Chemistry for Sustainable Development **17** (2009) 177–183

UDC 675.81+544.7

Intensification of Collagen Dissolution Process with the Help of Mechanochemical Treatment

V. A. POLUBOYAROV[1], E. V. VOLOSKOVA[1], V. V. YANKOVAYA[1] and T. I. GURYANOVA[2]

[1]*Institute of Solid State Chemistry and Mechanochemistry, Siberian Branch of the Russian Academy of Sciences, Ul. Kutateladze 18, Novosibirsk 6300128 (Russia)*

E-mail: sanych@solid.nsc.ru

[2]*Novosibirsk Institute of Technology, Branch of the Moscow State University of Design and Technology, Ul. Potaninskaya 5, Novosibirsk 630099 (Russia)*

(Received August 11, 2008; revised October 1, 2008)

Abstract

Different methods of mechanochemical intensification of the process of collagen dissolution are proposed: cavitation at the stage of preliminary treatment of collagen-containing material and mechanical mixing at the stage of dissolution in acetic acid. To obtain pure products of collagen dissolution unpolluted with chemical reagents for use in medicine and food industry, treatment with a complex of enzymatic preparations was used. Colloid solutions conserving fibrous structure necessary for further use of the products of collagen dissolution were obtained.

Key words: offal, processing of wastes, alkaline-salt treatment, acetic acid, ultrasound, enzyme preparations, molecular mass, collagen dissolution, intensification, mechanochemical action

INTRODUCTION

In view of the worsening ecological situation, the problem connected with utilization and processing of wastes becomes urgent. Thus, in tanning industry, up to 50 % of the mass of animal fell forms the waste product from currying and is not processed but dumped. At the same time, reasonable use of collagen-containing materials promotes enhancement of the efficiency of production and improves the state of environment.

One of the major components of wastes from tannery is fibrillar protein collagen, one of the most widespread in animals. Collagen is widely used in industry for manufacturing leather, fur, gelatine, a number of medical preparations. Collagen forms the fibres of connective tissues; it serves as the major substance of cutaneous covering, bones, tendons, cartilages and accounts for about 30 % of all the proteins of a living organism. The framework of an animal organism playing the parts of supporting, mechanical and protective means is formed with the participation of collagen [1].

The most efficient method to process collagen-containing wastes is to transform collagen in solution. Being a high-molecular protein, collagen forms a colloid solution during dissolution. The colloid solution may find application in many areas: in medicine (collagen sponges and films, material for artificial vessels), in food industry (obtaining sausage casing), in textile industry (clothes with the addition of collagen fibres are characterized by high hygienic, fire-proof properties) [2–8].

The structure of collagen is extremely complicated; there are bond of different character and strength inside it. Because of this, it is difficult to transfer collagen into solution conserving the fibrillar conformation. Under the action of different reagents, mechanochemical action and elevated temperatures, collagen fibres can change their conformation from

fibrils into globules. Forced solubility of collagen, size and properties of the fragments of native structure passing into solution are determined by the type of bonds and the depth of their distortion. The extent of solubility and the character of the formed dissolution products depend on the conditions of the preliminary treatment of collagen, the character of solvent, and dissolution mode. Good results were obtained with the joint alkaline-salt and acidic action on collagen without heating [9]. During alkaline-salt treatment, a number of cross intermolecular bonds get broken, the samples swell, their structure undergoes loosening and garneting. However, this method of collagen dissolution requires much time and material expenses (high consumption of chemical materials), so it is reasonable to optimize the process of collagen dissolution and to provide the possibility of obtaining more pure dissolution products.

Collagen dissolution process may be accelerated by using different chemical reagents, enzyme preparations and mechanochemical treatment.

Enzymatic methods of dissolution allow one to obtain collagen with good fibre-forming ability. However, the complicacy and labour-intensiveness of the preparation of enzymatic reagents, as well as the loss of rather substantial part of collagen as a result of its incomplete dissolution and denaturation under the effect of elevated temperature decrease the efficiency of this method substantially [10].

