Bulgarian Chemical Communications, Volume 49, Special Issue G (pp.118 –123) 2017

Fat soluble nutrients and fatty acids in skin and fillet of farmed rainbow trout

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Received November 12, 2016; Revised December 21, 2016

This study compares the fat soluble components in the muscle and edible skin parts of farmed rainbow trout (*Oncorhynchus mykiss W.*) fillets, sampled at two growth stages, from fish markets from Bulgaria. Insufficient information is available about the differential fat soluble pigments, cholesterol, vitamins and fatty acid compositions of rainbow trout fillets when eating them with or without the skin left on. Vitamins A, D_3 and E, β-carotene and cholesterol were analyzed simultaneously using HPLC system with UV and FL detection (vitamins A and E). Total lipids were extracted according to Bligh and Dyer method. Analysis of fatty acid methyl esters (FAME) were perform by GC/MS. The average lipid content, the cholesterol and vitamin E amounts and the saturated fatty acids were significantly higher in the skin than in the muscle, whereas the proportion of vitamin A and D_3, eicosapentaenoic acid (C20:5 ω-3) and docosahexaenoic acid (C22:6 ω-3) were higher in the muscle.

Key words: *Oncorhynchus mykiss, vitamins, carotenoids, cholesterol, PUFA*

INTRODUCTION

It is well-known that fish consumption has nutritional and health benefits in humans. Rainbow trout (*Oncorhynchus mykiss W.*) is one of the most consumed fish species in Bulgaria and also of interest to aquaculture because of the rapid growth rate and excellent nutritional qualities of the meat [1]. Nutritional quality of fish depends especially on tissue lipid composition including fat soluble vitamins, fatty acids, cholesterol and β-carotene. In the scientific literature, chemical composition of fish is investigated from different points of view. Recently a special interest in fish lipid composition has risen because of its advantageous effects of human health which depend on its fatty acid (FA) and fat soluble vitamins content. Moreover, the optimal quantities of polyunsaturated FA/saturated FA, ω-3/ω-6 FA ratios are considered as informative indices for nutritional quality. Comparative investigations on farmed rainbow trout lipids, FA, fat soluble vitamins content and cholesterol from Turkey were performed by Harlioglu A. (2012) [2]. Previous studies [3, 4] have reported data on the proximate and fatty acid composition of rainbow trout. Despite these facts, available information about the composition of different fat soluble pigments, cholesterol, vitamins and fatty acids of rainbow trout fillets (with or without the skin) is insufficient. It is important to investigate differences in fat soluble nutrients content in trout muscle and skin. Thus, the aim of the study was to investigate and compare β-carotene, vitamins (A, E, D_3), cholesterol and fatty acid profile of rainbow trout filets and skin. This is the first study on β-carotene, cholesterol, vitamins and FA composition of farmed rainbow trout filets and skin in Bulgaria. The presented results will be useful when determining what differences might exist in nutrient ingestion, depending on whether a rainbow trout fillet is consumed with or without the skin.

EXPERIMENTAL

Sample collection

Samples of rainbow trout were purchased from Varna fish market during March 2015. Fish was raised in two fish farms (Plovdiv region, Hvoina village and Dospat Dam Lake) and fed on commercial feed mixtures. Analyzed specimens were divided in two groups (with three specimens in each group): group I (Rainbow trout I) – weighing 300 - 400 g; group II (Rainbow trout II) – weighing 700 - 900 g. Each specimen was filleted, muscle tissue was separated from the skin. Two medium samples were prepared.

Vitamins, pigments and cholesterol analysis

Saponification and extraction: Two skin and two muscle tissue samples were homogenized and used for evaluation of all-trans-retinol, cholecalciferol, α-tocopherol, β-carotene and cholesterol contents. Sample preparation procedure was performed following the method of Dobreva *et al.* (2011) with some modifications [5]. An aliquot of the homogenized sample (1.000 ± 0.005 g) was weighed into a glass tube with a screw cap and 1% of methanolic L-ascorbic acid and 0.5M methanolic potassium hydroxide

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were added. Six parallel tests were prepared and subjected to saponification at 50°C for 30 min. After cooling the analytes were two times extracted from samples with *n*-hexane : dichloromethane = 2:1 (v/v) solution. The combined extracts was evaporated and redissolved in methanol : dichloromethane solution, filtrated (0.45 μm syringe filter) and injected (20μl) into the HPLC system.

