Research Article

Determination of Volatile Organic Compounds in Selected Strains of Cyanobacteria

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Microalgal biomass can be used in creating various functional food and feed products, but certain species of microalgae and cyanobacteria are known to produce various compounds causing off-flavour. In this work, we investigated selected cyanobacterial strains of *Spirulina*, *Anabaena*, and *Nostoc* genera originating from Serbia, with the aim of determining the chemical profile of volatile organic compounds produced by these organisms. Additionally, the influence of nitrogen level during growth on the production of volatile compounds was investigated for *Nostoc* and *Anabaena* strains. In addition, multivariate techniques, namely, principal component analysis (PCA) and hierarchical cluster analysis (HCA), were used for making distinction among different microalgal strains. The results show that the main volatile compounds in these species are medium chain length alkanes, but other odorous compounds such as 2-methylisoborneol (0.51–4.48%), 2-pentylfuran (0.72–8.98%), β -cyclocitral (0.00–1.17%), and β -ionone (1.15–2.72%) were also detected in the samples. Addition of nitrogen to growth medium was shown to negatively affect the production of 2-methylisoborneol, while geosmin was not detected in any of the analyzed samples, which indicates that the manipulation of growth conditions may be useful in reducing levels of some unwanted odor-causing components.

1. Introduction

Cyanobacteria (blue-green algae) among all microalgae represent some of the oldest living organisms and show great biological diversity [1, 2]. This evolutionary and phylogenetic diversity also means a great diversity regarding the chemical composition of these organisms, which makes them very attractive for use as sources of a wide range of biomolecules [3]. Microalgae are important sources of commercially produced high-value molecules including carotenoids [4], long-chain polyunsaturated fatty acids (PUFA), proteins [5, 6], and phycobilins [7–9]. The addition of microalgal biomass to food and feed products is an interesting option for providing nutritional supplementation with these biologically active compounds. Novel foods development requires selection of microalgal species with balanced nutritional profiles and proven health safety. The chemical composition of microalgae is often highly dependent on various environmental factors,

such as temperature, salinity, illumination, pH value, mineral content, CO₂ supply, population density, growth phase, and physiological status [10]. Beside many beneficial properties, microalgae also produce numerous volatile organic compounds which can cause musty, fishy, and mud-like odour. Blooms and scums of cyanobacteria can occur in various fresh and brackish water environments, causing musty odour and production of harmful toxins [11]. When microalgal biomass is added to food products, its odour and aroma can influence the sensory properties of the final products [12], and, in natural environment, it can also affect the sensory quality of the produced food (e.g., fish) [13].

In this work, we investigated selected strains of *Spirulina*, *Anabaena*, and *Nostoc* originating from Serbia, which were previously proven to be nontoxic under specified growing conditions [14]. As the investigated strains show a potential for incorporation in novel food products, being rich in proteins and PUFAs [15], the aim of this study was to

determine the chemical profile of volatile organic compounds produced by these organisms. Influence of nitrogen level during growth on the production of volatile compounds was also investigated for *Nostoc* and *Anabaena* strains. The mentioned work was undertaken in order to assess the best potential species of microalgae for incorporation in functional food products regarding their volatile compounds profile, as well as to determine the optimal growth conditions for minimizing undesirable sensory properties. In addition, multivariate techniques, principal component analysis (PCA), and hierarchical cluster analysis (HCA) were used to test whether they can be employed for making distinction among different microalgal strains.

2. Materials and Methods

2.1. Chemicals. A mixture of n-alkanes (Sigma-Aldrich, Germany) from n-octane (C8) to eicosane (C20) was used for calculation of retention indices (RI).

2.2. Samples. Samples of *Spirulina platensis* (labeled as S1 and S2), *Nostoc* spp. (labeled as 2S7B and 2S9B), and *Anabaena* spp. (labeled as C2 and C5) strains were obtained from the Department of Biology and Ecology at the Faculty of Sciences, University of Novi Sad. All of the investigated strains originated from Vojvodina region of Serbia [16]. Strains of the *Nostoc* and *Anabaena* genera were cultivated under laboratory conditions in synthetic mineral broth BG-11, with (+N) and without added nitrogen (−N) [17] while *Spirulina* strains were cultivated in mineral SOT broth [18]. All the investigated cyanobacteria were cultivated as steady cultures in Erlenmeyer flasks at temperature of 22–24°C and under illumination by cool white fluorescent light (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Daily light regimen was set to 12 hours of light and 12 hours of darkness. After 25 days of cultivation, the strains were lyophilized to obtain the dry algal biomass for analyses and kept well sealed at 5°C until usage.

