

RELEASE OF BIOACTIVE SUBSTANCES FROM FORMULATIONS CONTAINING *ARTHROSPIRA PLATENSIS* (*SPIRULINA PLATENSIS*)

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Abstract: *Arthrospira platensis* (*Spirulina platensis*) is a well-known microalga and has been utilized as a medicinal agent and foodstuff by humans since at least 16th century. The aim of this study was to determine zinc content as well as determine phenolic and indole compounds from commercial preparations containing *Arthrospira platensis* (lyophilizate, tablets, and capsules) before and after extraction with methanol and incubation with artificial digestive juices. The secondary aim of this study was to evaluate the quality of these preparations. The samples were incubated in artificial stomach juice and in intestinal juice. The samples were mineralized and their zinc(II) ions content was estimated using flame absorption atomic spectroscopy (F-AAS). The maximum zinc(II) ions content released into the digestive juices was found to be up to 1.6 mg/100 g of the preparation. Phenolic compounds identified in the examined extracts are as follows: gallic acid; protocatechuic acid; 3,4-dihydroxyphenylacetic acid; *p*-hydroxybenzoic acid; syringic acid; cinnamic acid; and quercetin. Furthermore, indole compounds identified were 5-hydroxy-L-tryptophan, 5-methyl-L-tryptophan, L-tryptophan, tryptamine, and 5-methyltryptamine. Consequently, it was also found that the distributed *Arthrospira platensis* in the form of tablets does not disintegrate in the artificial digestive juices. Among the examined preparations, only hard capsules met the requirements of the European Pharmacopoeia 8th ed.

Keywords: *Arthrospira platensis*, artificial digestive juices, indole compounds, phenolic compounds, zinc

Arthrospira platensis (*Spirulina platensis*) has been used as a food by the Aztecs since the 16th century in Mexico when it was fished from Lake Texaco and then dried and sold in the form of biscuits as described by Spanish soldiers. *A. platensis* is currently cultivated and is used in many countries as a dietary supplement because of its substantial nutritional value (1-3). *Arthrospira maxima* and *Arthrospira platensis* are the species classified as *Spirulina*, and most commonly these species are used in dietary supplements. The protein content in *A. platensis* is approximately 65–79% and is a complete source of protein and amino acids. About 7% of the weight of *A. platensis* contains lipids, mainly comprising γ -linolenic acid, α -linolenic acid, stearic acid, eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid. *A. platensis* also contains significant amounts of γ -linolenic acid (4, 5). *A. platensis* is a good source of water-soluble vitamins such

as C, PP (niacin) from group B (B₁, B₂, B₅, B₆, and B₁₂); fat-soluble vitamins, such as A, D, E, and K; and eicosapentaenoic acid. It is also a good source of macro and microelements, such as P, Ca, K, Na, Mg, Fe and also contains inositol and dyes (phycocyanins, carotenoids, and chlorophyll b) (6, 7, 8). Phycocyanin, belonging to the water-soluble pigments phycobilins (1% of *A. platensis* mass), is mainly responsible for its ability to neutralize free radicals. This blue dye gives *A. platensis* its characteristic, dark-turquoise color. It also contains carotenoids such as β -carotene and β -cryptoxanthin as well as chlorophyll a and b. *A. platensis* is used in the prevention and treatment of various diseases such as obesity, anemia, hypertension, hyperlipidemia, diabetes, some cancers, neurodegenerative diseases such as Alzheimer's disease, and so on (9–13). Due to the antioxidant, anti-inflammatory, antidepressant, and immunostimulatory properties

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of *A. platensis* described earlier in the scientific literature, it was decided to determine its content of zinc, phenolic, and indole compounds (14–16).

Zinc is essential for the proper development of human body. This element is not only an activator but is also a cofactor of about 300 enzymes. Zinc is responsible for the metabolism of nucleic acids, proteins, lipids, and carbohydrates (17). It affects the expression of genes during the replication and transcription of DNA and RNA (17). It is also responsible for the synthesis of red blood cells and affects the functions of inter alia respiratory, reproductive, and immune systems (18, 19) and also demonstrates an anti-inflammatory, regenerating and antidepressant activity.

Phenolic and indole compounds primarily exhibit an antioxidant, anti-inflammatory, and immunostimulant activity.

The aim of this study was to perform quantitative analysis of zinc ions as well as phenolic and indole compounds in preparations of commercial origin containing *A. platensis* before and after an extraction using artificial digestive juices. Flame absorption atomic spectroscopy (F-AAS) was used to analyze zinc, whereas phenolic and indole compounds were determined using reverse phase-high performance liquid chromatography (RP-HPLC).

The analysis was performed to evaluate the highest amounts of bioelements, phenolic, and indole compounds and zinc (II) ions released among the preparations containing *A. platensis* (lyophilizate, tablets, and capsules) after the extraction using artificial digestive juices. The final product will be a better source of these substances for human consumption. In addition, the next aim of the work was to evaluate whether the formulation containing *A. platensis* is suitable for the effective release of zinc and phenolic and indole compounds and whether it meets the pharmacopeia requirements.

EXPERIMENTAL

Reagents and standards

Standard zinc (II) ions solution at a concentration of 1000 ppm was obtained from OUM (Łódź, Poland); subsequent dilutions of 100, 10, and 1 ppm concentrations were prepared from the above solution. MgCl₂ was obtained from Chempur (Kraków, Poland); NaCl, KCl, and NaHCO₃ were obtained from PPH Golpharm (Kraków, Poland); pepsin and bile salts were obtained from BTL (Łódź, Poland); CaCl₂ was obtained from Pharma Zentrale GmbH (Germany); pancreatic extract, HCl, KCl, concentrated HNO₃ Suprapur®, and KNO₃, Suprapur® were

obtained from Merck (Darmstadt, Germany); C₆H₈O₇, ZnSO₄, KHCO₃, Na₂HPO₄, K₂HPO₄, and NaOH were purchased from Polish Company of Chemistry (Gliwice, Poland). Water (quadruple-distilled) with the conductivity of less than 1 μS/cm was obtained using an S2-97A2 distillation apparatus (ChemLand, Stargard Szczecin, Poland). The following standard phenolic compounds of HPLC grade were purchased from Fluka Chemie GmbH (Switzerland): *p*-coumaric acid; ferulic acid; *p*-hydroxybenzoic acid; vanillic acid; and 3,4-dihydroxyphenylacetate acid. Caffeic acid, chlorogenic acid, cinnamic acid, *o*-coumaric acid, protocatechuic acid, sinapic acid, gallic acid, and syringic acid and quercetin and standards of indole compounds, namely, L-tryptophan, 5-hydroxy-L-tryptophan, 6-methyl-L-tryptophan, serotonin, melatonin, tryptamine, and 5-methyl-tryptamine were purchased from Sigma-Aldrich (St. Louis, MO, USA); all were of HPLC grade. Methanol, acetic acid, and petroleum ether were purchased from Merck (Darmstadt, Germany) were also of HPLC grade.