HPLC analysis: The chromatographic analysis was performed on HPLC system (Thermo Scientific Spectra SYSTEM) equipped with UV2000 and FL3000 detectors. All-trans-retinol, ergocalciferol, cholecalciferol, α-tocopherol, β-carotene and total cholesterol were determined simultaneously using HPLC/UV/FL system equipped with RP analytical column Synergi 4μ Hydro-RP 80A pore 250 x 4.6 mm, through a mobile phase 75:20:5 = acetonitrile : methanol : 2-propanol (ACN:MeOH:iPrOH), at 1.1 mL/min. Detection of ergocalciferol (λ= 265 nm), cholecalciferol (λ= 265 nm), β-carotene (λ= 450 nm) and cholesterol (λ=208 nm) was performed by UV detector. Concentrations of all-trans-retinol (at λ_{ex} = 334 nm and λ_{em} = 460 nm) and α-tocopherol (at λ_{ex} = 288 nm and λ_{em} = 332 nm) were measured by fluorescence detection.

FA analysis

Lipid extraction: Portions of raw homogenate (5.000±0.001 g) were extracted according to Bligh and Dyer (1959) procedure [6]. Total lipid (TL) content was determined for each sample and the results were expressed as g per 100g wet weight ($g.100g^{-1}ww$). 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 [7]. After centrifugation (3500 rps), the hexane layer was separated and analyzed by GC-MS.

GS-MS analysis: The hexane layer was separated and analysed by GC-MS. Thermo Scientific FOCUS Gas Chromatograph, with Polaris Q MS detector coupled with TR-5 MS capillary column, (30 m, 0.25 mm i.d.) was used. For peaks identification mass spectra (ratio m/z) of FAME mix standard (SUPELCO 37 F.A.M.E. Mix C4 - C24) and internal Data Base (Thermo Sciences Mass Library, USA) was used. Results are expressed as a percentage of each FA with respect to the total FAs [8]. All chemicals used were of analytical and GC grade.

Statistical analysis

The results were expressed as a mean and standard deviation (mean ± SD). The obtained data was analyzed using Graph Pad Prism 6.0 software. An unpaired t-test statistical analysis was applied to estimate the differences between the analyzed samples. The comparison was made for total lipids, fat soluble vitamins, cholesterol and β-carotene and individual FA and FA groups. The differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Fat soluble vitamins, β-carotene and cholesterol contents

The amounts of the analyzed compounds are presented in Table 1 as microgram per 100 grams wet weight ($mg.100g^{-1}ww$). The results are expressed as average and standard deviation (mean ± SD).

Results for the different analytes in skin and muscle of the two groups are close. Data for all vitamins and cholesterol, presented in Table 1 showed similar correlation. In all cases the three vitamins and cholesterol contents in Rainbow trout group II samples were higher than Rainbow trout group I. β-carotene was detected neither in the skin nor in the muscle tissue of the samples in the first group. This fact can be attributed to feed composition. García-Chavarría and Lara-Flores considered that carotenoids play a major role in commercial aquaculture [9]. They are often added in food pellets, which reflect the skin and tissue coloration.

Other fat soluble analytes found in the two groups are vitamin A and D_3. Their amounts in skin and muscle of fish in group II were several times higher ($p<0.05$), than group I. Vitamin E ($p<0.01$ for skin, $p>0.05$ muscle) and cholesterol ($p<0.05$) amounts were also higher in group II, but the results are close. Cholesterol content in both groups is low. According to Ordinance № 23/19.07.2005 consuming less than 300 mg per day of cholesterol could help maintain normal blood cholesterol levels and prevent future cardiovascular disease. This characterizes the specimens from both grow stages as very suitable for healthy diet with about three times lower cholesterol amount compared to RDA [10].

