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VITAMIN CONTENT AND FATTY ACIDS COMPOSITION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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ABSTRACT

The aim of present study is to evaluate the composition and the content of fatty acids (FA) and fat soluble vitamins (A, E, D_3) in the edible tissue of farmed rainbow trout from the region of Central Bulgaria.

All-trans-retinol (vit. A), cholecalciferol (vit. D_3) and α-tocopherol (vit. E) were analyzed simultaneously using HPLC system with UV (vitamin A and D_3) and fluorescence detection (vitamin E). The sample preparation procedure includes saponification and liquid-liquid extraction of the unsaponifiable matter. Total lipids were extracted according to Bligh and Dyer method. Analysis of fatty acid methyl esters were performed using gas chromatography system with MS detection.

It was found that the lipid fraction contains substantial amounts of palmitic, palmitoleic, stearic, linolenic, arachidonic and docosahexaenoic fatty acids and fat-soluble vitamins. The retinol content in the fresh edible tissue of rainbow trout *(Oncorhynchus mykiss)* was 22.3±2.0 μg/100g; cholecalciferol – 6.0±0.29 μg/100g and α-tocopherol – 809.1± 56.0 μg/100g.

Linoleic acid (15.81%), docosahexaenoic acid (9.40%) and arachidonic acid (4.21%) were the most dominant polyunsaturated fatty acids, about 33% of total FA content. Palmitic acid (12.93%), tetracosanoic acid (3.76%) and oleic acid (3.57%) were found to be the dominant of the saturated and unsaturated FA in rainbow trout fillets.

Keywords: fat-soluble vitamins, PUFA, HPLC, GS/MS, trout

INTRODUCTION

Fish tissue is a good source of fats, proteins, vitamins and minerals and important component of balanced diet. Omega-3 and omega-6 fatty acids (FA), as well as fat-soluble vitamins are essential compounds of fish lipids and exclusively provided by the diet.

Being component of membrane lipids, the essential FAs maintain the integrity, flexibility and permeability of membranes, provide substrate for the biosynthesis of biologically active eicosanoids. It has been shown that omega-3 FAs exert antihypertensive, antiarrhythmic, antidepressive, and immunomodulatory effect. Acting as an-

tiatherogenic substances they can reduce the risk of ischemic heart disease. Along with omega-3 fatty acids, omega-6 fatty acids play a crucial role in brain function as well as normal growth and development. Polyunsaturated fatty acids (PUFAs), stimulate skin and hair growth, regulate metabolism, and maintain bone and reproductive health.

The fat-soluble vitamins are essential nutrients controlling a diversity of biologically important processes in human body. Vitamin A, also called retinol, takes place in photoreception and regulates gene expression and cell division, bone growth, teeth development, reproduction etc. Vitamin D_3 (cholecalciferol) plays crucial role in the regulation of calcium – phosphate balance stimulating calcium absorption by the small intestine and thus regulating bone metabolism. The biologically active isomer of vitamin E – alpha-tocopherol (a-TP) acts as an antioxidant, protecting membrane structures, essential fatty acids, and vitamins A and C from oxidation.

Being one of the major sources of omega-3 PUFA and fat-soluble vitamins A, D_3 and E, fish production by fish farming attains great economical importance. On the other hand, the worldwide decline of ocean fisheries stocks has provided impetus for rapid growth in fish and shellfish farming, or aquaculture [1].

Due to its economical importance, one of the most widely farmed fish in our country is the rainbow trout. Home of this fish is North America, where it inhabits cold and clear freshwater ponds. Trout is the preferred fish species for breeding and consumption because of its rapid growth and rich and diverse composition of the meat [2]. Rainbow trout (*Oncorhynchus mykiss*) is a predator with a varied diet – eats almost anything. Rainbows are not quite as piscivorous or aggressive as brown trout or lake trout (char). Larger fishes are active predators – they feed with fish including young brown trout, rainbow trout, shad and whitefish. They also eat small terrestrial animals that fall into the water.

Since the fish tissue is a valuable source of essential nutrients, a detailed analysis for evaluation the nutrient composition and content, and the effect of various factors (location, season of catch, water salinity, age, sex, part of the body, size and feeding) on fish lipids is needed.

The aim of present study is to evaluate the composition and the content of fatty acids (FA) and fat-soluble vitamins (A, E, D_3) in the edible tissue of farmed rainbow trout from the region of Central Bulgaria.

MATERIALS AND METHODS

Collection of fish species

Samples of rainbow trout fish species, caught from fish farm (Plovdiv region, Hvoina village), which were fed on commercial feed mixtures, were purchased from Varna fish market during March 2010. Three specimens of trout with average length of 21-28 cm and weight of 300 ± 30 g (three years old) were used as raw material for FA and vitamin analysis. Biological characteristics of fish species were determined and noted.

