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Antioxidant potential and fatty acid profile of fish fillet: effects of season and fish species

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Abstract

The objectives of the present study were to investigate the effect of season and fish species on the antioxidant capacity, fatty acids profile, and vitamin E content of fish fillets from Aras River. The antioxidant potential of hydrophilic and lipophilic extracts of fish fillets was evaluated. The fillet extracts of zander and bream in summer and common carp in winter had the highest antioxidant activity. Palmitic and oleic acids were the major saturated (SFA) and monounsaturated (MUFA) fatty acids, respectively. The fatty acids C22:6n3 and C20:5n3 were the most abundant polyunsaturated (PUFA) fatty acids in all the fishes. In summer, the highest levels of SFA (44.09), total PUFA (25.97), n3 PUFA (20.71) and n3/n6 ratio (4) were found in zander. In winter, the highest amounts of total PUFA and n3 PUFA were determined in silver carp, followed by zander. The highest n3/n6 ratio was also found in silver carp. The ratio of PUFA/SFA was higher in winter than in summer for all the fishes. Vitamin E content of fishes was largely varied. In conclusion, seasonal variation changed the antioxidant potential and fatty acid composition of fish fillets. Aras River fishes, especially zander, possess excellent antioxidant activity and high nutritional quality.

Introduction

Fish has been recognized as a valuable source of nutrients in the human diet due to its high- quality protein, polyunsaturated fatty acids (PUFA), essential minerals, and vitamins such as vitamins E and A.[1,2] PUFA are the essential components of the human diet and n3 PUFA such as eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) play protective effect against several diseases including colon cancer, cardiovascular diseases, and disorders of the immune system.[3] Given the beneficial effects of these fatty acids, it is recommended to consume two meals of fish per week.[4]

The fatty acid composition of fish may be influenced by many environmental and biological factors, such as fish species, nutritional condition, size, age, reproductive cycle, salinity, temperature, geographical location, and season.[5-9] Reportedly, the amount of lipids (total fatty acids) decreases in fish during the cold seasons. The studies also showed that the amount of unsaturated fatty acids varies significantly in the seasons of the year, which can affect the nutritional properties and storage conditions of fish.[6] Generally, a decrease in temperature results in an increase in the degree of unsaturation.[7] The fatty acid composition of many fish species from different regions of the world has been reported.[5,10-14] Some studies also evaluated the effect of season on the fatty acid profile of fish.[5,15,16] However, there is no report on the effect of season on the fatty acid profile of fish species from Aras River.

One of the most critical factors in decreasing the shelf life of food is the oxidation of lipids and fatty acids.[17] Antioxidants can inhibit the progress of oxidation. Antioxidants of fish not only can prevent lipid oxidation in fish but also may have health-promoting effects on the consumer.[18] Several natural antioxidants have been reported to be found in fish, including vitamin E, peptides, glutathione peroxidase, superoxide dismutase, ubiquinones, and catalase.[19] Although limited data are available on the antioxidant activity of muscle tissue of some fish species,[20-22] there is no data on the effect of season on the antioxidant potential of hydrophilic and lipophilic extracts of fish fillet.

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Considering the importance of the fishes caught from the Aras River in the diet of the human population in the northwest of Iran, we selected four commonly consumed fish species in this study. To the best of our knowledge, there is no report on the effect of season and fish species on the antioxidant potential of hydrophilic and lipophilic extracts of the fish fillet and fatty acid profile of Aras River fish species. Therefore, the aims of the present study were to investigate the effect of season and fish species on the antioxidant potential, fatty acid profile, and vitamin E content of four fish species from Aras River.

Materials and Methods

Chemicals. Butylated hydroxyl toluene (BHT), 2,2-azinobis-3-ethylenzothiazoline-6-sulphonic acid (ABTS), potassium persulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric chloride, potassium ferricyanide were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Disodium hydrogen phosphate (Na_2HPO4), sodium dihydrogen phosphate (NaH_2PO4), hexane, ethanol, methanol and trichloroacetic acid were obtained from Merck (Darmstadt, Germany).

Sample collection. Four fish species, including zander (*Sander Lucioperca*), common carp (*Cyprinus carpio*), bream (*Abramis brama* orientalis), and silver carp (*Hypophthalmichthys molitrix*) were selected to study. Five individual fishes from each species were caught from Aras River, located in the northwest of Iran, in summer 2019 and winter 2020. A total of 40 fishes were analyzed throughout the study. The approximate weights of the fishes were 300 g for bream and 700-800 g for other fishes. After cutting the head and tail, the abdominal contents of the fishes were discharged and washed with cold tap water and then transferred in ice to the laboratory. The samples were taken from dorsal muscle tissues and kept at – 20.00 ˚C until analyses.

Preparation of lipophilic extract of fish fillet. Fish fillet sample (5.00 g) was homogenized with 35.00 mL of n-hexane/ethanol (5:2), and then centrifuged at 4,000 rpm for 5 min. After that, two phases were formed; hexane in the higher and ethanol in the lower. The ethanol phase was used for the antioxidant tests.[23]

Preparation of hydrophilic extract of fish fillet. The sample (5.00 g) was added to 25.00 mL of distilled water in the falcon tubes and homogenized using a homogenizer (Ultra-Turrax T-25, Janke & Kunkel IKA-Labortechnik, Staufen, Germany) at 13,500 rpm for 2 min in an ice bath. Then, the tubes were centrifuged (4000 rpm) for 20 min at 4.00 ˚C. The supernatants were used for the antioxidant tests.

