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Proximate Composition, Fatty Acid Profile and Mineral Content of Wild Brown Trout (*Salmo trutta* sp.) From Munzur River in Tunceli, Turkey

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In present study, the proximate composition, fatty acid profile and mineral content of wild brown trout living in Munzur river (Tunceli/Turkey) were investigated. The average level of protein, lipid, moisture and ash content of muscle of the wild brown trout was calculated to be 17.48, 2.3, 77.8 and 1.5 %, respectively. Thirty fatty acids were identified in this study. Identification of the fatty acid was carried out with gas chromatography-mass spectrometry (GC-MS). The total polyunsaturated fatty acids (PUFA) were found the highest fatty acid followed by that of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Docosahexaenoic acid (DHA, C22:6n3) and eicosapentaenoic acid (EPA, C20:5n3), linolenic acid (LNA, C18:3n3) and linoleic acid (LA, C18:2n6) were found to be predominant fatty acids in PUFAs with the percentages of 11.25, 6.82, 6.30 and 4.88, respectively. The ratio of n3/n6 and DHA /EPA was calculated to be 4.55 and 1.65, respectively. Twelve elements were determined in this study. Determination of elements was performed with ICP-AES. P and K were the predominant element among the minerals analyzed and calculated to be 1305.56-2967.06 and 1908.68-1220.10 mg kg^{-1} in muscle-skin of the wild brown trout, respectively.

Key Words: Wild brown trout, Proximate, Fatty acid, Mineral content, Munzur river.

INTRODUCTION

Wild brown trout, belonging to the genus salmo, may be distinguished by their brownish-yellow colour with dark and red spots on olive background. It is easy to catch them in ponds, lakes, streams and rivers. These fish are became one of the most preferred and caught fish because of its unique aroma. However, the available information about proximate, fatty acids and mineral content of this species is very limited. Therefore, investigating these attributes should be useful in order to get information about the fish since, it is considered one of the convenient healthy diets for consumers.

In today's world, people are pretty interested in what they eat in order to both stay healthy and prevent some diseases. Thus, choosing to right meal becomes more crucial than ever for almost everyone. Fish is considered and suggested[1] as healthy diets because of its rich essential fatty acid content especially eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3). Some studies have just proved that fish oil have a protective role on cardiovascular mortality[2], decrease effect on coronary heart diseases such as hyperlipidemia, hypertension and heart attacks[3,4] and cancer treatment[5].

Fish digest minerals from both its diet and environment *via* its gills and skin[6]. Therefore, mineral content of fish muscle and skin reflects composition idea of its diet and surrounding water. Having knowledge of element composition of fish is very vital because it shows the raw material quality. Elements are required in our diets even though some elements present in very low amounts, they have some potential role in living organism[7].

Some previous studies were about only fatty acid in different type and body part of wild brown trout from different region[8], element content of different barbus, fresh water fish, from Dam Lake[9] and different freshwater fish[10]. However, to the best of our knowledge, no study has ever done on this kind of wild brown trout from Munzur river. Therefore, objective of this study is to search the level of proximate composition, fatty acid profile and mineral content of wild brown trout from Munzur river. It is also aimed to find out the element composition of the fish skin in order to figure out the available nutrition of environment.

EXPERIMENTAL

Fish were caught with electric fishing from Munzur river, Tunceli/Turkey in November, 2009 by our research team.

Fifteen representative fish samples were chosen and divided into three groups. The first group was used for proximate analysis, the second group was used for fatty acid analysis and the third group was used for mineral analysis. The samples were filleted and skinned. Both muscle and skin were homogenated separately with a laboratory blender. Triplicate subsamples were taken for proximate and fatty acid determination. Quintuple subsamples were taken for mineral analysis. The skin of the fish was only used for mineral analysis.

Proximate analysis: Determination of protein, lipid, moisture and ash content was carried on according to Official Methods of Analysis 39.1.15 (AOAC)[11], modified Bligh & Dyer Method[12], EEC-recommended oven drying method ISOR 1442[13] and Official Methods of Analysis 35.1.14[14], respectively.

