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VARIATIONS IN THE FATTY ACID COMPOSITION AND NUTRITIONAL VALUE OF ADRIATIC SARDINE (*Sardina pilchardus* Walb.) THROUGH THE FISHING SEASON [1]

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Variations in the fatty acid composition and nutritional value of Adriatic sardine (Sardina pilchardus Walb.) through the fishing season

We investigated the chemical composition, in terms of water, protein, ash, total fat and fatty-acid composition, of sardine meat, and estimated its nutritional value. The samples originated from Adriatic sardines (*Sardina pilchardus* Walb.) that were collected in the north Adriatic Sea through the winter, spring, summer and autumn seasons. The content of 20 fatty acids was determined by *in-situ* transesterification and capillary column gas-liquid chromatography, using nonadecanoic acid (19:0) as internal standard. The mean contents of the sardine meat were: 70.8% water, 21.0% protein, 2.5% ash and 6.4% fat. For the fatty-acid composition, 18.0% were mono-unsaturated, 42.6% polyunsaturated and 39.8% saturated. The total-fat content increased through the year, from winter to autumn (0.69 to 18.15 g/100 g meat). The fatty-acid composition in the sardine meat varied significantly, with the levels of the polyunsaturated fatty acids (4.6 g/100 g meat), and especially eicosapentaenoic acid (20:5*n*-3, 0.98 g/100 g meat) and docosahexaenoic acid (22:6*n*-3, 1.9 g/100 g meat), being the highest in autumn, before spawning. The *n*-6/*n*-3 ratio (0.13) and P/S ratio (7.6) show that sardine meat can and should be included in a balanced human diet. Considering the recommended daily intake of *n*-3 polyunsaturated fatty acids is 0.45 g per day for a healthy population, this would be consumed as 10 g sardine meat collected in the autumn or 100 g sardine meat collected in the winter.

Key words: human nutrition / food / fish / Adriatic sardine / *Sardina pilchardus* Walb. / composition / fatty acids / nutritional value / season

Maščobnokislinski profil in prehranska vrednost jadranske sardele (Sardina pilchardus Walb.) v odvisnosti od sezone ulova

Raziskovali smo kemijsko (vsebnost vode, beljakovin, maščob in mineralnih snovi) in še posebej maščobno kislinsko sestavo mesa sardel ter določili njeno prehransko vrednost. Jadranske sardele (*Sardina pilchardus* Walb.) so bile ujete v severnem Jadranskem morju v štirih različnih lovnih sezonah: pozimi, spomladi, poleti in jeseni. Z metodo *in situ* transesterifikacije in določitve na plinsko tekočinskem kromatografu smo določili 20 maščobnih kislin. Meso sardin povprečno vsebuje 70,8 % vode, 21,0 % beljakovin, 2,5 % mineralnih snovi in 6,4% maščob; od skupnih maščobnih kislin je 18,0 % enkrat nenasičenih, 42,6 % večkrat nenasičenih ter 39,8 % nasičenih. Vsebnost maščob je močno nihala med sezonami (naraščala od zime proti jeseni, od 0,69 % do 18,15 %) in statistično značilno vplivala na maščobnokislinsko sestavo mesa. Največ večkrat nenasičenih maščobnih kislin (skupaj 4,6 g/100 g mesa), predvsem eikozapentaenojske (20:5*n*-3, 0,98 g/100 g mesa) in dokozaheksaenojske (22:6*n*-3, 1,9 g/100 g mesa) so vsebovale sardele jesenskega ulova (pred drstenjem). Razmerja *n*-6/*n*-3 (0,13) in P/S (7,6) kažeta, da imajo sardine visoko prehransko vrednost. Priporočen dnevni vnos večkrat nenasičenih maščobnih kislin (0,45 g/dan za zdravo populacijo) dosežemo že z dnevnim zaužitjem 100 g mesa sardel, ujetih pozimi, in 10 g mesa sardel, ujetih jeseni.

Ključne besede: prehrana ljudi / živila / ribe / jadranska sardela / *Sardina pilchardus* Walb. / sestava / maščobne kisline / prehranska vrednost / letni čas

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1 INTRODUCTION

The Adriatic sardine (*Sardina pilchardus* Walb.) is a widespread fish along the European and African coasts of the Mediterranean Sea. It is also found in the English Channel, and in the Black Sea and North Sea. This pelagic oily fish was, and still is, an important species for the fish industry in Mediterranean countries.

