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Influencing of amino acid composition of green freshwater algae and cyanobacterium by methods of cultivation

[Kültivasyon metotlarının yeşil taze su algleri ve siyanobakter amino asitleri kompozisyonu üzerine etkisi]*

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ABSTRACT

Aim: To determine and evaluate amino acid profiles in algal biomass of green freshwater microalgae *Chlorella kessleri*, *Scenedesmus quadricauda* and *Chlorella* sp. and blue-green micro-alga (cyanobacterium) *Spirulina platensis* cultivated by various methods. To perform the comparison of amino acid contents in algal biomass originated from cultivation methods.

Material and Methods: Selected species of green freshwater micro-algae and blue-green microalgea were cultivated under the autotrophic cultivation in laboratory, in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer, in a solar photobioreactor and under the heterotrophic cultivation regime in a fermenter. Contents of seventeen amino acids in algal samples were determined by using ion-exchange chromatography.

Results: Autotrophic growth regime in a solar photobioreactor and in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer provided algal biomass with higher amino acid contents than cultivation in a laboratory regime and heterotrophic cultivation in a fermenter.

Conclusion: Microalgae are considered as an unconventional source of amino acids, so it is important to monitor their amino acid composition. This work provides amino acid profiles of investigated green freshwater microalgae and blue-green microalgea in dependence on their cultivation methods. The content of amino acids in algal biomass is directly associated with the selected cultivation method and growth conditions (e.g. chemical composition of culture medium, quality of light, pH, turbulence, salinity and temperature) of algal cells. Further studies are needed to elucidate the influence of individual growth conditions that may lead to changes in amino acid composition in algal biomass.

Key Words: Amino acids, blue-green micro-alga, cultivation methods, green freshwater micro-algae.

Conflict of Interest: The authors report no conflicts of interest.

ÖZET

Amaç: Alg biyokütle amino asit profilleri belirlemek vee değerlendirmek için yeşil tatlı su mikro algleri *Chlorella kessleri*, *Scenedesmus quadricauda* ve *Chlorella* sp. ve mavi-yeşil mikro-alg (siyanobakteri) *Spirulina platensis* çeşitli yöntemlerle yetiştirilmiştir. Alg biyokütle amino asit içeriğinin karşılaştırılması için kültivasyon yöntemlerinden yararlanılmıştır.

Metot: Yeşil tatlı su mikro-alglerinden ve mavi-yeşil mikro-alglerden seçilen türler, ototrofik kültivasyon laboratuvarında, güneş fotobiyoreaktör ve heterotrofik kültür rejimi altında ve bir ince tabaka açık dolaşım kaskad tipi yetiştirme aygıtında yetiştirilmişlerdir. Alg örneklerinde ki yedi amino asit içeriği iyon-değişim kromatografisi ile belirlenmiştir.

Bulgular: Fermentasyon laboratuvarında, güneş fotobiyoreaktörlü, bir ince tabaka açık dolaşım kaskad tipi yetiştirme aygıtı içinde ototrofik büyüme rejimi ile elde edilen alg biyokütle oranındaki amino asit içeriği, heterotrofik yetiştirmeye daha yüksek bulunmuştur.

Sonuç: Mikro-alg amino asitleri alışılmamış bir biyokütle kaynağı olarak kabul edilir. Bu nedenle bunların amino asit kompozisyonlarının elde edilmesi önemlidir. Bu çalışmada alglerin yetiştirme yöntemlerine bağımlılığı araştırıldı ve yeşil tatlı su mikroalglerinin ve mavi-yeşil mikroalglerinin amino asit profili bulundu. Alg biyokütlesinin amino asit içeriği doğrudan seçilen uygulama metodu ve alg hücrelerinin büyüme koşullarına (örneğin, kültür ortamı, kimyasal bileşimi, ışık, pH, türbülans, tuzluluk ve sıcaklık kalitesi) ile ilişkilendirildi. Amino asit kompozisyonun alg biyokütlesinde değişikliklere yol açabileceği önemini ve bireysel gelişim koşullarının buna etkisi aydınlatmak için ileri çalışmalarla ihtiyaç vardır.

Anahtar Kelimeler: Amino asitler, mavi-yeşil mikro algler, kültivasyon metotları, yeşil tatlısu miro algleri.

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Micro-algae are used by human for a long decade as a food, feed, therapeutics and fertilizers [1, 2]. These microorganisms are rich in the quantity of nutritious compounds as proteins, carbohydrates, lipids, vitamins, pigments, minerals and other nutraceuticals [2-6]. Micro-algae are used as an unconventional source of nutrition mainly because of high contents of amino acids and protein in their algal biomass [2-5, 7]. Nowadays, there has been an increasing interest in using of green freshwater micro-algae *Chlorella* sp., *Scenedesmus* sp. and cyanobacterium *Spirulina* sp. for producing protein products sold as health food and food supplements [2, 4-6]. Amino acid contents in algal biomass depend on some factors, such as the geographical area, species of algae, environmental conditions and mainly on the cultivation methods [1, 8-11].

