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Steam Explosion as Pretreatment to Increase Gelatin Extraction Yield from Chromium Tanned Leather Wastes

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Alkaline hydrolysis of chromium tanned leather wastes (CTLW) is a promising process that allows turning this waste into valuable materials, especially into protein, recovered as gelatin. Steam explosion, which is a fast, chemical-free, and efficient process, was tested in this work as a pretreatment to open up the fibrous structure of CTLW and ease gelatin extraction through subsequent alkaline hydrolysis, increasing the process yield. Two steam explosion temperatures (130 °C and 150 °C) and residence times (5 and 15 min) were tested. The gelatin extracted in aqueous medium was characterized according to its dry mass content, ash content, viscosity, protein content, chromium content, and helix-to-coil transition temperature. CTLW steam exploded in more aggressive conditions (150 °C, 15 min) allowed the extraction of three times more protein when compared to CTLW without any pretreatment. However, samples treated at 150 °C produced lower-quality (partially degraded) gelatin. Steam explosion performed at 130 °C for 15 min almost doubled protein extraction and resulted in a better quality gelatin. This is a promising process for the proper use of CTLW as raw material for the production of high added-value products. © 2018 American Institute of Chemical Engineers Environ Prog, 38: 367–373, 2019
Keywords: Waste management, Protein, Extraction

INTRODUCTION

Leather industry is known for its high potential to generate hazardous wastes. About 80–90% of this industry still uses chromium (III) as a tanning agent [1]. Vegetable tanning, a less environmentally harmful alternative, results in a lower quality leather, which cannot achieve physical properties as good as the ones provided by chromium tanning [2–4]. Therefore, more than 600,000 tons of chromium tanned leather wastes (CTLW) are produced every year worldwide [5].

CTLW is rich in protein, which can be extracted through hydrolysis as gelatin (higher molar mass: 50–200 kg/mol) or collagen hydrolysate (lower molar mass: 5–50 kg/mol) [6]. Higher protein extraction yield can be achieved when alkaline hydrolysis is conducted under higher pH values—higher than 12 [7,8], at higher temperatures—close to 100 °C [8,9], and for longer times—more than 6 h [8]. After alkaline hydrolysis, enzymatic hydrolysis may be used to extract residual protein [6,10,11].

To achieve higher pH values, higher amounts of alkalinizing agent are needed, which not only increases extraction cost but also increases ash content in gelatin, making its purification costlier and more challenging. When a higher-temperature process is used, more energy is needed, and longer processes are not desirable in today's industries. Finally, enzymatic hydrolysis demands good pH control to keep enzymes activity and usually does not last less than 1.5–2.0 h [6,9,11,12]. Therefore, all of the alternatives used to increase protein extraction yield have drawbacks.

Steam explosion may be defined as an adiabatic process capable of converting thermal energy into mechanical energy [13]. It is a well-known and widely used process for pretreatment of lignocellulosic biomass, replacing chemical agents and enhancing enzymatic hydrolysis, for example, in ethanol production [14,15]. It has also been tested as pretreatment for the production of nanofibrillated cellulose from wastes of the tobacco industry [16] and to improve biomethane production from *Miscanthus lutarioriparius* [17], among several other possibilities.

When it comes to reusing CTLW, however, steam explosion seems to be an unusual process. It has only been applied to extract protein from CTLW after its pretreatment in the alkaline aqueous medium for a long period of time (1–3 days) [18] and as pretreatment for subsequent biogas production [19].

Thus, in this work, the steam explosion was used as a pretreatment of CTLW with the aim of opening up the leather waste fibrous structure and increasing protein extraction yield in the subsequent alkaline hydrolysis.

Table 1. CTLW steam explosion conditions

Sample	Steam temperature (°C)	Steam pressure (bar)	Residence time (min)
130/5	130	2	5
130/15	130	2	15
150/5	150	4	5
150/15	150	4	15

MATERIALS AND METHODS

Materials

CTLW was collected from a wet blue shaving process (after leather tanning and before its retanning), from a local tannery (Peles Pampa, Brazil). It was milled before being steam exploded to increase surface area and sample homogeneity. Milling was carried out in a knife mill, without the need for a cooling system. It resulted in a reduction of average particles diameter from 3.04 mm before milling to 0.83 mm after milling.