Evidence suggests that fish consumption decreases the risk of cardiovascular disease, cancers and asthma, and particularly consumption of the oily fish that contain high levels of polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA, 22:6*cis*-4,7,10,13,16,19) and eicosapentaenoic acid (EPA, 20:5*cis*-5,8,11,14,17). Fish consumption also has positive influences on infant neurodevelopment, along with many other reported benefits (Simopoulos, 2002; FSA, 2004). However, although all fish are generally considered to be of similar nutritional value, it has been recognized that the PUFA composition can vary across different fish species. Therefore, when fish is suggested as a means of improving health, both the species and the PUFA composition should be taken into account (Osman *et al.*, 2001).

The role of lipids in nutrition is manifold. They are the carriers of the energy value and contain the essential FAs and PUFAs, and they are the precursors for the biosynthesis of the eicosanoids, which have important functions in the human body (Tapiero *et al.*, 2002; Nelson and Cox, 2005). Indeed, the most important value of fish meat and lipids are the PUFAs, and especially DHA and EPA, both of which are found mainly in fish lipids and in trace amounts in food of animal and plant origin.

Studies of the FA composition of fish are important when related to the above-mentioned benefits. From the nutritional point of view, there are different important indices, such as the P/S ratio, *n*-6/*n*-3 ratio and atherogenic index. The recommended ratio of PUFA to saturated FAs (P/S) should be above 0.4, with the normal P/S ratio for meat at around 0.1 (Wood *et al.*, 2003). Simopoulos (2002) concluded that the optimal ratio of *n*-6/*n*-3 varies from 1:1 to 4:1, depending on disease under consideration. A low ratio of *n*-6/*n*-3 PUFAs is more desirable to reduce the risks of many of chronic diseases of high prevalence in both Western societies and developing countries, which are being exported to the rest of the World. The World Health Organisation has recommended similar values for the *n*-6/*n*-3 PUFA ratio, as 5:1 to 10:1 (WHO, 1994). Attempts to develop a better index of the potential health attributes of foods containing a mixture of FAs have been reported by Ulbricht and Southgate (1991), with their indices of atherogenicity and thrombogenicity. The atherogenic index is recommended as between 0.70 and 0.72 (Ulbricht and Southgate, 1991), although Salobir (1997) recommended even lower levels, of under 0.5.

In view of these details, we have here followed the need for further studies of the nutritional value and lipid profiles of the most commonly consumed fish in Mediterranean countries throughout the four seasons of the year.

2 MATERIAL AND METHODS

The sardines (*Sardina pilchardus*) used in this study were caught in the north Adriatic Sea, off Slovenia and north Croatia, through the four seasons of the year: winter, spring, summer and autumn. Three samples were taken during each season, each of which consisted of eight sardines. The fish were prepared as ready-to-eat: they were beheaded, gutted and frozen (–80 °C), for a maximum period of one month before sampling.

The chemical composition of these sardines was determined at the end of the sample collection, in terms of their ash, water, proteins and fat content. The water content was determined on 5 g samples of the minced meat. These samples were dried in an oven at 105 °C according to the AOAC 950.46 (1997) recommendations. The total protein (crude protein, N × 6.25) content was determined by the Kjeldahl method, according to the AOAC 928.08 (1997) standard methodology. The ash content was determined by mineralisation of the samples at 550 °C, according to the AOAC 920.153 (1997) standard method.

The FA compositions of the samples were determined by gas-liquid chromatography (GLC). The method chosen was *in-situ* transesterification (Park and Goins, 1994). The contents of the FA methyl esters (FAMEs) were determined by GLC using an Agilent Technologies 6890 gas chromatograph with a flame ionisation detector and an SP™-2380 capillary column (Supelco, Cat.No. 24111) (60 m × 0.25 mm × 0.2 μm). The separation and detection were performed under the following conditions: temperature programme, 170 °C (hold 5 min); 4 °C/min to 250 °C (25 min); injector temperature, 250 °C; detector temperature, 280 °C; injector: split:splitless, 1:30, volume 1 μL; carrier gas: He, 1.0 mL/min; make-up gas: N_2, 45 mL/min; detector gases: H_2, 40 mL/min; synthetic air (21% O_2) 450 mL/min.

