

## Extraction and Characterization of "Chrome-Free" Protein from Chromium-Containing Collagenous Waste Generated in the Leather Industry

M. M. Taylor, E. J. Diefendorf, C. J. Thompson, E. M. Brown,  
and W. N. Marmer

Eastern Regional Research Center, Agricultural Research Service,  
U.S. Department of Agriculture, 600 East Mermaid Lane,  
Philadelphia, PA 19118

In the United States, almost 56,000 metric tons of chromium-containing solid waste are generated by the leather industry each year, and approximately ten times this amount is generated world-wide. Environmental concerns and escalating landfill costs are becoming increasingly serious problems to the leather industry and alternative disposal methods are needed. We have developed a process in which this waste is first treated with alkali to extract a high molecular weight gelable collagen protein. The sludge that remains is further treated with enzymes to recover a lower molecular weight protein hydrolyzate and a recyclable chromium product. The recovered protein fractions, practically devoid of chromium, could be used in a wide range of products, including adhesives, cosmetics, films, animal feed and fertilizer. The isolated chromium can be chemically treated and recycled into the tanning process.

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 alternatives to dumping. During the past 25 years, many investigators have developed some rather innovative methods to treat this waste product. Alkali hydrolysis has been one of the most investigated processes. For example, researchers used calcium hydroxide and steam for the purpose of chrome recovery and isolation of a protein fraction (1-2). Studies have been done with sodium hydroxide and pressure to improve the efficiency of the reaction (3), with ammonia to obtain fertilizers and with sodium carbonate and sodium hydroxide combinations to produce coagulants for natural rubber and leveling agents for leather dyeing (4).

Several investigators have hydrolyzed the waste products from chrome tanning with sulfuric acid and used the chromium-containing hydrolysate as a

retanning agent (5-6) or, after precipitation of the chromium, used the isolated amino acids as an animal feed supplement (7). The hydrolysates also may be used to produce fatliquors, surfactants and fillers for leather manufacture (8). Others have hydrolyzed with organic acids to obtain oligopeptides (9); acrylic acids have been used and the resulting hydrolysate was copolymerized with vinyl monomers to give fillers for leathers (10).

Many investigators have recovered the chromium by wet air oxidation (11), peroxide treatments (12), and incineration at a variety of temperatures (13-15). Chromium with the +6 oxidation state would be generated in these reactions and a reduction step would be needed.

Reaction of the chromium-containing waste product with monomers and polymers has been carried out by a number of investigators. The leather scraps have been reacted with polyisocyanates to make insulators and building materials (16). The substrate has been grafted with hydrophilic acrylates to make fibrous sheets (17), polymerized to make molded products fillers for leather (18), and, with vinyl acetate, formed into sheets for shoe soles (19).

Other uses include addition of the recovered chrome to cements and mortars (20). The waste product has been used in the manufacture of composite sheets for footwear (21). Leather substitutes have been made by a papermaking method (22) and mixtures of chrome leather fibers and cellulose pulp have been used as a paper substitute (23). Several researchers have detanned the chrome product for the purpose of gelatin preparation (24-25) and others have been able to isolate collagen fibers (26-27).

We have developed a process that can help the leather industry in solving this potentially difficult waste disposal problem. In this process, the chrome waste is treated with alkaline proteolytic enzymes at moderate temperatures for a short period of time. The process is unique because the pH at which the reaction takes place (8.3 to 10.5) prevents the chromium from going into solution, thus averting the poisoning of the enzyme by chromium and enabling the recovery of chromium as  $\text{Cr}(\text{OH})_3$  by filtration. The resulting protein solution may have commercial use as a feed or a fertilizer or could be discarded as sanitary sewage. The isolated residue containing chromium and organic matter (chrome cake) has the potential to be recycled into the tanning process by treatment with sulfuric acid. The tanning industry has begun collaborative efforts with us to assess the process on a commercial scale.

It had been documented (28-39) that chromium-containing waste can be treated enzymically, but only after denaturation of the collagen. The methods developed at this laboratory demonstrated that the collagen may be denatured in the presence of alkali at moderate temperatures and thus the direct addition of the enzyme to shavings already subjected to moderate pretreatment temperatures may be made. Maintenance of these temperatures throughout the entire digestion process eliminates the need to cool the reaction mixture.

