

# EFFECT OF VARIOUS ALKALINITY-INDUCING AGENTS ON CHEMICAL AND PHYSICAL PROPERTIES OF PROTEIN PRODUCTS ISOLATED FROM CHROMIUM-CONTAINING LEATHER WASTE\*

by

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## ABSTRACT

We have demonstrated that gelable and hydrolyzed protein products can be obtained from chromium-containing leather waste. Depending on the choice of alkalinity-inducing agent added, products with varying chemical and physical properties can be isolated. Also, we have shown that the ash content of these isolated protein products will be dependent not only on the choice of alkalinity-inducing agent, but also on the chemical make-up of the original substrate. One of the components of the ash is chromium and its content is also influenced by the choice of alkalinity-inducing agent. We found that adding magnesium oxide in combination with sodium or potassium hydroxide increases the chromium content in the *gelable* protein products over that obtained with magnesium oxide alone. Substituting carbonate salts for sodium or potassium hydroxide lowers the chromium content of the gelable protein products. Choice of alkalinity-inducing agent has little effect on the chromium content of the *hydrolyzed* protein products. With respect to Bloom value and viscosity of the gelable protein products, magnesium oxide alone gives the superior product, whereas the introduction of carbonates and hydroxides has a detrimental effect. Hydroxide is more detrimental than carbonate. In this study we have shown that a variety

of products can be isolated with varying properties and these properties will be dependent on the desired end use of the protein product.

## INTRODUCTION

Previously, we have demonstrated that chromium-containing leather waste can be enzymically treated to obtain a protein hydrolysate product and a recyclable chrome cake<sup>1-9</sup>. This original one-step process gave a low molecular weight protein hydrolysate with only limited commercial value as a fertilizer. The overall economic return from such a process would be attractive only if landfill fees were exorbitant or landfilling outright prohibited. Although it was demonstrated that the cost of the process could be reduced by recycling the solution and the enzyme, a higher return from a better quality higher molecular weight protein by-product would be desirable.

Extraction of gelatin from chromium leather waste has been described in the literature<sup>10</sup>. Nevertheless, a considerable amount of chromium sludge remains after this extraction and disposal of this sludge is necessary. We subsequently developed a two-step process that allowed a *gelable* protein product to be isolated in the first step followed by a lower molecular weight, *hydrolyzed* protein product obtained by enzymic treatment of the remaining chrome sludge<sup>11</sup>. Also, a recyclable chromium product is obtained.

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In this research we have characterized the chemical and physical properties of isolated protein fractions from the two-step process described above. Furthermore, we demonstrate that the choice of alkalinity-inducing agent added will produce protein products with a variety of properties. Moreover, these properties will determine the suitable end use of the protein product. We have chosen these various agents to be compatible with whatever chrome recycling system the tannery is using. These chemicals we have chosen are used in chrome precipitation<sup>12-13</sup>. Previously<sup>14</sup>, we described the effect of these agents on the ash content of the gelable and hydrolyzed protein products and, if the ash content was too high, how it can be reduced with the use of mixed-bed ion-exchange resins. In this paper, we describe the effects that these agents have on chromium concentration in the protein products and also on the physical properties of the gelable protein products. The effect that these agents have on the molecular weight distribution of the protein products has been described in another publication.<sup>15</sup>

## EXPERIMENTAL

### Materials

Chromium-containing leather waste (chrome shavings) was obtained from a commercial tannery.

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, standardized to contain 2.0 Anson Units/g (AU/g)), and as a solution (standardized to contain 2.5 AU/g). The solution form, Liquid Alcalase<sup>R</sup>, was used in these experiments.

