

# CHEMICAL MODIFICATION OF PROTEIN PRODUCTS ISOLATED FROM CHROMIUM-CONTAINING SOLID TANNERY WASTE AND RESULTANT INFLUENCE ON PHYSICAL AND FUNCTIONAL PROPERTIES\*†

by

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## ABSTRACT

Samples of gelatin extracted from chromium-containing leather waste by different protocols were chemically modified with glutaraldehyde, glyoxal and carbodiimide and compared to similarly modified commercial gelatins. Glutaraldehyde appeared to be a very efficient cross-linker, giving gels that would not melt at 70°C with the addition of only 2% glutaraldehyde but with a decrease in their rigidity when the amount of glutaraldehyde was increased. Foaming tests on these samples demonstrated that the addition of low amounts of glutaraldehyde increased both the foam capacity and the foam stability of the gelatins. Glyoxal modification did not have such an effect on the gel strength and melting point of the samples. It did not increase foam capacity but did increase foam stability, it gave better adhesive properties to these gelatins and it did increase dramatically their emulsification capacity. Carbodiimide had similar effects on gel strength as glyoxal; it did increase dramatically the melting point of commercial gelatin but had no effect on the melting point of the extracted gelatins. It decreased the foam and adhesive properties of all the samples, but it did increase the emulsification capacity of the extracted gelatins. The study demonstrates that the functional properties of

chemically modified gelatin extracted from chromium-containing leather waste were changed, and, as a result, new uses could be identified for these value-added products.

## INTRODUCTION

Gelatin is used commercially for one or more of the following properties: gel formation, emulsion stabilization, foam stabilization, flocculant formation, film formation, adhesive, and as a protective colloid. Any structural alteration to a reactive group could modify the physical behavior. This is often what is sought in the study of chemical modification. Reaction can take the form of substitution at one or more types of reactive sites, which is termed "chemical modification," or a reaction may be polyfunctional, in which case it can bridge adjacent protein chains. This is known as "cross-linking."

Cross-linking of gelatin is most commonly carried out in aqueous solution or on cast layers of the protein. Concentration is an important factor, as distance between molecules will determine whether linking will take place intermolecularly or intramolecularly. Intermolecular cross-linking increases the molecular weight, which in the case of a high grade gelatin is already large. In aqueous solution the intermolecular cross-linking is manifested by a rise in viscosity. If allowed to continue, the solution becomes viscoelastic. Further cross-linking causes the flow proper-

ties to disappear altogether and the material forms a rigid gel, which will either have an elevated melting point or fail to revert to a solution at all when heated. The progress of these effects depends upon the degree of cross-linkage. Cross-linked gelatin solutions generally give rise to weak gels on cooling, presumably because the covalent links introduced in the solution phase interfere with the freedom of the polypeptide chains to adopt the conformation necessary for the gel structure. On the other hand, if the gelatin is cross-linked in the gel state, dramatic increases in the rigidity occur.

Covalent cross-linking of gelatin has been extensively reported in the literature, and glutaraldehyde, glyoxal and carbodiimide have been broadly studied as hardening agents. While glutaraldehyde is fairly nonspecific in its reactivity, glyoxal modifies specifically arginine<sup>2</sup> and carbodiimide introduces new substituents at the carboxyl groups and amide cross-links.<sup>1</sup> The mechanisms of the modification of proteins by these reagents have been reported in the literature.<sup>3,4</sup>

Glutaraldehyde<sup>4</sup> cross-linking of proteins proceeds through the formation of  $\alpha,\omega$ -Schiff bases. One would expect this to be a readily reversible process in the absence of reducing agents such as sodium borohydride or sodium cyanoborohydride. An early study<sup>5</sup> showed that the reaction of proteins with glutaraldehyde was essentially irreversible even without reduction and resulted in a loss of available lysine. Later studies<sup>6</sup> showed that the chemistry of the reaction of glutaraldehyde is complex. Although glutaraldehyde has a fair degree of specificity for the  $\epsilon$ -amino group of lysine, reaction also occurred with other nucleophilic functional groups in proteins such as the sulfhydryl group of cysteine, the imidazole ring of histidine, and the phenolic hydroxyl group of tyrosine.<sup>7</sup>

Until approximately 20 years ago the specific chemical modification of arginine was relatively difficult to achieve.<sup>4</sup> It is reasonable to suggest that the recent advances in the study of the function of arginine residues in proteins stem from the work on the use of phenylglyoxal<sup>8</sup> as a reagent for the specific modification of arginine, though observations on the use of 2,3-butanedione<sup>9</sup> and glyoxal<sup>10</sup> appeared at approximately the same time. In the modification of arginyl residues with glyoxal, the specificity of reaction may be complicated by reaction also at primary amino groups and sulfhydryl groups.<sup>11</sup> For example, reaction of glyoxal with bovine serum albumin at pH 9.0 resulted in modification of greater than 80% of the arginine residues, with approximately 30% modification of lysine residues.<sup>11</sup>

The use of carbodiimide-mediated modification of carboxyl functional groups in proteins is by far the most widely used method for the study of such functional groups.<sup>4</sup> The most popular approach utilizes a water-soluble carbodiimide as the activating agent.<sup>12</sup> Despite the problems with side reactions, modification of carboxyl groups in proteins with a water-soluble carbodiimide and an appropriate nucleophile has proven extremely useful and is reviewed in several articles.<sup>13</sup>

In the present work, samples of gelatin extracted from chromium-containing leather waste by different protocols were treated with glutaraldehyde, glyoxal and carbodiimide and were compared to similarly modified commercial gelatin. Physical properties, gel strength and melting point, and functional properties, foamability, adhesive properties and emulsion capacity, were examined.

