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Research Article

Seasonal Differences in Lipid and Fatty Acid Composition of European Eels (*Anguilla anguilla*, Linnaeus 1758) from Orontes River, Turkiye

İdil Can Tunçelli[1], Özkan Özden[1], Nuray Erkan[2]

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ABSTRACT

Seasonal differences in the lipid contents and fatty acid composition of European Eels (*Anguilla anguilla*, Linnaeus 1758) caught from the Orontes River (Hatay, Turkey) were determined. High lipid levels, as well as ω-3 and ω-6 fatty acids, are important factors in product quality. High-content lipid values of European eels from the Orontes River differed between seasons ($p<0.05$). The fatty acid compositions of eels ranged from 5.91-8.03 g/100 g in saturated fatty acids (SFA), 10.59-14.08 g/100 g in monounsaturated fatty acids (MUFAs), and 2.19-3.52 g/100 g in polyunsaturated fatty acids (PUFAs). Those present in the highest proportions were palmitic acid (C16:0, 62-67.51%), palmitoleic acid (C16:1, 15.67-19.07%), stearic acid (C18:0, 11.04-17.2%), oleic acid (C18:1ω-9, 70.67-72.44%), eicosapentaenoic acid (EPA) (C20:5ω-3, 12.77-22.83%), and docosahexaenoic acid (DHA, C22:6ω-3, 4.35-11.93%). Some fatty acids' composition differed significantly ($p<0.05$) between seasons. In addition, the ratio of ω-3/ω-6 PUFAs varied between 1.14 and 1.72, reaching the highest value in autumn. The highest EPA+DHA contents were recorded during summer. In conclusion, analysis parameters show that commercially important European eels from the Orontes River are quite good sources of high-quality lipids and fatty acids.

Keywords: EPA, DHA, lipids, nutritional value, Orontes

ORCID IDs of the author:
İ.C.T. 0000-0002-9999-6658;
Ö.Ö. 0000-0001-8780-480X;
N.E. 0000-0002-0752-8495

[1]Istanbul University, Faculty of Aquatic Sciences, Department of Fisheries and Seafood Processing Technology, Seafood Processing Programme, Istanbul, Turkiye

[2]Istanbul University, Faculty of Aquatic Sciences, Department of Fisheries and Seafood Processing Technology, Food Safety Programme, Istanbul, Turkiye

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Correspondence:
İdil Can Tunçelli
E-mail:
idilcan@istanbul.edu.tr

INTRODUCTION

It is known that eels have a high oil content and are rich in ω-3 lipids, which is a high-value fish species preferred in gourmet cuisine (especially smoked products) due to its high-value nutrient content. It constitutes an important foreign exchange source input for the countries where eel production and fishing are carried out. If the fish, classified according to their size immediately after being caught, are to be consumed fresh, they are transferred to cooled, oxygenated packaging so that the skin does not dry out. Fish transferred for processing are usually delivered to the processing plant alive in water tanks (FAO, 2009).

Generally classified as warmwater fish, eels have a total of 19 species, which are distributed worldwide. They are found in freshwater sources in the Atlantic Ocean and the Mediterranean and are considered commercially important species, especially in the Eastern and Southern coasts of Turkey (Arai, 1991; Özogul et al., 2005; El-Obeid et al., 2018). The total production of European eels in Europe was 4478 tons (FEAP, 2020). In 2020, 320 tons of European eel were caught in Turkey (TurkStats, 2022). European eels in Turkey are distributed in the Orontes River, which flows to the East Mediterranean basin (Genc et al., 2008; Özden et al., 2020). The market demand for eels, which is also defined as gourmet seafood, is high in Europe and Asia, due to their taste and high flesh yield, resulting in increased eel exports (Ersoy, 2011).

Eels are a good source of FAs such as EPA, DHA and docosapentaenoic (C22:5ω-3, DPA),

which are essential for human health. Eels' high-quality fat content and composition are regarded as highly marketable quality traits that affect the wholesale price and consumer acceptance overall (Lie et al., 1990). Humans ingest long-chain ω-3-PUFAs, which cannot be synthesized inside the body, through diet (Alasalvar et al., 2002). Due to the link between human health and diet, consumers' interest in healthy food has increased, thus increasing the nutritional quality of food (Lie, 2001).

In this study, the seasonal differences in total fatty acid and lipid composition of European eels caught in the Orontes River, which are commercially important, were determined.

