GRASAS Y ACEITES 70 (1)
January–March 2019, e285
ISSN–L: 0017-3495
https://doi.org/10.3989/gya.0235181

Sex and seasonal variation in proximate composition and fatty acid profile of *Scomber scombrus* (L. 1758) fillets from the Middle East Coast of Tunisia

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Submitted: 01 March 2018; Accepted: 10 July 2018

SUMMARY: This study investigates the impact of season and sex variations on the total lipid contents and fatty acid composition of *Scomber scombrus* fillets from the Middle East Coast of Tunisia in order to dertermine the most favorable periods for consumption, and to see if the nutritional quality of the meat depends on the sex of the animal. The effect of fishing season induced significant changes in the lipid profile, and the highest values for total lipids were obtained in the spring for females with 13.2% and for males with 18.9%. The highest values for proteins were obtained in the summer for females with 22.0% and for males with 21.8%. Protein content variations were not significant ($p > 0.05$). The n-3/n-6 ratio showed a significant level, indicating a tendency toward n-3 fatty acid accumulation in mackerel fillets mainly represented by DHA, whose values were high during the study period for both sexes, except in autumun, which is the period of mackerel gonad maturation, when DHA decreased significantly ($p < 0.05$), reaching 23.2 and 34.0% for males and females, respectively. It was concluded that *Scomber scombrus* has high levels of proteins, lipids, and fatty acid contents mainly n-3 PUFA. DHA sex variations were not significant ($p > 0.05$). The Atherogenicity index (AI) and Thrombogenicity index (TI) were calculated. In our study the AI index was comprised between 0.3 and 0.6 for males and between 0.4 and 0.5 for females. The TI index values ranged from 0.6 to 0.8 for males, and was about 0.6 for females.

KEYWORDS*: Fatty acids; Nutritional quality; Proximate composition;* Scomber scombrus*; Seasons; Sex*

RESUMEN: ***Variación estacional y sexual en la composición y perfil de ácidos grasos en filetes de caballa,*** **Scomber scombrus** ***(L. 1758) de la costa este tunecina.*** Este estudio investiga el impacto de las variaciones estacionales y sexuales sobre el contenido total de lípidos y la composición de ácidos grasos de filetes de caballa, *Scomber scombrus,* de la Costa Este Tunecina, con objeto de determinar los períodos más favorables para su consumo y para ver si la calidad nutricional de la carne depende del sexo del animal. Los efectos de la temporada de pesca indujeron cambios significativos en el perfil de lípidos, así, los valores más altos de lípidos totales se obtuvieron en primavera para las hembras, 13,2% y para los machos, 18,9%. Los valores más altos de proteína se obtuvieron en verano para las hembras, 22,0% y para los machos 21,8%. Las variaciones en el contenido de proteínas no fueron significativas ($p <0.05$). La relación n-3/n-6 mostró un nivel significativo que indica una tendencia a la acumulación de ácidos grasos n-3 en filetes de caballa representados principalmente por DHA, cuyos valores fueron altos durante el período de estudio para ambos sexos, excepto en el otoño donde el DHA disminuyó significativamente ($p <0.05$), alcanzando el 23,2% y el 34,0% para machos y hembras respectivamente; período de maduración de las gónadas de la caballa. Se concluye que *Scomber scombrus* posee un alto contenido de proteínas, lípidos y ácidos grasos, principalmente AGPI n-3. La variación de sexo sobre el contenido de DHA no fue significativa ($p <0.05$). Se calcularon el índice de aterogenicidad (AI) y el índice de trombogenicidad (TI). En este estudio, el índice AI estaba comprendido entre 0,3 y 0,6 para los machos y entre 0,4 y 0,5 para las hembras. Los valores del índice TI oscilaron entre 0,6 y 0,8 para machos y fue de aproximadamente 0,6 para las hembras.

PALABRAS CLAVE*: Ácidos grasos; Calidad nutricional; Composición próxima; Estaciones;* Scomber scombrus*; Sexo*

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Citation/Cómo citar este artículo: El Oudiani S, Chetoui I, Darej C, Moujahed N. 2019. Sex and seasonal variation in proximate composition and fatty acid profiles in *Scomber scombrus* (L. 1758) fillets from the Middle East Coast of Tunisia. *Grasas Aceites* **70** (1), e285. https://doi.org/10.3989/gya.0235181

ABREVIATION: Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), Saturated fatty acids (SFA) Monounsaturated fatty acids (MUFA), Polyunsaturated fatty acids (PUFA).

1. INTRODUCTION

The blue species *Scomber scombrus* is an abundant pelagic species caught in different regions of Tunisia. This mackerel's catch represents about 15% of total pelagic fisheries. According to the General Direction of Fishing and Aquaculture in Tunisia (DGPA), the production of pelagic fisheries including mackerels fluctuates from year to year, and from port to port with a general decreasing tendency which reached 44,208 tons in 2010. The maximum production of mackerel in the port of Teboulba was about 3072 tons in 2005, with summer representing the highest catch season. Seafood products are the most beneficial to human health, and their consumption is recommended by nutritionists at least once a week, especially blueback fish.

