EVALUATION OF SOME CHEMICAL CHARACTERISTICS OF SPIRULINA FROM DIFFERENT MANUFACTURERS

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ABSTRACT

Some chemical characteristics of Spirulina from different countries (the USA, China, Belgium, and Bulgaria) were investigated. The study includes a comparative analysis of the content of some elements, tocopherols, and fatty acids in spirulina. The total lipids of the examined sample varied from 1.9 % (China) to 6.1 % (Belgium). Gas chromatography was used to determine the fatty acid composition of glyceride oil isolated from spirulina of different origins. Inductively coupled plasma-optical emission spectrometry and liquid chromatography were used to determine the elemental composition and tocopherol content. The fat content of Spirulina from Bulgaria approaches that of China and the USA, while that of Belgium is almost three times higher. It was found that palmitic acid is the main saturated fatty acid in all samples, and for spirulina from Bulgaria, it reaches 81.2 %. The content of unsaturated fatty acids is highest in that originating in the USA.

Keywords: Spirulina, fatty acid composition, chemical content.

INTRODUCTION

Cyanobacteria (*Spirulina platensis*) is considered a food source rich in proteins, vitamins, elements, essential amino acids, carbohydrates, fats, and some others [1, 2]. *Spirulina* also contains some more specific compounds such as phycocyanin beneficial for human health [3 - 5]. Consumption of *Spirulina platensis* in the form of food or dietary supplement has a number of beneficial effects on human health. It is known for its antimicrobial [6], antiviral, immunostimulating [7], anticoagulant [8], hepatoprotective [9], and antioxidant effects [10]. It has a positive effect on the condition of patients with anemia, obesity, accumulation of heavy metals in the body, malnutrition, diabetes, etc. [11, 12]. For these reasons, *Spirulina* is the object of development of different production technologies in different parts of the planet due to a number of favorable factors:

- no arable land is needed [13, 14];
- can be grown in lakes and saltwater reservoirs;
- gives a large yield of biomass per unit area [15];
- shows a good gelling, foaming, and emulsifying effect [16 - 18];

- improves the antioxidant and water adsorption properties of pasta products and their texture [19].

Microalgae and cyanobacteria possess a diverse lipid composition, giving a wide range of their applications from biofuels to food additives [20]. They are good sources of vitamins, minerals, proteins, polyunsaturated fatty acids, carotenoids, etc. An ω_3/ω_6 ratio of 1:1 to 1:4 is considered optimal in terms of health prevention of non-communicable and chronic diseases, and microalgae can produce a greater amount of omega-3 than those found in animal oils [21].

The aim of the present research is to study and analyze the fatty acid, elemental, and tocopherol composition of *Spirulina platensis* grown in a bioreactor in Bulgaria and to compare it with samples from other countries with the aim of developing nutritional supplements.

EXPERIMENTAL

Samples

Spirulina powder samples were purchased from organic stores in Bulgaria originating from the USA, Belgium and China, the Bulgarian *Spirulina* produced by the bioreactor in Varvara, Bulgaria, is conventionally dried and in powder form.

Determination of content of elements

A sample (0.3 g) is weighed on an analytical balance in Teflon vessels for a microwave digestion system, and 8 mL of 67 % HNO_3 (Suprapur) was added. Microwave digestion was carried out according to the following procedure: 10 minutes to reach 180°C and maintain this temperature for 10 minutes. After cooling solution was transferred into a 25 mL volumetric flask and diluted to the mark with deionized water. The blank sample was run through the entire analytical procedure. Samples were filtered through 0.45 μm cellulose membrane filters (Millipore) and kept at 4°C [22].

Apparatus for quantitative measurement of chemical elements

An ULTIMA 2 ICP-OES system, Jobin Yvon, (Longjumeau, France) was used to determine the content of Ba, Ca, Cr, Sr, and S. To prepare the diluted working standard solutions for instrument calibration, standard solutions were used: Ba - 1 g dm^{-3}, Ca - 1 g dm^{-3}, Cr - 1 g dm^{-3}, Sr - 1 g dm^{-3} and S - 1 g dm^{-3} for ICP (TraceCERT®, Merck).

Isolation of glyceride oil

The oil was extracted in a Soxhlet apparatus using n-hexane and the oil content was determined gravimetrically [23].

Fatty acids

The fatty acid composition of the glyceride oil was determined by gas chromatography (GC) [24]. Fatty acid methyl esters (FAMEs) were obtained by transesterification of the oil with methanol in the presence of sulfuric acid [25]. Determination was performed on Agilent 8860 gas chromatograph equipped with a capillary column DB-FastFAME column (30 m x 0.25 mm x 0.25 μm (film thickness) and a flame ionization detector (FID). The column temperature was programmed from 70°C (hold for 1 min), at 6°C min^{-1} to 180°C, and at 5°C min^{-1} to 250°C. The injector and detector temperatures were 270°C and 300°C; the carrier gas was nitrogen and the split ratio was 50:1. Identification was carried out by comparison of the retention times with those of a standard mixture of FAME (37 components, Supelco, USA).

