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Biochemistry

CAROTENOIDS IN FISH. XL. CAROTENOIDS IN FISH FROM THE FALKLANDS REGION

KAROTENOIDY U RYB. XL. KAROTENOIDY U RYB Z REJONU FALKLANDÓW

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The authors studied, by means of column and thin layer chromatography, the occurrence of various carotenoids in 8 fish species caught off Falkland Islands (*Dissostichus eleginoides, Genypterus blacodes, Macruronus magellanicus, Micromesistius australis, Merluccius merluccius, Raja georgiana, Salilota australis,* and *Iloucoetes fimbriatus*).

The presence of 20 carotenoids was found. The occurrence of ester forms of 2'-norastaxanthin was shown in fish for the first time.

The total carotenoid content was found to range from 0.012 (*Iloucoetes fimbriatus*) to 0.744 μg/g wet weight (*Macruronus magellanicus* liver).

INTRODUCTION

As shown by the analysis of resources of various groups of organisms inhabiting seas and oceans (Żmudziński, 1983), fish species play the most important role in the

nutrition of man. Numerous studies showed various populations of the same species to differ in their contents of different biologically active substances, depending on the fishing area. This is true, i.a., with respect to the carotenoid content (Czeczuga, 1982), some carotenoids being known as the vitamin A provitamin.

In this context we have thought it purposeful to analyse total carotenoid contents and percentages of individual carotenoids in some fish species caught by the Polish fishing fleet off Falkland Islands, an area rich in ichthyofauna.

MATERIALS AND METHODS

Five individuals of each fish species studied were collected in September 1982 off Falkland Islands (coordinates: 52°42'S; 058°45'W) at 150–175 m depth; water temperature and salinity were 6°C and about 35‰, respectively. Chromatographic assays were made on materials previously frozen to –20°C. Sample weight ranged from 30 to 50 g. Skin, muscles, and liver of *Dissostichus eliginoides* Smitt, *Genypterus blacodes* (Bloch et Schneider), *Macruronus magellanicus* Lönberg, *Micromesistus australis* Norman, *Merluccius merluccius hubbsi* Marini, *Raja georgiana* Norman, and *Salilota australis* Günter were analysed, while in *Iloucoetes fimbriatus* Jenyns the carotenoid content in the whole body was determined.

Carotenoid pigments were separated by means of column and thin layer chromatography. Prior to assays, the samples were hydrolysed for 24 h in 10% KOH in nitrogen at room temperature. After the hydrolysis, the extract was run through an Al_2O_3 –filled column (Quickfit, England), 15–25 cm long. Various fractions were eluted with different solvent combinations (Czeczuga and Czerpak, 1976), The eluent was then evaporated, the remainder being dissolved in an appropriate solvent to plot the absorption curve; its peaks were used, i.a., to identify carotenoids.

Regardless of column chromatography, the acetone extract obtained was separated by means of thin layer chromatography. Glass plates (15 x 40 cm) covered with silicone gel (Merck) were used. Acetone extracts were transferred with a micropipette into the starting line, different solvents being used here as well (Czeczuga and Czerpak, 1976). The R_f value was then determined according to the generally accepted principles.

Individual carotenoids were identified from absorption peaks in various solvents, from the R_f values compared to the standard, and from the epi- to hypophase ratios obtained. For β-carotene, β-cryptoxanthin, canthaxanthin, echinenone, lutein, zeaxanthin, tunaxanthin, and astaxanthin, standards manufactured by F. Hoffman-La Roche, Basle were used. Absorption peaks were determined in Spektromom-203 and Specol spectrophotometers.

Quantitative composition of various carotenoid groups were determined as in Czeczuga and Czerpak (1976).

RESULTS

Table 1 lists the carotenoids identified; the structure of carotenoids is given in Fig. 1. The materials studied revealed the presence of 20 carotenoids. A special mention is due to the detection of the ester and diester forms of 2′-norastaxanthin.