MATERIALS AND METHODS

Fish sampling

European eels *Anguilla anguilla* (Linnaeus, 1758) used in this study were caught from the Turkish waters of Orontes River, Samandag using fyke nets by professional local fishermen. Sampling was carried out monthly for a year and the values were examined seasonally (winter, spring, summer, and autumn). A total of 170 European eels of both sexes were investigated during this study. Samples were frozen in individual plastic bags immediately after capture. After transportation to the laboratory in a cold chain, physical data such as length and weight were recorded. Data concerning the sampling seasons, number of eels examined and their total body length and weight range are reported in Table 1. For fatty acid analyses, fish were eviscerated and filleted. Muscle tissues were analyzed after homogenization in the blender (Retsch, Grindomix GM200, Germany).

Lipid analysis

The modified acid hydrolysis method as in Erkan et al. (2020) was used for this analysis. Approximately 2 to 2.5 g of minced fillet were weighted into beakers and digested with 6 mL concentrated HCl by heating on a hot plate (approximately 80°C) for 90 minutes. The mixture was then transferred to a Mojonnier flask, after which the beaker was rinsed with 7 mL ethanol and 25 mL diethyl ether, and then vigorously shaken. Petroleum ether was added 3 times to the mixture and shaken again. The supernatant was taken and transferred to a pre-weighed flask. The petroleum ether in the flask was evaporated with a rotary evaporator and then dried in an oven for an hour at 105 °C. Flasks were allowed to cool and re-weighed. The amount of fat was calculated from the weight difference as below:

$$Fat\ (\%) = \frac{Fa - Fb}{W}$$

Fa: Weight of the flask after the oven

Fb: Weight of the flask before the oven

W: Weight of the sample

Fatty acid analyses

GC analyses were performed on Claurus 500 GC (Perkin-Elmer/Claurus 500 GC) equipped with an integrated autosampler and flame ionization detector (Awenud-Shelton, USA). Injector temperature was 220 °C and samples were injected with an autosampler (0.5 µL), in triplicate. The SGE BPX70 (SGE Analytic Science, Australia) capillary column had a length of 60 m, with an inner diameter of 0.25 mm, and with a film thickness of 0.25 µm. The temperature program was 120°C rising to 240 °C at a rate of 5 °C/min. The total program process time was 45 min. H_2 was used as carrier gas at a flow rate of 45 mL/min and air was at a flow rate of 450 mL/min. The flame ionization detector (FID) temperature was 240 °C. Lipids obtained after extractions (AOAC, 1998) were turned into corresponding methyl esters of fatty acids (FAMEs) (Ichihara et al., 2002). FAMEs were identified by analyzing a reference material (Menhaden Fish Oil, 47116 Supelco) and standards (Supelco 37 Component FAME Mixture, 47885-U Supelco). Results were expressed as g/100 g fish flesh. Greenfield and Southgate (2003) were used as a reference for the calculation of percent fatty acid contents, as in Özden et al. (2020).

Statistical analysis

Possible differences between means values were analyzed using ANOVA and *post-hoc* comparisons were done by Tukey›s test, with the statistical package SPSS version 28.0 (SPSS Inc., USA). The strength of lipid-length and lipid-weight relationship were evaluated from the determination coefficient (R^2).

RESULTS AND DISCUSSION

The muscle lipid content determined between seasons for the European eel from the Orontes River is shown in Table 2. Lipid values of eels were found to be significantly different in relation to seasons, especially in winter and summer ($p<0.05$).

Table 2. Lipid content of muscle from European eel caught from Orontes River.

Seasons	Lipids (w.w%)
Winter	30.32 ±0.54[*a]
Spring	27.90 ±0.46[ab]
Summer	24.09 ±0.35[b]
Autumn	23.12 ±0.47[b]

[*a,b]:Mean ± standard deviation (S.D), $p<0.05$.

Table 1. Sample characteristics of the study (n_{total}=170).

Seasons	n*	Length (Mean cm ± S.D.)	Weight (Mean g ± S.D.)
Winter	37	41.08±4.59[a]	182.33±114.02[a]
Spring	58	35.16±0.63[a]	90.41±4.74[a]
Summer	42	39.11±3.70[a]	140.97±57.76[a]
Autumn	33	38.63±1.01[a]	130.06±30.19[a]

* Fish sample (n); S.D. – standard deviation, different letters in the same column indicate differences ($p<0.05$).

