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Fatty Acid Composition in Phosphatidylcholine, Phosphatidylethanolamine, Triacylglycerol and Diacylglycerol of the Muscle and Skin Swordfish

(Xiphias Gladius)

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Abstract

The objective of this study was to quantify the phospholipids in swordfish that are present in both types of muscles, white and red, and in the skin. The content in total lipids was 5.1% in white muscle, 4.5% in the red muscle and 0.5% in the skin. The content in phospholipids was 52mg/100g, 84mg: 100g and 106mg/100g, respectively in the white muscle, the red muscle and the skin. Phosphatidylcholine (PC) dominated in the skin and in the red muscle with respective rates of 61mg and 51mg/100g.

The concentrations of phosphatidylethanolamine (PE) were 41 in the white muscle, 41mg in the red muscle and 44.8/100g within the skin. Polyunsaturated fatty acids (PUFA) were dominant in the PC red muscle with a rate of 16%. The most important were linoleic acid (LA) (5.6%) and EPA (3.4%). PUFAs were the most represented in the PE of the white muscle (9.4%) and the skin (8.8%). In the white muscle, PUFA were represented by linoleic acid (1.9%), while in the skin, they were represented by the EPA (4.7%).

Keywords: Phosphatidylcholine, Phosphatidylethanolamine, Triacylglycerol, Diacylglycerol, *Xiphias gladius*. **Introduction**

Fish is consumed for its high protein, poverty in saturated fat and richness in polyunsaturated fatty acids to support good health (Harper and Jacobson, 2001; Donadio and Grande, 2004; Holub and Holub, 2004; Blanchet et al., 2005).

In fact, the basic reference to classify fish is the capacity of the muscular tissue to stock lipids (Ackman, 1994). The lipid content and fatty acid profile of fish vary from one area to anathor and even between red muscle and white muscle. 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) (Nedjedly et al., 2011).

The polyunsaturated fatty acids (PUFAs) are recognized as essential biochemical components of human diet because of their beneficial effects on human health (Sushchik et al., 2007). The bioavailability of PUFA increases when carried by phospholipids (PL) such as hosphatidylcholine (lecithin) and triacylglycerol (TAG) (Payet et al., 2004). Phospholipids are important components of the cell membranes. They are found in vital organs such as the brain and liver. The main phospholipids of animal tissues, including fish, are phosphatidylcholine (PC) or lecithin, phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (SM) (Tocher et al., 2008). Neutral lipids (NL) serve mainly as lipid reserves used as an energy source (Henderson and Tocher, 1987).

PC, playing a beneficial anti-atherogenic role in the reduction of the cardiovascular risk, is -heavily involved in the functioning of the heart muscle cells (Chanussot, 2008).

Fish PC contains PUFA n-3 already metabolized by fish. It is a phospholipid with a significant amount of choline and phosphorus necessary for the reservation of cellular energy. The particularity of this PC is its animal origin which makes the closest to the human body (Dupont, 2006).

Among the large pelagic fish as the *X. gladius* a highly variable amounts of lipids are observed in the liver (26g), gonads (4.7 g) and red muscle (4.5 g) (Ben Smida et al., 2009). This fish could be a potential source of phospholipids. Not only knowledge of the lipid composition of fish in general and phospholipids in particular can provide essential information on possible sources of PC in the human diet, but it can also help to adjust the nutritional recommendations to consumer needs and patients. For this reason, we

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propose to determine the fatty acid composition of phosphatidylcholine, phosphatidylethanolamine, triacylglycerol, diacylglycerol in the skin, in the white and red muscles of swordfish.

Materials and methods

Fresh swordfish samples were collected at the wholesale market of Tunis in December 2013. They had not reached first sexual maturity yet, which is 140 cm in the Mediterranean Sea (Macias et al., 2005). The lower jaw fork length of our samples (n = 6) varied from 55cm to 104 cm while their gutted weight was between 1.4 and 10.5 kg. The temperature of the sea water surface (SST) varies between 16° and 18°C.

A body part of the white muscle (D1) and a skin fragment (P) have been taken from the first dorsal fin. Samples of red muscle (RM) were taken from the caudal fin. Thus, three samples of 1 g were taken from the right side of the fish.

