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Nutritional quality of biomass from four strains of *Nostoc* and *Anabaena* grown in batch cultures

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Abstract

The growing global demand for food has led the search for new sources of highly nutritious biomass. Cyanobacteria can be used not only as a source of compounds of biotechnological interest, but also as food for animals and humans, because of their high nutritional quality. The aim of this work was to evaluate the nutritional quality of biomass from four strains of nitrogen-fixing filamentous cyanobacteria in batch cultures. *Nostoc* LAUN015, *Nostoc* UAM206, *Anabaena* MOF015 and *Anabaena* MOF016 were cultured in diazotrophic conditions at laboratory scale. The biomass was harvested, dried and used for biochemical evaluation, proximal, microbiological and toxicological quality. Biomass protein has values between 25 and 40%, 43-48% of carbohydrates, 0.39 to 1.64% of fiber and 0.74-1.05% of lipids. Biomass is rich in amino acids, specially arginine, lysine, aspartate and glutamate. Pathogens such as *S. aureus*, total and fecal coliforms, *E. coli* and *Salmonella,* were undetected. Toxicology tests showed high LC50 for *Anabaena* MOF015, indicating no toxicity. In conclusion, *Anabaena* MOF015 stands within the group, for having an excellent biomass production, nutritional quality and no toxicity detected, and could be used as a protein source for human and animal nutrition.

Introduction

Prokaryotic cyanobacteria are a diverse group of organisms and can produce many biochemical compounds, such as proteins, lipids, pigments, polysaccharides, vitamins and other nutraceuticals (Borowitzka, 2013; Chen *et al*., 2016). In addition, because algae must adapt rapidly to the new environmental conditions, they produce a great variety of secondary metabolites, with biological activity, which cannot be found in other organisms (Plaza *et al*., 2008; Gouveia *et al*., 2010).

Limitations in cultivable land areas have led the search of new animal and human feed ingredients. During last two decades, microalgae have been considered promising sources of protein and lipids (Spolaore *et al*., 2006; Skrede *et al*., 2011). Humans have used these microorganisms for decades as food, feed, therapeutics and fertilizers (Spolaore *et al*., 2006; Samek *et al*., 2013).

Microalgae and cyanobacteria are used as alternative protein source, due its high content of amino acids in the biomass for producing protein products that are sold as health food and food supplements (Harun *et al.*, 2010; Batista *et al*., 2013). However, application of microalgae as nutrient source for the animal feed industry depends on detailed information on parameters such as chemical composition and digestibility (Skrede *et al*., 2011; Velazquez *et al*., 2016).

The most commonly grown microalgae and cyanobacteria for human dietary use are different strains of *Chlorella, Scenedesmus, Spirulina / Arthrospira* and *Dunaliella,* but there are many genera and strains that can be characterized, cultured and produced as food complement (Gantar and Svirčev, 2008; Chen *et al*., 2016). There have been numerous reports of cyanobacterial and microalgal products that possess numerous beneficial effects on human health. The list includes claims that these products are ‘‘super food for super health’’, with antiviral (Santoyo *et al.*, 2012; Yakoot and Salem, 2012), antineoplasic

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(Nobili *et al*., 2009), antibiotic (Caicedo *et al*., 2011), antifungal (Hernández and Gamboa, 2011), immune boosting (Anwer *et al.*, 2012), hypocholesterolemic (Rasmussen *et al.*, 2009), antioxidant (Goiris *et al.*, 2012), neuromodulator (Menvielle-Bourg *et al*., 2011) and many others (Gouveia *et al.*, 2010; Batista *et al*., 2013; Borowitzka, 2013; Wu *et al*., 2016).

The aim of this work is to characterize the proximate composition, amino acid profile, mineral content, microbiological analysis and toxicological tests of four filamentous cyanobacteria strains of *Anabaena* and *Nostoc*.

Materials and Methods

Filamentous cyanobacteria under study were: (1) Nostoc LAUN0015, isolated from a humid environment in Bogota, Colombia; (2) *Nostoc* UAM206, isolated from an inundated rice field in Valencia, Spain; (3) *Anabaena* MOF015, from activated sludge of a treatment plant after secondary treatment in Maracaibo, Venezuela; and (4) *Anabaena* MOF016 from a heavy oil pit in Venezuela.

Cultures by triplicate were maintained in 20 L flasks with 15 L of medium composed of sterilized tab water enriched BG-11 (Rippka *et al*., 1979) culture medium with no nitrogen added, under laboratory conditions, including irradiation of 156 µmol q m^{-2} s^{-1} bilaterally, light:dark cycle of 12:12 h and constant aeration to 5 mL s^{-1}, during 25 days. When stationary phase was reached, biomass was separated by filtering through a 70-µm mesh, dried and pulverized for further analysis.

