www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 10: 381-385 (2010) DOI: 10.4194/trjfas.2010.0311

Changes in Fatty Acid Compositions of Black Sea Anchovy (*Engraulis encrasicolus* L. 1758) During Catching Season

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Received 20 October 2009
Accepted 16 April 2010

Abstract

Monthly changes in proximate and fatty acid compositions of Black Sea anchovy were searched during catching seasons in this study. Moisture content of anchovy was at the lowest level (64.93%) in October, whereas it reached the maximum level in April (74.32%). In contrary to moisture, lipid level was at maximum level (16.32%) at the beginning and gradually declined (7.9%) towards the end of fishing season. Significant differences in lipid levels of anchovy was observed in all sampling months, whereas its ash content was differed significantly ($P<0.05$) only in December and April. In addition, the levels of C16:0, C14:0 and C18:0 in saturated fatty acids (SFA), C18:1n9 in monounsaturated fatty acids (MUFA) and C22:6n-3 (docosahexaenoic acid, DHA) and C20:5n-3 (eicosapentaenoic acid, EPA) in polyunsaturated fatty acids (PUFA) was the predominant fatty acids. EPA level gradually declined, while DHA increased from October to April. Furthermore, the average levels of n3 and n6 were found to be 30.33% and 4.43%, respectively. Significant changes were observed in fatty acid composition, particularly in DHA, of anchovy during the fishing season.

Keywords: Anchovy, proximate composition, fatty acids, EPA, DHA

Karadeniz Hamsisinde (*Engraulis encrasicolus*, L. 1758) Av Sezonu Boyunca Yağ Asitleri Komposizyonlarının Değişimi

Özet

Bu çalışmada Doğu Karadeniz bölgesi hamsilerinin avlama mevsimi süresince besin bileşenleri ve yağ asitleri komposizyonundaki aylık değişim incelenmesi amaçlanmıştır. Hamsilerin nem oranı ekim ayında en düşük (%64,93) iken nisan ayında ise en yüksek seviyeyede (%74,32) olduğu hesaplanmıştır ($P<0,05$). Buna bağlı olarak tüm aylardaki yağ seviyeleri ve aralık ve nisan aylarındaki kül miktarlarındaki değişim istatistiksel açıdan önemli bulunmuştur. İlaveten, avlanma mevsimi süresince doymuş yağ asitlerinden (SFA) C16:0, C14:0 ve C18:0, tekli doymamış yağ asitlerinden (MUFA) C18:1n9 ve çoklu doymamış yağ asitlerinden (PUFA) C22:6n-3 (DHA, dekosahekzanoik asit) ve C20:5n-3 (EPA, eikosapentanoik asit) en yüksek oranlarda bulunmuşlardır. Avlama mevsimi boyunca, ortalama omega–3 ve omega–6 değerleri sırası ile %30,33 ve %4,43 olarak hesaplanmıştır. EPA seviyesinde tedrici olarak bir azalma meydana gelirken, DHA seviyesinde ise ekim ayından nisan ayına kadar bir artış gözlenmiştir. Yağ asitlerinde av mevsimi boyunca yağ assitlerinde özellikle de DHA oranında belirgin bir şekilde bir değişimin olduğu gözlemlenmiştir.

Anahtar Kelimeler: Hamsi, besin bileşenleri, yağ asitleri, EPA, DHA.

Introduction

Anchovy is one of the most important fish species in the world. According to FAO (2004) report, anchovy by itself generated 10% of total captured fish in the world. Anchovy is also important and widely-consumed fish in Turkey, especially in the North-East Black Sea region of the country. The catching season for anchovy starts approximately in the middle of October and finishes almost at the end of April. It even extends to the early weeks of May. According to TURKSTAT (2008), anchovy had the highest catch for marine fish with 251,675 tons; this comprises 63.6% of catch sea fish in Turkey. Total export of anchovy (fresh and frozen) was almost 878 tons in 2008. Chilled, frozen, brined, canned, and even fresh anchovy caught in Black Sea is also exported to many other countries, among which such countries (Anonymous, 2010a) as USA, Germany and Holland (Anonymous, 2010b) can be listed.

