Collagen Hydrolysate Extraction from Chromed Leather Waste for Polymeric Film Production

by

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Abstract

The generation of hazardous wastes and the overuse of non-renewable sources in polymeric materials production are two major concerns for researchers worldwide. In this paper, an innovative approach to changing chromed leather waste (CLW) into a raw material for polymeric film production was studied. Collagen hydrolysate was extracted from CLW through alkaline hydrolysis. Time, temperature, agitation speed, type and mass of alkalinizing agent were tested. The optimum condition for collagen hydrolysate production (the one that results in a high protein and low chromium content) was determined. According to statistical analysis, hydrolysis performed for 6 h using a proportion of 4 g of MgO for each 50 g of CLW and 250 ml of water at 70°C and 180 rpm of agitation speed led to the best protein/chromium ratio in the collagen hydrolysate (TKN: 2,185.7 mg/L, Cr: <0.04 mg/L). The collagen hydrolysate obtained with optimum condition of production was then mixed with starch - a renewable source for polymeric material production - and glycerol - a byproduct of biodiesel production - to produce polymeric films by casting technique. Mechanical properties of four different compositions (with and without the presence of collagen hydrolysate and glycerol) of films were compared. The films produced with the addition of glycerol and collagen hydrolysate presented mechanical properties similar to the ones of commercial biodegradable films applied as mulches in agriculture. They also have in their composition a compound present in fertilizers - nitrogen - and possible traces of chromium, which is a micronutrient. Therefore, they can be an alternative to synthetic polymers.

Introduction

The role of leather industry in the world economy is indisputable: it is estimated that its global trade value is of approximately US$ 100 billion a year. From 80% to 85% of tanneries apply chromium III based processes to turn hides and skins into leather.[1] This is still the most efficient method of tanning, and the one that provides the best qualities to the final product. However, in a tannery, for each 1,000 kg of hides or skins processed, about 250 kg of chromed leather waste (CLW) and at least 500 kg of total solid waste are produced.[2, 3] This industry generates about 4 million tons of waste per year worldwide.[4]

Alternatives aiming the reuse of CLW have been studied. Some examples are the production of pigments, carbon ferrochromium alloy, basic chromium sulfate and polymeric microcapsules.[5-10] Also, hydrolysis was used to extract protein from CLW. Hydrolysis of CLW consists in reversing the reaction of the tanning agent (chromium) with the collagen, which is the responsible for the leather stability. High temperatures and the use of an agent that promotes the breaking of the chromium-collagen bonds are essential to the process. Hydrolysis can be performed in acid media, alkaline media or by enzymes.[9, 11-15]

When performed in alkaline media, hydrolysis produces protein (collagen hydrolysate or gelatin) in aqueous solution and a solid cake rich in chromium. Although collagen hydrolysate (and gelatin) extraction from CLW has been already studied,[11-15] its later possible applications have to be more explored since new alternatives for its use may be found. So far, the protein fraction has been suggested to be used in leather production or in animal feed.[12-15]

Not only management of wastes in industries like tanneries is an environmental issue to be widely discussed. The extensive employment of synthetic has encouraged researchers to find alternative renewable sources. Thermoplastic starch, for example, requires 68% less energy than polymers produced from petroleum. It also has a lower cost when it comes to raw material price and emits lower rates of CO_2 to the atmosphere during production process.[16, 17]

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Starch can be combined with protein products such as gelatin and collagen hydrolysate to produce polymeric films. When this mixture is made, different properties of the final material are obtained. Increasing in thickness and solubility in water, reduction in opacity and better mechanical properties were obtained when films were produced with the protein products.[18-20]

In order to reduce brittleness of the films, plasticizers may be added. A plasticizer broadly used in starch-gelatin films is glycerol. Biodiesel production generates great amounts of glycerol as a byproduct,[21] and applying it in polymeric films production is a simple alternative for its use. Glycerol, being a byproduct, can also reduce the cost of the films.

Although the production and application of gelatin-starch films and coatings has been covered by a variety of researches, generally food grade gelatin is used to produce edible and/or biodegradable materials.[22] Studies using protein fractions such as gelatin or collagen hydrolysate recovered from CLW in polymeric films production were not found in the literature. Films based on collagen hydrolysate recovered from CLW, starch and glycerol may be used in agriculture, as an alternative to petroleum-based polymers.

MATERIALS AND METHODS

Materials

The CLW used in this work was supplied by a local tannery (Peles Pampa, Portão, RS, Brazil). Magnesium oxide was acquired from Vetec Chemistry (Brazil), calcium oxide and glycerol were acquired from Cinética Chemistry (Brazil). Commercial cornstarch of the brand Yoki (Brazil) was used in film production.

Collagen Hydrolysate Extraction

For collagen hydrolysate extraction, 50 g of CLW were well mixed with the proper mass of alkalinizing agent and 250 ml of water in a 500 ml Erlenmeyer flask. Hydrolysis was conducted in an orbital shaker (Model MA 832, Marconi, Brazil) with control of speed, time and temperature. The mixture resultant of hydrolysis was then filtered. The collagen hydrolysate in aqueous solution was stored at 4°C.

