Isolation and characterization of gelatin obtained from chrome-tanned shavings

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Abstract:

The aim of the present work is to explore the potential of converting chrome-tanned shavings, into a high added value material for new applications. Various chemical and physical protocols were used to dechrome chrome tanned shavings: gelatin was obtained by chemical and thermal degradation. The most influential parameter on the final quality of the gelatin is hydrolytic agent (alkali/acid). Differences between alkali- or acid-based hydrolysis have been found. Alkali hydrolysis products obtained showed no gel strength and low molecular weight fragments, while gelatin obtained through thermal degradation showed good physical and chemical properties in terms of gel strength, swelling and thermal stability.

1. Introduction:

One of the most significant problems of the leather industry is waste generation. As environmental concerns increase, so does the importance of clean technologies and recycling methods. The tanning industry is a generator of liquid wastes as well as tanned and non-tanned solid wastes. One tonne of wet salted hide yields only 200kg of leather, but over 600kg of solid waste (125kg of which are chrome shavings[1]), and 50m^3 of waste water[2-4]. Conventional landfilling or incineration have limited application due to the potential of Cr(III) to being converted to Cr(VI)[4, 5]. Solid waste from tanning operations produce serious environmental impact: WHO describes Cr(VI) as toxic and carcinogenic[6]. The solid leather waste has potential for recycling into useful and high added value products due to its collagen substrate.

Previous studies have demonstrated that protein products can be isolated from chrome shavings using one and two-step enzymatic processes[2, 3, 7]. The present study shows that gelatin (a collagen by-product) can be isolated from pre-treated chrome shavings, using chemical and thermal hydrolysis.

Gelatin is obtained by chemical and thermal degradation of collagen, the main protein of connective tissue, by the breaking of the triple-helix structure into random coils. Gelatin presents better biological compatibility and degradation than other synthesised substances[8]. It has existing and potential uses in cosmetics, adhesives, printing, photography, encapsulation and film formation. The quality of any gelatin depends on the species from which is extracted and on the manufacturing method.

Gelatin presents different and multi-functional properties, which make it suitable for use as an ingredient to improve elasticity, consistency and stability; but one of the most important properties of gelatin is its ability to form reversible gels, which is important in film formation[9].

Gel formation is due to the development of collagen triple helices in solution during cooling. When a gelatin solution is cooled below gelation temperature, the protein coils start to form triple helices and progressively a 3D network is formed. The triple-helices are change wording of the native structure of collagen, though only partially. When the temperature is raised, the reverse transition helix to coil takes place and the gel becomes liquid[10, 11].

The conventional method of obtaining gelatin is based on a two-step process (maturation or ripening, also called maceration[4] and extraction). The aim of the maceration, an alkali/acid pre-treatment, is to weaken the collagen structure, solubilising the non-collagenic proteins and hydrolysing part of the peptide bonds, but keeping the consistency of the collagen fibres. The second step involves using hot water extraction[12-15]. The objective of the new methods is to make the process of obtaining gelatin from chrome-tanned shavings more cost-effective in terms of time, chemicals and waste generation.

Many variables (temperature, time, solvent, hydrolytic agent) and the origin of gelatin, play a role in determining the thermal and mechanical properties of the gels. The aim of this study is to investigate the effect of hydrolytic agents on the physical and chemical properties of gelatin isolated from dechromed shavings.

2. Materials and methods

Dechromed shavings were obtained after applied the dechroming process[16], [12], [13] on chrome-containing leather shavings supplied by BSLT. Finely grained hide powder was supplied by BSLT (Northampton, UK).

Ammonia solution (sp.gr. 0.880) was supplied by BDH Chemicals Ltd., UK; sodium hydroxide (NaOH) (pearl 98-100%) and glacial acetic acid glacial were supplied by Prime Chemicals, UK. All chemicals used were of analytical grade..

2.1. Hydrolysis

Dechromed shavings, 200g/l, were mixed by mechanical stirring in a temperature controlled bath supplied by Grant Instruments, UK, at a constant temperature for a fixed determined period of time. The solution hydrolysate was filtered and centrifuged at 10000rpm for 20min in a Jouan Gr20.22 centrifuge, UK, in order to separate the residue shavings which had not been solubilised.

