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Comparison of Polyunsaturated Fatty Acid and Fat-Soluble Vitamins Content of Cooked Shad (*Alosa Immaculata*)

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This study presents the evaluation of the effect of cooking (steaming and grilling) on lipids, fatty acids profile, nutritional quality indices (NQI) and fat soluble vitamins content of Black Sea shad (Alosa immaculata). The Bligh and Dyer's method was used for total lipid content determination. The fatty acid methyl esters were analysed by GC/MS and fat soluble vitamins - by RP-HPLC. The two cooking methods increased the levels of the saturated fatty acids (SFA), whereas grilling process decreased monounsaturated fatty acids (MUFA) quantity in shad tissue. Omega-3 polyunsaturated FAs (n-3 PUFA) levels significantly decreased after both thermal processes. Steaming doesn't affect the omega-6 (n-6) PUFA – their amounts remain almost unchanged. The vitamin A value decreases significantly after steaming, whereas vitamin D_3 and E remain almost unchanged. Among the three fat soluble vitamins, the grilling process affects significantly mainly vitamin A and E. In conclusion - both cooking methods are suitable for preserving the lipid nutrition quality of shad edible tissue.

Keywords: *Alosa immaculata*, Fatty acids, Fat soluble vitamins, Grilling, Steaming

Introduction

Various epidemiological studies have demonstrated the key role of fish consumption in prevention of coronary heart diseases (Kris-Etherton *et al.*, 2003; Ruxton, 2011). The nutritional benefits of fish consumption are mainly attributed to the effects of omega-3 Polyunsaturated Fatty Acids (n-3 PUFAs), which have several potential cardio protective effects along with their antithrombotic action. Numerous studies have explored and supported the antiatherogenic, antithrombotic, and antiarrhythmic effects of n-3 PUFAs (Lee *et al.*, 2006). The second essential nutrients of fish tissue are the fat soluble vitamins which control a diversity of biologically important processes in human body. It is well known that the marine fish fatty acid (FA) composition is characterized by high levels of n-3 PUFA (Eicosapentaenoic acid, EPA, C20:5

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n-3; Docosahexaenoic acid, DHA, C22:6 n-3) and vitamin D_3 content.

Furthermore, when fish are suggested as a means of improving health, lipid quality must be considered (Suloma, 2008). Usually fish lipid quality is evaluated using different FA ratios such as PUFA/SFA, n-3/n-6 PUFA, nutritional quality indices as Atherogenic index (AI), Thrombogenic index (TI), hypocholesterolaemic/ hypercholesterolaemic (HH) index based on FA content.

In addition, the fatty fish are a rich source of the above described biologically active substances. There are several oily fish species in the Black Sea, and the shad (Alosa immaculata) is one of them. Our previous investigations for Black Sea shad showed high nutrition quality of its edible tissue (Merdzhanova *et al.*, 2013; Stancheva *et al.* 2012). This fish inhabits the Black Sea but it is an anadromous species and migrates to the Danube River to spawn (Rozdina, 2015). Moreover, it is an economically important species - object of commercial fishing.

The consumption of raw fish fillets is very limited in Bulgaria and the Western society. However, in Bulgaria shad is generally consumed in a cooked state, but information about changes of nutrition quality of this species are not found in scientific literature. Therefore, information about FA composition and fat soluble vitamins' content in raw fish may have limited application for human health. These nutrients are considered to be especially susceptible to oxidation during cooking processes such as steaming, boiling, grilling and frying. Temperature processing of fish fillets inactivates pathogenic microorganisms and enhances its taste, but influences the amounts of fat soluble vitamins and PUFAs. According to Food Based Dietary Guidelines for Adults in Bulgaria (2006), the estimated daily intake of EPA, DHA and vitamin D_3 intake in Bulgaria is very low and significantly under the recommended daily intakes for EU countries. To our knowledge, no data is available in literature which describes the n-3 PUFA, nutritional quality indices (NQI) and fat soluble vitamin content changes after cooking in shad caught in the Bulgaria Black Sea waters. We expect that by providing information about shad lipid quality changes after cooking we will promote fish consumption in Bulgaria and the region.

Objectives: Having in mind all these facts, the aim of the present study was to evaluate the effect of the steaming and grilling processes on total lipids, FA profile, NQI and fat soluble vitamins in Black Sea shad edible tissue.