Tables I and II summarize the two different designs of experiments (DOE) used to define which collagen hydrolysate sample was the most proper for film production (the one with high protein/chromium ratio in a more sustainable process). In both, a 2^k factorial design with a center point was used. The experiments were run in the first DOE in duplicate and in the second DOE in triplicate.

The first DOE was used to determine optimum temperature and time of hydrolysis. Alkalinizing agent, MgO, and agitation speed, 150 rpm, were fixed in the first DOE. The samples of the first DOE were mixed with alkalinizing agent and water 24 h before hydrolysis, which was not done for the samples of the second DOE.

The second DOE determined optimum type of alkalinizing agent, mass of alkalinizing agent and also optimum agitation speed during the process. Time, 6 h, and temperature, 70°C, of hydrolysis were fixed in the second DOE.

Film Production

The filmogenic solution was obtained by dispersing starch and, when appropriated, glycerol, in 150 ml of water or collagen hydrolysate. The solution was then heated up to 85°C (starch gelatinization temperature previously determined through a differential scanning calorimetry run) for 10 min under constant stirring. The filmogenic solution was cooled to 20°C in room temperature. It was then casted in a 30 x 25 cm glass plate covered with a polytetrafluoroethylene (PTFE) layer. The films

TABLE I
Collagen hydrolysate samples codification for the first DOE: determination of optimum temperature and time of hydrolysis. Alkalinizing agent – MgO – and agitation speed – 150 rpm – were fixed parameters.

Collagen hydrolysate sample	Mass of alkalinizing agent (g)	Temperature (°C)	Time (h)
1A	1.2[a]	70.0[b]	4[a]
2A	1.2[a]	70.0[b]	6[b]
3A	1.8[b]	70.0[b]	4[a]
4A	1.8[b]	70.0[b]	6[b]
5A	1.2[a]	55.0[a]	4[a]
6A	1.2[a]	55.0[a]	6[b]
7A	1.8[b]	55.0[a]	4[a]
8A	1.8[b]	55.0[a]	6[b]
9A	1.5[c]	62.5[c]	5[c]

[a] Low level of the factor
[b] High level of the factor
[c] Center point

were allowed to dry at 20 ± 4°C and under approximately 50% ± 10% of relative humidity. Table III shows the composition of the four films, which were produced in duplicates.

Characterization Methods

CLW and Collagen Hydrolysate Characterization

Moisture content of CLW and of collagen hydrolysate was determined according to ASTM D3790-2012. Ash content was determined according to ASTM D2617-2012. Mass measurements were performed with an analytical balance (Model AUY220, Shimadzu, Japan).

Chromium in CLW was determined as chromic oxide through perchloric acid oxidation followed by sodium thiosulfate titration according to ASTM D2807-2009. For chromium content in collagen hydrolysate, samples were prepared according to Standard Methods for Examination of Water and Wastewater (SMEWW) Method 3030-E and then analyzed by atomic absorption spectroscopy (Equipment Model Analyst 200, Perkin Elmer, USA) according to SMEWW Method 3111-B.

TABLE II
Collagen hydrolysate samples codification for the second DOE: determination of optimum type and mass of alkalinizing agent an optimum agitation speed of hydrolysis. Time – 6 h– and temperature – 70°C – were fixed parameters.

Collagen hydrolysate sample	Alkalinizing agent	Mass of alkalinizing agent (g)	Agitation speed (rpm)
1B	CaO[b]	2.0[a]	180[a]
2B	CaO[b]	2.0[a]	280[b]
3B	MgO[a]	2.0[a]	180[a]
4B	MgO[a]	2.0[a]	280[b]
5B	CaO[b]	4.0[b]	180[a]
6B	CaO[b]	4.0[b]	280[b]
7B	MgO[a]	4.0[b]	180[a]
8B	MgO[a]	4.0[b]	280[b]
9B	MgO/CaO	1.5/1.5[c]	230[c]

[a] Low level of the factor
[b] High level of the factor
[c] Center point

Macro Kjeldahl method followed by titration was used for Total Kjeldahl Nitrogen (TKN) determination, which was used as a measure of protein content in the collagen hydrolysate. The analysis was carried out according to SMEWW - 4500N_{org}-B in collagen hydrolysate and according to ASTM D2868-2010 in CLW.

pH of CLW was determined according to ASTM D6657-2014 using a pH meter (Model 86502, AZ, Taiwan). pH of collagen hydrolysate was determined with the same instrument. A material balance was performed to determine the percent yield of protein and chromium. NTK concentration is converted into collagen hydrolysate with a conversion factor equal to 5.5.[23]

Film Characterization and Comparison

Tensile tests of films were performed according to ASTM D882-2012 in a universal testing machine (Model DL 2000, Emic, Brazil) to determine tensile strength and percentage of elongation at break. Thickness of films was measured in 20 different points with a micrometer (Model IP 65, Mitutoyo, Japan) capable of measuring from 0 to 25 mm with an accuracy of 0.001 mm.

