

PROCESSING OF LEATHER WASTE: PILOT SCALE STUDIES ON CHROME SHAVINGS. PART I. ISOLATION AND CHARACTERIZATION OF PROTEIN PRODUCTS AND SEPARATION OF CHROME CAKE

ABSTRACT

During the past years, we have demonstrated that it is possible to isolate protein products from chrome shavings, a waste from the tannery industry, using alkaline protease under mild conditions. This process has been patented and broadly described, though the treatment of the chrome cake has not been described in detail. The objective of this present work was to isolate protein products from chrome shavings, treat and purify the remaining chrome cake and tan hides with the recovered chromium. This first part discusses the digestion of commercial chrome shavings – with an alkali in a first step and with an alkaline protease in a second step – to isolate two different protein products: gelatin and hydrolysate. Chemical and physical properties of these samples were studied. Gelatin samples were deionized and chemical and physical analyses performed. Also, chemical properties of the solid residue from each step of the process were evaluated.

INTRODUCTION

Tanners handle the by-products, hides and skins, from the meat industry. Traditionally, bad odors, production of organic wastes and high water consumption were the factors contributing to the industry's reputation as highly polluting. To transform hides and skins from animals sacrificed for meat into leather, the tanner consumes water and chemicals; and produces wastewater and solid waste with the final leather.^{1,2}

In the leather industry it is accepted that 1 ton of wet salted hide yields only 200 kg of leather (about 20% of the initial hide weight) but over 600 kg of solid waste (over 60% of the initial hide weight). The waste consists of water, salt, unwanted skin proteins, hair, fat and surplus chemicals used in processing the raw hide into finished dry leather. Other wastes include off-cuts in trimming the hide to shape and in attaining thickness specification by splitting, shaving, or buffing, etc.³

Some of the waste may be saleable, but the remainder must be disposed. This may be a difficult and expensive task as

these wastes are considered undesirable in many environments due to their smell, noxious nature or adverse effect on the surrounding land or water and the local flora and fauna.³ Increased local restrictions on land disposal have encouraged the tanning industry to explore innovative methods to treat such solid waste products as shavings, trimmings and splits. Many papers have been published that describe how to treat or simply use these materials.⁴

During the past years, we have demonstrated that it is possible to isolate protein products from chrome shavings using alkaline protease under mild conditions. This process has been patented and broadly described,^{4,5} and has been used worldwide with some modifications.^{6,7,8} Although most of the experiments reported were performed on lab scale and their reproducibility was demonstrated,⁹ some pilot plant and industrial trials have also been done.¹⁰ The quality of the isolated protein products, gelatin and hydrolysate, has been studied and even functional properties have been described.^{11,12} Only the treatment of the chrome cake has not been described in detail. The objective of this present work was to isolate protein products from chrome shavings, treat and purify the remaining chrome cake and tan hides with the recovered chromium.

In this first part of this two-part report, we describe how commercial chrome shavings were digested – with an alkali in the first step and with an alkaline protease in the second – to isolate two different protein products: gelatin and hydrolysate. Chemical and physical properties, such as total solids, ash, chrome, and nitrogen content, gel strength, viscosity and density of these isolated samples, were studied. Gelatin samples were deionized and chemical and physical analyses were performed as described in a previous paper.¹³ Also, chemical properties, such as moisture, ash, chrome, nitrogen and fat content, of the solid residue from each step of the process were evaluated.

In the second part, we will describe how we purified the chrome cake remaining from the isolation process and how we then used the recovered chromium to tan hides using a matched sides comparison process.

EXPERIMENTAL

Materials

Chrome shavings were obtained from a commercial tannery in two different drums and kept at room temperature. Alcalase® (alkaline protease) was from Novo Nordisk, Inc. (Franklinton, NC); the solution form, Liquid Alcalase®, was

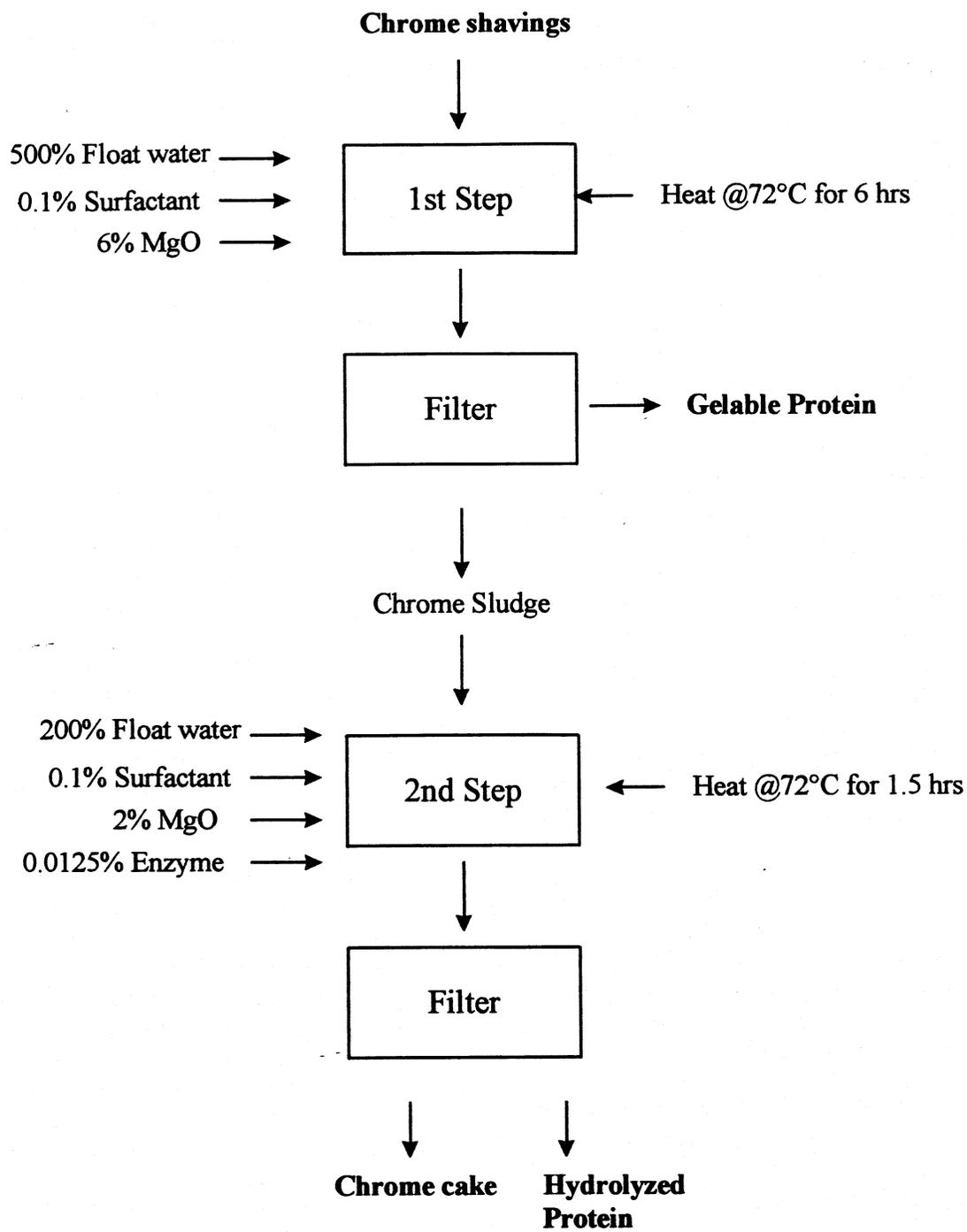
used in these experiments. Pluronic 25R2®, a non-ionic surfactant, was from BASF (Parsippany, NJ). Magnesium oxide was from Fisher Scientific (Fair Lawn, NJ).