Research material

The studies were conducted on the dietary supplements containing *Arthrospira platensis* (microalgae from *Microcoleaceae* family), two preparations in the powdered form and two in the tablet form, and one in capsules were evaluated. Both, the methanolic extracts of these preparations and the extracts obtained after incubation with artificial digestive juices were objects of the experiment. The selected preparations differed in preparation form and dosage and were derived from different manufacturers (Table 1).

Preparation of artificial digestive juices

Artificial saliva (pH = 6.8.) was prepared according to the method of Arvidson (20). Briefly, 100 mL KH₂PO₄ at a concentration of 25 mmol/L, 100 mL Na₂HPO₄ at a concentration of 24 mmol/L, 100 mL KHCO₃ at a concentration of 150 mmol/L, 100 mL MgCl₂ at a concentration of 1.5 mmol/L, 6 mL citric acid at a concentration of 25 mmol/L, 100 mL CaCl₂ at a concentration of 15 mmol/L were added to the flask and the volume was made up to 1000 mL with four-times distilled water.

Artificial stomach juice (pH = 2.0) was prepared according to the method described in Polish Pharmacopeia X. Briefly, 2.0 g NaCl and 3.2 g pepsin were dissolved in four-times distilled water. Then, 80 mL HCl at a concentration of 1 mol/L was added, and the volume was made up to 1 L using four-times distilled water (21).

Artificial intestinal juice (pH = 8.0) was prepared according to the method of Neumann (22). Briefly, 20 mg pancreatic extract, 120 mg bile salt, and 8.4 g NaHCO₃ were dissolved in four-times distilled water and the volume was made up to 1 L using four-times distilled water.

Sample preparation

Release studies were performed in the Gastroel-2014 apparatus, which was constructed at the Department of Inorganic and Analytical Chemistry at the Faculty of Pharmacy, Medical College, Jagiellonian University. This apparatus allows the study of substances released into the artificial digestive juices, imitates gastrointestinal motions, and also provides a constant temperature of 37°C (23).

Preparations containing *A. platensis* were weighed (at 0.5 g) and after performing mineralization three parts of the samples were made: one part of the samples was used to determine zinc (II) ions content, the second for methanol extraction (phenolic and indole compounds) while the other part was subjected to *in vitro* digestion using Gastroel-2014. The second part was used for three times extraction by 100 mL of methanol using ultrasound at a frequency of 49 kHz during 30 minutes (Sonic-2, Polsonic, Poland). For the last part, the weighed sample was transferred to 100 mL Erlenmeyer flasks and then wetted with 2 mL of artificial saliva; subsequently, 100 mL of stomach juice was added. The flasks were closed with a stopper and placed in the apparatus. The incubation process was continued for 15, 30, and 60 min. The resulting solutions were filtered using a Büchner funnel and a vacuum set. The residue was transferred to Erlenmeyer flasks together with the filter paper and 100 mL of intestinal juice was added (the extraction process lasted 150 min). Then, the contents were filtered again. The samples were divided into two lots, one of which was designated for zinc analysis and the other for the analysis of organic compounds (phenolic and indole compounds).

Analysis of Zn content before and after incubation with artificial digestive juices using the F-AAS method

The concentration of zinc (II) was determined using the F-AAS method. Mineralization of the preparations containing *A. platensis* was performed in the Magnum II microwave mineralizer ERTEC (Poland). Mineralization was performed for 1 h in three magnetron cycles: 15 min at 60% power, 15 min at 80% power, and 30 min at 100% power. Mineralization of solutions after incubation with artificial digestive juices using Gastroel-2014 was performed in the UV R-8 Mineral Poland mineralizer. This process was performed by UV irradiation of the mineralized test solution in a quartz reaction vessel in 5 cycles of 6–8 h.

Thermo Scientific AA Spectrometer iCE 3000 SERIES (USA) was used in all the measurements of zinc.

Reverse-phase high performance liquid chromatography (RP-HPLC) of phenolic compounds

The extracts obtained from methanol and after incubation with artificial digestive juices were analyzed for phenolic compounds using the method of RP-HPLC, which was performed based on the procedure developed by Sułkowska-Ziaja (24). The analysis was performed at 25°C, with a mobile phase consisting of A – methanol and B – methanol: 0.5% acetic acid 1 : 4 (v/v). The following gradient was applied: 100% B for 0–20 min; 100–80% B for 20–35 min; 80–60% B for 35–55 min; 60–0% B for 55–70 min; 0% B for 70–75 min; 0–100% B for 75–80 min; 100% B for 80–90 min at a flow rate 1 mL/min, $\lambda = 254$ nm (phenolic acids), $\lambda = 370$ nm (flavonoids). Quantification was performed by the measurement of peak area with reference to the standard curve derived from five concentrations (0.03125–0.5 mg/mL). The quantitative analysis of phenolic compounds was performed using a calibration curve with the assumption of the linear size of the area under the peak and the concentration of the

Table 1. Dietary supplements containing *Arthrospira platensis* which were used for determination of zinc content after an extraction to artificial digestive juices.

Name of the preparation	Form	Expiry date
Spirulina B	Powder	11.2016
Spirulina P	Powder	07.2012
Spirulina M	Capsules	10.2016
Spirulina N	Tablets	08.2016
Spirulina O	Tablets	03.2017

reference standard. The results were expressed in mg/100 g of dry weight (d.w.).

RP-HPLC analysis of indole compounds

The extracts obtained from methanol and after incubation with digestive juices were evaporated to dryness under pressure of 200 mBa at 40°C (Büchi evaporator, Germany). The concentrated analyte was dissolved in methanol transferred through Whatman No. 3 filter paper. The extracts were quantitatively dissolved in 1.5 mL of solvent system (methanol : water : ammonium acetate at 15 : 14 : 1 v/v/v) and subjected to separation by RP-HPLC using the Hitachi HPLC (Merck, Japan) equipped with a pump type L-7100, the Purospher® RP-18 (4 × 200 mm, 5 µm) column kept at 25°C and UV detector operated at $\lambda = 280$ nm. The isocratic separation was as follows: methanol/water/ammonium acetate (15 : 14 : 1 v/v/v) at a flow rate of 1 mL/min. The quantitative analysis of indole compounds was performed using a calibration curve with the assumption of the linear size of the area under the peak and the concentration of the reference standard. Quantification was performed by the measurement of peak area with reference to the standard curve derived from five concentrations (0.0625–1 mg/mL). The results were expressed in mg/100 g of d.w.

Properties of tablets

The tablets were evaluated as per standard procedure according to European Pharmacopeia 8th ed for uniformity of weight, hardness, friability, and disintegration time (25). Tablets were also tested for variation in thickness to determine any variability associated with the tablet press or the method of preparation.