## EXPERIMENTAL

### Materials

Gelatin, type B from bovine skin, 75 and 225 g Bloom, glyoxal and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) were obtained from Sigma Chemical Co. (St. Louis, MO). Glutaraldehyde was obtained from Eastman Kodak Co. (Rochester, NY).

Alkali-extracted and enzyme-extracted gelatin from chrome shavings were isolated by processes described in previous publications.<sup>14,15</sup> The alkali-extracted gelatin used in this study was obtained by treating the chromium-containing leather waste with 6% MgO (Fisher Scientific, Fair Lawn, NJ) in an aqueous solution for 6 hr at 70°C for the one labeled "high Bloom" and at 75°C for the one labeled "low Bloom." The enzyme-extracted gelatin was obtained by treating the chromium-containing leather waste with 0.01% pepsin (Sigma Chemical Co., Saint Louis, MO) in an aqueous solution at room temperature for 8 hr and, after an adjustment of the pH to 8 with MgO, at 70°C for 3 hr. The gelatin solutions were filtered and stored at 4°C. All samples were deionized using Amberlite MB-150 mixed bed resin (Sigma Chemical Co., Saint Louis, MO). The solutions were lyophilized and these dried samples were used for chemical modification.

### Chemical Modification

A 6.67% wt/wt concentration sample of gelatin was prepared and allowed to swell for 1 hr at ambient temperature. Then it was equilibrated at 50°C for 30 minutes. The modifier was added and the sample was shaken at 50°C for 1 hour when glutaraldehyde or carbodiimide were used and

4 hours when glyoxal was used. The sample was placed in a 10°C bath for 17-18 hours before any test was performed.

## METHODS

### Analyses

Moisture, ash, TKN, chrome content, gel strength, dynamic viscosity, density and molecular weight distribution of the extracted gelatins were determined as described in a previous publication.<sup>14</sup>

### Gel Strength

The Bloom Test<sup>16</sup> to determine gel strength was run using a TA.XT2 Texture Analyzer, designed and manufactured by Stable Micro Systems (Godalming, Surrey, UK) and marketed by Texture Technologies Corporation (Scarsdale, NY).

### Melting Point

The melting points of the gelatins were measured by the method BSI, 1975<sup>17</sup> with the modification as described by Rose.<sup>18</sup> Samples were prepared in test tubes as described above. A few drops of Bromophenol Blue indicator and a small solid sphere were added carefully to the top of the samples. The temperature of the bath was raised at 0.5°C per minute until samples melted as indicated by the drop of the sphere. As recommended, the temperature was recorded when the sphere was half way down the gel.<sup>19</sup>

### Foamability

Foam capacity and stability were determined as described in a previous publication.<sup>20</sup> Samples were prepared as described above and the next day were heated at 60°C for 15 min. Each sample was transferred to a Waring Blender with a mini mixing chamber attached. The sample was whipped for 15 seconds and then transferred to a 100 mL graduate cylinder. The initial foam volume was read at 0 time and every 5 minutes for 2 hrs while the samples stood in a 38°C water bath. The foam capacity or foam volume increase (volume after whipping minus the volume before whipping divided by the volume before whipping at 0 time and expressed as percent) and foam stability (foam capacity at specific time) over a 2 hour period were calculated.<sup>21-23</sup>

### Adhesive Test

Determination of the adhesive properties was carried out as described in previous publications<sup>20,24</sup> using a TA.XT2 Texture Analyzer, and its XT.RA Dimension software to set the conditions for the analysis, run the determinations and calculate the results. Samples were prepared as described

above and the next day were equilibrated at 35°C. The appropriate amount of sample, about 0.1 g, was weighed onto a lexan plate. The plate was then placed on the platform of the instrument and was screwed tightly in place. The appropriate parameters were programmed into the instrument and the test was begun. An acrylic probe of 1 inch diameter was used, approaching the sample at a speed of 1 mm/sec. When it sensed the sample, the speed increased to 2 mm/sec. A force of 100 grams was applied for 2 seconds and the probe was pulled away from the sample at a speed of 10 mm/sec for a distance of 5 mm. The software calculated the tackiness (peak, g) and the work of adhesion (area, g·s); the ratio of tackiness to the work of adhesion was calculated upon completion of the determinations, where smaller values would indicate higher grade adhesives.

