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Thermal analysis of collagen preparations

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The structural and phase transition and thermal stability of preparations in the form of hide powder and gelatin, obtained by several ways from untanned-collagen-containing materials of leather industry, have been investigated by differential, thermal and thermogravimetric analysis. In the conditions of temperature programmable change, the character of the thermal process has been ascertained, its thermal characteristics have been determined, and the presence of several stages of thermooxidative destruction has been confirmed.

The identity of temperatures of the fixed loss of the specimen mass as well as the high thermostability of gelatin and fine-grained Ukrainian hide powder have been revealed; coarse-grained Ukrainian hide powder is more sensitive to the influence of high temperature, whereas the foreign (Dutch) hide powder has the lowest thermostability. The regularities of thermal destruction of the analyzed collagen preparations can be explained by the peculiarities of their structure, including the amino acid composition, particle shape and size conditioned by their origin and method of manufacture. The obtained results will contribute to a more efficient use of primary and secondary collagen-containing raw materials.

Introduction

Fibrous collagen is the main component of the skin cover of animals, skin and fur skin. Due to the consecutive alternation of hydrophilic and hydrophobic zones in the polypeptide chain α [1], this protein can be regarded as a natural block copolymer in which different zones of the chain considerably differ in their structure and properties [1].

Nowadays, collagen as a typical biopolymer is more often applied practically in medicine, in the manufacture of cosmetics, food, materials used for the production and/or treatment of leather. For all these reasons, the collagen-inherent properties of the connective tissue, its fibrous and fibrillar structure, the capacity of water balance regulation and protection from mechanical damage can be also used [2–5].

The dominant area of collagen use is the manufacture of leather and fur products. The fact is that in the final product only less than half of the total collagen remains as compared with collagen content in the raw material from which the product has been produced. Therefore, the processing of collagen-containing materials has long since become an integral part of leather production, as well as a way of improving the economic situation and solving the environmental problems [2]. It should be noted that in many branches of industrial production collagen is used as a secondary raw material [3–5].

The search for rational ways of processing collagen-containing materials formed during the production of leather and the successful sale of the resulting products are possible after comprehensive analysis of their structure and physicochemical properties.

The aim of this work was to investigate the structural and phase transition, as well as the thermal stability of these preparations obtained from untanned collagen-containing materials.

Materials and methods

The following preparations from untanned collagen-containing materials of leather industry, differing in their producer and method of production, including various degrees of comminution, have been used for the analysis:

a) Ukrainian gelatin (state standard 11293-89) (sample 1);

b) foreign (Dutch) hide powder (sample 2);

c) highly dispersed Ukrainian hide powder (sample 3);

d) low-dispersed Ukrainian hide powder (sample 4).

The producer of samples 3 and 4 is “TOMIG” Co., Ltd enterprise in Nikolayev.

All preparations, gelatin, have a fibrous structure. According to particle size, samples can be arranged in the following order: sample 4 > sample 2 > sample 3 > sample 1 [10].

To investigate the amino acid composition of collagen preparations the method of ion-exchange liquid-column chromatography and an automatic analyzer (*339 M*, “Microtechna” Czech Republic) were used. As shown in Table 2,

according to amino acid composition the collagen-containing preparation did not differ much from gelatin which is the generally accepted model of collagen. At the same time, due to their fibrous structure, these preparations externally are similar to the ordinary hide powder and are identical rather to the collagen derma microstructure than to that of gelatin.

Table 1. Organoleptic evaluation and physico-chemical indices of collagen preparations

Index	Preparation			
	1	2	3	4
Exterior	Powdered homogeneous product			
Colour	Light yellow	Light cream, homogeneous along all mass		
Particle size, μm	2.94	6.60	3.17	11.13
Mass portion, %:				
moisture	12.0	12.1	8.3	12.1
hide substance*	91.8	93.8	90.0	91.0
incoherent fat*	0.1	1.4	0.2	0.2
mineral substances*	1.4	3.1	2.5	4.5

* In terms of absolutely dry substance.

