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Comparative Study on Fatty Acid Profiles of Anchovy from Black Sea and Mediterranean Sea (*Engraulis encrasicholus* L., 1758)

ABDULLAH ÖKSÜZ*, AYSE ÖZYILMAZ and CEMAL TURAN
Faculty of Fisheries and Aquaculture, Mustafa Kemal University, Iskenderun, Hatay, Turkey
Fax: (90)(326)6141877; Tel: (90)(326)6141693/306
E-mail: aoksuz@mku.edu.tr

Fatty acid profile of anchovies (*Engraulis encrasicholus*) from Black Sea and Mediterranean Sea were determined by using GC-MS. A total of 25 fatty acids were identified. The level of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were found as almost 38, 14.3 and 47.7 % in Mediterranean anchovy (MA), respectively. These levels were followed by 35.40 % (SFA), 29.5 % (MUFA) and 31.27 % (PUFA) in Black Sea anchovy (BSA). Geographical location has no effect on total saturated fatty acids of anchovy, whereas MUFA and PUFA changed significantly. BSA contained greater amount of MUFA and low level of PUFA. Conversely, MA had high level of PUFA and low level of MUFA compare to BSA. The ratio of DHA to EPA was calculated as 5.91 in MA and 1.64 in BSA. Palmitic acid, oleic acid and DHA (22:6 n3) were themost prominent fatty acid in both MA and BSA. DHA level may be considered as a key fatty acid to differentiate MA from the BSA.

Key Words: Fatty acids, Black Sea anchovy, Mediterranean anchovy.

INTRODUCTION

The anchovy, a family (*Engraulidae*) of small, common salt-water fish, is a small green fish with blue reflections because of a silver longitudinal stripe on it. They are found in very different areas throughout the world's oceans, but are concentrated in temperate waters and are very rare or absolutely absent in very cold or very warm seas. Anchovies are abundant and especially caught on the coast of the Black Sea as well as Mediterranean Sea. They are commercially important fish species in Turkey, since it has the largest market share. Anchovy contributes almost 42.5 % of total catch of marine fish in Turkey with a production of 138,569 tonnes per year[1].

Anchovies are widely consumed in Turkey, especially in winter when catch is abundant. It is also a significant food source for almost every predatory fish in its environment, extremely important to marine mammals and birds. They are very important for some dish, because of its popular and strong flavour. Consuming fish has some benefits since it is the main source of n-3 fatty acid. Some organization, such as The American Heart Association[2], the European Society for Cardiology[3], the Scientific Advisory Committee on Nutrition[4], the Australian Health and Medical Research council[5] *etc.* have recommended increasing n-3 fatty acid intake.

Anchovy lipid contains some essential fatty acids, which include linoleic acid and α-linolenic acid that are compounds of omega-6 and omega-3 acid series, respectively. In addition, eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) are the dominant n-3 fatty acids especially in marine fish[6]. The essential fatty acids are available in fish and consumption fish have several health benefits for human, such as reducing tooth loss[7], expected to reduce the incidence of coronary heart disease[8] brain disorders and cancer treatment[9], eye (retina) and brain development and functioning[10], prevention of coronary artery disease[11], nutrient for growth and development of human body[12] and prevention of some other diseases[13].

Black Sea Anchovies are widely consumed throughout the fishing season in Turkey. However, Mediterranean Anchovy are not in economic scale fishery products in North-eastern Mediterranean Sea yet. The aim of this study was to reveal the fatty acid profiles of anchovies living in north and south part of Turkish cost and investigate the effect of environmental factor on it.

EXPERIMENTAL

Black Sea anchovy (*Engraulis encrasicholus*) caught in coast of Trabzon was purchased from a local fisherman in Trabzon (Black Sea Region, Turkey) and Mediterranean Sea anchovy (MSA) (*Engraulis encrasicholus*) was caught in coast of Karatas, Adana Sea (Northern Eastern Mediterranean Sea, Turkey). Both of the samples were obtained in December 2007. The samples were transferred to the laboratory in a cooler. Representative fish samples were taken and the fish were immediately gutted and backbones were removed by hand. The samples were minced using with a laboratory type blender. Samplings were done in triplicate for each group.