Dry weight was determined using a Millipore© filtration system, with 0.45 µm fiberglass filter, by the method of Utting (1985). Moisture and ash were determined by the air oven method (AOAC, 2006). Total nitrogen was determined by Kjeldhal method, and crude protein was calculated by multiplying total nitrogen by the conventional conversion factor of 6.25 (Jones, 1931). Total carbohydrates were estimated by the anthrone colorimetric method and crude fiber was calculated by difference. Crude fat was determined by the Soxhlet extraction method (AOAC, 2006).

Chlorophyll a and carotenoids were obtained by methanolic extraction overnight and measured by spectrophotometry using length waves (480 and 665 nm for carotenoids and chlorophyll a, respectively) and equations by Marker *et al.* (1980) and Strickland and Parsons (1972). Phycobiliproteins were extracted by repeated freezing-thawing and length waves (562, 615, 652 nm) and equations by Bennet and Bogorad (1973).

Amino acids were analyzed by HPLC reverse phase as is described by Wu *et al.* (1997), and samples were treated following method 994.12 from AOAC (2006). Zinc (Zn), iron (Fe) and copper (Cu) determinations were carried out by flame atomic absorption spectrometry and cadmium (Cd) by graphite furnace by method 999.10 (AOAC, 2006). Chromium (Cr) and nickel (Ni) were determined by atomic absorption spectrometry using method 974.27 (AOAC, 2006). Sodium (Na) and potassium (K) were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES), using method 984.27 (AOAC, 2006).

Microbiological quality of culture and biomass was assessed by determining *E. coli*, total and fecal coliforms, total aerobic bacteria, fungi, *Staphylococcus aureus* and *Salmonella* following methods in the Bacteriological Analytical Manual (FDA, 1998). Toxicity was analyzed using liposoluble and hydrosoluble extracts from biomass on brine shrimp Artemia using the method proposed by Lee *et al.* (1998), and cyst decapsulation by Sorgeloos *et al.* (1977). Extract were evaluated at 50, 100, 500 y 1000 µg mL^{-1}. Software Probit Analysis version 1.5 from Environmental Protection Agency (EPA) was used to calculate $LC_{50.}$

Statistical analyses were performed with SPSS 20.0, using analysis of variance (ANOVA) and Sheffé's test to examine differences in biochemical composition between different cyanobacteria.

Results

Biochemical composition of four filamentous cyanobacteria is shown in Table 1. Moisture values were similar, between 11.73 and 12.80% ($p>0.05$). Ash content was higher for *Nostoc* UAM206 of 26.40 ±3.40% and with statistical differences ($p<0.05$). *Anabaena* strains produced more biomass than *Nostoc* strains. *Anabaena* MOF015 produced 1.55 ±0.2 mg mL^{-1}, followed by *Anabaena* MOF016 and *Nostoc* LAUN0015 with 1.21 ±0.1 and 0.98 ±0.1 mg mL^{-1}, respectively.

Carbohydrates are the major biochemical constituent for all cyanobacteria, with values between 43 and 48%. Fat and fiber were low in all samples. Fiber values were below 1.64% and fat values below 1.2%, with no differences ($p>0.05$).Total digestible nutrients (TDN) were higher for *Anabaena* strains with 69.51 ±1.32 and 69.52 ±2.70% for *Anabaena* MOF016 and *Anabaena* MOF015, respectively ($p>0.05$). Also, caloric value was higher for *Anabaena* strains, with 15.43 KJ g^{-1} (Table 1).

Crude protein content was higher for *Anabaena* strains. For *Nostoc* strains, protein values were

Table 1. Biochemical composition of *Nostoc* and *Anabaena* biomass

	Nostoc LAUN0015	*Nostoc* UAM206	*Anabaena* MOF015	*Anabaena* MOF016
Dry weight	0.98 ±0.1[a]	0.75 ±0.1[a]	1.55 ±0.2[b]	1.21 ±0.1[c]
Moisture	11.73 ±1.30[a]	12.80 ±1.79[a]	12.35 ±1.94[a]	12.73 ±1.78[a]
Ash	19.33 ±2.76[a]	26.40 ±3.40[b]	10.85 ±2.54[a]	11.93 ±0.62[a]
Crude Protein	31.23 ±3.07[a]	25.15 ±1.56[b]	39.59 ±5.57[c]	36.95 ±1.97[c]
Fiber	1.64 ±0.57[a]	1.31 ±0.76[a]	1.08 ±0.24[a]	0.39 ±0.08[a]
Fat	1.05 ±0.45[a]	0.74 ±0.49[a]	0.83 ±0.18[a]	1.05 ±0.34[a]
Carbohydrates	43.62 ±3.59[a]	46.40 ±0.59[a]	43.27 ±4.77[a]	48.30 ±1.48[a]
NDT	65.14 ±1.92[a]	58.53 ±2.92[b]	69.52 ±2.70[c]	69.51 ±1.32[c]
Caloric value	14.35	12.73	15.43	15.43

All in % dry matter. Dry weight in mg mL^{-1}, caloric value in KJ g^{-1}.Different letters in the same row correspond to significant differences ($p<0.05$).