The FAMEs were determined through comparison with the retention times of the FAMEs from standard mixtures (Supelco, 37-component FAME ester mix; Cat. No. 18919-1AMP; Supelco, PUFA No.1: Animal source, Cat. No. 47015-U; Supelco, Linoleic Acid Methyl Ester *cis*/*trans* Isomer Mix, Cat. No. 47791; Supelco, *cis*-7-octadecenoic methyl ester, Cat. No. 46900-U; Supelco, *cis*-11-

octadecenoic methyl ester, Cat. No. 46904; Fluka, methyl stearidonate, Cat. No. 43959; natural ASA CLA 10t, 12c in CLA 9c, 11t; NuChek standards: GLC-68D, GLC-85, GLC-411 and GLC-546). As an internal standard, 100 µL of a solution of nonadecanoic acid in hexane (10 g/L; C19:0) (Sigma, N4129) was added to the samples before saponification. The NuChek GLC-68D and GLC-85 standard mixtures were used to determine the response factor, Rf_i, for each FA. The weight portion of each FA in the sample was determined using Rf_i and the transformation factor of FA content from the FAME content. The reliability and accuracy of the analytical methods for the detection of the FAs was ensured by the use of the certified CRM 163 reference matrix (blended beef-pork fat; BCR), and these were in good agreement with the certified values. The FAs are expressed in % of total FAs (w/w) and as mg FA in 100 g of edible fish.

The data for the chemical composition of sardine meat were analysed by least squares analysis using the GLM procedure (SAS, 1999). The statistical model included the effects of season (S) and fish (F):

$$y_{ijk} = \mu + S_i + F_j + e_{ijk},$$

where y_{ijk} = the ijkth observation, μ = the general mean, S_i = the effect of the ith catching season (winter, spring, summer, autumn), F_j = the effect of the jth fish (1–24 fish), and e_{ijk} = the residual random term with a variance of σ^2_e.

The means of the experimental groups were obtained using the Duncan test, with relationships between the parameters assessed by Pearson correlation coefficients, using the CORR procedure (SAS, 1999).

3 RESULTS AND DISCUSSION

The fat content and the FA profiles of the sardine meat varied significantly according to the seasons of capture ($P \leq 0.001$). Table 1 gives the mean values for the chemical compositions, which are similar to other data reported in the literature (Castrillón *et al.*, 1997; Macciola *et al.*, 2003). The ash content was higher (2.5%) in comparison with other meats. The main reason for this was in the preparation of the samples, as they were prepared ready-to-eat: beheaded and gutted, but without the fish bones being removed as they are edible after cooking. The amount of total fat varied over the period of a year, in the range of 0.7% to 18.2% (Table 2).

Table 1: *Chemical composition of the sardine meat*
Preglednica 1: *Kemijska sestava mesa sardin*

Chemical composition, %	N	Mean ± SD	Min	Max	CV (%)
Protein Beljakovine	96	20.96 ± 0.98	19.62	22.63	4.68
Water Voda	96	70.79 ± 5.54	61.05	76.62	7.82
Fat Maščobe	96	6.42 ± 6.16	0.69	18.15	95.98
Ash Pepel	96	2.50 ± 0.25	2.13	2.83	9.86

N – number of observations / število vzorcev, – mean / povprečje, SD – standard deviation / standardni odklon, Min. – minimal value / minimalna vrednost, Max. – maximal value / maksimalna vrednost, SD – standard deviation / standardni odklon, CV (%) – coefficient of variation / koeficient variabilnosti.

Changes in fat content vary as the sardines drain or replenish their fat reserves in response to the availability of food, their spawning cycles and other factors in the sea (Hardy and Keay, 1972). Adriatic sardines spawn from the end of autumn to the end of winter, when little food is available, and therefore their fat stores are used up during this period. The sardines accumulate fat in their tissues for spawning and wintering in temperate seasons (summer, autumn), as is seen by the data given in Table 2.

