



Seasonal Variations of Proximate and Total Fatty Acid Composition of Wild Brown Trout in Munzur River, Tunceli-Turkey

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Abstract

Seasonal variations of proximate composition and fatty acid profiles of wild brown trout (*Salmo trutta macrostigma* Dumeril, 1858) caught from the Munzur River, Tunceli-Turkey were investigated in this study. It was determined that wild brown trout was high in mono-unsaturated, poly-unsaturated and saturated fatty acids, respectively. C18:1 ω -9 (oleic acid) was the most abundant fatty acid in all seasons ranging from 20.27 to 29.35 %. C22:6 ω -3 (docosahexaenoic acid-DHA), C18:3 ω -3c (cis-linolenic acid), C18:2 ω -6c (linoleic acid), and C20:5 ω -3 (eicosapentaenoic acid-EPA) were found to be predominant fatty acids in PUFAs. As a conclusion, wild brown trout living in Munzur river could be considered as an important protein and ω -3 fatty acid source according to the nutritional quality results evaluated in terms of proximate and fatty acid composition.

Keywords: *Salmo trutta macrostigma*, polyunsaturated fatty acids, nutritional quality

Tunceli Munzur Çayı'ndaki Doğal Kahverengi Alabalıktaki Besin Kompozisyonu ve Toplam Yağ Asidinin Mevsimsel Değişimi

Özet

Bu çalışmada Munzur Nehri, Tunceli-Türkiye'den yakalanan doğal kahverengi alabalıkların (*Salmo trutta macrostigma* Dumeril, 1858) vücut kompozisyonu ve yağ asidi profillerinin mevsimsel olarak değişimi araştırılmıştır. Doğal kahverengi alabalıkların en çoktan aza doğru sırasıyla mono-doymamış, çoklu-doymamış ve doymuş yağ asitlerini içerdiği tespit edilmiştir. C18:1 ω -9 (oleik asit) %20,27-29,35 arasında değişen oranlarla birlikte bütün mevsimlerde en baskın yağ asidi olarak bulunmuştur. C22:6 ω -3 (dokosaheksaenoik asit-DHA), C18:3 ω -3c (cis-linolenik asit), C18:2 ω -6c (linoleik asit) ve C20:5 ω -3 (eikosapentaenoik asit-EPA) çoklu doymamış yağ asitleri arasında en baskın olanlardır. Sonuç olarak, vücut ve yağ kompozisyonu açısından değerlendirilmiş besinsel kalite sonuçlarına göre, Munzur nehri'nde yaşayan doğal kahverengi alabalıkların önemli bir protein ve ω -3 yağ asidi kaynağı olduğu düşünülebilir.

Anahtar Kelimeler: *Salmo trutta macrostigma*, çoklu doymamış yağ asitleri, besinsel kalite.

Introduction

In recent years, since being beneficial for human health, fish lipids have been focused on due to its high contents of omega-3 polyunsaturated fatty acids (ω -3 PUFAs); in particular, eicosapentaenoic acid (EPA, C20:5 ω -3) and docosahexaenoic acid (DHA, C22:6 ω -3) which originate from phytoplankton and seaweed in the food chain (Arts *et al.*, 2001). Long chain polyunsaturated fatty acids (PUFAs) like linoleic acid (C18:2 ω -6), arachidonic (C20:4 ω -6) acids, α -linolenic acid-ALA (C18:3 ω -3), eicosapentaenoic acid (EPA, 20:5 ω -3) and docosahexaenoic acid (DHA, 22:6 ω -3) called as

essential fatty acids (EFA's) (Hunter and Roberts, 2000; Arts *et al.*, 2001) cannot be synthesised by humans and required in the diet (Sushchik *et al.*, 2007).

