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ORIGINAL PAPER

Chemometric Characterization and Classification of Selected Freshwater and Marine Fishes from Turkey Based on their Fatty Acid Profiles

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Abstract The fatty acid (FA) profiles of edible muscle of selected commercially important freshwater and marine fish species from Turkey were investigated. The fatty acid compositions of freshwater fish species were 23.00–29.60% saturated (SFA), 25.70–36.50% monounsaturated (MUFAs), and 26.59–31.92% polyunsaturated acids (PUFAs), whereas the fatty acid compositions of marine fish consisted of 21.08–36.90% (SFA), 18.03–51.45% MUFAs, and 20.92–53.17% PUFAs. There was a wide variation and significant ($P < 0.05$) differences among the fatty acid profiles of the freshwater and marine fish samples, including differences in the SFA, MUFA, PUFA, EPA, DHA, DHA/EPA, total n-3 PFAs, total n-6 PUFAs and n-3/n-6 values. In addition, the cheap marine fish species such as anchovy and European pilchard, bogue are better dietary sources of n-3 PUFAs than more expensive species such as bluefish, Atlantic mackerel, sea bream and sea bass. Through the application of two multivariate statistical methods, Principal Component and Hierarchical Analysis, fish species from Turkey waters were classified according to the geographical locations categorized in terms of fatty acid profiles. Clustering by fish species also gave rise to defined groups.

Keywords Fatty acid · Freshwater fish · Marine fish · EPA · DHA · Chemometri · Turkey

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Introduction

Total fish production in Turkey was reported as 662,991 ton in 2006, comprised of marine fisheries (73%, 488,966 ton), aquaculture (20%, 129,943 ton) and inland fisheries (7%, 44,082 ton). Anchovy, horse mackarel, bonito, sardine, bluefish, mullet and turbot are marine fish species.

Rainbow trout, seabass and sea bream are harvested important aquaculture species of Turkey. Anchovy is the most produced marine fish species (270,000 ton), followed by cultured brass (38,408 ton), bonito (29,960 ton), cultured sea bream (28,463 ton), sardine (15,586 ton), whiting (9,112 ton) and cultured rainbow trout (1,633 ton) [1]. The fish species used in the present study represent approximately 84% of total fish production in Turkey.

Fish lipids are well known to be rich in polyunsaturated fatty acids (PUFAs), especially the long chain (LC) n-3 PUFAs eicosapentaenoic acid (EPA C20:5n3) and docosahexaenoic acid (DHA C22:6n3). These fatty acids (FAs) play a vital role in human nutrition, disease prevention and health promotion. Results of clinical and epidemiological research suggest that EPA and DHA have beneficial properties for the prevention of human coronary artery disease. The benefits of n-3 PUFA (polyunsaturated fatty acids), are associated with the synthesis of eicosanoids such as prostaglandins, thromboxanes, and leukotrienes [2–5]. EPA has also been reported to be useful in brain disorders and cancer treatment [6].

In Turkey, in spite of there being a great variety of aquatic species, the contribution of fish to the diet is small with an annual per capita fish consumption of 7 kg [7]. Thus, it is very important to change the fish consumption habits of the population. In addition, knowledge of the fatty acid profiles of commercially important fish species is needed owing to post-mortem deterioration and changes of

the nutritional value of fish. The marine lipids also have applications in food, healthcare, pharmaceutical products and as an ingredients in feed, in agriculture and the aquaculture industry. In view of these facts, it seemed necessary to carry out a study on the nutritional value of highly consumed domestic fish species. The objective of this study was to determine the fatty acid (FA) profiles in commercially important freshwater and marine fish species of Turkey. The data obtained from FAs profiles of fish oil were subjected to the Principal component (PC) and Hierarchical cluster (HC) analysis, aimed to establishing differences in fatty acid profiles according to the fish species.

Materials and Methods

Material

Chemicals

All standards and reagents were of 99.9% purity and choromatographic grade (Merck, Darmstadt, Germany). The identification of FAME of the samples was performed by comparison to standard FAME (Sigma-Aldrich Chemicals 189-19, Diesenhofen, Germany).

Samples Selection

The fish species selected in the study were commonly consumed by Turkish people. Totally 15 fish samples were examined in this study. The first four of the samples were fresh water fish and other 11 samples were marine fish coming from the open sea (pelagic). All fish species were chosen and purchased from local fish markets under controlled official authories in Izmir. The properties of fish samples are given in Table 1.

Two lots of each species submitted daily fresh were sampled for the analysis. The individual fish (250–500 g lots of each) was washed, then carefully cut to separate the unedible portions (offals) including head, viscera and tail. These were stored in deep freeze at −18 °C after removal of their internal organs. The edible parts of each whole fish (with skin) were homogenized and about 50 g of homogenized sample, in triplicate, was mixed well with 15 g cleaned sea sand and 30 g anhydrous sodium sulphate, and then percolated overnight with hexane (100 ml) in a glass container. The hexane was removed with a rotary evaporator (40 °C) and then the residue was stored at −10 °C in dark until fatty acid composition could be determined. During the extraction and evaporation steps, the sample and oil were kept away from [8].

Esterfication of Fatty Acids

Methyl esters were prepared by transmethylation using 2 M KOH in methanol and *n*-hexane [9] with minor modifications. The extracted oil (10 mg) was dissolved in 2 ml hexane, followed by the addition of 4 ml of 2 M methanolic KOH. The tube was then vortexed for 2 min at

Table 1 The properties of some selected Turkish fish species examined in this study

The fish species and their codes	Origin	Cathing time	Latin name
The freshwater fish species			
Cultured rainbow trout (C.R. Trout-1)	Salihli-Manisa (Dam Lake)	December	*Oncorynchus mykiss* (Walbaum 1792)
Wild rainbow trout (W.R. Trout)	Salihli-Manisa	December	*Salmo trutta forma fario* (Walbaum 1792)
Cultured rainbow trout (C.R. Trout-2)	Bozdoğan/Aydin (Dam Lake)	September	*Oncorynchus mykiss* (Walbaum 1792)
Cultured hybrid striped bass (C.H.S. Bass)	Bozdoğan/Aydin (Dam Lake)	September	(*Morone saxatilis* × *M. chrysops*)
The marine fish species			
Atlantic bonito-1 (AB-1)	Marmara Sea	November	*Sarda sarda*
Atlantic bonito-2 (AB-2)	Black Sea	December	*Sarda sarda*
European pilchard Mediterannean (Pilchrad)	Aegean Sea	October	*Sardine Pilchardus* (Walbaum 1792)
European anchovy (Anchovy)	Black Sea	October	*Engraulis encrasicolus* (Linnaeus 1766)
Atlantic mackarel (Mackarel)	Aegean Sea	December	*Scomber scombrus* (Linnaeus 1758)
Bogue	Aegean Sea	February	*Boops boops* (Linnaeus 1758)
Bluefish (B. fish)	Marmara Sea	November	*Pomotamus saltator* (Linnaeus 1766)
Wild sea bream (W.S. Bream)	Aegean Sea	June	*Sparus aurata* (Linnaeus 1766
Cultured sea bream (C.S. Bream)	Aegean Sea	September	*Sparus aurata* (Linnaeus 1766)
Sea bass (S. Bass)	Aegean Sea	June	*Dicentrarchus labrax*
Whiting (White)	Aegean Sea	October	*Trisopterus minutus capelanus* (Lacepède 1800)

room temperature. After centrifugation at 4,000 rpm for 10 min, the hexane layer was taken for capillary GC analyses.

