Leather Retanning with Hydrolyzed Protein

by

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Abstract

Large quantities of solid waste containing chromium are generated by the leather industry. In recent years the specialized literature has described protein and chromium recovery processes. The molecular weight spectrum of the recovered protein shows that for retanning purposes the average molecular weight is too low. This study aimed to obtain an environmentally friendly protein modification process to generate high molecular weight protein to be used in the retanning step of leather wet processing. The aim was to test potential modifications to hydrolyzed protein using different tanning agents to generate retanning products. The retanning agents developed were applied in experiments on leather retanning, where the influence of the addition of vegetable tannin was also evaluated. The controlled variables were molecular weight of the tanning preparations, leather characteristics (compressibility, density, physical-mechanical resistance, grain firmness, and color) and the COD of the wastewater. The results obtained indicated the possibility to use hydrolyzed protein combined with glutaraldehyde as a retanning agent to obtain good quality leather.

Resumen

Grandes cantidades de residuos sólidos que contienen cromo son generados en la industria del cuero. En los últimos años la literatura especializada ha descrito la proteína y los procesos de recuperación de cromo. El espectro de valores del peso molecular de la proteína recuperada muestra que como agente recurtientes, el peso molecular promedio es demasiado bajo. Este estudio fue dirigido a obtener un proceso de una proteína modificada compatible con el medio ambiente para generar una proteína de alto peso molecular que se utilizaría en el proceso húmedo de recurtido. El objetivo era poner a prueba las posibles modificaciones a la proteína hidrolizada con diferentes agentes curtientes para generar productos de recurtido. Los agentes recurtientes desarrollados se han aplicado en experimentos en procesos de cuero húmedo, dónde la influencia de la adición de tanino vegetal también fue evaluada. Las variables controladas fueron el peso molecular de las preparaciones de curtientes, las características de cuero (compresión, densidad, resistencia físico-mecánica, la firmeza de la flor, y el color) y la DQO de las aguas residuales. Los resultados obtenidos indican la posibilidad de utilizar proteínas hidrolizadas combinadas con glutaraldehído como agente de recurtido para obtener cuero de buena calidad.

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INTRODUCTION

The literature is rich in research seeking alternatives for the treatment, recycling and reuse of leather wastes, such as the manufacture of reconstituted leather, waste incineration, manufacturing of composites and separation processes for recovery of chromium and protein. These studies include those involving the enzymatic hydrolysis or alkaline digestion of chromed leather wastes, generating a solid residue rich in chromium and liquid hydrolyzed protein.[1, 2, 3] The hydrolyzed protein solution could be used in various industries to produce cosmetics, food, medicines, animal feed and fertilizers. However, the presence of traces of chromium and the low molecular mass of the protein obtained make the use of these solutions unfeasible.

Research studies show that hydrolyzed collagen protein originated in leather waste treatment can be chemically modified to obtain a collagen of greater molecular weight and suggest that such material can be used as a retanning agent, providing good leather filling properties.

Collagen and Tanning

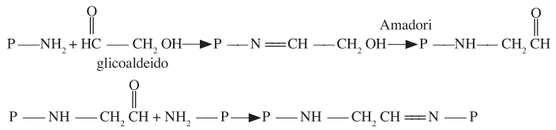
Collagen is the fibrous protein of the leather matrix. Its chemical structure is a repeating Gly-X-Y sequence, where Gly is the amino acid glycine at every third position and X and Y vary, but are not completely random. Another feature is the high content of proline and hydroxyproline amino acids in the X and Y positions. The collagen molecule arises from three polypeptide chains. Collagen molecules are stabilized by covalent intramolecular crosslinkers. These linkages are vital to the physiological role of the hide. The covalent bonds confer the tensile resistance and viscoelasticity to the matrix, needed to carry out the functions of the hide.[4] The packing of collagen fibrils gives rise to a structure known as elementary fiber, which in turn forms structures known as fiber bundles, which organize a structure known as the fiber network.

Generally, functional groups present in the structure of the hide are responsible for different leather processing reactions. The carboxylic groups, for example, connect to the chromium atoms through chemical reactions during the tanning process. The reaction mechanism between the collagen and tanning agents varies with their chemical characteristics. The functional groups of collagen protein react with tanning substances as follows:

- amino groups: location of linkage to organic molecules such as aldehydes or ionic bonds with sulfonic acid groups of synthetic tannins;
- carboxylic groups: locations of linkages to mineral tanning substances;
- peptidic groups: oxygen of the C = O bond as site of hydrogen bonding with vegetable tannins.

