Original Article

APPLIED FOOD BIOTECHNOLOGY, 2015, 2(1): 39-44

Journal's homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357

Evaluation of Carotenoids and Chlorophyll as Natural Resources for Food in Spirulina Microalgae

Ghaeni, M.[1], Roomiani, L.[1], Moradi, Y.[2]*

1 - Department of Fisheries, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.

2- Iranian Fisheries Research Organization, Tehran, Iran. P.O. Box: 14155-6116.

Abstract

Microalgae can produce various natural products such as pigments, enzymes, unique fatty acids and vitamins that benefit humans**.** The objective of the study was evaluation of carotenoids (beta carotene, zeathanthin, lutein, lycopene and astaxanthin) and chlorophyll a in spirulina microalgae. Spirulina powder has been produced by Jordan's method in Iran. Carotenoids were extracted from *Spirulina platensis* by adopting a method described by Reboul; then the sample was prepared and injected into a HPLC instrument with triplicate injection. Chlorophyll`s biomass content was determined by spectrophotometer. After assaying the curves of HPLC, the amount of chlorophyll a, astaxanthin, beta carotene, lycopene, zeaxanthin and lutein in spirulina was determined as 4.3±0.14, 0.21±0.02, 7393±2.76, 741±2.32, 6652±3.69 and 424±2.83 µg/ml respectively. Beta carotene account for 80% of the carotenoids present in spirulina after that zeaxanthin was most. At last, Spirulina was a good source for carotenoids as a pro-vitamin A in organisms.

Article Info

Article history
Received 6 Aug 2014
Revised 23 Oct 2014
Accepted 1 Nov 2014

Keywords
Spirulina Microalgae;
Carotenoid;
Chlorophyll a.

Correspondence to
Ghaeni, M. Department of Fisheries, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.
Fax: +98 21 2292 6718
Email:
ghaeni@iauahvaz.ac.ir

1. Introduction

Spirulina and its derivatives can be incorporated into many kinds of foods [1]. This useful microalgae consists of proper contents of macro and micro nutrients [2,3]. It may also improve the viability of probiotic bacteria in carrier food [4-6]. Due to its pharmacological and medical activities, several beneficial health benefits of spirulina have been documented [4, 7]. Carotenoids are tetraterpenoids and synthesized in plants, algae and other photosynthetic organisms as well as in some nonphotosynthetic bacteria, yeasts, and molds. Most of the carotenoids are composed of a central carbon chain of alternating single and double bonds, and carry different cyclic or acyclic end groups. Their major biochemical functions are determined by the extended system of conjugated double bonds, which is also responsible for their color. Carotenoids are unique constituents of a healthy diet, and play an important role in the network of antioxidant vitamins and phytochemicals. They are good blue light filters, and efficient quenchers of singlet oxygen and excited triplet state molecules. Lipophilicity of carotenoids determines their subcellular distribution. Carotenoids are enriched in membranes and other lipophilic compartments. Taken together, this makes them suitable photoprotectants, not only for plants but also for humans [8-10]. Microalgae produce various natural products such as pigments, enzymes, unique fatty acids and vitamins that benefit humans. The potential of microalgae to enhance nutritional content of conventional food preparations is great as they represent a valuable source of nearly all essential vitamins (e.g. A, B_1, B_2, B_6, B_{12}, C, E, nicotinate, biotin, folic acid and pantothenic acid). Microalgae can also be an important source of carotenoids for commercial use, because these compounds can be obtained in high yield [11-13]. *Spirulina platens* is a microscopic blue-green alga in the shape of a spiral coil, living both in sea and fresh water. It is widely used as health food due to its protein content, vitamins and active substances for the immune system [14]. Currently, the commercial production of spirulina in many countries is oriented mainly towards the health food market. Considerable interest has been invested in outdoor cultivation

of *Spirulina sp.* for commercial biomass production because of its potential source of protein and valuable chemicals [15].