Apart from their easy digestibility and high levels of protein and lipid contents, fish also are rich in vitamins and different minerals (Dan-Kishiya, 2013). The lipid characteristics of fish include a low cholesterol level (Hunter *et al.,* 2000) and ω3 polyunsaturated fatty acids, mainly eicosapentaenoic (C20:5n-3, EPA) and docosahexaenoic (C22:6n-3, DHA) fatty acids, which play an important role in the prevention of some diseases, such as cardiovascular, rheumatoid, arthritis, gastroenterologic and dermatologic pathologies (Simopoulos, 2002), along with several types of cancer, with anti-thrombotic effects which reduce cholesterolemia. In addition, a diet which is rich in these valuable nutrients reduces levels of triglycerides in plasma and the very low-density lipoprotein proportion (VLDL) (Harris, 1989).

Belonging to the Scombridae family, *Scomber scombrus* is a blueback fish species which is rich in polyunsaturated fatty acids. It is characterized by a high percentage of DHA and EPA (Rubio-Rodríguez *et al.,* 2010). According to Paulina *et al.,* (2016) the Atlantic mackerel caught during the summers of 2012 and 2013 from different sites of the Icelandic fishing area revealed a seasonal variation in its lipid contents and rancidity development.

The chemical composition of fish fillets is generally unstable, and may vary with season, water salinity and temperature, size, sex, fish age, maturity, the characteristics of the specific sea area, food availability and diet composition (Garrido *et al.,* 2008).

In order to valorize the quality of our fish flesh, some nutritional indexes were calculated. As signaled by nutritionists, the n-3/n-6 ratio has been suggested as a good standard to compare the nutritional value of oils present in fish meat; a n-3/n-6 ratio of 1:1, or 1:1.5 can contribute to a healthy diet for humans (Osman *et al.,* 2001).

Two other nutritional quality indexes, the atherogenicity index (AI) and thrombogenicity index (TI) were considered as healthy lipid indices and were evaluated from the fatty acid composition data (Ulbricht *et al.,* 1991).

This study evaluates with the effect of seasons and sex on the proximate and fatty acid compositions of the fillets of *Scomber scombrus,* caught from the Middle East Coast of Tunisia, in order to determine the most favorable periods for consumption and to determine whether the nutritional quality of the meat depends on the sex of the animal.

2. MATERIALS AND METHODS

2.1. Sampling strategy

Mackerel samples were collected monthly from the fishing off Teboulba, located on the Middle East Coast of Tunisia, during the period extending from June, 2010 to May, 2011. At landing, samples were transferred directly to the laboratory, where somatometric measurements were taken (total lenght (TL), total weight (TW)) and the fish were beheaded, washed, filleted, packed and then frozen at (-20 °C). The sample storage period before analysis did not exceed two days. During the study period, the analyses were based on samples with comparable body size and this was done monthly with parity between males and females (Table 1). In the present work, the analyzed muscle fragment cams from the left side of the animal and corresponded to the latero-dorsal part as indicated in Figure 1.

2.2. Proximate analysis

Crude protein (N×6.25) was analyzed by the Kjeldahl method AOAC (1990). The moisture content was determined by oven-drying for 4 h at 105 °C following the AOAC (1990) method, until a constant weight was reached. Lipid extraction was carried out using the Soxhlet method (AOCS, Ba 3-38) in triplicate, with 5 g of dry weight flesh powder with 200 ml of petroleum ether for 6 h. The extracted lipids were evaporated under vacuum at 65 °C in a rotary evaporator, and then placed in an oven at 45 °C for 1 h, before being transferred into desiccators and weighed again. The muscle fragment used for the analysis came from the left side of the fish and corresponded to the central part.

2.3. Fatty acids analysis

Methyl ester preparation was done by a direct transesterification according to the Mosers (1991) method. Mackerel lipid extract was vortexed with 1 ml (methanol/methylene chloride at 3:1); an internal standard (Heptadecanoic acid, C17:0), which did not exist in our sample and servied to quantify fatty acids, was added to the mixture. Then 200 µl of acetylchloride were added and vortexed and the mixture was incubated in an oven at 75 °C for one hour. After cooling for 15min at

TABLE 1. Number and body size of monthly Mackerel sampels.

Months	Sexes	Numbers (N)	Mean Total lenght (Lt)	Mean Total weight (Wt)
june-2010	**Males**	3	23.03 ± 0.92	110.65 ± 9.20
	Females	3	23.33 ± 1.04	116.19 ± 15.87
july-2010	**Males**	3	27.20 ± 1.56	170.48 ± 26.10
	Females	3	26.93 ± 1.37	172.91 ± 17.50
aug-2010	**Males**	3	25.66 ± 1.04	151.00 ± 23.73
	Females	3	26.33 ± 2.34	175.00 ± 51.44
sept-2010	**Males**	3	25.17 ± 2.65	158.00 ± 17.04
	Females	4	25.80 ± 3.58	174.80 ± 15.21
oct-2010	**Males**	3	24.63 ± 2.00	142.00 ± 29.87
	Females	3	25.37 ± 0.23	158.50 ± 6.60
nov-2010	**Males**	3	16.97 ± 0.25	41.50 ± 4.40
	Females	5	17.10 ± 0.14	41.00 ± 4.00
dec-2010	**Males**	3	26.10 ± 1.31	178.10 ± 9.05
	Females	3	28.60 ± 1.93	229.60 ± 13.80
jan-2011	**Males**	3	24.06 ± 1.00	211.50 ± 5.30
	Females	3	23.66 ± 1.60	118.70 ± 6.00
feb-2011	**Males**	3	29.00 ± 1.73	144.60 ± 19.00
	Females	3	29.37 ± 0.64	162.32 ± 5.40
mar-2011	**Males**	3	24.26 ± 0.46	118.83 ± 5.48
	Females	3	24.00 ± 1.08	104.22 ± 15.03
abr-2011	**Males**	3	22.66 ± 0.10	100.30 ± 13.86
	Females	3	21.70 ± 1.37	86.20 ± 16.88
may-2011	**Males**	4	21.00 ± 1.61	75.30 ± 23.00
	Females	6	21.10 ± 1.70	93.00 ± 25.10