Fatty acid methyl esters (FAME) preparation: Fatty acid methyl ester of wild brown trout was prepared according to the method as described below and fatty acids profile was determined with GC-MS. Lipid containing chloroform was transferred into Teflon capped tube. Chloroform was evaporated under the stream of nitrogen and remaining lipid material was weighted. 1.5 mL of 0.5M methanolic sodium hydroxide was added into the tube and capped tightly, mixed and heated in a heating block at 115 ºC for 7 min. Then mixture was cooled and 2 mL of methanolic boron trifluoride (14 %) mixture was added. The tube was capped tightly and the mixture was re-heated in the boiling water for further 5 min. Then mixture was cooled to about 30-40 ºC, fatty acid methyl esters were extracted with 2 mL of isooctane. Separation of fatty acid methyl esters were carried out as described by Oksuz *et al*.[15].

Chromatographic conditions: Column: HP-INNOWax polyethylene glycol capillary column, Model Number: HP 19091N-133, nominal length: 30.0 m, nominal diameter: 250 µm, nominal film thickness: 0.25 µm. Injection temperature was set at 250 ºC and detector temperature was set at 270 ºC with a split ratio: 20:1. Split flow was maintained at 9.9 mL/min; total flow: 13.9 mL/min; gas type: helium.

Oven temperature was programmed initially at 120 ºC and held for 3 min. The temperature was then increased to 250 ºC with a 10 ºC/min ramp rate and held at this temperature for 4 min. The identification of individual fatty acids was carried out by comparing those retention time of FAME standard (Supelco 47085U PUFA No: 3) and Supelco 37 component Fame mix (47885-U). Confirmation of FAME was also performed by using a MS data base library (FAMEDBWAX).

Extraction and determination of mineral elements: The wet ashing method was used for digestion of organic matter. This procedure was carried out according to (AOAC Method 975.03) with a minor modification. Known amount of fish flesh (2 g) and skin (0.5 g) were weighted into a pre-washed 100 mL flask with a 10 mL of 65 % HNO_3 (Merck) and 2 mL of hydrogen peroxide. Samples were heated gradually on a heating block until the sample digested completely. Digests were filtered into a 25 mL volumetric flask, using ash-free filter paper and the volume was made up to 25 mL with ultra pure water.

Determination and quantification of mineral elements were done by ICP-AES (Varian Model- Liberty series II). Calibration curves for each of the individual elements were prepared from ICP Multi element stocks (Merck; 1.70332.0100 for Mg and 1.11355.0100 for Na, Pb, Cd, Cr, Cu, Fe, Mn, Ca, Zn and K). The phosphorus standard solution was prepared by dissolving KH_2PO_4 in ultra pure water to obtain a 1000 ppm stock phosphorus standard. The standard stock solution was then acidified (100 mL/100 mL) with 65 % HNO_3.

Statistical analysis: Differences between muscle and skin of mineral content of wild brown trout were subjected to analysis of variance (ANOVA). Statistical analysis was performed with SPSS 13.0. Significance was established at $p < 0.05$.

RESULTS AND DISCUSSION

The mean value of proximate composition of wild brown trout was given in Table-1. The protein and lipid content of wild brown trout were 18.55 and 2.3 %, respectively. The wild brown trout was also contained relatively high amount of moisture (77.8 %) and ash (1.5 %).

TABLE-1
PROTEIN, LIPID, MOISTURE AND ASH CONTENTS OF WILD BROWN TROUT

Components	Wild brown trout	Ref. values
Protein (%)	18.55 ± 0.33	18.8-19.1*, 19**, 20.7-20.8***, 19.6****
Lipid (%)	2.3 ± 0.1	1.2-10.8*, 1.2-10.6**, 3.4***, 5.2****
Moisture (%)	77.8 ± 0.3	70-79*, 70-79**, 73.8-74.2***, 76.7****,
Ash (%)	1.5 ± 0.1	–

Values represent mean ± standard deviation (n = 3). *Murray and Burt [18]. **Church[19]. ***Rasco, Miller, King[20]. ****Holland, Brown & Buss[21].

The proximate composition of fish can be affected for different reasons, such as seasonal changes, stage of age, level of maturity and availability of food[16,17]. Fish may utilize the protein in its body in order to stay alive during long starvation periods. Nevertheless, main changes in the body composition occur in moisture and lipid content, which may show an inverse correlation. The average level of moisture of the wild brown trout was found to be 77.8 %. The moisture of the fish investigated in this study is in the range of lipid values in rainbow trout[18,19] however that value is higher than those previously reported by Rasco *et al*.[20] and Holland *et al*.[21].

