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Seasonal Effects on the Levels of Omega 3 Fatty Acids in Swordfish *(Xiphias Gladius)* Muscles According to Body Parts

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Abstract

Contents and total levels of polyunsaturated fatty acids of n-3 and n-6 fatty acids were determined for swordfish samples collected during the four seasons and at seven body parts of swordfish. Variability in the levels of total fatty acids and polyunsaturated fatty acids in n-3 and n-6 was observed during the seasons and between samples taken at different locations. Seasonal variability is greater than the variability between body parts of swordfish.

A content of 33.7g/100g FW was observed in spring in white muscle (D1) and an amount of 26.7g/100g FW at the red muscle (RM) of the caudal part in summer. For the n-3, percentages of 44.5% (of TFA) at V2 was observed in the spring, at 37% D2 and D3 was 34% at V1 and D3 fall to 40% level of D1 and D2 in the winter. For red muscle, the percentages ranged from 30.2% in summer and 36.6% in winter. For n-6, the highest percentages were obtained at D1 (spring) and at V2 (summer) with respectively 12% and 10.6% (of TFA). During the four seasons, concentrations vary between EPA + DHA 267 mg and 8865mg / 100g (FW). Arguably, whatever the season, each body part of the swordfish is a good source of EPA + DHA.

Keywords: Fatty acids, TFA seasonal variations, EPA+DHA, Swordfish body parts.

Introduction

The muscle represents 45-65% of the live weight of the fish and constitutes the part that is consumed most by humans (Ackman, 1990; Kolditz, 2008).The muscular tissues of fish are made up of two main types of muscular fibers, i.e. oxidative fibers (red muscle) and glycolytic fibers (white muscle) (Broadhurst et al., 1998). Fish meat is one of the most important nutriments of humans. It could be due to the presence of n-3 polyunsaturated fatty acids (PUFA) in fish meat (Sidhu, 2003). Seafood are good sources of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3).Several studies have reported an inverse relationship between the consumption of fish rich in omega 3 and its impact on cardiovascular disease (Breslow, 2006). Thus, a higher DHA intake is associated with a decrease in systolic and diastolic blood pressure (Rasmussen et al., 2006).

Consumption of one to two servings a week of fish, especially species rich in omega-3 acids (EPA and DHA), reduces mortality in patients with coronary disease by 36% and overall deaths by 17% (Mozafferian and Rimm, 2006). Currently, the American Heart Association, AHA (2006) recommends the consumption of at least two portions per week and by person associated to other food sources rich in acid alpha-linolenic. AHA (2006) he AHA (2006) recommends that patients with cardiovascular disease should consume about 1 gram per day of EPA and DHA.

The biochemical composition of sea organisms undergoes seasonal variations (Orban et al., 2002a). The fatty profile of fish depends on the season and the characteristics of the specific sea area (Bandarra et al., 1997). This fatty profile is determined by certain factors like salinity and temperature (El Cafsi et al., 2003), periods of sampling, fishing area and individual variability (Leblanc et al., 2006). According to Ackman (1980), the fish adapt the composition of their lipids to the requirements of the environment and to their own physiological requirements. Their behaviour and their food preferences are directed toward this objective Ackman (1980). Bouhlel et al., 2007 confirm seasonal variations in the fatty acid compositions in the red muscle and the white one of three Mediterranean coastal species of Sparidae (*Diplodus annularis, D. sargus, D. vulgaris*).

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The objective of this study is to determine firstly the seasonal impact on the total fatty acids composition according to different sections of the swordfish body as well in the red and white muscles. Secondly, to spot the periods which are favourable to the consumption of this fish in the purpose to benefit of a healthy diet.

Materials and methods

Sampling

Six samples (n=6) of swordfish were collected at the wholesale market of Tunis. The sampled individuals hadn't yet reached first sexual maturity which is considered to be 140 cm in case of Mediterranean swordfish (Macias et al., 2005) to avoid the phenomenon of muscle lipid mobilization by the gonads observed during sexual maturation (Shearer, 1994). The lower jaw fork length of our samples varied between 55 and 104cm, gutted weight (GW) between 5 and 10.5kg.The temperature data of the sea surface (SST) in the central Mediterranean were identified. The sample characteristics are shown in Table I.

