

ash content not only because of the alkali used to extract the gelable protein, but also because of the high mineral content in the original substrate. During the commercial preparation of gelatin, it is common practice to pass the protein solutions through ion-exchange resins in order to lower the ash content and improve the quality of the product.⁵⁰⁻⁵³ In this study we show the effects of deionizing these gelable protein solutions on their physical properties. This report discusses experiments that were designed to determine the yield of the protein products isolated from the chrome waste. From the data we were able to calculate material balances and determine the repeatability of the processes as well as the chemical and physical analyses.

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

Experimental

Materials

Alcalase* (alkaline protease) was obtained from Novo Nordisk Biochem, Inc. (Franklinton, NC). It is a proteolytic enzyme with optimal activity at pH 8.3-9.0 and 55-65°C. It is supplied either as a granular solid (adsorbed onto an inert carrier and standardized to contain 2.0 Anson Units/g (AU/g)), or 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). MgO was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ) and from Martin Marietta Magnesia Specialties (Hunt Valley, MD) as MagChem 50. NaOH (50% solution), KOH, Na₂CO₃ and K₂CO₃ were obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ).

Chromium containing leather waste used in the development of the one-step process 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). For development of the two-step process, chromium containing leather waste from a high exhaust chrome tannage was obtained from a commercial tannery (sample D). Two samples were received over a four month period.

Procedures

Recovery of hydrolyzed protein products from the one-step process

Each (11.5 kg) of the shavings samples (A, B and C) was pretreated at 67-69°C with 11.5 g of surfactant in 56L (500% float) of water for two hours. Bench type experiments determined the best pre-treatment for each individual sample prior to the pilot scale runs. This pre-treatment step is necessary to obtain the optimal pH for

the enzymatic digestion. Thus, Sample A was pretreated with 575 g MgO, and Sample B with 345 g NaOH and 230 g MgO. After several preliminary experiments, it was found that Sample C required 690 g MgO (C-1) or 345 g NaOH and 345 g MgO (C-2). The enzyme (345 ml) was added in three feeds (115 ml in each feed) to each of four reactions, over a three hour period. After the enzyme digestion (67-69°C for 3 h), the sample was pumped from the reaction vessel and allowed to settle overnight. The upper, protein hydrolysate, layer was decanted and the lower chromium layer was filtered through Whatman No. 1 filter paper. Protein layers were stored at 4°C. The unwashed chrome cake was collected and stored at 4°C.

Recovery of protein products: gelable and hydrolyzed protein from the two-step process

In the first step of the two-step process, 200 g of the chrome shavings (sample D) were shaken in 1 L of water (500% float), 0.2 g of a non-ionic surfactant and the alkali at 70-72°C for 6 h. The samples were then centrifuged at 70-72°C and the supernatant filtered through Whatman No. 1 filter paper. The chrome sludge and the filtered gelable protein solutions were stored at 4 °C. In the second step, the chrome sludge was warmed to room temperature and 200 to 300 ml water (100 to 150% float) and 0.2 g non-ionic surfactant were added. The samples were shaken at 70-72°C for 1.5 hours. The pH was adjusted with MgO to the optimal pH for the enzyme. The enzyme (0.025 to 0.2 g) was added and the samples were shaken at 70-72°C for 3.5 h. The solutions were filtered hot through Whatman No. 1 filter paper and the protein solutions were stored at 4°C. The chrome cake was air dried.

Treatment of the chrome cake

1 g of air dried chrome cake was dissolved in 50 ml of 3.6N (10%) H₂SO₄. 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 at ambient temperature and was then filtered. The residue was washed with 0.01N H₂SO₄ 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.

Deionization

The protein fractions were deionized batchwise using Bio-Rad Ag® 501-X8 (D) mixed bed resin (5g/100 ml of protein solution). The solution was stirred and additional resin was added until there was no further change in colour of the resin. This resin changed from blue when fully active to gold when exhausted. After treatment, the solutions were filtered through sintered glass funnels and freeze dried in preparation for chemical and physical analyses.

