

Properties of Collagen Hydrolyzates Obtained from Leather Shavings

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Hydrolyzates are low-molecular products obtained in the second stage of enzymatic hydrolysis of chrome-tanned leather shavings. It is necessary to know thermal properties of hydrolyzates, gels and films for their intended industrial applications, especially when processing them into packing materials. The object of the presented work is study of thermal properties of powdery hydrolyzates, gels of hydrolyzates prepared from 40% (w/w) solutions and films of hydrolyzates prepared from 12.5% (w/w) solutions with/without adding plasticizer (glycerin). Thermal properties (glass transition temperature, melting point of gels and melting point of films) were determined employing different scanning calorimetry and thermo mechanical analyzer.

Key Words: Collagen hydrolyzates, Thermal properties, Glass transition temperature, Melting temperature, Molecular mass.

INTRODUCTION

Tannery processing produces a considerable quantity of both liquid waste and solid chrome-tanned wastes which present a significant problem due to their chrome content. The problem of solid wastes has been solved so far by land filling. Nevertheless, ever increasing land filling costs pose an economic problem. In addition, the danger of chrome escaping into the ecosystem is also not negligible. Due to their containing a sizeable proportion of protein material, solid tanned wastes started to be processed. Enzymatic hydrolysis has being employed to considerable extent in latest years, with its particular advantages being mild reaction conditions and economic cost-effectiveness¹⁻⁵.

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In two-stage enzyme degradation of solid wastes, the cross-linked protein matrix undergoes alkaline disruption in the first stage and is subsequently degraded in the second stage by means of employed enzyme. The first stage yields high-molecular protein gelatin fractions (applicable to gels, adhesives, photographic substrates, printing, *etc.*) with molecular mass distribution ranging from 75,000 to 210,000 Da. Filter cake in the second stage undergoes enzymatic processing. A low-molecular peptide mixture (hydrolyzate) with molecular mass distribution ranging from 10,000 to 20,000 Da is obtained^{6,7}. The waste product (chrome sludge) almost free of protein remains, but has not yet found lucrative application. In contrast, on the one hand, applications of collagen hydrolyzate are broad, *e.g.*, as feed additive, nitrogenous fertiliser, raw material for preparing adhesives, corrosion inhibitors, *etc.* On the other hand, costs of obtaining protein hydrolyzates are not negligible and implementing obtained hydrolyzates in industry, in the present state of affairs, is more a problem of economy than of technology. Solution to the problem would undoubtedly be much aided by adequately lucrative industrial application of isolated hydrolyzates. Packing materials widely discussed lately are based on renewable raw material sources (of natural polymers), whose expected potential consists above all in easy, environmentally harmless disposal of packing material with their following use in food and non-food applications⁸⁻¹⁰. That would bring about reduced problems in waste management at many levels.

EXPERIMENTAL

Hydrolyzates were prepared from 5 different batches of chrome tanned-leather shavings. Average composition of chrome-tanned leather shavings is given in Table-1.

TABLE-1
AVERAGE COMPOSITION OF CHROME-TANNED
LEATHER SHAVINGS

Parameter	Value (%)
Total solids	69.0
Inorganic solids ^a	11.0
Total Kjeldahl nitrogen ^a	20.0
Cr ₂ O ₃ ^a	4.5

^amoisture-free basis

The schedule of enzymatic hydrolysis of leather shavings was carried out according to the scheme shown in Fig. 1 in a laboratory discontinuous reactor. Prior to enzymatic hydrolysis, shavings were cutted and milled using cutting mill Pulverisette 19 (Fritsch, GmbH, Idar-Oberstein, Germany) with a sieve cassette of 3 mm perforation. In the first step, a batch of 1500 g of industrial scraps, 800 mL of water, 75 g of MgO and 19 g of

KOH was processed under agitation of $70 \pm 2^\circ\text{C}$ for 6 h. pH value of the mixture ranged within 9-10. Then the mixture was filtered under the atmospheric pressure and ambient temperature using Filpap KA-1 paper (Filpap, The Czech Republic) yielding in 4035 g of filter cake and 4426 g of gelatinoprotein. In the second step, a batch of 4035 g filter cake, 2250 g of water 2.5 g of KOH, 0.75 g of Alcalase 2,4 L FG (NovoNordisk, Denmark) was processed under agitation at $70 \pm 2^\circ\text{C}$ for 1 h with pH adjustment to 9 (by adding 15% of H_3PO_4). Then, the mixture was filtered under the atmospheric pressure and ambient temperature using Filpap KA-1 paper yielding in 2075 g of chrome sludge and 3736 g of collagen hydrolyzate. Collagen hydrolyzate was spray-dried.

