Seasonal variation of Fatty Acid Composition of Trout (*Salmo opimus*)

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ABSTRACT

The changes of fatty acid (FAs) composition of muscle tissues of trout (*Salmo opimus*) caught from Fırnız and Törbüzek in the Ceyhan Basin Region were investigated by using GC. Palmitic acid and oleic acid were the most abundant FAs and significant differences ($p<0.05$) were observed between seasons. The composition of fatty acids showed that ω-3 poly unsaturated fatty acids (∑ω-3 PUFAs: 6.26-25.44%) were highest, followed by ω-6 (∑ω-6 PUFAs: 5.87-8.24%). The ratio of ω-3/ω-6 the samples were ranged from 1.06 to 3.13%. This study showed that the level of total PUFAs (mainly n-3 and n-6 fatty acids) increased significantly during the summer and autumn period in the muscle tissue of brown trout.

Key Words: Brown Trout, Ceyhan River Basin, Fatty Acids, *Salmo Opimus*, Seasonal Changes.

Dağ Alabalığının (*Salmo Opimus*) Yağ Asidi Kompozisyonunun Mevsimsel Değişimleri

ÖZ

Ceyhan Nehri'nin Fırnız ve Törbüzek kollarından yakalanan dağ alabalığının (*Salmo opimus*) mevsimlere bağlı yağ asidi (YAs) bileşimleri gaz kromatografi ile araştırılmıştır. Analiz sonuçlarına göre palmitik ve oleik asit en baskın yağ asidi olarak tespit edilmiş ve mevsimsel farklar çok anlamlı bulunmuştur ($p<0,05$). Yağ asidi kompozisyonlarına göre en yüksek ω-3 (∑ω-3 ÇDYAs: %6,26-25,44) grubu, daha sonra ω-6 (∑ω-6 ÇDYAs: %5,87-8,24) grubu olduğu gözlenmiştir. Örneklerdeki ω-3/ω-6 oranının %1,06 ile 3,13 arasında olduğu tespit edilmiştir. Dağ alabalığının kas dokusunda toplam ÇDYAs değerinin (esasen n-3 ve n-6 yağ asitlerinin) yaz ve sonbahar döneminde belirgin derecede arttığı gözlenmiştir.

Anahtar Kelimeler: Ceyhan Nehri, Dağ Alabalığı, Mevsimsel Değişiklik, *Salmo Opimus*, Yağ Asitleri.

1. INTRODUCTION

Fish is a major source of food for human, providing an important amount of the animal protein diet in many countries. Fish flesh is easily digestible due to its long muscle fibres as compared to red meat and fish lipids are acknowledged as being beneficial to health as good sources of long chain n-3 polyunsaturated fatty acids (n-3 PUFAs), especially eicosapentaenoic acid (EPA or 20:5n-3) and docosahexaenoic acid (DHA or 22:6n-3). Studies have been carried out with a view of improving the understanding the pathogenesis of diseases and association with food, along with the role of lipids. The fatty acid content of diet inflects the fatty acid profile in immune cells. Therefore, this may be an effective way to regulate the functionality of normal cells through nutrition. Fatty acids play major role in nutrition, disease prevention and health promotion (Kinsella, 1987; Lees and Karel, 1990; Simopoulos, 1991; Ulbricht and Southgate, 1991; Horrocks and Yeo, 1999; Mozaffarian et al., 2005). n-3 fatty acids are essential for neural development for infant in uterus and till the first few years after birth (Montaño et al., 2001) and n-3 PUFAs have beneficial effects on inflammation, hypertension, aggression, arrhythmias, inflammatory and auto-immune disorders, psoriasis, depression, coronary heart disease, and cancer (Candela et al., 1997; Pike, 1999).

Salmo opimus can be found around Europe, reaching southwards to the Atlas Range (Morocco, Algeria) and eastwards to the upper Amu-Darya drainage in Afghanistan. Until the late 1990's most European populations of trouts have been referred to as *Salmo trutta* (Turan et al, 2012).