N: number of specimens (males and females) in each month, **Lt**: mean total length (cm), **WT**: mean total weight (g)

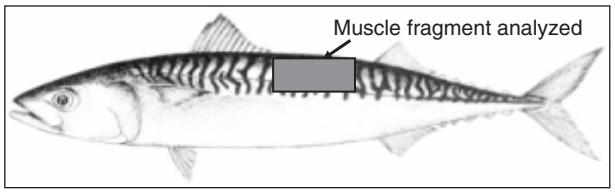

FIGURE 1. *Scomber scombrus* muscle fragment taken for analysis.

room temperature, 4 ml of (7%) potassium carbonate and hexane were added. The hexane layer was removed, and acetonitrile was added. Finally, the mixture was centrifuged and the hexane layer was dried under nitrogen. After that, 10 µl of hexane were injected into the tube and shaken again. Then 1 µl of the sample was extracted and used for the gas chromatography analysis.

Fatty acids were identified by gas chromatograph type HP series 6890 with a split/splitless injector and a flame ionization detector was used for the analysis. The device included a 30 m long HP Innowax capillary column with an internal diameter of 250 µm and a 0.25 µm film, the stationary polar phase of the column being polyethylene glycol. Comparison of the retention times of the fatty acids under study and those of standard fatty acids methyl esters (PUFA-3), allowed for identifying the different fatty acids contained in mackerel lipid extract.

2.4. Nutritional quality indexes

The atherogenicity index (AI) and thrombogenicity index (TI) were evaluated from the fatty acid composition data. AI indicates the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated. The main fatty acids of first class are designed as pro-atherogenic, which favors the adhesion of lipids to cells of the immunological and circulatory system; while the second-class fatty acids are considered as anti-atherogenic because they have an inhibiting effect on the aggregation of plaque and diminish the levels of esterified fatty acids, cholesterol, and phospholipids, thereby preventing the appearance of micro and macro-coronary diseases. These two indices were used in accordance with Ulbricht and Southgate (1991).

Atherogenicity Index (AI)

$$\mathbf{AI} = [(C12{:}0 + (4\times C14{:}0) + C16{:}0)] / [\Sigma MUFAs + \Sigma\ PUFA\ (n\text{-}6) + \Sigma\ PUFA\ (n\text{-}3)]$$

Thrombogenicity index (TI). TI is defined by the relationship between the pro-thrombogenetic and the anti-thrombogenetic fatty acids. It reflects the state of the blood vessels while indicating the training clots (Senso *et al.* 200; Ulbritch and Southgate, 1991)

$$\mathbf{TI} = [(C14{:}0+C16{:}0+C18{:}0) / (0.50*\Sigma MUFA) + (0.5*\Sigma PUFA\ (n\text{-}6) + (3*\Sigma PUFA\ (n\text{-}3) + (\Sigma PUFA\ (n\text{-}3) /\Sigma PUFA\ (n\text{-}6)]$$

2.5. Statistical Analysis

Statistical analysis of the effects of season and sex on total lipids and fatty acid composition was carried out with the SAS software program version 9.1. Different mean values were analyzed according to the Student Newman and Kull tests. Results are expressed as presentage (%) and g/100g of ww, and are considered significant at ($p < 0.05$).

3. RESULTS

3.1. Mackerel fillets proximate composition

As reported in Table 2, moisture percentages for males in autumn, winter and summer were not significantly different ($p > 0.05$); while spring presented the lowest moisture value at 66%. For females, moisture

TABLE 2. Seasonal variation in proximate composition for males and females of *Scomber scombrus*.

	Summer	Autumn	Winter	Spring
Moisture (%)				
Male	71.50±1.84 ^{a,A}	71.02±0.60^{a,A}	70.01±0.10^{a,A}	66.03±1.60^{b,A}
Female	72.01±0.40^{a,A}	70.05±0.50^{a,A}	66.9±0.76^{b,B}	67.90±1.12 ^{b,A}
Lipid (%)				
Male	5.37±1.30 ^{a,A}	5.70 ±0.68^{a,A}	6.05 ± 1.30^{a,A}	18.86±1.70^{b,A}
Female	3.39±0.20 ^{a,B}	9.01±1.22 ^{a,B}	11.92±0.80^{b,B}	13.21±2.10^{b,B}
Protein (%)				
Male	21.80±1.16 ^{a,A}	22.03±2.80 ^{a,A}	21.54±2.09 ^{a,A}	18.20 ± 1.80 ^{a,A}
Female	22.07±1.70^{a,A}	20.00 ±1.05 ^{a,A}	20.05±0.63 ^{a,A}	18.00 ±1.32 ^{a,A}
Ash (%)				
Male	1.33±0.23 ^{a,A}	1.10±0.35 ^{a,A}	1.30 ± 0.29 ^{a,A}	1.00 ± 0.30 ^{a,A}
Female	2.00±0.29 ^{a,B}	1.00 ±0.06 ^{a,A}	1.05 ±0.22 ^{a,A}	0.98±0.41^{a,A}

