
CHARACTERIZATION OF PRODUCTS ISOLATED BY ENZYME TREATMENT OF CHROMIUM-CONTAINING LEATHER WASTE*

by

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Abstract

Enzymic processing of chrome shavings and trimmings has previously been shown to be a viable treatment of this substantial waste product generated by the tanning industry. Characterization of the chrome cakes and the protein generated by these treatments was an important next step in this research. Tanneries use different types of processes to tan the leather and these varying processes are dependent on the type of leather to be produced. Also, because of environmental restrictions, a minimum amount of chrome should be present in the effluent. Chrome shavings were obtained from three tanneries. The blue stock had been processed by conventional, albeit different, tannages. For optimal solubility, an individual pretreatment was developed for each type of shavings. They were then treated enzymically and the chrome cake and the protein fraction were isolated. Analyses of the shavings, the chromium-containing cakes and the protein fraction showed that chromium containing leather waste from a variety of sources can be enzymically digested when the appropriate pretreatment is performed. The chemical composition of the isolated products is dependent on the type of treatment and on the composition of the original chromium-containing leather waste product.

Introduction

Enzymic processing of chrome shavings and trimmings has been shown to be a viable treatment of these substantial waste products generated by the tanning industry⁽¹⁻¹⁵⁾. Not all chromium-containing leather waste is the same. Tanneries use different types of 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 for environmental reasons. The protocol for the pretreatment of these shavings had to be adjusted for 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 and the characteristics of the isolated protein.

The treatments described in this paper are the subject of a patent⁽¹⁶⁾. Recent developments at this laboratory show that the process can be made more cost effective by reduction in enzyme concentration and isolation of a gelable protein; these new developments are the subject of a patent application⁽¹⁶⁾, and will be reported in a future publication.

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Experimental

MATERIALS

Chromium-containing leather waste was obtained from commercial tanneries. Sample A shavings 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).

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 AU/g (Anson Units/g)), and as a liquid (standardized to contain 2.5 AU/g).

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) was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ).

PROCEDURE

Eleven kg (24 lbs) of each of the shavings samples (A, B & C) were pretreated at 67-69°C 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 5% magnesium oxide (all percentages are based on the wet weight of the shavings), and Sample B with 3% NaOH and 2% magnesium oxide. After several preliminary bench experiments, it was found that Sample C needed to be treated with 6% magnesium oxide (C-1). Because of its acidity, another portion of this sample was pretreated with 3% NaOH and 3% magnesium oxide (C-2). Three percent Alcalase[®] was added in three feeds to each of the four reactions, over a three hour period. Upon completion of the digestion (67-69°C for 3 hours), the sample was pumped from the reaction vessel and allowed to settle overnight. The protein hydrolyzate 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.

ANALYSES

The chrome shavings and the isolated chrome cakes were analyzed for moisture, ash, calcium, magnesium, chromium, total nitrogen and fat as described in a previous publication⁽¹⁷⁾.

The protein hydrolyzate was analyzed for chromium using atomic absorption spectrophotometry⁽¹⁷⁾. The hydrolyzate was also analyzed for nitrogen, total solids and ash, and the hydrozylate was lyophilized and the dried product was analyzed for nitrogen and ash⁽¹⁷⁾. Amino acid analyses were carried out on a Beckman Model 119CL Analyzer.

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

Results and Discussion

The chrome shavings from each of the 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 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%. 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 data from this table indicate how great the protein content of the shavings is.

Table I
Analyses of Chrome Shavings

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

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

^bMoisture free basis.

^cTotal Kjeldahl nitrogen.

^dProtein content can be estimated by multiplying TKN by 5.51⁽¹⁹⁾.

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 hydrolyzed 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 recovered from
enzymic treatment of chrome shavings

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

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

^bMoisture free basis.

^cTotal Kjeldahl nitrogen.

^dProtein content can be estimated by multiplying TKN by 5.51⁽¹⁹⁾.

The isolated protein hydrolyzates were analyzed for chromium, Total Kjeldahl Nitrogen (TKN), total solids and ash. Data for samples A, B, C-1 and C-2 show that the chromium content is less than 1 ppm. This chromium concentration is similar to the concentrations we found, not only in testing of protein from our pilot studies, but also the protein solution recovered from industrial scale trials. The TKN, total solids and total ash averages about 11,000 ppm, 72,000 ppm and 8,000 ppm, respectively. Preliminary molecular weight studies show that the molecular weight of the hydrolyzed protein ranges from 1000-3000.