Lipid extraction and FAME preparation: Lipid extraction was carried out according to Modified Bligh and Dyer Method[14]. Approximately 10 g of samples were used for lipid extraction. Chloroform was evaporated by rotary evaporator under the vacuum at 40 ºC. Remaining fish lipids were dried at 105 ºC for 0.5 h in an oven. Approximately 30-35 mg of fish oil were converted to fatty acid methy ester (FAME) according to AOCS method Ce 1b-89 except with the following modifications: reaction times of 7 and 5 min were applied for methanolic alkali (NaOH) and acid (BF_3) catalyzed esterification, respectively and 2 mL of methanolic NaOH (0.5 N) and 1.5 mL BF_3 (14 %) were applied. Fatty acid methyl esters were extracted with total 4 mL of iso-octane with 2 step extraction.

Chromatographic conditions are as follows: Column: HP-Innowax, polyethylene glycol capillary column, Model Number: HP 19091N-133, nominal length: 30.0 m, nominal diameter: 250 µm, nominal film thickness: 0.25 µm. Injection temperature was set at 250 ºC and detector temperature was set at 270 ºC with a split ratio: 10:1. Split flow was maintained at 9.9 mL/min; total flow: 13.9 mL/min. Helium was used as a carrier gas.

Oven temperature was programmed with an initial temperature of 70 ºC and hold at this temperature for 1 min and increased to 180 ºC with a ramp rate of 20 ºC per min, then finally reached to 220 ºC with a 3 ºC/min increment and hold at this temperature for 10 min. Identification of individual fatty acid was made by comparing those retention time of FAME standards (Supelco 47085U PUFA No. 3) and Supelco 37 component Fame mix (47885-U). Confirmation of fatty acid methyl esters were also done by using MS database library (FAMEDBWAX).

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and statistical analysis was performed with SPSS 13.0.

RESULTS AND DISCUSSION

The fatty acid levels of Mediterranean Sea Anchovy (MSA) and Black Sea Anchovy (BSA) (*Engraulis encrasicholus*) are shown in Fig. 1. As it can be seen from Fig. 1, there are some obvious differences in fatty acid profie of both MA and BSA, particularly percentages of C14:0, C16:0, C18:0, C16:1 n9, C18:1 n9, C 22:6 n3. Differences in fatty acids profiles of same anchovy could be the result of effect of different environment, feeding habits, water temperature and available nutrient in their region. Apart from these facts, age[6], life stage and pollution could be the reason for this distinction.

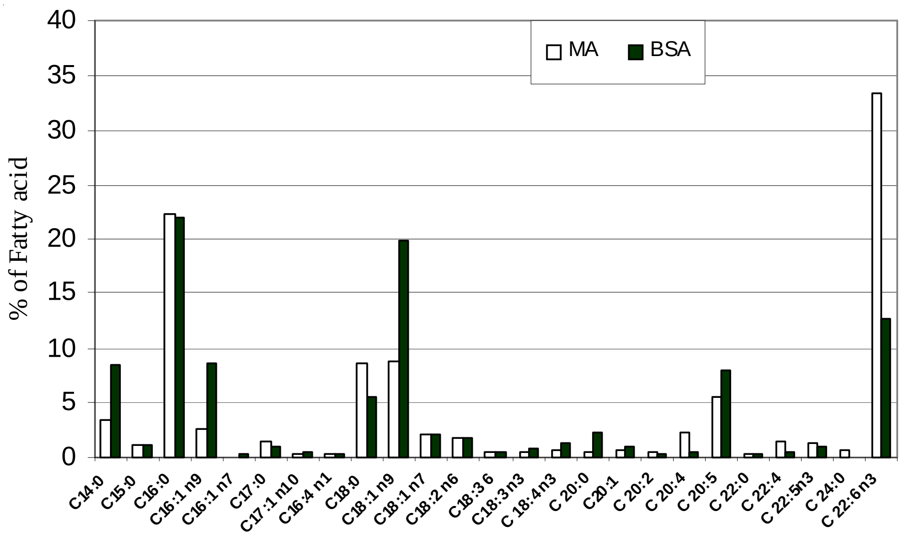

Fig. 1 Comparison of Mediterranean Sea anchovy (MSA) and Black Sea anchovy (BSA)