In Turkey, contrary to having a great variety of aquatic species, the contribution of fish to the diet is low. Thus, it is very important to change the diet habits. In view of these facts, it seems necessary to carry out a study on the nutritional value and lipid profile of highly consumed fish in two different seasons. The objective of this work was to characterize in terms of the fatty acid composition of trout in four different seasons in the Fırnız Stream, a tributary of the Ceyhan River which flowing into the east Mediterranean Sea.

2. MATERIALS AND METHODS

2.1. Fish Samples and Study Area

Study was carried out in the Fırnız Stream (37°45'N; 36°39'E; Altitude: 756 m) and Törbüzek Stream (38°04'N; 36°27'E; Altitude: 1390 m), a small tributary of the Ceyhan River near Kahramanmaraş in East Mediterranean, Turkey.

Trout samples were taken randomly for analysis in four seasons, spring (n:14), summer (n:15), winter (n:12), and autumn (n:12). The studies were conducted in three replicates. The samples were

collected in middle month of each season during 2013. Samples were immediately transported on ice to the laboratory and were measured and weighed, aged and frozen until the analyses.

2.2. Lipid Extraction and Fatty Acid Methyl Ester Analyses

Individual fishes were dissected and portions of muscle tissue below the dorsal fin, devoid of skin, sub-dermal fat and bone were kept in ice for less than 4 h before lipid extraction. For each analysis, about 10 g of edible white muscle were homogenized separately using a warring blender. Lipids were extracted following a modified from Bligh and Dyer method (Bligh and Dyer, 1959). by using a mixture of 70 ml methanol and 140 ml chloroform (1:2 v/v) during 3 min. The mixture was filtered by Whatman No 1 filter paper. After centrifugation, the lower chloroform phase was kept and dried with sodium sulphate for total lipid content determination. Fatty acids were prepared by saponification of total lipids (50 mg) with KOH-ethanol, 2 mol l^{-1} (1 ml), and acid-catalyzed methylation with methanolic hydrogen chloride as described by Christie (Christie, 1982).

2.3. Gas chromatographic condition

Fatty acid methyl esters (FAME) were analyzed on a HP (Hewlett Packard) Agilent 6890N model gas chromatograph (GC), equipped with a flame ionization detector (FID), with a DB-23 capillary column (60 m × 0.25 mm, ID × 0.25 μm). The temperature of oven was 140°C, held 5 min, raised to 200°C at a rate of 4 °C/min and to 220°C at a rate of 1°C/min, while the injector and the detector temperature were set at 220 °C and 280°C, respectively. Carrier gas was helium (2 ml/min) and split ratio was 30:1. Two replicate GC analyses were performed and the results were expressed in GC area % as a mean value and ± standard deviation (Güler et al., 2008).

2.4 Statistical Analysis

The data were subjected to ANOVA and the significant means were compared by Tukey's multiple range tests and were presented as mean ±SEM.

3. RESULTS AND DISCUSSION

The results of fatty acid composition of trout at different seasons and the results of fatty acid composition of male and female species of trout at different seasons were presented in Table 1 and 2. In autumn female fish samples and in winter male fish samples were not studied.

The mean length, weight and age of the specimens were 18.3 cm (range: 12.03-25.4 cm), 142.5 g (range: 60.3-232 g), 4.1 (range: III-VI), respectively.

The composition of saturated fatty acids (SFAs) were higher than those monounsaturated fatty acids (MUFAs) and polyunsaturated acids (PUFAs) among the seasons. The proportions of total of SFAs in spring, summer, autumn and winter were 51.25±6.34, 32.74±11.12, 35.84±0.15 and

58.01±0.01%, respectively, and the difference was significantly important among seasons ($p< 0.05$). ΣMUFAs in spring, summer, autumn and winter were 33.18±5.32, 30.04±5.38, 29.32±5.14 and 25.02±0.17%, respectively. Σn-3 PUFAs in spring, summer, autumn and winter were 6.26±3.83, 20.26±6.94, 25.44±3.29 and 11.29±0.69%. Σn-6 PUFAs were 5.87±2.50, 7.43±2.74, 8.24±1.05, 6.62±0.59% in spring, summer, autumn and winter (Table1).