In preliminary investigations using calcium hydroxide to control the pH (37-39), 78% solubilization of the shavings was achieved when 6% (based on wet

weight of shavings) of an alkaline proteolytic enzyme was used for hydrolysis. When magnesium oxide was used in conjunction with other alkaline agents (40-43), higher solubilization of protein was achieved with lower amounts of enzyme than previously reported, thus making the treatment more cost-effective.

More recently, we have found that if a two step process is used, a *gelable* protein product can be obtained that should provide a higher economic return. In this process, which is covered by a new patent (44), the chromium waste is treated with alkaline agents for six hours at 70-72°C and then filtered to recover a gelable protein. The chromium-containing sludge that remains is then treated with the bacterial enzyme as in the original process, resulting in a protein hydrolysate fraction and a chrome cake that can be chemically treated and subsequently recycled.

The protein products that result from these two treatments have many possible uses. Because of its high nitrogen content, the isolated protein has potential as a fertilizer and as an animal feed additive. The gelable protein has potential use in cosmetics, adhesives, printing or photography.

## Materials

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

**Recovery of Hydrolyzed Protein Products.** Chromium-containing leather waste was obtained from commercial tanneries. Sample A shavings came from a conventional chrome tannage. Sample B shavings came from a tannage in which a high exhaust chrome treatment had been used in order to reduce the chromium in the effluent. Sample C shavings came from a tannage in which the final pH was slightly more acidic (pH 3.6) than other chrome offal investigated (pH 3.8-4.2).

Each (11.5 kg) of the shavings samples (A, B and C) was pretreated with agitation at 67-69°C in 56 L (500% float) of water for two hours. Bench type experiments determined the best pretreatment for each individual sample prior to the pilot scale runs. This pretreatment step is necessary to obtain the pH that will be optimal for the enzymic digestion. Thus, Sample A was pretreated with 575 g magnesium oxide, and Sample B with 345 g NaOH and 230 g magnesium oxide.

After several preliminary bench experiments, it was found that Sample C needed to be treated with 690 g magnesium oxide (C-1). Because of its acidity, another portion of this sample was pretreated with 345 g NaOH and 345 g magnesium oxide (C-2). The enzyme (345 g) was added in three feeds (172.5 g in each feed) to each of the four reactions, over a three hour period. Upon completion of the digestion (67-69°C for 3 hr), the sample was pumped from the reaction vessel and allowed to settle overnight. The protein hydrolysate layer was decanted and the settled chromium layer was filtered through Whatman #1 filter paper. An aliquot of each protein layer was stored at 4°C. The unwashed chrome cake was collected and it, too, was stored at 4°C.

**Recovery of Gelable and Hydrolyzed Protein Products.** Chromium-containing leather waste was obtained from a commercial tannery. Two samples (A and B) were received over a four month time period.

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 hydrolyzed 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 and a flocculent precipitate formed. The solution was heated for several minutes at 60°C, 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, Total Kjeldahl Nitrogen (TKN), fat, calcium, magnesium, and chromium as described in a previous publication (45). Amino acid analyses were carried out on a Beckman Model 119CL Analyzer.

Protein molecular weights were estimated by SDS-PAGE (polyacrylamide gel electrophoresis in sodium dodecylsulfate) (46) using a PhastSystem by Pharmacia.

The instrument used to measure the Bloom value was the TA.XT2 Texture Analyzer from Texture Technologies Corporation, Scarsdale, NY. Gel strengths were measured by Bloom determinations (47). The dried gelatin (7.5 g) was weighed into a Bloom jar and 105 mL of water was added, to give a 6.67% weight/weight concentration. Water was absorbed for a set period of time (10 min to overnight) and then 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 hr. The sample was placed under a 0.5 inch analyzer probe and the probe was driven into the sample to a depth of 4 mm at a rate of 1 mm per sec. The force required for this was expressed as the Bloom value.

#### Recovery of Protein Solely as the Hydrolyzed Product (the "Original" Process)

Not all chromium-containing leather waste is the same. Tanneries use different processes to tan leather. These differences are introduced not only to affect the properties of the tanned leather, but also, in some cases, to allow high chrome exhaustion of the tanning liquor for environmental reasons. The protocol for the pretreatment of these shavings must be adjusted to achieve optimal solubility. The commercial value of this process depends not only on the savings from decreased landfill fees, but also on the value of the recovered reaction products. Thus, it is important to know the chemical composition of the isolated chrome cakes.

