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A rapid method for protein isolation from Chlorella vulgaris.

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Introduction:

Single-celled microalgae Chlorella is important for plant protection, wastewater treatment and rehabilitation of reservoirs. Chlorella also features a high content of high-grade protein, which can be considered as an additional food product for humans and farm animals. In this regard, the search for highly productive strains of protein producers and the development of effective methods for its isolation are of relevance.

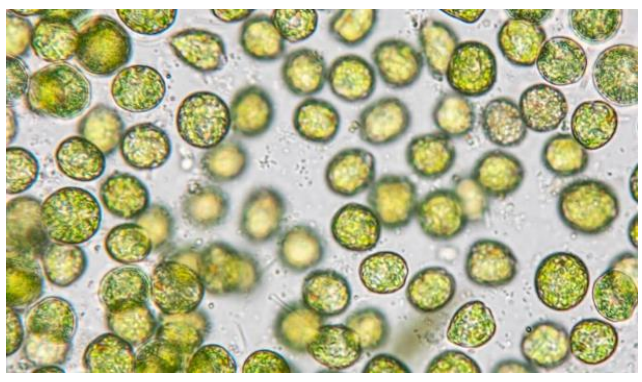

Fig.1 Chlorella vulgaris

Aim: to develop an effective and fast method of protein isolation from Chlorella vulgaris.

Objectives:

1. Isolation and characterization of protein from Chlorella vulgaris cell culture.
2. Obtaining purified protein using reverse phase HPLC.

Materials and Methods.

For isolation, 100 ml of culture was taken in the late logarithmic growth phase. The content of chlorella cells was 10 million cells/ml. After centrifugation (3500 rpm, 5 min), the precipitate was resuspended in 25 ml of sodium phosphate buffer solution (10 mm, pH =7.4) containing 6 M urea.

The suspension was exposed to ultrasound (ultrasonic disintegrator B.Braun, Germany) to destroy cells. 5 cycles of ultrasound exposure to cells were performed (1 minute exposure with 0.5 min breaks) The completeness of cell destruction was monitored using optical microscopy.

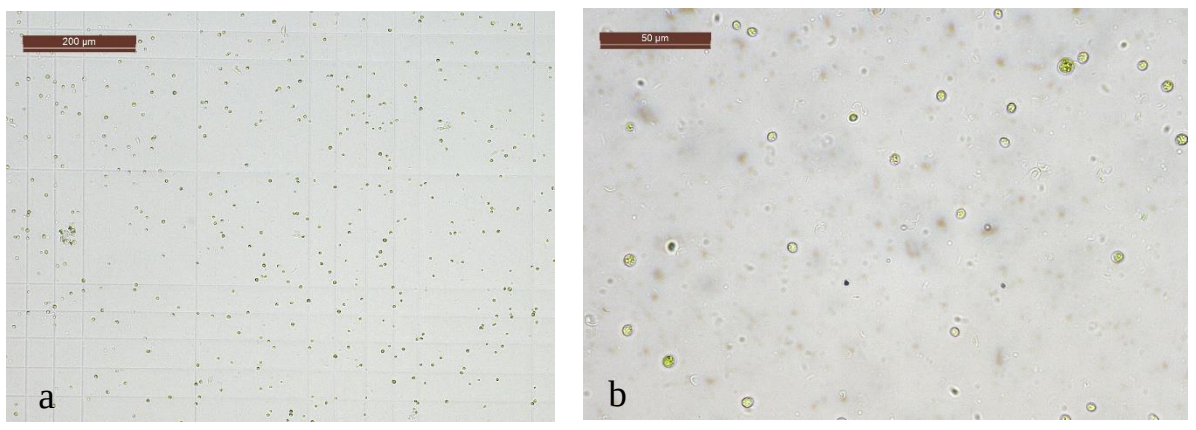

Fig. 2 Chlorella cell before (a) and after (b) ultrasound exposure

After ultrasonic treatment, the suspension was centrifuged for 10 min at 3500 rpm. The supernatant was dialized against two changes of sodium-phosphate buffer (10 mM, pH =7.4). The spectrum of the obtained proteins was studied using SDS electrophoresis. The final stage of protein purification was carried out using HPLC on a reversed phase column C_8. Protein elution was performed in a gradient of an acetonitrile solution containing 0.1% TFA. Protein peaks were collected and analyzed using SDS electrophoresis.

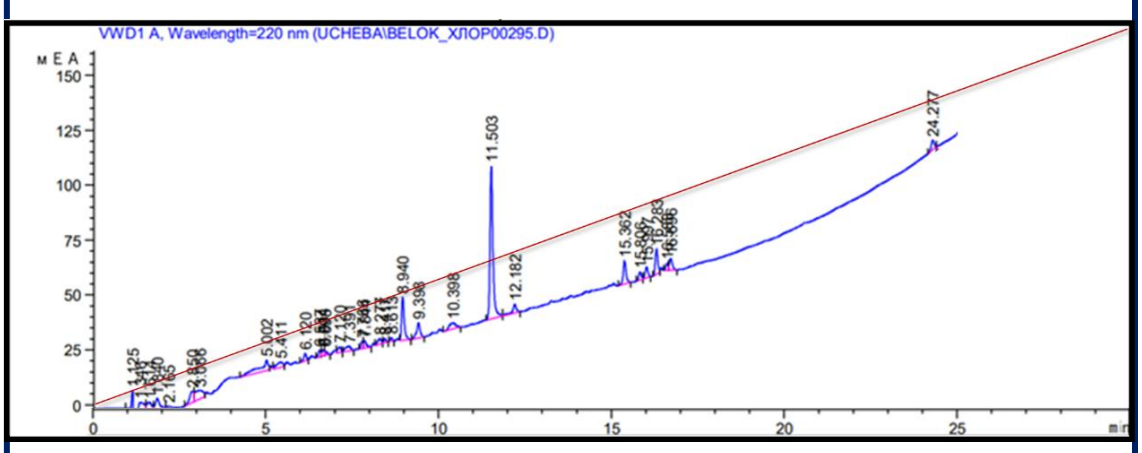

Fig.3 HPLC of the Chlorella proteins preparation on a reversed phase column C_8.

Results.

According to the SDS electrophoresis of the resulting preparation, the highest protein content was observed in the molecular weight range of 40-70 kDa. The protein concentration was 0.6 mg/ml according to the Bradford micro method. When using the proposed purification method, the protein yield was 1% of the wet mass of Chlorella vulgaris.

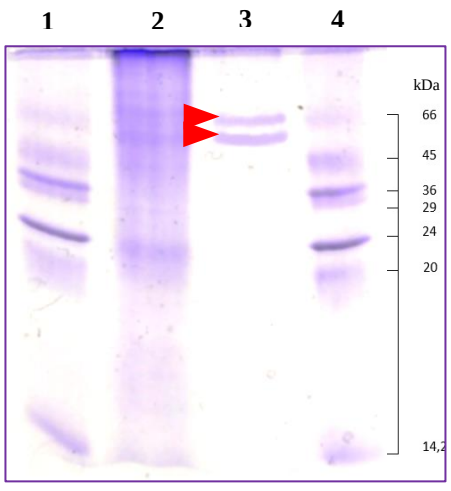

Fig.4 SDS electrophoresis in 12% PAGE of Chlorella preparation. 1,4 – standards; 2 – protein extract after dialysis; 3 – preparation after reversed phased HPLC.

Conclusion.

The proposed method makes it possible to obtain protein quickly and efficiently from Chlorella vulgaris with a high yield.