Carotenoids of Red, Brown, and Black Specimens of *Plectropomus leopardus*, the Coral Trout (*Suziara* in Japanese)

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**Abstract:** This study investigated the carotenoids occurring in the integument of *Plectropomus leopardus*, the coral trout. For a red specimen, the major carotenoids included astaxanthin diester and monoester, as well as α-cryptoxanthin ester, tunaxanthin diester, adonixanthin diester, adonirubin ester, and adonirubin; for brown and black specimens, tunaxanthin diester was the main carotenoid. ¹H-NMR and MS spectral analyses showed that docosahexaenoic acid was the sole fatty acid esterified with xanthophylls in the coral trout.

**Key words:** coral trout, *Plectropomus leopardus*, carotenoid ester, docosahexaenoic acid ester, carotenoid structure

**1 INTRODUCTION**

*Plectropomus leopardus*, or the coral trout (Japanese name: *Suziara*), belongs to the family Serranidae (Order Perciformes). Inhabiting the fringes of coral reefs in the South and East China Sea and Western Pacific Ocean, the coral trout is a carnivore that feeds on small fish belonging to Pomacentridae and Labridae, as well as squid and crustaceans. In Okinawa, Japan and Taiwan, Republic of China, the coral trout is considered a delicacy, consumed as sashimi, broiled with salt, and as fried fish. Its integument is olive brown, reddish brown, or bright red. The color is an important factor in determining the quality of the fish, with markets in Okinawa and Taiwan preferring a bright red or orange color (Fig. 1). Given the relative paucity of reports discussing the issue, we investigated the carotenoids that occur in the coral trout¹–³.

**2 EXPERIMENTAL**

2.1 Fish Samples

Red-colored coral trout (two specimens) were purchased at the Taipei Fishery Marketing Corporation (Taipei City, Taiwan, Republic of China) in April 2014. Black and brown-colored fish were also purchased at a local fish market in Ishigaki City, Okinawa Prefecture, Japan, in August 2014.

2.2 Carotenoid Extraction, Isolation, and Identification

We removed the integument from the body and used acetone to extract pigments. We then partitioned the acetone extract between *n*-hexane:Et₂O (1:1, v/v) and water and evaporated the organic layer to dryness. We dissolved the residue in acetone:*n*-hexane (2:8, v/v) and subjected it to HPLC on silica gel with acetone:*n*-hexane (2:8, v/v). Individual carotenoids were identified using our standard method⁴. UV-Visible spectra were recorded with a Hitachi U-2001 spectrophotometer in Et₂O. We performed LC/MS analysis of carotenoids⁵ using a Waters Xevo G2S Q Tof mass spectrometer (Waters Corporation, Milford, CT, USA) equipped with an Acuity UPLC system. We acquired electro-spray ionization (ESI) time-of-flight (TOF) MS spectra by scanning from *m/z* 100 to 1,500 with a capillary voltage of 3.2 kV, cone voltage of 40 eV, and source temperature of 120°C. Nitrogen was used as a nebulizing gas at a flow rate of 30 L/h. We measured MS/MS spectra using a Q-TOF MS/MS instrument with argon as a collision gas at a collision energy of 30 V and used a photodiode array detector (PDA) to record UV-Visible (UV-VIS) absorption spectra from 200 to 600 nm. We used an Acuity 1.7 μm BEH UPLC C18 column (Waters Corporation) as a stationary phase and MeOH as a mobile phase at a flow rate of 0.4
580 mL/min for the HPLC system. We measured the $^1$H-NMR (500 MHz) spectrum with a Varian Unity Inova 500 spectrometer in CDCl$_3$ with TMS as an internal standard. The CD spectrum was recorded in Et$_2$O at room temperature with a Jasco J-500C spectropolarimeter. Preparative HPLC was performed with a Hitachi L-6000 HPLC intelligent pump and Hitachi L-4250 UV-VIS detector set at 450 nm. The column used was a 250 × 4.6 mm i.d., 5 μm Cosmosil 5SL-II (Nacalai Tesque, Japan) with acetone:n-hexane (2:8, v/v) as a solvent at a flow rate of 1.0 mL/min. We identified the presence of optical isomers of astaxanthin and adonirubin using the Sumichiral OA-2000 chiral column with n-hexane:CHCl$_3$:EtOH (48:16:1, v/v) at a flow rate of 1.0 mL/min and detection at 470 nm$^{	ext{1}}$. Esterified adonirubin and astaxanthin underwent hydrolysis with lipase to form free adonirubin and astaxanthin, which were then submitted for chiral HPLC$^{	ext{6}}$.

