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CHEMICAL COMPOSITION OF *Spirulina platensis* CULTIVATED IN UZBEKISTAN

A. S. Babadzhanov,[1] N. Abdusamatova,[1] F. M. Yusupova,[1] N. Faizullaeva,[1] L. G. Mezhlumyan,[2] and M. Kh. Malikova[2]

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*Conditions for growing spirulina (*Spirulina platensis*) were developed. The amino-acid and carbohydrate compositions were determined. Lipids and vitamins of the culture biomass were studied.*

Key words: microalga, growth medium, proteins, essential amino acids, lipids, vitamins.

Spirulina (*Spirulina platensis*) is a photosynthetic cyanobacterium that possesses biological activity and is widely cultivated to produce biologically active food additives and to treat several diseases [1]. The cell wall of the microalga lacks cellulose but includes mycosaccharides. Therefore, spirulina is 85-95% assimilated by an organism [2].

Climatic conditions in Central Asia are favorable for normal growth and development of spirulina. Conditions for growing this culture on modified Zarrouk medium, which is highly standardized and ecologically pure, were developed at the "Nikhol" facility of the ROSC MH RUz. Spirulina grew best and accumulated biomass free of bacteria in 100 and 50% Zarrouk medium during 15 and 12 days, respectively. The nutrional value was due to an increased content of protein and other biologically active substances.

We investigated the chemical composition of ecologically pure high-quality raw material prepared under local conditions.

It should be noted that spirulina contains much more protein than many food products. Thus, the average value in spirulina is 65%; in meat and fish, 15-20%; in soy, 35%; in evaporated milk, 35%; in peanuts, 25%; in eggs, 12%; and in grains, 8-14% [3]. Spirulina protein is complete and contains all essential amino acids. Using the quantitative Kjeldahl method, we found a protein content of 68% in spirulina biomass samples grown during spring and summer. Table 1 shows that the total sum of amino acids in the culture consists of 60% essential ones.

Owing to the high-value proteins, essential amino acids, vitamins, β-carotene, minerals, polysaccharides, and essential fatty acids, spirulina is used as a biologically active additive. The microalga is know to contain 2-5% sulfolipids, which makes it active toward the AIDS virus [4]. Therefore, we then studied lipids of spirulina grown on modified Zarrouk medium.

The lipid content in the biomass was 14.3% (total) of air-dried mass. Of these, 10.9% were fatty acids; 3.4%, unsaponified substances. The carotinoid content was 6.25% (of the mass of unsaponified substances) and 0.21% (of the biomass). Table 2 presents the fatty-acid composition.

The yield of neutral, phospho-, and glycolipids was 14.3%. Total essential fatty acids (linoleic and gamma-linolenic) reached one third of their mass. These acids are precursors of prostaglandins, hormones that control many important functions.

The performed a chemical analysis of the carbohydrate composition of *S. platensis*. Carbohydrates are known to be necessary for hormonal exchange of proteins and fats. They in combination with proteins form certain hormones and enzymes and secretions of salivary and other glands [5]. Spirulina carbohydrates include polysaccharides that are readily assimilated by the organism with minimal losses of insulin [6].

1) Experimental facility "Nikhol," Republic Oncology Scientific Center, Ministry of Health, Republic of Uzbekistan, Tashkent, ul. Farabi, 383; 2) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 232-234, May-June, 2004. Original article submitted December 9, 2003.

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TABLE 1. Amino-acid Composition of *S. platensis*

Amino acids	%	Amino acids	%
Asp	0.9	Met	0.8
Thr	0.5	Yle	1.3
Ser	0.6	Leu	0.8
Glu	1.0	Tyr	3.3
Pro	0.3	Phe	2.5
Gly	0.6	His	4.7
Ala	1.0	Lyz	1.9
Val	1.3	Arg	2.1

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Not including Trp and Cys

TABLE 2. Properties of *S. platensis* Lipids

Fatty acids	mg/kg biomass	mass % of fatty acids
12:0	437	0.4
14:0	874	0.8
16:0	49031	44.9
16:1	2512	2.3
17:0	1310	1.2
18:0	2402	2.2
18:1	11029	10.1
18:2	12121	11.1
18:3	18672	17.1
others	10811	9.9

