PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

ESTUDO DE ESPECTROSCÓPIA-IV DA IMOBILIZAÇÃO DE COMPOSTOS DE SELÊNIO EM COLÁGENO BIOMODIFICADO

IR-SPECTROSCOPIC STUDY OF IMMOBILIZATION OF SELENIUM COMPOUNDS ON BIOMODIFIED COLLAGEN

ИК–СПЕКТРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ ИММОБИЛИЗАЦИИ СОЕДИНЕНИЙ СЕЛЕНА НА БИОМОДИФИЦИРОВАНОМ КОЛЛАГЕНЕ

GLOTOVA, Irina Anatolievna[1]; GALOCHKINA, Nadezhda Alekseevna[2*]; SELEMENEV, Vladimir Fedorovich[4] ; PEREGONCHAYA, Olga Vladimirovna[3] ; SOKOLOVA , Svetlana Anatol'evna[3].

[1] Voronezh State Agrarian University named after Emperor Peter the Great, The Faculty of Technology and Merchandizing, Department of Merchandizing Technique and Commodity Expertise.

[2] Voronezh State Agrarian University named after Emperor Peter the Great, The Faculty of Technology and Merchandizing, Department of technology of storage and processing of agricultural products.

[3] Voronezh State Agrarian University named after Emperor Peter the Great, The Faculty of Technology and Merchandizing, Department of chemistry.

[4] Voronezh State University, The Faculty of Technology and Merchandizing, Department of Analytic Chemistry, Chemical faculty

** Autor correspondente*
e-mail: galochkina.na@mail.ru

Received 12 June 2019; received in revised form 30 September 2019; accepted 19 October 2019

RESUMO

O objetivo do trabalho foi pesquisar as características espectrais de substâncias de colágeno produzidas por bioengenharia, utilizando preparações complexas de proteases colagenolíticas obtidas antes e após a imobilização de compostos de selênio em meios ácidos e alcalinos, e comparar o grau de interação das preparações de selênio com a matriz de colágeno após sua imobilização, e sua influência na conformação de moléculas de proteína. Os resultados da imobilização de preparações de selênio em colágeno biomodificado pelo método de espectroscopia no infravermelho foram apresentados, analisados e discutidos. O colágeno biomodificado foi obtido a partir de resíduos de corte de carne (veias e tendões) por hidrólise seqüencial de peróxido alcalino e enzimático com a preparação de colagenase alimentar. As seguintes fontes de selênio foram usadas como compostos com propriedades bioprotetoras para posterior imobilização em proteínas de colágeno biomodificadas: 4,4-di [3 (5-metildiprazolil)] selenida (DMDPS) com o conteúdo de 0,657g de DMDMS em 100 cm^3 e selenito de sódio . Os espectrogramas foram feitos para produtos de biomodificação de colágeno antes da sorção de compostos de selênio (à taxa de 1,2 g^{-6} de selênio em 1 g de colágeno) em ambientes ácido (pH = 5) e alcalino (pH = 10) em uma pesquisa de influência de compostos de selênio em espectros IR de produtos de biomodificação de colágeno. Foi estabelecido que a imobilização ocorreu por reação uma química de preparações de selênio com grupos funcionais das cadeias laterais de moléculas de proteína, e seu grau varia na faixa de Na_2SeO_3 (pH = 5) > 4,4-di [3 (5-metildiprazolil)] selenida (DMDPS) > Na_2SeO_3 (pH = 10). Demonstrou-se que, sob a interação de produtos de selênio com colágeno, não há alteração nas conformações de suas moléculas.

Palavras-chave: *Selênio, colágeno biomodificado, selenito de sódio, 4,4-di [3 (5-metildiprazolil)] selenida (DMDPS), imobilização*.

ABSTRACT

The aim of the work was to research the spectral characteristics of collagen substances bioengineered by using complex collagenolytic proteases preparation obtained before and after the immobilization of selenium compounds in acidic and alkaline media and to comparate the degree of interaction of selenium preparations

Downloaded from www.periodico.tchequimica.com

DOI: 10.52571/PTQ.v16.n33.2019.174_Periodico33_pgs_159_168.pdf

with collagen matrix upon immobilization and its influence on the conformation of protein molecules. The results of the immobilization of selenium preparations on biomodified collagen by the IR spectroscopy method are presented, analyzed, and discussed. Biomodified collagen was obtained from beef trimming waste (veins and tendons) by sequential peroxide-alkaline and enzymatic hydrolysis with the food collagenase preparation. The following sources of selenium were used as compounds with bioprotective properties for subsequent immobilization on biomodified collagen proteins: 4,4-di[3(5-methyldiprazolil)]selenide (DMDPS) with the content of 0,657g DMDMS in 100 cm^3 and sodium selenite. Spectrograms are carried out for products of biomodification of collagen before sorption of compounds of selenium, (at the rate of 1.2 g^{-6} of selenium on 1 g of collagen) in acid (pH =5) and alkaline (pH =10) environments at a research of influence of compounds of selenium on IR spectrums of products of biomodification of collagen. It was established, that the immobilization takes place by a chemical reaction of selenium preparations with functional groups of the side chains of protein molecules, and its degree varies in the range Na_2SeO_3 (pH=5) > 4,4-di[3(5-methyldiprazolil)]selenide (DMDPS) > Na_2SeO_3 (pH=10). It is shown that under the interaction of selenium products with collagen, there is no change in the conformations of its molecules occurred.