1. *Dissostichus eleginoides* has tasty meat but is not frequent in catches. As shown in Table 2, 10 carotenoids were found in the individuals of the species analysed. Such carotenoids as β-cryptoxanthin, canthaxanthin, lutein epoxide, zeaxanthin, astaxanthin, and 2′-norastaxanthin ester occured in all the body parts analysed. The liver was found to be the carotenoid-richest organ, muscles containing the lowest amount of total carotenoids.
2. *Genypterus blacodes,* with tasty meat as well. A total of 10 carotenoids were identified, β-cryptoxanthin, lutein epoxide, and astaxanthin occurring in all the body parts analysed (Table 3). Additionally, the presence of ε-carotene in the skin and idoxanthin in muscles should be mentioned. The liver was again the carotenoidrichest organ.
3. *Macruronus magellanicus:* 11 carotenoids were identified; β-cryptoxanthin, canthaxanthin, lutein epoxide, zeaxanthin, and astaxanthin were found in all the body parts tested. The skin was found to contain idoxanthin; the liver and skin are particularly carotenoid-rich (Table 4).
4. *Micromesistus australis,* very abundant in the area, caught to be processed for feedstuffs or fish meal. A total of 8 carotenoids were identified; β-cryptoxanthin, canthaxanthin, lutein epoxide, zeaxanthin, and 2′-norastaxanthin ester were present in all the body parts studied. Of all the organs examined, the liver proved to be the carotenoid-richest one (Table 5).
5. *Merluccius merluccius hubbsi,* fairly abundant in the fishing grounds of the area. The presence of 12 carotenoids was found in the organs tested (Table 6). β-cryptoxanthin, canthaxanthin, lutein epoxide, zeaxanthin, and α-doradexanthin were found to occur in all the body parts tested. The total carotenoid content ranged within 0.104 (muscles) – 0.127 μg/g wet weight (liver).
6. *Raja georgiana:* the body parts tested were found to contein 9 carotenoids (Table 7), 6 of which (β-cryptoxanthin, zeaxanthin, neothxanthin, tunaxanthin, astaxanthin, and 2′-norastaxanthin ester) occured in all the organs under study. Again, the liver was relatively carotenoid-rich (0.464 μg/g wet weight).
7. *Salilota australis:* 11 carotenoids were identified (Table 8); all the organs tested containd canthaxanthin, zeaxanthin, α-doradexanthin, tunaxanthin, and astaxanthin. Total carotenoid contents ranged from 0.042 (muscles) to 0.116 μg/g wet weight (liver).
8. *Ilucoetes fimbriatus:* Whole individuals were subject to assays; 6 carotenoids were identified (Table 9). The presence of echinenone and its derivative, 3′-hydroxyechinenone should be emphasised. The total carotenoid content amounted to 0.012 μg/g wet weight.

Table 1

List of the carotenoids found in the materials studied

No.	Carotenoid	Structure (see Fig. 1)	Semisystematic name
1.	ϵ – carotene	A – R – A	ϵ, ϵ – carotene
2.	β – carotene	B – R – B	β, β – carotene
3.	β – cryptoxanthin	B – R – D	β, β – caroten – 3 – ol
4.	echinenone	B – R – E	β, β – caroten – 4 – one
5.	3′ – hydroxyechinenone	D – R – E	3′ – hydroxy – β, β – caroten – 4 – one
6.	neothxanthin	A – R – C	ϵ, ϵ – caroten – 3 – ol
7.	lutein	C – R – D	ϵ, β – carotene – 3,3′ – diol
8.	lutein epoxide	C – R – I	5,6 – epoxy – 5,6 – dihydro – ϵ, β – carotene – 3,3′ – diol
9.	tunaxanthin	C – R – C	ϵ, ϵ – carotene – 3,3′ – diol
10.	zeaxanthin	D – R – D	β, β – carotene – 3,3′ – diol
11.	α – doradexanthin	C – R – G	3,3′ – dihydroxy – ϵ, β – caroten – 4 – one
12.	idoxanthin	G – R – F	3,3′,4′ – trihydroxy – β, β – caroten – 4 – one
13.	canthaxanthin	E – R – E	β, β – carotene – 4,4′ –dione
14.	astaxanthin	G –R – G	3,3′ – dihydroxy – β, β – carotene – 4,4′ – dione
15.	astaxanthin ester	G – R – H	3,3′ – β, β – carotene – 4,4′ – dione – 3 – acylate
16.	2′ – norastaxanthin ester	G – R – M	3,3′ – dihydroxy – 2-nor – β, β – carotene – 4,4′ – dione – 3 – acylate
17.	2′ norastaxanthin diester	H – R – M	3,3′ – dihydroxy – 2–nor – β, β – carotene – 4,4′ – dione – 3,3′ – diacylate
18.	mutatochrome	B – R_1 – K	5,8 – epoxy – 5,8 – dihydro – β, β – carotene
19.	mutatoxanthin	D – R_1 – L	5,8 – epoxy – 5,8 – dihydro – β, β – carotene – 3,3′ – diol
20.	diatoxanthin	D – R_2 – D	7,8 – didehydro – β, β – carotene – 3,3′ – diol

