

EFFECT OF DEIONIZATION ON PHYSICAL PROPERTIES OF GELABLE PROTEIN PRODUCTS RECOVERED FROM SOLID TANNERY WASTE

ABSTRACT

Extraction of gelatin or gelable protein products from solid tannery by-products has been demonstrated previously. During the commercial manufacture of gelatin from limed hides, skins and bones, it is common practice to pass the gelatin through a mixed-bed deionizing column in order to reduce the ash content of the protein. In the gelable protein products that we are isolating from chrome-tanned waste, the ash content is relatively high and is a function of both the alkali treatment and the original composition of the chrome waste. We have shown that deionizing the protein will reduce the ash. There is, however, a question as to what effect this treatment will have on the Bloom and the viscosity — commonly reported analyses for gel quality. Moreover, we have previously shown that the choice of alkali will affect the physical properties. In this study, chromium waste was treated with hydroxides and carbonates in combination with magnesium oxide to extract gelable protein products, and physical properties were measured before and after deionization. We found that the physical properties were improved in the deionized samples and this is probably due to the increased protein concentration with a small effect contributed by the removal of the ash. Protein yields were also calculated and we found that the type and concentration of alkali used to extract the gelable protein will affect the yield. We also demonstrated that these processes are

reproducible and that good material balances can be obtained.

INTRODUCTION

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 products¹⁻⁴. Cation, anion, or mixed-bed resins can be used. The gelable protein products that we are isolating in the treatment of chromium-containing leather waste have a high ash content not only because of the alkali that we use to extract the gelable protein, but also because of the high ash content in the original substrate. We have shown previously that the choice of alkali will affect the chemical and physical properties of the untreated isolated products⁵. In this study, we show the effect that deionizing these gelable protein solutions has on their physical properties.

Experiments were designed to determine the yield of the protein products that we isolated from the chrome waste and to show that yield is a function of the choice of alkali. From the data, we were also able to calculate material balances and determine the reproducibility of the processes.

EXPERIMENTAL

Materials

Chrome shavings were obtained from a commercial tannery and were kept at 4°C. Alcalase[†] (alkaline protease) was

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 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^R, 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 (MagChem 50, Hunt Valley, MD). Sodium hydroxide, potassium hydroxide, sodium carbonate and potassium carbonate were from J. T. Baker Chemical Co.

Procedure

The chrome shavings were treated as outlined in Figure 1. This process was described in a previous publication⁵. The only modification to the process was that the solution was centrifuged first and then filtered after the treatment to extract the gelable protein.

Deionization

The samples were deionized batchwise using Bio-Rad Ag^R 501-X8 (D) mixed-bed resin. This resin will change from blue when fully regenerated to gold when exhausted. Resin was added (5g/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. After treatment, the

