Amino acid composition and crude protein values of some Cyanobacteria from Çanakkale (Turkey)

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Abstract: Cyanobacteria (blue-green algae) form an important component of integrated nutrient managements in agriculture and are exploited in commercial biotechnological ventures. In this study, *Rivularia bullata* (Poir) Berkeley ex Bornet & Flahault, *Nostoc*s *pongiaeforme* C. Agardh ex Bornet & Flahault were researched for their amino acid composition and crude protein values. *R. bullata* was collected from coastal zones of the Gulf of Saros and *N.* s*pongiaeforme* from the Ayazma Stream. The levels of amino acids were measured in algae samples using EZ: fast kits (EZ: fast GC/FID Protein Hydrolysate Amino Acid Kit) by gas chromatography. The crude proteins of samples were determined by the Kjeldahl method and were calculated using a nitrogen conversion factor of 6.25. Thirty-two amino acids were investigated, for *N.* s*pongiaeforme* eight free essential amino acids (EAA), eight free non-essential amino acids (NEAA) and eleven other amino acids (OAA); for *R. bullata* eight EAA, eight NEAA and eight OAA were detected. Aspartic acid is the major constituent for both species. The total protein percents were determined for *N.* s*pongiaeforme* as % 19.83 and for *R. bullata* as % 6.15. When considering the increasing world population and reducing natural products; Cyanobacteria will benew feed sources for all living.

Keywords: Blue green algae, *Nostoc* s*pongiaeforme, Rivularia bullata,* gas chromatography.

INTRODUCTION

Microalgae that basic living groups in aquatic ecosystems can store the secondary compounds in their biomass, abundantly. Especially Cyanobacteria can store the primary compounds such as herbal proteins and other nutritional substances. Because of their characteristics, many microalgae have used as water bioremediation agents (Shekhawat *et al.,* 2012), as feed for aquaculture (Creswell, 2010), as food for humans and animals, in agriculture, in pigment production, in pharmaceutical industry (Gouveia *et al*., 2008) and in bioremoval of heavy metals (Wilde and Benemann, 1993; Ali *et al*., 2010). Parallelly, using of algaeincreases with the increasing algal researches (Jimenes-Escrig and Sanchez-Muniz, 2000; Lourenço *et al*., 2011). In recent years; researches are carried out in different areas such as detecting of alternative protein sources. Single cell protein (SCP) is the best-known alternative protein sources and obtained from fungi, bacteria, yeasts and especially microalgae (Ghasemi *et al.,* 2011).

Protein also other constituents of micro-algae as for instance nucleic acids, amines, glucosamides and cell wall materials contain nitrogen, this calculation results in an overestimation of the true protein amount (Becker, 2007). Proteins are composed of different amino acids and hence the nutritional quality of a protein is determined basically by the content, proportion and availability of its amino acids (Lourenço *et al.,* 2011; Taboada *et al.,* 2010).

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All living organisms must consume nitrogenous organic matter for building the basic components of life and the quality of protein is very important because of amino acid content. If a human or an animal cannot eat up all essential amino acid, amino acid deficiency and difficulties in the synthesis of structural proteins will be occurred.

Selected data on the amino acid profile of various algae are compared with some basic conventional food items and a reference pattern of a well-balanced protein, recommended by WHO/FAO (Becker, 2007). It can be seen that amino acid profiles of many algae studied to present are much higher than the recommended value.

Protein, crude lipid, and chlorophyll-a concentrations were found to be more dependent on the age and culture condition of the cyanobacterium than on colony form or origin (Briones-Nagata *et al.,* 2007).

Many analyses of gross chemical composition of different algae have been published but the species in present study were not found in the literature. In this research, the protein value and amino acid diversity of *Rivularia bullata* (Poir) Berkeley ex Bornet & Flahaultand *Nostoc* s*pongiaeforme* C. Agardh ex Bornet & Flahault were determined.