### Emulsion Capacity

Emulsion capacity was determined by the method described in a previous publication.<sup>20</sup> Samples were prepared as described above and equilibrated at 38°C. Each sample was processed using a Hamilton Beach hand blender for 30 sec and peanut oil was then slowly added, with blending, to the sample over a one hour time period. The oil was added until the emulsion broke. The grams of oil emulsified per gram of protein was calculated.<sup>25</sup>

## RESULTS AND DISCUSSION

For the production of Type B gelatin, both ossein and cattle hide pieces are subjected to lengthy treatment with an alkali (usually lime) and water at ambient temperature. Depending on previous treatment, the nature of the material, the size of the pieces, and the exact temperature, liming takes from 5 to 20 weeks. After conditioning, the raw material is thoroughly washed with cold water to remove excess lime, the pH adjusted with acid, and the product extracted with hot water to recover the soluble gelatin. The number of extractions varies; 3 to 6 is typical. The first extraction generally takes place at 50-60°C, subsequent extractions being made with successive increases in temperature of 5-10°C. The final extraction is carried out close to the boiling point. Extracts are kept separate, analyzed, and subsequently blended.<sup>26</sup> Two different Type B gelatins (225 g Bloom and 75 g Bloom) were used as references in the experiments described in this paper.

Non-commercial gelatins used in the experiments described in this paper were isolated from chrome shavings using two different methods. The alkali-extracted gelatin was isolated using 6% magnesium oxide in a 500% float at 70°C for

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6 hrs.<sup>14</sup> If the temperature is carefully controlled at 72°C a high Bloom gelatin is obtained, if the temperature is raised up to 75°C, a low Bloom gelatin is isolated. The enzyme-extracted gelatin was isolated, after a pretreatment with pepsin for 8 hrs at room temperature and pH 3, with an extraction at 72°C and pH 8.15 The chemical and physical properties of these products, after deionization and lyophilization, are presented in Table I.

As seen in Table I, the alkali-extracted gelatins show similar nitrogen and chrome content, about 18% TKN and 50 ppm of chromium, but the enzyme-extracted gelatin has a nitrogen content of 12% TKN and a chromium content of only 16 ppm. All the other chemical properties are very similar for the three samples used. The main differences in the physical properties are in the gel strength. The enzyme-extracted gelatin has the higher value, about 201 g Bloom; the high Bloom alkali-extracted sample has a value of 163

and the low Bloom of 96 g Bloom, which are comparable to the values of the commercial samples, labeled as 225 and 75 g Bloom, which read 238 and 122 g Bloom when tested.

Table I also presents the molecular weight distribution of these gelatins. The percentage of sample in the high range of molecular weight follows the pattern shown by the gel strength values, that is, the enzyme-extracted sample has the highest percentage, followed closely by the high Bloom alkali-extracted, and the low Bloom sample has a much lower value.

The variation of gel strength and melting point of gelatin treated with different amounts of the modifiers was studied. Figure 1 shows the changes in the gel strength of the gelatin when treated with a. glutaraldehyde, b. glyoxal and c. carbodiimide; Figure 2 presents these changes on the melting point.

**TABLE I**  
**Properties of Lyophilized Gelatins**

Parameter	Alkali-extracted		Enzyme-extracted
	High Bloom	Low Bloom	High Bloom
<b>Chemical†</b>			
pH	5.42	5.24	5.49
Moisture (%)	10.15	9.51	9.76
Total ash MFB (%)	<1.0	<1.0	<1.0
TKN M-AFB (%)	17.82	18.62	12.43
Chromium MFB (ppm)	48.0	50.0	15.73
<b>Physical‡, §</b>			
Gel Strength (g Bloom)	162.7	96.0	201.3
Dynamic Viscosity* (cP)	2.6345	2.1570	2.7968
Density* (g/mL)	1.0033	1.0006	1.0029
<b>Molecular weight distribution (%)</b>			
>208,000-85,000 D	47.1	21.0	57.9
85,000-50,000 D	21.6	24.8	19.5
50,000-<7,200 D	31.3	54.2	22.6

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

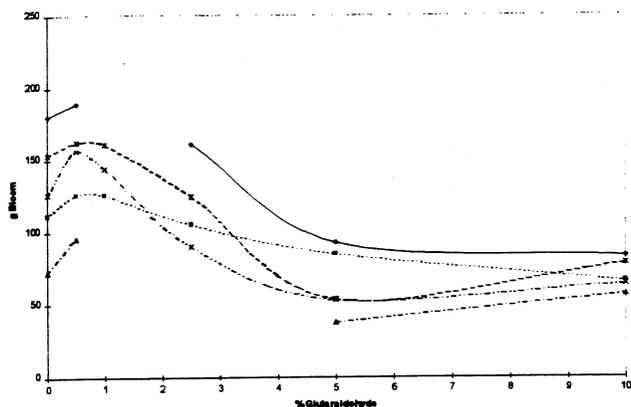
‡ N = 1 where N = number of replicates for each sample.