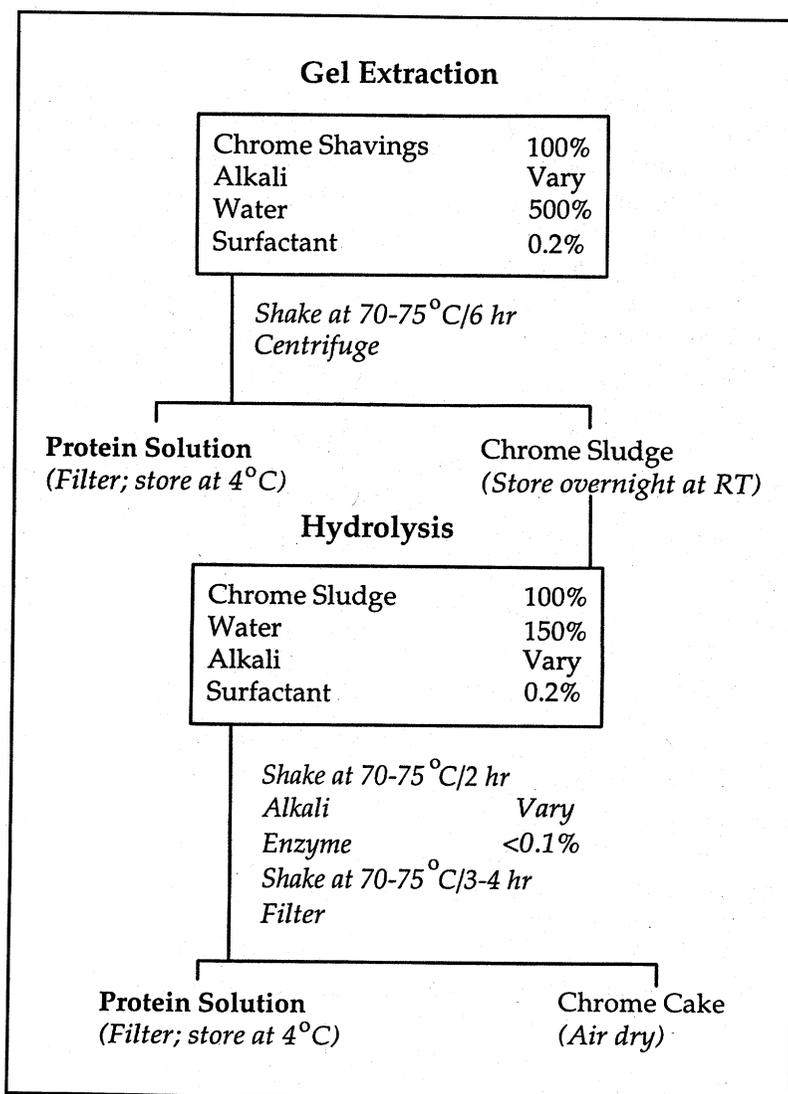


FIGURE 1. — Outline of two-step procedure for treatment of chromium-containing solid leather waste.

solutions were filtered through sintered glass funnels and were then freeze-dried in preparation for chemical and physical analyses.

Analyses

Moisture, ash, nitrogen, total solids and total ash were determined as described in a previous publication⁶. Bloom, viscosity and density of the gelable protein were determined as described in a previous publication⁵. The Jellygram determination² is similar to the Bloom determination, except that the concentration is 12.5% (w/w).

The yields of the the protein products and material balances were calculated by weighing the isolated products throughout the procedure and by determining the percent moisture, ash, nitrogen, total solids and total ash of these isolated products.

RESULTS AND DISCUSSION

In previous studies^{5,7,8}, we have demonstrated the effect of various alkali on the chemical composition of the isolated protein products. We have shown that the choice of alkali will affect the physical properties of the gelable protein fraction⁵. We also demonstrated that a gel that had been extracted with magnesium oxide, when passed through a mixed-bed deionizing column, had a significantly lower ash content and that its physical properties, such as the Bloom and viscosity, had improved. 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 that salt, protein concentration and even the choice of alkali have on the physical properties of the gelable protein products, an experiment was designed in which various alkalis were again used to extract gelable protein from the chrome waste (Figure 1). Then, portions of each of the solutions were deionized using a mixed-bed deionizing resin. The untreated and deionized solutions were lyophilized. 6.67% (w/w) concentrations of each of the dried samples were prepared. Bloom, viscosity and density determinations were run on the samples. Also, the sample that was not deionized was carefully treated with resin and physical tests were run on this sample; thus the protein concentration remained constant in this sample and one could observe the influence, if any, of the salt. From these data, an interpretation concerning the effect of salt, protein concentration and choice of alkali was made.

In the previous study, we passed the gelable protein solution through a mixed-bed deionizing column. In this present

study, we decided to batch-deionize the samples, for when larger amounts of samples were applied to the column, the column had a tendency to clog, even when the column was heated. This difficulty may be due to the solutions' gelling or high viscosity. An aliquot of the gelable protein solution was transferred to an Erlenmeyer flask and resin was added (5 g/100 ml of solution). Further additions of resin were made until no color change was observed (from blue to gold). The protein solution and resin were filtered through a sintered glass funnel. An aliquot of this deionized solution was analyzed for ash content and the results are shown in Table I. The ash content of all alkali treated samples decreased significantly and the values are within the 0 to 3% range reported for technical grade gelatin².

Physical tests were run on all samples before and after deionization and results can be seen in the following tables. In Table II, the effects of deionization on the physical properties of magnesium oxide-sodium carbonate-extracted gels are shown. One can see that the Bloom value increases slightly in the sample in which the protein concentration remained constant before and after deionization. However, the Bloom value increased significantly in the deionized sample in which the protein concentration is approaching 6.67% (w/w). These data indicate that the salt exerts a small influence on the Bloom value but that protein concentration is the governing factor. With respect to the viscosity, the samples are behaving predictably in that in the deionized sample of lower protein concentration, the viscosity drops and in the deionized sample of higher protein concentration, the viscosity increases significantly. Deionized samples had lower densities as would be expected.

