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Tocopherol composition of lipid in the carp (*Cyprinus Carpio* L.) grown in different production systems

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Tocopherol composition of lipids isolated from carp meat grown in different production systems was investigated by HPLC with fluorescence detection. The total quantity of tocopherols was found to be between 70 - 430 mg/kg. α-Tocopherol predominates in the lipids of carp breeding in earthen ponds (Fish-Farming Experimental Facility in Tri voditsi village-pond No 10; Fish-Farming "Tundzha 79" Ltd - pond No 4 and No 5), in free aquatic environment of Tzarimir 1 reservoir, Tzarimir 2 reservoir, "40 springs" reservoir, Bistritsa reservoir and in net-cages farm situated in the Bistritsa reservoir and in net-cages farm situated in the Kardzhali reservoir. In lipids from carps grown in Bistritsa reservoir and in net-cages situated in the same reservoir a significant amount of γ-tocopherol (65.0% and 38.0% respectively) was established. δ - Tocopherol was detected only in lipids from carp grown in earth pond of Fish-Farming Experimental Facility in Tri voditsi village while β - tocopherol was found in lipids from carp of the Bistritsa reservoir and net-cages situated in the same reservoir.

Keywords: *Cyprinus carpio* L.; fat soluble vitamins; tocopherols, HPLC

INTRODUCTION

The interest in the commercial fish species, subject of the fresh water aquaculture, is determined by their nourishment significance as protein food for people, as well as by the content of the full complex of essential amino acids, fatty acids, vitamins, macro and microelements in their meat. Fish fat is a main supplier of considerable quantities of important for the human organism vitamins, i.e. A, D, E, B_1, B_2, B_{12}, niacin (PP), which are involved in important processes in the human organism.

The lack of enough scientific information about the composition and content of tocopherols in the fats of the carp fish is the reason for this research. Thanks to its antioxidant properties, vitamin E prevents the development of atherosclerosis[1] and the destruction of erythrocytes, and provides the free inflow of oxygen into all cells of the human organism. The biologically active isomer of vitamin E - α-tocopherol acts as antioxidant protection of the membrane structures against to oxidation [2,3]. According to Bramley et al.[1] the antioxidant stability of vitamin E is due to the phenol hydroxyl group and the number of methyl groups of the aromatic ring. Among all isomers and analogues α-tocopherol is characterized with greatest biological activity [2].

In relation to the said by far, the purpose of this research is to determine the quantitative and qualitative composition of tocopherols of fats in the carp (*Cyprinus carpio* L.), grown in different production systems.

EXPERIMENTAL

The quantitative and individual tocopherol composition of fish fat in scaly carp (*Cyprinus carpio* L.) of market size bred in nine production systems, with different level of intensity and feeding, which characteristics are presented on Table1 is studied.

During the investigation in the period May-October 2012-2013 the physicochemical characteristics of the aquatic environment are recorded on monthly intervals. The measured water temperature, pH and concentration of dissolved oxygen were within the technological norms for carp ponds ensuring proper gowning conditions.

For the purposes of the study a representative number of fishes are taken at random principle of selection [3], from each production system, whereas the total processed number consists of 45 samples. The individual samples subject to analysis are

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Table 1. Characteristics of investigation production systems

Type of production systems	**Investigation production systems**								
	Earthen ponds -Fish -Farming			Reservoirs - free aquatory				Net-cages farm situated in reservoirs	
Sites	*Tri voditsi pond No 10*	*„Tundzha 79" pond No 4*	*„Tundzha 79" pond No5*	*"Bistritsa"*	*"Tzarimir 1"*	*"Tzarimir 2"*	*"40 springs"*	*"Bistritsa"*	*„Kardzhali"*
Area, dka	45	750	200	204	500	40	489	80	156
Volume, m^3	58 500	1125000	300000	714000	1250000	80000		320000	936000
Depth, m	1.3	1.5	1.5	3.5	2.5	2.0		4.0	6.0
Degree of technology intensification	Semi-intensive carp rearing	Intensive carp rearing	Intensive carp rearing	Semi-intensive carp rearing	Semi-intensive carp rearing	Intensive carp rearing	Extensive carp rearing	Intensive carp rearing	Intensive carp rearing
Feeds for fish feeding	Sunflower groats and grain	Extruded feed	Pelleted feed	Extruded feed and grain	Extruded feed and grain	Extruded feed	No feed	Extruded feed	Pelleted feed

prepared from the muscular tissue (lateral muscle) of the fish, from one and the same location by separating the skin with the subcutaneous fat and subsequent grinding and homogenization (meat).

