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Proximate (lipid, moisture, ash) levels and fatty acid profiles in edible parts of common cuttlefish (*Sepia officinalis*, L., 1758) from different geographical areas

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Proximate (lipid, moisture, ash) levels and fatty acid compositions of common cuttlefish (*Sepia officinalis*) from different geographical areas along the Eastern Mediterranean coasts of Turkey (Fethiye, Antalya, Gazipasa, Anamur, and Iskenderun) were evaluated. The average total saturated fatty acids (SFA) ranged from 30.5% to 33.2%. The major SFA components were C16:0 (ranging from 18.2 to 20.74%) and C18:0 (ranging from 7.46 to 8.70%) for all sampling points. The average levels of mono unsaturated fatty acids (MUFA) of specimens from Iskenderun, Fethiye, Antalya, Gazipasa, and Anamur were calculated to be 12.17%, 12.05%, 10.75%, 10.32%, and 10.05%, respectively. Among major fatty acids of the MUFA, the highest and lowest levels of C18:1n9 were calculated to be 4.26% in Fethiye and Anamur, respectively. Polyunsaturated fatty acids (PUFA) level from all of the mantle part of common cuttlefish was calculated to be higher than 55%. The highest amounts of arachidonic acid (ARA, C20:4n6), eicosapentaenoic acid (EPA, C20:5n3), and docosahexaenoic acid (DHA, C22:6n3) were observed in Anamur, Fethiye, Gazipasa with the values of 6.76%, 16.57%, and 29.69%, respectively. It was concluded that the proximate (lipid, moisture, ash) levels and fatty acid components of the cuttlefish had been affected from different geographical areas and have high quality unsaturated fatty acids (e.g., PUFA, DHA, EPA) which are described as essential fatty acids required for a healthy life.

Keywords: *Sepia officinalis*, Mediterranean, fatty acid, DHA, EPA

INTRODUCTION

European common cuttlefish is the best known cephalopod native in all European and Mediterranean waters [1]. Cephalopods are not only very valuable food items for human beings but also very important nutritional source for the predators in the sea. Common cuttlefish is the mainly fished cephalopod in the Mediterranean [2,3].

Compared to other sea creatures, common cuttlefish has a relatively short life span around two years. They have the ability to grow fast because of their carnivore diet and metabolic activity [4]. Mature common cuttlefish die after spawning, generally in spring (Boletzky 1983; Dunn 1999 cited in [14]).

Of marine invertebrates, common cuttlefish is considered to be one of the commercially important fish with high prices in Turkish coastal waters, as well as all around the world. According to reports of Fisheries statistics [5], approximately 1.163, 1.396, and 1.244 tons of cuttlefish were captured in Turkey in 2011, 2012, and 2013, respectively.

Nowadays, because common cuttlefish contain the omega 3 fatty acids such as C20:5n3 (EPA) and C22:6n3 (DHA), they are appreciated for their many beneficial health effects.

Fish lipids are constituents of different lipid classes such as phospholipids, partial glycerides, free fatty acids, and esters. They are found in fish and in other sea products including shrimp, octopus, squids, and cuttlefish. Previous research has pointed out that cuttlefish is low in fat. Major part of the lipids is a valuable source of essential fatty acids. Although there are a few research studies on cuttlefish [6-10], there exists very limited information about the biochemical characteristics of cuttlefish from different geographical areas along coasts of the Mediterranean. The objective of the present study is to investigate the possible effects of different geographical areas along the coasts of eastern Mediterranean on lipid contents, moisture amounts, ash levels, and fatty acid compositions of common cuttlefish (*Sepia officinalis*).

EXPERIMENTAL

Sample collection

Five different coasts which are also important bays (Fethiye, Antalya, Gazipasa, Anamur, and Iskenderun) along Eastern Mediterranean in Turkey (Figure 1) were selected to obtain cuttlefish for the present study.

