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Seasonal variation of lipid content and fatty acid composition of *Sardinella aurita* from the Tunisian coast

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This study was conducted to clarify the seasonal variation of the chemical composition of the Tunisian Sardinella aurita *with a focus on the total lipid content. The chemical composition showed a large fluctuation over years in response to various factors. For the entire fish, lipid content was lower in July (2.50%), but higher in November (10.25%). It varies with seasons in inverse proportion to water content. Interestingly, it was found that red muscle have much higher lipid content than white muscle and the entire fish body. The major fatty acids in* S. aurita *lipids were palmitic acid, palmitoleic acid, eicosapentaenoic acid, docosahexaenoic acid and myristic acid. Palmitic acid comprised the main proportion (23.9%). The high amounts of saturated and monounsaturated fatty acids in the screened species are almost in agreement with other studies. Moreover, the percentage of omega-3 fatty acids (25%) was very similar to that in oil production commercial fish.*

Keywords: pelagic fish, *Sardinella aurita*, fish oil, lipid content, PUFAs, ω-3 fatty acids

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INTRODUCTION

Tunisia is a major producer and exporter of seafood and the fishing sector is still of great economic, social and environmental importance. Tunisia seafood includes: tuna fish, shrimp, cuttlefish and squid, shellfish and mussels, octopus, sea bass and sea bream, sardines, anchovy, etc. (DGPA, 2004). The country's pelagic fish sources have been evaluated around 100,000 tons/year. However, the production has never exceeded 35% of the estimated value. Among the pelagic fish considered as products of low value, *Sardinella aurita* and *Sardina pilchardus* constitute the important proportion. Because of their abundance and lower price, these species should be used as often as possible. Therefore, these products could be utilized to produce oil of quality suitable for human consumption. According to Ackman (1990), generally, fish oils contain higher concentration of ω-3 polyunsaturated fatty acids (PUFAs). The major ω-3 fatty acids present are the eicosapentaenoic acid (EPA, C20:5 ω-3) and the docosahexaenoic acid (DHA, C22:6 ω-3). PUFAs receive intense interest in the scientific and industrial communities because of their positive effects on human health. Specifically, ω-3 fatty acids are known to exert therapeutic attributes on human cardiovascular diseases, hypertension autoimmune disorders and proper neural development (Uauy-Dagach & Valenzuela, 1996). Interestingly, many studies were conducted on the beneficial health properties of PUFAs. For example, it was demonstrated that fish oil supplementation has valuable effects on the electrophysiology of the heart, thereby reducing heart rate, a major factor for sudden death from cardiac disease (Mozaffarian *et al.*, 2005). Also, fish oil lowered blood lipid levels and reduced the risk of cardiac disease mortality (Studer *et al.*, 2005). Moreover, the addition of vitamin B_{12} and folic acid to fish oil reduces the risk of congenital birth defects, and is more effective in reducing menstrual pain (Deutch, 1995; Harel *et al.*, 1996). The most available fish oil on the market is fish body oil produced by pressing–heating of entire pelagic fish (Pigott, 1996). Up to now, there are no detailed studies of lipid and fatty acid composition carried out on pelagic fish from the Tunisian coast. Therefore, in order to start a preliminary feasibility analysis regarding the possibility of producing Tunisian fish oil, the need for future studies aiming to elucidate many factors such as the amount of fish and fish species available, and the variation of the composition over the year becomes a necessity. Hence, the objective of this study was to examine the seasonal changes over the year in lipid content and to analyse the fatty acids composition in the case of *Sardenella aurita* caught in the Gulf of Gabès area (Tunisia).

MATERIALS AND METHODS

Sardinella aurita species was caught from the Gulf of Gabès area (Tunisia). Fresh fish were sliced into pieces and homogenized by a meat grinder and used to determine the chemical composition. According to the AOAC (1990) methods, water content was quantified by drying samples at 100°C until weight constant, lipid by the Soxhlet extraction, proteins by the Kjeldahl procedure, and ash by incineration in a muffle furnace at 550°C (for 1 hour). Water and lipids were also determined for both red and white muscles separately.