Materials and methods

Samples collection and preparation

Black Sea Shad specimens (n=9) were filleted with the skin. The parts were separated into three groups: the first six fillets was analysed in raw state, the second six was analysed after steaming (10 min, 90^{o}C) and the third group - after grilling (15 min, 220^{o}C). The cooked and raw samples were weighed to obtain the changes.

Moisture analysis

Portions of homogenized raw and cooked samples (2.000±0.005 g) were dried at 105±2^{o}C in an air oven to constant weight (AOAC: 950.46, 2002). All samples were cooled in desiccator and weighted. The moisture was calculated as weight loss and presented as persetiges.

Lipid extraction and fatty acid analysis

Portions of raw and cooked homogenate (5.000±0.001 g) were extracted according to Bligh and Dyer (1959) procedure. 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 dry residue was methylated according to (BDS EN ISO 5509:2000, 2000). 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, (30m, 0.25mm 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 (BDS EN ISO 5508:2004, 2004).

Nutrition quality indices (NQI)

Nutrition qualities were estimated by several indices and ratios based on FA composition: the indices of atherogenicity (AI), thrombogenicity (TI), cholesterolemic index (HH); n-6/n-3 and PUFA/SFA ratios, according to (Hosseini et al., 2014; Santos-Silva et al., 2002; Ulbritcth and Southgate, 1991). AI and TI indices might better describe the pro- and anti- atherogenic and pro- and anti-thrombogenic potential of different unsaturated FA. HH presents the

functional effects of different PUFAs of cholesterol metabolism (hypo- and hyper-cholesterolemic effect).

Fat soluble vitamin's analysis

The fat soluble vitamin's extraction was performed by the method of Dobreva et al. (2011). The parallel samples of each group (1,000±0.005 g) were subjected to alkaline saponification. Vitamins A, D_3 and E were analysed simultaneously using HPLC/UV/FL system (Thermo Scientific Spectra SYSTEM) equipped with analytical column ODS2 Hypersil™ 250x 4,6mm, 5u. The mobile phase was 97:3 = methanol:water, flow rate 1ml/min. The results were expressed as μg per 1g total lipid [$\mu g.g^{-1}$TL].

Statistical analysis

The data were analysed using Graph Pad Prism 5 software. The results were expressed as means and standard deviations. One-way ANOVA (nonparametric test) analysis was employed for the calculation of differences between raw and cooked states (significant at $p<0.05$).

Results and discussions

Moisture and total lipid content

The raw samples of shad showed highest moisture content (56.5%). During the process of cooking an insignificant decrease compared to raw tissue was observed – with 3.3 % ($p<0.05$) after steaming and 2.5% ($p<0.05$) after grilling (table 1). These results are consistent with Sikorski and Kolakowska (2007) for trout and Larsen et al (2010) for King salmon, who also reported water loss in fish tissues during the cooking processes. The result for TL content in raw Black Sea Shad was 14.1 $g.100g^{-1}$ww. This is typical for shad which is usually classified as oily species. Our data are in good agreement with those of the USDA Nutrient Database (2015) for shad (13.8 $g.100g^{-1}$ww).

Table 1. Moisture [%] and total lipids [$g.100g^{-1}$ww] content in raw, steamed and grilled Black Sea shad (mean ±SD)

Specification	Black Sea shad		
	raw	steamed	grilled
Moisture	56.48±2.50	$54.6\pm2.20^{**++}$	$55.10\pm1.90^{**}$
Total lipid	14.10±1.10	$9.00\pm0.70^{***}$	$11.10\pm1.00^{++}$

*** $p<0.001$, ** $p<0.05$ raw vs steamed and grilled; $^{+++}$ $p<0.001$, $^{++}$ $p<0.05$ steamed vs grilled

After cooking a significant decrease in TL content in fillets was observed – with 36.2% (steamed, $p<0.001$) and 21.3% (grilled, $p<0.05$). Our previous results for steamed Black Sea bluefish showed lower TL decreases (15%) compared to this investigation (Stancheva *et al*., 2014). The presented results are in conformity with those by other authors, who observed loss of lipids after heat treatment in Rainbow trout and King salmon edible tissue (Larsen *et al*. 2010; Gladyshev *et al*. 2007). Moreover, the same authors supposed that the changes in the lipid amounts after steaming and roasting depend on the fish species, processing temperature, sample size and heatable surface area.

