Carotenoid Composition of *Spirulina maxima**¹Wataru Miki,*² Katsumi Yamaguchi,*² and Shoji Konosu*²

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Commercial *Spirulina* powder differs in the carotenoid composition from preparation to preparation. In order to elucidate the reason we analyzed carotenoids in *Spirulina maxima* spray-dried at 80°C (SmI) and 200°C (SmII). The contents of carotenoids in SmI and SmII were 648 and 6.0 mg/100 g, respectively. The main components identified in SmI were zeaxanthin (25%), myxoxanthophyll (13–17%), β -carotene (15%), and echinenone (11–13%), and a rare carotenoid, 3'-hydroxyechinenone, was also detected at 7–11%. In SmII, only two carotenoids, zeaxanthin and β -cryptoxanthin were detected as major components, and presumably decomposed products were observed in appreciable proportions. Thus the marked discrepancies in the composition of carotenoids of dried *Spirulina* preparations are most probably due to their thermolability.

Blue green algae *Spirulina* spp. are employed at large as a feed ingredient for pigmentation of goldfish and fancy red carp and were used in some research work for improvement of integumentary color of cultured fishes such as tilapia¹⁾ and sweet melt.*³ Several papers have already been published for carotenoids of the dried algae^{1–3)} but the contents reported therein varied widely from 10 to more than 400 mg/100 g with marked discrepancies in their composition. In the present study, we intended to find out reasons of these discrepancies.

Materials and Methods

Materials

Spirulina maxima spray-dried at 80°C (SmI) and at 200°C (SmII) were used as materials. Pigments were extracted with acetone/methanol (4:6), and transferred into ethylacetate by the addition of distilled water. After drying over anhydrous sodium sulfate, pigment samples were prepared by evaporation under reduced pressure.

Analyses

Total carotenoid content was determined as follows: The pigments including carotenoid glycosides and chlorophylls were subjected to silica gel column chromatography with hexane/benzene (1:9) to separate carotenes from other pigments

and the content of carotenes was measured as usual.⁶⁾ The other pigments were eluted with methanol/dichloromethane (1:4) and again combined with the carotenes. The total combined pigments were saponified by the usual procedure⁷⁾ to get rid of chlorophylls and then developed on silica gel 60 TLC plate using acetone/petroleum ether (3:17) and ethylacetate/dichloromethane (1:4). Relative percentages of each carotenoid were measured by densitometry and the total carotenoid content was calculated on the basis of the content and percentage of carotenes.

Isolation and identification of each carotenoid were conducted in the following manner. The carotenoids were separated into nonpolar and polar groups by passing silica gel column with acetone. Individual carotenoids were isolated by silica gel column chromatography with ethylacetate/dichloromethane (1:9) for the nonpolar group, and with methanol/chloroform (1:9) for the polar one. Further purification was carried out by HPLC on ERC CN using hexane/dichloromethane/ethanol (70:30:0.5) and on CQ-3 using methanol/dichloromethane (5:95) for nonpolar and polar groups, respectively. Identification of each carotenoid was achieved by means of co-TLC and co-HPLC with authentic specimens, spectroscopic and instrumental analyses, and chemical treatments as usual.⁸⁾

Visible absorption and infrared (IR) spectra

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were taken on a Hitachi 330 recording spectrophotometer and a Jasco IR-S infrared spectrophotometer, respectively. Mass spectra (MS) were run on a Hitachi RMU-6L mass spectrometer, and ^1H and ^{13}C NMR spectra were measured with a Nihon Denshi FX-100 FT-NMR spectrometer.

Results and Discussion

Fig. 1 shows the separation on TLC of the nonpolar and the polar carotenoid groups in SmI, and the content and composition of carotenoids in SmI and SmII are shown in Table 1. The content in SmI was more than one hundred times as much as that in SmII, suggesting that the high

Fig. 1. Thin layer chromatograms of nonpolar (A) and polar (B) carotenoids of *Spirulina maxima* spray-dried at 80°C , on silica gel 60 using ethyl-acetate/dichloromethane (1:4) and methanol/dichloromethane (1:9) in A and B, respectively.

Table 1. Carotenoid content and composition in *Spirulina maxima*

	Sm I	Sm II
Content (mg/100 g)	648	6.0
Composition (%)		
β -Carotene	15	—
β -Carotene-5,6-epoxide	+	—
Echinone	11–13	—
β -Cryptoxanthin	6–8	22–23
3'-Hydroxyechinenone	7–11	—
Zeaxanthin	25	35
Diatoxanthin	5	—
Cynthiaxanthin	5	—
Myxoxanthophyll	13–17	+
Oscillaxanthin	3–5	+
Total unidentified	3–4	38–41

+, trace; —, not detected.

temperature treatment during the drying process in SmII should have decomposed most of the carotenoids.