Determination of FA Composition

The FA compositions of samples were carried out by an official capillary gas choromatographic method [10]. Analysis of the fatty acids were carried out on a GC (HP 6890) using a capillary column (DB-23; J & W Scientific, Folsom, USA) having the following specifications: (30 m × 0.25 mm ID and 0.25 μm film thickness of 50% cyanopropyl). The GC conditions were as follows: Initial temperature 100 °C, 100–175 °C at 5 °C/min, 175–210 °C at 10 °C/min, and 210 °C for 15 min; the injector and FID were set at 250 °C; Carrier gas (He) 0.5 ml/min; split ratio 100:1; hydrogen flow 30 ml/min; make-up flow (Nitrogen) 24.5 ml/min; dry air flow 300 ml/min. Each sample was injected in triplicate ($n = 3$). FA standards had linear calibration curves through the origin ($R^2 = 0.99$). The GC method used were validated for FA determination of fish samples within the 95% confidence limits. Mean analytical recoveries determined from individual FA in fish samples changed from 99.7% to 100%. The results were calculated using the HP 3365 Chemistation program and recorded as percentage peak area. The identification of FAMEs of samples was performed using a standard FAMEs mixture (Sigma-Aldrich Chemicals 189-19).

Lipid quality indices were calculated according to Ulbricht and Southgate [11]. The atherogenic index (AI) was calculated as follows:

$$\mathrm{AI} = \frac{[12:0 + 4(14:0 + 16:0)]}{[(\mathrm{n6} + \mathrm{n3})\mathrm{PUFA} + 18:1 + \sum \mathrm{MUFA}]}$$

The index of thrombogenicity (IT) was calculated as follows:

$$\mathrm{IT} = \frac{(14:0 + 16:0 + 18:0)}{[(0.5 \times 18:1) + 0.5(\sum \mathrm{MUFA}) + 0.5(\mathrm{n6PUFA}) + 3(\mathrm{n3PUFA}) + (\mathrm{n3PUFA}/\mathrm{n6PUFA})]}$$

[C 12:0 Lauric, C14:0 Myristic, C16:0 Palmitic, C 18:1 Oleic].

Stastistical Analysis

The Tukey's multiple range test was applied when the variance analysis indicated significant differences in mean FA profile values of fish groups. Stastistical analysis was performed using the SPSS 10 stastistical software [12].

Multivariate Analysis

Characterization and classification of fish species taken from different locations of Turkey were carried out using widely used chemometric methods, Principal Component Analysis (PCA, Ward Method) and Hierarchical Cluster Analysis (HCA, Euclidian Distance). Multivariate analysis was performed using the Matlab 7.5.0 (R2007b) [13].

Results and Discussion

The DB-23 column gave clear and excellent separation in all of short and long chain FAs on fish oil sample (Fig. 1). A total of 31 FAs were determined by capillary GC in fish samples. The fatty acid profiles of the 15 fish samples collected from different geographic origins of Turkey is shown as percentages of total lipids in Table 2.

The FA contents in the 15 fish species are presented in Tables 2, 3, 4 and 5. These tables show the amount of FAs varied widely among the fish species. There was a wide variation and significant ($P < 0.05$) differences among the FAs profiles of the freshwater and marine fish samples in terms of total and individual saturated and unsaturated fatty acids.

As seen in Table 2, among saturated fatty acids (SFAs), the largest concentration in freshwater and marine fish species was for palmitic acid (C16:0), varying from 15.20–17.87% and 13.52–20.19%, respectively. Palmitic acid consisted of 60–66% and 55–66% of the total SFAs content of lipids for freshwater and marine fish species, respectively. Stearic acid (C18:0) was the other predominant SFA of freshwater and marine fish species, varying from 2.90–4.27% and 3.57–6.61%, respectively.

Palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9 *cis*) were the MUFAs (Table 3). Freshwater fish species were 5.84–8.00% palmitoleic acid and 25.07–36.50% oleic acid, whereas these FA profiles of marine fish consisted of 1.86–6.90% and 8.84–48.69%,of these fatty acids, respectively. Oleic acid (C18:1n9) was the major MUFA accounting for 70–79% of total MUFAs for freshwater fish while it contributes 61–95% of total MUFAs for marine fish. SFAs and MUFAs results of freshwater and marine fish species are in agreement with previous studies [4, 8, 14–25].

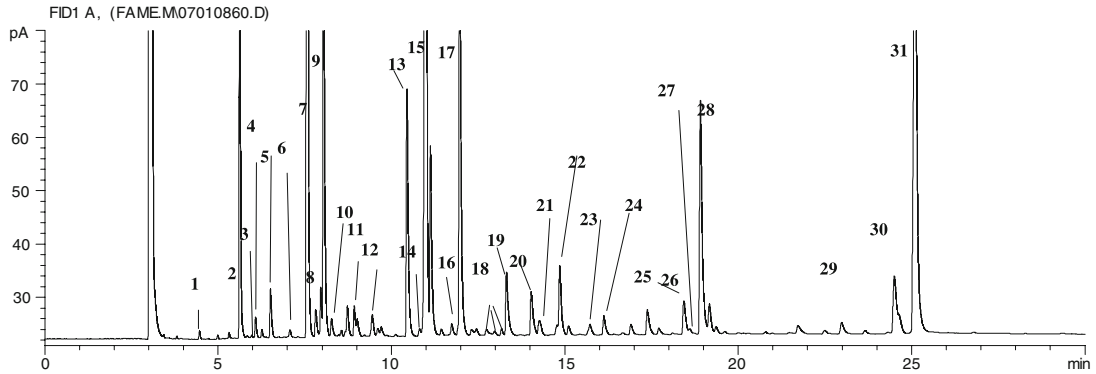

Fig. 1 A typical choromatogram of cultured rainbow trout, *Oncorynchus mykiss* (Walbaum 1792), fatty acids from Salihli-Manisa *1* C12:0, *2* C14:0, *3* C14:1, *4* C14:1 *t*, *5* C15:0, *6* C15:1, *7* 16:0, *8* 16:1 *t*, *9* C16:1, *10* C16:2, *11* C17:0, *12* C17:1, *13* C18:0, *14* C18:1 *t*, *15* C18:1, *16* C18:2 *t*, *17* C18:2, *18* C18:3 *t*, *19* C18:3, *20* C18:4, *21* C20:0, *22* C20:1, *23* C20:2, *24* C20:3 n3, *25* C20:4, *26* C20:3n6, *27* C22:0, *28* C20:5 n3 (EPA), *29* C24:0, *30* C22:5 n3, *31* C22:6n3 (DHA)

PUFAs in fish oils can be divided into groups (n-3 PUFA) and (n-6 PUFA), which have different physiological functions and effects. The main n-3 PUFAs are C18:3n-3 and its metabolites, eicosapentaenoic acid (EPA C22:6n-3) and docosahexaenoic acid (DHA C22:6n-3). The C18:2n-6 and its metabolite (C20:4n-6) were the main n-6 PUFAs. For all fish samples, the major n-3 PUFA was mainly DHA and this followed by EPA, DPA (C22:5n-3), linolenic acid (C18:3n-3) and stearidonic acid (18:4n-3) (Table 4). Compared with freshwater fish, marine fish contain higher levels of PUFAs especially DHA and EPA. EPA and DHA are the predominant n-3 PUFAs due to the marine fish staple diets comprise mainly of zooplankton that are rich in PUFAs [19]. DHA values were higher than EPA values in all fish samples. The EPA and DHA results found on marine fish species agreed previous researchers [4, 8, 17, 19–25].

