ISOLATION OF BIOACTIVE PREPARATIONS FROM ALCOHOLIC EXTRACTS OF *Chlorella vulgaris*

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The vitamin composition of the micro alga *Chlorella vulgaris* has been studied fairly fully [1-3]. However, the question of the isolation of individual biologically active substances remains open. The development of methods and conditions for isolating these substances will create the prerequisites for the more complete and comprehensive utilization of *Chlorella* biomass.

We have studied the possibility of isolating fat-soluble vitamins from alcoholic extracts of *Chlorella*, these forming intermediates in the treatment of the biomass by the methods described previously.

We investigated extracts of two samples of *Chlorella*: biomass in the form of a paste (time of storage 3 days at − 3°C), and biomass after freeze-drying (time of storage more than a year at 15-17°C).

The amount of dry matter in the extract from the pasty *Chlorella* was 20-25%, and from the freeze-dried material 10-12% (results calculated to the absolutely dry substance of the initial biomass).

PROVITAMINS OF THE A GROUP (CAROTENOIDS)

The amount of carotenoids in any plant material changes according to the conditions and methods used for its storage. In order to determine the suitability of the extracts investigated for further processing, we determined their content of vitamins and, in the first place, of carotenoids. The ethanol was distilled off from the extract, and then the residue was extracted with hexane.

The amount of carotenoids in the extracts from the pasty *Chlorella* was 423 mg-%, and from the freeze-dried material 52 mg-%. It must be mentioned that the amount of carotenoids in the fresh biomass is considerably higher (536 mg-%). The total carotenoids were determined by paper chromatography (according to I. K. Mur) and TLC (according to Shmit [4, 5]). Four carotenoid zones were obtained. Each zone was eluted. The carotenoids were identified and determined quantitatively by a spectrophotometric method. This shows that the amount of β-carotene in the extract from the pasty *Chlorella* was 83 mg-% on the absolutely dry *Chlorella* biomass. β-Carotene was present in only trace amounts in the extract from the freeze-dried biomass.

The composition of the carotenoid extracts was as follows (mg-% on the absolutely *Chlorella*):

Zone	Carotenoid	λ, nm (chloroform)	Biomass in the form of a paste	Biomass after drying
I	Lutein	430, 455, 485	110	20,5
II	Neoxanthin	448, 478	49,5	9,5
III	Violaxanthin	452, 481	142	21,8
I	β-Carotene	465, 496	83	0,2

Thus, when the biomass is stored changes take place both in the total carotenoids and in the relative amount of β-carotene in them.

Because of the very small amount of carotenoids in the extracts from the freeze-dried *Chlorella*, the separation of the fat-soluble bioactive substances was studied only with the extract from the pasty biomass.

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The group of carotenoids was isolated by their extraction with type BR-1 gasoline from previously saponified alcoholic extract. The yield of unsaponifiable substances from 1 kg of dry *Chlorella* biomass was 1.84%, including 75.6 mg-% of β-carotene. The characteristics of the carotenes are given for a preparation of β-carotene purified on alumina.

VITAMINS OF GROUP F (HIGHER UNSATURATED FATTY ACIDS)

The saponified fraction of the extract after the isolation of the carotenoids consisted of a mixture of fatty acid derivatives and chlorophyll. To decompose the saponified product, the mixture was treated with sulfuric acid, and then the fatty acids were extracted from the solution with type BR-1 gasoline. The gasoline extract was then subjected to vacuum evaporation.

The qualitative and quantitative composition of the fatty acids isolated were determined by GLC after their esterification with diazomethane. The results obtained showed that the proportion of myristic acid was 5.5% of the total acids, of palmitic 34.0%, of hexadecadienoic 13.7%, of hexadecatrienoic 8.5%, of oleic 4.6%, of linoleic 27.9%, and of linolenic 10.3%. The total yield of acids on the dry chlorella biomass was 7.3%, and the vitamins of group F made up about 40% of the acids isolated.

SODIUM CHLOROPHYLLIN

Sodium chlorophyllin remains in the residue after the isolation of the fatty acids. It was freed from accompanying impurities by washing it with gasoline and water. Then the sodium chlorophyllin was dissolved in ethanol and the solution was filtered. The yield of sodium chlorophyllin on the dry *Chlorella* biomass was 1.4%. The preparation isolated was identified by comparing its spectral characteristics, obtained on a SF-4 spectrophotometer, with those given in the literature [6].