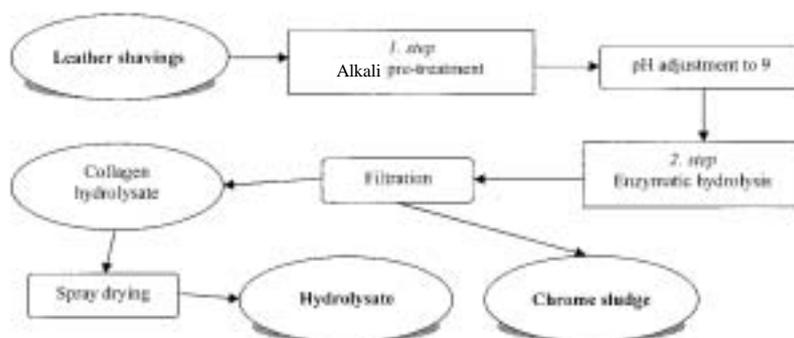


Fig. 1. Scheme of 2-step enzymatic hydrolysis of leather shavings

Chemical composition of hydrolyzates is presented in Table-2. Solid was determined¹¹ according to ISO PWI 4684, inorganic solids¹² according to ISO 4047:1998, nitrogen¹³ according to ISO 5397:1984, chromium¹⁴ according to ISO 9174:1998, primary amino groups after reaction with ninhydrine¹⁵ and number average relative molecular mass by osometry using Osmomat 050 Colloid Osmometr (Gonotec GmbH, Berlin, Germany).

TABLE-2
CHEMICAL COMPOSITION OF HYDROLYZATES

Parameter	Hydrolyzate No.				
	1	2	3	4	5
Total solids (%)	91.44	88.930	92.86	91.80	90.590
Inorganic solids ^a (%)	5.29	4.180	4.94	3.61	4.770
Total Kjeldahl nitrogen ^a (%)	14.89	15.270	14.97	14.75	15.090
Chrome ^a (ppm)	205.20	139.000	28.2	158	69.700
Primary-NH ₂ groups ^a (mmol g ⁻¹)	0.22	0.342	0.216	0.138	0.254
Number average relative molecular mass (Da)	12800	16900	23400	27400	24400

^aMoisture-free basis

Thermal properties of hydrolyzates: Prior to thermal study, hydrolyzates were conditioned in a desiccator filled with dried silica gel for 60 h at 40°C. Thermal characteristics (glass transition temperature - T_g , melting point - T_m and degradation temperature - T_d) were determined immediately after their conditioning by commercial differential scanning calorimeter DSC 2010 (TA Instruments, New Castle, DE 1970, USA) in open aluminium crucibles with a charge of 10 mg under nitrogen atmosphere at a flow rate of 150 mL min⁻¹. Measurements were performed in a temperature interval from a temperature of 20 to 310°C at a temperature growth rate of $dT/dt = 10^\circ\text{C min}^{-1}$. Each test was performed threefold and arithmetic mean calculated, standard deviation ranged within $\pm 1.5\%$.

Thermal properties of gels: Gels whose properties were assessed were prepared from 40 % aqueous solutions of hydrolyzates and their subsequent cooling at 10°C in a refrigerator for 16 h. Then, a charge of approximately 40 mg was weight onto an aluminium DSC crucible and kept in a closed vessel at 10°C in refrigerator for another 4 h. Melting point of gels (T_{m1}) was determined immediately on their cooling by employing DSC. Measurements were performed in a temperature interval from 10 to 45°C at a temperature growth rate of $dT/dt = 1^\circ\text{C min}^{-1}$ in an open aluminium crucibles under nitrogen atmosphere at a flow rate of 150 mL min⁻¹. Each test was performed theefold and arithmetic mean calculated, standard deviation ranged within $\pm 5.0\%$.

Thermal properties of films: Thermal properties of films were tested at hydrolyzate No.4. Films were prepared from 12.5 % aqueous solutions of hydrolyzates containing 0, 1, 5, 10, 20 and 40 % (weight per weight of hydrolyzate) of plasticizer (glycerine) by cold casting onto silicone plates and their subsequent drying at 30°C for 24 h. Then, specimens 2.3×1.0 cm were cut and subsequently conditioned in a desiccator filled with a dried silica gel for 24 h. Crack characteristics expressed by creep temperature of film (T_c) were tested on thermomechanical analyzer Du Pont 990 (Wilmington, USA) under 5 g load from a starting temperature of $20 \pm 0.5^\circ\text{C}$ at a temperature growth rate of $dT/dt = 5^\circ\text{C min}^{-1}$. From graphical reading (dimensional change of films in dependence onto a temperature) the temperature corresponding to a crack of a specimen (creep) was determined. Procedures used to evaluate creep behaviour of polymers are described in¹⁶. Each test was performed threefold and arithmetic mean calculated, standard deviation ranged within $\pm 5.0 \%$.