Results are given as mean ± SD (n = 6: 3 males + 3 females by month),
Different letters for the same line (lower case) indicate significant differences among seasons, and different letters for the same column (capital) indicate significant differences between sexes ($P < 0.05$), according to the Student Newman and Kull tests.
Values with the same superscripts are not significantly different at ($P < 0.05$), according to the Student Newman and Kull tests.

TABLE 3. Seasonal variation in *Scomber scombrus* fatty acid composition for males (% of total fatty acids)

Fatty acids	Summer	Autumn	Winter	Spring
C14:0	1.42 ±0.90^{a}	2.04 ±0.32^{a}	1.11± 0.43^{a}	2.14±0.45^{a}
C16:0	22.18±1.63^{a}	24.13±2.75^{a}	22.43±2.11^{a}	23.97±1.38^{a}
C18:0	11.42±1.04^{a}	10.39 ± 0.95^{b}	10.68±0.67^{b}	9.26 ±0.91^{b}
C16:1	2.22± 0.27^{a}	3.93 ± 0.04^{a}	2.72 ± 0.15^{a}	3.45 ±0.07^{a}
C18:1	9.77±1.09^{a}	12.01 ± 2.51^{b}	9.51±1.03^{a}	7.83±1.14^{a}
C18:2 *n*-6	1.15±0.27^{a}	1.47± 0.04^{b}	1.43±0.15^{b}	1.03±0.07^{a}
C18:3 *n*-6	0.25±0.07^{a}	0.19 ± 0.08^{a}	0.20 ±0.05^{a}	0.14±0.05^{a}
C20:2 *n*-6	0.18 ±0.03^{a}	0.26 ± 0.06^{b}	0.11±0.06^{a}	0.17±0.03^{a}
C20:3 *n*-6	0.44 ±0.08^{a}	0.32 ± 0.03^{b}	0.20 ±0.04^{c}	0.29 ±0.07^{c}
C20:4 *n*-6	2.88 ±0.25^{a}	3.55 ± 0.50^{b}	3.68 ±0.28^{b}	3.07±0.26^{b}
C18:3 *n*-3	0.36±0.08^{a}	0.42± 0.16^{a}	0.48±0.07^{a}	0.67±0.03^{b}
C20:5 *n*-3	5.04±0.67^{a}	5.80± 0.28^{a}	4.76±0.12^{a}	6.58±0.56^{a}
C22:5 *n*-3	1.51±0.05^{a}	1.30 ± 0.20^{a}	1.16 ±0.30^{b}	1.70±0.03^{c}
C22:6 *n*-3	41.19±1.21^{a}	34.17± 1.96^{b}	41.52±0.32^{a}	39.66±1.90^{ab}

Results are given as mean ± SD (n = 3 males in each month)
Different letters indicate significant differences among seasons ($P < 0.05$), according to the Student Newman and Kull tests.
Values with the same superscripts (a and c) are not significantly different ($P < 0.05$), according to the Student Newman and Kull tests.

seasonal variation was significant ($p < 0.05$), and the lowest values were obtained in winter and spring, with 67 and 68%, respectively. Mackerel fillet composition showed a high lipid level in spring for both males and females with 18.9 and 13.2%, decreasing significantly ($p < 0.05$) in summer to 5.4 and 3.4%, respectively. Season and sex variations in lipid contents were highly significant ($p < 0.05$). Protein contents ranged from 18 to 22%; the highest levels for females and males were obtained in summer with 22 and 21.8%, respectively. No significant differences in protein content between seasons and sex were observed ($p > 0.05$). Ash content ranged from 1 to 2% and seasonal variations were significant ($p < 0.05$). There was no significant difference between sexes ($p > 0.05$). High levels were obtained in summer for females and males with 2 and 1.3%, respectively.

3.2. Fatty acids composition

Gas chromatography (GC) analysis allowed the identification of diffrent categories of fatty acids as reported in Tables 3 and 4. The saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA); PUFA/SFA and n-3/n-6 ratios for both sexes are summarized in Table 5.