Some studies have been conducted to various cultivation methods and their influence on the chemical composition of algal biomass, but only in one type of cultivation. Morist *et al.* (2001) [7] describes chemical composition of *Spirulina platensis* produced by continuous photobioreactor and its composition after various post-producing treatment by freeze-drying, spray drying and after pasteurisation, further the influence of temperature and pH during laboratory cultivation on nutrition composition of *Spirulina* sp. was investigated by Ogbonda *et al*. (2007) [12]. Various cultivation methods of *Chlorella pyrenoidosa* on its growth, energetics and carbon metabolisms were described by Ogbonna *et al*. (1998) [13] and Yang *et al*. (2000) [14]. Heterotrophic production of *Chlorella protothecoides* on various nitrogen sources was studied by Shi *et al.* (2000) [15]. While mentioned studies have been focused on the growth conditions of algal cells under cultivation method and chemical composition of algal biomass, there is still a lack of information about direct comparison and evaluation of amino acid contents in algal biomass of blue-green and green freshwater micro-algae originating from various cultivation methods.

The aims of this research were to determine basic chemical characteristics of algal biomass, further amino acid profiles in algal biomass of the selected green freshwater micro-algae (*Chlorella* sp., *Chlorella kessleri*, *Scenedesmus quadricauda*) and blue-green micro-alga (*Spirulina platensis*) cultivated by the autotrophic cultivation methods in the laboratory conditions, in a solar photobioreactors, outdoor open circulating cascade-type cultivation apparatus in a thin-layer and by the heterotrophic cultivation method in a fermenter. Further aims of this work were to evaluate differences in amino acid contents of algal biomass depending on the selected cultivation methods and to indicate the cultivation regime providing algal biomass abundant in amino acid contents. In addition, the present research extends the current knowledge of amino acid contents in micro-algae and could be potentially useful for more abundant usage of these organisms in various branches of industry.

Materials and methods

Reagents and chemicals

Chemicals for amino acid analyses such as hydrochlorid acid, citric acid monohydrate, sodium citrate dihydrate, sodium chloride, thiodiglycol, boric acid, sodium azide and sodium hydroxide were supplied by Ingos (Prague, Czech Republic). Ninhydrine was also purchased from Ingos (Prague, Czech Republic) as a kit for ninhydrine reagent for post-column derivatization. All reagents and chemicals were of p.a. purity.

Algal materials

The series of species of green freshwater micro-algae *Chlorella kessleri*, *Scenedesmus quadricauda* and blue-green micro-alga *Spirullina platensis* were obtained from Academic and University Center of Nové Hrady, Institute of Physical Biology, Nové Hrady, Czech Republic; and micro-alga *Chlorella* sp. was obtained from Academy of Science of the Czech Republic, Institute of Microbiology, Department of Phototrophic Microorganisms in Třeboň. All investigated samples were acquired in dried forms.

The samples of micro-algae (*C. kessleri*, *S. quadricauda, S. platensis*) from Institute of Physical Biology in Nové Hrady were cultivated autotrophically in a solar photobioreactor (PBR) and simultaneously, the autotrophic cultivation in the laboratory conditions was performed. *Chlorella* sp. obtained from Department of Phototrophic Microorganisms in Třeboň was autotrophically cultivated in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer, then the heterotrophic cultivation in a fermenter was performed. Summary and detailed description of the investigated samples is shown in Table 1.

Autotrophic cultivation under the laboratory conditions

The micro-algal species *C. kessleri*, *S. quadricauda, S. platensis* were cultivated in a 1000 mL glass flasks which were gently bubbled by a mixture of air and 2.2 % carbon dioxide at the temperature of 30°C, the light illumination was provided by fluorescence tubes (Philips, Osram Dulux L, 55W/12-950) at the intensity of 479 $\mu mol.m^{-2}.s^{-1}$. Blue-green alga was cultivated in Zarrouk´s culture medium [16], other algal species in Šetlík and Simmer culture medium [17]. The algal biomass was harvested by centrifugation at 6500 rpm for 10 min at a room temperature (Hettich 200R centrifuge, Germany) after one-week cultivation, subsequently the biomass was lyophilized (Alpha 1-4 LSC, Christ, Germany).

Table 1. Summary and description of investigated samples of blue-green and green freshwater algae, autotrophic cultivation in solar photobioreactor (AP), autotrophic laboratory cultivation (AL), autotrophic cultivation in outdoor open circulating cascade-type cultivation apparatus in thin-layer (AO), heterotrophic cultivation in fermenter (HF).