Chrome shavings from various tanneries were analyzed for moisture, ash, chromium, nitrogen, fat, calcium, and magnesium. The results of these analyses allow a prediction of the chemical composition of the chrome cakes. Table I shows the results of these analyses. Each of the shavings contained about the same

Table I. Analyses of Chrome Shavings

Parameter % <sup>a</sup>	A	B	C
Moisture	53.51 ± 0.28	53.47 ± 1.04	51.47 ± 0.36
Ash <sup>b</sup>	14.32 ± 0.10	8.40 ± 0.48	14.95 ± 0.37
Chromic Oxide <sup>b</sup>	4.21 ± 0.03	4.28 ± 0.09	3.99 ± 0.11
TKN <sup>b,c,d</sup>	14.54 ± 0.48	14.56 ± 0.24	14.13 ± 0.16
Fat <sup>b</sup>	0.09 ± 0.01	1.51 ± 0.36	1.79 ± 0.22
Calcium <sup>b</sup>	0.34 ± 0.01	0.40 ± 0.01	0.48 ± 0.01
Magnesium <sup>b</sup>	0.33 ± 0.02	0.08 ± 0.01	0.16 ± 0.01

<sup>a</sup> N = 3 where N = number of replicates for each sample.

<sup>b</sup> Moisture free basis.

<sup>c</sup> Total Kjeldahl nitrogen.

<sup>d</sup> Protein content can be estimated by multiplying TKN by 5.51.

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amount of moisture, from 51.5 to 53.5%. Ash content ranged from 8 to 15%. Chromic oxide content ranged from 3.99 to 4.28%. The nitrogen content was 14.1 to 14.6%, and may be correlated to the protein content of the shavings (roughly 80% on a moisture-free basis). The fat content varied from 0.1 to 1.8%. Calcium values ranged from 0.34 to 0.48% and magnesium from 0.08 to 0.33%.

The chemical composition of the recovered chrome cakes from each treatment is shown in Table II. The fat contents reflect the amount of fat found in the untreated shavings (Table I). The fat content in Sample B may also reflect the compounds that had been used in the high exhaust chrome treatment. These compounds appeared to be lipophilic, for the extracts from these samples, dark brown and viscous, were different from the other two. The cakes were not washed during filtration; the nitrogen content reflects the protein that remains and is dependent on the efficiency of the filtration process. The chromic oxide content reflects the amount of chrome in the original shavings. The magnesium content reflects the amount of magnesium used in the pretreatments. The value for calcium found in the cakes may reflect the approximately 1% calcium impurity in the magnesium oxide.

**Table II. Analyses of Chrome Cakes from Enzymic Treatment of Chrome Shavings**

Parameter % <sup>a</sup>	A	B	C-1	C-2
Moisture	85.42 ± 0.17	85.54 ± 0.22	82.93 ± 0.60	82.53 ± 0.94
Ash <sup>b</sup>	35.45 ± 0.08	32.55 ± 0.49	34.14 ± 0.83	36.99 ± 0.38
Chromic Oxide <sup>b</sup>	7.76 ± 0.30	11.82 ± 0.54	8.74 ± 0.10	11.44 ± 0.03
TKN <sup>b,c,d</sup>	7.51 ± 0.09	8.40 ± 0.66	6.66 ± 0.24	8.09 ± 0.55
Fat <sup>b</sup>	1.37 ± 0.10	6.31 ± 0.38	4.26 ± 0.07	4.93 ± 0.06
Calcium <sup>b</sup>	0.35 ± 0.01	0.82 ± 0.02	0.75 ± 0.06	1.18 ± 0.08
Magnesium <sup>b</sup>	9.96 ± 0.12	5.00 ± 0.06	9.47 ± 0.16	6.73 ± 0.22

<sup>a</sup> N=3 where N=number of replicates for each sample.

<sup>b</sup> Moisture free basis.

<sup>c</sup> Total Kjeldahl nitrogen.

<sup>d</sup> Protein content can be estimated by multiplying TKN by 5.51.

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The isolated protein hydrolysates were analyzed for chromium, total Kjeldahl nitrogen (TKN), total solids and ash (Table III). Average values for samples A, B, C-1 and C-2 show that the chromium content was less than 1 ppm. This chromium concentration is similar to the concentrations found, not only in testing of protein from pilot studies, but also the protein solution recovered from

industrial scale trials. The TKN, total solids and total ash averaged about 11,000 ppm, 72,000 ppm and 8,000 ppm, respectively. The molecular weight distribution of the hydrolyzed protein ranged from 1000-3000.