Procedures

Isolation of protein products and chrome cake. The diagram of the procedure used can be found in Figure 1. In the first step, chrome shavings (22.65 kg; well mixed from the two drums) were tumbled in tap water (114 kg; 500% float), a non-ionic surfactant (25 g; 0.1%; added only in the first two batches) and MgO (1.36 kg; 6%) at 72°C for 6 hours in a Dosemat drum (Dose Maschinenbau GmbH, Lichtenau, Germany) at 16 rpm. The reaction mixture was then filtered warm through a conventional filter press (Model AA Manual Filter Press, Serfilco, LTD., Glenview, IL). After filtering, samples were taken from the chrome sludge and from the gelable protein. The gelable protein was discharged and the chrome sludge was kept in a covered bucket at room temperature for further treatment. In the second step, the chrome sludge was tumbled with tap water (46 kg; 200% float), a non-ionic surfactant (25 g; 0.1%), MgO (470 g; 2%) and enzyme (2.84 g; 0.0125%) at 72°C for 1.5 hours at 16 rpm. The reaction mixture was then filtered warm through a conventional filter press. After filtering, samples were taken from the chrome cake and from the hydrolyzed protein. The hydrolyzed protein was discharged and the chrome cake was kept in a covered bucket at room temperature for further treatment.

Filter Press Operation. The capacity of the filter press was less than that of the drum, so that at the end of each run the material from the drum was filtered in two batches and labeled "Filtration 1" and "Filtration 2." After each filtration, before stopping the filter press, water was introduced to wash out the system. All material discharged from the filter after addition of wash water was kept in a separate container labeled "Wash." Finally, pressurized air was forced through the cake to remove as much water as possible. Material removed in this step was labeled "Exhaustion."

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



% based on initial weight of chrome shavings

FIGURE 1. — Flow diagram of the procedure for treatment of chrome shavings.

Analyses

For moisture determination, the samples were weighed into dry, tared porcelain dishes. The samples were dried for 17 hrs at 105°C. The samples were cooled in a desiccator, weighed and the percent moisture determined. For ash determination, the dried samples were ashed at 600°C for two hours. The samples were cooled in a desiccator and weighed to determine ash content. These analytical methods were described previously.¹⁴

Chromium was determined using a Perkin Elmer Atomic Absorption Spectrophotometer, Model 3300 (Norwalk, CT).¹⁵ Solid samples were weighed into appropriate flasks that were fitted with standards joints, 2N HCl (75 mL) was added, and the samples were hydrolyzed for 4 hrs. The hydrolyzed samples were filtered into volumetric flasks and made up to volume and diluted so that the chromium concentration would be between 1 and 10 ppm. Percent chromium and percent chromium oxide from original weight were calculated. For liquid samples, the solutions were well mixed and chromium was determined by aspirating the solution, original or diluted, directly into the flame of the Atomic Absorption Spectrophotometer. For gelatin samples, freeze-dried samples were dried for 17 hrs at 105°C. The samples were weighed into test tubes, 2N HCl (10 mL) was added, and the samples were hydrolyzed for 4 hrs. These hydrolyzed samples were treated the same as the hydrolyzed solids.

Total Kjeldahl Nitrogen (TKN) was determined by the semi-micro Kjeldahl method. Solid samples were weighed to the nearest 50 mg and liquid samples were measured to 1 mL and transferred to a 30 mL digestion flask. Digestion catalyst (1.2 g), a few boiling chips and sulfuric acid (2 mL) were added. The samples were digested for two hours. The samples were carefully transferred to the filling funnel and NaOH solution (10 mL) was added. The mix was distilled to a 125 mL Erlenmeyer flask containing boric acid saturated solution (10 mL). The samples were titrated with standardized HCl to the gray endpoint.

For fat determination, samples were weighed into appropriate flasks and 6N HCl (75 mL) was added. The samples were hydrolyzed for 2 hrs. The hydrolysate was transferred to a separatory funnel and the fat was extracted with chloroform. The chloroform layer was put in dry, tared crystallizing dishes, the chloroform was evaporated and the samples held at 60°C for 16 hrs. The samples were cooled in a desiccator and then weighed.

Gel strengths were measured by Bloom determinations with a TA-XT2 Texture Analyzer from Texture Technologies

Corporation (Scarsdale, NY).⁹ Dried gelatin (2.5 g) was weighed into a 39 mm internal diameter jar and water (35 mL) was added to give 6.67% w/w concentration. The gelatin and water were allowed to stand for a set period of time (10 minutes to overnight) until total water absorption, then heated in a 65°C bath for 15 min, cooled at room temperature for 15 minutes and kept in a 10°C bath for 17-18 hrs. The sample was placed under a 1.27 cm (0.5 inch) diameter analytical probe which was driven into the sample to the depth of 4 mm, at 1 mm per sec. The measured grams force corrected with the factor 1.398 is expressed as the Bloom value.

Viscosities were measured in a Cannon Manning viscosimeter.¹⁶ The samples, at 6.67% w/w concentration, were heated in a Cannon Instrument Company (State College, PA) constant temperature bath, and held at 60°C. Kinematic viscosity was calculated by multiplying the time, in seconds, by the viscosimeter constant (0.00368). The dynamic viscosity was calculated by multiplying the kinematic viscosity by the solution density at 60°C.

Protein molecular weights were estimated by SDS-PAGE (polyacrylamide gel electrophoresis in sodium dodecyl sulfate) using a Phast-Gel System by Pharmacia Biotech Inc. (Piscataway, NJ).¹⁷ Individual gels are 43 x 50 mm and are precast with a 13 mm stacking gel (4.5% acrylamide) and 32 mm separating gel containing a continuous 4-15% polyacrylamide gradient on a polyester backing. For a calibration standard the Bio-Rad (Richmond, CA) broad range SDS-Standard (BRM), which contains a mixture of eight proteins ranging in size from aprotinin at 7,200 Da to myosin at 208,000 Da, was used. Samples of lyophilized protein (1 mg), were dissolved in sample buffer (50 µL; 10 mM Tris-HCl at pH 8.0 containing 1 mM EDTA, 2.5% SDS, 5% β-mercaptoethanol and 0.01% bromphenol blue) and heated at 40°C for 4 hrs. An eight slot applicator was used to load 1.2 µL of each sample and the standard. Separation required about 30 minutes and was achieved at 250 V, 10.0 mA, and 3.0 W for 65 Vh at 15°C in the Phast System apparatus. Gels were stained with Coomassie Blue (Pharmacia) following the manufacturer's directions. The gels were scanned with a Personal Densitometer SI and analyzed using ImageQuaNT v:4.1 software from Molecular Dynamics, Inc. (Sunnyvale, CA).