§ 6.67% (w/w) solution

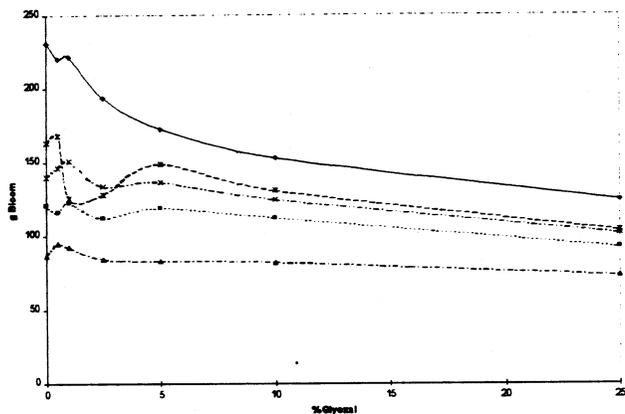
\* @ 60°C

MFB = Moisture Free Basis AFB = Ash Free Basis TKN = Total Kjeldahl Nitrogen

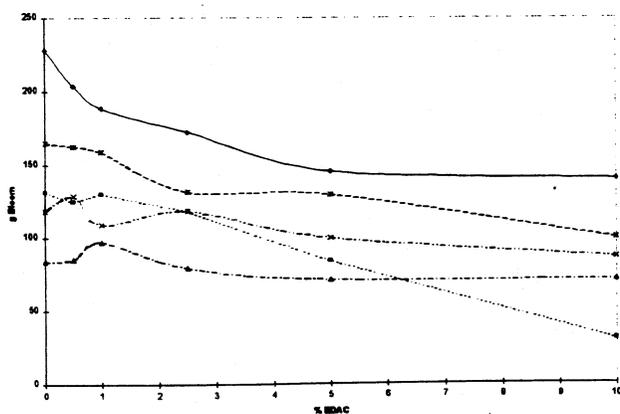
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a. Effect of glutaraldehyde



b. Effect of glyoxal



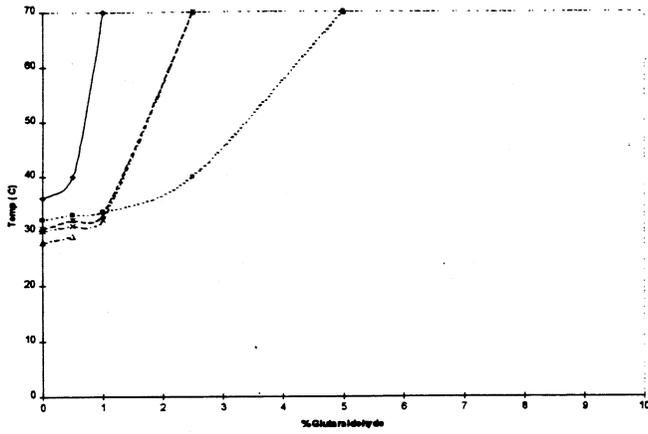
c. Effect of carbodiimide

## Gelatin

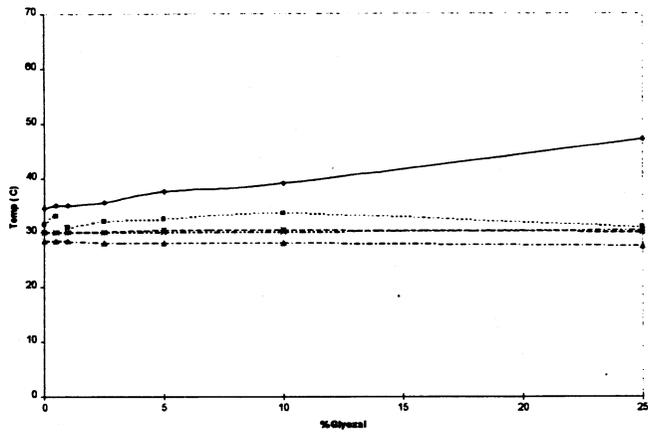
- ◆— 225 g Bloom commercial gelatin
- 75 g Bloom commercial gelatin
- ▲- MgO-ext low Bloom gelatin
- ×- MgO-ext high Bloom gelatin
- ✱- Pepsin-ext high Bloom gelatin

FIGURE 1. — Effect of chemical modification on the gel strength (measured as g Bloom) of the treated gelatins.