Fish oil has been utilised by humans for hundred's of years to help relieve medical problems (Hunter and Roberts, 2000). A regular consumption of EFA (EPA+DHA) may play an important role in the prevention of cardiovascular diseases and neural disorders (Arts *et al.*, 2001; Lauritzen *et al.*, 2001; Kris-Etherton *et al.*, 2002), has beneficial effects on bone formation and metabolism (Watkins *et al.*, 2003), and decreases high blood pressure and cholesterol, prevents certain cardiac arrhythmias,

lowers the occurrence of diabetes, and appears to reduce symptoms associated with rheumatoid arthritis. Also, ω -3 PUFAs play a vital role in the development and function of the nervous system, photoreception, and the reproductive system. (Hunter and Roberts, 2000; Sidhu, 2003; Tapiero *et al.*, 2002). ω -3 fatty acids have protective effect of on breast, colon, gastrointestinal and lung cancer (Rose, 1997; Hunter and Roberts, 2000). Eicosapentaenoic acid (EPA, 20:5 ω -3), docosahexaenoic acid (DHA, 22:6 ω -3) and arachidonic acid (C20:4 ω -6; ARA) are necessary to maintain cell membrane structure integrity and particularly proved as the precursors of composite hormones known as eicosanoids which involve in several metabolic processes of the human body (Sargent *et al.*, 1999).

Since, the main part of fish consumed by human is generally muscle, analysis of the fatty acid profiles of the muscle from fish living in their natural ecosystem, can yield valuable information (Akpınar *et al.*, 2009). A rare fish species inhabited the Munzur River is the wild brown trout belonging to the genus *salmo*, may be distinguished by their brownish-yellow colour with dark and red spots on olive background. Trout that lives in great number in the Munzur River became one of the most preferred and caught fish because of its unique aroma and makes up an important economic value for the region (Kayım *et al.*, 2011). Arslan *et al.* (2012) mentioned that, brown trout can be considered an important fish species in efforts to increase diversity in Turkish aquaculture production since, it is highly regarded in Turkey in recent years although the local market value is higher than rainbow trout, and the growth rate is relatively low comparingly rainbow trout.

Since fish is the most important source of the essential ω -3 fatty acids for human nutrition, and due to the known variation of proximate and FA composition in different ecosystems, investigating chemical composition of fish species from various locations is very important.

A few authors have ascertained that *Salmo trutta macrostigma* from two different rivers of Turkey contain lipids rich in these ω -3 LC-PUFAs (Aras *et al.*, 2003; Akpınar *et al.*, 2009; Bayır *et al.*, 2010). There is only one study (Kayım *et al.*, 2011) determining the fatty acids of *S. trutta macrostigma* in Munzur River, Turkey. But, the seasonal fatty acid dynamics of this wild species is not known. The aim of this study was to determine the seasonal changes of proximate and fatty acid composition in the muscle of *S. trutta macrostigma* caught from Munzur River, Tunceli-Turkey.

Materials and Methods

Fish and Sampling

Wild Brown trout (*S. trutta macrostigma* Dumeril, 1858) samples were caught with electric

fishing from Munzur River from two different stations for three times in the dates of October-2009, January-2010 and March-2010 representing the middle months of seasons of autumn (3 individuals), winter (4 individuals) and spring (5 individuals) respectively. Immediately after catching, they were stored on ice in an insulated box and transferred to the National Food Reference Laboratory, Ministry of Food, Agriculture and Livestock, Ankara, Turkey.

Two stations through the Munzur River named as Upper Torunoba and Koyungölü (Ovacık) were located at the latitude of 39°17.510' North, longitude of 39°22.997' East and altitude of 1140 m, and the latitude of 39°20.804' North, longitude of 39°7.073' East and altitude of 1277 m, respectively. Munzur River rises from the Munzur mountain located in the North of Ovacık. It combines with Pülümür stream in the city center of Tunceli and then pour into Keban Dam Lake. The main part of river flows from Tunceli province. The river is very rich in terms of red-spotted trout (*S. trutta macrostigma* Dumeril, 1858). Munzur valley is full of natural plants and step slopes, waterfalls, canyons and interesting rock formations, and taken into protection as Munzur natural park.

Proximate Analysis (Chemical Determinations)

Chemical composition of fish samples was analyzed by standard methods following AOAC (1995). Prior to analyses, edible parts of the fish samples were homogenized. Moisture content was calculated on 3 g of sample, dried in a thermoventilated oven at 105°C overnight. Crude protein was analyzed using the Kjeldahl method ($N \times 6.25$) and ash by incineration at 550°C in a muffle furnace. Lipid was extracted from the fish samples with cyclohexane, 2-propanol, and water (Smedes and Thomasen, 1996). All chemical determinations were carried out in duplicate.