Nowadays, there is a growing concern in healthy diets among people. Fish is one of the most preferred and recommended food items by virtue of its rich oil content. According to American Heart Association (AHA), fish (particularly fatty fish) is recommended eating at least two times (two servings) a week (Anonymous, 2010c). Fish oil may be considered as one of the richest source of n-3 PUFA among the other lipid containing foods. Therefore, it can prevent the occurrence of such diseases as depressive disorder (Mischoulon *et al*., 2009), cardiovascular mortality (Anderson *et al*., 2009), rheumatoid arthritis (Galarraga *et al*., 2008), tooth loss (Hamazaki *et al*., 2006), cardiovascular diseases (Calder, 2004), and diabetes (Woodman *et al.,* 2003).

Previous researches have mainly focused on the proximate composition or fatty acid compositions of anchovy (Oksuz *et al*., 2009; Zlatanos and Laskaridis, 2007; Saglik and Imre, 2001; Tanakol *et al*., 1999). However, to the best of the authors' knowledge, no study has ever investigated monthly changes on the proximate composition and fatty acid compositions of anchovy (*Engraulis encrasicolus*, L. 1758) during the catching season. Therefore, the objective of this present study is to investigate monthly changes on the proximate composition and fatty acid content of anchovy. Due to the high variability in the proximate composition particularly in lipid, moisture, ash, and fatty acid profiles of anchovy during the fishing season, it requires different preserving methods for manufacturers in processing, packaging, and storing and cooking for anchovy consumers. Therefore, it is very crucial to know monthly changes in muscle composition, particularly in lipid and fatty acids in order to apply right preservation method.

Material and Methods

Anchovies were collected monthly during the catching season from the Southeast Black Sea from 2008 to 2009 in this present study. The samples were frozen and brought to the laboratory of Faculty of Fisheries and Aquaculture at Mustafa Kemal University in Hatay-Turkey. They were taken out of the freezer before the analyses and defrosted in the fridge. Then, anchovies were beheaded, eviscerated, filleted, and minced for further analyses. Analyses of lipid, moisture, and ash content were carried out in the same laboratory. Determination of fatty acids was performed by GC-MS in Natural Science and Application Centre at Mustafa Kemal University.

Lipid, Moisture and Ash Determinations

Levels of crude lipid, moisture, and ash of anchovy were determined by modified Bligh & Dyer Method (Hanson and Olley,1963), EEC recommended oven drying method ISOR 1442 (Commission of European Communities EE, 1979), and A.O.A.C., 35.1.14 (2000) method no 938.08, respectively. Determinations were done in triplicates. The results were expressed as a mean and standard deviation of the triplicates samples.

Preparation of Fatty Acid Methyl Esters

Approximately 30-35 mg of anchovy oil was saponified with 1.5 ml of 0.5 N methanolic NaOH for 7 min./115°C. After cooling, 2 ml of BF3% was added and heated for another 5 min. at the same temperature above. Then reaction tubes was cooled, 2 ml of iso-octane and 3 ml of saturated NaCl solution were added, and mixed for 30 second then allowed to separate organic phase. FAME's were extracted from the top layer, and transferred into the amber vial for further GC analysis. FAME extracts were kept at the freezer at -20°C until GC analysis.

Chromatographic Conditions

Fatty acids were analysed with 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 HP-INNOWAX Polyethylene Glycol Capillary Column (Model number HP 19091N-133, 0.25 mm * 30m * 0.25 µm) and HP 6890 automatic injection system was used. Injection and detector temperatures were set at 250°C and 270°C, respectively. Split ratio was 1:20 with a total injection volume of 1 µl. Injector was washed three times with iso-octane and with the FAME containing isooctane prior to injection. Post injection, injector program was also set to triple wash of injector for next injection.

