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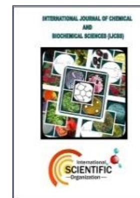

Study of biochemical parameters of *Chlorella* microalgae

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Abstract

The microalgae industry has been undergoing rapid growth in recent years. This study aimed to perform comprehensive biochemical studies on the *Chlorella* microalgae. It was found that the biomass accumulated 5.425% protein, 68.4 g/L fat, 0.025 g/L carbohydrates, and 0.1150 µg/g iodine. A study of the vitamin composition of *Chlorella* biomass revealed a high content of vitamins B6 (2.66 µg/mL), B2 (2.02 µg/mL), and C (0.99 µg/mL). The polysaccharide profile of the *Chlorella vulgaris* biomass was investigated. The total dietary fiber content of *Chlorella* cell walls was found to be 143.5 g/kg dry weight. The estimation of uronic acid and neutral sugar content revealed that the concentration of uronic acids in the studied microalgae was 127.8 g/kg dry weight and the concentration of neutral sugars was 90.3 g/kg dry weight. It was demonstrated that the main monosaccharide residues are D-glucose and L-rhamnose residues, with both α- and β-glycosidic bonds between them. Among the side substituents identified were methyl, amide, and aromatic groups. The limiting acid, palmitic acid, was found to account for 55.2% of the total fatty acids in the sample. The essential amino acid content was found to be 41.9%. A record amount of glycine (22.9% of the total amino acid content) was discovered. According to the results of the evaluation of the profile of antioxidant compounds in the biomass of *C. vulgaris*, the maximum concentration of polyphenolic substances (15.6 mg/g dry weight) from microalgae biomass can be extracted with 70% ethyl alcohol at extraction temperature 40°C, hydromodule 1:50, and process duration 15 minutes. It was found that lutein (90.9% of the total carotenoid content) predominated in the carotenoids profile of *C. vulgaris* biomass. These components can be used for the production of biofuels, biologically active food additives, functional foods, pharmaceuticals, and cosmetics.

Keywords: *Chlorella vulgaris*, Chemical composition, Lipid fraction, Protein fraction, Polysaccharides

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1. Introduction

The microalgae industry has been growing rapidly in recent years. Microalgae biomass is used in aquaculture as feed and food ingredients, growth stimulants and immunomodulators, and biofuels. One of the widely distributed microalgae species is *Chlorella vulgaris*, due to its diverse biochemical composition [1]. *C. vulgaris* is a green unicellular microalga (family Chlorophyta). It can grow under autotrophic, heterotrophic, and mixotrophic conditions [2]. The biochemical composition of the microalgae *C. vulgaris* is affected by several factors, including the chemical composition of the nutrient medium, pH, salt content, temperature, intensity, and duration of light exposure [3]. The nutritional value of this microalgae cannot be overstated; it contains up to 61.6% protein, 12.5% fat, 13.7% carbohydrates, trace elements (iron, potassium, calcium, phosphorus, magnesium), vitamins B, C, E, D, K, and pigments [1]. All essential amino acids are synthesized by *C. vulgaris* microalgae [4, 5]. In terms of dry biomass, the total protein content of mature *Chlorella* ranges from 42 to 58%. Around 20% of all proteins are linked to the cell wall, 50% are intrinsic, and 30% migrate outside the cell. The molecular mass of proteins produced by *C. vulgaris* ranges from 12 to 120 kDa [6].

Under optimal growth conditions, *Chlorella* biomass can accumulate from 5 to 40% of lipids in terms of dry biomass. The lipids of *C. vulgaris* mainly consist of waxes, glycolipids, phospholipids, and small amounts of free fatty acids [7, 8]. The fatty acid profile may vary depending on the growing conditions of the algae. According to [9], the biomass of *C. vulgaris* grown under mixotrophic conditions accumulates 60-68% of saturated and unsaturated fatty acids consisting of palmitic, stearic, palmitoleic, and oleic acids. Such fatty acid profile is more suitable for biodiesel production [10]. However, when microalgae are grown under favorable conditions, it accumulates polyunsaturated fatty acids

(linoleic, linolenic, eicosapentaenoic acids), which can be most appropriately used for food purposes. The main polysaccharides of *C. vulgaris* microalgae biomass are starch, cellulose, and glucan [5, 11]. *C. vulgaris* is rich in chlorophylls (a and b, 1-2 % of dry weight) and carotenoids, the main ones being β-carotene, lutein, astaxanthin, zeaxanthin, violaxanthin, and neoxanthin [5, 12]. The concentration of chlorophyll a in *Chlorella* biomass ranges from 2.5 to 17.5 mg/g of dry weight [13]. Lutein is the most abundant carotenoids in *Chlorella* biomass, accounting for up to 0.45% of the dry weight of the cells [14]. Microalgae pigments have a wide range of therapeutic properties, including antioxidant, immunomodulatory, cardioprotective activity, and the ability to regulate blood cholesterol levels [15].

The mineral profile of *C. vulgaris* includes the macronutrients sodium, potassium, calcium, magnesium, phosphorus, and the trace elements iron, chromium, copper, zinc, manganese, selenium, and iodine. Biotin, cyanocobalamin, folic acid, niacin, ascorbic acid, riboflavin, pyridoxine, thiamine, and pantothenic acid were identified among the vitamins [5]. Considering the diverse biochemical profile of microalgae *C. vulgaris*, the application spectrum is quite broad: biofuel production, food industry (production of food, biologically active additives, dyes, food emulsions), animal feed production, wastewater treatment (due to carbon dioxide fixation, nitrogen and phosphorus absorption), application in agrochemistry as plant growth stimulators. Currently, the world leader in the production of *Chlorella* is Japan, which uses it, among other things, in the pharmaceutical industry due to the presence of microalgae with proven immunomodulatory and antitumor properties [17-20]. This study aimed to perform comprehensive biochemical studies on the microalgae *Chlorella* microalgae.

2. Materials and methods

The object of research was the biomass of microalgae *C. vulgaris* grown in the laboratory of microbiology and biotechnology of I. Kant BFU at room temperature (21-23 °C) and constant illumination of 30-50 μU by fluorescent lamps with warm white light for 7 days. The microalgae were cultured on Tamiya medium, the composition of which is presented in Table 1. The nutrient medium was sterilized by autoclaving. At the end of cultivation, the resulting suspension was filtered under vacuum using a Bunsen flask and a Buchner funnel. After filtration, the filters were washed with distilled water and then dried in a BINDER ED 53 desiccator (BINDER, Germany) at (50±5) °C to constant weight. The proportion of microalgae in the mixture, mass fraction of crude protein, crude ash, carbohydrate, fat, and iodine content of dried *Chlorella* biomass were all determined. The proportion of microalgae in the mixture was determined by centrifugation followed by weighing the biomass and expressed as g/L of the mixture. Determination of mass fraction of moisture is based on the removal (evaporation) of water from the sample of microalgae under study at a temperature of (103±2) ° C and determining the change in its mass by weighing. The microalgae biomass was dried to constant weight in a BINDER ED 53 desiccator (BINDER, Germany).

Determination of the mass fraction of crude protein was performed using the Kjeldahl method based on mineralization of organic matter of the sample with subsequent determination of nitrogen by the amount of ammonia formed. The crude protein content is calculated by multiplying the mass fraction of nitrogen by a factor of 6.25. The method, which consists in determining the mass of the residue after combustion in a muffle furnace at a temperature of (525±25) °C and subsequent calcination of the sample, was used to determine the mass fraction of crude ash in the biomass of the microalga *Chlorella* microalgae.

The crude fat content of microalgae biomass was determined by extracting crude fat from the analyzed sample with petroleum ether, removing the solvent through distillation and drying, and weighing the resulting residue.

The content of carbohydrates in microalgae biomass was studied by determining the mass fraction of soluble carbohydrates according to the Bertrand's method. The essence of the method is the ability of reducing sugars to reduce divalent copper into copper (I) oxide in an alkaline environment, which is then oxidized with ferric ammonium alum before titration of reduced divalent iron with potassium permanganate solution.

A colorimetric method based on the formation of a colored complex compound of iodine with sodium nitrate in acidic medium and its colorimetric determination at a wavelength of 490 nm using a UV-3600 spectrophotometer (Shimadzu, Japan) was used to determine the iodine content in microalgae biomass. Alginic acids were quantified using a method based on the reverse titration with sulfuric acid of the excess sodium hydroxide remaining after its interaction with alginic acid in microalgae biomass. The method of aqueous extraction of mannitol, formation of its complex compound with copper sulfate, and determination of the amount of the target compound by optical density at a wavelength of 597 nm was used to determine the mannitol content. A calibration curve was preliminarily plotted using pure mannitol.