Calcium oxide (CaO) analytical grade (Cinética, Brazil) was used for alkaline hydrolysis. CaO is cheaper than other alkalinizing agents (as MgO) and the lime milk produced by it adsorbs the small $Cr(OH)_3$ particles, facilitating chromium removal through filtration [9,20].

Methods

CTLW Characterization

CTLW was characterized according to its moisture content (ASTM D3790-79: Standard test method for volatile matter—moisture of leather by oven drying); total ash content (ASTM D2617-12: Standard test method for total ash in leather); chromium content (ASTM D2807-93: Standard test method for chromic oxide in leather—perchloric acid oxidation); pH (ASTM D6657-14a: Standard test method for pH of wet blue); and protein/nitrogen content (semi-micro Kjeldahl method).

Steam Explosion Pretreatment

About 100 g of CTLW were placed in the steam-explosion reactor, fed with saturated steam, and kept under the conditions specified in Table 1. After the residence time, CTLW was suddenly released to the blow tank through a nozzle. The steam-exploded CTLW was characterized according to its moisture content, total ash content, and pH (following the same test methods specified for CTLW). Thermogravimetry of these samples was performed in a simultaneous thermal analyzer (STA, Jupiter 449, Netzsch, Germany). A sample of 10 mg was heated at 10 °C/min from 20 °C to 600 °C under N_2 atmosphere (50 mL/min).

Alkaline Hydrolysis

CTLW and steam-exploded CTLW, in a proportion equal to 25 g of sample (dry mass basis) to each 2 g of CaO and 275 mL of total water (sum of water from the sample – moisture content – and water added to the process), were hydrolyzed. At the beginning of the process, pH values were close to 11, which were reduced, as hydrolysis occurred, to values close to 10. Hydrolysis was conducted in an orbital shaker (MA 832, Marconi, Brazil) at 70 °C (maximum temperature reached by the equipment), 180 rpm of agitation speed, and for 6 h. All the tests were performed in triplicates. The two resultant phases—gelatin in an aqueous medium and solid cake—were separated through vacuum filtration and later characterized.

Table 2. Characterization of CTLW before steam explosion process

Parameter	Result
pH	3.4 ± 0.1
Dry mass (%)	48.1 ± 0.3
Ashes (% dry basis)	6.54 ± 0.05
Chromium (Cr_2O_3, % dry basis)	4.56 ± 0.29
Nitrogen (TKN, % dry basis)	14.6 ± 0.6
Onset temperature of main degradation event (°C)	300
Temperature of maximum degradation rate (°C)	321

Gelatin Characterization

Gelatin's pH was measured with a pH meter (86502, AZ, Taiwan) right after extraction. Its dry mass was determined by drying a preweighted 5 g mass sample at 105 °C until constant mass. For total ash content analysis, 5 g of gelatin sample were oven-dried at 105 °C and put in a muffle furnace at 600 °C for 2 h. All mass measurements were made in an analytical balance (AUY220, Shimadzu, Japan).

Viscosity of the samples was measured with a N° 1 Ubbelohde viscometer for transparent liquids at 25 °C, according to ASTM D446-07 (Standard specifications and operating instructions for glass capillary kinematic viscometers). Temperature control was performed in a water bath designed for viscosity measurements (Model Q303SR, Quimis, Brazil).

Protein content was determined through two different methods: as total nitrogen by Kjeldahl method (Standard Methods for Examination of Water and Wastewater—SMEWW 4500-Norg C) and as protein content by Lowry method [21]. Hexavalent chromium content was analyzed through the colorimetric dinitrophenylhydrazine method using the sample as extracted (American Society for Testing and Materials—ASTM D 1687-92). Total chromium content was determined through the same method after oxidation of trivalent chromium to hexavalent using hydrogen peroxide in alkaline medium. Signal measurements were performed in a spectrophotometer (Ionlab IL-226).

Differential scanning calorimetry (DSC) of gelatin was performed in a DSC 60 (Shimadzu, Japan). A sample of 8 mg was subjected to a heating rate of 10 °C/min from −40 °C to 80 °C under N_2 atmosphere (50 mL/min).

