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THE EFFECT OF STEAMING PROCESS ON FAT SOLUBLE VITAMINS' CONTENT AND FATTY ACID PROFILE IN BLUEFISH AND RAINBOW TROUT FILLETS

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ABSTRACT

Fatty acid composition and all-trans-retinol, alpha-tocopherol, and cholecalciferol content was determined and compared in raw and steamed Bluefish and Rainbow trout. Total lipids were extracted by Bligh and Dyer method followed by GC-MS. All-trans-retinol, cholecalciferol and alpha-tocopherol were analyzed simultaneously using HPLC. In comparison with raw fish fillets, analyzed fat soluble vitamin's content in steamed fish filletsfor the Trout and Bluefish decreased significantly to about 54.2% and 49.8% for retinol and 32.6% and 43.5% for alpha-tocopherol, respectively. After steaming, the cholecalciferol amounts in processed fillets decreased significantly only in Rainbow trout (23.5%), whereas in Bluefish the losses were non-significant. After cooking, the polyunsaturated fatty acid content changed significantly in the Rainbow trout (45.8%), whereas the variations in the Bluefish were minor. The major PUFA in all samples were linoleic acid (LA) and docosahexaenoic acid (DHA). PUFA/SFA ratios were between 1.01 and 1.68 for both species. Steaming increases PUFA/SFA ratio by 8.33% in Rainbow trout, but does not affect this ratio in Bluefish.

Keywords: Black sea, Fish nutrition, Human health, Oncorhynchus mykiss, Pomatomus saltatrix, Thermal processing.

1. INTRODUCTION

The Rainbow trout and Bluefish are commercially important fish species in Bulgaria. The Rainbow trout (*Oncorhynchus mykiss*) is one of the most widely farmed fishes in our country. It is a predator which inhabits cold and clear freshwater ponds. Because of its rapid growth and rich and diverse composition of meat, the trout is preferred fish for breeding and consumption [1]. The Bluefish (*Pomatomus saltatrix*) is important pelagic fish for the Black Sea fish markets. This species feeds primarily on fish (anchovy, horse mackerel) and on crustaceans (shrimps) [2]. On the other hand, the distinctive flavor of Rainbow trout and Bluefish makes them favorite dishes.

Fish is a rich source for essential nutrients such as fat soluble vitamins and Polyunsaturated Fatty Acids (PUFA) [3-5]. Fatty fish is the most important source of vitamin D and long-chain omega-3 (n3) PUFAs, and is favorable with respect to both cardiovascular disease and fetal

development. It is well known that fat soluble vitamins control the diversity of biologically important processes in the human body. All-trans-retinol (vitamin A) play role in photoreception, bone growth, reproduction etc. Alpha-tocopherol (vitamin E) acts as an antioxidant, protecting membrane structures, essential fatty acids, and vitamin A from oxidation. Cholecalciferol (vitamin D_3) plays a crucial role in the regulation of the calcium-phosphate balance stimulating calcium absorption and thus regulating bone metabolism.

The majority of researches on fish and human health have focused on the link between cardiovascular diseases and the consumption of fish or long chain n3 PUFAs as eicosapentaenoic acid (C20:5, EPA) and docosahexaenoic acid (C22:6, DHA) [6]. Consumption of at least two fish servings per week is recommended by the American Heart Association and FAO/WHO [3, 5]. In Bulgaria the consumption of fish is very low (4.5kg annual per capita) compared to the average European levels (23 kg annual per capita) [7]. Meanwhile, in the Western society and Bulgaria consumption of raw fish is rare. Temperature processing of fish tissue enhances its taste, inactivates pathogenic microorganisms and increases its shelf life. During cooking, chemical and physical reactions occur and the content of thermo labile compounds as fat-soluble vitamins and PUFA in fish tissue is reduced. Steaming is known as a mild and often recommended cooking method used in healthy diets. There are no research data in Bulgaria on the effect of steaming on fat soluble vitamins' content, total lipids and fatty acids (FA) composition of these fish species.

The aim of the present study is to evaluate the effect of the steaming process on the total lipids, the fat soluble vitamins' content and the fatty acid composition in these two traditionally consumed in Bulgaria fish species – the Rainbow trout and the Bluefish.