The possibility of increasing the level of some bioactive compounds (beta carotene, phycopiliprotein and total lipids) in *Spirulina sp.* isolated from Wadi El Natron lake (Egypt) was studied [16].

The low productivity of algal cultures in the production of high-value compounds is the most significant bottleneck for commercialization of this technology. Production and selective extraction of carotenoids in two-phase bioreactors from *Spirulina platensis* as an alternative algal technology to traditional organic water solvent extraction systems can be done by using several methods [17]. Also, chemical characterization of the extracts was performed using liquid chromatography with Diode Array Detector while the fractions were also functionally characterized by *in vitro* antioxidant activity assays (using the b-carotene bleaching method) [18]. The amounts of total carotenoids, chlorophylls-derived and phenolic compounds were associated inversely with the concentration of nitrogen in growth media [19]. Different conditions of culture and drying can also affect the biochemical composition of spirulina [20, 21]; for this reason, the objective of the study is evaluation of carotenoids (beta carotene, zeathanthin, lutein, lycopene and astaxanthin) and chlorophyll a in *Spirulina platensis* microalgae that has been produced in Iran for the first time.

2. Materials and Methods

2.1 Preparation of spirulina microalgae

Spirulina platensis was cultured by Jourdan's method and harvested with net filtration in maximum growth in Persian Gulf Biotechnology Center [22, 17]. Slurry of spirulina was dried by freeze drier (VACO2-E model, ZIRBUS) and milled to produce spirulina powder [17]. Extraction and determination of carotenoids and chlorophyll a have been carried out in Shahid Beheshti University, Tehran, Iran.

2.2 Determination of chlorophyll a

The cellular concentration was determined by measurements of optical density at 560 nm. The chlorophyll biomass content was determined spectrophotometrically at 661 nm from a fresh biomass on a D5 spectrophotometer following the methods of Mackinney [23, 24].

2.3 Extraction of carotenoids

Carotenoids were extracted from *Spirulina platensis* by adopting a method described by Reboul et al. [25]. In brief, 0.5 g of microalgae powder was added to 10 ml of methanol containing 0.57 (w/v) magnesium carbonate. Then, the samples were homogenated for 30 seconds using a vortex. Next, 10 ml of trichloromethane, which contained 0.005 (w/v) butylated hydroxyl toluene, was added. The samples were then homogenised for a further 30s in the vortex blender. Ten millilitres of distilled water was added after 15 minutes. The samples were centrifuged (2000 ×g for 10 minutes) by using Hettich Universal 32R centrifuge (Hettich, Tuttlingen, Germany). The lower phases of the samples were collected, and the upper phases were repeatedly extracted over three rounds. The extracts of the lower layer were then pooled, evaporated to dryness, and re-dissolved in 8 ml of acetonitrile/dichloromethane (50:50; v/v). Then, the extract was filtered with Whatman polytetrafluoroethylene (PTFE) 0.22 μm syringe filter, and the filtrate was injected into a HPLC valve with 1 ml syringe. To obtain dried extracts, the dried microalgae extracts after evaporation were dissolved in 5 ml trichloromethane and flushed with nitrogen in a dark room until dried [13].

2.4 HPLC

A Waters` HPLC equipped with a Model 440 fixed-wavelength detector fitted with a 436-nm wavelength kit at an attenuation of 0.02 absorbance units full scale (AUFS), a Waters` Guard-Pak pre-column, and a stainless steel (30 cm×3.9 mm 1.0. 10-llm IlBonda-pak CUl column) were used for the chromatographic separation. Sample injection volumes (50 to 100 μL(were dispensed with a Rheodyne 7125 injector. A Water`s 6000A solvent delivery system was used to deliver the mobile phase (acetonitrile-methanol-ethyl acetate, 88:10:2, v Iv) at the rate of 2.0 ml/min. Peak areas were quantitated with a Water`s 730 Data Module [26]. Hexane in the test solution was first evaporated off on a water-bath with the aid of nitrogen gas. The residue was immediately re-dissolved in a suitable volume of the mobile phase. After passing through an OA5-l1m regenerated cellulose membrane filter, suitable volumes were chromatographed using the conditions described above. Quantitation of the carotenoids was carried out by comparing with the reference standards. Peak areas of the sampl-

es and standards used for calculation were based on the mean values obtained from at least three injections.