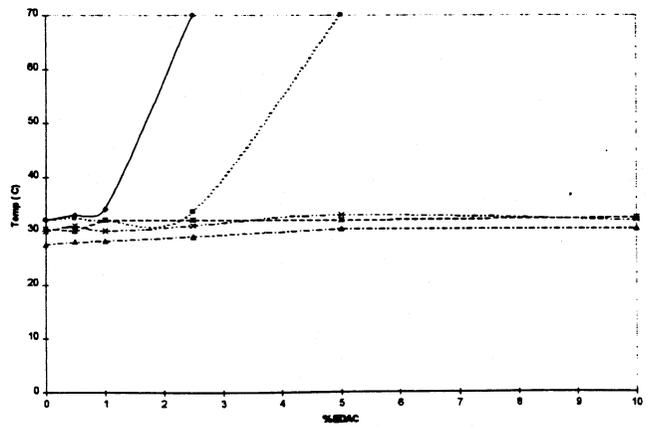
CHEMICAL MODIFICATION OF RECOVERED GELATIN



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Gelatin

- ◆— 225 g Bloom commercial gelatin
- -■- - 75 g Bloom commercial gelatin
- -▲- - MgO-ext low Bloom gelatin
- ...×... MgO-ext high Bloom gelatin
- \* - Pepsin-ext high Bloom gelatin

FIGURE 2. — Effect of chemical modification on the melting point of the treated gelatins.

As seen in Figure 1a, all the samples followed the same tendency, showing a slight increase of gel strength when a very low amount of glutaraldehyde was added and a decrease when more than 1% was added. As described in the introduction, the addition of cross-links in the gel decreases the degrees of freedom of the molecules to rearrange and give a high gel strength. The cross-linking of the gelatin after the treatment with glutaraldehyde is shown again in Figure 2a, where the melting point of the samples is presented. With the addition of about 2% glutaraldehyde, the samples did not melt even at 70°C, while the controls had a melting point of 28-35°C.

Figure 1b shows the effects of different levels of glyoxal modification on the samples of gelatin; in these experiments the gel strength did not significantly increase, but did in fact decrease particularly after the addition of at least 10% glyoxal (except for the 225 g Bloom commercial gelatin). The melting point (Figure 2b) did not change after modification with this aldehyde.

The gel strengths of carbodiimide modified gelatins (Figure 1c) were similar to the gel strengths of glyoxal modified gelatins. The gel strength of the gelatin decreased after the addition of at least 2% carbodiimide. The effect on the melting point (Figure 2c) is different for the commercial gelatins than for the extracted gelatins. The modified commercial gelatins did not melt at 70°C if more than 1% carbodiimide was used to modify the 225 g Bloom or more than 2.5% was used to modify the 75 g Bloom; the extracted gelatins did melt at about 30°C even with the use of 10% carbodiimide.

It has been reported that the amino acid composition of gelatins prepared by the alkaline process (Type B gelatin) differs from those produced by the acid process (Type A gelatin).<sup>27</sup> Also, the heterodisperse nature of the molecular weights of gelatins has been discussed, highlighting the advantage of a high molecular weight gelatin in matters such as cross-linkage and chemical functionality.<sup>1</sup> All these differences are obvious between the gelatins studied, not only between commercial and non-commercial, but also between the non-commercial gelatins, which were extracted with different methods.

To see the effect of chemical modification with glutaraldehyde, glyoxal and carbodiimide on the functional properties of the different gelatins, each gelatin was tested unmodified, and modified with 2% glutaraldehyde, 25% glyoxal or 2.5% carbodiimide, and the resultant functional properties, foamability, adhesiveness and emulsion capacity were measured.

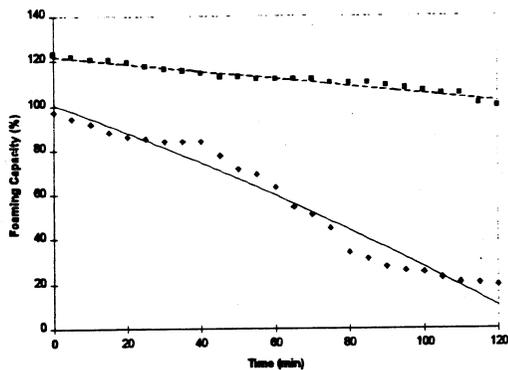
Figure 3 presents the results of the foaming test of the different gelatins used, comparing the different treatments. Figure 3a shows the foaming test results for the commercial sample 225 g Bloom. The unmodified gelatin shows a good foam capacity, but the foam stability is not so good, since after 1 hour the foam capacity has decreased 50%. The results of the modification of this sample with glutaraldehyde and carbodiimide are not presented, because the gelatin did not melt at 60°C as required for the foaming test. The modification with glyoxal increased both the foam capacity and stability of the 225 Bloom commercial gelatin.

The high Bloom alkali-extracted gelatin (Figure 3b) has a higher foam capacity than the 225 g Bloom commercial gelatin and also an excellent foam stability, since after two hours, the foam capacity has decreased less than 10%. Again, the results of the glutaraldehyde modification of this sample are not presented, because the gelatin did not melt at 60°C as required for the foaming test. The glyoxal modification had little effect on foaming properties but the carbodiimide modification decreased the foam capacity about 20% and also the foam stability of this sample.