Symbol of sample	Origin of sample	Specification of sample Cultivation medium	Alga
SpAP	1	BG11	*Spirulina platensis*
SpAL	1	Zarrouk	*Spirulina platensis*
ScAP	1	BG11	*Scenedesmus guadricauda*
ScAL	1	Šetlík and Simmer	*Scenedesmus guadricauda*
ChKAP	1	BG11	*Chlorella kessleri*
ChKAL	1	Šetlík and Simmer	*Chlorella kessleri*
ChAO	2	for autotrophic cultivation[a]	*Chlorella* sp.
ChHF	2	for heterotrophic cultivation[a]	*Chlorella* sp.

1 - Academic and University Center of Nove Hrady, Institute of Physical Biology, Nové Hrady, Czech Republic
2 - Academy of Science of the Czech Republic, Institute of Microbiology, Department of phototrophic microorganisms, Třeboň, Czech Republic
[a] Composition of cultivation medium described in method section.

Autotrophic cultivation in a solar photobioreactor (PBR)

The micro-algal species *C. kessleri*, *S. quadricauda*, and *S. platensis* were autotrophically cultivated in a solar PBR described in the study [18]. BG11 culture medium [19] was used for the cultivation of micro-algae. After the cultivation, the algal biomass was lyophilized (Alpha 1-4 LSC, Christ, Germany).

Autotrophic cultivation in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer

Micro-alga *Chlorella* sp. was autotrophically cultivated in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer described in the study [20]. Briefly, cells were maintained in the medium with the composition as follows (per liter): 182 mg $(NH_2)_2CO$, 41 mg KH_2PO_4, 29 mg $MgSO_4.7H_2O$, 5.1 mg $FeSO_4.7H_2O$; trace element solution I (per liter): 141 µg H_3BO_3, 160 µg $CuSO_4.5H_2O$, 559 µg $MnCl_2.4H_2O$, 105 µg $CoSO_4.7H_2O$, 455 µg $ZnSO_4.5H_2O$; trace element solution II (per liter): 29.1 µg $(NH_4)_6Mo_7O_{24}$, 2.37 µg NH_4VO_3. The medium was sterilized by autoclaving. The algal biomass was harvested and spray dried after the cultivation.

Heterotrophic cultivation in a fermenter

Chlorella sp. was heterotrophically cultivated in a fermenter (the volume of 450 l), at the temperature of 35-37 °C in the dark. Cells were maintained in the medium with the composition as follows (per liter): 77.8 g $C_6H_{12}O_6$, 7.11 g $(NH_2)_2CO$, 16.44 g KH_2PO_4, 1.22 g $MgSO_4.7H_2O$, 97.3 mg $FeSO_4.7H_2O$, 22.2 mg H_3BO_3, 6.22 mg $CuSO_4.5H_2O$, 8.66 mg $ZnSO_4.7H_2O$, 7.55 mg $CoSO_4.7H_2O$, 10.11 mg $MnCl_2.4H_2O$, 3.55 mg $(NH_4)_6Mo_7O_{24}.4\ H_2O$, 54 mg $CaCl_2$. The medium was sterilized by autoclaving. The cultivation medium in a fermenter was stirred by blender and was simultaneously aerated by using hypertensive air which was lead through a flowmeter and microbial filter inside the fermenter. After the cultivation, the suspension of algal biomass was transferred into a washing tank, where it was diluted by water and further the suspension was concentrated in a disc centrifuge. This concentrated suspension of algal biomass was spray dried and prepared for analyses.

Basic chemical analyses of algae

Content of dry matter, ash and crude protein in the investigated samples was analyzed according to AOAC (1995) approved methods [21]. Crude protein content was calculated using a conversion factor of 6.25.

Determination of amino acid

The content of seventeen amino acids: aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), tyrosine (Tyr), histidine (His), lysine (Lys), arginine (Arg), methionine (Met) and cysteine (Cys) in algal samples was determined by using ion-exchange chromatography by Amino Acid Analyser AAA400 (Ingos, Prague, Czech Republic) after acid hydrolysis or previous oxidation of sulphur amino acids cysteine and methionine. Sulphur amino acids were partially or completely destroyed during acid hydrolysis. Hence, cysteine and methionine were first oxidized and then transformed to cysteic acid and methionine sulphone which were determined after acid hydrolysis. Amino acids glutamine and asparagine were converted by HCl into glutamic and aspartic acid, respectively [22]. Hydrolysis procedures of algal samples (20 – 25 mg)

were treated for 24 h by different ways of hydrolyses under the conditions described in the method [22].