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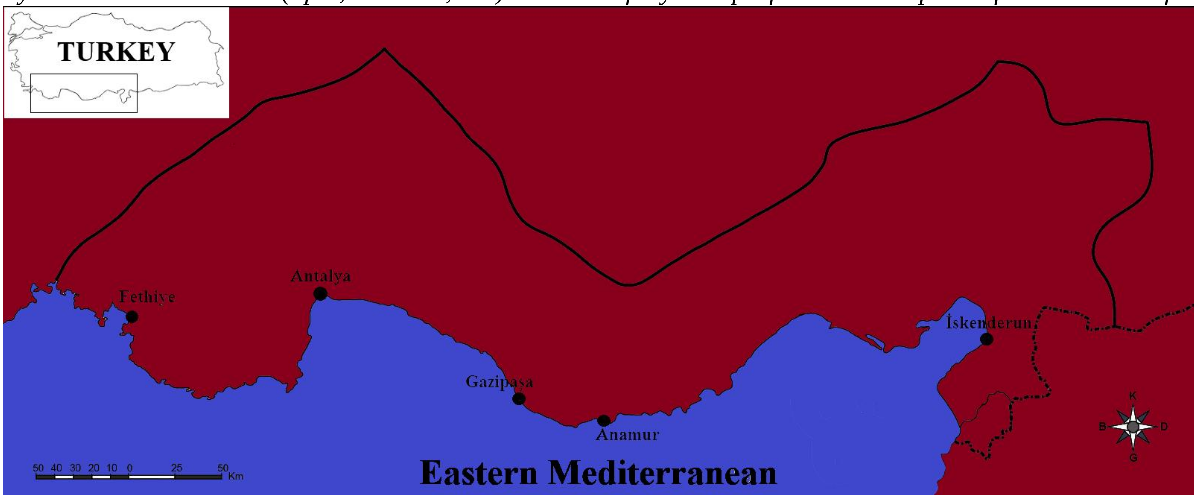

Figure 1. Sampling area (Fethiye, Antalya, Gazipasa, Anamur, and Iskenderun) along the Eastern Mediterranean.

Of a total of 50 samples used in the present study, only five randomly selected samples from each coast were measured morphometrically, the measurements of which are shown in Table 1. The common cuttlefish are highly captured and the most frequently consumed fish in the selected areas.The fish were bought from fish markets in April 2014 on the same day when they were caught, placed on ice, and immediately transferred to the laboratory. The mantle parts (the main edible portion) were removed for the analyses right after body measurements. The mantle length (ML) was measured with a digital Caliper (0.01mm, Mitutoyo/Japan). The body weights were determined with a precision close to 0.01.

Moisture and ash contents

Moisture and ash contents of the cuttlefish were determined according to the recommended method (Commission of European Communities) ISOR 1442 (CEC, 1979) [20] and AOAC 35.1.14 (2003) [21] method no 938.08, respectively.

Lipid extraction, fatty acid methyl esters (FAME) preparation and column conditions

Lipid extraction was carried out according to the modified Bligh and Dyer method [11]. A detailed information about lipid extraction is given in Özyılmaz and Öksüz (2015) [19]. The conversion and separation of FAMEs was performed as described in Ozyilmaz (2016) [12]. Fatty acid profiles of cuttlefish from Fethiye, Antalya, Gazipaşa, Anamur, and Iskenderun were determined by GC-MS (gas chromatography-mass spectrometry) using a Hewlett Packard GC (model 6890) and coupled with Hewlett Packard (model 5972A, HP 6890 system) MS detector. Separations of fatty acids were achieved with an HP-INNOWAX. Polyethylene glycol capillary column model number: HP 19091N-136, nominal length: 60.0 m, nominal diameter: 250 µm, nominal film thickness: 0.25 µm) was used in this experiment. Split flow was maintained at 9.9 ml/min; total flow: 13.9 ml/min helium. The separation was performed using helium as carrier gas. The injector was maintained at 270°C with an injection volume of 1 µL in splitless mode with a total injection volume of 1 µl. The injector was washed three times with iso-octane post-injection, the injector program was also set to triple-wash before next injection. The initial column temperature was set at 120°C and held for 3 min, and then increased to 180°C with a 10°C per min ramp rate and held at this temperature for ten min. Then the temperature was increased again to 250°C with a 10°C per min ramp rate and held at this temperature for 17 min. The separation of the total fatty acids took a total of 43 min.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's test. Significance was established at $P<0.05$.