ABTS radical scavenging activity. The ABTS solution was prepared according to the method of Re *et al.*[24] First, 50.00 µL of the lipophilic extract or 100 µL of the hydrophilic extract was transferred into tubes containing 2.00 mL of ABTS solution and mixed. After 5 min, the tubes were centrifuged at 4,000 rpm for 5 min. Finally, the absorbance was measured at 734 nm using a spectrophotometer (Novaspec II; Pharmacia LKB, Uppsala, Sweden). The following equation was used to calculate ABTS radical scavenging activity (RSA):

$$RSA\ (\%) = [(A_b - A_s) / A_b] \times 100$$

where, A_b was the absorbance of the blank (containing ABTS solution and extracting solvent), and A_s was the absorbance of the sample.

DPPH radical scavenging activity. Two milliliter of methanol solution of DPPH (24.00 µg mL^{-1}) was mixed with 50.00 µL of lipophilic extract or 200 µL of hydrophilic extract. The samples were stored at 25.00 ˚C for 30 min. After that, the absorbance of the samples was determined at 517 nm using the spectrophotometer.[25] The DPPH radical scavenging activity was calculated according to the equation presented in ABTS assay.

Reducing power. For this test, 1.00 mL of each extract was poured into the test tube and 1.00 mL of phosphate buffer (pH = 6.60) and 1.00 mL of potassium ferricyanide solution (1%) was added to the tube, and then the tube was placed in a water bath of 50.00 ˚C for 20 min. After that, 1.00 mL of trichloroacetic acid (10.00%) solution was added to the mixture and then centrifuged at 4,000 rpm for 5 min. Then, 1.00 mL of the upper layer of the mixture was taken and mixed with 0.50 mL distilled water and 0.50 mL of ferric chloride (0.10%). After 10 min, the mixture was centrifuged at 2,000 rpm for 2 min. Finally, the absorbance was read at 700 nm using the spectrophotometer.[26]

Analysis of fatty acid profile. The fish fillet sample was first weighed, and after adding 3.00 mL of pure ethyl alcohol, dried at 105 ˚C for 1 hr. The weight of the samples was noted before and after drying. Then, 5.00 g of dried samples were taken in the test tube and carefully weighed. The sample was saponified with 3.00 mL methanolic sodium hydroxide (2.00 M) (Merck, Darmstadt, Germany) and the fatty acids were esterified with 5.00 mL methanolic sulfuric acid. One mL of hexane used for fatty acid methyl ester (FAME) extraction, and then 1.00 µL of the organic phase was injected into the gas chromatography.[27] An Agilent 6890N Gas Chromatograph (Agilent Technologies, Palo Alto, California, USA) equipped with an FID detector and a split/splitless injector was used. Separations of the analytes were carried out on HP-88 (88.00% Cyanopropyl aryl-polysiloxane) capillary column (100 m, 0.25 mm inner diameter, film thickness 0.20 µm). The initial column temperature was maintained at 140 ˚C for 5 min and then raised at 4.00 ˚C per min to 240 ˚C and held for 15 min. Nitrogen was used as the carrier and makeup gas with flow rates of 1.00 mL per min and 45.00 mL per min, respectively. The injector and detector temperatures were set at 260 ˚C and 280 ˚C, respectively.

Injection of analytes was carried out in splitless mode. ChemStation Software (Rev. A.10.01; Agilent Techonologies, Waldbronn, Germany) was used for processing. Individual FAME was identified by comparison with known standards (Sigma-Aldrich, St. Louis, USA).[27]

Analysis of vitamin E. For sample preparation, the fish fillet was weighed out to be homogenized and transferred to the test tube. 3.00 mL of metaphosphoric acid 5.00% was added to the tube and was interrupted in the ultrasonic apparatus for 10 min. Extraction was carried out with 2.00 mL hexane containing BHT 1.00% by vortexing for 10 min, followed by centrifugation at 3,000 rpm for 3 min. Hexane containing vitamin E was transferred to another test tube and was evaporated under nitrogen gas. After hexane drying, the residues were dissolved in 250 µL of acetonitrile. Then, 50.00 µL of the sample was injected into HPLC for analysis.[28] The study was carried out using a HPLC apparatus (Agilent 1100; Agilent Technologies) equipped with a quaternary pump, inline degasser, and diode array detector. Separations were carried out on a C18-ODS column from Supelco (250 × 4.60 mm inner diameter, 5.00 mm particle size; Bellefonte, Pennsylvania, USA) by using mixture of 65:34:1 acetonitrile: methanol: water (mobile phase) under isocratic conditions with a flow of 1.00 mL per min. The UV detector was used at 296 nm.[28]

Statistical analysis. All experiments were performed in triplicate and results were presented as mean ± SD. Statistical analyses were conducted using SPSS (version 18.0; IBM Corp., Armonk, USA). The antioxidant potential of the fish extracts and fatty acid composition among fish species were analyzed by one-way analysis of variance (ANOVA) followed by Duncan post-test. The antioxidant potential and fatty acid composition of each fish in summer and winter were compared by *t*-test. The significance level was set at 0.05.