The action of elastic oscillations within the ultrasonic frequency range allows one to intensify diffusion processes to a much higher extent than usual chemical mixing do. In the majority of cases the action of ultrasonic oscillations on a substance or a reaction should be considered as a consequence of cavitation phenomena and intense mixing action. Cavitation causes the hydraulic shock, which promotes rupture of intermolecular bonds necessary for the transfer of collagen-containing material into solution. As a result of such an action, the duration of obtaining the products of collagen dissolution (PCD) decreases.

The goals of the present work were intensification of the process of collagen dissolution with the help of mechanochemical action; obtaining PCD with the conservation of fibrillar structure; processing of unused collagen-containing materials with the minimal formation of secondary wastes.

EXPERIMENTAL

Almost in all the branches of industry, collagen is used as a colloid solution from which the ready product is obtained.

In the present work, we chose a known method of complete dissolution of dermal collagen in acetic acid with preliminary alkaline-salt treatment [11]. We studied the dissolution of wastes from tanning industry – offal split and split cut-off pieces that are formed in lime yards of tannery works in offal breaks and after splitting process.

We considered the possibility of intensifying the dissolution process with the help of mechanochemical action at different stages of obtaining the solution. The following versions of collagen dissolution were carried out:

1. Intensification of the dissolution of collagen-containing material through cavitation action at the stage of alkaline-salt treatment;

2. Preparation of collagen solution by mechanical mixing of offal without preliminary alkaline-salt treatment in acetic acid with different concentration with the help of a homogenizer;

3. The joint alkaline-salt treatment in activator and mechanical mixing at the stage of dissolution in acetic acid;

4. Dissolution of the collagen-containing material with preliminary enzymatic treatment;

5. Obtaining collagen solution with the help of the set of above-listed intensifying actions.

To accelerate the process of dissolution, we used the ultrasonic apparatus [12]. For comparison, we also considered the effect of the treatment in a laboratory mixer (homogenizer) of ML-4 type [13].

Determination of the viscosity of collagen solutions (to calculate the molecular mass of PCD) was carried out with a capillary glass viscosimeter VPZh-1. The diameter of the capillary of viscosimeter was 0.73 mm.

To reveal the regularities of the transition of collagen-containing material into solution,

quantitative analysis was carried out. To determine the humidity of collagen-containing material and concentration of solutions, the samples were dried in a drier at 105 °C to the constant mass.

To study the changes occurring in offal during alkaline-salt treatment and under the action of cavitation, offal microsections were preliminarily coloured using special dyes and observed a the microscope.

RESULTS AND DISCUSSION

A series of solutions was obtained during the investigation; the characteristics of solutions are listed in Table 1. Molecular mass of the PCD varies within the range 120 000–360 000 a.m.u., which is the evidence of the fibrillar structure of the obtain suspensions of collagen.

One can see in the data shown in Table 1 that the preparation of the colloid using the conventional procedure (exp. No. 1) is a long-term process taking 15 days. This process was successfully activated using cavitation action at the stage of preliminary alkaline-salt treatment. The hydraulic shock arising in the activator [14] leads to bond rupture in collagen structure and allows one to reduce the duration of alkaline-salt treatment from 46 h to 6–12 min. Correspondingly, the duration of dissolution process decreases from 15 to 6 days. It should be noted that we failed to reveal any definite regularity in changes of the molecular mass with an increase in the time of treatment in the activator (Fig. 1). Nevertheless, it is evident that treatment for 6 min in the activator is insufficient for the penetration of the alkaline-salt solution into the derma thickness. It is impossible to carry out treatment for more than 12 min because of voluminous foaming and sharp temperature rise. It is likely that the action of the hydraulic shock during a longer alkaline-salt treatment results in splitting not only intermolecular but also intramolecular bonds, which causes the destruction of collagen. A decrease in the intensity of cavitation action allows one to avoid the rupture of chemical intramolecular bonds and helps to increase the molecular mass but this is impossible to implement due to the design of the device.

Even more substantial effect on the duration of dissolution process is produced by the joint preliminary treatment in the activator and dissolution in acetic acid under mechanical mixing (see Table 1). Under the action of mechanical action, the diffusion of acetic acid into the derma accelerates, so the fibres pass into solution more rapidly.