Keywords: *Selenium, biomodified collagen, sodium selenite, 4,4-di[3(5-methyldiprazolil)]selenide (DMDPS), immobilization.*

АННОТАЦИЯ

Цель работы – исследовать спектральные характеристики коллагеновых субстанций, биомодифицированных с применением комплексного препарата коллагенолитической протеиназы, полученные до и после иммобилизации соединений селена в кислой и щелочной средах, на основе чего дать сравнительную оценку степени взаимодействия селеновых препаратов с коллагеновой матрицей при иммобилизации и их влияния на конформацию белковых молекул. Представлены, проанализированы и обсуждены результаты иммобилизации препаратов селена на биомодифицированном коллагене методом ИК-спектроскопии. Биомодифицированный коллаген был получен из отходов жиловки говядины (жилки и сухожилия) путем последовательного перекисно-щелочного и ферментативного гидролиза с использованием ферментного препарата “Коллагеназа пищевая”. В качестве источника селена были использованы искусственно синтезированная органическая форма селена для последующей иммобилизации на биомодифицированных коллагеновых белках: 4,4-ди [3 (5-метилдипиразолил)]селенид (ДМДПС) с содержанием 0,657 г ДМДПС в 100 $см^3$ и селенит натрия , Спектрограммы проведены для продуктов биомодификации коллагена после сорбцией соединений селена (из расчета 1,2 мкг селена на 1 г коллагена) в кислой (pH = 5) и щелочной (pH = 10) средах при исследовании влияния соединений селена на ИК-спектры продуктов биомодификации коллагена. Установлено, что иммобилизация проходит путём химического взаимодействия препаратов с функциональными группами боковых цепей молекул белка, а его степень изменяется в ряду Na_2SeO_3 (pH=5) > ДМДПС > Na_2SeO_3 (pH=10). Показано, что при взаимодействии селеновых препаратов с коллагеном не происходит изменения конформаций его молекул.

Keywords: *селен, биомодифицированный коллаген, селенит натрия, 4,4-ди[3(5-метилдипиразолил)]селенид (ДМДПС), иммобилизаци*

1. INTRODUCTION

The development and application of sorption biomaterials as carriers of biologically active substances is a promising direction in biotechnology (Dyankova and Solak, 2014; Benavides *et al.*, 2012; Holyavka *et al.*, 2014; Kovaleva, *et al.* 2011; Olshannikova *et al.*, 2018; Holyavka *et al.*, 2017). Proteins have Wide possibilities of using as polysorbates, in connection with the presence of a large number of potential binding, which is located in the side radicals of amino acids (Kuznetsova and Glushko, 2007; Chi H Lee, 2001; Yamada *et al.*, 2014; Dharmendra, 2013).

In some works, the collagen is used as a carrier of biologically active substances. The authors of these works apply enzymology engineering methods using general proteolytic activity enzyme preparations (Protosubtilin, Savinase, Neutrase 1.5 MG) and collagenolytic activity enzyme preparation (Collagenase from hepatopancreas of the Kamchatka crab) to improve the sorption capacity of collagen (Kovaleva *et al.*, 2011; Kovaleva *et al.*, 2011; Galochkina *et al.*, Glotova *et al.*, 2015)

Selenium is one of the essential microelements. It is involved in the immune, antioxidant and detoxification systems of the body. The protective property of selenium from ionizing radiation, toxic effects of nitrates and

Downloaded from www.periodico.tchequimica.com

nitrites and heavy metals is well known. Selenium has a positive effect on the quality of life of organisms, including increases resistance to stress, reduces the rate of development of various age-related diseases.The development of more improving technologies for food fortification with essential micronutrients, including selenium, is a promising method of correcting diet (Glotova, *et al.*, 2014; Galochkina *et al.*, 2012).

The aim of the work is to research the spectral characteristics of collagen substances bioengineered by using complex collagenolytic proteases preparation obtained before and after the immobilization of selenium compounds in acidic and alkaline media. On the base of these data, we can give a comparative evaluation of the degree of interaction of selenium preparations with collagen matrix upon immobilization and its influence on the conformation of protein molecules.

2. MATERIAL AND METHODS

To obtain a functional collagen substance were used: veins and tendons, which were allocated in the trimming of cattle in the sausage department of the meat processing plant (JSC "Donskoy", Voronezh, PE "Four penguins", Voronezh) in cutting of beef according to GOST 779; the enzyme preparation "Food Collagenase" (Technical Conditions 2639-001-4554109-98, manufacturer CJSC "Bioprogress", Shchelkovo, Moscow region).

The hepatopancreas of the Kamchatka crab is the source of the enzyme preparation "Food Collagenase". It is the organ, which combines the functions of the liver and pancreas in the crab's digestive tract. The hepatopancreas is a complex of collagenolytic proteases, the molecular weight of which is in the range 23-36 κDa, this complex is adapted to the biodegradation of native collagen according to the physiological characteristics of crab nutrition (Glotova et al., 2014).

