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ScienceDirect
Food Chemistry 103 (2007) 725–728
Food Chemistry
www.elsevier.com/locate/foodchem

Seasonal variation in the fatty acid composition of three Mediterranean fish – sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*)

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Received 6 January 2006; received in revised form 26 June 2006; accepted 8 September 2006

Abstract

The total fat contents and the fatty acid compositions of three common Mediterranean fish, namely sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*) were determined at bimonthly intervals for a one-year period. The purpose of this work was to study the seasonal variation of the fatty acids in the three fish that are some of the best sources of $n-3$ fatty acids. The fat and fatty acid content of the investigated fish species show a significant seasonal dependency. Two of the fish (anchovy and picarel) have the highest fat content during the late winter – spring period. On the other hand, sardine shows the highest fat concentrations during the spring-early summer period. The fish that showed the highest variation in fatty acid composition was the anchovy. The sardine was found to be the best source of $n-3$ fatty acids during the one-year period (35.35 g/100 g fatty acids). Finally the picarel had the highest oleic acid content (on average, 13.89/100 g fatty acids).

Keywords: Fatty acid composition; Sardine; Anchovy; Picarel; Seasonal variation; $n-3$ Fatty acids

1. Introduction

Marine food and especially marine fish are an important part of the Mediterranean diet (Greek Ministry of Agriculture, 2004). The beneficial effect of fish consumption on human health has been related, among other factors to the high content of $n-3$ fatty acids, especially eicosapentaenoic acid (C20:5$n-3$) and docosahexaenoic acid (C22:6$n-3$). The effect of these fatty acids is well documented in numerous investigations, as were reviewed by Horrocks and Yeo (1999) and Leaf et al. (1999).

Fish consumption is common in Greece. The most important (in quantitative terms) fish are the sardine (*Sardina pilchardus*), the anchovy (*Engraulis encrasicholus*) and the picarel (*Spicara smaris*) (Bareltzis, 1997). According to Zlatanos and Sagredos (1993), these fish are among the best sources of $n-3$ fatty acids. However, the fat content and the fatty acid composition of the fish are not constant. They are related to the life cycle of the fish and external factors, like temperature, salinity and fatty acid composition of their food (Bandarra, Batista, Nunes, & Empis, 2001; Gockse, Tasbozan, Celik, & Tabakoglu, 2004). In fact studies that have investigated the $n-3$ fatty acid content of Mediterranean fish (Guner, Dincer, Alemdag, Colak, & Tufekci, 1998; Karakoltsidis, Zotos, & Constantinides, 1995; Saglik & Imre, 2001; Zlatanos & Sagredos, 1993) do not always show similar results. Great variations are also reported in the fat content and fatty acid composition of several marine organisms during the year (Gockse et al., 2004; Luzia, Sampaio, Castellucci, & Toreres, 2003; Shirai, Terayama, & Takeda, 2002).

In the present study the influence of seasonality on the fat content and the fatty acid composition of the aforementioned three fish was investigated in order to find the best source of $n-3$ fatty acids during the year.

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doi:10.1016/j.foodchem.2006.09.013

2. Materials and methods

Fish were purchased from the Central Market of Thessaloniki (northern Greece) every two months. The edible part of four fish from each species was removed, cut into pieces and homogenized. The homogenized samples were divided into three parts and the fat of each part was extracted according to Bligh and Dyer (1959). The fat content was determined gravimetrically.

The fat was methylated according to Zlatanos and Sagredos (1993). Margaric acid (C17:0) was added as an internal standard. The determination of fatty acids was conducted in a Perkin–Elmer Sigma 2B gas chromatograph, equipped with split injector in a 1:50 ratio, FID and a 60 m J&W Scientific (model DB-3) capillary column (film 0.25 μm, diameter 0.32 mm). The temperature of the injector port and detector was held at 250 °C and 300 °C, respectively. The injected volume was 3 μl. The temperature of the column was held at 80 °C for 5 min, raised to 180 °C at 5 °C/min, held at 180 °C for 16 min, raised again to 220 °C at 5 °C/min and finally held at 220 °C for 20 min.