RESULTS AND DISCUSSION

The process used in this paper has been studied for several years and several patents have been issued.^{5,9} The repeatability of this process was studied at lab scale with respect to

TABLE IX
Physical Properties of Gelatin

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>Gel Strength</i> ^{a,b,c}						
Filtration 1	—	81.8	83.3	73.2		
Filtration 2	—	55.5	69.5	68.4		
Average	51.7	68.7	76.4	70.8	66.9	10.6
<i>Dynamic viscosity</i> ^{a,c,d,e}						
Filtration 1	—	2.4144	3.2987	1.9077		
Filtration 2	—	2.2900	1.9790	2.3208		
Average	1.7446	2.3522	2.6389	2.1143	2.2125	0.3785
<i>Density</i> ^{a,c,d}						
Filtration 1	—	0.9981	1.0116	1.0187		
Filtration 2	—	1.1470	1.0061	1.0111		
Average	0.9955	1.0726	1.0089	1.0149	1.0230	0.0340

a N = 1 where N = number of replicates for each sample.

b g Bloom.

c 6.67% (w/w) solution.

d @ 60°C.

e cP.

Figure 2 and Table XV present the molecular weight distribution of the protein products isolated in the process. The percentages of the different molecular weight range distributions of gelatin and wash of gelatin are very similar, showing no significant differences, but the two samples analyzed of the exhaustion of the gelatin present different values, indicating that this step of the process helps in the separation of the gelatin from the chrome sludge differently in each batch. However, the samples of hydrolyzed protein were reproducible from one batch to the other. These results demonstrate that very different ranges of molecular weight distribution of collagen degradation products can be evaluated by SDS-PAGE.

Four gelatin samples, one from each batch, were deionized batchwise. The ash content of the samples decreased significantly to 1% or less and so the values are within the 0 to 3% range reported for technical grade gelatin.¹⁸ Chemical (Table XVI) and physical (Table XVII) tests were run on all samples before and after deionization. The pH of the gelatin decreases until the isoionic point of gelatin is reached, at which point deionization was complete as shown in Table XVI. The total solids showed a slight decrease as expected from the removal of the ash. The values of chrome content reported in Table XVI on a

moisture free basis are higher after deionization than before, because though the chrome content is lower after deionization, at this point all the ash of the gelatin is chrome. The gel strength of the gelatin increased an average of about 135% and the viscosity about 22%, whereas the density decreased.

Tables XVIII, XIX, XX and XXI summarize the data collected from the chemical analyses of the solid products. These results were tabulated for four different batches. For each analysis the mean and the standard deviation are given and for each parameter the average and the standard deviation of the four batches were calculated. The analyses *per se* and the repeatability of the analyses in these experiments were quite good, as indicated by the standard deviations.

The moisture of the chrome cake was higher than the moisture of the chrome sludge because the latter was much easier to filter due to its fibrous nature. As one would expect, the ash content of the chrome cake increases and the TKN decreases because protein has been removed from the chrome sludge in the enzymatic step of the procedure. The fat and the chrome content are the same for chrome sludge and chrome cake, showing that they remain in the chrome and are not removed with the protein.

TABLE I
Analyses of Chrome Shavings^a

Parameter	A	B
<i>pH</i>	3.45 (0.01) ^{b,c}	3.87 (0.05) ^{b,c}
<i>Moisture</i> ^d	53.12 (0.62)	50.52 (0.54)
<i>Ash</i> ^{d,e}	10.33 (0.40)	10.52 (0.29)
<i>TKN</i> ^{d,e,f}	16.45 (0.72)	16.59 (2.74)
<i>Cr₂O₃</i> ^{d,e}	3.27 (0.38)	3.01 (0.24)
<i>Fat</i> ^{d,e}	0.65 (0.06)	0.86 (0.17)

a Chrome shavings A and B came from a conventional chrome tannage in two different drums.

b Mean (Standard Deviation).

c N = 3 where N = number of replicates for each sample.

d Expressed as percentage.

e Moisture free basis.

f Ash free basis.

TABLE II
Repeatability of Pilot Plant Process

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>Gel extraction step</i>						
Material Balance ^a	85.52	95.95	97.80	92.60	92.97	5.41
Gelatin Yield ^a	37.88	42.32	41.99	39.68	40.97	2.80
<i>Hydrolysis step</i>						
Material Balance ^a	84.89	83.60	92.89	92.02	88.35	4.78
Hydrolysate Yield ^a	43.49	49.27	49.28	42.22	46.07	3.74
Total material balance ^a	83.01	89.47	95.26	91.17	89.73	5.09
Total protein recovery ^{a,b}	72.91	83.59	82.93	71.22	77.66	6.51
Ash recovery ^a	71.80	79.62	78.59	72.68	75.67	4.00

a Expressed as percentage.

b Protein recovered as gelatin and hydrolysate.

TABLE VI
Chemical Properties of Hydrolysate

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>pH</i>						
Filtration 1	9.66	9.69	9.80	9.38		
Filtration 2	9.78	9.60	9.64	9.31		
Average	9.72	9.65	9.72	9.35	9.61	0.18
<i>Total solids</i> ^{a,b,c}						
Filtration 1	5.13 (0.01)	5.47 (0.04)	5.22 (0.07)	4.64 (0.02)		
Filtration 2	4.73 (0.01)	5.09 (0.02)	4.90 (0.03)	4.39 (0.01)		
Average	4.93	5.28	5.06	4.52	4.95	0.32
<i>Total ash</i> ^{a,b,c,d}						
Filtration 1	4.65 (0.08)	4.32 (0.05)	3.79 (0.07)	4.81 (0.08)		
Filtration 2	4.65 (0.04)	4.20 (0.08)	3.84 (0.03)	4.72 (0.01)		
Average	4.65	4.26	3.82	4.77	4.37	0.43
<i>TKN</i> ^{a,b,c,d,e}						
Filtration 1	21.14 (0.40)	18.80 (0.13)	19.75 (0.26)	18.19 (0.68)		
Filtration 2	16.36 (0.42)	19.89 (0.61)	19.00 (0.42)	18.97 (0.31)		
Average	18.75	19.35	19.38	18.58	19.01	0.41
<i>Chromium</i> ^{a,b,d,f}						
Filtration 1	2.0 (0.7)	14.7 (0.5)	6.5 (1.9)	25.5 (2.6)		
Filtration 2	10.4 (0.6)	5.3 (1.9)	3.6 (0.1)	26.2 (3.9)		
Average	6.2	10.0	5.1	25.9*	7.10	2.57

a Mean (Standard Deviation).

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

c Expressed as percentage.

d Moisture free basis.

e Ash free basis.

f ppm.

* Not included in average.