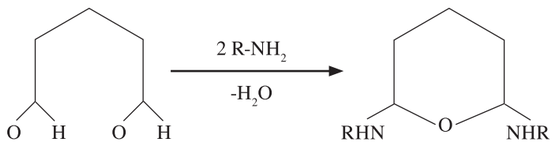
Classic tanning theory postulates that tanning substances should be bifunctional and with the appropriate molecule size to carry out crosslinking in the collagen structure. It is known that the synergistic effect can overcome insufficient ability of certain substances through their combination with others.[5]

In the case of tanning using aldehyde or other similar chemical substances a stable covalent bond forms with the amino group of collagen, but the crosslinks are polymeric and non-rigid. The tanning reactions occur only with short chain aldehydes, where the reaction may be based on an Amadori rearrangement. The simplest aldehyde, which has considerable tanning power, is formaldehyde, but the tanning effect of hydroxyaldehydes is comparable to that of formaldehyde. Figure 1 shows a scheme of the aldehyde bond formation in the case of glycolaldehyde (the simplest hydroxyaldehyde structure) with collagen.[6]



Formation of bond between aldehyde and collagen

Known hydroxyaldehydes include glycolaldehyde, 3-hydroxy-propionaldehyde and glutaraldehyde. The effectiveness of the tanning bonds of these aldehydes is considerably higher than that of other aldehydes, and the shrinkage temperatures obtained are also higher. Glutaraldehyde is today one of the most important commercial aldehydes used in the leather industry. It has a greater tanning ability than formaldehyde, can be employed under acid or alkali conditions, and has a peak performance in terms of fixing at close to pH 7.0. Figure 2 shows the reaction between cyclic glutaraldehydes and amino groups of collagen.[6]



Bond building between cyclic glutaraldehydes and collagen

The reaction of phenolic substances with collagen is based primarily on hydrogen bonds. Therefore, vegetable tannins, resins and synthetic tannins may have geometries appropriate for irreversible interaction with collagen, however, if the interaction is only due to hydrogen bonding at both crosslinker terminals, this is a reversible connection.

Dechroming of leather wastes

The separation and recovery of chromium and proteins by hydrolysis is an important alternative for the treatment of

waste generated in the leather shaving operation (thickness adjustment of leather). Authors[7] studied the isolation of proteins present in wet-blue leather residue through the action of peroxochromates based on chromium oxidation, from Cr^{+3} to Cr^{+6}, under alkaline conditions. This process produces gelatins with good yield and quality and the residual chromium content can be eliminated by ultra filtration techniques, reverse osmosis or ion exchange resins.

According to Brown and collaborators,[8] the waste market value depends on the molecular size of the protein fragments obtained, their conformation and their thermal stability, which are important factors in determining the potential use of this material. In their experiments the protein fragments were extracted with magnesium oxide alone or in combination with sodium hydroxide, potassium hydroxide, sodium carbonate or potassium carbonate. The results indicated a large dispersion of molar mass in all samples.

Taylor and collaborators[9] studied the effect of deionization on the properties of proteins recovered from tanning solid waste, which were treated in ion exchange columns to reduce the ash content. It was noted that the protein deionization reduced the ash content and that the physical properties measured showed better results for the deionized protein due to its higher purification grade.

Collagen hydrolysates obtained from shavings of chrome tanned sheep skins were used in a pretanning process for sheepskin. Wet-blue leathers pretanned with 5% protein by-product showed increased chromium take-up and better physical characteristics.[10] A retanning agent was synthesized from chrome-shaving hydrolysate modified with vinyl monomers. The chrome-shavings were hydrolyzed without de-chroming and were then modified by grafting with vinyl monomers. Good stretch and filling properties were obtained from leather retanned with this agent.[11] Cantera and collaborators[12] developed a copolymer collagen hydrolysate-acrylic acid retanning agent that show good properties in the development of full grain leather and splits. Chemical modifications to protein products isolated from tanned leather wastes were carried out and the influence in their functional properties was evaluated.[13] The samples were modified with glutaraldehyde, glyoxal and carboimide and compared with similarly modified commercial gelatin. The glutaraldehyde was found to be a powerful crosslinker, and with 2% a gel was obtained which did not melt at 70°C and which increased the ability to form foam and the protein stability.