Microalgae are interesting because of their vitamin composition. In this regard, the content of water-soluble vitamins (riboflavin, nicotinic acid, pyridoxine hydrochloride, ascorbic acid) was analyzed in the obtained *Chlorella* biomass. The capillary electrophoresis method and the Kapel-105M system (Lumex, Russia) were used to analyze the vitamin composition. The capillary electrophoresis method is based on the migration and separation of the analyzed components' ionic forms due to their different electrophoretic mobility under the action of an electric field, with subsequent registration by a spectrophotometric detector at a wavelength of 254 nm and temperature of 20 °C. The vitamin mixture is separated in a capillary with an inner diameter of 75 μm and a length of 60 cm. Samples were prepared as follows. Aqueous and aqueous-alcoholic (80% ethyl alcohol) extracts of dried and crushed biomass were prepared (extraction temperature 40-60 °C, mixing time 15-20 min), 25 mL of which were placed in an Eppendorf tube and centrifuged for 5 minutes at 5000-6000 rpm (centrifuge-vortex MSC-6000, BioSan, Latvia), the solution above the precipitate was transferred to a clean

Eppendorf tube. The sample was then filtered through a membrane filter, discarding the first 1-1.5 mL of solution.

For analysis, the capillary was washed for 10-15 minutes with a leading electrolyte (0.05 M sodium tetraborate solution, 0.2 M boric acid solution). Next, a tube with the same buffer solution was placed at the outlet in the working position, and a tube with the sample was placed at the inlet and the sample was injected, and the electrophoregram was recorded. An electrophoregram of a standard solution of vitamins was obtained beforehand and a graduation graph was plotted. Separation conditions: background electrolyte is borate, pH 8.9. As a result, a higher content (by 20%) of vitamins in aqueous-alcoholic extracts of microalgae biomass compared to aqueous extracts was found, so it was decided to use aqueous-alcoholic solutions for vitamin extraction. In addition, the content of organic and inorganic carbon, as well as elemental composition, were determined when studying the chemical composition of *Chlorella* microalgae biomass. The Enviro TOC elemental analyzer (Elementar, Germany) was used to determine organic and inorganic carbon. The operating principle of the analyzer is based on catalytic high-temperature (up to 1200 °C) combustion of analyzed samples.

To determine the elemental composition (manganese, nickel, zinc, bromine, strontium, rubidium, iron, calcium) of *Chlorella* microalgae biomass, the X-ray fluorescence spectrometry (XRF) method and the Spectroscan max-6054 instrument (NPO Spektron, Russia) were used. The biomass sample with culture fluid was centrifuged and the solid part (sediment) was used for analysis. Pre-dried in a BINDER ED 53 desiccator (BINDER, Germany) at 60 °C to a constant mass, the precipitate was ground to a powdery state on a laboratory mill with a metal beaker LZM-M1/M2 (Russia). A 0.5 g suspension was pressed into a tablet on a boric acid substrate at a pressure of 100 kN. Anode – Ag, crystal analyzer – LiF (200), voltage 40 kV, current intensity 0.1 A, exposure – 100 seconds, for iron – 50 seconds.

Characteristics of polysaccharides accumulated in *C. vulgaris* biomass such as total dietary fiber content; content of easily hydrolyzable polysaccharides (EHPS) and hardly hydrolyzable polysaccharides (HHPS); content of uronic acids and neutral sugars; molecular weight of polysaccharides; structure of polysaccharides (including non-carbohydrate substituents, functional groups, and spatial configuration of glycosidic bonds) were studied. A method based on enzymatic hydrolysis using α-amylase, protease and amyloglucosidase was used to determine the total dietary fiber content in *C. vulgaris* biomass [19]. Dried microalgae biomass samples (about 1 g) were suspended in phosphate buffer and incubated with α-amylase at 60°C for 30 minutes. The pH of the mixture was then adjusted to 7.5 and protease was added, and the samples were incubated at 60°C for 30 minutes. The pH was then adjusted to 4.5 and hydrolyzed with amyloglucosidase at 60°C for 30 minutes. The dietary fibers were then precipitated with 96% ethanol at 60°C and filtered, the resulting precipitate was dried in a desiccator at 105°C and weighed [20].

The contents of easily hydrolyzable polysaccharides (EHPS) and hardly hydrolyzable polysaccharides (HHPS) were estimated using the Bertrand's method. The content of reducing agents was determined using a method based on the reduction of 3,5-dinitrosalicylic acid (DNS) by reducing sugars (glucose as a standard) to 3-amino-5-nitrosalicylic acid [21, 22]. The content of uronic acids in *Chlorella* biomass was estimated using the method proposed by van den Hoogen et al. [23]. The method is based on the interaction of uronic acids with m-hydroxydiphenyl in dimethyl sulfoxide mixed with sulfuric acid to form colored solutions, which were photometrically analyzed at 540 nm using a MultiskanSpectrum tablet photometer (Thermo Fisher Scientific, USA). A calibration curve was preliminarily plotted using the uronic acid standard. The content of neutral sugars in the microalgae biomass was determined using the microcolorimetric resorcin-sulfuric acid method based on the dehydration of hydrolyzed sugars to furfural derivatives, which form colored complexes with resorcin in the presence of 75% sulfuric acid [24]. Polysaccharides were extracted from the obtained *C. vulgaris* biomass to determine their molecular weight. Several extraction methods were used, including alkaline extraction, hot water extraction, microwave extraction, and enzymatic extraction [25]. When investigating the safety of the developed products positioned as poultry feed additives, they were tested for the presence of toxic substances. Nitrite was determined using the photometric method. The determination of benz(a)pyrene was also performed on these products, and the studies were carried out in accordance with the method described in [26].

Organophosphorus compounds (OPCs) (diazinon, dimethoate, melathion, parathion-methyl, pyrimiphos-methyl, phosalone, phosmet, chlorpyrifos, fenitrothion, etc.) were determined. Different compounds of this group were determined as described in [26]. Organochlorine compounds (OCs) (HCCH and sum of isomers, DDT and metabolites, hexachlorobenzene, aldrin, heptachlor, endrin, endosulfan, etc.) were measured in order to assess the toxicity of feed additives. The sum of HCCH and isomers was determined as described in [25]. DDT and its metabolites were determined following [27]. This study used enzymatic extraction method. The extracted polysaccharides were separated into fractions by ultrafiltration using membranes with pore diameters of 1 kDa, 3 kDa, 5 kDa, 10 kDa, 20 kDa, 30 kDa, 50 kDa, and 100 kDa. The molecular weight of the isolated polysaccharide fractions was determined using high-performance liquid chromatography and LC-20AB chromatograph (Shimadzu, Japan) equipped with UV detector. The chromatograph was pre-calibrated against a standard glucosan solution. A method based on a nuclear magnetic resonance (NMR) system was used to determine the structure of polysaccharides accumulated in *Chlorella* biomass. Previous studies have shown that the use of 1H NMR, when preceded by the Zeman hydrolysis procedure [26], can identify and quantify the composition of the ozide monomers of basic polysaccharides [27].

The following signals can be detected in proton magnetic resonance spectra: carboxyl 12-10 ppm, amide 8.5-8.0 m. ppm, aromatic 7.0-8.0 m. ppm, carbinol with intramolecular hydrogen bonds 5.5-6.5 m. ppm, β-anomeric

5.2-5.4 m. ppm, α-anomeric 4.4-4.6 m. ppm, carbinol 5.2-3.5 m. ppm, methyl in acyl 2.1-1.9 ppm, methyl of other functional substituents outside the polymer chain, aliphatic (fatty) 1.8-0.7 ppm, and other signals, on the basis of which the studied polysaccharide components can be assigned to the corresponding monomeric units, and their ratios evaluated. Bruker Avance III 400 MHz NMR spectrometer (Bruker, Germany) in DMSO-d6/C6H5CF3 mode was used for this method. Reference (δ, ppm, corresponding to TMS), 1H: 2.50- DMSO-d6. Polysaccharide samples were dissolved in deuterated dimethyl sulfoxide. Water signal suppression was also performed during the experiment. Fatty acid content was determined by gas chromatography using GCMS-QP2010Ultra gas chromatography-mass spectrometer (Shimadzu, Japan). A modified spectrophotometric technique developed by El-Sheekh and Fathy [28] was used to determine pigments in the biomass of *Chlorella* microalgae. To accomplish this, 1 g of dried ground biomass was suspended in 50 mL of acetone and vigorously stirred with an IKA C-MAG HS 7 magnetic stirrer. The solutions were then placed in the dark at 4°C and centrifuged at 4000 rpm for 10 minutes. The supernatants obtained were used to determine the concentration of chlorophyll a (Chl a), chlorophyll b, (Chl b) and total carotenoids (Car). The concentrations of Chl a, Chl b, and Car were determined spectrophotometrically at wavelengths of 662, 645, and 470 nm, respectively, using a UV-3600 dual-beam spectrophotometer (Shimadzu, Japan). The content (mg/g) of each pigment was quantified using the formulas:

$$Chl\ a = 11{,}75 \cdot A_{662} - 2{,}35 \cdot A_{645}, \quad (1)$$

$$Chl\ b = 18{,}61 \cdot A_{645} - 3{,}96 \cdot A_{662}, \quad (2)$$

$$Car = \frac{1000 \cdot A_{470} - 2{,}27 \cdot Chl\ a - 81{,}40 \cdot Chl\ b}{227}, \quad (3)$$

The analysis of fractional and amino acid composition of proteins is of particular interest when studying the protein profile of *C. vulgaris* biomass. The fractional composition of protein isolated from microalgae biomass was determined by Laemmli vertical electrophoresis. Protein extraction from microalgae biomass was done in advance using the method described in [29] and [30]. Microalgae biomass samples were suspended in lysis buffer composed of 0.5M Tris-HCl, 8M urea, 5% (w/v) sodium dodecyl sulfate (SDS), 20% (v/v) glycerol, and 10% (v/v) β-mercaptoethanol (pH 6.8) and centrifuged at 4°C for 20 minutes at 10,000 rpm.