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

### Procedure

#### RECOVERY OF GELABLE AND HYDROLYZED PROTEIN PRODUCTS

Two hundred grams of chrome shavings were shaken in 1 L of water (500% float), 0.2 g of a non-ionic surfactant and the appropriate alkali at 70-72° C for six hours. The samples were first centrifuged to isolate the gelable protein solution, which was then filtered warm through Whatman #1 filter paper. The chrome sludge and the filtered gelable protein solutions were stored at 4° C. The chrome sludge was warmed to room temperature and 200 ml water (100% float) and 0.2 g non-ionic surfactant were added. The samples were shaken at 70-72° C for 1.5 hrs. The pH was adjusted with magnesium oxide to optimal pH for the enzyme. The enzyme (0.2g) was added and the samples were shaken at 70-72° for 3.5 hrs. The solutions were filtered hot through Whatman #1 filter paper and the protein solutions were stored at 4° C. The chrome cake was air dried. The gelable protein and hydrolyzed protein solutions were lyophilized and the protein products were stored in the dried state.

#### ANALYSES

Chromium in the gelable and hydrolyzed protein products was determined by Perkin-Elmer Atomic Absorption Spectrophotometer, Model 3300 (Norwalk, CT), as described previously<sup>16</sup>. Moisture in the dried gelable protein products was determined by heating the sample at 105° C for 17 hrs<sup>10</sup>. Ash in the dried gelable protein products was determined by heating the sample at 600° C for two hours<sup>16</sup>.

Gel strengths were measured by Bloom determinations<sup>17</sup> with a TA.XT2 Texture Analyzer from Texture Technologies Corporation (Scarsdale, NY). For most experiments, the dried gelatin (7.5g) 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. The water was allowed to absorb for a set period of time (10 min. to overnight), the sample was heated in a 65° C

**TABLE I**  
**Residual Chromium in Proteinaceous Products Derived From**  
**Treatment of Chromium-Containing Leather Waste**

Alkalinity-Inducing Agent: weight (g) per 100 g wet shavings			Cr Concentration (ppm) in Proteinaceous Products	
NaOH	MgO	pH <sup>a</sup>	Gel	Hydrolysate
0	6	8.90	55	5
1	4	9.10	67	5
2	3	9.30	126	14
<b>KOH</b>				
0	6	8.90	55	5
1	4	8.72	89	4
2	3	8.78	100	10
<b>Na<sub>2</sub>CO<sub>3</sub></b>				
0	6	8.90	55	5
1	5	8.75	46	2
2	4	8.65	40	4
<b>K<sub>2</sub>CO<sub>3</sub></b>				
0	6	8.90	55	5
1	5	8.40	35	6
2	4	8.40	20	4

<sup>a</sup> pH of reaction mixture after 6 hours.

**TABLE II**  
**Determination of Correction Factor for Modified Bloom Determination**

Sample	Jar Diameter (mm)	Weight (g)	Bloom (gm force)	Correction Factor <sup>a</sup>
6% MgO	59	7.5	121.3	1.000
6% MgO	39	2.5	169.6	1.398
Wootton & Kenchington <sup>b</sup>	30	0.833	—	1.480

<sup>a</sup> Determined by dividing Bloom value obtained from sample run in 59 mm jar into Bloom value obtained from sample run in 39 or 30 mm jar.

<sup>b</sup> Wootton, J. and Kenchington, A. W. GGRA Bulletin 10, No. 3, 12-14 (1959) (cited from "The Science and Technology of Gelatin" Ward, A. G. and Courts, A. eds., Academic Press, New York, 1977, page 516).

bath for 15 min., cooled at room temperature for 15 min. and then placed in a 10° C bath for 17-18 hrs. 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 per sec. The grams force required for this is expressed as the Bloom value.

Viscosities were measured in a Cannon Manning viscometer<sup>18</sup>. 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

In order to identify markets for the gelable and hydrolyzed protein products isolated from treatment of chromium-containing leather waste, the chemical and physical properties need to be determined. For example, because there is concern that the character and quantity of the ash in the protein products would have an adverse effect on marketing of these products, the use of different proportions of alkaline agents on the ash content of the protein products was investigated<sup>14</sup>. We recently looked at one of the components of the ash, chromium, and determined the effect of treatment parameters on its concentration in the protein products. Depending on its physical properties, the gelable protein has potential use in cosmetics, adhesives, printing or photography and the effect of alkalinity-inducing agents on the Bloom (gel strength) and the viscosity also was examined.