The average weight was obtained according to pharmacopeia limits by weighing randomly selected 20 tablets on an analytical balance (OHAUS Adventurer Pro). The hardness of the tablets was

determined for at least 10 tablets using the Erweka TBH 20 hardness tester (Erweka GmbH), and adopting a minimum hardness of 40 N as the acceptance criterion. For each formula, friability was evaluated from the percentage weight loss of 20 tablets tumbled in Erweka TAR 120 friabilator (Erweka GmbH, Hausenstamm, Germany) at 25 rpm for 4 min. The tablets were dedusted and the loss in weight caused by fracture or abrasion was recorded as the percentage weight loss. Friability < 1% was considered acceptable. Respective disintegration times of the prepared tablets were measured in 900 mL of purified water with disks at 37°C using an Erweka ZT 222 tester (Erweka GmbH, Hausenstamm, Germany). Six tablets were randomly selected from each formulation and were put into basket-rack. The disintegration time was recorded till all the fragments of the disintegrated tablet passed through the screen of the basket. For non-modified tablets, the disintegration time should not be longer than 15 min. The thickness of the tablets was determined for 20 tablets using digimatic Vernier caliper (0–150 mm).

Statistical analysis

Statistical analysis of the data was performed using one-way ANOVA with Tukey-Kramer *post hoc* analysis of multiple comparisons. A value $p < 0.05$ was accepted as the level of statistical significance.

RESULTS AND DISCUSSION

This is the first study where the content of zinc, phenolic and indole compounds in commercial preparations of *A. platensis* from different manufacturers were analyzed. Furthermore, the level of these compounds release was investigated using Gastroel-2014 apparatus which imitates conditions in the human digestive tract. Selected commercially available preparations as dietary supplements differed in

Table 2. Zinc content in selected preparations containing *Arthrospira platensis*.

Preparation	Zinc content in preparations containing spirulina (mg/100 g ± SD)
Spirulina B	1.87 ± 0.05
Spirulina P	0.38 ± 0.02
Spirulina M	0.63 ± 0.03
Spirulina N	1.17 ± 0.06
Spirulina O	1.15 ± 0.07

The data presented was mean ± SD (standard deviation); n = 6

Table 3. The content of zinc released to artificial digestive juices from selected *Arthrospira platensis* – containing preparations.

Digestive juice	Stomach juice			Intestinal juice (150 min)		
	15	30	60	after 15 in stomach juice	after 30 in stomach juice	after 60 in stomach juice
Amount of Zn released (mg 100 g ¹ of preparation)						
Spirulina B (powder)						
	0.60 ± 0.04 ^a	1.56 ± 0.50 ^a	0.33 ± 0.02 ^a	0.14 ± 0.05 ^a	0.14 ± 0.05 ^a	0.39 ± 0.03 ^a
Spirulina P (powder)						
	0.91 ± 0.07 ^{abc}	1.14 ± 0.04 ^{abc,d}	0.67 ± 0.04 ^{abc,d}	0.16 ± 0.01 ^{bd}	0.20 ± 0.00 ^{bc,d}	1.05 ± 0.01 ^{abc,d}
Spirulina M (capsules)						
	0.50 ± 0.17 ^b	0.62 ± 0.04 ^{ab}	0.35 ± 0.06 ^{ab}	0.24 ± 0.0 ^{ab}	0.16 ± 0.02 ^b	0.12 ± 0.00 ^{ab}
Spirulina N (tablets)						
	0.26 ± 0.03 ^{abc}	0.27 ± 0.02 ^{ac}	0.403 ± 0.08 ^{bc}	0.13 ± 0.00 ^{bc}	0.15 ± 0.02 ^c	0.15 ± 0.00 ^{abc}
Spirulina O (tablets)						
	0.21 ± 0.02 ^{abd}	0.40 ± 0.08 ^{cd}	0.22 ± 0.02 ^{abc,d}	0.27 ± 0.04 ^{ac,d}	0.05 ± 0.01 ^{abc,d}	0.09 ± 0.02 ^{abc,d}

Data are presented as the mean ± standard deviation (SD); n = 6 repetitions Tukey–Kramer test was used to reveal the differences between paired groups of zinc in rows, the same letters (a, b, c, d, e) are marked for the content whose differences are statistically significant (for p values < 0.05) (GraphPad InStat)

Spirulina species, preparation form (powdered lyophilizates, capsules, and tablets), dosage, and were derived from different manufacturers.

F-AAS was used to evaluate the content of zinc (II) ions in *A. platensis*–containing preparations that were incubated with artificial digestive juices. The elaborated conditions of lyophilized material mineralization and the analytical method allowed to determine the zinc(II) ions concentration in preparations and extracts of incubated preparations.

Zinc content in the examined preparations was found to be in the range of 0.38–1.87 mg/100 g of preparation (Table 2). The highest content of zinc (II) ions was determined in the powder form of preparation (1.87 mg/100 g of preparation). The amount of zinc (II) ions released for artificial digestive juice varies within a narrow range of values and it depends on the degree of powdering the raw material in preparations. The content of zinc (II) ions in the dry matter of *A. platensis* according to previous studies has been reported as 2 mg/100 g (26). But, in this study, was found a considerably lower content of zinc (II) ions, 0.38 mg/100 g of preparation, in the powdered *A. platensis* (cell lyophilizate). For tablets, very similar amounts were determined, that is, in the range of 1.15–1.17 mg/100 g of preparation.

Al-Dhabi (2013) determined zinc (II) ions in *A. platensis*–containing preparations and obtained the results at a similar level (0.05–0.6 mg/100 g of preparation) (27).

In order to estimate the actual quantities of this element available to the human body, incubation of preparations containing *A. platensis* with artificial digestive juices (in Gastroel-2014 apparatus) was performed under conditions imitating those in the human body (temperature 37°C and movements mimicking peristalsis of the human digestive tract). On the basis of F-AAS analysis was found that zinc (II) ions are better released from lyophilized *A. platensis* than that from tablets or capsules. The amount of zinc (II) ions released into the artificial digestive juices ranged from 0.05 to 1.56 mg/100 g of the preparation (Figs. 1a, b). The highest amount of zinc (II) determined in artificial stomach juice was in the range of 0.2 to 1.6 mg/100 g of the preparation, after 30 min of incubation (Table 3). In addition, these amounts in artificial intestinal juice were significantly lower, regardless of the incubation time in the stomach juice, which ranged from 0.1 to 0.4 mg/100 g of the preparation. The highest amounts were determined for Spirulina B, that is, preparation in the form of a cell lyophilizate (powder), and 1.6 mg/100 g of preparation was released into the stomach juice after 30 min of incubation (Figs. 1a, b). This preparation proved to be the most optimal due to the amount of zinc (II) ions found in both types of artificial digestive juices (stomach and intestinal) and in any time variant. Zinc (II) ions were more efficiently released from capsules than that from tablets, despite the demonstrated lower

content of this element in the product before incubation with the artificial digestive juices. The degree of zinc (II) ions released into the artificial stomach juice was found to be lowest in stomach juice (in case of 15 min incubation). The daily human demand for this element is about 12 mg, that is, the amount released from the preparation can constitute zinc supplement to the human diet (28).