Differences among of PUFAs profiles wild and cultured fish species were observed. For instance, the proportions of EPA and DHA in wild rainbow trout samples were higher than cultured fish sample. Also, EPA and DHA levels in wild goldbrasse sample was lower than cultured marina fish samples (Table 4). Similar results on freshwater fish species were reported by Suzuki et al. [14]. However, both freshwater and marine fish were good sources of EPA and DHA. The consumption of freshwater fish contributes significantly to the amount of n-3 PUFAs in the diet of the Turkish people. In addition, the cheap marine fish species such as anchovy and European pilchard, bogue are better dietary sources of n-3 PUFAs than more expensive species such as bluefish, Atlantic mackerel, sea bream and sea bass. According to these results, Atlantic bonito, European anchovy and European pilchard consumed commonly in Turkey are concluded to be good sources of EPA and DHA, n-3 PUFA series, and should be recommended for dietary inclusion to reduce risks of cardiovascular disease. The level of n-6 PUFAs of marine fish were found to be high (Table 4). Among n-6 PUFAs, freshwater contained higher levels of linoleic acid (C18:2n-6) than marine fish species whereas freshwater fish samples gave lower arachidonic acid (C20:4n-6) than marine fish. The n-6 series obtained were in agreement with the findings of other researchers [4, 19, 21, 22].

The n-3/n-6 FA ratio, the best index in comparing relative nutritional values of fish oils from different species, varied widely among the fish species. The ratio ranged from 1.29 to 2.22 for freshwater fish species and the values of marine fish species were between 0.73 and 9.78 (Table 4). The results on n3/n6 FA ratio on freshwater fish were similar to the findings of other studies [4, 14, 20]. In the present study, high n-3/n-6 ratios were found in marine fish species, Atlantic bonito 1, European pilchard Mediterannean, European anchovy, bogue, bluefish. Our experiments show that marine fishes have higher n-3/n-6 FA rates than fresh water fishes. The results found on marine fish species agreed with previous authors [21, 22, 24, 25].

There was no relationship between market price and n-3/n-6 ratio for the 11 marine species studied. For example, although bluefish, goldbrasse, brown meager and Atlantic mackerel were the most expensive fish studied, the n-3/n-6 ratio were lower than in cheaper species such as anchovy, European pilchard, bogue and Atlantic bonito. In other words, some cheap fish species have better nutritional values in terms of FAs. Similar observations on marine fish species from the Mediterranean were reported by other researchers [22, 25].

A minimum value of PUFA/SFA ratio recommended is 0.45 [26], which was lower than those obtained from all freshwater and marine fish species studied. As seen in Table 4, the highest PUFA/SFA ratios in freshwater and marine fish species were 1.16 (cultured hybrid striped

Table 2 The saturated fatty acid (SFA) profiles of some selected freshwater and marine fish species from different geographical locations of Turkey

Codes of FAMEs	Samples FAs	The freshwater fish species				The marine fish species			
		C.R. Trout	W.R. Trout	C.R. Trout	C.H.S. Bass	AB-1	AB-2	E. Pilchard	E. Anchovy
LAU	12:0	0.09 ± 0.02 A	0.11 ± 0.00 a	0.05 ± 0.00 a	ND	0.09 ± 0.01 a	0.07 ± 0.01 a	0.08 ± 0.00 a	0.16 ± 0.03 a
MA	14:0	4.86 ± 0.41 Bc	5.72 ± 0.43 c	3.50 ± 0.04 ab	3.43 ± 0.24 ab	3.47 ± 0.04 ab	4.09 ± 0.03 b	4.28 ± 0.06 b	5.81 ± 0.04 c
PDA	15:0	0.63 ± 0.03 ab	0.69 ± 0.02 ab	0.51 ± 0.02 a	0.50 ± 0.00 a	0.73 ± 0.04 ab	0.75 ± 0.01 ab	1.07 ± 0.00 b	1.76 ± 0.02 bc
PA	16:0	17.23 ± 0.28 bc	17.87 ± 0.34 cd	16.87 ± 0.25 bc	15.20 ± 0.89 ab	15.89 ± 0.24 b	18.87 ± 0.43 cd	19.21 ± 0.48 de	20.19 ± 0.16 e
MG	17:0	0.68 ± 0.02 b	0.73 ± 0.04 bc	0.54 ± 0.04 a	0.44 ± 0.00 a	0.32 ± 0.01 a	0.55 ± 0.03 ab	0.42 ± 0.03 a	1.70 ± 0.05 a
SA	18:0	4.05 ± 0.02 bc	3.96 ± 0.03 b	4.27 ± 0.04 bc	2.90 ± 0.03 a	3.57 ± 0.04 a	5.00 ± 0.07 c	4.61 ± 0.18 bc	5.47 ± 0.01 c
AA	20:0	0.39 ± 0.01 a	0.37 ± 0.00 a	0.43 ± 0.02 a	0.40 ± 0.01 a	0.33 ± 0.01 a	0.88 ± 0.04 c	0.52 ± 0.03 ab	0.66 ± 0.02 b
BA	22:0	0.14 ± 0.02 a	0.11 ± 0.02 a	ND	ND	0.24 ± 0.01 a	0.14 ± 0.01 a	0.21 ± 0.01 a	0.20 ± 0.03 a
LG	24:0	0.08 ± 0.00 a	0.07 ± 0.00 a	0.08 ± 0.00 a	0.13 ± 0.00 a	0.11 ± 0.00 a	0.45 ± 0.03 b	0.09 ± 0.00 a	0.95 ± 0.04 c
SFA	SFA	28.15 ± 0.10 c	29.63 ± 0.08 d	26.25 ± 0.04 bc	23.00 ± 0.16 a	24.75 ± 0.04 b	30.80 ± 0.09 de	30.49 ± 0.10 d	36.90 ± 0.04 f

Codes of FAMEs	Samples FAs	The marine fish species						
		Mackarel	Bogue	Bluefish	W.S. Bream	C.S. Bream	Seabass	Whiting
LAU	12:0	0.45 ± 0.05 ab	0.06 ± 0.01 a	0.04 ± 0.01 a	ND	0.01 ± 0.00 a	0.09 ± 0.00 a	ND
MA	14:0	2.86 ± 0.26 ab	4.68 ± 0.80 b	4.19 ± 0.41 b	3.54 ± 0.81 ab	2.06 ± 0.02 a	1.38 ± 0.00 a	1.03 ± 0.39 a
PDA	15:0	0.78 ± 0.02 ab	1.13 ± 0.12 b	0.69 ± 0.04 ab	0.49 ± 0.01 a	0.33 ± 0.02 a	0.41 ± 0.03 a	ND
PA	16:0	15.37 ± 1.50 ab	15.19 ± 0.77 ab	19.51 ± 1.10 ab	21.33 ± 1.56 e	16.26 ± 0.04 b	13.52 ± 0.28 a	14.03 ± 2.71 a
MG	17:0	0.76 ± 0.05 bc	0.93 ± 0.03 c	0.76 ± 0.05 bc	0.35 ± 0.01 a	0.30 ± 0.03 a	0.67 ± 0.03 b	0.56 ± 0.06 ab
SA	18:0	6.61 ± 0.35 e	5.98 ± 0.11 cd	5.35 ± 0.01 c	6.21 ± 0.40 de	5.64 ± 0.18 cd	6.05 ± 0.12 d	4.67 ± 0.05 bc
AA	20:0	0.64 ± 0.04 b	0.84 ± 0.04 c	0.72 ± 0.01 bc	0.86 ± 0.05 c	0.51 ± 0.01 ab	0.79 ± 0.07 bc	0.79 ± 0.50 bc
BA	22:0	0.14 ± 0.00 a	0.11 ± 0.01 a	0.16 ± 0.01 a	ND	0.29 ± 0.02 ab	ND	ND
LG	24:0	0.19 ± 0.01 a	0.22 ± 0.01 a	0.14 ± 0.01 a	0.44 ± 0.10 b	0.18 ± 0.01 a	0.22 ± 0.05 a	ND
SFA	SFA	27.80 ± 0.05 cd	29.14 ± 0.03 de	31.56 ± 0.06 e	33.22 ± 0.06 ef	25.67 ± 0.04 b	23.13 ± 0.12 a	21.08 ± 0.13 a