MATERIALS AND METHODS

Preparation of samples

R. bullata and *N. spongiaeforme* are members of Cyanobacteria or blue-green algae. *R. bullata* was

collected from intralittoral zones of the Gulf of Saros (Çanakkale, Turkey) in September 2011 and *N. spongiaeforme* was collected from the Ayazma Stream (Çanakkale, Turkey) in September 2011 and they were identified by Dr. Rıza AKGÜL. Algae samples were washed with sterile water to remove any associated sandy particles. They were then pressed between filter papers and subsequently dried at room temperature, in the shade. After drying the sample, it was ground thoroughly to powder form, filled in plastic bags and stored at -20^{o}C. The powder was then used for the primary estimation of amino acids and total protein.

Analysis of samples

The crude proteins of samples were determined by the Kjeldahl method and were calculated using a nitrogen conversion factor of 6.25 (AOAC 981.10; Ortiz *et al.,* 2006; Yaich *et al*., 2011). The results were expressed as percent of dry weight.

In order to determine amino acid analysis, between 90 mg and 450mg of the dried cells according to contents of crude protein, which should be contain 30% was hydrolysed at 110^{o}C for 24 hours with 6.0 M hydrochloric acid. The hydrolysates of all samples was filtered through a 0.20µm polytetrafluoroethylene (PTFE) syringe filter, and then was evaporated all the hydrochloric acid in the hydrolysates. After evaporation, all hydrolysates samples were dissolved in citrate-sodium citrate buffer (0.1 M, pH 2.2) (Chi *et al.,* 2008; Srivastava *et al*., 2006).

The levels of amino acids were measured in algae samples using EZ: Fast kits (EZ: fast GC/FID Protein Hydrolysate Amino Acid Kit) by gas chromatography (Badawy *et al.,* 2008). The procedure of amino acids analysis consists of a solid phase extraction step, followed by a derivatization procedure and a liquid/liquid extraction step (Badawy *et al.,* 2008; Kale *et al.,* 2006). Prepared and derivatized samples are then analyzed by gas chromatography. Norvaline is used as internal standard. The concentration of the internal standard (IS; Norvaline) in the sample prepared for GC analysis was used as 200 nmoles/mL. Gas chromatography (Finnigan Trace GC Ultra AI 3000 Thermo Finnigan analyzer, Milan, Italy) was used to determine amino acids. The column was a Zebron Zebron™ ZB-HAAC 10 m x 0.25 mm capillary GC column. GC conditions were performed as injection: Split 1: 15 at 250ºC, 2.0µL; carrier gas: helium 1.0mL/min; oven program: 35ºC/min from 110ºC to 320ºC, hold at 320ºC for 1 min; Detector: FID at 320ºC. The instrument was calibrated with standard solution of multi amino acids (EZ: Fast SD solutions).

Chemical name and abbreviation of measured amino acids were given in table 2 and table 3.

RESULTS

The crude protein values of blue-green algae samples are shown in table 1.

Table 1: The amount of total amino acid and crude protein

	N	R
Total AA (mg/g dry algae)	186,73	58,91
The crude protein (% dry algae)	19.83	6.15

The crude protein value of *N. spongiaeforme* is higher than *R. bullata*. The protein amount of *N. spongiaeforme* is 19.83% of the dry weight algae.

The protein amount of *R. bullata* is 6.15% of the dry weight algae; this value is very low according to the other Cyanobacteria such as *Spirulina platensis*.

The amino acid composition and values of algae samples presented in table 2 and table 3. Thirty-two amino acids was identified and estimated. The analysis revealed the presence of nine free essential, eight free non essential and fifteen other amino acids. Varieties and percents of amino acids vary according to algae.

Total EAA amounts in crude protein are 0.2722g/g (weight protein) for *R. bullata*; 0.3039g/g (weight protein) for *N. spongiaeforme.*

NEAA amounts are much higher than EAA amounts for both species. Total NEAA amounts in crude protein are 0.4851 g/g (weight protein) for *R. bullata*; 0.4988 g/g (weight protein) for *N. spongiaeforme,* these values are almost half the total protein.

Due to an EAA tryptophan was hydrolyzed during the analysis and a NEAA arginin is a alkaline amino acid could not researched.

OAA amount in *N. spongiaeforme* (27.57 mg/g dry weight algae) is much higher than *R. bullata* (12.01 mg/g dry weight algae).

