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BAZYLI CZECZUGA

Karotenoidy u ryb. 5. *Anguilla anguilla* (L.)

Carotenoids in fish. 5. *Anguilla anguilla* (L.)

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Abstract — Using column and thin-layer chromatography the occurrence of separate carotenoids in fins, skin, gills, muscles, liver, and intestines of the eel *Anguilla anguilla* (L.) was investigated. The presence of such carotenoids as carotene, canthanxanthin, tunaxanthin, lutein, isozeaxanthin, zeaxanthin, astaxanthin, and astacene was recorded. In all the investigated parts of the eel astaxanthin was the dominant carotenoid.

In the preceding works of this series the occurrence of separate carotenoids in the roe of *Acipenser ruthenus* (Czeczuga 1972), in seven species of the coastal part of the Black Sea (Czeczuga 1973), and the occurrence and quantitative relations of carotenoids in three species of phytophagous fish imported into the warmed waters of Poland (Czeczuga 1972 a) were discussed. The results of the carotenoid investigations in sperm (Czeczuga 1974) and in roe (Czeczuga 1975) in some species of fish were also published.

The present work contains data on the occurrence of separate carotenoids and their quantitative relations in the individual parts of the body of the eel *Anguilla anguilla*.

Up to now the literature on the occurrence of carotenoids in *Anguilla anguilla* is scanty. The first report on the presence of pigments of the carotenoid type in the individuals of this species was found in Lönnberg's work (1931), these data were then repeated in the work from 1939 (Lönnberg 1939). According to Goodwin (1951), the carotenoid described by Lönnberg (1931, 1939) was lutein. This opinion was also shared by Fox (1957), who reviewed the literature on the occurrence of carotenoids in the individual species of fish. Moreover, in the work of Hirao et al. (1957) the data on the occurrence (among others) of astacene in the Japanese eel — *Anguilla japonica* have been also published. This is all that has appeared in the literature on the occurrence of carotenoids in the eel.

Material and methods

The specimens of eel *Anguilla anguilla* (L.) with a body length of 50—60 cm were taken from the lakes of the Ełk Lake District. The fins, skin, gills, muscles, liver, and intestines were analysed. In order to ascertain the occurrence of the individual carotenoids in the skin and muscles of smoked specimens the analysis was carried out on the basis of the market material. The collected material was treated with 95% acetone in dark glass bottles and kept in a refrigerator in nitrogen atmosphere to the moment of analysis. The separation of particular carotenoid pigments was carried out using column and thin-layer chromatography. Before chromatography the material was hydrolysed in nitrogen atmosphere at room temperature throughout 24 hrs. After hydrolysis the extract was passed through a column filled with Al_2O_3, the lenght of the column being 15—25 cm. The separate fractions were eluted using various systems of solvents (Czeczuga 1971), then the eluate was evaporated and after evaporation the residue was dissolved in a suitable solvent in order to draw the curve of absorption maxima which, among other uses, served for identification of particular carotenoids. The absorption maxima were determined using a Unicam spectrophotometer or Specol spectrocolorimeter.

Independently of the column chromatography the obtained acetone extract was separated into individual strains using thin-layer chromatography. The glass plates 15—45 cm in size were covered with silica gel and the acetone extract was then placed with a micropipette on the starting line, various solvent systems being used as well (Czeczuga 1973). The R_f value was determined according to the generally accepted rules.

The identification of the individual carotenoids was carried out on the basis of the absorption maxima of separate fractions, on the R_f values, on the epoxide test, and also on the obtained epiphase and hypophase relations (Czeczuga 1975). The quantitative relations of the individual carotenoids were determined according to Davies's method (Czeczuga 1975).

Results

The absorption maxima of separate carotenoids in various solvents and their coefficients of the epiphase and hypophase are given in Table I. The results of the quantitative investigation of carotenoids in the different parts of the body of *Anguilla anguilla* specimens are presented in Table II. On the basis of the chromatographic analysis the presence of β-carotene, canthaxanthin, tunaxanthin, lutein, isozeaxanthin, zeaxanthin, astaxanthin, and astacene was determined in the specimens of *Anguilla anguilla* (Table I).

