Fatty acid profile and assessment of heavy metals content of *Sardina pilchardus* captured in the Algerian coast

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Received: April 2016 Accepted: September 2016

Abstract

Total lipids, fatty acid composition and heavy metal content of *Sardina pilchardus* fillet samples captured in February 2014 in Beni saf, Mostaganem, Ghazaouet, Algiers and Jijet coast were evaluated. Total lipid content was related to the five sites of catch (p<0.05), ranging from 7.18 g $100g^{-1}$ for Algiers to 10.07g $100g^{-1}$ for Beni saf. The fatty acid composition of *S. pilchardus* shows a high content of polyunsaturated fatty acids (PUFAs) dominated by n-3 fatty acids eicosapentaenoic acid (EPA C20:5 n-3) and docosahexaenoic acid (DHA C22:6n-3) with maximum rates of 15.75% and 33.42%, respectively (p<0.05). The saturated fatty acid (SFA) content was important in different samples of sardines, ranging from 35.50% to 41.32% according to sites of capture (p<0.05). Concerning the heavy metals, the levels of lead (Pb) ranged from 0.013mg to 0.024mg, however those of mercury (Hg) varied from 0.080 mg to 0.130 mg (p<0.05), which affects the health value of the fish species.

Keywords: Algerian coast, *Sardina pilchardus*, Fatty acids, Lipids, Heavy metals

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Introduction

Fish is considered as an essential amino acid and protein source for the global population (Ghaly *et al*., 2013). In Algeria, the amount of seafood is estimated at 100,000 tons per year (Algerian Ministry of Fisheries, 2013). In addition, marine fish contain other important nutrients for human consumption such as trace elements, lipid-soluble vitamins and polyunsaturated fatty acids (PUFAs). It should be noted that linoleic (C18:2, n-6) and linolenic (C18:3, n-3) acids are present in low amounts in marine fish, while eicosapentaenoic (EPA, C20:5, n-3) and docosahexaenoic (DHA, C22:6, n-3) acids are in high quantities (Artemis and Simopoulos, 1988). The steady growth of human populations results in higher fish consumption partly due to their omega 3–PUFA (ω-3) that confer a decisive role in the prevention of cardiovascular diseases (Bouderoua *et al*., 2011) and neurodegenerative syndromes such as Alzheimer's disease (Moyad, 2005). However, there is a wide variation in the fatty acid composition of fish of the same species. The nature of diet, seasonal variation and location are the fundamental factors affecting the fatty acid composition (Özogul and Özogul, 2007).

Currently, industries and human activities intensify the emission of various environmental pollutants (Yabanli, 2013) such as heavy metals for which the interest of determination in the aquatic environment and fish became necessary (Kayhan *et al*., 2010). Naturally, sea water contains low concentrations of heavy metals but their rates increase due to anthropogenic pollutants (Kargin and Dönmez, 2001). Fish and other aquatic life are continuously in contact with these pollutants (Burger *et al*., 2002) which accumulate in large quantities in fish (Olaifa *et al*., 2003). Some heavy metals such as cadmium and lead damage the kidneys, causing symptoms of chronic toxicity and other diseases such as cancers (tumors), and hepatic dysfunction (Abou-Arab *et al*., 1996).

The aim of this study was to determine the fatty acid profile of *Sardina pilchardus* fished in five different locations of the Algerian coast (Beni Saf, Gazahouet, Mostaganem, Algiers and Jijel) and to assess the levels of two heavy metals (lead and mercury), which may impair the health value of this fish species.