Zlatanos and Laskaridis (2007) reported that sardines collected during winter had the highest lipid content (10.6%). Our findings are not in agreement with their results, whereby our data show that the sardines collected at the end of winter, and at the end of spring to the beginning of summer, had the lowest fat content (Table 2). The highest fat content was seen in sardines collected in late summer and autumn, and the values were much higher than those reported for the Greek study (8.3% to 15.1%, *vs.* 5.9% to 8.5%). Similar results for the fat content of marinated sardines (highest in late summer and autumn,

Table 2: *Fat content (%) of the sardine meat (N = 24) with respect to catching season*
Preglednica 2: *Vsebnost maščob (%) v mesu sardin v odvisnosti od sezone ulova*

Parameter, %	Winter /Zima	Spring / Pomlad	Summer / Poletje	Autumn / Jesen	P-value / p-vrednost
Fat / Maščoba	0.97 ± 0.22^{c}	1.26 ± 0.43^{c}	8.33 ± 2.38^{b}	15.11 ± 3.08^{a}	< 0.0001

Mean values ± standard deviation. *N* – number of sardines in each season. ^{a,b,c} – mean values of seasons with different letters are statistically significant different ($P < 0.05$)
Povprečne vrednosti ± standardni odklon. *N* – število vzorcev v vsaki sezoni. ^{a,b,c} – srednje vrednosti z različnimi nadpisanimi črkami se statistično značilno ($p \leq 0.05$) razlikujejo.

and lowest in winter and spring) were reported by Macciola *et al.*, (2003), while Hardy and Key (1972) saw lower levels of fat during spawning, due to the fat mobilisation associated with gametogenesis.

The FA composition of the fish investigated in the present study are given in Table 3. The data show remarkable and significant changes ($P \leq 0.05$ or less) in the individual FAs during this one-year period.

On average, the sardine meat contained 6.4% fat, and for the FA composition, 18.0% was mono-unsatu-

Table 3: *Fatty acid composition (% of total fatty acid) of the sardine meat (N=24) with respect to catching season*
Preglednica 3: *Maščobnokislinski profil (% od skupnih maščobnih kislin) maščobe v mesu sardin v odvisnosti od sezone ulova*