A different influence of seasonality on the various kinds of fish from the same geographic area was studied and it concluded that different genetic cycle of the fish species could cause the differentiation[15,16]. Based on this previous study, it can be added with results of this study that the same fish species in different region may also show some certain differences in fatty acid profiles. In addition, as it mentioned by Gruger[17], fatty acids compositions of same species may vary due to environment. Results of this present study agree with this previous study.

The percentage of total fatty acid composition of Mediterranean Anchovy (MA) and Black Sea anchovy (BSA) is presented in Table-1. Although MA contained higher level of SFA than BSA, this level was not significantly different in MA and BSA ($p > 0.05$). Total unsaturated fatty acid also were not significantly different in MA and BSA. However, the percentage of total unsaturated fatty acids were greater than saturated fatty acids in both MA and BSA.

TABLE-1
FATTY ACID COMPOSITIONS OF BLACK SEA AND MEDITERRANEAN ANCHOVY

Fatty acid	MA % FA	BSA % FA	Fatty acid	MA % FA	BSA % FA
C14:0	$3.26^{a}\pm0.13$	$7.21^{b}\pm0.49$	C18:2 n6	$1.74^{a}\pm0.08$	$1.68^{a}\pm0.06$
C15:0	$1.14^{a}\pm0.08$	$1.01^{a}\pm0.08$	C18:3 n 6	$0.28^{a}\pm0.25$	$0.52^{b}\pm0.01$
C16:0	$22.13^{a}\pm0.82$	$19.19^{b}\pm0.51$	C 20:4 n6	$2.34^{a}\pm0.25$	$0.71^{b}\pm0.24$
C17:0	$1.25^{a}\pm0.87$	$0.83^{b}\pm0.34$	C 20:2 n6	$0.52^{a}\pm0.07$	$0.27^{b}\pm0.07$
C18:0	$8.69^{a}\pm0.61$	$4.82^{b}\pm0.34$	**Σ n6**	**$4.65^{a}\pm0.82$**	**$3.17^{b}\pm0.39$**
C 20:0	$0.45^{a}\pm0.04$	$2.00^{b}\pm0.20$	C18:3 n3	$0.39^{a}\pm0.02$	$0.87^{b}\pm0.07$
C 22:0	$0.43^{a}\pm0.15$	$0.36^{a}\pm0.01$	C 18:4 n3	$0.48^{a}\pm0.07$	$1.58^{b}\pm0.15$
C 24:0	0.60 ± 0.10	ND	C 20:5 n3	$5.65^{a}\pm0.39$	$10.03^{b}\pm1.15$
Σ SFA	**$37.96^{a}\pm2.79$**	**$35.41^{a}\pm1.98$**	C 22:4 n3	$1.44^{a}\pm1.25$	$0.58^{a}\pm0.05$
C16:1 n9	$2.61^{a}\pm0.18$	$7.80^{b}\pm0.57$	C 22:5 n3	$1.27^{a}\pm0.77$	$1.36^{a}\pm0.17$
C16:1 n7	$0.08^{a}\pm0.14$	$0.27^{b}\pm0.04$	C 22:6 n3	$33.40^{a}\pm2.24$	$16.40^{b}\pm2.21$
C17:1 n10	$0.12^{a}\pm0.21$	$0.43^{b}\pm0.04$	**Σ n3**	**$42.63^{a}\pm4.74$**	**$30.82^{b}\pm3.80$**
C18:1 n9	$8.79^{a}\pm0.36$	$18.29^{b}\pm1.38$	**Σ Unsat.**	$61.77^{a}\pm6.94$	$63.87^{a}\pm7.98$
C18:1 n7	$2.18^{a}\pm0.10$	$1.97^{b}\pm1.51$	**Σ PUFA**	$47.47^{a}\pm5.89$	$34.41^{b}\pm4.31$
C20:1 n9	$0.53^{a}\pm0.06$	$0.70^{a}\pm0.12$	**Σ n3/n6**	9.17^{a}	9.71^{a}
Σ MUFA	**$14.3^{a}\pm1.05$**	**$29.46^{b}\pm3.67$**	**DHA/EPA**	5.91^{a}	1.64^{b}
C16:4 n1	$0.19^{a}\pm0.33$	$0.42^{a}\pm0.11$	**UNSFA:SFA**	1.63^{a}	1.80^{a}