2.3 Identification of Individual Carotenoids

**Figure 2** shows the HPLC of the carotenoids in the integument of the red specimen of coral trout. The identification of individual carotenoids was as follows.

**α-Cryptoxanthin** ester: UV-VIS: 425, 444, 474 nm. ESI TOF MS: $m/z$ 863.6681 (M + H$^+$), calcd. for C$_{62}$H$_{87}$O$_2$; 885.6540 (M + Na$^+$), calcd. for C$_{62}$H$_{86}$O$_2$Na. Saponification of this ester with 5% KOH/MeOH provided α-cryptoxanthin, identified by LC/MS with comparison of an authentic sample$^6$.

**Tunaxanthin diester**: UV-VIS: 415, 439, 468 nm. ESI TOF MS: $m/z$ 1189.8915 (M + H$^+$), calcd. for C$_{84}$H$_{117}$O$_4$; 1211.8790 (M + Na$^+$), calcd. for C$_{84}$H$_{116}$O$_4$Na. Saponification of this ester with 5% KOH/MeOH provided tunaxanthin as identified by LC/MS. The tunaxanthin consisted of tunaxanthin A$^3$S,6$^5$S,3$^6$S, and tunaxanthin B$^3$R,6$^5$S,3$^6$S-tunaxanthin, and tunaxanthin C$^3R,6S,3'R,6'S$-tunaxanthin in the ratio (1:4:5). Lutein was identified as a minor component of this fraction.

**Adonirubin** ester: UV-VIS: 470 nm. ESI TOF MS: $m/z$ 891.6306 (M + H$^+$), calcd. for C$_{62}$H$_{83}$O$_4$; 913.6123 (M + Na$^+$), calcd. for C$_{62}$H$_{82}$O$_4$Na. $^1$H-NMR δ (CDCl$_3$): 0.98 (t, J = 7.5 Hz, CH$_3$ in fatty acid

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Fig. 1 Wild coral trout *Plectropomus leopardus*: upper: red specimen; middle: brown specimen; lower: black specimen.

Fig. 2 HPLC of carotenoids in the integument of a red coral trout specimen.

1: α-Cryptoxanthin ester, 2: Tunaxanthin diester, 3: Adonixanthin diester, 4: Astaxanthin diester, 5: Adonirubin ester, 6: Astaxanthin monoester, 7: Adonirubin, 8: Astaxanthin HPLC Cosmosil 5SL-II with acetone:n-hexane (2:8, v/v) as solvent at a flow rate of 1.0 mL/min, detection at 450 nm.
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Adonixanthen diester: UV-VIS: 460 nm. ESI TOF MS: m/z 1203.8766 (M+H⁺, calcd for C₆₄H₁₁₀O₆Na), m/z 1225.8559 (M+Na⁺, calcd for C₆₄H₁₁₀NaO₆, 1225.8564). 