Statistical Design and Analysis

Statistical analysis was performed to determine if the factors varied in the steam explosion had a significant effect on gelatin extracted through alkaline hydrolysis. A 2^2 factorial design was analyzed by analysis of variance (ANOVA) using 99% as confidence interval in the Software Statistica 12 (StatSoft Inc., Tulsa, OK). Therefore, P values lower than 0.01 indicate that a factor is statistically significant.

RESULTS AND DISCUSSION

CTLW Characterization

CTLW characterization is presented in Table 2. Reproducibility of steam explosion and alkaline hydrolysis tests depend on the characteristics of the CTLW. Most of the results here found are in accordance to the ones presented by other authors, who found pH values between 2.4 and 4.7, dry mass between 45.9% and 49.5%, TKN between 13.0% and 16.6% (dry basis), Cr_2O_3 between 2.4 and 4.7% (dry basis). Only ash content is lower than the values found by other authors, which range between 7.4% and 14.9% (dry basis) [7,11,12,22,23].

Table 3. Characterization of CTLW after the steam explosion

Sample	pH	Dry mass (% m/m)	Ash (% m/m dry basis)	Onset temperature of main degradation event (°C)	Temperature of maximum degradation rate (°C)
130/5	3.30 ± 0.04	23.4 ± 0.4	7.0 ± 0.3	301	322
130/15	3.20 ± 0.01	17.7 ± 0.6	6.1 ± 0.5	301	321
150/5	3.12 ± 0.02	17.8 ± 1.3	7.1 ± 0.2	296	314
150/15	3.08 ± 0.02	13.3 ± 1.2	7.1 ± 0.3	293	312

Table 4. Statistical analysis results indicating statistical significance of steam explosion temperature and time on CTLW characteristics.

|	P value		
Factor	Dry Mass	Ashes	pH
Temperature	0.002	0.030	0.001
Time	0.003	0.022	0.009

CTLW Steam Explosion

When CTLW is steam exploded after different residence times and at different temperatures, its dry mass changes significantly (Tables 3 and 4). Longer times and higher temperatures probably allowed the leather fibrous structure to be more opened up, which resulted in more water absorbed by it and in a consequent reduction in dry mass. This will affect gelatin extraction, because a more opened-up structure eases water and alkalinizing agent penetration into CTLW fibrous structure, resulting in higher extraction yield, which is discussed in the next section. The highest steam explosion temperature provided more energy to ease the breakage of the collagen-chromium chemical bonds, which stabilize CTLW structure. The longest residence time, on its turn, allowed steam to deeply penetrate into CTLW fibrous structure, causing the same effect of collagen-chromium chemical bonds breakage.

During the steam explosion, just water is added to CTLW, which means that, on a dry basis, its ash content should not change when compared to the one of CTLW with no pretreatment. In CTLW with no pretreatment, ash content is equal to 6.54% (m/m, dry basis). It changes to 6.1–7.1% (m/m, dry basis) after CTLW is steam exploded (Table 3), which may be attributed to analysis standard error.

pH of steam-exploded CTLW significantly reduced when higher steam explosion time and temperature were employed (Tables 3 and 4). It may have two causes: acids release or detanning (when cross-links between chromium (III) and protein chains from collagen are broken, reversing tanning process) [24,25]. More than just reducing pH, which happens due to the exposure of carboxylic groups from glutamic and aspartic acids, detanning would also reduce thermal stability of CTLW [25].

Thermal degradation behavior of the samples steam exploded at 130 °C did not expressively change when compared to CTLW without any pretreatment, as it can be seen in Figure 1. Therefore, when 130 °C is used, pH reduction of steam-exploded CTLW probably happens mainly due to acids release.

