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Full Length Research Paper

Amount and qualities of carotenoids in fillets of fish species fed with natural feed in some fisheries of West African Coast

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Using column (CC), thin- layer (TLC) and high- performance liquid chromatography (HPLC), carotenoid content was examined in the fillets (muscles with skin) of 16 fish species from the fisheries of West African Coast. 15 carotenoids, including 6 ketocarotenoids (4'- hydroxyechinenone, canthaxanthin, phoenicopterone, phoenicoxanthin, astaxanthin, 7,8- didehydroastaxanthin) were found, with a predominance of 3,4- dihydro- α- carotene, zeaxanthin, phoenicopterone, canthaxanthin, phoenicoxanthin and astaxanthin. Total carotenoid content in the examined material ranged from 0.086 (*Merluccius merluccius*) to 0.352 µg g^{-1} wet mass (*Macrurus aequalis*). Also, the increase of different environmental factors- especially food, parasites and organochlorine pollution on carotenoid content in fishes and their role in human's life was discussed.

Key words: Carotenoids, fish, fillets, fisheries, West Africa.

INTRODUCTION

Carotenoids are wide spread and important pigments in nature. They occur in all families of flora and fauna. Only bacteria, fungi and plants are able to synthesize them *de novo*; animals have to obtain them from food. Fish meat owes its nutrient value not only to protein and fats, but also to other biologically active substances- including carotenoids, which plays an important role in humans' life. Carotenoids serve as a source of vitamin A, antioxidant and pro- oxidant (Yeum et al., 2009), play a cancer- protective role (Rock, 2009) and increase the immune response in mammals (Chew and Park, 2009). Carotenoids may also be significant in the coronary heart disease (Johnson and Krynski, 2009). Therefore, many authors have been carrying out extensive studies on the presence and biological function of carotenoids in plants, animals and in humans. Thus, the knowledge about the carotenoid content in meat or respective fish species in fisheries seems to be of a great importance.

We have already investigated some of the species from the Baltic Sea (Czeczuga and Klyszejko, 1996), Black Sea (Czeczuga, 1973), fishing areas of the Antarctic (Czeczuga, 1978 a,b; 1982; Czeczuga and Klyszejko, 1978, 1986) and from the fisheries of New Zealand (Czeczuga et al.,2000). Tsukuda and Amono (1966), Matsuno et al. (1974, 1979), Matsuno and Katsuzama (1976) and Miki et al. (1982) have investigated the carotenoids in species from the waters washing the Japanese islands. Later, the analyses of carotenoids found in some species from the ocean near the coasts of California have been made (Crozier, 1967). Tanaka et al. (1978) have investigated the carotenoids in marine yellow fish from tropical regions. Carotenoids content in particular species

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of Pacific salmons have also been investigated (Jarzombek, 1970; Czeczuga, 1979; Kitahara, 1984) and some species from an ocean ranching farm near Islands they were analysed by Czeczuga et al. (2005).

Reviewed papers about carotenoids in fishes are generally dated prior to 2000 (Fox, 1957; Simpson et al., 1981); more recent works cover the carotenoids in general (Bjerkeng, 2008).

The present paper discusses the results of the analysis of carotenoid content in fishes from some fisheries of West African Coast.

MATERIAL AND METHODS

The study population included 16 fish species (Table 2) from the fisheries of the West African Coast (17°05'- 22^{0}35'S; 0.11°20'- 0.13°30'E southern Africa and 22°0'- 17°10'E northern Africa), caught in July and August.

50 g fillets (muscles with skin) have been used in the investigations. We analysed the material from three specimens. After three weeks of storage, the refrigerated material (-4°C) which has been used for the analysis, was sent by air mail to the department laboratory where they were analysed a week later. The carotenoid pigments were isolated using column (CC), thin- layer (TLC) and high- performance liquid chromatography (HPLC).