At least 10 and 4 kinds of carotenoids were found in SmI and SmII, respectively, and the following carotenoids were isolated and identified. The numbers after the name of carotenoids are the spot numbers on TLC in Fig. 1.

β -Carotene (1). Detected in appreciable amounts in only SmI, and identified by direct comparison with authentic specimen obtained from the integument of rainbow trout *Salmo gairdneri*.⁷⁾

β -Carotene-5,6-epoxide (2). Detected as a trace component in only SmI, and identified through epoxide test,⁸⁾ and by measuring the visible absorption spectrum.

Echinone (3). Detected in only SmI at more than 10%, and identified by direct comparison with authentic carotenoid obtained from the ovary of sea urchin *Strongylocentrotus nudus*.¹⁰⁾

β -Cryptoxanthin (4). Detected as a major component in SmII and as a minor one in SmI. Identification was carried out by direct comparison with the authentic specimen obtained from mandarin orange.¹¹⁾

3'-Hydroxyechinenone (5). Detected as one of the major components in only SmI. It showed a single absorption maximum at 469 nm in benzene, and peaks at 462 and 491 nm in the same solvent after NaBH_4 reduction, indicating to be β -carotene type. On reduction with NaBH_4 it migrated between isozeaxanthin and zeaxanthin on silica gel TLC. The carotenoid was proved to have one hydroxy group at C-3 and one oxo group at the allylic position by acetylation⁹⁾ and allylic OH test⁹⁾ of the intact carotenoid and its NaBH_4 reduction product. MS of this carotenoid showed a peak at m/z 566 indicating the chemical formula to be $\text{C}_{40}\text{H}_{54}\text{O}_2$, and the ^1H NMR spectrum gave the signal at δ 4.0 (m, 1H) showing cyclohexene linked to hydroxy group at C-3 without oxo group at C-4. From these data, it was identified to be 3'-hydroxyechinenone which was formerly isolated from some species of blue green algae.^{12,13)}

Zeaxanthin (6). Detected as a major carotenoid in both SmI and SmII. It was identified by direct comparison with authentic carotenoid obtained from the ovary of yellowtail *Seriola quinqueradiata*.⁸⁾

Diatoxanthin (7). Detected as a minor component in only SmI, and identified by direct comparison with authentic carotenoid obtained from

the integument of rainbow trout.⁷⁾

Cynthiaxanthin (8). Detected as a minor carotenoid in only SmI. Identification was carried out by direct comparison with authentic specimen obtained from the mantle of sea squirt *Halocynthia roretzi*.¹⁴⁾

Myxoxanthophyll (1'). Detected as a major component in SmI and as a trace one in SmII. It gave positive phenol/sulfuric acid reaction, and showed absorption maxima at 476 and 507 nm in acetone. IR spectrum showed absorption at 3300 (—OH), 2800 (C=C), around 1600 (ring), 1140 and around 910 cm⁻¹ (tertiary —OH). By LiAlH₄ treatment in tetrahydrofuran, it gave an aglycon and a nonpolar derivative. From these data, it was identified to be myxoxanthophyll,¹⁵⁾ and this was supported by the data of ¹H and ¹³C NMR spectra.

Oscillaxanthin (2'). Detected as a minor carotenoid in SmI and as a trace one in SmII. It also gave positive reaction in phenol/sulfuric acid test, and showed absorption maxima at 469, 497 and 531 nm in acetone. By LiAlH₄ treatment in tetrahydrofuran, it gave an aglycon and two nonpolar derivatives. After hydrolysis with 6 N HCl at 110°C, it released rhamnose. From these data, it was identified to be oscillaxanthin,¹⁶⁾ and this identification was further confirmed by the data of ¹H and ¹³C NMR spectra.

In addition to the carotenoids described above, small amounts of unidentified carotenoids were present in SmI. In SmII, however, unidentified carotenoids amounted to approximately 40% and most of them were supposed to be decomposed products formed during the drying process and the subsequent storage. In this connection, we also analyzed *S. maxima* which was spray-dried at 170°C for 5 seconds and found that the content of carotenoids was about 400 mg/100 g and that their composition was almost the same as in SmI but with much more unidentified components. Thus, we conclude that the marked discrepancies in the content and composition of carotenoids of *Spirulina* spp. reported so far are largely due to their thermolability. On account of such great

differences in the carotenoid composition of dried *Spirulina* spp., care should be taken in their utilization for the pigmentation of fishes.

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