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Experimental Study of New Collagen-containing Preparations

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Authors' contributions

This work was carried out in collaboration between both authors. Author OA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author LM performed the statistical analysis, managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Leather industry is one of the main sources of collagen-containing raw materials. Therefore, effective recycling of these raw materials into marketable products is a pressing problem not only for leather industry but also for other branches of national economy. In the present work, we have studied into new collagen-containing preparations, derived from untanned by-products of leather industry. The size of particles in collagen preparations was assessed using microscopy. Chemical analysis was carried out under chemical research technique. IR spectroscopy has revealed the presence of various functional groups in the structure of developed preparations. Based on the results of experimental data, we concluded that new collagen-containing preparations can be used in many sectors of national economy.

Keywords: Collagen; leather wastes; IR-spectroscopy; properties.

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1. INTRODUCTION

Collagen is the basic fibrous protein in animal skin and connective tissue [1,2]. Since animal skin for thousands years remains the basic resource for gelatin, genuine leather, fur and footwear manufacturing, the technical importance of collagen is crucial. Thus, there is an urgent need for collagen-containing raw materials.

Previous research [3] has shown that nearly 40% of collagen-containing raw products are turned into wastes during leather production, and then garments and footwear manufacturing (i.e. in the production chain «animal skin-leather-leather product»). According to [4], the figure is almost 50%. Thus, the problem of reprocessing of collagen-containing materials and their further use is an extremely important task to be solved. This goal can be achieved by either developing a waste-free recycling technology for collagen-containing raw materials or by converting them into homogenous state for further usage.

Of between studies a multicomponent and multipurpose system, such as connective tissue can be conducted due to the development of scientifically based methods of native collagen extraction which preserve the molecular architecture and biological activity of the protein as well as to provide the maximum level of protein purification from concomitant biopolymers. At the same time, collagen-containing products can be obtained both in the solid and salted state and in solution that significantly extends the scope of application of industrial wastes containing collagen. For example, split hide cutting of cattle skin is the main protein-containing waste product that is actively being used by processing plants as a source for production of different forms of soluble collagen.

The soluble collagen finds application in biomedical and pharmaceutical fields as well as in veterinary and food industries that allows to develop new medicines and biomaterials [5,6]. Therefore, in recent years, collagen-containing waste products have become valuable raw materials for processing plants, i.e. they already have the customer value as well as the status of industrial goods. As regards the soluble forms of collagen derived from collagen-containing waste products, they are high-demand goods in spite of high price, small assortment and strict quality requirements. As collagen-containing waste products and their derivatives have a set of characteristics that makes them valuable both for producers and consumers, the study of their qualities at different stages of stocking, recycling and further industrial use is very actual [5,6].

Leather industry is one of the main sources of collagen-containing raw materials [7-9]. Recent research works shows [3,10], that nowadays there are various application areas of collagen-containing raw materials and their by-products. For example: The production of additives and emulsions containing of protein and fat component; the production of multifunctional drugs and structured products (like extrudate and crisps); gelatin manufacturing; production of preparations for fragrance and cosmetics industries, veterinary medicine, medicine, leather manufacturing etc.

Taking into account all mentioned above, detailed study of new preparations, obtained from untanned collagen-containing materials, was carried out. The technology for the production of collagen-containing preparation has been developed by Co. Ltd «TOMIG» (Ukraine) [11-12]. This paper is only a small part of comprehensive research in this area. It complements the data on physico-chemical properties of new preparations such as dispersion, chemical composition (moisture, ash and hide substances content, content of substances extracted by using organic solvents and presence of active groups responsible for interaction with various chemical substances.

2. MATERIALS AND METHODS

In present study, Ukrainian protein preparations with particles of different size, developed by Co. Ltd «TOMIG», were used. All preparations were derivatives of untanned collagen-containing materials originating from cattle hides. Gelatin was selected as a model compound to control all measurements. It is difficult to extricate pure collagen, because this protein, insoluble under normal conditions, is always mixed with other proteins. For this reason, collagen preparations are used for research. Gelatin is an example of such preparations. Its aminoacid composition is close to collagen. When collagen is being transformed into gelatin, its polypeptide chains shorten as a result of breakage of the part of peptide and some other covalent links, spiral structure breaks up, conformation transformations such as «spiral-globule» take place.

Table 1. Size measurements of collagen-containing preparations

Preparation	Measurement quantity	Particles size, mm		*E*, %
		longitudinal	transversal	
1	15	0.124±0.007	0.118±0.001	0.258
2	15	0.131±0.005	0.122±0.004	0.258
3	13	0.139±0.001	0.138±0.001	0.258
4	15	0.548±0.046	0.093±0.028	0.258
Gelatin	12	0.591±0.061	0.566±0.065	0.289

Testing of collagen preparations was performed according to the recommendations for standardization [13-14]. An averaged sample for measurement was chosen only after being blended in bag.

To get statistically significant results, all measurements were carried out no less than 3-5 times. Control studies were performed simultaneously with the data correction process. For example, to determine the content of hide substances and the consumption of chemicals, a control measurement was done. It means that all tests are being performed under the same conditions, however, the sample of preparations isn't used.