The conditions applied to obtain gelatin using sodium hydroxide as hydrolytic agent (0.3M NaOH solution, for 1h at 80ºC) have been described previously[17].

The conditions applied to obtain gelatin using ammonia (42% (v/v), for 1h at 80ºC) and acetic acid (10% (v/v), for 2.5h at 80ºC) as hydrolytic agents were obtained by a initial study [not presented in this work] of the different variables (concentration, time and temperature) in order to obtain a gelatin with the optimum characteristics of yield, gel strength and film formation.

A gelatin, obtained by thermal hydrolysis, without the addition of further chemicals, was investigated. An initial study into the influence of temperature and time to obtain gelatin showed that the optimum thermally hydrolysed gelatin was obtained after stirring a solution of dechromed shavings in water for 5h at 80ºC.

The same protocol was applied using stardard hide powder as a reference.

2.2. Film preparation

A solution of gelatin (10ml, 120mg/ml protein) was placed in a petri dish and allowed to air dry at room temperature (25ºC)[18].

2.3. Determination of Chromium (VI) content (IUC18)[19]

The determination of the percentage of chromium (VI) in each gelatin was carried out according to the International Standard method (IUC18).

International Standard specifies a method for determining chromium (VI) in solutions leached from leather under defined conditions; the chromium content is quantified photometrically at 540nm.

2.4. Yield

The yield was calculated as the percentage of leather material converted to gelatin and is calculated according to the following formula:

$Yield(\%) = 100(1-W_{res}/W_{shav})$

Where W_{res} is the residual weight of gelatin after filtration and/or centrifugation, and W_{shav} is the initial weight of shavings.

2.5. Gel Strength

Gel strength was measured by Bloom determinations on 100ml of gelatin. The test was carried out according the International Standard (ISO 9665); using a MT-LQ plus Materials Tester designed by Stable Micro Systems, with 1kg load cell and a 0.5inch radius cylinder probe (P/0.5R).

2.6. Melting Point

The melting point of the gelatins was determined according to a modified[20] B.S. 757, 195 Method[15]. Samples were prepared in test tubes and were kept overnight in a Brookfield TC500 bath at 10ºC. A small coloured plastic sphere was placed in each tube. The bath temperature was raised at 1ºC/min until the gelatin samples melted allowing the balls to move freely down the gel. As recomended, the melting point temperature was recorded when the sphere was half way down the gel[15, 20]

2.7. Differential Scanning Calorimetry (DSC)

Samples were stored in a conditioning room for 48h (20ºC; 60% humidity). A known amount of dry gelatin film (10mg) was hermetically sealed in aluminium pans and subjected to analysis using a Mettler Toledo DSC822^e calorimeter. Heating was carried out at 5ºC/min in the temperature range from 20ºC to 100ºC.

2.8. Swelling

Gelatin films were analysed for their water absorption or swelling. The films were weighed and immersed in a physiological solution for different periods of time. Wet samples were blotted with tissue to remove excess liquid and re-weighed. The amount of absorbed water was calculated as:

$$\text{Swelling}(\%) = 100 \cdot (W_{wet} - W_{dried})/W_{dried}$$

Where W_{wet} is the weight of the sample wet and W_{dried} is the weight of the dry sample.

2.9. SDS-PAGE (Polyacrylamide Gel Electrophoresis)

The samples were denatured at 90ºC for a specified period of time and loaded in appropriate concentration onto a vertical acrylamide gel (4% stacking gel, 5.5% resolving gel). The gels were run at a determined voltage (50-60V). The gels were stained with Coomassie Brilliant Blue and destained with a methanol and acid acetic solution, prior to analysis.

3. Results and discussion

The proposed gelatin production process consists of a one-phase process using as a "maturators" or hydrolytic agents: sodium hydroxide, ammonia and acetic acid; some gelatins were obtained by thermal degradation, without further use of chemical agents.

The possibility of producing gelatin from dechromed shavings by thermal hydrolysis lay in the previous treatment of the chromed shavings to remove the chromium (dechroming process). If the pretreated raw material is brought to a high temperature in contact with water, a gelatin solution with moderate gel forming properties will gradually be obtained[15]. Thus the previous treatment (dechroming) could be understood as a "maturation or maceration" step in the conventional two-step gelatin process; so the thermal hydrolysis could be assimilated as the second step of the conventional gelatin process (hot water extraction)[21].