2. MATERIALS AND METHODS

2.1. Sample Collection and Cooking Method

A total of twelve fresh Rainbow trout (fish farm, Plovdiv region) and Bluefish specimens were purchased from Varna local fish market during the autumn season. Biological and biometrical characteristics of the species were determined and noted (Table 1).

Table-1. Biometric and biologic characteristics of studied fishes (mean ± SD)

Fish species	Mean total weight [g]	Mean total length [cm]	Habitat	Food habits
Bluefish (n=6)	60.5 ± 2.3	16.0 ± 2.1	Pelagic	Carnivorous
Rainbow trout(n=6)	325.0 ± 15.0	28.0 ± 3.4	Pelagic	Omnivorous

All fishes were immediately frozen and stored at -20°C. Prior to analysis the frozen samples were thawed at 4°C, for 12 hours. The edible tissue was filleted with the skin. The fillets were totally randomized and then divided in two batches: first group of fillets (n=6 for each fish species) were analyzed in raw state, a second group was analyzed after steaming (n=6 for each fish species). The fish fillets from the first group were homogenized at 800 rpm for 3 minutes using Moulinex blender. The homogenized tissue was used to prepare the parallel samples. The

second group was placed in a steamer above a glass pot of boiling water (500 ml) and cooked for 10 minutes. The steamed fillets were placed for 5 minutes on absorbent papers and then processed as described for group one. The Steamed samples were weighed and the observed losses were noted.

2.2. Moisture Analysis

Test portions of homogenized fish tissue (2.000±0.005g) were dried at 105±2°C in an air oven, to constant weight for 16-18 hours [8]. All samples were cooled in desiccator and weighted. The change in the moisture [%] was calculated as weight loss.

2.3. Extraction of Fat Soluble Vitamins and HPLC Analysis

The sample preparation was performed using the method of Dobreva, et al. [9]. 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 1M methanolic potassium hydroxide was added. Six parallel samples of edible fish tissue were prepared and subjected to saponification at 80°C for 20 min. The components of interest were extracted with n-hexane and the extract was evaporated under nitrogen. The dry residue was dissolved in methanol and injected (20µl) into the liquid chromatography system. The three fat soluble vitamins were analyzed simultaneously using HPLC system (Thermo Scientific Spectra SYSTEM) equipped with RP analytical column ODS2 Hypersil™ 250x 4,6mm, 5um. All-trans retinol and cholecalciferol were detected by UV, alpha-tocopherol by fluorescence detection. The mobile phase composition was 97:3 = MeOH:H_2O and the flow rate was 1ml/min. The qualitative analysis was performed by comparing the retention times of pure substances: at λ_{max} = 325nm for retinol; λ_{max} = 265nm for cholecalciferol and alpha-tocopherol fluorescence at λ_{ex} = 288nm and λ_{em} = 332nm. The quantitation was done by the method of external calibration comparing the chromatographic peak areas of the corresponding standards (Retinol solution, Fluka; DL-alpha Tocopherol, Supelco; Cholecalciferol, Supelco). The results are expressed as µg per 100 g wet weight (µg.100g^{-1}ww).

2.4. Lipid Extraction and Fatty Acid Analysis

Portions of freshly prepared homogenate (5.000±0.001g) were extracted in triplicate with chloroform: methanol (1:2 v/v) according to Bligh and Dyer procedure [10]. BHT (2-terth-Butyl-4-hydroxyanisole) was added to all samples as antioxidant. After phase separation the chloroform layers were evaporated on a rotary vacuum evaporator (Bushi 5200) until dryness and quantified gravimetrically. The total lipid (TL) content of edible tissue was determined for each group (n=6) and the results are presented as g per 100g wet weight (g.100g^{-1}ww). The dry residue of the chloroform fraction was methylated by base-catalyzed transmethylation using 2M KOH in methanol and n-hexane [11]. The hexane layer was separated and analyzed by GC-MS. Gas chromatography was performed by a model FOCUS Gas Chromatograph with auto sampler A3000, equipped with Polaris Q MS detector (Thermo Scientific, USA). The capillary column

used was a TR-5 MS, 30m length, 0.25mm i.d. Helium was used as a carrier gas at a flow rate 1 ml/min. Peaks were identified according to two parameters: Retention Time (RT) based on available FAME mix standard (SUPELCO 37 F.A.M.E. Mix C4 - C24) and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA). Results are expressed as the percentage of each fatty acid with respect to the total fatty acids [12].