Taking into account the need to minimize the number and duration of technological operations, the consumption of chemical reagents and biocatalysts to obtain collagen hydrolyzate with a large number of ionogenic groups for immobilizing selenium in DMDPA, it is advisable to carry out operations for the isolation, purification and biomodification of collagen from the veins and tendons of cattle in accordance with supports sequence presented in Figure 1.

The raw material - vein, tendon, fascia, were allocated at the stage of trimming, after that they were washed with running water and were placed in a peroxide-alkaline solution. This solution contained sodium hydroxide with a concentration of 10 % and hydrogen peroxide – 3 %. The processing for 6-10 h in hydromodule 1:2-2,5 provides a uniform interaction of the peroxide-alkaline solution with the feedstock. After that, the liquid fraction was separated by decantation or centrifugation, and the solid residue of collagen was washed with water and neutralized to pH 8,0-8,5. This interval is in the border region of pH, favorable for the collagenolytic activity's manifestation of the preparation "Food Collagenase". Collagen hydrolyzate was obtained by exposure for 2.5-3.0 hours at 36-38°C with the preparation of "Food Collagenase" in the amount of 0.02 % by the weight of collagen (Glotova and Galochkina, Patent, 2015; Glotova *et al.*, 2015; Majorov A. F. *et al.*,1992) .

The following sources of selenium were used as compounds with bioprotective properties for subsequent immobilization on biomodified collagen proteins: 4,4-di[3(5-methyldiprazolil)]selenide (DMDPS) Technical Conditions 9291-007-59582032 with the content of 0,657g DMDMS in 100 cm^3 (artificially synthesized organic form of selenium, the manufacturer – Limited Liability Company "Safron", Moscow, sanitary-epidemiological conclusion №77.99.13.003.T.000518.03.06). The aggregate state is white powder, in 2002, allowed as a dietary supplement. According to state scientific institution All-Russian veterinary research institute of pathology, pharmacology and therapy of the Russian Academy of agricultural sciences DMDPS currently is the most low-toxic compound of selenium with low cumulative (Glotova et al., 2013); sodium selenite (FSP 42-0250-1024-01) the manufacturer is the firm "MCD chemicals" ("MCD", Moscow);

We studied the impact of selenium compounds on the IR spectra of the products of biomodification collagen. We conducted a survey of these products' spectrograms before and after sorption of selenium compounds, (at the rate of 1.2 mcg of selenium per 1 g of collagen (Glotova and Galochkina, 2015) in the acidic (pH = 5) and alkaline (pH = 10) environments.

The samples of the researched substances with collagen immobilized selenium products were pre-dried at a temperature of 36 °C for 24 hours to obtain IR spectra. After that the

Downloaded from www.periodico.tchequimica.com

samples were carefully ground in an agate mortar to obtain a homogeneous fine powder, and then tablets were made with pre-dried and ground powder of optically pure single-crystal KBr in the ratio of 0.1 mg of sample in 100 mg potassium bromide (Ramasamy Sripriya *et al.*, 2015; Gudkov *et al.*, 2018).

IR spectra of the collagen substances were obtained on a spectrometer with Fourier transform (with ATR) "Vertex-70" (Germany), for subsequent processing, the program GRAMS 4/32 was used.

3. RESULTS AND DISCUSSION

IR spectra of collagen before and after immobilization of DMDPS are presented in fig. 2. The spectra contain two distinct spectral ranges with the wavenumber of 900-1800 cm^{-1} (long-wave) and 2800-3700 cm^{-1} (short-wave). The first region characterizes the fluctuations of collagen molecules' fragments and bonds between the atoms in these molecules. The second area characterizes the valence vibrations of C-H-bonds, OH-bonds in the hydration shells of functional groups, specifies the presence of free water with the normal network of hydrogen bonds and additionally includes the wide absorption spectral band of the vibrations in N-H bonds.

These maximums of the original sample spectra of collagen (fig. 2a, curve 1) adequately explain the structure and functional composition of the protein (Ramasamy Sripriya *et al.*, 2015; Shkutina *et al.*, 2004). Spectral bands 2923 and 2852 cm^{-1} characterize valence vibrations of C-H bonds in the main chain and side radicals of protein molecules stretching. The peak 1453cm^{-1} characterizes their deformational vibrations. The frequencies 1549 and 1636 cm^{-1} correlate to vibrations of the peptide bonds (respectively, the "amide II and amide I»). The maximum 1746 cm-1 correlate to valence vibrations C=O bonds. The salt form of carboxyl groups can be identified by the presence of 1549 and 1333 cm^{-1} absorption bands, which characterize asymmetric and symmetric vibrations of the carboxylate anions of aspartic and glutamic acids. Frequency 1638 cm^{-1} simultaneously corresponds to the salt form of carboxyl groups valence vibrations and the deformation vibrations of amino groups, which are included in the amino acid diaminocarbenes residues. The 3080 cm^{-1} band indicates vibrations of C-H in the aromatic nuclei of phenylalanine and tyrosine residues.