Table 2. Amino acids profile of *Nostoc* and *Anabaena* biomass

	Nostoc LAUN0015		*Nostoc* UAM206		*Anabaena* MOF015		*Anabaena* MOF016	
	mg g^{-1}	%PF	mg g^{-1}	%PF	mg g^{-1}	%PF	mg g^{-1}	%PF
Alanine	7.65	2.38	9.53	3.79	13.16	3.11	13.50	3.57
Tyrosine	NC	NC	NC	NC	NC	NC	NC	NC
Aspartate	28.46	8.84	24.76	9.84	35.86	8.49	34.88	9.24
Glutamate	28.97	9.00	32.07	12.75	39.62	9.38	39.44	10.44
Glicine	12.68	3.94	13.71	5.45	18.83	4.46	18.71	4.95
Proline	14.06	4.37	14.28	5.68	17.66	4.18	19.16	5.07
Serine	10.20	3.17	10.29	4.09	14.30	3.38	14.34	3.80
Arginine	30.10	9.35	33.93	13.49	50.60	11.98	54.25	14.36
Lisine	11.30	3.51	10.88	4.33	15.23	3.60	17.62	4.67
Leucine	19.79	6.15	21.03	8.36	25.83	6.11	27.66	7.32
Isoleucine	13.34	4.15	13.59	5.40	17.81	4.22	18.63	4.93
Treonine	11.49	3.57	12.36	4.92	16.99	4.02	17.61	4.66
Valine	NC	NC	NC	NC	NC	NC	NC	NC
Histidine	NC	NC	NC	NC	NC	NC	NC	NC
Fenilalanine	9.59	2.98	10.14	4.03	13.53	3.20	13.54	3.58

Values expressed in mg g-1dry weight. ND: No detected.%PF: % protein fraction. Amino acids in bold correspond to essential amino acids.

between 25 and 31%. Higher content was achieved for *Anabaena* MOF016 with 39.59 ±2.57% with statistical difference, compared to the rest of the strains ($p<0.05$) (Table 1). Production of liposoluble and hydrosoluble pigments is shown in Figure 1. Higher values of chlorophyll a were achieved by *Anabaena* MOF015 and *Anabaena* MOF016 with 1.00 ±0.23 and 1.23 ±0.14 mg g^{-1} dry weight, with no statistical differences ($p>0.05$). *Anabaena* MOF016 produced higher quantities of phycoerithrin, phycocianin and total phycobiliproteins with 4.88 ±0.81, 8.27 ±1.44 and 15.61 ±2.36 mg g^{-1} dry weight ($p<0.05$).

Biomass was analyzed for 15 out 20 amino acids that form proteins, and results are shown in Table 2. All cyanobacterial biomass were rich in arginine, glutamate, aspartate and leucin, to concentrations higher than 20 mg g^{-1} dry weight. Biomasses from all cyanobacteria were characterized by high zinc, copper and iron concentrations, and the results are shown in Table 3. Chromium, cadmium and nickel concentrations were between 0.1 and 10 mg Kg^{-1} dry weight. Microbiological analysis on *Nostoc*

Table 3. Mineral composition of *Nostoc* and *Anabaena* biomass

	Nostoc LAUN0015	*Nostoc* UAM206	*Anabaena* MOF015	*Anabaena* MOF016
Zinc*	420,81	448,01	378,45	589,17
Copper*	296,82	351,65	110,13	142,33
Chromium*	0,10	0,10	0,10	0,10
Nickel*	4,11	10,05	1,72	1,27
Cadmium*	0,59	0,82	0,50	0,50
Iron*	698,81	941,27	288,62	395,58
Sodium**	26,90	20,40	21,80	3,23
Potassium**	6,10	3,79	2,96	21,08

*Values expressed in mg Kg-1dry weight. ** Values expressed in g Kg^{-1} dry weight

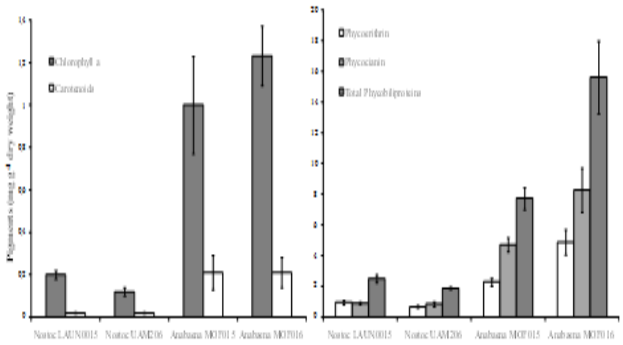

Figure 1. Pigment content of *Nostoc* and *Anabaena* biomass. Values expressed in mg g^{-1} dry weight

LAUN015 and *Anabaena* MOF015 biomass showed high quantity of heterotrophic bacteria, but absence of molds, *S. aureus, Salmonella, E. coli,* total and fecal coliforms (Table 4).