Phenolic and indole compounds were determined using RP-HPLC and the results of the calculations were converted to the amount of compounds released from 100 g of the preparation.

Commercial preparations of *A. platensis* are a good source of phenolic and indole compounds. In this study, we identified seven phenolic compounds (gallic acid; protocatechuic acid; 3,4-dihydroxyphenylacetic acid; *p*-hydroxybenzoic acid; syringic acid; cinnamic acid; and quercetin) in almost all commercial preparations (Table 4). Furthermore, 3,4-dihydroxyphenylacetic acid and gallic acid were found to be highest among the examined phenolic compounds. It was best extracted from the powder form of preparation released in the artificial stomach juice, which was up to 38.1 mg/100 g of preparation. In addition, gallic acid was found to be released highest in the intestinal juice from capsules (Spirulina M) containing lyophilized *A. platensis*, up to a maximum of 7.3 mg/100 g of preparation. Other phenolic compounds were determined in both stomach and intestinal juices at a similar level of 0.01 to

2.2 mg/100 g of preparation. It is estimated that 0.1 to 1.0 g daily dose of phenolic compounds is required in the human diet. This range is broad and depends largely on the diet, including the amount of consumed fruits, vegetables, coffee, or tea (29, 30). All the analyzed preparations were found to be a source of phenolic compounds. After conversion of the recommended daily dosage by manufacturers for the product, the total amount of phenolic compounds was determined, and on this basis, was demonstrated that the preparations may be a source of phenolic compounds up to 60.7 mg/day for Spirulina P (lyophilizate).

The indole compounds determined in the preparations include 5-hydroxy-L-tryptophan, tryptamine, 5-methyltryptamine, 6-methyl-tryptophan, and L-tryptophan. They were determined in a significantly lower number of experimental variants than phenolic compounds. Compared to methanolic extracts, indole compounds were determined at higher concentrations in artificial digestive juices (Table 5). In addition, 5-hydroxy-L-tryptophan was indole compound found in the highest amounts in the digestive juices, up to 156.2 mg/100 g of preparation. Due to their numerous health benefits (antioxidant, anti-inflammatory, antidepressive etc.), indole compounds should be supplemented. The daily doses of indole compounds supplied from *A. platensis* – containing preparations (converted to the doses recommended by manufacturers) were the

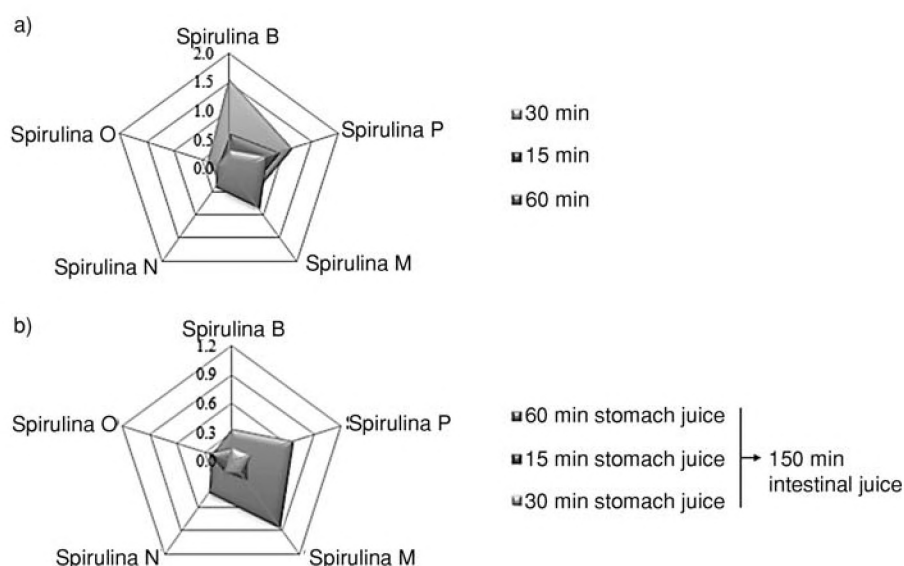


Figure 1. a) Total zinc content (mg/100 g of preparation) after extraction to stomach juice over the time of 15, 30, and 60 min. b) Total zinc content (mg/100 g of preparation) after extraction to intestinal juice over the time of 15, 30, and 60 min incubation in stomach juice

Table 4. The content of phenolic compounds released to artificial digestive juices from selected *Arthrospira platensis*-containing preparations.