TABLE IV
Chemical Properties of Wash of Gelatin in Filter Press

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>pH</i>						
Filtration 1	—	8.73	9.18	9.22		
Filtration 2	—	9.12	9.20	9.28		
Average	9.26	8.93	9.19	9.25	9.16	0.16
<i>Total solids</i> ^{a,b,c}						
Filtration 1	—	2.08 (0.26)	2.26 (0.00)	2.32 (0.01)		
Filtration 2	—	2.11 (0.11)	2.06 (0.04)	2.89 (0.00)		
Average	2.18 (0.00)	2.10	2.16	2.61	2.26	0.23
<i>Total ash</i> ^{a,b,c,d}						
Filtration 1	—	18.59 (0.12)	19.87 (0.14)	20.79 (0.14)		
Filtration 2	—	19.16 (0.16)	20.43 (0.16)	19.62 (0.06)		
Average	18.85 (0.07)	18.88	20.15	20.21	19.52	0.76
<i>TKN</i> ^{a,b,c,d,e}						
Filtration 1	—	15.64 (0.28)	17.23 (0.62)	17.05 (0.44)		
Filtration 2	—	16.29 (0.52)	17.06 (0.19)	16.65 (0.05)		
Average	15.05 (0.54)	15.97	17.15	16.85	16.25	0.95
<i>Chromium</i> ^{a,b,d,f}						
Filtration 1	—	5.4 (0.7)	6.5 (0.8)	10.2 (3.5)		
Filtration 2	—	6.1 (0.2)	7.1 (1.7)	13.4 (5.5)		
Average	5.2 (0.9)	5.8	6.8	11.8	7.4	3.0

a Mean (Standard Deviation).

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

c Expressed as percentage.

d Moisture free basis.

e Ash free basis.

f ppm.

TABLE V
Chemical Properties of Exhaustion of Gelatin in Filter Press

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>pH</i>						
Filtration 1	—	8.14	8.24	8.18		
Filtration 2	—	7.87	8.13	8.43		
Average	9.32	8.01	8.19	8.31	8.45	0.59
<i>Total solids</i> ^{a,b,c}						
Filtration 1	—	0.97 (0.01)	1.52 (0.01)	1.52 (0.02)		
Filtration 2	—	0.90 (0.01)	0.57 (0.01)	2.16 (0.01)		
Average	3.69* (0.01)	0.94	1.04	1.84	1.27	0.49
<i>Total ash</i> ^{a,b,c,d}						
Filtration 1	—	25.53 (0.15)	24.52 (0.19)	26.29 (0.51)		
Filtration 2	—	28.22 (0.09)	34.91 (0.52)	22.74 (0.16)		
Average	16.90* (0.01)	26.88	29.72	24.52	27.04	2.60
<i>TKN</i> ^{a,b,c,d,e}						
Filtration 1	—	15.27 (1.09)	14.76 (0.29)	14.94 (0.49)		
Filtration 2	—	14.04 (0.66)	8.54*(0.05)	15.91 (0.12)		
Average	16.69 (0.52)	14.66	14.76	15.43	15.38	0.94
<i>Chromium</i> ^{a,b,d,f}						
Filtration 1	—	13.4 (1.4)	4.9 (0.0)	12.2 (1.0)		
Filtration 2	—	11.2 (0.0)	11.1 (0.1)	10.9 (0.3)		
Average	11.5 (0.7)	12.3	8.0	11.6	10.8	1.9

a Mean (Standard Deviation).

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

c Expressed as percentage.

d Moisture free basis.

e Ash free basis.

f ppm.

* Not included in average.

TABLE VI
Chemical Properties of Hydrolysate

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>pH</i>						
Filtration 1	9.66	9.69	9.80	9.38		
Filtration 2	9.78	9.60	9.64	9.31		
Average	9.72	9.65	9.72	9.35	9.61	0.18
<i>Total solids</i> ^{a,b,c}						
Filtration 1	5.13 (0.01)	5.47 (0.04)	5.22 (0.07)	4.64 (0.02)		
Filtration 2	4.73 (0.01)	5.09 (0.02)	4.90 (0.03)	4.39 (0.01)		
Average	4.93	5.28	5.06	4.52	4.95	0.32
<i>Total ash</i> ^{a,b,c,d}						
Filtration 1	4.65 (0.08)	4.32 (0.05)	3.79 (0.07)	4.81 (0.08)		
Filtration 2	4.65 (0.04)	4.20 (0.08)	3.84 (0.03)	4.72 (0.01)		
Average	4.65	4.26	3.82	4.77	4.37	0.43
<i>TKN</i> ^{a,b,c,d,e}						
Filtration 1	21.14 (0.40)	18.80 (0.13)	19.75 (0.26)	18.19 (0.68)		
Filtration 2	16.36 (0.42)	19.89 (0.61)	19.00 (0.42)	18.97 (0.31)		
Average	18.75	19.35	19.38	18.58	19.01	0.41
<i>Chromium</i> ^{a,b,d,f}						
Filtration 1	2.0 (0.7)	14.7 (0.5)	6.5 (1.9)	25.5 (2.6)		
Filtration 2	10.4 (0.6)	5.3 (1.9)	3.6 (0.1)	26.2 (3.9)		
Average	6.2	10.0	5.1	25.9*	7.10	2.57

a Mean (Standard Deviation).

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

c Expressed as percentage.

d Moisture free basis.

e Ash free basis.

f ppm.

* Not included in average.

TABLE VII
Chemical Properties of Wash of Hydrolysate in Filter Press

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>pH</i>						
Filtration 1	9.68	9.62	9.80	9.39		
Filtration 2	9.70	9.65	9.81	9.26		
Average	9.69	9.64	9.81	9.33	9.61	0.21
<i>Total solids</i> ^{a,b,c}						
Filtration 1	5.09 (0.03)	5.42 (0.03)	4.68 (0.01)	4.38 (0.02)		
Filtration 2	3.68 (0.03)	5.65 (0.01)	5.18 (0.01)	4.66 (0.00)		
Average	4.39	5.54	4.93	4.52	4.84	0.52
<i>Total ash</i> ^{a,b,c,d}						
Filtration 1	4.57 (0.09)	4.53 (0.51)	3.93 (0.07)	4.76 (0.16)		
Filtration 2	5.08 (0.04)	4.28 (0.05)	3.80 (0.04)	4.40 (0.04)		
Average	4.83	4.41	3.87	4.58	4.42	0.41
<i>TKN</i> ^{a,b,c,d,e}						
Filtration 1	20.54 (0.35)	19.69 (0.47)	19.23 (0.03)	18.75 (0.28)		
Filtration 2	18.26 (0.18)	18.61 (0.38)	19.89 (0.20)	18.06 (0.65)		
Average	19.40	19.15	19.56	18.41	19.13	0.51
<i>Chromium</i> ^{a,b,d,f}						
Filtration 1	4.0 (0.6)	4.5 (2.0)	7.7 (0.6)	2.4 (0.0)		
Filtration 2	2.0 (0.7)	2.8 (0.8)	0.0 (0.0)	8.4 (1.1)		
Average	3.0	3.7	3.8	5.4	4.0	1.0

a Mean (Standard Deviation).

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

c Expressed as percentage.

d Moisture free basis.

e Ash free basis.

f ppm.