Dissolution in acetic acid without preliminary treatment causes only partial transition

TABLE 1

Dependence of molecular masses of collagen solutions on treatment procedures

Experiment No.	Brief description of experiment	Content of collagen dissolution products, g/L	Molecular mass, ±20 000 a.m.u.
1	Dissolution in acid under static conditions after alkaline-salt treatment for 48 h	7.0	386 180
2	The same? under dynamic conditions after alkaline-salt treatment for 6 min in the ultrasonic apparatus	13.63	184 316
3	The same, under static conditions after 8 min	9.99	183 223
4	The same, under dynamic conditions after 8 min	10.15	188 463
5	The same, under static conditions after 10 min	12.03	245 539
6	The same, under dynamic conditions after 10 min	11.22	215 245
7	The same, under static conditions after 12 min		
8	The same, under dynamic conditions after 12 min	8.09	167 121
9	Treatment in distilled water in ultrasonic apparatus, with a complex of enzymes, dissolution in acetic acid	19.8	302 125

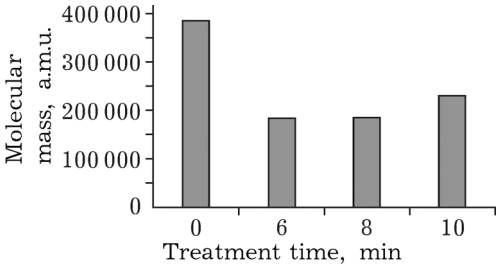

Fig. 1. Dependence of the molecular mass of collagen on the duration of alkaline salt treatment in ultrasonic apparatus.

of collagen into solution. It was shown that the yield of collagen in this process is 1.5 %. We studied the effect of such factors as treatment time and concentration of acetic acid on the transition of collagen-containing material into solution. One can see in the data presented in Table 2 that the molecular mass of the obtained products of collagen dissolution changes insignificantly with an increase in treatment time. Mechanical action causes only the surface destruction and detachment of the bundles of collagen fibres but does not promote destruction of chemical bonds in deeper structures. A substantial increase in the time of mechanical action may cause a change of the conformation of collagen molecules from fibrillar to globular [1] and therefore the disappearance of valuable fibre-forming properties that cannot be reproduced in collagen by artificial means.

It was shown that an increase in the concentration of acetic acid causes an increase in the molecular mass and concentration of PCD, acceleration of the diffusion of acetic acid, so a larger mount of collagen fibres may pass into solution (Table 3). An increase in the molecular mass is likely to be due to the fact that stronger chemical bonds in collagen structure may get destroyed with a change of the concentration of the acid.

So, complete dissolution of collagen during 2 days is provided only as a result of preliminary treatment with cavitation followed by dissolution in acetic acid under mechanical mixing. Thus obtained colloids only slightly differ in molecular mass and concentration from the colloids prepared by dissolution under static conditions (see Table 1).

The products of collagen dissolution are used to obtain fibrous materials for food industry and medicine, so the absence of chemical reagents in them is necessary. In the present work we studied the possibility of collagen dissolution with the help of enzyme preparations. In addition, the use of enzymatic treatment, unlike for alkaline-salt treatment, allows achieving collagen dissolution with smaller changes of

TABLE 2

Dependence of the molecular mass of collagen solutions on the duration of treatment

Experiment No.	Brief description of experiment	Content of collagen dissolution products, g/L	Molecular mass, ±20 000 a.m.u.
1	Treatment in 1 M CH_3COOH under dynamic conditions for 1.5 h without alkaline-salt treatment	8.8	163 800
2	The same for 2 h	3.48	165 919
3	The same for 4 h	5.8	161 042

TABLE 3

Dependence of the molecular mass of collagen solutions on the concentration of acetic acid

Experiment No.	Brief description of experiment	Content of collagen dissolution products, g/L	Molecular mass, ±20 000 a.m.u.
1	Treatment in 0.5 M CH_3COOH under dynamic conditions for 4 h without alkaline-salt treatment	6.76	107 457
2	The same in 1 M CH_3COOH	5.80	161 042
3	The same in 1.5 M CH_3COOH	10.0	182 994