Oven temperature was programmed initially at 120°C and hold for 3 minute. Then, the temperature was increased to 250°C with a 10°C per minute ramp rate and hold at this temperature four minutes. Total separation was achieved in 20 minutes. Identification of individual fatty acids was made by comparing those retention time of FAME standard (Supelco 47085U PUFA No: 3) and Supelco 37 component Fame mix (Supelco 47885-U). Confirmation of fatty acid methyl esters was also performed by using MS data base library (FAMEDBWAX).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and mean comparison of the monthly changes was performed by using Duncan test. Statistical analysis was performed with SPSS 15.0. Significance was established at $P<0.05$.

Results

Proximate compositions of anchovies were presented in Table 1. As it can be observed in the Table 1, monthly changes in moisture, lipid and ash content in the muscle of anchovies were observed during the catching season. The average levels of moisture, lipid, and ash were calculated in the ranges of 64.93-74.32%, 6.49-16.32%, and 1.35-1.68%, respectively. The fluctuations in these values could be the consequence of the anchovy's life cycle.

The initial levels of moisture, lipid, and ash in anchovy muscle were calculated to be 64.93%, 16.32%, and 1.41%, at the beginning of the catching season while they were 74.32%, 6.49%, and 1.68% in April, at the end of the catching season, respectively. The average levels of moisture in October, November, and December were found close to each other ($P>0.05$) and differed from the other months ($P<0.05$). On the other hand, the lipid levels of anchovy muscle in each sampling month were differed. Differences in lipid content throughout the catching season were found statistically significant ($P<0.05$). Moreover, the lipid level of anchovy in October had the highest level among the sampling months and started to decrease from the same month to January and then started to increase in February again, lasting to the end of the catching season, never reaching to the lipid level in October again. The highest and lowest ash levels were observed in December and April ($P<0.05$).

A total of 23 fatty acids were determined by using GC-MS. A comparison of fatty acid compositions of Black Sea anchovy caught in from October to April was shown in Table 2. The ranges of SFA, MUFA, and PUFA were found to be in the ranges of 33.40-37.91%; 25.91-31.51%, and 34.00-

Table 1. Proximate compositions of anchovies during catching season

Proximate (%)	October	November	December	January	February	March	April
Moisture	64.93±0.17^{a}	66.34±0.6^{a}	66.25±0.97^{a}	73.72±0.49^{c}	71.56±0.14^{b}	70.61±0.7^{b}	74.32±0.6^{c}
Ash	1.41±0.09^{ab}	1.48±0.05^{ab}	1.35±0.06^{a}	1.49±0.04^{ab}	1.54±0.05^{b}	1.38±0.09^{a}	1.68±0.14^{c}
Lipid	16.32±0.19^{a}	15.68±0.07^{b}	14.21±0.11^{c}	8.85±0.11^{d}	10.65±0.09^{e}	7.89±0.33^{f}	6.49±0.08^{g}

(n=3), values are mean± standard deviation.
Means followed by different letters in a same row are significantly different ($P<0.05$).