Prior to chromatography, the material was homogenized and hydrolyzed for 24 h in a 10% solution of nitrogen, at room temperature. The extract was subsequently placed onto an Al_2O_3- filled Quickfit Co. column. The individual fractions were eluted using various solvent systems (Czeczuga, 1988). The eluent was evaporated and the residue was dissolved in appropriate solvent to draw the maximum of absorption. This was necessary, among other reasons, to identify particular carotenoids. In addition to column chromatography, the acetone extract has been divided into fractions with thin- layer chromatography. Silicon- gel- covered glass plates (Merck Co.) and various solvent systems were used. The R_f values were established according to commonly accepted criteria (Schiedt and Liaaen- Jensen, 1995).

Pigments were also determined by ion- pairing in reverse- phase HPLC. The HPLC equipment consisted of
a Shimadzu SCL- 6B gradient programmer and a Reodyne 7125 injector equipped with a 20 µl loop. Detection was achieved in a Shimadzu SPD-6AV UV- VIS spectrophotometric detector set at 440 nm and a Shimadzu RF- 535 fluorescence detector.

Carotenoids were identified through comparison with the standards from: a) the behaviour in CC; b) their UV- UIS spectra; c) their partition between n- hexane and 95% ethanol; d) their R_f- values in TLC; e) the presence of the allylic OH group determined by the acid $CHCl_3$ test; f) the epoxide test; g) the mass spectrum (Vetter et al., 1971).

Carotenoid pigment standards were purchased from the Hoffman-La Roche Company, Switzerland, the International Agency for the ^{14}C Determinations, Denmark, and Sigma Chemical Company, USA.

Carotenoids recorded in investigated fishes belong to three groups (hydrocarbon, hydroxcarotenoids and ketocarotenoids) which chemical, structural and semisystematical characteristics have been placed in monographs of Foppen (1971), Isler (1971) and Straub (1987). We have also used those monographs in our investigations. Quatitative analyses were performed with UV- UIS spectroscopy according to Davies methods (Czeczuga, 1988). The structure of carotenoids was described by Straub (1987). Chromatography methods (CC, TLC, HPLC) were described in detail by Bernhard (1995), Schiedt (1995) and Pfander and Riesen (1995) respectively. The results were evaluated with Scheffe Test (Winer, 1997).

RESULTS AND DISCUSSION

Fifteen (15) carotenoids were found in the examined material (Table 1, Figure 1). Most of them were common in fish, but some, such as 3,4- didehydro- α- carotene, 4-hydroxy- α- carotene, phoenicopterone and 7,8- didehydroastaxanthin were rather rare. In most of the species, lutein, zeaxanthin, phoenicopterone and astaxanthin were found (Table 2). In the investigated material, 3,4-didehydro- α- carotene, zeaxanthin, phoenicopterone, phoenicoxanthin, canthaxanthin and astaxanthin were the predominant group. The total carotenoid content in the investigated fish species ranged from 0.086 (*Merluccius merluccius*) to 0.424 µg g^{-1} wet mass (*Pterothrissus belloci*). Mean value for all investigated species averaged 0.200 µg g^{-1} wet mass.

The total carotenoid content in the fillets of the species from African fishing areas in comparison with species from other fisheries were considered (mean 0.200 µg g^{-1} wet mass). The mean value for nine species from the region of the Falkland Islands was 0.08 (Czeczuga and Klyszejko, 1978) and for 10 species from the Szczecin Lagoon of Baltic Sea- was 0.230 µg g^{-1} wet mass of fillets (Czeczuga and Klyszejko, 1996). Similar values were observed in fillets of fishes from Black Sea fisheries (Czeczuga, 1973), New Zealand fishing areas (Czeczuga et al., 2000) and from the waters washing the Japanese Islands (Tsukuda and Amono, 1966; Matsuno et al., 1974; Matsuno and Katsuyama, 1976; Miki et al., 1982).