Total SFA level of MA and BSA were calculated as 37.96 and 35.41 %, respectively. Among the saturated fatty acid, the amounts of myristic (C14:0) and arachidic acid (C20:0) of BSA were found 7.21 and 2.0 % which are significantly ($p < 0.01$) higher than those of MA with the value of 3.26 and 0.45 %, respectively. The level of palmitic (C16:0), heptadecanoic (C17:0), and stearic acid (C18:0) of BSA were found lower than those of MA ($p < 0.05$). In addition, the amount of pentadecanoic (C15:0), behenic acid (C22:0), were found almost same for both d BSA ($p > 0.05$). Palmitic acid was the dominant fatty acid for both MA and BSA in terms of saturated fatty acids and followed by stearic acid. Percentage of strearic acid in MA was found almost twice as much of that in BSA. Moreover, only low amount of lignoceric acid (C24:0) was detected in MA, whereas it was not present in BSA.

Saglik and Imre[18] were found C16:0 and C14:0, C18:0 as predominant fatty acids in SFA which are in parallel with present findings and in the range of with findings Zlatanos and Laskaridis[19] except this order changed as C16:0, C18:0 and C14:0 in MSA.

Total MUFA level of MA and BSA were found as 14.3 and 29.46 %, respectively. The MUFA's percentage of BSA is almost twice as much as that of MA ($p < 0.01$). Palmitoleic (C16:1 n9), vaccenic (C18:1 n7), and oleic acid (C18:1 n9) levels of BSA were found higher than those of MA ($p < 0.01$), while the amount of C16:4 n1 and C20:1n9 were not significantly different in MA and BSA. According to Osborn and Akoh[20], n-9 fatty acids, found as oleic acids (C18:1 n-9) play a moderate role in the body. Moreover, n-6 fatty acids can not be synthesized by humans and are therefore considered essential fatty acids. Palmitoleic acid (C16:1 n7) and *cis*, 10 heptadecenoic acid (C17:1) were present at the lowest level in both MA and BSA. Based on data of this present study, it may be concluded that MUFA content of BSA as twice as MA.

Total PUFA level of MA and BSA were calculated 47.47 and 34.41 %, respectively. Eicosapentaenoic (EPA, C 20:5 n3) and docosahexaenoic acid (DHA, C 22:6 n3) level of MA were found as 5.65 and 33.4 %, whereas those of BSA were as 10.03 and 16.4 %, respectively. DHA was the major PUFA and higher than EPA for both anchovy ($p < 0.01$). These results agree with the fact that marine fish is rich in EPA and DHA[6]. These 2 fatty acids have a great importance to human health[7,10]. Nettleton[21] stated that although relative amount of EPA and DHA among different fish species varies, DHA is more abundant than EPA in most species.