2.5. Statistical Analysis

The obtained data were analyzed using Graph Pad Prism 5 software. Column statistics were used for calculation of means and standard deviations and results are presented as average ± SD. To estimate the differences between two groups - raw and steamed samples unpairedt-test statistical analysis was applied. Thus the comparison was made between moisture, total lipids, fat soluble vitamins and individual FA and FA groups. The differences were considered significant at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Moisture Content

The raw samples of Rainbow trout fillets showed high moisture content (78.5%) followed by the Bluefish (62.6%). During the process of steaming a significant decrease in moisture, compared to raw tissue was revealed (16.2%) for Rainbow trout (65.8%, $p<0.001$) and 5.6% for Bluefish fillets (59.1%, $p<0.001$). These results are consistent with Sikorski and Kolakowska for trout, and possibly due to the fact that the fish food experienced water loss in its tissues during the cooking process [13]. Larsen et al. presented a similar result for moisture content of steamed King salmon [14].

3.2. Fat Soluble Vitamins' Content

All-trans-retinol and alpha-TP are known to be unstable when heated in the presence of air. The steaming method of cooking affects strongly the content of these vitamins. Retinol and alpha-TP contents in steamed fish fillets for both Rainbow trout and Bluefish decrease significantly $p<0.001$, compared to their content in the raw fish samples - by about 54.2% and 49.8% for retinol and 32.6% and 43.5% for alpha-TP, respectively (table 2).

Table-2. Vitamin contents in raw and steamed fish fillets, μg.100g^{-1}ww, (mean ± SD)

Fish species	All-trans-retinol		Alpha-tocopherol		Cholecalciferol	
	raw	steamed	raw	steamed	raw	steamed
Rainbow trout	58.95±2.6	26.99±1.4***	1648.90±68.8	1112.17±40.3***	14.92±1.1	11.42±0.6**
Bluefish	38.48±2.4	19.35±1.7***	427.08±37.1	241.38±24.7***	11.18±1.2	10.40±0.5

*** $p<0.001$ steamed vs raw; ** $p<0.01$ steamed vs raw

Rainbow trout and Bluefish are amongst the fishes comprising highest amounts of cholecalciferol. Our data for cholecalciferol content in raw fish tissue (table 2) are in accordance with the data given by Mattila et al. for vitamin D_3 content (0.28 - 47.7 μg.100g-1 raw tissue) in fishes [15]. The amount of cholecalciferol decreased significantly ($p<0.01$) by 23.5% only in Rainbow trout, whereas in Bluefish fillet the losses were non-significant, after steaming (table 2). There is a discrepancy in the literature regarding the effect of various types of cooking on the fat soluble vitamin contents in fish tissue [16-18]. Erkan et al. found out losses of about 75% for retinol and 55% for alpha-tocopherol in steamed horse mackerel [18]. Our results, regarding retinol and alpha-TP changes after steaming, are comparable with those of Erkan, et al. [18]. Mattila et al. reported losses below 10% for cholecalciferol in fish samples undergoing a baking process [16]. We also established slight non-significant losses (6.9%) for cholecalciferol in Bluefish fillets after steaming. In contrast Ersoy and Ozeren reported no significant differences in fat soluble vitamins A and E in edible tissue of African catfish after various types of cooking - baking, grilling, microwaving and frying [17].