RESULTS AND DISCUSSION

Most water contained in hydrolyzates goes away at temperatures below 100°C which is showed on thermograms (Figs. 2-6) by a distinct endothermic peak with a minimum in the 42.3-54.2°C range (depending on

the batch of hydrolyzate). Hydrolyzates exhibits on them thermograms a turn in the 144.0-167.4°C range, which is attributed to glass transition temperature of hydrolyzates (2nd order transition - T_g). In the case of hydrolyzate No. 2, T_g was not detected. Glass transition temperature is associated with melting of crystalline structure. The curve at this phase is downtrending. According to Yannas¹⁷, T_g lies in the section of strongly polar residues and aminoacids. Observations of DSC curves showed that melting point (T_m) of hydrolyzates lies at least 44°C higher than glass transition temperature which is characterized by endothermic peaks. Regarding procedures common in synthetic polymer processing, the difference between T_g and T_m should be as great as possible. Heat processing of a material is recommended at temperatures approx. 10°C higher than observed T_g , along with sufficient temperature range to melting point at which plastic processing is unnecessary. Endothermic effect attributed to degradation (T_d) which was observed in a narrow temperature range, 294.6-299.8°C. Thermal coordinates of characteristics turns and peaks on thermograms of powdered hydrolyzates are summarized in Table-3. DSC curves of all hydrolyzates are showed in Figs. 2-6.