In order to characterise the gelatins, different properties such as yield, gel strength, melting point, molecular weight distribution, swelling and thermal properties were measured.

3.1. pH

Table 1 shows the pH values of the gelatins obtained using the different hydrolytic agents:

Table 1: pH values of gelatin derived from different hydrolytic agents

Gelatin	Hydrolytic agent	pH
SH	Sodium hydroxide	12
Am	Ammonia	10
AA	Acetic acid	2
TH	Thermal degradation	3.5
HP	Thermal degradation (hide powder)	9

3.2. Cr content

The sodium hydroxide, ammonia and acid acetic derived gelatins contained 1.162, 1.117 and 0.015ppm of Cr(VI), respectively.

The acetic acid hydrolysis protocol produced a gelatin with traces of chromium (VI) much lower than the alkaline hydrolysed gelatins, as the most of the chromium was present as Cr (III) due to the acid pH.

3.3. Yield

The yield, percentage of shavings converted to gelatin, is an important property in terms of recycling. As the aim of recycling is to maximise the conversion of waste into a potentially useful product, to create more and new waste is not desirable[22].

Figure 1 shows gelatins obtained by thermal or acid hydrolysis were found to have the highest value whereas the obtained by alkali hydrolysis presented a slightly lower yield value.

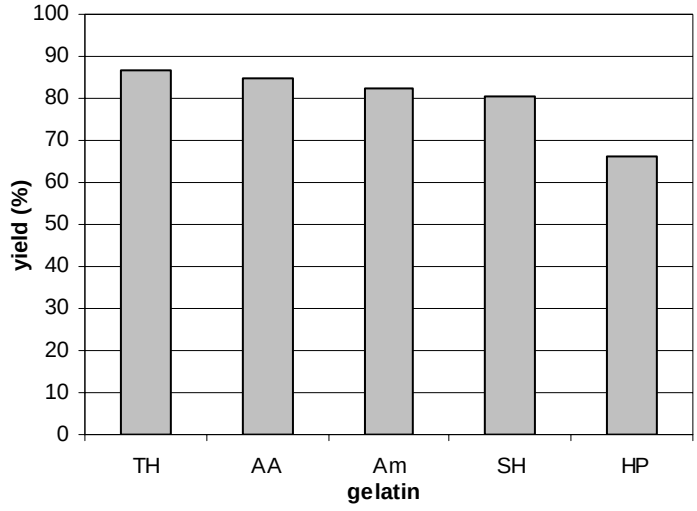

Figure 1: Effect of various hydrolytic agents on the total yield.

3.4. Gel strength (bloom)

Figure 2 presents the bloom values of the gelatins. Thermally hydrolysed gelatin showed the highest value of gel strength, whereas for gelatins SH and Am the value of gel strength was undetectable due to the sensivity of the instrument.

The gel strength of the gelatins was again measured after one month. The results gave an indication of the stability of the gelatins. There were not big differences in the bloom values after one month, except that the gelatin obtained from hide powder had solubilised.

The bloom value gives an indication of the level of hydrolysis that a protocol of hydrolysis can achieve. A low value of gel strength means that the degradation of the collagen fibres is high[18, 23].

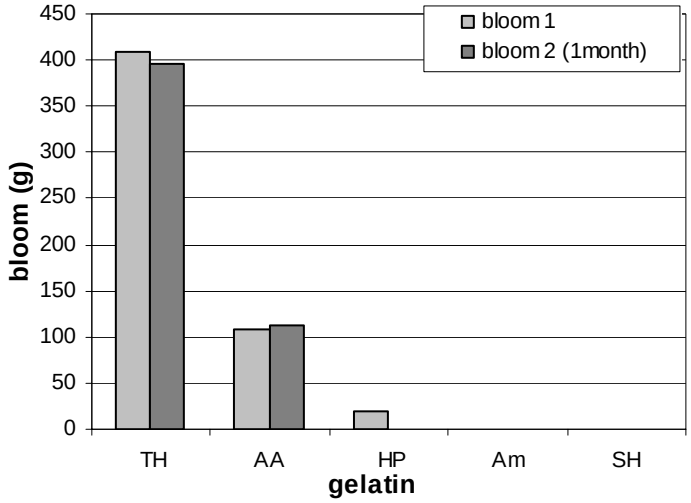

Figure 2: Effect of various hydrolytic agents on the gel strength of the gelatins.