As described in previous publications<sup>1-9</sup>, the alkalinity-inducing agents used to treat chromium-containing leather waste were chosen so that the process could be worked into whatever chrome recycling system the tannery would be using. Since magnesium oxide, sodium carbonate and sodium hydroxide have been used in chrome precipitation<sup>12-13</sup>, their effects were evaluated first. Potassium hydroxide and potassium carbonate were chosen to enhance the value of the hydrolysate as a fertilizer.

Chrome shavings from different tannery processes were found to have different pH values, ranging from 3.50 to 4.20. The shavings being used in these experiments had a pH range of 3.95 to 4.00 and the concentrations of alkalinity-inducing agents shown in Table I were arrived at experimentally, so that the pH would be optimal, not only for the

gel extraction but also for the subsequent enzymic treatment. Also, the pH of the reaction was not allowed to fall below 8.0 to prevent solubilizing chromium. The end use for the protein products determines whether the presence of chromium is a problem. Therefore, we designed an experiment to determine the effect of the alkalinity-inducing agents on the concentration of chromium in these products.

In Table I, the effect of sodium and potassium hydroxide concentrations on chromium content of protein products is shown. Zero percent indicates the use of 6% magnesium oxide alone. The chrome content of the gelable protein product increases significantly when sodium and potassium hydroxide, partially substituted for magnesium oxide, are in the pretreatment. The 2% concentration of both sodium and potassium hydroxide appears to have a significant effect on the chromium content of the hydrolyzed protein products.

The effect of increasing sodium and potassium carbonate concentrations on chromium content of protein products is shown in Table I. The carbonate ion appears to have an opposite effect of hydroxide on the chrome content of the gel, reducing it to less than with the magnesium oxide alone. The chrome content in the gelable protein product decreases with increasing carbonate concentration. The chrome content in the hydrolyzed protein products does not appear to show a significant change.

Curiously, the chrome contents of the gels are much higher than the hydrolyzed products. Perhaps the chromium is being protected, for when these same alkalinity-inducing agents were used in the one-step process with the enzymic treatment, the chrome concentration of these products were similar to those shown for the hydrolyzed protein products. In the future we intend to investigate passing the isolated gelable protein solutions through chelating resins as a method of removing this chromium.

We looked at the physical properties of the isolated gelable products. The Bloom or gel strength and viscosity often are examined when evaluating a gel<sup>10</sup>. We had to modify the AOAC method<sup>17</sup> for determining the Bloom because we had a limited amount of sample. According to F. W. Wainwright in "The Chemistry and Technology of Gelatin", the Bloom test is dependent on the dimensions of the bottle<sup>18</sup>. The Standard Methods of the Gelatin Manufacturers Institute of America specify bottles with internal diameters of  $59 \pm 1$  mm. The overall height of the bottle is not critical. Also described in this chapter is a method developed by Wootton and Kenchington<sup>19</sup> for determining the Bloom of small gelatin samples, in which 0.833 g of gelatin is dissolved in 11.66 ml of water and the

Bloom is measured in a jar 30 mm diameter and then compared to the larger sample. By dividing the values obtained from the sample run by the standard method into the values obtained from the small sample, a correction factor of 1.48 was calculated. In Table II, the Bloom values we obtained from magnesium oxide extracted gel, run on both the standard AOAC size sample and on a smaller sample, are shown. The correction factor (1.398) was calculated and this value was used to correct our small sample test. One can see that the value is lower than the British determination, which one would expect because the dimensions of their jar is smaller.