Toxicity test on *Artemia* showed that hydrosoluble extracts from biomass possess low toxicity, due to high LC_{50} values of 13.51 and 3690.79 mg mL^{-1} for *Nostoc* LAUN0015 and *Anabaena* MOF015 (Table 4). Survival from control cultures was always higher than 80%. Lower survival was achieved for *Anabaena* MOF015 with highest concentration of liposoluble extract of 1000 µg mL^{-1}.

Table 4.Microbiological quality and LC_{50} of *Nostoc* and *Anabaena* biomass

	Nostoc LAUN0015	*Anabaena* MOF015
Aerobial bacteria	3,23 x10^5CFU g^{-1}	4,37 x10^5 CFU g^{-1}
S. aureus	< 10 CFU g^{-1}	<10 CFU g^{-1}
Molds and yeast	< 10 CFU g^{-1}	<10 CFU g^{-1}
TC, FC and *E. coli*	< 3 MPN/100 g	<3 MPN/100 g
Salmonella	Absent	Absent
LC50		
Hydrosoluble fraction	13,51	3960,79
Liposoluble fraction	5,22	0,31

CFU g^{-1}: Colony forming units pergram dry weight. TC: total coliforms. FC: fecal coliforms. MNP/100 gr: More probable number per hundred grams dry weight. LC_{50} in mg mL^{-1}

Discussion

For algal biomass, it is required that moist levels do not overpass 10%, because higher levels could create an ideal environment for insects, bacteria and fungi (Becker, 2004). Previous studies have shown ash contents ranging from 5% to 30% for *Spirulina* and *Nostoc* (Dai *et al.*, 1991; Ortega-Calvo *et al.*, 1993; Gao, 1998; Shimamatsu, 2004; Batista *et al.*, 2013). High ash content is related to marine cultures, for its higher salt concentrations. For moist and ashes, results from biomass of *Anabaena* and *Nostoc* strains overpass 10% of dry weight, though they were cultured in non-marine systems.

Fiber content was low, around 1% dry weight, related to previous works. It has been reported values from 3% and up to 20% for *Spirulina* (Shimamatsu, 2004; Matos *et al*., 2016). Those low values are due to the peptidoglycan present in the cell wall of cyanobacteria and not due to presence of cellulose (Vincent, 2009). Similarly, fat content was low, related with most of microalgae, with values from 10% up to 70% dry weight (Chisti, 2007; Skrede *et al*., 2011; Batista *et al*., 2013). Some strains of *Nostoc, Arthrospira* and *Spirulina* have been reported to produce lipid content to 5% and 20% dry weight, respectively (Dai *et al*., 1991; Ortega-Calvo *et al*., 1993; Gao, 1998; Danesi *et al.*, 2002; Colla *et al*., 2007; Panyakampol *et al.*, 2016).

In contrast, carbohydrate content was high, between 40-50% dry weight, over passing values found in other microalgae and cyanobacteria (Brown *et al.*, 1997; Ogbonda *et al.*, 2007; Batista *et al*., 2013). This high content is related not just with intracellular reserves, but also to the presence of capsular exopolysaccharide in these strains, composed by glucose, mannose, rhamnose, xylose and uronic acids (Parikh and Madamwar, 2006). There are few reports in *Nostoc*, with similar values, around 50% dry weight (Dai *et al*., 1991; Gao, 1998).

Nutritional value of microalgae and cyanobacteria has been documented extensively elsewhere (Plaza *et al*., 2008; Gouveia *et al*., 2010; Skrede *et al*., 2011; Batista *et al.*, 2013). Both digestibility and nutritional value of these food sources are influenced not just by

genetic traits, but also by technical process used for its production (Gantar and Svirčev, 2008).

Under normal cultures conditions, biomass from microalgae must achieve a caloric value between 18 and 21 KJ g^{-1}, and this value have to increase up to 42 KJ $g^{-1}$for biodiesel proposes (Illman *et al.*, 2000; Scragg *et al*., 2002). Low caloric values obtained in these results are directly related to low lipid content in *Nostoc* and *Anabaena* biomass, which is commonly low in cyanobacteria.

