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Characterization of organic *Spirulina* spp. and *Chlorella vulgaris* as one of the most nutrient-dense food

Charakterisierung von bio form Spirulina spp. und Chlorella vulgaris als eines der nährstoffreichsten Lebensmittel

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Summary

The microalgae *Spirulina* spp. and *Chlorella vulgare* are commercially produced and distributed worldwide as dietary supplements. In this paper, commercial products dried organic form of *Spirulina* spp., and *Chlorella vulgaris* were evaluated in terms of nutritional value, safety parameters, and antioxidant potential. The protein content was slightly higher in *Spirulina* spp., while *Chlorella vulgaris* was richer in terms of dietary fiber content. *Chlorella vulgaris* showed to be a source of omega-3 fatty acids, polyunsaturated fatty acdis (PUFA) and had a favourable ratio of omega-6/omega-3 fatty acids. In addition, *Chlorella vulgaris* had slightly higher content of total amino acids (TAA) and essential amino acids (EAA) than *Spirulina* spp., with leucine, glutamic and spartic acid being the most abundant in both algae. The tested microalgae showed a high content of essential minerals and trace elements required for human nutrition. When analysed antioxidative potential of the microalgae it was found that *Chlorella vulgaris* had a higher phenolic content and antioxidant activity determined by DPPH and RP assays while *Spirulina* spp. showed a higher antioxidant capacity measured by ABTS test. The established values of microbiological parameters as well as heavy metals do not violate regulatory limits that support the fact that most products *Spirulina* and *Chlorella* from several large producers have "GRAS" designations.

Keywords: microalgae, *Spirulina* spp., *Chlorella vulgaris,* antioxidant value, nutritional value, safety parameters

Introduction

The growing world's population is expected to reach a whopping 9.8 billion by 2050 (https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html 2017). In that sense, great attention has been given to meet the population's need for a more sustainable protein supply for food and feed. Changes in consumer behavior and interest in alternative sources of protein, partly due to health and environmental concerns as well as animal welfare, have made a growing interest in the non-meat-based protein. Alternatives are protein-rich ingredients sourced from plants, insects, fungi, but single cells (Hadi and Brightwell 2021).

Microalgae are considered a sustainable source of high-valuable nutrients with health benefits. They contain up to 70% of proteins, along with rich polyunsaturated fatty acids (PUFA), vitamins, minerals, fiber, polysaccharides, enzymes, photosynthetic pigments (carotenoids and chlorophylls), and sterols (Dineshkumar et al. 2017, Gómez-Zorita et al. 2020, Khan et al. 2018). In this respect, microalgae appear as a promising supplement in the era of functional food and feed production.

Owing to their composition, as well as their simplicity of cultivation, microalgae have received considerable attention as potential supplements in the food, feed, and pharmaceutical industry. Due to the plentiful of bioactive compounds in microalgae, during the past decades, microalgal biomass has been predominately used in the health food market, with more than 75% of annual microalgal biomass production being employed for the manufacture of powders, tablets, capsules, or pastilles (Chacón-Lee 2010, Galasso et al. 2019). Instantaneously, approximately 30% of the current global production of microalgae has found application in the feed industry (Levasseur et al. 2020, Milledge 2010). Microalgal biomass can be incorporated into the diet of a wide variety of animals ranging from fish to livestock (Levasseur, Perré and Pozzobon 2020, Madeira et al. 2017) since valuable nutritional compositions of microalgae justify their usefulness as feed ingredients. Hence, they could positively influence the nutritional value of the most worldwide consumed meats (pork and poultry meat), through their enrichment in PUFA, carotenoids as important antioxidants, as well as iodine content (El-Bahr et al. 2020). Additionally, the supplementation of distinct microalgae species in diets for farm animals can successfully improve animals' health and productivity by increasing their growth performance parameters (Świątkiewicz et al. 2019).

The perspective of microalgae utilization as food and feed supplements lies in the diversity of biomass composition. This can be achieved either by strain selection or by growth condition manipulation. The chemical composition of microalgae depends on the species and cultivation conditions, such as temperature, illumination, pH, CO_2 supply, as well as salt and nutrients content (Mimouni et al. 2012). Indeed, microalgae can modulate their biochemical composition in response to environmental changes. As a consequence, researchers have developed strategies based on metabolic imbalances that divert the electron energy towards the selected target (Gifuni et al. 2019). These are usually referred to as stressing procedures and feature nutrient depletion, high light irradiance, extreme pH, temperature, high salinity, or metal concentration.