Tabela I. Maksima absorpcji i stosunki epifazy do hipofazy poszczególnych karotenoidów u węgorza

Table I. Absorption maxima and epiphase to hypophase relations of individual pigments in the eel

Nazwa karotenoidu Name of carotenoid	Maksimum absorpcji w Maximum absorption in nm				Stosunek Ratio
	Eter naftowy Petroleum ether	Heksan Hexane	Etanol Ethanol	Benzen Benzene	
β - karoten β - carotene	421,451,478				100 : 0
Kantaksantyna Canthaxanthin		467	477		55 : 45
Tunaksantyna Tunaxanthin		415,435,466			20 : 80
Luteina Lutein		420,445,475	420,445,475		12 : 88
Izozeaksantyna Isozeaxanthin	446,475	451,481	451,478		22 : 78
Zeaksantyna Zeaxanthin			425,451,482		11 : 89
Astaksantyna Astaxanthin	470	472		485	10 : 90
Astacyna Astacene			478	495	23 : 77

As the data in Table II show, in the fins of the investigated specimens of *Anguilla anguilla* the presence of canthaxanthin, tunaxanthin, lutein, zeaxanthin, astaxantin, and astacene was found. Astaxanthin occurred in the greatest amounts, constituting 40 per cent of all the carotenoids. As far as the presence of carotenoids in the skin is concerned, all those found in the fins also occurred here, with the exception of canthaxanthin and zeaxanthin which were not detected. Moreover, the presence

Tabela II. Zawartość stwierdzonych karotenoidów w badanych częściach ciała węgorza

Table II. Content of carotenoids found in the investigated parts of the body of the eel

Nazwa karotenoidu Name of carotenoid	% zawartości poszczególnych karotenoidów w różnych częściach ciała węgorza Percentage content of separate carotenoids in various parts of the body of the eel					
	Płetwy Fins	Skóra Skin	Skrzela Gills	Mięśnie Muscles	Wątroba Liver	Jelita Intestines
β - karoten β - carotene		6.5	10.5	3.9	12.2	8.6
Kantaksantyna Canthaxynthin	11.4		17.1			14.0
Tunaksantyna Tunaxanthin	13.5	14.5	2.3	9.6	16.5	9.9
Luteina Lutein	9.9	2.9	1.8	2.8	28.8	11.3
Izozeaksantyna Isozeaxanthin		3.2				
Zeaksantyna Zeaxanthin	6.0		15.7	8.7		
Astaksantyna Astaxanthin	40.0	45.9	37.6	47.1	37.6	56.2
Astacyna Astacene	19.2	26.4	15.0	27.9		
Nierozpoznane Unknown		0.6			4.9	

of β-carotene and of one unidentified carotenoid was observed. In petroleum ether this carotenoid showed the absorption maximum at 485 nm and a hypophase coefficient. In the skin the dominant carotenoids were astaxanthin (45.9%) and

Tabela III. Występowanie poszczególnych karotenoidów w skórze i w mięśniach wędzonych osobników węgorza

Table III. Occurrance of the separate carotenoids in the skin and muscles of smoked specimens of the eel

Nazwa karotenoidu Name of carotenoid	Skóra Skin	Mięśnie Muscles
β - karoten β - carotene	+	+
Kantaksantyna Canthaxanthin	+	+
Tunaksantyna Tunaxanthin	+	+
Luteina Lutein	+	
Izozeaksantyna Isozeaxanthin	+	+
Zeaksantyna Zeaxanthin	+	
Astaksantyna Astaxanthin	+	+
Astacyna Astacene	+	+

astacene (26.4%). In the skin of smoked specimens all carotenoids specific for this species were detected (Table III).