2.3 Statistical analysis

The experiments were done in triplicate. Data are expressed as the means±SD. Results were analyzed by one-way ANOVA and Student's t-test at significance level of 5%. All analyses performed using spss software version 16.

3. Results and Discussion

The amount of chlorophyll a, astaxanthin, beta carotene, lycopene, zeaxanthin and lutein in spirulina was 4.3±0.14, 0.21±0.02, 7393±2.76, 741±2.32, 6652±3.69, 424±2.83 µg/ml, respectively.

Fig. 1 shows the separation of 4 standards in chromatogram and fig. 2 presents the chromatogram of extracted spirulina solvent in methanol. Maximum absorption of UV-Vis in carotenoids in dynamic phase (nm) and concentration of carotenoids and Equation adjustment in spirulina are shown in Tables 1 and 2, respectively.

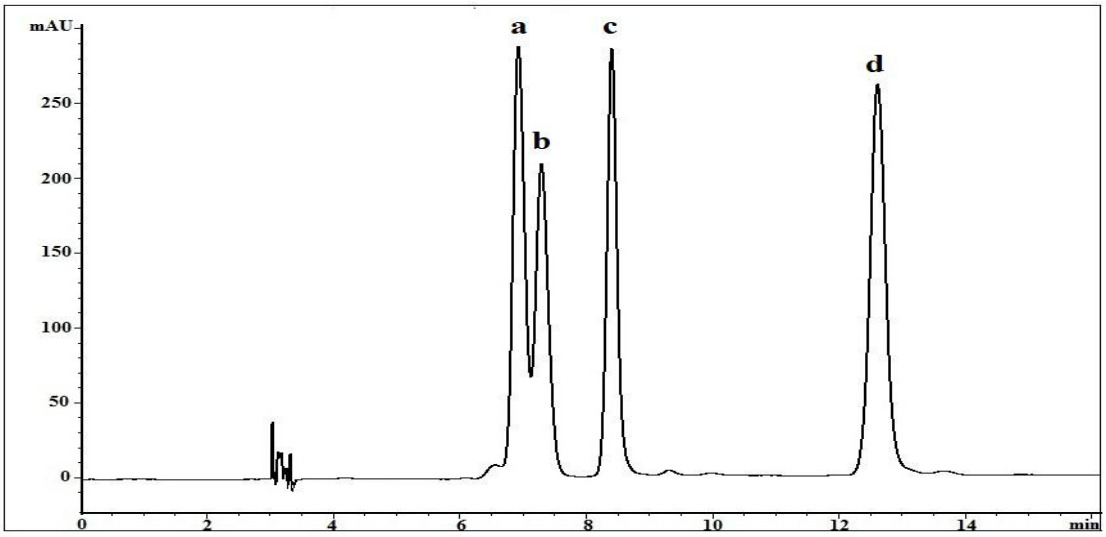

Figure 1. Chromatogram of a) lutein b) zeaxanthin c) lycopene and d) beta carotene