FA ANALYSIS

Lipid extraction

Prior to analysis, the head, tail, fins, and viscera of the fish were removed. The edible tissue was filleted with the skin left on and homogenized. Aliquots of freshly prepared homogenate (5,00g) were extracted by the method of Blight and Dyer [3] using chloroform/methanol/water in a ratio 2:2:1. After phase separation, the chloroform extracts were evaporated to dry residue and were quantified by weight. The total lipid content was determined gravimetrically.

Preparation of FA methyl esters

The dry residue of the chloroform fraction was methylated by base-catalyzed transmethylation using 2M KOH in methanol and n-hexane [4]. After centrifugation (3500 rps), the hexane layer was separated and analyzed by GC-MS.

GS-MS analysis

Gas chromatography was performed by a model FOCUS Gas Chromatograph with autosampler A 2000, equipped with Polaris Q MS detector (Thermo Scientific,USA). The capillary column used was a TR-5 MS (Thermo Scientific,USA) universal column 30 m length and 0.25mm i. d., with a wide range of applications of food analysis. Helium was used as a carrier gas at flow rate 1 ml/min. Peaks were identified according to two parameters: Retention Time (RT) based on available fatty acid methyl esters (FAME) mix standard (SUPELCO F.A.M.E. Mix C4-C24) and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA). FAMEs were identified and quantified by comparison with the RT and peak areas of SUPELCO standards. All chemicals used were of analytical and GC grade (Sharlau, Spain).

ANALYSIS OF FAT-SOLUBLE VITAMINS

Saponification and extraction

The sample preparation was performed using the method of Sanchez-Machado et al [5] with slight modifications.

An aliquot of the homogenized sample (1,00g) was weighed into a glass tube with a screw cap and 1% of methanolic L-ascorbic acid and 1M methanolic potassium hydroxide were added. Three parallel samples of fish edible tissue were prepared and subjected to saponification at 80°C for 20 min. The non-saponified components were extracted with n-hexane and the extract was evaporated under nitrogen. The dry residue was dissolved in MeOH and injected (20 μl) into the liquid chromatograph system.

HPLC conditions

We used reversed-phase high-performance liquid chromatographic (HPLC) for the vitamin analysis. Three fat-soluble vitamins A, D_3 and E were analyzed simultaneously using HPLC system (Thermo Scientific Spectra SYSTEM) equipped with

analytical column ODS2 Hypersil™ 250 x 4, 6 mm, 5u, UV and fluorescence (vitamin E) detection. The mobile phase was composed of 97:3 = MeOH : H_2O, flow rate 1ml/min. The qualitative analysis was performed by comparing the retention times of pure substances vitamin A and D_3 absorbing at λ_{max} = 325nm for vitamin A; λ_{max} = 265 nm for vitamin D_3 and alpha-TP fluorescence at λ_{ex} = 288 nm and λ_{em} = 332 nm. The quantitation was done by the method of external calibration on the basis of comparing the chromatographic peak areas of the corresponding standards (Retinol solution, Fluka; DL-alpha Tocopherol, Supelco; Cholecalciferol, Supelco).

Statistical analysis

Standard curves for all-trans-retinol, cholecalciferol and α-tocopherol were obtained using six different concentrations of standard solutions in triplicate. Reproducibility, estimated by the coefficients of variation for determinations of ten parallel samples was calculated. For the determination of the analytical recoveries, samples of homogenized fish tissue were spiked with a methanolic solution containing known amounts of three fat soluble vitamins. All samples were analyzed in triplicate. The results were expressed as average and standard deviation (mean ± SD). Total lipid content of edible tissue was determined for each group (n = 5) and the results were present as g per 100 g raw tissue.

RESULTS AND DISCUSSION

Fat-soluble vitamin content

Our results showed that rainbow trout edible tissue contains highest amounts of alpha-tocopherol, followed by retinol and cholecalciferol (table 1). Data from the Danish data base [6] for fat-soluble vitamin content of farmed rainbow trout are in good agreement with our results. On the other hand Dias M. G. et al, (2003) [7] was found substantially lower content of the vitamin E in wild rainbow trout fresh tissue (table 1).

Table 1. *Fat-soluble vitamin content in edible tissue of rainbow trout (mean ± SD)*

Rainbow trout		
α-tocopherol [μg/100g]	all-trans-retinol [μg/100g]	Cholecalciferol [μg/100g]
809.1±56.0	22.3±2.0	6.0±0.3
Danish data base		
1100.0	12.3	5.1
[Dias M.G. et al]		
130	8.8	19

The rainbow trout remains the highest source of alpha-tocopherol when compared with some Black sea fish species such as European sprat and goby with alpha-tocopherol content found to be 284.85 ± 44.50 μg/100g and 614.90 ± 40.30 μg/100g, respectively [8].

Since the analytical method revealed good reproducibility (3.42–7.44%, estimated by the coefficients of variation for ten parallel samples) and analytical recoveries (ranges 72.8–92.7%), evaluated after spiking samples with known amounts of standards) and since we used samples of farmed fish species, the only possible reason for the higher content of fat-soluble vitamins is the diet enriched with fat-soluble vitamins.