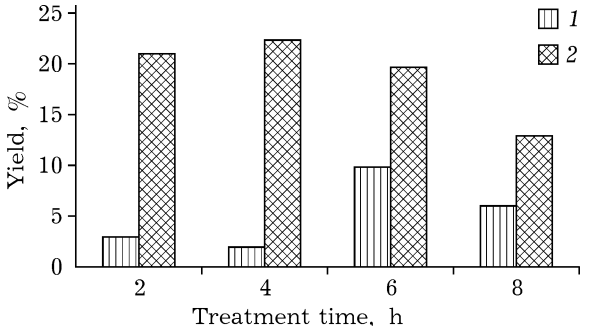

Fig. 2. Dependence of the yield of collagen-containing material released into solution on the time of enzyme treatment with protease (*1*) and amylosubtilin (*2*).

its chemical composition and physicochemical properties [15]. For this purpose, we used alkaline proteases, amylosubtilin and pepsin.

During the preliminary treatment of offal with enzyme preparations, we used the enzymes possessing proteolytic and amylolytic activity. We studied the action of each enzyme on collagen-containing material, and their joint action as well. It was established that amylosubtilin and protease exhibit different kinds of action on collagen. Quantitative analysis showed that amylosubtilin, unlike protease, promotes more complete transition of collagen into solution (Fig. 2).

The obtained data provide evidence that there is a large number of carbohydrate bonds at the supramolecular level; they fix and cement the adjacent collagen molecules. Protease affects the available structures, the end groups. It was established on the basis of these data that the enzymes under investigation when acting separately do not cause the necessary garneting effect, so it was proposed to use a mixture of proteolytic and amylolytic enzymes to enhance the content of acid-soluble fraction.

The data on the molecular mass of the obtained solutions (Fig. 3) only confirm the above statements. Enzyme preparations promote the release of acid-soluble forms of collagen from its supramolecular structures without affecting the main chains of collagen molecules.

It is known that up to 90 % of collagen of the derma may be transformed into the acid-soluble form as a result of the action of pepsin at a temperature of 25 °C for 15–40 h [15]. An increase in the content of the acid-soluble fraction is promoted only by the treatment with a large amount of the enzyme (10 % of the protein mass), which may lead to the rupture of not only intermolecular but also intramolecular bonds. In this connection, it was proposed to use enzyme preparations – protease and amylosubtilin for the preliminary treatment of collagen, and to carry out subsequent dissolution in acetic acid in the presence of pepsin but with smaller concentration (5 % of the offal mass).

In addition, we studied the possibility of preliminary treatment in the ultrasonic apparatus in distilled water, then with a complex of amylolytic and proteolytic enzymes, followed by the treatment in pepsin and final dissolution in acetic acid under mechanical mixing (see Table 1, exp. No. 9). As a result of this complex enzymatic treatment, we succeeded in achieving complete dissolution and obtaining highly concentrated solution within 5 days. This version may be considered optimal.

To determine changes occurring in the offal structure during different kinds of treatment, we carried the microscopic studies. The images of microsections of the initial offal (Fig. 4, *a*) show coarse, thick bundles of collagen fibres interlacing at different angles; they are to be split into thinner fibres during subsequent treatment. After alkaline-salt treatment for 48 h (see Fig. 4, *b*) cross splitting of the coarse bundles occurs; as a consequence, the treated offal becomes soluble in the acid. The ultrasonic action in the alkaline-salt solution also promotes the cross splitting of the collagen fibres of the derma; with an increase in the duration of treatment with ultrasound, the bun-

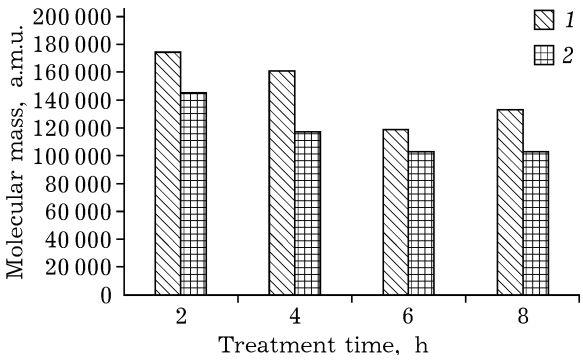

Fig. 3. Dependence of the molecular mass of solutions on the time of preliminary enzyme treatment with protease (*1*) and amylosubtilin (*2*).