Results

Antioxidant potential. The results of ABTS radical scavenging activity of lipophilic and hydrophilic extracts of zander, common carp, bream, and silver carp fillets in summer and winter are illustrated in Figure 1. In summer, the lipophilic extracts of zander and bream had the highest ABTS radical scavenging activity. Similarly, the hydrophilic extract of bream showed the highest activity ($p < 0.05$). In winter, the lipophilic extract of common carp and zander had significantly higher radical scavenging activity than that of other fishes ($p < 0.05$). Although radical scavenging activity of hydrophilic extracts of bream, common carp, and zander were higher than that of silver carp, there were no significant differences among their radical scavenging activity ($p > 0.05$). The ABTS radical scavenging activity of lipophilic extracts of bream and silver carp in summer were significantly higher than their activity in winter ($p < 0.05$).

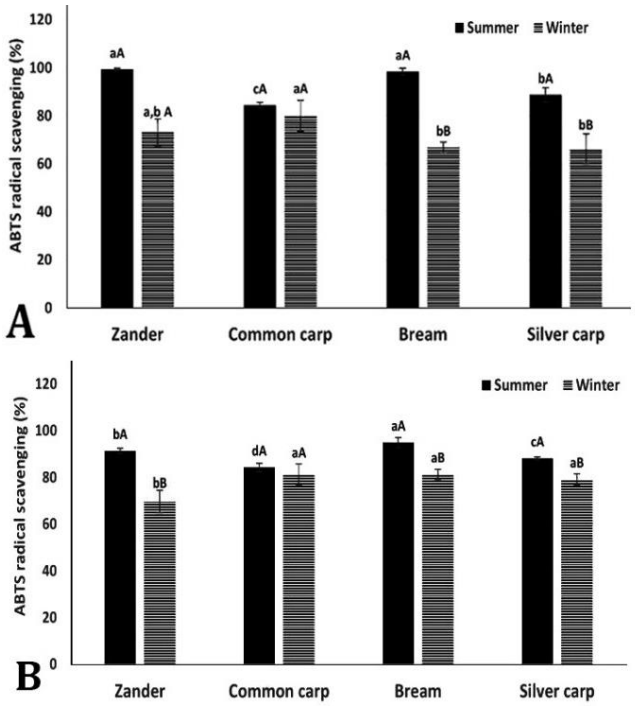

Fig. 1. The ABTS radical scavenging activity of **A)** lipophilic and **B)** hydrophilic extracts of Aras River fish fillets in summer and winter. Different small letters indicate significant differences among ABTS radical scavenging activities of the extracts of four fish species in each season ($p < 0.05$). Different capital letters indicate significant differences between ABTS radical scavenging activities of the extracts of each fish in summer and winter ($p < 0.05$).

However, the season had no significant effect on the radical scavenging activity of lipophilic extracts of zander and common carp. Meanwhile, ABTS radical scavenging activity of hydrophilic extracts of all fishes except for common carp were significantly higher in summer. Considering the sample volume used in ABTS assay, radical scavenging activity of the lipophilic extracts of fish fillets was 2-fold higher than that of hydrophilic extracts.

Figure 2 shows the DPPH radical scavenging activity of lipophilic and hydrophilic extracts of fish samples in summer and winter. In summer, both lipophilic and hydrophilic extracts of bream and zander showed significantly higher radical scavenging activity than other samples. In winter, lipophilic and hydrophilic extracts of zander had the highest radical scavenging effect.

The DPPH radical scavenging activity of lipophilic extracts of zander and common carp were significantly higher in winter than in summer ($p < 0.05$) while the radical scavenging effect of silver carp was higher in summer than in winter ($p < 0.05$). However, the season had no significant effect on the radical scavenging activity of lipophilic extract bream. Meanwhile, DPPH radical scavenging effects of hydrophilic extracts of all fishes in summer were significantly higher than those of in winter ($p < 0.05$).

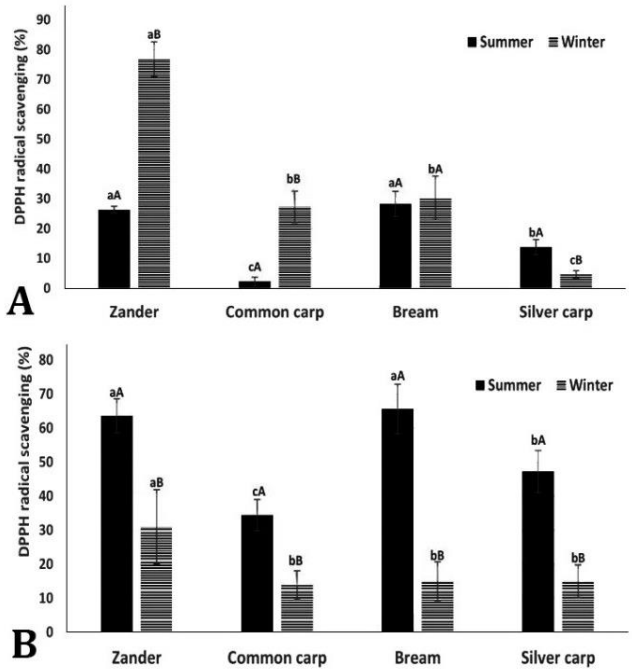

Fig. 2. DPPH radical scavenging activity of **A)** lipophilic and **B)** hydrophilic extracts of Aras River fish fillets in summer and winter. Different small letters indicate significant differences among DPPH radical scavenging activities of the extracts of four fish species in each season ($p < 0.05$). Different capital letters indicate significant differences between DPPH radical scavenging activities of the extracts of each fish in summer and winter ($p < 0.05$).