In the gills the presence of all carotenoids, with the exception of isozeaxanthin, was observed, astaxanthin being dominant also in this case (37.6%). In the muscles the occurrence of neither isozeaxanthin nor canthaxanthin was traced, the dominant carotenoids being astaxanthin (47.1%) and astacene (27.9%). In the liver, besides the canthaxanthin and isozeaxanthin, also zeaxanthin and astacene were absent while similarly as in the skin of the eel an unidentified carotenoid occurred. In the case of the liver the dominant carotenoids were also astaxanthin (37.6%) and lutein (28.8%). The intestines of the eel contained β-carotene, canthaxanthin, tunaxanthin, lutein, and astaxanthin, the last carotenoid being dominant here (56.2%).

Discussion

In the reviews on the occurrence of carotenoids in fish (Goodwin 1951, Fox 1957) only the presence of lutein in the skin of *Anguilla anguilla* was mentioned. As the obtained data suggest, a number of other carotenoids which have been shown in other species of fish occur both in the skin and in other organs of the eel (Czeczuga 1972, 1972 a, 1973, 1974). Above all, the occurrence of astaxanthin in all parts of the body of the eel and of astacene in the majority of cases should be stressed. Only in the liver and in the intestines of the eel was astacene not found. The content of astaxanthin varied from 37.6% (gills and liver) to 56.2% (intestines) and that of astacene from 15.0% (gills) to 27.9% (muscles). Astaxanthin is fairly often among

the dominant carotenoids in the representatives of other aquatic organisms. As it is, many species of fresh-water (Czeczuga 1971) andsea-water (Czeczuga 1974 a) crustaceans astaxanthin is a dominant carotenoid. Astaxanthin gives a red coloration to the aquatic species of *Arachnoidea* (Czeczuga 1972 b) and it is also a dominant carotenoid in the representatives of *Cyclostomata — Lampetra planerii* (Czeczuga 1973 a). Moreover, in certain species of sea- and fresh-water fish the roe, sperm, and other organs contain astaxanthin in the greatest amounts. This carotenoid gives a red coloration in the spawning period, especially with Salmonids (Crozier 1970) and some authors attribute to it an essential role in reproduction (Gilchrist, Lee 1972). According to Grangaud et al. (1962) astaxanthin is converted into vitamin A in the wall of the intestines. As the investigations have shown (Hata, Hata 1972, Katayama et al. 1972, 1974), a number of carotenoids found so far in fish, in consequence of numerous conversions, are transformed into astaxanthin which accumulates in these or in other organs of various species of fish. It should be stressed here that in certain species of fish other carotenoids dominate, perhaps β-carotene or canthaxanthin (Webber et al. 1973), while Hata and Hata (1971) report the domination of zeaxanthin in the liver of *Carassius auratus*. On the other hand, Matsuno et al. (1973) report the dominance of tunaxanthin in certain species of *Gobio*. The accumulation of great amounts of this or other carotenoids is greatly influenced by the type of food, as was shown by Saito and Rigler (1970), who investigated the content of carotenoids in specimens of *Salvelinus fontinalis*, the data of Katayama et al. (1972 a) suggesting a selective accumulation of carotenoids from the food eaten by the fish.

Moreover, the occurrence of tunaxanthin in all the investigated body organs of the eel should be stressed, since it has been chiefly reported as a carotenoid of sea fish (Crozier (1974). The investigations of the present author (Czeczuga 1974) have shown that this carotenoid also occurs in certain species of fish living only in fresh waters.

STRESZCZENIE

Stosując chromatografię kolumnową i cienkowarstwową badano występowanie poszczególnych karotenoidów w płetwach, skórze, skrzelach, mięśniach, wątrobie i w jelitach węgorza — *Anguilla anguilla* (L.).

W wyniku badań ustalono obecność takich karotenoidów, jak: β-karotenu, kantaksantyny, tunaksantyny, luteiny, izozeaksantyny, zeaksantyny, astaksantyny i astacenu. We wszystkich badanych częściach węgorza dominującym karotenoidem okazała się astaksantyna.

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Adres autora — Author's address

prof. dr hab. Bazyli Czeczuga

Zakład Biologii Ogólnej, Instytut Biostruktury, Akademia Medyczna, ul. Kilińskiego 1, 15-089 Białystok

ERRATA

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