Materials and methods

Sample preparation

Sardina pilchardus samples were captured from five different sites along the Algerian coast during the month of February 2014. Concerning the west coast the fishing regions selected were: Beni Saf (latitude: 35° 18′ 08″ N; longitude: 1° 23′ 01″ W), Mostaganem (latitude: 35° 56′ 00″ N; longitude: 0° 05′ 00″ E) and Ghazaouet (latitude: 35° 05′ 38″ N; longitude: 1° 51′ 37″ W), while from the central and east coasts sardines were captured in Algiers (latitude: 36° 46′ 34″ N; longitude: 3° 03′ 36″ E) and Jijel (latitude: 36° 49′ 00″ N; longitude: 5° 46′ 00″ E) locations, respectively. 60 sardine

samples for each site of capture were maintained in polystyrene boxes containing ice and transported to the laboratory where metric measurements were carried out. The initial mean body of sardine samples ranged from 22 g to 34 g and the length from 13 cm to 18 cm. The fish were gutted and the head, viscera, and backbone were removed while the edible flesh was chopped and stored at -20^0C for further analysis.

Total lipids and gas chromatographic analysis

Total lipids were extracted by a mixture of chloroform/methanol (2:1, by volume) according to Folch *et al.* (1957).

Fatty acid methyl esters of all sardine samples were prepared using a solution of 0.5N KOH in CH_3OH (KOH–CH_3OH method) and extracted with 5 ml n-hexane (Nasopoulou *et al*., 2012). Fatty acid methyl esters were separated and quantified by gas chromatographer Shimadzu CLASS VP (GC-17A) (Kyoto, Japan) equipped with a split/splitless injector, flame ionisation detector and fused silica capillary column (60 mx 0.251 mm i.d ., 0.25 μm; Agilent, Santa Clara California, USA). The oven temperatures were programmed as follows: 120°C, held for 5 min, raised to 180°C at 10°C·min−1, then to 220°C at 20°C·min^{-1} and finally isothermal at 220°C for 30 min. The temperatures of the injector port and detector were held at 220°C and 225°C respectively. The carrier gas was high purity helium with a linear flow rate of 1 mL min^{-1} and split ratio of 1:50. Fatty acids are expressed as percentage of identified fatty acids and total amount calculated using an internal standard (C17:0).

Digestion and heavy metal assessment

One gram of each sardine sample was digested with a mixture of 10 ml of sulfuric acid (H_2SO_4, Sigma-Aldrich, USA) and 5 ml of nitric acid (HNO_3, Sigma-Aldrich, USA) for 60 min at 250 °C in an Automatic Kjeldahl Digestion Unit VELP (DKL, 8 Series) until the solution became transparent. Each solution volume was raised to 50 ml with distilled water and all the digests were filtered through a Whatmann filter paper to remove the impurities. The filtrates were transferred to glass tubes rinsed with 5% HNO_3, capped with polyethylene films and stored at room temperature until further analysis.

Heavy metals were determined using an atomic absorption spectrophotometer (AAS) Shimadzu AA – 7000. Metal concentrations (Pb and Hg) were determined as milligram of metal per kilogram of filets of sardine samples. Limits of detection were 0.05 and 0.06 $mgkg^{-1}$ for Pb and Hg, respectively. Lead and Mercury levels were recorded from the digital scale of AAS and calculated according to the following equation: C=R x (D/W). Where, C: concentrations of element ($mgkg^{-1}$), R: reading of digital scale of AAS, D: Dilution of prepared sample and W: Weight of the sample (Shaltout *et al*., 2015).

Statistical analysis

Statistical analysis was performed using ANOVA analysis (IBM SPSS software® version 20) followed by the Duncan test. Data were expressed as mean±SD and differences were considered significant for $p<0.05$.

Results

According to Table 1, the lipid content of all sardine samples was ranged from 7.18 % to 10.07 % for Algiers and Beni saf fillets sardine, respectively ($p<0.05$). Also, Table 1 gives the % of 12 FA for *Sardina pilchardus* caught in Algerian coast. The fatty acid compositions of sardine fillets ranged between 35.50 % and 41.32 % for saturated (SFA), 14.22–22.27% for monounsaturated (MUFAs) and 36.63-47.96 % for polyunsaturated acids (PUFAs).