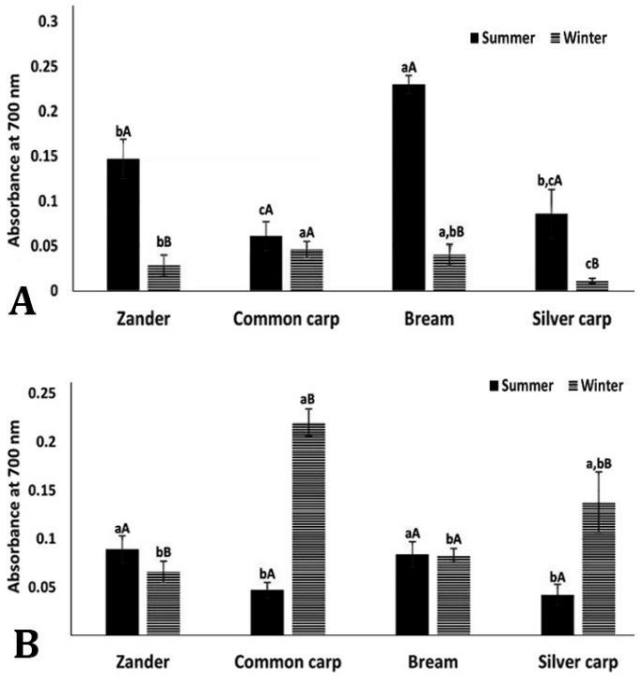

Fig. 3. Reducing power of **A)** lipophilic and **B)** hydrophilic extracts of Aras River fish fillets in summer and winter. Different small letters indicate significant differences among reducing power of the extracts of four fish species in each season ($p < 0.05$). Different capital letters indicate significant differences between reducing power of the extracts of each fish in summer and winter ($p < 0.05$).

Reducing power of lipophilic and hydrophilic extracts of fish samples in summer and winter are presented in Figure 3. In summer, the lipophilic extract of bream showed the highest reducing power. After bream, zander had higher reducing power. Similarly, hydrophilic extracts of bream and zander had significantly higher reducing power than other fish extracts ($p < 0.05$). In winter, lipophilic extracts of common carp and bream had significantly higher reducing power ($p < 0.05$). Reducing power of hydrophilic extracts of common carp and silver carp were significantly higher that of other fish extracts ($p < 0.05$). Unlike common carp, reducing power of lipophilic extracts of all fish samples were significantly higher in summer than in winter ($p < 0.05$). In the case of hydrophilic extract, reducing power of zander was higher in summer than in winter. Reducing power of hydrophilic extract of common carp and silver carp was significantly higher in winter than in summer ($p < 0.05$). However, the season had no significant effect of reducing power of hydrophilic extract of bream.

Fatty acid profile. Fatty acid profiles of zander, common carp, bream, and silver carp in summer are given in Table 1. The total saturated fatty acids (SFA) ranged from 27.68% to 44.09%. The bream and zander contained the lowest and the highest levels of total SFA, respectively.

The level of monounsaturated fatty acids (MUFA) in common carp, bream and silver carp was comparable (31.50 - 36.50%) but significantly higher than in zander (22.91%), ($p < 0.05$). The fatty acid C18:1n9c (oleic acid) was the most abundant MUFA in all species examined. The level of total polyunsaturated fatty acids (PUFA) in zander (25.97%) was significantly higher compared to other species (19.57 - 20.51%), ($p < 0.05$). The level of n3 PUFA in zander (20.71%) was significantly higher than that in other fishes examined, ($p < 0.05$). The n3 PUFA levels of other fishes were similar. Our results also showed that there were no significant differences in n6 PUFA contents of four fish species. The ratio of n3/n6 was found to range from 2.13 for common carp to 4.00 for zander. The PUFA/SFA ratio ranged from 0.57 in common carp to 0.72 in bream. However, there were no significant differences in the n3/n6 and PUFA/SFA ratios among the fish species ($p > 0.05$). Table 2 shows fatty acid profiles of zander, common carp, bream, and silver carp in winter. Zander, common carp, and silver carp had similar levels of SFA (26.43 - 26.88%), but bream showed the lowest content of SFA (22.45%). Palmitic acid (C16:0) was the major saturated fatty acid in all fishes. The level of MUFA ranged from 26.46 to 35.40%. Bream (35.40%) and common carp (30.09%) had significantly more MUFA as compared to zander and silver carp ($p < 0.05$).