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These analyses were performed over the year. In order to determine fatty acid composition, a homogenized sample of sliced flesh of fresh fish (caught in February) was extracted using water and methanol/chloroform (2v/1v) according to Kates (1972) method. Fatty acid methyl esters (FAMEs) were prepared by saponification with methalonic–NaOH mixture followed by transetherification with BF_3 in methanol (Morrison & Smith, 1964). Acid methyl ester (C23:0) was added as an internal standard. The determination of fatty acids was conducted using gas chromatography (Perkin-Elmer) equipped with flame ionization detector and Omegawax 320 fused silica capillary column (length 30 m × internal diameter 0.25 mm). The separation was carried out with helium as the carrier gas (1.13 ml/minute). The temperatures of the injector port and detector were held at 250°C and 260°C, respectively. The initial temperature of the column was held at 190°C for 20 minutes, then, increased at a rate of 4°C/minute to be maintained at 210°C for the last 25 minutes. The injected volume was 3 µl. FAMEs were identified by comparing the retention times with the standard 37 component FAME mixture and a menhaden oil standard (Supelco). Peaks areas were determined using the Varian software.

Data were subjected to Student's *t*-test to determine whether there is significant difference between means at the 5% level of probability or not. Each sample lot consisted of twenty-five fish. Values are expressed as mean ± SEM (standard error of mean). The software used was SPSS (version 11.0).

RESULTS

The water, ash, crude protein and total lipid contents of the entire *Sardinella aurita* are illustrated in Table 1. The moisture content over the year ranged from 67.78% ± 0.79 in November to 74.48% ± 0.65 in July with significant differences ($P < 0.05$). From November to February the moisture value remained almost constant and a significant enhancement was observed in April, June and July ($P < 0.05$). However, a significant decrease was shown from July to October, respectively.

The protein content varied slightly from 19.59% ± 0.56 to 20.36% ± 0.10 in November and in July respectively without significant differences over the year. The total lipids content ranged from 2.50 ± 0.65 to 10.25% ± 1.40 and the highest level was found in November. However, this value was not significantly different while compared to values obtained in December and February, respectively. The lowest lipid content (2.50% ± 0.65) was found in July with significant differences while compared to values obtained over the year ($P < 0.05$). Ash content varied also over the year, however it remained under a maximum of 2.32% ± 0.13 obtained in July.

The variation of lipids and water contents of *Sardinella aurita* (entire body, red and white muscles) are presented in Figure 1. Lipids content varies widely with seasons, and in inverse proportion to water content. Variations of total lipids content over the year were also observed in white and red muscles with minimum and maximum values in July and in November, respectively. Lipids varied from 1.32 to 5.55% in white muscle, while in the red muscle the observed variation ran from 6.99 to 18.50%. Interestingly, red muscle indicated significant higher lipids content ($P < 0.05$) than white muscle and entire body fish (almost 3 to 4 times). Additionally, the entire body of this species indicated significant higher lipids than white muscle ($P < 0.05$).

The fatty acids profile in *Sardinella aurita* analysed in February is presented in Table 2. The fatty acids are almost 90% of the total oil. Higher concentrations of palmitic acid (C16:0), palmitoleic acid (C16: 1n-7), eicosapentaenoic acid (C20:5n-3), docosahexaenoic acid (C22:6n-3) and myristic acid (C14:0) were observed. The main fatty acid having a concentration of 23.9% ± 0.66 was palmitic acid (C16:0) with significant differences ($P < 0.05$) in comparison to the other fatty acids. The fatty acids with the highest percentage after C16:0 were C16:1n-7, C20:5n-3 and C14:0 with values of 11.8% ± 0.39, 11% ± 0.25 and 7.4 ± 0.34, respectively. These values are significantly different ($P < 0.05$). Interestingly, in the Tunisian *Sardinella aurita*, the total SFAs, MUFAs and PUSFAs were 37.5, 21.2 and 31.6% of the total lipids, respectively.

DISCUSSION

From the first part of this work, devoted to specimens of *Sardinella aurita* collected from the Gulf of Gabès area (Tunisia), it can be concluded that the general composition (water, ash, proteins and lipids) undergoes large fluctuation in response to a variety of factors. The protein content remains constant and at high values over the year. This tendency supports the reports of many studies (Emokpae, 1983; Njinkoue *et al.*, 2002) according to which the protein content of fish changes very little with season. Recently, Tzikas *et al.* (2007) reported the same observation for the Mediterranean horse mackerel. Elsewhere, the total lipids content showed high variation (ranging from 2.50 to 10.25%). According to Rajasilta (1992), these changes are likely associated with the preparation for reproductive

Table 1. Seasonal variation of entire *Sardinella aurita* chemical composition (% wt/wt).