TABLE 3. Water-Soluble Vitamins in Spirulina Biomass, %

Vitamins	Ser. 150102 (sample collected in winter)	Ser. 240402 (sample collected in spring)	Ser. 200702 (sample collected in summer)	Ser. 21002 (sample collected in spring)
Ascorbic acid	42.8	42.0	106.2	195.3
Nicotinamide	5.3	0.6	0.4	0.9
Pyridoxine	4.0	0.6	0.3	0.5
Riboflavin	0.8	0.7	0.9	0.2
Thiamine	11.6	15.4	0.8	0.8
Cyanocobalamine	0.4	0.8	0.3	0.3
Folic acid	0.6	0.4	0.2	0.6

We treated the culture biomass with $CHCl_3$ to isolate the carbohydrates by removing the noncarbohydrate components. Sugars soluble in alcohol (SSA, 5.14%) were extracted by boiling in ethanol (82%). Then, water-soluble polysaccharides (WSPS, 2.0%) and hot-water-soluble polysaccharides (HWSPS, 2.8%), which contained starch according to a positive reaction with I_2, were isolated in succession. Pectinic substances (PS) were extracted by a mixture of oxalic acid and ammonium oxalate (2.0%). Hemicellulose (HC, 4.6%) was determined by extraction with alkali (5%). The quantitative yields of SSA and HC were highest in the isolated carbohydrates.

Vitamins are the principal factor in the biological properties of spirulina. We identified the following vitamins (mg/100 g) in the biomass: vitamin C (42.0-195.3 mg), nicotinamide (0.6-5.3), B_1 (0.8-15.4 mg), B_2 (0.2-0.9 mg), B_6 (0.3-4.0 mg), B_c (0.2-0.6 mg), B_{12} (0.3-0.8 mg), β-carotene (64-140 mg), vitamin E (10-19 mg), and vitamin D (12,000 U). Biomass grown in spring-summer had a high vitamin content. Spirulina contains the most important vitamins, especially B vitamins, in the optimal ratios (Table 3).

Thus, we developed local strains of *S. platensis* by collecting samples, growing cultures, cloning, and determining the optimal conditions for growth and development of the alga. The protein content in dry biomass of *S. platensis* grown in Uzbekistan is 68% and has a complete set of amino acids, including essential ones. The biomass contains a large amount of essential fatty acids and gamma-linolenic acid. Spirulina carbohhydrates have a high content of SSA and HC. *S. platensis* has an optimally balanced complex of B vitamins.

EXPERIMENTAL

The nutrient medium for growing spirulina was 50% modified Zarrouk medium (Zarrouk, 1966) with continuous sparging of air. The growth temperature was 25-27°C. The illumination on the culture surface was 7000-8000 lux. Daylight lamps (LDS-125) were used.

Spirulina growth and development were determined by microscopy and by the change of dry biomass. It was separated by filtration through caproic cloth, dried, and then used for the analyses.

Proteins were isolated by the literature method [8] and determined by the Kjeldahl method [7]; amino acids, on a T-339 amino-acid analyzer (Czech Rep.) after hydrolysis in HCl (5.7 N) at 110°C for 24 h.

Isolation of SSA. Raw material (5 g) was boiled in $CHCl_3$ (40 mL) twice for 2 h, removed by filtration, dried, and extracted twice with alcohol (50 mL, 82%) on a water bath at 90°C for 1.5 h. Yield 0.257 g.

Isolation of WSPS. The remaining raw material was extracted twice with H_2O (30 mL) with constant stirring at room temperature. The extracts were combined and evaporated to a small volume. Addition of alcohol (1:5) produced a precipitate that was separated by centrifugation and dried. Yield 0.1 g.

Preparation of HWSPS. The remaining raw material was extracted twice with hot water at 80-85°C (1:3) for 1.5 h. The extracts were evaporated. WSPS were precipitated by adding alcohol (1:5), separated, and dried. Yield 0.14 g.