DISCUSSION

Similar or different results were found by the other researchers like our results. Briones-Nagata *et al.* (2007) reported that the protein amount of *Nostoc commune* varied from 23 to 29 % of the dry mass and *N. commune* has been used as food in the Philippines and in Japan, its popularity is localized and determined by its conspicuous abundance and traditional consumption. Wen *et al.* (2006) reported that the protein amount of *Nostoc flagelliforme* species is 25.47% of dry mass. And we found the protein amount of *N. spongiaeforme* is 19.83% of the dry weight algae.

The protein amount of *R. bullata* is very low (table 1). The reasons of this situation are important properties of *Rivularia* sp.; calcification and production a large amount

Table 2: Essential and non-essential amino acid composition and values

Essential AA (EAA)	N (mg/g dry algae)	N (g/100g protein)	R (mg/g dry algae)	R (g/100g protein)	S (g/100g protein) (Habib *et al*., 2008)	E (g/100g protein) (Fleurence, 1999)
Histidine (HIS)	1.518	0.765	0.313	0.508	2,81	4.1
Isoleucine (ILE)	10.381	5.235	3.152	5.125	3,85	4.8
Leucine (LEU)	12.479	6.293	3.268	5.313	8,37	6.2
Lysine (LYS)	5.170	2.607	1.513	2.460	4,63	7.7
Methionine (MET)	1.525	0.769	0.309	0.502	2,75	3.1
Phenylalanine (PHE)	7.075	3.568	2.290	3.723	4,10	4.1
Threonine (THR)	10.021	5.053	2.623	4.265	3,35	3.0
Valin (VAL)	12.091	6.097	3.583	5.826	4,02	5.4
Tryptophan(TRP)	N.D.	N.E.	N.D.	N.E.	1,98	1.0
Total (EAA)	60.27	30.39	17.05	27.22	38.86	39.4
Nonessential (NEAA)						
Alanine (ALA)	11.178	5.636	3.065	4.983	10,81	6.7
Aspartic Acid (ASP)	36.945	18.630	12.046	19.586	5,37	6.2
Glutamic Acid (GLU)	18.131	9.143	6.847	11.133	7,04	9.9
Glycine (GLY)	9.389	4.734	2.740	4.4552	6,66	3.4
Proline (PRO)	6.722	3.389	1.676	2.7252	4,11	2.8
Serine (SER)	6.115	3.083	2.521	4.0991	3,84	6.8
Tyrosine (TYR)	10.021	5.053	0.938	1.5252	3,42	1.8
Cystine (C-C)	0.383	0.193	N.D.	N.E.	0,6	
Arginine(ARG)	-	-	-	-	4,94	11.7
Total (NEAA)	98.88	49.88	29.84	48.51	46.79	49.3
*EAAI	0.611	N.E.	0.571	N.E.		

N.D.: investigated but Not Detected; N.E: Not Estimated -: Not investigated; *: Essential amino acid index (EAAI= EAA/NEAA); N: *N. spongiaeforme;* R*: R. bullata;* S: *Spirulina sp.;* E: Egg

exopolisaccarides such as thick mucilage sheath. Pentecost (2005) indicated that all *Rivulariahaematites* colonies were heavily calcified and two patterns of calcification. Wehr and Sheath (2003) expressed that mucilage in *Rivularia* sp., a compound consisted with exopolisaccaride, plays an important role in colony formation and specified that these species live in calcium-rich water.

EAA/NEAA is known as EAA index (EAAI) (Chi *et al.,* 2008; Feng and Zhao, 1997; Dawczynski *et al.,* 2007). Amino acid values can be classified according to EAAI≥0.95: indicate high quality; 0.86-0.95: good quality; 0.75-0.86: useful; ≤0.75: inadequate. EAAIs of *N. spongiaeforme* and *R. bullata* are 0.611 and 0.571, respectively. These values are low but acceptable.

Spirulina sp.contains high levels of protein (50-70%) that is associated with health food, pharmaceuticals and nutraceuticals (Cohen *et al.,* 1987). If the algae in our study compare to egg and *Spirulina* sp. with regard to EAA amounts, it can be seen that our samples have lower values, but with regard to NEEA amounts, nearly equal.

When looking at the composition and values of EAA. In *N. spongiaeforme* the most abundant amino acids are LEU (12.479mg/g), VAL (12.091mg/g), ILE (10.381 mg/g), and the amount of total EAA is 60.27mg/g (dry weight algae). In *R. bullata* the most abundant EAA are VAL (3.583mg/g), LEU (3.268mg/g), ILE (3.152mg/g), and the amount of total EAA is 17.05mg/g (dry weight algae).