Total lipid was extracted with chloroform/methanol (2:1 v/v) containing butylated hydroxytoluene (BHT) as antioxidant (Folch et al., 1957). The organic solvent was evaporated under a stream of nitrogen. Samples were stored in a freezer at -28° C.

Analysis of the three body parts, the white muscle (D1), the red muscle (MR) and the skin (P) would determine the levels of PC, PE, TAG, DAG and fatty acids (FAs) present in lipids classes. These lipid classes were separated by thin layer chromatography (20cm x 20cm x 0.2 mm silica gel 60 Merck, Darmstadt, Germany).

Three body parts, the white muscle (D1), the red muscle (RM) and the skin (P) will be characterized by their levels of PC, PE, TAG, DAG and fatty acids (FA) present in these lipid classes. Lipid classes were separated by a thin chromatography layer (20cm x 20cm x 0.2 mm, silica gel 60, Merck, Darmstadt, Germany).

For polar lipids, PC and PE classes were separated according to the method of Lepage (1967). For neutral lipids, the separation of DAG and TAG classes were performed according to the method of Wood et al. (1969). The revelation of lipid classes was made by exposure to UV plates after spraying dichloro-fluorescein. Reference standards were used to identify PE, PC, DAG and TAG present in the three body parts.

For further analysis, the fatty acids were transformed into methyl esters according to the Cecchi and al. (1985) method. A gas chromatograph type HP 6890 with a split/splitless injector with electronic pressure control and a flame ionization detector was

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used for analysis. Separation was performed with a 30 m HP Innowax capillary column with an internal diameter of 250μ m and a 0.25μ m film thickness, stationary polar phase of the column being polyethylene glycol.

The different fatty acids swordfish were obtained by the retention times of the fatty acids under study and those of a mixture of methyl esters (SUPELCO PUFA-3). The quantification of the fatty acids is based on an internal standard not present in our samples, methyl nonadecanoate or $C_{19:0}$ (Sigma). The results represented the average of six replicates (n = 6). Mean comparison was performed by analysis of variance (ANOVA) followed by Duncan test at the significance level of p<0.05. All statistical analyses were carried out using the software program SPAD 4.

Results and discussion

In swordfish, contents (g/100g fresh weight) in TFA muscle area veried from 3.98g to 4.62 g. While in the skin, this content is 0.57g. There is no significant variation between white muscle and the red one. Ackman (1994) confirmed that fish with a medium lipidic storage rate contain 4-8 out of 100g of muscular tissue. The rate of total fatty acids (TFA) contained both in swordfish and in other species depends on the location in the different parts of tissues (Kitchell et al., 1997). The results of this study show that consumption of swordfish provides consumers with important amounts of TFA (Table 1).

Among the phospholipids (PL), five were classified: PI, PS, PC, PE and diphosphatidylglycerol or cardiolipin (DPG). PC and PE were the major compounds in the PL in the three body parts (D1, RM and P). The results are shown in table 2. Among the neutral lipids (NL), six lipid classes were identified. These were MAG, free cholesterol (Chol. L), diacylglycerols (DAG), free fatty acids (FFA), TAG and choletérol esterified (Chol. East). Only TAG and DAG were used (Table 2).

According to Table 2, the white muscle (D1) is twice richer in neutral lipids than the red muscle (RM). This result is explained by the content of DAG of the white muscle which is equivalent to 10.5 times the amount of DAG in the red muscle. The phospholipids content of the red muscle is 1.6 times as important as that of the white muscle. This result is explained by the presence of the PC in the red muscle whose content is four times the amount of the white muscle. The skin, considered as coproduce, is 2.8 times more concentrated in neutral lipids than the white muscle. The skin contains five times as much TAG as the white muscle. The variability of the muscle lipid content, therefore, results from the

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changes in the content of TAG (Ando et al., 1993). This result has been verified

in the red muscle. In the white muscle, TAG and DAG contents are very high (Table 2).