The thermal analysis of the preparations was performed on a *Paulick–Erdey* derivatograph (MOM Company, Hungary) at a temperature ranging from 18 to 800 °C in an atmosphere with a simultaneous removal of gaseous decomposition products at the temperature rise rate of 10 deg/min. The initial mass of samples was 100 mg.

Table 2. Aminoacid composition of collagen preparations

Aminoacid	% μmol/mol			
	1	2	3	4
Glycine	29.47	29.20	29.15	29.41
Alanine	11.57	11.50	11.38	10.89
Valine	1.69	1.61	1.66	1.81
Leucine	2.36	2.57	2.41	2,63
Isoleucine	0.72	0.94	0.87	0.95
Aspartic acid	5.22	5.15	5,03	4.88
Glutamic acid	8.50	8.74	8.25	8.06
Arginine	4.74	5.17	4.79	4.70
Lysine	2.95	3.05	2.96	2.93
Oxylysine	0.53	0.00	0.62	0.68
Serine	3.29	3.70	3.40	3.38
Threonine	1.87	2.02	1.92	1.86
Tyrosine	0.30	0.54	0.31	0.34
Phenylalanine	1.34	1.48	1.31	1.28
Histidine	0.32	0.57	0.43	0.47
Cysteine	0.13	0.12	0.12	0.13
Methionine	0.01	0.01	0.01	0.03
Proline	15.79	15.27	15.33	14.3
Oxyproline	9.21	8.35	10.06	10.97

Results and discussion

The temperature interval of the individual destruction stages was evaluated from differential weight loss curves (DTG), considering the fact that the peak area of a curve is proportional to the mass loss at the corresponding stage (Fig. 1, Table 3).

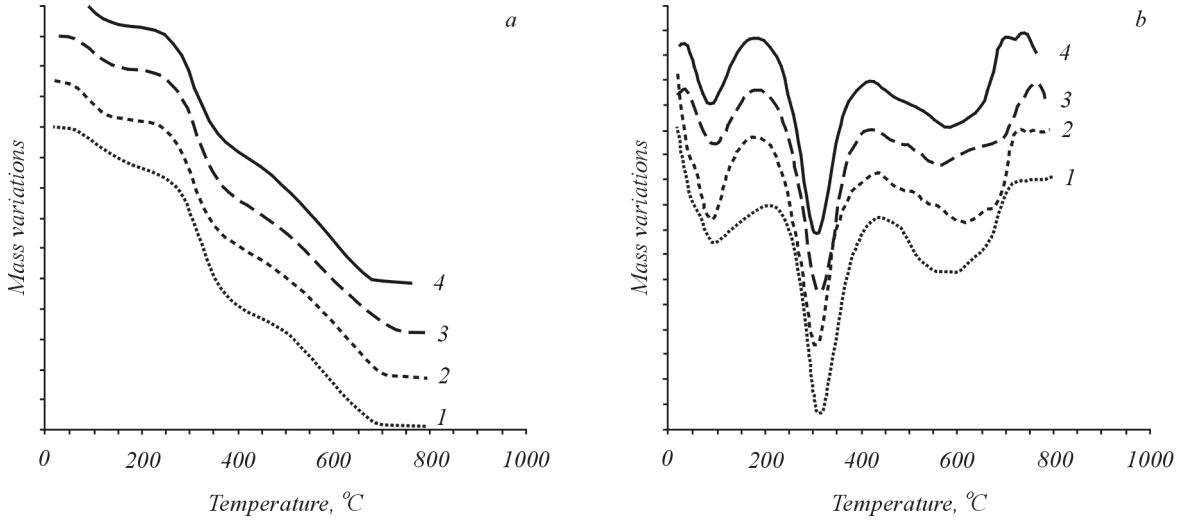

Fig. 1. TG (*a*) and DTG (*b*) curves of the test preparations (*1–4* – sample numbers)