TABLE VIII
Chemical Properties of Exhaustion of Hydrolysate in Filter Press

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>pH</i>						
Filtration 1	9.51	9.34	9.57	9.69		
Filtration 2	9.57	9.33	9.72	9.21		
Average	9.54	9.34	9.65	9.45	9.49	0.13
<i>Total solids</i> ^{a,b,c}						
Filtration 1	4.99 (0.13)	5.08 (0.00)	3.59 (0.03)	2.85 (0.01)		
Filtration 2	2.70 (0.02)	5.71 (0.02)	4.32 (0.00)	3.74 (0.01)		
Average	3.85	5.40	3.96	3.29	4.12	0.90
<i>Total ash</i> ^{a,b,c,d}						
Filtration 1	4.58 (0.05)	4.26 (0.08)	4.43 (0.23)	6.02 (0.08)		
Filtration 2	5.70 (0.06)	4.25 (0.06)	4.12 (0.23)	5.18 (0.04)		
Average	5.14	4.26	4.28	5.60	4.82	0.67
<i>TKN</i> ^{a,b,c,d,e}						
Filtration 1	19.50 (0.81)	19.37 (0.80)	18.46 (0.55)	20.40 (0.13)		
Filtration 2	17.72 (0.40)	18.37 (0.52)	19.53 (0.64)	18.04 (0.34)		
Average	18.61	18.87	19.00	19.22	18.92	0.25
<i>Chromium</i> ^{a,b,d,f}						
Filtration 1	5.2 (0.6)	8.2 (1.4)	6.8 (0.8)	10.2 (2.7)		
Filtration 2	3.3 (0.7)	4.4 (0.6)	3.3 (0.7)	5.7 (3.0)		
Average	4.3	6.3	5.1	8.0	5.9	1.6

a Mean (Standard Deviation).

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

c Expressed as percentage.

d Moisture free basis.

e Ash free basis.

f ppm.

TABLE IX
Physical Properties of Gelatin

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>Gel Strength</i> ^{a,b,c}						
Filtration 1	—	81.8	83.3	73.2		
Filtration 2	—	55.5	69.5	68.4		
Average	51.7	68.7	76.4	70.8	66.9	10.6
<i>Dynamic viscosity</i> ^{a,c,d,e}						
Filtration 1	—	2.4144	3.2987	1.9077		
Filtration 2	—	2.2900	1.9790	2.3208		
Average	1.7446	2.3522	2.6389	2.1143	2.2125	0.3785
<i>Density</i> ^{a,c,d}						
Filtration 1	—	0.9981	1.0116	1.0187		
Filtration 2	—	1.1470	1.0061	1.0111		
Average	0.9955	1.0726	1.0089	1.0149	1.0230	0.0340

a N = 1 where N = number of replicates for each sample.

b g Bloom.

c 6.67% (w/w) solution.

d @ 60°C.

e cP.

Figure 2 and Table XV present the molecular weight distribution of the protein products isolated in the process. The percentages of the different molecular weight range distributions of gelatin and wash of gelatin are very similar, showing no significant differences, but the two samples analyzed of the exhaustion of the gelatin present different values, indicating that this step of the process helps in the separation of the gelatin from the chrome sludge differently in each batch. However, the samples of hydrolyzed protein were reproducible from one batch to the other. These results demonstrate that very different ranges of molecular weight distribution of collagen degradation products can be evaluated by SDS-PAGE.

Four gelatin samples, one from each batch, were deionized batchwise. The ash content of the samples decreased significantly to 1% or less and so the values are within the 0 to 3% range reported for technical grade gelatin.¹⁸ Chemical (Table XVI) and physical (Table XVII) tests were run on all samples before and after deionization. The pH of the gelatin decreases until the isoionic point of gelatin is reached, at which point deionization was complete as shown in Table XVI. The total solids showed a slight decrease as expected from the removal of the ash. The values of chrome content reported in Table XVI on a

moisture free basis are higher after deionization than before, because though the chrome content is lower after deionization, at this point all the ash of the gelatin is chrome. The gel strength of the gelatin increased an average of about 135% and the viscosity about 22%, whereas the density decreased.

Tables XVIII, XIX, XX and XXI summarize the data collected from the chemical analyses of the solid products. These results were tabulated for four different batches. For each analysis the mean and the standard deviation are given and for each parameter the average and the standard deviation of the four batches were calculated. The analyses *per se* and the repeatability of the analyses in these experiments were quite good, as indicated by the standard deviations.

The moisture of the chrome cake was higher than the moisture of the chrome sludge because the latter was much easier to filter due to its fibrous nature. As one would expect, the ash content of the chrome cake increases and the TKN decreases because protein has been removed from the chrome sludge in the enzymatic step of the procedure. The fat and the chrome content are the same for chrome sludge and chrome cake, showing that they remain in the chrome and are not removed with the protein.

TABLE X
Physical Properties of Wash of Gelatin in Filter Press

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>Gel Strength</i> ^{a,b,c}						
Filtration 1	—	66.7	57.4	52.6		
Filtration 2	—	74.1	54.5	73.0		
Average	48.6	70.4	56.0	62.8	59.4	9.3
<i>Dynamic viscosity</i> ^{a,c,d,e}						
Filtration 1	—	2.2557	1.7411	1.8916		
Filtration 2	—	2.1872	1.7466	2.1183		
Average	1.9934	2.2215	1.7439	2.0050	1.9909	0.1953
<i>Density</i> ^{a,c,d}						
Filtration 1	—	0.9963	1.0028	1.0105		
Filtration 2	—	1.0028	1.0125	1.0171		
Average	1.0128	0.9996	1.0077	1.0138	1.0085	0.0065

a N = 1 where N = number of replicates for each sample.

b g Bloom.

c 6.67% (w/w) solution.

d @ 60°C.

e cP.

TABLE XI
Physical Properties of Exhaustion of Gelatin in Filter Press

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>Gel Strength</i> ^{a,b,c}						
Filtration 1	—	40.5	18.2	33.8		
Filtration 2	—	41.6	5.0	51.5		
Average	75.0	41.1	11.6	42.7	42.6	25.9
<i>Dynamic viscosity</i> ^{a,c,d,e}						
Filtration 1	—	1.6493	1.8889	1.3737		
Filtration 2	—	1.6610	0.8969	1.7255		
Average	3.9779*	1.6552	1.3929	1.5496	1.5326	0.1320
<i>Density</i> ^{a,c,d}						
Filtration 1	—	1.0047	1.0157	1.0190		
Filtration 2	—	1.0125	1.0199	1.0228		
Average	1.0036	1.0086	1.0178	1.0209	1.0127	0.0080

a N = 1 where N = number of replicates for each sample.

b g Bloom.

c 6.67% (w/w) solution.

d @ 60°C.

e cP.

* Not included in average.