'H-NMR: δ (CDCl₃) 0.98 (t, J = 7.5 Hz, CH₃ in fatty acid moiety), 1.08 (3H, s H-17), 1.11 (3H, s H-16'), 1.23 (3H, s, H-17), 1.34 (3H, s H-16), 1.58 (1H, dd, J = 12, 12 Hz, H-2' ax), 1.72 (3H, s, H-19'), 1.91 (3H, s, H-18), 1.97 (3H, s, H-19), 1.99 (3H, s, H-19'), 2.00 (6H, s, H-20, 20'), 2.01 (1H, overlapped, H-2eq), 2.07 (1H, dd, J = 12, 6 Hz, H-2ax), 2.08 (m, CH₃ in fatty acid moiety), 2.45 (1H, ddd, J = 17, 6, 1.5 Hz, H-4 eq), 2.40∼2.50 and 2.80∼2.85 (m, CH₃ in fatty acid moiety), 5.06 (1H, m, H-3'), 5.37 (about 12H, m, -CH=CH- in fatty acid moiety), 5.54 (1H, dd, J = 13.5, 5.5 Hz, H-3), 6.10 (1H, d, J = 16 Hz, H-7'), 6.13 (1H, d, J = 16 Hz, H-8'), 6.16 (1H, d, J = 11.5 Hz, H-10'), 6.20 (1H, d, J = 16 Hz, H-7), 6.25 (1H, d, J = 11 Hz, H-14'), 6.31 (1H, d, J = 11.5 Hz, H-10), 6.33 (1H, d, J = 11 Hz, H-14), 6.36 (1H, d, J = 15.5 Hz, H-12'), 6.40 (1H, d, J = 16 Hz, H-8), 6.45 (1H, d, J = 15.5 Hz, H-12), 6.65 (2H, m, H-15, 15'), 6.65 (1H, dd, J = 15.5, 11.5 Hz, H-11), 6.66 (1H, dd, J = 15.5, 11.5 Hz, H-11'), 6.70 (2H, m, H-15, 15'). HPLC analysis with a chiral column showed the astaxanthin consisted of 3R,3'S and, 3S,3'S optical isomers in the ratio (13:7:80).

Astaxanthin monoester: UV-VIS: 470 nm. ESI TOF MS: m/z 907.6254 (M+H⁺, calcd for C₆₃H₁₀₁O₅Na, 907.6241), m/z 929.6052 (M+Na⁺, calcd for C₆₃H₁₀₁NaO₅, 929.6060).

'H-NMR: δ (CDCl₃) 0.98 (t, J = 7.5 Hz, CH₃ in fatty acid moiety), 1.21 (3H, s, H-17'), 1.23 (3H, s, H-17), 1.32 (3H, s, H-16'), 1.34 (3H, s H-16), 1.82 (1H, dd, J = 13, 13 Hz, H-2 ax), 1.91 (3H, s, H-18), 1.95 (3H, s, H-18'), 1.99 (6H, s, H-19, 19'), 2.00 (6H, m, H-20, 20'), 2.01 (1H, overlapped, H-2eq), 2.07 (1H, dd, J = 12, 6 Hz, H-2ax), 2.08 (m, CH₃ in fatty acid moiety), 2.16 (1H, dd, J = 13, 5.5 Hz, H-2 eq), 2.40∼2.50 and 2.80∼2.85 (m, CH₃ in fatty acid moiety), 3.69 (1H, d, J = 15 Hz, OH-3'), 4.33 (1H, ddd, J = 13.5, 5.5, 1.5 Hz, H-3'), 5.37 (m, -CH=CH- in fatty acid moiety), 5.54 (1H, dd, J = 13.5, 5.5 Hz, H-3), 6.15 (1H, s, meso), 6.45 (2H, d, J = 15.5 Hz, H-12, 12'), 6.66 (2H, dd, J = 15.5, 11.5 Hz, H-11, 11'), 6.70 (2H, m, H-15, 15'). HPLC analysis with a chiral column showed the astaxanthin consisted of 3R,3'R, meso, and 3S,3'S optical isomers in the ratio (13:7:80).

Adonirubin: UV-VIS: 470 nm (Et₂O). ESI TOF MS: m/z 581.3980 (M+H⁺, calcd for C₆₃H₁₀₁O₅Na, 581.3995), m/z 603.3799 (M+Na⁺, calcd for C₆₃H₁₀₁NaO₅, 603.3814). HPLC analysis with a chiral column showed the adonirubin consisted of 3'R and 3'S optical isomers in the ratio (14:86).