Amino acids were determined by using ion-exchange chromatography by the device Amino Acid Analyser AAA400 under the measurement conditions described in detail in the study [22]. Briefly, the amount of a 100 µL of the hydrolysate extract in loading buffer (14.0 g/L of citric acid monohydrate; 11.5 g/L of sodium chloride; 0.1 g/L of sodium azide; 5.0 ml/L of thiodiglycol) was automatically injected into an Amino Acid Analyser AAA400 equipped with a column (370 mm x 3.7 mm filled with an ion exchanger Ostion LG ANG – Ingos, Prague, Czech Republic), post-column ninhydrine derivatization and spectrophotometric detection (440 nm for proline and 570 nm for other amino acids). Amino acids were eluted according to the programme: 0-5 min buffer A (11.11 g/L of citric acid monohydrate; 4.04 g/L of sodium citrate dihydrate; 9.29 g/L of sodium chloride; 0.1 g/L of sodium azide; 2.5 ml/L of thiodiglycol), 5-32 min buffer B (10.0 g/L of citric acid monohydrate; 5.6 g/L of sodium citrate dihydrate; 8.36 g/L of sodium chloride; 0.1 g/L of sodium azide; 2.5 ml/L of thiodiglycol), 32-44 min buffer C (7.53 g/L of citric acid monohydrate; 9.06 g/L of sodium citrate dihydrate; 18.0 g/L of sodium chloride; 0.1 g/L of sodium azide; 2.5 ml/L of thiodiglycol), 44-75 min buffer D (19.6 g/L of sodium citrate dihydrate; 52.6 g/L of sodium chloride; 2.05 g/L of boric acid; 0.1 g/l of sodium azide; 0.5 g/L of sodium hydroxide). Then the column was regenerated by 0.2 mol/L NaOH for 10 min and stabilized for further 17 min by buffer A. The temperature of the column was set to 60 °C (0-60 min and 90-102 min) and 74 °C (60-90 min), respectively. A flow rate was 0.3 mL/min for buffers and 0.2 mL/min for ninhydrine reagent. A standard of analyzed amino acids was obtained from Ingos, Prague, Czech Republic.

Statistical analysis

The results of basic chemical analyses (dry matter, ash, crude protein contents) were statistically evaluated by means of Wilcoxon test; Unistat® 5.5 software (Unistat, London, UK) was used for the statistical evaluation, P values less than 0.05 were considered to be significant ($P < 0.05$). Means within a column (the difference of dry matter content, ash content and crude protein content between cultivation methods of individual micro-algae) followed by different superscript letter differ ($P < 0.05$); values of individual parametres (dry matter content, ash content and crude protein content) and individual algae were evaluated separately. The results of amino acid analyses were statistically evaluated by Unistat® 5.5 software (Unistat, London, UK), results were evaluated as significantly different if P values were less than 0.05 ($P < 0.05$).

Results and Discussion

Dry matter, ash and crude protein contents

Contents of dry matter, ash and crude protein are shown in Table 2. The values of dry matter in all algal samples of strain *Chlorella* cultivated under the different conditions were not significantly different ($P < 0.05$). Similarly ash contents in the samples of *C. kessleri* (ChKAL) from the autotrophic open circulating cascade-type cultivation apparatus in a thin-layer and *Chlorella* sp. (ChHF) from the heterotrophic cultivation did not show significant differences ($P < 0.05$). However, all other results of dry matter, ash and especially crude protein were determined as significantly different ($P < 0.05$), among the samples originated from different cultivations across various strains of determined algal samples. Mostly, higher content of mentioned parametres were established in the algal samples from the autotrophic cultivation in a solar photobioreactor (PBR), except for *S. quadricauda* (ScAL) from the autotrophic cultivation under the laboratory regime that showed significantly higher content ($P < 0.05$) of crude protein as compared to the sample of the same micro-alga from the autotrophic cultivation in a solar PBR. The highest difference of crude protein content was evaluated in *S. platensis*; the result related

Table 2. Results of basic chemical analysis (dry matter content, ash content and crude protein content) in blue-green and green freshwater micro-algae (Sp – *Spirulina platensis*; ChK – *Chlorella kessleri*; Sc – *Scenedesmus quadricauda*; Ch – *Chlorella* sp.) after the autotrophic cultivation in a solar photobioreactor (AP), the autotrophic laboratory cultivation (AL), the autotrophic cultivation in an outdoor open circulating cascade-type cultivation apparatus in thin-layer (AO) and the heterotrophic cultivation in a fermenter (HF); mean ± SD (n = 3).