The fat content of the fish was determined according to a Schmid-Bondzynski-Ratzlaff method [5]. Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) on a Merck-Hitachi (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector Merck-Hitachi F 1000. The operating conditions were as follows: mobile phase of n-hexane:dioxan (96:4, v/v), flow rate 1.0 mL/min, excitation 290 nm, emission 330 nm [6]. 20 μL 1% solution of oil in hexane were injected. The individual tocopherols were identified by comparing the retention times with those of standards (reference individual pure tocopherols – DL-α-, DL-β-, DL-γ- and DL-δ-tocopherol with purity ≥98%) purchased from Merck (Darmstadt, Germany). The content of tocopherols in the oils were calculated by comparing the peak areas obtained for the relevant tocopherol in the sample with those obtained for the standard solutions with known concentrations.

RESULTS AND DISCUSSION

The content of lipids in the tested carp fish from different production systems varies from 0.67% (pond No 5 in Fish-Farming "Tundzha 79") to 8.25% (net - cages farm situated in the Bistritsa reservoir) (Table 2). It can be seen that the fat content in the carp fish bred in floating net cages and in dams is higher than that of the carp fish bred in ponds. The obtained results about the fat content of the carp are similar to the data published in earlier researches (3.08 – 6.76%) [7-10], whereas in literature sources there are quoted even higher values for the total lipid content of the carp fish (12.74g/100g) [11].

The average values of the reported quantity of tocopherols (Table 2) in carp fish fat vary significantly from 70 mg/kg (Tzarimir 1 reservoir) to 430 mg/kg (Fish-Farm "Tundzha 79" – pond No 5). The comparison of the data that we have obtained with such by other authors [12,13] indicates that the carp fish fat contains considerably higher quantity of tocopherols, compared to other fish species, both sea and fresh water.

Table 2. Average weight, fats and tocopherols of fat in the carp

Parameters	**Investigation production systems**								
	Earthen ponds- Fish -Farming			Reservoirs - free aquatory				Net-cages farm situated in reservoirs	
	Tri voditsi pond No 10	*„Tundzha 79" pond No 4*	*„Tundzha 79" pond No5*	*"Bistritsa"*	*"Tzarimir 1"*	*"Tzarimir 2"*	*"40 springs"*	*"Bistritsa"*	*„Kardzhali"*
Weight, g	1159	989	1206	909	1469	2217	505	810	1533
Fats, %	0.74	1.03	0.67	2.45	5.43	5.10	1.03	8.25	5.03
Tocopherols, mg/kg	149	227	430	260	70	103	281	98	196

Table 3. Tocopherol composition of lipids in the carp meat

Tocopherols, %	Investigation production systems								
	Earthen ponds –Fish -Farming			Reservoirs - free aquatory				Net-cages farm situated in reservoirs	
	Tri voditsi pond No 10	*„Tundzha 79" pond No 4*	*„Tundzha 79" pond No5*	*"Bistritsa"*	*"Tzari-mir 1"*	*"Tzari-mir 2"*	*"40 springs"*	*"Bistritsa"*	*„Kardzhali"*
α-T	73.7	100.0	96.2	27.0	95.4	84.7	91.4	50.6	99.0
β-T	-	-		8.0	-	-	-	11.4	-
γ-T	10.2	-	3.8	65.0	4.6	15.3	8.6	38.0	1.0
δ-T	16.1	-	-	-	-	-	-	-	-

Legend: α-T – α- Tocopherol; β-T – β-Tocopherol; γ-T – γ- Tocopherol; δ-T – δ- Tocopherol

Stancheva et al.[12] inform that the vitamin E content in pike perch is about 0.5 mg/100g, while in herring is respectively 0.76 mg/100g. Stancheva et al.[13] provide values about the content of tocopherols in sprat fish and gobies about 284.85 ± 44.50 μg/100g, while for the rainbow trout 809.1 μg/100g. According Merdzhanova et al.[8] vitamin E content in bighead carp fillets is 1097.03±44.06 $\mu g.100g^{-1}$ww during the spring and 1051.80±37.11 $\mu g.100g^{-1}$ww during the autumn.