On the other hand, the onset temperature and the maximum degradation rate temperature (data shown in Tables 2 and 3 and Figure 1) of samples treated at 150 °C significantly reduce. For these samples, pH reduction may have happened both due to acids release and due to dettaning effect, as previously presented. As detanning implies in the breakage of chromium-collagen bonds, it may also result in the breakage of collagen polypeptide chains. Therefore, gelatin extracted from CTLW steam exploded at 150 °C will have lower molar mass, which may be seen by a reduction on helix-to-coil
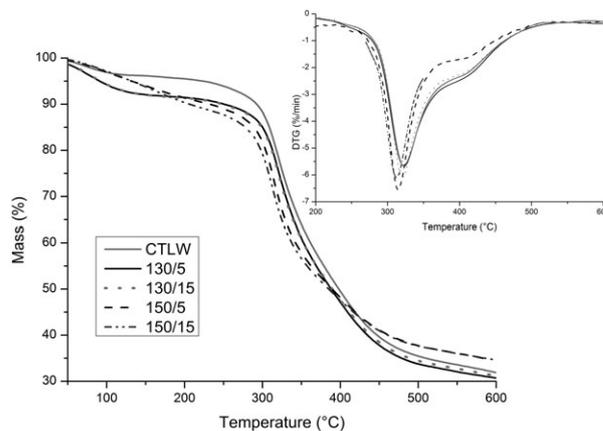
Figure 1. Thermogravimetry of CTLW without any treatment (CTLW), steam-exploded at 130 °C with residence time of 5 min (130/5), steam-exploded at 130 °C with residence time of 15 min (130/15), steam-exploded at 150 °C with residence time of 5 min (150/5), and steam-exploded at 150 °C with residence time of 15 min (150/15)

transition temperature, viscosity and Lowry/TKN rate in gelatin samples, as discussed in the next section.

TG curves for CTLW and steam-exploded CTLW follow the pattern shown by other authors. The curves show a main mass loss event between 300 °C and 350 °C followed by another decomposition step at about 430 °C, which is detected in DTG curves as a shoulder. Under more aggressive steam explosion conditions (130/15, 150/5, and 150/15), this shoulder appears more defined and separated from the main peak, as had been previously shown [5].

Alkaline Hydrolysis

Characterization of gelatin extracted through alkaline hydrolysis of CTLW and of steam-exploded CTLW is shown in Table 5. Table 6 shows material balance for alkaline hydrolysis and Table 7 presents the results of statistical analysis, indicating which steam explosion parameters (residence time/temperature) had significant effects on gelatin characteristics.

Gelatin's pH did not significantly change due to more or less aggressive steam explosion conditions. pH changes noticed for steam-exploded CTLW did not reflect in changes to gelatin's pH. pH of steam-exploded CTLW varied by 0.22 (from 3.08 to 3.30). Standard deviations for pH values found in gelatin were as high as 0.20. Therefore, the pH changes seen for CTLW as a result of different steam explosion conditions may not have been observed in gelatin pH.

The differences found in ash contents among the samples are due to the fact that these values are on dry basis. As dry mass in gelatin increased with more extreme CTLW steam explosion conditions, ash content decreased on dry basis. When on wet basis, ash content of the samples are 0.71%, 0.77%, 0.78%, 0.77%, and 0.80%, for CTLW/Gel, 130/5/Gel, 130/15/Gel, 150/5/Gel, and 150/15/Gel, respectively. Substantial increase in ash content is

Table 5. Characterization of gelatin—in aqueous medium—extracted from CTLW through alkaline hydrolysis with and without steam explosion as pretreatment

Sample	pH	Dry Mass (%)	Ashes (% dry basis)	Viscosity (cP)	Glass transition temperature (°C)	Helix-to-coil transition temperature (°C)	Protein Lowry Method (g/L)	Protein TKN Method (g/L)	Lowry Protein/TKN Protein Ratio	Total Chromium (mg/L)	Hexavalent Chromium (mg/L)
CTLW/Gel	9.83 ± 0.07	3.1 ± 0.7	22.8 ± 2.4	2.9 ± 0.03	13	60	21.0 ± 1.8	4.2 ± 0.5	5.0	7.7 ± 2.4	1.4 ± 0.2
130/5/Gel	9.88 ± 0.10	4.1 ± 0.5	18.8 ± 1.9	4.2 ± 0.04	12	60	29.4 ± 0.4	5.9 ± 0.3	5.0	21.3 ± 3.9	1.8 ± 0.2
130/15/Gel	9.76 ± 0.20	4.6 ± 0.6	17.0 ± 2.1	6.0 ± 0.16	12	56	32.1 ± 1.1	6.3 ± 0.8	5.2	38.3 ± 6.6	2.1 ± 0.2
150/5/Gel	9.89 ± 0.11	6.2 ± 1.0	12.4 ± 1.6	4.3 ± 0.01	11	53	41.1 ± 2.8	8.8 ± 0.8	4.7	33.7 ± 6.4	2.0 ± 0.2
150/15/Gel	9.75 ± 0.06	7.2 ± 0.7	11.1 ± 1.0	3.9 ± 0.03	10	43	44.8 ± 0.9	11.1 ± 0.5	4.1	43.4 ± 2.2	1.4 ± 0.2