The mechanical properties of the films were compared to the ones of commercial biodegradable films used as mulches in agriculture. As an example, ecovio® F Mulch produced by BASF, presents elongation at break of 180-380% and tensile strength of 18-22 MPa. This commercial material is made of a copolyerster and polylatic acid.

TABLE III
Films formulation.

Film Sample[A]	Solvent employed in the filmogenic solution production[b]	Glycerol (g)
Collagen hydrolysate +Starch+Glycerol	Collagen hydrolysate[c]	0.45
Collagen hydrolysate +Starch	Collagen hydrolysate[c]	0.00
Water+Starch+Glycerol	Water	0.45
Water+Starch	Water	0.00

[a] All samples were produced with 3.5 g of starch
[b] All samples were produced with 150 ml of solvent
[c] Gelatin in aqueous media extracted from chromed leather waste through alkaline hydrolysis

Statistical Analysis

Statistical analysis of the experiments was performed through Analysis of Variance (ANOVA) to determine statistical significance of the factors studied.

RESULTS AND DISCUSSION

CLW Characterization

Table IV shows chemical composition of the CLW used in this work. Whilst some differences are expected, composition of CLW used in this work is similar to the ones of CLW used by other authors.[12-14]

Reproducibility of collagen hydrolysate extraction depends on the characteristics of the CLW employed in the process. Some slightly differences can be found among CLWs due to the origin of raw hides and skins and also because of the tanning process used.

Collagen Hydrolysate Extraction

First DOE for Collagen Hydrolysate Extraction

The effects of time, temperature and mass of MgO used as alkalinizing agent were evaluated in the first DOE for collagen hydrolysate extraction to determine which conditions would be employed in the second DOE for collagen hydrolysate extraction.

The chemical properties, volume of collagen hydrolysate produced and protein and chromium yield of collagen hydrolysate samples obtained in the first DOE for collagen hydrolysate extraction are shown in Table 5.

The process resulted in a diluted collagen hydrolysate product with moisture content higher than 99%. Chromium was detected only in the sample produced with the high level of all factors (1.8 of MgO, 70°C and 6 h of hydrolysis). Latter tests determined that chromium was dissolving during the period of 24 h between the beginning of hydrolysis and the time when alkalinizing agent, water and CLW had been mixed. Samples produced with the same conditions of sample 4A, except for the absence of the 24 h between the two processes, presented chromium contents <0.04 mg/L. Consequently, for the second DOE for collagen hydrolysate extraction, the mixture of the material used in hydrolysis was performed immediately before the samples were subjected to the process itself.

Table VI presents the analysis of variance used to evaluate statistical significance of the factors tested in the first DOE for collagen hydrolysate extraction. With a probability of 95%, all the factors studied (mass of MgO, temperature and time of hydrolysis) had a positive significant statistical effect ($p<0.05$) on TKN and chromium concentration. Therefore, between the limits studied in this paper, increasing mass of alkalinizing agent, increasing time and increasing temperature of hydrolysis led to higher TKN and chromium amounts in the collagen hydrolysate in aqueous solution.

Figure 1 presents the graphic of factor effects on TKN concentration. It indicates that the highest TKN value was achieved when the high levels of all factors were employed in hydrolysis: 1.8 g of MgO, 6 h and 70°C. It resulted in sample 4A, which presented TKN concentration of 1,088.8 mg/L.

When temperature is increased, more energy is provided to disrupt cross-link bonds as well as bonds that stabilize collagen structure and peptide bonds of α-chains.[24, 25] A temperature of 55°C cannot provide enough energy for collagen hydrolysate extraction. However, temperatures higher than 70°C (thus higher than the ones used in this work) can hydrolyze protein, lowering its molecular weight and forming hydrolyzed collagen. Furthermore, temperatures higher than 70°C can also promote dissolving of chromium in the collagen hydrolysate.[15] On that account, temperature of 70°C was chosen to be used in the second DOE for collagen hydrolysate extraction.

Time of extraction presented similar behavior to extraction temperature: higher time led to higher TKN concentration between the time limits studied in this work. According to other researchers, increasing hydrolysis time from 3 to 6 hours results in 21.2% higher protein yield, but increasing hydrolysis time from 6 to 24 h resulted in only 5.6% higher protein yield.[14] From 3 to 6 h, water in the presence of alkalinizing agent is still acting to disrupt chromium-collagen bonds and to reverse tanning process, which allows collagen hydrolysate to be extracted. After 6 h under the same temperature and pH, hydrolysis seems to have reached its limit, not extracting any more collagen hydrolysate and only allowing chromium to dissolve. Therefore, in the second DOE of collagen hydrolysate extraction, hydrolysis was performed for 6 hours.