In fillets from Antarctic fish species, especially in representatives of Nototheniidae and Chaenichthyidae (white blooded fish) families, total carotenoid content was low (Czeczuga, 1978a, b; 1982). In Atlantic (Torrissen et al., 1989; Czeczuga et al., 2005) and Pacific salmonids (Jarzombek, 1970; Crozier, 1970; Kitahara, 1984), the biggest amounts of carotenoids are retained in the fillets.

Apart from carotenoids commonly occurring in fishes, some rare types have also been found. One of them is 3,4- didehydro- α- carotene. It was first isolated from the body of sheatfish *Silurus glanis* (Czeczuga, 1977). Although it has been found in material from six examined species, only in *Merluccius merluccius* was it a predominant carotenoid. 4- hydroxy- α- carotene- also called β,ε- carotene- 4-ol, was first observed in plant material by Zechmeister in 1958 (Isler, 1971). It also occurred in sheatfish (Czeczuga, 1977a). We found it only in 2 of the 16 investigated species; in *Belone belone* and *Centrolophus niger*. Phoenicopterone and 4- keto- α-carotene derivative is frequently called 4- keto- α-carotene. It was observed in nine species and in *Belone belone* and *Dentex macrophtalmus* specimens as predominant carotenoid. Phoenicopterone was found for the first time in certain green algae as extra-plastidic pigment (Goodwin, 1980). 7,8- didehydroastaxanthin, astaxanthins' derivative, was being frequently called asterinic acid in previous studies and has been noted only in *Belone belone* and *Clupea pilchardus* individuals. This acetylenic Zeaxanthin occurs in fillets of investigated 13

Table 1. Carotenoid list from investgated material

Carotenoid	Summary formula	Structure (Figure 1)	Semisystematic name
α- Carotene	$C_{40}H_{56}$	A-R-B	β,ε- Carotene
β- Carotene	$C_{40}H_{56}$	B-R-B	β,β- Carotene
ε- Carotene	$C_{40}H_{56}$	A-R-A	ε,ε- Carotene
3,4- Didehydro- α- carotene	$C_{40}H_{54}$	A-R-C	3,4- Didehydro- β,ε- carotene
β- Cryptoxanthin	$C_{40}H_{56}O$	B-R-D	β,β- Carotene- 3- ol
4- Hydroxy- α- carotene	$C_{40}H_{56}O$	A-R-E	β,ε- Carotene- 4- ol
Lutein	$C_{40}H_{56}O_2$	F-R-D	β,ε- Carotene- 3,3'- diol
Tunaxanthin	$C_{40}H_{56}O_2$	F-R-F	ε,ε- Carotene- 3,3'- diol
Zeaxanthin	$C_{40}H_{56}O_2$	D-R-D	β,β- Carotene- 3,3'- diol
Phoenicopterone	$C_{40}H_{54}O$	A-R-G	β,ε- Carotene- 4- ol
4'- Hydroxyechinenone	$C_{40}H_{54}O_2$	E-R-G	4'- Hydroxy- β,β- caroten- 4-one
Canthaxanthin	$C_{40}H_{52}O_2$	G-R-G	β,β- Carotene- 4,4'- dione
Phoenicoxanthin	$C_{40}H_{52}O_3$	G-R-H	3- Hydroxy- β,β- carotene- 4,4'-dione
Astaxanthin	$C_{40}H_{52}O_4$	H-R-H	3,3'- Dihydroxy- β,β- carotene- 4,4'-dione
7,8- Didehydroastaxanthin	$C_{40}H_{50}O_4$	I-R_1-H	3,3'- Dihydroxy- 7,8- dihydro- β,β- carotene- 4,4'-dione