TABLE 4. Seasonal variation in *Scomber scombrus* fatty acid composition for females (% of total fatty acids)

Fatty acids	Summer	Autumn	Winter	Spring
C14:0	1.42 ±0.90[a]	2.04 ±0.32[a]	1.11± 0.43[a]	2.14±0.45[a]
C16:0	22.18±1.63[a]	24.13±2.75[a]	22.43±2.11[a]	23.97±1.38[a]
C18:0	11.42±1.04[a]	10.39 ± 0.95[b]	10.68±0.67[b]	9.26 ±0.91[b]
C16:1	2.22± 0.27[a]	3.93 ± 0.04[a]	2.72 ± 0.15[a]	3.45 ±0.07[a]
C18:1	9.77±1.09[a]	12.01 ± 2.51[b]	9.51±1.03[a]	7.83±1.14[a]
C18:2 *n*-6	1.15±0.27[a]	1.47± 0.04[b]	1.43±0.15[b]	1.03±0.07[a]
C18:3 *n*-6	0.25±0.07[a]	0.19 ± 0.08[a]	0.20 ±0.05[a]	0.14±0.05[a]
C20:2 *n*-6	0.18 ±0.03[a]	0.26 ± 0.06[b]	0.11±0.06[a]	0.17±0.03[a]
C20:3 *n*-6	0.44 ±0.08[a]	0.32 ± 0.03[b]	0.20 ±0.04[c]	0.29 ±0.07[c]
C20:4 *n*-6	2.88 ±0.25[a]	3.55 ± 0.50[b]	3.68 ±0.28[b]	3.07±0.26[b]
C18:3 *n*-3	0.36±0.08[a]	0.42± 0.16[a]	0.48±0.07[a]	0.67±0.03[b]
C20:5 *n*-3	5.04±0.67[a]	5.80± 0.28[a]	4.76±0.12[a]	6.58±0.56[a]
C22:5 *n*-3	1.51±0.05[a]	1.30 ± 0.20[a]	1.16 ±0.30[b]	1.70±0.03[c]
C22:6 *n*-3	41.19±1.21[a]	34.17± 1.96[b]	41.52±0.32[a]	39.66±1.90[ab]

Results are given as mean ± SD (n=3 females in each month)
Different letters indicate significant differences among seasons ($P < 0.05$), according to the Student Newman and Kull tests.
Values with the same superscripts (a and c) are not significantly different at ($P < 0.05$), according to the Student Newman and Kull tests.

The SFA are represented by myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids. Palmitic acid (C16:0) was the major fatty acid in the SFA. The highest values were obtained in autumn with 25 and 24.1% for males and females, respectively. The lowest values were obtained in summer with 20 and 22.2% for males and females, respectively. There were no significant differences among seasons and sexes ($p > 0.05$). The highest values for myristic acid (C14:0) were registered in autumn for males with 3.6% and in spring for females with 2.1%. The lowest levels were observed in summer and in winter with 0.9 and 1.1% for males and females, respectively. Myristic acid was significantly different among seasons ($p < 0.05$) but not between females and males ($p > 0.05$). The highest percentages for stearic acid (C18:0) were obtained in autumn for males (11.2%) and in summer for females (11.4%). Nevertheless, the lowest values were obtained in spring with 8.6 and 9.3% for males and females, respectively. Stearic fatty acid was significantly different among seasons ($p < 0.05$), but not between females and males ($p > 0.05$). SFAs mean values for males and females were 34.9 and 35.3%, respectively; there was no significant difference among seasons and sexes for SFAs ($p > 0.05$).

The MUFA are represented by palmitoleic (C16:1 *n-7*) and oleic (C18:1 *n-9*) acids. Oleic acid (C18:1) was the major fatty acid in MUFA. The highest values were obtained in autumn for males and females with 15.8 and 12%, respectively. The lowest levels were obtained in summer with 5.8% for males and in spring for females with 7.8%. There were no significant differences among seasons and sexes ($p < 0.05$). The highest values were obtained for palmitoleic acid in autumn for males at 5.2% and for females at 4%. The lowest levels were registered in summer for males at 1.7% and females at 2.2%. There were no significant differences among seasons and sexes ($p > 0.05$). The mean MUFA values for males and females were 12.9 and 13.5%, respectively; whereas MUFA average values during the study period ranged from 11.6% in autumn to 14% in winter. There were no significant differences among seasons and sexes ($p < 0.05$).

The PUFAs are divided into two families, those of the *n-3* and *n-6* series. The n-3 fatty acids are mainly represented by linolenic (C18:3*n-3*), docosapentaenoic (DPA, C20:5, *n-3*) and docosahexaenoic (DHA, C22:6 *n-3*). The n-6 fatty acids are mainly represented by linoleic (C18:2n-6) and arachidonic (C20:4 *n-6*). The PUFAs were the most prominant in *Scomber scombrus* lipid contents. Seasonal variations in these FAs were significant ($p < 0.05$), although the sex variation was not ($p > 0.05$). The highest PUFA levels were related to those of the n-3 family, mainly represented by the EPA and the DHA. The highest levels for EPA were registered in spring with 8.3 and 6.6% for males and females, respectively. The lowest values were obtained in winter with 5.1 and 4.8% for males and females, respectively. DHA was the most abundant fatty acid in the *n-3* family; its highest values were noted in summer with 48.6% for males and in winter for females with 41.5 %. We also noted that females were rich in DHA in summer with 41.2%. The lowest levels were obtained in autumn with 23.2 and 34.2% for males and females, respectively.

TABLE 5. Seasonal variations in EPA, DHA, SFA, MUFA, PUFA (*n*-3), PUFA (*n*-6) and ratio for *Scomber scombrus* males and females.