Lipid Extraction and Fatty Acid Analysis

Edible parts of fish samples were homogenized, and 40 g of samples were taken. Next, 2-propanol and cyclohexane were added, homogenized via ultra turrax, and then distilled water was added; it was homogenized again via ultra turrax and then centrifuged. The supernatant was pipetted to the funnel filled with anhydrous sodium sulphate fitted to a flask tared before. This procedure was repeated 2 more times. After that, the solvent was evaporated via the rotary evaporator and then the flask was left to stay in the oven overnight (Smedes and Thomasen, 1996). Afterwards, extracted lipids were further prepared for fatty acid analysis. For preparation of fatty acid methyl esters, 2-3 drops fish oil was taken in a tube, and mixed with 10 ml n-heptane and 0.5 ml 2 N KOH in methanol. And then the mixture was homogenized by using vortex and waited for an hour. After that, the supernatant was pipetted and filtered

from 0.45 µm filter disc and taken into 2 ml vial, and injected to the Agilent GC-FID (EN ISO 5509, 2000).

Fatty acids methyl esters (FAME) were analyzed on a Hewlett Packard (HP) Agilent 6890N model gas chromatograph (GC), equipped with a flame ionization detector (FID) and fitted with a JW 122-2362, DB-23 capillary column (60 m x 0.25 mm i.d. x 0.25 µm thickness). Helium was used as the carrier gas at a constant pressure of 27.56 psi with an initial flow of 1.0 mL/min, and an average velocity of 23 cm/sec. Injection port was maintained at 220°C, and the sample was injected in split mode with a split ratio of 20:1. Detector temperature was 250°C. Column temperature was started at 150°C, and then programmed at 1°C/min to 155°C, ramped at 1°C/min to 160°C, 2°C/min to 180°C, 0.70°C/min to 194°C, 3°C/min to 200°C, and 10°C/min to 230°C, and held for 13 min. Helium was used as the make up gas at a constant flow of 30 mL/min, and hydrogen and dry air were used as detector gases (EN ISO 5508, 1990).

Identification of fatty acids was carried out by comparing sample FAME peak relative retention times with those obtained for Supelco standards (Supelco 37 Compounds FAME mix 10 mg/ml in CH₂Cl₂ – 47885 U, Supelco 1819-1 Ampule FAME mix C4-C24). Results of each fatty acids were expressed as FID response area relative percentages of the total fatty acids determined (EN ISO 5508, 1990).

Results and Discussion

The proximate composition of wild brown trout is shown in Table 1. The values of protein and lipid were determined as 18.85% and 2.84%, 19.47% and 1.48%, and 19.19% and 2.24% for autumn, winter and spring respectively. The values of dry matter and ash were determined as 21.67% and 1.15%, 21.51% and 1.18%, and 22.63% and 1.20% for autumn, winter and spring respectively. As also shown in Table 1, the average length and weight of red-spotted trout were found as 20.2 cm and 99.8 g, 23.6 cm and 157.4 g, and 17 cm and 66.1 g for autumn, winter and spring respectively. For this species while total protein content in winter was determined to be higher than in other seasons, total lipid content in autumn was determined to be higher comparatively. Data on proximate composition of wild brown trout were

found almost same with the results of the study of Kayım *et al.* (2011) and generally in agreement with the results of the study of Turchini *et al.* (2003). The proximate composition of *S. trutta macrostigma* showed variability throughout the year due to the factors like season, temperature, age, maturity level, fluctuations in availability and compositions of food as mentioned before by Love (1997).

Lipid content ranged between 1.48%-2.84% showing that the species belong to low fat fish (2-4 % lipid) as indicated by Ackman, 1989 and Bayır *et al.*, 2010. Lipid results in this study were found lower than the results of the study of Arslan *et al.* (2012), and were found in agreement with the studies of Kaushik *et al.* (2006) and Bayır *et al.* (2010) since the range is comprised between 1.0 % and 4.5 % in wild salmonids as mentioned by Bayır *et al.* (2010).