Previous studies in *Nostoc* strains from Southeast Asia, have obtained protein values between 20 and 30% (Briones et al., 1997; Gao, 1998), and between 30 and 50% for *Nostoc* and *Anabaena* strains from South America (Gómez-Silva et al., 1994). *Spirulina/ Arthrospira* is really known for high protein productions, around 60-70% (Shimamatsu, 2004; Rasmussen and Morrissey, 2007; Chen *et al*., 2016). However, there are several studies with protein contents similar to our results, between 40% and 50% (Ogbonda *et al.*, 2007; Batista *et al*., 2013; Samek *et al*., 2013). All cyanobacteria are not good protein producers; some prefer to produce large amounts of carbohydrates, as *Calothrix, Gleocapsa, Lyngbya, Scytonema* and *Oscillatoria* with protein production lower than 10% (Rajeshwari and Rajashekhar, 2011).

Chlorophyll content in microalgal biomass is generally found between 0.5 and 1.5% of dry weight (Becker, 2004). *Nostoc* strains produce low chlorophyll levels, between 0.1 and 0.2 mg g^{-1}, as found in our results and by Deng *et al.* (2008). *Anabaena* values are similar to those found in *Spirulina* and *Chlorella* (Ortega-Calvo *et al.*, 1993; Batista *et al*., 2013). In relation to phycocianin, studies have shown values between 4 and 20 mg g^{-1} in *Spirulina*, similar to our results, around 8 mg g^{-1} (Shimamatsu, 2004).

High values of amino acids leucine, glutamate and aspartate obtained in *Nostoc* and *Anabaena* have been found in some macroalgae, microalgae and cyanobacteria (Ortega-Calvo *et al*., 1993; Menvielle-Bourg *et al*., 2011; Skrede *et al.*, 2011; Samek *et al.*, 2013). Whereas, high arginine and lysine concentrations, with maximum of 54.25 and 17.62 mg g^{-1}, respectively; found in our results are higher than those obtained in *Porphyridium, Chaetoceros, Nitzschia, Tetraselmis, Isochrysis, Chlorella, Dunaliella, Haematococcus, Nannochloris, Synechococcus, Oscillatoria*; and even higher than some foods as eggs and soy (Brown *et al*., 1997; Becker, 2004; Jegan *et al*., 2013). Obtained values of lysine, leucine and isoleucine meet reference values established for Food and Agriculture Organization and World Health Organization for protein sources (FAO/WHO, 1973).

Zinc, chromium and nickel content were similar in several studies to our results, carried out in *Chlorella, Spirulina, Oscillatoria* and *Scytonema* (Ortega-Calvo *et al*., 1993; Rajeshwari and Rajashekhar, 2011). Cadmium and copper content were higher than most of previous studies in *Spirulina, Chlorella, Calothrix, Gloeocapsa, Lyngbya, Nostoc, Scytonema* and *Oscillatoria* (Ortega-Calvo *et al.*, 1993; Rajeshwari and Rajashekhar, 2011; Batista *et al*., 2013). Subhashini *et al*. (2003) found in *Anabaena,* copper content similar to our results.

Iron content in *Nostoc* and *Anabaena*, between 288 and 941 mg Kg^{-1}, are lower than those found in some microalgae, such as *Chlorella, Haematococcus, Diacronema,* and other cyanobacteria: *Lyngbya, Oscillatoria, Scytonema, Spirulina*, another *Nostoc* strains, among others (Ortega-Calvo *et al*., 1993; Shimamatsu, 2004; Plaza *et al*., 2008; Rajeshwari and Rajashekhar, 2001; Batista *et al.*, 2013). Iron in cyanobacteria is not present in form of hemo groups, which gives higher absorption in mammals (Ortega-Calvo *et al*., 1993).

Microalgae and cyanobacteria cultures possess low concentrations of heterotrophic bacteria; due to algal culture media do not include carbon sources. In marketed products of *Spirulina* and *Chlorella*, most of the samples were bacteria-free or present in low quantities (Görs *et al.*, 2010). Acceptable microbial levels on biomass, depends on specific regulations of each country. In Japan, bacterial count cannot exceed 5 x 10^4 CFU g^{-1} (Shimamatsu, 2004), whereas in France and Sweden, maximal values are 0.1 and 10 x 10^6 CFU g^{-1} (Becker, 2004).

Bacteria-free biomass is not easy to obtain. Delicate treatments on the biomass may preserve some valuable substances such as pigments or fatty acids, but generally will not lead to a reduction in bacterial contamination, and microalgae and cyanobacteria culture under sterile conditions on an industrial scale, is virtually impossible (Görs *et al*., 2010). Grobbelaar (2004) explains that there are no regulations and standards regarding the quality of products derived from microalgae, so the quality criteria are mostly defined by the producers or distributors.