RESULTS AND DISCUSSION

The results of the morphological measurements and lipid analyses are presented in Table 1. The average mantle weight of cuttlefish from Fethiye, Antalya, Gazipasa, Anamur, and Iskenderun which are along the coasts of Eastern Mediterranean varied from 258.80 to 72.40 g. The average mantle length of cuttlefish from Fethiye, Antalya, Gazipaşa, Anamur, and Iskenderun was 14.10, 12.02, 8.10, 11.38, and 12.00 cm, respectively.

Table 1. Morphological measurements and proximate contents of the cuttlefish

Locations	Mantle Length (cm)	Body Weight (g)	Lipid (%)	Ash(%)	Moisture(%)
Fethiye	14.10±1.56	258.80±71.02	1.38±0.01 [a]	1.60±0.06 [a]	75.42±0.72 [a]
Antalya	12.02±3.12	204.60±78.85	1.82±0.07 [b]	1.50±0.04 [a]	76.18±1.66 [b,c]
Gazipaşa	8.10±1.60	72.40±49.80	1.80±0.14 [b]	1.56±0.07 [a]	75.23±1.53 [b,c]
Anamur	11.38±2.21	169.00±49.95	1.70±0.05 [b]	1.76±0.08 [a]	77.28±1.02 [c]
İskenderun	12.00±0.80	178.00±27.43	0.89±0.09 [c]	1.64±0.06 [a]	75.82±0.34 [a,b]

Data represent means± standard deviation (n=5 for mantle length and body weight; n=3 for lipid, moisture, and ash)
Means followed by different letters within the same row are significantly different (P<0.05).

Previous research set a standard on the age of cuttlefish. For example, 8.7 cm and 10.0 cm male and female cuttlefish from Antalya can be considered as adult cuttlefish [13, 14]. The standard sexual maturity lengths were calculated to be 12.04 cm for females and 10.30 cm for males in Iskenderun. Based on these standards set in previous studies, all cuttlefish collected from all sampling points in this study can be considered as adults.

The average moisture and ash contents of all sampling points (cuttlefish from Fethiye, Antalya, Gazipaşa, Anamur, and Iskenderun) were measured to be between 75.42±0.72% and 77.28±1.02% and between 1.50±0.04% and 1.76±0.08%, respectively (Table 1). The differences in the moisture contents of the cuttlefish were found to be statistically significant (P<0.05), except for ash contents (P>0.05). Similar findings regarding moisture and ash contents were reported by Ayas *et al.* (2012) [15] for male and female cuttlefish from Mersin in winter and spring.

Even though there seem to be slight differences among lipid levels, these differences were found to be statistically significant for cuttlefish collected from all five sampling points in terms of lipid levels regarding sampling points differences (P<0.05) in the present study. The mean total lipid contents of the cuttlefish's mantle from five different sampling points ranged from 1.82% to 0.89%. According to Ackman (1989) [16], fish having lipid lower than 2% can be classified as lean fish. Accordingly, all cuttlefish caught from 5 different sampling points in the current study can be considered as lean fish. On the other hand, Tir *et al*. (2015) [10] reported higher lipid levels for the same species from a different part of the Mediterranean (Rades, located in northern Tunisia). Fatty acid profiles (% of total fatty acids) of cuttlefish from the five sampling points are presented in Table 2. The fatty acids of cuttlefish from each sampling point were in the range of 30.48-33.21% for saturated fatty acids (SFA), 10.05-12.17% for monounsaturated fatty acids (MUFA), and 55.23-58.14% for polyunsaturated fatty acids (PUFA). The percentages of PUFA in cuttlefish from all sampling points were calculated to be higher than the sum of the average levels of SFA and MUFA.