The recorded quantities of tocopherols in the carp fish fat ranging 103 - 430 mg/kg for some production systems give us the grounds to define this fish as a good source of vitamin E.

The content of the indentified classes of tocopherols (α-, β-, γ- и δ- tocopherol) is shown in Table 3.

In the tested production systems α-tocopherol varies from 27.0 % (free aquatory of Bistritsa reservoir) to 100% (Fish-Farming "Tundzha 79" - pond No 4). Content of β- tocopherol is found out only in the samples of fish fat from the free aquatory of Bistritsa reservoir, and net -cages situated in the same reservoir, with quantities from 8.0 % to 11.4 %. Presence of γ-tocopherol is found to be in all the studied production systems, with the exception of Fish-Farming "Tundzha 79", pond No 4. The quantity of γ- tocopherol varies from 1.0 % (net cages situated in the Kardzhali reservoir) to 65.0 % (free aquatory of Bistritsa reservoir). δ-Tocopherol was detected only in the lipids of carp fish bred in earth type pond No 10 of Fish-Farming Experimental Facility in Tri voditsi (16.1%).

The analysis of the data shows that the carp fish fats contain predominantly α-tocopherol and γ-tocopherol. In the seven of the researched fisheries (earthen ponds of Fish-Farming Experimental Facility in Tri voditsi and in Fish-Fatming "Tundzha 79"; Tzarimir 1 reservoir, Tzarimir 2 reservoir, „40 springs" reservoir; net cages in Kardzhali reservoir) α-tocopherol was detected in quantity over 70% of their total content. This shows that the studied fish fat is predominated by the biologically active α- tocopherol, so therefore the fish oil obtained from these fisheries may be classified as α- type. The carp fish lipids, isolated from the fish bred in free aquatory of Bistritsa reservoir are predominated by γ- tocopherol (65.0%) so the oil is classified as γ- type. In the carp fish lipids from fish bred in net cages situated in the same reservoir - the quantity of α-tocopherol is 50.6%, while of γ- tocopherol is 38.0%.

CONCLUSION

It has been found out that the carp fish fat is dominated by the contents of α-tocopherol and γ-tocopherol. For seven of the studied fisheries (earthen ponds of Fish-Farming Experimental Facility in Tri voditsi and in Fish-Fatming "Tundzha 79"; Tzarimir 1 reservoir, Tzarimir 2 reservoir, „40 springs" reservoir; net cages in Kardzhali reservoir) α-tocopherol comprise of over 70% of their total content.

The recorded quantities of tocopherols in the carp fish fat is within the range 103 - 430 mg/kg so it can be defined as good source of vitamin E for the human organism.

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ТОКОФЕРОЛОВ СЪСТАВ НА ЛИПИДИ ОТ ШАРАН (*CYPRINUS CARPIO* L.) ОТГЛЕЖДАН В РАЗЛИЧНИ ПРОИЗВОДСТВЕНИ СИСТЕМИ

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(Резюме)

Изследван е токфероловия състав на липиди, изолирани от месо на шаран (*Cyprinus Carpio* L.), отглеждан в различни производствени системи чрез високоефективна течно-течна хроматография. Общото съдържание на токфероли в липидите, изолирани от шараните варира от 70 до 430 mg/kg. α- Токоферолът доминира в липидите от шаран, отглеждан в земните басейни на рибно-експерименталната база в с. Три водици, басейн №10, рибовъдно стопанство „Тунджа 79", басейни №4 и №5; язовири „Царимир 1", „Царимир 2" и язовир „40 извора", както и в садковите установки на язовир „Бистрица" и язовир „Кърджали". В липидите от шаран, отглеждан в язовир „Бистрица" и в садките на същия язовир е установено значително количество на γ-токоферол (65,0% и 38,0% съответно). δ – Токоферол е установен само в липидите от шаран, отглеждан в басейн № 10 на рибно-експерименталната база в с. Три водици, а β – токоферол е установен в липидите на шаран, отглеждан в язовир „Бистрица" и в садките на същия язовир.