On the other hand; for NEAA, the ASP is the highest amount amino acid for both of algae and second one is GLU. The amount of total EAA is 98.8mg/g (dry weight algae) for *N. spongiaeforme* and 29.8mg/g (dry weight algae) for *R. bullata*.

OAA, GPR is the most abundant amino acid in two algae. And the amount of total OAA is 27.57mg/g (dry weight algae) for *N. spongiaeforme* and 12.01mg/g (dry weight algae) for *R. bullata*.

Dusheiko *et al*. determined the presence of EAA in blue-green algae and proteins of algae might be considered biologically valuable for animal feeding as well as for microbiological purposes (Heiba *et al*., 1993).

Briones-Nagata *et al.,* (2007) stated that all *N. commune* samples were characterized by relatively greater concentration of aspartic acid, arginine, and glycine but

Table 3: Other amino acid composition and values

Other Amino Acids (OAA)	N (mg/g dry algae)	N (g/100g protein)	R (mg/g dry algae)	R (g/100g protein)
4-Hydroxyproline (HYP)	1.160	0.584	0.278	0.452
Hydroxylysine HLY)	N.D.	N.E.	N.D.	N.E.
Sarcosine SAR)	5.848	2.949	3.204	5.209
α-Aminobutyric acid ABA)	0.316	0.159	N.D.	N.E.
β-aminoisobutyric acid (BAIB)	3.907	1.970	1.498	2.435
allo-Isoleucine (AILE)	0.367	0.185	N.D.	N.E.
Asparagine (ASN)	0.268	0.135	0.115	0.186
Thiaproline (TPR)	N.D.	N.E.	N.D.	N.E.
α-Aminoadipic acid (AAA)	2.763	1.393	0.808	1.313
Aminopimelic acid (APA)	1.789	0.902	0.473	0.769
Glutamine (GLN)	2.707	1.365	1.543	2.508
Ornithine (ORN)	0.106	0.053	N.D.	N.E.
Glycine-proline (GPR)	8.333	4.202	4.089	6.648
Proline-hydroxyproline(PHP)	N.D.	N.E.	N.D.	N.E.
Cystathionine CTH	N.D.	N.E.	N.D.	N.E.
Total (OAA)	27.57	13.90	12.01	19.52

N.D.: investigated but Not Detected; N.E: Not Estimated; N: *N. spongiaeforme;* R*: R. bullata*

low concentration of methionine. *Nostoc commune* is also an important source of amino acids with variation of amino acid concentration most likely influenced by culture conditions and age of the organism (Martinez and Querijero, 1986). The nutritional value of algal proteins depends not only on its amino acid content but also on its digestibility, which consequently determines the availability of amino acids (Briones-Nagata *et al.,* 2007).

The differences in the levels of specific amino acids between both microalgae *(Isochrysis galbana* and *Chaetoceros calcitrans*) were very minor, and only few significant differences were detected. Phenylalanine, alanine, glutamic and aspartic acid were found in high concentrations in both microalgae, with values ranging from 6.3% to 0.2%. In the meantime, the lowest amino acid amount<4% for both microalgae were for histidine and valine, with values ranging from 1.3% to 3.6%. The highest amino acid amounts were determined for*I. galbana;* glutamic acid (10.2±1.3%) and for *C. calcitrans;* alanine (10.0±1.4%) and they stated that microalgae with high antioxidant activity have high nutritive value (Natrah *et al.,* 2007).

Considering the following point that nutrient deficiency within the next 20-30 years and further increasing the algae studies about their nutritional value; algae may be used for animal and human food. With this study, new data will be obtained about alternative protein source for animal and human consumption.

CONCLUSION

Based on the data presented, it is clear that the prospects of Cyanobacteria as sources of natural products are very promising. The findings show that they could serve as vast sources of potential organic matter for the living things. But while consuming algae for human or animal food, should be very careful; because lots of Cyanobacteria have enormous toxicological affect. So firstly, the toxic degree of algae should be researched and then nutritional value. All of these properties make microalgae worth exploring for industrial utilization in the future.

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