Regardless of the target application (either for food or feed production), a word of caution is necessary since microalgae can easily accumulate heavy metals, such as arsenic and lead, which are potentially deleterious for human or animal health, thus requiring monitoring to avoid toxic effects (Rzymski et al. 2018). Moreover, recent reports also point out that microalgae can be a source of polycyclic aromatic hydrocarbons (PAHs) (Grosshagauer et al. 2020). Another limiting factor of using large quantities of microalgae for human consumption is the presence of the high content of nucleic acids that are metabolized to uric acid and might result in adverse health effects, such as gout or kidney stones Gantar and Svircev (2008).

Since microalgae have the great potential for the production of high value-added food/feed products, research efforts should be directed into their chemical, nutritive, and safety characterization. Therefore, the present study aimed to investigate the nutritional and safety parameters, as well as the antioxidant potential of organic *Chlorella vulgaris* and *Spirulina* spp. in the role of a new generation food and feed supplements.

Material and methods

Samples

Commercial products produced by "Hemp products and other super foods" Serbia, containing a dried organic form of *Spirulina* spp., and *Chlorella vulgaris,* which were purchased in a pharmacy of Serbia, were used for all the analysis. Samples imported from the United Kingdom with the origin of China, the number of lots: 160124 for *Chlorella vulgaris* and O SP-JY-160427 for *Spirulina* spp.

Methods

Nutritive value

The nutritional analysis of *Spirulina* spp. and *Chlorella vulgaris* were performed in triplicate. The moisture content was determined according to AOAC Method 934.01, while crude protein content was determined by Kjeldahl method (AOAC Method 978.04). Determination of crude ash content was done using AOAC Method 942.05, crude fat according to the Soxhlet extraction (AOAC Method 920.39), while crude fiber was determined using AOAC Method 978.10. Macro (Ca, P, Mg, Na, K) and micro (Fe, Mn, Zn) element contents were determined according to EN ISO 6869:2008 method based on atomic absorption spectrometry. Heavy metal analysis (Pb, Hg, Cd, and As) was performed by atomic absorption spectrometry after dry ashing according to FINSLab Method 5.4-3M-004/13 which is a documented method based on EN 14082:2003.

The sample preparation for amino acids analyses included hydrolysation in 6M HCl (Merck, Germany) at 110 °C for 24 h, followed by cooling to room temperature (Ponka et al., 2016). Thereafter, samples were filtered and made up to 25 mL in sodium loading buffer (pH 2.2) (Biochrom, Cambridge, UK). Subsequently, prepared samples were filtered through 0.22 µm pore size PTFE filter (Plano, Texas, USA), and the filtrate was transferred to an HPLC vial (Agilent Technologies, USA). Amino acid profile of prepared samples was performed using ion-exchange chromatography with utilization of Automatic Amino Acid Analyzer Biochrom 30+ (Biochrom, Cambridge, UK) according to Spackman et al. (1958) and (Ponka et al. 2016). The technique was based on amino acid separation using strong cation exchange chromatography, followed by the ninhydrin colour reaction and photometric detection at 570 nm and 440 nm (for proline). The amino acid peaks

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were identified by comparison of retention times with those of reference Amino Acid Standard Solution (Sigma-Aldrich, St. Louis, USA). The results were expressed as grams of amino acid in 100 g of sample.

Lipids from the microalgae samples were extracted by cold extraction with chloroform: methanol mixture (2:1) according to the method described by Folch et al. (1957). Fatty acid methyl esters (FAMEs) were then prepared by transmethylation using 14% boron trifluoride methanol solution. FAMEs were separated and analysed using an GC equipped with a flame ionization detector FID (Agilent Series, 7890 Series, USA). The separation was done in a SP-2560 fused silica capillary column (100 m x 20 mm i.d. and 0.20 µm thickness). Helium was used as the carrier gas. The split ratio was 50:1 and the injected volume was 1 µL. The injector and detector temperatures were set up to 250 °C. An initial column temperature of 140 °C was maintained for 5 min, followed by increasing it to 230 °C at a rate of 3 °C/min, and holding it for 5 min. Then, the temperature was increased to 240 °C at a rate of 3 °C/min, and held constant for 10 min. The FAMEs were identified by comparison of retention times with those of reference standard Supelco 37 FAME Mixture. The results were expressed as the mass of fatty acid or fatty acid group (g) in 100 g of total fatty acids.