Artificial juice	Artificial stomach juice (mg/100 g of preparation)			Artificial intestinal juice (mg/100 g of preparation)			
	15	30	60	150	150	150	
Preparation	(after 1 min incubation in artificial saliva)			(after incubation in artificial stomach juice)			
Extract to artificial digestive juice							Methanolic extract (control)
Gallic acid							
Spirulina B	0.12 ± 0.05 ^a	0.14 ± 0.00 ^a	0.13 ± 0.00 ^a	0.66 ± 0.00 ^a	0.12 ± 0.00 ^a	0.33 ± 0.00 ^a	0.79 ± 0.02 ^a
Spirulina P	0.18 ± 0.00 ^b	0.38 ± 0.00 ^{ab}	0.07 ± 0.2 ^{ab}	2.13 ± 0.33 ^b	0.89 ± 0.09 ^b	1.38 ± 0.00 ^a	0.67 ± 0.04 ^b
Spirulina M	0.33 ± 0.03 ^{abc}	0.69 ± 0.04 ^{abc}	0.10 ± 0.01 ^{abc}	7.22 ± 1.11 ^{abc}	4.56 ± 1.78 ^{abc}	1.41 ± 0.30 ^{ac}	2.44 ± 0.17 ^{bc}
Spirulina N	0.01 ± 0.00 ^{bc,d}	0.02 ± 0.03 ^{bc,d}	0.01 ± 0.00	0.62 ± 0.58	0.59 ± 0.61	0.42 ± 0.40	1.06 ± 0.06
Spirulina O	0.10 ± 0.00	0.15 ± 0.00	-	7.31 ± 0.81 ^{abd}	2.32 ± 0.38 ^{bc}	2.09 ± 0.74 ^{cd}	0.81 ± 0.02 ^{cd}
Protocatechuic acid							
Spirulina B	1.46 ± 0.14 ^a	0.28 ± 0.05 ^a	1.97 ± 0.05 ^a	0.93 ± 0.10 ^a	0.03 ± 0.00 ^a	0.16 ± 0.06 ^a	0.01 ± 0.001 ^a
Spirulina P	1.44 ± 0.14 ^b	0.01 ± 0.00 ^{ab}	0.71 ± 0.05 ^{ab}	0.01 ± 0.00 ^a	0.30 ± 0.02 ^b	0.50 ± 0.05 ^{ab}	0.01 ± 0.00 ^b
Spirulina M	0.40 ± 0.03 ^{abc}	0.39 ± 0.05 ^{bc}	2.22 ± 0.60 ^{bc}	0.04 ± 0.005	0.01 ± 0.001	0.01 ± 0.01	0.01 ± 0.01
Spirulina N	0.20 ± 0.002 ^{abd}	0.25 ± 0.06 ^{bd}	0.64 ± 0.04 ^{bc}	0.05 ± 0.001 ^a	0.05 ± 0.003 ^{bc}	0.01 ± 0.00 ^{ab}	0.05 ± 0.003 ^c
Spirulina O	1.25 ± 0.27 ^{cd}	0.79 ± 0.13 ^{bc,d}	0.93 ± 0.0 ^{bc}	0.02 ± 0.002 ^a	0.06 ± 0.001 ^{abc}	0.01 ± 0.00 ^{ab}	0.01 ± 0.002 ^d
3,4-Dihydroxyphenylacetic acid							
Spirulina B	26.47 ± 8.94 ^a	20.27 ± 6.01 ^a	20.90 ± 2.66 ^a	2.05 ± 0.08 ^a	2.29 ± 0.25	4.49 ± 0.20 ^a	1.13 ± 0.11 ^a
Spirulina P	38.14 ± 2.25 ^{ab}	1.83 ± 0.00 ^b	7.05 ± 0.13 ^{ab}	2.27 ± 0.19 ^b	2.80 ± 0.30 ^b	9.48 ± 2.11 ^{ab}	0.71 ± 0.07 ^b
Spirulina M	5.27 ± 0.89 ^{ab}	6.55 ± 1.27 ^a	25.15 ± 2.12 ^{bc}	3.86 ± 0.21 ^{abc}	2.39 ± 0.2	1.95 ± 0.04 ^{ab}	1.61 ± 0.13 ^c
Spirulina N	2.05 ± 0.00 ^{ab}	3.15 ± 0.77 ^a	7.96 ± 0.24 ^{bc}	2.16 ± 0.00 ^{cd}	2.00 ± 0.16 ^b	2.14 ± 0.28 ^{ab}	5.77 ± 0.38 ^{cd}
Spirulina O	6.62 ± 1.30 ^{ab}	8.60 ± 1.72 ^a	7.64 ± 1.24 ^{bc}	3.26 ± 0.15 ^{abc}	2.23 ± 0.15	1.99 ± 0.34 ^{ab}	0.71 ± 0.01 ^{bc,d}
<i>p</i> -Hydroxybenzoic acid							
Spirulina B	0.16 ± 0.01 ^a	0.12 ± 0.06 ^a	0.11 ± 0.05 ^a	0.09 ± 0.01 ^a	0.09 ± 0.01 ^a	0.11 ± 0.04 ^a	0.03 ± 0.01 ^a
Spirulina P	0.45 ± 0.07 ^{ab}	0.22 ± 0.03 ^b	0.29 ± 0.02 ^{ab}	0.09 ± 0.01 ^b	0.12 ± 0.03	0.22 ± 0.05 ^{ab}	0.05 ± 0.01 ^b
Spirulina M	0.11 ± 0.01 ^{bc}	0.13 ± 0.05 ^c	0.14 ± 0.02 ^b	0.22 ± 0.03 ^{abc}	0.11 ± 0.03 ^c	0.08 ± 0.00 ^{ab}	0.09 ± 0.01 ^{abc}
Spirulina N	0.09 ± 0.01 ^{bd}	0.08 ± 0.00 ^{bd}	0.18 ± 0.06	0.34 ± 0.02 ^{abcd}	0.20 ± 0.19	0.07 ± 0.01 ^b	0.37 ± 0.01 ^{abcd}
Spirulina O	0.30 ± 0.04 ^{abcd}	0.44 ± 0.01 ^{abcd}	0.25 ± 0.06 ^a	0.08 ± 0.01 ^{cd}	0.10 ± 0.05 ^d	0.09 ± 0.01 ^b	0.05 ± 0.01 ^{cd}
Syringic acid							
Spirulina B	0.37 ± 0.03 ^a	0.21 ± 0.02 ^b	0.28 ± 0.07 ^a	0.10 ± 0.01 ^a	0.08 ± 0.01 ^a	0.07 ± 0.005 ^a	0.02 ± 0.001 ^a
Spirulina P	0.40 ± 0.02 ^b	0.38 ± 0.02 ^{ab}	0.29 ± 0.05 ^b	0.07 ± 0.02 ^b	0.17 ± 0.01 ^{ab}	0.21 ± 0.01 ^{ab}	0.03 ± 0.00 ^{ab}
Spirulina M	0.04 ± 0.0 ^b	0.21 ± 0.06 ^{abc}	0.45 ± 0.06 ^{abc}	0.29 ± 0.001 ^{abc}	0.14 ± 0.01 ^{bc}	0.05 ± 0.005 ^{bc}	0.05 ± 0.01 ^{abc}
Spirulina N	0.02 ± 0.00 ^{ab}	0.02 ± 0.00 ^{abc}	0.05 ± 0.01 ^{abc}	0.19 ± 0.02 ^{abcd}	0.09 ± 0.01 ^{bc,d}	0.05 ± 0.01 ^{bd}	0.07 ± 0.01 ^{abcd}
Spirulina O	0.04 ± 0.00 ^{ab}	0.05 ± 0.0 ^{abc}	0.05 ± 0.01 ^{abc}	0.31 ± 0.01 ^{abd}	0.15 ± 0.03 ^{cd}	0.13 ± 0.04 ^{abcd}	0.01 ± 0.00 ^{cd}
Cinnamic acid							
Spirulina B	0.11 ± 0.01 ^a	0.01 ± 0.00 ^a	0.07 ± 0.01 ^a	0.03 ± 0.00 ^a	0.08 ± 0.005 ^a	0.10 ± 0.01 ^a	0.01 ± 0.00 ^a
Spirulina P	0.04 ± 0.005 ^{ab}	0.07 ± 0.00	0.05 ± 0.00 ^b	0.11 ± 0.003 ^{ab}	0.06 ± 0.01 ^{ab}	0.06 ± 0.001 ^{ab}	0.01 ± 0.00 ^b
Spirulina M	0.07 ± 0.003 ^{abc}	0.04 ± 0.001 ^c	0.11 ± 0.02 ^{bc}	0.11 ± 0.01 ^{bc}	0.07 ± 0.005 ^c	0.07 ± 0.00 ^{bc}	0.04 ± 0.002 ^{abc}
Spirulina N	0.09 ± 0.002 ^{abc}	0.12 ± 0.005 ^{bc}	0.07 ± 0.00 ^{cd}	0.01 ± 0.00 ^{abcd}	0.01 ± 0.00 ^{abcd}	0.05 ± 0.001 ^{abcd}	0.08 ± 0.005 ^{abcd}
Spirulina O	0.04 ± 0.005 ^{acd}	0.12 ± 0.06 ^{cd}	0.03 ± 0.003 ^{acd}	0.04 ± 0.005 ^{acd}	0.05 ± 0.005 ^{acd}	0.07 ± 0.00 ^{cd}	0.02 ± 0.00 ^{abcd}

Table 4. Continued.

