INVESTIGATION OF PHYSICOCHEMICAL AND BIOLOGICAL CHANGES IN THE COLLAGENS UNDER THE INFLUENCE OF THE PROKARYOTIC ORGANISMS-ACIDS SYSTEM

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Great attention is being paid nowadays to the ecological safety and rational technologies of leather production. New technologies of waste processing make it possible to get products that could find wide application in different industries. Products of collagen dissolution (PCD) are among them. To get a high quality product with specified properties it is necessary to research the characteristics of the modified collagen and to analyze the influence of proteins present both in leather tissue and combined whey. The objective of the research is to study the collagen-chemical and thermo-dynamic properties of modified collagen (PCD) produced on the basis of different nature organic acids and combined whey. In the paper physical and chemical properties of colloid system PCD-acid were investigated. Adsorption and dampening ability, structural, mechanical and rheological characteristics of modified collagen were also defined. There has been found out that the surface activity of the modified collagen produced on the basis of combined whey is the result of symbiotic influence of proteins present both in derma (collagen) and in the combined whey (casein). Research of the mechanism of collagen - acid interaction on properties of collagen dissolution products could make it possible to better understand collagen structure.

Keywords: modified collagen, prokaryotic organisms-acids system, combined whey

INTRODUCTION

The current stage of the human society development witnesses global use of natural resources. The characteristics feature of any resource cycle is not only the increasing amount of the resource being taken out but also environment contamination. Material resources are applied in production processes where some part of them is processed into final products while the other one forms waste.

Both liquid and solid waste is formed in great amount in leather and fur processing. In the course of processing one ton of raw material (hides and skins) using the traditional technology the amount of final material (leather) is 200 kg., i.e. leather industry produces a great deal of waste. Waste accumulation in great amount influences negatively the environment, its utilization costs much. Great attention is being paid nowadays to the ecological safety and rational technologies of leather production.

New technologies of waste processing make it possible to get products that could find wide application in different industries. Products of collagen dissolution are among them. The objective of the research is to study the collagen-chemical and thermo-dynamic properties of modified collagen (products of collagen dissolution – PCD) produced on the basis of different nature organic acids and combined whey.

EXPERIMENTAL AND RESULTS

The Study of the Influence of Solvents' Nature on the Quality of Produced Modified Collagen

To produce modified collagen (PCD) there have been applied collagen-containing hide waste being contoured and subjected to partial biological decay.

After being selected the specimens were subjected to soaking and sharpened liming made according to the traditional technology of hide treatment for the footwear upper. Modified collagen has been produced by alkali-salt technology.

Hides having been limed were washed in the running water (t = 8°C) for 24h. then cut pieces of the size 2x2 cm were salted for 4h at the temperature 18±2°C, LC = 3, Na_2SO_4 concentration – 40g/dm^3. After that the specimens were subjected to alkali-salt treatment in sodium hydroxide and sodium sulphate. The specimens were again salt-treated in the solution of sodium sulphate for 4h with LC = 5 and the temperature of 18±2°C.

To remove the alkali surplus the neutralization by the solution of ammonia sulphate for 3h with pH4 was used. The rest of alkali was controlled by the lack of pink colour of the cut treated by the indicator of phenolphthalein. Finally hide waste was thoroughly washed in the running water (t = 8±2°C) until there was no sulphate-ions (quality reaction with boron chloride is the lack of amorphous white sediment) in the washing water. Acid treatment of collagen-containing raw material was made in different organic acids with pH2 – 3: lactic acid (pH2.3), aminic acid (pH2.85), acetic acid (pH2.5), combined whey (mixture of curds and cheese whey) with pH3.04 and 269°T.

The time of destruction of acid-liable binds of collagen-containing raw material conducted with LC = 5 and the temperature of 20±2°C was different depending on the acid type and amounted for the system with: lactic acid – 144h or 6 days and nights, aminic acid – 480h or 20 days and nights, acetic acid – 48h or 2 days and nights, combined whey – 384h or 16 days and nights. To produce the monodisperse system of PCD the Braun type 4191 blender was applied for 2 min (v = 10 r/s). To remove the acid surplus from the colloid system-PCD-organic acid the dialysis was performed for 7 days and nights (with lactic acid), 9 days and nights (with aminic acid), 6 days and nights (with acetic acid), 7 days and nights (with combined whey). The temperature of the running water for all the treatment variants was 8±2°C.