The maximum lipid content of the *S.t.macrostigma* was determined in the autumn and the lowest lipid content in the winter. In the study of Aras *et al.* (2009); it was found that the highest gonado-somatic index values were determined in the autumn, and they dramatically decreased in the winter indicating that reproduction period was between the second half of autumn and first half of winter for *S.t.macrostigma*. Lowest lipid content in winter can be attributed to the spawning period. Lipids and proteins are mobilised from the muscle and transferred to the gonads in the reproductive period (Love, 1997). Also it is known that fish use lipids preferentially rather than carbohydrates as an energy source and need them during sexual maturation (Bayır *et al.*, 2010).

Crude protein and ash results were found similar with Turchini *et al.* (2003), lower than Arslan *et al.* (2012) and higher than Şahin *et al.* (2011). The proximate composition differences between this study and the other studies could be the result of different fish species, feeding regime, environment, season and age. The fatty acid composition of the wild brown trouts which were captured in different seasons were presented in Table 2. In general, the fatty acid composition of the fish analysed is in agreement with the data available on the fatty acid composition of the *Salmo trutta* sp. in the literature (Akpınar *et al.*, 2009; Kayım *et al.*, 2011).

The major fatty acids in the trout were identified as C18:1 ω-9 (oleic acid), C16:0 (palmitic acid),

Table 1. Proximate composition of *Salmo trutta macrostigma* in Munzur River in different seasons (%)

Sample Component	Seasons		
	Autumn	Winter	Spring
N	3	4	5
Moisture (%)	78.33	78.49	77.37
Protein (%)	18.85 (86.98)*	19.47 (90.52)	19.19 (84.80)
Lipid (%)	2.84 (13.11)	1.48 (6.88)	2.24 (9.90)
Ash (%)	1.15 (5.31)	1.18 (5.49)	1.20 (5.30)
Length (cm)	20.2±1.9	23.6±1.8	17±1.7
Weight (g)	99.8±28.5	157.4±38	66.1±27

* Values in parentheses represent percentage on dry matter basis

Table 2. Fatty acid composition of *Salmo trutta macrostigma* in Munzur River in different seasons (% of total fatty acids)

<i>Salmo trutta macrostigma</i> Fatty acids	Seasons		
	Autumn (%)	Winter (%)	Spring (%)
C12:0	-	0.26	0.27
C13:0	-	-	0.05
C14:0	3.18	1.94	2.13
C15:0	0.26	0.39	0.52
C16:0	23.29	17.57	18.27
C17:0	0.51	1.02	1.03
C18:0	3.99	3.74	3.98
C20:0	0.21	0.14	0.14
C21:0	0.57	1.02	1.05
C22:0	0.17	0.11	0.18
C24:0	0.08	0.11	0.11
ΣSFA	32.26	26.3	27.73
C14:1 ω-5	0.19	0.10	0.11
C15:1 ω-5	-	0.03	-
C16:1 ω-7	11.54	6.94	8.11
C17:1 ω-7	0.62	0.33	0.19
C18:1 ω-9	20.27	28.66	29.35
C20:1 ω-9	0.26	1.04	1.41
C22:1 ω-9	0.04	0.14	0.17
C24:1 ω-9	0.09	0.25	-
ΣMUFA	33.01	37.49	39.34
C18:3 ω-3 c	10.68	8.98	5.47
C18:3 ω-3 t	-	0.46	-
C20:3 ω-3	0.63	1.98	2.66
C20:5 ω-3	7.79	4.46	4.43
C22:6 ω-3	10.02	12.80	11.22
Σ ω-3 PUFA	29.12	28.68	23.78
C18:2 ω-6 c	4.71	6.23	7.00
C18:2 ω-6 t	0.13	-	-
C18:3 ω-6	0.29	-	-
C20:2 ω-6	0.21	0.88	1.53
C20:4 ω-6	0.26	0.40	0.61
Σ ω-6 PUFA	5.60	7.51	9.14
Σ PUFA	34.72	36.19	32.92
ω-3/ω-6 ratio	5.20	3.82	2.60
EPA+DHA	17.81	17.26	15.65

C22:6 ω-3 (docosahexaenoic acid, DHA), C16:1 ω-7 (palmitoleic), C18:3 ω-3c (linolenic acid), C18:2 ω-6c (linoleic acid), C20:5 ω-3 (eicosapentaenoic acid, EPA), C18:0 (stearic acid), C14:0 (myristic acid) respectively, in all seasons. These findings are in accordance with the results of Kayım *et al.* (2011), Akpınar *et al.* (2009), Bayır *et al.* (2010) and Aras *et al.* (2003).