Table III shows the effects of deionization on the physical properties of magnesium oxide-potassium carbonate extracted gels. With respect to viscosity and density, the values appear to follow the same trend as does the magnesium oxide-sodium carbonate samples. However, the Bloom value of the deionized samples in which the protein concentration remained constant did not change, possibly indicating that the sodium ion has a slight effect on the Bloom readings.

Table IV shows the effect of deionizing the magnesium oxide-sodium hydroxide gel samples. The Bloom values are much lower than found in a previous study⁵ in which the Bloom values for the 4% MgO-1% NaOH and 3% MgO-2% NaOH samples were 91.3 and 74.1, respectively. The reactions were run again and the Bloom value for the 4% MgO-1% NaOH sample was 79.3, which is midway between the other two runs. The sample that had been

TABLE I
Effect of Batch Deionization on the Ash Content of Gelable Protein

Alkali Treatment	Ash Content ^a	
	Before	After ^b
6% MgO	13.90	0.94
5% MgO-1% Na ₂ CO ₃	18.45	0.55
4% MgO-2% Na ₂ CO ₃	15.66	0.32
5% MgO-1% K ₂ CO ₃	16.96	0.52
4% MgO-2% K ₂ CO ₃	16.32	0.35
4% MgO-1% NaOH	16.00	0.19
3% MgO-2% NaOH	13.36	0.50
4% MgO-1% KOH	17.77	0.55
3% MgO-2% KOH	18.06	0.82

^a Expressed in percent on a MFB (moisture-free basis)

^b Used Bio-Rad AG^R 501-X8 (D) resin to deionize samples

TABLE II
Effect of Deionization on Physical Properties of Gelable Protein
Extracted with MgO-Na₂CO₃

Alkali Treatment	Protein Conc. (w/w)	Bloom (g)	Dynamic Viscosity (cP) ^a	Density ^a
Before Deionization^b				
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
After Deionization^c				
5% MgO-1% Na ₂ CO ₃	5.51	112.4	1.8963	0.9979
4% MgO-2% Na ₂ CO ₃	5.69	115.6	2.0489	0.9978
After Deionization^{bd}				
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

^a @60°C

^b 6.67% (w/w) solutions

^c Samples above were deionized and the protein concentration remained constant

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

TABLE III
Effect of Deionization on Physical Properties of Gelable Protein
Extracted with MgO-K₂CO₃

Alkali Treatment	Protein Conc. (w/w)	Bloom (g)	Dynamic Viscosity (cP) ^a	Density ^a
Before Deionization^b				
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
After Deionization^c				
5% MgO-1% K ₂ CO ₃	5.60	96.1	1.9130	0.9996
4% MgO-2% K ₂ CO ₃	5.64	106.3	2.0384	1.0010
After Deionization^{bd}				
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

^a @60°C

^b 6.67% (w/w) solutions

^c Samples above were deionized and the protein concentration remained constant

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

TABLE IV
Effect of Deionization on Physical Properties of Gelable Protein
Extracted with MgO-NaOH

Alkali Treatment	Protein Conc. (w/w)	Bloom (g)	Dynamic Viscosity (cP) ^a	Density ^a
Before Deionization^b				
4% MgO-1% NaOH	5.66	70.0	2.1662	1.0082
3% MgO-2% NaOH	5.84	34.0	1.6490	1.0092
After Deionization^c				
4% MgO-1% NaOH	5.66	89.5	1.8584	0.9996
3% MgO-2% NaOH	5.84	35.8	1.4780	1.0010
After Deionization^{bd}				
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 Samples above were deionized and the protein concentration remained constant

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

extracted with the higher concentration of sodium hydroxide, however, had an even lower Bloom value of 31.0. When these two samples were deionized, the 4% MgO-1% NaOH sample increased to 116.4 but the higher hydroxide sample fell to 28.2. These data indicate the difficulty of controlling the reaction when sodium hydroxide is used. Lack of control leads to more degraded gelable protein with poorer physical properties.