Authors[14] proposed modifications to hydrolyzed protein in order to obtain a product of greater molar mass distribution for use in the leather retanning step. These experiments showed that a treatment with 12% glutaraldehyde at 50°C for one hour generates a modified protein with larger molar mass. In addition, experiments of wet-blue leather retanning using 12% modified protein with glutaraldehyde showed an increase of 4.7% in leather thickness compared with the non-modified protein, and that, while the non-modified protein was removed by washing, 52% of the modified protein remained absorbed by leather.

This study investigated changes in a hydrolyzed protein, in order to generate a product of higher molar mass and consequently greater tanning power. The main objective was to study the use of a modified hydrolyzed protein with different substances for application in leather retanning by evaluating the properties conferred to the leather and the wastewater generated.

EXPERIMENTAL

For the leather retanning experiments the hydrolyzed collagen protein was provided by a manufacturer of this material (Protein Trading). The hydrolyzed collagen was produced by enzymatic treatment of leather wastes without chromium and their characteristics (as specified by the manufacturer) are given in Table 1.

TABLE 1

Characteristics of hydrolyzed collagen protein

Characteristics	
Appearance	White or beige powder
Odor	Neutral
Ash	< 2,0%
Dry material	94-98%
pH (5% in water)	5.0 – 6.0
Protein (Kjeldahl method)	92 – 96%
Solubility	Cold water soluble

To study and evaluate the modifications to the hydrolyzed protein, as well as to assess their leather retanning ability, the followings (re)tanning agents were used:

- Solution of glutaraldehyde at 50% (GT);
- Solution based on modified glutaraldehyde at 43% (MGT);
- Aluminum sulfate at 53%; and
- Acacia vegetable tannin extract at 72%.

The following solutions were prepared under agitation at 50° C for 1 h:

- Aqueous solution of 10% hydrolyzed protein by weight (HP);
- Aqueous solution of 10% hydrolyzed protein and 12% glutaraldehyde (HP-GT);
- Aqueous solution of 10% hydrolyzed protein and 12% modified glutaraldehyde (HP-MGT);
- Aqueous solution of 10% hydrolyzed protein and 10% aluminum sulfate (HP-AL); and
- Aqueous solution of 10% hydrolyzed protein and 20% vegetable tannin extract.

Attempts to modify the protein solution with vegetable tannin extract were unsuccessful, since the reaction product was obtained as a precipitate. Therefore, an experiment was performed with the addition of vegetable tannin after the hydrolyzed protein, directly to the leather during the retanning. The goal of the protein modification with these tanning substances was firstly to increase the molar mass of these products to give them a leather filling property. Another goal of these changes was to create links between the collagen structure of the leather and the hydrolyzed (collagen) protein, since the tanning substances was first to increase crosslinks with the collagen.

To assess the outcome of the different chemical treatments, the molar mass distribution of the products obtained was verified in aqueous solutions by permeation gel chromatography (GPC). The samples of the prepared solutions of hydrolyzed proteins were dissolved in the proportion of 1 ml in 100 ml of distilled water, and 1 µL of each sample was injected into the GPC equipment. The retanning experiments were carried out with wet-blue leather of Zebu cattle hide. The characterization of the leather is given in Table 2.

Strips of wet-blue leather (approximately 90.0 cm x 25.0 cm) were cut out from two leathers halves (left and right) of the same animal hide and were marked in accordance with the region from which they were cut. Experiments were carried out in stainless steel drums with two strips for each retanning test, where the strips were taken carefully from two different halves and not from close regions. A complete formulation of leather retanning which could be used to produce trade leather was employed (Table 3). The amounts of chemicals used were based on the leather mass.

The two experiments were named I and II and each was composed of a group of four tests as follows (with addition of vegetable tannin only in the tests of experiment II):

TABLE 2

Characterization of wet-blue leather

Characteristics	
Thickness	1.0 – 1.2 mm
Chromium oxide	4.3%
pH of aqueous extract	3.3%
Differential pH Cifer	0.45
Calcium	0.08%
Extractable with dichlormethane	0.40%

- without the retanning step: Blank test (BL);
- retanning with hydrolyzed protein (HP) as the tested product;
- retanning with hydrolyzed protein and glutaraldehyde (HP-GT) solution as the tested product;
- retanning with hydrolyzed protein and modified glutaraldehyde (HP-MGT) solution as the tested product;

The wet finished leather samples were dried in industrial leather dryer under vacuum at 50 ° C for 2 min, hung in an air dryer, and then evaluated.