The polar lipids are the major components of the cell membranes; their content and composition are relatively constant (Médale, 2009). They consist mainly of PC (50 to 60% of PL) and PE (20 to 30% of PL) (Aursand et al., 1994). According to table 2, the PC displays a relative percentage of 61% (of PL) in the red muscle (RM),- 58% (of PL) in the skin (P) and against 25% in the white muscle (D1). For PE, the percentages shown are significantly higher and are 48% (RM), 42% (P) and 78% (D1). The net decrease in amount of phospholipids (PC+PE) in the white muscle during winter indicates that components were catabolized during this period. The net increase in the relative percentage of phospholipids in the red muscle suggests that the rate of degradation was slower than that of the TAG. Changes in the composition and metabolism of phospholipids in the biological membranes are responses to environmental factors, particularly temperature (Hazel and Williams, 1990).

A change in the lipid structure is from adaptive response which provides an organism with the ability to survive under conditions of physiological limits (Svetlana et al., 2013). This change in structure is a compensatory mechanism for maintaining the membrane such as fluidity, permeability, the mobility of membrane components, the transmission activity and the activity of the ionic membrane enzymes. In consequence, the membranes are able to optimize the activity of their various functions (Arts et al., 2009).

The PC/PE ratio is 1.37, 1.26, and 0.32 respectively in the skin, the red muscle and the white muscle. It is known that the PC/PE ratio of a body decreases during thermal adaptation and acclimatization (Murzina et al., 2013). There is a preservation and / or synthesis of PE, as indicated in cod's eggs that are rich in phospholipids (Fraser et al. 1988). The gas chromatography evaluates the trace of each molecular species of different lipid classes in the swordfish (Fig. 1).

Table 3 shows the fatty acid composition of lipid classes of three different body parts of swordfish. Palmitic (C16: 0), stearic (C18: 0), oleic (C18: 1n-9), octadecatrienoic (C18: 3n-4), and eicosapentaenoic (EPA, C20: 5n-3) are the major fatty acids of this fish with respective percentages of 14% -61.2% 0.4% - 34.2% 5.3% -32.6% 1.5% -24.4% and 0.2% -5.6% (of TFA). The SFA dominate the PC white muscle (75.3%), red muscle (40.9%) and skin (73%). In PE, they dominate in the red muscle (66.2%) and the skin (63.8%) of swordfish.

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Monounsaturated fatty acids (MUFA) were the main constituents of unsaturated fatty acids (Table 3). The highest percentages were observed in the PC red muscle (31.6%) and PE white muscle (44.2%). Oleic acid (C18: 1n-9) and complex C16: 1n-7+1n-9 were among the most abundant MUFA compound with significant differences between, the body parts in the PC and PE. The highest percentages of oleic acid were observed in the red muscle PC (19.3%) and in the white muscle PE (32.6%). For the complex C16: 1n-7+1 n-9, the percentages of 7.3% in the DAG and 10.6% in the TAG were observed in the skin.

The distribution of polyunsaturated fatty acids (PUFA) of PL or TAG is a criterion for the selection of potential sources of PL. The bioavailability of PUFA (n-3) increased when the food vehicle is made by PL rather than TAG (Cansell et al., 2003). The ideal sources that could be used in human food should contain PUFA (n-3) (ALA, EPA, DHA) and PUFA (n-6) (LA, ARA). These PUFAs might be attached to PL that is more easily assimilated by the body through the TAG (Parmentier et al., 2007). PC red muscle contains 16.3% PUFA (Table 3), fatty acids such as linoleic (C18: 2n-6) and EPA were abundant with percentages of 5.6% and 3.4%. In PE white muscle and skin, PUFAs were present in percentages of 9.4% and 8.8%. The percentages of the linoleic acid in D1 and the EPA of the skin were 1.9% and 4.7% respectively.

Muscle metabolism could influence the composition of fatty acids (FA) of phospholipids and neutral lipids. Muscles that have oxidative metabolism (red muscle) have higher levels of polyunsaturated fatty acids in the two types of lipids (Muriel et al., 2002). In accordance with table 3, the PC displays a red muscle percentage PUFA 16.3% against 63.3% in the TAG of the same muscle. In white muscle (D1), the respective percentages of 3.4% and 5.8% were observed. It is important to know the lipid composition of fish in general and that of phospholipids in particular to adapt dietary recommendations to the needs of patients and consumers. PC or lecithin does not accumulate in the body unlike TAG and cholesterol whose pathologies of their accumulation are known (Chanussot, 2008). Of the three swordfish body parts, the skin was richer in PC. Lecithin or PC dominated in the red muscle and PE dominated in the white muscle. Fatty acids which were conveyed by the PC were linoleic acid and EPA.