	Summer	Autumn	Winter	Spring
C20:5 *n*-3 (EPA %)				
Male	$5.33 \pm 0.33^{a,A}$	$7.07 \pm 0.43^{b,A}$	$5.12 \pm 0.03^{a,A}$	$8.29 \pm 1.54^{b,A}$
Female	$5.04 \pm 0.67^{a,A}$	$5.80 \pm 0.28^{a,B}$	$4.76 \pm 0.12^{a,A}$	$6.58 \pm 0.56^{a,A}$
C22:6 *n*-3 (DHA %)				
Male	$48.58 \pm 1.97^{a,A}$	$23.25 \pm 1.56^{b,A}$	$40.14 \pm 2.55^{c,A}$	$38.78 \pm 2.17^{c,A}$
Female	$41.19 \pm 1.21^{a,A}$	$34.17 \pm 1.96^{b,B}$	$41.52 \pm 0.32^{a,A}$	$39.66 \pm 1.90^{ab,A}$
Σ SFA (%)				
Male	$31.60^{a,A}$	$39.92^{a,A}$	$34.70^{a,A}$	$33.20^{a,A}$
Female	$35.02^{a,A}$	$36.56^{a,A}$	$34.12^{a,A}$	$35.37^{a,A}$
ΣMUFA (%)				
Male	$7.40^{a,A}$	$20.99^{b,A}$	$12.98^{c,A}$	$12.98^{c,A}$
Female	$11.99^{a,B}$	$15.94^{b,B}$	$12.23^{a,A}$	$11.28^{a,A}$
Σ PUFA *n*-6 (%)				
Male	$5.16^{a,A}$	$6.13^{a,A}$	$5.06^{a,A}$	$4.41^{a,A}$
Female	$4.46^{a,A}$	$5.47^{a,A}$	$5.42^{a,A}$	$4.41^{a,A}$
Σ PUFA *n*-3 (%)				
Male	$55.83^{a,A}$	$32.94^{b,A}$	$47.27^{a,A}$	$49.41^{a,A}$
Female	$48.54^{a,B}$	$42.01^{a,B}$	$48.12^{a,A}$	$48.93^{a,A}$
PUFA/SFA				
Male	$1.93^{a,A}$	$0.97^{a,A}$	$1.50^{a,A}$	$1.62^{a,A}$
Female	$1.51^{a,A}$	$1.29^{a,A}$	$1.56^{a,A}$	$1.50^{a,A}$
n-3/n-6				
Male	$10.81^{a,A}$	$5.37^{b,A}$	$9.34^{a,A}$	$11.20^{a,A}$
Female	$10.88^{a,A}$	$7.68^{a,A}$	$8.87^{a,A}$	$11.09^{a,A}$
EPA (mg/100g of w/w)				
Male	$23.37 \pm 1.48^{a,A}$	$16.94 \pm 3.82^{b,A}$	$14.42 \pm 0.45^{b,A}$	$25.49 \pm 1.72^{b,A}$
Female	$31.96 \pm 1.49^{a,B}$	$26.74 \pm 5.27^{a,B}$	$59.36 \pm 6.56^{a,B}$	$15.74 \pm 3.04^{a,B}$
DHA (mg/100g of w/w)				
Male	$163.73 \pm 18.47^{a,A}$	$60.70 \pm 7.79^{b,A}$	$113.1 \pm 1.03^{c,A}$	125.70 ± 7.12^{cA}
Female	$279.27 \pm 7.32^{a,B}$	$113.30 \pm 15.20^{a,B}$	$185.37 \pm 16.6^{a,B}$	$100.89 \pm 7.90^{a,A}$
ΣPUFA *n-6* (mg/100g of w/w)				
Male	$22.84^{a,A}$	$20.00^{a,A}$	16.78^{bA}	$14.97^{b,A}$
Female	$25.96^{a,A}$	$18.55^{a,A}$	$31.78^{a,B}$	$11.58^{a,B}$
Σ PUFA *n-3* (mg/100g of w/w)				
Male	$191.20^{a,A}$	$82.66^{b,A}$	$133.28^{c,A}$	$152.18^{d,A}$
Female	$333.70^{a,B}$	$146.00^{b,B}$	$230.00^{c,B}$	$122.00^{d,B}$

Results are given as mean ± SD (n=6: 3 males and 3 females in each month),
Different letters for the same line (lower case) indicate significant differences among seasons, and different letters for the same column (capital) indicate significant differences between sexes ($P < 0.05$), according to the Student Newman and Kull tests.
Values with the same superscripts are not significantly different at ($P < 0.05$), according to the Student Newman and Kull tests.
SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids

PUFA n-3 mean values for males and females were 47 and 45.8%, respectively; whereas seasonal variation was significant ($p < 0.05$). Average values during the study period ranged from 42% in winter to 49% in autumn. PUFA n-3 series was significantly different among seasons ($p < 0.05$) but not between sexes ($p > 0.05$).

The highest proportion of n-6 PUFAs was arachidonic acid (C20:4 *n-6*), whose values varied significantly with season ($p < 0.05$); the maximum level was obtained in autumn for males with 3.8% and in winter for females with 3.7%. PUFA n-6 mean values for males and females were 5.5 and 5.3%, respectively. Average values during the study period

ranged from 4.7 in summer to 6% in winter. PUFA n-6 was significantly different among seasons ($p < 0.05$) but not between sexes ($p > 0.05$).