Table 3. Basic parameters of the thermal decomposition of preparations

Sample	Temperature interval, °C			Mass loss, %	Decay rate, mg/min	Activation energy, KJ/mol	Residue, %
	$T_{initial}$	T_{max}	T_{final}				
1	51	95	207	13.6	0.20	23.1	0.95
	207	313	441	48.4	0.73	128.3	
	441	608	729	35.9	0.29	77.6	
2	34	88	176	12.4	0.28	41.0	2.17
	176	308	428	44.3	0.67	132.8	
	428	618	728	40.2	0.13	75.6	
3	45	96	186	10.7	0.21	13.9	1.,91
	186	314	424	45.1	0.68	132.4	
	424	565	763	41.3	0.26	57.3	
4	33	88	175	11.5	0.23	17. 7	2.97
	175	310	417	43.1	0.63	145.2	
	417	583	727	41.4	0.29	63.9	

On the basis of the obtained thermograms, the temperature at which a fixed sample mass had been lost when heated to a temperature of 800 °C as well as the mass loss of samples at a fixed temperature, were determined (Table 4).

Table 4. Thermostability of the preparations under investigation

Sample	Temperature, °C							
	Fixed mass loss						Decomposition	
	$T_{1\%}$	$T_{5\%}$	$T_{10\%}$	$T_{20\%}$	$T_{50\%}$	$T_{80\%}$	$T_{initial}$	T_d
1	62	100	146	272	354	574	51	797
2	53	85	116	269	366	601	34	795
3	59	95	149	280	370	594	45	791
4	54	87	127	276	374	585	33	767
Sample	Mass loss, %							
	Fixed temperatures							General
	m_{100}	m_{200}	m_{300}	m_{400}	m_{500}	m_{600}	m_{750}	
1	5.0	13.7	28.4	59.0	68.0	84.6	98.5	99.1
2	7.9	13.1	29.8	54.2	64.6	79.8	97.2	97.8
3	5.8	11.4	26.0	53.8	65.2	80.9	97.4	98.1
4	7.0	12.2	28.1	53.1	65.3	82.9	96.5	97.0

The temperature of the start ($T_{initial}$), finish (T_{final}) and the maximum speed of destruction process (T_{max}) were used as the thermoanalytical characteristics of the samples. In addition, the mass loss of the sample at the maximum rate of the destruction process (Δm_{max}) was measured, and by the Freeman–Caroll method the activation energy of the thermooxidative destruction of *E*a (activation energy) was determined [11]. The relative error was ±3 ºC.

On the TG and DTG curves we can recognize three main sections corresponding to the three main stages of thermodestruction process, which are accompanied by the change (loss) of sample mass Δm (Fig. 1, Table 3).

At the first stage, the mass loss of the samples begins at 33–51 ºC and ends at 175–207 ºC, the maximum rate of the destruction process being observed at 88–96 ºC. The lowest final temperature (175–176 ºC) was determined while heating low-dispersed (coarse-grained) hide powder (samples 3, 5) and the higher one (207 and 186 °C) while heating gelatin and highly dispersed (fine-grained) hide powder (samples 1, 4). At this stage, the mass loss was negligible and for most samples

(1, 3, 5) did not exceed 11.5–13.6%; for sample 4 (fine-grained Ukrainian hide powder) this index was even lower – 10.7%. The decrease of biopolymer mass at this stage can be explained by the rupture of hydrogen bonds [1] and the removal of water – first of unbound and then of firmly bound one (on the basis of the results of thermal analysis of leather waste [12]). The endothermic effect of evaporation from collagen of the last water molecules was recorded at about 175−207 °C (Fig. 2). The peculiarities of dehydration were evidently conditioned by moisture content (the water content was lowest in samples 4, 1) and the nature of water bond with protein.