Palmitic acid (C16.0) was the primary saturated fatty acid (SFAs), ranged between 16.59-29.40%. In summer the composition of palmitic acid of male fish samples (24.54%) were approximately twice as higher than female samples (11.29%). Palmitic acid content was higher in winter and autumn than in summer and spring, this result was not important statistically. Aras et al., (2003), reported similar results in *S. trutta macrostigma* in Yeşildere Creek in the Karasu Basin for summer season.

Palmitoleic (16:1), oleic (n-9, 18:1) and eicosenoic (n-9, 20:1) acids were the main acids in monounsaturated fatty acids. Palmitoleic acid composition showed statistically important variation among seasons ($p< 0.01$).

Stearic acid (18.0) composition, in total SFA, was 5.19-8.29%, and variation between the seasons was statistically important ($p< 0.05$). The ratio of myristic acid (C14.0) in winter was higher than the other seasons ($p< 0.05$).

It is reported that the ratio of n-3/n-6 in fresh water fish is lower than the sea water fish (Borlongan and Benitez, 1992; Sheikh-Eldin et al., 1996). In summer and autumn n-3/n-6 ratio was in high amounts (3.05±1.39 and 3.13±0.80) (Table1) in trout male and female samples (Table2). But Haliloğlu (2001), reported that the ratio of n-3/n-6 in fresh water rainbow trout fish was nearly one.

According to the results the ratio of n-3PUFA was higher amounts in autumn (25.44±3.29%) and in summer (20.26 ±6.94%). The ratio of n-6 PUFA was high in summer and autumn (7.43±2.74 and 8.24±1.05%) than spring and winter (5.87±2.50 and 6.62±0.59%) which can be explained by reproduction season, temperature adaptation, and nutritive facts. The amount of PUFA could be high in cold water fish (Dey et al., 1993; Wodtke, 1991). The act of PUFA in plasticity and membrane permeability may be one of the factors of these differences (Brenner, 1984; Czesny et al., 2000).

Akpınar et al. (2009) studied the fatty acid compositions of liver and muscle of male and female *S. trutta macrostigma*, in the Tohma River, Turkey. There were quantitative differences between individual fatty acids in the tissues investigated, depending on the sex. The most abundant fatty acids in both tissues of both sexes were palmitic acid (C16:0; 19.0-21.6%), stearic acid (C18:0; 5.32-11.3%), C18:1 ω7 (5.65-9.38%), oleic acid (C18:1 ω9; 15.6-22.4%), eicosapentaenoic acid (EPA;C20:5 ω3; 6.34-7.88%) and docosahexaenoic acid (DHA; C22:6 ω3; 7.38-15.6%). The ω3/ω6 ratio in tissues were found to be 2.89 (male) and 1.97 (female) in liver, and 2.59 male) and 2.26 (female) in muscle. These results showed quite similarity with this study.

Blanchet et al. (2005) studied the fatty acid composition of wild and farmed Atlantic salmon and rainbow trout. Results showed that lipid and n-3 highly unsaturated fatty acid contents of farmed and

wild Atlantic salmon were similar. Total n-3 and n-6 PUFA were significantly higher in farmed Atlantic salmon than in wild. Farmed rainbow trout contained more fat and less n-3 PUFA than wild rainbow trout. Our results showed that farmed salmonids provide high levels of n-3 HUFA to consumers.

Görgün and Akpınar (2007) studied the Liver and muscle fatty acid composition of mature and immature rainbow trout (*Oncorhynchus mykiss*). The amounts of C22:6 n-3 were higher in the liver (29.04±0.06 - 27.41±0.17%) and muscle (13.05±0.40-11.37±0.21%) of immature fish than in mature fish and depended on the composition of the diet. Results of this study showed that fatty acid composition in fish tissues can considerably vary, depending on the age of fish and their diet.