FA / % total FAs MK / % skupnih MK	Winter Zima	Spring Pomlad	Summer Poletje	Autumn Jesen	Mean ± SD Povprečje ± so	P-value p-vrednost
12:0	0.03 ± 0.01^{c}	0.06 ± 0.01^{b}	0.07 ± 0.01^{b}	0.08 ± 0.00^{a}	0.07 ± 0.01	< 0.0001
14:0	1.42 ± 0.07^{c}	5.67 ± 1.03^{b}	6.68 ± 0.30^{ab}	7.26 ± 0.37^{a}	5.94 ± 1.65	< 0.0001
15:0	0.46 ± 0.01^{c}	0.87 ± 0.09^{b}	0.83 ± 0.06^{b}	1.01 ± 0.04^{a}	0.86 ± 0.15	< 0.0001
16:0	23.13 ± 0.76^{c}	27.48 ± 1.37^{a}	24.73 ± 1.18^{bc}	26.30 ± 1.07^{ab}	26.24 ± 1.85	0.0057
16:1*cis*-9	1.36 ± 0.04^{c}	4.45 ± 1.07^{ab}	4.18 ± 0.14^{b}	5.39 ± 0.21^{a}	4.37 ± 1.22	0.0009
17:0	0.79 ± 0.01^{c}	0.95 ± 0.06^{b}	1.09 ± 0.04^{a}	1.14 ± 0.07^{a}	1.01 ± 0.12	< 0.0001
18:0	6.08 ± 0.39^{a}	4.99 ± 0.36^{b}	4.92 ± 0.24^{b}	5.79 ± 0.30^{a}	5.24 ± 0.53	0.0002
18:1*trans*-9	0.13 ± 0.03^{c}	0.14 ± 0.02^{c}	0.25 ± 0.01^{b}	0.36 ± 0.03^{a}	0.22 ± 0.09	< 0.0001
18:1*cis*-9	4.97 ± 0.01^{d}	6.87 ± 0.62^{c}	11.77 ± 1.54^{b}	14.38 ± 0.90^{a}	9.59 ± 3.56	< 0.0001
18:2*cis*-9,12	2.62 ± 0.02^{b}	3.03 ± 0.34^{a}	2.49 ± 0.07^{b}	2.99 ± 0.10^{a}	2.86 ± 0.33	0.0028
18:2 CLA	0.96 ± 0.01^{c}	1.99 ± 0.16^{b}	2.17 ± 0.04^{a}	2.04 ± 0.05^{ab}	1.96 ± 0.32	< 0.0001
18:3*cis*-9,12,15	0.73 ± 0.00^{c}	2.39 ± 0.28^{b}	3.52 ± 0.13^{a}	3.74 ± 0.06^{a}	2.83 ± 0.89	< 0.0001
20:1*cis*-11	0.50 ± 0.13^{d}	2.73 ± 0.23^{c}	4.03 ± 0.25^{a}	3.12 ± 0.19^{b}	2.95 ± 0.91	< 0.0001
22:1*cis*-13	1.82 ± 0.17^{a}	0.91 ± 0.11^{b}	0.59 ± 0.11^{c}	0.96 ± 0.15^{b}	0.92 ± 0.32	< 0.0001
18:4*cis*-6,9,12,15	0.85 ± 0.17^{c}	1.16 ± 0.08^{a}	1.04 ± 0.04^{ab}	0.95 ± 0.03^{bc}	1.06 ± 0.13	0.0027
20:5*cis*-5,8,11,14,17 (EPA)	6.94 ± 0.07^{b}	8.92 ± 1.23^{a}	7.78 ± 0.66^{ab}	7.23 ± 0.63^{b}	8.12 ± 1.21	0.0692
22:3*cis*-13,16,19	0.62 ± 0.04^{c}	1.63 ± 0.15^{a}	1.20 ± 0.11^{b}	1.03 ± 0.09^{b}	1.31 ± 0.35	< 0.0001
22:4*cis*-10,13,16,19	1.52 ± 0.03^{a}	0.95 ± 0.13^{b}	0.77 ± 0.10^{bc}	0.74 ± 0.08^{c}	0.90 ± 0.23	< 0.0001
22:5*cis*-7,10,13,16,19	1.04 ± 0.06^{a}	1.03 ± 0.07^{a}	1.03 ± 0.11^{a}	1.13 ± 0.12^{a}	1.05 ± 0.10	0.2041
22:6*cis*-4,7,10,13,16,19 (DHA)	44.03 ± 0.56^{a}	23.77 ± 4.40^{b}	20.88 ± 2.61^{b}	14.39 ± 1.39^{c}	22.50 ± 8.04	< 0.0001
SFA / nasičene MK	31.92 ± 1.24^{c}	40.02 ± 2.31^{ab}	38.32 ± 1.59^{b}	41.58 ± 1.79^{a}	39.36 ± 3.11	0.0007
MUFA / enkrat nenasičene MK	8.78 ± 0.27^{d}	15.10 ± 1.50^{c}	20.82 ± 1.76^{b}	24.20 ± 1.20^{a}	18.03 ± 4.88	< 0.0001
PUFA / večkrat nenasičene MK	59.30 ± 0.96^{a}	44.88 ± 3.50^{b}	40.87 ± 3.23^{b}	34.22 ± 1.99^{c}	42.60 ± 7.12	< 0.0001
ΣEPA+DHA	50.97 ± 0.63	32.69 ± 5.63	28.66 ± 3.27	21.62 ± 2.02	30.62 ± 9.25	< 0.0001
Σ*n*-3	55.73 ± 0.93^{a}	39.85 ± 3.82^{b}	36.31 ± 3.32^{b}	29.20 ± 2.07^{c}	37.78 ± 7.46	< 0.0001
Σ*n*-6	3.58 ± 0.03^{c}	5.02 ± 0.32^{a}	4.66 ± 0.10^{b}	5.02 ± 0.11^{a}	4.83 ± 0.46	< 0.0001
n-6/*n*-3	0.07 ± 0.00^{c}	0.13 ± 0.02^{b}	0.13 ± 0.01^{b}	0.17 ± 0.02^{a}	0.13 ± 0.03	< 0.0001
P/S	9.23 ± 0.28^{a}	7.26 ± 0.29^{c}	7.28 ± 0.21^{c}	7.92 ± 0.29^{b}	7.57 ± 0.62	< 0.0001
IA	0.43 ± 0.02^{b}	0.86 ± 0.11^{a}	0.86 ± 0.06^{a}	0.98 ± 0.07^{a}	0.85 ± 0.16	< 0.0001

Mean value ± standard deviation. N – number of sardines in each season. a, b, c, d – mean values of seasons with different letters are statistically significant different ($p < 0.05$). Σn-3 – sum of 18:3cis-9,12,15, 18:4cis-6,9,12,15, 20:5cis-5,8,11,14,17, 22:3cis-13,16,19, 22:4cis-10,13,16,19, 22:5cis-7,10,13,16,19 and 22:6cis-4,7,10,13,16,19. Σn-6 – sum of 18:2cis-9,12 and 18:2 CLA. IA – index of atherogenicity = (12:0 + 4 × 14:0 + 16:0)/(Σ(n-6) + Σ(n-3) + 18:1cis-9 + other MUFA) (Ulbricht and Southgate, 1991).