Artificial juice	Artificial stomach juice (mg/100 g of preparation)			Artificial intestinal juice (mg/100 g of preparation)			
	15	30	60	150	150	150	
Time [min]	(after 1 min incubation in artificial saliva)			(after incubation in artificial stomach juice)			
Preparation							Methanolic extract (control)
Extract to artificial digestive juice							
Quercetin							
Spirulina B	0.06 ± 0.01 ^a	0.03 ± 0.00 ^b	0.04 ± 0.00 ^b	0.01 ± 0.00 ^b	0.02 ± 0.00 ^b	0.01 ± 0.00 ^b	0.01 ± 0.00 ^b
Spirulina P	0.04 ± 0.01 ^{ab}	0.21 ± 0.02 ^{ab}	0.11 ± 0.04 ^{ab}	0.01 ± 0.00 ^b	0.03 ± 0.005 ^{ab}	0.18 ± 0.001 ^{ab}	0.03 ± 0.00 ^{ab}
Spirulina M	0.02 ± 0.00 ^{ab}	0.03 ± 0.002 ^b	0.11 ± 0.02 ^{bc}	0.02 ± 0.002 ^{bc}	0.01 ± 0.00 ^{ab}	0.01 ± 0.00 ^b	0.03 ± 0.02 ^{bc}
Spirulina N	0.01 ± 0.00 ^{ab}	0.01 ± 0.00 ^b	0.01 ± 0.00 ^{bc}	0.01 ± 0.00 ^b	0.01 ± 0.00 ^{ab}	0.01 ± 0.00 ^b	0.11 ± 0.00 ^{ab,cd}
Spirulina O	0.01 ± 0.00 ^{ab}	0.01 ± 0.00 ^b	0.02 ± 0.00 ^{bc}	0.01 ± 0.00 ^b	0.01 ± 0.00 ^{ab}	0.01 ± 0.00 ^b	0.02 ± 0.00 ^{ab,cd}

Data are presented as the mean ± standard deviation (SD); n = 6 repetitions Tukey–Kramer test was used to reveal the differences between paired groups of phenolic compounds in rows, the same letters (a, b, c, d) are marked for the content whose differences are statistically significant (for p values < 0.05) (GraphPad InStat)

highest for capsules (up to 6.8 mg/day) and the lowest for lyophilizate (up to 2.6 mg/day).

In summary, the release of zinc and organic compounds from preparations containing *A. platensis* depended on the time of incubation and the degree of degradation in artificial digestive juices. Artificial digestive juices did not break down *A. platensis*-containing tablets even after a maximum incubation period of 60 min in artificial stomach juice and 150 min in artificial intestinal juice. During extractions was observed the formation of a gelatinous layer on the surface to prevent liquid penetration into the interior and further disintegration of the tablet mass. After the tablet was cut into two parts, the completely dry core was clearly visible inside.

Thereby, was decided to study the physical properties of tablets containing *A. platensis* according to European Pharmacopeia 8th ed (25). The physical properties of prepared tablets are shown in Table 6.

The controlled tablets were elegant in appearance. The thickness of the tablets ranged from 4.74 to 4.99 mm. For formulation Spirulina O percent deviations of thickness exceeded 5% (acceptable range of thickness is ± 5%). Average weight, hardness, and friability were within pharmacopeia specification. The variation in weight ranged from 255.3 to 401.5 mg (acceptable range of weight variation is ± 5%). The hardness of tablets ranged from 62.4 to 64.3 N (acceptable range of hardness is > 40 N) and friability ranged from 0.15 to 0.22% (acceptable range of friability is < 1%). The disintegration times of investigated tablets exceed 15 min. Tablet

formulations had excessively long disintegration times which amounted from 72.3 to 81.2 min. The average disintegration time of investigated tablets was found to be up to 76.7 min. According to European Pharmacopeia 8th ed., the disintegration times for these preparations should be less than 15 min. Hence, on the basis of the conducted studies, was found that *A. platensis* in the form of tablets cannot be a good source of zinc and organic compounds in the human diet. Of the three preparations containing *A. platensis* tested in accordance with the pharmacopeia regulations, only capsule form complied with the pharmacopeia standards. The deviations of weight in capsule ranged from 0.2 to 3.6% and the disintegration time was found to be 11.5 min.

Chemometric analysis

Chemometric tools were used to obtain deeper information of the obtained dataset (results of indole and phenolic compounds performed for preparations containing *A. platensis*). The analysis was based on Table 4. and 5. The objects of analysis were preparations containing *A. platensis* in various forms (powder, tablets, and capsules), described by parameters – analyzed indole and phenolic compounds. Chemometric analysis for such a dataset, characterized by a wide range of variability, allowed the extraction of additional information on the correlations that occur between the analyzed objects preparations containing *A. platensis*. Two methods for chemometric analysis were used in the study: cluster analysis (CA) and principal component analysis (PCA). CA method indicated the similarity

Table 5. The content of indole compounds released to artificial digestive juices from selected *Arthrospira platensis* – containing preparations.