Analyses

The chromium containing shavings were analysed for moisture, ash, total solids, total ash, total Kjeldahl nitrogen (TKN), fat, calcium, magnesium, and chromium as described in a previous publication.⁵⁴ Amino acid analyses were carried out on a Beckman Model 119CL Analyzer

* Reference to brand or firm does not constitute endorsement by the U.S. Department of Agriculture and the authors over others of a

Chromium in the gelable and hydrolyzed protein products were determined on a Perkin-Elmer Atomic Absorption Spectrophotometer, Model 3300 (Norwalk, CT) as described previously.⁵⁴ Moisture in the dried gelable protein products was determined by heating the sample at 105°C for 17 h.⁵⁰ Ash in the dried protein products was determined by heating the sample at 600°C for two hours.⁵⁴

Protein molecular weights were estimated by SDS-PAGE (polyacrylamide gel electrophoresis in sodium dodecylsulphate)⁵⁵⁻⁵⁶ on PhastSystem by Pharmacia. Gel strengths were measured by Bloom determinations⁵⁷ with TA.XT2 Texture Analyzer from Texture Technologies Corporation (Scarsdale, NY). For most experiments, the dried gelatin (7.5 g) was weighed into a Bloom jar (59 ± 1 mm, inside diameter) and 105 ml of water was added, to give a 6.67% weight/weight concentration. For comparison some experiments were done by a modification of the Bloom method utilizing a 39 mm jar (inside diameter) with 2.5 g sample and 35 ml of water.⁵⁸ The water was allowed to absorb for a set period of time (10 min to overnight), the sample was heated in a 65°C bath for 15 min, cooled at room temperature for 15 min and then placed in a 10°C bath for 17-18 h. The sample was placed under a 0.5 inch diameter analytical probe and the probe was driven into the sample to a depth of 4 mm at a rate of 1 mm sec⁻¹. The grams force required for this is expressed as the Bloom value.

Viscosities were measured in a Cannon Manning viscometer.⁵⁹ The samples, which were 6.67% weight/weight concentration, were heated in a Cannon Instrument Company (State College, PA) constant temperature bath. The determinations were carried out at 60°C. Kinematic viscosity was calculated by multiplying the time in seconds by the viscometer constant at 60°C (0.00368). The dynamic viscosity was calculated by multiplying the kinematic viscosity by the density at 60°C.

Results and Discussion

Recovery of protein solely as a hydrolyzed product from the one-step process

Not all chromium containing leather waste is the same. Tanneries use different processes to tan the 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 preliminary 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 reaction products. Thus, it is important to know the chemical composition of the isolated chrome cakes.

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

TABLE I
Analyses of chrome shavings^a

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

^a Chrome shavings A came from a conventional chrome tannage, B, from a high exhaust chrome tannage, and C, from a tannage in which the final pH was slightly more acidic (3.6) than other chrome offal investigated (3.8-4.2).

^b N = 3 where N = number of replicates for each sample.

^c Moisture free basis.

^d Total Kjeldahl nitrogen.

^e Protein content can be estimated by multiplying TKN by 5.11.

0.1 to 1.8%. Calcium values ranged from 0.34 to 0.48% and magnesium from 0.08 to 0.33%.

The chemical compositions of the chrome cakes recovered from one-step treatments 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. Because the cakes were not washed during filtration, the nitrogen content reflects the hydrolyzed protein that remains and the amount is dependent on the efficiency of the filtration process. 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 MgO.

The isolated protein hydrolysates were analyzed for chromium, total Kjeldahl nitrogen (TKN), total solids and ash (Table II). Data 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 in protein from pilot studies, and also in the protein solutions recovered from industrial scale trials. The TKN, total solids and total ash averaged about 11 000 ppm, 72 000 ppm and 8000 ppm, respectively. The molecular weight distribution of the hydrolyzed protein ranged from 1000-3000. Amino acid analyses for each of the dried protein samples are shown in Table III. When the amino acid 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 enzymatically hydrolyzed. In this treatment the alkali pre-treatment 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 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 to almost one-fifth.