The lipid content of eels tends to increase with age (Van Ginneken et al., 2018). In this study, lipid contents ranged between 23.12% ±0.47 and 30.32% ±0.54 in yearlong sampling. Our findings were comparable to those of Parzanini et al. (2021). According to Ackman (1990), samples with more than 8% fat content are classified as high fat. All eels sampled according to this classification are in the high-fat category. In this study, no correlations were found between lipid values and weight (R^2=0.03) or lipid values and length (R^2=0.06).

Table 3. Seasonal changes in the fatty acid composition of European Eels (*Anguilla anguilla)* from Orontes River.

Anguilla anguilla (g/100 g)	**Seasons (Mean ± SD)**			
Fatty acids	**Winter**	**Spring**	**Summer**	**Autumn**
Lauric acid C12:0	0.12±0.18^{a}	0.17 ± 0.08^{a}	0.14±0.20^{a}	0.06±0.05^{a}
Tridecanoic acid C13:0	0.05±0.04^{a}	0.04±0.01^{a}	0.02±0.03^{a}	0.03±0.00^{a}
Myristic acid C14:0	1.00±0.21^{a}	0.91±0.14^{a}	0.81±0.07^{a}	0.72±0.08^{a}
Pentadecanoic acid C15:0	0.09±0.03^{a}	0.07±0.02^{b}	0.07±0.02^{a}	0.03±0.00^{a}
Palmitic acid C16:0	5.19±0.32^{a}	4.65±0.47^{b}	4.02±0.49^{ab}	3.99±0.60^{a}
Heptadecanoik acid C17:0	0.13±0.05^{a}	0.12±0.01^{a}	0.11±0.03^{a}	0.09±0.00^{a}
Stearic acid C18:0	1.15±0.27^{a}	1.29±0.13^{b}	1.02±0.14^{b}	0.67±0.06^{ab}
Behenic acid C20:0	0.24±0.10^{a}	0.21±0.08^{a}	0.14±0.07^{a}	0.22±0.04^{a}
Tricosylic acid C22:0	*nd*	*nd*	0.03±0.06^{a}	*nd*
Lignoceric acid C24:0	0.07±0.09^{a}	0.06±0.04^{a}	0.04±0.05^{a}	0.11±0.03^{a}
SFAs (Total)	8.03±0.69^{a}	7.50±0.54^{b}	6.41±0.63^{bc}	5.91±0.58^{ac}
Myristoleic acid C14:1	0.11±0.16^{a}	0.13±0.11^{a}	0.14±0.21^{a}	0.03±0.04^{a}
Pentadecanoic acid C15:1	0.05±0.03^{a}	0.05±0.01^{a}	0.05±0.04^{a}	0.04±0.05^{a}
Palimiteloic acid C16:1	2.43±0.25^{a}	2.12±0.06^{a}	2.02±0.37^{a}	1.83±0.46^{a}
Heptadecanoic C 17:1	0.11±0.08^{a}	0.10±0.09^{a}	0.12±0.07^{a}	0.08±0.01^{a}
Oleic acid C18:1 (ω-9)	9.95±1.16^{a}	9.57±0.19^{a}	6.99±1.00^{ab}	8.07±2.13^{ac}
Vaccenic acid C18:1 (n-7)	0.88±0.05^{a}	1.10±0.35^{a}	0.89±0.27^{a}	0.71±0.04^{a}
Gadoleic acid C20:1 ω-9	0.30±0.03^{a}	0.29±0.01^{ab}	0.21±0.04^{ab}	0.26±0.05^{ac}
Erucic acid C22:1 (ω-9)	0.14±0.07^{a}	0.10±0.05^{a}	0.07±0.08^{a}	0.09±0.04^{a}
Nervonic acid C24:1 (ω-9)	0.10±0.04^{a}	0.05±0.06^{a}	0.10±0.07^{a}	0.03±0.01^{a}
MUFAs (Total)	14.08±1.25^{ab}	13.53±0.51^{a}	10.59±1.35^{ab}	11.14±2.62^{b}
Hexadecadienoic acid C16:2 (n-4)	0.15±0.08^{a}	0.13±0.04^{a}	0.10±0.05^{a}	0.06±0.09^{a}
Hexadeca-6,9,12-trienoic acid C16:3 (n-4)	0.01±0.01^{a}	*nd*	*nd*	0.03±0.00^{b}
Linoleic acid C18:2 (ω-6)	1.01±0.09^{a}	0.94±0.97^{a}	1.05±0.25^{a}	0.48±0.28^{a}
alfa-Linolenic acid C18:3 (ω-6)	0.04±0.01^{a}	0.08±0.02^{ab}	0.03±0.01^{b}	0.03±0.04^{a}
alfa-Linolenic acid C18:3 (ω-3)	0.18±0.09^{a}	0.13±0.06^{b}	0.17±0.03^{a}	0.05±0.03^{b}
Eicosadienoic acid C20:2 (ω-6)	0.16±0.04^{a}	0.07±0.0^{a}	0.17±0.07^{a}	0.10±0.03^{a}
Eicosatrienoic acid C20:3 (ω-6)	0.10±0.08^{a}	0.12±0.08^{a}	0.14±0.03^{a}	0.01±0.00^{a}
Eicosatrienoic acid C20:3 (ω-3)	0.29±0.17^{a}	0.28±0.09^{ab}	0.49±0.22^{ab}	0.09±0.03^{b}
Arachidonic acid C20:4 (ω-3)	0.18±0.31^{a}	*nd*	0.04±0.06^{a}	0.33±0.13^{a}
Docasadienoic acid C22:2 (ω-6)	0.01±0.00^{a}	0.01±0.01^{a}	0.01±0.00^{a}	0.01±0.01^{a}
Eicosapentaenoic acid C20:5 (ω-3)	0.42±0.11^{a}	0.56±0.34^{a}	0.49±0.03^{a}	0.50±0.09^{a}
Clupanodonic acid C22:5 (ω-6)	0.05±0.03^{a}	0.01±0.02^{a}	0.05±0.03^{a}	0.02±0.00^{a}
Clupanodonic acid C22:5 (ω-3)	0.36±0.15^{a}	0.30±0.17^{a}	0.37±0.19^{a}	0.32±0.03^{a}
Docosahexaenoic acid C22:6 (ω-3)	0.32±0.22^{a}	0.12±0.05^{a}	0.42±0.28^{a}	0.17±0.03^{a}
PUFAs (Total)	3.29±0.29^{a}	2.76±0.47^{ab}	3.52±0.69^{ab}	2.19±0.48^{b}
Unidentified	1.88±0.32^{a}	1.32±0.39^{a}	1.16±0.27^{a}	1.56±0.26^{a}
Fatty Acids (Total)	27.29±1.78^{a}	25.11±0.40^{b}	21.68±2.76^{ab}	20.80±2.98^{a}
EPA+DHA	0.74±0.31^{a}	0.68±0.39^{a}	0.91±0.26^{a}	0.67±0.06^{a}
ω-3 (Total)	1.57±0.47^{a}	1.40±0.52^{a}	1.94±0.60^{a}	1.12±0.09^{a}
ω-6 (Total)	1.37±0.19^{a}	1.23±1.03^{a}	1.44±0.35^{a}	0.65±0.35^{a}
ω-3/ ω-6	1.14^{a}	1.14^{a}	1.38^{a}	1.72^{a}