3. Results and discussion

The fat content of the analysed species shows an important dependency on season (Table 1). The fat content of the sardine was minimal at the end of winter and maximal at the end of spring – beginning of summer. A high fat content in the summer and a low fat content in the winter has also been reported for the Japanese sardine *Sardinops melanostictus* (Shirai et al., 2002). On the other hand, the fat content of the anchovy and picarel was maximal at the end of winter – beginning of spring and minimal at the end of the summer. A different influence of seasonality on the various kinds of fish from the same geographic area is common and is probably affected by the different genetic cycle of the fish species (Karakoltsidis et al., 1995; Luzia et al., 2003).

The fatty acid compositions of the investigated fish are presented in Tables 2–4. The results show remarkable changes in the individual fatty acids during the one-year period. In all investigated fish the main fatty acids were palmitic acid (C16:0) and C22:6$n-3$. In particular, the sardine has more C22:6$n-3$ than C16:0 in two out of the six investigated months. These results are in agreement with the literature, where C16:0 was reported as the most abundant fatty acid in three studies on the Mediterranean sardine (Karakoltsidis et al., 1995; Leonardis & Macciola, 2004 & Saglik & Imre, 2001) and C22:6$n-3$ in one study (Zlatanos & Sagredos, 1993).

In the present study, C16:0 was found to be the most abundant fatty acid for anchovy and picarel in four and

Table 1
Fat contents (g/100 g dry sample) of the three investigated fish

	February	April	June	August	October	December	Mean
Sardine	3.88 ± 0.2	11.86 ± 0.6	11.47 ± 0.5	5.88 ± 0.2	8.46 ± 0.5	6.92 ± 0.2	8.08
Anchovy	5.71 ± 0.4	3.41 ± 0.3	1.32 ± 0.2	0.94 ± 0.2	2.99 ± 0.3	2.85 ± 0.2	2.87
Picarel	4.42 ± 0.4	4.52 ± 0.3	2.95 ± 0.2	0.93 ± 0.2	1.85 ± 0.3	3.06 ± 0.3	2.96

Table 2
Seasonal variation in the fatty acid composition of the sardine (g/100 g fatty acids)

	February	April	June	August	October	December	Mean
C12:0	0.21 ± 0.02	0.09 ± 0.03	0.08 ± 0.04	0.13 ± 0.02	0.15 ± 0.05	0.08 ± 0.02	0.12
C14:0	6.45 ± 0.79	8.00 ± 0.62	7.52 ± 0.58	6.98 ± 0.47	6.69 ± 0.80	6.52 ± 0.71	7.03
C14:1	0.30 ± 0.04	0.22 ± 0.05	0.15 ± 0.02	0.29 ± 0.04	0.21 ± 0.03	0.18 ± 0.02	0.23
C15:0	0.67 ± 0.09	0.88 ± 0.14	0.82 ± 0.13	0.95 ± 0.08	0.89 ± 0.11	0.93 ± 0.13	0.86
C16:0	24.76 ± 1.55	22.5 ± 1.67	22.47 ± 1.82	20.56 ± 1.48	25.69 ± 2.20	23.32 ± 1.78	23.22
C16:1	5.75 ± 0.48	7.84 ± 0.37	6.65 ± 0.40	5.38 ± 0.56	5.11 ± 0.47	5.83 ± 0.39	6.09
C17:1	0.30 ± 0.02	0.14 ± 0.03	0.6 ± 0.03	0.21 ± 0.02	0.30 ± 0.05	0.23 ± 0.02	0.30
C18:0	4.16 ± 0.78	2.08 ± 0.47	4.28 ± 0.59	3.39 ± 0.40	2.60 ± 0.37	3.69 ± 0.52	3.37
C18:1	3.53 ± 0.57	4.33 ± 0.44	9.47 ± 0.74	10.56 ± 1.09	9.84 ± 0.88	4.65 ± 0.61	7.06
C18:1$n-7$	1.85 ± 0.28	3.31 ± 0.31	2.01 ± 0.48	2.42 ± 0.17	2.00 ± 0.31	2.3 ± 0.27	2.32
C18:2$n-6$	1.24 ± 0.17	1.54 ± 0.25	1.19 ± 0.17	2.22 ± 0.31	1.16 ± 0.18	1.78 ± 0.24	1.52
C18:3	1.14 ± 0.22	3.01 ± 0.34	1.08 ± 0.18	2.02 ± 0.27	1.54 ± 0.19	2.96 ± 0.35	1.96
C18:4$n-3$	1.02 ± 0.25	0.61 ± 0.12	0.47 ± 0.14	0.84 ± 0.23	0.71 ± 0.26	1.12 ± 0.17	0.80
C20:1	1.02 ± 0.19	1.38 ± 0.26	1.14 ± 0.24	1.13 ± 0.27	1.22 ± 0.16	1.37 ± 0.25	1.21
C20:4$n-6$	0.84 ± 0.17	3.03 ± 0.24	0.68 ± 0.14	0.60 ± 0.09	0.80 ± 0.11	1.26 ± 0.18	1.20
C20:5$n-3$	11.92 ± 1.18	10.33 ± 0.94	10.32 ± 0.86	11.20 ± 1.52	11.23 ± 1.19	8.99 ± 0.79	10.67
C22:1	0.83 ± 0.14	0.94 ± 0.23	0.65 ± 0.13	0.88 ± 0.09	0.71 ± 0.08	0.93 ± 0.14	0.82
C22:5$n-3$	1.46 ± 0.04	0.99 ± 0.05	0.95 ± 0.06	1.08 ± 0.02	1.14 ± 0.07	1.01 ± 0.04	1.11
C22:6$n-3$	25.32 ± 2.29	18.07 ± 1.72	17.67 ± 1.58	18.36 ± 2.33	21.2 ± 2.38	24.36 ± 3.21	20.83
$\sum n-3$	40.86	33.01	30.49	33.50	35.82	38.4	35.35
$\sum n-6$	2.08	4.57	1.87	2.82	1.96	3.04	2.72
$\sum$MUFA	13.58	18.16	20.67	20.87	19.39	15.49	18.03
$\sum$SFA	36.25	33.55	35.17	32.01	36.02	34.54	34.59