3.3. Total Lipid Content

The TL content in raw Rainbow trout was 11.50 $g.100g^{-1}ww$ and 15.54 $g.100g^{-1}ww$ for Bluefish. After steaming a significant ($p<0.001$) decrease in TL content was observed for both species - 9.02 $g.100g^{-1}ww$ for Rainbow trout and 13.34 $g.100g^{-1}ww$ for Bluefish. According to other authors, changes in the lipid amounts after steaming depend on fish species, temperature treatment, portion size and heatable surface area [13, 19]. The obtained results are consistent with those quoted by other authors, who observed loss of fat due to spreading during heat treatment in Rainbow trout and steamed King salmon fillets [14, 20]. To provide more useful and precise information of the actual lipid losses after steaming in the samples it was necessary to calculate the True Retention Factor (%TR). %TR is based on the measuring of weight change and proportion of TL in steamed and raw fish samples. This factor is calculated by the True Retention Method as follows: %TR = (nutrient content per g of cooked food X g of food after cooking) / (nutrient content per g of cooked food X g of food before cooking) X 100 [21]. In this study the observed weight changes for Bluefish after heat treatment were higher (from 100 g raw sample to 87 g after steaming) in comparison to these for Rainbow trout (from 100 g raw sample to 90 g after steaming). On this basis %TR were calculated - 72.1% for Rainbow trout and 71.1% for Bluefish. These results are in good agreement with the data for average retention factor for fat in steamed carp (70%) presented by Kalyoncu, et al. [22].

3.4. Fatty Acid Composition

Twenty-eight FA (from C12:0 to C 24:1) were identified and compared among the different species. There was a wide variation and significant differences ($p<0.05$) among the FA profiles of fish species and after steaming in terms of total and individual saturated and unsaturated FAs. The FA pattern in raw trout followed the order: PUFA>SFA>MUFA. Statistically compared to

the raw trout, steamed fillets showed significant increase in the values of total PUFA and MUFA ($p<0.001$), whereas the SFA amounts decreased slightly ($p<0.05$). The FA pattern has changed in the following way PUFA>MUFA>SFA after steaming. Only minor increase in MUFAs ($p<0.05$) was observed in Bluefish and PUFA>SFA>MUFA pattern did not change after steaming. Therefore we may assume that the Bluefish FAs content is more stable after steaming than the Rainbow trout FAs content. Gladishev et al. reported increased PUFA levels after heat treatment in four fish species and suggested that this may be due to the higher degree of hydrolysis of fish tissue lipids [19]. The FA profiles of analyzed fish species before and after steaming are shown in table 3. As a result the steaming leads to significant changes in the levels of the individual FAs within the FA groups.

Table-3. FA profiles (% total FA) of raw and steamed Rainbow trout and Bluefish (mean ± SD)