In all cuttlefish's fatty acid total amount, the highest proportions of two fatty acids were palmitic acid (C16:0, 18.20-20.74%), stearic acid (C18:0, 7.46-8.70%) in SFA, oleic acid, and 20:1n9 in MUFA. Additionally, the highest amounts of three fatty acids in cuttlefish were ARA, EPA, and DHA in PUFA. Regarding the highest levels of fatty acids in SFA, MUFA, and PUFA, the results in this study are in agreement with previously reported studies on cuttlefish fatty acids of cephalopods [6-10].

The cuttlefish from all sampling points were found to be rich in PUFA, which is in accordance with the previously reported values of PUFA [6-10]. Although the sums of PUFA contents of cuttlefish from five different sampling points differed from each other, especially the percentages of arachidonic acid (ARA, C20:4n6), eicosapentaenoic acid (EPA, C20:5n3), and docosahexaenoic acid (DHA, C22:6n3) were found to be very close to each other. The DHA levels of cuttlefish from Gazipasa and the EPA levels of cuttlefish from Gazipasa and Anamur statistically differed from all other sampling points (P<0.05). The levels of ARA in cuttlefish from all sampling points significantly differed from each other (P<0.05).

Ozogul *et al*. (2008) [8] studied cuttlefish from eastern Mediterranean in an area close to the sampling area of the present study in Iskenderun. Accordingly, seasonal changes of fatty acid compositions of cuttlefish along with other cephalopods data regarding levels of DHA were reported to be higher than those of DHA in the current study. Additionally, fatty acid level (in spring 29.5%, DHA) of cuttlefish reported by Ozyurt *et al.* (2006) [7] was higher than that of cuttlefish reported in the present study, except for cuttlefish from Gazipasa which was very close to the findings presented here. DHA levels of cuttlefish from all sampling points were calculated to be higher than 27%. Ozogul *et al*. (2009) [17] investigated fatty acid profiles of 34 marine water fish species from northeast Mediterranean.

Table 2. Fatty acids profiles (% of total fatty acids) of cuttlefish from five locations (Fethiye, Antalya, Gazipaşa, Anamur, and Iskenderun)

