction is dependent on the process time allocated, enzyme concentration, pH and temperature. The bate enzyme conditions for optimal activity was replicated for the hydrolysis of chrome shavings. Under these conditions: temperature of 33-37°C and pH of 8.3-8.5, substantial hydrolysis of the protein remaining in the cake after alkaline hydrolysis is triggered. The bate enzyme breaks down the molecular bonds between the individual collagen strands and peptides into the aqueous solution. The pH was maintained at optimum by the ammonium salts already present in the bate enzyme as the diluent. The aqueous fraction containing the solubilised protein hydrolysate was separated from the insoluble chromium residue by centrifugation. A light yellowish hydrolysate on visual observation was obtained.

The characteristics of the collagen hydrolysate obtained after bate enzyme hydrolysis are as listed in Table III.

TABLE III
Composition of hydrolysate from bate enzyme treatment

	[a] Mean ± SD
% Inorganic ash	1.35 ± 0.05
% TKN	12.69 ± 0.44
Chromium (ppm)	1.28 ± 0.21
% Dry matter	7.73 ± 0.03

[a] Triplicate measurements expressed on a dried in basis

The dry matter content was 7.73% consisting of 1.35% ash, 12.69% TKN and 1.28 mg/L chromium. Previous studies have employed the use of heat stable alkaline proteases, commercially available as Alcalase enzyme. Reported values in the reviewed literature give mean TKN levels in the hydrolysate range of 15-19%,[13,16,23] which is higher than reported in our study. This could be attributed to the enzyme purity and specificity. The aforementioned factors are also responsible for the longer hydrolysis time as well as the high offer of the bate enzyme, 1% against a maximum of 0.1% used in the case of Alcalase, in the hydrolysis. Commercial bate powders are not pure enzyme. They are packed as formulations of a mixture of proteases with very low enzyme concentrations. Measurement of TKN in the hydrolysate is an important parameter in assessing the protein extraction yield. Protein extraction yield of 50.76% was realised from the bate hydrolysis and is in good agreement with the expected range of 50-60% for enzyme hydrolysis.[9,23] Combined hydrolysis involving the use of MgO and bating enzyme therefore realised 79.45% protein recovery which is shy of the expected 80% yield.[3]

The quantity of ash is consistent with other literature in which values less than 2% have been reported for enzyme catalysed hydrolysis.[16,22] In our study, the ash content consists of the trace residues of and Cr^{3+} transferred from the previous pretreatment with alkali as well as inorganic residues present in the matrix. Little effect is expected on chromium because of the pH of operation and the stabilising effect of ammonium salts. At an alkaline pH the chromium remains insoluble in the solution. Thus, poisoning of the enzyme is averted while at the same time recovery of the chromium as $Cr(OH)_3$ is made possible through filtration.[21] The presence of the divalent cation of magnesium serve as a cofactor for the bate enzyme activity.[12,25,26] Enzyme hydrolysis of chrome shavings gives low molecular weight degradative products compared to alkaline hydrolysis.[21] This is reflected through the reduction in the dry matter content of the collagen hydrolysate.

Chrome residue

The filter cake remaining after bate enzyme hydrolytic degradation was subjected to similar quality parameters as the collagen hydrolysate. The results are presented in Table IV.

TABLE IV
Characteristics of the chrome residue

	[a] Mean ± SD
% Inorganic ash	44.71 ± 0.66
% TKN	8.83 ± 0.09
% Chromium	16.84 ± 0.46
% Dry matter	15.14 ± 0.11

[a] Triplicate measurements expressed on a dried in basis

The filter cake had 15.14% dry matter containing 44.71% ash, which includes 16.84% chromium and other inorganic residues. The chromium can be re-processed for use in leather production. Other uses of this chrome cake include addition to cement and mortar.[16] The high moisture content of the filter cake is as a result of filtration technique employed, and is the direct consequence of the low dry matter reported in the filter cake.[16] The dry matter also exhibited 8.83% TKN. Lower values may be attained if the residue is subjected to a series of washing to deprive the chrome cake of entrained residues of protein.[15] This data shows that, there could be recalcitrant bonds in the collagen-chromium complex that are difficult to break with the action of bating enzyme. This also reflects the efficacy of the bating enzyme to completely degrade the protein fraction in the alkaline denatured chrome shavings. Comparatively, higher hydrolysing power of enzyme could increase the deproteination rates. As noted from a similar study, deproteination using enzymes Esperase and trypsin achieved greater efficiencies with lower dry matter content in the resulting sludge.[27]

CONCLUSION

The results of this study show that conventional bate enzyme powder can be successfully employed in the two-step hydrolysis of chrome shavings. This could be important in the reduction and management of tanned solid waste in the tannery as well as reduction in cost for the hydrolysis step. The protein fraction has potential for use as fertilisers, leather fillers and additive for use in the film and cosmetic industries. The chrome cake can be reprocessed for use in tanning or as an additive to mortar and cement. This is a step forward in improving the sustainability and clean processing aspects of leather manufacture. Hydrolysis dechroming with bating enzyme offers ease of operation and low cost alternative for the effective treatment of tanned solid waste. However, longer hydrolysis time and lower yields of protein in the hydrolysate are the limiting factors.

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