Those occurring in the highest proportions were palmitic acid (C16:0, 20.10–27.84%), palmitoleic acid (C16:1 ω-7, 2.23–6.10%), oleic acid (C18:1 ω-9 cis, 5.57–16.07%), linoleic acid (C18:2 ω-6, 1.45–5.89%), eicosapentaenoic acid (EPA, C20:5 ω-3, 7.60–15.75%) and docosahexaenoic acid (DHA, C22:6 ω-3, 16.83–33.42%). It was also observed that the levels of these fatty acids changed significantly between different sites of catch ($p<0.05$).

The profile of fatty acids presents a dominance of the two classes, SFAs and PUFAs (Table 1). The proportions of PUFAs (ω-3) (ranging from 29.4% for Ghazaouet sardine to 42.4% for Mostaganem sardine) were higher than those of PUFAs (ω-6) (ranging from 2.58% Algiers sardine to 7.23% for Ghazaouet sardine). Concerning total PUFAs, the highest content was found for Mostaganem sardine (47.96%).

In this study, the first saturated fatty acid, contributing to 56.23-67.37% of the total saturated fatty acids (SFAs) was Palmitic acid (C16:0). Oleic acid (18:1 cis ω-9) was the most represented of the total MUFAs, particularly in Algiers (72.91% of total MUFAs) and Jijel sardine (72.15% of total MUFAs). For PUFAs, the major fatty acids identified were eicosapentaenoic acid (EPA, C20:5ω-3) and docosahexaenoic acid (DHA, C22:6ω-3). The highest EPA values were obtained in Beni saf and Ghazaouet sardine, accounting for 38.36% and 31% of total PUFAs, respectively ($p<0.05$). The high rates of DHA ($p<0.05$) were found approximately similar in Algiers, Mostaganem and Jijel sardine (69% of total PUFAs), whereas Beni saf (52% of total PUFAs) and Ghazaouet sardine (46% of total PUFAs) showed lower DHA contents.

Concerning the ratio n6/n3, this parameter varied from 0.06 to 0.24 and showed significant differences ($p<0.05$) between sites of catch of this study. At the same time the ratio PUFA/SFA of Mostaganem (1.13), Beni saf and Jijel sardine (1.15) were highest, whereas, the lowest values were registered for Algiers sardine (1.01) and Ghazaouet sardine (0.88).

Table 1: Total lipids (g 100g^{-1}) and fatty acids profile of *Sardina pilchardus* fillets caught in Algerian coasts (in % of identified FA).