The antioxidant quality

The total phenolic content was determined spectrophotometrically by Folin-Ciocalteau method adapted to microscale (Gonzalez-Molina et al. 2008). Results were expressed as mg gallic acid equivalents (GAE) per 100 g.

The chlorophyll a and chlorophyll b contents were determined spectrophotometrically by the method adapted to microscale (Lichtenthaler 1987). Results were expressed as mg per 100 g.

The antioxidant activity of *Chlorella vulgaris* and *Spirulina* spp. algae were investigated using three in vitro antioxidant tests (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays, and reducing power). DPPH$^{\bullet}$ assay (DPPH) was performed according to Girones-Vilaplana et al. (2014), reducing power (RP) was tested as described by Oyaizu (1986), and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS$^{\bullet+}$) method was performed following (Mena et al. 2011). The antioxidant activities were expressed as µmol of Trolox equivalents per 100 g (µmoLTE/100g).

Microbiological parameters

Microbiological safety parameters were determined by enumeration of the total count of bacteria (ISO:4833-1 2013), the total count of yeasts and molds (ISO:21527-2 2011), the total number of *Enterobacteriaceae* (ISO:21528-2 2017), *Salmonella* spp. (ISO:6579-1 2017), beta-glucuronidase-positive *Escherichia coli* (ISO:16649-2 2008), coagulase-positive staphylococci (ISO:6888-1 2021) and *Clostridium perfringens* (ISO:7937 2004).

Results and discussion

The chemical composition of dried powder *Spirulina* spp. and *Chlorella vulgaris* is summarized in Table 1.

Moisture is an important factor for assessing the microalgae quality. The moisture content of *Spirulina* spp. and *Chlorella vulgaris* were 5.63% and 5.75%, respectively. Obtained values of moisture content are in the range of general recommendations for their quality, which is less than 10% (Becker 1994). Reduced moisture content and water activity consequently stabilize microalgae-based products by inhibiting microbial growth and enzymatic activity and slowing chemical reactions. Powdered microalgae present advantages that are easy to store and transport and have a longer shelf life. However, it should also take care regarding drying of microalgae, since extremely drying could change the structure of living cells which would degrade their physiochemical properties. Furthermore, microalgae are characterized by a high ash content (Liu 2017), while some can have ash up to 70% of dry matter. The mineral contributions to ash are important for the health and performance of the animal. By their nature, ash or minerals are devoid of protein, calories, energy, or nutrients that, and as such the high ash content in animal feed does not provide any nutritional value, while taking place in the diet for other more valuable nutrients to be consumed. The ash contents of *Spirulina* spp. and *Chlorella vulgaris* were 7.77% and 4.82%, respectively. Obtained results were consistent with findings of previous study conducted by (Liu 2017) , who reported ash content of 7.9% for *Spirulina* spp. and 6.4% for *Chlorella vulgaris.* Similar values for ash content in different microalgae have been reported by (Tokuşoglu and üUnal 2003).

Fibers play an important role as structural components of the cell wall in microalgae. On the other hand, a high portion of dietary fiber in human nutrition regulates transit time, but delays stomach emptying, thus improving nutrient and mineral absorption and retarding hunger pangs (de Jesus Raposo et al. 2016). Additionally, dietary fibers reduce blood cholesterol (Praznik et al. 2015), improve the levels of blood glucose, and also regulate insulin secretion (de Jesus Raposo, de Morais and de Morais 2016). Based on the results obtained in this study, *Chlorella vulgaris* was richer in terms of dietary fiber content (4.52%) compared to the *Spirulina* spp. (0.61%). Depending on the human age and gender, nutrition experts recommend eating at least 21 to 38 grams of fiber per day for optimal health, which could be easily obtained with appropriate food ingredients, including microalgae as well.

The fat content of many microalgae species ranging from 1 to 70% is well documented (Spolaore et al. 2006). Factors that can affect the production of different lipids are microalgae species, cultivation conditions, i.e., growth phase, nutrient availability, salinity, light intensity, temperature, and pH (Guschina and Harwood 2006). In this study, both algae were characterized by small fat content: 0.91% in *Chlorella vulgaris,* whereas *Spirulina* spp. contained 0.54% of fat.