Tocopherols

Tocopherols were determined on a Merck Hitachi high-performance liquid chromatograph with Nucleosil Si 50 - 5 column (250 × 4 mm, particle size: 5 μm) and fluorescent detection at 295 nm excitement and 330 nm emission. The mobile phase was hexane: dioxane, 96:4 (v/v) and the flow rate - 1 mL min^{-1} [26].

RESULTS AND DISCUSSION

Knowing the fatty acid, tocopherol and elemental composition of spirulina samples gives reason to believe that the cyanobacterium can be used as an alternative to synthetic vitamins, mineral supplements, and others used in various diets and fitness programs.

The oil content of the *Spirulina* ranges from 1.9 % to 6.1 %. The highest total lipids are found in the sample from Belgium and the lowest is observed in that from China. The *Spirulina* produced by the bioreactor has 2.3 % lipid content, which is similar to that of the sample from the USA (2.2 %).

The distribution of fatty acids in the studied samples is presented in Fig. 1 and Fig. 2a, b, c, d.

The differences in the fatty acid composition of the

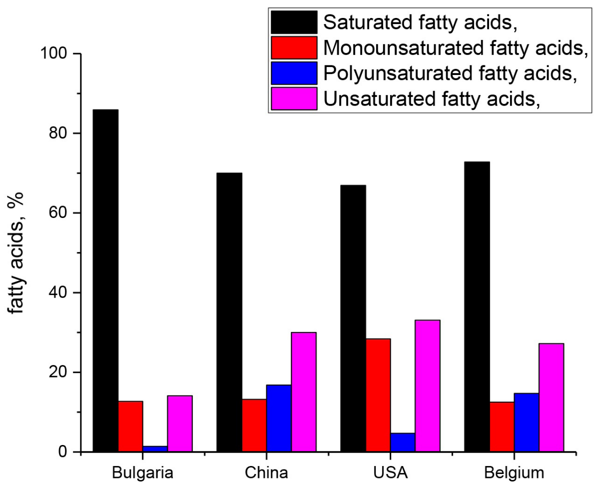

Fig. 1. Distribution of fatty acids in the studied samples.

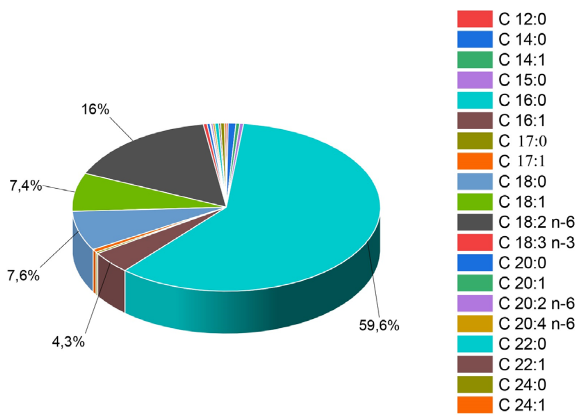

Fig. 2 (a). Fatty acid composition of the *Spirulina* samples from Bulgaria.

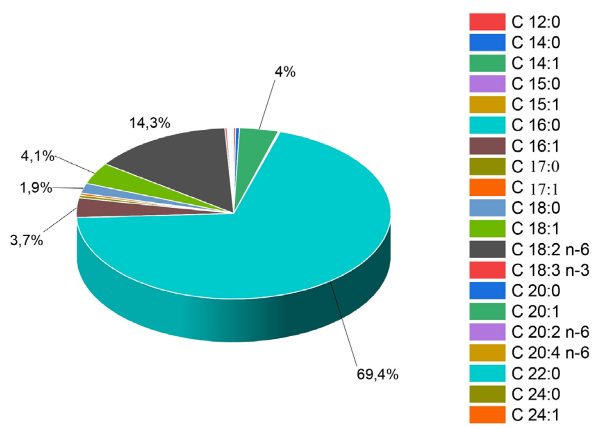

Fig. 2 (b). Fatty acid composition of the *Spirulina* samples from China.