	Locations				
Fatty Acids	Fethiye	Antalya	Gazipaşa	Anamur	İskenderun
C14:0	1.72±0.01^{a}	1.68±0.05^{a}	1.43±0.03^{b}	1.15±0.00^{c}	1.17±0.02^{c}
C15:0	0.89±0.02^{a}	1.08±0.01^{b}	0.66±0.01^{c}	0.53±0.01^{d}	0.70±0.00^{e}
C16:0	19.92±0.67^{ab}	20.62±0.51^{b}	20.74±0.18^{c}	19.41±0.12^{d}	18.20±0.05^{c}
C17:0	1.57±0.01^{a}	1.71±0.04^{b}	1.37±0.01^{c}	1.16±0.02^{a}	1.71±0.06^{b}
C18:0	7.46±0.19^{a}	7.75±0.11^{b}	8.59±0.07^{c}	7.92±0.02^{b}	8.70±0.09^{c}
C20:0	0.42±0.10^{a}	0.36±0.01^{ab}	0.22±0.11^{b}	0.33±0.02^{ab}	0.49±0.08^{a}
Total SFA	31.98	33.21	33.00	30.48	30.97
C15:1	ND	0.22±0.01^{a}	0.08±0.11^{b}	0.21±0.04^{a}	0.20±0.05^{a}
C16:1n9	0.75±0.01^{ac}	0.77±0.02^{b}	0.27±0.27^{b}	0.56±0.00^{a}	0.69±0.06^{c}
C16:1n7	0.24±0.01^{ac}	0.21±0.01^{ac}	0.06±0.08^{b}	0.17±0.02^{a}	0.28±0.01^{c}
C17:1	0.42±0.20^{a}	0.42±0.23^{a}	0.25±0.04^{a}	0.45±0.08^{a}	0.65±0.14^{a}
C18:1n9	4.26±0.12^{a}	3.66±0.14c^{b}	3.49±0.03^{c}	3.05±0.08^{d}	3.75±0.06^{b}
C18:1n7	2.12±0.24^{ab}	1.92±0.14^{ac}	1.62±0.01^{c}	1.81±0.05^{ac}	2.34±0.00^{a}
C20:1n9	3.52±0.09^{a}	2.97±0.00^{b}	4.21±0.04^{c}	3.17±0.02^{d}	3.48±0.03^{a}
C22:1n9	0.69±0.04^{a}	0.48±0.08^{b}	ND	0.34±0.11^{b}	0.78±0.10^{a}
C24:1n9	0.06±0.08^{a}	0.08±0.12^{ab}	0.36±0.06^{a}	0.32±0.13^{b}	ND
Total MUFA	12.05	10.75	10.32	10.05	12.17
C16:2n4	0.24±0.01b^{a}	0.26±0.06b^{a}	0.14±0.00^{b}	0.19±0.01^{bc}	0.28±0.00^{a}
C16:3n4	0.28±0.13^{ab}	0.31±0.01^{b}	0.15±0.01^{a}	0.14±0.01^{a}	0.24±0.03^{ab}
C16:4n1	1.21±0.18^{a}	1.79±0.02^{b}	1.33±0.08^{ac}	2.14±0.03^{a}	1.60±0.16^{cb}
C18:2n6	0.67±0.21^{ab}	0.66±0.16^{ab}	0.35±0.01^{c}	0.52±0.11^{c}	0.84±0.24^{b}
C18:3n6	0.46±0.29^{a}	0.38±0.13^{a}	0.25±0.01^{a}	0.22±0.01^{a}	0.52±0.19^{a}
C20:3n6	0.53±0.08^{a}	0.52±0.05^{a}	0.50±0.01^{a}	0.55±0.06^{a}	0.58±0.05^{a}
C20:4n6	3.94±0.19^{a}	3.67±0.06^{b}	5.66±0.08^{c}	6.76±0.05^{d}	4.78±0.06^{e}
C22:2n6	ND	0.14±0.21^{a}	0.12±0.16^{a}	0.32±0.01^{a}	ND
C22:4n6	0.32±0.01^{a}	0.40±0.07^{a}	0.61±0.04^{b}	0.55±0.03^{b}	0.40±0.03^{a}
C22:5n6	0.78±0.03^{a}	0.87±0.01^{b}	1.06±0.12^{b}	1.60±0.02^{c}	0.81±0.01^{b}
C18:3n3	0.21±0.01^{a}	0.22±0.01^{a}	0.19±0.01^{a}	0.17±0.08^{a}	0.20±0.02^{a}
C18:4n3	0.19±0.27^{a}	0.08±0.11^{a}	ND	0.20±0.01^{a}	ND
C20:3n3	0.56±0.01^{a}	0.64±0.03^{a}	0.54±0.05^{a}	0.52±0.06^{a}	0.57±0.00^{a}
C20:5n3	16.57±0.68^{a}	16.13±0.41^{a}	13.42±0.25^{b}	14.25±0.21^{b}	15.61±0.10^{a}
C22:5n3	1.38±0.03^{a}	1.46±0.06^{a}	1.63±0.04^{b}	1.85±0.05^{c}	1.23±0.01^{d}
C22:6n3	27.90±0.17^{a}	27.70±0.47^{a}	29.69±0.84^{b}	28.20±0.18^{a}	27.62±0.40^{a}
Total PUFA	55.23	55.25	55.59	58.14	55.28
Total n6	6.70	6.65	8.53	10.50	7.94
Total n3	46.80	46.23	45.45	45.17	45.22
n6/n3	0.14	0.14	0.19	0.23	0.18
DHA/EPA	1,69	1,72	2,21	1,98	1,77
PUFA/SFA	1.73	1.66	1.68	1.91	1.78

Data represent means ± standard deviation (n=2). ND not detected.
Means followed by different letters within the same row are significantly different (P<0.05).