The PUFA/SFA ratio ranged from 1 to 2. The maximum values were obtained in summer for males with 2 and in winter for females with 1.6. Season and sex variations in the PUFA/SFA ratio were insignificant ($p > 0.05$). Mean values for males and females were 1.6 and 1.5, respectively; whereas average values during the study period ranged from 1.3 in winter to 1.6 in autumn. The highest levels for the n-3/n-6 were registered in spring with 11.2 and 11 for males and females, respectively. There were no significant differences in the n-3/n-6 ratio among seasons and sexes ($p > 0.05$). Mean values for males and females were 8.8; whereas average values during the study period ranged from 7.2 in winter to 10.4 in summer (Table 5).

For both sexes, EPA, DHA, PUFA *n-3* and PUFA *n-6* results are expressed by mg/100g of ww and are indicated in Table 5. For EPA and DHA, a significant difference among sexes and seasons was observed only for males ($p < 0.05$). The highest EPA quantity was obtained in spring for males, with 25.5 mg/100 g of ww and in winter for females with 59.4 mg/100gww. The highest DHA values were recorded in summer for males and females with 163.8 mg/100g of ww and 279.3mg/100g of ww, respectively.

For both sexes, the highest PUFA *n-3* values were observed in summer with 191.2 mg/100g of ww for males and 333.7 mg/100g of ww for females. There were significant diferences among seasons and sexes ($p < 0.05$).

3.3. Nutritional quality index

The Atherogenicity index (AI) and the Thrombogenicity index (TI) were calculated for the both sexes and all seasons (Table 6). The AI index was comprised between 0.3 and 0.6 for males and between 0.4 and 0.5 for females. Duncan's test was significant ($p < 0.05$) for both sexes. The values for the TI index ranged from 0.6 to 0.8 for males, and was about 0.6 for females. For both sexes, Duncan's test was significant ($p < 0.05$) acording to season but it was not significant ($p > 0.05$) between sexes.

4. DISCUSSION

4.1. Scomber scombrus fillets' proximate composition

The highest moisture percentages for males and females were associated with low levels of lipid contents, indicating that there is an inverse relationship among these compounds. Protein content for each sex showed a positive relation to moisture. The results for moisture are similiar to those of El Oudiani *et al*., (2016) in their study on Atlantic mackerel from the South East of Tunisia. Moreover, they are also in accordance with those indicated by Orban *et al.,* (2011) on their study on *Boops boops* and *Trachurus trachurus*; and by Ben Rebah *et al.,* (2014), who reported on the golden-grey mullet *Liza aurata* from the Tunisian coast; and by Anthony *et al*., (2000) who studied forage fish from the northern Gulf of Alaska, and demonstrated that a high-lipid fish has less water and more protein than low-lipid fish.

Mackerel flesh lipids were abundant and ranged from 3.4 to 19%. They varied significantly ($p < 0.05$) according to sex and season. Similar trends were obteined by Guizani and Moujahed (2015) and El Oudiani *et al*. (2016) in their studies on Atlantic mackerel from the North East and the South East of Tunisia, with values ranging from 4.35 to 11.53%. Other authors such as Chanet *et al.,* (2011) reported that lipids are abundant in fresh mackeral at more than 15%, ranging from 5 to 30% depending on the season.

Seasonal variation in protein content was not significant ($p>0.05$) and was in agreement with Tzikas *et al.,* (2007), who studied the Mediterranean horse mackerel *Trachurus mediterraneus* muscle from the North Aegean Sea. Moreover, according to Chanet *et al*., (2011) protein levels were about 19%, and this value is included in the range obtained in the present data (18 to 22%).

The observed variations may be explained by the impact of both exogenous and endogenous factors, including environmental parameters and the physiological state of the fish. However, during reproductive periods, lipids and proteins are mobilized from

TABLE 6. Nutritional quality indexes of *Scomber scombrus* males and females

Indexes	Sexes	Summer	Autumn	Winter	Spring
AI	**Male**	$0.45\pm0.03^{a,A}$	$0.35\pm0.03^{a,A}$	$0.67\pm0.15^{b,A}$	$0.44\pm0.09^{a,A}$
	Female	$0.50\pm0.04^{a,A}$	$0.43\pm0.09^{a,A}$	$0.51\pm0.04^{a,A}$	$0.41\pm0.07^{a,A}$
TI	**Male**	$0.60\pm0.05^{a,A}$	$0.54\pm0.02^{a,A}$	$0.80\pm0.30^{a,A}$	$0.64\pm0.09^{a,A}$
	Female	$0.65\pm0.05^{a,A}$	$0.65\pm0.11^{a,A}$	$0.69\pm0.10^{a,A}$	$0.62\pm0.08^{a,A}$

Results are given as mean ± SD (n=6: 3 males +3 females in each month),
Different letters for the same line (lower case) indicate significant differences among seasons, and different letters for the same column (capital) indicate significant differences between sexes ($P < 0.05$), according to Duncan's tests.
Values with the same superscripts are not significantly different at ($P < 0.05$), according to Duncan's tests.
AI: Atherogenicity index; TI: Thrombogenicity index.

muscle and transferred to the gonads, thus influencing its lipid content (Børresen, 1992; Pirini *et al.,* 2010). Moreover, according to Wallace (1991), a study on mackerel from the western English Channel demonstrated that lipid content is correlated with the spawning period and its gonad maturation state. However, its fat fillet contents varied with season, at 25 to 30% in December when fish is well-fed, and around 5% in May when the fish spawns.