Values are percentage of total fatty acid expressed as mean ± SD of four separate determinations. The values in the same row with different letters show stastistically significant differences ($P < 0.05$)

ND not detected, *SFA* saturated fatty acids

Table 3 The monounsaturated fatty acid (MUFAs) profiles of some selected freshwater and marine fish species from different geographical locations of Turkey

Codes of FAMEs	Samples FAs	The freshwater fish species				The marine fish species			
		C.R. Trout	W.R. Trout	C.R. Trout	C.H.S. Bass	AB-1	AB-2	E. Pilchard	E. Anchovy
MOA	14:1n9	0.26 ± 0.02 a	0.29 ± 0.00 a	0.18 ± 0.02 a	0.31 ± 0.00 a	0.18 ± 0.01 a	0.29 ± 0.01 a	0.22 ± 0.00 a	0.35 ± 0.00 a
PDDA	15:1	0.12 ± 0.00 a	0.11 ± 0.00 a	0.07 ± 0.00 a	0.08 ± 0.00 a	0.09 ± 0.01 a	0.15 ± 0.02 a	0.11 ± 0.00 a	0.16 ± 0.02 a
PAO	16:1n7	7.51 ± 0.02 d	8.00 ± 0.00 d	5.84 ± 0.04 c	6.70 ± 0.32 d	4.25 ± 0.04 b	5.75 ± 0.15 c	3.51 ± 0.00 b	2.89 ± 0.05 a
MGO	17:1	0.40 ± 0.00 a	0.39 ± 0.00 a	0.32 ± 0.01 a	0.40 ± 0.00 a	0.49 ± 0.01 a	0.61 ± 0.02 b	0.34 ± 0.02 a	0.31 ± 0.00 a
OA	18:1n9	26.26 ± 0.09 c	25.06 ± 0.10 c	33.37 ± 0.14 d	36.50 ± 0.82 e	12.37 ± 0.04 a	24.13 ± 0.08 c	12.23 ± 0.02 a	8.84 ± 0.09 a
GA	20:1n9	1.38 ± 0.03 c	1.46 ± 0.04 c	1.35 ± 0.04 d	2.43 ± 0.11 e	0.51 ± 0.02 a	2.57 ± 0.02 e	1.44 ± 0.03 c	1.20 ± 0.04 d
DMA	22:1n6	0.68 ± 0.01 b	0.69 ± 0.00 b	0.70 ± 0.04 b	0.77 ± 0.01 b	0.14 ± 0.04 a	3.05 ± 0.20 d	0.32 ± 002 a	0.40 ± 0.02 a
NRA	24:1n9	ND	ND	ND	ND	ND	ND	0.07 ± 0.01 a	0.24 ± 0.03 a
MUFA	MUFA	36.61 ± 0.10 c	36.01 ± 0.12 c	41.83 ± 0.06 d	45.85 ± 0.14 de	18.03 ± 0.08 a	36.55 ± 0.07 c	18.24 ± 0.08 a	14.39 ± 0.04 a

Codes of FAMEs	Samples FAs	The marine fish species						
		Mackarel	Bogue	Bluefish	W.S. Bream	C.S. Bream	Seabass	Whiting
MOA	14:1n9	0.21 ± 0.00 a	0.28 ± 0.03 a	0.32 ± 0.01 a	0.50 ± 0.03 b	0.11 ± 0.01 a	ND	ND
PDDA	15:1	ND	0.14 ± 0.01 a	0.14 ± 0.01 a	0.13 ± 0.01 a	0.06 ± 0.00 a	ND	ND
PAO	16:1n7	2.55 ± 0.50 a	3.93 ± 0.20 a	6.90 ± 0.40 b	5.20 ± 0.41 bc	3.95 ± 0.10 b	1.93 ± 0.15 a	1.86 ± 0.77 a
MGO	17:1	0.27 ± 0.02 a	0.44 ± 0.01 a	0.52 ± 0.01 b	0.42 ± 0.01 a	0.30 ± 0.01 a	0.29 ± 0.02 a	0.45 ± 0.04 a
OA	18:1n9	38.11 ± 1.51 e	17.63 ± 0.23 b	25.42 ± 0.09 c	35.76 ± 1.16 de	31.30 ± 0.58 d	48.69 ± 0.87 f	45.26 ± 1.78 f
GA	20:1n9	1.11 ± 0.03 c	1.63 ± 0.08 cd	3.92 ± 0.05 f	1.17 ± 0.01 c	1.66 ± 0.04 cd	0.26 ± 0.00 a	1.56 ± 0.25 cd
DMA	22:1n6	0.30 ± 0.00 a	0.48 ± 0.02 ab	0.18 ± 0.01 a	0.49 ± 0.08 ab	1.50 ± 0.32 c	0.28 ± 0.04 a	ND
NRA	24:1n9	0.12 ± 0.02 a	ND	ND	ND	0.05 ± 0.00 a	ND	ND
MUFA	MUFA	42.67 ± 0.09 d	24.53 ± 0.02 b	37.40 ± 0.01 c	43.67 ± 0.08 d	38.93 ± 0.05 cd	51.45 ± 0.09 e	49.13 ± 0.50 e

Values are percentage of total fatty acid expressed as mean ± SD of four separate determinations. The values in the same row with different letters show statistically significant differences ($P < 0.05$)

ND not detected, *MUFA* monounsaturated fatty acids

Table 4 The polyunsaturated fatty acid (PUFAs) profiles and some parameters of some selected freshwater and marine fish species from different geographical locations of Turkey