2.3. Headspace and GC-MS Analysis Procedure. Static headspace sampling was performed with the headspace sampler, CombiPAL System (CTC Analytics, Zwingen, Switzerland). A 2.5 mL headspace syringe for CombiPAL was used for the injection of 2 mL from the 10 mL headspace vials with 1 g of measured dry sample or 50 μL of n-alkane mixture. The autosampler conditions were set as follows: incubation temperature, 80°C; incubation time, 10 min; syringe temperature, 100°C; agitator speed, 500 rpm; fill speed, 100 $\mu\text{L/s}$; pullup delay, 1 s; injection speed, 500 $\mu\text{L/s}$; pre- and postinjection delay, 500 ms; flush time, 10 s. After each injection, carryover in the syringe was eliminated by automatic flush of the syringe with carrier gas.

Chromatographic separation was achieved by Agilent Technologies GC-MS Model 7890 A Series gas chromatograph coupled to 5975 C mass selective detector. A HP 5 MS (30 m $\times$ 0.25 mm i.d.) (J & W Scientific, USA) fused silica capillary column with a 0.25 μm film thickness was used with helium as carrier gas (purity > 99.9997 vol% and flow rate = 1.1 mL/min). Oven temperature program was started at 60°C

(not held) and linear temperature gradient was applied at rate of 3°C/min to final temperature of 260°C and held for 5 minutes (total run time: 65 min). The ion source temperature was kept at 230°C, the quadrupole was at 150°C, and the mass spectra were obtained in 50 to 500 m/z range, at an electron energy of 70 eV.

ChemStation software (Agilent Technologies) was used for data analysis, and curves used for experimental estimation of retention indices were plotted and drawn using SciDaVis (<http://scidavis.sourceforge.net/>) software. The identification of the compounds was based on comparison of their retention indices (RI) calculated against mixture of n-alkanes, their retention times (RT), and mass spectra with NIST 05/Adams libraries spectra and literature [19].

2.4. Statistical Analysis. The means of two replicates were subjected to PCA and HCA using XLSTAT (Addinsoft, 2013, NY, USA).

3. Results and Discussion

The obtained results of the volatile compounds determination are shown in Table 1. All the results are shown as relative % of chromatographic peak abundance in total ion chromatograms. Temperature at which samples were analyzed (90°C) was chosen by the authors in order to represent conditions of the thermal treatment during cooking. The results show that unbranched alkanes represent the main group of volatile compounds in these species. It has been shown that hydrocarbon production in cyanobacteria is mainly achieved by metabolic pathways connected with fatty acids. Research has identified two enzyme families that are responsible for alkane production in cyanobacteria: an acyl–acyl carrier protein reductase (AAR) and an aldehyde decarbonylase (AAD), which play crucial role in converting fatty acid intermediates to alkanes and alkenes [20]. Heptadecane is shown to be present in all of the analyzed samples, with its content ranging from 27.39% in C5+N sample to 82.21% in S1. Hexadecane and pentadecane were also detected in all samples, although in lower amounts compared to heptadecane (0.90–5.45% for hexadecane and 0.22–8.81% for pentadecane). Tetradecane and 6,9-heptadecadiene were detected only in samples of *Spirulina*. Other significant hydrocarbons were 8-heptadecene which was detected in one *Nostoc* (2S9B) and two *Anabaena* (C2 and C5) strains and 8-methylheptadecane which was detected in 2S7B and C2 strains. 3-Octadecene was detected only in one (2S9B) *Nostoc* strain. The obtained results are in accordance with work of other authors [21, 22], indicating that, although C16 and C18 are the most abundant types of fatty acids in cyanobacteria, the main hydrocarbons produced are from C15 to C17 chain length. It should be noted, however, that higher chain hydrocarbons may also be present in the investigated samples but were not detected due to relatively low temperature used during the headspace sampling procedure and not utilizing previous extraction of samples with organic solvents.

2-Methylisoborneol (MIB) and geosmin are among the most important odorous compounds in cyanobacteria and are often cited as sources of unpleasant earth-like and

TABLE 1: Volatile organic compounds as relative % of chromatographic peak abundance in total ion chromatograms in the investigated cyanobacterial samples.

