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Carotenoids in the sea bream. *Chrysaphrys major Temminck* and *Schlegel* — III.

The carotenoids in mysis and the internal organs of squid as the food for sea bream

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Abstract

The carotenoids in mysis and the internal organs of squid were extracted, purified on the columns, characterized by absorption spectra, the behavior on the columns, absorption maxima of the reduction and co-chromatography with authentic samples.

In the mysis, the existence of β-carotene, echinenone, isocryptoxanthin, lutein, zeaxanthin, canthaxanthin, astaxanthin, and crustaxanthin was confirmed. In the internal organs of squid, astaxanthin was found.

The content of astaxanthin was most abundant amoung each pigment in mysis and the internal organs of squid. It was clarified that mysis and internal organs of squid were good additives to the food for sea bream in order to improve their reddish brightness.

It was assumed that in mysis β-carotene would be converted to astaxanthin through the steps of isocryptoxanthin, echinenone, and canthaxanthin.

Though the function of the carotenoids in fish has not yet been solved clearly, it is often observed that the bright red color of natural sea bream fades or becomes rather dark color while they are in captivity at a fish farm. In the previous papers[1),2),3)] it was clarified that the faded color of the cultured sea bream was caused by the extremely small amount of astaxanthin contained in them. The stomach of the natural sea bream was examined and half digested Squilla ortoria and other crustacea were found. It was clarified that most of astaxanthin in the integument of natural sea bream was brought about by their food[4)].

The present investigation was undertaken to confirm the contents of astaxanthin in mysis and internal organs of squid, and to decide their value in order to improve their reddish brightness and to confirm the interconversion of plant carotenoids (β-carotene, zeaxanthin, lutein) into fish carotenoids (astaxanthin) in mysis. The existence of β-carotene, echinenone, isocryptoxanthin, lutein, zeaxanthin, canthaxanthin and astaxanthin was confirmed. It was assumed that astaxanthin in mysis was converted from β-carotene. It was clarified that both mysis and the internal organs of squid are excellent food additives for sea bream and also golden carp in order to improve their reddish brightness.

Materials and Methods

I. Carotenoids in mysis : Mysis were purchased from a local fish market, and

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their carotenoids were extracted with acetone in Waring blender untile no further pigment could be obtained. The acetone solutions were combined, transferred to petroleum ether by the addition of water and washed repeatedly with water to remove acetone.

Petroleum ether solution of the pigments was concentrated under vacuum and dried over sodium sulfate. The absorption spectra of the extracted carotenoids in petroleum ether is shown in Fig. 1.

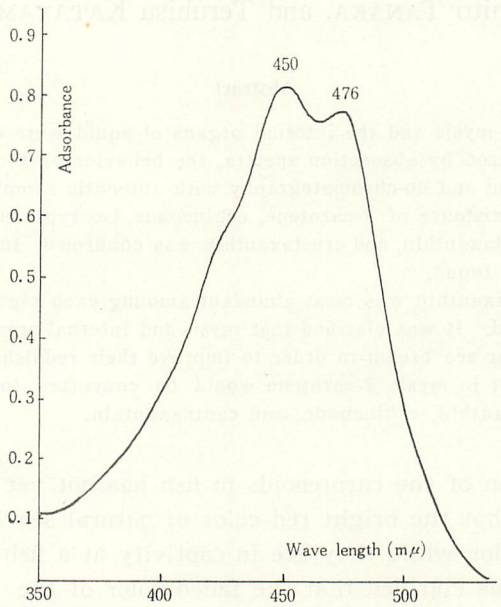

Fig. 1. The absorption spectrum of the crude carotenoids obtained from mysis in petroleum ether.

The pigments were separated chromatographically on a column of magnesium oxide (magnesium oxide: hyflosupercel=1: 2), using petroleum ether as developing solvent. Two bands were obtained: Band-I and Band-II.

The pigments of Band-I were saponified by using the same method mentioned in the previous paper[3]. After saponification, the pigments were purified on an alumina column (grade II) by using 15 % acetone in petroleum ether as developing solvent.

Two bands were obtained: Band-I-A (lower band), and Band-I-B (upper band).

β-carotene: The pigments of Band-I-A (lower band) were repurified on a magnesium oxide column by using 0.5 % acetone in petroleum ether. Two bands were obtained, the pigment of lower band was confirmed to be β-carotene.

Echinenone: The pigment of upper band (Band-I-A) was repurified on a magnesium oxide column, the absorption spectra in petroleum ether and the behavior on the column were all in agreement with those of echinenone.

The pigment of Band-I-B were repurified on a magnesium oxide column using 6

% to 25 % acetone in petroleum ether. Four bands were obtained: Band-I-B-a (lowest band), Band-I-B-b (middle band-1st), Band-I-B-c (middle band-2nd) and Band-I-B-d (upper band).

Canthaxanthin: This pigment of Band-I-B-a was eluted from the column, increasing the amount of acetone in petroleum ether. The absorption spectrum and the behavior on the column were identical with those of pure canthaxanthin.

Isocryptoxanthin: The pigment of Band-I-B-b was eluted from the column with acetone. The absorption spectra and the behavior on the column were all in agreement with those of pure isocryptoxanthin obtained from prawn[5].

Lutein: The pigment of Band-I-B-c was eluted from the column with acetone and transferred to petroleum ether. The absorption spectra and the behavior on the column were all identical with those of pure lutein obtained from Benibuna[6]. This pigment was confirmed to be lutein.

Zeaxanthin: The pigment of Band-I-B-d from the column with acetone and transferred to petroleum ether by adding water. The absorption spectra and the behavior on the column were all in agreement with those of pure zeaxanthin obtained from red carp[7].