The fatty acids profiles of investigated microalgae are presented in Table 2. As it could be observed, *Spirulina*

TABLE 1: *Chemical composition of Spirulina spp., and Chlorella vulgaris powder (% dry matter basis).*

Nutrients	*Spirulina* spp.	*Chlorella vulgaris*
Moisture	5.63 ± 0.025	5.75 ± 0.020
Crude protein	64.70 ± 0.200	61.19 ± 0.300
Crude fat	0.54 ± 0.005	0.91 ± 0.030
Fatty acids	0.10 ± 0.000	0.10 ± 0.000
Crude fiber	0.61 ± 0.010	4.52 ± 0.050
Ash	7.77 ± 0.010	4.82 ± 0.025

Data present mean value of three replicates ± SD

TABLE 2: *Fatty acid profile of Spirulina spp., and Chlorella vulgaris powder (g/100 g of total fatty acids).*

Fatty acid	*Spirulina* spp.	*Chlorella vulgaris*
C14:0	1.4 ± 0.050	4.3 ± 0.100
C16:0	49.2 ± 0.100	26.0 ± 0.150
C16:1	5.6 ± 0.100	6.2 ± 0.050
C17:0	0.5 ± 0.000	1.1 ± 0.050
C17:1	0.8 ± 0.050	0.5 ± 0.000
C18:0	1.4 ± 0.000	3.3 ± 0.050
C18:1n9c	3.5 ± 0.050	6.2 ± 0.100
C18:2n6c	19.0 ± 0.100	30.9 ± 0.150
C18:3n3	–	21.6 ± 0.100
C18:3n6	18.6 ± 0.100	–
∑SFA	52.5 ± 0.150	34.6 ± 0.150
∑MUFA	9.9 ± 0.014	12.9 ± 0.050
∑PUFA	37.6 ± 0.140	52.5 ± 0.250
∑ω-6	37.6 ± 0.071	30.9 ± 0.300
∑ω-3	–	21.6 ± 0.200
∑ω-6/∑ω-3	–	1.43 ± 0.005

Data present mean value of three replicates ± SD; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

spp. had a higher content of saturated fatty acids (SFA) in comparison to *Chlorella vulgaris.* SFA content in *Spirulina* spp. was 52.5%, while that in *Chlorella vulgaris* was 34.6 %. Among all SFA, palmitic acid was the most dominant fatty acid in both algae, while myristic, margaric, and stearic acid were distributed in lesser amounts. *Chlorella vulgaris* was characterized by a higher amount of mono- (MUFA) and polyunsaturated fatty acids (PUFA) in comparison to *Spirulina* spp. Among PUFA, *Spirulina* spp. contained linoleic acid (19.0%) and gamma-linolenic acid (GLA) (18.6%). The obtained results are in agreement with the values reported by others (Otleş and Pire 2001). GLA is a commercially important PUFA being used as a dietary supplement for various health conditions such as coronary disease, zinc deficiency, obesity, etc. (Otleş and Pire 2001). Although no GLA was found in *Chlorella vulgaris,* high amounts of linoleic acid (30.9%) and α-linolenic acid (ALA) (21.6%) were found. *Chlorella vulgaris* was already reported to be a good source of omega-3 fatty acids (Sayeda et al. 2015). PUFA, especially omega-3 and omega-6 fatty acids are essential fatty acids that have a crucial role in human nutrition and health. These fatty acids cannot be synthesized in body and, therefore, must be derived from the diet. They are important in therapeutics and dietetics as they counteract cardiovascular and metabolic disorders, reduce blood cholesterol levels, decrease risk of diabetes, atherosclerosis, some autoimmune disorders, and some types of cancer (Adarme-Vega et al. 2012, EFSA 2010). They have also demonstrated positive effects on the nervous system and brain function (Adarme-Vega, Lim, Timmins, Vernen, Li and Schenk 2012). Being abundant in omega-3 and omega-6 fatty, *Spirulina* spp. and *Chlorella vulgaris* can help in meeting nutritional requirements for PUFA and thus be valuable in the prevention of certain diseases. Furthermore, *Spirulina* spp. and *Chlorella vulgaris* showed to be valuable feed supplements when administrated into animal diets. It was found that omega-3 fatty acids in chlorella may act as an anti-inflammatory agent thus improving the immune system of animals (Abdelnour et al. 2019). Omega-3 fatty acids from algae can influence milk fat composition, alter muscle fatty acid composition in ruminants (Shingfield et al. 2013) and affect designing of functional "omega-3" eggs (Omri et al. 2019).