Compound	RI	SI	S2	2S7B+N	2S7B-N	2S9B+N	2S9B-N	C2+N	C2-N	C5+N	C5-N
2-Pentylfuran (I)*	992	0.80	5.25	3.69	6.05	1.27	1.17	1.81	8.56	8.98	0.72
2-Ethyl-1-hexanol (II)	995	0.77	n.d.	0.51	5.59	0.98	5.56	n.d.	n.d.	n.d.	n.d.
2,2,6-Trimethylcyclohexanone (III)	1086	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.73	4.99	0.29	0.90
1,1,3-Trimethyl-2-cyclohexanone (IV)	1096	n.d.	0.77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,6-Dimethylcyclohexanol (V)	1030	2.16	2.66	3.95	4.24	1.69	1.92	1.24	3.06	3.48	0.61
β -Cyclotriol (VI)	1204	0.20	1.09	0.43	1.17	0.25	0.50	0.33	1.34	n.d.	n.d.
2-Methylisoborneol (VII)	1262	0.90	1.06	0.80	2.88	0.51	4.42	0.94	4.48	1.77	2.18
Tetradecane (VIII)	1413	0.14	0.39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β -Ionone (IX)	1457	1.42	1.75	1.96	2.47	1.75	1.47	1.15	2.72	1.72	1.20
Pentadecane (X)	1512	4.15	8.81	0.83	0.68	0.22	0.74	0.52	0.84	0.63	0.44
Diisobutyric acid											
1-tert-butyl-2-methyl-1,3-propanediyl ester (XI)	1560	0.90	n.d.	0.67	2.45	0.61	0.68	n.d.	n.d.	n.d.	n.d.
Hexadecane (XII)	1612	5.45	4.79	1.29	1.07	1.35	1.55	1.11	1.34	1.46	0.90
6,9-Heptadecadiene (XIII)	1727	0.90	0.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8-Heptadecene (XIV)	1719	n.d.	n.d.	n.d.	n.d.	29.38	30.87	0.45	0.00	54.29	56.72
Heptadecane (XV)	1711	82.21	73.12	43.49	45.19	59.81	44.51	64.82	64.65	27.39	36.32
8-Methylheptadecane (XVI)	1746	n.d.	n.d.	42.37	28.21	n.d.	n.d.	26.91	8.01	n.d.	n.d.
3-Octadecene (XVII)	1818	n.d.	n.d.	n.d.	n.d.	2.18	6.60	n.d.	n.d.	n.d.	n.d.

*Roman numerals in brackets are assigned for the purpose of simplification of PCA analysis maps.

n.d.: not detected.

RI: retention index; SI and S2: samples of *Spirulina platensis*; 2S7B and 2S9B: samples of *Nostoc* spp. (+N and -N stands for with and without added nitrogen to the medium); C2 and C5: samples of *Anabaena* spp. (+N and -N stands for with and without added nitrogen to the medium).

musty odour, especially in various aquatic environments [23–25]. MIB was detected in all samples in the range from 0.51% in sample 2S9B+N to 4.48% in sample C2–N. It is important to note that, to our knowledge, there have been no previous reports of MIB production by *Spirulina* species. Concentrations of MIB seem to be higher in all *Anabaena* and *Nostoc* strains grown without added nitrogen (–N) when compared to the same strains where nitrogen was added to the growing medium (+N), which may indicate that the presence of added nitrogen suppresses biosynthesis of MIB. The results of increases or decreases in MIB production related to the amount of nitrogen in the growth medium are weakened by relying solely on relative % of chromatographic peak abundance, since changes in the amounts of other peak areas can directly affect the % of MIB peak abundance. Regarding this issue, further studies, using MIB standard in order to more accurately quantitate the differences in concentrations in samples, should be undertaken to assess the observed differences. Interestingly, geosmin was not detected in any of the tested samples in this work, which may be explained by observations of Saadoun et al. [24], who concluded that addition of copper ions to growing medium at level above 6.92 mg Cu^{2+} /L had inhibitory effect on the production of geosmin. Since copper ions were added to the growing medium of all tested strains in our work in concentrations which were higher than previously mentioned, it may explain why geosmin was not detected in any of the investigated samples. However, it is also possible that none of the cyanobacterial strains in this study naturally produces geosmin. Also, the previously mentioned group of authors had similar observations regarding the effect of added nitrogen to the medium on production of geosmin but, to our knowledge, a similar effect was not previously investigated concerning the levels of MIB.