The fatty acid C18:1n9c was the primary MUFA in all fishes. The level of PUFA was ranged from 26.79 to 37.96%. Silver carp and zander had significantly higher levels of PUFA as compared to other fishes ($p < 0.05$). The most abundant PUFA was C22:6n3, followed by C20:5n3 in all fish species tested. The highest level of n3 PUFA was found in silver carp, followed by zander. The n6 PUFA levels of four fishes were similar. The ratio of n3/n6 was found to range from 1.93 for bream to 3.75 for silver carp. The PUFA/SFA ratio ranged from 1.19 in bream to 1.39 in silver carp.

The effect of season on the fatty acid composition of Aras River fish species is presented in Table 3. Total SFA levels of all fish species examined were significantly ($p < 0.05$) higher in summer than in winter. The season had no significant effect on total MUFA levels of zander, common carp, and bream, but total MUFA level of silver carp was significantly higher in summer than in winter ($p < 0.05$).

In our study, total PUFA levels all fishes except zander were higher in winter than in summer. The n3 PUFA levels of common carp and silver carp were also significantly higher in winter than in summer ($p < 0.05$). Meanwhile, the amounts of n6 PUFA in all fishes except common carp were higher in winter than in summer. In the case of n3/n6 ratio, only zander showed significantly ($p < 0.05$) higher ratio in summer than in winter and seasonal variation of this ratio in other fishes was not significant. Finally, the ratio of PUFA/SFA was significantly higher in winter than in summer for all fish species examined in this study ($p < 0.05$).

Vitamin E. The vitamin E contents of fish species were examined in this study. Large variations were observed in vitamin E content among four fish species and different samples of the same fish. Vitamin E content of zander was ranged from 0.38 to 143.68 mg per 100 g of fillet in

Table 1. Fatty acid composition (%) of four fish species from Aras River in summer.

Fatty acid	Zander	Common carp	Bream	Silver carp
C14:0	1.05 ± 0.13	1.75 ± 0.46	2.07 ± 0.23	2.95 ± 1.20
C15:0	0.43 ± 0.03	0.55 ± 0.07	0.40 ± 0.13	0.51 ± 0.32
C16:0	27.29 ± 1.77	17.91 ± 0.57	15.03 ± 2.00	19.91 ± 2.61
C17:0	1.28 ± 0.15	1.06 ± 0.17	0.71 ± 0.30	0.66 ± 0.14
C18:0	10.83 ± 1.52	8.25 ± 1.29	4.47 ± 1.86	5.77 ± 4.03
C20:0	0.38 ± 0.08	0.35 ± 0.45	0.17 ± 0.13	0.45 ± 0.16
C21:0	0.85 ± 0.57	0.54 ± 0.37	0.78 ± 0.78	1.21 ± 0.26
C22:0	0.45 ± 0.04	3.89 ± 1.26	3.30 ± 0.40	0.70 ± 0.58
C23:0	ND	ND	0.01 ± 0.02	0.01 ± 0.01
C24:0	1.47 ± 0.12	0.55 ± 0.11	0.51 ± 0.11	0.49 ± 0.30
Total SFA	44.09 ± 3.42^{a}	34.84 ± 2.10^{b}	27.68 ± 3.55^{c}	32.64 ± 4.92^{bc}
C14:1	0.31 ± 0.04	0.41 ± 0.26	0.30 ± 0.10	0.12 ± 0.18
C15:1	ND	ND	0.06 ± 0.04	0.01 ± 0.01
C16:1	2.69 ± 0.50	5.67 ± 1.25	7.88 ± 1.49	8.45 ± 3.31
C17:1	0.44 ± 0.07	0.62 ± 0.30	0.81 ± 0.34	2.09 ± 1.05
C18:1n9t	0.24 ± 0.07	0.44 ± 0.60	0.38 ± 0.06	1.17 ± 1.50
C18:1n9c	13.63 ± 3.08	15.85 ± 2.14	16.93 ± 1.58	18.67 ± 2.83
C18:1n7c	3.20 ± 0.17	5.82 ± 0.97	7.36 ± 1.26	2.88 ± 3.14
C20:1	0.51 ± 0.09	0.13 ± 0.28	1.20 ± 1.06	0.87 ± 0.58
C22:1n9	0.56 ± 0.07	0.62 ± 0.22	0.45 ± 0.44	0.01 ± 0.02
C24:1n9	1.32 ± 0.16	1.94 ± 0.46	1.13 ± 0.16	2.10 ± 0.57
Total MUFA	22.91 ± 3.55^{b}	31.50 ± 3.76^{a}	36.50 ± 4.58^{a}	36.37 ± 4.91^{a}
C18:2n6t	0.35 ± 0.08	0.30 ± 0.18	0.18 ± 0.14	0.14 ± 0.15
C18:2n6c	1.24 ± 0.16	4.58 ± 1.30	2.78 ± 0.48	1.96 ± 0.39
C18:3n6	0.39 ± 0.78	3.16 ± 2.29	1.38 ± 1.59	1.23 ± 0.43
C18:3n3	1.74 ± 0.19	1.38 ± 1.13	4.34 ± 1.31	3.30 ± 0.97
C20:2	0.08 ± 0.06	0.09 ± 0.15	0.32 ± 0.59	0.01 ± 0.02
C20:3n6	3.20 ± 0.47	ND	0.16 ± 0.36	0.53 ± 0.71
C20:3n3	0.02 ± 0.04	0.39 ± 0.87	0.02 ± 0.02	0.05 ± 0.09
C20:4n6	ND	ND	0.88 ± 1.84	1.21 ± 0.12
C20:5n3	4.48 ± 1.05	4.09 ± 0.59	5.46 ± 1.51	7.02 ± 1.74
C22:2n6	ND	ND	ND	0.14 ± 0.31
C22:6n3	14.48 ± 2.85	5.73 ± 0.73	4.41 ± 1.33	4.49 ± 1.38
Total PUFA	25.97 ± 4.27^{a}	19.57 ± 1.57^{b}	19.80 ± 4.61^{b}	20.51 ± 2.57^{b}
n3 PUFA	20.71 ± 3.99^{a}	11.59 ± 2.29^{b}	14.23 ± 3.68^{b}	15.28 ± 2.68^{b}
n6 PUFA	5.18 ± 0.47^{a}	8.04 ± 3.49^{a}	5.25 ± 2.94^{a}	5.21 ± 0.97^{a}
n3/n6	4.00 ± 0.64^{a}	2.13 ± 2.16^{a}	3.20 ± 1.43^{a}	3.04 ± 0.93^{a}
PUFA/SFA	0.60 ± 0.15^{a}	0.57 ± 0.07^{a}	0.72 ± 0.17^{a}	0.64 ± 0.16^{a}