As indicated by Ackman (1989), fish are relatively low in SFA (<30 %) generally, and our results are also in accordance with this finding except with the result of 32.26 % in autumn.

Eleven fatty acids were determined in terms of SFA. The major SFAs identified were C16:0 (palmitic acid), C18:0 (stearic acid), C14:0 (myristic acid), C21:0 (heneicosanoic acid) and C17:0 (margaric acid) however some trace amounts of SFAs, like C15:0 (pentadecanoic acid), C20:0 (arachidic acid), C22:0 (behenic acid), C24:0 (lignoceric acid), C12:0 (lauric acid) and C13:0 (tridecanoic acid) were also present. The finding of predominance of C16:0,

C18:0, C14:0 fatty acids is in accordance with Aras *et al.* (2003), Akpınar *et al.* (2009), Bayır *et al.* (2010), and Kayım *et al.* (2011).

Eight fatty acids were determined in terms of MUFA. The levels of C18:1 ω-9 (oleic acid) and C16:1 ω-7 (palmitoleic acid) in the wild brown trout were found as the highest two fatty acids in MUFA within the range of 20.27% - 29.35%, and 6.94% - 11.54%, respectively. Although C18:1 ω-9 (oleic acid) values were found higher in this study, the findings indicating the dominance of these two fatty acids are in accordance with the studies of Aras *et al.* (2003), Kayım *et al.* (2011) and Bayır *et al.* (2010). In the study of Bayır *et al.* (2010) apart from the fatty acids mentioned above, also C20:1 ω-9 (eicosanoic acid) were found as dominant.

Ten fatty acids were determined in terms of PUFA. Major fatty acids were determined as C22:6 ω-3 (docosahexaenoic acid-DHA), C18:3 ω-3c (cis-linolenic acid), C18:2 ω-6c (linoleic acid), and C20:5 ω-3 (eicosapentaenoic acid-EPA). This finding is in

accordance with the finding of Akpınar *et al.* (2009) and Kayım *et al.* (2011), but different from Aras *et al.* (2003) and Bayır *et al.* (2010). Trace amounts of ω -3 fatty acids named as C18:3 ω -3t (trans-linolenic acid) and C20:3 ω -3 (methyl cis 11, 14, 17 eicosatrienoic acid) respectively. Also trace amounts of ω -6 fatty acids named as C18:2 ω -6t (trans-linoleic acid), C18:3 ω -6 (gamma-linolenic acid), C20:2 ω -6 (cis 11, 14-eicosadienoic acid), C20:3 ω -6 (methyl cis 8, 11, 14 eicosatrienoic acid), C20:4 ω -6 (arachidonic acid), C22:2 ω -6 (methyl cis 13,16 docosadienoic acid). Although other PUFAs like C22:5 ω -3 and C20:3 ω -6 were determined in other studies we did not determine these fatty acids.

Fatty acid composition of fish muscle is influenced by its diet. The lipids of fresh water feeds are characterized by linoleic (C18:2 ω -6) and linolenic (C18:3 ω -3) acids and EPA (Çelik *et al.*, 2005). The finding of the dominance of linoleic, linolenic and EPA in our study is similar with this finding since trout is a freshwater fish, but apart from these fatty acids, DHA was found as the most abundant ω -3 fatty acid. Also, the fatty acid composition of fresh water fish is characterized by high contents of ω -6 PUFA (Çelik *et al.*, 2005), and it means that generally lower ω -3/ ω -6 PUFA ratio is found in freshwater species in comparison to marine species (Hunter and Roberts, 2000). As indicated by Henderson and Tocher (1987); the ratio of ω -3/ ω -6 PUFAs in total lipids of freshwater fishes ranges between 0.5 and 3.8, whereas it is 4.7–14.4 for marine fish species. The total ω -3 fatty acid concentrations were found higher than the total ω -6 fatty acid concentrations, and the ratio of ω -3/ ω -6 PUFAs ranged between 2.60-5.20 in our study. This finding may be explained by the study of Hunter and Roberts (2000) indicating that; carnivores, due to their consumption of other fish in which chain elongation and desaturation is completed, were rich in long chain ω -3 PUFA's but lower in linolenic acid. Results of total ω -6 PUFA percentages were found in accordance with the Ackman (2000) study indicating that freshwater fish contain 5-10 % ω -6 PUFA.