*$^{a, c}$: Mean ± standard deviation (S.D.), different letters in the same column indicate differences ($p<0.05$).

As opposed to other fish species, eels store their lipids in between fibers of muscle tissue, and fat can comprise up to 55% of the eel's body (dry) weight (Lovern, 1938; Henderson & Tocher, 1987). Also, for the success of migration and spawning, eels require high-fat content in their muscular tissue (Larsson et al., 1990) and they accumulate these lipids before migration (Durif et al., 2005; Parzanini et al., 2021). This situation is also compatible with our lipid values. As seen in Table 2, before migration, in winter, eels accumulated more lipids (30.32% ±0.54) than in summer and autumn.

Seasonal differences in the total fatty acids composition of samples are presented in Table 3. The major fatty acids in European eels at all seasons were C18:0, C16:0, C22:6 ω-3 (DHA), C18:1 (ω-9), C18:2 (ω-6), and C16:1. In winter and summer, stearic acid (C18:0) values differed (p<0.05).

Similarly, McKenzie et al. (2000) stated that the most common FAs in muscle tissue were C16:0 and C18:1 (ω-9). According to our results, palmitic acid was the highest corresponding SFA, contributing approximately 62-67.51% to the total SFA content for eels in all seasons. The highest contents of MUFA was oleic acid [C18:1 (ω-9)] in winter, with 9.95 ± 1.16 g/100 g, and PUFA was linoleic acid [C18:2 (ω-6)] in summer, with 1.05 ± 0.25 g/100 g. Soriguer et al. (1997) reported lower values in European eels than in our study in ω-3 (0.71 g/100 g), ω-6 (0.61 g/100 g), MUFA (3.93 g/100 g), and SFA (2.23 g/100 g). Also, some significant variances in MUFA and SFA values between seasons (p<0.05) were observed.