TABLE II
Analyses of products isolated from the one-step treatment of chrome shavings

Parameter (%) ^b	Chrome cake ^a			
	A	B	C-1	C-2
Moisture	85.42 ± 0.17	85.54 ± 0.22	82.93 ± 0.60	82.53 ± 0.04
Ash ^c	35.45 ± 0.08	32.55 ± 0.49	34.14 ± 0.83	36.99 ± 0.38
Chromic Oxide ^c	7.76 ± 0.30	11.82 ± 0.54	8.74 ± 0.10	11.44 ± 0.03
TKN ^{c,d,e}	7.51 ± 0.09	8.40 ± 0.66	6.66 ± 0.24	8.09 ± 0.55
Fat ^c	1.37 ± 0.10	6.31 ± 0.38	4.26 ± 0.07	4.93 ± 0.06
Calcium ^c	0.35 ± 0.01	0.82 ± 0.02	0.75 ± 0.06	1.18 ± 0.08
Magnesium ^c	9.96 ± 0.12	5.00 ± 0.06	9.47 ± 0.16	6.73 ± 0.22

Protein hydrolysate			
<i>Protein hydrolysate (liquid)^f</i>			
Chromium		(AV)	<1
TKN		(AV)	11 000
Total Solids		(AV)	72 000
Total Ash		(AV)	8 000
<i>Protein hydrolysate (dried)^g</i>			
TKN			13.8–15.0
Ash			9.7–18.9
Molecular weight distribution ^h			1000–3000

^a Chrome cake A isolated from chrome shavings treated with 575 g MgO, B, from shavings treated with 345 g NaOH and 230 g MgO, C-1, from shavings treated with 690 g MgO, and C-2, from shavings treated with 345 g NaOH and 345 g MgO.

^b N=3 where N=number of replicates for each sample.

^c Moisture free basis.

^d Total Kjeldahl nitrogen.

^e Protein content can be estimated by multiplying TKN by 5.51.

^f Expressed in PPM.

^g Expressed as percent.

^h Daltons.

Recycling of the protein solution containing the enzyme was attempted. The enzyme was still active after being subjected to high temperatures and pH 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 high and the enzyme lost its activity. A 1% concentration of the enzyme initially is recommended if one is recycling.

TABLE III
Amino acid composition of hydrolyzed protein^a

Residue	Collagen (Type I) ^b	Hyrolyzate	Std. Dev. ^c
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	

^a Expressed as mole percent.

^b Piez, K.A., *Extracellular Matrix Biochemistry* (Piez, K.A. and Reddi, A.H. eds.), Elsevier, New York, (1984), p 1.

Recovery of gelable and hydrolyzed protein products from the two-step process

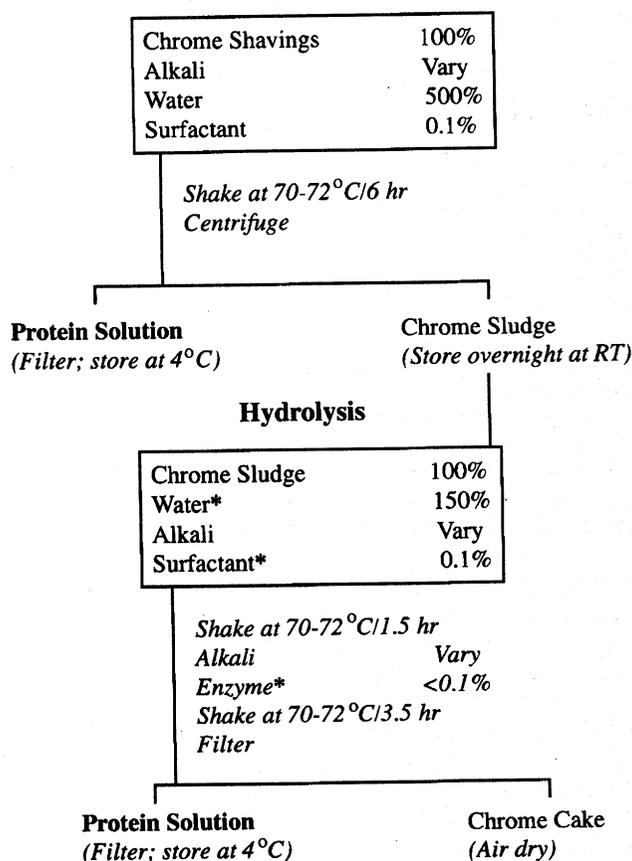
The next important step in the investigations was to obtain a higher molecular weight protein fraction than was previously isolated. The original one-step process gave a re-cyclable chromium product but also gave a low value protein hydrolysate that could possibly be used as animal feed and fertilizer. Even though we 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.⁵¹ 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 enzymatic treatment of the remaining chrome sludge. A filterable and recyclable chromium product would also be obtained.