Sample	Dry matter content (%)	Ash (%)	Crude protein (%)
SpAP	91.49 ± 0.15^a	17.81 ± 0.42^a	55.56 ± 0.65^a
SpAL	83.56 ± 1.78^b	13.12 ± 0.99^b	22.66 ± 0.61^b
ScAP	96.03 ± 0.49^a	5.24 ± 0.13^a	43.37 ± 0.70^a
ScAL	92.86 ± 1.55^b	5.92 ± 0.11^b	52.49 ± 1.93^b
ChKAP	94.00 ± 0.10^a	6.72 ± 0.61^a	53.30 ± 0.72^a
ChKAL	93.48 ± 0.81^a	5.19 ± 0.42^b	49.48 ± 0.19^b
ChAO	93.80 ± 0.42^a	4.81 ± 0.26^a	54.93 ± 0.42^a
ChHF	94.68 ± 0.82^a	4.80 ± 0.29^a	31.64 ± 0.87^b

to the sample from the autotrophic cultivation in a solar PBR was nearly 50 % higher than in the sample from the autotrophic cultivation under the laboratory regime.

Statistically significant difference ($P < 0.05$) in crude protein content was evaluated also in micro-alga *Chlorella* sp. (ChAO) originated from the autotrophic cultivation in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer and in algal biomass from the heterotrophic cultivation (ChHF). Autotrophically cultivated *Chlorella* sp. contained more than 40 % higher amount of crude protein content in comparison with the heterotrophically cultivated algal biomass in a fermenter. These results are in agreement with published data in the study [10] in which higher content of proteins in micro-alga *Chlorella vulgaris* originated from the autotrophic cultivation than from heterotophic cultivation was also reported. Nevertheless, these results could not be generalized because the information showed in the study [23] indicated that some cultures grown under the heterotrophic regime could provide a higher level of proteins than under the autotrophic conditions. Higher content of protein in some heterotrophic cultures is mainly dependent on the species and strain of micro-algae. This situation is probably caused by dissimilar sensitivity of various micro-algal strains to different conditions during the autotrophic and heterotrophic cultivation (intensity of illumination, chemical composition of substances in culture medium or temperature during cultivation).

Amino acids

Contents ($g.16g^{-1}N$) of total amino acids (∑AAs), total essential amino acids (∑EAAs), total non-essential amino acids (∑NEAAs) and total sulphur amino acids (∑Met+Cys) in green freshwater and blue-green micro-algae are illustrated in Fig. 1. It is evident that the highest contents of ∑AAs (86.30 $g.16g^{-1}N$), ∑EAAs (32.67 $g.16g^{-1}N$) and ∑NEAAs (53.64 $g.16g^{-1}N$) were evaluated in *Chlorella* sp. (ChAO) cultivated under the autotrophic regime in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer, as well as higher amounts of ∑AAs (80.01; 81.62 $g.16g^{-1}N$), ∑EAAs (29.53; 30.79 $g.16g^{-1}N$) and ∑NEAAs (50.48; 50.83 $g.16g^{-1}N$) in *C. kessleri* (ChKAP) and *S. quadricauda* (ScAP) cultivated autotrophically in a solar photobioreactor, respectively. Blue-green micro-alga *S. platensis* seems to be the most sensitive to the cultivation conditions. *S. platensis* (SpAL) autotrophically cultivated in the laboratory regime was evaluated as the poorest source of amino acids in this study. It contained the lowest amounts of ∑AAs 47.80 $g.16g^{-1}N$, ∑EAAs 18.00 $g.16g^{-1}N$ and ∑NEAAs 29.80 $g.16g^{-1}N$. Additionally, significant differences ($P < 0.05$) between amino acids composition of algal biomass of *Chlorella* sp. cultivated by the autotrophic and heterotrophic method were established. Autotrophically cultivated *Chlorella* sp. (ChAO) in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer contained higher amounts of amino acid totals than heterotrophically cultivated micro-alga in a fermenter (ChHF). Further, differences in contents of ∑AAs, ∑EAAs and ∑NEAAs between algal biomass originated from the autotrophic and heterotrophic cultivation were expressed in percentage 26.1 %, 24.8 % and 19.7 %, respectively. This fact is in accordance with the results from the study [10] in which it has been observed that *Chlorella vulgaris* cultivated under the autotrophic regime was three times more abundant in protein content than heterotrophically cultivated micro-alga. *C. kessleri* (ChKAP) grown under the autotrophic regime in a solar PBR was the most abundant source (7.02 $g.16g^{-1}N$) of sulphur amino acids (∑Met+Cys) among the all investigated micro-algae. On the other hand, approximately a half of the content of sulphur amino acids in comparison with alga originated from the autotrophic cultivation in a fermenter was evaluated in heterotrophically cultivated *Chlorella* sp. (ChHF).