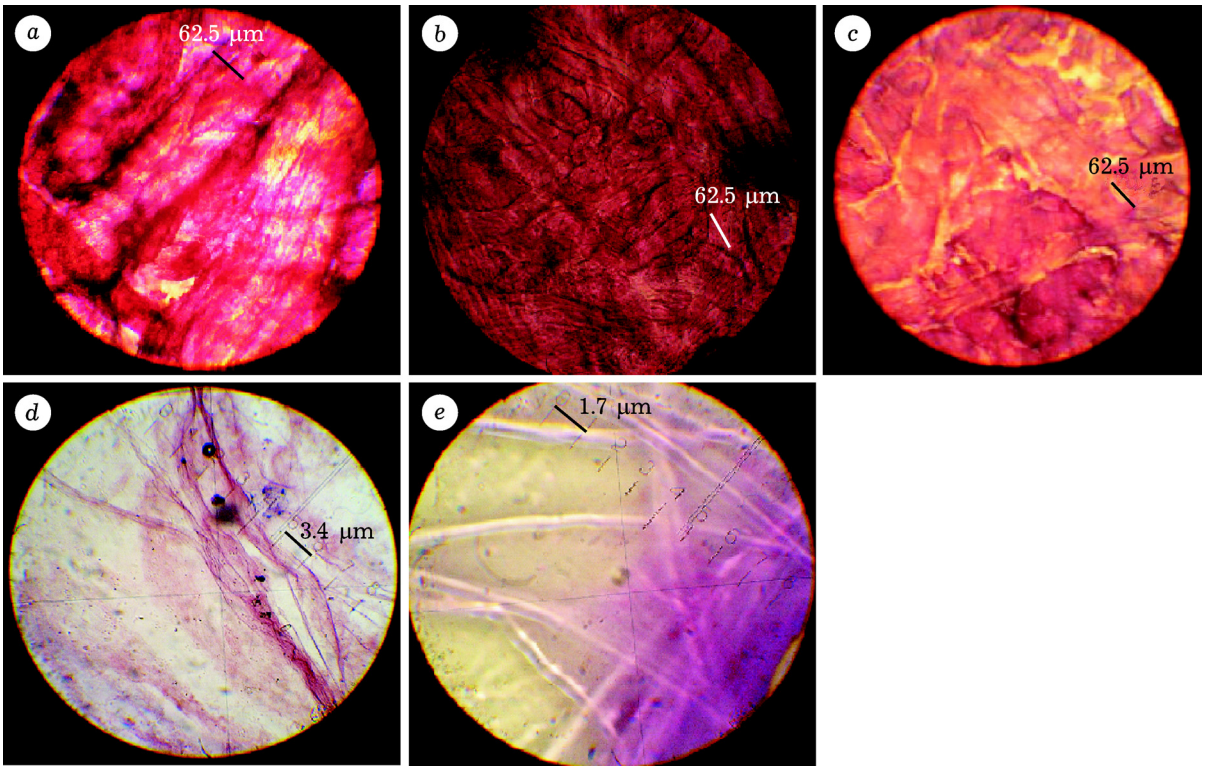

Fig. 4. Micrographs of microsections: *a* – initial offal, magn. 150; *b* – offal after alkaline-salt treatment for 48 h, magn. 150; *c* – the same, in the ultrasonic apparatus for 8 min, magn. 150; *d* – collagen fibre (from the collagen solution obtained by intense mechanical mixing of the offal in acetic acid for 2 h without preliminary treatment), magn. 300; *e* – the same, obtained by treatment in alkaline-salt solution in the ultrasonic apparatus for 10 min and dissolution during mechanical mixing for 25 min, magn. 600.

dles become thinner (see Fig. 4, *c*). One can see that collagen in suspensions has fibrillar structure, namely, infinite fibres (see Fig. 4, *d, e*).