The absolute amount of omega-6 and omega-3 fatty acids in the diet is not a crucial factor as much as their ratio. Namely, the properly balanced ratio of these fatty acids in the diet is highly important for multiple biological processes and maintaining metabolic homeostasis. On the other hand, the unbalanced ratio in favour of omega-6 fatty acids is considered to have prothrombotic and proinflammatory effects, which increases the incidence of obesity, diabetes, and atherosclerosis (Simopoulos 2016). It was documented that the ratio of dietary omega-6/omega-3 of about 1-2:1 is optimal and consistent with recommended adequate intakes (Simopoulos 2002). In that respect, *Chlorella vulgaris* showed to have a favourable ratio of omega-6/omega-3 fatty acids (1.43). The balanced omega-6/omega-3 ratio is also essential in animal diets for numerous physiological, biological, reproductive, developmental and beneficial health effects (Alagawany M et al. 2019). Based on the previously

TABLE 3: *Amino acid profile of Spirulina spp., and Chlorella vulgaris powder (g/100 g of total protein).*

Amino acid	*Spirulina* spp.	*Chlorella vulgaris*	FAO/WHO[1] recommendations	Egg[1]	Soya[1]
Leu	7.35 ± ± 0.02	7.99 ± 0.12	7.0	8.8	7.7
Val	5.39 ± 0.11	5.65 ± 0.09	5.0	7.2	5.3
Thr	5.04 ± 0.12	5.37 ± 0.03	N/A	5.0	4.0
Iso	4.64 ± 0.17	3.40 ± 0.14	4.0	6.6	5.3
Lys	4.28 ± 0.12	6.09 ± 0.10	5.5	5.3	6.4
Met	1.40 ± 0.09	1.30 ± 0.08	N/A	3.2	1.3
His	1.14 ± 0.01	1.47 ± 0.01	N/A	2.4	2.6
Phe	n.d.	n.d.	N/A	5.8	5.0
Total EAAs	29.24 ± 0.53	31.27 ± 0.66			
Glu	13.02 ± 0.08	11.23 ± 0.01	N/A	12.6	19.0
Asp	9.43 ± 0.03	9.41 ± 0.08	N/A	11.0	11.3
Pro	6.51 ± 0.02	8.82 ± 0.07	N/A	4.2	5.3
Ala	6.50 ± 0.01	7.29 ± 0.04	N/A	N/A	5.0
Arg	5.45 ± 0.12	5.10 ± 0.03	N/A	6.2	7.4
Gly	4.53 ± 0.02	5.62 ± 0.05	N/A	4.2	4.5
Ser	4.05 ± 0.01	3.76 ± 0.13	N/A	6.9	5.8
Tyr	3.57 ± 0.07	3.14 ± 0.15	1.0	1.7	1.4
Cys	1.36 ± 0.04	1.44 ± 0.05	N/A	2.3	1.9
Try	0.47 ± 0.03	0.47 ± 0.01	1.0	1.7	1.4
Total NEAAs	54.89 ± 0.77	56.28 ± 0.92			
TAAs	84.13 ± 1.26	87.55 ± 1.40			

Data present mean value of three replicates ± SD; n.d. – not detected; N/A – not avaliable; [1] FAO/WHO (1973); Leu – Leucine, Val – Valine, Thr – Threonine, Iso – Isoleucine, Lys – Lysine, Met – Methionine, His – Histidine, Phe – Phenilalanin, Glu – Glutamic acid, Asp – Aspartic acid, Pro – Proline, Ala – Alanine, Arg – Arginine, Gly – Glycine, Ser – Serine, Tyr – Tyrosine, Cys – Cysteine, Try – Tryptophan, EAAs – essential amino acids, NEAAs – non-essential amino acids, TAAs – total amino acids

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described facts, it could be said that these microalgae could contribute to the health status of the human, but the animal too, and enrich the end product resulting in the higher added value.

