

Abstract

Enzymic processing of chromium-containing leather waste is an alternative to dumping in landfills. In prior research, we demonstrated that chrome shavings as well as chrome splits and trimmings may be hydrolyzed enzymically. In our new two step process chrome shavings were treated under alkaline conditions to obtain a high-value, high molecular weight gelable protein fraction, then the sludge that remained was treated enzymically to isolate a hydrolyzed protein fraction with 10,000-20,000 molecular weight distribution, and a recyclable chromium cake. Because there is concern that the character and quantity of the ash in the protein products would have an adverse effect on marketing of these products, we investigated the use of different proportions of alkaline agents, i.e., magnesium oxide with varying amounts of sodium hydroxide, sodium carbonate, potassium hydroxide or potassium carbonate. Increasing proportions of magnesium oxide resulted in lower ash content. Further reduction in ash content was achieved by use of ion-exchange resins. Finally, since it is imperative that the chrome cake that is being recovered can be recycled, a chemical treatment was developed in which the chrome cake is dissolved in acid and the extraneous materials are eliminated by precipitation with base.

Introduction

Historically, shavings, trimmings and splits from the chrome tanning of hides and skins have been disposed of in landfills. Recently, tighter local restrictions have caused the tanning industry to seek out alternatives to dumping. Utilization of these waste products has been described extensively in the literature. For example, the shavings have been utilized in preparation of building materials and composites with polymers have been molded into sheets. Acidic and basic hydrolysis have yielded animal feed and fertilizer. The main disadvantage of such hydrolysis is the low molecular weight value and the small economic return of the recovered protein products.

Enzymic hydrolysis, under acidic and basic conditions, has also been documented¹⁻⁸. At this

laboratory we have demonstrated that the chrome shavings as well as the splits and trimmings may be enzymically hydrolyzed to obtain an almost chrome-free protein product and a chromium cake that may be chemically treated and recycled⁹⁻¹⁷. In our original one-step process, we were able to dissolve the chrome waste with less than one percent enzyme. However, even at this low enzyme concentration, the isolated protein products had a low molecular weight distribution and thus the economic return was negligible.

In a new two-step process¹⁸, we are recovering a high molecular weight gelable protein in the first step and in the second step we have reduced the amount of enzyme needed to extract the protein remaining in the chrome sludge (Fig. 1). The process has thus become economical not only because of the high quality protein products that are being recovered but also because a minimum of enzyme is needed; the enzyme is the most expensive reagent used in the reaction.

In this paper, we will show the effects of various alkali treatments on the total solids and total ash contents of the final products. We will also show how the ash content of the original chromium substrates contributes to the ash in the recovered products. We will also suggest a method to lower the ash content of the products if the concentration is too high. It is imperative that the chromium that we are recovering can be recycled and we will propose a method that will remove extraneous materials from the chrome cake. A detailed characterization of the gelable and hydrolyzed protein products and effects of processing parameters on chromium content of the protein will be the subjects of future publications.

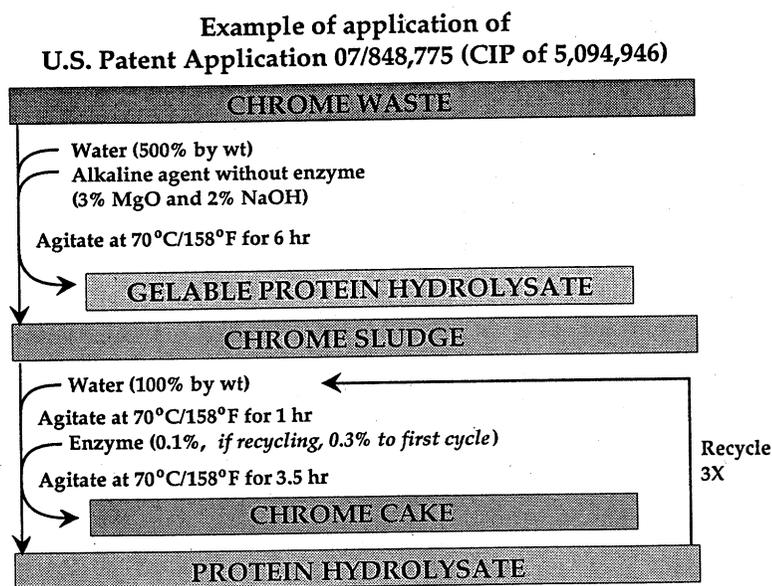


FIG. 1 — Example of two step process to treat chromium-containing leather waste. A variety of alkalinity-inducing agents can be used to extract gelable protein products.