Table 6. Material balance for alkaline hydrolysis of CTLW and of steam-exploded CTLW

	IN				OUT				Chromium			Protein (TKN)		
Sample	CTLW* (g)	Water (g)	CaO (g)	Total (g)	Gelatin (g)	Cake (g)	Total (g)	Mass Loss (g)	CTLW** (mg)	Gelatin (mg)	Solubilized (%)	CTLW (g)	Gelatin (g)	Extracted (%)
CTLW/Gel	51	249	2	302	135	113	247	55	775	1.0	0.1	3.7	0.6	15.5
130/5/Gel	107	193	2	302	144	105	248	54	775	3.1	0.4	3.7	0.8	23.2
130/15/Gel	141	159	2	302	161	99	261	41	775	6.2	0.8	3.7	1.0	27.8
150/5/Gel	141	158	2	301	162	95	257	44	775	5.5	0.7	3.7	1.4	39.1
150/15/Gel	197	102	2	301	154	90	244	58	775	6.7	0.9	3.7	1.7	46.8

*CTLW mass presented in this column represents CTLW mass after the steam explosion, which has a different moisture content. Higher CTLW mass indicates higher moisture content in CTLW. Its dry mass, however, was kept equal to 25 g in each test.

**To determine the amount of chromium in CTLW, it was considered chromium content in CTLW as Cr_2O_3 (4.56%, dry basis), the molar mass of Cr_2O_3 equal to 152 g/mol, chromium molar mass equal to 52 g/mol, the percentage of chromium in Cr_2O_3 equal to 68%. This indicates 3.1% of chromium in CTLW represented as Cr. It was also considered CTLW dry mass employed in the process, which was equal to 25 g in all samples

Table 7. Statistical analysis results: statistical significance of steam explosion temperature and time on gelatin's characteristics.

Factor	Protein—Lowry Method	Protein—TKN Method	Total Chromium	Hexavalent Chromium	Dry Mass	Ashes	Viscosity	pH
				P value				
Temperature	<0.001	<0.001	0.112	0.021	0.002	0.006	<0.001	0.80
Time	0.004	0.009	0.122	0.171	0.034	0.076	<0.001	0.09

seen only when the sample extracted from CTLW with no pretreatment is compared to the ones extracted from steam-exploded CTLW. This increase in ash content is of about 0.07% or 700 mg/L. Taking into consideration that chromium content increase in gelatin samples is of up to 35.7 mg/L (from 7.7 mg/L for CTLW/Gel to 43.4 mg/L for 150/15/Gel), it may be concluded that ash content increase (700 mg/L) is not only due to chromium dissolution. When CTLW fibrous structure is opened up through the steam explosion, the inorganic components remaining from the leather processing (especially NaCl) are dissolved, increasing gelatin's ash content.

Dry mass in gelatin increased for CTLW samples treated at the highest temperature and/or longest residence time in a steam explosion. This increase is mainly related to higher protein extraction, as seen in Table 5. Protein extraction increases as steam explosion temperature and/or residence time increase. It is here confirmed what had been predicted from steam-exploded CTLW characterization. The more opened-up the fibrous structure of steam-exploded CTLW was, the higher protein extraction was achieved.