The number of peaks (1243, 1133, 1080, 1021 cm^{-1}) is evident on the left part of the long-wave region of the spectrum. They are due to oscillations of the carbon skeleton of protein, deformed OH, valence CO, and other vibrations. The dependence of the absorption value on the wavenumber for collagen adsorbed selenium preparation (Figure 2A, curve 2) is not qualitatively different from the curve for the original sample. Some differences are in the deviation of the maxima by a few cm^{-1}, which corresponds to the accuracy of the IR spectroscopy method. The form of the spectra remains practically unchanged by varying the pH. Spectrograms were almost identical for the samples with different pH values. Presumably, the interaction of collagen and selenium preparation DMDPS is absent. This can not be confirmed unequivocally since the intensity of the absorption bands on spectrograms is different. To obtain comparable data, we applied the method of processing spectra using the baseline method (Ewing and Kazarian, 2017) . As standard, the maxima (1453 cm^{-1} in the long-wavelength region and 2923 cm^{-1} in the short-wavelength) were chosen. The most appropriate data baseline method is given if the bands that are close in frequency to the analyzed bands are taken as standard. In Fig. 3 shows the relative heights of the maxima in different regions of the spectra.

Immobilization of sodium selenite on collagen in an acidic medium causes a sharp decrease in the intensity of the 1743 cm^{-1} band of C = O vibrations in undissociated carboxyl groups. Immobilization of sodium selenite on collagen also leads to a significant increase in the absorption intensity of asymmetric (1551 cm^{-1}) and less pronounced increase in symmetrical (1397 cm^{-1}) vibrations of carboxylate ions (Fig. 2b, curves 1, 3). This is due to the course of reactions between the functional groups of collagen and sodium selenite (figure 4). In a weakly acidic medium (pH = 5) the process proceeds:

The hydroselenite of sodium formed as a result of the reaction (Figure 4) reacts with the amino groups of the protein to form a mixed sodium-ammonium salt:

$$R\text{-}NH_2 + NaHSeO_3 \rightarrow R\text{-}NH_3 + SeO_3\text{-}Na^+$$

As a result, selenium is fixed on the protein matrix. A certain contribution to the maximum value of 1551 cm^{-1} is made by the vibrations of the $–NH_3^+$ groups (Cherkasov and Pasechnik, 1991).

In an alkaline medium (pH = 10), reaction (fig. 4) can not proceed, since the carboxyl groups of the protein are completely

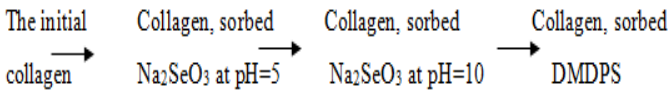
Downloaded from www.periodico.tchequimica.com

deprotonated, and strong immobilization of selenite on the protein is difficult. Apparently, the selenium ion weakly interacts with the collagen matrix. This fact is indicated by a slight increase in the relative heights of the peaks (h / h_{st}) in comparison with the original sample under these conditions (Figures 3b No. 3 and No. 1, maxima of 1635 and 1397 cm^{-1}).

The immobilization of DMDS on collagen (Fig. 3b, No. 4), as well as the immobilization of sodium selenite, causes a sharp decrease in the content of free carboxyl groups (1746 cm^{-1}) with the simultaneous increase in carboxylate ions (1638, 1549 cm^{-1}). This indicates a possible chemical interaction of the selenium preparation with the protein. The process follows the scheme (figure 5):

In this case, a carboxylate ion of the protein and charged quaternary ammonium are formed. The presence of the quaternary ammonium ion is confirmed by the presence of maxima of 3308 cm^{-1} (corresponding to the hydrated water of the amino group) and 1638 cm^{-1}. The relative height of the peaks h / h_{st} in Fig. 3b, which corresponds to asymmetric and symmetric vibrations of carboxylate ions, in the case of DMDS is less than in the sample that adsorbed sodium selenite at pH = 5 but greater than the one sorbed at pH = 10. This is due to the fact that reaction (3) is less intense than in reaction (1) because of the spatial difficulties in the sorption of a large-size DMDPS molecule compared to sodium selenite.

It is necessary to take into account the following results that we obtained. First, the calculation of h / h_{st} at a maximum of 3308 cm^{-1} relatives to the standard band of 2983 cm^{-1} showed that these values in the series:



change as 0.25-2.36-0.62-0.76. This fact points to the highest water content in collagen samples with immobilized sodium selenite at pH = 5. The reason for this is an increase in the water content in the hydrated shells of carboxylate ions and of quaternary nitrogen of collagen.

Secondly, the almost complete coincidence of the values of h/h_{st} in the region of «fingerprints» for all the samples under study makes it possible to assume that the immobilization of selenium preparations has an insignificant effect on the conformation of collagen molecules.

4. CONCLUSIONS

The immobilization of selenium preparations on the collagen occurs by their chemical interaction with the carboxyl and amino groups of the protein molecules side chains, with the formation of oppositely charged ions.
The degree of interaction between selenium preparations with collagen varies among: Na_2SeO_3 (pH=5) > DMDPS > Na_2SeO_3 (pH=10).
The immobilization of selenium preparations on the collagen causes an increase in the content of the water of hydration and does not affect the conformation of protein molecules.