3.5. Melting point

The melting point test is used to determine the temperature when gelatin starts to melt. Figure 3 shows that gelatin SH and Am, alkali-derived gelatins, exhibited characteristics of a viscous liquid. Gelatin SH and Am could be considered as a collagen hydrolysate[24] instead of gelatins since they did not exhibit a “gel state” even at low temperatures.

Gel strength and melting point values were expected have similar characteristics as both methods are related to the level of degradation of the collagen fibres[22]. Differences between alkali or acid based hydrolysis have been found in both methods. The

conversion for collagen into gelatin is accelerated at moderate temperatures by extraction under acid conditions, but this extraction do not promote substantial secondary reactions to the same extent; however, extraction under alkaline conditions likewise speeds up conversion, but, at the same time it also promotes other degradation processes, whereby the gel forming properties are impaired[15].

Although the behaviour of most of the gelatins confirmed the relationship between gel strength and melting point, hide-powder based gelatin showed a high melting point and low bloom value. However, thermally hydrolysed gelatin had the highest melting point and alkali-derived gelatins (Am and SH) the lowest values.

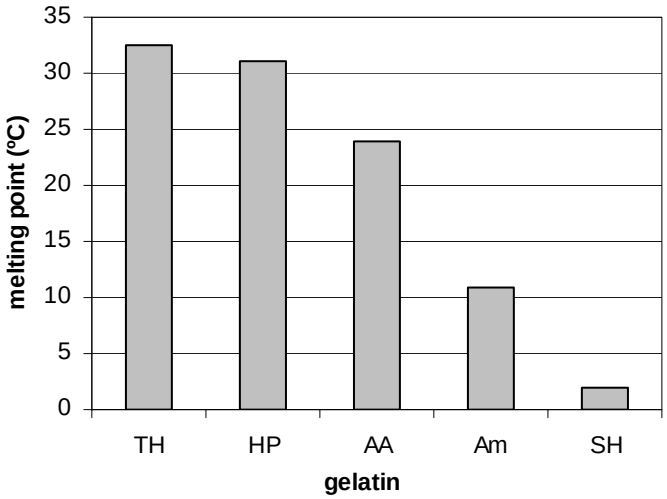

Figure 3: Effect of various hydrolytic agents on the melting point of the gelatins.

3.6. Swelling

Gelatin films were analysed for their water absorption or swelling. The swelling is an indicator of the matrix network characteristics and stability of the gelatin films, as well as the level of hydrolysis achieved with each process used[23, 25]. If the level of hydrolysis is low, the network permits less absorption of water therefore the stability is higher.

There is no value of swelling for SH gelatin film since the film was completely absorbed in water. TH gelatin formed a more stable film, as it showed the lowest swelling value.

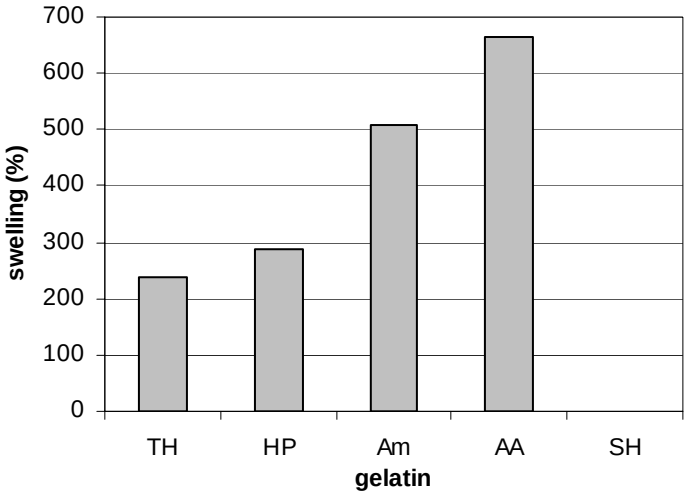

Figure 4: Effect of various hydrolytic agents on the swelling.