Experimental

MATERIALS

Chromium-containing leather waste was obtained from a commercial tannery. Two samples (A and B) were received over a four month time period.

Alcalase^{®***} (alkaline protease) was obtained from Novo Nordisk Bioindustrials, Inc. (Danbury, CT). It is a proteolytic enzyme with optimal activity at pH 8.3-9.0 and 55-65°C. It is supplied both as a granular solid (adsorbed onto an inert carrier and standardized to contain 2.0 Anson Units/g (AU/g)), and as a liquid (standardized to contain 2.5 AU/g). Liquid Alcalase[®] was used in these experiments.

Pluronic 25R2[®], a non-ionic surfactant, was obtained from BASF (Parsippany, NJ). Magnesium oxide was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ) and from Martin Marietta Magnesia Specialties (Hunt Valley, MD) as MagChem 50[®]. Sodium hydroxide (50% solution), potassium hydroxide, sodium carbonate and potassium carbonate were obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ).

PROCEDURE

Treatment of chrome waste (Figure 1): Two hundred grams of either of the chrome shavings samples (A and B) were shaken in 1 L of water (500% float), 0.2 g of a non-ionic surfactant and the appropriate alkali at 70-72°C for six hours. The samples were filtered hot through Whatman #1 filter paper. The chrome sludge and the filtered gelable protein solutions were stored at 4°C. The chrome sludge was warmed to room temperature and 200 mL water (100% float) and 0.2 g non-ionic surfactant were added. The samples were shaken at 70-72°C for 1.5 hrs. The pH was adjusted with magnesium oxide to optimal pH for the enzyme. The enzyme (0.2 g) was added and the samples were shaken at 70-72°C for 3.5 hrs. The solutions were filtered hot through Whatman #1 filter paper and the protein solutions were stored at 4°C. The chrome cake was air dried.

Treatment of chrome cake: One gram of air dried chrome cake was dissolved in 50 mL of 3.6N (10%) sulfuric acid. The pH was <1.0. The pH of the solution was slowly raised to 1.85 - 2.00 with 0.25N NaOH. A flocculated precipitate formed that coagulated when the solution was heated for several minutes at 60°C. The solution was allowed to stand overnight and was then filtered. The residue was washed with 0.01N sulfuric acid to remove trapped chromium. The residue was dried overnight at 60°C and then weighed; the percent residue was calculated. The residue was ashed at 600°C in a Muffle furnace and percent ash and volatile solids were calculated.

ANALYSES

The chrome shavings were analyzed for moisture, ash, total solids, total ash and chromium as described in a previous publication¹⁹.

Protein molecular weights were estimated by SDS-PAGE (polyacrylamide gel electrophoresis in sodium dodecylsulfate)²⁰ on PhastSystem by Pharmacia.

Gel strengths were measured by Bloom determinations²¹. The dried gelatin (7.5 g) is weighed into a Bloom jar and 105 mL of water is added, to give a 6.67% weight/weight concentration. The water is absorbed for a set period of time (10 min to overnight), the sample is heated in a 65°C bath for 15 minutes, cooled at room temperature for 15 min and then placed in a 10°C bath for 17-18 hrs. The sample is placed under a 0.5 inch probe and the probe is driven into the

sample to a depth of 4 mm at a rate of 1 mm per sec. The force required for this is expressed as the Bloom. The instrument used to measure the Bloom was the TA.XT2™ Texture Analyzer from Texture Technologies Corporation, Scarsdale, NY.

Results and Discussion

When treating solid chrome-containing leather waste it is imperative that value added by-products be recovered. Previous findings⁹⁻¹⁷ from this laboratory have shown that a low molecular weight protein product, usable in fertilizer, may be isolated along with a recyclable chromium cake from enzymic hydrolysis of the chromium-containing waste. The economic return from these products would not make this process viable unless the landfill fees were exorbitant or there would be no place or outlet for disposal of this waste product. Extraction of gelatin from chromium leather waste has been described in the literature²². However, a considerable amount of chromium sludge remains after this extraction and disposal of this sludge is necessary. We proposed a new two-step process that would isolate a gelable protein in the first step and a lower molecular weight, hydrolyzed protein after enzymic treatment of the remaining chrome sludge. A recyclable chromium product was also obtained.