Contents of individual amino acids ($g.16g^{-1}N$) of investigated micro-algae are shown in Table 3. Non-essential amino acids Asp and Glu were presented in the highest amounts ($P < 0.05$) among all investigated algal species, in contrast to the amino acid His which was presented in the lowest concentration ($P < 0.05$). The most abundant essential amino acid ($P < 0.05$) was Leu in majority of investigated micro-algae, except for *S. quadricauda* (ScAP) from the cultivation in a solar photobioreactor and *Chlorella* sp. (ChHF) originated from the heterotrophic cultivation, in which Lys was evaluated as the most abundant ($P < 0.05$) essential amino acid. On the other hand, Thr and Ile were established in the lowest concentrations ($P < 0.05$) within a group of essential amino acids in *S. platensis* and *S. quadricauda* from all cultivations. In the algal biomass of *C. kessleri* (ChKAP) from the autotrophic cultivation in a solar PBR, Ile was in the lowest concentration ($P < 0.05$), while Thr was established in the minor content ($P < 0.05$) in the sample of the same micro-alga originated from the autotrophic laboratory cultivation.

Micro-alga *Chlorella* sp. (ChAO) autotrophically cultivated in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer was evaluated as the richest source ($P < 0.05$) of essential (Val, Leu, Phe and Thr) and non-essential (Asp, Gly, Ala and Tyr) amino acids among all the investigated algae. Individual amino acids as well as other nutritional factors (e.g. protein) were also present in significantly lower amounts ($P < 0.05$) in micro-alga *Chlorella* sp. originated from the heterotrophic cultivation in comparison with autotrophically cultivated alga in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer.

Contents of individual amino acids (Val, Phe, Met and His) in autotrophically cultivated *S. platensis* (SpAP) in a PBR were almost at the same levels as in micro-alga

Table 3. Contents of amino acids (g.16g^{-1}N) in blue-green and green freshwater microalgae (*Spirulina platensis* - Sp; *Chlorella kessleri* - ChK; *Scenedesmus quadricauda* - Sc; *Chlorella* sp. - Ch) after the autotrophic cultivation in a photobioreactor (AP), the autotrophic laboratory cultivation (AL), the autotrophic cultivation in an outdoor open circulating cascade-type cultivation apparatus in thin-layer (AO) and the heterotrophic cultivation in a fermenter (HF); mean ± SD (n = 8)[a]; a the values reflecting a significant difference between amino acid content in algae cultivated by various methods are designated with an asterisk * ($P < 0.05$; individual algae and each amino acid were evaluated separately).

Amino acids	*Spirulina platensis*		*Scenedesmus quadricauda*		*Chlorela kessleri*		*Chlorela* sp.	
	SpAP	SpAL	ScAP	ScAL	ChKAP	ChKAL	ChAO	ChHF
Essential AAs								
Val	3.26 ± 0.20	3.06 ± 0.09	4.68 ± 0.22*	3.11 ± 0.13*	4.19 ± 0.18	4.28 ± 0.15	4.82 ± 0.29*	3.46 ± 0.14*
Ile	2.48 ± 0.11*	2.90 ± 0.12*	2.84 ± 0.17*	2.00 ± 0.13*	3.45 ± 0.16	3.35 ± 0.13	3.07 ± 0.10*	2.07 ± 0.09*
Leu	4.03 ± 0.26*	4.74 ± 0.07*	5.89 ± 0.50*	4.72 ± 0.20*	6.32 ± 0.40	6.43 ± 0.25	7.69 ± 0.21*	5.28 ± 0.32*
Phe	3.17 ± 0.24*	1.70 ± 0.05*	4.32 ± 0.19*	2.99 ± 0.12*	3.89 ± 0.19	3.88 ± 0.09	4.72 ± 0.22*	2.74 ± 0.09*
Lys	3.61 ± 0.13*	2.38 ± 0.09*	6.07 ± 0.16*	4.34 ± 0.19*	4.29 ± 0.22	4.39 ± 0.09	5.13 ± 0.14*	5.74 ± 0.24*
Met	2.49 ± 0.14*	1.66 ± 0.07*	4.06 ± 0.33	3.94 ± 0.11	3.72 ± 0.16	3.74 ± 0.23	2.81 ± 0.16*	1.50 ± 0.05*
Thr	2.28 ± 0.08*	1.56 ± 0.04*	2.93 ± 0.21	2.83 ± 0.13	3.67 ± 0.18	3.22 ± 0.26	4.40 ± 0.21*	2.96 ± 0.13*
Non-essential AAs								
Asp	5.14 ± 0.19*	3.91 ± 0.16*	8.36 ± 0.36*	6.01 ± 0.38*	7.42 ± 0.38	6.57 ± 0.75	9.85 ± 0.64*	6.21 ± 0.19*
Ser	4.22 ± 0.27*	1.20 ± 0.06*	2.53 ± 0.15	2.64 ± 0.08	3.53 ± 0.16	3.28 ± 0.16	3.83 ± 0.18*	2.73 ± 0.11*
Glu	5.33 ± 0.18*	7.05 ± 0.20*	9.85 ± 0.52*	6.75 ± 0.30*	8.52 ± 0.42	7.84 ± 0.72	9.49 ± 0.50*	7.29 ± 0.27*
Pro	3.98 ± 0.25*	1.89 ± 0.12*	5.77 ± 0.23*	3.44 ± 0.04*	5.26 ± 0.29	5.39 ± 0.40	4.37 ± 0.16*	3.62 ± 0.19*
Gly	3.29 ± 0.20*	2.67 ± 0.11*	5.21 ± 0.44*	3.80 ± 0.20*	4.35 ± 0.21	4.43 ± 0.14	5.04 ± 0.42*	3.60 ± 0.19*
Ala	3.69 ± 0.19	3.96 ± 0.17	6.42 ± 0.51*	4.55 ± 0.22*	5.55 ± 0.24	6.01 ± 0.47	6.97 ± 0.50	6.91 ± 0.25
Tyr	2.18 ± 0.11*	1.70 ± 0.04*	3.29 ± 0.25*	1.89 ± 0.01*	2.71 ± 0.15	2.89 ± 0.05	3.55 ± 0.27*	2.32 ± 0.11*
Cys	2.56 ± 0.08*	2.78 ± 0.12*	2.65 ± 0.08	2.57 ± 0.18	3.30 ± 0.21	3.21 ± 0.15	2.00 ± 0.05*	1.77 ± 0.08*
Arg	4.35 ± 0.17*	3.79 ± 0.10*	4.88 ± 0.38*	3.70 ± 0.09*	8.32 ± 0.49*	6.19 ± 0.21*	6.69 ± 0.31*	5.19 ± 0.05*
His	1.28 ± 0.08*	0.88 ± 0.06*	1.88 ± 0.01*	1.32 ± 0.09*	1.54 ± 0.05	1.62 ± 0.02	1.84 ± 0.06*	1.44 ± 0.03*