Electrophoretic separation of proteins was performed in 4-12% polyacrylamide gel (PAGE) under denaturing conditions according to a standard protocol using a Protean II xi Multi-Cell vertical electrophoresis chamber (Bio-Rad Laboratories, Inc., USA). Samples were prepared in the presence of 1% NP-40 detergent. After electrophoretic separation, the PAAG was stained with a protein-specific dye, 0.1% toluidine blue in distilled water. The gels were viewed and photographed using an ECX-F20 transilluminator (Vilber Lourmat, France) at an emission wavelength of 312 nm. Protein electrophoresis data were stored and processed using a Vitran-Photo gel-documentation system.

The amino acid composition of *Chlorella* microalgae biomass was determined using high-performance liquid chromatography (HPLC) and LC-20AB chromatograph (Shimadzu, Japan) equipped with a double pump, autosampler, and diode matrix detector. Qualitative and quantitative analysis of amino acids was performed on a Zorbax Eclipse XDB-C18 4.6x150mm, 5μm column (Agilent, USA) in gradient elution mode from 1% to 65% eluent B in 45 minutes. The flow rate was 1.4 mL/min, eluent A was 0.02M sodium acetate solution, and eluent B was methanol. The temperature was 35 °C, and the wavelength of signal registration was 338 nm. The pre-dried microalgae biomass was ground and hydrolyzed with 2 H hydrochloric acid solution at 50 °C for 15 minutes.

The Folin-Ciocalteu method was used to determine the polyphenolic content of the *Chlorella* biomass. Microalgae biomass extracts were prepared in ethyl alcohol with different solvent concentrations of 30%, 50% and 70%. The extraction hydromodule was 1:50, extraction temperature was 40 °C, and the extraction was performed under stirring for 15 minutes. At the end of extraction, the samples were centrifuged for 15 minutes at 2000 rpm. Quantitative analysis of polyphenolic substances was performed as follows: 1.0 mL of extracts were diluted with 4 mL of 95% ethanol, centrifuged at 1000 rpm for 5 minutes. Thereafter, 1 mL of the supernatant was mixed with 1 mL of 95% ethanol, 5 mL of distilled water, 1 mL of 5% sodium carbonate, and 0.5 mL of 50% Folin-Ciocalteu reagent. The resulting mixture was incubated at 35 °C in the dark for 1 hour before the optical density at 725 nm was measured with a UV-3600 spectrophotometer (Shimadzu, Japan). A calibration curve was previously plotted using gallic acid as a standard. The content of substances of polyphenolic nature was expressed in mg of gallic acid equivalents per 1 g of dried microalgae biomass [31].

The total flavonoid content of microalgae *C. vulgaris* biomass samples was determined using a modified method with aluminum chloride. For this purpose, 1.0 mL of extracts were diluted with 4 mL of 95% ethanol, centrifuged at 1000 rpm for 5 minutes. Then 1 mL of the supernatant was mixed with 0.5 mL of 10% aluminum chloride, 0.5 mL of 1 M sodium acetate, and 2 mL of deionized water. The optical density was measured at 415 nm using a UV-3600 spectrophotometer (Shimadzu, Japan) after incubating the mixture in the thermostat at 35 °C in the dark for 40 minutes. A calibration curve was preliminarily plotted using quercetin as a standard. The content of substances of polyphenolic nature was expressed in mg of quercetin equivalents per 1 g of dried microalgae biomass [31]. High-performance liquid chromatography (HPLC) and LC-20AB chromatograph (Shimadzu, Japan) were used to study the chemical composition of the antioxidant fraction isolated from the algae biomass for the content of individual carotenoid representatives. The extractant was dimethyl sulfoxide.

Standard mathematical statistics methods were used to process the data. Each experiment was performed in three

replicates. The gathered data were expressed as means ± standard deviation. The correspondence of the used samples to the normal distribution was assessed using the t-test (mathematical expectations) for independent samples and Fisher's test (variance). The Levene test was used to ensure that the variances of the isolated samples were equal. The results were analyzed using Duncan multiple range test at $P<0.05$ to identify samples that were significantly different from each other. Data were subjected to analysis of variance (ANOVA) using Statistica 10.0 software (StatSoft Inc., 2007, Tulsa, OK, USA).\

3. Results and Discussions

The results of determining the proportion of microalgae in the mixture, mass fraction of moisture, crude protein, crude ash, carbohydrate, fat, iodine, alginic acids, mannitol content in the biomass of *Chlorella* microalgae are presented in Table 2. According to Table 2, 2.237 g of *Chlorella* microalgae biomass is accumulated in 1 liter of culture liquid. The protein content of the obtained biomass was 5.425%, which was significantly lower than that reported in the literature. For example, there is information about the accumulation of up to 64.61 % of protein in the *Chlorella* biomass [32]. In [25], the crude protein content of *Chlorella* biomass was estimated to be 53.0 %. Publications by different authors report protein content in *Chlorella* biomass ranging from 42 to 58 % [33-35]. Nonetheless, there is a paper [36] where the protein content of *C. vulgaris* biomass was found to be 36.56 mg/g of dry weight, which corresponds to 3.6%. The low protein content found in this study is most likely due to incomplete extraction of intracellular protein due to *Chlorella*'s strong cell wall, as described in [33]. Cell lysis, such as mechanical action, ultrasound, chemical, enzymatic treatment, thermal or osmotic shock, would be appropriate to increase the yield of protein substances [37, 38]. In addition to protein, lipids are accumulated in microalgae cells in large quantities (crude fat content is 68.4 g/L). This value is lower than in other published studies evaluating the biochemical composition of microalgae. For example, the accumulation of 12.0 to 26.0 % fat in *C. vulgaris* cells [39-42], 19.0 % fat in *C. sorokiniana* biomass [43] were reported. At the same time, data on *C. pyrenoidosa* cell fat content at the 2.0% level have been reported [40], which is consistent with the data from the current study.

The content of crude ash in the biomass of *Chlorella* microalgae was 3.70 %, which is in agreement with the data of literature sources: 3.1-4.5 % [44]. The biomass was characterized by a low carbohydrate content of 0.025 g/L. Similar studies reported the accumulation of up to 42.13 mg/g of dry weight of carbohydrates by *C. vulgaris* cells [36]. According to [45], the *C. vulgaris* biomass produced 23.43 % of carbohydrates. The results of iodine content determination in *Chlorella* biomass (0.1150 μg/g) and their comparison with the data obtained by other researchers indicate that microalgae biomass can serve as a promising source of organically bound iodine [46]. However, in order to enrich *Chlorella* biomass with organic forms of iodine, it is necessary to use nutrient medium containing iodine in inorganic form for its cultivation. The vitamin content of the aqueous-alcoholic extracts of *Chlorella* microalgae biomass is summarized in Figure 1 and Table 3. Table 4 shows that the major part of carbon (347.1 mg/L) in the *Chlorella* biomass is organic carbon, which is a part of proteins, carbohydrates, lipids, vitamins, and polyphenols of microalgae. The results of determining the elemental composition of *Chlorella* microalgae biomass are presented in Table 5.

The content of macronutrients (calcium), trace elements (iron, manganese, strontium), and heavy metals (zinc, nickel) in the biomass of *Chlorella* microalgae was determined. The calcium content (0.6 %) correlates with the literature data. In particular, the concentration of this macronutrient in *Chlorella* biomass has been reported to be 0.16-0.59 % [2,47,48]. The leading trace element is iron (1152.0 mg/kg, or 0.115 %). According to the literature [5], the iron content in *Chlorella* samples ranged from 0.20 to 0.68 %. Manganese accumulated in significant amounts (106.0 mg/kg, or 0.01 %), although this value was lower than in the papers of other authors – 0.40 % [5, 47]. Among heavy metals, zinc dominates (263.0 mg/kg, or 0.03%), while nickel was found in lower concentration (13.0 mg/kg). Authors [47] found zinc at the level of 0.55 % in *Chlorella* biomass.