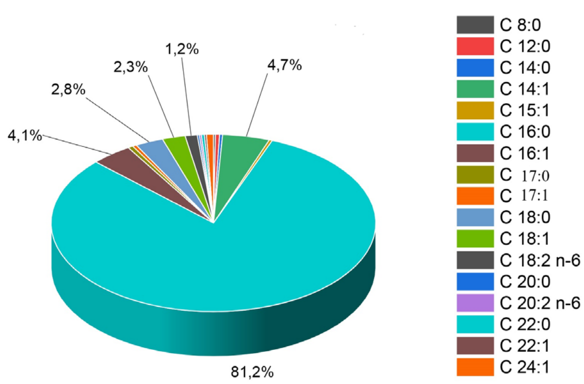

Fig. 2 (c). Fatty acid composition of the *Spirulina* samples from USA.

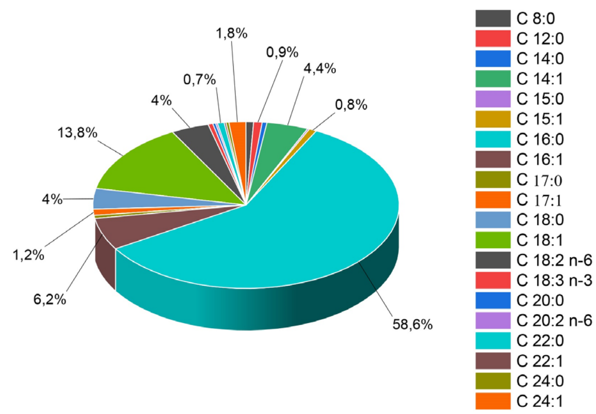

Fig. 2 (d). Fatty acid composition of the *Spirulina* samples from Belgium.

studied samples may be due to the environment, habitat, growing season, the presence of light, temperature, pollution, and finally, extraction methodology during the analysis, etc.

22 types of fatty acids have been identified, classified into three groups saturated, polyunsaturated, and monounsaturated. Saturated fatty acids predominate in all samples, with the sample from the Bulgarian bioreactor having the highest value (85.9 %), and the sample from the USA (66.9 %) the lowest. The sample from the USA has the highest amount of unsaturated fatty acids (33.1 %), followed by the one from China (30 %). The high amount of saturated fatty acids is associated with a higher growth temperature of the cyanobacterium [27 - 29]. If unsaturated fatty acids predominated, the samples were lyophilized, otherwise, the samples were convection dried [30].

There is a difference between the distribution of polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids. For the samples from Bulgaria and the USA, the distribution of fatty acids is according to the following inequality: SFA > MUFA > PUFA, which is different from the distribution in the samples from Belgium and China: SFA > PUFA > MUFA.

There is evidence in the literature that cyanobacteria are a source of ω-6 but not ω-3 fatty acids [31]. This statement is confirmed in the samples from Belgium and China, which contain from 14 % to 16 % ω-6, the American one contains less than 4 % of it, and it is not found in the Bulgarian one.

In the group of saturated fatty acids, there is the following inequality C16:0 > C18:0 > C14:0 ≈ C15:0 ≈ C12:0 ≈ C17:0 ≈ C20:0 ≈ C22:0. In the studied samples, palmitic acid (C16:0) is the most abundantly distributed. Differences exist for the other fatty acids with a higher content in the analyzed samples. In the sample from China, after palmitic acid (C16:0) in content is linoleic (C18:2) followed by oleic (C18:1) and stearic (C18:0), palmitoleic acid (C16:1). In the US sample, the distribution is C16:0 > C18:1 > C16:1 > C18:0 ≈ C18:2. Similar results in fatty acid composition are reported [31, 32]. The predominant fatty acids in s*pirulina* samples can be used as a basis for the development of various nutritional supplements. Monounsaturated fatty acids oleic acid (C18:1) and palmitoleic acid (C16:1) can be associated with improving the function of the cardiovascular system and reducing bad cholesterol in the body. The reduction of oxidative stress and protection of cells from the action of free radicals can be achieved under the action of stearic acid (C18:0) and linoleic acid (C18:2). The palmitic acid pathway plays an important role in the digestive process.

In addition to the fatty acid composition, the tocopherol content was also investigated, presented in Fig. 3a and Fig. 3b.

No tocopherols were detected in the Bulgarian sample, the highest total content of tocopherols was measured in the sample from China. Distribution of the types of tocopherols as a percentage of the total mass was made. The high content of α-tocopherols, in the samples

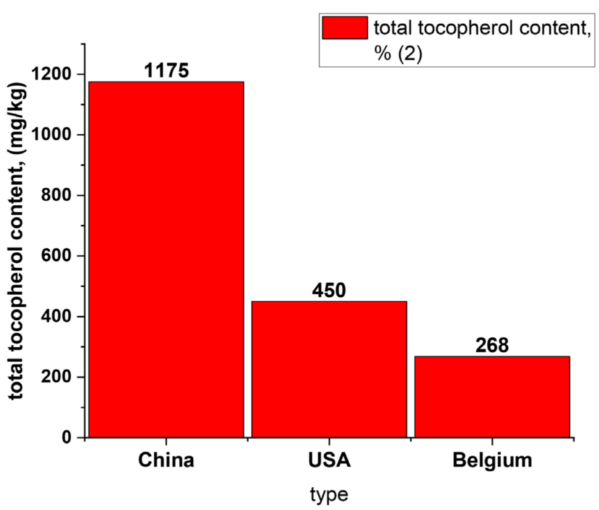

Fig. 3 (a). Total tocopherol content of Spirulina samples.