Samples for analysis

Three different zones to undergo analysis were selected: a frontal section (A) just under the first dorsal fin, a second central section (B) before the first anal fin and a third (C) near the second dorsal fin(Fig. 1).

We took two one-gram-samples of white muscles in each one of the three sections A, B and C, i.e : one in the dorsal area, and the remaining two from the ventral area, i.e.: D_1 and V_1 from section A, D_2 and V_2 from section B and D_3 and V_3 from section C. One sample of red muscle (RM) belongs to the dorsal area of section C. Only lipids of muscular tissue (white and red muscles) were analysed, disregarding any other type of lipids such as dermal lipids. We analysed seven anatomical samples of muscular tissue, i.e. D_1, V_1, D_2, V_2, D_3, V_3, RM. The anatomical areas as well as the levels of practised sections are recapitulated in figure 2. The muscular fragments were selected from the right side of the fish. Samples were stored in a freezer at -28° C.

Extraction of total fatty acids

The extraction of total fatty acids (TFAs) was performed in the presence of chloroform-methanol (2:1 v/v) (Folch et al., 1957). The total lipids obtained were stored in chloroform-methanol- butylated hydroxytoluene (BHT) at -28°C. For further analysis, the fatty acids were transformed into methyl esters, according to Cecchi et al. (1985). The quantification of the fatty acids is based on an internal standard not present in our samples, methyl nonadecanoate or $C_{19:0}$ (Sigma Aldrich, Corporate Headquarters, St Louis, MO).

Identification and quantification of fatty acids

Methyl esters of TFAs were separated, identified, and quantitated by gas chromatography using a HP 6890 gas chromatograph with a split/splitless injector with electronic pressure control and a flame ionisation detector was used for the analysis. Separation was performed with a 30 m HP Innowax capillary column with an internal diameter of 250 lm and a 0.25 lm film thickness, the stationary polar phase of the column being polyethylene glycol.

Statistics

The results represent the average of six fish (n = 6) per season. In total, 24 fish have been analysed. The statistical analyses were carried out with the SAS software program version 6.12. Different mean values were analysed according to the Duncan's multiple range test. The result is considered significant if $p < 0.05$.

Results and discussion

The cycles of storing lipid in fish are directly connected with food abundance. If there is scarcity of food in their environment the variation is low, but if it is abundant, the variation is higher during the year (Mute *et al.*, 1989). In terms of percentages, the overall seasonal levels vary between 4.3% and 17.3% (Table 2). These results are similar to those of Luzia et al (2003) who observed values ranging from 0.94% to 10.6%.

Specimens of swordfish taken per season during one year, revealed that the weight of gutted fish were lowest in winter and autumn compared to those collected in spring and summer having the highest weight (table 2). Meanwhile, there was also a significant increase in the amount of total fatty acids (TFA) per 100g of gutted fish in the spring (17.3g/100g GW) which continued until the summer (16.5g/100g). The percentage of body fat of the fish depends on the stage of its life cycle and its energy consumption (Jobling, 1994). It is also shown that *Diplodus puntazzo* has a faster growth rate when the water temperature is high (Garcia et al. 2001). (Garcia et al. 2001).

The lipid content and fatty acid profile of fish vary depending on the species, the body parts and on the type of the muscles (red or white). These variations are attributed, in addition to temperature and the life

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cycle stages of the fish, to the prey availability and their abundance (Saito *et al*., 1999). The total fatty acid contents TFA (g / g GW 100) contained in each swordfish muscle area (D1, V1, D2, V2, D3, and RM V3) according to the seasons are listed in table 3. The TFA content in both the white and red muscles of swordfish varies significantly according to the origin of the sample (D_1, D2, D3, V1, V2, V3 and RM) and the season. The mean levels of TFA in the muscle, varying from 12.3g (D1), 10.5g (MR) to 3.7g (V3), show a different distribution of TFA according to the areas. In addition, TFAs contents of each zone vary depending on the season. Thus, areas D1 (33.7g), V1 (10.2g), D2 (7.8g) and V2 (11.8g) show a high concentration of TFA in the spring while the D3 (6.1g), V3 (11.5g) and MR (26.8g) areas are characterized by their higher amounts in summer.