Spirulina sp. described in the study [12]. On the other hand, amino acids Thr, Glu, Gly, Ala and Tyr were presented in lower concentrations and finally, remaining amino acids were in higher amounts than in micro-alga *Spirulina* sp. What is more, data in the study [12] did not confirm the presence of sulphur amino acid Cys in *Spirulina* sp., in contrast with the research [7]. Moreover, *S. platensis*, evaluated in our study, was the abundant source of Cys. The work [7] showed the concentrations of Lys and Leu that were similar to our presented values, nevertheless significantly lower values of contents of these amino acids were published in the study [12]. The content of total sulphur amino acids in *S. platensis* (SpAP) was about two times higher in comparison with the concentration determined in the studies [7, 12], beyond the sum of Phe and Tyr which was at a half level in opposite to value evaluated in the research [12].

Concentrations of individual amino acids in *S. quadricauda* autotrophically cultivated in a PBR (ScAP) and under the laboratory regime (ScAL) were predominantly at lower levels than the values of amino acid contents in *Scenedesmus obliquus* presented in the study [3]. In *S. quadricauda*, were higher amounts of Met, Cys (under laboratory regime) and Lys, Met, Pro, Tyr and Cys were determined in micro-alga cultivated in a PBR, in contrast with *Sc. obliquus* [3]. The values of ∑Met+Cys in *S. quadricauda* exceeded the concentration of this parameter in *S. obliquus* three times [3].

Higher contents of amino acids Pro and Arg were determined in autotrophically cultivated *C. kessleri* in a PBR (ChKAP) and the under laboratory cultivation (ChKAL) in comparison with concentrations of these amino acids that were determined in *C. vulgaris* in the studies [3, 6]. On the other hand, lower contents of Val, Ile, Leu, Lys, Thr, Asp, Glu, Gly, Ala and His were evaluated in the investigated *C. kessleri* than in *Ch. vulgaris* according to [3, 6]. Content of Ser in micro-alga *C. kessleri* from the both autotrophic and heterotrophic cultivation, was in accordance with the values determined in *C. vulgaris* [6]. Green micro-alga *C. kessleri* presented in this study was evaluated as a rich source ($P < 0.05$) of sulphur amino acids; this alga was abundant in this character approximately two times or more, conversely to *C. vulgaris* presented in the studies [3, 6]. However, *Chlorella* sp. grown heterotrophically in a fermenter (ChHF) contained lower amounts of induvidual amino acids in comparison with micro-alga *C. vulgaris* described in the studies [3, 6], except for amino acid Cys [3]. In *Chlorella* sp. (ChAO) autotrophically cultivated in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer, higher values of Arg and ∑Met+Cys were determined in contrast with concentrations evaluated in *C. vulgaris* [3, 6]. Further, higher concentration of Ser was assessed in ChAO in opposite with the research [4] and abundant contents of Met Asp, Tyr and Cys unlike the work [3].