Fatty Acid	Rainbow trout		Bluefish	
	raw	steamed	raw	steamed
C 12:0[1]	0.40±0.02	1.92±0.12***	0.42±0.03	0.51±0.02
C 14:0	3.35±0.65	4.13±0.75***	4.17±0.36	4.47±0.34**
C 16:0	12.95±1.23	14.09±1.34***	22.65±1.57	23.00±1.55**
C 17:0	0.51±0.01	0.35±0.04	0.47±0.05	0.43±0.03
C 18:0	3.35±0.51	2.24±0.12***	3.46±0.55	3.40±0.15
C 20:0	2.68±0.35	2.00±0.10	0.54±0.02	0.46±0.02
C 21:0	nd	nd	0.33±0.01	0.27±0.01
C 22:0	1.95±0.40	0.75±0.05***	0.57±0.02	0.47±0.03
C 23:0	0.00	0.28±0.02	0.30±0.01	0.25±0.01
C 24:0	3.20±0.60	1.50±0.33***	0.54±0.03	0.45±0.04
Σ SFA	**28.90**	**27.28***	**33.73**	**33.93**
C 14:1	3.16±0.52	3.40±0.51	0.33±0.02	0.27±0.01
C 16:1	4.15±0.45	5.57±0.84***	6.92±0.54	7.01±0.45
C 17:1	0.50±0.03	0.35±0.05	0.47±0.02	0.42±0.02
C 18:1 n 9	11.50±1.22	13.62±1.06***	15.45±1.35	16.15±1.25***
C 20:1	2.03±0.67	2.68±0.54	2.81± 0.55	2.71±0.20
C 22:1 n 9	2.74±0.53	2.69±0.50	3.70±0.41	3.71±0.61
C 24:1	1.30±0.37	0.51±0.04	0.91±0.06	0.83±0.04
Σ MUFA	**25.39**	**28.82*****	**30.59**	**31.04***
C 18:3 n6	3.19±0.64	0.56±0.03***	0.40±0.01	0.30±0.01
C 18:2 n6	13.56±1.15	21.66±1.67***	16.60±1.15	16.10±1.05
C 18:3 n3	6.16±0.80	1.22±0.31***	1.50±0.20	1.78±0.21
C 20:5 n3	1.56±0.10	0.60±0.02***	0.43±0.02	0.35±0.04
C 20:4 n6	3.73±0.22	5.33±0.54***	2.82±0.34	2.82±0.35
C 20:2	1.46±0.14	0.45±0.01	0.40±0.02	0.35±0.01
C 20:3 n3	nd	0.41±0.02	0.38±0.01	0.45±0.01
C 20:3 n6	1.98±0.51	0.45±0.01	0.35±0.01	0.25±0.01
C 22:6 n3	11.23±1.05	14.45±0.98***	11.30±1.12	11.60±1.26
C 22:2	1.90±0.08	0.70±0.05	0.50±0.03	0.40±0.02
Σ PUFA	**44.47**	**45.82*****	**34.60**	**34.23**
Σ n3	18.95±1.53	16.68±1.34***	13.96±1.25	14.10±1.23
Σ n6	22.46±1.67	28.00±1.84***	20.04±1.71	19.45±1.64
EPA+DHA	12.79±0.92	15.05±0.85***	11.73±1.03	11.95±1.10
Σ n3/ Σ n6	0.84±0.06	0.60±0.05***	0.70±0.06	0.73±0.05
PUFA/SFA	1.54±0.05	1.68±0.07	1.03±0.08	1.01±0.04

*** $p<0.001$ steamed vs raw; ** $p<0.01$ steamed vs raw; * $p<0.05$ steamed vs raw

3.4.1. Saturated Fatty Acids

The SFA group of steamed Rainbow trout shows increased levels of palmitic C16:0, myristic C14:0 and lauric acid C12:0 ($p<0.001$), whereas the levels of stearic acid C18:0 and the very long-chain FAs – lignoceric acid C24:0 and behenic acid C22:0 were significantly reduced ($p<0.001$). These changes reflect the overall reduction of SFAs in the steamed trout. A similar trend was observed in the Bluefish SFA levels after steaming, but the differences were very small.

3.4.2. Monounsaturated Fatty Acids

The amounts of unsaturated FA as MUFAs vary especially in wild fish [18, 23, 24]. Oleic acid (C18:1 n9) is the main MUFA in both species Fish are able to biosynthesize this FA, but the oleic acid also has an exogenous origin and usually its content reflects the type of fish diet. [23, 24] In our study the levels of C18:1 n9 were increased significantly ($p<0.001$) after steaming in both species. The second abundant MUFA was palmitoleic acid C16:1 n7. Steaming results in significant increase in the levels of C16:1 n7 only for trout ($p<0.001$). The increase of both C18:1 n9 and C16:1 n7 acid levels after steaming contributed to higher extent to the elevation of total MUFA content in the Rainbow trout. Larsen et al. reported insignificant effect of the steaming on King salmon MUFA contents, while Su and Babb found significant changes in MUFA levels in steamed scallops [14, 25]. There are several possible reasons for the discrepancies and differences among the reported results, but one of the most important is the lack of standardized times and temperatures for any cooking method.