The effect of various combinations of magnesium oxide with sodium hydroxide and potassium hydroxide on the Bloom and viscosity of the gelable products is shown in Table III. The magnesium oxide extracts give the highest Bloom and viscosity whereas, increasing the hydroxide concentration decreases the Bloom value and the viscosity. Potassium hydroxide combinations decreases the Bloom value and the viscosity less than the sodium hydroxide in combination with magnesium oxide. These viscosities were run at 60° C as were the densities used to convert the kinematic viscosity to dynamic viscosity.

Table IV shows the effect of various combinations of magnesium oxide with sodium carbonate and potassium carbonate. The carbonate ion does not appear to have as much of a detrimental effect on the physical properties of the gel as does the hydroxide ion, but the values are lower than with MgO alone. The carbonates are convenient to work with because of better pH control.

According to Rose in the chapter on Inedible Gelatin and Glue in the book on "Inedible Meat By-Products" by Pearson and Dutson<sup>10</sup>, the Bloom values of gelatin should range from 75-300 g. The values we obtained for our isolated gelable protein samples, except for the 3% MgO-2% NaOH sample, were within this range. Rose states that viscosities should range from 2 to 10 mPa s(cP) and our samples are within this range. He also reports that the ash content of a good gel should be between 0-3% and that the salt content will affect the Bloom and may affect the viscosity. As shown in Table V, the ash content of our samples ranged from 11.8 to 18.8%. As shown in a previous publication, this ash was found to contain magnesium and calcium ions as well as the more soluble sodium and potassium salts<sup>9</sup>. This ash content reflects the ash content of the original

shavings in addition to the alkali used to extract the gelable protein.

Typically, in commercial gelatin preparation, the solutions are passed through ion-exchange columns to lower the ash content. In a previous publication<sup>14</sup>, we demonstrated that by passing the gelable protein solutions through mixed-bed ion-exchange columns of two different compositions, the ash content of the samples was reduced to about 0.4 to 0.5%. The ash content of these treated samples of gelable proteins were within the criteria set for gelatin. Both resins worked equally well. We deionized one of the isolated gels (6% MgO extracted gel) and determined the effect on the Bloom and the viscosity. A solution of the gelable protein sample was passed through a Bio-Rad AG<sup>R</sup> 501-X8 mixed bed resin. Shown in Table VI are effects of deionization on the ash content, Bloom, viscosity and density. The reduction in the ash content, from 11.8% to 0.38%, was similar to that found in previous experiments<sup>14</sup>. After the sample was deionized, an improvement was seen in the physical properties. This improvement may be due to a higher concentration of protein since the inorganic salts were removed or may be due to the fact the salts may interfere. Presently, we are deionizing all samples from the study, and, after looking at all the data, an interpretation will be made.

## CONCLUSION

We have demonstrated that depending on the choice of alkalinity-inducing agent, one can obtain protein products with varying chemical and physical properties. Compared to the use of magnesium oxide alone, the use of magnesium oxide in combination with sodium or potassium hydroxide increases the chromium content in the gelable protein products, and, in the higher hydroxide concentration has a significant effect on the chromium content of the hydrolyzed protein products. Substituting sodium and potassium carbonates for the hydroxides lowers the chromium content of the gelable protein products but does not have a significant effect on the chromium content of the hydrolyzed protein products, when compared to the use of magnesium oxide alone. With respect to physical properties of the gelable protein products, such as the Bloom and viscosity, magnesium oxide alone gives the superior product, whereas the introduction of carbonates or hydroxides has a detrimental effect, with the latter having the most significant effect.