The present study confirms the concept of inverse relationship between lipid and moisture content in fish muscle. Lipid content of fish is considered as crucial nutritional quality criteria. As a comparison, brown trout contained considerable amount of less lipid than farmed rainbow trout.

Some reference values are presented in Table-1. The mean level of protein in the wild brown trout in this study is similar to that of protein in rainbow trout[18] whereas it is lower than those previously reported by Rasco *et al.*[20] and Holland *et al*.[21] and Church[19]. This could be the result of different fish species, environment and stage of age.

The lipid values of wild brown trout in this current study is in the range of lipid values in rainbow trout[18,19] however it is lower than those previously reported by Rasco *et al*.[20] and Holland *et al*.[20]. Aditionally, Oksuz[22] reported the monthly lipid changes in farmed trout all year long and found out those lipid

levels of trout were in the range of 3.1 and 7.2 %. These values are higher than that of lipid in wild trout investigated in present study.

A chromatogram of FAME from the wild brown fish is represented in Fig. 1. The peaks of particular interest, such as C16:0, C16:1n7, C18:1n7,C18:1n9, C:18:2n6, C18:3n3, C20:4n3, C20:5n3 and C22:6n3 were well separated and separation of long chain PUFAs were achieved in twenty minutes. Some peaks are vulnerable to overlap during to chromatographic process, such as C18:1 n9 and C18:1 n7 however those peaks were clearly separated.

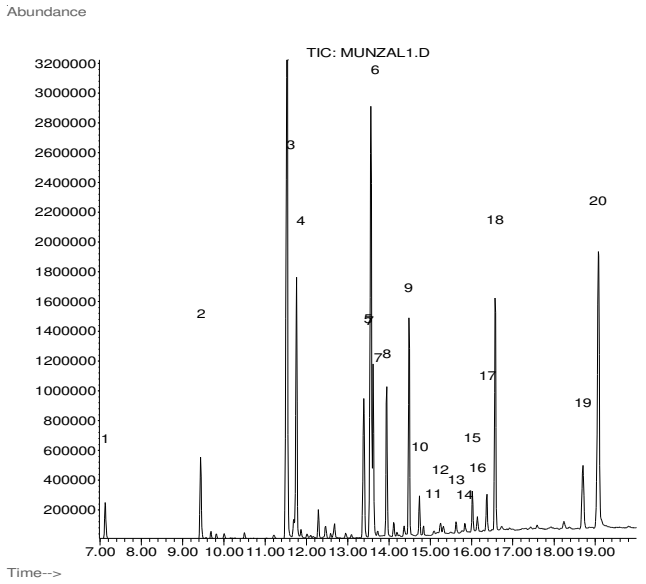

Fig. 1. Typical GC profile of FAME obtained from brown trout oil sample peak ID: 1 = C12:0; 2 = C14:0; 3 = C16:0; 4 = C16:1n-7; 5 = C18:0; 6 = C18:1n-9 7 = C18:1n-7;8 = C18:2n-6; 9 = C18:3n-3; 10 = C18:4n-3; 11 = 20:0; 12 = C20:1n-9; 13 = 20:2 14 = C20:3n-6 15 = C20:4n-6; 16 = C20:3n-3; 17 = C20:4n-3; 18 = C20:5n-3; 19 = C22:5n-3; 20 = C22:6n-3

The fatty acid composition of the wild brown trout is presented in Table-2. The level of total PUFA had the highest percentage in all fatty acid composition in muscle of wild brown trout with the percentage of 37.85, followed by SFA and MUFA, respectively. The total n3 (30.44 %) was found higher than total n6 (6.68 %). These results are different from fatty acid compositions of muscle of *Salmo trutta macrostigma*, a sub-species of the *Salmo trutta*[8]. Vliet and Katan[23] found that wild fish are better source of n3 than farmed fish. Similarly to previous study[23], findings about n3 in this study is in parallel. Based on the findings, the wild brown trout, wild fish, investigated in this current study could be considered as a good source of n3 fatty acid.

Seven fatty acids were determined in terms of SFA. The major SFA C16:0 (palmitic acid), C18:0 (stearic acid), C14:0 (myristic acid) and C12:0 (lauric acid) however some trace SFA, such as C17:0 (heptadecanoic acid), C15:0 (pentadecanoic acid) and C20:0 (arachidic acid) were present. As it was expected, the level of C16:0 in wild brown trout in this study was found predominant fatty acid in SFA followed by C18:0 and C14:0, respectively. Aras *et al*.[8] found same pattern in fatty acid compositions of muscle of *Salmo trutta macrostigma*.