TABLE XII
Physical Properties of Hydrolysate

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>Gel Strength</i> ^{a,b,c}						
Filtration 1	—	—	—	2.5		
Filtration 2	—	—	—	2.4		
Average	—	—	—	2.5	—	—
<i>Dynamic viscosity</i> ^{a,c,d,e}						
Filtration 1	1.0007	0.9417	0.9799	1.2128		
Filtration 2	0.9704	0.9147	0.9709	1.2498		
Average	0.9856	0.9282	0.9754	1.2313	1.0301	0.1364
<i>Density</i> ^{a,c,d}						
Filtration 1	1.0056	1.0108	1.0004	1.0051		
Filtration 2	1.0063	1.0053	1.0046	1.0003		
Average	1.0060	1.0081	1.0025	1.0027	1.0048	0.0027

a N = 1 where N = number of replicates for each sample.

b g Bloom.

c 6.67% (w/w) solution.

d @ 60°C.

e cP.

TABLE XIII
Physical Properties of Wash of Hydrolysate in Filter Press

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>Gel Strength</i> ^{a,b,c}						
Filtration 1	—	—	—	—		
Filtration 2	—	—	—	—		
Average	—	—	—	—	—	—
<i>Dynamic viscosity</i> ^{a,c,d,e}						
Filtration 1	1.0373	0.9464	0.9058	1.2102		
Filtration 2	0.9495	0.9134	0.9855	1.2459		
Average	0.9934	0.9299	0.9457	1.2281	1.0243	0.1385
<i>Density</i> ^{a,c,d}						
Filtration 1	1.0022	1.0056	1.0019	1.0063		
Filtration 2	1.0028	1.0055	1.0038	1.0077		
Average	1.0024	1.0056	1.0029	1.0070	1.0045	0.0022

a N = 1 where N = number of replicates for each sample.

b g Bloom.

c 6.67% (w/w) solution.

d @ 60°C.

e cP.

TABLE XIV
Physical Properties of Exhaustion of Hydrolysate in Filter Press

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No. 4	Aver.	StdDev
<i>Gel Strength</i> ^{a,b,c}						
Filtration 1	—	—	—	—		
Filtration 2	—	—	—	—		
Average	—	—	—	—	—	—
<i>Dynamic viscosity</i> ^{a,c,d,e}						
Filtration 1	0.9835	0.8657	0.9893	1.2012		
Filtration 2	0.9581	1.0481	1.2950	1.1482		
Average	0.9708	0.9569	1.1422	1.1747	1.0611	0.1133
<i>Density</i> ^{a,c,d}						
Filtration 1	1.0063	1.0068	1.0114	1.0110		
Filtration 2	1.0007	1.0094	1.0055	1.0112		
Average	1.0035	1.0081	1.0085	1.0111	1.0078	0.0032

a N = 1 where N = number of replicates for each sample.

b g Bloom.

c 6.67% (w/w) solution.

d @ 60°C.

e cP.

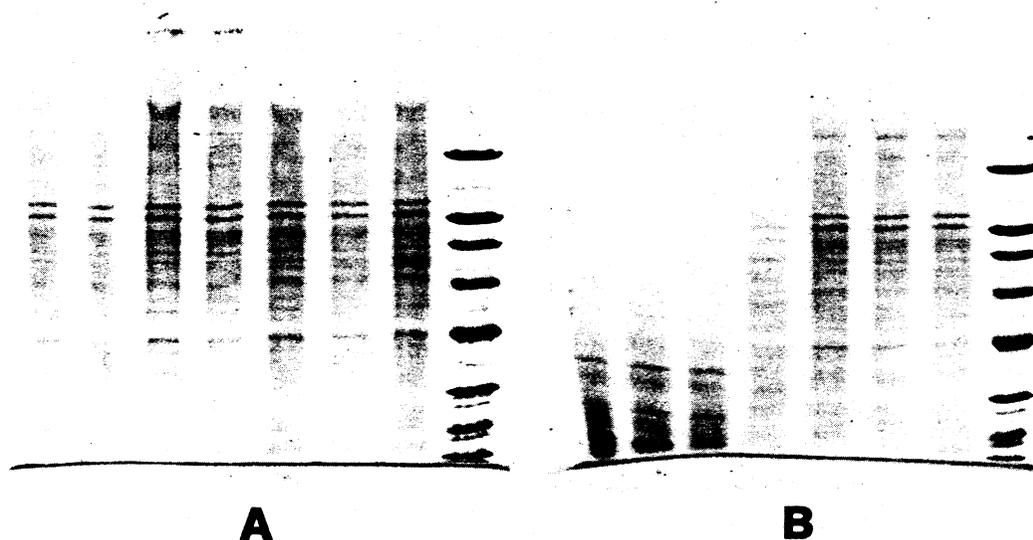


FIGURE 2. — SDS-PAGE gels for molecular weight distribution evaluation of the protein products. Gel A, from right to left, shows standard, gelatin from batch 1, gelatin wash in the filter press from batch 1, gelatin exhaustion in the filter press from batch 1, gelatin from filtration 1 of batch 2, gelatin from filtration 2 of batch 2, gelatin from filtration 1 of batch 3, and gelatin from filtration 2 of batch 3. Gel B, from right to left, shows standard, gelatin from filtration 1 of batch 4, gelatin from filtration 2 of batch 4, gelatin wash in the filter press from filtration 1 of batch 3, gelatin exhaustion in the filter press from filtration 1 of batch 3, hydrolysate from filtration 1 of batch 3, hydrolysate wash in the filter press from filtration 1 of batch 3, and hydrolysate exhaustion in the filter press from filtration 1 of batch 3.

TABLE XV
Molecular Weight Distribution of Gelatin and Example of
Molecular Weight Distribution of Other Protein Products

Sample	<i>Molecular Weight Range^a</i>		
	>208,000-85,000 D	85,000-50,000 D	50,000-<7,200 D
Gelatin			
<i>Batch 1</i>	44.1	21.2	34.7
<i>Batch 2</i>			
Filtration 1	54.0	22.0	24.0
Filtration 2	52.8	23.6	23.6
<i>Batch 3</i>			
Filtration 1	45.6	22.5	31.9
Filtration 2	38.6	22.3	39.1
<i>Batch 4</i>			
Filtration 1	44.3	22.6	33.1
Filtration 2	49.8	20.6	29.6
<i>Average</i>	47.0	22.1	30.9
<i>Std.Dev.</i>	5.5	1.0	5.6
Wash of gelatin in filter press			
<i>Batch 1</i>	42.1	22.0	35.9
<i>Batch 3</i>			
Filtration 1	47.1	21.9	31.0
Exhaustion of gelatin in filter press			
<i>Batch 1</i>	45.4	22.4	32.2
<i>Batch 3</i>			
Filtration 1	25.0	22.3	52.7
Hydrolysate			
<i>Batch 3</i>			
Filtration 1	10.3	11.2	78.5
Wash of hydrolysate in filter press			
<i>Batch 3</i>			
Filtration 1	7.2	9.6	83.2
Exhaustion of hydrolysate in filter press			
<i>Batch 3</i>			
Filtration 1	5.6	8.0	86.4

^a Expressed in percentage.