Polysaccharides can be synthesized by different organisms (bacteria, fungi, macroalgae, microalgae, plants, etc.). Polysaccharides are polymeric carbohydrate molecules consisting of elongated linear or branched chains of monosaccharides (rhamnose, fucose, galactose, glucose, xylose, uronic acids, etc.) linked by glycosidic bonds. These macromolecules exist in two forms: homopolysaccharides, which contain only one type of monosaccharide, and heteropolysaccharides, which contain two or more types of monomeric links [50, 51]. Microalgae polysaccharides have unique biological properties such as antioxidant, antitumor [52], antibacterial, antiviral, hypocholesterolemic [51], and anti-inflammatory [53], making them a promising functional ingredient for value-added products in various industries (pharmaceutical, food, and feed) [54]. However, the biological properties of polysaccharides depend on the monosaccharide composition, spatial configuration of the molecule, degree of sulfation, and polysaccharide substituents [51].

A number of scientists have reported that *Chlorella* microalgae can contain both cellulose [55], hemicellulose, and starch [56, 57] and polysaccharides associated with other organic matter (glycoproteins, chitin-like compounds) in their composition [57]. It should be noted that the monomers (glycan units) of these compounds are glucose, galactose, and rhamnose. Sun L. proved in his experiments that changing the cultivation conditions of a microalgae can induce polysaccharide overproduction, as in the case of nutrient limitation, but can also induce significant changes in the polymer composition, thus modulating its biological activity [52]. Similar results were obtained by Gaignard C. et al. They found that polysaccharide composition depends on cultivation conditions and algae taxonomy [51]. The results of studying the spatial structure of polysaccharides isolated from the biomass of the *C. vulgaris* microalgae are presented in Figures 2 and 3. Analysis of the results presented in Figures 3 and 4 revealed that four anomeric hydrogen signals are

present in the 1H NMR of the polysaccharide sample under study: δ 1.09 ppm, δ 1.18 ppm, δ 4.45 ppm, and δ 5.25 ppm. Two hydrogen signals (δ 1.09 ppm, δ 1.18 ppm) indicate the presence of methyl groups in the PS molecule. According to literature data, this may represent a doublet of 6-deoxysaccharide [52]. The δ 4.45 ppm and δ 5.25 ppm signals (Figure 3) correspond to monosaccharides and, according to the literature, they can be identified as D-glucose and L-rhamnose [52].

In addition, H atom signals in the region of 4.4-4.6 ppm indicate the presence of an α-anomeric configuration, whereas signals at 5.2-5.4 ppm indicate the presence of a β-anomeric configuration. The spatial structure analysis also revealed that the polysaccharide sample isolated from the microalga *Chlorella* is a polymer of monosaccharide residues linked by both α- and β-glycosidic bonds. According to Figure 3, aromatic (signals in the δ 7.0-8.0 m.d. region) and amide (signals in the δ 8.0-8.5 m.d. region) protons are present in trace amounts in the sample. Signals in the δ 1.8-0.7 m.d. region indicate the presence of alkyl protons (Figure 4). The results of total dietary fiber, EHPS and HHPS in *Chlorella* biomass are presented in Figure 4. According to the data obtained, the total content of dietary fiber in *Chlorella* biomass (mainly in cell walls) is 143.5 g/kg of dry weight, which corresponds to 14.4%. Of them, the share of easily hydrolyzable polysaccharides, the main representatives of which are hemicelluloses, water-soluble polysaccharides, pectin substances, accounts for 8.6% of the total content of dietary fiber (12.4 g/kg of dry weight), and the share of hard-hydrolyzable PS (fiber) - 91.4% (131.0 g/kg of dry weight).

The correlation of the results obtained with the information published by other authors was established when analyzing the literature review. Thus, the total dietary fiber content of *C. vulgaris* biomass was reported to be 192 g/kg of dry matter, soluble dietary fiber content to be 12.5 g/kg of dry matter and insoluble dietary fiber content to be 179 g/kg of dry matter [58]. In another microalgae Tetradesmus obliquus, for comparison, the values of these values were 237 g/kg, 7.5 g/kg and 229 g/kg, respectively [58, 59]. Authors [20] reported a total dietary fiber content of 7.0 % in the biomass of *C. vulgaris*. The results of determining the content of uronic acids and neutral sugars in the biomass of *C. vulgaris* are presented in Table 6.

The data presented in Table 6 allowed concluding that the cells of the microalgae *C. vulgaris* accumulate uronic acids 29.3 % more (127.8 g/kg dry weight) than neutral sugars (90.3 g/kg dry weight). The analysis of the literature data revealed that the obtained results do not contradict the data obtained by other authors. For example, [58] found the content of neutral sugars in dried *Chlorella* biomass to be 164 g/kg of dry weight. The concentrations of neutral sugars, uronic acids in the cell walls of different *Chlorella* strains (*C. vulgaris* UTEX 259, *C. sorokiniana* UTEX 2805, *C. minutissima* UTEX, 2341, and *C. variabilis* NC64A were reported by [60]. The content of neutral sugars ranged widely from 4.80 to 15.47 % of cell wall mass, while the content of uronic acids ranged from 9.16 to 26.41 % of cell wall mass. The same authors in [61] evaluated the concentration of neutral sugars and uronic acids in the cell wall of *C. variabilis* strain NC64A grown under other conditions. Researchers demonstrated that the composition of cell wall sugars includes mainly uronic acids (15-27% of cell wall mass), followed by neutral sugars (8-15% of cell wall mass) and then amino sugars (7-12% of cell wall mass). Thus, the ratio of uronic acid, neutral sugar, and aminosugar ranged from approximately 1.5:0.9:1 to 2.1:1.2:1. The results of the separation are presented in Figure 5. Figure 5 shows that the maximum yield is characterized by polysaccharide fractions cut off by membranes with pore diameters of 30 kDa (25.5-34.0 wt%), 50 kDa (19.8-24.0 wt%), and 100 kDa (16.7-20.0 wt%) in all extraction variants. The highest yield of high molecular weight fractions (30, 50, and 100 kDa) was observed for polysaccharides isolated from microalgae biomass by enzymatic extraction. The results on molecular weight of polysaccharide fractions are summarized in Table 7.

The results are partially in agreement with data published by other researchers. For example, [62] presented the results of ultrafiltration separation of polysaccharides isolated from *C. pyrenoidosa* biomass. The authors found that the polysaccharide extract was dominated by fractions screened by a membrane with a pore diameter of 30 kDa, accounting for 53.8 wt%. The molecular masses of two fractions, 69.7 kDa and 109.4 kDa, were determined using the HPLC method. Thus, the analysis of the spatial structure using NMR method allows suggesting a complex structure of the studied polysaccharides. Basic characteristics of the spatial structure of polysaccharides isolated from *Chlorella* biomass:

– monosaccharide residues - D-glucose and L-rhamnose;
– α- and β-glycosidic bonds;
– the presence of alkyl (particularly methyl), amide and aromatic groups.

The results obtained are in agreement with the literature data. Y. Shi et al. [63] found that mannose (78.0 %) and glucose (13.2 %) are the dominant monosaccharides in C. pyrenoidosa polysaccharides. Homopolysaccharides of microalgae of the genus *Chlorella* were described in [64]. Two fractions of glucans from C. pyrenoidosa were isolated. One of these was a starch-like glucan composed of repeating α-(1→4)-D-glucose residues with α-(1→6)-branched glucose. The other contained both linear and cyclic β-(1→2)-D-glucans in a ratio of 64:36 [28,65]. According to [25], the presence of monosaccharide residues such as glucose, galactose, rhamnose, xylose, and mannose was found in polysaccharide extracts obtained from *C. vulgaris* biomass. For example, in the alkaline extract of microalgae biomass, the concentration of glucose is 1.0%, galactose 0.92%, mannose 0.41%, rhamnose 0.38%, and xylose 0.15%. In the aqueous extract, the values of these indices are 1.0 %; 0.75 %; 0.30 %; 0.19 %; and 0.12 %, respectively; in the extract obtained under the action of microwave radiation - 1.0 %; 2.15 %; 1.59 %; 0.99 %; and 0.73 %; in the enzymatic extract - 1.0 %; 0.69 %; 0.32 %; 0.27 %; and 0.16 %. The authors [66] showed the predominant content of such monosaccharides as D-glucose, D-galactose, and D-mannose in the composition of polysaccharides of the microalga *C. pyrenoidosa*. Additional studies are required to better characterize the spatial structure of polysaccharides accumulating in *C. vulgaris* biomass.