The pigments of Band II were saponified by using the same method reported in the previous paper[8]. The saponified pigments were rechromatographed on a Microcel-C column, using 10 % acetone in petroleum ether as developing solvent. Two bands were obtained: Band-I-a (lower band) and Band-II-b (upper band).

Astacin: The pigment of Band-II-a (lower band) was repurified on a sugar column, using 0.8 % acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectrum and the behavior on the column were all identical with those of pure astacin obtained from lobster[9]. This pigment was identified to be astacin.

Crustaxanthin: The pigment of Band-II-b (upper band) was rechromatographed on a sugar column, using 5 % acetone in petroleum ether. One band was obtained. The absorption spectra and behavior on the column were all in agreement with those of crustaxanthin.

II. The carotenoids in the internal organs of squid : The carotenoids in the internal organs of squid were completely extracted with acetone in a Waring blender. The pigments in acetone solution were transferred to petroleum ether by the addition of water. The deeply colored acetone solution of pigments was diluted with water and washed with petroleum ether in a separatory funnel. The petroleum ether phase was evaporated under reduced pressure. The absorption spectra of the crude carotenoids is shown in Fig. 2.

The crude carotenoids were saponified by using the same method reported in the previous paper[3]. The saponified pigments were chromatographed on a silica gel column, using 2.5 % acetone in petroleum ether as developing solvent. Two bands were obtained: Band-I (lower band) and Band-II (upper band).

β-carotene: The pigment of Band-I (lower band) was rechromatographed on an aluminum oxide column (grade-II), using 0.2 % acetone in petroleum ether as de-

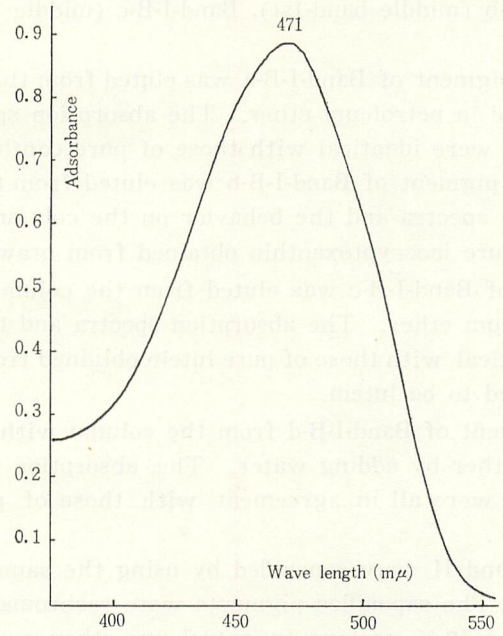

Fig. 2. The absorption spectrum of the crude carotenoids obtained from the internal organs of squid in petroleum ether.

veloping solvent. Only one band was obtained. The absorption spectra and the behavior on the column were all identical with those of pure β-carotene. The pigment was co-chromatographed on an aluminum oxide column (grade II) with pure β-carotene and formed a unitary zone. These results show this pigment to be β-carotene.

Astacin: The pigment of Band-II (upper band) was repurified on a sugar column, using 1% acetone in petroleum ether as developing solvent. Only one band was obtained. The absorption spectrum and behavior on the column were all in agreement with astacin. The pigment was also co-chromatographed with pure astacin obtained from prawn and formed a unitary zone. These results show this pigment to be astacin.

Result and Discussions

The carotenoids pigments in mysis are listed in Table 1 in the order which they were eluted from the columns and the relative amounts of each pigment are given as a percentage of the total.

The carotenoids in the internal organs of squid are also listed in Table 2.

In mysis the existence of β-carotene, isocryptoxanthin, echinenone, canthaxanthin astaxanthin and crustaxanthin was confirmed. It was assumed that in mysis β-carotene would be converted to astaxanthin through the steps of isocryptoxanthin,

Table 1. The spectral characteristics and relative abundances of the carotenoids in mysis

Pigments	Spectral characteristics			Relative abundance (%)	Concn. (mg/kg)
	λ max (mμ) in petroleum ether	λ max (mμ) in chloroform	λ max (mμ) after reduction		
β-Carotene	426, 449, 476	437, 463, 488		5.0	0.20
Echinenone	454	467	428, 450, 476	0.6	0.02
Isocryptoxanthin	424, 447, 472	435, 458, 485		3.9	0.15
Lutein	419, 447, 471	431, 455, 484		11.0	0.43
Zeaxanthin	424, 448, 474	438, 461, 480		38.0	1.48
Astacin	471	485	425, 450, 476	38.9	1.52
Canthaxanthin	453	468		2.0	0.08
Crustaxanthin	425, 449, 476	435, 463, 488		3.3	0.12
Pigment-427	427			0.6	0.02
Unknown				1.9	0.07

Table 2. The spectral characteristics and relative abundances of carotenoids in the internal organs of squid.

Pigments	Spectral characteristics			Relative abundance (%)	Concn (mg/kg)
	λ max (mμ) in petroleum ether	λ max (mμ) in chloroform	λ max (mμ) after reduction		
β-Carotene	426, 449, 476,	436, 463, 485		13.7	0.5
Astacin	471	485	425, 450, 476	86.3	3.2

echinenone and canthaxanthin.

The content of astaxanthin was most abundant amoung each pigment. It was clarified that mysis is good additives to the food for sea bream in order to improve their reddish brightness, because in the previous paper it had been confirmed that sea bream transferred astaxanthin from their food to their body astaxanthin.[4]

It was confirmed that the internal organs of squid contained abundant amount of astaxanthin. It is also good additives to the food for sea bream to improve their reddish brightness by the same reasons mentioned above.

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