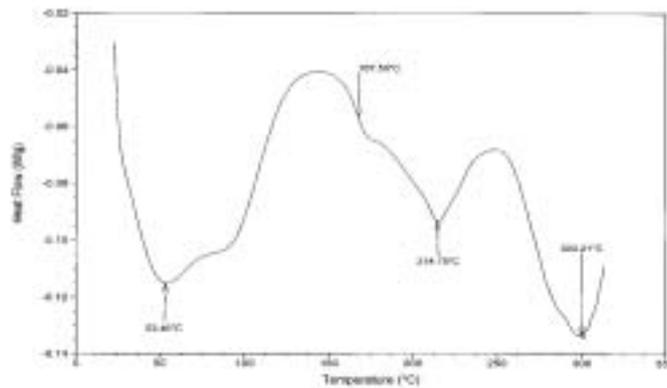


Fig. 2. Model DSC curve of hydrolyzate No.1

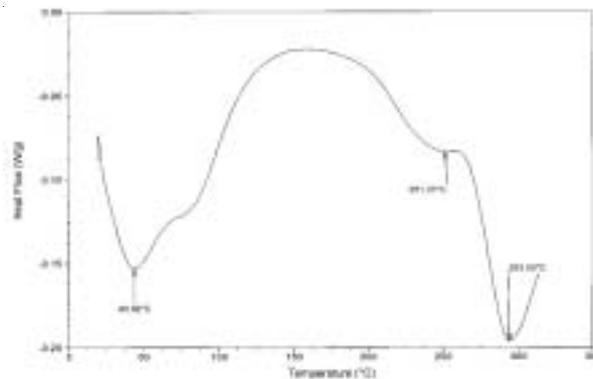


Fig. 3. Model DSC curve of hydrolyzate No.2

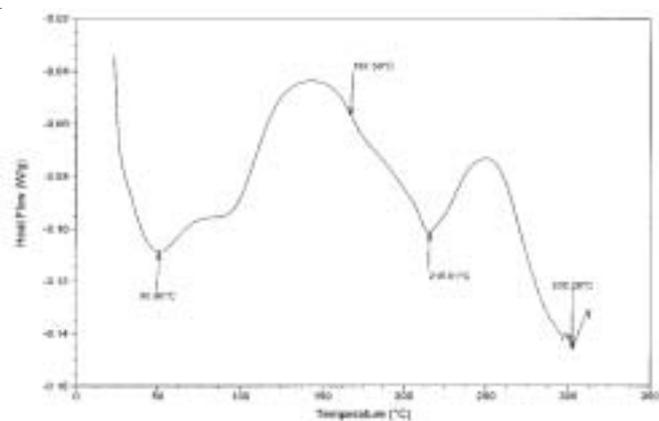


Fig. 4. Model DSC curve of hydrolyzate No.3

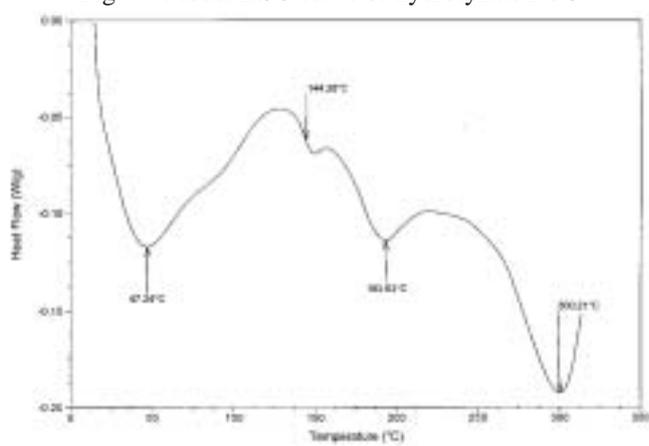


Fig. 5. Model DSC curve of hydrolyzate No.4

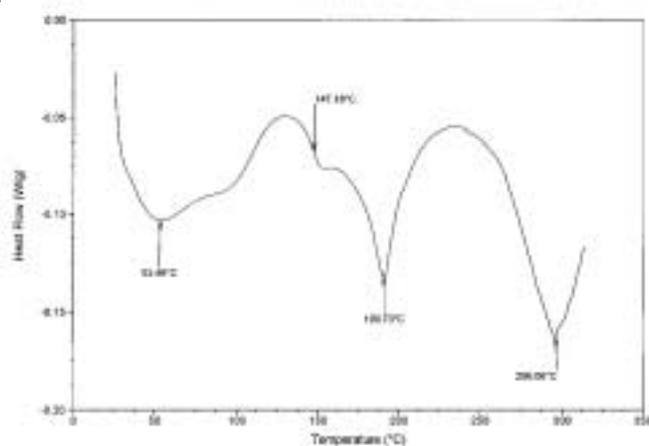


Fig. 6. Model DSC curve of hydrolyzate No.5

TABLE-3
THERMAL COORDINATES OF CHARACTERISTIC TURNS AND PEAKS ON DSC CURVES OF HYDROLYZATES

Hydrolyzate No.	Endo peak (°C)	T _g (°C)	T _m (°C)	T _d (°C)
1	54.52	167.35	215.57	299.80
2	42.26	–	250.24	294.61
3	49.32	166.91	215.20	299.17
4	47.86	144.03	193.22	299.80
5	52.22	146.63	190.94	298.55

Endo peak: Temperature coordinate of minimum of DSC endothermic peak relating to evaporation of free water from sample, T_g : Glass transition temperature (2nd order transition), T_m : Melting point (1st order transition), T_d: Temperature coordinate of degradation, – : Was not found out

Gels exhibit on their thermograms a characteristics endothermic peak which is attributed to melting point of gel. The results are presented in Table-4 and model DSC curve of melting point of gel is showed in Fig. 7. Melting point of gels was found in the 19.1-34°C range.

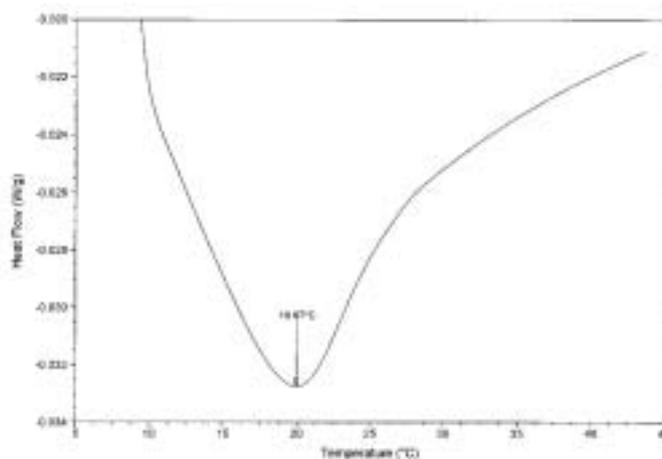


Fig. 7. Model DSC curve of gel prepared from hydrolyzate No.3

TABLE-4
MELTING POINT OF GELS (T_{m1}) PREPARED FROM 40% (w/w) SOLUTIONS OF HYDROLYZATES

Hydrolyzate No.	T _{m1} (°C)
1	33.99
2	33.52
3	19.06
4	22.19
5	21.45

Summarized results of thermal properties of films prepared from hydrolyzate No.4 with/without addition of glycerine are shown in Table-5. Temperature corresponding to the creep was found out in the 32.1-41.1°C range depending on glycerine content (Fig. 8). The decrease of glass transition temperature (T_g) of films at higher content of glycerine is presented in Fig. 9. On thermograms a typical turn in the 35.6-122.5°C range (depending on the glycerine content) was found. Melting point (T_m) was determined to be 168.6°C in the case of film without adding of plasticizer and observations showed that T_m increases with higher content of glycerine up to the value of 201.4°C in the case of 40 % of glycerine (w/w) addition. Endothermic effect attributed to degradation (T_d) was observed in the 271.9-305.5°C temperature range.