All analyzed samples are very good sources of the three fat soluble vitamins (A, D_3 and E). Vitamin A and D_3 contents in skin and muscle of Rainbow trout in group I are close to the recommendations for daily intake of those vitamins in Bulgaria [10]. While results for the corresponding vitamins in group II were several times higher than RDA.

Table 1. Fat soluble analytes contents in raw skin and muscle of rainbow trout in two growth stages (mg.100g^{-1} ww)

	Rainbow trout I*		Rainbow trout II*	
	Skin	**Muscle**	**Skin**	**Muscle**
Vitamin A	0.43±0.019	0.31±0.017	1.62±0.24***	1.26±0.21***
Vitamin D$_3$	0.07±0.001	0.06±0.001	0.20±0.011**	0.26±0.015**
Vitamin E	7.89±0.63	28.22±1.21	16.80±1.03**	30.42±2.72
β-carotene	nd	nd	0.24±0.04	0.10±0.003
Cholesterol	66.65±7.92	46.13±4.81	75.65±6.25	47.32±4.30

* - Rainbow trout I - with weigh 300-400 g; Rainbow trout II - with weigh 700-900 g
*** p<0.001, ** p<0.01 and * p<0.05 groups I vs II

Data in Table 1 is in good agreement with those published by other authors and our previous studies [11]. Harlioğlu presented close amount of investigated compounds in rainbow trout fillet - 12.4 μg·100 g^{-1} ww for vitamin A, 13.2 μg·100 g^{-1} ww for vitamin D$_3$, 714 μg·100 g^{-1} ww for vitamin E and 40.2 mg·100 g^{-1} ww cholesterol [2].

Total lipid and fatty acid composition

Table 2 presents information for total lipid (TL, g.100^{-1}g wet weight) content and FA profile as well as the levels of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the skin and muscle of the trout meat. PUFA/SFA and ω-6/ω-3 ratios, EPA and DHA content (g.100^{-1}g wet weight) are shown too. Both trout groups presented 70.9% higher TL (group I) and 159% (group II) in the skin compared to muscle (p<0.001). One possible reason could be the inclusion of subcutaneous adipose tissue fixed to the skin in the analyzed samples. Our findings shown that fat depot is not evenly distributed in edible parts of analyzed trout. With increasing fish weight presented results showed proportionally increase of TL content mainly in skin, whereas in muscle the inverse correlation was observed. Rebole *et al*. (2015) reported similar results for skin TL in farmed trout from Granada, Spain [3]. A common practice to lower fat intake (especially SFA) is to remove the skin, due to its higher TL content. However, in this study it was found that fish skin lipids contained higher unsaturated FA than SFA (Table 2).

Presented results showed similar FA distribution in muscle: PUFA>SFA>MUFA and in skin tissue: MUFA>SFA>PUFA, despite fish weight. Most significant differences between the muscle and skin were found for MUFA – 4.2 times (group I) and 2.2 times (group II) higher in skin compare to muscle. The content of individual FAs varied between skin and muscle samples in both analyzed groups. The major SFAs in muscle and skin were C16:0 and C18:0 acids, but C16:0 content was significantly higher in the skin (p<0.001) than in muscle. In this study oleic (C18:1 ω-9) and linoleic acid (C18:2 ω-6) were found to be the dominant FAs in the skin in both fish groups. One possible reason for relatively higher levels of C18:2 ω-6 could be due to the necessity of fish to retain some degree of skin permeability - epidermal cell membranes should be more fluid to facilitate transport. Moreover, the predominance of C18:2 ω-6 in the farmed fish lipids has been attributed to the commercial diet where the major FA is C18:2 ω-6 [2]. These results agree with those reported by Rebole *et al*., (2015), who observed a similar order of the major unsaturated FA in the muscle and skin of farmed rainbow trout form Spain [3]. In contrast, other researchers found that C22:6 ω-3 followed by C16:0 were the predominant FA in the skin of wild *Sardinella* species from Senegalese coast [12]. This is probably due to the use of different lipid sources in the diet; however FA composition of the farmed fish does not only depend on the feed, but on the fish metabolism as well. As a whole, the growth stage affects significantly (p > 0.05) C22:6 ω-3 (DHA) content in the fillet parts. In addition a significant interaction (p<0.001) between fillet part vs growth stage was detected. An increase in the muscle and a decrease in the skin levels of DHA with increased fish weight were observed. Rebole *et al*, (2015) supposed that a possible reason is the greater amount of triacylglycerol-rich storage lipids deposited mainly in the skin. European Food Safety Authority (EFSA, 2012) recommends daily intake (RDI) of 0.500 g EPA + DHA [13]. The percentage values of these FAs were recalculated to g.100g^{-1} edible tissue in order to evaluate the nutrition lipid quality based of ω-3 PUFAs content. A 100 g of edible filet portion from both analysed groups contains average 0.660-0.700 g of EPA+DHA ω-3 PUFA (Table 2) and provides 135% of EPA+DHA RDI.