The fact that soluble fraction from *Anabaena* has obtained a high LC_{50} provides an excellent indication of the absence of toxic factors. Microcystins, major toxins present in filamentous cyanobacteria, are a group of monocycle heptapeptides of hydrophilic nature, highly toxic to animals and humans (Babica *et al*., 2006). Soluble extracts of various species of *Cylindrospermopsis raciborskii,* have reached

mortalities of 100% and LC_{50} values between 3 and 5 mg mL^{-1} (Metcalf *et al*., 2002). Some studies have detected lethality over *Artemia* of hydrosoluble and liposoluble extracts of Hydrocoleus, *Cylindrospermum, Petalonema, Myxosarcina, Lyngbya, Nostoc, Tolypothrix* and *Scytonema*. In most cases, toxicity exceeded 80% (Jaki *et al.*, 1999; Mian *et al*., 2003).

Liposoluble extracts of both cyanobacteria show much lower values of LC_{50}, and low toxicity is related to the presence of free lipids, such as free fatty acids and terpenes. These lipid molecules, seem to have a major toxic effect against microcrustaceans as *Artemia*, and hence are not related to production of any toxin by cyanobacteria (Yamada *et al*., 1993).

Conclusion

Anabaena MOF015 shows the higher biomass production, with high protein content, presence of essential amino acids, low bacterial load and absence of toxic factors. Moreover, processes of harvest and dry of biomass could be done easily for *Anabaena* cultures, which are more complex for *Nostoc* cultures, due the presence of capsular polysaccharides that hinders these processes. Therefore, it could be recommended to establish a protocol for the permanent production of biomass enriched with compounds of commercial interest and food supplement.

References

Anwer, R., Khursheed, S. and Fatma, T. 2012. Detection of immunoactive insulin in *Spirulina*. Journal of Applied Phycology 24(3): 583-591.

Association of the Official Analytical Chemists (AOAC). 2006. Official Methods of Analysis. 18thed. Gaithersburg, Maryland, USA: AOAC International.

Babica, P., Blaha, L. and Marsalek, B. 2006. Exploring the natural role of microcystins - A review on photoautotrophic organisms. Journal of Phycology 42: 9-20.

Batista, A., Gouveia, L., Bandarra, N., Franco, J. and Raymundo, A. 2013. Comparison of microalgal biomass profiles as novel functional ingredient for food products. Algal Research 2: 164-173.

Becker, W. 2004. Microalgae in human and animal nutrition. In Richmond, A. (Ed.). Handbook of microalgal culture, p. 312-351. Oxford: Blackwell Publishers.

Bennet, A. and Bogorad, L. 1973. Complementary chromatic adaptation in a filamentous blue-green alga. Journal of Cell Biology 58: 419-435.

Borowitza, M. 2013. High-value products from microalgae - their development and commercialization. Journal of Applied Phycology 25: 743-756.

Briones, M., Hori, K., Martínez-Goss, M., Ishibashi, G. and Okita, T. 1997. A comparison of physical properties, oxalate-oxalic acid soluble substances, protein content, and in vitro protein digestibility of the blue-green alga *Nostoc commune* Vauch. from the Philippines and Japan. Plant Foods for Human Nutrition 50: 287-294.

Brown, M., Jeffrey, S., Volkman, J. and Dustan, G. 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151: 315-331.

Caicedo, N. H., Heyduck-Söller, B., Fischer, U. and Thöming, J. 2011. Bioproduction of antimicrobial compounds by using marine filamentous cyanobacterium cultivation. Journal of Applied Phycology 23: 811-818.

Chen, J., Wang, Y., Benemann, J., Zhang, X., Hu, H. and Qin, S. 2016. Microalgal industry in China: challenges and prospects. Journal of Applied Phycology 28: 715-725.

Chisti, Y. 2007. Biodiesel from microalgae. Biotechnology Advances 25: 294-306.

Colla, L., Oliveira Reinehr, C., Reichert, C. and Vieira Costa, J. 2007. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioresource Technology 98: 1489-1493.

Dai, Z., Cao, Y. and Wang, T. 1991. Determination of the nutrients and chemical composition of *Nostoc flagelliforme* of Ningxia. Journal of AgroForestry Science and Technology 3: 42-43.

Danesi, E., Rangel-Yagui, C., de Carvalho, J. and Sato, S. 2002. An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by Spirulina platensis. Biomass and Bioenergy 23: 261-269.

Deng, Z., Yan, C., Lu, F., Hu, Q. and Hu, Z. 2008. Growth kinetics of 1–2 mm and 3–4 mm colonies of *Nostoc sphaeroides* (Cyanophyta) in outdoor culture. Biotechnology Letters 30: 1741-1746.

Food and Agricultural Organization / World Health Organization (FAO/WHO). 1973. Energy and protein requirement. Report of a joint FAO/WHO ad hoc expert committee. WHO Technical Report Series 522. Genève: World Health Organization.

Food and Drugs Administration (FDA). 1998. Bacteriological Analytical Manual. 8th Ed. Washington D.C.: AOAC International.

Gantar, M. and Svirčev, Z. 2008. Microalgae and cyanobacteria: food for thought. Journal of Phycology 44: 260-268.