Fig. 1 is a flow diagram of the new process. After isolation of the gelable protein product by filtration, the chrome sludge is prepared for enzymatic hydrolysis. The pH is measured and adjusted if necessary for optimal enzyme activity and the alkaline protease is added. The reaction is carried out for 3.5 h. If one has whole splits or large trimmings, chipping or grinding is recommended before the first step is carried out. These splits and trimmings have been dissolved in their intact state, with 1% enzyme or less, but the protein product is low molecular weight.

In the one-step process, the chromium containing waste was pre-treated with a variety of alkalis at

Gel Extraction



*Based on weight of chrome shavings

Figure 1. Outline of two-step procedure for treatment of chromium-containing solid leather waste.

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 MgO, Ca(OH)₂ and various combinations of MgO, NaOH, Na₂CO₃ and Ca(OH)₂ 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.^{60–61}

The effect that MgO alone and in combination with varying amounts of NaOH, Na₂CO₃, KOH and K₂CO₃ has on the chemical and physical properties of the gelable and hydrolyzed protein products has been investigated. As has been shown in previous publications,^{41–42} careful control of the concentrations of the alkali-inducing agents will give 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 used in these experiments had a pH range of 3.95–4.00. The concentrations of alkalis to be added were arrived at experimentally in small bench trials prior to larger scale runs.

Table IV 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 can range

TABLE IV
Characterization of protein products from the two-step process

Parameters	Gelable Protein	Hydrolysed Protein
Total solids	1.75–4.00%	6.00–9.50%
Moisture	4.00–13.00%	—
Ash ^a	8.90–20.00%	3.30–7.70%
Chromium ^a	0.005–0.013%	0.0005–0.005%
Molecular weight distribution ^b	75 000–>200 000	10 000–20 000
Bloom value ^c	34–200	—

^a Moisture-Free Basis (MFB).

^b Daltons.

^c Grams.

from 1.75 to 4%. 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 used. The Bloom values, or gel strengths, range from 34 to 200 g.

Table IV also summarizes the properties of the hydrolyzed protein products, 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 one-step process; this reflects the small amount of enzyme used in the sludge digestion. Also shown are the range of total solids and total ash content of the protein solutions along with the range for the chromium concentration. The total ash content of the hydrolyzed protein products is much lower than those found in the one-step 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 ash in the protein products will effect the marketing of these products, the use of different proportions of alkaline agents on the ash content of the protein products was investigated.^{46,62–65} Increasing proportions of MgO resulted in lower ash content.

We have shown that the choice of alkali affects the physical properties of the gelable protein fraction.⁴⁸ We also demonstrated (Table V) that treating a gelable protein fraction with a mixed-bed deionizing resin, significantly lowered the ash content and improved the physical properties, such as the Bloom and viscosity, of the extracted gel. This improvement could be a function of the increased protein concentration or lack of interference from the salt or a combination of these parameters. To better understand the effect of protein concentration and even the choice of alkali on the physical properties of the gelable protein products, an experiment was designed in which various alkalis were used to extract gelable protein from the chrome waste (Fig. 1). Then, portions of each of the solutions were deionized using a mixed-bed deionizing resin. The untreated and deionized solutions were lyophilized. Solutions of each of the dried samples were prepared at 6.67% (w/w) concentrations. Bloom, viscosity and density determinations were run on the samples. From these data, an interpretation concerning the effect of protein concentration and choice of alkali was made.