Table 2

Carotenoid content in some parts of the body of *Dissostichus eleginoides* (in %)

Carotenoid	skin	muscles	liver
β – carotene		6.3	
β – cryptoxanthin	30.7	10.6	trace
canthaxanthin	32.6	trace	36.2
lutein epoxide	5.8	11.6	14.9
zeaxanthin	3.8	21.1	9.3
tunaxanthin		20.6	
α – doradoxanthin		22.8	
astaxanthin	16.5	trace	32.4
2′ – norastaxanthin ester	7.2	7.0	7.2
2′ – norastaxanthin diester	3.4		
Total content of carotenoids in μg/g wet weight	0.092	0.063	0.112

Table 3

Carotenoid content in some parts of the body of *Genypterus blacodes* (in %)

Carotenoid	skin	muscles	liver
ε – carotene	14.3		
β – cryptoxanthin	trace	12.3	19.7
canthaxanthin		2.9	
lutein		10.5	
lutein epoxide	38.0	12.4	38.3
idoxanthin		4.3	
neothxanthin			10.9
tunaxanthin	19.5		
astaxanthin	28.2	50.2	24.8
2′ – norastaxanthin ester		7.4	6.3
Total content of carotenoids in μg/g wet weight	0.041	0.037	0.066

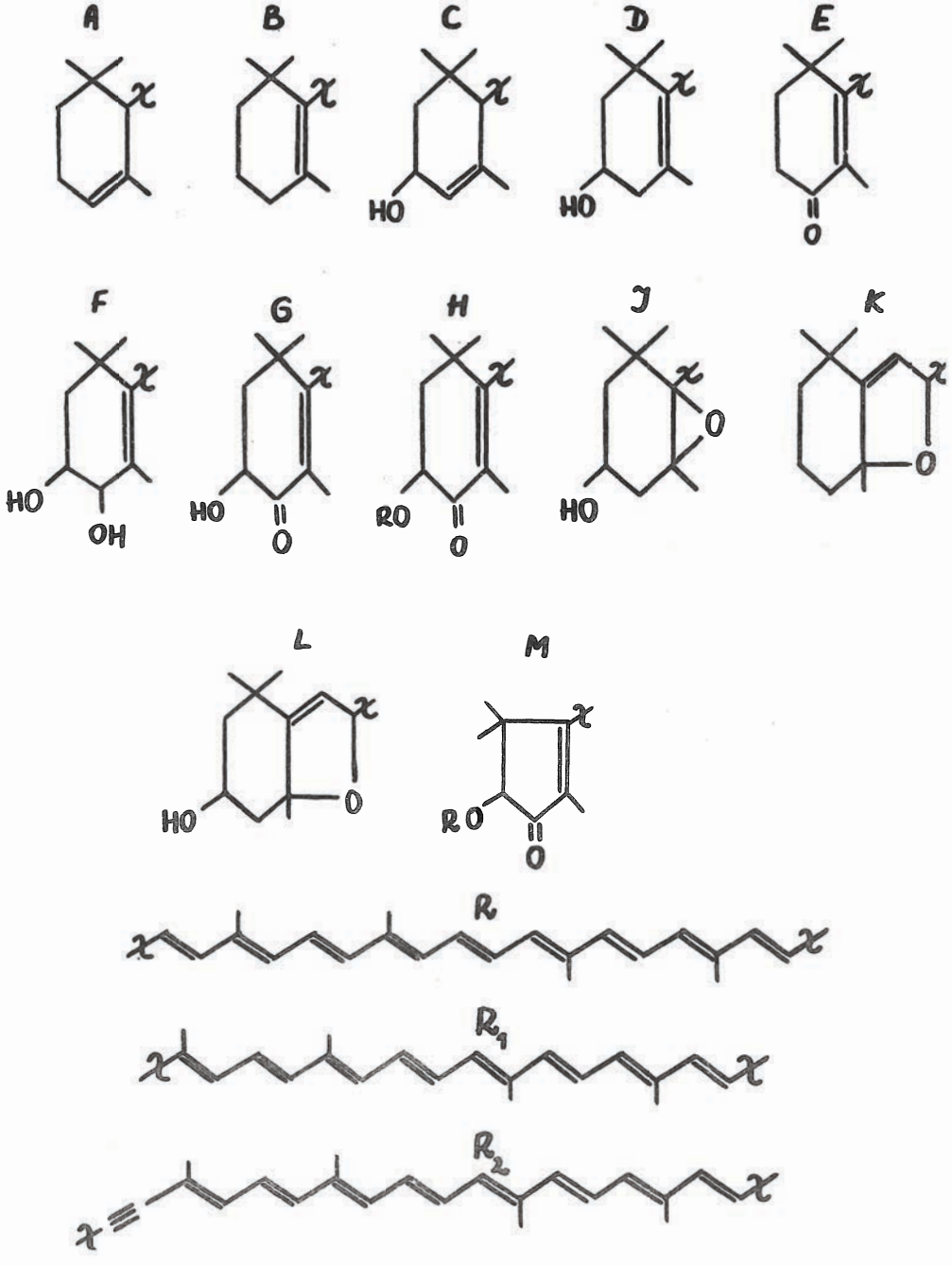

Fig. 1. Structural features of carotenoids from investigated materials

Table 4

Carotenoid content in some parts of the body of *Macruronus magellanicus* (in %)

Carotenoid	skin	muscles	liver
β – cryptoxanthin	7.1	21.4	trace
canthaxanthin	45.2	31.3	trace
lutein epoxide	26.1	trace	12.7
zeaxanthin	6.4	19.1	18.0
idoxanthin	2.1		
neothxanthin			38.5
α – doradoxanthin		7.0	
astaxanthin	7.9	trace	30.8
astaxanthin ester		18.2	
2′ – norastaxanthin ester	5.2		
2′ – norastaxanthin diester		3.0	
Total content of carotenoids in μg/g wet weight	0.152	0.519	0.744

Table 5

Carotenoid content in some parts of the body of *Micromesistus australis* (in %)

Carotenoid	skin	muscles	liver
β – carotene			8.5
β – cryptoxanthin	8.3	32.2	15.3
canthaxanthin	5.9	41.4	33.3