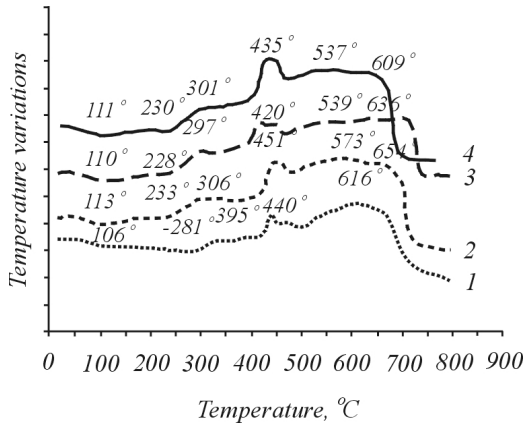

Fig. 2. Thermograms (DTA curves) of the study preparations (samples *1–4*)

At the second stage ($T_{initial}$ = 175–207 ºC, T_{final} = 417–441 ºC), the further mass loss of all samples occurred (43.1–48.4% at a temperature of 308–314 °C) as a result of the formation of gaseous products during the melting process and transition into a viscous-flow state with the signs of destruction (up to the temperature of 308–314 ºC) and its redoubling with the following heating [13, 14]. It is known [15] that during the thermal processing of amino acids from which collagen is generated, CO_2 and water are lost. The rate and heating the technique influence the course of pyrolysis, so during a rapid heating the reaction of decarboxylation dominates, while during a gradual heating dehydration prevails, leading to the formation of a piperazine derivative. Thus, with this in mind [13], we may suggest that the decarboxylation and rupture of other bonds (those with a similar energy) occur simultaneously with the formation of thermal destruction products of the study preparations: with CO_2, also CO, NH_3, and C may be present. As compared with gelatin, the structural and phase transition of hide powder at this stage occurs at a lower temperature. If the first stage of decomposition proceeds slowly (0.20–0.29 mg/min), the second one proceeds three times faster (0.63–0.73 mg/min) (Table 3).

At the further temperature increase, sample mass decreases by 35.9–41.4% (DTG curves of the third segment) because of the final destruction of biopolymers [15]. At stage II, the initial temperature of gelatin is higher by 6–18 °C than that of hide powder, regardless of how it is generated. The highest final decomposition temperature Td, which is equal to the complete disintegration, is the peculiarity of the fine-grained hide powder (767 °C vs. 791–797 °C for other samples). The total sample mass loss was 97.0–99.1% in all cases (Table 4).

The thermostability of the samples was also estimated by such thermoanalytical characteristics as the temperature of the fixed mass loss and the mass loss at a fixed temperature in the same test conditions (heating rate, environment, etc.). Data in Table 4 show the identity of the fixed mass loss temperature of gelatin (sample 1) and fine-grained Ukrainian hide powder (sample 4), as well as the identity of the fixed mass loss of the coarse-grained hide powder, regardless of the manufacturer (samples 3, 5), when heated to 180–200 °C, i.e. prior to the melting of collagen. The coarse-grained-fiber hide powder, especially the foreign (Dutch) one, was proven to be most sensitive to the temperature.

At the further temperature rise this trend was leveled, and if the temperature was ≥ 400 °C, the mass loss of a hide powder at a fixed temperature, regardless of the generation technique, was by 1.1–5.9% less than the mass loss of gelatin, which is consistent with the activation energy *E*a index of the thermooxidative destruction process.

In our opinion, the difference between the thermal characteristics of the test biopolymers can be explained by the peculiarities of their origin and manufacture, i.e. structural features. The influence of the chemical nature and particle size of the reagents on the reaction rate is known from the literature [16]: the larger and the more complex the molecules of the reagents, the slower the reaction rate.

The hide powder differs from gelatin both in its structure and amino acid composition [10, 17]. For example, the foreign (Dutch) hide powder had the lowest content of hydroxyproline (8.35% vs. 9.21–10.97% in gelatin and Ukrainian hide powder), the highest content of tyrosine (0.54% vs. 0.30–0.34%) and a slightly higher content of dicarboxylic acids (13.9% vs. 12.94–13.72%). These amino acids are essential to the strength of peptide bonds in the structure of collagen (hydroxyproline, dicarboxylic acids), to the activity of telopeptide areas of polypeptide chains (tyrosine, dicarboxylic acids), as well as to the amount of bound water (for example, the carboxyl groups of dicarboxylic acids are capable of connecting 3–4, whereas hydroxyl groups of hydroxyproline and tyrosine 2–3 water molecules) [1].