^{abc} Different small letters in each row indicate significant differences ($p < 0.05$). ND: not detected.

Table 2. Fatty acid composition (%) of four fish species from Aras River in winter.

Fatty acid	Zander	Common carp	Bream	Silver carp
C14:0	1.61 ± 0.56	1.64 ± 0.55	1.73 ± 0.34	1.31 ± 0.48
C15:0	0.49 ± 0.09	0.46 ± 0.07	0.33 ± 0.06	0.35 ± 0.05
C16:0	17.15 ± 2.60	15.49 ± 2.07	12.84 ± 0.65	16.58 ± 1.30
C17:0	0.70 ± 0.08	0.56 ± 0.32	0.64 ± 0.36	0.51 ± 0.27
C18:0	3.83 ± 0.97	4.56 ± 2.22	3.39 ± 0.57	4.04 ± 0.86
C20:0	0.35 ± 0.12	0.32 ± 0.11	0.42 ± 0.04	0.41 ± 0.17
C21:0	1.06 ± 0.41	1.99 ± 1.43	1.26 ± 0.40	2.60 ± 2.52
C22:0	0.62 ± 0.12	0.682 ± 0.18	0.78 ± 0.10	0.50 ± 0.20
C23:0	0.01 ± 0.02	0.04 ± 0.04	0.10 ± 0.04	0.02 ± 0.04
C24:0	1.08 ± 0.29	1.01 ± 0.28	0.97 ± 0.19	1.10 ± 0.54
Total SFA	26.88 ± 2.60^{a}	26.76 ± 2.48^{a}	22.45 ± 1.48^{b}	26.43 ± 3.38^{a}
C14:1	0.09 ± 0.05	0.17 ± 0.05	0.19 ± 0.06	0.08 ± 0.06
C15:1	0.04 ± 0.04	0.09 ± 0.12	0.02 ± 0.03	0.02 ± 0.04
C16:1	6.61 ± 2.11	3.45 ± 1.93	7.52 ± 1.02	5.29 ± 1.20
C17:1	0.88 ± 0.21	0.88 ± 0.22	0.91 ± 0.14	0.83 ± 0.19
C18:1n9t	0.20 ± 0.02	0.29 ± 0.05	0.23 ± 0.03	0.22 ± 0.04
C18:1n9c	14.45 ± 4.47	14.45 ± 1.21	17.18 ± 2.80	12.85 ± 2.74
C18:1n7c	3.50 ± 2.01	7.17 ± 5.36	6.40 ± 0.75	4.50 ± 0.89
C20:1	0.64 ± 0.18	1.24 ± 0.49	1.02 ± 0.07	0.65 ± 0.15
C22:1n9	0.01 ± 0.01	0.09 ± 0.06	0.11 ± 0.07	0.02 ± 0.05
C24:1n9	1.53 ± 0.76	2.26 ± 0.39	1.83 ± 0.19	1.99 ± 0.22
Total MUFA	27.91 ± 5.26^{b}	30.09 ± 5.41^{ab}	35.40 ± 3.15^{a}	26.46 ± 4.47^{b}
C18:2n6t	0.20 ± 0.03	0.26 ± 0.11	0.33 ± 0.05	0.19 ± 0.06
C18:2n6c	4.06 ± 1.07	3.89 ± 1.39	3.39 ± 1.07	2.05 ± 0.45
C18:3n6	0.20 ± 0.13	0.09 ± 0.08	0.26 ± 0.02	0.20 ± 0.13
C18:3n3	4.44 ± 0.17	6.32 ± 2.68	3.83 ± 0.88	3.76 ± 0.99
C20:2	0.14 ± 0.04	0.24 ± 0.07	0.20 ± 0.02	0.19 ± 0.29
C20:3n6	3.58 ± 0.80	4.47 ± 1.95	3.81 ± 0.74	3.85 ± 1.47
C20:3n3	ND	ND	0.05 ± 0.06	ND
C20:4n6	1.14 ± 0.22	0.98 ± 0.41	1.34 ± 0.33	1.70 ± 1.05
C20:5n3	7.27 ± 1.17	7.20 ± 1.80	6.05 ± 0.57	9.70 ± 0.74
C22:2n6	ND	ND	ND	ND
C22:6n3	13.83 ± 5.53	8.29 ± 2.57	7.82 ± 1.29	16.49 ± 6.81
Total PUFA	34.86 ± 6.79^{ab}	31.77 ± 2.23^{bc}	26.79 ± 1.43^{c}	37.96 ± 4.92^{a}
n3 PUFA	25.54 ± 6.33^{ab}	21.87 ± 3.72^{bc}	17.47 ± 1.87^{c}	29.65 ± 5.61^{a}
n6 PUFA	9.18 ± 0.96^{a}	9.67 ± 2.28^{a}	9.12 ± 0.79^{a}	7.99 ± 0.56^{a}
n3/n6	2.78 ± 0.66^{ab}	2.45 ± 1.03^{b}	1.93 ± 0.33^{b}	3.75 ± 0.76^{a}
PUFA/SFA	1.30 ± 0.23^{a}	1.19 ± 0.11^{a}	1.19 ± 0.02^{a}	1.39 ± 0.16^{a}