TABLE IV
Characterization of the CLW used in collagen hydrolysate extraction.

Parameter	Result
pH	3.55
Moisture (%)	51.5
Ash[a] (%)	8.5
TKN[a] (%)	13.00
Cr_2O_3[a] (%)	2.39
Cr[a] (%)	0,82

[a] Moisture free basis

Second DOE for Collagen Hydrolysate Extraction

In the second DOE, it was determined the optimum condition for collagen hydrolysate extraction, which was later employed in film production. Optimum condition is the one that results in the highest TKN/Cr ratio in the collagen hydrolysate (the one with high protein/chromium ratio in a more sustainable process).

Table VII presents the chemical properties, volume of collagen hydrolysate produced and protein and chromium yield of collagen hydrolysate samples obtained in the second DOE for collagen hydrolysate extraction. An increase in TKN content and a decrease in moisture content were found in the second DOE when compared to the results of the first DOE. It indicates that better conditions were selected for hydrolysis after the analysis of the first DOE.

As it can be seen in Table VII, higher pH, TKN and Cr concentrations were found in collagen hydrolysate extracted with CaO. When an alkaline metal oxide reacts with water, a hydroxide is produced. The higher is the quantity of OH^- ions released to the system, the higher is its pH. CaO and $Ca(OH)_2$ are more soluble than MgO and $Mg(OH)_2$, so CaO releases more OH^- to the system than MgO. It seems to be the reason why the same mass of MgO and CaO produces a different pH, being CaO stronger than MgO as alkalinizing agent. As reported elsewhere ash contents on collagen hydrolysate samples extracted with MgO are higher than the ones on collagen hydrolysates extracted with CaO.[14]

Table VIII presents the analysis of variance used to evaluate statistical significance of the factors testes in the second DOE for collagen hydrolysate extraction. According to it, for the

TABLE V
Chemical characterization, volume of collagen hydrolysate produced and protein and chromium yield of collagen hydrolysate samples obtained in the first DOE for collagen hydrolysate extraction.

Collagen hydrolysate sample[a]	Moisture (%)	pH	Total Ash[b] (%)	TKN (mg/L)	Chromium[c] (mg/L)	Volume of collagen hydrolysate[d] (ml)	Protein yield[e] (%)	Chromium yield[e] (%)
1A	99.4	7.3	14.8	112.7	<0.04	189	0,7	--
2A	99.3	7.6	12.8	165.2	<0.04	155	0,8	--
3A	99.3	8.8	14.8	279.3	<0.04	172	1,5	--
4A	99.3	8.6	16.5	1,088.8	0,14	166	5,8	0,01
5A	99.3	8.0	13.9	123.5	<0.04	192	0,8	--
6A	99.3	8.1	16.9	117.1	<0.04	176	0,7	--
7A	99.3	8.6	12.0	130.0	<0.04	186	0,8	--
8A	99.3	8.8	13.7	149.7	<0.04	181	0,9	--
9A	99.3	8.2	14.8	202.5	<0.04	173	1,1	--

[a] Samples conditions of production: **1A**: 1.2 g of MgO, 70 °C, 4 h, 150 rpm / **2A**: 1.2 g of MgO, 70 °C, 6 h, 150 rpm / **3A**: 1.8 g of MgO, 70 °C, 4 h, 150 rpm / **4A**: 1.8 g of MgO, 70 °C, 6 h, 150 rpm / **5A**: 1.2 g of MgO, 55 °C, 4 h, 150 rpm / **6A**: 1.2 g of MgO, 55 °C, 6 h, 150 rpm / **7A**: 1.8 g of MgO, 55 °C, 4 h, 150 rpm / **8A**: 1.8 g of MgO, 55 °C, 6 h, 150 rpm / **9A**: 1,5 g of MgO, 62,5 °C, 5 h, 150 rpm.

[b] Moisture free basis

[c] Detection limit of the method = 0.04 mg/L

[d] Collagen hydrolysate extracted from CLW in aqueous media

[e] Protein and chromium yield in the collagen hydrolysate are based on the total mass of protein (TKN) and chromium present on the CLW used for alkaline hydrolysis and on the total mass of protein (TKN) and chromium present in the collagen hydrolysate in aqueous media.

second DOE, the type of alkalinizing agent and its mass have a significant effect on TKN/Cr ratios.

When 2 or 4 g of MgO were used for hydrolysis, lower pH values were found in collagen hydrolysate (from 8.9 to 10.2). In this band of pH, chromium hydroxide precipitates more easily with the cake, which reduces its concentration in the collagen hydrolysate. When pH is increased to values around 11-12, situation that happens when 2 g of CaO are used for hydrolysis, chromium hydroxide solubility increases, thus it is found in greater amounts in the collagen hydrolysate. However, when pH reaches values close to 14 (when 4 g of CaO are used for hydrolysis), polynuclear chromium compounds, less soluble than chromium hydroxides, are formed, reducing chromium content in the collagen hydrolysate.[26]

Figure 2 presents the graphic the significant factors effects on TKN/Cr ratio in the collagen hydrolysate. Agitation speed is not significant to the process. In order to have lower energy cost and to cause less equipment maintenance, agitation speed of 180 rpm (the lowest one tested in the second DOE) was chosen to be used as the optimum one.

