Chemistry of Natural Compounds, Vol. 48, No. 1, March, 2012 [Russian original No. 1, January-February, 2012]

CAROTINOID COMPOSITIONS OF FIVE MICROALGA SPECIES

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UDC 547.912+577.115+582.263

Microalgae are promising subjects for creating medicinal, therapeutic, and preventative preparations and biologically active food additives and for producing biofuels and other biotechnology products [1, 2]. Several microalgae are used to produce carotinoids, in particular, *Dunaliella salina* to produce *β*-carotene and *Haematococcus pluvialis*, astaxanthin [2, 3].

The subjects of our study were five species of green alga (Division Chlorophyta) that were promising sources for producing biodiesel fuel and polyunsaturated fatty acids. Herein we present data on the carotinoid compositions of these microalgae grown under the conditions used by us earlier to obtain their oils.

Green algae *Chlorella vulgaris* KCTC AG10032 and *Scenedesmus quadricauda* KCTC AG10308 were obtained from the Biological Resource Center (BRC, Korea); *Ankistrodesmus gracilis* SAG 278-2, from the German Culture Collection of Algae (SAG, Gottigen); *C. protothecoides* UTEX 250 and *Neochloris oleoabundans* UTEX 1185, from the Culture Collection of Algae at the University of Texas (UTEX). Standard lutein and *β*-carotene were purchased from Sigma; astaxanthin, violoxanthin, zeaxanthin, canthaxanthin, neoxanthin, and echinenone, from DHI LAB (Denmark).

The first three of these five strains were grown in BG-11 medium [14] for two weeks in a 5-L photobioreactor with constant illumination by luminescent daylight lamps of intensity 120 $\mu E \cdot m^{-2} \cdot s^{-1}$. The other two strains were cultivated in SGM medium [5] for seven days under the same lighting conditions. Cultures were continuously purged with air and held at 25°C. Microalga cells were settled by centrifugation, rinsed with distilled H_2O, lyophilized, and thoroughly ground with a pestle in an agate mortar. Pigments were isolated using ultrasonic extraction by CH_2Cl_2:MeOH (1:3, v/v) for 30 min at 20°C in a VCX 500 apparatus (Sonic & Materials, Newtown, USA). The contents of individual carotinoids were analyzed by HPLC over a Supelco Discovery C18 reversed-phase column (250 × 4.6 mm, 5 μm) using mobile phases a) MeOH:MeCN:H_2O (78:10:12, v/v) and b) CH_2Cl_2:MeOH:MeCN:H_2O (22.0:28.0:45.5:4.5, v/v) at flow rate 1 mL/min. Gradient elution was carried out as follows: 100% a (10 min), from 100% to 10% a (25 min), 10% a (5 min), from 10% to 0% a (5 min), 0% a (5 min), from 0% to 100% a (5 min), 100% a (10 min). Detection was made at wavelengths 455 and 665 nm using a UV730D detector (Young Lin Instrument, Korea). Pigments were identified using retention times, absorption spectra in the visible region, and comparison of them with standard samples. The concentrations of individual pigments were determined from calibration curves that were constructed for each of the standard carotinoids under the HPLC conditions described above.

Table 1 shows that the principal carotinoid detected in all strains was lutein. This was characteristic of many green algae [3]. Three other pigments, namely *β*-carotene, neoxanthin, and violoxanthin, were also present in all studied microalga species but in smaller quantities. The comparatively high content of the aforementioned carotinoids in *N. oleoabundans* was interesting. Astaxanthin and echinenone were detected only in *A. gracilis* although in smaller quantities than the other carotinoids. Hydrolysis of the total extracts by the literature method [6] increased the amount of astaxanthin by 10.5% only for *A. gracilis*, indicating that small quantities of esters of this carotinoid were present in the total extract. Thus, *N. oleoabundans* had the highest carotinoid content under the used cultivation conditions; *A. gracilis*, the highest qualitative variety of them.

The supplemental production of valuable side products such as carotinoids is considered one pathway for reducing the cost of biofuel from algae [7]. Therefore, the results for the carotinoid compositions of these microalgae under the cultivation conditions used to isolate their oils may be useful for estimating their biotechnological potential as sources of biodiesel fuel.

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0009-3130/12/4801-0141 ©2012 Springer Science+Business Media, Inc.

TABLE 1. Carotinoid Content in Microalgae

Carotinoids	Ret. time, min	Carotinoid content, mg/g of lyoph. biomass				
		A. gracilis	*C. protothecoides*	*C. vulgaris*	*N. oleoabandans*	*S. quadricauda*
Neoxanthin	6.8	0.68	0.53	1.26	1.25	1.65
Violoxanthin	8.0	0.54	0.48	0.50	0.63	1.02
Astaxanthin	11.8	0.24	–	–	–	–
Lutein	14.9	2.05	1.54	2.77	4.61	2.12
Echinenone	36.9	0.11	–	–	–	–
β-Carotene	48.9	0.42	0.73	0.77	1.79	1.57
Total pigments		4.04	3.28	5.30	8.28	6.36

ACKNOWLEDGMENT

The work was supported financially by the Korea Research Center for Industrial Science & Technology (Grant 10-11-149) and the Korea Brain Pool Program (2011, Grant 111S-4-5-0032), Republic of Korea.

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