Povprečje ± standardni odklon. N – število vzorcev v vsaki sezoni. a,b,c,d – srednje vrednosti z različnimi nadpisanimi črkami se statistično značilno ($p \leq 0.05$) razlikujejo. Σn-3 – vsota 18:3cis-9,12,15, 18:4cis-6,9,12,15, 20:5cis-5,8,11,14,17, 22:3cis-13,16,19, 22:4cis-10,13,16,19, 22:5cis-7,10,13,16,19 in 22:6cis-4,7,10,13,16,19. Σn-6 – vsota 18:2cis-9,12 in 18:2 CLA. IA – indeks aterogenosti = (12:0 + 4 × 14:0 + 16:0)/(Σ(n-6) + Σ(n-3) + 18:1cis-9 + druge MUFA) (Ulbricht in Southgate, 1991).

rated, 42.6% polyunsaturated, and 39.4% saturated. The lipids of this sardine meat contained large proportions of palmitic acid (16:0; 26.2%) and DHA (22.5%). Oleic acid (18:1*cis*-9; 9.6%), EPA (8.1%), myristic acid (14:0; 5.9%), stearic acid (18:0; 5.2%), palmitooleic acid (16:1*cis*-9; 4.4%), α-linolenic acid (18:3*cis*-9,12,15; 2.8%) and docosapentaenoic acid (22:5*cis*-7,10,13,16,19; 1.1%) were all present as minor components.

The saturated FAs in the sardine fat ranged from 31% to 45%; the highest proportions being seen as palmitic (26.2%) and myristic (5.9%) acids. In spring, summer and autumn, the fat had a significantly higher weight percentage in the 16:0 palmitic acid than in winter ($P < 0.05$), presumably due to the spawning season and wintering. The content of the 14:0 myristic acid increasing during the year by more than five-fold, with its peak in autumn. In our study, these weight percentages of the 14:0 and 16:0 FAs were more variable in comparison with the values reported by Bandarra *et al.* (1997) and Zlatanos and Laskaridis (2007), which were seen to be particularly constant throughout the year and did not appear to be influenced by the diet of the sardines. This phenomenon could be explained according to the sea temperature and its oscillations: in the north Adriatic Sea, the temperatures oscillate from 8 °C in winter to 29 °C in summer and at the beginning of autumn. More food is available in the warmer periods, which the sardines accumulate as fat for the colder periods. In warmer seas, food is more uniformly available and therefore less fat accumulation is needed, which would explain the more constant saturated FA composition seen by others.

The mono-unsaturated FAs in the lipids ranged from 8.8% to 24.2%; with the highest proportions seen for oleic acid (18:1*n*-9C). Here, these 18:1*n*-9C levels (5.0% to 14.4%) were similar than those reported by Bandarra *et al.* (1997) in their Portugese study (7.4% to 14.3%) and Zlatanos and Laskaridis (2007) in a Greek study (3.5% to 10.6%).

The PUFAs in the sardine lipids ranged from 34.2% to 59.3%, with the highest proportions seen for DHA (mean, 22.5%) and for EPA (mean, 8.1%). The DHA content decreased from winter (44%) to autumn (14%), and generally the EPA content increased significantly during spring and summer, when compared to the winter-autumn period (8.9% and 7.8% *vs.* 6.9% and 7.2%, respectively).

Our data for the *n*-3 PUFAs are in agreement with the literature (Bandarra *et al.*, 1997), although they were higher (mean, 37.8%) than those reported by Zlatanos and Laskaridis (2007) in the Greek study (35.3%) and much higher than those reported by Luzia, Sampaio, Castellucci, and Torres (2003) in the Brazilian study (13.4% in summer). In the present study, there was a negative correlation between the fat content and that of the *n*-3 PUFAs: the *n*-3 PUFAs were low during the months with a high fat content ($R = -0.84$, $P = 0.0001$) (not presented in tables). In contrast, the saturated FA content increased during the months with a high fat content, in summer and autumn ($R = 0.81$, $P = 0.0001$). These data are in agreement with other studies (Bandarra *et al.*, 1997; Macciola *et al.*, 2003; Zlatanos and Laskaridis, 2007).