Artificial juice	Artificial stomach juice (mg/100 g of preparation)			Artificial intestinal juice (mg/100 g of preparation)			Control
	Time [min]	15	30	60	150	150	
Preparation	(after 1 min incubation in artificial saliva)			(after incubation in artificial stomach juice)			Control
5-Hydroxy-L-tryptophan							
<i>Extract</i>							Control
Spirulina B	124.14 ± 10.3 ^a	18.10 ± 0.81 ^a	88.73 ± 12.0 ^a	45.74 ± 1.79 ^a	35.56 ± 4.21 ^a	50.02 ± 8.78 ^a	11.47 ± 0.71 ^a
Spirulina P	117.35 ± 2.41 ^{ab}	–	25.02 ± 1.21 ^{ab}	33.81 ± 5.67 ^b	29.07 ± 4.31 ^b	21.25 ± 1.41 ^{ab}	9.54 ± 0.15 ^b
Spirulina M	27.17 ± 2.98 ^{ab}	80.28 ± 1.60 ^{abc}	–	156.20 ± 17.2 ^{abc}	96.69 ± 11.06 ^{ab}	28.70 ± 1.71 ^a	–
Spirulina N	22.07 ± 1.93 ^{ab}	3.47 ± 0.04 ^{abc}	46.46 ± 3.96 ^{abcd}	33.02 ± 3.97 ^b	60.51 ± 5.84 ^{abc}	23.39 ± 1.18 ^a	29.49 ± 1.61 ^{abc}
Spirulina O	27.19 ± 1.02 ^{ab}	42.23 ± 3.62 ^{abcd}	27.71 ± 2.43 ^{abc}	42.03 ± 2.24 ^a	34.56 ± 3.43 ^{cd}	33.29 ± 2.92 ^{ab}	–
Tryptamine							
<i>Extract</i>							Control
Spirulina B	–	–	–	1.54 ± 0.00 ^a	1.57 ± 0.01 ^a	5.37 ± 0.80 ^a	–
Spirulina P	1.66 ± 0.01	–	–	1.57 ± 0.00 ^b	1.83 ± 0.03 ^b	1.71 ± 0.20 ^{ab}	–
Spirulina M	–	2.37 ± 0.00	–	3.66 ± 0.38 ^{abc}	2.94 ± 0.38 ^{abc}	4.23 ± 0.17 ^{abc}	–
Spirulina N	–	1.68 ± 0.15	1.36 ± 0.01	–	–	–	–
Spirulina O	–	–	–	3.18 ± 0.08 ^{abcd}	2.37 ± 0.37 ^{cd}	2.49 ± 0.09 ^{cd}	–
5-Methyltryptamine							
<i>Extract</i>							Control
Spirulina B	–	–	3.27 ± 0.00	3.43 ± 0.01	3.21 ± 0.01	3.21 ± 0.01	0.69 ± 0.01
Spirulina P	–	–	–	–	–	–	–
Spirulina M	–	–	–	–	–	–	–
Spirulina N	–	–	–	–	–	–	–
Spirulina O	–	–	–	–	–	–	–
6-Methyl-L-tryptophan							
<i>Extract</i>							Control
Spirulina B	1.57 ± 0.08 ^a	1.03 ± 0.00 ^b	1.40 ± 0.10 ^b	1.12 ± 0.01 ^a	0.92 ± 0.08 ^a	–	–
Spirulina P	3.71 ± 0.18 ^{ab}	3.92 ± 0.21 ^{ab}	1.41 ± 0.22 ^b	1.92 ± 0.33 ^{ab}	2.94 ± 0.11 ^{ab}	5.00 ± 0.26 ^{ab}	0.76 ± 0.04 ^b
Spirulina M	3.06 ± 0.21 ^{abc}	10.23 ± 1.74 ^{abc}	4.91 ± 0.08 ^{ab}	–	–	1.04 ± 0.01 ^{abc}	–
Spirulina N	2.44 ± 0.11 ^{abcd}	–	–	0.26 ± 0.01 ^{ab}	0.74 ± 0.09 ^{bcd}	–	1.38 ± 0.01 ^b
Spirulina O	1.00 ± 0.08 ^{abcd}	1.88 ± 0.83 ^c	1.13 ± 0.20 ^{cd}	–	0.74 ± 0.04 ^{abd}	0.58 ± 0.04 ^{abcd}	–
L-Tryptophan							
<i>Extract</i>							Control
Spirulina B	2.39 ± 0.78 ^a	27.7 ± 0.32 ^a	1.69 ± 0.42 ^a	1.50 ± 0.10	0.50 ± 0.01	–	–
Spirulina P	2.61 ± 0.25 ^b	12.03 ± 0.46 ^{ab}	0.46 ± 0.00 ^b	–	–	–	–
Spirulina M	9.60 ± 0.78 ^{abc}	18.45 ± 1.75 ^{abc}	1.26 ± 0.05 ^{abc}	2.92 ± 0.00	1.08 ± 0.10	–	–
Spirulina N	0.76 ± 0.06 ^{abc}	–	0.83 ± 0.04 ^{abc}	–	–	–	–
Spirulina O	–	1.09 ± 0.40 ^{bc}	0.75 ± 0.03 ^{bc}	–	–	–	–

Data are presented as the mean ± standard deviation (SD); n = 6 repetitions Tukey–Kramer test was used to reveal the differences between paired groups of phenolic compounds in rows, the same letters (a, b, c, d) are marked for the content whose differences are statistically significant (for p values < 0.05) (GraphPad InStat)

between the examined objects (preparations containing *A. platensis*). In case of this analysis, the similarity of objects is demonstrated by their close relative position in multidimensional space. The graphical image of the CA analysis is the dendrogram (Fig. 2), in which the objects characterized by considerable similarity form the clusters. In this instance, the x and y -axes do not correspond to the Cartesian numerical axes (31, 32). The analyzed objects (*A. platensis* preparations) were marked on the x -axis, whereas the distance between the examined objects calculated on the basis of Ward's agglomeration method on the y -axis. Three major clusters were observed based on the similarity analysis (CA – Fig. 2). The first cluster formed preparations in the form of capsules, while the remaining clusters were formed from preparations in

the form of tablets or powders. Their membership in particular clusters indicates their similarity within particular clusters. This similarity may be related to the composition of the examined preparations (content of indole and phenolic compounds) as well as the degree of release of the examined metabolites into the digestive juices.

Short dendrogram arms in case of preparations in the form of tablets show the highest correlation similarity within these preparations (31, 33). In addition, the differences between the preparations (capsules and tablets and powdered form) belonging to other clusters may result from unequal conditions of *A. platensis* culture and depend on the type of *A. platensis* used in the preparations; therefore, there are discrepancies for metabolites released into the digestive juices.

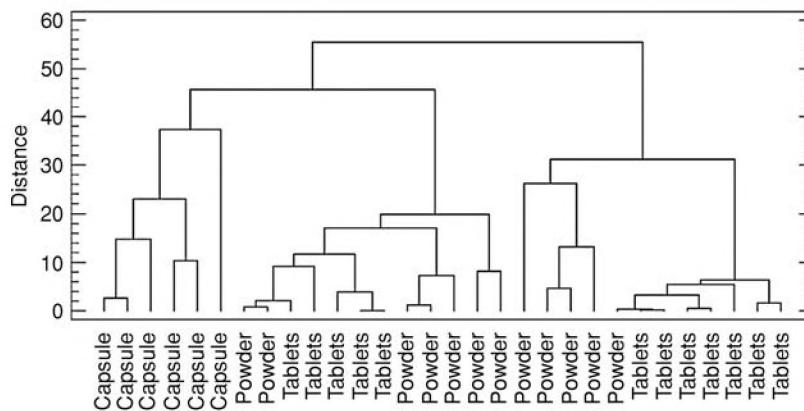


Figure 2. Dendrogram presenting similarity between the objects (preparations containing *Arthrospira platensis*) depending on the form of preparation (City-Block, Ward's algorithm). The analysis was performed in Statgraphics Centurion XVII program