U.S. experts targeting choline argued that the appropriate dose (adequate intake) of lecithin is 6g/day and that of choline is 550 mg/day for an adult male weighing 70 kg, 425mg for an adult female and a little less amount for children (Zeisel et al., 2003).

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	White muscle	Red muscle	Skin
TFA	3.98 ± 0.75^{a}	4.62±0.71 ^a	0.57 ± 0.07^{b}

mean \pm *SE* ; *n* = 6; *superscript letters indicate inter group statistical differences, p* < 0.05.

	D1	RM	Р
PC	13.1±5.5 ^a	51.6±17.4ª	61.7±20.3ª
PE	41.1±8.4 ^a	41.0±9.7 ^a	44.8±7.7 ^a
Phospholipids (PC+PE)	52.3	84.2	106.5
TAG	94.7±40.9 ^b	62.9±24.5 ^b	462.3±109.6 ^a
DAG	74.3±15.4 ^a	6.9±3.2 ^b	11±3.7 ^b
Neutra lipids (TAG+DAG)	169.5	69.5	473.3

Table 2. Contents (mg/100g FW) of each lipid class in the swordfish body parts.

Mean \pm SE; n = 6; superscript letters indicate inter group statistical differences, p < 0.05

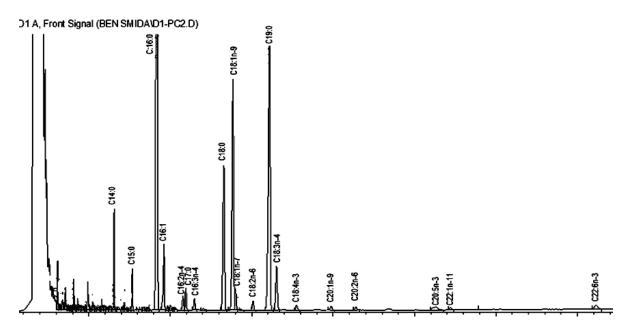


Table 3. Percentages of fatty acids in lipid fractions of the swordfish body parts.