Fatty acid composition

The fatty acids compositions of rainbow trout are given in Table 2.

Table 2. *Fatty acids profile in edible fish tissue (mean ± SD)*

Unsaturated FA	Mean± SD	Monounsaturated FA	Mean± SD	Polyunsaturated FA	Mean± SD
C 12:0	2.98±0.04	C 14:1	3.64±0.32	C 18:3 n3	4.03±0.28
C 13:0	0.00	C 16:1	4.76±0.8	C 20:5 n3	2.21±0.05
C 14:0	2.03±0.04	C 17:1	0.00	C 20:4 n6	4.21±0.39
C 16:0	12.93±1.02	C 18:1 n 9 t	0.76±0.01	C 20:3 n3	0.00
C 17:0	0.00	C 18:1 n9 c	11.03±0.99	C 20:3 n6	2.31±0.2
C 18:0	3.57±0.26	C 20:1	2.40±0.23	C 20:2	2.09±0.05
C 20:0	3.07±0.1	C 22:1 n9	3.17±0.32	C 22:6 n3	9.40±1.2
C 21:0	0.00	C 24:1	2.78±0.1	C 22:2	0.50±0.01
C 22:0	1.05±0.05	**Total MUFA**	**28.54**	**Total PUFA**	**43.13**
C 23:0	0.00	C 18:3 n6	3.11±0.24	**n – 3**	**15.64**
C 24:0	3.76±0.01	C 18:2 n6 t	2.63±0.3	**n – 6**	**25.44**
Total SFA	**28.33**	C 18:2 n6 c	13.18±2.24	**n – 3/n – 6**	**0.62**

The present study indicated that the analyzed trout (12.30 g/100 g raw tissue) could be characterized as highly fat fish.

The FA composition of tissue lipids in fish is strongly influenced by the FA in their dietary lipids [9]. It has been shown that the amount of FA varies widely among the species but in most studies the palmitic (C 16:0) and stearic (C 18:0) acids are the predominant SFA. The total SFA content in Bulgarian rainbow trout (28.33%), was lower than the amount of SFA (25.39%) found by Haliloglu (2002) [10] probably due to the differences in the amounts of palmitic and stearic acids. Our results showed that 80% of total SFA were composed of palmitic acid. In contrast to others our results for some Bulgarian Black sea fish species [8], freshwater fish, as brown trout, shows lower concentration of SFA group. Availability of low level of this fatty acid supports the results published in many similar studies conducted on freshwater fish.

Significant differences were also observed in monounsaturated fatty acid (MUFA) among the fresh water fish species investigated by others. For example,

Haliloglu described 41.90% of total MUFA which is higher than our results – 28.54%. In the other side our results is similar to the result presents in Danish date base for SFA content in rainbow trout (28.50%) [6]. In contrast to other publications we observed similar amounts of SFA and MUFA groups. Among MUFAs the highest content was found for oleic acid (40%) followed by palmitoleic acid (16,12%). These results are in a good agreement with the data of Haliloglu [10].

The long chain omega-3 and omega-6 fatty acids commonly called PUFA and their ratios (omega-3/omega-6) are also considered important for the FA composition. The total sum of Omega-6 acids series of the analyzed fish samples was 25.44% of total FA while the sum of omega 3 FA was only 15.64% of total FA. The linoleic acid (cis+trans) was the most dominant FA in the group of PUFAs – 15.81%. Fishes are unable to synthesize any fatty acids of the omega-6 and omega-3 series unless a precursor with this structure is present in the diet. The ability to elongate and desaturate fatty acids is not the same in all fish species. The Omega-3/Omega-6 ratio has been suggested to be a useful indicator for comparing the relative nutritional value of fishes [11]. A ratio between 0.2–1.6 would constitute a healthy human diet. Our results revealed an omega-3/omega-6 ratio of 0.62 which is within the recommended range. Hearn T. showed that in freshwater fishes n 3/n 6 ratio ranged from 0.55 to 5.60 and our results confirm this. [12]

In modern nutrition studies, namely the values of EPA (C20:5) and DHA (C22:6) were used as one of the key biochemical characteristics of products consumed by human population. At the Workshop on the Essentiality of and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids held in 2000 [13], there was consensus about the importance of reducing the omega-6 and increase the omega-3 PUFA in diet of both adults and newborns for optimal brain and cardiovascular health and function.

CONCLUSION

Total lipids, fatty acids profile and fat soluble vitamin content in the rainbow trout from central Bulgaria were defined and compared. SFA was the group with the lowest level in this fish, which corresponds to their total lipid content. The highest PUFA values related to the high-level concentrations of LA, ARA and DHA. The n3/n6 ratio was found to be 0.62.

The results for the fat-soluble vitamins content in the analyzed fishes are in the same order of magnitude with those reported by other groups.

Regarding to the lipid contents, n3/n6 ratio and high level of all analyzed fat soluble vitamins we may conclude that Rainbow trout have good nutritional quality.

Like marine fish, freshwater fish constitute a healthy addition to the human diet.

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