Steam explosion temperature is more relevant for dry mass and protein extraction increase than steam explosion time is. Comparing the sample 130/5/Gel to the sample 130/15/Gel, and comparing the sample 150/5/Gel to the sample 150/15/Gel, increases of 1.12 and 1.16 times are found for dry mass and 1.06 and 1.26 times are found for protein (measured as TKN), respectively. On the other hand, comparing the sample 130/5/Gel to the sample 150/5/Gel, and comparing the sample 130/15/Gel to the sample 150/15/Gel, increases of 1.51 and 1.56 times are found for dry mass and 1.49 and 1.76 times are found for protein (measured as TKN), respectively. *P* values confirm these results, being lower for steam explosion temperature (<0.001 and 0.002 for TKN content and dry mass content, respectively) than for steam explosion residence time (0.004 and 0.034 for TKN content and dry mass content, respectively).

As for gelatin viscosity, it may be affected mainly by two factors: concentration and molar mass/Bloom value. An increase in gelatin concentration or in its Bloom value results in a higher viscosity. Higher Bloom values indicate that the molecular structure of gelatin is more preserved, therefore its molar mass is higher and hydrolysis degree is lower [26,27]. Chain scission, higher extraction temperatures and longer times of extraction lead to lower viscosity [28]. Therefore, being gelatin's molar mass equal or similar, its viscosity should increase as its concentration increases. However, this behavior is observed only when the steam explosion pretreatment is performed at 130 °C (Table 5). For samples pretreated at 150 °C, viscosity values decrease while its concentration increases. It indicates that the protein chain was hydrolyzed, therefore its molar mass reduced when CTLW was treated at 150 °C.

Another indicative of protein degradation is found when DSC curves of gelatin samples are studied (Figure 2). Classical thermal behavior for gelatin with high water content in DSC runs consists in the presence of a second-order transition—glass transition (T_g), followed by a first-order transition—an endothermic peak that indicates melting temperature, also related to the helix-to-coil transition of gelatin (T_m) [29,30].
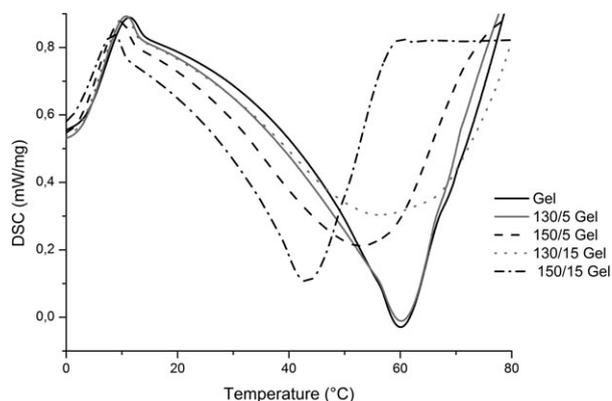
Figure 2. DSC curves of gelatin samples extracted from CTLW without pretreatment and pretreated with the steam explosion in two different temperatures (130 °C and 150 °C) and for two different residence times (5 and 15 min)

Lower T_m values indicate lower thermal stability, which is an indication of protein degradation [31]. T_m of gelatin extracted from CTLW with no pretreatment and from CTLW pretreated at 130 °C for 5 min is equal (60 °C). However, it reduces as steam explosion pretreatment is performed for longer periods of time and at higher temperatures. According to these results, not only the samples extracted from CTLW pretreated at 150 °C but also the one extracted from CTLW pretreated at 130 °C for 15 min was partially degraded due to steam explosion.

Finally, protein content in gelatin was measured through two different methods to identify possible degradation and amino acids release to gelatin. While TKN method measures directly the total amount of nitrogen in gelatin, Lowry method measures a combination of peptide bonds and two amino acids: tryptophan and tyrosine [32]. Tryptophan is not present in gelatin, while tyrosine represents less than 1% of its amino acids [9,33]. This indicates that Lowry method should detect almost only amino acids linked by peptide bonds, while TKN method should detect any nitrogen atom in the sample. It is known that, to convert nitrogen content (TKN) into gelatin content (for not degraded samples), a conversion factor of 5.55 is used [34]. Therefore, when dividing Lowry protein content by TKN content values similar to 5.55 should be found.

Table 6 results indicate, however, lower values (from 4.1 to 5.2), with a significant decrease to 4.1 when the sample is extracted from CTLW pretreated at 150 °C for 15 min. It not only confirms the degradation of the samples extracted from pretreated CTLW but also indicates that the sample extracted from CTLW with no pretreatment was also partially degraded. This is a favorable result as it indicates that steam explosion can be used at lower temperatures (130 °C), increasing protein extraction yield while not degrading gelatin more than a traditional process (with no steam explosion pretreatment) would.