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Fatty acid	PC			PE		
	D1	RM	Р	D1	RM	Р
SFA	75.3±1.8ª	40.9±13.7 ^b	73 ±7.9 ⁿ	44.1±1.4 ^b	66.2±27.0 ^a	63.8±3.8
MUFA	19.1±1.3ª	31.6±8.2ª	20.2±2.8ª	44.2±2.3ª	17.7±7.2 ^b	13.2±2.6 ^t
PUFA	3.4±0.2 ^b	16.3±8.4ª	2.1±1.4 ^b	9.4±3.4ª	4.1±1.7 ^a	8.8±2.1ª
Σ n-3	2.0±0.2a	6.8±3.3ª	1.6±1.1ª	2.1±0.2 ^b	1.7±0.7 ^b	6.8±1.7ª
Σ n-6	1.4±0.2 ^b	9.5±5.1ª	0.5±0.3 ^b	7.3±3.3ª	2.3±0.9 ^a	1.9±0.9ª
n-3 / n-6	1.6 ^{ab}	0.7 ^b	3.1 ^a	0.5 ^a	0.8 ^a	3.5ª
DHA/EPA	0.7 ^a	0.2 ^a		0.4	-	-
C14: 0	2.0±0.2 ^b	4.9±0.7ª	5.8±0.9 ^a	3.2±0.5 ^b	2.5±0.4 ^b	5.5±0.7ª
C15:0	1.3±0.1 ^b	2.8±0.3ª	2.6±0.3 ^a	0.9±0.1ª	1.5±0.3ª	1.5±0.2ª
C16:0	61.2±2.3 ^a	14.0±9.5 ^b	48.6±3.1ª	38.6±1.6 ^a	24.4±7.5ª	35.8±1.5
C16 :1n-7+1n-9	2.8±0.3 ^a	7.6±2.7ª	5.8±0.6 ^a	2.5±0.5 ^b	4.4±1.1 ^{ab}	5.6±1.1ª
C16:2n-4	0.7±0.1 ^b	2.7±0.4ª	0.6±0.3 ^b	0.7±0.1ª	2.2±0.6 ^a	4.4±2.0ª
C17: 0	0.6±0.0 ^b	2.0±0.5ª	0.5 ± 0.2^{b}	1.0±0.2 ^b	3.5±0.5ª	0.9±0.3 ^b
C16:3n-4	trace	0.3±0.2 ^a	trace	0.1±0.1 ^a	1.1±0.6 ^a	2.7±2.7ª
C16:4n-3	0.2±0.0 ^{ab}	0.8 ± 0.4^{a}	trace	0.3±0.1ª	0.3±0.2ª	0.1±0.1ª
C18: 0	10.2±1.0 ^a	17.1±2.7ª	15.6±3.4ª	0.4±0.1 ^b	34.2±8.1ª	20.1±5.1
C18:1n-9	14.8±0.8 ^a	19.3±4.0 ^a	13.4±1.8ª	32.6±3.2ª	10.2±4.3 ^b	6.5±1.2 ^b
C18:1n-7	1.2±0.0 ^b	4.1±0.9 ^a	0.9±0.4 ^b	8.7±2.6 ^a	3.1±0.5 ^b	1.1±0.5 ^b
C18:2n-6	1.1±0.2 ^a	5.6±3.1ª	0.5 ± 0.3^{a}	1.9±0.3 ^a	1.1±0.6 ^{ab}	0.3±0.2 ^b
C18: 3n-6	trace	0.6±0.4ª	trace	0.4±0.3 ^a	0.2±0.2 ^a	0.2±0.2 ^a
C18:3n-4	1.5±0.5 ^b	8.1±2.9 ^a	4.1±1.0 ^{ab}	1.3±0.5 ^b	8.8±2.0 ^a	7.2±0.9ª
C18:3n-3	trace	trace	trace	trace	0.6±0.4ª	trace
C18:4n-3	0.7±0.1 ^b	1.8±0.5 ^a	$0.4{\pm}0.0^{b}$	1.6±0.4 ^a	0.8±0.3 ^a	2.1±0.5ª
C20:1n-9	0.2±0.1ª	0.2±0.2 ^a	trace	0.3±0.2 ^a	trace	trace
C20:2n-6	0.2±0.1 ^b	3.2±1.3ª	trace	5.0±3.1ª	1.1±0.5 ^a	1.4±0.6 ^a
C20:4n-6	0.1 ± 0.0^{a}	0.2±0.2*	trace	trace	trace	trace
C20:4n-3	trace	0.2±0.2ª	trace	trace	trace	trace
C20:5n-3	0.6±0.1ª	3.4±1.7ª	1.1±0.2 ⁿ	0.2±0.1 ^b	trace	4.7±1.2ª
C22:1n-11	0.1±0.0 ^a	0.4±0.4ª	trace	0.2±0.2 ^a	trace	trace
C22:1n-9	trace	trace	trace	trace	trace	trace
C22: 4n-6	trace	trace	trace	trace	trace	trace
C22:5n-6	trace	trace	trace	trace	trace	trace
C22:5n-3	trace	trace	trace	trace	trace	trace
C22:6n-3	0.6±0.2 ^a	0.6±0.6ª	trace	0.2±0.2ª	trace	trace