**Table III. Analyses of Protein Hydrolysates**

Solubilization <sup>a</sup> with 1% Enzyme		80
Protein Hydrolysate (Liquid) <sup>b</sup>		
Chromium	(AV)	< 1
TKN	(AV)	11,000
Total Solids	(AV)	72,000
Total Ash	(AV)	8,000
Protein Hydrolysate (Dried) <sup>a</sup>		
TKN		13.8-15.0
Ash		9.7-18.9
Molecular Weight Distribution		1,000-3,000

<sup>a</sup> Expressed as percent.

<sup>b</sup> Expressed in PPM.

A composite of the amino acid analyses for each of the dried protein samples is shown in Table IV. The values are expressed as mole percent. When the profile of the protein hydrolysate is compared to the profile of collagen, the results are quite similar, suggesting that no modification of amino acids occurred during processing.

It was demonstrated that full splits and trimmings could be enzymically hydrolyzed. In this treatment, the alkali pretreatment time was extended to three hours and the temperature was increased to 70-72°C. The structure of the hides was so totally disrupted that upon addition of the enzyme, the samples were readily digested. It was decided to apply this extended holding time and higher temperature to chrome shavings and it was found that 0.3% of an alkaline protease was successful in digesting the shavings and giving a clean cake. Thus, the amount of enzyme that was suggested previously had been reduced almost five times.

Recycling of the protein solution containing the enzyme was attempted. The enzyme did not denature after being subjected to the high temperatures and pH's and it was found that one could successfully recycle the protein solution and enzyme, not once, but four times. The salt concentration eventually became quite

**Table IV. Amino Acid Composition of Hydrolyzed Protein <sup>a</sup>**

<i>Residue</i>	<i>Collagen (Type I)</i>	<i>Hydrolysate</i>	<i>Std Dev <sup>b</sup></i>
Gly	32.7	33.0	1.7
Hyp	8.6	10.0	1.2
Pro	13.0	12.5	0.5
Ala	11.4	8.4	0.6
Arg	5.2	4.8	0.3
Asp	4.6	5.1	0.1
Cys	0.0	0.0	0.0
Glu	7.5	7.7	0.3
His	0.5	0.9	0.6
Ile	1.2	1.4	0.2
Leu	2.5	2.6	0.1
Lys	2.8	2.7	0.2
Met	0.6	0.2	0.3
Phe	1.3	1.3	0.0
Ser	3.1	4.1	0.9
Thr	1.6	2.1	0.7
Tyr	0.4	0.5	0.1
Val	2.3	2.4	0.1
Total	99.3	100.0	

<sup>a</sup> Expressed as mole percent.

<sup>b</sup> Hydrolysate samples.

high and the enzyme lost its activity. A 1% concentration of the enzyme initially is recommended if one is recycling.

#### Recovery of Gelable and Hydrolyzed Protein Products (the "New" Process).

The next important step in the investigations would be to obtain a higher molecular weight protein than was previously isolated. The original one-step process gave not only a recyclable chromium product but also gave a low return protein hydrolysate that could possibly be used as animal feed and fertilizer. The economic return from these products would not make this process viable unless the landfill fees were exorbitant or there were no outlets for disposal of this waste product. Even though it was demonstrated that this protein solution and enzyme

could be recycled in order to reduce the cost of the process, a higher return from a better quality by-product would be desirable.

Extraction of gelatin from chromium leather waste has been described in the literature (24-25). However, a considerable amount of chromium sludge remains after this extraction and disposal of this sludge is necessary (48). A new two-step process was proposed 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 filterable and recyclable chromium product would also be obtained.

Figure 1 is a flow diagram of the new process and illustrates one of the many alkali-inducing combinations that can be used to extract the gelable protein. After isolation of the gelable protein product by filtration, the chrome sludge is prepared for enzymic hydrolysis. The pH is measured and adjusted if necessary for optimal enzyme activity and the alkaline protease is added. The reaction was carried out for 3.5 hours. The protein hydrolysate solution can be recycled to reduce enzyme costs, and a 0.3% initial feed of enzyme is recommended. If one has whole splits or large trimmings, chipping or grinding is recommended before the first step is carried out. These substrates have been dissolved in their intact state, with 1% enzyme or less, but the protein product is low molecular weight.