According to Figure 2, when compared the two alkalinizing agents, MgO led to higher TKN/Cr ratios and, thus, it is the optimum alkalinizing agent for the process. Still according to Figure 2, the two different masses of MgO employed in the process did not result in significant TKN/Cr ratio changes. Therefore, in order to reduce the amount of chemicals used during collagen hydrolysate extraction and turn the process into a more environmentally friendly one, the lower mass (2 g) was chosen as the optimum mass of alkalinizing agent for the hydrolysis. On that account, optimum condition for collagen hydrolysate extraction is the one that uses: 50 g of CLW, 250 ml of water, 2 g of MgO in a hydrolysis process conducted at 70°C, 180 rpm for 6 h. This is the process used in collagen hydrolysate 3B, which contains 2,185.7 mg/L of NTK (equivalent to 12 g/L of collagen hydrolysate) and chromium content <0.04 mg/L.

Film Production

The films produced with collagen hydrolysate and starch, with or without the addition of glycerol were easy to handle and visually homogeneous. Thickness of films is presented in

TABLE VI
ANOVA table showing positive significant effect ($p<0.05$) of all factors on TKN and chromium concentrations in the first DOE for collagen hydrolysate extraction.

	ANOVA for TKN concentrations					ANOVA for chromium concentrations				
	Sums of Squares ($x10^{-5}$)	Degrees of Freedom	Means of Squares ($x10^{-5}$)	F ratio	p-Value	Sums of Squares ($x10^{3}$)	Degrees of Freedom	Means of Squares ($x10^{3}$)	F ratio	p-Value
(1)Temperature[a]	3.16	1	3.16	438	<0.001	2.33	1	2.33	14.9	<0.001
(2)Time[a]	1.91	1	1.91	265	<0.001	2.33	1	2.33	14.9	<0.001
(3)Mass of MgO[a]	3.18	1	3.18	441	<0.001	2.33	1	2.33	14.9	<0.001
1 by 2	1.80	1	1.80	249	<0.001	2.33	1	2.33	14.9	<0.001
1 by 3	2.76	1	2.76	382	<0.001	2.33	1	2.33	14.9	<0.001
2 by 3	1.53	1	1.53	212	<0.001	2.33	1	2.33	14.9	<0.001
1 by 2 by 3	1.33	1	1.33	185	<0.001	2.33	1	2.33	14.9	<0.001
Error	0.06	9	0.007			1.40	9	0.16		
Total	15.85	17				1.80	17			

[a] All the three factors presented positive effects on TKN and chromium concentrations

Figure 3. It varied from 0.09 mm to 0.16 mm. Addition of collagen hydrolysate and glycerol increased thickness. High contents of solid matter (protein and chemicals used in collagen hydrolysate extraction) that do not evaporate when films dry are the responsible for higher thickness in collagen hydrolysate films. Glycerol addition to the film led to higher thickness as well. As a typical plasticizer, glycerol reduces chain to chain interactions, which decreases intermolecular attraction and packing and could explain higher thickness.[27] Since glycerol is hydrophilic, it retains higher water contents which may also increase thickness of the film.[22]

The mechanical properties of the films are presented in Figure 4 and 5. Glycerol, as expected, had a pronounced plasticizing effect: it reduced film brittleness, resultant of high intermolecular forces, by increasing the mobility of polymer chains.[28,29] Therefore, lower tensile strength and higher elongation at break values were found in films produced with glycerol. This relation had been presented by other authors who studied starch- collagen hydrolysate films.[19,30]

Presence of collagen hydrolysate reduced tensile strength in both films, plasticized and non-plasticized. It results from the interaction of hydroxyl groups between starch and protein that may reduce the interaction between starch chains.[18] Consequently, elongation at break increased in the presence of collagen hydrolysate, reaching a value higher than 60% when glycerol was added.

Both mechanical properties changes, with addition of collagen hydrolysate and glycerol, may also be explained by the high horoscopy degree of these compounds. They produce films with higher water contents. Since water is a natural plasticizer for starch films, its presence also reduces tensile strength and increases elongation at break of the material.[18]

TABLE VII
Chemical characterization, volume of collagen hydrolysate produced and protein and chromium yield of collagen hydrolysate samples obtained in the second DOE for collagen hydrolysate extraction.