The content of exogenous oleic acid is related to the content of fatty acids in the diet and depends on the metabolism of individual fish species (Ackman, 1990). Most of the MUFA in this study was identified as oleic acid, highest in winter. This is consistent with the hypothesis that in winter, the eels accumulate energy stores before migration (Parzanini et al., 2021). In contrast to this, total PUFAs were not as high as expected in winter. This decrease has been associated with eels' PUFA utilization for gonad maturation (Pérez et al., 2000; Zhou et al., 2011; Kaçar & Başhan, 2017). The contents of C16:3 (n-4) and alfa-Linolenic acid C18:3 (ω-6) PUFAs between winter and summer were significantly different (p<0.05). PUFAs such as EPA and DHA in eels are directly associated with their feeding behavior. Vasconi et al. (2019) also reported that lipids from a Mediterranean coastal lagoon appear to be richer in SFA and ω-6 PUFA if compared to fish from lagoons and the open seafood web, which are richer in ARA (p<0.05). The fact that some fatty acid values in our samples did not differ significantly according to the seasons is thought to be related to the nutritional habits throughout the year. This has been seen in the case of Japanese eels, which are characterized by the presence of MUFAs as the main fatty acid category instead of PUFAs (Oku et al., 2009).

Marine organisms have relatively smaller amounts of ω-3 PUFAs than freshwater organisms. Freshwater fish are rather better than marine fish at elongating and desaturating shorter fatty acids into longer DHA and EPA and upturning food of low nutritional value into higher value (Moreira et al., 2001). Specifically, freshwater fish contain higher amounts of C-16, C-18, EPA, and DHA and lower amounts of C-20 and C-22 acids compared with marine fish (Ackman, 1967; Wang et al., 1990). In this study, the lowest amount of EPA was in winter with 0.42 ± 0.11 g/100 g. EPA, DHA, linoleic acid and clupanodonic acid were dominant in the PUFAs in all seasons. EPA contributing to the total SFA in muscle tissue of European eels was found to be 12.77%, 20.29%, 13.92%, and 22.83% in winter, spring, summer, and autumn, respectively. Thus, among the ω-3 series, it was determined that European eels are good sources of EPA and DHA throughout all seasons. Gómez-Limia et al. (2021) reported lower values of EPA and DHA for European eels than our study. In this study, no significant difference was determined in the content of EPAs and DHAs between seasons. It was reported that there is also a coordinate relationship between eel size and the fat content of the muscle (Garcia-Gallego & Akharbach, 1998).

The ω-3/ω-6 ratio is used to compare the relative nutritional value of fish oils. Generally, this proportion is extended from 1 to 4 in freshwater fish species, and 5 to 10 in marine fish species (Valfré et al., 2003). This is mainly due to the fact that freshwater fish species feed on plants and marine fish species feed on zooplankton, which are rich in PUFAs (Vlieg and Body, 1988; Gómez-Limia et al., 2021). All of the eels in our study were caught in the freshwater Orontes River. The present data show that the ω-3/ω-6 ratio was 1.14 in winter and spring and 1.38 in summerm and that the highest value 1.72 was in autumn. Gómez-Limia et al. (2021) observed ω-3/ω-6 ratio levels between 1.80 to 2.07 depending on the eel size.

CONCLUSION

Changes in lipids and fatty acids were examined in this year-long study of European eel from the Orontes River. Lipid content was determined to be in the range of 23-30% between seasons and significant differences were observed (p<0.05). The ω-3/ ω-6 ratio differed between 1.14 to 1.72, from winter to autumn during sampling. The essential FAs, such as EPA and DHA, are important in a healthy human diet. There were some significant variances (p<0.05) between the SFA, MUFA, and PUFA profiles of the muscle tissues. The findings of our research showed that European eels from the Orontes River have high nutritional benefits with their high-quality lipid and fatty acid content.

The overall results of this study revealed that European eels caught from the Orontes River showed consistent high-quality lipid content throughout the year in terms of nutritional content. Stable nutrient content throughout the year is also important in terms of product quality in this fish, which offers delicious and attractive products with its high and quality lipid content. In particular, the measures taken against lipid oxidation enable products with a longer shelf life. In addition, the results of this study will be decisive and informative in terms of revealing the expected nutrient composition in eel culture, which is at the forefront of new species trials in rising trends in aquaculture.

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