In this experimental study, a range of different methods have been applied. The particles size of collagen preparations were measured using Bresser Researcher Bino optical microscope with increasing 40-1000 (Bresser, Germany). To identify optical density of absorption and identification of collagen preparations active groups the infrared absorption spectra on spectrophotometer TENSOR 37 (Brucer, Germany) was applied. Statistical analysis was performed using standard statistical software [15].

Those characteristics that allow to describe precisely the physico-chemical properties of preparations were determined. They include the particle size, mass fraction of humidity, content of fat, hide substance and mineral components etc.

To describe the error degree of indices, i.e. at which degree a particular index deviates from its average, variation coefficient (E) must be identified. The smaller the value of E is, the more uniform the data are. In our work, we have calculated the standard deviation for particles size of collagen preparations. No less than 12 tests were done to calculate its value. According to the Table 1 above, the «particles size» characteristics demonstrated great uniformity and homogeneousness for all collagen preparations tested ($E < 5\%$).

3. RESULTS AND DISCUSSION

In this paper, we have studied the structure and main physico-chemical properties of the new collagen preparations. They are light, homogenous and scentless products that look like small fibers. We have identified their particles size using optical microscopy. This criterion allows to divide them into two groups (fine-fibrous and coarse-fibrous products), and put in the following particle size sequence: *preparation 4 > preparation 3 > preparation 2 > preparation 1.*

Chemical analysis (moisture content, ash, hide substances, substances extracted with organic solvents) was conducted in accordance with ISO 4047:1977, ISO 5397:1984, ISO 4048:2008 guidelines. The identification of unbound fatty substances includes: their removal from the test preparation with an organic solvent (in this case, carbon tetrachloride), extraction of fatty components, samples drying and weighing.

Because the investigated preparations are the derivatives of fibrous collagen of derma, their chemical composition corresponds to the described in previous studies [1-2]. High content of hide substances (>80 %) is one of the advantages of preparations that indicates a high protein content. The content of mineral and fatty substances is much smaller. It is equal to 3,9-4,9 and 1,0-1,6%, respectively.

Hide substance (collagen) is the main component of preparations under test. Its content exceeds 80%. Content of mineral substances and fat is much lower – about 3.9-4.9 and 1.0-1.6% correspondingly. The difference in chemical composition of the new preparations can be explained by the influence of primary test conditions, notably, the degree of hydrolysis of initial collagen-containing raw materials (Table 2).

Table 2. The main characteristics of collagen-containing preparations

Preparation	Mass fraction, %			
	Humidity	Hide substance*	Mineral substances*	Substances, extracted with organic solvents*
1	5.4±0.2	86.4±0.4	4.9±0.2	1.2±0.3
2	5.5±0.2	85.7±0.4	3.9±0.1	1.1±0.3
3	5.7±0.2	90.1±0.4	3.9±0.1	1.0±0.3
4	5.2±0.2	81.5±0.4	4.8±0.2	1.6±0.3
Gelatin	12.2±0.2	85.3±0.4	4.8±0.2	1.2±0.3

** Per completely dry substance*

Spectrograms of the new preparations (Fig. 1) show the bands the most distinctly at the following frequencies: 3398, 1649, 1540, 1237 and 555 cm^{-1}. These frequencies correspond to oscillation of amines, carboxylic acids and amides A, I, II, III, VI with different content of constituents. Also, at the frequency of 1448 cm^{-1}, that corresponds to deformative oscillations of hydroxyl group. There is also a wide band of 2500-3800 cm^{-1}, that corresponds to valent oscillations of carboxylic acids hydroxyl groups (Table 3).

IR spectroscopy has been used to identify the structural peculiarities of the preparations.

Presence of these groups can be explained by the fact that the preparations are collagen derivatives. Thus, they underwent the hydrolytic influence of alkaline and other reagents during their production.

Difference in peaks intensity of investigated preparations is stipulated by the presence of great amount of active groups in fine-dispersed preparations samples due to intensive destruction of polypeptide protein chains.

Thus, we have identified a range of functional groups in the structure of collagen preparations under test (mostly amide ones they comprise –NH, –C=O, CN, amine, carboxylic and hydroxyl). This is due to multifunctional nature of these materials and their ability to interact with various chemical materials.