5. ACKNOWLEDGMENT

The studies were carried out using the equipment of Common Use Center at Voronezh State University.

6. REFERENCES

1. DYANKOVA S., SOLAK A., Biopolymer matrix systems for incorporation of biologically active substances. Agricultural science and technology; v. 6, p.104-110, 2014. (in Russian)
2. BENAVIDES S., VILLALOBOS-CARVAJAL R., REYES J.E. Physical mechanical and antibacterial properties of alginatefilm: Effect of the cross linking degree and oregano essential oil concentration. Journal of Food Engineering; v.110(2), p.232-239, 2012.
3. HOLYAVKA M.G., KOVALEVA T.A., KARPOV S.I., SEREDIN P.V., ARTYUKHOV V.G Investigation of mechanisms of interaction between inulinase from kluyveromyces marxianus and the matrices of ion exchange resins and fiber. Biophysics; v.59(2), p. 223-229, 2014. (in Russian)
4. KOVALEVA T.A., BELENOVA A.S., SHATALOV G.V. Immobilization of lipase on poly(N-Vinyl pyrrolidone). Bulletin of Experimental Biology and Medicine; v.151(4) , p. 415-417, 2011. (in Russian)
5. OLSHANNIKOVA S.S., KOROLEVA V.A., KHOLYAVKA M.G., PANKOVA S.N., BELENOVA A.S., KONDRATEV M.S., SAMCHENKO A.A., KABANOV A.V., ARTYUKHOV V. G. The adsorption immobilization of bromelain on chitosan matrices. Journal of Biotechnology; v. 280(5) , p. 68, 2018. (in Russian)
6. HOLYAVKA M. G., ARTYUKHOV V. G.,

Downloaded from www.periodico.tchequimica.com

KONDRATYEV M. S., SAMCHENKO A. A., KABANOV A. V., KOMAROV V. M., TERENTYEV V. V. The molecular mechanism of adsorption immobilization of inulinase on polymer matrices. Biophysics; v.62(1) , p. 5-11, 2017. (in Russian)
7. KUZNETSOVA L. S., GLUSHKO A. A., Technological research noninvasive of wound coverings with the juice of plantain and analysis of the adsorption of biologically active substances juice on collagen. Modern problems of science and education; v.6, URL: https://science-education.ru/en/article/view?id=11775 , 2013. (in Russian)
8. CHI H LEE, ANUJ SINGLA, YUGYUNG LEE Biomedical applications of collagen. International Journal of Pharmaceutics; v.221, p. 1–22, 2001.
9. YAMADA S., YAMAMOTO K., IKEDA T., Yanagiguchi K., Hayashi Y. Potency of Fish Collagen as a Scaffold for Regenerative Medicine. BioMed Research International; URL: http://dx.doi.org/10.1155/2014/302932 , 2014
10. DHARMENDRA KUMAR A review on collagen based drug delivery systems. International Journal of Pharmacy Teaching & Practices; v. 4(4), p. 811-820, 2013.
11. KOVALEVA T. A., MAKAROVA T. A., SLIVKIN A. I., KOROTKOVA E. V. Adsorbcionnaya immobilizaciya , iz Aspergillus awamori na kollagene. Voprosy biologicheskoj, medicinskoj i farmacevticheskoj himii; v.11, p.19-22, 2011. (in Russian)
12. KOVALEVA T. A., MAKAROVA E. L., KOROTKOVA E.V. Sorbciya glyukoamilazy iz Aspergillus awamori na kollagene. Vestnik Voronezhskogo gosudarstvennogo universiteta. Seriya: Himiya. Biologiya. Farmaciya; v.1, p. 32-36, 2011. (in Russian)
13. GALOCHKINA N. A., LITOVKIN A. N., GLOTOVA I. A., SVESHNIKOVA I. A. Issledovanie molekulyarno-massovogo raspredeleniya belkovyh frakcij pri modifikacii pishchevyh biopolimernyh system. Sovremennye naukoemkie tekhnologii; v.5(1), p.187-187, 2014. (in Russian)
14. GLOTOVA I. A., GALOCHKINA N. A., SHAKHOV S. V., MATEEV E. Z., KADIRBAEV M. K. Biocatalytic Properties and Substrate Specificity of Proteinase Preparations From Different Sources. Research Journal of Pharmaceutical, Biological and Chemical Sciences; v.6(1), p.1640-1645; URL: http://www.rjpbcs.com/2015_6.1.html, 2015. (in Russian)
15. GLOTOVA I. A., GALOCHKINA N. A. Effect of selenium sources on biochemical processes during swelling and germination of wheat grain. Himija rastitel'nogo syr'ja; 4 DOI: 10.14258/jcprm.2017041849, 2017. (in Russian)
16. GALOCHKINA N. A., GLOTOVA I. A., PARSHIN P. A., PRYANISHNIKOV V. V. Improving the technology of enrichment of food of animal origin. Myasnaya industriya; v.10, p.35-38, 2012. (in Russian)
17. GLOTOVA I. A., GALOCHKINA N. A. Justification of the conditions for obtaining functional biomodified collagen substances. Bulletin of biotechnology and physico-industrial biology. Yu.A. Ovchinnikov; v.10(1), p.10-19, 2014. (in Russian)
18. Patent 2542123 Rossijskaya Federaciya, MPK51 A23L 8/0.3 Sposob polucheniya polifunkcional'noj dobavki dlya obogashcheniya selenom pishchevyh produktov / Glotova I.A., Galochkina N.A.; Voronezh State Agrarian University named after Emperor Peter the Great. - № 2013134911/13; zayavl. 24.07.13; opubl. 20.02.2015, Byul. № 5. (in Russian)
19. GLOTOVA, I. A., GALOCHKINA N. A., KURCHAEVA E. E. Selenium-deficient population conditions and ways of their nutritional correction. Food Industry; v. 12, p.74-77, 2013. (in Russian)
20. RAMASAMY SRIPRIYA, RAMADHAR KUMAR A Novel Enzymatic Method for Preparation and Characterization of Collagen Film from Swim Bladder of Fish Rohu. Food and Nutrition Sciences; v. 6, p.1468-1478, 2015. (in Russian)
21. GUDKOV M., GLOTOVA I., SHAKHOV S., PUGACHEVA I., GALOCHKINA N., BOKADAROV S., LOBACHEVA N Investigation of polymer interaction with nanoscale modifier and development of its identification algorithm. Actual Issues of Mechanical Engineering; p.187-192, 2018. (in Russian)
22. SHKUTINA I.V. STOYANOVA O.F.,