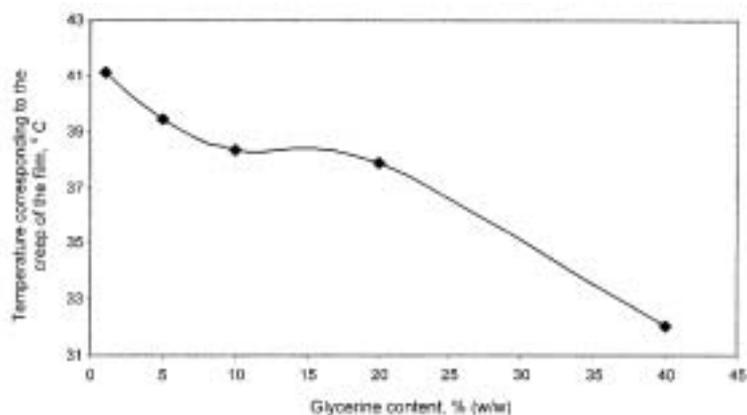


Fig. 8. Temperature corresponding to the creep of the film in dependence on glycerine content

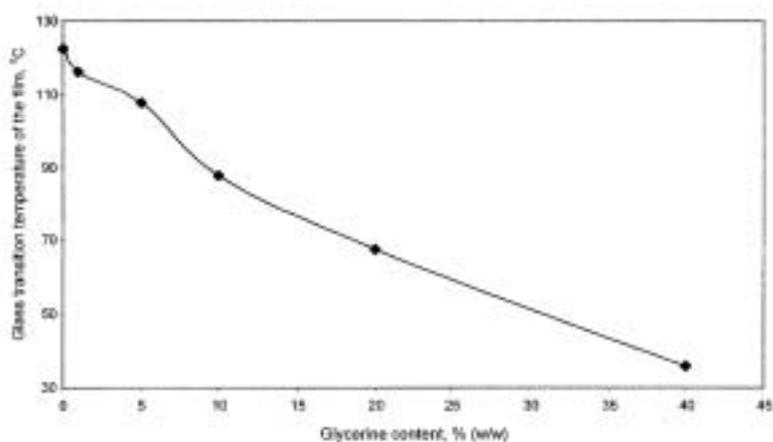


Fig. 9. The decrease of glass transition temperature of the film in dependence on glycerine content

TABLE-5
RESULTS OF THERMAL PROPERTIES OF FILMS PREPARED
FROM HYDROLYZATE NO. 4 WITH/WITHOUT
ADDITION OF GLYCERINE

Specimens	T _c (°C)	T _g (°C)	T _m (°C)	T _d (°C)
Hydrolyzate	-	147.74	191.97	300.48
Film	#	122.54	168.61	271.87
Film + 1 % of glycerine	41.12	116.18	175.80	298.03
Film + 5 % of glycerine	39.46	107.74	178.29	305.05
Film + 10 % of glycerine	38.36	87.79	182.99	305.46
Film + 20 % of glycerine	37.87	67.48	192.52	303.66
Film + 40 % of glycerine	32.06	35.62	201.37	305.19

T_c : Temperature corresponding to the creep of the film

Conclusion

Thermal properties of hydrolyzates, gels and films are a point of interest because processing of hydrolyzates into biodegradable packing materials, films and foils is possible. The present paper deals with thermal properties of hydrolyzates obtained from leather shavings. Melting point of gels prepared from 40 % (w/w) solutions of hydrolyzates and creep of films prepared from 12.5 % (w/w) solution of hydrolyzate with and without adding of plasticizer were tested as well. Addition of plasticizer not only positively effects properties of prepared films but also make their thermal treatment by plastic procedures much more easier. Enzymatic hydrolyzate of chrome-tanned leather shavings thus has a way opened up to being applied as biodegradable packaging materials for a number of industrial products. Nevertheless, modifying hydrolyzates by cross linking enabling to regulate their mechanical and permeation characteristics have to be studied.

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