Collagen hydrolysate sample[a]	Moisture (%)	pH	Total Ash[b] (%)	TKN (mg/L)	Cr[c] (mg/L)	TKN/Cr Ratio	Volume of collagen hydrolysate[d] (ml)	Protein yield[e] (%)	Chromium yield[e] (%)
1B	93.3	11.6	12.2	9,483.9	38.70	257	162	48.7	3.21
2B	92.7	11.4	11.3	10,805.6	74.85	145	178	60.4	6.82
3B	97.6	9.2	21.6	2,185.7	<0.04	54,643	131	9.2	--
4B	97.4	8.9	21.5	2,713.0	0.25	11,295	124	10.7	0.02
5B	92.6	13.8	11.7	10,787.0	0.44	30,439	218	74.5	0.05
6B	92.1	14.0	11.9	11,075.0	0.43	30,052	215	75.1	0.05
7B	97.7	9.9	25.5	2,148.6	<0.04	53,714	120	8.3	--
8B	97.2	10.2	18.7	2,671.2	<0.04	66,780	130	11.1	--
9B	91.6	13.2	11.5	6,457.3	0.76	8,524	174	35.3	0.07

[a] Sample conditions of production: **1B**: 2.0 g of CaO, 70 °C, 6 h, 180 rpm / **2B**: 2.0 g of CaO, 70 °C, 6 h, 280 rpm / **3B**: 2.0 g of MgO, 70 °C, 6 h, 180 rpm / **4B**: 2.0 g of MgO, 70 °C, 6 h, 280 rpm / **5B**: 4.0 g of CaO, 70 °C, 6 h, 180 rpm / **6B**: 4.0 g of CaO, 70 °C, 6 h, 280 rpm / **7B**: 4.0 g of MgO, 70 °C, 6 h, 180 rpm / **8B**: 4.0 g of MgO, 70 °C, 6 h, 280 rpm / **9B:** 1.0 g of MgO + 1.0 g of CaO, 70 °C, 6 h , 230 rpm

[b] Moisture free basis

[c] Detection limit of the method = 0.04 mg/L

[d] Collagen hydrolysate extracted from CLW in aqueous media

[e] Protein and chromium yield was based on the total mass of protein (TKN) and chromium present in the CLW used for alkaline hydrolysis and on the total mass of protein (TKN) and chromium present in the collagen hydrolysate in aqueous media.

Table IX presents statistical analysis that evaluated significance of the two factors studied (presence or absence of glycerol and type of solvent used: water or collagen hydrolysate in aqueous solution). Significance of the factors was evaluated for elongation at break and tensile strength. Presence of collagen hydrolysate and glycerol had significant positive effects on elongation at break and negative significant effects on tensile strength. It confirms that increasing collagen hydrolysate and glycerol contents will increase elongation at break and decrease tensile strength of the films.