Downloaded from www.periodico.tchequimica.com

SELEMENEV V.F. IK spektroskopiya dlya issledovaniya kompleksa inulaza-nositel'.Vestnik VGU. Seriya: Himiya. Biologiya. Formaciya; v.1: p.110 – 113, 2004. (in Russian)
23. EWING A. V., KAZARIAN S. G. Infrared spectroscopy and spectroscopic imaging in forensic. Analyst; v.142(2), p.257-272, 2017.
24. CHERKASOV A. N. PASECHNIK V. A. Membrany i sorbenty v biotekhnologii. Himiya. – 239 p. 1991. (in Russian)
25. Patent 2112036 Rossijskaya Federaciya, MPK C12N 9/64 Method for producing collagenase/ Sposob poluchenija kollagenazy / MAJOROV A.F., ALBULOV A.I., SAMUJLENKO A. Ja.; 5067815/13, zayavl.09.10.1992 opubl. 27.05.1998 (in Russian)

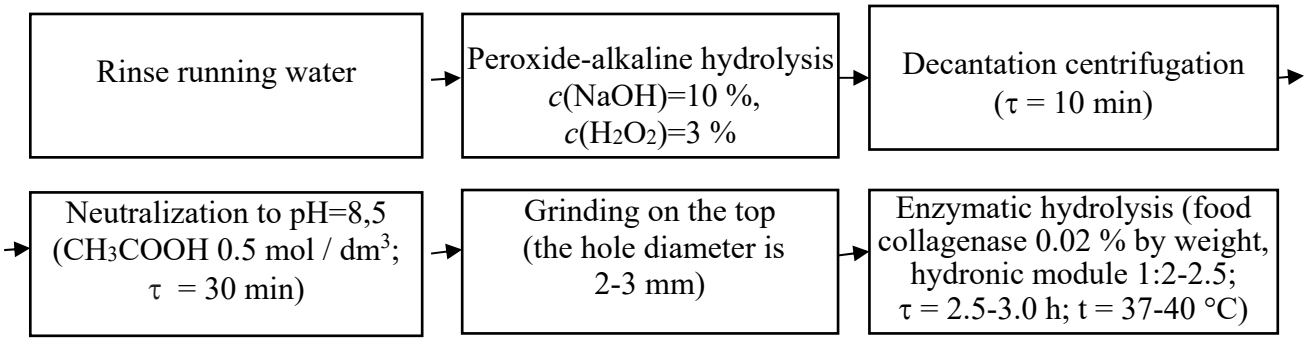


Figure 1 *Sequence of operations in the preparation of collagen biomodination product for the subsequent modification of DMDPS*