3.4.3. Polyunsaturated Fatty Acids

In this study PUFA in both fish species were dominated by linoleic acid C18:2 (LA) from n6 and DHA from n3 series. In the steamed Rainbow trout LA content increases up to 47.30 % of total PUFAs ($p<0.001$), whereas in Bluefish its value was unchanged EPA and DHAn3 are highly polyunsaturated and more vulnerable to the oxidation even at ambient temperature compared to LA n6 and their contents strongly depend on the storage conditions. Conversely, the levels of the second abundant n6 FA - arahidonic acid (AA) were constant in raw and steamed Bluefish (8.15 % of total PUFAs), whereas the Rainbow trout showed slight increase by 3.22 % ($p<0.01$) compared to raw samples. Su and Babb reported increase in DHA and AA contents and decrease of EPA content in steamed scallops [25]. Some discrepancies were found when comparing the obtained results with the results involving amounts of EPA and DHA quoted by other authors. In the present study the sum of EPA plus DHA in raw trout and Bluefish accounted to 28.8 % and 34.0% of total PUFA respectively (table 3). The results obtained by Kalyoncu et al. and Steffens and Wirth for these FAs in trout were lower (with 10%) [22, 26]. The feeding habits, the method of fish farming and the different locations are probably the main reasons for the discrepancies in the results. Significant changes in EPA and DHA levels were observed in the steamed trout (32.8 % of total PUFA) due to the increase of DHA value ($p<0.001$), while no differences were found for the Bluefish (table 3). The Lack of negative

influence of the cooking procedure on the DHA levels of trout fillets has high practical importance. Due to this fact, compared with Bluefish, the steamed trout is a better source of these n3 PUFAs. Kolakowska et al. suggested that the different heat treatment procedures had no substantial influence on the percentage of EPA and DHA in Baltic herring [20]. In other researches the same authors reported that heating for 20 min at 160°C could reduce DHA and EPA contents in the Rainbow trout [13]. The reported changes in EPA + DHA levels for both species could be caused by the changes in their lipid extractability. Other important results in this study showed that the total sum of n6 PUFAs was elevated in steamed Rainbow trout (up to 61.00 % of total PUFA) in comparison with the raw one (50.5% of total PUFA, $p<0.001$), whereas the opposite trend was observed in Bluefish ($p<0.001$) after steaming (table 3). The n3 series showed significantly decreasing (below 6.0%, $p<0.001$) in steamed Rainbow trout, while in Bluefish this series remains unaffected. As a result, steaming can be preferred without significant loss of n3 PUFAs in Bluefish. The n3/n6 PUFA ratio is a key factor for balanced synthesis of eicosanoids in the organism [27]. Previous studies revealed that n3/n6 ratio in freshwater fishes varies between 0.5 and 5.6, whereas in marine fishes it is between 0.7 and 14.4 [26, 28]. The obtained results in this study for raw species (from 0.7 up to 0.84) are in agreement with the above mentioned results. The observed differences in omega PUFA levels result in a decrease of n3/n6 ratio only in Rainbow trout, while it remains stable in Bluefish after steaming (table 3). Gladishev et al. and Su and Babb reported a decrease in n3/n6 ratio in steamed and boiled fish species and thus support our results [19, 25]. According to the current WHO recommendations, n3/n6 PUFA should not be lower than 0.2 [2, 27]. In both analyzed fish species before and after steaming this ratio remains significantly above the cut-of value. The Department of Health recommended PUFA/SFA ratios greater than 0.45 [29]. Simopoulos and Cleland [27] reported that several studies had found inverse association of the PUFA/SFA ratios with cardiovascular diseases [27]. In our study, the most balanced PUFA/SFA ratio was obtained for Bluefish (table 3). We found that the steaming increases PUFA/SFA ratio by 8.33 % in the Rainbow trout, whereas this parameter remains unchanged in the Bluefish. Our results reveal that steaming does not induce a reduction of PUFA/SFA ratio below 0.45 in both species.

4. CONCLUSIONS

The Nutritional quality of the Rainbow trout and the Bluefish are fairly similar, with relatively high levels of unsaturated FAs and fat-soluble vitamins. No significant changes in PUFA amounts, FA distribution and cholecalciferol content for the Bluefish compared to the Rainbow trout were observed after steaming. The positive beneficial effect of fish lipids based on EPA and DHA n3 PUFA, n3/n6 and PUFA/SFA ratios, and fat soluble vitamins content are preserved after that treatment. The steaming method can be recommended as a mild and less aggressive cooking method suitable for healthy dietetic regimes. Since FA composition and fat soluble vitamins' content are important components of the nutritional value of fishes, their

changes during the various regimens of processing need special interest and further investigations.

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