It is known that microalgae are a promising source of lipid fractions with biological activity, as well as pigments characterized by antioxidant properties. This chapter presents the results of studying the content of fatty acids and pigments (chlorophylls a and b, carotenoids) in the biomass of the *Chlorella* microalgae. The fatty acid content was expressed as a percentage of the sum of all fatty acids in the sample. The results obtained are presented in Table 8. Based on the results presented in Table 8, it was concluded that the dominant fatty acid in the analyzed biomass is hexadecanoic (palmitic) acid, which belongs to the limiting acids, its content was 55.2 % of the sum of all fatty acids in the sample. *Chlorella* microalga also accumulates unsaturated fatty acids, namely 9,12,15-octadecatrienoic acid/α-linolenic acid (25.3%) and 9,12-octadecadienoic acid/linoleic acid (19.5%), in the process of life activity. The content of 9-octadecenoic (oleic) acid in the studied samples could not be determined, which is probably due to the interfering influence of other components of microalgae, in particular, the diterpene phytol. The findings do not contradict the results obtained by other authors. In [67], the fatty acid composition of the microalga *Chlorella kessleri* was studied and it was shown that the dominant fatty acids are palmitic, stearic, oleic, linoleic, and α-linolenic acids, totaling 78.7-91.4% of the total FA. Similar results have been obtained by other authors [68].

The data on the pigment composition of *Chlorella* microalgae biomass are presented in Table 9. According to Table 9, the pigments chlorophyll a (6.68 mg/g) and carotenoids (3.36 %) accumulate in notable amounts in the biomass of *Chlorella* microalgae. Chlorophyll b is present in lower amounts, 1.58 mg/g. There is information in the literature about the accumulation of chlorophyll a in the biomass of the microalgae *C. kessleri* at the level of 32.7 mg/g in mixotrophic conditions of cultivation compared to 13.1 mg/g in heterotrophic conditions [68]. Thus, varying the conditions of microalgae cultivation makes it possible to regulate the level of photosynthetic pigments in the cells.
Chlorophyll a content in *C. vulgaris* biomass has been reported to be 0.25-9.6 mg/g dry weight, chlorophyll b 0.07-5.8 mg/g [69], β-carotene 0.007-0.012 mg/g [70], astaxanthin 0.55 mg/g, lutein 0.052-3.8 mg/g, and violaxanthin 0.01-0.04 mg/g [8, 71]. The results of molecular weight composition of *Chlorella* biomass proteins are summarized in Table 10. Table 10. Molecular weight composition of *C. vulgaris* biomass proteins According to the data presented in Table 10, the protein isolated from *Chlorella* biomass shows fractions with molecular masses ranging from 10 to 75 kDa. The maximum content is typical for fractions 16-20 kDa (21.6 %), 21-25 kDa (19.2 %), and 28-32 kDa (18.3 %). The minimum content (4.2 %) is typical for the fraction with the range of molecular weights 72-75 kDa. Comparing the results obtained with the data established by other authors, we concluded that there were no serious discrepancies. Thus, the authors [72] detected bands with molecular masses of 14, 17, 18, 19, 20, 22, 23, 25 kDa on the electrophoregamma of protein isolated from *C. vulgaris* grown in autotrophic mode. In the case of photoheterotrophic and mixotrophic cultivation, fractions corresponding to molecular masses of 30, 32, 35, and 39 kDa were also recorded on electrophoregrams.

In [73], the peptide profile of protein isolated from *C. vulgaris* grown under different conditions was determined. The authors [73] showed that *Chlorella* cultured under different conditions is characterized by bands on protein electrophoregrams corresponding to molecular masses of 23, 26, 28, 36, 55, 75, 76 kDa. In addition, fractions with molecular masses of 99-101 kDa were detected. The electrophoregram of the protein isolated from *C. vulgaris* biomass presented in [74] revealed only two bands corresponding to molecular masses of 39 and 75 kDa.
The results of amino acid composition of *Chlorella* microalgae biomass are summarized in Table 11 and Figure 6. According to the results obtained, amino acids such as glycine (2.690 μg/mL, 22.9 %), glutamic acid (1.224 μg/mL, 10.4 %) and leucine (1.142 μg/mL, 9.7 %) prevail in the biomass of *Chlorella* microalgae. Lysine (0.976 μg/mL, 8.3%), valine (0.746 μg/mL, 6.3%), arginine (0.693 μg/mL, 5.9%), threonine (0.645 μg/mL, 5.5%), and phenylalanine (0.620 μg/mL, 5.3%) also accumulate in sufficient amounts. The minor amino acid is alpha-aminobutyric acid and its concentration is 0.038 μg/mL. The high content of essential amino acids in the studied sample should be noted – 41.9 %. Our results are in agreement with those obtained by other researchers. For example, A.-V. Ursu et al. determined the accumulation of essential amino acids in the biomass of *C. vulgaris* at the level of 38.0% [4]. The leading amino acids are alanine (10.7 %), glutamic acid (10.3 %), aspartic acid (8.6 %), leucine (8.2 %), and glycine (7.0 %) [4].

According to different authors [75-77], *C. vulgaris* proteins contain from 9.08 to 13.70 % glutamic acid, 7.49-9.50 % leucine, 6.40-6.83 % lysine, 3.09-7.00 % valine, 6.22-7.38 % arginine, 5.15-6.09 % threonine, 5.50-5.81 % phenylalanine, and 6.30-8.60 % glycine. These data correlate with the results presented in this paper. The presence of antioxidant properties in the biomass of the microalgae *C. vulgaris* is well documented in the literature, owing to the presence of polyphenolic compounds (including flavonoids), chlorophylls, carotenoids, and tocopherols [31, 48, 49]. In this regard, it was of interest to evaluate the content of antioxidant compounds in the *C. vulgaris* biomass. Figure 7 presents the results of determining the polyphenol content in ethanolic extracts of the *C. vulgaris* microalgae at various solvent concentrations. According to the data presented in Figure 7, the maximum concentration of substances of polyphenolic nature (15.6 mg/g of dry weight) is achieved during extraction of C. *vulgaris* biomass with 70% ethyl alcohol at a hydromodule of 1:50, temperature 40 °C, for 15 minutes.

Table 1: Tamiya nutrient medium composition

Component	Content, g/L
Potassium nitrate (KNO_3)	5.00
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	2.50
Potassium dihydrophosphate (KH_2PO_4)	1.25
Fe+EDTA solution	1.00 mL
Micronutrient solution of the following composition:	1.00 mL
NH_4VO	0.023
H_3BO_3	2.860
$MnCl_2 \cdot 4H_2O$	1.810
$ZnSO_4 \cdot 7H_2O$	0.222
MoO_3	0.018
distilled water	up to 1.0 L

Table 2. Chemical composition of *Chlorella* microalgae biomass

Indicator	Value
Fraction of microalgae in the mixture, g/L	2.237±0.067
Mass fraction of water, %	14.45±0.73
Mass fraction of crude protein, %	5.425±0.624
Mass fraction of crude ash, %	3.70±0.11
Carbohydrate content, g/L	0.025±0.001
Crude fat content, g/L	68.400±2.052
Iodine content, µg/g	0.1150±0.0005
Alginic acid content, %	BLOD
Mannitol content, %	BLOD

BLOD – below the limit of detection

Table 3. Water-soluble vitamins in water-alcohol extracts of *Chlorella* microalgae

Vitamin	Vitamin content, µg/mL
B_2 (riboflavin)	2.02±0.26
B_5 (nicotinic acid)	BLOD
B_6 (pyridoxine hydrochloride)	2.66±0.34
C (ascorbic acid)	0.99±0.11

BLOD – below the limit of detection

Table 4. Organic and inorganic carbon content in the *Chlorella* microalgae

Indicator	Value
Organic carbon content, mg/L	347.1±10,4
Inorganic carbon content, mg/L	39.2±1.1

Table 5. Elemental composition of *Chlorella* microalgae

Indicator	Value, mg/kg
manganese (Mn)	106.0±2.0
nickel (Ni)	13.0±0.5
zinc (Zn)	263.0±3.0
bromine (Br)	BLOD
strontium (Sr)	6.0±0.3
rubidium (Rb)	BLOD
iron (Fe)	1152.0±14.0
calcium (Ca), %	0.60±0.05

BLOD – below the limit of detection

Table 6. Uronic acids and neutral sugars content in *C. vulgaris* biomass

Indicator	Value
Uronic acid content, g/kg of dry weight	127.8±6.1
Content of neutral sugars, g/kg of dry weight	90.3±4.5

Table 7 – Molecular weight of polysaccharide fractions of *C. vulgaris* isolated by ultrafiltration method

Method of PS extraction from biomass	Molecular mass of PS, kDa		
	30 kDa fraction	50 kDa fraction	100 kDa fraction
Alkaline extraction	47.8±2.4	77.9±3.9	112.6±5.6
Hot water extraction	37.9±1.9	80.9±4.0	107.9±5.4
Microwave extraction	39.2±2.0	75.1±3.8	115.4±5.8
Enzymatic extraction	44.1±2.2	76.4±3.8	121.4±6.1