	Water	Lipids	Proteins	Ash
December	68.42 ± 0.32^a	9.45 ± 0.46^a	19.65 ± 0.02^a	2.03 ± 0.06^a
February	68.60 ± 0.20^a	9.33 ± 0.64^a	19.62 ± 0.77^a	2.02 ± 0.04^a
April	70.17 ± 0.19^b	7.42 ± 0.15^b	19.91 ± 0.40^a	2.01 ± 0.03^a
June	72.36 ± 0.76^c	4.87 ± 0.35^c	20.07 ± 0.44^a	2.24 ± 0.03^b
July	74.48 ± 0.65	2.50 ± 0.65	20.36 ± 0.10^a	2.32 ± 0.10^b
September	71.61 ± 0.36^c	5.77 ± 0.40^c	19.79 ± 0.32^a	2.22 ± 0.11^b
October	70.04 ± 0.40^b	7.65 ± 0.08^b	19.82 ± 0.30^a	2.02 ± 0.03^a
November	67.78 ± 0.79^a	10.25 ± 1.40^a	19.59 ± 0.56^a	1.95 ± 0.07^a

Values are expressed as mean ± SEM (N = 3). Values in a column with the same superscripts (a–c) are not significantly different at the 5% level.

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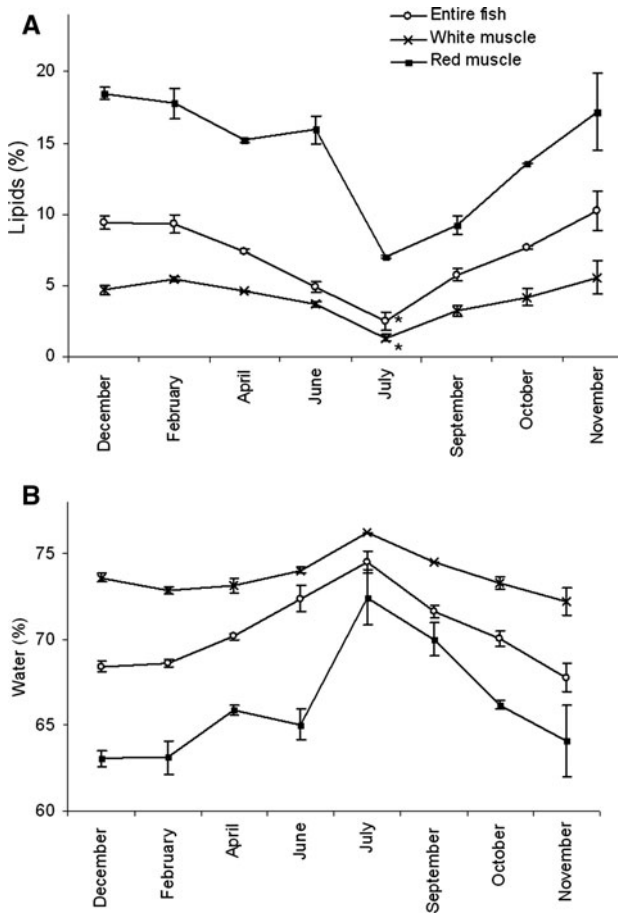

Fig. 1. Seasonal variation of (A) lipids and (B) water contents (% wt/wt) of *Sardinella aurita* (entire fish, red and white muscles). Values are expressed as mean ± SEM (N = 3); * values are not significantly different at the 5% level.

Table 2. February fatty acids profile in *Sardinella aurita* (% wt/wt of total lipids).