From a nutritional point, the EPA (20:5, n-3) + DHA (22:6, n-3) contents are of interest due to the incidence of these fatty acids in the therapy and prevention of cardiovascular diseases (Uauy and Valenzuela, 1992). Sargent (1996), reported that n-3 PUFA, DHA, has a role in maintaining the structure and functional integrity of fish cells and has important role in neural cell membranes, i.e. the brain and eyes along with the nutritional and health benefits. Study indicated DHA levels were higher than EPA levels and winter season decreased EPA and DHA levels significantly ($p< 0.01$).

It is known that the fatty acid composition of the fish is not constant. They are related to the life cycle of the fish and external factors, like temperature, salinity and fatty acid composition of their food, reproduction season (Gallagher et al, 1991; Bandarra et al., 2001). This could be also valid for trout samples in the streams of Ceyhan River basin.

Significant differences in lipid content and fatty acid composition can be observed between species and season of collect. This study showed that the level of total PUFAs (mainly n-3 and n-6 fatty acids) increased significantly during the summer and autumn period in the muscle tissue of brown trout.

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Table 1. Fatty acid composition of muscle tissue of trout.

Fatty acids	Spring (n:14)	Summer (n:15)	Autumn (n:12)	Winter (n:12)
C6:0	3.58±1.35	0.71±0.37[b]	0.18±0.01[b]	0.21±0.01[b]
C8:0	4.97±1.65	1.08±0.70[b]	0.19±0.01 [b]	ND
C10:0	1.23±1.58	0.19±0.07	0.08±0.01	0.13±0.01
C11:0	0.27±0.13	0.10±0.06	0.08±0.36	ND
C12:0	3.87±2.50	1.72±1.52	0.35±0.23	0.23±0.01
C13:0	0.33±0.07	0.23±0.34	0.05±0.01	0.10±0.01
C14:0	4.48±5.65	2.32±0.29	2.18±0.24	14.10±1.51[a,d,f]
C14:1	0.39±0.22	0.40±0.33	0.31±0.02	0.53±0.05
C15:0	0.99±0.37	1.48±1.56	0.62±0.01	1.40±0.21
C15:1 Cis-10	2.79±1.06	0.60±0.46[b]	0.06±0.001[b]	0.16±0.04[b]
C16:0	18.70±3.39	16.59±10.62	23.56±0.36	29.40±0.67
C16:1	10.25±2.10	6.96±1.05[a]	4.99±1.66 b	3.96±0.43[b]
C17:0	1.85±0.38	1.37±0.48	1.49±0.06	0.94±0.06
C17:1 Cis-10-	3.57±1.53	1.58±0.70	0.78±0.26 [a]	0.43±0.11[a]
C18:0	7.77±1.06	5.19±0.81[b]	6.11±0.62	8.29±0.68[e]
C18:1 n-9 trans	0.69±0.08	0.52±0.33	0.13±0.05[a]	ND
C18:1 n-9 cis	10.95±3.45	16.60±2.67[a]	17.64±2.87	17.59±0.65
C18:2 n-6 trans	0.29±0.04	0.42±0.70	0.26±0.13	ND
C18:2 n-6 cis	3.56±2.19	3.54±0.67	4.55±0.90	5.13±0.31
C18:3 n-6	0.13±0.03	0.30±0.27	0.19±0.001	ND
C20:0	0.56±0.33	0.41±0.20	0.30±0.04	0.41±0.01
C18:3 n-3α	1.50±2.25	10.29±4.0[b]	11.83±1.47[b]	8.82±0.50[a]
C20:1 n-9	1.61±0.97	1.86±0.49	1.25±0.11	0.68±0.03
C21:0	0.12±0.06	0.14±0.14	0.14±0.12	0.34±0.05
C20:2 cis 11,14	0.90±0.57	1.00±1.02	0.52±0.13	0.38±0.05
C20:3 n-3 cis-11,14,17	0.19±0.10	0.34±0.29	0.23±0.04	ND
C22:0	0.16±0.08	0.304±0.238	0.20±0.02	0.19±0.01
C20:4 n-6	1.14±0.10	0.86±0.07[a,g]	2.62±0.17 [c]	0.50±0.01[c,d,g]
C22:2 cis 13,16	1.16±0.71	1.96±0.591	0.07±0.01 [d]	ND
C22:1 n-9	0.63±0.26	1.58±0.89	1.54±0.16	0.65±0.01
C23:0	0.11±0.04	0.12±0.18	0.07±0.02	ND
C20:5 n-3 cis-5,8,11,14,17	2.92±1.16	4.27±1.38	4.99±0.42	0.46±0.10[d,f]
C24:0	0.36±0.24	0.24±0.06	0.23±1.63	ND
C24:1 n-9	1.29±0.46	2.34±1.02	2.33±0.16	0.86±0.16
C22:6 n-3 cis-4,7,10,13,16,19	1.83±0.84	5.36±2.76[a]	8.37±1.39[b]	1.94±0.001[f]
Σ SFAs	51.25±6.34	32.74±11.12[a]	35.84±0.15	58.01±0.01[d]
Σ MUFAs	33.18±5.32	30.04±5.38	29.32±5.14	25.02±0.17
Σ n-3 PUFAs	6.26±3.83	20.26±6.94[b]	25.44±3.29[b]	11.29±0.69
Σ n-6 PUFAs	5.87±2.50	7.43±2.74	8.24±1.05	6.62±0.59
Σ n-3 / n-6	1.06±0.18	3.05±1.39[a]	3.13±0.80	1.71±0.04