Table 3
Seasonal variation in the fatty acid composition of the anchovy (g/100 g fatty acids)

	February	April	June	August	October	December	Mean
C12:0	0.18 ± 0.03	0.17 ± 0.03	0.30 ± 0.04	0.27 ± 0.03	0.17 ± 0.02	0.15 ± 0.04	0.21
C14:0	8.41 ± 0.87	6.38 ± 0.94	5.89 ± 0.59	9.16 ± 1.13	4.34 ± 0.87	6.59 ± 0.63	6.80
C14:1	0.23 ± 0.03	0.27 ± 0.02	0.53 ± 0.04	0.86 ± 0.05	0.22 ± 0.03	0.36 ± 0.03	0.41
C15:0	0.9 ± 0.18	1.05 ± 0.14	1.09 ± 0.26	1.81 ± 0.17	0.51 ± 0.09	1.32 ± 0.17	1.11
C16:0	31.18 ± 2.39	17.86 ± 2.14	19.85 ± 1.85	28.22 ± 1.48	35.63 ± 3.22	29.68 ± 2.78	27.07
C16:1	4.79 ± 0.54	5.91 ± 0.62	2.79 ± 0.36	7.86 ± 0.75	1.1 ± 0.97	2.77 ± 0.36	4.20
C17:1	0.33 ± 0.12	0.75 ± 0.14	1.23 ± 0.23	0.99 ± 0.23	0.65 ± 0.11	0.56 ± 0.13	0.75
C18:0	5.9 ± 0.41	3.67 ± 0.57	4.35 ± 0.32	6.75 ± 0.78	4.28 ± 0.39	6.32 ± 0.64	5.21
C18:1	9.29 ± 1.36	7.55 ± 0.72	4.67 ± 0.63	9.89 ± 1.15	10.48 ± 0.71	9.35 ± 0.89	8.54
C18:1$n-7$	1.11 ± 0.27	1.26 ± 0.33	0.83 ± 0.48	1.52 ± 0.26	1.51 ± 0.16	1.66 ± 0.31	1.32
C18:2$n-6$	1.08 ± 0.41	1.24 ± 0.32	2.25 ± 0.35	1.71 ± 0.24	2.23 ± 0.48	2.60 ± 0.47	1.85
C18:3	0.95 ± 0.14	1.62 ± 0.25	2.01 ± 0.34	1.57 ± 0.17	1.7 ± 0.23	1.36 ± 0.25	1.54
C18:4$n-3$	0.32 ± 0.04	0.47 ± 0.08	0.74 ± 0.15	0.81 ± 0.14	0.63 ± 0.09	0.51 ± 0.08	0.58
C20:1	1.87 ± 0.16	2.79 ± 0.29	1.3 ± 0.09	1.63 ± 0.14	1.55 ± 0.19	1.08 ± 0.13	1.70
C20:4$n-6$	0.51 ± 0.12	0.60 ± 0.08	1.05 ± 0.17	0.67 ± 0.05	0.35 ± 0.02	0.77 ± 0.06	0.66
C20:5$n-3$	11.86 ± 2.19	12.4 ± 1.22	6.76 ± 0.78	3.64 ± 0.67	2.46 ± 0.51	9.36 ± 1.19	7.75
C22:1	0.57 ± 0.17	0.63 ± 0.24	0.55 ± 0.13	0.22 ± 0.02	0.41 ± 0.09	0.67 ± 0.13	0.51
C22:5$n-3$	0.57 ± 0.14	0.88 ± 0.28	0.69 ± 0.16	0.43 ± 0.08	0.21 ± 0.04	0.76 ± 0.15	0.59
C22:6$n-3$	12.23 ± 1.22	26.41 ± 2.24	32.46 ± 2.79	14.32 ± 1.82	23.75 ± 2.21	17.33 ± 2.50	21.08
$\sum n-3$	25.93	41.78	42.66	20.77	28.75	29.32	31.98
$\sum n-6$	1.59	1.84	3.30	2.38	2.58	3.37	2.34
$\sum$MUFA	18.19	19.16	11.90	22.97	15.92	16.45	17.63
$\sum$SFA	46.57	29.13	31.48	46.21	44.93	44.06	39.66