We have begun the characterization of the isolated protein fractions with respect to molecular weight distribution, chemical analysis and gel strength as measured by the Bloom value. The ranges that one can attain are summarized in Tables I and II. The effect of treatment variables on the physical and chemical properties of these products will be discussed in a future publication. These ranges are shown to demonstrate that a variety of properties can be achieved and these will be dependent on the desired end use of the protein product.

TABLE I
Characterization of Gelable Protein Products

Parameters	Range
Total solids	1.74-4.20%
Moisture	4.00-13.00%
Ash ^a	8.90-20.70%
Chromium ^a	0.005-0.013%
Molecular weight distribution	75,000->200,000
Bloom value	80-150

^aMoisture-free basis (MFB).

TABLE II
Characterization of Hydrolyzed Protein Products

Parameters	Range
Total solids	5.20-9.30%
Ash ^a	3.20-7.75%
Chromium ^a	0.0005-0.005%
Molecular weight distribution	10,000-20,000

^aMoisture-free basis (MFB).

In our original process, the chromium-containing waste was pretreated with a variety of alkalis at 69-71°C, not only to aid in the denaturing of the collagen but also to prepare the system for the optimal pH for the enzyme. At that time we used magnesium oxide, calcium hydroxide and various combinations of magnesium oxide, sodium hydroxide, sodium carbonate and calcium hydroxide. We used these various agents so that the process could be worked into whatever chrome recycling system the tannery would be using, since all these chemicals have been used in chrome preparation²³⁻²⁴.

We now have investigated the effect that magnesium oxide, alone and in combination with varying amounts of sodium hydroxide, sodium carbonate, potassium hydroxide and potassium carbonate, has on the ash contents of the gelable and hydrolyzed protein products. We also have used an ion-exchange method to remove excess ash from the protein solutions.

An experiment was designed to isolate gelable proteins and hydrolyzed proteins using varying combinations of magnesium oxide and sodium hydroxide. As has been shown in previous publications¹⁶⁻¹⁷, careful control of the concentrations of these two alkalis will give the optimal pH range for enzyme hydrolysis and—as will be shown—the optimal range for gelable protein extraction. Also, the pH of the reaction should not fall below 8.5, for then there would be the risk of solubilizing the chromium. Shavings from different tannery processes have different pH values, ranging from 3.50 to 4.20. The shavings being used in these experiments had a pH range of 3.95-4.00. The concentration of alkalis to be added were arrived at experimentally in small bench trials previous to larger scale runs.

Table III shows the effect that magnesium oxide alone and with varying concentrations of sodium hydroxide has on the pH, total solids and total ash content of the gelable protein. This

TABLE III
Effect of MgO-NaOH Concentrations of Character of Gelable Protein and Hydrolyzed Protein Products^a

Parameter	Alkali Concentration			
	6% MgO ^b	5% 0.5% MgO-NaOH ^c	4% 1% MgO-NaOH ^d	3% 2% MgO-NaOH ^e
Gelable Protein				
Final pH	8.85±0.12	8.88±0.08	9.05±0.04	9.30±0.16
% Total Solids	2.08±0.35	2.33±0.08	2.50±0.14	3.63±0.52
% Total Ash	0.21±0.02	0.27±0.01	0.31±0.00	0.41±0.04
% Ash (MFB) ^f	10.44±1.52	11.64±0.06	12.37±0.81	11.44±0.55
Hydrolyzed Protein				
Final pH	8.87±0.30	8.75±0.15	8.71±0.07	8.80±0.10
% Total Solids	6.05±0.54	7.37±0.41	6.30±0.06	5.68±0.47
% Total Ash	0.22±0.05	0.26±0.00	0.30±0.01	0.30±0.02
% Ash (MFB) ^f	3.72±0.54	3.56±0.16	4.68±0.08	5.33±0.51

^aIsolated from treatment of chrome shavings, sample A (cf. Table IV).

^bN=7 experiments.

^cN=2 experiments.

^dN=2 experiments.

^eN=3 experiments.

^fMoisture-free basis.

table shows that as the hydroxide concentration is increased, the percent total solids and total ash content increases accordingly. When expressed on a moisture-free basis, the percent ash does not show a significant difference.

Table III also shows the ash content of the hydrolyzed protein from the second step of the process. These values are much lower than those found in the gelable protein and were as expected. As has been described in previous publications²⁵⁻²⁸, chrome shavings contain soluble salts that reflect the type of processing carried out as the raw hide is processed through to the blue; these salts would be extracted in the first step. The salts would be added to maintain pH and those trapped in the chrome sludge would appear in the protein from the second step. As can be seen in Table III, the higher the hydroxide concentration, the higher the percent total ash content.