Fatty acid composition

The effect of heat treatment on FA composition of different fish species varies among studies (Gladishev et al., 2014; Gülgün et al., 2013; Larsen et al., 2010; Mnari Bhouri et al., 2010; Gladyshev et al., 2007). Our study reported that in raw samples, SFA was the most abundant FA group (35.70%) followed by MUFA (32.22 %) and PUFA (32.08 %). The same pattern was observed in the processed fish fillets (SFA>MUFA>PUFA). At steam temperature (100 ℃, 10 min) the SFA levels increased slowly (with 2.8 %), while the content of SFA at 220 ℃ increased up to 11.5 % ($p<0.05$) when the cooking period increased up to 14 min. The discrepancy of this FA pattern was observed for MUFA group, which changed in a different way under the influence of temperature. At cooking temperature of 100 ℃, the MUFA were found to increase up to 8.6%, whereas the losses in MUFA values slightly decreased at 220 ℃ and 14 minutes cooking time (1.5%). The PUFA group at both cooking methods was also found to decrease up to 11.9% ($p<0.05$) at 100 ℃ and whit 14.6% ($p<0.001$) at 220 ℃ compared to raw samples. As indicated, the maximum decrease of MUFA and PUFA levels was observed at high cooking temperature of 220 ℃ by longer residence time. This reduction of FAs might be due to reduction in overall fat contents of the examined samples. Similar trends were reported by other researchers. Larsen et al. (2010) found decreasing of MUFA and PUFA levels in steamed salmon. However, the conventional cooking method (Gladishev et al., 2014) resulted in a higher SFA and MUFA with lower proportions of PUFA. This has been explained in light of the higher affinity of PUFA to the oxidation compared with SFA and MUFA. The

comparison of FA profiles (FA % of total FA) of raw, steamed and grilled shad is shown in table 2.

Table 2. Fatty acids profile in raw and cooked shad (mean ± SD)

Fatty acid, [% of total FA]	**Black Sea shad**		
	raw	**steamed**	**grilled**
C 12:0	0.60±0.05	0.65±0.03	0.60±0.04
C 14:0	1.30±0.08	1.16±0.11	1.50±0.10$^{**,+}$
C 16:0	28.50±1.80	29.30±2.05*	33.60±2.25$^{***,+++}$
C 17:0	0.30±0.03	0.35±0.04	0.30±0.01
C 18:0	3.10±0.15	2.64±0.15**	2.20±0.12$^{***,+}$
C 20:0	0.50±0.07	0.68±0.03	0.52±0.03
C 21:0	0.12±0.02	0.17±0.01	0.20±0.01
C 22:0	0.50±0.03	0.50±0.02	0.64±0.04
C 23:0	0.15±0.01	0.18±0.01	0.15±0.01
C 24:0	0.50±0.03	0.70±0.03	0.50±0.02
Σ SFA	***35.70***	***36.33***	***40.22***$^{***,++}$
C 14:1 n-5	0.25±0.01	0.22±0.01	0.26±0.01
C 16:1 n-7	15.20±1.35	16.46±1.30**	14.80±1.05$^{***,++}$
C 17:1 n-8	0.26±0.01	0.32±0.02	0.25±0.01
C 18:1 n-9	11.66±1.50	13.50±1.55$^{***,++}$	12.10±1.00**
C 20:1 n-9	1.95±0.10	1.80±0.08	1.75±0.09
C 22:1 n-9	2.30±0.12	2.10±0.10	2.05±0.11
C 24:1 n-9	0.60±0.03	0.60±0.01	0.55±0.01
Σ MUFA	***32.22***	***35.00***$^{***,+++}$	***31.76***
C 18:3 n-6	0.25±0.01	0.30±0.01	0.35±0.01
C 18:2 n-6	7.20±0.70	6.40±0.65	6.30±0.50***
C 18:3 n-3	0.85±0.05	0.60±0.02	0.75±0.04
C 20:5 n-3	0.70±0.03	0.75±0.01	0.65±0.03
C 20:4 n-6	2.40±0.14	2.20±0.10	1.95±0.09**
C 20:2 n-6	0.55±0.02	0.35±0.01	0.50±0.02
C 20:3 n-3	0.86±0.03	0.78±0.05	0.78±0.01
C 20:3 n-6	nd	0.60±0.03	0.50±0.02
C 22:6 n-3	18.81±1.70	16.05±1.25	15.46±1.15***
C 22:2	0.50±0.01	0.64±0.03	0.53±0.02
Σ n 3	21.22±1.80	18.18±1.55	17.53±1.37***
Σ n 6	9.85±1.00	9.50±0.85	8.30±0.80$^{**,++}$
Σ PUFA	***32.08***	***28.67***	***28.02******