The following physical-mechanical measurements and analyses were carried out:

- Leather samples: compressibility, apparent density, grain distension and grain breaking load in the lastometer test, tear load to progressive break, tensile strength, elongation, grain firmness and color;
- Process wastewaters: biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

For the tests performed on the leather samples four measurements were taken, two of them from samples cut in the vertical direction and two from samples cut in the horizontal direction, and the standard deviation was calculated. The compressibility test is used to measure the reduction of the thickness of a leather sample when subjected to a weight applied on its surface. The difference in the leather thickness with and without a standard weight (500g) is expressed in percentage terms. Thus, the effect of the leather filling is evaluated since the lower the compressibility, the lower the amount of empty spaces in the fibrous leather structure. The lastometer test was carried out according to DIN 53325 and is used to assess

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TABLE 3

Formulation of retanning

Step	%	Product	Time (min)	Temperature (°C)	pH
Washing	300	Water	15	25	
		drain			
Deacidification	50	Water		25	
	1.0	Sodium formate			
	0.5	Sodium bicarbonate	60		5.5
		Drain/washing			
Retanning*	50	Water		25	
	25	Tested product	45 (or 30*)		
	5**	Vegetable tannin**	30*		
Dyeing	3	Black dye	60		
Fat liquoring	200	Water		60	
	5	Fat liquor (sulfochloride natural oil)	45		
Fixation	1.0	Formic acid	15		
	1.0	Formic acid	15		3.8
		Drain/washing			

*retanning step: not for the blank test
**addition only in experiment II

the expandability of the grain, which is the thermostable outer layer of the hide. This is carried out using an apparatus in which a ball-ended rod is forced against the centre of a diaphragm of the material until it cracks and breaks and the distension and force are measured.

The progressive break resistance test is carried out according to DIN EN ISO 3377-1 and is used to assess the capacity of leather to bear multidirectional tensile strengths to which the leather will be submitted during use. The tests for tensile strength and the percentage of elongation are described in standard DIN EN ISO 3376, where the goal is to verify the elasticity and viscoelastic behavior of leather.

Apparent density is determined from the quotient of mass and volume measurements of a given sample of leather. Grain firmness is determined through evaluation of the adherence between the grain layer on the surface and the underlying reticular layer. The leather is considered to have firm grain when the two layers do not separate on folding. The assessment of grain is made visually by leather professionals as follows: 1 = leather with loose grain; 2 = leather with regular grain; and 3 = leather with firm grain.

The evaluation of the leather color following dyeing and retanning was carried out on a Gretag Macbeth CE2180 spectrophotometer, equipment that emits a wave of light in the direction of the sample and reads the reflected wavelength. This color reading is transformed by software into a three-dimensional coordinate system. The luminescence used in the experiment was a D65 lamp, which imitates the luminosity of sunlight. The system allows a comparison between the color of a standard substrate and that of the samples, transforming the differences between them into numbers expressed as difference in lightness (Dl), difference of tonality and difference in saturation. The standards used for the comparisons were the blank tests (BL) of each experiment. The average of these differences is transformed into a total color difference De.

In order to determine the effect of the formulations and products tested, for the analytical results and standard deviations the variances were analyzed using Minitab Statistical Software (Minitab Statistical Software Inc). A 95% level of confidence with 1 degree of freedom for the factor formulation and 3 degrees of freedom for the factor retanning of the tested product was used. Thus, when the value of F was lower than 0.05 the factor was considered significant.

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RESULTS AND DISCUSSION

The GPC chromatograms in Figure 1 show the molar mass distribution of the hydrolyzed protein solutions prepared. In this type of chromatographic column, larger molecules of greater molar mass appear first. It was observed that changes due to both glutaraldehyde agents displace smaller molar mass bands of the hydrolyzed protein to greater molar mass regions. Modifying HP with aluminum sulfate (where the chromatogram had greater peaks) was not successful, since the chromatogram shows that there is virtually no increase in molar mass, i.e., there was no chemical binding of the HP with aluminum sulfate. This solution was therefore not applied in the retanning experiments.

Table 4 shows all of the results for the measurements taken and analyses carried out. The F values calculated for the factors formulation and tested products, as well as the analysis of significance, are shown in Table 5. The formulations were compared in order to verify the influence of the addition of vegetable tannin in the retanning of leather carried out with variations in the hydrolyzed protein solutions prepared.