Table 2. Total lipid and fatty acid composition of total lipids in the skin and muscle from farmed rainbow trout

	Rainbow trout I[+]		Rainbow trout II[+]	
	Skin	Muscle	Skin	Muscle
Total lipid (g.100^{-1}g)	***5.64±0.40***	***3.3±0.30***	***6.37±0.50***	***2.46±0.20***
FA, % of total FA				
6:0	0.80±0.07	0.70±0.05	0.70±0.06	0.40±0.03
8:0	1.00±0.08	0.53±0.02	0.90±0.05	0.80±0.04
10:0	1.30±0.10	0.70±0.03	0.90±0.06	0.74±0.03
12:0	1.70±0.15	0.93±0.05	1.30±0.11	1.50±0.15
14:0	2.10±0.22	1.00±0.07	1.87±0.20	1.75±0.12
16:0	22.00±1.10	20.00±1.00	23.40±1.25	17.52±0.80***
18:0	5.55±0.40	7.50±0.60**	7.30±0.55	3.40±0.30***
20:0	1.00±0.07	0.25±0.01	0.60±0.03	0.50±0.01
21:0	0.15±0.01	0.10±0.01	0.20±0.01	0.10±0.01
22:0	0.60±0.02	0.20±0.01	0.70±0.03	0.30±0.02
23:0	Nd	0.15±0.01	0.10±0.01	0.34±0.02
24:0	0.55±0.02	0.30±0.02	0.45±0.02	0.22±0.01
SFA	**36.65±2.50**	**32.38±2.20**	**38.50±2.40**	**27.57±2.00*****
14:1ω-5	0.45±0.02	0.60±0.03	1.05±0.08	1.00±0.06
16:1ω-7	3.95±0.25	2.95±0.20	3.00±0.32	1.60±0.13**
18:1ω-9	31.10±2.50	3.50±0.30***	34.00±2.40	14.54±1.05***
22:1ω-11	1.16±0.08	1.10±0.07	0.25±0.01	0.26±0.02
24:1ω-9	1.00±0.06	0.65±0.03	0.80±0.02	0.50±0.01
MUFA	**37.69±2.20**	**8.85±0.55*****	**39.10±2.60**	**17.90±1.55*****
18:3ω-6	0.90±0.04	1.80±0.20	1.20±0.07	1.00±0.08
18:3ω-3	2.90±0.25	3.80±0.25**	nd	2.72±0.10***
18:2ω-6	12.50±1.10	20.25±1.55***	13.73±0.30	14.45±0.55*
20:5ω-3	1.00±0.04	7.95±0.50	5.20±0.25	4.70±0.20
20:4ω-6	0.70±0.02	3.90±0.28***	Nd	4.36±0.15***
20:3ω-6	2.00±0.07	2.50±0.20	Nd	1.45±0.05
20:3ω-3	0.50±0.01	2.00±0.15	0.20±0.01	0.22±0.01
20:2ω-6	0.40±0.01	0.70±0.06	Nd	0.75±0.02
22:6ω-3	4.00±0.30	14.87±0.95***	1.45±0.06	24.08±2.05***
22:2ω-6	0.80±0.02	1.00±0.08	0.60±0.02	0.80±0.05
PUFA	**25.70±1.70**	**58.77±2.50*****	**22.38±1.65**	**54.53±2.45*****
ω-3	8.40±0.55	28.62±2.10***	8.65±0.60	31.72±2.60***
ω-6	17.30±1.10	30.15±2.50***	15.53±1.50	22.81±1.70***
ω-6/ω-3	2.06±0.15	1.05±0.07	1.79±0.08	0.72±0.03
PUFA/SFA	0.70±0.03	1.82±0.10	0.58±0.02	1.98±0.15
EPA	0.055±0.002	0.238±0.020***	0.320±0.022	0.110±0.005***
DHA	0.220±0.015	0.455±0.030***	0.090±0.004	0.550±0.050***