In terms of nutrition and according to the season, muscle areas that are most benefit to the consumer i.e. the richest in lipids are the areas D1, V1, D2 and V2 in spring and the caudal areas, D3, V3 and MR in summer. Fish is a highly nutritious food, it is an excellent source of protein, vitamin D, iodine, taurine and polyunsaturated fatty acids n-3 PUFA (Rossano et al., 2005). In contrast, several studies have reported an inverse relationship between the consumption of fish rich in n-3 PUFA and its impact on cardiovascular disease (Breslow, 2006).

Today it is known that PUFA n-3, or a balanced n-3/n-6 ratio in the diet, are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, diabetes, hypertension and cancer (Gökçe et al., 2004). It is important for human health to increase the consumption of fish or fish products, which are rich in PUFA n-3 family and poor in PUFA n-6 family (Sargent, 1997).The seasonal variations of the composition of PUFA n-3 of swordfish are illustrated in table 4.

Throughout the year, the percentages of n-3 PUFA exceed largely those of the n-6 series (table 4). The four season averages for the n-3 PUFA varied from 33% to 36% of TFA. For the n-6 PUFA, a significant variation is observed between the muscle areas. The highest percentage was observed in V2 area (8%) and the lowest in the red muscle (4.8%). The highest ratios of n-3/n-6 were observed in spring in V3 area (9.4) and in summer in V3 area (12.1). While the lowest value, was found in the D3 area (7.4) in autumn and in V1 area (7.5) in winter.

An increase in the n-3/n-6 ratio is essential to help the body use n-3 fatty acids. A low ratio indicates that the enzymes that convert fatty acids to their active forms are likely to be used by n-6 PUFAs (Hossain, 2011).

Usually, fish with high fat content has been considered to be an important nutritional species since they have a relatively high content of n-3 FAs. Figure 3 shows a high correlation between TFA and EPA + DHA. Similar results were found in teleosts and elasmobranchs from deep sea (Okland et al., 2005). However, it has been demonstrated that there is an inverse relationship between the amount of n-3 FAs and the total fat content in the cod liver (Breivik et al., 1997).

It is widely recognized that n-3 fatty acids, especially those with very long chain such as DHA and EPA are important for human nutrition. To provide consumers with information on fatty acids having an impact on the nutritional, levels of EPA + DHA are expressed in mg/100 g fresh weight (FW). Table 5 expresses the seasonal variations for the seven muscle areas.

It appears from the analysis of the results shown in Table 5 that the seasonal averages of EPA + DHA (mg/100 g FM) vary so significantly ($p <0.05$) in the swordfish muscle. The highest concentration was observed in the spring (3045 mg) and lowest in autumn (981) and winter (955mg). From this analysis, we retain that the storage of fatty acids varies significantly ($p <0.05$) between the different muscle areas. The most important storage areas are D1 and the red muscle with respective levels of 2733mg and 2569mg

This study confirms that swordfish is a suitable source in the human diet when the amounts of TFA, PUFA n-3, EPA + DHA and the ratio of n-3 / n-6 are taken into consideration.

A small fish consumption (100g) would be necessary for the consumer if he wishes to acquire the recommended daily intake: 200-1000 mg of EPA + DHA (Inhamuns and Franco, 2008).