They found out that DHA levels of the fish were in the range of 3.31 to 31.03%. Only 2 fish species exceeded 27% and few ones were close to that level. Comparing this previous information with the ones of the present study, one can say that cuttlefish from Fethiye, Antalya, Gazipaşa, Anamur, and

Iskenderun have higher DHA levels than many marine fish species. Based on the results of the present study and previous studies on fatty acid profiles of cuttlefish, lower lipid levels and higher DHA levels are distinctive characteristics for common cuttlefish.The average levels of EPA in cuttlefish from Fethiye (16.57%), Antalya (16.13%), and Iskenderun (15.61%) were close to each other (P>0.05) and higher than those of EPA in cuttlefish from Gazipaşa (13.42%) and Anamur (14.25%). EPA was the second-highest fatty acid in PUFA for the cuttlefish from all sampling points in this study. Similar results regarding EPA levels were observed in previously reported studies [7-10].

Additionally, the total MUFA levels of cuttlefish from all sampling points were calculated to be the lowest ones compared to the SFA and PUFA levels. In MUFA, oleic acid is the most widely distributed fatty acid and generally the highest fatty acid and supposed to be the deterministic fatty acid level of MUFA, especially, in muscle and liver oils of bony and cartilaginous fish [12,17-19].

The levels of 20:1n9 in MUFA were found to be slightly higher than those of oleic acid in lipids of all cuttlefish from two sampling points (Gazipasa and Anamur). The levels of oleic acid found in lipids of all cuttlefish from three sampling points (Fethiye, Antalya, and Iskenderun) are still the highest fatty acid levels found in MUFA. However, the levels of oleic acid in cuttlefish are exceptionally low compared to bony fish oil. Similar results were observed in some cephalopods especially in cuttlefish [7,8]. Having low oleic acid compared to that of fish lipid could be a distinctive characteristic for cuttlefish. Additionally, the levels of stearic acid in cuttlefish from all sampling points were found to be higher than those of oleic acid. However, the levels of oleic acid are generally higher in bony fish oil. Similar results were observed in some cephalopods, especially in cuttlefish [7,8]. Having higher stearic acid compared to oleic acid level could be another distinctive characteristic for cuttlefish.

Moreover, the major fatty acids in SFA of cuttlefish from all sampling points were C16:0 (palmitic acid), C18:0 (stearic acid), C17:0 (heptadecanoic acid), and C14:0 (myristic acid). These results are in agreement with the results of previous studies on fatty acid composition of common cuttlefish [7,9]. Total average SFA and PUFA levels of all cuttlefish from all sampling points were also found to be higher than 30% and 55%, respectively. The ratio of PUFA/SFA was calculated to be 1.66-1.91, which is higher than the recommended minimum PUFA/SFA ratio by HMSO (1994) but in agreement with the previously reported study of Ozogul *et al*. (2008) [8].

In conclusion, lipid, moisture, ash levels, and fatty acid compositions of the cuttlefish from all sampling points diverged. Almost all sampling points in the current study affected the tested biochemical characteristics. These are probably the results of the biological and geographical conditions, e.g., environmental conditions, food availability and life stages. Stearic acid level in cuttlefish is considerably higher than that of oleic acid compared to bony fish. In general, the cuttlefish from eastern Mediterranean have low fat and rich unsaturated fatty acids, especially EPA and DHA. Different geographical areas seem to have an effect on proximate and fatty acids contents. Even if some individual fatty acids of cuttlefish were different or very close to each other, they are all good for health.

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