Table 4
Seasonal variation in the fatty acid composition of the picarel (g/100 g fatty acids)

	February	April	June	August	October	December	Mean
C12:0	0.09 ± 0.02	0.09 ± 0.04	0.08 ± 0.02	0.15 ± 0.03	0.20 ± 0.06	0.11 ± 0.03	0.12
C14:0	4.81 ± 0.68	4.65 ± 0.37	5.74 ± 0.65	4.73 ± 0.94	3.7 ± 0.51	4.09 ± 0.38	4.62
C14:1	0.88 ± 0.22	0.76 ± 0.17	0.98 ± 0.25	0.61 ± 0.10	0.48 ± 0.08	0.53 ± 0.14	0.71
C15:0	0.96 ± 0.17	1.13 ± 0.08	0.62 ± 0.18	1.22 ± 0.16	1.01 ± 0.22	0.81 ± 0.19	0.96
C16:0	22.36 ± 2.26	20.39 ± 1.73	21.97 ± 2.13	22.76 ± 1.82	23.15 ± 1.93	25.31 ± 3.03	22.66
C16:1	7.59 ± 0.94	6.47 ± 0.43	6.38 ± 0.77	4.8 ± 1.08	8.89 ± 0.78	6.09 ± 0.84	6.70
C17:1	0.62 ± 0.12	0.29 ± 0.06	0.31 ± 0.04	0.41 ± 0.11	0.47 ± 0.07	0.32 ± 0.03	0.40
C18:0	4.01 ± 0.59	4.19 ± 0.35	4.4 ± 0.42	4.62 ± 0.58	3.47 ± 3.7	4.46 ± 0.53	4.19
C18:1	13.22 ± 0.74	15.92 ± 1.61	13.27 ± 1.27	12.2 ± 0.89	14.3 ± 1.18	14.41 ± 1.33	13.89
C18:1$n-7$	1.32 ± 0.16	1.63 ± 0.37	1.74 ± 0.25	1.28 ± 0.21	1.68 ± 0.27	1.31 ± 0.16	1.49
C18:2$n-6$	1.41 ± 0.022	0.78 ± 0.29	1.34 ± 0.11	1.56 ± 0.17	0.83 ± 0.16	1.74 ± 0.17	1.28
C18:3	1.53 ± 0.25	2.78 ± 0.37	1.3 ± 0.16	1.64 ± 0.15	1.77 ± 0.24	0.47 ± 0.16	1.58
C18:4$n-3$	1.24 ± 0.17	2.06 ± 0.25	1.47 ± 0.17	1.36 ± 0.15	1.3 ± 0.18	1.97 ± 0.12	1.57
C20:1	1.24 ± 0.15	2.54 ± 0.26	2.23 ± 0.23	2.68 ± 0.29	2.11 ± 0.17	2.31 ± 0.26	2.19
C20:4$n-6$	0.88 ± 0.14	0.62 ± 0.09	0.93 ± 0.07	0.75 ± 0.06	0.95 ± 0.08	1.21 ± 0.08	0.89
C20:5$n-3$	9.63 ± 1.21	8.38 ± 1.18	8.77 ± 1.22	7.57 ± 0.87	9.57 ± 1.18	11.63 ± 1.13	9.26
C22:1	0.21 ± 0.02	0.25 ± 0.04	0.26 ± 0.05	0.14 ± 0.03	0.36 ± 0.08	0.33 ± 0.03	0.26
C22:5$n-3$	0.75 ± 0.25	0.84 ± 0.16	1.02 ± 0.17	0.87 ± 0.22	1.24 ± 0.18	0.99 ± 0.14	0.95
C22:6$n-3$	14.23 ± 1.17	21.89 ± 1.62	19.07 ± 2.31	18.95 ± 2.57	19.02 ± 2.40	17.45 ± 2.02	18.44
$\sum n-3$	27.38	35.95	31.63	30.39	32.90	32.51	31.65
$\sum n-6$	2.29	1.40	2.27	2.31	1.78	2.95	20.1
$\sum$MUFA	25.08	27.86	25.17	22.12	28.29	25.30	25.70
$\sum$SFA	32.23	30.45	32.81	33.48	31.53	34.78	32.10