In a previous publication¹⁷, we described the effect that the chemical composition of the chrome substrates would have on the isolated protein products and chrome cakes. Table IV shows the effect of a higher ash content in the chrome shavings on the total ash in the gelable protein and hydrolyzed protein samples. In this experiment, only magnesium oxide was used to treat the two shavings samples.

TABLE IV
Effect of Ash Content of Chrome Shavings on Character of Gelable Protein and Hydrolyzed Protein Products^a

Parameter ^b	Chrome Shavings	
	Sample A	Sample B
% Moisture	52.03±0.28	53.75±0.16
% Ash	3.40±0.12	4.24±0.02
% Ash (MFB) ^c	7.09±0.19	9.17±0.04
Gelable Protein		
Final pH	8.94	8.90
% Total Solids	2.12±0.01	2.20±0.02
% Total Ash	0.23±0.01	0.30±0.01
% Ash (MFB) ^c	10.94±0.14	13.71±0.18
Hydrolyzed Protein		
Final pH	8.80	8.60
% Total Solids	5.78±0.01	6.90±0.00
% Total Ash	0.22±0.00	0.36±0.00
% Ash (MFB) ^c	3.86±0.03	5.27±0.02

^aChrome shavings (A and B) were treated with 6% MgO.

^bN = 3 analyses.

^cMoisture-free basis.

Table V shows the effect of varying concentrations of magnesium oxide, sodium hydroxide and sodium carbonate on the total solids and total ash. These treatments were applied to chrome shavings, Sample B (Table IV). One can see a higher ash in the sodium hydroxide samples when compared to the results found in Table III in which sodium hydroxide was used on a substrate with a lower ash content (Sample A, Table IV). In both cases, increasing the concentrations of

the hydroxide and carbonates increases the ash content in both the gel and the hydrolysate. However, the use of carbonates in these reactions gives a more controllable pH.

TABLE V
Effect of MgO and NaOH or Na₂CO₃ Concentrations
on Character of Gelable Protein and Hydrolyzed Protein Products^a

Parameter ^b	Alkali Concentrations				
	6% MgO	4% 1% MgO-NaOH	3% 2% MgO-NaOH	5% 1% MgO-Na ₂ CO ₃	4% 2% MgO-Na ₂ CO ₃
Gelable Protein					
Final pH	8.90	9.10	9.30	8.73	8.65
% Total Solids	2.20±0.01	2.25±0.03	3.10±0.01	2.05±0.04	2.40±0.00
% Total Ash	0.30±0.01	0.42±0.01	0.52±0.01	0.38±0.02	0.46±0.00
% Ash (MFB) ^c	13.71±0.18	18.88±0.24	16.72±0.26	18.59±1.28	19.09±0.06
Hydrolyzed Protein					
Final pH	8.60	8.60	8.85	8.82	8.84
% Total Solids	6.90±0.00	8.46±0.01	8.46±0.01	8.78±0.44	8.28±0.01
% Total Ash	0.36±0.00	0.45±0.02	0.65±0.01	0.40±0.02	0.42±0.01
% Total Ash (MFB) ^c	5.27±0.02	5.28±0.16	7.67±0.06	4.60±0.08	5.13±0.04

^aIsolated from treatment of chrome shavings, Sample B (cf. Table IV).

^bN=3 analyses.

^cMoisture-free basis.

TABLE VI
Effect of MgO and KOH or K₂CO₃ Concentrations on Character of Gelable Protein and
Hydrolyzed Protein Products^a

Parameter ^b	Alkali Concentrations				
	6% MgO	4% 1% MgO-KOH	3% 2% MgO-KOH	5% 1% MgO-K ₂ CO ₃	4% 2% MgO-K ₂ CO ₃
Gelable Protein					
Final pH	8.90	8.72	8.78	8.40	8.40
% Total Solids	2.20±0.01	2.48±0.00	2.29±0.00	2.30±0.01	2.17±0.01
% Total Ash	0.30±0.01	0.37±0.01	0.44±0.02	0.35±0.02	0.44±0.01
% Ash (MFB) ^c	13.71±0.18	14.74±0.28	19.36±0.82	15.12±0.56	20.46±0.24
Hydrolyzed Protein					
Final pH	8.60	8.61	8.65	8.75	8.70
% Total Solids	6.90±0.00	7.03±0.01	7.27±0.01	7.46±0.01	8.36±0.01
% Total Ash	0.36±0.00	0.37±0.00	0.43±0.01	0.41±0.01	0.43±0.01
% Ash (MFB) ^c	5.27±0.02	5.29±0.04	5.97±0.10	5.51±0.12	5.12±0.07

^aIsolated from treatment of chrome shavings, sample B (cf. Table IV).