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Table 1.Size and weight of the Swordfish specimens collected per season and the temperature of the sea water

		Lower jaw fork length (cm)	Gutted fish (weight in kg)	Water temperature (°C)
2010-2011	Winter	85–104	5–10.5	18-16
	Spring	95–100	9–10	16-18
	Summer	90–96	7– 8	22-27
	Autumn	85–90	5–7	25-21

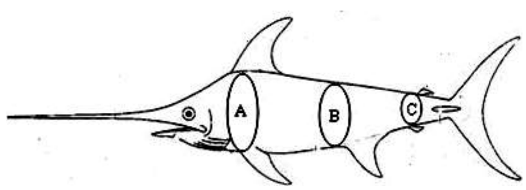

Figure 1. The sampling zones across the sections

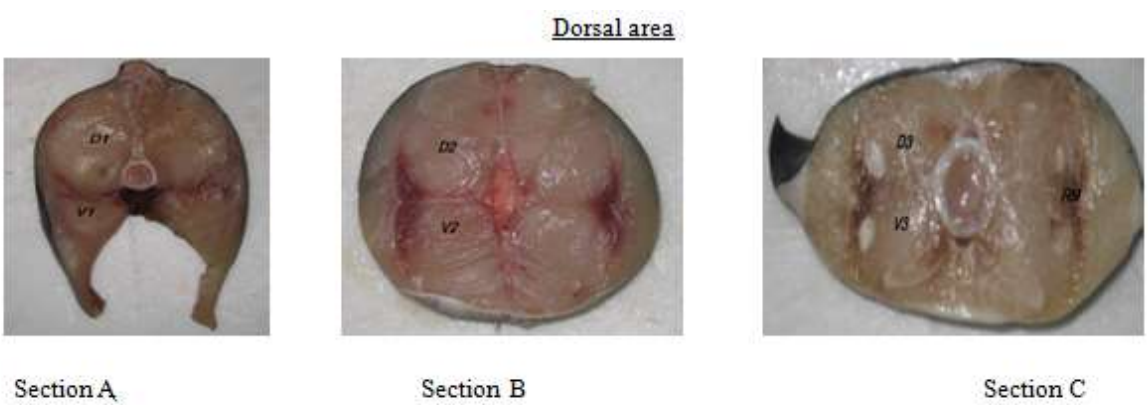

Figure 2. Sections A, B and C with their anatomical areas

Table 2. The seasonal variations of the total fatty acid (mean ± SE; TFA values with different subscripts were significantly different at p < 0.05)

	Lower jaw fork length (cm)	Gutted fish	Total fatty acid

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		(weight, kg)	(g/100g GW)
Winter	85–104	5–10.5	4.3±0.5^{b}
Spring	95–100	9–10	17.3±6.0^{a}
Summer	90–96	7– 10	16.5±3.6^{a}
Autumn	85–90	5–7	8.8±1.4^{b}

Table 3. Seasonal variations in Total fatty acid (g / 100 g GF) per anatomical (mean ± SE;TFA values with different subscripts were significantly different at p < 0.05)

	D_1	V_1	D_2	V_2	D_3	V_3	MR
Winter	5.1±1.1^{b}	3±0.8^{bc}	2.7±0.7^{b}	1±0.1^{b}	3.3±0.9^{a}	1.4±0.6^{b}	4.5±1^{b}
Spring	33.7±7.3^{a}	10.2±2.9^{a}	7.8±2.3^{a}	11.8±1.9^{a}	4.9±1.2^{a}	1.5±0.6^{b}	3.5±1.8^{b}
Summer	9.1±3.1^{b}	8.3±3.2^{ab}	6.±1.5^{ab}	2.3±1.9^{b}	6.1±2^{a}	11.5±3.2^{a}	26.8±7.7^{a}
Autumn	8.4±3.7^{b}	2.1±0.3^{c}	2.6±0.9^{b}	2.1±0.6^{b}	4±0.9^{a}	1.2±0.1^{b}	8.2±0.8^{b}

Table 4.Seasonal variations in n-3 (% of TFA), n-6 (% of TFA) and n-3/n-6 ratios per anatomical (mean ± SE; values with different subscripts were significantly different at p < 0.05)