five of the sampling months, respectively. In the literature C16:0 has been reported to be the most abundant fatty acid in three of four studies on the anchovy (Guner et al., 1998; Karakoltsidis et al., 1995; Saglik & Imre, 2001; Zlatanos & Sagredos, 1993) and in one of two studies on the picarel (Karakoltsidis et al., 1995; Zlatanos & Sagredos, 1993).

The fatty acids with the highest percentages, next to C16:0 and C22:6$n-3$, were C20:5$n-3$, oleic acid (C18:1$n-9$) and myristic acid (C14:0). These results are also in agreement with the literature (Guner et al., 1998; Karakoltsidis et al., 1995; Leonardis & Macciola, 2004; Saglik & Imre, 2001; Zlatanos & Sagredos, 1993). On the other hand, the third most abundant fatty acid in the picarel was C18:1$n-9$.

The picarel had the most stable fatty acid composition during the whole year. On the other hand, the anchovy

showed the greatest variations. In fact, the anchovy exhibited the two highest values of $n-3$ fatty acids among all investigated samples at the months of April and June, and four of the five lowest values of these fatty acids at the other four months. Sardine has a high C20:5$n-3$ and C22:6$n-3$ content, and the picarel had the highest C18:1$n-9$ content during the whole year. Because of its high C18:1$n-9$ content, the picarel exhibited the highest monounsaturated fatty acid (MUFA) content.

The comparison of the seasonal fat variation with the seasonal fatty acid variation showed a negative correlation between the fat content and the $n-3$ fatty acid content for sardine and anchovy, that means that the percentages of $n-3$ fatty acids were low during the months of high fat content. The opposite relation was found for the saturated fatty acids (SFA), which increased during the months of high fat content. This suggests different biological functions for the various fatty acids in the fish. SFA are probably used for energy storage. Therefore, their concentration increases during periods of enhanced feeding activity (Gockse et al., 2004; Shirai et al., 2002). On the other hand, the $n-3$ fatty acid and SFA percentages of the picarel showed no relation to the fat content.

The sardine and anchovy belong to the Clupeiformes order. The negative correlation of the fat content with the $n-3$ fatty acid percentage is probably a characteristic of this order since Shirai et al. (2002) reported a similar correlation for *S. melanostictus*.

4. Conclusions

The anchovy showed the highest $n-3$ fatty acid content in two of the six months studied (April and June), and the sardine in the other four. However, the fat content of the sardine, during April and June is so high, that it still has the highest $n-3$ fatty acid content (with reference to the edible part of the fish). Thus sardine appears to be the richest source of $n-3$ fatty acids among the three fish during the whole year. However, all three examined species are rich sources of the essential $n-3$ fatty acids during the whole year, and therefore are recommended as part of a diet aiming at improving the intake of these fatty acids.

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