In the original process, the chromium-containing waste was pretreated with a variety of alkalis at 67-69°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 magnesium oxide, calcium hydroxide and various combinations of magnesium oxide, sodium hydroxide, sodium carbonate and calcium hydroxide were used. These various agents were used 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 precipitation (49-50).

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 chemical and physical properties of the gelable and hydrolyzed protein products has been investigated. As has been shown in previous publications (40-41), careful control of the concentrations of the alkali-inducing agents 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 to 4.00. The concentrations of alkalis to be added were arrived at experimentally in small bench trials prior to larger scale runs.

Table V summarizes the chemical and physical properties of the gelable protein that has been extracted using various combinations of the above-mentioned alkali treatments. The percent total solids in the solutions can range from 1.75 to 4%, depending on the choice of alkali. The chromium content of the protein products can range from 0.005 to 0.0126%. These gelable protein solutions were freeze-dried to a white solid with a moisture content ranging from 4 to 13 % and

the ash content from 8.9 to 21%. Molecular weight distribution can range from 75,000 to more than 200,000, depending on the alkali treatment that is used. The Bloom values, or gel strengths, range from 80 to 150 g.

Example of U.S. Patent 5,271,912 (CIP of 5,094,946)

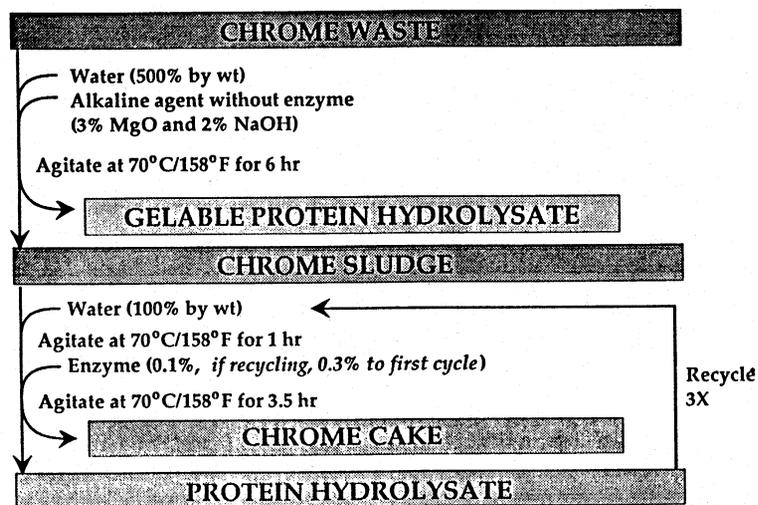


Figure 1. Example of two step process to treat chromium-containing leather waste. A variety of alkalinity-inducing agents can be used to extract gelable protein products.

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Table V. Characterization of Gelable Protein Products

Parameters	Range
Total Solids	1.75-4.00%
Moisture	4.00-13.00%
Ash <sup>a</sup>	8.90-20.00%
Chromium <sup>a</sup>	.005-.013%
Molecular Weight Distribution	75,000-> 200,000
Bloom Value	80-150 g

<sup>a</sup> Moisture-free basis (MFB).

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Table VI summarizes the chemical properties of the hydrolyzed protein products isolated from the chrome sludge, the character of which will vary depending on the choice of alkali. The molecular weight ranged from 10,000 to 20,000, values much higher than those protein products isolated in the original process; this reflects the small amount of enzyme used in the sludge digestion.

Table VI. Characterization of Hydrolyzed Protein Products

Parameters	Range
Total Solids	6.00-9.50%
Ash <sup>a</sup>	3.30-7.70%
Chromium <sup>a</sup>	0.0005-0.005%
Molecular Weight Distribution	10,000-20,000

<sup>a</sup> Moisture-free basis (MFB).

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Also shown are the range of total solids and total ash content of the protein solutions along with the range of the chromium concentration. The total ash content of the hydrolyzed protein products is much lower than that found in the original process.

Possible uses of the gelable protein fractions include graft polymerized products, waste-water treatment, encapsulation, powdered filler for skid resistant tires and thermal printing materials. Possible uses for the hydrolysates include fertilizer, animal feed, and adhesives.

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, the use of different proportions of alkaline agents on the ash content of the protein products was investigated. Increasing proportions of magnesium oxide resulted in lower ash content.