The degradation mainly observed for CTLW samples pretreated at 150 °C directly reflects in gelatin's degradation. The

energy added to the process due to temperature, or the use of longer residence time, resulted not only in collagen-chromium bonds breakage but also in peptide bonds breakage, reducing gelatin's molar mass.

As for hexavalent chromium in gelatin, some authors indicate that this hazardous oxidation state of chromium would not be created in the hydrolysis of CTLW [8,10]. However, all gelatin samples produced in this work, including the ones produced from CTLW with no pretreatment, were contaminated with hexavalent chromium in concentrations that varied from 1.4 to 2.1 mg/L. No tendency of increasing or decreasing concentrations, depending on the steam explosion condition applied, was observed. Different characteristics of CTLW used in this work and in the papers presented by the previously cited authors may have led to chromium (VI) formation. Nevertheless, the results here presented indicate that this issue must be considered in future studies. To reduce chromium (VI) to chromium (III), gelatin produced in this work was treated with ferric sulfate, resulting in a solution free of chromium (VI).

As it can be seen in the hydrolysis material balance (Table 6), as samples pretreated under different steam explosion conditions have different moisture contents, different total CTLW and steam-exploded CTLW masses were used in alkaline hydrolysis. Dry mass of the waste used in every test, however, was kept the same: 25 g. These different moisture contents resulted in different amounts of water added to the process, resulting in the same total mass entering the system in all tests. Mass loss, which varies from 13.6% to 19.6%, occurs mainly due to water evaporation—as it is expected for samples maintained at 70 °C for 6 h. Some mass, however, may be lost during filtration stage.

The percentage of protein extraction from CTLW considerably increased with steam explosion pretreatment. Protein extraction yield increased 1.5, 1.8, 2.5, and 3.0 times when comparing 130/5/Gel, 130/15/Gel, 150/5/Gel, and 150/15/Gel to CTLW/Gel sample.

The extraction of gelatin from CTLW pretreated at 150 °C resulted in the highest protein extraction. However, if a better quality gelatin (with lower degradation degree) is desired, samples extracted at 130 °C should be considered good options, as their viscosity, Lowry/TKN ratio, and helix-to-coil transition temperature results indicate. When compared to CTLW with no pretreatment, using steam explosion at a temperature as low as 130 °C would still represent a gain of up to 80% in protein extraction yield.

Percentage of chromium solubilized also increased due to steam explosion use, but no statistically significant differences were found when comparing the four different pretreatment conditions. Chromium removal from CTLW gelatin may be performed, as presented by Wionczyk, Apostoluk [35], through solvent extraction using Aliquat 336, a quaternary ammonium salt. The cited authors removed 99% of chromium from CTLW hydrolysate.

It should be highlighted that, while protein extraction yield from pretreated CTLW ranges from 23.2% to 46.8%, chromium extraction is lower than 0.9%. It means that the process is efficient at extracting the protein in an aqueous medium with a low percentage of chromium dissolution.

CONCLUSIONS

Steam explosion used as pretreatment for subsequent alkaline hydrolysis of CTLW resulted in protein extraction yield increase of up to three times (from 15.5% to 46.8%) when compared to gelatin extracted through alkaline hydrolysis of CTLW without any pretreatment. It also led to considerable reduction in gelatin quality when performed at 150 °C. If performed at 130 °C, however, higher molar mass/better quality gelatin (with higher viscosity, Lowry/TKN ratio, and glass transition temperature) is produced, and still, an increase of 1.8

times in protein extraction is obtained. As for hexavalent chromium, future studies, possibly with the addition of ferric sulfate during hydrolysis, should be performed to avoid its formation.

Steam explosion is a simple and fast process with low environmental impact. It was proved here that it increases protein extraction yield by facilitating the opening up of CTLW fibrous structure. In future studies, it may also be used to reduce reagent consumption and to shorten hydrolysis time. The resultant gelatin still has to be purified to reduce ash and chromium content, but this purification step is a common posttreatment of any gelatin. Steam explosion certainly is a promising process for adequate use of CTLW as raw material for protein products.

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