Table 2. Fatty acid compositions of monthly caught Black Sea anchovy

C chain	October	November	December	January	February	March	April	Mean value
C14:0	9.10±0.06^{a}	8.31±0.10^{ab}	8.06±1.21^{b}	7.99±0.16^{b}	7.77±0.03^{b}	6.48±0.21^{c}	6.02±0.03^{c}	7.67
C15:0	1.43±0.09^{a}	1.42±0.06^{a}	1.28±0.29^{ab}	0.97±0.06d	1.26±0.04^{abc}	1.13±0.01^{bcd}	1.04±0.02^{cd}	1.22
C16:0	20.89±1.43^{a}	19.16±0.56^{b}	19.21±1.18^{b}	17.35±0.62^{c}	18.06±0.43^{bc}	17.25±0.26^{c}	18.63±0.01^{bc}	18.65
C17:0	1.14±0.13^{abc}	0.98±0.15a	1.19±0.14^{bc}	1.01±0.06^{ab}	1.23±0.03^{c}	1.11±0.03^{abc}	1.04±0.02^{abc}	1.10
C18:0	4.48±0.33^{a}	4.62±0.04^{a}	5.11±0.28^{bc}	5.01±0.08^{b}	5.26±0.05^{bcd}	5.38±0.08^{cd}	5.51±0.13^{d}	5.05
C20:0	0.65±0.57^{a}	1.53±0.54^{b}	1.62±0.59^{b}	1.59±0.10^{b}	1.92±0.06^{b}	1.61±0.04^{b}	1.40±0.04^{b}	1.47
C 22:0	0.21±0.36^{a}	1.10±1.00^{b}	0.15±0.26^{a}	0.12±0.20^{a}	0.41±0.35^{ab}	0.45±0.07^{ab}	0.39±0.35^{ab}	0.40
Total SFA	37.91^{a}	37.12^{a}	36.62^{a}	34.03^{bc}	35.92^{ab}	33.40^{c}	34.04^{bc}	35.58
C16:1	7.83±0.24^{ab}	7.60±0.04 ^{a}	8.21±0.17^{bc}	7.93±0.36^{abc}	8.25±0.28 ^{c}	6.82±0.03 ^{d}	6.66±0.08 ^{d}	7.61
C17:1	0.25±0.43^{ab}	0.33±0.01^{ab}	0.45±0.40^{ab}	0.00±0.00^{b}	0.59±0.51^{b}	0.71±0.01^{b}	0.60±0.03^{b}	0.42
C18:1n9	14.67±0.70^{a}	16.25±0.76^{b}	16.34±1.79^{b}	16.80±0.70^{b}	16.91±0.29^{b}	18.71±0.42^{c}	20.26±0.28^{d}	17.14
C20:1n9	1.56±0.25^{a}	1.45±0.91^{a}	2.15±0.48^{ab}	3.51±0.31^{c}	2.12±0.04^{ab}	2.55±0.07^{b}	2.16±0.03^{ab}	2.21
C22:1 n9	1.59±0.11^{abc}	1.37±0.73^{ab}	2.24±0.53^{c}	3.27±0.44^{d}	1.66±0.15^{abc}	1.96±0.25^{bc}	1.20±0.10^{a}	1.90
Total MUFA	25.91^{a}	27.00^{a}	29.39^{b}	31.51^{c}	29.53^{b}	30.74^{bc}	30.87^{bc}	29.28
C16:2n4	0.34±0.33^{ab}	0.72±0.05^{b}	0.60±0.52^{ab}	0.55±0.47^{ab}	0.49±0.43^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	0.38
C18:2n6	3.30±0.12^{a}	3.11±0.06^{ab}	2.58±0.76^{b}	2.01±0.16^{c}	2.98±0.15^{ab}	2.86±0.16^{ab}	2.52±0.06^{bc}	2.76
C20:2n6	0.14±0.24^{a}	0.25±0.09^{ab}	0.10±0.16^{a}	0.46±0.06^{b}	0.00±0.00^{a}	0.09±0.16^{a}	0.00±0.00^{a}	0.15
C20:4n6	1.80±0.19^{a}	1.59±0.06^{abc}	1.32±0.31^{c}	1.27±0.25^{c}	1.56±0.21^{abc}	1.69±0.06^{ab}	1.39±0.11^{bc}	1.52
Total n6	5.23^{a}	4.94^{a}	3.99^{b}	3.74^{b}	4.54^{ab}	4.64^{ab}	3.91^{b}	4.43
C18:3n3	1.57±0.13^{a}	1.15±0.67^{a}	1.45±0.17^{a}	1.07±0.10^{a}	1.25±0.07^{a}	1.11±0.06^{a}	1.05±0.03^{a}	1.23
C18:4n3	1.72±0.06^{a}	1.77±0.09^{a}	1.45±0.25^{a}	1.07±0.10^{b}	0.93±0.10^{b}	0.42±0.36^{c}	0.00±0.00^{d}	1.05
C20:3n3	0.00±0.00^{a}	0.26±0.46^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.04
C20:4n3	0.57±0.49^{a}	0.54±0.47^{a}	0.69±0.05^{a}	0.49±0.42^{a}	0.69±0.07^{a}	0.52±0.01^{a}	0.50±0.01^{a}	0.57
C20:5n3	11.55±0.24^{a}	10.82±0.21^{b}	10.12±0.52^{c}	10.92±0.46^{b}	10.01±0.26^{cd}	9.92±0.08^{cd}	9.48±0.04^{d}	10.40
C22:5n3	1.17±0.16^{ab}	1.17±0.04^{ab}	1.21±0.05^{ab}	1.32±0.11^{b}	1.25±0.03^{ab}	1.19±0.09^{ab}	1.09±0.02^{a}	1.20
C22:6n3	14.03±1.05^{a}	14.50±0.46^{ab}	14.49±0.47^{ab}	15.29±0.32^{b}	15.38±0.27b	18.07±0.58^{c}	19.04±0.44^{c}	15.83
Total n3	30.61^{ab}	30.21^{ab}	29.42^{a}	30.17^{ab}	29.51^{a}	31.21^{b}	31.16^{b}	30.33
Total PUFA	36.18^{a}	35.88^{a}	34.00^{b}	34.45^{ab}	34.54^{ab}	35.85^{a}	35.07^{ab}	35.14
n3/n6	5.85^{a}	6.11^{ab}	7.38^{bc}	8.07^{c}	6.50^{abc}	6.72^{abc}	7.97^{c}	6.94
DHA/EPA	1.21^{a}	1.34^{b}	1.43^{bc}	1.40^{b}	1.54^{c}	1.82^{d}	2.01^{e}	1.46