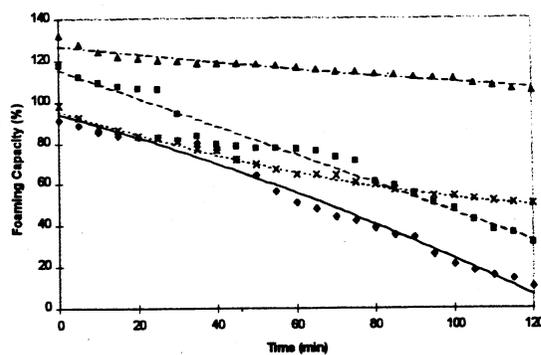
Figure 3c presents the results of the foaming test of the enzyme-extracted gelatin. The foam capacity and stability of this sample are very similar to the 225 g Bloom commercial gelatin (Figure 3a), suggesting that the commercial gelatin was probably extracted with pepsin, as usual in commercial production. Glutaraldehyde cross-linking of the enzyme-extracted gelatin increased the foam capacity about 20%, and also the foam stability. The glyoxal modification did not change the foaming properties of these samples and the carbodiimide modification decreased them, similarly to what is shown by the high Bloom alkali-extracted gelatin.

Figure 3d shows the results of the foaming test for the 75 g Bloom commercial gelatin. The foam capacity and stability of this sample are very similar to the results from the 225 g Bloom commercial gelatin. When the 75 g Bloom commercial gelatin was cross-linked with glutaraldehyde, the foam capacity increased about 50%, reaching the values shown by the 225 g Bloom gelatin, and the foam stability was also improved. The glyoxal modification did not change the foam capacity of the sample, but increased the foam stability, while carbodiimide modification increased the foam capacity, but did not change the foam stability. It is important to realize that commercially, gelatin is not a uniform product; the desired Bloom value is achieved by blending different gelatins with different gel strengths. The blending influences the way the gelatin is cross-linked or modified.

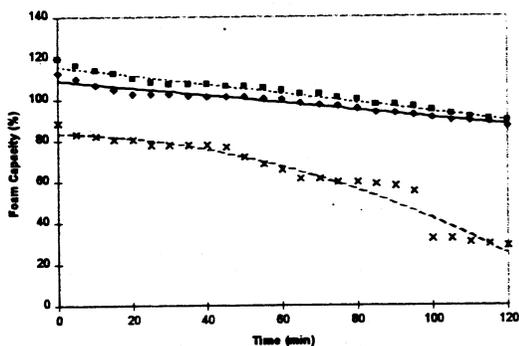
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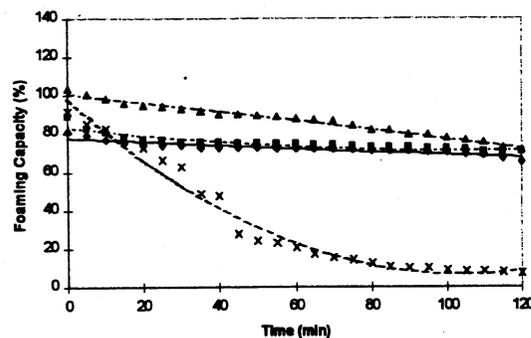
a. 225 g Bloom commercial gelatin



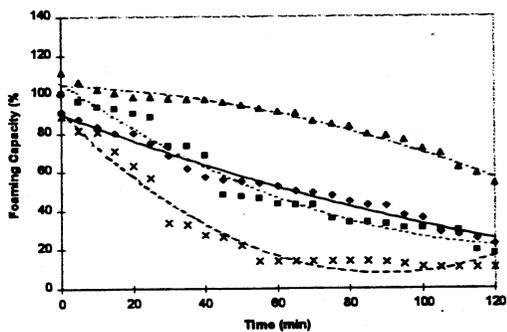
d. 75 g Bloom commercial gelatin



b. MgO-ext high Bloom gelatin



e. MgO-ext low Bloom gelatin



c. Pepsin-ext high Bloom gelatin

### Modification

- ◆— as is
- -▲- - + 2% glutaraldehyde
- -■- - + 25% glyoxal
- -×- - + 2.5% carbodiimide

FIGURE 3. — Effect of chemical modification on the foamability of the treated gelatins.

Finally, Figure 3e presents the results of the foaming test for the low Bloom alkali-extracted gelatin. As in the samples with high Bloom, the alkali-extracted gelatin had a much better foam stability than the commercial gelatin, and the foam capacity of both samples was similar. The change in the foam capacity of the low Bloom alkali-extracted gelatin after glutaraldehyde cross-linking was about 20%, and the foam stability did not show a big change. Again, glyoxal modification did not change the foam capacity of the sample, and carbodiimide modification decreased the foaming properties of the sample.

Table II shows the results of the adhesive test of the studied samples. Looking at the samples as-is, the high Bloom alkali-extracted gelatin had adhesive properties very similar to the 225 g Bloom commercial sample, while the low Bloom alkali-extracted gelatin had adhesive properties very similar to the 75 g Bloom commercial sample; the enzyme-extracted gelatin showed adhesive properties in between the two commercial samples.