lutein epoxide	trace	19.3	27.8
zeaxanthin	65.2	trace	3.6
α – doradoxanthin			6.2
astaxanthin	14.3		
2′ – norastaxanthin ester	6.3	7.1	5.3
Total content of carotenoids in μg/g wet weight	0.208	0.118	0.437

Table 6

Carotenoid content in some parts of the body of *Merlucius merlucius* (in %)

Carotenoid	skin	muscles	liver
β – cryptoxanthin	24.0	3.8	trace
canthaxanthin	31.6	28.3	29.9
lutein		2.9	
lutein epoxide	2.4	11.4	13.5
zeaxanthin	37.5	29.9	trace
α – doradoxanthin	trace	19.4	15.4
tunaxanthin			10.9
mutatochrome		1.5	
mutatoxanthin		2.8	
astaxanthin			23.0
2′ – norastaxanthin ester	4.5		3.2
2′ – norastaxanthin diester			4.1
Total content of carotenoids in μg/g wet weight	0.126	0.104	0.127

Table 7

Carotenoid content in some parts of the body of *Raja georgiana* (in %)

Carotenoid	skin	muscles	liver
β – cryptoxanthin	8.2	trace	20.6
lutein epoxide	7.4		
zeaxanthin	28.4	47.6	34.1
α – doradoxanthin		2.7	1.5
neothxanthin	3.2	trace	6.2
tunaxanthin	trace	36.6	15.6
astaxanthin	45.6	4.4	14.7
2′ – norastaxanthin ester	7.2	4.2	7.3
2′ – norastaxanthin diester		4.5	
Total content of carotenoids in μg/g wet weight	0.221	0.070	0.464

Table 8

Carotenoid content in some parts of the body of *Salitota australis* (in %)

Carotenoid	skin	muscles	liver
β – cryptoxanthin		16.2	
canthaxanthin	trace	18.3	31.3
lutein epoxide			9.1
zeaxanthin	21.6	30.2	trace
diatoxanthin	29.2		
α – doradoxanthin	12.5	trace	25.4
tunaxanthin	trace	20.3	9.2
mutatochrome	14.6		
mutatoxanthin	12.6		
astaxanthin	5.3	9.9	17.4
2′ – norastaxanthin ester	4.2		4.5
2′ – norastaxanthin diester		5.1	3.1
Total content of carotenoids in μg/g wet weight	0.051	0.042	0.116

Table 9

Carotenoid content in the body of *Iloucoetes fimbriatus* (in %)