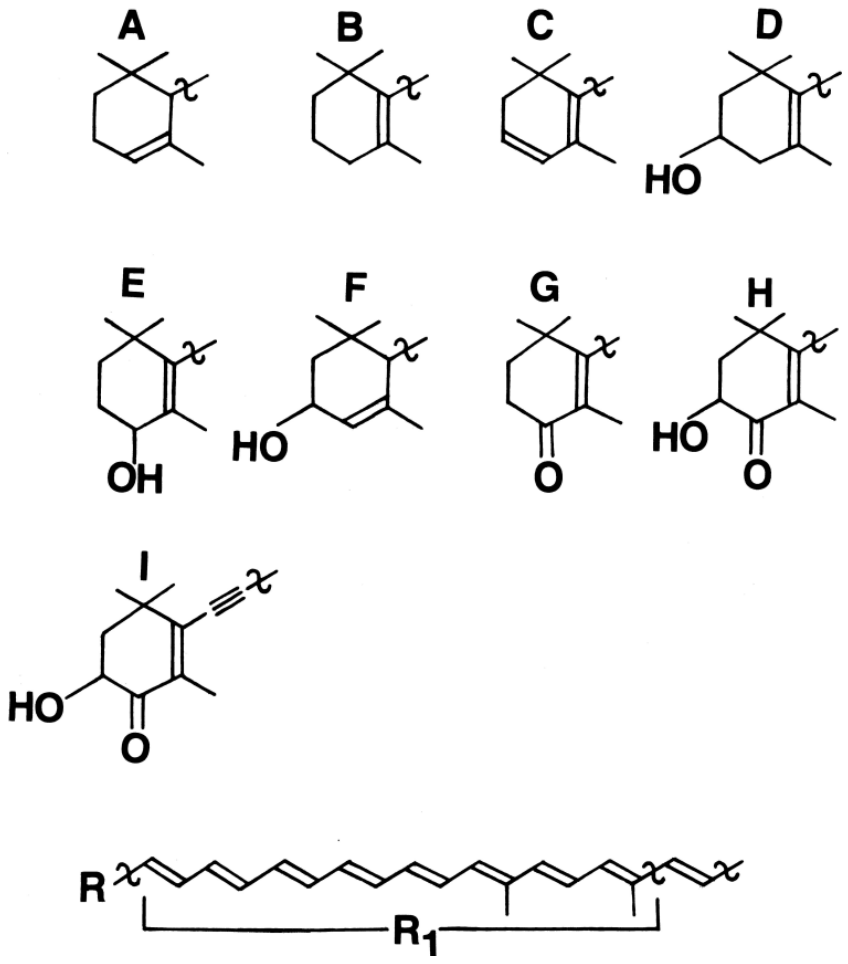

Figure 1. Structural features of carotenoids from the analysed materials. A- I, end group designation of carotenoids; R, R_1 polyene chain.

Table 2. Carotenoid content in the investigated material.