Table 8 – Results of determination of fatty acid composition of *Chlorella* microalgae biomass

Fatty acid	Value, %
Hexadecanoic acid	55.2±1.6
9,12-octadecadienoic acid	19.5±0.6
9,12,15-octadecatrienoic acid	25.3±0.8
9-octadecenoic acid	BLOD

The fatty acid total was calculated without taking 9-octadecenoic acid into account. BLOD – below the limit of detection due to the interfering influence of other components of microalgae (phytol)

Table 9. Pigment content in the biomass of *Chlorella* microalgae

Pigment	Value
Chlorophyll *a,* mg/g	6.68±0.10
Chlorophyll *b,* mg/g	1.58±0.03
Carotenoids, %	3.36±0.12

Table 10. Molecular weight composition of *C. vulgaris* biomass proteins

Molecular mass range, kDa	Value, %
10–15	11.5±0.6
16–20	21.6±1.1
21–25	19.2±1.0
28–32	18.3±0.9
35–39	16.8±0.8
52–55	8.4±0.4
72–75	4.2±0.2

Table 11. Amino acid composition of *Chlorella* microalgae biomass

Amino acid	Amino acid content, µg/mL	Amino acid content, % of total amino acid content
Aspartic acid	0.411±0.013	3.5
Glutamic Acid	1.224±0.037	10.4
Serine	0.447±0.014	3.8
Glycine	2.690±0.081	22.9
Histidine	0.212±0.007	1.8
Threonine	0.645±0.020	5.5
Cystine	0.512±0.016	4.4
Arginine	0.693±0.021	5.9
Tyrosine	0.605±0.019	5.1
Alpha-aminobutyric acid	0.038±0.002	0.3
Alanine	0.191±0.006	1.6
Methionine	0.297±0.009	2.5
Valine	0.746±0.023	6.3
Phenylalanine	0.620±0.019	5.3
Isoleucine	0.321±0.010	2.7
Leucine	1.142±0.010	9.7
Lysine	0.976±0.030	8.3
Total	11.77±0.55	100.0

Table 12. Results of carotenoid content determination in extracts of *C. vulgaris* biomass in DMSO

Carotenoid	Carotenoid content in extract, mg/mL of extract
α-carotene	0.00030±0.00002
β-carotene	0.00020±0.00001
lutein	0.0050±0.0003

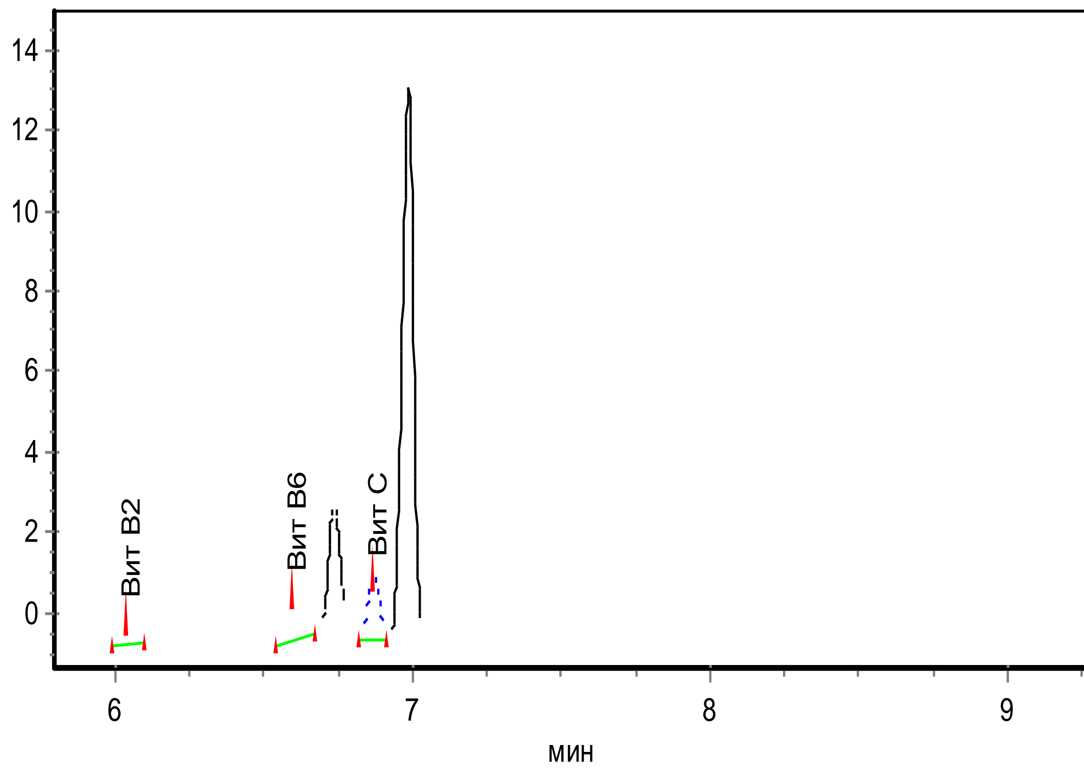

Fig. 1. Electrophoregram of *Chlorella* microalgae biomass extract

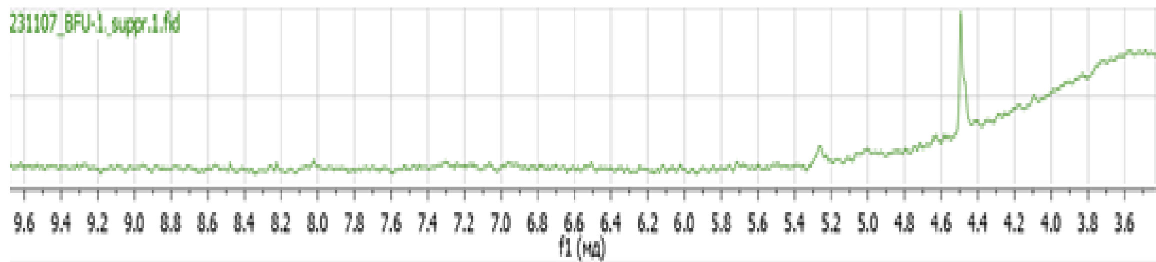

Fig. 2. The 10-3.5 ppm region of PMR of a polysaccharide isolated from the *C. vulgaris* microalgae

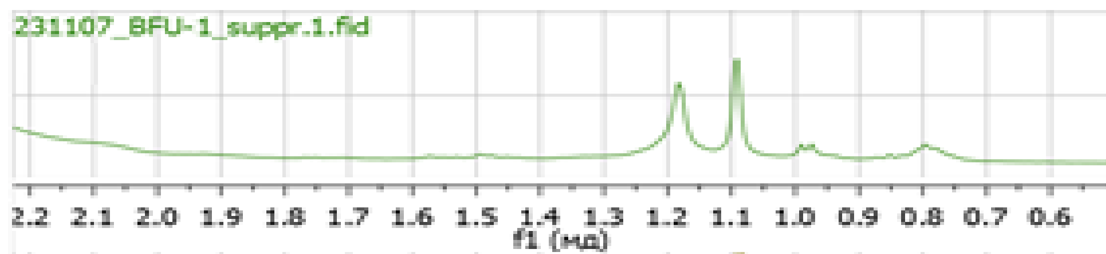

Fig. 3. The 2.5-0.5 ppm region of PMR of a polysaccharide isolated from the *C. vulgaris* microalgae

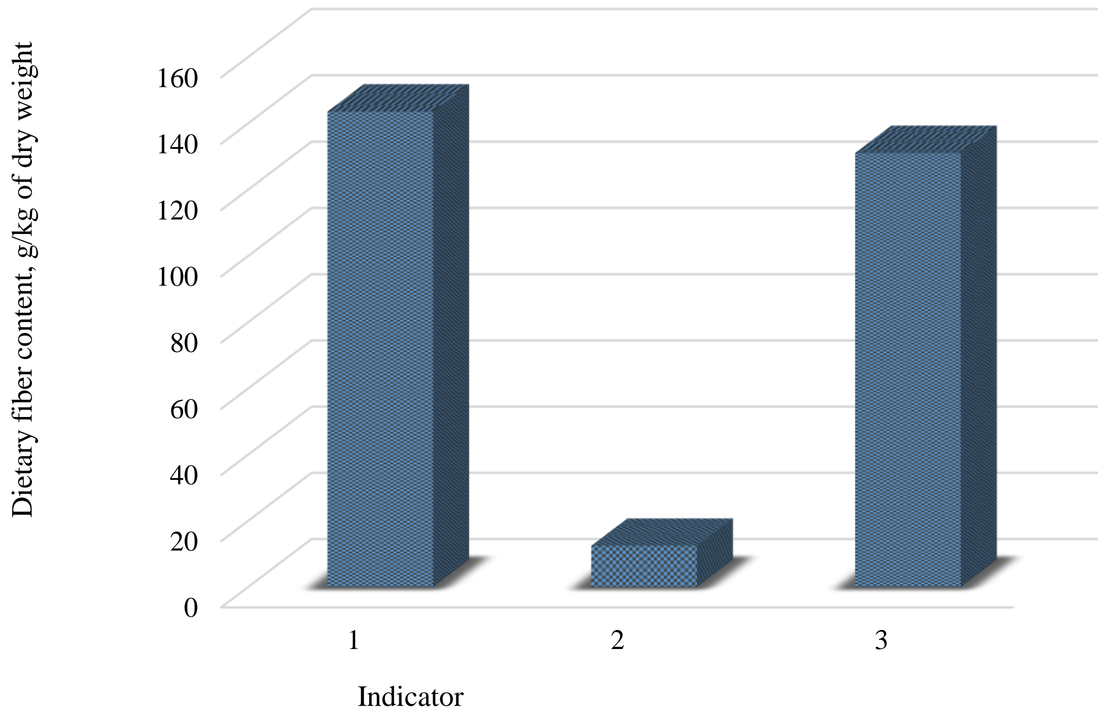

Fig. 4. Results of determination of total dietary fiber (1), easily (2) and hardly hydrolyzable (3) polysaccharides in the *C. vulgaris* biomass