In Table VII, 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 of the gelable fraction significantly. The total solids and total ash content of the hydrolysates increases with higher concentrations of hydroxide and carbonate.

Table VII. Effect of MgO and KOH or K<sub>2</sub>CO<sub>3</sub> Concentrations on Character of Gelable Protein and Hydrolyzed Protein Products<sup>a</sup>

Parameter <sup>b</sup>	6%	4%	1%	3%	2%	5%	1%	4%	2%
	MgO	MgO-KOH	MgO-KOH	Mg-CO <sub>3</sub>	Mg-CO <sub>3</sub>				
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) <sup>c</sup>	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) <sup>c</sup>	5.27±0.02	5.29±0.04	5.97±0.10	5.51±0.12	5.12±0.07				

<sup>a</sup> Isolated from treatment of chrome shavings, sample B (cf. Table VIII).

<sup>b</sup> N=3 analyses.

<sup>c</sup> Moisture-free basis.

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The effect that the chemical composition of the chrome substrates would have on the isolated protein products and chrome cakes has been described (42-43). Table VIII shows the effect of a higher ash content in the chrome shavings on the

Table VIII. Effect of Ash Content of Chrome Shavings on Character of Gelable Protein and Hydrolyzed Protein Products

Parameter <sup>a</sup>	Sample A	Sample B
% Moisture	52.03±0.28	53.75±0.16
% Ash	3.40±0.12	4.24±0.02
% Ash (MFB) <sup>b</sup>	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) <sup>b</sup>	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) <sup>b</sup>	3.86±0.03	5.27±0.02

<sup>a</sup> N=3 analyses.

<sup>b</sup> Moisture-free basis.

<sup>c</sup> Chrome shavings (A & B) were treated with 6% MgO.

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total ash in the gelable protein and hydrolyzed protein samples. In this experiment, only magnesium oxide was used to treat the two shavings samples.

It has been reported (48) that the ash content of a good gel should be between 0-3%. The ash content of the samples ranged from 8.9-21% (MFB). As shown in a previous publication, these ashes contain magnesium and calcium ions as well as the more soluble sodium and potassium salts (51-54). 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. The gelable solutions were passed through mixed bed ion exchange columns of two different compositions. Table IX 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 IX. Reduction of Ash Content by Mixed Bed Ion-Exchange Resins**

Resin	Percent Ash <sup>a</sup>	
	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

<sup>a</sup> Moisture-free basis (MFB).

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The chrome cake may be treated chemically to give a recyclable chrome product, using a reported method (55) in which the chrome cake is dissolved in acid and the extraneous materials are eliminated by precipitation with base. Table X reports the percent residue that remains after the chrome cake is chemically treated. Samples (a) through (e) were also analyzed for the percent non-chrome insoluble ash. The low percent ash indicates that the bulk of the residue is organic, i.e. unextracted protein and/or the resins that are used in the high exhaust chrome tannages.

**Table X. Characterization of Residue from Treatment of Chrome Cake**

Sample	Final pH	% Residue <sup>a</sup>	% Ash <sup>b</sup>
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

<sup>a</sup> % residue in chrome cake, moisture-free basis (MFB).

<sup>b</sup> % insoluble ash in chrome cake (MFB).

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A cost and return estimate of the described treatments has been calculated. The cost of chemicals, energy, labor, and equipment and the return from chrome cake, savings on landfilling, and the recovered protein were factored into the equation. It was found that the tanner should realize a \$1.77 return from the total chrome-containing solid waste generated from each cattlehide, when using the

technology of the newer two-step process and recycling the enzyme-containing solution twice. The new treatment is more profitable and this is influenced by the return on the gelable protein. Recycling the enzyme will increase profits slightly in the two-step treatment (from \$1.61 to \$1.77), but will definitely improve the cost effectiveness of the original one-step treatment (from a loss of \$0.17 to a profit of \$0.11). These savings are mainly the result of lowered costs of evaporation.

In conclusion, high quality gelable protein and hydrolyzed protein products can be isolated from chromium-containing leather waste. Depending on choice of alkali, the process can be varied to give a desired end product, such as molecular weight distribution and Bloom value. It has been shown that the choice of alkali for treatment of chromium-containing waste 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 percentage 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 alkalinity-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 recyclable product can be recovered. A cost estimate has been calculated and indicated that a profit can be achieved if the new two-step process is run and the enzyme is recycled.

Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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