Colloid systems produced with different acids looked differently: PCD – lactic acid was non-transparent; viscid-fluid yellowish system with the density of 1000 kg/m^3 and pH 6.44; PCD-aminic acid was non-transparent, viscid, grey-yellowish system with the density of 997 kg/m^3 and pH 6.90; PCD-acetic acid was grayish, non-transparent, viscid, non-homogenous system with the density of 1055 kg/m^3 and pH 7.5, and PCD-combined whey was non-transparent, viscid, beige-colour system with the density of 1013 kg/m^3 and pH 6.31.

The content of the amino nitrogen, dry sediment and protein concentration were defined in the PCD. Physical and chemical properties of PCDs produced with the help of organic acids are given in Table 1.

Digital ionometer U-135 was applied to measure pH, the density was measured by aerometer.

Table 1. Physical and Chemical properties of colloid system PCD-acid

Acid used for dissolving	pH	Density kg/m^3	Dry remnant, %	Amino Nitrogen, g/dm^3	Protein concentration, g/dm^3
Aminic	6,90	997	7,96	0,126	2,37
Lactic	6,44	995	22,20	0,196	2,74
Acetic	7,50	1055	4,70	0,390	4,78
Combined whey	6,31	1013	28,40	0,462	5,48

Minimum content of amino nitrogen, protein and dry remnant is characteristic for the system PCD-aminic acid, while the maximum one – for the system PCD-combined whey (Table 1). Such high content of protein in the colloid system PCD-combined whey could be explained probably by the presence of proteins from not only hide tissues but also from casein containing secondary waste.

The produced modified collagen for all the treatment variants contained only ammonia cation.

A very important characteristic of PCD is the molecular mass according to which it is possible to define the survival of the three-spiral collagen structure and identify it. Molecular mass was calculated on the equation of Mark-Khuvink, where $K=1{,}34\ 10^{-4}$, $\alpha=0{,}71$ at the temperature 22-25°C.

Molecular mass of PCD depended greatly upon the type of acid treatment. PCD on the basis of cultured milk compositions coefficient (CMC) had smaller molecular mass than PCD on the basis of pure acids. It might be connected with the following: applying CMC as an acid agent the disordering of collagen fibres is caused not only by the acidic but also ferment dissolution.

The greatest molecular mass of PCD was registered after being treated by SMC 4 ($72.4 – 74.6 \times 10^3$ c.u.). increasing the concentration of PCD the molecular mass for all the acidic treatments increased on 2000 conventional units.

The Study of Thermodynamic Properties of Modified Collagen

The most important characteristic of the inter-phase surface of the system bio-polymer-solvent is surface tension that characterizes the surplus of free surface energy per 1m^2 of the inter-phase surface as its presence is characteristic for disperse systems. Adhesion refers to surface phenomena (adsorption and dampening) and means molecular interaction of contacting surfaces of condensed phases of different nature. Adhesion provides certain connecting strength between two surfaces thanks to the molecular forces of different nature. Adhesion work characterizes the strength of adhesion connection. It corresponds to the possible connection break per the unit of area.

To study the thermo-dynamic properties there have been used modified collagen solutions (PCD), produced by dissolving collagen-containing raw material in lactic, aminic and acetic acids and combined whey.

Surface tension was defined by stalagmometric method. Solutions with different PCD concentration were applied to define surface tension and dampening angle. Protein concentration was defined by Yarosh method.

Analysing the influence of modified collagen concentration on the degree of surface tension it can be pointed out that the increase of PCD concentration in the system results

in the decrease of the degree of surface tension for all the studied variants. The maximum decrease of surface tension degree is for the colloid system PCD-combined whey. It could be connected with the symbiotic influence of proteins present both in derma (collagen) and combined whey (casein).

Adsorption process takes place due to the decrease of surface tension. Adsorption isotherm was built according to the surface tension isotherm using Gibb's equation. To calculate Gibb's quantity the tangent method that made it possible to define the surface activity was applied (Figure 1).