Fatty acids	Concentration (% wt/wt of total lipids)*
SFAs:	
C14:0	7.4 ± 0.22
C15:0	0.7 ± 0.02^{bc}
C16:0	23.9 ± 0.42
C17:0	0.7 ± 0.05^{bc}
C18:0	4.1 ± 0.05
C20:0	0.3 ± 0.01^{c}
C22:0	0.2 ± 0.01^{c}
C21:0	0.2 ± 0.01^{c}
∑ SFAs	37.5
MUFAs:	
C16:1n-7	11.8 ± 0.25
C18:1n-9	6.3 ± 0.11
C18:1n-7	3.1 ± 0.04
C20:1n-9	0.4 ± 0.06^{c}
∑ MUFAs	21.2
PUFASs:	
C16:2n-4	1.0 ± 0.01^{ab}
C16:3n-4	1.3 ± 0.02^{ab}
C18:2n-6	1.6 ± 0.02^{a}
C18:3n-6	0.5 ± 0.01^{c}
C18:3n-3	0.8 ± 0.02^{b}
C18:4n-3	1.5 ± 0.02^{a}
C20:2n-6	0.1 ± 0.01^{c}
C20:4n-6	2.1 ± 0.03
C20:3n-3	0.1 ± 0.00^{c}
C20:4n-3	0.5 ± 0.01^{c}
C20:5n-3	11.0 ± 0.16
C21:5n-3	0.3 ± 0.01^{c}
C22:5n-3	1.0 ± 0.01^{ab}
C22:6n-3	9.8 ± 0.11
∑ PUSFAs	31.6
∑ ω-3 FAs	25.0

(*), values are expressed as mean ± SEM (N = 4). Values with the same superscripts (a–c) are not significantly different at the 5% level. SFAs, saturated fatty acids; MUFAS, monounsaturated fatty acids; PUFAS, polyunsaturated fatty acids; ω-3 FAs, omega-3 fatty acids.

activities. It was reported that sexual maturity stage of the fish affects the lipid content due to increased consumption of fat reserves during the spawning period. Moreover, food availability and environmental water temperature are also among the affecting factors. Therefore, fish will have various lipid contents, depending on the breeding cycle and time of year (Beltran & Moral, 1991; Aidos *et al.*, 2002). Commonly, the variation of the general chemical composition has been described for different fish species, and this variation was reported to be related to many factors such as season, temperature, location, breeding cycle, diet, age, size and sex (Rajasilta, 1992; Bandarra *et al.*, 1997).

The variation of lipids and water contents of *Sardinella aurita* (entire body, red and white muscles) showed that lipids content varies widely with seasons, and in inverse proportion to water content. It is a fact widely recognized that water content in pelagic fish was inversely related to lipid content (Love, 1980; Méndez & González, 1997; Grigorakis *et al.*, 2002). For the entire fish, it is clear that in the warm season, when water was poor in nutrient and mineral salts, lipids contents decreased considerably dropping to 2.50% in July. However, Shirai *et al.* (2002) found that the lipids content of the Japanese sardine (*Sardinops melanostictus*) was higher in summer than in winter. Moreover, *Sardina pilchardus* showed a minimal fat content at the end of winter and a maximal value at the end of spring–beginning of summer (Zlatanos & Laskaridis, 2007).

Generally and as indicated above, lipids content variation is a function of many factors (season, temperature, breeding cycle, diet, age, size, sex, location, etc.). Nevertheless, the different influence of seasonality on the various kinds of fish from different geographical areas is probably affected by the different genetic cycle of the fish species (Karakoltsidis *et al.*, 1995; Luzia *et al.*, 2003).

Significant variations of total lipids content over the year were observed between white muscle, red muscle and the entire body of the fish. The significant higher content of lipids in the entire body could be explained by the fact that viscera and head contained lower percentage of the total lipids. The difference in lipids content between red and white muscles was reported for many fish species (*Sardina pilchardu, Clupea harengus*, *Gadus virens*, *Salmo solar*, *Scomber scombrus*, *Thunnus thynnus*, etc.) caught in different areas of the world (Love, 1980). These findings supported the hypothesis that, for many fish species, red muscle is used for

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Table 3. *Sardinella aurita* general fatty acids compared to others (%wt of total fatty acids).