**TABLE III**  
**Effect of Increasing Hydroxide Concentration On**  
**Gel Strength and Viscosity of Gelable Protein Products**

Alkalinity- Inducing Agent	Bloom <sup>a</sup> in gm <sup>b</sup>	Kinematic <sup>c</sup> Viscosity (cS)	Density at 60° C	Dynamic <sup>d</sup> Viscosity (cP)
6% MgO	121.3	2.5900	1.0076	2.6097
4% MgO-1% NaOH	91.3	2.2755	1.0125	2.3039
3% MgO-2% NaOH	74.1	2.0868	1.0098	2.1072
4% MgO-1% KOH	110.6	2.4504	1.0076	2.4690
3% MgO-2% KOH	89.5	2.3107	1.0115	2.3372

<sup>a</sup> Concentration 6.67% wt/wt.

<sup>b</sup> Corrected Bloom value (see Table II).

<sup>c</sup> Time in sec. (at 60° C) x viscometer constant.

<sup>d</sup> Kinematic viscosity x density at 60° C.

**TABLE IV**  
**Effect of Increasing Carbonate Concentration On**  
**Gel Strength and Viscosity of Gelable Protein Products**

Alkalinity- Inducing Agent	Bloom <sup>a</sup> in gm <sup>b</sup>	Kinematic <sup>c</sup> Viscosity (cS)	Density at 60° C	Dynamic <sup>d</sup> Viscosity (cP)
6% MgO	121.3	2.5900	1.0076	2.6097
5% MgO-1% Na <sub>2</sub> CO <sub>3</sub>	97.6	2.1801	1.0099	2.2017
4% MgO-2% Na <sub>2</sub> CO <sub>3</sub>	100.6	2.4990	1.0106	2.5254
5% MgO-1% K <sub>2</sub> CO <sub>3</sub>	100.4	2.3828	1.0094	2.4052
4% MgO-2% K <sub>2</sub> CO <sub>3</sub>	99.8	2.2134	1.0094	2.2342

<sup>a</sup> Concentration 6.67% wt/wt.

<sup>b</sup> Corrected Bloom value (see Table II).

<sup>c</sup> Time in sec. (at 60° C) x viscometer constant.

<sup>d</sup> Kinematic viscosity x density at 60° C.

**TABLE V**  
**Effect of Various Alkalinity-Inducing Agents on Moisture  
and Ash Content of Gelable Protein Products**

<b>Alkalinity- Inducing Agent</b>	<b>% Moisture<sup>a</sup></b>		<b>% Ash<sup>a</sup></b>	
		<b>As is</b>		<b>MFB<sup>b</sup></b>
6% MgO	10.00	11.80		13.16
4% MgO-1% NaOH	9.87	16.90		18.85
3% MgO-2% NaOH	8.94	16.49		18.14
4% MgO-1% KOH	11.61	11.87		13.38
3% MgO-2% KOH	10.14	17.28		19.15
5% MgO-1% Na <sub>2</sub> CO <sub>3</sub>	10.84	17.82		20.11
4% MgO-2% Na <sub>2</sub> CO <sub>3</sub>	10.04	17.04		18.96
5% MgO-1% K <sub>2</sub> CO <sub>3</sub>	11.32	13.33		15.06
4% MgO-2% K <sub>2</sub> CO <sub>3</sub>	9.94	18.82		21.00

<sup>a</sup> N = 2

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

**TABLE VI**  
**Effect of Deionization on Chemical and Physical Properties of Gelable Protein Product**

<b>Property</b>	<b>Gelable protein product</b>	
	<b>Before Treatment</b>	<b>After Treatment<sup>a</sup></b>
Moisture %	10.00	8.31
Ash %	11.80	0.38
Ash (MFB) <sup>b</sup> %	13.16	0.42
Bloom (gm)	121.30	176.80
Kinematic Viscosity <sup>c</sup> (cS)	2.5900	3.2949
Dynamic Viscosity <sup>c</sup> (cP)	2.6097	3.3018
Density <sup>c</sup> (gm/ml)	1.0076	1.0021

<sup>a</sup> Used Bio Rad AG<sup>R</sup> 501-X8 (D) mixed bed resin.

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

<sup>c</sup> at 60° C.

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