Microalgae as a source of proteins pay great attention to the food and feed industry, mainly due to their high protein and essential amino acids (EAAs) contents. Bearing in mind that *Spirulina* spp. and *Chlorella vulgaris* protein contents amounted 64.70% and 61.19% on a dry matter (DM) basis, respectively (Table 1), it is proved that investigated microalgae produce more proteins than plant-based foods, and as such, they are recognised as a vegan source of protein. Investigations carried out by other authors (Becker 2007, Tokuşoglu and üUnal 2003, Wells et al. 2017), has shown that microalgae such as *Chlorella* sp. and *Spirulina* spp. contain up to 70% protein on DM basis. The contents of crude protein are comparable and even higher than some conventional feed ingredients such as soybean, maize, and wheat (Lum et al. 2013). Amino acid profile of *Spirulina* spp. and *Chlorella vulgaris* are presented in Table 3. Total amino acids (TAA) content was slightly higher in *Chlorella vulgaris* (87.55%) compared to *Spirulina* spp. (84.13%), while similar results were obtained regarding the total EAAs content. Seven of the eight EAAs (except for phenylalanine) were recorded in both microalgae samples. EAAs decreasing content in *Spirulina* spp. sample is: Leu>Val>Thr>Iso>Lys>Met >Hys, while in *Chlorella vulgaris* was as follows: Leu> Lys >Val>Thr>Iso>Hys>Met. Tested microalgae also have the amino acid profile comparable to other protein sources such as egg and soybean (Table 3), notably containing all of the EAA that humans cannot synthesize and must obtain from food sources or supplements. Regarding the EAAs, in both of the microalgae samples, leucine was detected in the highest amount, while glutamic and aspartic acid represents the highest proportions of non-essential amino acids (NAA) in the tested samples. These findings are in agreement with data published by Kolmakova and Kolmakov (2019). Content of EAAs represents the first criterion in estimating the nutritional quality of protein. In this respect, owing to microalgae higher protein quality, when compared to other protein sources such as eggs and soy, *Spirulina* spp. and *Chlorella vulgaris* could be considered as sources of high valuable protein, with a well-balanced amino acid profile according to the FAO/WHO (FAO/WHO 1973) recommendations regarding human's daily requirements of EAA (Table 3). Furthermore, microalgae biomass can be considered as a feed ingredient with proper nutritional quality, and as such could replace conventional protein like soybean meal. Based on the previously mentioned, it could be said that *Spirulina* spp. and *Chlorella vulgaris* could be safely used as partial replacers of conventional protein sources of the poultry diet up to an inclusion level of about 5–10%.

TABLE 4: *Mineral profile of Spirulina spp., and Chlorella vulgaris powder.*

	Spirulina spp.	Fulfiled recommendations for female*	Fulfiled recommendations for male*	RDA female/male
Macroelements (mg/100g)				
Ca	418.6 ± 0.150	41.9 %	41.9 %	1000/1000
P	1211.0 ± 1.000	173.0 %	173.0 %	700/700
Mg	339.8 ± 0.100	128.2 %	97.1 %	265/350
Na	77.6 ± 0.141	5.2 %	5.2 %	1500/1500
K	811.3 ± 0.150	17.3 %	17.3 %	4700/4700
Microelements (mg/100g)				
Fe	1.5 ± 0.015	8.3 %	18.75 %	18.0/8.0
Mn	36.2 ± 0.100	2011.1 %	1573.91 %	1.8/2.3
Zn	34.8 ± 0.200	435.0 %	316.4 %	8.0/11.0
Toxic elements (mg/kg)				
Pb	<0.5	–	–	–
Cd	<0.1	–	–	–
Hg	0.00501 ± 0.000	–	–	–
As	<0.5	–	–	–
	Chlorella vulgaris	**Fulfiled recommendations for female***	**Fulfiled recommendations for male***	**RDA female/male**
Macroelements (mg/100g)				
Ca	127.4 ± 0.250	12.7 %	12.7 %	1000/1000
P	1100.0 ± 5.000	157.1 %	157.1 %	700/700
Mg	280.8 ± 0.750	106.0 %	80.23 %	265/350
Na	57.53 ± 0.035	3.8 %	3.8 %	1500/1500
K	107.6 ± 0.250	2.3 %	2.3 %	4700/4700
Microelements (mg/100g)				
Fe	73.26 ± 0.105	407.0 %	915.7 %	18.0/8.0
Mn	5.70 ± 0.100	316.7 %	247.8 %	1.8/2.3
Zn	1.97 ± 0.010	24.6 %	17.9 %	8.0/11.0
Toxic elements (mg/kg)				
Pb	<0.5	–	–	–
Cd	<0.1	–	–	–
Hg	0.003599 ± 0.000	–	–	–
As	<0.5	–	–	–

Data present mean value of three replicates ± SD; RDA – Recommended daily allowances of minerals (mg/day) for an adult female/male aged between 31–50 years; *The percentage of fulfiled RDA recommendations for female/male aged between 31–50 years