n: number of sample, a: p< 0.05 vs. spring, b: p< 0.01 vs. spring, c: p< 0.001 vs.spring; d: p< p< 0.05 vs. summer, e: p< 0.01 vs.summer; f: p< 0.05 vs. autumn, g: p< 0.001 vs. autumn, ND: not detected

Table 2. Fatty acid composition of male and female species of muscle tissue of trout among seasons

Fatty acids	Spring (n:14) ♀	Spring (n:14) ♂	Summer (n:15) ♀	Summer (n:15) ♂	Autumn (n:12) ♂	Winter (n:12) ♀
C6:0	4.71±0.04[a]	2.46±0.70	0.72±0.40	0.70±0.03	0.19±0.02	0.21±0.02
C8:0	6.39±0.28[b]	3.55±0.35	0.49±0.20[c]	1.68±0.06	0.17±0.13	ND
C10:0	0.56±0.03	1.90±2.39	0.20±0.13	0.19±0.01	0.08±0.01	0.13±0.07
C11:0	0.27±0.02	0.27±0.22	0.12±0.10	0.09±0.03	0.08±0.03	ND
C12:0	6.16±0.223	2.33±1.92	1.99±1.97	1.34±1.02	0.35±0.02	0.28±0.07
C13:0	0.40±0.030	0.27±0.02	0.317±0.460	0.12±0.09	0.05±0.01	0.10±0.08
C14:0	1.59±0.10	6.41±7.07	2.29±0.413	2.37±0.02	2.18±0.24	14.10±1.56
C14:1	0.23±0.01	0.50±0.23	0.52±0.413	0.23±0.06	0.31±0.02	0.53±0.05
C15:0	1.11±0.04	0.90±0.49	1.94±2.02	0.79±0.09	0.62±0.00	1.40±0.21
C15:1 cis10	3.69±0.09	2.19±0.96	0.59±0.52	0.63±0.58	0.06±0.04	0.16±0.21
C16:0	20.56±0.66	17.50±4.17	11.29±10.62	24.54±3.85	23.56±0.36	29.40±0.67
C16:1	8.52±0.43	11.40±1.94	6.42±0.99	7.77±0.55	4.99±0.16	3.96±0.43
C17:0	1.60±0.22	2.02±0.41	1.479±0.654	1.22±0.01	1.49±0.06	0.94±0.06
C17:1 cis10	4.56±0.05	2.91±1.75	1.51±0.98	1.70±0.24	0.78±0.26	0.43±0.11
C18:0	8.72±0.30	7.14±0.86	4.76±0.48	5.84±0.89	6.11±0.62	8.29±0.68
C18:1 n-9 trans	0.71±0.01	0.67±0.13	0.81±0.06**	0.23±0.06	0.13±0.05	ND
C18:1 n-9 cis	11.0±0.49	10.92±4.87	15.27±2.46	18.61±1.7	17.64±2.87	17.59±0.65
C18:2 n-6 trans	0.31±0.00	0.28±0.06	0.65±0.91	0.09±0.03	0.26±0.13	ND