Table V shows the effect of deionization on gelable protein samples isolated from a magnesium oxide-potassium hydroxide 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 sodium hydroxide was used. When the salt was removed from these low protein concentration samples, the effect on the Bloom was negligible. When the deionized samples with the high protein concentration were examined, the increase in the Bloom value was significant. The sodium hydroxide samples were apparently too degraded, so that even at a higher protein concentration, no improvement could be seen. This phenomenon will also be observed in the product isolated by enzymic hydrolysis, as shown on Table VI. If the sodium hydroxide is to be 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 type protein could be isolated from the chrome sludge after it was treated with an enzyme. In this experiment, in which 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. A jellygram determination, which is similar to the Bloom determination except that a 12.5% (w/w) concentration is used², was run on this sample. Jellygram values are usually 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. A sample in which the protein concentration is approaching 12.5% is also shown and the value is similar to the deionized lower protein concentration sample. However, the viscosity of this 12.5% concentration sample has increased significantly. 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. Rose² 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 sludges with careful control of the enzyme concentration.

The yields of the isolated protein products and the material balances were calculated for all the experiments in which the protein products were isolated for determination of the physical properties. Figure 1 shows a general description of the process that we used. 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 appropriate. After the enzymic treatment, filtration was easy and proceeded quickly.

All isolated fractions were weighed; moisture and ash were run on the chrome substrates while total solids and total ash determinations were performed on the isolated protein fractions. Table VII summarizes the data collected from the analyses of the extracted gelable protein. These results were tabulated from 27 separate experiments. For each parameter, the standard deviation was calculated. The reproducibility of the moisture, ash and total solids analyses in these experiments was quite good, as indicated by the standard deviation. The standard deviations for total ash were higher and this was due to the varying alkalis that were used in the extractions. The gelable protein yield will be dependent on the type of alkali used; this varies and the standard deviation for this parameter would be meaningless and therefore was not calculated.

Table VIII shows the results from the analyses of the products isolated from the hydrolysis of the chrome sludges. The standard deviation values for moisture and total solids were quite close; again the ash in the chrome cake and hydrolyzed protein products would be dependent on the pretreatment and thus there would be more of a variation. The standard deviation value for the yield of the hydrolyzed protein products was high, and this may be due to the variation in the yield of the gelable protein products. The standard deviation for the overall protein yield will reflect the efficiency of the alkali treatments. The ash balances were calculated to check the overall precision of the analyses.

Table IX shows the results from two experiments in which 5% MgO-1% Na₂CO₃ was used as the alkali to treat the chrome shavings. The standard deviations for the material balance and for the individual analyses are quite good; these data indicate the reproducibility of the process as well as the precision of weighings, sampling and analyses.

TABLE V
Effect of Deionization on Physical Properties of Gelable Protein
Extracted with MgO-KOH

Alkali Treatment	Protein Conc. (w/w)	Bloom (g)	Dynamic Viscosity (cP) ^a	Density ^a
Before Deionization^b				
4% MgO-1% KOH	5.55	94.2	2.2236	1.0087
3% MgO-2% KOH	5.53	68.7	1.9550	1.0116
After Deionization^c				
4% MgO-1% KOH	5.55	93.6	1.9348	1.0002
3% MgO-2% KOH	5.53	75.8	1.7330	1.0014
After Deionization^{bd}				
4% MgO-1% KOH	6.64	180.3	2.9109	1.0014
3% MgO-2% KOH	6.61	149.5	2.5094	1.0015

^a @60°C

^b 6.67% (w/w) solutions

^c Samples above were deionized and the protein concentration remained constant

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

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 ^d	12.0	14.8	2.1759	1.0136
After Deionization ^{be}	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 Samples above were deionized and the protein concentration remained constant

^e These samples were first deionized and a 12.5% (w/w) solution was examined

TABLE VII
Data from Extraction of Gelable Protein

Parameter	Average ^a	Std. Dev.
Material Balance	96.09 ^b	2.83
Chrome Sludge		
Moisture	84.11	1.20
Ash (MFB)	19.64	1.27
Gelable Protein		
Total Solids	2.84	0.59
Total Ash (MFB)	15.13	2.41
Gelable Protein Yield	Varies	