^{abc} Different small letters in each row indicate significant differences ($p < 0.05$). ND: not detected.

summer and from 18.39 to 63.58 mg per 100 g of fillet in winter. In common carp, it was ranged from 1.20 to 121.37 mg per 100 g of fillet in summer and from 7.98 to 157.64 mg per 100 g in winter. The highest content of vitamin E (305.40 mg per 100 g) was found in one sample of bream in summer while it was ranged from 10.01 to 151.92 in winter. In the case of silver carp, vitamin E level was varied from 26.85 to 117.39 mg per 100 g in summer and from 5.65 to 95.18 mg per 100 g in winter. However, there were no statistical differences among vitamin E contents of fish species examined in this study.

Discussion

The antioxidant potential of lipophilic and hydrophilic extracts of four fish species fillets from Aras River was determined. Several studies evaluated the antioxidant systems in different fish species, but there is no report on the effect of season on the antioxidant potential of lipophilic and hydrophilic extracts of fish. A previous study showed that DPPH radical scavenging activity of keli (*Clarias batrachus*) was higher than those of other fishes.[22] Another study indicated that sardine and horse mackerel muscles had the highest radical scavenging activity. Sardine muscle also had the highest content of α-Tocopherol.[29] Similarly, the highest radical scavenging activity was found in cutlass fish.[20]

Fatty acid profile of fish fillets was also analyzed in this study. The results showed that the fillets of bream and zander had the lowest and the highest levels of total SFA, respectively. Similarly, it has been shown that bream had the lowest amount of total SFA.[11] The fatty acid C16:0 (palmitic acid) was the dominant saturated fatty acid in all fish species examined. The result is in agreement with the

Table 3. Effect of season on the fatty acid composition of Aras River fishes in summer and winter.

Fish	Fatty acid	Summer	Winter
Zander	Total SFA	44.09 ± 3.42[a]	26.88 ± 2.60[b]
	Total MUFA	22.91 ± 3.55[a]	27.91 ± 5.26[a]
	Total PUFA	25.97 ± 4.27[a]	34.86 ± 6.79[a]
	n3 PUFA	20.71 ± 3.99[a]	25.54 ± 6.33[a]
	n6 PUFA	5.18 ± 0.47[a]	9.18 ± 0.96[b]
	n3/n6	4.00 ± 0.64[a]	2.78 ± 0.66[b]
	PUFA/SFA	0.60 ± 0.15[a]	1.30 ± 0.23[b]
Common carp	Total SFA	34.48 ± 2.10[a]	26.76 ± 2.48[b]
	Total MUFA	31.50 ± 3.76[a]	30.09 ± 5.41[a]
	Total PUFA	19.57 ± 1.57[a]	31.77 ± 2.23[b]
	n3 PUFA	11.59 ± 2.29[a]	21.87 ± 3.72[b]
	n6 PUFA	8.04 ± 3.49[a]	9.67 ± 2.28[a]
	n3/n6	2.13 ± 2.16[a]	2.45 ± 1.03[a]
	PUFA/SFA	0.57 ± 0.07[a]	1.19 ± 0.11[b]
Bream	Total SFA	27.68 ± 3.55[a]	22.45 ± 1.48[b]
	Total MUFA	36.50 ± 4.58[a]	35.40 ± 3.15[a]
	Total PUFA	19.80 ± 4.61[a]	26.79 ± 1.43[b]
	n3 PUFA	14.23 ± 3.68[a]	17.47 ± 1.87[a]
	n6 PUFA	5.25 ± 2.94[a]	9.12 ± 0.79[b]
	n3/n6	3.20 ± 1.43[a]	1.93 ± 0.33[a]
	PUFA/SFA	0.72 ± 0.17[a]	1.19 ± 0.02[b]
Silver carp	Total SFA	32.64 ± 4.92[a]	26.43 ± 3.38[b]
	Total MUFA	36.37 ± 4.91[a]	26.46 ± 4.47[b]
	Total PUFA	20.51 ± 2.57[a]	37.96 ± 4.92[b]
	n3 PUFA	15.28 ± 2.68[a]	29.65 ± 5.61[b]
	n6 PUFA	5.21 ± 0.97[a]	7.99 ± 0.56[b]
	n3/n6	3.04 ± 0.93[a]	3.75 ± 0.76[a]
	PUFA/SFA	0.64 ± 0.16[a]	1.39 ± 0.16[b]