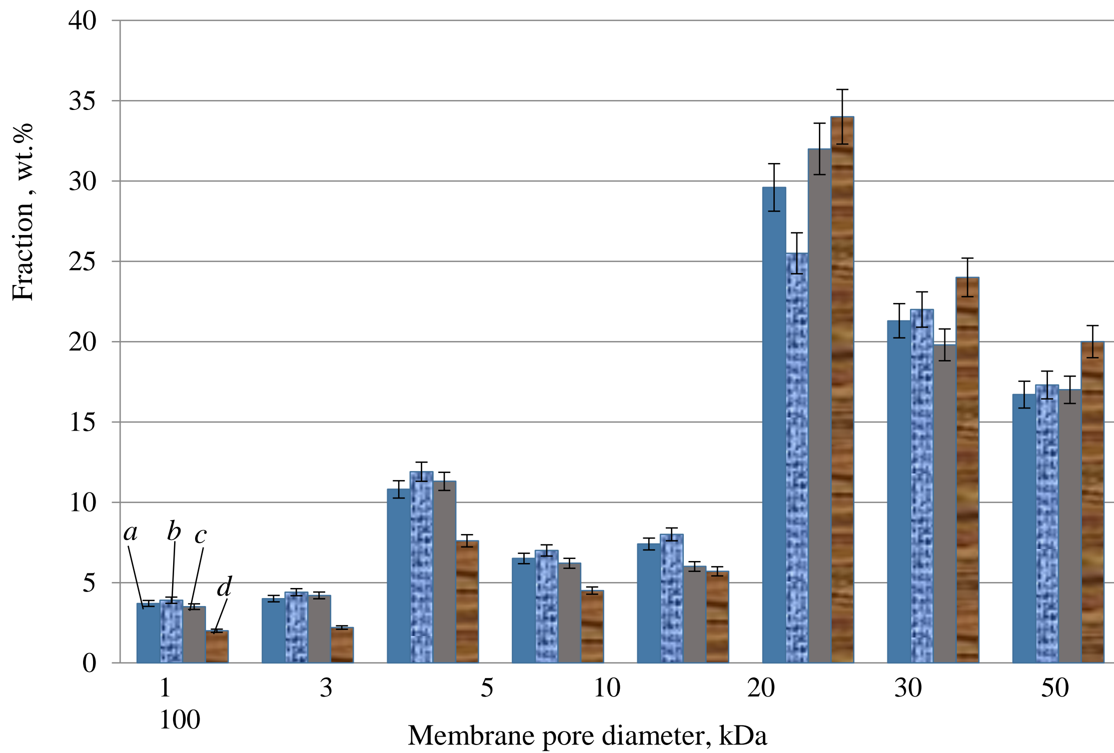

Fig. 5. Results of ultrafiltration separation of polysaccharides isolated from *C. vulgaris* biomass using different methods: a – alkaline extraction, b – hot water extraction*, c – microwave extraction, d –* enzymatic extraction

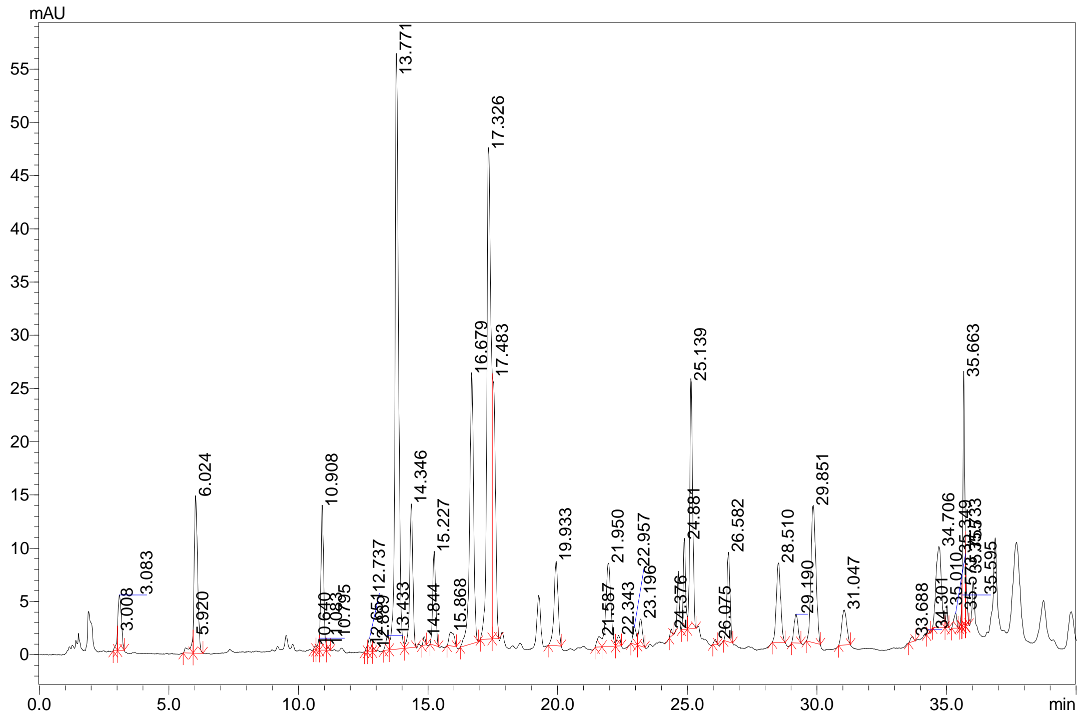

Fig. 6. Chromatogram of a hydrolyzed sample of *Chlorella* microalgae biomass; retention time for glycine 13.8 minutes, threonine – 15.2 minutes, alanine – 16.9-17.0 minutes, tyrosine – 18.8-18.9 minutes, phenylalanine – 24.9 minutes, isoleucine – 33.9-34.0 minutes, leucine – 34.6-34.7 minutes, lysine – 35.7 minutes, aspartic acid – 3.08 minutes, glutamic acid – 8.0 minutes, serine – 10.9 minutes, histidine – 13.7 minutes, cystine – 12.73 minutes, arginine – 19.93 minutes, alpha-aminobutyl acid – 21.58 minutes, methionine – 23.19 minutes, valine – 24.37 minutes.

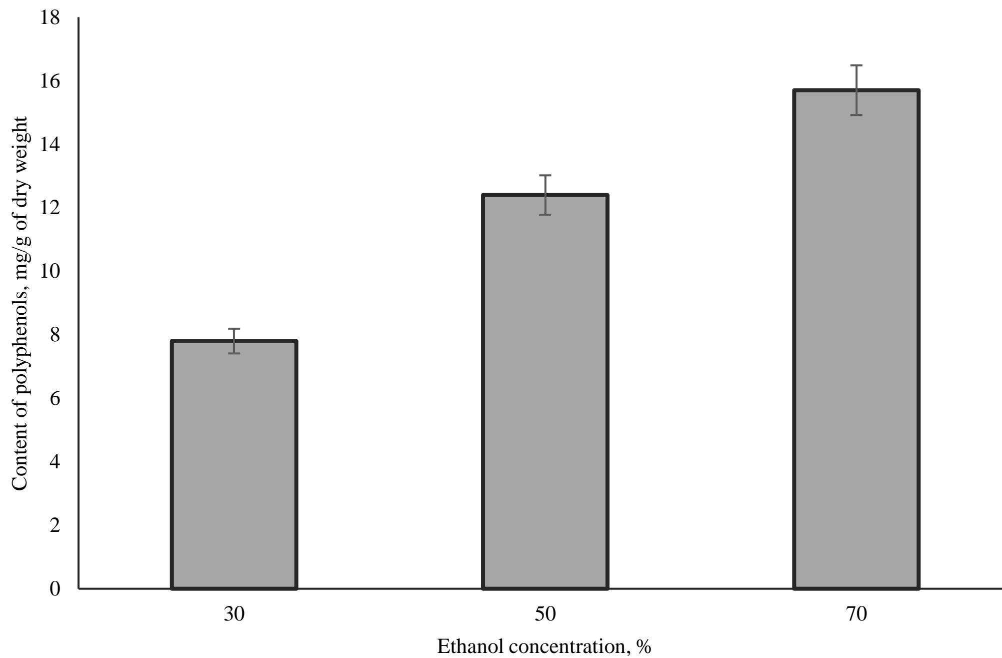

Fig. 7. Results of polyphenol content determination in ethanolic extracts of *C. vulgaris* microalgae