^bN=3 analyses.

^cMoisture-free basis.

In Table VI, the effects of potassium hydroxide and potassium carbonate with magnesium oxide and their effect on the ash content of the gels and hydrolysates are shown. Potassium is used in fertilizers and would be advantageous if the hydrolysate product could be used in this market. Increasing the hydroxide and carbonate concentrations increases the ash content significantly of the gelable fraction. The total solids and total ash content of the hydrolysates increases with higher concentrations of hydroxide and carbonate.

It has been reported in the literature²² that the ash content of a good gel should be between 0-3 percent. Our samples range from 8.9-21 percent (MFB). As shown in a previous publication, these ashes contain magnesium and calcium ions as well as the more soluble sodium and potassium salts¹⁷. As has been shown above, this ash content reflects not only the ash content of the original shavings, but also the type of alkali used to extract the gel. Typically, in commercial gelatin preparation, the solutions are passed through ion-exchange columns to lower the ash content. We took about 50 mL of our gelable solutions and passed them through mixed bed ion-exchange columns of two different compositions. Table VII shows the results. The first column gives the ash of the original sample and the second column gives ash contents of the deionized sample. These treated samples of gelable proteins are within the criteria set for gelatin. Both resins worked equally well. A cation exchanger may be sufficient, for it is tedious to regenerate the mixed beds; one must separate the two resins, which though not difficult is time consuming.

TABLE VII
Use of Mixed Bed Ion-Exchange Resins to Reduce Ash Content
of Gelable Protein Products

Resin	Percent Ash ^a	
	Before	After
Amberlite MB-1		
Sample 1	12.77	0.50
Bio-Rad AG [®] 501-X8 (D)		
Sample 1	12.77	0.43
Sample 2	17.48	0.40

^aMoisture-free basis (MFB).

We treated the chrome cake chemically using a reported method²⁹, to give a recyclable chrome product. Table VIII reports the percent residue that remains after the chrome cake is dissolved in 3.6N sulfuric acid and the residue is precipitated with 0.25N sodium hydroxide to a pH of about 1.85. When the residue from Sample a was first analyzed, an ash content of 14.5% (or 1.33%, based on dried weights of the chrome cake) was found. It appeared that there was chromium in the ash. Apparently, when filtering, chromium can be trapped in the residue, and if the residue is washed with 0.01N sulfuric acid, this chromium will be removed. As shown in Table VIII, when Sample a was washed with the dilute acid, the percent ash dropped accordingly. Samples b through e were also analyzed for percent residue in the chrome cakes as well as the percent non-chrome insoluble ash, and the results are shown in this table. The low percent ash indicates that the bulk of the residue is organic and could be unextracted protein and/or the resins that are used in the high exhaust chrome tannages.

TABLE VIII
Characterization of Residue from Treatment of Chrome Cake

Sample	Final pH	%Residue ^a	% Ash ^b
a	1.84	9.13	0.37
b	1.85	6.33	0.18
c	1.84	7.33	0.28
d	1.85	7.46	0.25
e	1.85	10.14	0.23

^a% Residue in chrome cake, Moisture-free basis (MFB).

^b% Insoluble ash in chrome cake (MFB).

Conclusions

It has been shown that the choice of alkali for treatment of chromium-containing waste generated in the leather industry influences the chemical composition of the isolated protein products. The chemical composition of the original chromium waste product also contributes to the chemical makeup of the protein products. A higher percent of the ash is extracted with the gelable protein, and if this ash is too high for the desired end product, it can be removed by ion-exchange resins. This study has also shown that a variety of alkali-inducing agents can be used to treat the waste, depending on the desired composition of the end product or compatibility with the chemicals used in chrome recycling in the tannery system. It has also been shown that the chrome cake isolated in these treatments can be chemically treated to remove undissolved protein or the resins used in the high exhaust chrome tannages so that a product can be recovered that can be recycled. The effect that these systems have on the chromium contents of the proteins and on the physical and other chemical properties of the gels and hydrolysates will be the subject of future publications.

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