Contents of some essential amino acids such as valine (Val), isoleucine (Ile), leucine (Leu), lysine (Lys) and threonine (Thr) were compared to the concentrations of these amino acids - 5.0, 4.0, 7.0, 5.5 and 4.0 g.16g^{-1}N, respectively in an "ideal" protein according to WHO [24]. The Leu and Thr contents were over WHO recommendations in *Chlorella* sp. (ChAO) cultivated autotrophically in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer. *S. quadricauda* (ScAP) grown under the autotrophic regime in a solar PBR and *Chlorella* sp. (ChHF) heterotrophically cultivated in a fermenter were over WHO recommendation in Lys content. In remaining algae, the values of individual amino acid contents were similar or lower than WHO values. The values of amino acids contents of Val, Ile, Leu, Lys and Thr determined in this study were lower in comparison with the values presented in the studies [3, 7] according to WHO recomendations.

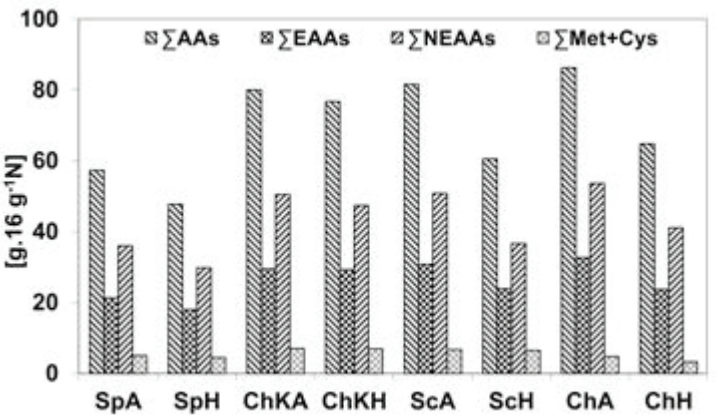

Figure 1. Contents of total (∑AAs), essential (∑EAAs), non-essential (∑NEAAs) and sulphur (∑Met+Cys) amino acids in blue-green and green freshwater micro-algae (Sp – *Spirulina platensis*; ChK – *Chlorella kessleri*; Sc – *Scenedesmus quadricauda*; Ch – *Chlorella* sp.) after autotrophic cultivation in solar photobioreactor (AP), autotrophic laboratory cultivation (AL), autotrophic cultivation in outdoor open circulating cascade-type cultivation apparatus in thin-layer (AO) and heterotrophic cultivation in fermenter (HF) in g.16g^{-1}N.

From the obtained results of amino acid contents of investigated blue-green and green freshwater micro-algae it could be deduced that algal biomass is a good source of essential and non-essential amino acids. It was observed that the culture method and its conditions may significantly affect the amino acid composition of algal biomass. It was documented that micro-algae originated from the autotrophic cultivation in a solar photobioreactor contained significantly higher ($P < 0.05$) amount of total amino acids, total essential and non-essential amino acids than algal biomass cultivated under the autotrophic regime in the laboratory conditions. Further, it was assessed that heterotrophically cultivated *Chlorella* sp. in a fermenter contained significantly lower concentrations ($P < 0.05$) of amino acids than micro-alga originated from the autotrophic regime in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer.

This study and its results presents a fact that various cultivation methods could provide algal biomass containing different amounts of amino acids, further it demonstrates that blue-green and green freshwater micro-algae are an important source of amino acids. This work gives basis of further studies dealing with an investigation of cultivation conditions affecting amino acid compositions of algal biomass. Further research may be focused on the optimization of cultivation methods that could provide algal biomass with abundant amino acid contents.

Conclusion

The amino acid profiles in green freshwater micro-algae and blue-green micro-alga were determined in dependence on various methods of cultivation. Data from this research confirm the direct correlation of cultivation methods to the contents of amino acids in algal biomass. Autotrophic growth regime in a solar photobioreactor and in an outdoor open circulating cascade-type cultivation apparatus in a thin-layer provided algal biomass abundant in amino acid contents. Cultivations in laboratory regime and algal growth under the heterotrophic conditions in a fermenter were less suitable for providing algal biomass with a high content of amino acids. Perspective of this study is to provide information on the amino acid composition of investigated green freshwater micro-algae and blue-green micro-alga from different cultivation methods, which give an overview of the changes in amino acid contents due to the selected regime of cultivation. This work may also be used as a basis of more effective commercial production of micro-algae. Nevertheless, further research is needed to confirm the degree of influence of cultivation factors, e.g. chemical composition of culture medium, quality of light, pH, turbulence, salinity and temperature during algal cultivation that lead to changes in amino acid composition in algal biomass.

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