[+] - Rainbow trout I - with weigh 300-400 g; Rainbow trout II - with weigh 700-900 g

*** p<0.001, ** p<0.01 and * p<0.05 groups I vs II

PUFA/SFA and ω-3/ω-6 ratios are indices widely used to evaluate the nutritional quality of edible fat for human consumption. According to Department of Health (1994), PUFA/SFA ratio in human diets should be above 0.45 and ω-6/ω-3 ratio should not exceed 4.0 [13]. In this study ω-6/ω-3 ratio was between 2 – 2.5 times higher in the skin than in the muscle in both groups, due to a greater content of C18:2 ω-6 and smaller proportion of C22:6 ω-3 in the lipid fraction of the skin. In addition our results showed differences in the PUFA/SFA ratio. It was 2.6 – 3.4 times higher in the muscle than PUFA/SFA ratio in the skin tissue for both fish groups. In any case, ω-6/ω-3 PUFA and PUFA/SFA ratios are within recommended levels for a healthy diet. Moreover, the information presented in this investigation confirms that the public perception that fish skin is healthier is justified.

CONCLUSIONS

In conclusion, despite its weight, rainbow trout was characterized by high contents fat soluble vitamins and low content of cholesterol in the skin and in the muscle tissue. On the other hand, muscle and skin exhibited different TL and fatty acid composition. Muscle tissue contained significantly higher levels of unsaturated ω-3 PUFA and lower levels of lipids than the skin. Based on the PUFA/SFA and ω-6/ω-3 PUFA ratios, the nutritional quality of the muscle is better than that of the skin. A 100 g of filet portion from both analysed groups contains average 0.660-0.700 g of EPA+DHA ω-3 PUFA and provides over than 130% of RDI. In any rate, it could be summarized that farmed rainbow trout filets, with or without the skin are rich sources of analysed fat soluble nutrients. Moreover, the assessment of the quality and quantity of fat soluble components of commercially important trout species could lead to raising consumers' awareness and help them make better informed choices when choosing healthier food.

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МАСТНО-РАЗТВОРИМИ НУТРИЕНТИ И МАСТНИ КИСЕЛИНИ В КОЖА И ФИЛЕ НА КУЛТИВИРАНА ДЪГОВА ПЪСТЪРВА

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Постъпила на 11 ноември 2016 г.; приета на 21 декември 2016 г.

(Резюме)

Представеното изследване сравнява съдържанието на мастноразтворими компоненти в мускулната тъкан и кожата на дъгова пъстърва (*Oncorhynchus mykiss W.*), предмет на аквакултура. Екземплярите, пробонабирани в две групи според теглото им, са закупени от рибни търговски обекти във Варна. Установена е липсата на информация относно разликите при диетичния внос на мастноразтворими биологично активни вещества чрез консумацията на филета от пъстърва – с и без кожата. Витамини A, D_3 и E, β-каротен и холестерол са съвместно количествено определени чрез използване на BETX система с UV и FL детектори. Общите липиди са извлечени от рибната матрица по метода на Блайт и Даер. Анализът на метиловите естери на мастните киселини беше извършен чрез използване на ГХ/МД. Данните от извършените анализи показват, че общото липидно съдържание, количеството на витамин Е и холестерол, както и това на наситените мастни киселини са значително по-високи в кожата, отколкото в мускулната тъкан. Противоположно – с по-високо в мускулната тъкан съдържание се представят витамини А и D_3, ейкозапентанова киселина (C20:5 ω-3) и докозахексанова киселина (C22:6 ω-3).

Ключови думи: *Oncorhynchus mykiss, витамини, каротеноиди, холестерол, ПНМК*