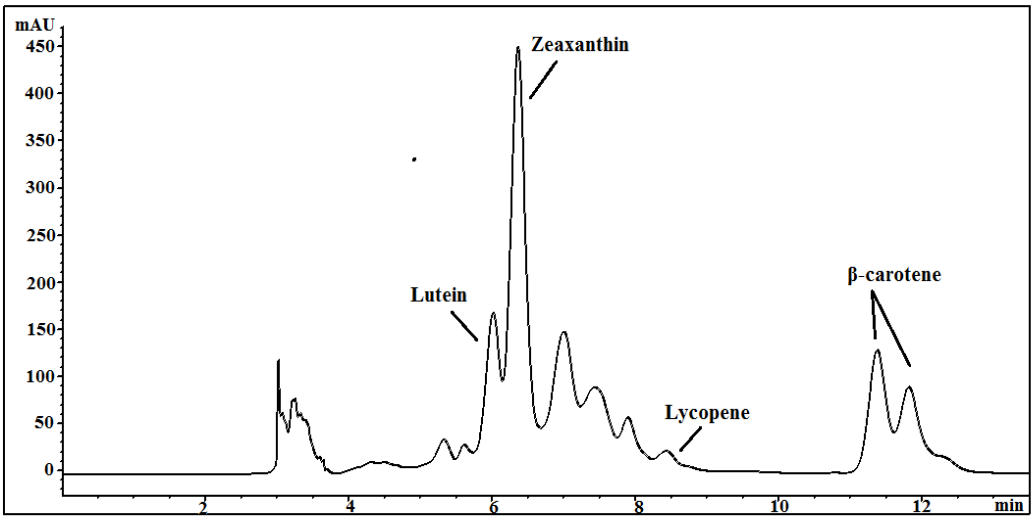

Figure 2. Chromatogram of the sample extracted by methanol

Table 1. Maximum absorption of UV-Vis in carotenoids in dynamic phase (nm) in spirulina powder

Carotenoid	λ_{max1}(nm)	λ_{max2}(nm)	λ_{max3}(nm)
Beta carotene	425	455	484
Lycopene	448	474	505
Lutein	422	448	475
Zeaxanthin	425	454	480

Table 2. Concentration of carotenoids and Equation adjustment in spirulina powder

Carotenoid	Amount of pigment (μg/g Dry weight)	Equation adjustment	R^2	RSD (%)	Recovery (%)
Beta carotene	7393±2.76*	y = 2103.2x + 68.4	0.997	0.45	90
Lycopene	741±2.32	y = 1077.x +78.09	0.998	0.58	92
Lutein	424±3.69	y = 13110x + 255.1	0.999	1.5	93
Zeaxanthin	6652±2.83	y = 4945.9x + 151	0.994	0.72	93

*Mean±CL (confidence limit 95%)

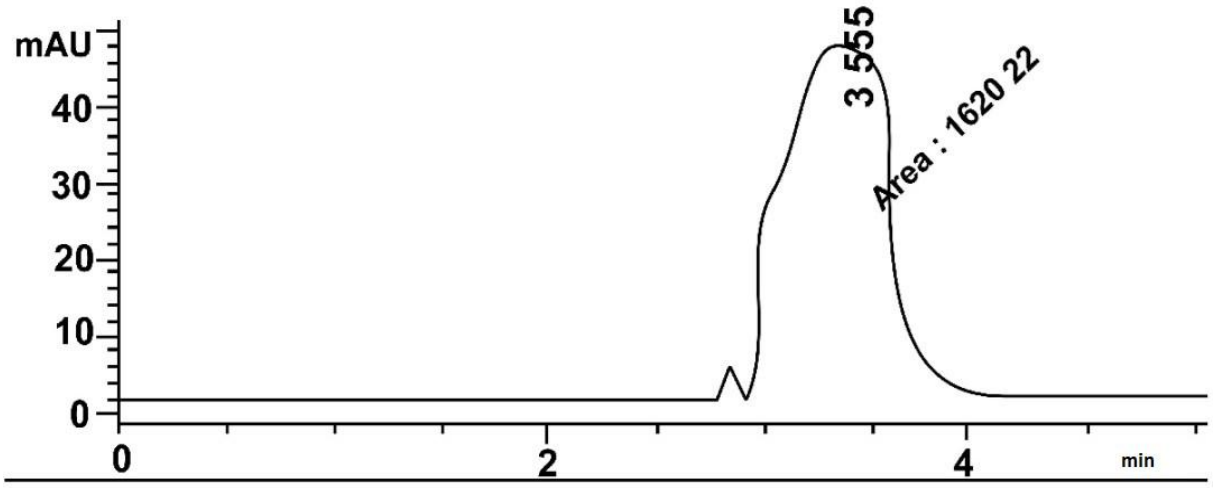

Figure 3. Chromatogram of astaxanthin in spirulina powder

The carotenoid content in the extract strongly depends on sample preparation and extraction conditions, and the solvent properties markedly favor the extraction of carotenoids [27]. Spirulina organic extracts showed that carotenoids and derived chlorophyll were present as the main constituents, and their quantity was changed significantly based on the culture conditions [19].