2-Pentylfuran is a volatile organic compound which is an important product of lipid degradation and is responsible for licorice-like and beany sensory attributes in various food products [26]. It was detected in all samples in concentrations from 0.72% to 8.98%. β -Cyclocitral and β -ionone can also be considered as important volatile odour compounds and are commonly used in food industry as components of artificial flavourings. β -Ionone was detected in all the investigated strains of cyanobacteria in relatively high concentration range (1.15–2.72%), while β -cyclocitral was also detected in all samples, except in C5 strain of *Anabaena*, however, in lower amounts (0.20–1.34%). β -Cyclocitral, known to be produced by *Microcystis* cyanobacterial strains, is a product of enzymatic degradation of β -carotene, and its presence can indicate death of cyanobacterial cells [25]. Although the analyzed samples were previously lyophilized, they were considered biologically viable after rehydration. The detected levels of β -cyclocitral may indicate that the applied thermal treatment of such cells causes cell damage and degradation of β -carotene.

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are among the most popular multivariate explanatory methods used to reduce number of parameters retaining only those which are the most

FIGURE 1: PCA: projection of the variables on the factor plane.

FIGURE 2: PCA: projection of the cases on the factor plane.

significant for the explanation of some phenomena. These two techniques complement one another and have been widely used in solving classification problems [27]. The data matrix constructed of 10 rows (investigated samples) and 17 columns (volatile compounds) was subjected to PCA analysis. The first two components explained 55.53% of variance, with component PC1 contributing 30.77% and component PC2 24.75%.

The first plot (Figure 1) shows a projection of the initial variables in the factor space. The horizontal axis (PC1) is related with hydrocarbons of alkane and alkene type (with an exception of compound IV) since it is highly correlated with compounds IV, VIII, X, XII, XIII, and XV. The second, vertical axis is well linked with compounds I, V, VI, and IX, which are of cyclic or bicyclic structure. Compound XIV (8-heptadecene) is on the opposite side of the centre, implying that it is negatively correlated with PC2 axis, correctly indicating that this compound does not possess cyclic structure.

The algae samples were completely separated by the determined organic compounds (Figure 2). Samples of *Spirulina*, labeled as S1 and S2, are quite unique and can be distinguished mostly by their content of alkanes, while *Anabaena* and *Nostoc* samples labeled as C2–N and 2S7B–N were separated mostly by high concentration of compounds V, VI, and

FIGURE 3: Dendrogram for the HCA results using Ward's clustering algorithm.

IX (2,6-dimethyl-cyclohexanol, β -cyclocitral, and β -ionone, resp.).

According to these graphs, we can assume that samples can be categorized in three or four different groups. But for the purpose of more detailed determination of similarities between samples, data are subjected to a hierarchical cluster analysis (HCA). Figure 3 shows the horizontal hierarchical tree plot, called dendrogram, for the cluster analysis results using Ward's algorithm. All samples are divided into three separate groups. First and third clusters contain four samples and the second only two. Dendrogram shows that the samples constituting first two groups are more homogenous than in the third one. The results are confirmed by variance within the class, for Cluster 1: 274.11, Cluster 2: 121.62, and Cluster 3: 452.66. It appears that S1 and S2 *Spirulina* samples are very similar to C2 strains of *Anabaena* (C2-N and C2+N) and, going back to Table 1, it can be noticed that similarity is derived from the lack of 8-heptadecene and 3-octadecene in these samples.

4. Conclusion

The obtained results of the volatile compounds determination show that medium length alkanes and alkenes represent the main volatile components of the investigated strains of cyanobacteria. Presence of odorous compounds such as 2-methylisoborneol, 2-pentylfuran, β -cyclocitral, and β -ionone was also observed in the samples in various concentrations. Content of 2-methylisoborneol was lower in samples which were grown in medium with higher added nitrogen levels, although further studies using standard of 2-methylisoborneol should be undertaken to more precisely assess the observed differences. Presence of geosmin, which is very important component of odour, was not detected in any of the tested samples, which may be explained by the presence of relatively high concentrations of copper ions to the growth medium. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) have shown

good separation of analyzed samples regarding the type and concentration of their volatile compounds and samples of *Spirulina* were characterized as notably different from other cyanobacterial strains with regard to their high content of alkanes. It can also be concluded that growing conditions have significant impact on production of volatile and odorous compounds in cyanobacteria, and altering these conditions may be useful in obtaining cyanobacterial biomass with favorable sensory properties for potential use in formulation of food and feed products.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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