TABLE XVI
Effect of Deionization on Chemical Properties of Gelatin

Parameter	Batch 1	Batch 2 Filtration 1	Batch 3 Filtration 1	Batch 4 Filtration 1	Aver.	StdDev
<i>pH</i>						
Before	9.09	9.12	9.04	9.19	9.11	0.06
After	6.19	6.16	6.37	6.87	6.40	0.33
<i>Total solids</i> ^{a,b,c}						
Before	3.46 (0.01)	3.67 (0.01)	3.31 (0.07)	3.55 (0.00)	3.50	0.13
After	2.54 (0.02)	2.75 (0.01)	2.42 (0.01)	2.51 (0.00)	2.56	0.14
<i>Total ash</i> ^{a,b,c,d}						
Before	17.62 (0.16)	16.42 (0.18)	17.34 (0.03)	18.69 (0.26)	17.52	0.81
After	0.06 (0.03)	0.41 (0.02)	0.62 (0.13)	1.09 (0.04)	0.55	0.43
<i>Chromium</i> ^{a,b,d,e}						
Before	16.0 (1.0)	5.2 (0.9)	17.2 (1.1)	8.6 (0.2)	11.8	5.0
After	37.0 (4.0)	18.0 (0.0)	32.0 (0.3)	48.0 (0.3)	33.8	12.5

a Mean (Standard Deviation).

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

c Expressed as percentage.

d Moisture free basis.

e ppm.

TABLE XVII
Effect of Deionization on Physical Properties of Gelatin

Parameter	Batch 1	Batch 2 Filtration 1	Batch 3 Filtration 1	Batch 4 Filtration 1	Aver.	StdDev
<i>Gel Strength</i> ^{a,b,c}						
Before	51.7	81.8	83.3	73.2	72.5	14.6
After	146.9	173.3	185.1	162.7	167.0	16.2
<i>Dynamic viscosity</i> ^{a,c,d,e}						
Before	1.7446	2.4144	3.2987	1.9077	2.3414	0.6991
After	2.4369	3.4569	2.9350	2.6345	2.8658	0.4441
<i>Density</i> ^{a,c,d}						
Before	0.9955	0.9981	1.0116	1.0187	1.0060	0.0110
After	0.9851	0.9857	0.9958	1.0033	0.9925	0.0087

a N = 1 where N = number of replicates for each sample.

b g Bloom.

c 6.67% (w/w) solution.

d @ 60°C.

e cP.

TABLE XVIII
Chemical Properties of Chrome Sludge

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No.4	Aver.	StdDev
<i>Moisture</i> ^{a,b,c}						
Filtration 1	73.73 (0.81)	81.82 (0.04)	78.93 (0.21)	79.28 (0.22)		
	75.06 (0.73)	79.52 (0.10)	79.91 (0.22)	74.01 (2.60)		
Filtration 2	82.36 (0.38)	75.32 (1.80)	72.29 (0.76)	74.40 (1.64)		
	85.06 (0.19)	77.66 (1.02)	76.87 (0.57)	81.71 (0.24)		
Average	79.05	78.58	77.00	77.35	78.00	0.98
<i>Total ash</i> ^{a,b,c,d}						
Filtration 1	24.73 (1.32)	22.74 (0.17)	21.95 (0.39)	22.13 (0.49)		
	22.33 (0.01)	23.97 (0.77)	21.81 (0.33)	22.50 (1.68)		
Filtration 2	22.45 (0.32)	23.75 (0.77)	23.04 (0.06)	23.73 (0.68)		
	22.87 (1.02)	23.15 (0.11)	21.10 (1.39)	23.44 (0.13)		
Average	23.10	23.40	21.98	22.95	22.86	0.62
a Mean (Standard Deviation).			c Expressed as percentage.			
b N = 3 where N = number of replicates for each sample.			d Moisture free basis.			

TABLE XIX
Chemical Properties of Chrome Sludge

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No.4	Aver.	StdDev
<i>TKN</i> ^{a,b,c,d,e}						
Filtration 1	13.72 (0.45)	13.45 (0.64)	15.13 (0.71)	15.70 (0.91)		
	14.22 (0.59)	16.78 (1.40)	13.90 (0.45)	15.68 (0.17)		
Filtration 2	13.92 (0.24)	14.98 (0.37)	13.47 (0.19)	13.89 (1.04)		
	14.30 (0.68)	14.82 (0.84)	15.29 (0.21)	15.15 (0.90)		
Average	14.04	15.01	14.45	15.11	14.65	0.50
<i>Fat</i> ^{a,b,c,d}						
Filtration 1	0.67 (0.11)	0.10 (0.06)	0.22 (0.09)	0.54 (0.11)		
	0.48 (0.11)	0.41 (0.02)	0.18 (0.04)	0.74 (0.14)		
Filtration 2	0.75 (0.05)	0.38 (0.14)	0.37 (0.04)	0.64 (0.08)		
	0.33 (0.17)	0.41 (0.02)	0.27 (0.09)	0.55 (0.05)		
Average	0.55	0.33	0.26	0.62	0.44	0.17
<i>Chrome oxide</i> ^{a,b,c,d}						
Filtration 1	3.59 (0.08)	6.99 (0.06)	6.78 (0.29)	6.33 (0.25)		
	6.35 (0.12)	6.79 (0.57)	6.33 (1.01)	4.53 (0.29)		
Filtration 2	6.61 (1.20)	6.37 (0.05)	6.05 (0.27)	10.09 (0.40)		
	6.72 (0.46)	6.75 (0.10)	5.62 (0.28)	5.33 (0.28)		
Average	5.82	6.73	6.20	6.57	6.33	0.41
a Mean (Standard Deviation).			c Expressed as percentage.		e Ash free basis.	
b N = 3 where N = number of replicates for each sample.			d Moisture free basis.			

TABLE XX
Chemical Properties of Chrome Cake

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No.4	Aver.	StdDev
<i>Moisture</i> ^{a,b,c}						
Filtration 1	82.37 (0.26)	82.97 (0.89)	81.09 (1.76)	79.81 (0.71)		
	81.89 (0.09)	82.56 (0.40)	82.36 (0.29)	81.67 (0.56)		
Filtration 2	85.52 (0.75)	82.71 (0.35)	84.15 (0.29)	83.97 (0.28)		
	84.72 (0.12)	83.06 (0.23)	83.80 (0.48)	84.20 (0.60)		
Average	83.63	82.83	82.85	82.41	82.93	0.51
<i>Total ash</i> ^{a,b,c,d}						
Filtration 1	38.15 (0.87)	41.99 (1.57)	40.46 (1.34)	40.14 (0.76)		
	39.83 (0.31)	43.47 (0.39)	41.25 (0.58)	40.37 (0.66)		
Filtration 2	41.69 (2.71)	40.52 (0.06)	41.58 (0.28)	42.12 (1.62)		
	44.03 (0.32)	42.27 (0.61)	41.86 (0.45)	40.25 (0.23)		
Average	40.93	42.06	41.29	40.72	41.25	0.59

a Mean (Standard Deviation).

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

c Expressed as percentage.

d Moisture free basis.

CONCLUSIONS

In this work, we have demonstrated that the reproducibility of this process, the isolation of gelatin and hydrolysate proteinous products from chrome shavings, in pilot plant scale is very good and better yields and product purity was obtained than the lab-scale results. The material balances indicated good repeatability of the process.