As can be seen from Fig. 1, the main absorption maxima are located in the blue-violet (400-500 mμ) and red (600-700 mμ) regions of the spectrum. The spectrum of the preparation of sodium chlorophyllin isolated from *Chlorella* is similar to the spectra of the green pigments isolated from higher plants. The least absorption is observed in the 500-600 mμ region. However, the absorption spectrum of the preparation investigated has a number of differences from that of chlorophyll. Thus the absorption maximum in the blue-violet region is displaced in the short-wave direction (410 mμ) and the second maximum in this region is less pronounced. The maximum in the red region of the spectrum is also shifted in the short-wave direction.

A similar shift in the main absorption maxima is observed in the spectrum of a preparation of sodium chlorphyllin isolated from coniferous needles. A comparison of the spectral characteristics of the preparations of sodium chlorophyllin isolated from coniferous needles and *Chlorella* shows considerable similarity of their chemical compositions.

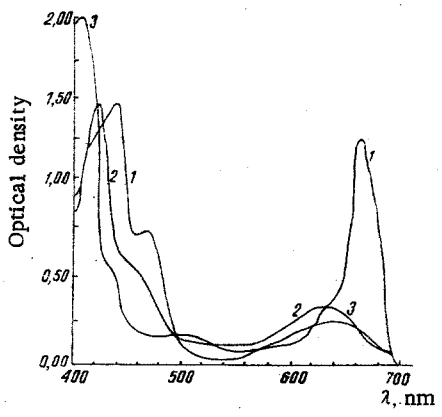

Fig. 1. UV spectra of chlorophyll preparations: 1) chlorophyll; 2) sodium chlorophyllin from coniferous needles; 3) sodium chlorophyllin from *Chlorella*.

EXPERIMENTAL

Preparation of the Extract. The *Chlorella* biomass (1 kg) was extracted with ethanol. To increase the degree of extraction of the fat-free substances, the extraction process was combined with the destruction of the *Chlorella* cell membrane. This destruction was carried out for 25-30 min in a mill with a profiled lining, with four changes of solvent. Then the extract was freed from undissolved residue by filtration or centrifugation. The combined extract was taken for further treatment.

Isolation of the Carotenoids. The extract after the ethanol had been distilled off was heated to 60°C and treated with a 30% solution of caustic soda to give a distinctly alkaline reaction. Treatment was carried out with stirring for 1.5 h. After saponification, the solution was diluted with water and extracted with type BR-1 gasoline. The gasoline extract of the carotenoids was then fed to a vacuum evaporator.

The carotenoids were determined and identified by paper chromatography and thin-layer chromatography using standard methods [4, 5]. The spectral characteristics of the substances isolated were obtained on a SF-4 spectrophotometer.

Isolation of the Fatty Acids. The fatty acids were extracted from the saponified residue remaining after the isolation of the carotenoids, this residue being first decomposed with 10% sulfuric acid at 60-70°C for 30 min. After settling for 10-12 hours, the acid products were washed with gasoline and the gasoline solution of fatty acids was sent for vacuum evaporation.

The composition of the fatty acids was determined by the GLC method on a LKhM-72 chromatography with a thermal conductivity detector. The stationary phase — Apiezon L — was deposited on Chromaton N-AW in the amount of 15%. The column was 2 m long and 4 mm in diameter, and the carrier gas was helium at a rate of flow of 45 ml/min.

Isolation of Sodium Chlorophyllin. The unextractable residue after the isolation of the fatty acids consisted of crude chlorophyllin. It was washed in two stages: I) three times with gasoline, and II) with water until the wash-waters were neutral. The washed sodium chlorophyllin was dissolved in ethanol and the solution was neutralized with sodium carbonate. After cooling, the solution was filtered and the ethanol was partially distilled off to give a solution of the required concentration.

The sodium chlorophyllin was identified by spectral methods [6].

SUMMARY

Three groups of bioactive substances (provitamins of the A group, vitamins of the F group, and sodium chlorophyllin) have been isolated from alcoholic extracts of the micro alga *Chlorella vulgaris*. The conditions of separating these substances are given. The qualitative and quantitative compositions of the groups isolated have been investigated by paper chromatography, gas—liquid chromatography, and spectral analysis.

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