Carotenoid	%
echinenone	8.1
3′ – hydroxyechinenone	4.0
lutein	16.8
zeaxanthin	52.5
astaxanthin	12.0
2′ – norastaxanthin diester	6.6
Total content of carotenoids in μg/g wet weight	0.012

DISCUSSION

Out of 20 carotenoids identified in the fish species under study, the presence of ε-carotene, echinenone and its derivative 3′-hydroxyechinenone, neothxanthin, idoxanthin as well as ester and diester forms of 2′-norastaxanthin deserves mention. These carotenoids, apart from 2′-norastaxanthin esters, have already been, albeit occasionally identified in some fish species. ε-carotene was found, i.a., in *Silurus glanis* (Czeczuga, 1977), *Coregonus albula* (Czeczuga, 1977), and in some species of *Salmo* (Czeczuga, 1979). On the other hand, echinenone has been so far identified in certain salmonids (Czeczuga, 1977a; b; Matsuno et al., 1980; Czeczuga and Chełkowski, 1984); its derivative 3′-hydroxyechinenone is found nost often in the salmonids as well (Matsuno et l., 1980). Echinenone was also found in specimens of *Misgurnus fossilis* (Czeczuga, 1980a).

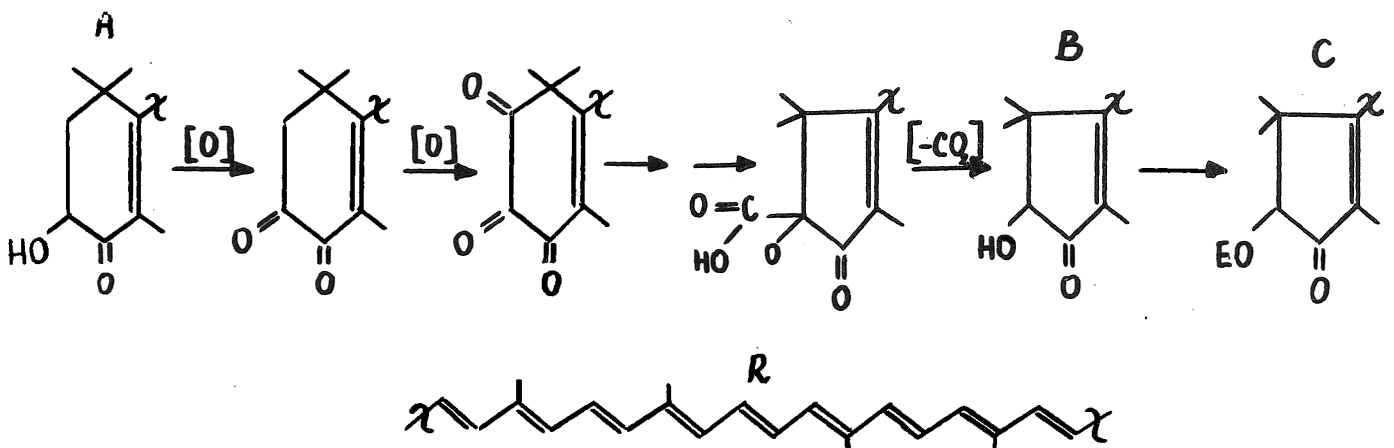

Fig. 2. Possible pathway for the transformation of astaxanthin to 2′ – norastaxanthin ester

Neothxanthin was found for the first time in *Neothunnus albacora* (Tanaka et al., 1977) and subsequently in *Limanda limanda* (Czeczuga, 1980) and *Micropterus salmoides* (Czeczuga, 1981). With respect to the presence of idoxanthin in fish, the carotenoid was revealed in *Cyprinus carpio* (Nagata and Matsuno, 1979; Czeczuga and Dąbrowski, 1984), *Micropoterus salmoides* (Czeczuga, 1981), and in bream *Abramis brama* (Czeczuga, 1982).

Particularly noteworthy is the identification of 2′-norastaxanthin esters in the fish species under study, the carotenoid having not been found previously in fish. Generally, esters of 2′-norastaxanthin were found in coelenterates (Hertzberg and Liaaen-Jensen, 1968; LeBoeuf et al., 1981 a, b) and in a few copepod species (Bandaranayake and Gentien, 1982). Most probably, 2′-norastaxanthin and its esters are formed by oxidation of astaxanthin (Fig. 2). The presence of 2′-norastaxanthin esters in the fish species under study is food-related rather than indigenous to the organisms themselves (formed in them by oxidation of astaxanthin), although the latter possibility cannot be excluded at the present stage of research.