Samples treated with 2% glutaraldehyde were prepared to test adhesive and emulsification properties, but these samples did not melt at 35°C, the temperature described in the experimental part as necessary for these tests.

Samples treated with 25% glyoxal were prepared and their adhesive properties are presented in Table II. While the sample prepared with 225 g Bloom commercial gelatin did not melt at 35°C, the 75 g Bloom commercial gelatin had better adhesion after glyoxal modification (it presented a decrease of about 7% in the ratio, influenced by the big change in the work of adhesion). All the extracted gelatins also improved the adhesive properties after glyoxal modification, showing a decrease between 13 and 19% in their ratio.

The adhesive properties of samples prepared with 2.5% carbodiimide are also shown in Table II. Again, the sample prepared with 225 g Bloom commercial gelatin did not melt at 35°C. The 75 g Bloom commercial gelatin had the same adhesion properties after carbodiimide modification, while the extracted gelatins decreased their adhesiveness between 3 and 20%.

Table III shows the results from the emulsification capacity test. When unmodified samples were tested, both commercial gelatins presented a value above 92 g of oil emulsified per g of protein, while the alkali-extracted gelatins presented a value between 74 and 78 g oil emulsified per g of protein, showing that the gel strength does not have any

**TABLE II**  
**Adhesive Properties of Modified Gelatins**

Sample <sup>‡</sup> , §	Unmodified			Glyoxal			Carbodiimide		
	Peak*	Area <sup>§</sup>	Ratio <sup>¶</sup>	Peak*	Area <sup>§</sup>	Ratio <sup>¶</sup>	Peak*	Area <sup>§</sup>	Ratio <sup>¶</sup>
<b>Commercial</b>									
225 Bloom	1888.1	37.57	50.61	—	—	—	—	—	—
75 Bloom	1189.8	20.42	58.44	1565.9	28.99	54.49	1190.4	21.03	57.02
<b>Alkali-extracted</b>									
High Bloom	1730.0	33.80	51.30	2301.6	51.83	44.50	923.7	15.00	61.70
Low Bloom	1052.1	15.87	66.84	1513.6	28.14	53.81	789.9	12.17	64.90
<b>Enzyme-extracted</b>									
High Bloom	1332.1	23.30	57.24	2006.7	43.20	46.50	830.9	13.06	63.62

<sup>‡</sup> N = 5 where N = number of replicates of each sample.

<sup>‡</sup> Samples equilibrated at 35°C

\* Tackiness in g

§ Work of adhesion in g s

¶ Low ratio (peak/area) indicates good adhesive properties.

influence on this functional property. The enzyme-extracted gelatin emulsified 81 g of oil, demonstrating again that the way the gelatin was extracted has more influence on this property than the physical characteristics of the sample.

When the gelatins were modified with glyoxal, the emulsification capacity increased considerably in the low Bloom alkali-extracted gelatins but not much in the commercial low Bloom alkali-extracted and in the high Bloom extracted gelatin. Glyoxal modified high Bloom enzyme-extracted gelatin did not show any change. Carbodiimide modification decreased the emulsification capacity of the 75 g Bloom commercial gelatin and increased the emulsification capacity of all the extracted gelatins.

### CONCLUSIONS

Three different samples of gelatin extracted from chromium-containing leather waste each were chemically modified with glutaraldehyde, glyoxal and carbodiimide. Glutaraldehyde appeared to be a very efficient cross-linker, giving gels that would not melt at 70°C with the addition of only 2% glutaraldehyde but with a decrease in their rigidity when the amount of glutaraldehyde increases above 2%. Foaming tests on these samples demonstrated that the addition of low amounts of glutaraldehyde increases both foam capacity and foam stability of the gelatins. Glyoxal modification did not have such an effect on the gel strength and melting point of the samples. It did not increase foam capacity but did increase foam stability, it gave better adhesive properties to these gelatins and it increased

dramatically their emulsification capacity. Carbodiimide had similar effects on gel strength as glyoxal; it dramatically increased the melting point of commercial gelatin but did not have any effect on the melting point of the extracted gelatins. It decreased the foam and adhesive properties of all the samples, but it increased the emulsification capacity of the extracted gelatins. The results indicate that depending on the application of the gelatin, a different modifier can be used to get the desired functional properties.

### ACKNOWLEDGMENTS

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### REFERENCES

1. Clark, R. C. and Courts, A.; *The Chemical Reactivity of Gelatin*. in *The Science and Technology of Gelatin*. (A. G. Ward and A. Courts eds.) Academic Press, New York, pp. 209-247, 1977.
2. Barker, R.; *Organic Chemistry of Biological Compounds*, Prentice-Hall, Inc., Englewood, New Jersey, pp. 66, 121, 1971.
3. Robinson, I. D.; Rate of Cross-linking of Gelatin in Aqueous Solution. *J. Appl. Polymer Sci.* **8**, 1903-1918, 1961.