	Sites				
	Algiers	**Mostaganem**	**Jijel**	**Beni Saf**	**Ghazaouet**
TL	7.18 ± 0.50^{d}	8.69 ± 0.32^{b,c}	7.80 ± 0.84^{d}	10.07 ± 0.74^{a}	9.17 ±0.93^{b}
C14:0	3.13 ± 0.19^{b}	3.06 ± 0.18^{b}	2.55 ± 0.40^{b}	6.73 ± 0.11^{a}	6.47 ± 0.48^{a}
16:0	27.27± 0.67^{b}	20.10 ± 1.83^{c}	27.63 ± 0.42^{a}	23.79 ± 0.23^{b}	27.84 ± 0.91^{a}
16:1 (ω-7)	2.23 ± 0.28^{b}	2.98 ± 0.16^{b}	2.57 ± 0.07^{b}	6.10 ± 0.34^{a}	5.79 ± 0.06^{a}
18:0	9.85 ± 4.94^{a}	12.58 ± 2.72^{a}	5.56 ± 0.03^{a}	4.98 ± 0.10^{a}	6.74 ± 0.16^{a}
18:1 cis (ω-9)	13.14± 0.83^{b}	5.57 ± 0.79^{e}	16.07 ± 0.20^{a}	7.18 ± 0.31^{d}	8.82 ± 0.32^{c}
18:1 trans (ω-9)	1.47 ± 0.17^{d}	2.44 ± 0.26^{d}	2.33 ± 0.05^{c}	3.62 ± 0.03^{b}	4.08 ± 0.06^{a}
18:2 (ω-6)	2.12 ± 0.23^{b}	2.83 ± 0.42^{b}	2.75 ± 0.02^{c}	1.45 ± 0.10^{b}	5.89 ± 0.08^{a}
18:3 (ω-3)	2.29 ± 1.15^{a}	1.13 ± 0.27^{a}	1.40 ± 0.01^{a}	1.09 ± 0.03^{a}	1.23 ± 0.02^{a}
20:1 (ω-6)	1.18 ± 0.09^{c}	3.23 ± 0.47^{b}	1.30 ± 0.04^{b}	3.72 ± 0.19^{a}	1.45 ± 0.67^{b}
20:4 (ω-6)	0.46 ± 0.12^{c}	2.73 ± 0.46^{a}	0.70 ± 0.01^{c}	1.38 ± 0.02^{b}	1.34 ± 0.01$^{a.b}$
20:5 (ω-3) EPA	7.74 ± 0.51^{c}	7.85 ± 1.74^{c}	7.60 ± 0.13^{c}	15.75 ± 0.15^{a}	11.34 ± 0.06^{b}
22:6 (ω-3) DHA	28.34± 0.53^{b}	33.42 ± 1.12^{c}	28.71 ± 0.82^{a}	21.38 ± 0.27^{d}	16.83 ± 0.74^{e}
Total SFA	40.25± 5.80^{a,b}	35.74 ± 4.73^{c}	35.74 ± 0.85^{b}	35.5 ± 0.44^{b}	41.32 ± 1.55^{a}
Total ω-9 FA	14.61± 1.00^{b}	8.01 ± 1.05^{d}	18.4 ± 0.25^{a}	10.8 ± 0.34^{c}	12.9 ± 0.38^{b}
Total MUFA	18.02 ± 1.37^{c}	14.22 ± 1.68^{d}	22.27 ± 0.36^{a}	20.62 ± 0.87^{b}	20.14 ± 1.11^{a,b}
Total ω-3 PUFA	38.37±2.19^{b}	42.40 ± 3.13^{a}	37.71 ± 0.96^{a}	38.22 ± 0.45^{a}	29.4 ± 0.82^{a}
Total ω-6 PUFA	2.58±0.35^{c}	5.53 ± 0.88^{b}	3.45± 0.03^{b,c}	2.83 ± 0.12^{b}	7.23 ± 0.09^{a}
Total PUFA	40.95 ± 2.54^{c}	47.96 ± 4.01^{d}	41.16 ± 1.00^{a}	41.05 ± 0.57^{b}	36.63 ± 0.91^{b,c}
PUFA/SFA	1.01±0.43^{b,c}	1.34 ± 0.84^{a}	1.15 ± 1.17^{b}	1.15 ± 1.29^{b}	0.88 ± 0.58^{c}
ω-6/ω-3	0.06 ± 0.15^{d}	0.13 ± 0.28^{b}	0.09 ± 0.03^{c,d}	0.07 ± 0.26^{b,c}	0.24 ± 0.10^{a}
At*	0.67±0.10^{b}	0.52±0.21^{b,c}	0.59±0.31^{b}	0.82±0.51^{a}	0.94±0.11^{a}

Results are means ± SD; n = 3. The values in the same line with different superscript letter (a-b-c-d-e) are significantly different (p<0.05).

At*: Index of atherogenicity, according to Ulbricht and Southgate (1991); calculated as: (4×C14:0+C16:0)/(ΣMUFA + Σ PUFA).

About heavy metals, the levels of mercury (Hg) and lead (Pb) in sardine fillets (*Sardina pilchardus*) captured from different sites of Algerian coast are shown in Table 2.

Lead levels in sardine ranged from 0.013 mg kg^{-1} in Ghazaouet to 0.024mg kg^{-1} in Mostaganem (p<0.05).