Codes of FAMEs	Samples FAs	The freshwater fish species				The marine fish species			
		C.R.Trout	W.R. Trout	C.R. Trout	C.H.S. Bass	AB-1	AB-2	E. Pilchard	E. Anchovy
SDA	18:4n-3	0.96 ± 0.00 a	0.95 ± 0.00 a	0.77 ± 0.04 a	0.41 ± 0.03 a	2.49 ± 0.01 ab	1.23 ± 0.02 a	1.95 ± 0.04 ab	2.37 ± 0.09 ab
ESA	20:3n-3	0.40 ± 0.02 a	0.26 ± 0.01 a	0.30 ± 0.01 a	0.62 ± 0.02 a	0.22 ± 0.00 a	0.16 ± 0.01 a	0.20 ± 0.00 a	0.51 ± 0.04 a
EPA	20:5n-3	5.01 ± 0.05 b	5.08 ± 0.05 b	3.79 ± 0.07 a	3.24 ± 0.04 a	11.85 ± 0.06 d	5.05 ± 0.01 b	10.55 ± 0.03 d	10.40 ± 0.07 d
DPA	22:5n-3	2.03 ± 0.05 a	2.02 ± 0.08 a	1.60 ± 0.04 a	1.43 ± 0.01 a	1.98 ± 0.03 a	2.65 ± 0.16 b	2.57 ± 0.03 ab	1.83 ± 0.04 a
DHA	22:6n-3	13.07 ± 0.09 d	13.21 ± 0.11 d	11.76 ± 0.05 d	9.30 ± 0.04 c	31.70 ± 0.08 h	15.81 ± 0.07 de	26.00 ± 0.20 g	24.63 ± 0.55 g
n-3 PUFA	n3 PUFA	21.47 ± 0.05 d	21.52 ± 0.03 d	18.22 ± 0.02 c	15.00 ± 0.07 bc	48.24 ± 0.02 h	24.90 ± 0.11 e	41.27 ± 0.06 g	39.74 ± 0.10 g
HDDA	16:2n-6	0.28 ± 0.00 a	0.26 ± 0.02 a	0.21 ± 0.00 a	0.20 ± 0.02 a	0.22 ± 0.00 a	0.24 ± 0.01 a	0.38 ± 0.01 ab	0.33 ± 0.01 a
LO	18:2n-6	7.18 ± 0.05 c	6.98 ± 0.07 c	6.44 ± 0.04 c	9.39 ± 0.21 d	1.80 ± 0.01 a	2.18 ± 0.01 a	1.69 ± 0.02 a	1.79 ± 0.03 a
LnO	18:3n-6	1.34 ± 0.03 a	1.29 ± 0.04 a	1.12 ± 0.04 a	1.08 ± 0.04 a	1.67 ± 0.04 ab	1.05 ± 0.03 a	1.15 ± 0.04 a	1.30 ± 0.01 a
EDA	20:2n-6	0.29 ± 0.02 a	0.16 ± 0.00 a	0.14 ± 0.01 a	0.18 ± 0.00 a	0.09 ± 0.00 a	0.24 ± 0.01 a	0.33 ± 0.01 a	0.20 ± 0.00 a
ARA	20:4n-6	0.60 ± 0.04 a	0.54 ± 0.03 a	0.48 ± 0.00 a	0.34 ± 0.01 a	0.98 ± 0.01 a	0.63 ± 0.00 a	0.95 ± 0.03 a	1.21 ± 0.03 a
DGLA	20:3n-6	0.76 ± 0.05 a	0.64 ± 0.05 a	0.74 ± 0.03 a	0.40 ± 0.00 a	0.17 ± 0.00 a	0.82 ± 0.01 a	1.19 ± 0.00 a	0.82 ± 0.04 a
n-6 PUFA	n-6 PUFA	10.45 ± 0.02 c	9.69 ± 0.02 bc	9.13 ± 0.04 b	11.59 ± 0.04 c	4.93 ± 0.04 a	5.16 ± 0.05 a	5.69 ± 0.04 a	5.65 ± 0.04 a
PUFA	Total PUFA	31.92 ± 0.05 cd	31.21 ± 0.04 c	27.35 ± 0.04 bc	26.59 ± 0.50 b	53.17 ± 0.10 g	30.06 ± 0.17 c	46.96 ± 0.13 f	45.39 ± 0.21 f
n3n-6	n-3n-6	2.05 ± 0.02 b	2.22 ± 0.02 b	1.99 ± 0.01 b	1.29 ± 0.01 a	9.78 ± 0.03 f	4.82 ± 0.02 d	7.25 ± 0.02 e	7.03 ± 0.04 e
PUFA SFA	PUFA SFA	1.13 ± 0.02 a	1.05 ± 0.02 a	1.04 ± 0.04 a	1.16 ± 0.01 a	2.15 ± 0.02 b	0.98 ± 0.01 a	1.54 ± 0.01 ab	1.23 ± 0.04 a
DHA EPA	DHA EPA	2.61 ± 0.02 bc	2.60 ± 0.02 bc	3.10 ± 0.02 c	2.87 ± 0.00 c	2.67 ± 0.01 bc	3.13 ± 0.03 c	2.46 ± 0.01 b	2.37 ± 0.01 b
AI	AI	0.95 ± 0.01 a	0.78 ± 0.02 a	0.80 ± 0.02 a	0.68 ± 0.01 a	0.95 ± 0.02 a	1.03 ± 0.01 a	1.24 ± 0.00 a	1.58 ± 0.01 b
TI	TI	0.25 ± 0.00 a	0.23 ± 0.00 a	0.25 ± 0.01 a	0.23 ± 0.00 a	0.13 ± 0.00 a	0.25 ± 0.01 a	0.19 ± 0.01 a	0.22 ± 0.00 a

Codes of FAMEs	Samples FAs	The marine fish species						
		Mackarel	Bogue	Bluefish	W.S. Bream	C.S. Bream	Seabass	Whiting
SDA	18:4n-3	0.73 ± 0.02 a	1.37 ± 0.04 a	1.07 ± 0.04 a	1.11 ± 0.03 a	0.87 ± 0.05 a	0.93 ± 0.11 a	ND
ESA	20:3n-3	0.52 ± 0.02 a	0.52 ± 0.05 a	0.39 ± 0.02 a	0.33 ± 0.01 a	0.40 ± 0.03 a	0.31 ± 0.01 a	1.33 ± 0.50 a
EPA	20:5n-3	2.81 ± 0.14 a	4.53 ± 0.36 ab	8.88 ± 0.63 c	2.90 ± 0.10 a	3.98 ± 0.37 a	3.87 ± 0.25 a	4.56 ± 0.84 ab
DPA	22:5n-3	2.10 ± 0.13 a	3.54 ± 0.10 b	2.44 ± 0.04 ab	2.45 ± 0.08 ab	2.13 ± 0.21 a	1.65 ± 0.14 a	2.42 ± 0.43 ab
DHA	22:6n-3	11.10 ± 0.10 d	20.66 ± 0.02 e	10.61 ± 0.88 d	6.82 ± 0.10 b	7.07 ± 0.12 b	2.08 ± 0.09 a	11.29 ± 0.57 d
n-3 PUFA	n3 PUFA	17.26 ± 0.04 c	30.62 ± 0.13 f	23.39 ± 0.14 de	13.61 ± 0.10 b	14.45 ± 0.04 b	8.84 ± 0.11 a	19.60 ± 1.10 d
HDDA	16:2n-6	0.22 ± 0.02 a	0.37 ± 0.01 ab	0.35 ± 0.01 ab	0.44 ± 0.01 b	0.22 ± 0.01 a	0.39 ± 0.08 ab	ND
LO	18:2n-6	4.73 ± 0.08 b	1.72 ± 0.08 a	1.92 ± 0.01 a	8.02 ± 0.58 d	12.45 ± 0.21 e	6.20 ± 0.24 c	4.40 ± 1.20 b
LnO	18:3n-6	0.51 ± 0.02 a	1.00 ± 0.03 a	1.08 ± 0.01 a	0.80 ± 0.04 a	2.83 ± 0.15 b	1.08 ± 0.04 a	1.03 ± 0.90 a
EDA	20:2n-6	0.20 ± 0.00 a	0.16 ± 0.01 a	0.31 ± 0.01 a	0.28 ± 0.01 a	0.24 ± 0.02 a	0.49 ± 0.04 a	ND
ARA	20:4n-6	1.20 ± 0.05 a	1.65 ± 0.03 ab	0.48 ± 0.02 a	2.64 ± 0.08 b	0.78 ± 0.04 a	3.40 ± 0.02 bc	ND
DGLA	20:3n-6	0.78 ± 0.04 a	0.89 ± 0.03 a	0.80 ± 0.01 a	0.39 ± 0.03 a	0.88 ± 0.03 a	0.52 ± 0.00 a	ND