The percentages of total n3 fatty acids were found higher than those of total n6 fatty acids in the total fatty acid composition of MA and BSA. Both anchovies contained high level of n3 series of fatty acids compare to n6 series and other MUFA's. Omega 3 fatty acids were significantly higher in MA anchovy than BSA. Fatty acids profile in this present study showed that percentage of n-3: n-6 was 9.17 (MA) and 9.71 (BSA). The ratio of n-3: n-6 PUFA's in total lipids of marine fish changes mostly between 4.7 and 14.4 %[22]. The findings for MA and BSA in this study are in between the ranges. Epidemiological data showed[23] that, increasing n-6:n-3 PUFA ratio led to the rapid increase in mortalities from Western-type cancers and allergies in Japan. Therefore, Okuyama *et al.*[23] recommended that n-6: n-3 PUFA ratio to be less than 2. Because of high n-3 fatty acids level both MA and BSA n-6:n3 ratio was even less than one in this study. Several clinical studies support the view that decreasing the n-6/n-3 fatty acid ratio results in an increased protection against degenerative diseases[24]. Seafood may be thought as a better way of lowering n-6:n3 ratio, since it contains high level of n3 fatty acids.

In conclusion, result of this study shows the fact that fatty acid profile of anchovy species living in Black sea and Mediterranean Sea varied greatly. Traceability point of view, fatty acid profile can be used for tracing back the fish was originated from. Among the fatty acids, the level of DHA may be considered the most distinctive fatty acid to distinguish Mediterranean anchovy from Black sea anchovy.

REFERENCES

1. TURKSTAT, Fishery Statistics (2005).
2. P.M. Kris-Etherton, W.S. Harris and L.J. Appel, *Circulation*, **106**, 2747 (2002).
3. G. De Backer, E. Ambrosioni, K. Borch-Johnsen, C. Brotons and R. Cifkova, *Eur. Heart J.*, **24**, 1601 (2003).
4. Anonymous-2, http://www.food.gov.uk/news/newsarchive/2004/jun/fishreport (2004).
5. Anonymous-3, http://www.nhmrc.gov.au/publications/synopses/n35syn.htm.
6. R.G. Ackman, *Food Nutr. Sci.*, **13**, 161 (1989).
7. K. Hamazaki, M. Itomura and S. Savazaki, *Med. Hypotheses*, **67**, 868 (2006).
8. W.S. Harris, *Atheroscler Rep.*, **6**, 447 (2004).
9. W.S. Fenton, J. Hibbeln and M. Knable, *Biological Psychiatry*, **47**, 8 (2000).
10. W.E. Conner, *Am. J. Clin. Nutr.*, **17**, 171S (2000).
11. W.E. Conner, M. Neuringer and S. Reisbick, *Nutr. Rev.*, **50**, 21 (1992).
12. A.P. Simopolous, *Am. J. Clin. Nutr.*, **54**, 438 (1991).
13. C.A. Drevon, *Nutr. Rev.*, **50**, 38 (1992).
14. S.W.F. Hanson and J. Olley, *Proc. Biochem. Soc.*, **89**, 101 (1963).
15. P.A. Karakoltsidis, A. Zotos and M. Costantinides, *J. Food Comp. Anal.*, **8**, 258 (1995).
16. L.A. Luzia, G.R. Sampaio, C.M.N. Castellucci and E.A.F.S. Torres, *Food Chem.*, **83**, 93 (2003).
17. E.H. Gruger, in ed.: M.E. Stansby, Fish Oils/3, Westport CT: AVI Publishing Co., Ch. 1 (1967).
18. S. Saglik and S. Imre, *J. Food Sci.*, **66**, 210 (2001).
19. S. Zlatanos and K. Laskaridis, *Food Chem.*, **103**, 725 (2007).
20. H.T. Osborn and C.C. Akoh, *Comp. Rev. Food Sci. Food Safety*, **1**, 93 (2002).
21. J.A. Nettleton, Omega-3 Fatty Acid and Health, Chapman and Hall, p. 19 (1995).
22. R.J. Henderson and D.R. Tocher, in eds.: R.T. Holman and W.W.H. Sprecher, The Lipid Composition and Biochemistry of Freshwater Fish, New York, Pergamon Press, Vol. 26, pp. 281-437 (1987).
23. H. Okuyama, T. Kobayashi and S. Watanabe, *Prog. Lipid Res.*, **35**, 409 (1996).
24. G.L. Russo, *Biochem. Pharmacol.*, (2008) in press.

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