Specie	Carotenoid (Table 1)	Content of particular carotenoids (%)	Total content (µg g^{-1} wet mass) ± SD
Acanthias acanthias L.	2,3,7,8,9,14	9 (33.6), 2(21.3), 14 (19.2), 7 (9.5), 8 (9.5), 3 (7.5)	0.089
Belone belone (Brünn.)	6,7,9,10,15	10 (21.9), 9 (21.5), 15 (20.9)l, 6 (18.4), 7 (17.3)	0.143
Centrolophus niger (Gmelin)	5,6,7,11,12,14	12 (23.1), 11 (22.8), 14 (20.9), 5 (15.7), 6 (10.2), 7 (7.3)	0.121
Clupea pilchardus Walb.	7,9,10,14,15	14 (47.6), 10 (28.5), 15 (8.2), 9 (8.1), 7 (7.6)	0.259
Dentex macrophtalmus (Bloch.)	7,8,10,14	10 (43.0), 7 (39.2), 14 (10.1), 8 (7.7)	0.153
Engraulis encrasicholus L.	2,3,7,9,10,14	14 (33.2), 10 (30.8), 2 (20.5), 3 (8.6), 7 (4.2), 9 (2.7)	0.258
Genypterus capensis (Smith.)	1,2,4,5,7,9,14	9 (22.6), 14 (21.8), 5 (18.4), 2 (15.0), 1 (12.4), 4 (6.8), 7 (3.0)	0.231
Macrurus aequalis (Ginter)	1,3,4,5,9,10,12	12 (19.4), 5 (18.3), 9 (16.5), 10 (14.9), 1 (12.8), 3 (11.6), 4 (6.5)	0.352
Merluccius merluccius L.	4,5,7,8,9,10,14	4 (26.7), 7 (24.8), 8 (21.4), 5 (18.8), 9 (4.4), 10 (2.7), 14 (1.2)	0.086
Myliobatis aquila L.	3,7,9,13,14	13 (36.0), 7 (28.2), 14 (25.4), 9 (7.6), 3 (2.8)	0.202
Pterothrissus belloci (Cadenat)	3,4,5,9,10,12	12 (34.4), 5 (20.6), 10 (19.2), 9 (18.6), 3 (5.4), 4 (1.8)	0.424
Sarda sarda (Bloch)	2,5,7,10,14	14 (44.5), 10 (30.2), 2 (18.4), 5 (6.2), 7 (0.7)	0.305
Sebastes dactyloptera (Dela Roche)	2,4,5,7,9,12,14	9 (28.6), 2 (26.4), 5 (18.8), 7 (12.2), 4 (10.3), 14 (2.5), 12 (1.2)	0.265
Trachurus trachurus L.	1,2,3,5,9,14	14 (22.8), 2 (21.4), 1 (20.8), 3 (18.4), 5 (14.2), 9 (2.4)	0.108
Trigla lyra L.	4,5,9,10,12,14	12 (24.2), 5 (22.2), 10 (21.4), 14 (20.4), 4 (8.2), 9 (3.6)	0.090
Zeus faber L.	2,7,8,9,14	14 (25.4), 2 (24.8), 7 (21.3), 9 (17.6), 8 (10.9)	0.111

species, while lutein was found in 12 of them. Zeaxanthin appears predominant in *Acanthias acanthias*, *Genypterus capensis*, and *Sebastes dactyloptera*.

These are the carotenoids belonging to the yellow group and are the principle carotenoids of the lens and the macula of humans and primate eye (Schalch et al., 2009). The role of this dihydroxy compounds in risk reduction of macular degeneration and cataract of the eye are important. From all of the environmental factors such ones as food (Simpson et al., 1981; Latscha, 1990) increases the carotenoid content in fish.

The studies included 17 species of invertebrates (food of fish) belonging to Porifera, Coelenterata, Annelida, Crustacea, Mollusca and Echinodermata which were caught along the coast of West Africa (Czeczuga and Klyszejko, 1977). The investigations showed that they have been the richest in carotenoids. 27 particular carotenoids have been identified. Total carotenoid content in this material varied from 0.039 to 1.529 µg g^{-1} fresh weight. The lowest amount of carotenoids was found in the representatives of Coelenterata and the highest in the representatives of Crustacea. In natural conditions, the total carotenoid content and the mount of keto- and dihydroxy-carotenoids in the respective parts of the fishes' body depends not only on the type of food it consume but also on the parasites and the organochlorine pollutants. The effect of ecto- and edoparasites on the carotenoid content in fish in natural infection was also described (Czeczuga et al., 2009). This study was conducted using pairs of the host- parasite with ectoparasites and endoparasites. In all examined pairs of host and parasite, the parasitic organism has always had the higher total carotenoid content in comparison to the host. Carotenoid content in healthy and infected fishes ranged from 3.1 (*Mollienisia latipinna*) to 51.9 (intestines of *Tinca tinca*). Obtained data indicates that some carotenoids are selectively accumulated by respective parasites.