Gao, K. 1998. Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review. Journal of Applied Phycology 10: 37-49.

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., De Brabanter, J. and De Cooman, L. 2012. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. Journal of Applied Phycology 24: 1477-1486.

Gomez-Silva, B., Mendizabal, C., Tapia, I. and Olivares, H.

1994. Microalgas del norte de Chile IV. Composición química de *Nostoc commune* (Llaita). Revista de Investigación Científica y Tecnológica. Serie Ciencias Marinas 3: 15-20.

Görs, M., Schumann, R., Hepperle, D. and Karsten, U. 2010. Quality analysis of commercial *Chlorella* products used as dietary supplement in human nutrition. Journal of Applied Phycology 22: 265-276.

Gouveia, L., Marques, A., Sousa, J., Moura, P. and Bandara, N. 2010. Microalgae - source of natural bioactive molecules as functional ingredients. Food Science and Technology Bulletin 7: 21-37.

Grobbelaar, J. 2004. Algal nutrition. In Richmond, A. (Ed.). Handbook on microalgal culture, p. 97-115. Oxford: Blackwell Science.

Harun, R., Singh, M., Forde, G. M. and Danquah, M. K. 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. Renewable and Sustainable Energy Reviews 14: 1037-1047.

Hernandez, B. and Gamboa-Angulo, M. 2011. Metabolites from freshwater aquatic microalgae and fungi as potential natural pesticides. Phytochemical Reviews 10: 261-286.

Illman, A., Scragg, A. and Shales, S. 2000. Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. Enzyme Microbial Technology 27: 631-635.

Jaki, B., Orjala, J., Bürgi, H. and Sticher, O. 1999. Biological screening of cyanobacteria for antimicrobial and molluscicidal activity, brine shrimp lethality, and cytotoxicity. Pharmaceutical Biology 37: 138-143.

Jegan, G., Mukund, S., Nayagam, S., Senthilkumar, N. and Sivasubramanian, V. 2013. Amino acid content and biochemical analysis of the methanolic extract of *Oscillatoria terebriformis.* International Journal of Pharmaceutical Research and Development 5: 22-27.

Jones, D. B. 1931. Factors for converting percentages of nitrogen in food and feeds into percentages of protein. United States Department of Agriculture Circular 183: 1-21.

Lee, T.H., Chen, Y. and Chou, H. 1998. Toxicity assay of cyanobacterial strain using *Artemia salina* in comparison with the mouse bioassay. Acta Zoologica Taiwanica 10: 1255-1263.

Marker, A., Nusch, E., Rai, H. and Riemann, B. 1980. The measurement of photosynthetic pigments in freshwater and standardization of methods: conclusions and recommendations. Archives of Hydrobiology, Egebn Limnology 14: 91-106.

Matos, A., Feller, r., Siegel Moecke, E., de Oliveira, J., Furigo Junior, A., Bianchini Derner, R. and Sant'Anna, E. 2016. Chemical characterization of six microalgae with potential utility for food application. Journal of the American Oil Chemists' Society 93: 963-972.

Menvielle-Bourg, F. J., Vitaccal, A. and Scoglio, S. 2011. L'algue Klamath et ses proprieties nutritionnelles. Un extrait spécifique aux effets neuromodulateurs et neuroprotecteurs. Phytothérapie 9: 165-171.

Metcalf, J., Lindsay, J., Beattie, K., Birmingham, S., Saker, M., Torokné, A. and Codd, G. 2002. Toxicity of cylindrospermopsin to the brine shrimp *Artemia salina*: comparisons with protein synthesis inhibitors and microcystins. Toxicon 40: 1115-1120.

Mian, P., Heilmann, J., Bürgi, H. and Sticher, O. 2003. Biological screening of terrestrial and freshwater cyanobacteria for antimicrobial activity, brine shrimp lethality, and cytoxicity. Pharmaceutical Biology 41: 243-247.

Nobili, S., Lippi, D., Witort, E., Donninic, M., Bausi, L., Minia, E. and Capaccioli, S. 2009. Natural compounds for cancer treatment and prevention. Pharmacological Research 59: 365-378.

Ogbonda, K., Aminigo, R. and Abu, G. 2007. Influence of temperature and pH on biomass production and protein biosynthesis in a putative *Spirulina* sp. Bioresource Technology 98: 2207-2211.

Ortega-Calvo, J., Mazuelos, C., Hermosin, B. and Saiz-Jiménez, C. 1993. Chemical composition of *Spirulina* eukaryotic algae food products marketed in Spain. Journal of Applied Phycology 5: 425-435.

Panyakampol, J. Cheevadhanarak, S., Senachak, J., Dulsawat, S., Siangdung W., Tanticharoen, M. and Paithoonrangsarid K. 2016. Different effects of the combined stress of nitrogen depletion and high temperature than an individual stress on the synthesis of biochemical compounds in *Arthrospira platensis* C1 (PCC 9438). Journal of Applied Phycology 28: 2177-2186.