Fish species	*Sardinella aurita*	*Sardinella aurita**	*Sardina pilchardus* W.	*Sardina pilchardus*	*Sardinops melanostictus*
Month of sampling	February	January	June	February	February
Region of sampling	Gulf of Gabès, Tunisia	Dakar, Senegal	Castellon, Spain	Northern Greece	Hyuga-Nada, Japan
SFAs	37.5	40.6	31.81	36.25	34.3
MUFAs	21.2	26.6	26.56	13.58	15.1
PUFAs	31.6	31.4	27.73	43.65	49.4
ω-3 FAs	25	29.1	26.62	40.86	42
References	Present study, 2008	Njinkoue *et al.*, 2002	Beltran & Moral, 1991	Zlatanos & Laskaridis, 2007	Shirai *et al.*, 2002

(*), results of fatty acids of red muscle; SFAs, saturated fatty acids; MUFAS, monounsaturated fatty acids; PUFAS, polyunsaturated fatty acids; ω-3 FAs, omega-3 fatty acids.

long, sustained swimming motion, and that lipid, rather than glycogen, is the main energy source for this activity (Yuan *et al.*, 2005). Moreover, it was reported that muscles of highly active species are richer in lipids as compared with those in species which are characterized by lower functional activity. In this perspective, it was reported that lipids are much concentrated in liver in the case of *Cephalopholis taeniops* (Njinkoue *et al.*, 2002). Generally, tissue concentration of lipids is closely related to the growth and the reproduction patterns (degree of maturity of gonads) of each species, as well as their exploitation in food resources, which are highly influenced by the dynamics of upwelling systems (Rajasilta, 1992). Furthermore, ecological and physiological peculiarities of fish significantly affect lipid characteristics of their tissues (Shchepkin & Shulman, 1978).

In the second part of this work, we analysed the fatty acids composition of *Sardinella aurita* lipids in February. The result is in agreement with the literature reporting C16:0 as the most abundant fatty acid in other studies on Mediterranean fish (Karakoltsidis *et al.*, 1995; Saglik & Imre, 2001; Zlatanos & Laskaridis, 2007). This observation was typical because palmitic acid is the key metabolite in fish (Andrade *et al.*, 1995). Zlatanos & Laskaridis (2007) reported that C16:0 was found to be the most abundant fatty acid for anchovy and picarel and this depends on the period of sampling. In the case of anchovy, a higher value of C16:0 was observed in February, August, October and December. However, for picarel it was also observed in February, August, October and December.

The fatty acids C16:1 N−7 (11.8% ± 0.49) and C20:5 N−3 (11% ± 0.31) were reported to have the highest percentage after C16:0. These results were different from those reported for other fish species (Karakoltsidis *et al.*, 1995; Guner *et al.*, 1998). For example, according to Zlatanos & Laskaridis (2007), the second most abundant fatty acid in the anchovy and in picarel was C22:6 N−3 with value of 12.23 and 14.23%, respectively (obtained in February). This difference in fatty acid profile among species suggests different biological functions for the various fatty acids in fish (Zlatanos & Laskaridis, 2007). Moreover, it is very important to note that fatty acid profile differs from one organ to another. Hooper *et al.* (1973) reported that in red muscle and liver, lipids undergo more enzymatic activities than in smooth muscle, allowing large amounts of free fatty acids in oils. In the Tunisian *Sardinella aurita*, the high amounts of saturated and monounsaturated fatty acids are almost in agreement with data obtained from other studies (Table 3). Generally, SFAs and MUFAs are abundant in fish from warm or temperate regions, compared to PUFAs which show high levels in fish from cold regions (Dey *et al.*, 1993). It is also important to mention that the total omega-3 content of the total fatty acids (25%) was comparable to many commercial marine fish oils such as Atlantic mackerel, *Scomber scombrus* (18, 8%) and Japan sardine, *Sardinops melanosticta*, (25, 9%) (Akman, 1990). Therefore, Tunisian *Sardinella aurita* seems to be a good source of omega-3 fatty acids recognized for their health benefits.

CONCLUSION

In this work, the obtained data on lipid extracted from *Sardinella aurita* were a first step towards the possibility of valorization of Tunisian marine products into production of fish oil, thereby contributing to a good utilization of the available pelagic sources. However, more investigations are needed to determine the whole inventory concerning the availability and the composition of the other lower value fish products suspected or expected to be used in fish oil process. A preliminary feasibility study and cost estimation of Tunisian fish oil production should be carried out taking in consideration many factors, especially the amount of the available fish products, the variation of the oil quality over the year and the possibility of reducing the cost of the process by producing, at the same time, other higher value added products (fish foods, media for bacterial growth, bioactive peptides, etc.) and by using as well by-products generated from fish processing industries.

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