		D1	D2	D3	V1	V2	V3	MR
Spring	n-6	12.3±3.8	6±1.2	7.1±1.1	5.2±0.9	11.6±4	4±0.7	4.5±0.4
	n-3	35.5±2.4	40.9±1	38.9±4	36±2.4	44.5±2.6	32.8±2.7	31.9±2.1
	n-3/n-6	4.1	7.9	6.4	8.3	6.5	9.4	7.1
Summer	n-6	4.8±0.6	5.8±0.8	4.6±0.6	3.7±0.3	10.5±4	3.8±0.7	4.6±0.6
	n-3	29.9±3.1	36.9±2.7	37.7±1.6	25.5±4.4	32.2±4.1	33±4.5	30.2±3
	n-3/n-6	6.7	7.4	8.6	6.6	5.7	12.1	6.7
Autumn	n-6	5.4±0.7	4.8±0.3	5±0.3	5.7±0.8	5.1±0.4	5.8±0.2	4.9±0.6
	n-3	33.4±2.1	30.1±2.9	34±2.3	34.2±1.7	31.9±3.1	32.6±1.62	32.6±1.2
	n-3/n-6	6.8	6.2	7.2	7	6.5	5.7	6.9
Winter	n-6	6.3±0.5	7±0.8	5.1±0.1	4.7±0.1	6.8±0.6	5.1±0.5	4.8±0.3
	n-3	40±2.4	39.8±1.6	36.6±1.5	35.1±4.5	33.1±1.7	35.1±0.7	36.6±3.3
	n-3/n-6	6.9	6.2	7.2	7.5	5.3	7.3	8

Table 5.Seasonal variations in EPA+DHA content per anatomical area (mg/100g GF)

	D1	D2	D3	V1	V2	V3	MR
Spring	8865	2586	1366	2629	3654	267	2324
Summer	1992	1400	1815	970	817	3300	7084
autumn	1741	728	1201	618	546	416	1621
Winter	1252	930	1056	877	319	883	1318

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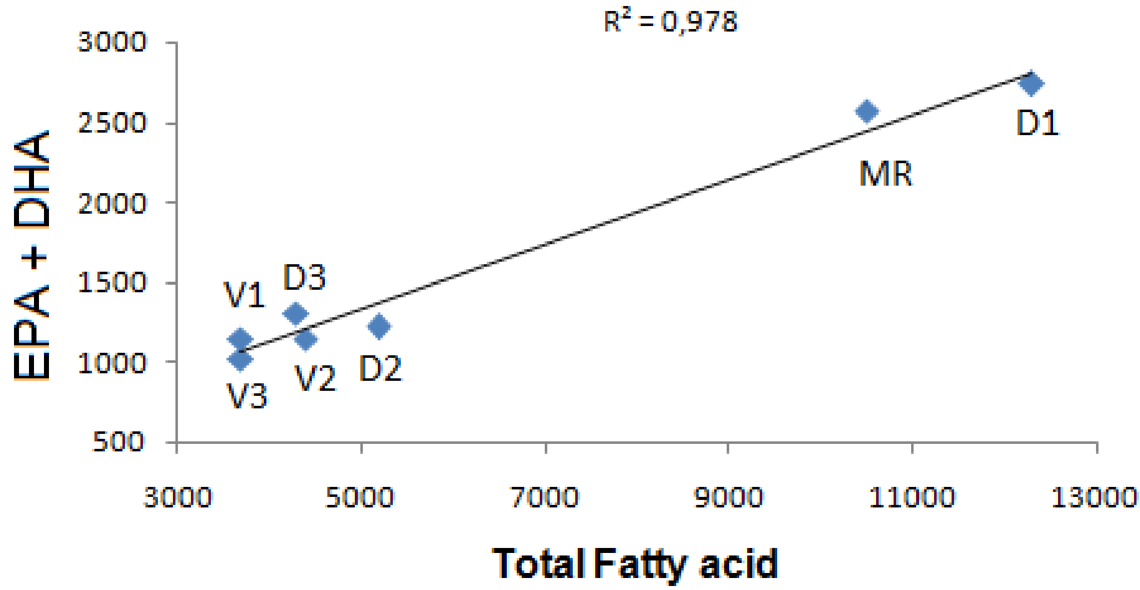

Figure 3. Relationship between Total fatty acid and PUFA n-3 in muscles of Tunisian swordfish (expressed as % total fatty acids)