On the other hand, we should not exclude the influence of the thermal stability of the amino acids that make up the collagen-containing materials. A comparative analysis of the amino acid composition and thermoanalytical characteristics of the test preparations was made to clarify this factor, taking into account only the curves in which the value of approximation reliability was at least 0.75. The inclusion of amino acids into a particular group was based on information in the literature concerning the thermal destruction of amino acids [13–15]. We found that, as compared with gelatin, hide powder contained by 1.9–12.8% more thermally

unstable amino acids (serine, threonine, lysine, tyrosine, phenylalanine, histidine). This fact is reflected in the final temperature of the second phase decomposition: with increasing the concentration of thermally unstable amino acids in a biopolymer, the melting process begins and ends at a lower temperature (Fig. 3).

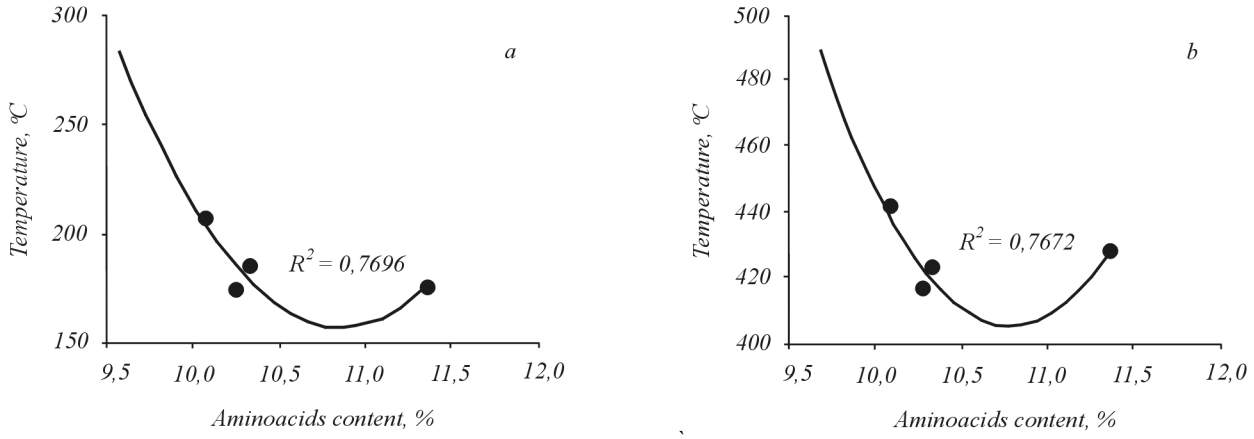

Fig. 3. Influence of thermally unstable amino acid content on the initial (*a*) and final (*b*) temperature of phase II destruction of preparations

With increasing the concentration of thermostable amino acids in biopolymers (alanine, glutamic acid, glycine, isoleucine, leucine, proline, valine), the final temperature of decomposition stage II rises (Fig. 4, b). At this stage, the content of thermostable amino acids has no effect on the initial temperature (R^2 = 0.4489; Fig. 4, a).