Fatty acid	DAG			TAG		
	Dl	RM	P	D1	RM	P
SFA	69.1±6.6*	46.1±2.1*	58.4±3.3**	50.2±6.3*	19±2.9*	52.0±1.9*
MUFA	10.7±3.6 ^b	9.4±1.0 ^b	23.9±1.7ª	29.7±6°	14±6.5*	16.8±3.2*
PUFA.	16.5±7.0 [≥]	12.7±3.3°	12.1±4.0*	5.8±2 ^b	63.3±10.2*	22.0±5.3 ^b
Σ n-3	11.4±5.8*	4.6±0.8ª	8.9±2.9*	4.2±1.4°	7.8±1.3ª	5.9±0.5*
Σ n-6	5.1±1.9°	8.1±2.7ª	3.2±1.1°	1.6±0.6 ^b	55.4±11.1*	16.1±5.0 ^b
n-3 / n-6	3.9*	0.6 ^b	2.8 ^{ab}	2.6ª	0.7 ^b	1.1 ^b
DHA/EPA	-	-	-	7.1*	2.1 ^b	8.2*
C14: 0	4.6±0.8 ^b	7.2±0.3*	9.3±0.9°	6.8±0.6ª	6.2±2.1*	9.6±1.3*
C15:0	7.2±5.6ª	1.7±0.2*	2.5±0.3*	1.6±0.1*	1.3±0.4*	2.0±0.2*
C16:0	30.9±5.9 ^{ab}	21.3±1.3 ^b	34.4±2.5*	31.5±4*	5.4±2.0 ^b	32.5±1.7
C16 :1n-	2.5±0.6 ^b	4.0±0.4 ⁶	7.3±0.7ª	7.3±0.8ª	5.7±2.6*	10.6±1.0
7+ln-9	0.8±0.2*	1.7±0.7*	0.8±0.2*	1.5±0.4°	1.2±0.3*	1.8±0.2*
C16:2n-4	0.4±0.2*	3.0±1.7*	0.1±0.1*	1.1±0.1*	0.7±0.2*	1.2±0.2*
C17:0	0.1±0.1*	trace	0.2±0.2*	0.2±0.0 ^a	0.1±0.1*	0.3±0.2*
C16:3n-4	6.4±6.3*	1.3±0.6*	6.0±3.1*	0.3±0.1*	0.3±0.1*	0.3±0.0ª
C16:4n-3	26.0±5.8°	12.9±0.8 ^b	12.1±1.4 ^b	9.3±1.6ª	5.4±1.2ª	6.7±1.9*
C18: 0	7.7±3.2 ^b	5.4±0.7°	15.3±1.0*	19.6±5.5*	7.2±3.7⁵	5.3±2.5
C18:1n-9	0.5±0.2**	trace	1.2±0.4°	1.7±0.6°	1.1±0.5*	0.8±0.4ª
C18:1n-7	2.3±1.5*	0.2±0.2*	0.6±0.3*	0.9±0.2*	0.9±0.2*	0.8±0.1*
C18:2n-6	2.0±1.8°	trace	trace	0.1±0.0 ^b	53.6±11.4*	14.9±5.1 ¹
C18: 3n-6	1.9±1.1 ^b	24.4±5.3°	4.7±0.6 ^b	3.7±2.8°	2.5±0.7*	5.9±3.9*
C18:3n-4	1.8±0.5°	trace	trace	0.1±0.0 ^b	0.3±0.1 ^{ab}	0.4±0.1*
C18:3n-3	1.4±0.6 ^b	3.3±0.3*	0.7±0.4 ^b	0.2±0.0°	0.5±0.1*	0.4±0.1*
C18:4n-3	trace	trace	trace	0.2±0.1*	0.1±0.1*	0.4±0.1°
C20:1n-9	0.8±0.5 ^b	7.9±2.9ª	2.6±1.2 ^{ab}	0.2±0.1 ^b	0.6±0.1*	0.2±0.1 ^b
C20:2n-6	0.1±0.1*	trace	trace	0.3±0.2*	0.3±0.1*	0.3±0.1*
C20:4n-6	0.3±0.3*	trace	trace	0.1±0.1ª	0.5±0.3*	0.8±0.3*
C20:4n-3	trace	5.6±1.1*	2.1±0.7 ^b	1.0±0.7*	1.9±0.4*	0.8±0.2*
C20:5n-3	trace	trace	trace	0.9±0.3°	trace	0.1±0.1 ^b
C22:1n-11	trace	trace	trace	trace	trace	trace
C22:1n-9	trace	trace	trace	trace	trace	trace
C22: 4n-6	trace	trace	trace	0.1±0.1°	trace	0.1±0.0*
C22:5n-6	trace	trace	trace	0.3±0.1ª	0.5±0.2*	0.4±0.0a
C22:5n-3	1.6±0.9°	trace	trace	3.2±1.3°	4.0±0.8*	3.6±0.3*
C22:6n-3	VENDES CORTES		100000000000000000000000000000000000000	2-4/5/9/5/03/200/	1960 - COLEMA (1960) (11-1-10-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-10-17-

Table 3 (continued). Percentages of fatty acids in lipid fractions of the swordfish body parts

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