[ab] Different small letters in each row indicate significant differences ($p < 0.05$).

findings of previous studies.[10,12,14,15,30,31] It has been pointed out that the level of palmitic acid in fish was not affected by diet.[32] It was found that oleic acid was the most abundant MUFA in all species examined. Oleic acid has also been reported as the main MUFA for zander,[5,30,31] and carp.[6,12,14,31]

The total PUFA level in zander was lower than that reported by Guler *et al*.,[5] but similar to findings of Uysal and Aksoylar.[33] The fatty acids C22:6n3 (DHA) in zander and common carp, and C20:5n3 (EPA) in bream and silver carp were the dominant PUFA. Similarly, DHA has been reported as the major PUFA in zander by other researchers.[6,10,15] Zander can be considered as a good source of DHA. DHA in wild carp filet and linoleic acid in farmed one was the major PUFA.[14] DHA and EPA has been found as the predominant n3 PUFA in bream and carp.[12] DHA and EPA have beneficial effects in the prevention of human cardiovascular diseases.[34] DHA also possesses an essential role in neural cell membranes, i.e., the brain and eyes.[35]

The results indicated that the level of n3 PUFA in zander was significantly higher than that in other fishes examined, while the n3 PUFA levels of other fishes were similar. However, it has been reported that the *n*-3 PUFA content in bream was significantly higher than that in carp.[12] Similar to our finding, the high level of n3/n6 ratio has been found in zander by others.[15] However, this ratio is higher than those reported by other researchers.[5,30] The low level of n3/n6 ratio (0.4 and 0.44) has been reported for carp.[11,12] It has been suggested that the n3/n6 ratio can be used as a good indicator to compare the relative nutritional value of fish oil.[36]

Contrary to our results, other researchers[15] reported that total PUFA in zander was higher than total SFA, because they found a higher level (38.56 - 41.66%) of n3 PUFA in zander. The recommended minimum value for PUFA/SFA ratio is 0.45,[37] therefore, this ratio in all fish examined was higher than the recommended minimum value.

It was found that the season had no significant effect on total MUFA levels of zander, common carp, and bream, but total MUFA level of silver carp was significantly higher in summer than in winter. Contrary to our result, other researchers[15] reported that the total MUFA level in common carp was higher in summer than in winter. However, similar to our results, another study showed that the total MUFA level of zander in the summer was similar to winter.[15]

Seasonal variation in the fatty acid composition of carp has been reported.[16] Palmitic acid and oleic acid were identified as the main SFA and MUFA in all seasons, respectively. DHA (C22:6, n3), linoleic acid (c18:2, n6), and EPA (C20:5, n3) were the most abundant PUFA. The n3/n6 ratios were 1.43 and 1.60 in summer and winter, respectively. Another similar study reported the seasonal changes in the fatty acid composition of common carp muscle lipids.[6] It was found that palmitic acid and oleic acid were the main SFA (14.6 - 16.6%) and MUFA (15.10 - 20.30%) in all seasons, respectively. DHA was determined as the major PUFA in summer and winter. Effect of season on the fatty acid composition of common carp and zander muscle lipids has been reported.[15] The results showed that the PUFA levels of common carp and zander were higher in winter than in summer.[15] The DHA was the predominant PUFA in zander in both seasons. In the case of common carp, DHA was the major PUFA in winter. The ratios of n3/n6 in zander were 3.89 and 3.84 in summer and winter, respectively.[15] However, another study showed that n3/n6 ratios in zander were 0.72 and 1.22 in summer and winter, respectively.[5]

Daily vitamin E requirement for healthy humans (Recommended Dietary Allowances) is 3.00 - 4.00 mg per day in the UK and 8.00 - 10.00 mg per day in the USA.[38] Then, the consumption of 100 - 200 g per day of fish examined in this study may meet the daily needs for vitamin E. Vitamin E not only has health promotion effects for humans,[39] but also it is the primary antioxidant for oxidative stability of fish fillet during storage.[40,41]

The results of this study suggest that the fish fillets examined could be considered as significant sources of antioxidants in the diet. Season affected the antioxidant activity of the fish extracts. The antioxidant potential of the extracts could be used as an index of potential health benefits to consumers and as a predictive index of the oxidative stability of the fish fillet. Further studies are needed to investigate the individual contributions of the antioxidant compounds such as antioxidant enzymes, peptides, amino acids, and vitamins. The results also showed that the fatty acid composition of the fishes depended on fish species and season. The PUFA/SFA ratio was higher than 0.40 (minimum recommended value from WHO/FAO organization) in all the fishes. This ratio in winter was significantly higher than that in summer for all fishes. The n3 PUFA levels in all fish species were found to be higher than the n6 PUFA levels. Thus, fish consumption could exert health benefits due to antioxidant potential and essential fatty acids, especially n3 PUFA.

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Conflict of interest

The authors confirm that they have no conflicts of interest with respect to the work described in this study.

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