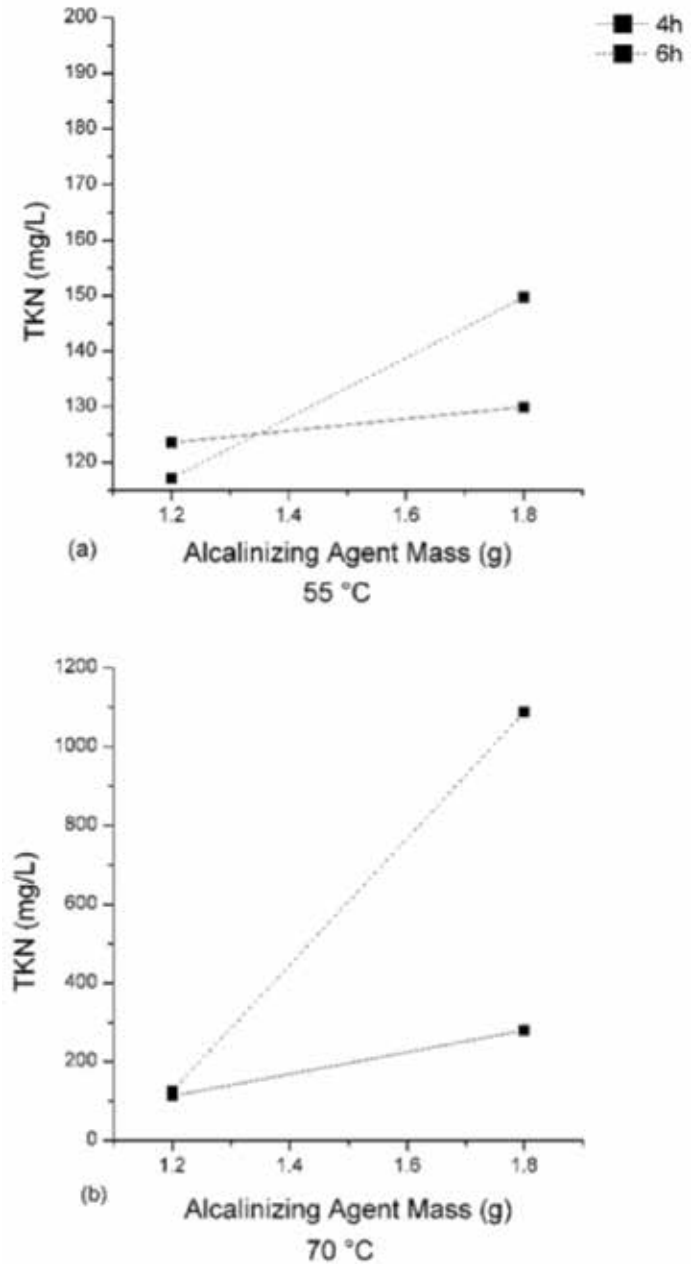

Figure 1. Graphic of factor effects on TKN concentrations (y axis) of the first DOE for collagen hydrolysate extraction. Hydrolysis performed at 55°C (a) and 70°C (b) for 4 h and 6 h with 1.2 g and 1.8 g of MgO as alkalinizing agent.

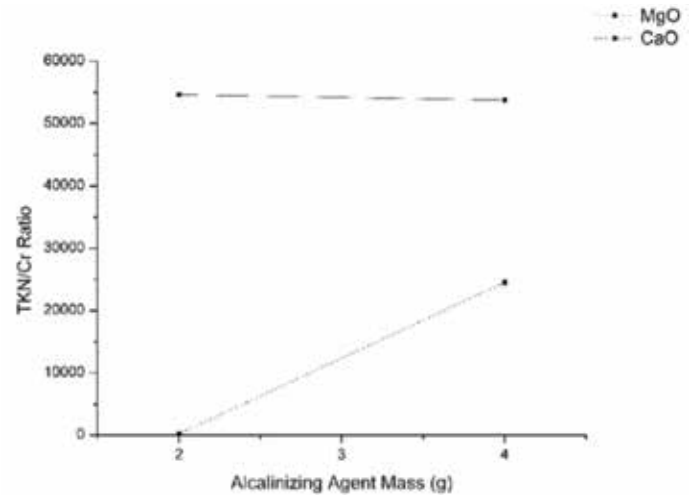

Figure 2. Graphic of factor effects on TKN/Cr ratios (y axis) of the second DOE for collagen hydrolysate extraction. Hydrolysis performed with MgO and CaO as alkalinizing agent for 6 h at 70°C and 180 rpm of agitation speed.

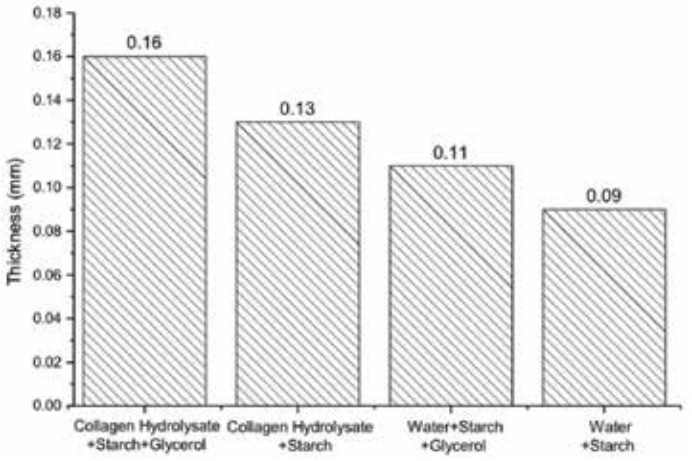

Figure 3. Thickness of the four different films produced with starch, collagen hydrolysate recovered from chromed leather waste in aqueous solution or water and in the presence or absence of glycerol.

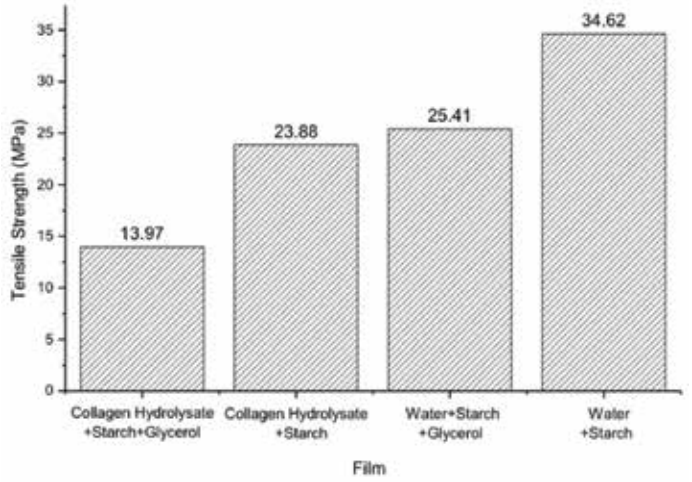

Figure 4. Tensile strength of the four different films produced with starch, collagen hydrolysate recovered from chromed leather waste in aqueous solution or water and in the presence or absence of glycerol.

TABLE VIII
ANOVA table showing significance (p<0.05) of alkalinizing agent and alkalinizing agent mass on TKN/Cr ratios for the second DOE for collagen hydrolysate extraction.