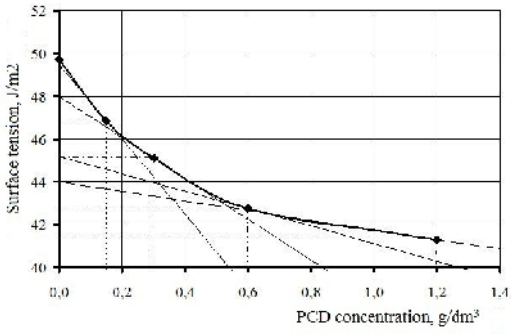

Figure 1. Isotherm of surface tension for colloid system PCD-combined whey

On the basis of experimental data has been found out that Gibb's quantity increased with the increase of PCD concentration in the system biopolymer-organic acid, while in the combined whey it did not change. That is why we believe the application of combined whey could provide greater disordering of collagen macromolecules.

Thus, according to the results achieved in the research it can be stated that adsorption ability of the organic acids under study increases in the line aminic acid – acetic acid – combined whey – lactic acid.

Study of PCD Influence upon Dampening Ability of Organic Acids (on the Example of Wax Film)

To study the dampening ability of the system PCD-acid there have been measured dampening angles θ. Ten parallel measurements have been made for every concentration under study. The experimental data show that with the increase of PCD concentration in the organic acid solution the edge angle decreases which demonstrates the dampening ability. The maximum dampening effect is characteristic for the system PCD-lactic acid, and the least one – for the PCD – combined whey. This might be connected with the presence of basic hydroxide group in the molecule of lactic acid that contributed to the development of inter-phase interaction. Increase in the dampening ability of the colloid system PCD – aminic acid can be explained by the presence of aldehyde group in the molecule of the given acid.

Study of Adsorption and Dampening Ability, Structural, Mechanical and Rheological Characteristics of Modified Collagen

It has been defined that the increase of modified collagen concentration in the colloid system PCD – organic acid leads to the decrease of the quantity of surface tension for all the studied variants. The maximum decrease of surface tension was characteristic for the colloid system PCD – combined whey, which might be connected with the symbiotic influence of proteins present both in leather tissue and combined whey.

That is why there was applied the combined whey being a by-product consisting of lactic and curds whey as a reagent for the acidic hydrolysis of the collagen-containing raw material in further research. To study the influence of a specific type of modified collagen on some thermodynamic properties of the colloid system PCD – combined whey there has been made a number of solutions with the decreasing concentration of PCD, g/dm^3: 3.0; 2.0; 1.0; 0.5; 0.25; 0.17, and 0.05.

To estimate surface activity and dampening ability experimental data for the change in the quantity of surface tension and dampening angle has been achieved. A drop of the liquid under research was placed on the covered with wax glass and the dampening angle was measured. Experimentally there has been proved that lactic acid concentration in combined whey does not greatly influence the thermo-dynamic characteristics of the modified collagen produced.

Unique dependence is characteristics for all the variants of substances having surface properties, and namely, with the influence of PCD concentration in the colloid system PCD – combined whey the quantity of surface tension decreases for all the systems being studied. The maximum decrease of the surface tension quantity has been achieved for the colloid system modified collagen – combined whey with the lactic acid content of 20 g/dm^3 and made up 59,14 j/m^2. It might be connected with the fact that the collagen-containing product, was characterized by a great number of highly-molecular segments that were formed as a result of preserving in its structure peptide binds.

Structural and mechanical properties of modified collagen with respect to viscidity have been studied to confirm the results achieved. Viscidity was defined on the viscometer VPZh-2 (SS 10028-67) with the diameter of capillary d = 0.99 mm. Solutions density was measured by aerometer.

On the basis of the data it can be stated that putting modified collagen into the colloid system PCD – combined whey results in the viscidity decrease for the variant where the acidic hydrolysis was made applying combined whey with 20 g/dm^3 lactic acid.

CONCLUSIONS

On the basis of the results achieved the following conclusions could be made:

1) maximum decrease of surface tension and correspondingly, high dampening ability is provided by modified collagen produced while applying in acidic hydrolysis the combined whey with the lactic acid concentration of 30 g/dm^3;

2) there have been studied adsorption and dampening abilities, structural and mechanical, rheological characteristics of modified collagen;

3) there has been found out that the surface activity of the modified collagen produced on the basis of combined whey is the result of symbiotic influence of proteins present both in derma (collagen) and in the combined whey (casein).

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