Astaxanthin: UV-VIS: 470 nm. ESI TOF MS: m/z 597.3940 (M+H⁺, calcd for C₆₃H₁₀₁O₅Na, 597.3944), m/z 619.3730 (M+Na⁺, calcd for C₆₃H₁₀₁NaO₅, 619.3763). HPLC analysis with a chiral column showed the astaxanthin consisted of 3R,3'R, meso, and 3S,3'S optical isomers in the ratio (13:7:80).

Tunaxanthin diester was identified as a major carotenoid in brown and black specimens of coral trout. Carotenoids identified in these specimens are shown in Table 1.

2.4 Quantification of Carotenoids

The total carotenoid content in the acetone extract of the integuments of the red specimen of coral trout was calculated employing an extinction coefficient of E₅₂₀=2100⁴ at λ max. Similarly, those of the brown and black specimens were calculated employing an extinction coefficient of E₅₂₀=2500⁴ at λ max. Carotenoid compositions were estimated by the peak area of the HPLC on silica gel with acetone–hexane (2:8) monitored at 450 nm.

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3 RESULTS AND DISCUSSION

The overall carotenoid content in the integument of the red-colored coral trout was 0.18~0.23 mg/g (Integument).

Figure 2 shows the HPLC of the carotenoids found in the integument of the red-colored coral trout. Eight peaks are observed, and identified as: \( \alpha \)-cryptoxanthin ester (peak 1), tunaxanthin diester (peak 2), adonixanthin diester (peak 3), astaxanthin diester (peak 4), adonirubin monoester (peak 5), astaxanthin monoester (peak 6), adonirubin (peak 7), and astaxanthin (peak 8). Table 1 shows the total carotenoid content and percentage compositions of individual carotenoids estimated by the peak area of the HPLC monitored at 450 nm in the integument of these coral trout. Figure 3 illustrates the structures of the carotenoids identified.

As in other red marine fish\(^7,8\), we found astaxanthin to be a major component (84.3% of total carotenoids, the sum of diester, monoester, and free forms) along with yellow xanthophylls such as tunaxanthin (7.3%). Adonirubin (3.7% sum of ester and free forms) was also present. This carotenoid composition resembles that of other red marine Perciformes, such as the red seabream. As in other marine fish, astaxanthin and adonirubin were also present as their optical isomers\(^7,9\).

Reversed phase LC/MS analysis of the astaxanthin diester of the red coral trout showed one peak indicating the presence of one kind of astaxanthin fatty acid diester. Figures 3 and 4 show the ESI TOF MS and \(^1\)H-NMR spectrum of the astaxanthin diester. ESI TOF MS indicated molecular-weight related ions at \( m/z \) 1217.8562 (M + H\(^+\)) calcd. for C\(_{84}\)H\(_{113}\)O\(_6\), 1217.8537, \( m/z \) 1239.8386 (M + Na\(^+\)) calcd. for C\(_{84}\)H\(_{112}\)O\(_6\)Na, 1239.8357. This indicates that the astaxanthin diester consists of only docosahexaenoic acid. Furthermore, \(^1\)H-NMR signals of the fatty acid part of this

### Table 1 Carotenoid content and percentage composition in the integument of the coral trout.

| Body color | Red | Brown | Black |
|------------|-----|-------|-------|
| Total Carotenoid mg/g | 0.18~0.23 | 0.01 | 0.02 |
| Percentage Composition | % | % | % |
| \( \alpha \)-Cryptoxanthin ester | 0.2 | | |
| Tunaxanthin diester | 7.3 | 72.3 | 99.2 |
| Lutein diester | 0.8 | | |
| Adonixanthin diester | 3.3 | | |
| Astaxanthin diester | 52.7 | 25.1 | 0.8 |
| Adonirubin ester | 3.6 | 0.2 | |
| Astaxanthin monoester | 28.5 | 0.2 | |
| Canthaxanthin | 0.1 | | |
| Adonirubin | 0.4 | | |
| Astaxanthin | 3.1 | 0.2 | |

Fig. 3 Structures of identified carotenoids in the integument of coral trout.