Table 4 continued

Codes of FAMEs	Samples FAs	The marine fish species						
		Mackarel	Bogue	Bluefish	W.S. Bream	C.S. Bream	Seabass	Whiting
n-6 PUFA	n-6 PUFA	7.64 ± 0.03 b	5.79 ± 0.12 a	4.94 ± 0.05 a	12.57 ± 0.04 d	17.40 ± 0.04 e	12.08 ± 0.04 d	5.43 ± 0.56
PUFA	Total PUFA	24.90 ± 0.05 b	36.41 ± 0.05 e	28.33 ± 0.31 bc	26.18 ± 0.09 b	31.85 ± 0.10 cd	20.92 ± 0.10 a	25.03 ± 0.00 b
n3n-6	n-3n-6	2.26 ± 0.02 b	5.29 ± 0.01 d	4.73 ± 0.01 cd	1.08 ± 0.01 a	0.83 ± 0.01 a	0.73 ± 0.00 a	3.61 ± 0.60 c
PUFA SFA	PUFA SFA	0.90 ± 0.03 a	1.25 ± 0.01 a	0.90 ± 0.01 a	0.79 ± 0.01 a	1.24 ± 0.03 a	0.90 ± 0.01 a	1.19 ± 0.04 a
DHA EPA	DHA EPA	3.95 ± 0.03 d	4.56 ± 0.04 d	1.19 ± 0.01 a	2.35 ± 0.02 b	1.78 ± 0.01 ab	0.54 ± 0.00 a	2.48 ± 0.03 bc
AI	AI	0.77 ± 0.00 a	1.03 ± 0.03 a	1.05 ± 0.02 a	0.93 ± 0.02 a	0.72 ± 0.01 a	0.50 ± 0.00 a	0.50 ± 0.02 a
TI	TI	0.24 ± 0.01 a	0.21 ± 0.02 a	0.27 ± 0.00 a	0.35 ± 0.01 a	0.17 ± 0.00 a	0.25 ± 0.00 a	0.18 ± 0.00 a

Values are percentage of total fatty acid expressed as mean ± SD of four separate determinations. The values in the same row with different letters show stastistically significant differences ($P < 0.05$)

ND not detected, *SFA* saturated fatty acids, *PUFA* polyunsaturated fatty acids

bass), and 2.15 (Atlantic bonito-1) respectively. In contrast, the lowest values were for wild rain trout (1.05) and (wild sea bream (0.79), respectively. The results on PUFA/SFA ratio were accordance with to the findings of Zuraini et al. [23].

With respect to the analysis, total *trans* fatty acids (TFAs) levels of freshwater fish species were 0.92–1.27% and the highest level of elaidic acid (C18:1*t* n9) was determined in cultured hybrid striped bass as 0.21% (Table 5). Total TFAs contents of marine fish ranged from 0.90% to 2.38% and the highest level of elaidic acid (C18:1*t* n9) was in Atlantic mackarel as 0.62%. In fish, the total amount of TFA was below 2% [27]. The TFAs of Turkish fish species were lower than findings of Luzia et al. [17], who found that elaidic acid and linoeladic acid (C18:2 *t*) ranged from 0.90–17.0% and 0.23–8.95%, respectively.

Ulbricht and Southgate [11] proposed that an atherogenicity index (AI) for the composition of a fat based on current information about the effect of various fatty acids on serum cholesterol that is low-and high-density lipoprotein concentrations. According to this equation, only saturated fatty acids with chain lengths of 12–16 are atherogenic and myristic acid is considered four times more atherogenic than the other two. All unsaturated fatty acids, regardless of their double bond number, position, or configuration, are considered equally effective in decreasing atherogenicity. In the present study, the change of atherogenicity index (AI) values for freshwater and marine fish species were 0.68 (cultured hybrid striped bass) −0.95 (cultured rainbow trout) and 0.50 (sea bass and whiting) −1.58 (anchovy) respectively, whereas the change of thrombogenicity index (IT) values for freshwater and marine fish species were between 0.23 (cultured hybrid striped bass) −0.25 (cultured rainbow trout), and 0.13 (Atlantic bonito-1) −0.35 (wild sea bream) respectively (Table 4). Valfre et al. [28] reported that IT and AI values were 0.45 and 1.35 for anchovy, 0.32 and 0.94 for eel, 0.37 and 0.57 for rainbow trout, and 0.25 and 0.45 for seabass.

There was a wide variation and significant ($P < 0.05$) differences among the fatty acid profiles, total SFAs, MUFAs, PUFAs, EPA, DHA, DHA/EPA, TFAs, total n-3 PUFAs, total n-6 PUFAs and n-3/n-6, among freshwater and marine fish samples (Table 2, 3, 4 and 5). Differences in fatty acids of freshwater and marine fishes may originate from many factors such as species (herbivorous, omnivorous or carnivorous), sex, sexual maturity, size, age, reproductive status of fish, geographical location of the catch, water temperature, salinity, feeding and season [4, 29–32].

Based on the chemometric analysis (PCA), it was observed that stearic acid (SA), palmitoleic acid, DHA/EPA, myristic acid, stearidonic acid, DPA (C22:5n3),

Table 5 The *trans* fatty acid (TFAs) profiles of some selected freshwater and marine fish species from different geographical locations of Turkey

Codes of FAMEs	Samples FAs	The freshwater fish species				The marine fish species			
		C.R. Trout	W.R. Trout	C.R. Trout	C.H.S.Bass	AB-1	AB-2	E. Pilchard	E. Anchovy
14:1 *t*	0.03 ± 0.00 a	0.04 ± 0.00 a	0.03 ± 0.00 a	ND	0.03 ± 0.00 a	0.03 ± 0.00 a	0.04 ± 0.00 a	0.06 ± 0.00 a	ND
PAO *t*	16:1 *t*	0.43 ± 0.00 a	0.47 ± 0.00 a	0.35 ± 0.04 a	0.33 ± 0.00 a	0.54 ± 0.03 a	0.51 ± 0.02 a	1.00 ± 0.01 a	1.11 ± 0.05 b
EA	18:1 *t*	0.13 ± 0.02 a	0.12 ± 0.02 a	0.07 ± 0.00 a	0.21 ± 0.01 a	0.09 ± 0.04 a	0.08 ± 0.00 a	0.18 ± 0.00 a	0.10 ± 0.04 a
t-PUFA	18:2 *t* + 18:3 *t*	0.68 ± 0.04 ab	0.58 ± 0.02 ab	0.47 ± 0.04 a	0.56 ± 0.10 a	0.24 ± 0.04 a	0.43 ± 0.05 a	0.52 ± 0.08 a	1.11 ± 0.07 b
TFA	Total TFAs	1.27 ± 0.01 a	1.21 ± 0.01 a	0.92 ± 0.04 a	1.10 ± 0.06 a	0.90 ± 0.04 a	1.05 ± 0.04 a	1.74 ± 0.02 ab	2.38 ± 0.02 b

Codes of FAMEs	Samples FAs	The marine fish species						
		Mackarel	Bogue	Bluefish	W.S. Bream	C.S. Bream	Seabass	Whiting
14:1 *t*	0.03 ± 0.00 a	0.03 ± 0.00 a	0.02 ± 0.00 a	ND	0.01 ± 0.00 a	ND	ND	ND
PAO *t*	16:1 *t*	ND	1.27 ± 0.03 b	0.62 ± 0.05 a	0.42 ± 0.04 a	0.39 ± 0.04 a	0.12 ± 0.01 a	ND
EA	18:1 *t*	0.62 ± 0.04 b	0.17 ± 0.03 a	0.12 ± 0.01 a	0.33 ± 0.01 a	0.10 ± 0.01 a	0.33 ± 0.02 a	ND
t-PUFA	18:2 *t* + 18:3 *t*	0.38 ± 0.05 a	0.83 ± 0.04 b	0.35 ± 0.01 a	0.74 ± 0.05 ab	0.91 ± 0.08 b	0.78 ± 0.03 ab	ND
TFA	Total TFAs	1.00 ± 0.01 a	2.30 ± 0.02 b	1.11 ± 0.02 a	1.49 ± 0.02 ab	1.41 ± 0.01 ab	1.23 ± 0.03 a	ND