The samples were lyophilized and these dried samples were analyzed for total nitrogen and ash. The results of these analyses are shown in Table III. The nitrogen content of the dried protein ranges from 13.8% to 15.0% and the ash content ranges from 9.7 to 18.9 percent. This ash content not only reflects the pretreatment but also the ash content of the original shaving samples. This protein or collagen hydrolyzate is extremely deliquescent.

Amino acid analysis was run on each of the dried protein samples and the results are shown in Table IV. The values are expressed as mole percent. When the profile of the protein hydrolyzate is compared to the profile of collagen, the results are quite similar, suggesting that no modification of amino acids occurred during processing.

During the enzymic digestion of Sample C-2, which had been subjected to the sodium hydroxide-magnesium oxide pretreatment, samples of the reaction were taken at 10, 30, 60, 90, 120, 150 and 180 minutes. The samples were immediately heated at 90°C for 5 minutes to kill enzyme activity. To prevent further hydrolysis from taking place, the reaction mixture was then immediately cooled to about 25-30°C. The samples were stored at 4°C. Each of the samples was filtered and the isolated chrome cake and protein solution were again stored at 4°C. The chrome

Table III
Analyses of lyophilized protein hydrolyzate

Parameter % ^a	Sample			
	A	B	C-1	C-2
TKN ^{b,c,d}	14.35 ± 0.02	14.99 ± 0.01	14.21 ± 0.03	13.75 ± 0.06
Ash ^b	9.74 ± 0.01	10.89 ± 0.59	12.91 ± 0.58	18.94 ± 0.52

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

^bMoisture free basis.

^cTotal Kjeldahl nitrogen.

^dProtein content can be estimated by multiplying TKN by 5.51⁽¹⁹⁾.

cake was subjected to the protocol outlined above with respect to drying and TKN. Table V shows the results of those analyses. The nitrogen content of the unwashed cake was about 7% after only 10 minutes of enzymic digestion. The fluctuations of the nitrogen content seen in this table only reflect the efficiency of the filtraton.

We have done preliminary experiments into cleaning the cake in preparation for recycling. In the first set of experiments, we repeatedly washed the cake with water, monitoring the decrease in protein by Kjeldahl nitrogen. Ten grams of each cake was weighed into a centrifuge tube. Two volumes (200%) of water, based on the net weight, were added, the samples were mixed well and then centrifuged for 10 minutes. The supernatant was decanted. Depending on the sample, the procedure was repeated up to four more times for each of the cakes. The washed cakes were transferred to vials and dried at 60°C for 48 hours. Upon cooling in a dessicator they were weighed and analyzed for TKN. The results are (shown in Fig. 1).

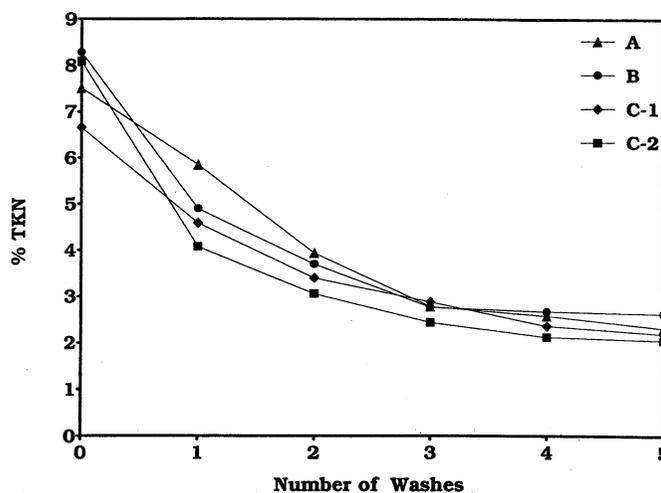


FIG. 1. — The effect of repetitive washing on the nitrogen content of chrome cakes. These cakes were recovered from chrome shavings that were subjected to a variety of alkaline pretreatment steps and then were enzymically treated for three hours. The cakes were washed and then analyzed for nitrogen.

Table IV
Amino acid composition of hydrolyzed proteins^a

Residue	Collagen (Type I)	Hydrolyzate	StdDev ^b
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	

^aExpressed as mole percent.

^bHydrolyzate samples.

As demonstrated in this figure, repeated washing of the cakes will decrease the nitrogen content from about 7% to less than 3%. Differences in the pretreatment may be affecting the percent nitrogen remaining. On an industrial scale, there may be more efficient ways of washing this cake, such as vacuum filtration.