Downloaded from www.periodico.tchequimica.com

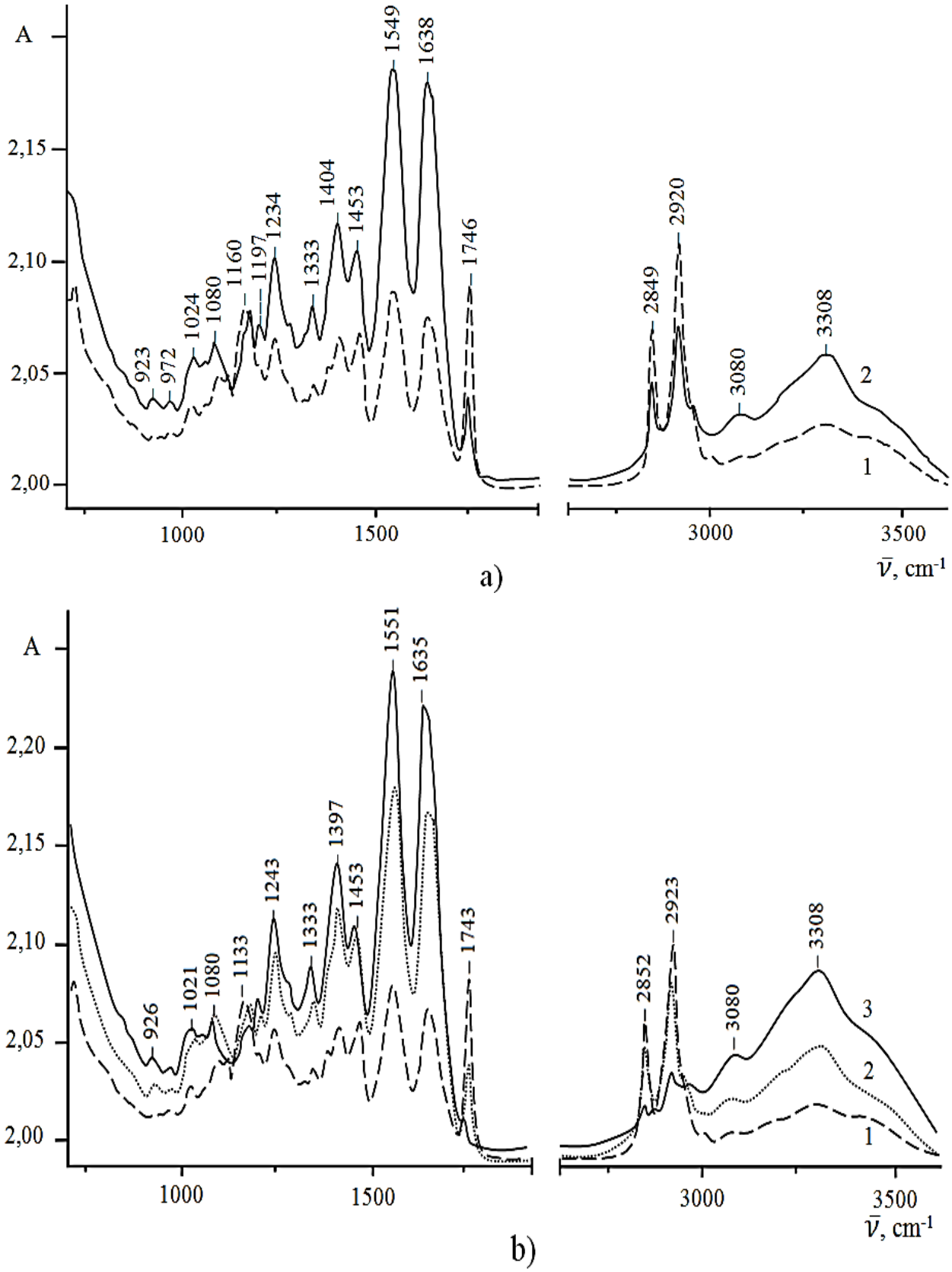


Figure 2 *IR spectra of collagen substances before and after adsorption of DMDPS (a): 1 – initial sample, 2 – sample after adsorption of DMDPS; and during immobilization of sodium Selenite (b): 1 – initial sample, 2 – sample after sorption of sodium selenite (pH=10), 3 – sample after sorption of sodium selenite (pH=5)*