In this research, the amount of spirulina chlorophyll a was 4.3 μg/ml, but it was 6.6-9.2 g/kg in Sethu`s study [28], and another study has reported it as 1.35 (% DW) The chlorophyll productivity depended on temperature variable in an analog manner to what occurred for the total chlorophyll [29]. Beta carotene account for 80% of the carotenoids present in spirulina after that zeaxanthin was most. At last, Spirulina was a

good source for carotenoids as a pro-vitamin A in organisms.

4. Conclusion

Scientists in India, China, Japan, USA and other countries are working on this remarkable food to unlock its potential. However, it is already clear that this safe and natural food provides concentrated nutritional support for optimum health and wellness. The multifunctional role of spirulina species makes it an ideal natural drug with immense prophylactic and therapeutic properties variable.

Carotenoids are unique constituents of a healthy diet and play an important role in the network of antioxidant vitamins and phytochemicals. In some studies, the concentration of beta carotene, astaxanthin, luteine, zeaanthin and cryptoxanthin in *Spirulina platensis* was reported as 39.12, 5.61, 0.30, 1.56 and 1.69 µg/g, respectively; however, in our study, it was more than this. Astaxanthin has been determined 0.21 (%DW) in India for *Spirulina platensis.*This result is in agreement with our study. In previous researches it was achieved 6 and 7.9 g/kg for carotenoid and chlorophyll, respectively in spirulina, but in our study, it was less than this.

Acknowledgments

The authors are very thankful from Mrs. Hashtroodi for preparation of standards, and HPLC analysis and Mrs. Ghaderi for spirulina culture.

References

1- Beheshtipour H, Haratian P, Mortazavian AM, Khosravi-Darani K. Effects of *Chlorella vulgaris* and *Arthrospira platensis* addition on viability of probiotic bacteria in yogurt and its biochemical properties. Eur Food Res Technol. 2012; 235: 719–728.

2- Hoseini SM, Khosravi-Darani K, Mozafari MR. Nutritional and medical applications of spirulina microalgae. Mini Rev Med Chem. 2013; 13: 1231-1237.

3- Hosseini SM, Shahbazizadeh S, Khosravi-Darani K, Mozafari MR. *Spirulina paltensis*: Food and function. Curr Nutr Food Sci. 2013; 9: 189-193.

4- Fadaei V, Eslami-Moshkenani F, Kosravi-Darani K. Effect of *spirulina platensis* powder on viability of *lactococcus* strains in probiotic yoghurt. Int J Biotechnol. 2013; 10: 511-513.

5- Fadaei V, Mazinani S, Khosravi-Darani K. Influence of *Spirulina platensis* powder on viability of lactococcus strains in probiotic of feta cheese containing *mentha longifolial*. Int Jou Bio and Biotech. 2013; 10 (3): 475-478.

6- Fadaei V, Mohamadi-Alasti F, Khosravi-Darani K. Influence of *Spirulina platensis* powder on the starter culture viability in probiotic yoghurt containing spinach during cold storage. Eur. J. Exp. Bio. 2013; 3: 389-393.

7- Soehili M, Mazinani S, Khosravi-Darani K. The potential health benefits of algae and micro algae in medicine: A Review on *Spirulina Platensis*. Curr Nutr Food Sci. 2011; 7: 279-285.

8- Stahl W, Sies H. Bioactivity and protective effects of natural carotenoids. Biochim Biophys Acta. 2005; 101– 107.