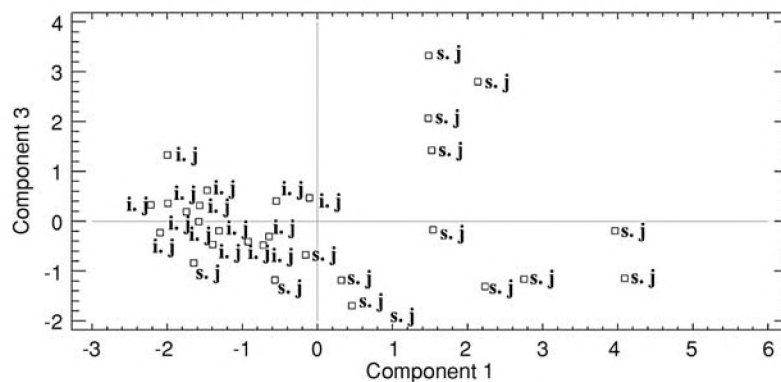


Figure 3. Scatterplot – similarity of the analyzed preparation with *Arthrospira platensis* with respect to the site of release phenolic and indole compounds (s. j – stomach juice, i. j – intestinal juice). The analysis was performed in Statgraphics Centurion XVII program

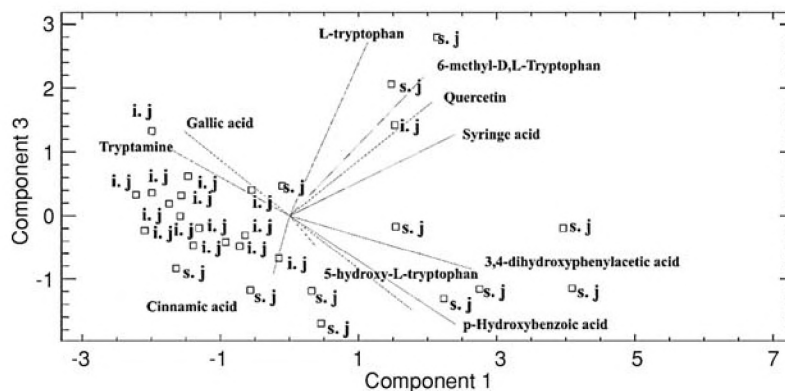


Figure 4. Biplot diagram presenting the correlation between the analyzed organic compound present in the preparations containing *Arthrospira platensis* and the site of their release in human digestive tract (s, j – stomach juice, i, j – intestinal juice). The analysis was performed in Statgraphics Centurion XVII program

Table 6. Evaluation of physical properties of prepared tablets (mean \pm standard deviation).

Formulation	Weight variation (mg)	Thickness (mm)	Friability (%)	Breaking force (N)	Disintegration time (min)
Spirulina M	443.0 \pm 4.62	7.5 \pm 1.10	--	–	11.5 \pm 1.05
Spirulina N	401.45 \pm 4.21	4.74 \pm 0.03	0.219	62.4 \pm 7.06	81.17 \pm 5.56
Spirulina O	255.3 \pm 5.09	4.99 \pm 0.12	0.15	64.3 \pm 6.38	72.33 \pm 4.59

Table 7. Factor loads for three first main principal components (PC1, PC2, and PC3).

Compounds	PC1	PC2	PC3
3,4-Dihydroxyphenylacetic acid	0.450	0.217	-0.170
5-Hydroxy-L-tryptophan	0.061	0.581	-0.090
6-methyl-D,L-Tryptophan	0.314	-0.070	0.415
Cinnamic acid	-0.038	0.226	-0.173
Gallic acid	-0.242	0.495	0.249
L-Tryptophan	0.186	-0.137	0.528
p-Hydroxybenzoic acid	0.279	0.047	-0.277
Protocatechuic acid	0.422	0.084	-0.355
Quercetin	0.326	-0.061	0.333
Syringic acid	0.381	0.352	0.240
Tryptamine	-0.303	0.397	0.216

The second method complementary to the CA used in this study is PCA. PCA allows the reduction of the measurement data space to the minimum necessary to characterize the interactions between them. A large number of parameters (measured content of indole and phenolic compounds) describing ana-

lyzed objects (preparations with *A. platensis*) made the direct visualization of multidimensional data space complicated. In order to facilitate it, the number of data was reduced by correlating them. Output parameters were replaced by new variables – primary components. PCA analysis demonstrated that

61.92% of the variations in the examined data set can be described by the three primary components (PC1, PC2, and PC3). Consequently, the remaining components were not considered for further analysis. The three primary components (PC1, PC2, and PC3) are a linear combination of primary variables multiplied by corresponding charges. The values of the charges that correspond to the correlation coefficient with the primary variables are summarized in Table 7. For the PC1 component, its magnitude is considerably affected by the measured value of the phenolic compound, that is, 3,4-dihydroxyphenylacetic acid. The other main components, PC2 and PC3, can be interpreted in the same way. Consequently, the reduction of the input data area to the three primary components allowed for analysis in two-dimensional space (Fig. 3) (34, 35). Two distinct clusters were distinguished analyzing the examined objects (preparations with *A. platensis*) in relation to the site in the digestive system, where phenolic and indole compounds were released (Fig. 3). These compounds are to a various degree released into an artificial stomach and intestinal juices, as evidenced by their belonging to distinct groups. Such division indicates that the examined organic components are released into the digestive juices to varying degrees, which in turn, depends on the release site in the condition imitated human digestive tract. In addition, considering the Biplot diagram (Fig. 4) based on the two primary components (PC1 and PC3), the preference of components to the site where they were released into the artificial digestive tract was analyzed. On this basis, was found that the release of indole and phenolic compounds from preparations containing *A. platensis* into artificial digestive juices depended on the type of artificial digestive juice. The organic compounds from the preparations were to the highest degree released into stomach juices, which confirms the direction of the arms in the Biplot diagram (Fig. 4). Only sparse phenolic compounds (gallic acid and cinnamic acid) and indole ones (tryptamine) were released from the preparations mostly to intestinal juice.

CONCLUSIONS

The experiment presented that the active substances in the commercial preparations are not completely released into the artificial digestive juices and hence may not be potentially available to human. The quantities of released zinc (II) ions from preparations containing *A. platensis* are insufficient for human body according to the daily requirement for this element. In the case of phenolic compounds, their amounts were found to be

higher in the extracts of artificial digestive juices than that of methanolic extracts of the tested preparations. In this way, it has been adequately shown that the content of active substance in medicinal preparations is not the same as its amount released into digestive juices which suggest the need for such analyzes. The experiment further showed that only hard capsules met the European Pharmacopeia 8th ed standards whereas tablets did not. Due to this fact, an important element of research on dietary supplements is the evaluation of their formulations' quality and the definition of a form that allows the release of biologically active compounds in the most effective manner.

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