(n=3), values are mean± standard deviation.
Means followed by different letters in a same row are significantly different ($P<0.05$).

36.18%, respectively. The levels of SFA in anchovy lipid were determined higher than those of MUFA and PUFA in October, November, December, and February while levels of PUFA had the highest in March and April. Although the monthly levels of DHA and EPA differed in the range of 14.03-19.04% and 9.48-11.55%, respectively. Both EPA and DHA were the dominant fatty acid in PUFA in anchovy during the catching season. Additionally, the average ratio of n3/n6 and DHA/EPA was found to be 6.94 and 1.46, respectively. The lowest percentage of DHA/EPA was found to be 1.21% in October while the highest percentage was 2.01% in April.

Discussion

The lipid levels of anchovy greatly varied (16.32-6.49%) during the fishing season. This variation was mostly found statistically significant ($P<0.05$). The highest lipid level of anchovy was found in October, whereas the lowest level was calculated in April. Differentiation in lipid levels of monthly caught anchovies could be result of the season, life stage or availability of nutrition in the sea in this study. The lipid level of European anchovy was reported to be 14.68% by Saglik and Imre (2001). This finding is parallel to our finding in December specimens. On the other hand, the lipid amount of anchovy from Black Sea region in this present study was found much lower than that of anchovy reported by Zlanatos and Laskaridis (2007). This could be the effects of different environment and season.

The proportions of moisture content in anchovy during the catching season in this study also differed (in the ranges of 64.93-74.32%). The level of moisture in fish can also change depending on species, ages, and sex. Some previous studies showed that there was an inverse relation between level of lipid and moisture in fish muscle and this relation can vary among fish species (Stroud, 1972 and Lupin 1980). According to Lupin (1980) this relationship can be predicted when lipid level is known or vice versa. Similar to former studies on this issue in the literatures, an inverse correlation between moisture and lipid content was also observed in the present study.

Lipid content of anchovy may affect the preserving and processing methods. Thus, when the anchovy contained high lipid, it may be suitable for baking, grilling, steaming, smoking, canning, while in low lipid season frying may be suggested. Apart from the cooking method, lipid content of anchovy should be considered when canning or smoking process of anchovy.

The amount of ash content of anchovy did not change as much as that of moisture and lipid content. The levels of ash content in October, November, December, January, and March were different from those in February and April. The highest and lowest level of ash in anchovy was observed in April and December with a value of 1.68% and 1.38%, respectively. This differentiation was found statistically significant ($P<0.05$).