3.7. Calorimetric analysis

The denaturation temperature of gelatin was determined by DSC and is usually associated with the physico-chemical characteristics of the material. The DSC plot of collagenous materials exhibits an endothermic peak which is generally accepted as associated with the helix-coil transition of collagen[23, 25, 26], the rupture of hydrogen bonds and a rearrangement of the triple helix into a random configuration[25].

Figure 5 compares the DSC of gelatin films obtained through different hydrolytic protocols. The denaturation temperature of the dechromed shavings was 86ºC; in all cases, the hydrolysis decreases the denaturation temperature and therefore the stability since hydrolysis involves the breaking of the triple-helix structure into random coils. Gelatin films derived from NaOH hydrolysis (SH) show a lower denaturation temperature (30ºC) whereas TH gelatin shows a denaturation temperature of 82ºC.

The values of the denaturation enthalpy associated with the endothermic peaks are related to the relative amount of triple helical structure in the samples[23], and are approximate 10 times lower for gelatin compared to chrome shavings.

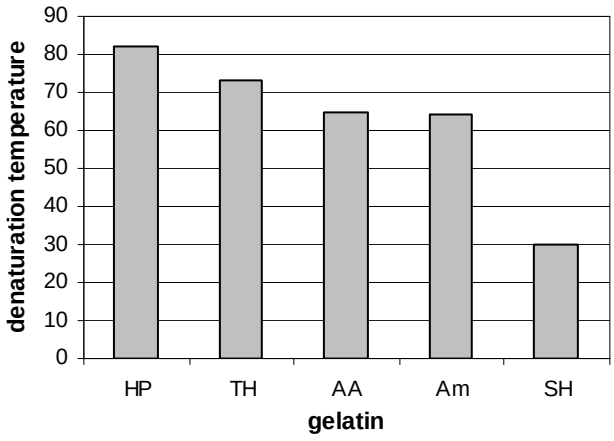

Figure 5: Effect of various hydrolytic agents on the thermal stability.

3.8. SDS-PAGE

Previous studies[27] have shown that collagen displays one β band (200kDa) and two α bands (100kDa for α_1 α_2), for the unfolding polypeptide chains of the triple helix. The molecular weight usually ranges within limits of 15-50kDa for hydrolysates[28] and 50-200kDa for gelatin[29]. The average of molecular weight of a gelatin is largely responsible for its functional properties and gelling behaviour[22, 30]

Figure 6 shows the electrophoresis patterns of the gelatins Am, AA and SH. The molecular weights of gelatins Am and SH were less than approximately 60 and 40kDa, respectively. The lower molecular weight of SH and Am gelatins confirms that these products should be treated as collagen hydrolysate rather than gelatin. The molecular weight distribution of all the samples were wide ranging. Gelatin and collagen hydrolysate were obtained under severe conditions of medium of reaction (acid/alkali), time and temperature (above the denaturation temperature of collagen). Most of the triple helices of gelatin and part of their peptide bonds were destroyed randomly, therefore this material is a composite of different molecular weight polypeptides and presents a wide molecular weight distribution[27].

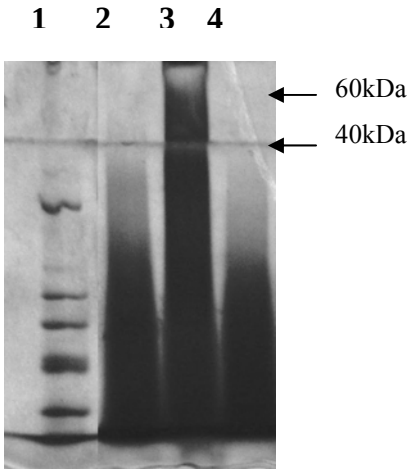

Figure 6: SDS-PAGE analysis of molecular weight standards (lane1), and gelatins: Am (lane2), AA (lane3) and SH(lane 4)

4. Conclusion

It has been demostrated that valuable products with different chemical and physical properties can be recovered from chrome shavings. The thermal hydrolysis, a relatively simple treatment, permits obtain a valuable and useful product solving both economic and environmental problems. The introduction of a crosslinker or a plasticiser could improve the stability and mechanical properties of the gelatin, making the collagenic solid waste a material with a potential for new applications such as encapsulation, binding or finishing agent for the leather industry.

5. References

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