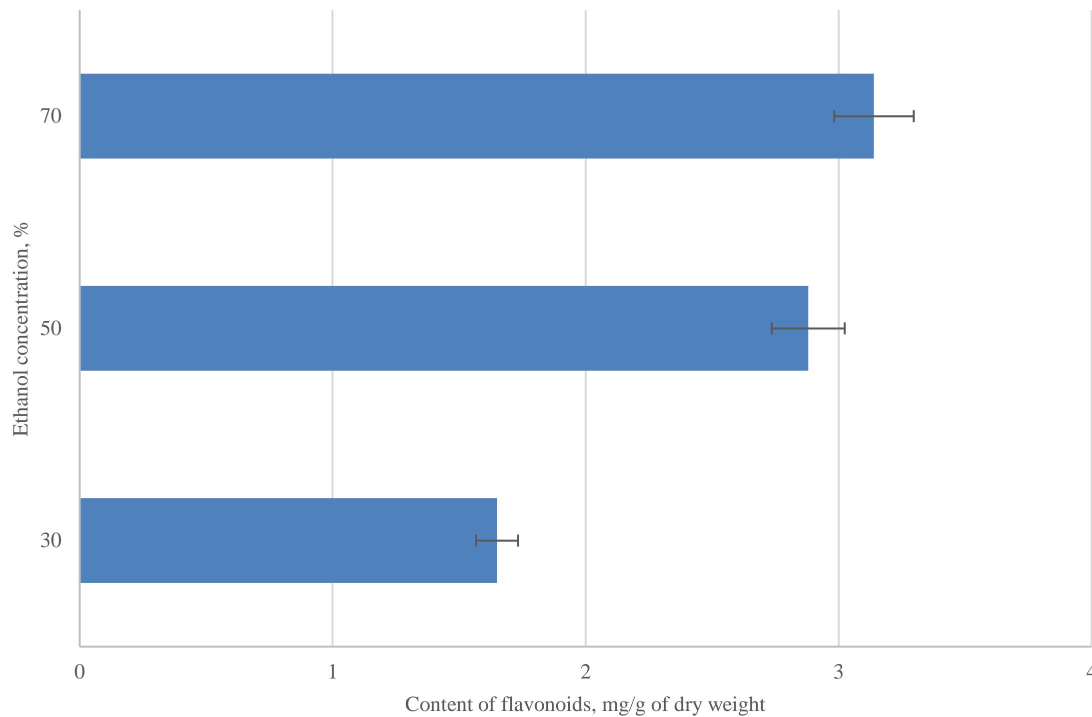

Fig. 8. Results of polyphenol content determination

Figure 8 demonstrates the accumulation of flavonoids in ethanolic extracts of the *C. vulgaris* microalgae. Analyzing Figure 8, it was concluded that the values of flavonoids content in *C. vulgaris* biomass extracts obtained using 50% and 70% ethyl alcohol were within statistical error: 2.9±0.2 mg/g and 3.1±0.2 mg/g, respectively. The results of the evaluation of the accumulation of polyphenolic substances and flavonoids in the *C. vulgaris* biomass agree with data from literature sources. Thus, polyphenols accumulate at 8.5-9.5 mg of gallic acid equivalent/g of dry matter (extractant - 80% ethanol) in *C. sorokiniana* and S. acuminatus biomass under zinc-induced stress, whereas this value is 4.0-6.0 mg of gallic acid equivalent/g dry matter in the absence of stress. The flavonoid content for these microalgae species under Zn-induced stress is 2.3 mg of quercetin equivalent/g of dry biomass versus 1.25-2.25 mg/g of dry biomass in the absence of stress [79].

In [78], the extraction efficiency of antioxidant components, including polyphenols, from the biomass of *Spirulina*, *Chlorella*, and *Phaeodactylum tricornutum* microalgae was studied using pressurized liquid extraction under the influence of different concentrations of dimethyl sulfoxide (DMSO). The content of polyphenols in the extracts was shown to increase with increasing solvent concentration (from 25 to 100%) from 3.0 to 10.0 mg/g of dry biomass.

The extraction efficiency of polyphenols from the biomass of *Scenedesmus* sp. and *Chlorella* sp. microalgae was studied with different solvents (ethanol, methanol, ethyl acetate). It was found that in ethanol extracts of Scenedesmus sp. the concentration of substances of polyphenolic nature ranged from 17.58 to 20.37 mg/g, in methanol extracts of Scenedesmus sp. – from 20.56 to 28.11 mg/g, in ethyl acetate extracts of Scenedesmus sp. – 5.22 to 8.12 mg/g. In ethanol extracts of *Chlorella* sp., the concentration of substances of polyphenolic nature ranged from 15.59 to 21.35 mg/g; in methanol extracts of *Chlorella* sp., it ranged from 19.81 to 31.26 mg/g; and in ethyl acetate extracts of *Chlorella* sp., it ranged from 1.05 to 5.25 mg/g [80]. Apart from polyphenolic substances, other compounds with antioxidant characteristics are carotenoids, which make up 3.36±0.12% of *Chlorella* sp. biomass.

The results obtained for caratinoid content in *Chlorella* extracts are shown in Table 12. According to the results presented in Table 12, the dominant carotenoid in the DMSO extract of *C. vulgaris* biomass is lutein (0.005 mg/mL extract). Carotenes (α- and β-) are found in lower concentration (0.0003 and 0.0002 mg/mL of extract, respectively). The results obtained correlate with the data obtained by the authors [78]. The authors of this study showed that in DMSO-extracts of *Chlorella* biomass, lutein accounted for 83.4% of the total carotenoid content, α-carotene for 10.3%, and β-carotene for 5.0%. In [81], a carotenoid profile of native *C. vulgaris* strain including lutein (1.853 mg/g dry weight), β-carotene (0.585 mg/g), phytoin (0.252 mg/g), neoxanthin (0.181 mg/g), violaxanthin (0.033 mg/g), and zeaxanthin (0.010 mg/g) was presented.

4. Conclusions

Thus, the chemical composition of *Chlorella* microalgae biomass was analyzed. It was demonstrated that 5.425 % of protein, 68.4 g/L of fat, 0.025 g/L of carbohydrates, and 0.1150 µg/g of iodine were accumulated in the biomass. The study of vitamin composition of *Chlorella* biomass revealed a high content of vitamins B6 (2.66 µg/mL), B2 (2.02 µg/mL) and C (0.99 µg/mL). The calcium content in the studied biomass was found to be 0.6 %. Among trace elements, iron (1152.0 mg/kg) and manganese (106.0 mg/kg) are the leaders. The polysaccharide profile of the *C. vulgaris* microalgae biomass was studied.

The total dietary fiber content of *Chlorella* cell walls was found to be 143.5 g/kg of dry weight. Among them, easily hydrolyzable polysaccharides account for 8.6% of the total dietary fiber content (12.4 g/kg of dry weight), and the share of hardly hydrolyzable polysaccharides is 91.4% (131.0 g/kg of dry weight).

The pigment-lipid profile of the *Chlorella* microalgae biomass was studied. It was found that 55.2% of the sum of all fatty acids in the sample accounted for palmitic acid.

Experimental data on the content of amino acids in the *Chlorella* microalgae biomass were obtained, indicating that the protein profile of the sample is characterized by the content of essential amino acids at the level of 41.9%.

The profile of antioxidant compounds (polyphenols, carotenoids) in the *C. vulgaris* microalgae biomass was investigated. It was demonstrated that the maximum concentration of substances of polyphenolic nature (15.6 mg/g of dry weight) from microalgae biomass can be extracted with 70% ethyl alcohol at an extraction temperature of 40 °C, a hydromodule of 1:50, and a process duration of 15 minutes.

Unique biochemical composition of *Chlorella* microalgae provides possibilities for obtaining polyunsaturated fatty acids, photosynthetic pigments, lipid-pigment complexes, essential amino acids, macro-, microelement, and vitamin complexes from it. These components can be used in the production of biofuels, biologically active food additives, functional foods, pharmaceuticals, and cosmetics.

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