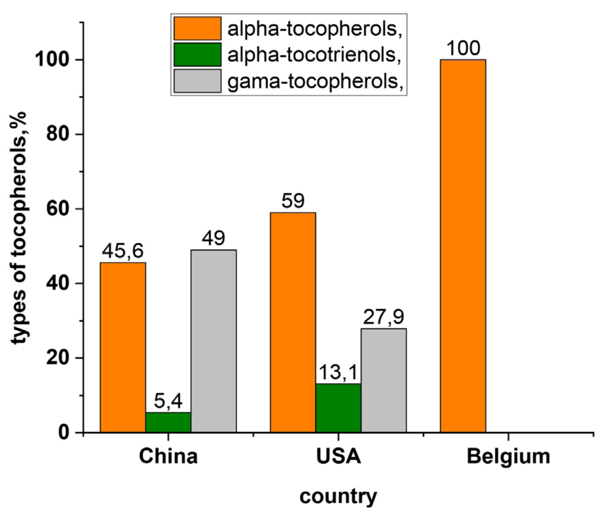

Fig. 3 (b). Percent of tocopherol content of dry mass.

Table 1. Concentrations of Ba, Ca, Cr, Sr and S in *Spirulina* samples (RSD = 2 - 5 %, n = 3).

Elements	Ba, mg kg^{-1}	Ca, mg kg^{-1}	Cr, mg kg^{-1}	Sr, mg kg^{-1}	S, mg kg^{-1}
Bulgaria	4.16	5132	2.53	25.3	4833
China	2.49	1483	2.49	33.3	6500
USA	2.35	2666	3.35	25.2	5833
Belgium	2.34	2315	2.51	141	8583

from the USA (59 %) and Belgium (100 %), makes these sources of *Spirulina* potentially useful for cosmetic and pharmaceutical products. α-tocopherol is known for its antioxidant properties and its ability to protect the skin from the action of free radicals. This would provide an opportunity to incorporate cyanobacteria extracts into cosmetic products that reduce premature aging of the skin and improve its elasticity and fresh appearance. The presence of γ-tocopherol in some samples is also associated with the anti-inflammatory effect on the skin.

The content of chemical elements Ba, Ca, Cr, Sr and S in four studied samples is shown in Table 1.

As can be seen, both elements Ca and S are macro components and Ca content in Bulgarian *Spirulina* is statistically different showing almost twice higher results compared to all other samples while S is with the lowest concentration. The lowest Ca content is determined in *Spirulina* from China. The highest S concentration is measured in *Spirulina* from Belgium. The concentration range for Ca reported in the literature varied between 0.64 to 63.5 g kg^{-1} and for S between 3.60 to 9.92 g kg^{-1} [32]. Similar Ca concentrations to those found for BG S*pirulina* were found in tablets from India and China and *Spirulina* powder from China. In the *Spirulina* powder from Taiwan, the sulfur content is close to that found in the sample from China [32]. In dietary supplements from various pharmaceutical companies containing *Spirulina*, researchers from Argentina obtained Ba concentrations from 3.79 to 36.2 μg g^{-1}, these results are higher than ours, except for that of BG *Spirulina* [33]. Other authors reported for the samples of *Spirulina* powder, mean concentrations of Ba - 1.25 ± 0.65 mg kg^{-1} dw [34]. For Sr concentrations are from 25.2 to 141 mg kg^{-1}, the reported values for this element in the literature vary in a very wide interval: 4.39 - 478 mg kg^{-1} [32], therefore, the obtained results are comparable to those already known. Results for the traceable elements follow an approximate distribution S > Ca > Sr and Ba ≈ Cr.

CONCLUSIONS

It was found that spirulina from Bulgaria differs from the other samples with the highest values of saturated fatty acids, followed by monounsaturated fatty acids. In addition, no tocopherols were detected in Bulgarian samples. Surprisingly, Belgian samples of *Spirulina* showed 100 % α-tocopherol. However, Bulgarian *Spirulina* showed the highest Ba and Ca content and the lowest Sr and S values.

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