Chemical and physical analyses of the protein products isolated showed a low variation between the four batches. The deionization of the gelatin gave a product with excellent physical properties, demonstrating that the process allows the isolation of high quality protein products.

The chemical analyses of the solid products confirmed the good reproducibility shown in earlier lab experiments. The low protein and fat content, and the high chrome content of the chrome cake, final product of this process, indicate that the product can be treated chemically to provide a chrome product able to be reused in the tannery.

ACKNOWLEDGMENTS

The authors acknowledge P. Sams, T. Bosch, M. Tornos, S.M. Clauson and T.A. Dunn for their valuable contribution to the work presented in this paper.

REFERENCES

1. Salmerón, J.; Contaminación. in *Generación y Tratamiento de Residuos en la Industria del Curtido Valenciana*, Confederación Empresarial Valenciana, Valencia, Spain, pp. 15-30, 1995.
2. Alexander, K. T. W., Corning, D. R., Cory, N. J., Donohue, V. J. and Sykes, R. L.; Environmental & safety issues - Clean technology and environmental auditing. *Industrial Waste Management* **76**, 17-23, 1991.
3. Sharphouse, J. H.; Effluent, By-products and the Environment. in *Leather Technician's Handbook*, Leather Producers' Association, Northampton, U.K., pp. 452-469, 1995.

TABLE XXI
Chemical Properties of Chrome Cake

Parameter	Batch No. 1	Batch No. 2	Batch No. 3	Batch No.4	Aver.	StdDev
<i>TKN</i> ^{a,b,c,d,e}						
Filtration 1	11.87 (0.51)	10.21 (0.47)	11.11 (0.81)	11.45 (1.09)		
	12.79 (0.30)	9.64 (0.85)	11.26 (0.85)	7.91 (0.21)		
Filtration 2	11.99 (0.83)	12.41 (0.42)	10.27 (0.92)	10.11 (1.32)		
	10.86 (0.39)	12.82 (0.46)	11.07 (0.52)	8.27 (0.21)		
Average	11.88	11.27	10.93	9.44	10.88	1.04
<i>Fat</i> ^{a,b,c,d}						
Filtration 1	0.82 (0.06)	0.49 (0.01)	0.49 (0.06)	0.40 (0.02)		
	0.79 (0.02)	0.53 (0.07)	0.34 (0.01)	0.44 (0.05)		
Filtration 2	0.33 (0.05)	0.59 (0.00)	0.41 (0.14)	0.38 (0.03)		
	0.38 (0.02)	0.49 (0.10)	0.27 (0.03)	0.37 (0.09)		
Average	0.58	0.53	0.38	0.40	0.47	0.10
<i>Chrome oxide</i> ^{a,b,c,d}						
Filtration 1	7.21 (0.33)	8.46 (0.06)	7.84 (0.30)	7.49 (0.13)		
	6.51 (0.05)	8.58 (0.16)	8.08 (0.03)	7.09 (0.32)		
Filtration 2	8.23 (0.08)	10.37 (0.05)	7.46 (0.93)	7.14 (0.23)		
	7.19 (0.16)	10.19 (0.82)	8.58 (0.21)	6.78 (0.14)		
Average	7.29	9.40	7.99	7.13	7.95	1.04

a Mean (Standard Deviation).

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

c Expressed as percentage.

d Moisture free basis.

e Ash free basis.

4. Taylor, M. M., Diefendorf, E. J., Thompson, C. J., Brown, E. M. and Marmer, W. N., Cabeza, L. F.; Extraction of Value Added Byproducts from the Treatment of Chromium Containing Collagenous Leather Industry Waste. *J. Soc. Leather Technol. Chem.* **81**, 5-13, 1996.
5. Taylor, M. M., Diefendorf, E. J., Brown, E. M. and Marmer, W. N.; Enzymatic Processing of Materials Containing Chromium and Protein. U.S. Patent 5,271,912, 1993.
6. Kolomaznik, K., Langmaier, F., Mladek, M. and Taylor, M.; Industrial Treatment of Chrome Tanned Solid Waste. Proceedings of the IULTCS Centenary Congress, **1**, 238, 1997.
7. Cantera, C. S., Angelinetti, A. R., Escobar, R., Gaita, G. and De Giusti, M.; Hydrolysis of Shavings. Application of Collagen Hydrolysate and of "Acrylic-Protein" in Post-Tanning Processes. Proceedings of the IULTCS Centenary Congress, **1**, 355-366, 1997.
8. Mucka, P., Kopny, J. and Matyasovsky, J.; Chrome Shaving Processing. Proceedings of the IULTCS Centenary Congress, **1**, 427-432, 1997.
9. Taylor, M. M., Diefendorf, E. J., Thompson, C. J., Brown, E. M., Marmer, W. N. and Cabeza, L.F.; Extraction of value-added by-products from the treatment of chromium-containing collagenous waste generated in the leather industry. *Bol. Téc. AQEIC* **47**, 124-150, 1996.
10. Taylor, M. M., Diefendorf, E. J., Thompson, C. J., Brown, E. M. and Marmer, W. N.; Isolation and characterization of value-added by-products from chromium-containing leather waste. *The Leather Manufacturer* **112(7)**, 14-18, 1994.
11. Taylor, M. M., Cabeza, L. F., Marmer, W. N., and Brown, E. M.; Computer-assisted method to measure the adhesive properties of hydrolysis products from collagen. *JALCA* **92**, 28-37, 1997.
12. Taylor, M. M., Kolomaznik, K., Cabeza, L. F., Marmer, W. N. and Brown, E. M.; Functional properties of hydrolysis products from collagen. *JALCA* **93**, 40-50, 1998.
13. Taylor, M. M., Diefendorf, E. J., Marmer, W. N., and Brown, E. M.; Effect of deionization on physical properties of gelable protein products recovered from solid tannery waste. *JALCA* **90**, 365-374, 1995.
14. Taylor, M. M., Diefendorf, E. J., Phillips, J. G., Feairheller, S. H., and Bailey, D. G.; Wet process technology I. Determination of precision for various analytical procedures. *JALCA* **81**, 4-18, 1986.
15. Taylor, M. M., Diefendorf, E. J., Marmer, W. N., and Brown, E. M.; Effect of various alkalinity-inducing agents on chemical and physical properties of protein products isolated from chromium-containing leather waste. *JALCA* **89**, 221-228, 1994.
16. Wainwright, F. W.; Physical Tests for Gelatin and Gelatin Products. in *The Science and Technology of Gelatin* (A. G. Ward and A. Courts, eds.) Academic Press, New York, pp. 507-534, 1977.
17. Brown, E. M., Thompson, C. J., and Taylor, M. M.; Molecular size and conformation of protein recovered from chrome shavings. *JALCA* **89**, 215-220, 1994.
18. Rose, P. I.; Inedible Gelatin and Glue. in *Inedible Meat By-Products*, Advances in Meat Research, Vol. 8, (A. M. Pearson and T. R. Dutson, eds.) Elsevier Applied Science, London and New York, pp. 217-263, 1992.