The tunaxanthin consisted of tunaxanthin A: tunaxanthin B: tunaxanthin C (1:4.5). The adonirubin consisted of 3R and 3S optical isomer in the ratio (14:86). The astaxanthin consisted of with 3R,3'R, meso, and 3S,3'S optical isomers in the ratio (13:7:80). The major steroisomer of adonixanthin was 3S,3'R.
esterified xanthophyll, 0.98 (t, J = 7.5 Hz, CH₂ in fatty acid moiety), 2.08 (m, CH₃ in fatty acid moiety), 2.40 ~ 2.50 and 2.80 ~ 2.85 (m, CH₂ in fatty acid moiety), and 5.37 (m, -CH = CH- in fatty acid moiety), clearly indicate that the fatty acid part consists solely of poly-unsaturated fatty acids. Similarly, using ESI TOF MS, we identified the molecular formulas of astaxanthin monoester, adonixanthin diester, adonirubin ester, tunaxanthin diester, and α-cryptoxanthin ester, respectively, as C₆₂H₈₂O₅, C₈₄H₁₁₄O₅, C₆₂H₈₂O₄, C₈₄H₁₁₆O₄, and C₆₂H₈₆O₂. This indicates that only docosahexaenoic acid is esterified with astaxanthin, adonixanthin, adonirubin, tunaxanthin, and α-cryptoxanthin in the integument of the coral trout. We found no xanthophyll esters esterified with other fatty acids.

As is widely known, fatty acids esterified with xanthophylls in green algae, plants, and animals are mixtures, with the exception of certain species of bacteria and algae. The fatty acids of the astaxanthin diester in the integument of the red seabream Pagrus major consist of docosahexaenoic, arachidonic, eicosapentaenoic, stearic, oleic, palmitic, and palmitoleic acids (Maoka et al., unpublished data). The only fatty acid found to be esterified with xanthophylls in the coral trout was docosahexaenoic acid. To the best of our knowledge, no carotenoid ester in animals consisting of a single fatty acid has been reported.

Soltan and Gibson (2008) reported on the fatty acid content of the flesh of the coral trout. Docosahexaenoic acid (34.4%) was found to be a major component, along with eicosapentaenoic acid (2.2%), arachidonic acid (3.5%), and mono-unsaturated fatty acids.
acid (12%), and saturated fatty acid (32.5%). The only fatty acid esterified with carotenoids in the coral trout was docosahexaenoic acid. The other fatty acids present in this fish were not esterified with carotenoids. While the carotenoid esterase in the coral trout may demonstrate a specificity for fatty acids, no specificity for fatty acids esterified with carotenoids has been reported for shrimp, clam, lobster, or red seabream [Maoka et al., unpublished data]. The reasons for this are currently unknown. Enzymatic and genetic studies of carotenoid esterase may shed light on this issue.

In the same way, we investigated carotenoids in the integument of the black and brown coral trout specimens (Table 1). The carotenoid contents of these fish were some 10- to 20-fold lower that of the red fish. We also found that tunaxanthin was a major carotenoid in these fish. We conclude that the color of the integument of the coral trout reflects the carotenoid content of the individual fish, especially regarding astaxanthin.

4 CONCLUSION

The color of the integument of the coral trout reflects the carotenoid content of the individual fish, especially regarding astaxanthin. In the integument of a red specimen of the coral trout, we identified astaxanthin as the main carotenoid, along with adonirubin and adonixanthin. In brown and black specimens, we identified tunaxanthin as the main carotenoid. These xanthophylls in the integument were present mainly in their fatty acid esterified forms. While fatty acids esterified with xanthophylls in green algae, plants, and animals tend to be mixtures, the only fatty acid esterified with xanthophylls in the coral trout was docosahexaenoic acid. To the best of our knowledge, no carotenoid ester consisting of a single fatty acid has been found in animals.

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