Water polluted with organochlorine substances causes the so called M74 syndrome in fish, especially in salmons (Vuorinen et al., 1997). Afflicted are the females, especially the eggs which are pale yellow as they have a low red carotenoid content (especially low astaxanthin level) (Pettersson and Liguell, 1999; Czeczuga et al., 2002, 2005). Most of the larvae which develop from such eggs die when they begin the active feeding process. M74 syndrome occurs not only in Baltic salmon, but has also been noted in the sea trout *Salmo trutta* m. *trutta* (Czeczuga et al., 2005). The effect of chemical water pollution on the larval form of other fishes is well known in

other latitudes. The high mortality of early life- stage salmonids including Pacific salmon from some of the Great Lakes of North America has been reported under the name of Early Mortality Syndrome (EMS) (McDonald, 1995). Clinical symptoms were similar to those noted in M74 in salmons from the Baltic Sea or in EMS in other salmonid species from the North American Great Lakes; were observed in Atlantic salmon specimens with Cayuga syndrome in the New York Finger Lakes (Fisher et al., 1996). All three of those disorders responsible for mortality in early life- stage salmonids have been characterized (in females and eggs) through the low level of astaxanthin and thiamine (Fitzsimons et al., 1999).

Fish, like other animals, do not synthesize carotenoids *de novo*, which only plants, bacteria and fungi are able to synthesize. Those substances get to animals organisms only through food and some of them can be converted into other carotenoids via oxidation or reduction and into vitamin A. Carotenoids catered with food are being released in the intestines during digestion and are absorbed along the alimentary tract in fish (March et al., 1990). As revealed by studies on fish, the free vitamin A is formed not only from carotenoids which have three beta end groups (β- ring) from the β- carotene type (Simpson et al., 1981, Latscha, 1990), but also from a number of xantophylls, including those from dihydroxy compounds-such as lutein, zeaxanthin or tunaxanthin (Katsuyama and Matsuno, 1987) as well as astaxanthin and canthaxanthin belonging to ketocarotenoids compounds (Guillou et al., 1989). Both, dihydroxy compounds and ketocarotenoids are quite common in fish species from the fisheries of Africa.

The shift of carotenoids in the pre- and postreproductive period occurs in fishes. At first, the shift has been reported only from the salmonids in the Pacific (Crozier, 1970; Kitahara, 1983). Further studies have showed, that this phenomenon is also typical to salmonid species from the *Salmo* genus (Czeczuga and Chelkowski, 1984) and freshwater fish species (Czeczuga and Czeczuga-Semeniuk, 2002). Differences are noted in the shift between sexes. Both, in males and females, the liver is the main reservoir of carotenoids, and on the second range, the intestines. In the females, in prespawning season, carotenoids mostly shift to the gonads and in males mainly to the skin and fins giving the mating colouration. A similar pattern occurs in amphibians (Czeczuga et al., 2006).

Conclusions

In the fillets of 16 fish species from the fisheries of West African coast, 15 carotenoids were found. Total carotenoids content ranged from 0.086 to 0.424 with mean value of 0.200 μg g^{-1} wet mass. Seven out of 15 carotenoids such as α- carotene, β- carotene, β- cryptoxanthin, lutein, zeaxanthin, canthaxanthin and astaxanthin play a significant role in the humans' health. They all have been postulated to increase an immune (Chew and Park, 2009) and cancer protective role (Rock, 2009) in mammals. B- carotene and other carotenoids from this group behave as antioxidant factors at low oxygene pressure and as pro- oxidants at higher oxygene partial pressure (Yeum et al., 2009).

B- carotene and lutein have been postulated to have the antiproliferative effect on the cells (Palozza et al., 2009). Known are the effects of those carotenoids on the cell signalling and communication including cell cycle, apoptosis, cell differentiation and growth factors (Wang, 2009). Also, such derivatives of β- carotene as the β-apocarotenales are biologically active. Those carotenoid metabolites can offer protection against chronic diseases and certain cancers. The photoprotective effects of those carotenoids especially β- carotene towards skin damage induced by UVA and UVB have been reviewed (Goralczyk and Wertz, 2009). Concluding, α- carotene, β-carotene, β- cryptoxanthin, lutein and zeaxanthin may play a significant role in the coronary heart disease (Johnson and Krinsky, 2009). This all carotenoids are quite common in fish species from the fisheries of West Africa.

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