9- Mortensen A. Carotenoids and other pigments as natural colorants. Pure Appl Chem. 2006; 78: 1477–1491.

10- Spolaore P, Joannis-Cassan C, Duran E. Isambert A. Commercial applications of microalgae. J Biosci Bioeng. 2006; 101: 87–96.

11- Moorhead K, Capelli B. Nature's Superfood. Aerial view of microalgae farm of Cyanotech Corporation, Kailua-Kona, Hawaii, 2011.

12- Goh LP, Loh SP, Fatimah MY, Perumal K. Bioaccessibility of carotenoids and tocopherols in marine microalgae, *Nannochloropsis sp*. and *Chaetoceros sp.* Malays J Nutr. 2009; 15: 77-86.

13- Gaurav D, Preet S, Dua K. Antioxidative and antiperoxidative effects of *Spirulina platensis* against cadmium induced hepatotoxicity in rats. Ann Biolog Res. 2012; 2: 121-127.

14- Bogahawatte C. Production and application of *Spirulina platensis* rich in fatty acids, and vitamins. Int J Agr Env. 2013;1: 39-52.

15- Khan Z, Bhadouria P, Bisen PS. Nutritional and therapeutic potential of spirulina, Curr Pharm Biotechnol. 2005; 6: 373-379.

16- Hifney A, Issa AA, Fawzy MA. A biotic Stress induced production of beta carotene, allophycocyanin and total lipids in *Spirulina sp*. J Biol Earth Sci. 2013, 3: 54–64.

17- Mala R, Karthik V, Sakthiselvan S, Saravanababu S. Milking of *Spirulina platensis* for the production of carotenoids by aqueous two phase bioreactor, Int J Chem. Sci. 2010; 8: 584-591.

18- Mendiola JA, Señoráns FJ, Ibañez E, Jaime L, Reglero G. Supercritical fluid of microalgae *Spirulina platensis* chemo-

functional characterization. MSc. Degree Thesis in Madrid University,pp. 98, 2008.
19- El-Baky HHA, El Baz FK, El-Baroty GS. Characterization of nutraceutical compounds in blue green alga Spirulina maxima. J Med Plants Res. 2008; 2: 292-300.
20- Rafiqul IM, Hassan A, Sulebele G, Orosco C, Roustaian P. Influence of temperature on growth and biochemical composition of *Spirulina platensis* and *S. fusiformis*. Iran Int J Sci. 2003; 4: 97-106.
21- Gaese H. Chemical composition and potential application of *Spirulina platensis* biomass. Int J Agr Envir. 2012; 4:35-40.
22- Jourdan P. Manual of small scale spirulina culture, Antenna Technologies, 2001.
23- Chauhan UK, Pathak N. Effect of different conditions on the production of chlorophyll by *Spirulina platensis*. J Alg Biom Utln. 2010; 1: 89–99.
24- Mackinney G. Absorption of light by chlorophyll solution. J Biol Chem. 1941; 140: 466-469.
25- Reboul E, Richelle M, Perrot E, Desmoulins-Malezet C, Pirisi V, Borel P. , Bioaccessibility of carotenoids and vitamin E from their main dietary sources,J Agric Food Chem. 2006; 15;54(23):8749-55.
26- Valderrama JO, Perrut M, Majewski W. Extraction of astaxantine and phycocyanine from microalgae with supercritical carbon dioxide. J Chem Eng Data. 2003; 48: 827-830.
27- Sethu P. Studies on important phytochemicals and genetic transformation of the *Cyanobacterium spirulina*, Thesis of Mysore University, India. 2007.
28- Kumar M, Kulshreshtha J, Singh GP. Growth and Pigment Profile of *Spirulina platensis* isolated from Rajasthan India. Res J Agr Sci. 2011; 2: 83-86.
29- El-Baky HHA, El Baz F, El-Baroty GS. Enhancement of antioxidant production in Spirulina plantensis under oxidative stress. Am-Eurasian J Sci Res. 2007; 2: 170-179.