From the nutritional point of view, the P/S ratio, *n*-6/*n*-3 ratio and the atherogenic index were calculated. The P/S ratio was high (7.6 in average) due to presence of large content of DHA (22:6*cis*-4,7,10,13,16,19) and EPA (20:5*cis*-5,8,11,14,17) (Table 3). The *n*-6/*n*-3 ratio in the present study (mean, 0.13) was favourable mainly because of the low *n*-6 FA content. This ratio is considered to be a risk factor in cancers and coronary heart disease, and it is recommended that it is less than 10.0, and even less than 4.0 (WHO, 1994; Simopoulos, 2002; FSA, 2004).

The introduction of a correct combination of sardines and other food into the diet can assure a good balance for human nutrition. On the other hand, in the present study, the atherogenic index values of the sardine varied, from 0.43 to 0.98 (mean, 0.85), which is higher than recommended (lower than 0.72). The main reason for these high atherogenic index values appears to lie in the presence of 14:0 myristic acid. The myristic acid value according to the atherogenic index is enhanced because of the large influence of cholesterol in the blood (Ulbricht and Southgate, 1991). Aside from the relatively high atherogenic index, there can be further benefits from the high amounts of *n*-3 PUFAs that are seen, because they balance the *n*-6/*n*-3 ratio, which in a modern western diet is generally greater than 15:1 to 16.7:1 (Simopoulos, 2002).

The Food Standards Agency (FSA, 2004) has published recommendations for the daily intake of *n*-3 PUFAs: 0.45 g daily for the protection of the adult population. Chapkin (1992) recommends 0.8 g of EPA and DHA daily for a healthy adult population, while Simopoulos (2002) recommends 0.65 g of EPA and DHA daily (calculated on a 8,400 kJ diet). This should be increased twofold or more for the *n*-3 PUFA intake for a population with cardiovascular disease: from 0.9 g to 1.5 g of *n*-3 PUFAs (FSA, 2004), and up to 2 g to 4 g of EPA and DHA daily (AHA, 2003).

As can be seen from the present study, a healthy adult can satisfy their daily *n*-3 PUFA intake (0.45 g; FSA, 2004) by eating only 10 g of the sardine meat collected in the autumn, or 100 g of the sardine meat when the sardines are lean. Similarly, people with different cardiovascular diseases can cover the recommended amounts of the *n*-3 PUFAs by eating just 30 g of the sardine meat collected in autumn, and up to 330 g of the sardine meat

Table 4: *Mean FA levels (mg FA/100 g meat) in the sardine meat (N = 24) with respect to catching season*
Preglednica 4: *Povprečne vrednosti maščobnih kislin (mg FA/100 g mesa) v mesu sardine (N = 24) v odvisnosti od sezone ulova*