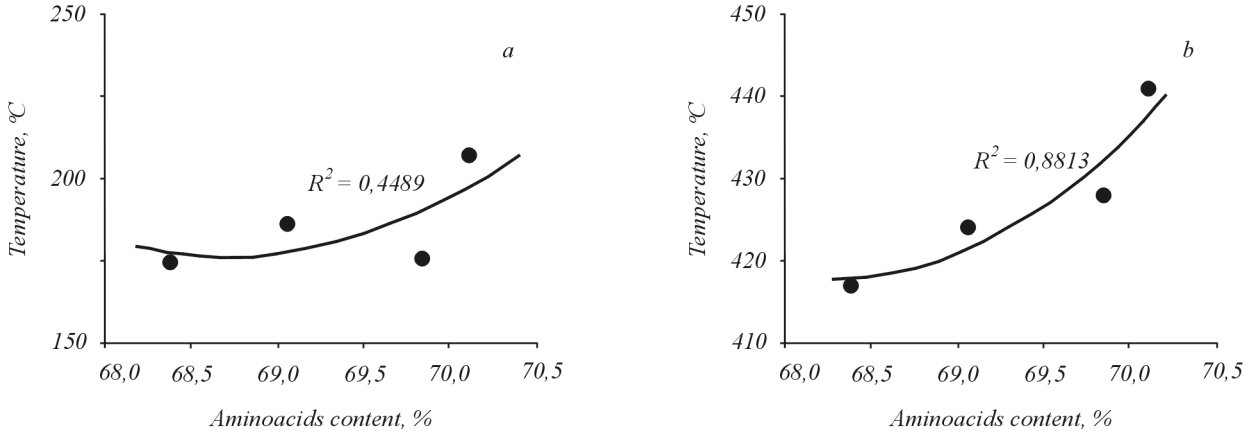

Fig. 4. Influence of thermostable amino acid content on the initial (*a*) and final (*b*) temperature of phase II destruction of preparations

Conclusions

In the conditions of the programmable temperature change, the character of the thermal process has been ascertained, and its thermal characteristics at different stages of thermooxidative destruction have been defined. Thus, it was found that:

– in the temperature range from 33–51 to 175–207 ºC, the dehydratation process occurs, accompanied by minimal mass loss (11–14%) and speed (up to 0.28 mg/min);

– in the temperature range from 175–207 to 417–441 ºC, there occur crystalline softening and the melting of thermally unstable amorphous areas of collagen; actually, the processes of thermal and oxidative destruction begin, including the transition into viscous-flow state from 175–207 to 308–314 ºC. They are characterized by a significant mass loss (4–45%) and a more rapid decomposition (0.64–0.74 mg/min);

– in the temperature range from 417–441 to 727–763 ºC, the last stage of destruction takes place, accompanied by burning out of the coke residue, accompanied by a little, almost constant, mass loss.

The identity of the temperature of the fixed mass loss of samples, as well as the high thermostability of gelatin and fine-grained Ukrainian hide powder have been revealed; coarse-grained Ukrainian hide powder is more sensitive to the influence of high temperature, and the foreign (Dutch) hide powder has the lowest thermostability index.

The established regularities of thermal destruction of the test preparations can be explained by structural peculiarities, including amino acid composition, particle shape and size conditioned by their origin and method of manufacturing.

The obtained results are planned to be used in further research aimed at a more efficient use of primary and secondary collagen-containing raw materials in various fields of science and technology.

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ТЕРМИЧЕСКИЙ АНАЛИЗ ПРЕПАРАТОВ ИЗ КОЛЛАГЕНСОДЕРЖАЩИХ МАТЕРИАЛОВ

Р е з ю м е

Методами дифференциально-термического и термогравиметрического анализа исследованы структурно-фазовые превращения и термостабильность препаратов, в виде гольевого порошка и желатина, полученных разными способами из недубленых коллагенсодержащих материалов кожевенного производства.

В условиях программируемого изменения температуры установлен характер теплового процесса, определены его термические характеристики, подтверждено наличие нескольких стадий термоокислительной деструкции. Выявлена идентичность температур фиксированных потерь массы образцов, высокая термостабильность для желатина и мелкого отечественного гольевого порошка; крупный отечественный порошок более чувствителен к действию высокой температуры, а у иностранного гольевого порошка наиболее низкие показатели термостабильности. Закономерности термической деструкции анализируемых коллагенсодержащих препаратов можно объяснить особенностями их строения, в том числе аминокислотным составом, формой и размером частиц, обусловленными их происхождением и способом изготовления.

Полученные результаты будут способствовать более рациональному использованию первичных и вторичных коллагенсодержащих сырьевых ресурсов.