Fatty acids compositions of anchovies caught from the coast of Southeast Black Sea were depicted in Table 2. The levels of C16:0 (palmitic acid) were found predominant fatty acid not only in SFA but also all the fatty acid determined in this study during the catching season. Similar findings were reported by Oksuz *et al.* (2009); Zlatanos and Laskaridis (2007), Saglik and Imre, (2001). In addition, remaining highest two fatty acids in SFA were the C14:0 (myristic acid) and C18:0 (Stearic acid) and calculated to be 7.67 and 5.05%, respectively.

The mean value of total MUFA content in anchovy muscle was found to be averagely 29.28% throughout sampling months. C16:1 n-7 (palmiteoleic acid), C18:1n-9 (oleic acid), C17:1 (heptadecanoic acid), C20:1n9 (eicosenoik asid) and C22:1 n9 (docosenoic acid) were investigated in terms of MUFA. The monthly changes in the levels of the MUFA component throughout the catching season showed some differences. The total MUFA levels were in the range of 25.91-31.51%. The amount of C18:1n9 and C16:1 in anchovy muscle was determined as the major two fatty acids in MUFA in this present study. Saglik and Imre (2001) and Tanakol *et al.,* (1999) were found similar result for European anchovy and Black Sea anchovy, respectively. Oksuz *et al.,* (2009) were also reported presence of C22:1 fatty acid only in Black Sea anchovy compare to its Mediterranean counterpart.

On the other hand, the levels of EPA started with the average level of 11.55% (in October) and ended that of 9.48% (in April) and almost continuously degreased from the beginning to the end of the catching season. Based on findings this study in the terms of EPA levels, there could be a positive correlation between lipid level and EPA contents of anchovy muscle.

A total of 11 PUFA were determined in this present study. The DHA (C22:6n-3) and EPA (C20:5n-3), these two novel fatty acids, were the predominant fatty acids during the sampling seasons. The level of DHA was calculated as 14.03% when the anchovy muscle had the highest amount of lipid at the beginning of the catching season. It incrementally increased to the level of 19.04% by the end of the catching season. Based on our findings, it seems that there was a possible inverse correlation between lipid and DHA levels of anchovy muscle. On the other hand, the levels of EPA started with the average level of 11.55% (in October) and ended that of 9.48% (April) and almost continuously decreased from the beginning to the end of the catching season.

The levels of DHA were always found higher than that of EPA in Black Sea anchovy in this study during catching seasons. Similar findings were observed in Black Sea and Mediterranean anchovy (Oksuz *et al.,* 2009); sardine, anchovy, and pilchard (Zlatanos and Laskaridis, 2007); anchovy and sardine

(Saglik and Imre, 2001) and sardine (Tanakol *et al.,* 1999).

Additionally, anchovy contains a high level of PUFA (including EPA and DHA). Fish is recommended to consume in order to reduce the risk for heart diseases (Anonymous, 2010c). In addition to reducing the risk for heart disease, regular consumption of fish can be advisable in preventing, treating, or improving a wide variety of diseases and disorders, including mental health (Mischoulon *et al*., 2009), chronic, systemic inflammatory disorder (Galarraga *et al*., 2008), dental health (Hamazaki *et al.,* 2006) and even positive effects on different stages of cancer (Augustsson *et al*., 2003).

In conclusion, aanchovy is a fatty fish species and has got considerable amount of DHA and EPA in the whole catching seasons. This content makes anchovy lipid a novel one. Therefore, anchovy can be recommended to consume especially when it is high in lipid. That's why anchovy could be considered as a source of high quality fatty acids. Besides, the cost of anchovy is affordable for almost anyone. Monthly changes in lipid should be taken into the consideration by processors, fish meal producers, and consumers in order to decide to utilise it in a better way.

Acknowledgements

Authors would like to thank Dr. Hacer Sağlam and R. Sema Nadir for providing the materials, and Mustafa Kemal University of Natural Science and Application Centre for the fatty acid analyses.

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