	Sums of Squares ($x10^{-6}$)	Degrees of Freedom	Means of Squares ($x10^{-6}$)	F ratio	p-Value
(1)Type of Alkalinizing Agent[a]	1,337	1	1,337	9.4	<0.001
(2)Mass of Alkalinizing Agent[a]	5,910	1	5,910	41.6	<0.001
(3)Agitation Speed	4,928	1	4,928	34.7	0.131
1 by 2	355	1	355	2.5	0.779
1 by 3	11	1	11	0.1	0.143
2 by 3	332	1	332	2.3	0.010
1 by 2 by 3	1,181	1	1,181	8.3	0.010
Error	1,205	9	1,205	8.5	
Total	2,557	18	2,557		

[a] The use of MgO and higher masses of alkalinizing agent had a positive effect on TKN/Cr ratio.

TABLE IX
ANOVA table showing significance (p<0.05) of presence of glycerol and of the different solvents employed in the filmogenic solution on the mechanical properties (elongation at break and tensile strength) of the polymeric films produced.

	Elongation at break					Tensile Strength				
	Sums of Squares	Degrees of Freedom	Means of Squares	F ratio	p-Value	Sums of Squares	Degrees of Freedom	Means of Squares	F ratio	p-Value
(1)Presence of glycerol	842.9	1	842.9	158.2	<0.001[b]	295.6	1	295.6	44.8	<0.001[c]
(2) Solvent employed in the filmogenic solution[a]	2364.5	1	2364.5	443.8	<0.001[b]	196.1	1	196.1	29.7	<0.001[c]
1 by 2	675.3	1	675.3	126.8	<0.001	11.3	1	11.3	1.7	0.26
Error	21.31	4	5.3			26.4	4	6.6		
Total	3904.	7				529.4	7			

[a] Collagen hydrolysate extracted from CLW in aqueous solution or water

[b] Presence of collagen hydrolysate extracted from CLW in aqueous solution and glycerol had a positive effect on elongation at break.

[c] Presence of collagen hydrolysate extracted from CLW in aqueous solution and glycerol had a negative effect on tensile strength.

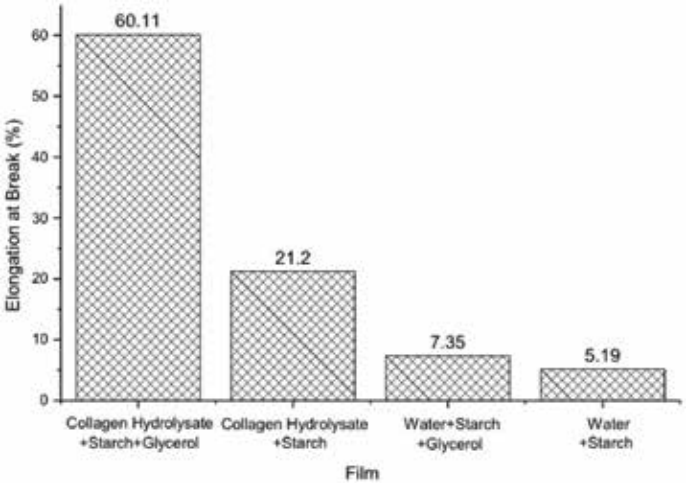

Figure 5. Elongation at break of the four different films produced with starch, collagen hydrolysate recovered from chromed leather waste in aqueous solution or water and in the presence or absence of glycerol.

The films studied in this paper show mechanical properties (elongation at break of 60.1% and tensile strength of 14.1 MPa) that are not so different when compared to the ones of commercial biodegradable films used as mulches in agriculture. In addition, the films produced in this paper, when degraded, release nitrogen, which is a fertilizer for the soil. Possible traces of chromium (under the detection limit of the method used for its analysis in the collagen hydrolysate) may be available as micronutrients for plants. Even though more studies are necessary to improve properties and completely characterize films produced with collagen hydrolysate recovered from CLW, this paper show that they are a promising alternative to petroleum-based films.

Conclusions

Collagen hydrolysate can be successfully extracted from CLW through alkaline hydrolysis. Optimum condition for collagen hydrolysate extraction - the one that led to a high TKN/Cr ratio in a more sustainable process - was obtained when samples were extracted with 2 g of MgO during 6 h of hydrolysis at 70°C and 180 rpm of agitation speed. This collagen hydrolysate, obtained in aqueous solution, presented 2,185.7 mg/L of TKN (approximately 12 g/L of collagen hydrolysate) and chromium content <0.04 mg/L.

Films made with collagen hydrolysate recovered from CLW, starch and glycerol are not only more environmentally friendly, when compared to petroleum-based films, due to using renewable and recycled raw materials, but are also promising materials. The presence of collagen hydrolysate and glycerol led to less brittle films, with mechanical properties similar to commercial biodegradable films used as mulches in agriculture.

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