Mineral profiles of *Spirulina* spp. and *Chlorella vulgaris* are summarized in Table 4. The contents of evidenced macro elements decreased in the order K>Na>Ca>Mg for *Spirulina* spp., while for *Chlorella vulgaris* decreased as follows: Mg>Ca>K>Na. Significantly higher content of K, Na, Ca, Mg, and P was observed for *Spirulina* spp. Regarding microelements, *Spirulina* spp. was characterized with higher content of Mn and Zn, whilst *Chlorella vulgaris* showed to have a higher content of Fe. Generally, microalgae are considered to have a high content of essential minerals and trace elements required for human nutrition. It should be noted that microalgae can be a source of iodine. It is known that iodine represents a critical element for thyroid hormones. Therefore, iodine deficiency in human food or animal feed may lead to serious disorders associated with growth and development of mammals, in which functions iodine is complemented by selenium. On contrary, extremely high iodine levels can trigger an over-functioning (iodine-induced hyperthyreosis) of the thyroid gland

with life-threatening effects on metabolism (Gómez-Jacinto et al. 2010, Kotrbáček et al. 2015).

According to Recommended Dietary Allowances (RDA) of minerals (mg/day) for an adult female/male aged between 31–50 years (Table 4), it could be noted that examined microalgae fulfill the RDA recommendations for females for P, Mg, and Mn. *Chlorella vulgaris* additionally fulfills RDA recommendation for Fe, while *Spirulina* spp. fulfills RDA recommendation for Zn. Regarding the recommendations for males, *Chlorella vulgaris* fulfills RDA recommendations for P, Fe, Mn, while *Spirulina* spp. fulfills RDA recommendations for P, Mn, and Zn. Consequently, this study confirms that *Chlorella's* and *Spirulina's* benefits may be attributed to both mineral and trace element contents.

In Europe, there is no regulation on the maximum levels of toxic metals in seaweeds as food. Having in mind a huge interest in microalgae and their application in the food industry, there is a great need for toxic metals monitoring to set the maximum levels for arsenic, lead, cadmium, and mercury for seaweeds as well as providing more data to improve the risk assessments regarding the consumption of this food. The only maximum level is defined for cadmium (3.0 mg/kg wet weight) in food supplements consisting exclusively or mainly of dried seaweed or products derived from seaweed' (European Commission, 2008). From this point of view, it could be said that investigated microalgae are safe for human consumption.

Only a few scientific studies have investigated the role of phenolic compounds in algae and microalgae (Hajimahmoodi et al. 2010, López et al. 2011). *Spirulina* and *Chlorella* are safe and natural sources of bioactive molecules and have a long history of use as healthy food and feed supplements (Zakaria et al., 2020). Results in Table 5 show that *Chlorella vulgaris* had higher total phenolic content (398.68 mg GAE/100 g) obtained by spectrophotometric method than *Spirulina* spp. (287.97 GAE/100 g), while *Spirulina* spp. showed slightly higher chlorophyll a (40.84 mg/100 g) and b (71.40 mg/100 g) content. Hajimahmoodi, Faramarzi, Mohammadi, Soltani, Oveisi and Nafissi-Varcheh (2010) evaluated the total phenolic contents of 12 soil-isolated strains of microalgae including *Chlorella* sp. The total phenolic contents varied among different microalgae strains, with *Chlorella* sp. showing values ranging from 0 to 19.15 mg/g (Hajimahmoodi et al., 2010). The amounts of phenolic compounds in *Spirulina* published in the study by Kepekçi and Saygideger (2012) were as follows: 6.32, 25.73, and 49.83 mg GAE/g dry weight in cultures incubated at 40, 60, and 120 µmol photons/ms, respectively.

In this work, the free radical scavenging capacities of *Chlorella vulgaris* and *Spirulina* spp. were determined using DPPH•, RP, and ABTS•+ assays. DPPH radical scavenging assay determines the ability of samples to donate an electron and scavenge DPPH radicals. RP of phytochemicals is associated with antioxidant capacity since it is related to their ability to transfer electrons. One of the most often used organic radicals for the evaluation of antioxidant efficiency of pure substances and complex systems are stable synthetic ABTS•+. The antioxidant activities of samples are mainly due to their redox properties, which can play an important role in neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. *Chlorella vulgaris* had higher antioxidant activity determined by DPPH and RP assays (1448.92 and 97.99 µmol Trolox/100g, respectively) than *Spirulina* spp. (1304.40 and 83.64 µmol Trolox/100g, respectively) (Table 5). It was already reported that antioxidant activity in algae is directly influenced by the phenolic content and, which suggests that phenolic compounds in *Chlorella vulgaris* contribute to the total antioxidant capacity in the algae (Wu et al. 2005). On the other hand, *Spirulina* spp. showed a higher antioxidant capacity measured by ABTS test (10629.57 µmol Trolox/100g) regardless of lower phenolic content. This might be explained by the fact that phenolic compounds are not the only compounds in algae with antioxidant potentials. In fact, algae can generate a wide range of compounds that contribute to the total antioxidant potential such as carotenoids, polysaccharides, and polyunsaturated fatty acids (Li et al. 2007).