Downloaded from www.periodico.tchequimica.com

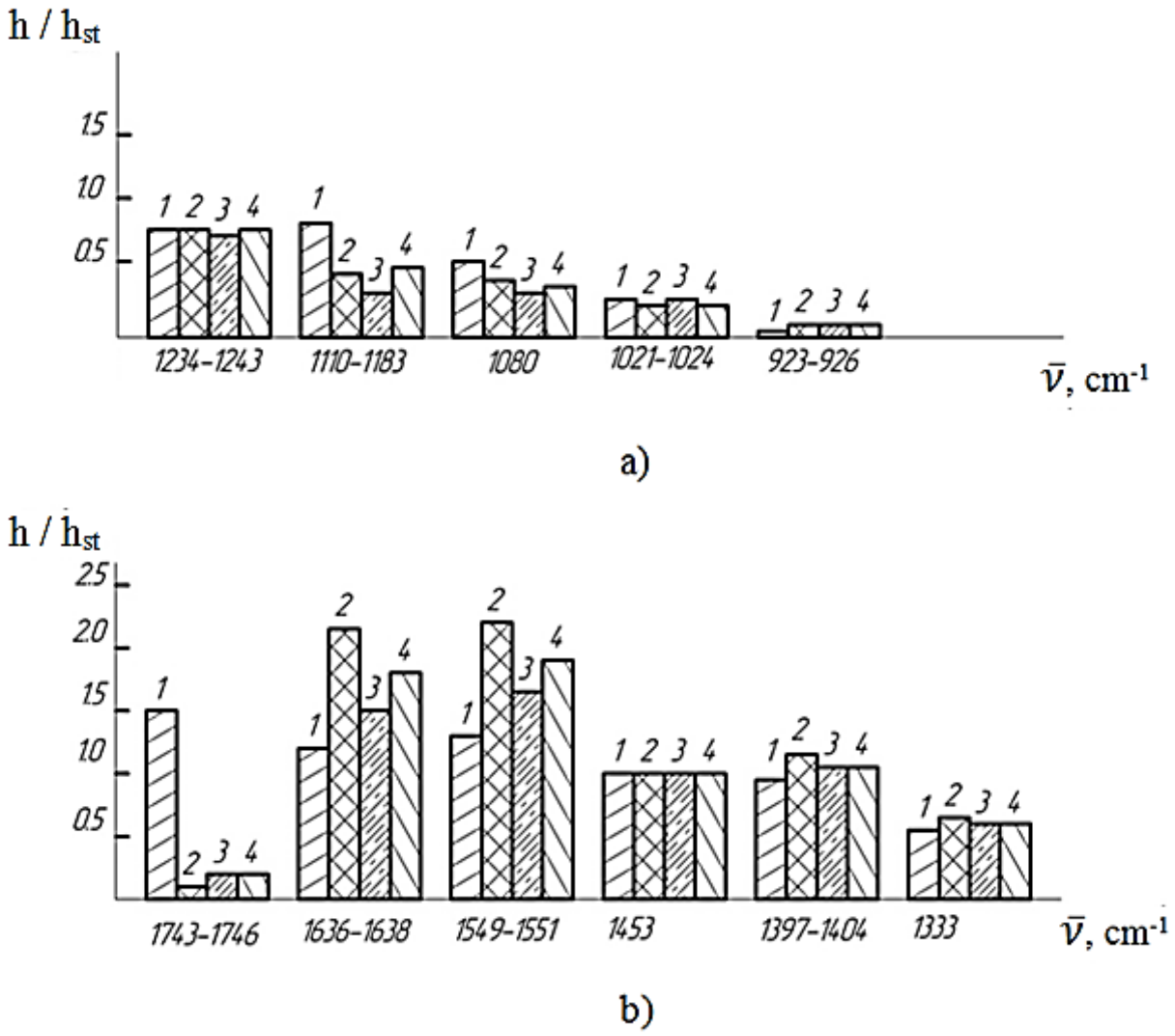


Figure 3 *The ratio h / h_{st} of the peaks of the IR spectra of the initial collagen (1); collagen with immobilized Na_2SeO_3 at pH = 5 (2); and at pH = 10 (3); and DMDPS (4): a - in the wavelength range 923-1243 cm^{-1}; b - in the wavelength interval 1333-1746 cm^{-1}*

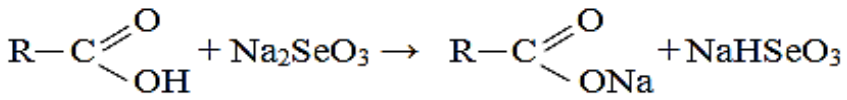


Figure 4 *The scheme of interaction of sodium selenite with functional groups in the collagen matrix, R is the matrix of collagen*

Downloaded from www.periodico.tchequimica.com

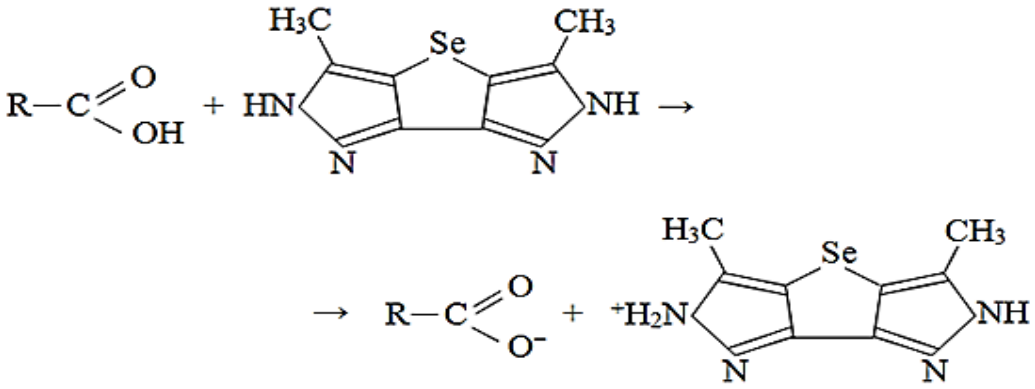


Figure 5 *Possible interaction of DMDPS with functional groups in the collagen matrix*

The Periódico Tchê Química (ISSN: 1806-0374; 2179-0302) is an open-access journal since 2004. Journal DOI: 10.52571/PTQ. http://www.tchequimica.com.
This text was introduced in this file in 2021 for compliance reasons.
© The Author(s)
OPEN ACCESS. This article is licensed under a Creative Commons Attribution 4.0 (CC BY 4.0) International License, which permits use, sharing, adaptation, distribution, and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third-party material in this article are included in the article 's Creative Commons license unless indicated otherwise in a credit line to the material . If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.