Values are percentage of total fatty acid expressed as mean ± SD of four separate determinations. The values in the same row with different letters show stastistically significant differences ($P < 0.05$)

ND not detected, *TFA* total trans fatty acids

arachidonic acid, linolenic acid and other parameters were used as features in classification of E. pilchard (Figs. 2, 3). The n-3/n-6 ratio and EPA content played a role in discrimination of E. anchovy. The linoleic acid and n6-PUFAs levels were responsible in classification of Atlantic bonito −2. The bogue fish from Aegean was characterized with palmitic acid. The DHA, n3-PUFAs, total PUFAs aided in characterization of Atlantic Bonito-1, cultured rainbow trout-1, bluefish, wild rainbow trout, cultured sea bream and wild sea bream. The SFA level was discriminative in classification of Mackarel, cultured hybrid stripped bass and cultured rainbow trout-2. The whiting and seabass from Aegean Sea were characterized with oleic acid and MUFAs. The variance levels explained by the PCA of fish samples were 87.03 and 11.46% for PC1 and PC2, respectively. The values for fatty acid profiles were determined as 87.58 and 5.91% for PC1 and PC2, respectively.

In this study, Turkish fish species from different geographical locations were classified by chemometric methods. As seen in Fig. 4, the dendogram on the HCA results (Euclidian method) of economically important Turkish fish species showed to be separated into five groups based on their fatty acid profiles. Cultured and wild rainbow trout (Salihli, Manisa), Atlantic bonito-2 (Black Sea), and bluefish (Marmara) were grouped (group 1). Group 2 consisted of only bogue (Aegean Sea) while group 3 consisted of cultured rainbow trout (Bozdogan, Aydin), mackarel (Aegean Sea), cultured hybrid stripped bass (Bozdogan, Aydin), wild sea bream (Aegean Sea), and cultured sea bream (Aegean Sea). Sea bass and whiting (Aegean Sea) composed group 4 whereas Atlantic bonito-1 (Marmara Sea), E. pilchard (Aegean Sea) and E. anchovy (Black Sea) made up group 5.

As seen in Fig. 5, 21 fatty acids and 9 chemical parametres were used to identity of fish species from different locations of Turkey. Long chain FAs (especially MUFAs, PUFAs and their parameters) were classified in same groups. The EPA and 3/n6 ratio was grouped together with n-6 PUFAs and linoleic acid in same cluster. The DHA, palmitic acid, SFA, PUFAs, n-3PUFA, oleic acid and MUFAs were classified in the right cluster. DPA (C 22:5n3), from characteristics PUFAs in fishes, appeared in the right-hand cluster together with DHA/EPA, GA, ARA, TFA, SDA PUFA/SFA. Linolenic acid was classified in middle whereas MUFAs and oleic acid grouped to the left of dendogram.

Conclusions

Although there are a differences in FA profiles of freshwater and marine fish species, this study showed that fatty

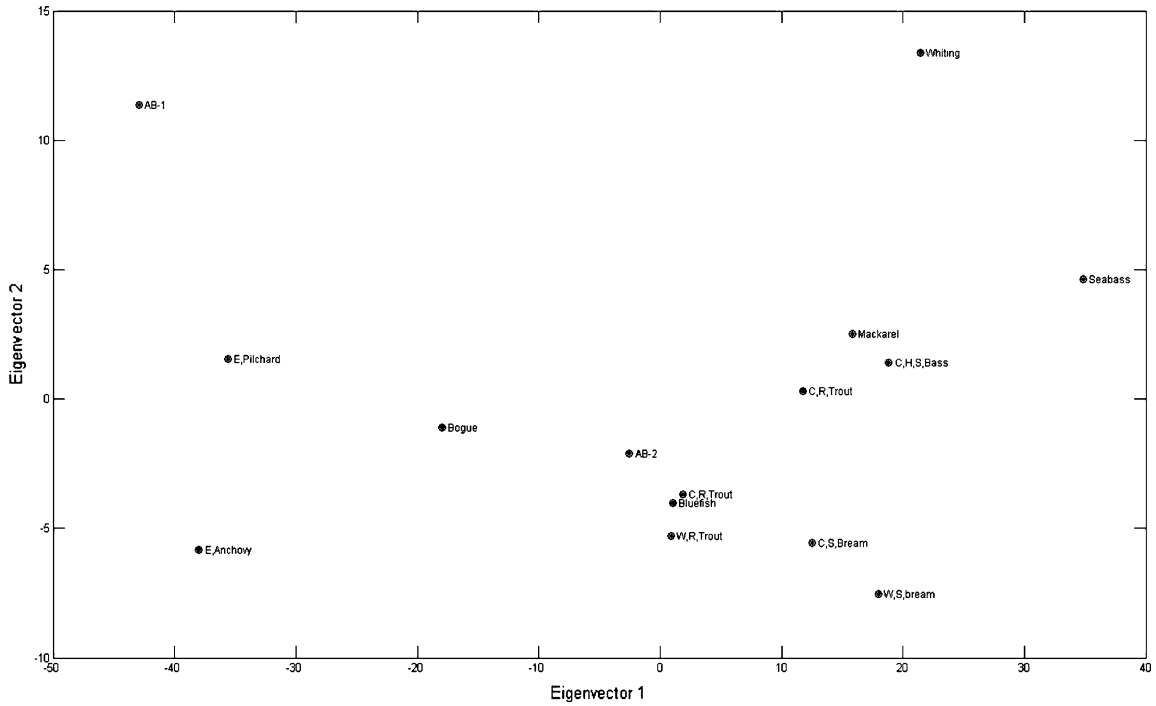

Fig. 2 The Principal Component Analysis (PCA) results concerning the distribution of fish species from different geographical locations of Turkey based on their fatty acid profiles