In our study the range of the concentrations of the fatty acids is in the order of MUFA>PUFA>SFA. This finding is similar with Akpınar *et al.* (2009), but different from the other studies (Aras *et al.*, 2003; Kayım *et al.*, 2011; Bayır *et al.*, 2010) since being of MUFA concentrations dominant in our study. But finding of PUFA>SFA is in accordance with the findings of Aras *et al.* (2003) and Kayım *et al.* (2011) but, not similar with the study of Bayır *et al.* (2010) mentioning SFA>MUFA.

In our study; fatty acid concentrations showed variation in seasons, also individual and total fatty acid concentrations varied between our study and the other studies. The type and amount of fatty acids may vary among species and within a species, also even between tissues of fish (Akpınar *et al.*, 2009) due to the factors like season, the type and availability of

feed, water temperature, pH, salinity, reproduction cycle, size, sex or age of fish, geographic location and whether fish are wild or farm-raised (Hunter and Roberts, 2000; Kris-Etherton *et al.*, 2002; Gökçe *et al.*, 2004; Kaushik *et al.*, 2006; Ackman, 1989; Henderson and Tocher, 1987).

As indicated by Kris-Etherton *et al.* (2002); The American Heart Association dietary advisory recommends that persons with coronary heart disease should consume about one gram of fish oil (EPA and DHA) daily. If one meal is considered as 227 g, as indicated by Sidhu (2003), wild brown trout living in Munzur River provides adequate amount of omega-3 PUFAs (0.58-1.15 g per meal).

The low fat nature of seafood plus the presence of eicosapentaenoic acid (EPA, C20:5 ω -3) and docosahexaenoic acid (DHA, C22:6 ω -3) provide additional health benefits (Hunter and Roberts, 2000). Although, chain elongation and desaturation processes are more efficient in freshwater fish than in marine fish, research indicates that freshwater fish generally have lower levels of ω -3 PUFA; (Akpınar *et al.*, 2009; Çelik *et al.*, 2005); especially C20 and C22 PUFA's and higher levels of saturated fats and ω -6 PUFA's than marine fish (Hunter and Roberts, 2000). Cold and deep sea fish species have higher levels of long chain highly unsaturated ω -3 fatty acids in their diets than warm-water species having higher levels of ω -6 and ω -9 fatty acids (Hunter and Roberts, 2000). As indicated by Çelik *et al.* (2005); fish need PUFA to provide the lower water temperature adaptation and the melting temperatures of ω -3 fatty acids are lower than ω -6 fatty acids (Çelik *et al.*, 2005). Generally a decrease in temperature results an increase in the degree of unsaturation (Hunter and Roberts, 2000) and, it is estimated that ω -3 PUFA contents of fish in warm regions are lower (Çelik *et al.*, 2005). Our results are in accordance with these findings.

This study investigated the proximate composition and fatty acid profile of wild brown trout captured from Munzur River. Chemical analysis of freshwater fish is important, due to the dietary and medical interest on the role of fatty acids in human health, and being relatively a cheaper source of animal protein of high biological value as Jabeen and Chaudry (2011) mentioned. In conclusion, wild brown trout living in Munzur river could be considered as an important protein and ω -3 fatty acid source in the human diet according to the nutritional quality results evaluated in terms of proximate and fatty acid composition.

Since ω -3 PUFAs, such as eicosapentaenoic acid (20:5 ω -3, EPA) and docosahexaenoic acid (22:6 ω -3, DHA), are effectively synthesized only by aquatic organisms (Sushchik *et al.*, 2007), and considering all the beneficial effects on human health; it may be advised to consume a variety of fish at least 1 to 2 times a week, avoiding deep-fried fish or fast-food fish, in order to maintain or enhance our present-day health by obtaining these essential fatty acids (Arts *et*

al., 2001).

It appears that only one study (Kayim *et al.*, 2011) on the chemical compositions and fatty acid profiles of this fish species in the study area is available. Therefore this study on seasonal variation of chemical and fatty acid composition of *S.t.macrostigma* will form the basis for further research in this field of fish chemistry for the benefits of human beings.

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