C18:2 n-6	2.80±0.02	4.06±2.94	3.57±0.40	3.51±1.21	4.55±0.90	5.13±0.31
C18:3 n-6	0.11±0.01	0.14±0.03	0.38±0.360	0.19±0.01	0.19±0.04	ND
C20:0	0.27±0.01	0.75±0.30	0.47±0.27	0.33±0.02	0.30±0.04	0.41±0.01
C18:n 3a	0.60±0.25	2.10±2.96	9.89±5.59	10.90±0.52	11.83±1.47	8.82±0.50
C20:1 n-9	1.01±0.07	2.02±1.14	1.81±0.65	1.95±0.32	1.25±0.11	0.68±0.03
C21:0	0.11±0.01	0.13±0.07	0.18±0.19	0.07±0.01	0.14±0.12	0.34±0.05
C20:2 cis 11,14	0.66±0.09	1.06±0.74	1.26±1.36	0.61±0.01	0.52±0.13	0.38±0.05
C20:3 n-3 cis 11,14,17	ND	0.19±0.10	0.41±0.39	0.23±0.03	0.23±0.04	ND
C22:0	0.10±0.01	0.20±0.08	0.38±0.29	0.17±0.01	0.20±0.02	0.19±0.01
C20:4 n-6	1.13±0.11	1.15±0.12	0.90±0.03	0.81±0.08	2.62±0.17	0.54±0.05

C22:2 cis 13,16	1.11±0.01	1.19±1.01	2.08±0.54	1.77±0.83	0.07±0.01	ND
C22:1 n-9	0.51±0.18	0.74±0.35	1.96±1.02	1.0±0.15	1.54±0.16	0.65±0.01
C23:0	ND	0.11±0.04	0.18±0.23	0.04±0.01	0.07±0.02	ND
C20:5 n-3 cis 5,8,11,14,17	2.27±0.10	3.35±1.42	4.98±0.93	3.20±1.45	4.99±0.42	0.46±0.10
C24:0	0.27±0.04	0.42±0.32	0.24±0.08	0.25±0.03	0.23±0.16	ND
C24:1 n-9	0.93±0.02	1.53±0.47	2.81±0.90	1.62±0.90	2.33±0.16	0.86±0.16
C22:6 n-3 cis 4,7,10, 13,16, 19	1.33±0.03	2.17±0.99	6.31±2.65	3.93±3.13	8.37±1.39	2.13±0.26
Σ SFAs	57.04±0.14	47.39±4.96	28.24±11.64	39.49±8.49	35.84±0.15	58.01±0.04
Σ MUFAs	30.96±0.06	34.64±6.98	27.55±5.76	33.77±1.81	29.32±5.14	25.02±0.17
Σ n3 PUFAs	3.91±0.02	7.83±4.49	21.60±8.76	18.25±5.06	25.44±3.29	11.29±0.69
Σ n6 PUFAs	4.09±0.17	7.06±2.69	8.26±3.20	6.18±2.11	8.24±1.05	6.62±0.59
n-3 / n-6	1.10±0.15		3.10±1.96	2.98±0.20	3.13±0.80	1.71±0.04

n: number of sample, ♀: female, ♂: male, ND: not detected, [a]: $p<0.01$ vs. male, [b]: $p<0.001$ vs. male, [c]$p<0.05$ v.s male, -: not studied