FA ratios and Lipid quality indices			
n-3/n-6	2.15±0.45	1.91±0.40	$2.11 \pm 0.55^{+}$
PUFA/SFA	0.90±0.05	0.79±0.05	$0.69 \pm 0.05^{**}$
AI	0.54	0.55	$0.70^{**,++}$
TI	0.38	0.42	$0.80^{***,+++}$
HH	1.40	1.30	$1.08^{***,++}$
PUFA groups, EPA and DHA n-3 PUFA in [g.100^{-1}g w.w.]			
PUFA	4.17±0.25	2.37±0.15	2.86±0.10
n-6	1.28±0.08	0.78±0.03	0.85±0.07
n-3	2.76±0.12	1.50±0.07	1.79±0.10
EPA	0.091±0.006	$0.062 \pm 0.005^{***}$	$0.066 \pm 0.005^{**}$
DHA	2.450±0.090	$1.320 \pm 0.060^{***}$	$1.570 \pm 0.050^{***,+}$
EPA+DHA	2.541±0.100	1.382±0.070	1.636±0.080

*** p<0.001 raw vs cooked; $^{+++}$ p<0.001 steamed vs grilled; AI - atherogenic index; TI - thrombogenic index; HH - hypocholesterolaemic/hypercholesterolaemic index EPA –eicosapentaenoic acid (20:5, n-3); DHA – docosahexaenoic acid (22:6, n-3)

Saturated and monounsaturated fatty acid

Fatty acids responded differently to heat treatments. Generally, SFA are fairly heat stable in temperatures encountered during common cooking methods. Steamed shad showed an increase of palmitic acid C16:0 (p<0.001), whereas the levels of myristic (C14:0) and stearic acid (C18:0) were significantly reduced (p<0.001). Grilled samples presented different trend - C16:0 and C14:0 levels significantly increased (p<0.001), while C18:0 levels decreased (p<0.001) in comparison to raw and steamed samples. These changes reflect the overall increase of SFAs in both heat treated shad (especially in grilled samples, table 2). The main MUFA's as palmitoleic (C16:1 n-7) and oleic (C18:1 n-9) acid showed the similar changes after thermal processing. The C16:1 n-7>C18:1n-9 pattern remains unchanged in both cases. Furthermore, steam treatment causes a significant increase in the levels of these acids, especially of C18:1 n-9 levels (p<0.001), which contributed to higher extent to the elevation of total MUFA content. Larsen et al. (2010) reported insignificant effect of steaming on King Salmon MUFA contents, while Merdzhanova et al., (2013) and Stancheva et al. (2014) found significant changes in MUFA levels in steamed Black Sea horse mackerel and bluefish. There are several possible reasons for 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. Moreover,

observed changes in C18:1 n-9 levels could be caused by changes in lipid extractability.

Polyunsaturated fatty acid

Results show that n-3 PUFA value in raw samples was 21.22% and was predominately due to the long chain PUFA (LCPUFA) as DHA and EPA, compared to levels of linolenic (C18:2 n-6, LA) and arachidonic (C 20:4 n-6, AA) acids from n-6 PUFAs series. In this study the n-3 PUFA content of Black Sea shad was of particular interest. In general, in this research EPA levels were found significantly lower than those of DHA in all analyzed samples. It is well known that highly unsaturated FA as EPA and DHA are more vulnerable to the oxidation even at ambient temperature compared to LA n-6 (Gladishev et al., 2014; Gülgün et al., 2013; Kolakowska et al., 2001). However the grill treatment leads to significant reduce on DHA (18.40%, $p<0.001$), whereas EPA levels remain unchanged compared to raw samples. Our results also showed that when we eat grilled shad we will ingest 95% of the amount of EPA and 82% of DHA observed in raw shad. These results are in agreement whit those presented for oven cooking see bas (Mnari Bhouri et al., 2010). This study found that both thermal processes resulted in significant decrease in n-3 LCPUFA levels – from 14.33% (steaming, $p<0.001$) up to 17.40% (grilling, $p<0.001$). The similar trend was observed for n-6 PUFA content, which was significantly lower in grilled shad (15.74, $p<0.001$). Some discrepancies were found when comparing the obtained results with results involving amounts of EPA and DHA quoted by other authors. About 25% higher amounts of EPA and DHA, in comparison with boiled humpback salmon raw fillets and trout fillets were observed by Gladyshev et al (2007). Kolakowska et al. (2001) suggested that different heat treatment procedures had no substantial influence on the percentage of EPA and DHA in Baltic herring. In other research the same authors reported that heating for 20 min at 160 ℃ could reduce DHA and EPA contents in sprat and mackerel (Gladishev et al., 2014). The possible reason is that high temperature may shatter the FAs with long chains by means of lipolitic enzymes. This is reflected in decrease of n-6 and n-3 PUFAs contents due to the increase of cooking temperature and time.