So, the dissolution process may be intensified by means of the complex treatment by dissolving in acetic acid under intense mechanical mixing with preliminary alkaline-salt treatment in the activator or with the treatment with a mixture of proteolytic and amylolytic enzymes. The choice of the version of treatment is determined by the area of further application of the products of collagen dissolution.

The obtained colloid solutions may be used to prepare fibrous materials – collagen films, porous materials, or cylindrical protein shells.

CONCLUSIONS

1. The possibility to dissolve the offal split of the fell of bovine animals was studied. About 50 different versions of the dissolution of untanned collagen-containing were considered: a) in acetic acid with preliminary alkaline-salt treatment; b) with the help of ultrasonic apparatus; c) under mechanical mixing; d) by means of preliminary enzyme treatment.

2. An optimal version of obtaining collagen solution may be considered to be the procedure with the preliminary treatment with the complex of enzyme preparations possessing amylolytic (amylosubtilin G3x) and proteolytic (alkaline protease JW-2) activity, subsequent treatment with pepsin in the acidic medium and final dissolution in acetic acid under mechanical mixing. In this case, the duration of collagen dissolution was 5 days. The content of collagen dissolution products reaches 19.5 g/L, which is much higher than their content in solutions prepared using the conventional procedure.

3. The products of collagen dissolution were studied. The molecular masses of the obtained solutions correspond to the literature data and vary within the range 120 000–360 000 a.m.u.,

which is the evidence of the conservation of fibrillar structure of collagen in the colloid. The optimal version of dissolution (with the use of enzyme treatment) allows obtaining collagen solutions with molecular mass about 300 000 a.m.u., which corresponds to the molecular mass of tropocollagen particle, which proves that the conformation of the obtained PCD has not changes.

REFERENCES

1 S. A. Pavlov, I. S. Shestakova, A. A. Kasyanova, Khimiya i Fizika Vysokomolekulyarnykh Soyedineniy v Proizvodstve Iskusstvennoy Kozhi i Mekha, 2nd Ed., Lyogkaya Industriya, Moscow, 1976.
2 E. Zhukhovskiy, *Kozh.-Obuv. Prom.*, 12 (1984) 13–15.
3 K. Benkovich, U. Gzhegozhevskaya, S. Pilyavskiy, *Ibid.*, 12 (1984) 15.
4 URL: http://www.leathernet.ru/index.php
5 B. Felitsiyanyak, V. Petzhikovskiy, B. Tsislo, *Kozh.-Obuv. Prom.*, 12 (1984) 9.
6 A. A. Fridland, G. M. Nikitin, Dopolnitelnaya Produktsiya iz Otkhodov Kozhevennogo i Mekhovogo Proizvodstva, Lyogkaya Industriya, Moscow, 1965.
7 M. Mladek, Pererabotka Otkhodov Kozhevennoy Promyshlennosti, Lyogkaya Industriya, Moscow, 1976.
8 URL: http://www.securpress.ru
9 I. S. Shestakova, N. V. Chernov, A. A. Golovteeva, *Izv. Vuzov. Tekhnol. Lyogkoy Prom.*, 4 (1966) 84.
10 G. Raykh, Kollagen (Problemy, Metody Issledovaniya, Rezultaty), Lyogkaya Industriya, Moscow, 1969.
11 A. A. Golovteeva, D. A. Kutsidi, L. B. Sankin, Laboratorny Praktikum po Khimii i Tekhnologii Kozhi i Mekha, Lyog. i Pishch. Prom., Moscow, 1982.
12 KB 583.00.000-06 Apparat Ultrazvukovoy. Tekhnicheskoye Opisaniye i Naznacheniye Apparata, Novosibirsk, 1998.
13 Vremennaya Instruktsiya po Obsluzhivaniyu. Laboratornaya Meshalka Tipa ML-4, Novosibirsk, 2000.
14 I. P. Golyamina, Ultrazvuk, Sov. Entsiklopediya, Moscow, 1990.
15 I. S. Shestakova, L. V. Moiseeva, T. F. Mironova, Fermenty v Kozhevennom i Mekhovom Proizvodstve, Leprombytizdat, Moscow, 1990.