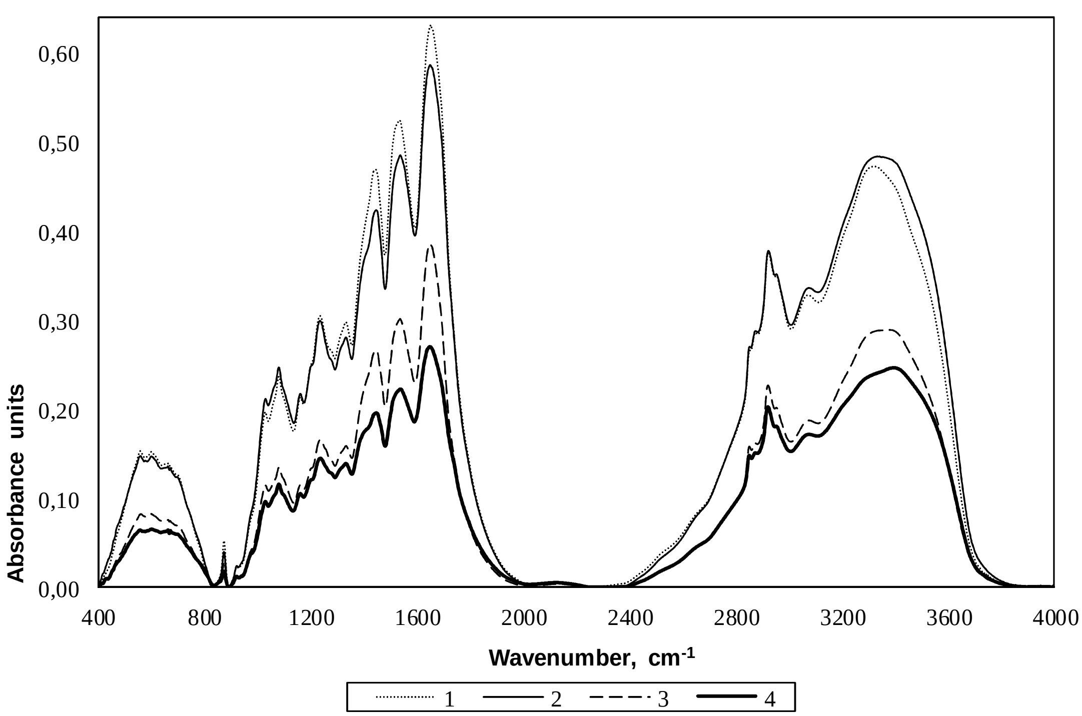

Fig. 1. IR-spectrograms of collagen-containing preparations

Table 3. The most typical bands of IR spectra of collagen-containing preparations

[16-18]			Preparation				
Frequency, cm^{-1}	**Intensity***	**Type of compound, group****	**1**	**2**	**3**	**4**	**Gelatin [19]**
3400-3500		Amines (Primary) (vNH_2)	3325	3342	3398	3375	3320
3350-3400	m	Amines (Secondary) (vNH_2)					
3300		Amide A (100% *v*NH)					
2500-3800 broad band		Carboxylic acids (*v*CO)	Available				
3100		Amide B (100% *v*NH)	3074				3083
2926	w	Alkanes (vCH_2)	2925	2926	2923	2923	
2850-2860	m	Alkanes (vCH_2)			2853	2853	2880
1597-1672		Amide I (80% *v*CO; 10% *v*CN; 10% *d*NH)	1653	1650	1649	1650	1650
1650-1850	s	Carbonyl compounds, carboxylic acids and its derivatives (*v*C=O)					
1480-1575		Amide II (60% *d*NH; 40% *v*CN)	1533	1538	1540	1537	1554
1400-1500	s, m, w	Alcohols (*d*OH)	1445	1446	1448	1446	1454
1460	m	Alkanes (dCH_3)					
1330-1350	w	Alkanes (dCH_2)	1333	1333	1333	1334	1333
1229-1301		Amide III (30% *v*CN; 30% *d*NH; 10% *v*CO; 10% O=CN)	1235	1235	1237	1237	1240
1180-1250	m	Amines (Secondary, tertiary) (*v*C-N)					
1125-1220	s	Alcohols (secondary, tertiary) (*v*C-O)	1161	1161	1161	1161	1162
1050-1085	s, m	Alcohols (Primary) (*v*CO)	1081	1081	1081	1081	1083
625-767	w	Amide IV (*d*O=CN planar)	1030	1030	1030	1030	1033
640-800	w	Amide V (*d*NH nonplanar)	874	874	874	874	648
537-606	m	Amide VI (*d*CO nonplanar)	558	556	555	558	

** s – strong, m – medium, w – weak; ** v – valent, d – deformative*

Based on the results of the experimental data, we have concluded that new collagen-containing preparations can be used as secondary raw materials in many sectors of national economy.

Due to a set of the properties (non-toxicity, compatibility, similarity to collagen [12], multifunctionality) as well as their ability to preserve the fibrous structure, new protein-containing preparations can be used in food industry, agricultural sector, medicine, veterinary medicine, cosmetology, light industry etc.

4. CONCLUSION

We have analyzed the properties of the new protein preparations, derived from untanned collagen-containing materials, produced by Ukrainian manufacturer.

Using the scope of basic methods, we have identified main physical, chemical and structural properties of these preparations.

It has been shown that preparations consist of tiny fibers, which helps us divide them into fine-fibrous and coarse-fibrous component; they also contain a lot of proteins, a little fat and some of the minerals. They are of multifunctional nature – they have various functional groups (amide, amine, carboxylic and hydroxyl) in their structure.

We have concluded that new collagen-containing preparations can be used in many sectors of national economy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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