The European Food Safety Authority (EFSA, 2012) recommended daily intake (RDI) of 0.500 g EPA + DHA (EFSA, 2012). To evaluate the nutrition

lipid quality based of n-3 LCPUFAs content the percentage values of these FAs were recalculated to $g.100g^{-1}$ww of shad edible tissue according to FAO/INFOOD (2012). A 100 g of edible portion of raw shad filets contains 2.54 g of EPA+DHA n-3 PUFA (table 2) and provides 500% of EPA+DHA RDI. We considered that when presenting the LCPUFA content in absolute amounts [$g.100g^{-1}$wet weight], it is possible to provide more useful and accurate information for PUFA changes after heat treatments. In this study, it was observed that both cooking methods significantly decreased EPA+DHA content. Nevertheless, a 100 g portion of cooked shad can supply over than 270% (276% steamed, 327% grilled) of RDI.

PUFA/SFA and n-3/n-6 ratios are indices widely used to evaluate the nutritional value of edible fat for human consumption. According to some nutritional recommendations (Department of Health, London, 1994), the PUFA/SFA ratio in human diets should be above 0.45 and the n-6/n-3 ratio should not exceed 4.0. The n-3/n-6 ratio in row shad is 2.15, and PUFA/SFA ratio is 0.9 (table 2). Both cooking methods have no significant effect on this ratio and all fish samples had an n-3/n-6 ratio within the recommended range. Grilling affected mostly PUFA/SFA ratio ($p<0.05$) due to decrease of PUFA and increase of SFA levels. Hosseini et al. (2014) reported different effects of boiling and baking on *kutum roach* tissue, whereas Neff et al., (2014) presented similar trend for four freshwater fishes from the Laurentian Great Lakes region. Possible reason of discrepancy is that the effects of heat treatment are species-specific. The nutritional value of cooked shad is also determined by NQI (table 2). Higher values of AI and TI (> 1.0) are detrimental to human health, whereas higher HH levels (> 1.0) are recommended (Ulbritcth and Southgate, 1991). With regard to the quality indices considered, AI showed insignificant statistical differences ($p=0.05$) between raw and steamed shad, whereas grilling increased AI ($p<0.01$) level. Both thermal processes affected significantly TI, especially grilling ($p<0.001$) due to higher C14:0 values, which have most proatherogenic potential. HH index of raw shad shows value 1.4, but its levels decreased in both cooking samples up to 1.08. Considering the specific effects of FA on cholesterol metabolism indicates that Black Sea shad have a good nutritional value. Presented values (Table 2) are beneficial for human nutrition and clearly showed that both cooking methods do not affect significantly the nutrition lipid quality of shad tissue.

Fat soluble vitamins' contents

The raw and processed fish samples were analysed for the contents of fat soluble vitamins and the data were shown in table 3. The results were expressed as μg per 1g total lipid [$\mu g.g^{-1}TL$]. It is known that some of analysed vitamins are very susceptible on presence of UV light, oxygen and high temperature, especially vitamin A. This was confirmed by the received results - the grilling method of cooking strongly affects ($p<0.001$) the vitamin A level (table 3).