Parikh, A. and Madamwar, D. 2006. Partial characterization of extracellular polysaccharides from cyanobacteria. Bioresource Technology 97: 1822-1827.

Plaza, M., Cifuentes, A. and Ibañez, E. 2008. In the search of new functional food ingredients from algae. Trends in Food Science and Technology 19: 31-39.

Rajeshwari, K. and Rajashekhar, M. 2011. Biochemical composition of seven species of cyanobacteria isolated from different aquatic habitats of Western Ghats, southern India. International Journal of Current Trends in Science and Technology 2: 240-251.

Rasmussen, H., Blobaum, K., Jesch, E., Ku, C., Park, Y., Lu, F., Carr, T. and Lee, J. 2009. Hypocholesterolemic effect of *Nostoc commune* var. sphaeroides Kützing, an edible blue-green alga. European Journal of Nutrition 48: 387-394.

Rasmussen, R. and Morrissey, M. 2007. Marine biotechnology for production of food ingredients. Advances in Food Nutrition Research 52: 237-292.

Rippka, R., Deruelles, J., Waterbury, J., Herdman, M. and Stanier, R. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology 111: 1-61.

Samek, D., Mišurcová, L., Machů, L., Buňka, F. and Fišera, M. 2013. Influencing of amino acid composition of green freshwater algae and cyanobacterium by methods of cultivation. Turkish Journal of Biochemistry 38: 360-368.

Santoyo, S., Jaime, L., Plaza, M., Herrero, M., Rodriguez-Meizoso, I., Ibañez, E. and Reglero, G. 2012. Antiviral compounds obtained from microalgae commonly used as carotenoid sources. Journal of Applied Phycology

24: 731-741.

Scragg, A., Illman, A., Carden, A. and Shales, S. 2002. Growth of microalgae with increased calorific values in a tubular bioreactor. Biomass and Bioenergy 23: 67-73.

Shimamatsu, H. 2004. Mass production of *Spirulina*, an edible microalga. Hydrobiologia 512: 39-44.

Skrede, A., Mydland, L., Ahlstrøm, Ø., Reitan, K., Gislerød, H. and Øverland M. 2011. Evaluation of microalgae as sources of digestible nutrients for monogastric animals. Journal of Animal Feed Science 20: 131-142.

Sorgeloos, P., Bossouyt, E., Laviña, E., Baeza-Mesa, M. and Persoone, G. 1977. Decapsulation of *Artemia* cysts: A simple technique for the improvement of the use of brine shrimp in aquaculture. Aquaculture 12: 311-315.

Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert A. 2006. Commercial applications of microalgae. Journal of Bioscience and Bioengineering 101: 87-96.

Strickland, J. and Parsons, T. 1972. A practical handbook of seawater analysis. Ottawa, Canada: Fisheries Research Board of the Canadian Bulletin.

Subhashini, R., Kumar, K. and Kannaiyan, S. 2003. Intrinsic antibiotic resistance and biochemical characteristics of *Anabaena azollae* isolated from Azolla– cultures. Indian Journal of Microbiology 43: 165-169.

Utting, S. 1985. Influence of nitrogen availability on the biochemical composition of three unicellular marine algae of commercial importance. Aquaculture Engineering 4: 175-190.

Velazquez, S., Chan, M., Abisado, R., Ferdinand, R., Tayamen, M., Maliwat, G. and Ragaza, J. 2016. Dietary *Spirulina (Arthrospira platensis)* replacement enhances performance of juvenile Nile tilapia *(Oreochromis niloticus)*. Journal of Applied Phycology 28: 1023-1030.

Vincent, W. 2009. Cyanobacteria. In Likens, G. (Ed.). Encyclopedia of Inland Waters, p. 226-232. Amsterdam: Elsevier.

Wu, G., Davis, P., Flynn, N., Knabe, D. and Davidson, J. 1997. Endogenous synthesis of arginine plays an important role in maintaining arginine homeostasis in postweaning growing pigs. Journal of Nutrition 127: 2342-2349.

Wu, Q., Liu L., Miron, A., Klímová B., Wan, D. and Kuča, K. 2016. The antioxidant, immunomodulatory, and anti inflammatory activities of *Spirulina*: an overview. Archives of Toxicology 90: 1817-1840.

Yakoot, M. and Salem, A. 2012. *Spirulina platensis* versus silymarin in the treatment of chronic hepatitis C virus infection. A pilot randomized, comparative clinical trial. BMC Gastroenterology 12: 32

Yamada, N., Murakami, N., Morimoto, T. and Sakakibara, J. 1993. Autogrowth inhibitory substances from the freshwater cyanobacterium *Phormidium tenue*. Chemical and Pharmaceutical Bulletin 41: 1863-1865.