**TABLE III**  
**Emulsification Capacity (g oil/g Protein) of Modified Gelatins**

Sample <sup>†</sup>	Unmodified	Glyoxal	Carbodiimide
<b>Commercial</b>			
225 Bloom	96.6	—	—
75 Bloom	92.4	97.7	77.4
<b>Alkali-extracted</b>			
High Bloom	73.9	88.8	83.1
Low Bloom	78.2	108.8	84.7
<b>Enzyme-extracted</b>			
High Bloom	81.4	80.8	89.6

<sup>†</sup> N = 1 where N = number of replicates of each sample.

4. Lundblad, R. L. and Noyes, C. M.; Chemical Reagents for Protein Modification, Vol. 2, CRC Press, Inc., Boca Raton, Florida, 1984.
5. Richards, F. M. and Knowles, J. R.; Glutaraldehyde as a Protein Cross-Linking Reagent. *J. Mol. Biol.* **37**, 231-233, 1968.
6. Monsan, P., Puzo, G. and Mazarguil, H.; Etude du mécanisme d'établissement des liaisons glutaraldéhyde-protéins. *Biochimie* **57**, 1281-1292, 1975.
7. Habeeb, A. F. S. A. and Hiramoto, R.; Reaction of Proteins with Glutaraldehyde. *Arch. Biochem. Biophys.* **126**, 16-26, 1968.
8. Takahashi, K.; The Reaction of Phenylglyoxal with Arginine Residues in Proteins. *J. Biol. Chem.* **243**, 6171-6179, 1968.
9. Yankeelov, J. A., Jr., Mitchell, C. D. and Crawford, T. H.; A Simple Trimerization of 2,3-Butanedione Yielding a Selective Reagent for the Modification of Arginine in Proteins. *J. Am. Chem. Soc.* **90**, 1664-1666, 1968.
10. Nakaya, K., Horinishi, H. and Shibata, K.; States of Amino Acid Residues in Proteins. XIV. Glyoxal as a Reagent for Discrimination of Arginine Residues. *J. Biochem.* **61**, 345-351, 1967.
11. Jonas, A. and Weber, G.; Presence of Arginine Residues at the Strong, Hydrophobic Anion Binding Sites of Bovine Serum Albumin. *Biochemistry* **10**, 1335-1339, 1971.
12. Hoare, D. G. and Koshland, D. E., Jr. A Procedure for the Selective Modification of Carboxyl Groups in Proteins. *J. Am. Chem. Soc.* **88**, 2057-2058, 1966.
13. Carraway, K. L. and Koshland, D. E., Jr. Carbodiimide Modification of Proteins. *Meth. Enzymol.* **25**, 616-623, 1972.
14. Taylor, M. M., Cabeza, L. F., DiMaio, G. L., Brown, E. M., Marmer, W. N., Carrió, R., Celma, P. J. and Cot, J.; Processing of Leather Waste: Pilot Scale Studies on Chrome Shavings. Part I. Isolation and Characterization of Protein Products and Separation of Chrome Cake. *JALCA* **93**, 61-82, 1998.
15. Cabeza, L. F., Taylor, M. M., Brown, E. M. and Marmer, W. N.; Isolation of Protein Products from Chromium-Containing Leather Waste Using Two Consecutive Enzymes and Purification of Final Chromium Product – Pilot Plant Studies *JSLTC*, **83**, 14-19, 1999.
16. AOAC Method 948.21.
17. 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.
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, New York, pp. 218, 1992.
19. Stainsby, G. and Taylor, J. T.; GGRA Research Report A20 1958.
20. Taylor, M. M., Cabeza, L. F., Marmer, W. N., Brown, E. M. and Kolomaznik, K.; Functional Properties of Hydrolysis Products from Collagen. *JALCA* **93**, 40-50, 1998.
21. Lawhon, J. T., Cater, C. M., and Mattil, K. F.; A Comparative Study of the Whipping Potential of an Extract from Several Oilseed Flours. *Cereal Science Today* **17**, 240-244, 246, 294, 1972.
22. Coffmann, C. W. and Garcia, V. V.; Functional Properties and Amino Acid Content of a Protein Isolate from Mung Bean Flour. *J. Food Technol.* **12**, 473-484, 1977.
23. Eke, O. S. and Akobundu, E. N. T.; Functional Properties of African Yam Bean (*Sphenostylis-Stenocarpa*) Seed Flour as Affected by Processing. *Food Chemistry* **48**, 337-340, 1993.
24. 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.
25. Narayana, K. and Narasinga, R.; Functional Properties of Raw and Heat Processed Winged Bean (*Psophocarpus Tetragonolobus*) Flour. *Journal of Food Science* **47**, 1534-1538, 1982.
26. Anonymous; Gelatin. Gelatin Manufacturers Institute of America, Inc., New York, pp. 6, 1993.
27. Eastoe, J. A. and Leach, A. A.; Chemical Constitution of Gelatin. in *The Science and Technology of Gelatin*, (A.G. Ward and A. Courts eds.) Academic Press, New York, pp. 73-107, 1977.