TABLE V
Effect of deionization on physical properties of gelable protein

Alkali Treatment	Protein conc (w/w)	Bloom (g)	Dynamic viscosity (cP) ^a	Density ^a
<i>Before Deionization^b</i>				
5%MgO-1%K ₂ CO ₃	5.60	97.7	2.2745	1.0094
4%MgO-2%K ₂ CO ₃	5.64	106.4	2.3373	1.0082
5%MgO-1%Na ₂ CO ₃	5.51	108.2	2.2450	1.0106
4%MgO-2%Na ₂ CO ₃	5.69	100.5	2.3331	1.0108
4%MgO-1%KOH	5.55	94.2	2.2236	1.0087
3%MgO-2%KOH	5.53	68.7	1.9550	1.0116
4%MgO-1%NaOH	5.66	70.0	2.1662	1.0082
3%MgO-2%NaOH	5.84	34.0	1.6490	1.0092
<i>After Deionization^{bc}</i>				
5%MgO-1%K ₂ CO ₃	6.63	178.3	3.1556	1.0039
4%MgO-2%K ₂ CO ₃	6.65	167.2	3.0809	1.0045
5%MgO-1%Na ₂ CO ₃	6.64	200.8	3.0959	0.9997
4%MgO-2%Na ₂ CO ₃	6.65	182.0	3.0324	1.0010
4%MgO-1%KOH	6.64	180.3	2.9109	1.0014
3%MgO-2%KOH	6.61	149.5	2.5094	1.0015
4%MgO-1%NaOH	6.66	89.1	2.6284	1.0012
3%MgO-2%NaOH	6.64	46.0	1.9426	1.0048

^a @ 60°C.

^b 6.67% (w/w) solutions.

^c These samples were first deionized and then a 6.67% (w/w) solution was examined.

In this present study the samples were batch-deionized. The ash content of all alkali treated samples decreased significantly to less than 1% and so the values are within the 0 to 3% range reported for technical grade gelatin.⁵¹ Physical tests were run on all samples before and after deionization and the results can be seen in Table V. The effects of deionization on the physical properties of gels extracted with various alkalis are shown. Bloom values increased 30% to 100% in the deionized samples where the concentration of the protein itself approached 6.67% (w/w). The viscosity in the deionized samples increased 20% to 40%, whereas the deionized samples have lower densities as would be expected.

Table V shows the effects of deionization on the physical properties of gels extracted with MgO-K₂CO₃ and MgO-Na₂CO₃ mixtures. The choice of sodium or potassium carbonate has little effect on either viscosity or density of the gelable protein fractions. The Bloom values are similar, but the Na₂CO₃ extracted gels appear to have slightly higher values than the K₂CO₃ extracted gels.

Table V also shows the effect of deionizing MgO-KOH and MgO-NaOH gel samples. The Bloom values of the untreated NaOH samples (70.0, 34.0) are lower than found in a previous study⁴⁸ where the Bloom values for the 4% MgO-1% NaOH and 3% MgO-2% NaOH samples were 91.3 and 74.1, respectively. These reactions were run again and the Bloom value for the 4% MgO-1% NaOH sample was 79.1, which is midway between the other two runs. The sample that had been extracted with the higher concentration of NaOH, however, had an even lower Bloom value of 31.0. When the third set of samples were deionized, the 4% MgO-1% NaOH sample increased to 116 but the higher hydroxide sample fell to 28.1. These data indicate the difficulty of

TABLE VI
Effect of deionization on physical properties of hydrolyzed protein^a

Parameter	Protein conc.(w/w)	Jellygram (g) ^b	Dynamic viscosity(cP) ^c	Density ^c
Before deionization	12.0	12.8	2.2260	1.0254
After deionization ^{bd}	12.4	15.1	2.6278	1.0268

^a Alkali treatment: 4% MgO-1% KOH

^b 12.5% (w/w) solution.

^c @ 60°C.

^d These samples were first deionized and a 12% (w/w) solution was examined.

controlling the reaction when NaOH is used. Lack of control leads to more degraded gelable protein with poorer physical properties.

Table V also shows the effect of deionization on gelable protein samples isolated from a MgO-KOH extraction. The Bloom values for the untreated samples are slightly lower than those examined in a previous study but are not as low as those found when NaOH was used. When the deionized samples with the high protein concentration were examined, we found that the increase in the Bloom value was significant. The NaOH samples were apparently too degraded, so that even at a higher protein concentration no improvement could be seen. This phenomenon was also observed in the product isolated by enzymatic hydrolysis, as shown in Table VI. If NaOH is used, it should only be used at low concentrations if one wants to obtain a higher quality product.