TABLE 4

Physical-mechanical resistance of leather and results of analysis of samples from the leather retanning experiments

Variables	Experiment I				Experiment II			
	BL	HP	HP-GT	HP-MGT	BL	HP	HP-GT	HP-MGT
Compressibility (%)	6.04	6.75	8.41	7.29	8.09	7.87	6.97	8.53
Standard Deviation	1.88	2.04	0.83	0.64	0.72	0.19	0.34	0.63
Apparent Density ($g.cm^{-3}$)	0.577	0.591	0.532	0.542	0.561	0,558	0.560	0.522
Standard Deviation	0.015	0.027	0.014	0.004	0.011	0.015	0.016	0.015
Grain Distension (mm)	9.4	8.0	8.2	7.2	8.6	7.5	7.2	7.0
Standard Deviation	0.5	1.1	0.2	0.0	1.1	0.4	0.1	0.5
Grain Breaking Load (kgf)	21	17	18	14	20	16	14	11
Standard Deviation	1	3	0	2	4	2	2	2
Tear Load to Progressive Break (kgf)	65.5	64.8	67.8	63.7	70.1	66.1	63.9	52.9
Standard Deviation	6.6	4.2	3.4	11.5	2.8	5.0	0.4	4.4
Tensile Strength ($N.mm^{-2}$)	15.4	13.1	13.8	13.1	14.6	11.6	11.0	9.9
Standard Deviation	3.1	1.3	0.6	3.7	1.8	2.0	2.2	2.2
Elongation (%)	66.8	61.2	60.8	51.0	53.7	52.0	44.9	44.1
Standard Deviation	15.1	14.3	21.1	7.6	3.5	5.8	9.3	5.9
Grain Firmness	2.0	1.0	2.0	2.5	2.0	1.0	2.5	3.0
Color (De)	-	1.46	2.49	1.45	-	2.21	2.14	1.61
Color (Dl)	-	1.45	2.23	1.20	-	-2.13	-1.83	-1.18
BOD ($mg.L^{-1}$)	< 40	< 40	< 40	< 40	< 40	< 40	< 40	< 40
COD ($mg.L^{-1}$)	3624	3750	2405	3014	1828	2012	1085	1458

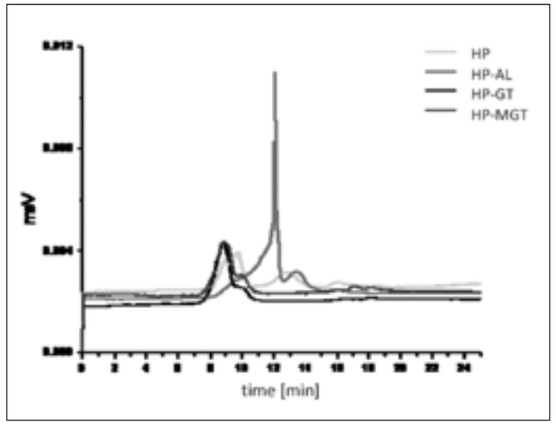

Figure 1—GPC chromatograms of hydrolyzed protein solutions

The values obtained in the determination of compressibility, tear load to progressive break and tensile strength were not significant for the formulation or for the tested product, as shown in Table 5. This difficulty in finding significant differences between formulations I and II is attributed to the similarity between the formulations due to the addition of other products and the influence of the raw material hide, which has a high degree of heterogeneity. Nevertheless, it can be observed in Table 4 that for formulation I there is a tendency toward higher values for tear load to progressive break and tensile strength and lower values for compressibility.

The results for elongation were significant for the factor formulation, with higher average values for the samples of experiment I. In formulation I vegetable tannin was not added and thus it is clear that this type of retanning, although important for obtaining a greater filling of the leather and grain firmness, reduces the flexibility of the leather, decreasing its physical performance. The apparent density was found to be statistically significant for the factor tested product. The analysis of the average values showed higher

TABLE 5

Analysis of variance of the physical-mechanical resistance and properties of leather samples from retanning experiments

Variables	Factor	F calculated	Significance
Compressibility	Formulation	0.492	No
	Tested Product	0.993	No
Apparent Density	Formulation	0.143	No
	Tested Product	0.000	Yes
Grain Distension	Formulation	0.050	Yes
	Tested Product	0.005	Yes
Grain Breaking Load	Formulation	0.025	Yes
	Tested Product	0.002	Yes
Tear Load to Progressive Break	Formulation	0.460	No
	Tested Product	0.171	No
Tensile Strength	Formulation	0.068	No
	Tested Product	0.154	No
Elongation	Formulation	0.050	Yes
	Tested Product	0.377	No
Grain Firmness	Formulation	0.166	No
	Tested Product	0.000	Yes