Isolation of PS. PS were extracted twice by a mixture of oxalic acid and ammonium oxalate (both 0.5%, 1:1) at 70-80°C in a 1:3 ratio for 1.5 h. Yield 0.1 g.

Isolation of HC. HC were extracted twice from the remaining raw material by NaOH solution (5%, 1:3) at room temperature for 1 h. The extracts were dialyzed and evaporated. Addition of alcohol (1:5) produced a precipitate. Yield 0.23 g.

Total lipids were extracted thrice by $CHCl_3$:CH_3OH (2:1).

The total extract was purified of nonlipid components by washing with aqueous $CaCl_2$ (0.05%). Then, the solvent was evaporated in a rotary evaporator. The lipids were dried in a vacuum drying chamber.

The contents of fatty acids and unsaponified substances were determined as before [9]; of carotinoids, colorimetrically using the "Addendum to the USSR State Pharmacopeia" (Moscow, Xth Ed., Vol. 3, 321, 1986).

GC of fatty-acid methyl esters was performed on a Chrom-5 instrument using a steel column (2.5 M) packed with Reoplex 400 (5%) on Inerton N-AW (0.16-0.20 mm) at column temperature 190°C and N_2 flow rate 30 mL/min.

Methyl esters were prepared by treating fatty acids with diazomethane [10].

Determination of Water-soluble Vitamins in *S. platensis* Biomass. Air-dried biomass (0.1 g) was suspended in C_2H_5OH:CH_3CN:H_2O:F_3CCO_2H (TFA) (15:15:85:0.1 by vol.) and heated on a water bath at 60-70°C for 5 min. The mixture was centrifuged at 10,000 rpm for 3 min. Supernatant was collected for quantitative determination of vitamins.

Identification and Quantitative Determination of Vitamins by HPLC on Reversed-phase Sorbents.

Vitamin standards: solutions of ascorbic acid, nicotinamide, riboflavin, thiamine hydrochloride, and pyridoxine hydrochloride in the aforementioned solvent at 0.01 mg/mL.

Column: Ultrasphere ODS (0.46 × 25 cm) or analogous column with octadecylsilyl sorbent.

Mobile phase: the aforementioned solvent at flow rate 1 mL/min.

Detection: at 254 nm.

Chromatography of standard mixtures of water-soluble vitamins gave the retention time for each vitamin and the corresponding peak area at the given concentration. Then, chromatography of the biomass and tablet extracts was performed under these same conditions. Vitamins were identified by their retention times and were determined quantitatively using the formula:

$$X = (S_{sam} \times C_{std} \times 1000)/S_{std},$$

where X is the amount of vitamin (mg/100 g of air-dried biomass), S_{sam} is the peak area of the determined vitamin from the chromatogram of the tested biomass extract, S_{std} is the peak area of the same vitamin from the chromatogram of the standard vitamin solution, C_{std} is the vitamin concentration in the standard solution, and 1000 is a conversion factor to give mg of vitamin per 100 g of biomass.

REFERENCES

1. L. P. Blinkova, O. B. Gorobets, and A. P. Baturo, *Zh. Mikrobiol.*, 2, 114 (2001).
2. *Earth food Spirulina: A new food to restore our health and planet*, Spirulina Health Library, July 1994.
3. R. Herehson, *Earth Food Spirulina*, Konore Press (1977).
4. N. N. Besednova et al., *Zh. Mikrobiol.*, **12**, 75 (1979).
5. O. K. Voronova, *Al ′gologiya*, **4**, 2, 76 (1994).
6. Yu. N. Loenko et al., *Rastit. Resur.*, **27**, 3, 150 (1991).
7. G. A. Kochetov, *Practical Handbook of Enzymology* [in Russian], Vysshaya Shkola, Moscow (1980).
8. T. Devenyi and J. Gergely, *Amino Acids, Peptides, and Proteins: Biochemical and Immunochemical Techniques in Protein Chemistry*, Elsevier-Scientific, New York (1974).
9. M. Kates, *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Elsevier, New York (1973).
10. L. Fieser and M. Fieser, *Reagents for Organic Synthesis*, Wiley, New York (1967).