Table 2: The heavy metal concentrations (Pb, Hg) in fillets sardines (*Sardina pilchardus*) caught in Algerian coasts as mg/kg.

	Sites				
	Algiers	**Mostaganem**	**Jijel**	**Beni Saf**	**Ghazaouet**
Pb	$0.016\pm 0.01^{c,d}$	0.024 ± 0.02^{a}	$0.017\pm 0.01^{b,c}$	$0.018\pm 0.01^{a,b}$	0.013 ± 0.00^{d}
Hg	$0.091\pm 0.01^{c,d}$	$0.117\pm 0.03^{a,b}$	0.130 ± 0.01^{a}	$0.101\pm 0.02^{b,c}$	0.080 ± 0.00^{d}

Results are means±SD; n=3. The values in the same line with different superscript letter (a-b-c-d) are significantly different at 5% ($p<0.05$)

In this study, the levels of mercury varied from 0.080 mg/kg (Ghazaouet sardine) to 0.130 mg/kg (Jijel sardine) with a difference of 38.50% between these two sites ($p<0.05$).

Discussion

Generally, fish lipid content is affected by different factors such as fish diet, the different fish species, the season and the geographical origin (Rasoarahona *et al*., 2005). Lipid content obtained shows points of similarity to the study carried out by Bandarra *et al*. (1997); who found the value of 8 and 3g $100g^{-1}$ on *Sardina pilchardus*.

Concerning total PUFAs, the highest content was found for Mostaganem sardine (47.96%), analogous results were found by Bandarra *et al*. (1997) and Okada and Morrissey (2007) with levels total PUFAs of 42% and 45% respectively. Nevertheless, the results of this study appear distinctly higher than those found by Zlatanos and Laskaridis (2007) at the rate of 37.5% for the sardine caught in the east of the Mediterranean Sea. In this study, the eicosapentaenoic acid (EPA, C20:5ω-3) and docosahexaenoic acid (DHA, C22:6ω-3) were found in higher proportions. These PUFAs are considered fundamental for human and animal nutrition (Innis 2004). In the human diet, EPA is the most essential fatty acid of the ω-3 series because it is the precursor to the 3-series eicosanoids (Chen *et al*., 1995). DHA proportions obtained are clearly superior to that found by Bouderoua *et al.* (2011) within sardines caught in the Algerian coast (56.22% of total PUFAs). According to Watanabe *et al*. (1991), the DHA is considered more efficient than EPA as an essential fatty acid; which improves the health value of the sardine. Also, this research noticed a decrease of EPA at the expense of DHA. Similar results were obtained by Bouderoua *et al.* (2011) on Algerian *S. pilchardus* fillets with rates of 9.85% for EPA and 26.66% for DHA. According to Saito *et al*. (1997), the variation of fatty acid composition of fish depends on fishing ground, being influenced by environmental conditions and geographical effects.

For the ratio PUFA/SFA, the values obtained in this work (0.88-1.13) were higher than that (0.8) obtained by Özogul and Özogul (2007) for *Sardinella aurita* (Sardine).

Finally, the tests on heavy metal contents showed that the mercury concentrations (0.080 - 0.130 mg kg^{-1}) were higher than lead (0.013 - 0.024mg kg^{-1}). This heavy metal may be directly ingested by man or indirectly by fish (Olaifa *et al*., 2003).

Yabanli (2013) reported a mean lead concentration of 0.140 mg kg^{-1} in the sardine from İzmir (Turkey). According to the "South African Department of Health", the maximum lead level permitted in fresh and processed fish is 0.50 mg kg^{-1}, however, the "Commission of the European Communities" tolerates the maximum of 0.30 mg/kg (Bosh *et al*., 2015). In addition, mercury can be found in the human food chain from seafood (Plessi *et al*., 2001). In the literature, the maximum mercury content of sardine was estimated as 0.090 mg kg^{-1} (Shiber, 2010). These results revealed that mercury concentrations in different sardine samples were found to be higher than the legal limits; except in Ghazaouet sardine.

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