We designed an experiment to see if a gelable protein could be isolated from the chrome sludge after it was treated with an enzyme. In this experiment, the chrome shavings were first treated with 4% MgO-1% KOH to extract the gel, only 0.0125% enzyme was used to treat the sludge. Indeed, a rather viscous, clear, gelable-like solution was isolated. The Jellygram value (similar to the Bloom value except that a 12.5% (w/w) concentration is used⁵¹) of this sample was determined. Jellygram values are typically four times the Bloom values and are used to evaluate hide glues. As shown in Table VI, the value obtained was quite low and did not change after deionization. However, the viscosity of this deionized sample was increased by 16%. These data indicate that the product will not give an increased gel strength at the higher protein concentration but will give a higher viscosity, suggesting that the structure of the protein has been degraded. Rose⁵¹ reported, in his chapter, "Inedible Gelatin and Glue", that there are gelatins produced in the United States that have very low or no gelling ability but do have a variety of applications. We have demonstrated that a higher quality gel-like hydrolyzed product can be obtained from the chromium sludge with careful control of the enzyme concentration.

An experiment was designed to determine the repeatability of the two-step process with respect to chemical and physical analyses and material balance and yields. MgO was the alkali used to extract the gelable protein. Fig. 1 shows a general description of the process. After the treatment to extract the gelable protein, the reaction mixture was centrifuged to separate the protein solution from the chrome sludge. This centrifugation was necessary for these bench scale experiments because of the difficulty in filtering the viscous solutions. In pilot and industrial scale operations, a filter press or a continuous centrifugation apparatus may be appro-

TABLE VII
Repeatability of process using 6% MgO as alkali

Parameter(%)	Run No. 1	Run No. 2	Run No. 3	Run No. 4	Run No. 5	Run No. 6	Average	Std. Dev.
Gel extraction step								
Material balance	94.8 ^a	98.0 ^a	97.2 ^a	98.5 ^a	96.9 ^a	97.8 ^a	97.2 ^a	1.19
<i>Chrome sludge</i>								
Moisture	83.71	84.01	83.72	83.80	83.61	84.20	83.84	0.20
Ash (MFB)	20.10	20.36	20.74	21.21	20.90	21.65	20.83	0.51
<i>Gelable protein</i>								
Total solids	2.71	2.98	2.67	3.12	2.78	2.96	2.87	0.16
Total ash (MFB)	9.92	9.23	11.88	10.55	10.25	9.48	10.22	0.86
Gel yield ^b	32.14	37.00	32.66	39.30	35.05	38.18	35.72	2.68
Hydrolysis step								
Material balance	92.2	92.9	91.1	91.5	95.5	95.1	93.0	1.69
<i>Chrome cake</i>								
Moisture	80.73	80.79	79.71	80.65	79.34	80.10	80.22	0.55
Ash (MFB)	34.58	35.52	35.18	35.59	35.90	36.61	35.56	0.62
<i>Hydrolysed protein</i>								
Total solids	6.07	5.80	5.85	5.70	6.12	5.92	5.91	0.15
Total ash (MFB)	3.03	3.18	3.09	3.47	3.10	3.27	3.19	0.15
Hydrolyzed prot. yield ^b	59.96	58.14	59.12	58.34	62.67	60.08	59.72	1.51
Total protein recovery ^b	77.55	79.12	74.84	77.35	80.67	77.96	77.92	1.78
Ash recovery	87.92	85.57	104.2	102.6	100.2	100.2	96.78	7.26

^a All values expressed as percent.

^b Based on 92.3% protein (moisture-free, ash-free basis) in chrome shavings.