Additionally, safety parameters of *Spirulina* spp., and *Chlorella vulgaris* powder are presented in Table 6.

In general, the value of water activity (aw) is correlated with the potential for growth and metabolic activity of microorganisms, and therefore, is used as an indicator of the presence of free water for microbiological activity. From the results shown in Table 7, it can be seen that the established aw values of the tested samples were lower than 0.5 (0.337 and 0.365). Since the growth and development of microorganisms cannot occur at this aw value (except extreme xerophilic microorganisms), it could be stated that there is no possibility of changing the microbiological status of the tested samples. A comprehensive view of the obtained results supports the fact that the most products *Spirulina* and *Chlorella* from several large producers have "GRAS" designations [Generally Recognized As Safe (FDA 2016).

TABLE 5: *Phenolic and chlorophyll content, and antioxidant activitiy of Spirulina spp., and Chlorella vulgaris powder.*

	Spirulina spp.	*Chlorella vulgaris*
Phenolic content (mgGAE/100g)	287.97 ± 22.99	398.68 ± 22.65
Chlorophyll a (mg/100g)	40.84 ± 1.27	37.92 ± 1.02
Chlorophyll b (mg/100g)	71.40 ± 2.20	66.24 ± 1.77
Antioxidant activity (µmoLTrolox equivalents (TE)/100 g)		
DPPH assay	1304.40 ± 38.51	1448.92 ± 91.04
RP test	83.64 ± 12.15	97.99 ± 13.96
ABTS assay	10629.57 ± 61.44	6594.46 ± 28.56

Data present mean value of three replicates ± SD

TABLE 6: *Safety parameters of Spirulina spp., and Chlorella vulgaris powder (CFU/g).*

	Spirulina spp.	*Chlorella vulgaris*
Total viable count	<10	<10
Total count of yeast and molds	<100	<100
Enterobacteriaceae	<10	<10
Salmonella spp.	not detected in 25 g	not detected in 25 g
E. coli	<10	<10
Coagulase-positive staphylococci	<100	<100
Clostridium perfringens	<10	<10

TABLE 7: *AW value.*

	Spirulina spp.	*Chlorella vulgaris*
Water activity (aw)	0.337 ± 0.008	0.365 ± 0.000

Data present mean value of three replicates ± SD

Conclusion

Due to its chemical composition, rich nutritional value, as well as antioxidant activities, *Spirulina* spp. and *Chlorella vulgaris* powder have important nutraceutical potential as a source of protein, EAAs, essential fatty acids, carotenoids and can be considered as the species with nutrient-rich profiles. The protein content and well-balanced amino profile of the investigated microalgae make them superior compared to other protein sources. However, despite its high content of nutritious protein, the cost-price of microalgae, powder-like consistency, dark green colour, and its slightly fishy smell, could be limiting factors for application into conventional food and feedstuff, which should take particular care. It should be emphasized that only scarce information is available about concentrations of PAH residues, although the outcomes of recent studies indicated the need for more profound analysis about the contamination risk in algae products for humans as well as for animal consumption. The potential exposure risk of these, undesirable components from microalgae, was not evaluated in this study and requires further investigation. To unrestrictedly promote algal products as dietary supplements for regular use, cultivation methods have to be optimized to prevent the growth of potential cyanotoxin producers, which implies strict monitoring as a key for improving the quality and safety of microalgal products. Thus, controlled cultivation can be used to enrich microalgal biomass with organically bound trace elements to form a complete food/feed supplement in biologically usable forms. In addition, it is necessary to check the metabolism and bioavailability of nutraceutical potential of microalgae foods in humans and establish possibilities for potential use as food and feed.

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Conflict of interest

The authors declare that no conflict of interest among authors.

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