TABLE-2
FATTY ACID COMPOSITION OF WLLD BROWN TROUT

Fatty acids	Mean (%)	Fatty acids	Mean (%)
C12:0	1.58 ± 0.29	C18:3n3	6.30 ± 0.24
C14:0	3.01 ± 0.40	C18:4n3	1.26 ± 0.12
C15:0	0.14 ± 0.16	C20:2n6	0.30 ± 0.20
C16:0	21.00 ± 1.67	C20:3n3	0.59 ± 0.08
C17:0	0.39 ± 0.26	C20:3n6	0.16 ± 0.19
C18:0	5.01 ± 0.28	C20:4n6	1.12 ± 0.18
C20:0	0.08 ± 0.16	C20:4n3	1.43 ± 0.20
Total SFA	31.20	C20:5n3	6.82 ± 0.38
C14:1	0.13 ± 0.15	C22:2	0.10 ± 0.20
C16:1n7	8.65 ± 1.29	C22:5n3	2.79 ± 0.35
C16:1n9	0.75 ± 0.24	C22:6n3	11.25 ± 1.38
C17:1	0.00 ± 0.00	Total PUFA	37.85
C18:1n7	4.58 ± 0.48	Total n3	30.44
C18:1n9	15.20 ± 1.21	Total n6	6.68
C20:1n9	0.34 ± 0.23	n3/n6	4.55
C22:1n9	0.46 ± 0.93	DHA/EPA	1.65
Total MUFA	30.15	–	–
C16:4n1	0.49 ± 0.35	–	–
C16:2n4	0.23 ± 0.47	–	–
C18:2n6	4.88 ± 0.35	–	–
C18:3n6	0.22 ± 0.25	–	–

Values represent mean ± standard deviation (n = 3).

This similarity could be one of the distinguishable characteristics of the wild brown trout species. Additionally, the levels of C16:0 (21.00 ± 1.67 %) and in wild brown trout in present study was found to be similar to C16:0 in muscle of *Salmo trutta macrostigma*[8] (19.27 ± 1.53 %) while the levels of C14:0 and C18:0 were found lower.

The levels of C18:1n9 (oleic acid) and C16:1n7 (palmitoleate) in the wild brown trout were found the highest two fatty acid in MUFA with the values of 15.20 ± 1.21 and 8.65 ± 1.29 %, respectively. These results were parallel to that reported by Aras *et al*.[8]. In addition the levels of the C18:1 n9 and C16:1 n7, the level of the C18:1n7 in wild brown trout in this current study was found to be 4.58 ± 0.48 % and the ratio of C18:1n9 and C16:1n7 was about 3.3.

Fifteen fatty acids were determined in terms of PUFA. The levels of DHA (C22:6n-3), EPA (C20:5n-3), LA (C18:2n6) and LNA (C18:3n3) in wild brown trout muscle were the predominant fatty acids. The presence high level of LNA and LA shows that diets of the wild brown trout include both animal and plant originated diets. Additionally, the level of LNA in muscle of wild brown trout in this study was found higher than that of LA in muscle of *Salmo trutta* macrostigma[8] whereas the levels of DHA and EPA the wild brown trout were found lower[8].

The wild brown trout contains considerably a high level of PUFA (including DHA, EPA, LNA and LA). Fish is an excellence food item to lower the risk of many diseases[1,24,25]. In this point of view, the wild brown trout from Munzur river has considerable amount of PUFA.

The element composition of skin and muscle of the wild brown trout from Munzur river was shown in Table-3. The levels of elements in muscle and skin of wild brown trout investigated in this current study were found different from each other. The amounts of K in muscle of wild brown trout and P in skin wild brown trout were calculated as the predominant element in this present study. The levels of both P and K in