TABLE VIII
Repeatability of chemical and physical properties in a two-step process using 6% MgO as alkali

Parameter	Run No. 1	Run No. 2	Run No. 3	Run No. 4	Run No. 5	Run No. 6	Aver.	Std. Dev.	Mixture
Gel extraction step									
<i>Chemical properties</i>									
Total solids ^a	2.71	2.98	2.67	3.12	2.78	2.96	2.87	0.16	2.87
Total ash (MFB) ^a	9.92	9.23	11.88	10.55	10.25	9.48	10.22	0.86	11.18
TKN (AFB) ^a	15.80	15.95	16.60	16.83	17.04	16.79	16.50	0.46	17.11
Chrome (ppm)	8.70	6.25	8.05	8.75	6.10	5.45	7.22	1.33	8.30
<i>Physical properties</i>									
Bloom (g)	129.5	117.7	105.0	83.3	90.3	90.7	103.6	15.5	106.5
Dyn. visc. (cP)	2.7219	2.5679	3.4821	3.2799	2.6057	2.2991	2.8261	0.4164	2.5542
Density	1.0080	1.0090	1.0101	1.0090	1.0079	1.0051	1.0082	0.0016	1.0040
Hydrolysis step									
<i>Chemical properties</i>									
Total solids ^a	6.07	5.80	5.85	5.70	6.12	5.92	5.91	0.15	5.84
Total ash (MFB) ^a	3.03	3.18	3.09	3.47	3.10	3.27	3.19	0.15	3.42
TKN (AFB) ^a	17.80	18.15	17.90	8.38	17.79	18.34	18.07	0.24	18.50
Chrome (ppm)	0.35	1.05	0.75	0.65	0.80	0.70	0.72	0.21	1.05
<i>Physical properties</i>									
Bloom (g)	—	—	—	—	—	—	—	—	—
Dyn. visc. (cP)	0.9559	0.9220	1.1634	1.0042	0.8605	0.8732	0.9632	0.1018	0.9022
Density	1.0111	1.0064	1.0102	1.0091	1.0059	1.0096	1.0087	0.0019	1.0076

^a All values expressed as percent.

prate. After the enzymatic treatment, filtration was easy and proceeded quickly. Table VII shows the material balances and yields from 6 experiments. Also shown are the average values and the standard deviation. The data indicate the high repeatability of the process.

Total solids, total ash, TKN and chromium determinations were performed on the isolated protein fractions. Table VIII summarizes the data collected from the analyses of the extracted gelable protein. These results were tabulated from 6 separate experiments and for the mixture of all. For each parameter the average and standard deviation were calculated. The repeatability of

the analyses in these experiments was quite good, as indicated by the standard deviation and comparing the 6 single experiments with the mixture. Also examined were Bloom, dynamic viscosity and density (Table VIII). The repeatability of viscosity and density were quite good. Although the standard deviation for Bloom was high, the average Bloom was very close to the Bloom of the mixture.

The chrome cake may be treated chemically, using a reported method,²⁸ to give a recyclable chrome product. Table IX reports the percent residue that remains after the chrome cake is chemically treated. Residues of

TABLE IX
Characterization of residue from treatment of chrome cake

Sample	Final pH	% Residue ^a	% Ash ^b
1	1.84	9.13	0.37
2	1.85	6.33	0.18
3	1.84	7.33	0.28
4	1.85	7.46	0.25
5	1.85	10.14	0.23

^a % residue in chrome cake, Moisture-Free Basis (MFB).

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

samples (1) through (5) were also analyzed for the percent non-chrome insoluble ash. The low ash content indicates that the bulk of the residue is organic, i.e. residual protein and/or the resins that are used in the high exhaust chrome tannages.

To develop an economic profile of these processes, a variety of costs, savings and returns must be considered. The cost estimate will be unique for each potential processor. We would expect the costs of the chemicals and equipment to be similar for all, but energy and labour costs will be specific to each location. Savings will depend on the costs of current tipping fees or other disposal arrangements as well as on the extent to which the recovered products may be used on site. Return on the processes will depend on the quality of products and the development of suitable markets. The cost of the additional separation step to obtain a gelable protein should be more than balanced by the higher value of this product.

Conclusions

High quality gelable and hydrolyzed protein products can be isolated from chromium containing leather waste. Depending on the choice of alkali and the starting material, the process can be varied to give an end product, with a desirable 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 level 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. We have demonstrated that the products of these processes are repeatable in their chemical and physical properties and that good and repeatable material balances are obtained. 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. Suggestions for the development of an economic profile are presented and show that a profit can be achieved if the new two-step process is run and the enzyme is re-cycled.

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