In all the fish species studied, the highest total carotenoid contents were typical of the liver. Among muscles, those of *Macruronus magellanicus* were the carotenoid-richest ones (0.519 μg carotenoids/g wet weight). The value found is high for a fish. It should be reminded here that values of this order of magnitude were recorded in *Salmo trutta* (Czeczuga and Chełkowski, 1984) having, as it is widely known, reddish muscles. The lowest carotenoid contents were found in muscles of *Genypterus blacodes* (0.037 μg/g); values of this order of magnitude are most common among both freshwater (Czeczuga, 1982) and marine fish species (Czeczuga and Kłyszejko, 1978).

As seen from Table 10, the muscles richest in those carotenoids constituting the vitamin A provitamin are those of *Macruronus magellanicus* (0.055 μg/g wet weight) and *Micromesistius australis* (0.019 μg/g wet weight); muscles of the remaining species contained much lower amounts of those carotenoids.

Table 10

Provitamin A in muscles fish species investigated

Species	in %	in μg/g wet weight
Dissostichus eleginoides	11.6	0.007
Genypterus blacodes	6.2	0.002
Macruronus magellanicus	10.7	0.055
Micromesistus australis	16.1	0.019
Merlucius merlucius	2.7	0.003
Raja georgiana	0.5	0.003
Salilota australis	88.1	0.003
Iloucoetes fimbriatus	4.1	0.001

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KAROTENOIDY U RYB XL.
KAROTENOIDY U RYB Z REGIONU FALKLANDÓW

STRESZCZENIE

Stosując chromatografię kolumnową i cienkowarstwową autorzy badali występowanie poszczególnych karotenoidów w skórze, mięśniach i wątrobie 8 gatunków ryb z łowisk w okolicy Falklandów. Badaniami objęto takie gatunki jak: *Dissostichus eleginoides, Genypterus blacodes, Macruronus magellanicus, Micromesistus australis, Merlucius merlucius, Raja georgiana, Salilota australis* oraz *Iloucoetes fimbriatus.*

W wyniku badań ustalono obecność takich karotenoidów jak ε- iβ-carotene, β-cryptoxanthin, echinenone, 3′-hydroxyechinenone, neothxanthin, lutein, lutein epoxide, tunaxanthin, zeaxanthin, α-doradexanthin, idoxanthin, canthaxanthin, astaxanthin, astaxanthin ester, 2′-norastaxanthin ester, 2′-norastaxanthin diester, mutatochrome, mutatoxanthin oraz diatoxanthin.

Podano również ogólną zawartość karotenoidów oraz stosunki procentowe poszczególnych z nich. Ogólna zawartość karotenoidów wahała się od 0,012 (*Iloucoetes fimbriatus*) do 0.744 μg/g świeżej masy (wątroba *Macruronus magellanicus*). Jeśli chodzi o badane części ciała, to najzasobniejszymi w karotenoidy okazały się wątroby badanych gatunków ryb.

Чечуга Б., Клышейко Б.

КАРОТИНОИДЫ У РЫБ. XL. КАРОТИНОИДЫ У РЫБ ИЗ РАЙОНА ФАЛКЛАНДОВ

Р е з ю м е

С помощью колоночной и тонкослойной хроматографий авторами исследовалось присутствие отдельных каротинов в коже, мышцах и печени у 8 видов рыбы происходящих из ловли в окресностях Фалкландов. Исследовались следующие виды: Dissostichus eleginoides, Genypterus blacodes, Macruronus magellanicus, Micromesistus australis, Merlucius merlucius, Raja georgiana, Salilota australis и Iloucoetes fimbriatus.

На основании результатов исследований установлено наличие следующих каротиноидов: ε и β - carotene, β - cryptoxanthin, echinenone, 3´-hydroxyechinenone, neothxanthin, lutein, lutein epoxide, tunaxanthin, zeaxanthin, α - doradexanthin i doxanthin, canthaxanthin, astaxanthin, astaxanthin ester, 2´- norastaxanthin ester, 2´- norastaxanthin diester, mutatochrome, mutatoxanthin и diatoxanthin.

Установлено общее содержание каротиноидов, а также процентные соотношения между отдельными каротинами. Общее содержание каротиноидов составляло предел с 0,012 (Iloucoetes fimbriatus) до 0,744 μг/г сырого вещества (печень Macruronus magellanicus). Самые большие содержания каротиноидов присутствовали в печени исследованных видов рыбы.

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