Table 3. Fat soluble vitamin's content in raw and cooked fish fillets, [$\mu g.g^{-1}TL$], (mean ± SD)

	Black Sea Shad		
Vitamin	**raw**	**steamed**	**grilled**
A	0.31 ± 0.01	0.30 ± 0.01	$0.12 \pm 0.01^{***,+++}$
D_3	3.20 ± 0.25	$5.53 \pm 0.46^{**}$	$4.0 \pm 0.26^{++}$
E	139.79 ± 14.5	$214.62 \pm 15.9^{***}$	$156.08 \pm 14.6^{+++}$

*** $p<0.001$ and ** $p<0.01$ raw vs cooked; $^{+++}$ $p<0.001$ and $^{++}$ $p<0.01$ steamed vs grilled

The amount of that analyte remains almost unchanged after the process of steam treatment. It was not observed a decrease of the levels of the vitamins in the steamed sample compared to the raw one. This correlate to the stability of moisture and total lipid contents in the same samples. The little changes of the three fat soluble vitamins in the raw compared to the processed samples are cost by the insignificant differences in moisture contents.

As opposed to vitamin A, the results for vitamins E and D_3, showed a different trend – they are substantially more resistant to two different cooking methods. Both analytes presented the lowest values in raw and the highest contents in steamed fillets. The observed differences were based on the significant decrease in lipids on treated sample. They showed low changes on vitamin's E and D_3 contents in wet samples tissues. Amounts of these vitamins also increased after grilling process, but in lesser extent, compared to the steaming. The SFA and MUFA groups are characterized by such behaviour. Similar studies were performed with different fish species by other research groups (Erkan *et al.,* 2010; Ersoy and Ozeren, 2009; Erickson, 1991; Mattila *et al.,* 1991). Their results show a discrepancy regarding the effect of various

types of cooking on the fat soluble vitamin contents in fish tissue, represented as μg.100g^{-1} wet weight.

Erkan *et al*. (2010) reported losses of about 75% for vitamin A and 55% for vitamin E after steaming on horse mackerel tissue. Our results for vitamin A losses after steaming are comparable with those of Erkan e*t al*. (2010) and Hosseini *et al*. (2014), when considering the amount of vitamin A in 100 g fish tissue. The data in table 3 shows relative stability of vitamin E after cooking methods, which was reported by Erickson (1991) for channel catfish processed fillets. It is known that the antioxidant function of vitamin E is critical for the prevention of oxidation of tissue PUFA. According to Nordic Nutrition Recommendations (2012) and Raederstorff *et al.* (2015), the relationship between vitamin E and PUFA intake (for adults), presented as over 0.4 ratio of mg vitamin E/ g total PUFA could also be used as a criterion for evaluation of nutrition quality. In our study minor changes were observed after both cooking methods: the ratio ranged between 0.47-0.82. Therefore we can conclude that Black Sea shad fillet preserves a good nutrition quality during thermal processes.

Mattila *et al.* (1999) presented slight variations for vitamin D_3 (below 10%) in fish samples after a baking process, which was similar to our data. Hosseini *et al.* (2014) reported more significant decrease of vitamin D_3 for baking *kutum roach* fish, which confirms the thesis that thermal processes affect vitamin levels in a different way depending on the fish species. These results confirm the stability of vitamin D_3 compared with vitamins A and E. A different conclusion about the effect of thermal processing on fish was presented by Ersoy and Ozeren (2009). They 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.

Conclusion

This study showed that the Black Sea shad is a very good source of n-3 LCPUFA and fat soluble vitamins D_3 and E regardless of cooking method. Significant decrease of TL content after steaming and grilling method was found. SFA groups showed significant increase, whereas PUFAs slowly decreased at both investigated cooking methods. Regardless of observed

changes of FA groups ratios, the shad tissue FA pattern: SFA>MUFA>PUFA was unaffected after thermal processes. On the other hand, investigated cooking methods significantly affected the n-3/n-6, PUFA/SFA ratios, AI, TI and HH indices. Nevertheless, the nutritional quality of the Black Sea Shad tissue is characterized by a very good beneficial effect of fish lipids based on EPA + DHA n-3 PUFA, fat soluble vitamins' content, which was well preserved after heat treatments. Based on the observed results, it can be concluded that the low cooking temperature is more preferable for the preserving of the unsaturated FA and vitamin D_3 contents. Consequently the steaming method can be recommended as a mild and less aggressive cooking method suitable for healthy dietetic regimes. The evaluation of the quality and the quantity of the shad nutritional components may promote their consumption and enable the consumers in making healthy food choices. When this information is available to whole populations and applied as a public health measure, the possibility for a healthier food choice may prevent several chronic diseases related to seafood consumption.

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