FA / mg FA/100 g meat MK / mg MK/100 g mesa	Winter Zima	Spring Pomlad	Summer Poletje	Autumn Jesen	Mean Povprečje
12:0	0 ± 0	1 ± 0	5 ± 0	11 ± 0	4 ± 1
14:0	12 ± 1	64 ± 12	501 ± 23	988 ± 50	343 ± 95
15:0	4 ± 0	10 ± 1	63 ± 5	137 ± 5	50 ± 9
16:0	202 ± 7	312 ± 16	1855 ± 88	3578 ± 145	1516 ± 107
16:1*cis*-9	12 ± 0	51 ± 12	314 ± 10	733 ± 28	252 ± 71
17:0	7 ± 0	11 ± 1	82 ± 3	155 ± 9	58 ± 7
18:0	53 ± 3	57 ± 4	369 ± 18	787 ± 41	303 ± 30
18:1*trans*-9	1 ± 0	2 ± 0	18 ± 1	49 ± 5	12 ± 5
18:1*cis*-9	43 ± 0	78 ± 7	883 ± 115	1956 ± 122	554 ± 206
18:2*cis*-9,12	23 ± 0	34 ± 4	187 ± 5	406 ± 13	165 ± 19
18:2 CLA	8 ± 0	23 ± 2	163 ± 3	277 ± 7	113 ± 19
18:3*cis*-9,12,15	6 ± 0	27 ± 3	264 ± 10	509 ± 8	164 ± 52
20:1*cis*-11	4 ± 1	31 ± 3	302 ± 19	424 ± 25	170 ± 53
22:1*cis*-13	16 ± 1	10 ± 1	44 ± 8	130 ± 20	53 ± 19
18:4*cis*-6,9,12,15	7 ± 1	13 ± 1	78 ± 3	129 ± 4	61 ± 7
20:5*cis*-5,8,11,14,17 (EPA)	61 ± 1	101 ± 14	584 ± 49	983 ± 85	469 ± 70
22:3*cis*-13,16,19	5 ± 0	19 ± 2	90 ± 8	140 ± 13	76 ± 20
22:4*cis*-10,13,16,19	13 ± 0	11 ± 2	58 ± 8	100 ± 11	52 ±13
22:5*cis*-7,10,13,16,19	9 ± 1	12 ± 1	77 ± 8	154 ± 16	61 ± 6
22:6*cis*-4,7,10,13,16,19 (DHA)	385 ± 5	270 ± 50	1566 ± 196	1957 ± 189	1300 ± 464
SFA / nasičene MK	279 ± 11	455 ± 26	2874 ± 119	5656 ± 244	2275 ± 179
MUFA / enkrat nenasičene MK	77 ± 2	172 ± 17	1561 ± 132	3291 ± 163	1042 ± 282
PUFA / večkrat nenasičene MK	519 ± 8	510 ± 40	3065 ± 243	4654 ± 270	2462 ±412
ΣEPA+DHA	446 ± 6	371 ± 64	2150 ± 245	2940 ± 274	1769 ± 534
Σ*n*-3	487 ± 8	453 ± 43	2723 ± 249	3971 ± 282	2183 ± 431
Σ*n*-6	31 ± 0	57 ± 4	349 ± 8	683 ± 16	279 ± 26
ΣFA/100 g meat / ΣMK/100 g mesa	874 ± 23	1137 ± 134	7500 ± 581	13602 ± 797	5778 ± 1273
g fat/100 g meat / g maščobe/100 g mesa	0.97 ± 0.03	1.26 ± 0.15	8.33 ± 0.65	15.11 ± 0.89	6.42 ± 1.41

Mean value ± standard deviation. N – number of sardines in each season. Σn-3 – sum of 18:3cis-9,12,15, 18:4cis-6,9,12,15, 20:5cis-5,8,11,14,17, 22:3cis-13,16,19, 22:4cis-10,13,16,19, 22:5cis-7,10,13,16,19 and 22:6cis-4,7,10,13,16,19. Σn-6 – sum of 18:2cis-9,12 and 18:2 CLA.
Povprečje ± standardni odklon. N – število vzorcev v vsaki sezoni. Σn-3 – vsota 18:3cis-9,12,15, 18:4cis-6,9,12,15, 20:5cis-5,8,11,14,17, 22:3cis-13,16,19, 22:4cis-10,13,16,19, 22:5cis-7,10,13,16,19 in 22:6cis-4,7,10,13,16,19. Σn-6 – vsota 18:2cis-9,12 in 18:2 CLA.

collected in spring. Also, for the DHA and EPA intake for a healthy population, 20 g of the sardine meat collected in autumn or 220 g of the sardine meat collected in winter would cover the daily recommendations, with higher amounts of the sardine meat covering the EPA and DHA recommendations for cardiovascular patients. The full data for the analysis of the individual FAs (expressed as mg FA/100 g edible sardine meat) are given in Table 4.

4 CONCLUSIONS

Although *n*-3 PUFAs are not only found in fish, as they are also present in flax seeds and nuts in particular, the EPA and DHA from fish fat are much more efficiently incorporated into the human body. α-linolenic acid, as a short *n*-3 PUFA, must be converted in the body to DHA and EPA, a process that is not particularly efficient in many people (and especially not so in the elderly), thus indicating that the direct consumption of DHA and EPA

is preferable. In conclusion, due to the increasing importance of the *n*-3 FAs for our health, the aim of this study was achieved: the definition of the FA composition of sardine collected in the Adriatic sea throughout the year, to provide this specific information for food specialists to include sardines in their menus for different kind of diets. The differences in the FA composition through the different periods of the year should also be taken into account in these diets.

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