In the experiment in which we took samples at different times during the enzymic digestion (C-2), the resulting cakes were washed five times, for, as found in the previous experiment, it was after this many washings that no further protein would be removed. Fig. 2 shows some very interesting results. The nitrogen is significantly reduced for the sample digested for 10 minutes

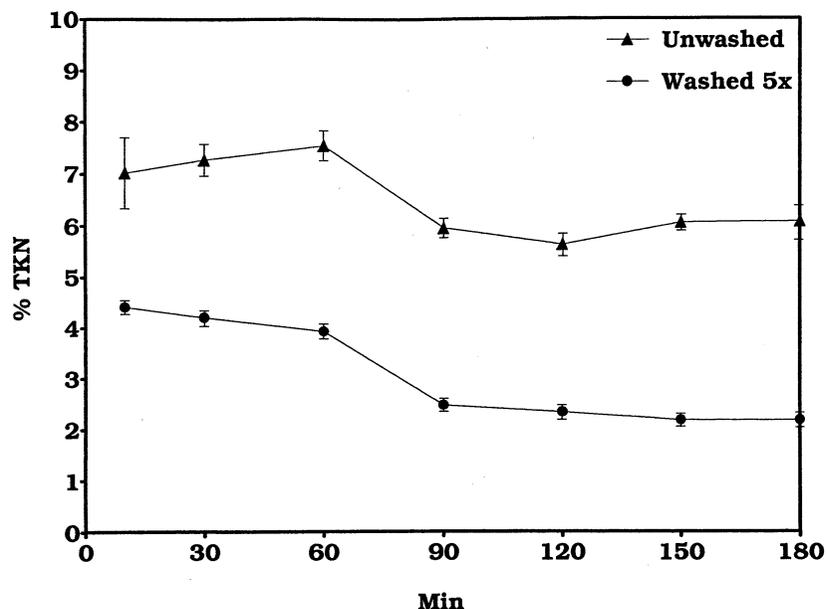


FIG. 2. — Nitrogen content of chrome cakes isolated at various times during the treatment. These cakes were recovered from chrome shavings that were subjected to a sodium hydroxide-magnesium oxide pretreatment step and then were enzymically treated with three feeds of Alcalase[®] over a three hour period. The cakes were washed and then analyzed for nitrogen.

and secondly, it appears that after the 2% feed of enzyme (at 2 hours), no further depletion of nitrogen takes place, suggesting that less enzyme could be used. When the reaction was run on an industrial scale, we found that, in fact, the quantity of enzyme could be substantially decreased.

At present, experiments are being performed to further clarify the enzymic reactions. We are interested in the effects of time, temperature and enzyme concentration on the molecular weight distribution of the protein. We have developed methods to reduce the amount of enzyme needed for an efficient treatment and, at the same time, we are recovering a higher quality gelable protein⁽¹⁶⁾. We have also developed methods to clean the chrome cakes in preparation for chrome recycling⁽¹⁸⁾. Cost estimates have been calculated and show that a return can be made from these treatments⁽¹⁸⁾. These data will be the subject of future publications.

Treatments on other types of solid chrome-containing tannery waste such as splits and trimmings have been carried out successfully. Preliminary treatments on crust leather have begun.

Table V
Analyses of unwashed chrome cakes (C-2) from rate study

Parameter % ^a	Enzymic digestion time (minutes)					
	10	30	60	90	120	180
Moisture	86.45 ± 0.10	85.56 ± 0.06	84.05 ± 0.27	84.43 ± 0.28	84.44 ± 0.18	83.97 ± 0.38
Ash ^b	35.49 ± 0.58	36.49 ± 0.48	34.89 ± 0.14	38.97 ± 0.93	39.48 ± 0.26	40.57 ± 0.84
Ash ^c	4.79 ± 0.05	5.25 ± 0.01	5.51 ± 0.03	6.03 ± 0.16	6.13 ± 0.04	6.35 ± 0.02
TKN ^{b,de}	7.02 ± 0.68	7.28 ± 0.30	7.55 ± 0.29	5.93 ± 0.19	5.61 ± 0.21	6.07 ± 0.32

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

^bMoisture free basis.

^cAs is basis.

^dTotal Kjeldahl nitrogen.

^eProtein content can be estimated by multiplying TKN by 5.51⁽¹⁹⁾.

Conclusion

We have shown that chromium-containing leather waste from a variety of sources can be enzymically treated when the appropriate modification of the pretreatment step is performed. Secondly, the chemical composition of the isolated products will be dependent on the type of treatment and on the composition of the original chromium-containing leather waste product.

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