^a N = 27

^b All values expressed as percent

TABLE VIII
Data from Hydrolysis of Chrome Sludge

Parameter	Average ^a	Std. Dev.
Material Balance	95.24 ^b	3.13
Chrome Cake		
Moisture	81.78	1.33
Ash (MFB)	31.66	2.12
Hydrolyzed Protein		
Total Solids	6.13	0.35
Total Ash (MFB)	5.21	1.48
Hydrolyzed Protein Yield ^c	62.09	5.29
Total Protein Yield ^c	79.83	6.91
Ash Material Balance	101.29	4.59

^a N = 27

^b All values expressed as percent

^c Based on 92.3% protein (MF-AFB) in chrome shavings

TABLE IX
Reproducibility of Process Using 5% MgO-1% Na₂CO₃ as Alkali

Parameter	Run #1	Run #2	Average	Std. Dev.
Gel Extraction:				
Material Balance	92.60 ^a	98.60 ^a	95.60 ^a	3.08
Chrome Sludge				
Moisture	84.02	83.77	83.90	0.13
Ash (MFB)	19.64	19.49	19.57	0.07
Gelable Protein				
Total Solids	2.10	1.88	1.99	0.11
Total Ash (MFB)	17.40	18.45	17.93	0.52
Gel Yield ^b	22.61	21.26	21.94	0.67
Hydrolysis:				
Material Balance	94.70	95.50	95.10	0.40
Chrome Cake				
Moisture	81.18	80.92	81.05	0.13
Ash (MFB)	32.36	31.76	32.06	0.30
Hydrolyzed Protein				
Total Solids	6.07	6.20	6.17	0.09
Total Ash	4.10	3.91	4.01	0.10
Hydrolyzed Protein				
Yield ^b	62.10	66.21	64.16	2.05
Total Protein Rec ^b	72.88	76.60	74.75	1.86
Ash Recovery	93.79	100.04	96.92	3.13

^a All values expressed as percent

^b Based on 92.3% protein (MF-AFB) in chrome shavings

Table X shows the effect of alkali on the gelable protein yield, the hydrolyzed protein yield and total protein yield. With respect to yield of the gelable protein, it appears that an increase in the alkalinity will increase the yield. With respect to the hydrolyzed protein products, the alkali treatment in the first step does not appear to affect the yield of the protein from the hydrolysis step. An exception to this is the 3% MgO-2% NaOH treatment, in which a higher amount of protein is extracted in the first step. The overall yield is a calculated value and is not a total of the gelable and hydrolyzed protein product yields. Gelable protein remains in the chrome sludge before hydrolysis and the amount that remains must be taken into account in the calculation.

CONCLUSION

The gelable protein products extracted from chromium-containing solid tannery waste have a high ash content which is due not only to the type of alkali that is used but also to the high ash content of the original waste product. Treating the protein solution with a mixed-bed deionizing resin, a common practice in gelatin production, decreased the ash to less than 1% on a moisture-free basis. The physical properties, such as Bloom and viscosity, were improved in the deionized samples and this is probably due to the increased protein concentration with a small effect contributed by the removal of the ash. Also, the type and concentration of the alkali will affect the physical

TABLE X
Effect of Alkali on Protein Yield

Alkali Treatment	Protein Yield ^b		
	Gel	Hydrolysate	Total
MgO	24.62	67.13	79.44
5% MgO-1% Na ₂ CO ₃	21.26	66.21	76.61
4% MgO-2% Na ₂ CO ₃	31.70	67.72	82.78
5% MgO-1% K ₂ CO ₃	24.81	66.15	77.71
4% MgO-2% K ₂ CO ₃	30.92	63.34	78.10
4% MgO-1% NaOH	30.21	63.73	79.38
3% MgO-2% NaOH	51.79	58.32	84.41
4% MgO-1% KOH	26.91	65.65	78.95
3% MgO-2% KOH	30.58	65.61	80.39

^a Based on 92.3% protein (MF-AFB) in chrome shavings

^b Expressed in percent

properties. The physical properties of the products are similar to those obtained from technical grade gelatin, and the reported uses for gelatin could apply to these products obtained from the solid waste. We have also demonstrated that by adjustment of the enzyme concentration when treating the sludge, one can obtain a product with gel-like properties, indicating that a higher-value product can be obtained from the hydrolysis step. We have shown that the type and concentration of alkali used to extract the gelable protein from the solid chrome waste will affect the yield of the protein. Finally, we have demonstrated that these processes can be carried out reproducibly and that good material balances are obtained.

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