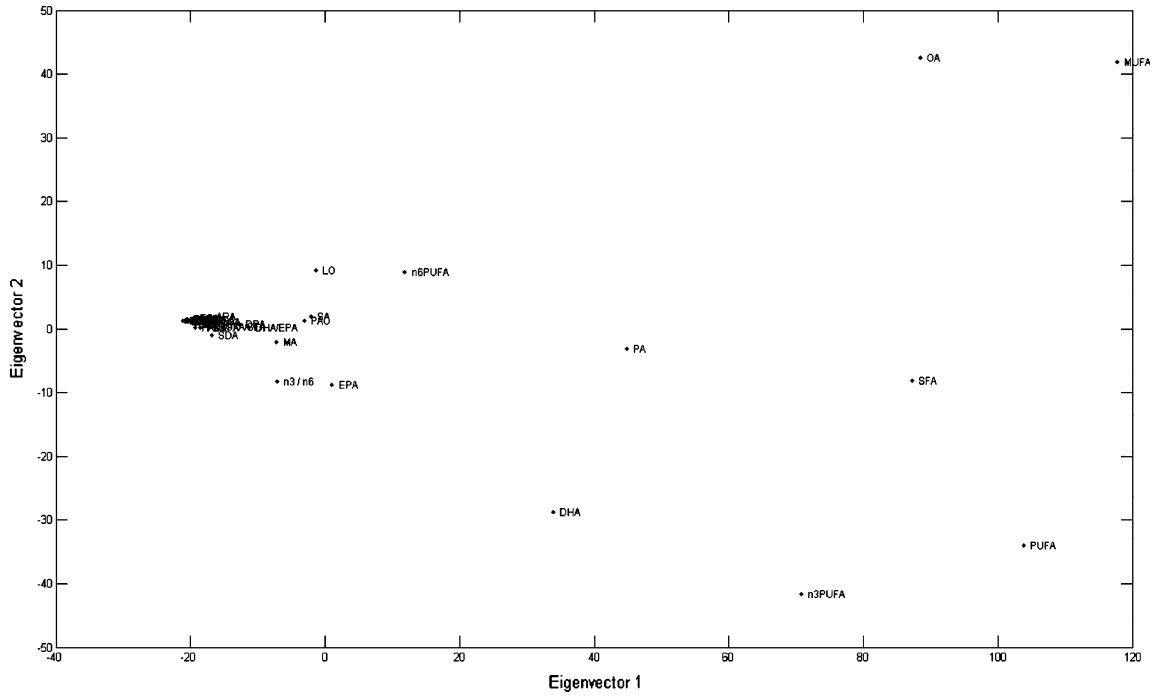

Fig. 3 The Principal Component Analysis (PCA) results concerning the distribution of the fatty acid profiles of fish species from different geographical locations of Turkey

acid profiles of freshwater fish are comparable to those of seawater fish as sources of PUFAs, especially EPA and DHA. It showed that some cheap marine fish species (Atlantic bonito, anchovy, pilchard and bogue) by commonly consumed in Turkey are better dietary sources of n-3 PUFAs than more expensive species (bluefish, Atlantic

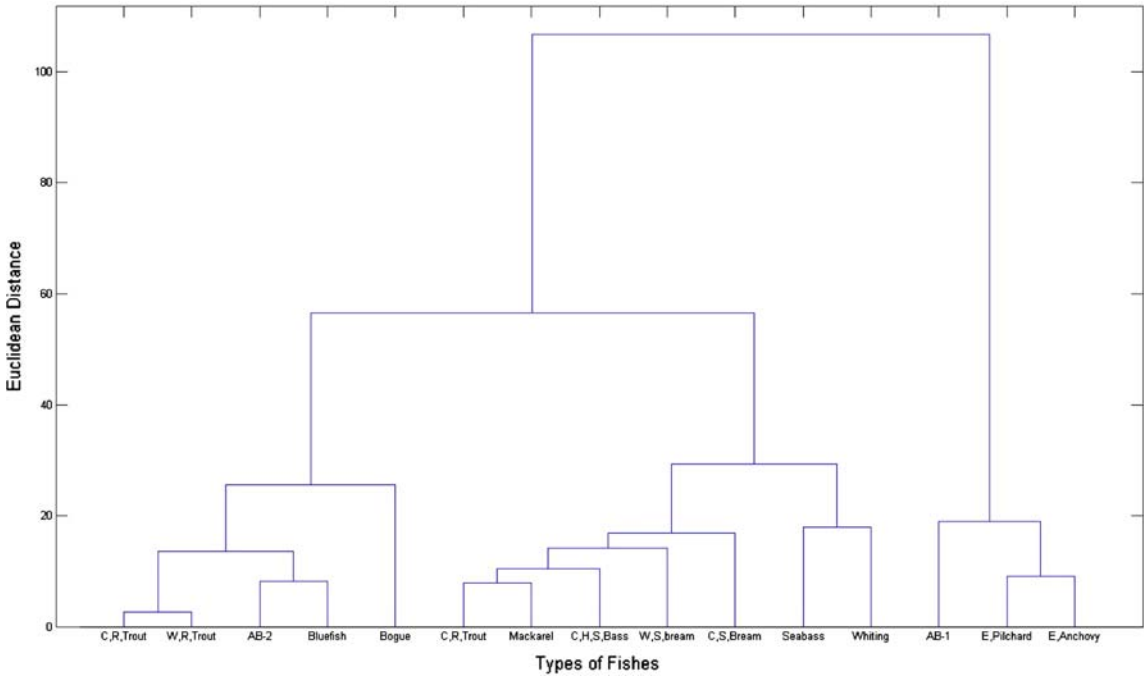

Fig. 4 The dendogram on Hierarchical Cluster Analysis (HCA) results concerning the classification of fish species from different geographical locations of Turkey based on their fatty acid profiles

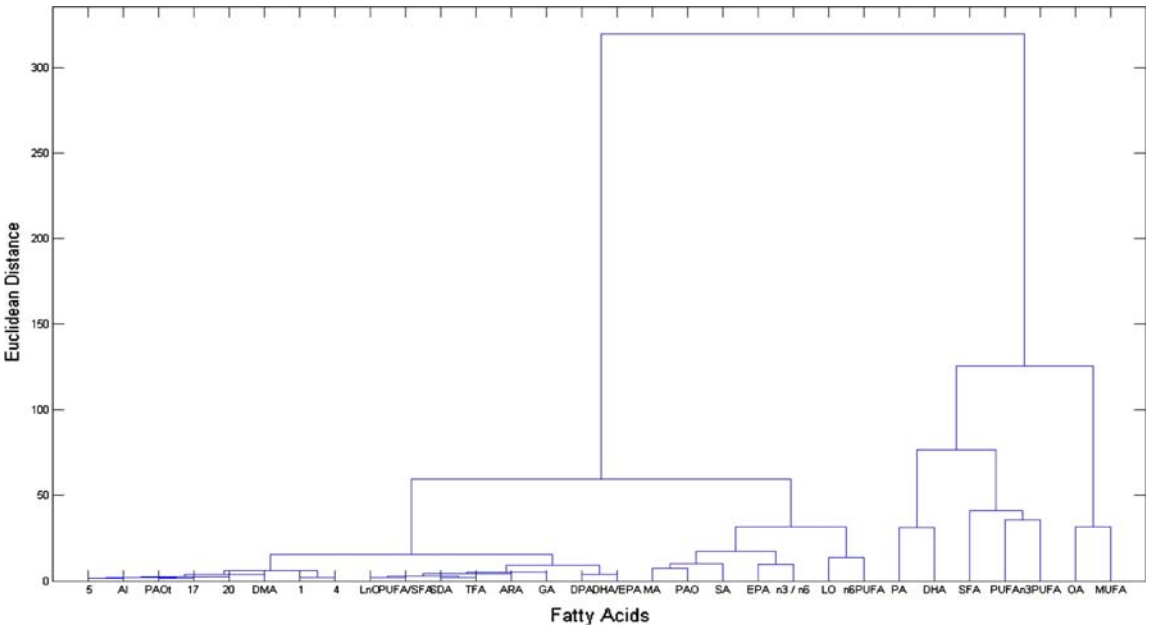

Fig. 5 The dendogram on HCA results concerning the distribution of the fatty acid profiles of fish species from different geographical locations of Turkey

mackerel, sea bream and sea bass). Additionally, consumers must be made aware that sometimes there is no relation between price and nutritional value of fish species. More detailed data on the relationship between price and nutritional value of fish species in Turkey must be taken into consideration. In addition, the information presented in this study may be valuable for the pharmaceutical and food industries in the selection of marine and freshwater fish oils for chemical studies.

As a result, this study shows that the FA profiles could be used for identification and characterization of fish species by chemometric analysis (PCA and HCA). The present

study is a step towards the subjective characterization and classification of economically important some fish species for utilization in the Turkish food and aquaculture industry.

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