TABLE-3
ELEMENT COMPOSTION OF MUSCLE AND SKIN OF WLLD BROWN TROUT

Elements	Muscle	Skin	*p*-Value
Cd	0.05 ± 0.01	0.07 ± 0.04	0.360
Cr	0.08 ± 0.03	0.18 ± 0.02	0.010
Mn	0.15 ± 0.05	0.90 ± 0.31	0.015
Cu	0.58 ± 0.10	0.99 ± 0.18	0.026
Pb	0.89 ± 1.22	0.24 ± 0.26	0.420
Zn	8.63 ± 12.32	22.37 ± 2.23	0.130
Fe	12.33 ± 2.64	29.78 ± 6.25	0.011
Ca	53.78 ± 34.04	574.48 ± 280.53	0.033
Mg	231.92 ± 24.93	307.09 ± 58.47	0.110
Na	294.14 ± 53.83	247.75 ± 39.74	0.296
P	1305.56 ± 122.31	2967.06 ± 735.95	0.018
K	1908.68 ± 190.59	1220.10 ± 27.59	0.003

Values represent mean ± standard deviation (n = 3).

muscle and skin of wild brown trout were differed from each other. This differences in values was found statistically significant ($p < 0.05$).

The levels of Cd among the 12 elements investigated in this study in both muscle and skin of the wild brown trout were found to be lowest amount with the values of 0.05 and 0.07 mg kg^{-1}, respectively. Moreover, the amount of Cr, Mn and Cu in muscle of the wild brown trout were found lower than that those of the same elements in skin ($p < 0.05$). Furthermore, the level of Cr in muscle and skin of wild brown trout were found to be 0.08 and 0.18 mg kg^{-1}, respectively. Similar findings were observed by Alhas *et al.*[9], for two barbus species, *Barbus xanthopterus* and *Barbus rajanorum mystaceus* in Ataturk Dam Lake. The average level of Mn in muscle of the wild brown trout was found lower than that of two Barbus. whereas the level of Cu was higher.

The mean level of Fe in muscle and skin of the wild brown trout were calculated to be 12.33 and 29.78 mg kg^{-1}. These findings were higher than those reported by Erdogrul and Erbilir[10] for muscle of thorn-bream, nose-carp and carp from Sir Dam Lake. Fe is one of the essential trace elements and should be included a certain of amount in daily diet. According to FAO and WHO, this amount for Fe for a 70 kg person should be 0.8 mg/day.

Even though, the average levels of Zn in muscle and skin of wild brown trout differed from each other, this difference were found statistically not significant ($p > 0.05$). The levels of Zn in both muscle and skin in the wild brown trout were found higher than that reported by Alhas *et al*., for both *Barbus xanthopterus* and *Barbus rajanorum mystaceus*. Higher level of Zn may cause health problems[26]. According to FAO and WHO, daily intake of Zn by for a 70 kg person should be less than 0.8 mg/day.

In contrast to other micro elements (Cd, Cr, Mn, Cu, Zn) investigated in this current study, only the level of Pb was found higher than that of other micro elements ($p > 0.05$). Additionally, the level of Pb in muscle of the wild brown trout was found more than three times higher than that of Pb in skin of the fish. Accumulation of Pb in muscle of the fish could be much higher than that of skin. Lead is known to have some negative effect on health, such as lowering intellectual performance in children and increasing blood pressure and cardiovascular disease in adults[27]. According to FAO and WHO, tolerable daily intake of Pb by for a 70 kg person is 0.24 mg/day.

The average levels of Ca, Mg and P in muscle of the wild brown trout were found lower than those of the same elements in skin of the fish while the mean amounts of Na and K were higher. In addition, the macro elements (Ca, Mg, Na, P and K) in both muscle and skin of the wild brown trout studied in this present study mostly differed from each other. These difference in values were found statistically significant ($p < 0.05$) for Ca, P and K while they were statistically not significant for Mg and Na. Ca is responsible for some regulatory functions in the body. P is directly involved in energy producing cellular reaction. K has a major role in maintaining fluid and electrolyte balance and cell integrity. K requirement for human is about 2 g/day. Magnesium is necessary for energy metabolism[28].

Conclusion

The results reflect a positive evaluation of nutritional quality and safety for the wild brown trout living in Munzur river. Lower amount of heavy metals, such as Cd, Cr and Pb in the muscle showed that the aquatic environment of the fish seems to be not polluted by heavy metals. The fish investigated in this study was characterized by a comparable nutritional quality, low level of lipid, high amount of protein and mineral contents. The lipid structure was characterized by a high proportion of n3 PUFA, great amount of DHA, EPA, LNA and LA which are considered as important parameters for a fish to consume.

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