Results of ash contents were in agreement with other fish species, as reported by Caponio *et al.,* (2004). High ash levels are probably related to the size of fish and its feeding habit composition. Seasonal variation in ash contents was significant ($p < 0.05$) as observed by Kacem *et al.,* (2011) in other fish species such as *Sardinella aurita*, *Sarpa salpa*, and *Sepia officinalis* from the Tunisian coast.

4.2. Scomber scombrus fatty acids profiles

Gas chromatography analysis indicated the presence of three categories of fatty acids, essentially saturated SFAs, monounsaturated MUFAs and polyunsaturated PUFAs, whose compositions varied with season. This result is in line with Guizani and Moujahed (2015) and El Oudiani *et al.* (2016), who studdied Atlantic mackerel, and with other studies dealing with the viscera and edible parts of fish (Ben Rebah *et al.,* 2009).

In the SFA family, palmitic acid (C16:0) was the most abundant fatty acid. This finding is in agreement with other studies carried out on different Mediterranean fish as reported by Ben Rebah *et al.,* (2009). In fact, according to Andrade *et al.,* (1995), palmitic acid is the key part of fish lipid metabolism. There were significant differences among seasons and sexes ($p>0.05$), which is in accordance with the results found by Bulla *et al.,* (2011) on raw sardines (*Sardinella Brasiliensis*).

For all samples, myristic acid (C14:0) exhibited the lowest proportion. This result is in accordance with those of Rioux *et al.,* (2001), who claimed that the lowest proportion in the animal body is represented by myristic acid, ranging between 0.5 and 2% of total fatty acids (TFAs).

In this data, the highest MUFA levels were registered in autumn for both males and females. This trend contradicts Soriguer *et al.,* (1997), who studied Atlantic mackerel from Spain and found that the highest MUFA levels were found in winter. This difference may be explained by the effect of environmental parameters, essentially temperature, and probably the physiological state of the fish. In fact, Dalsgaard *et al.* (2003) signaled that high MUFA levels are an indicator of a high degree of carnivory of the species. However, according to Lee *et al.,* (2006), MUFA levels are correlated with the mackerel's diet composition and its high degree of carnivory. Pepin *et al.,* (1988) reported that Atlantic mackerel is an opportunistic fish species, whose diet composition is based on zooplankton, crustaceans such as copepods (copepod nauplii and adult copepod at its larval stage and at juvenile stage, amphipods, mysid shrimp) and mollusks. In our study the highest proportions of MUFAs were oleic acid. This result is in accordance with Ben Rebah, *et al.,* (2014) on males of *liza aurata* from the Tunisian coast. In fact, oleic acid is characteristic of fish tissue and is actively synthesized by cells; under the action of ACAT (acyl CoA-cholesterol acyl transferase), oleic acid binds to cholesterol. The formed cholesterol esters are evidence of the form of transport of cholesterol in lipoproteins (Shirai *et al.,* 2002; Legrand, 2007).

In comparison with SFAs and MUFAs, PUFAs constitute the largest part of mackerel lipid muscles. This result is in accordance with Özogul *et al.* (2007), who studied *Scomber scombrus* from the Marmara Sea and with Guizani and Moujahed (2015) and El Oudiani *et al.* (2016), who studied the Atlantic mackerel from the North and South East of Tunisia. The highest PUFA levels are those of the *n-3* PUFA family, mainly represented by (EPA, C20:5 *n-3*) and (DHA, C22:6 *n-3*). Concerning the *n-6* PUFA family, the highest proportion was that of arachidonic acid (C20:4 *n-6*); which varied significantly among seasons ($p < 0.05$), but not between sexes ($p > 0.05$).

The seasonal variability in PUFA peaks may be explained by the impact of temperature, food availability, competition for food during each catching season and the physiological state of the fish. The effect of temperature on PUFA was studied by Paulina *et al*., (2016), who claimed that the unsaturation of the fatty acids in mackerel depends on geographical location. In fact, the ocean temperature was probably responsible for these changes. Therefore, the highest temperatures in the East of Iceland can explain the high saturation degree; while the lowest temperatures in the Northeast of Iceland are responsible of the lowest saturation and elevation in unsaturated degree of fatty acids. Moreover, according to Dwyer *et al.,* (2003), in a trophic relationship the predator's fatty acid composition depends on its feeding preferences. This linkage provides an opportunity for the identification of trophic relationships in the marine environment and the correlation between fatty acids and diet composition, leading to the identification of the climatic process and to better knowledge on ecosystem dynamics (Dalsgaard *et al.,* 2003).

In our study, the decrease in PUFA levels for both males and females was observed in autumn with 39 and 47.5%, respectively. This decrease may be explained by the mobilization of fish fat reserves serving for gonad maturation. According to Garrido *et al.,* (2008), food availability during the reproduction resting stage influences the amount of fat content accumulated by sardines before the spawning season and hence impacts the reproduction process.

In our study PUFA/SFA ratio values varied from 1 to 2 for males and from 1.3 to 1.6 for females. The highest levels were obtained in summer for males and in winter for females; whereas lowest levels were obtained in autumn for both sexes. The mínimum ratio value recommended is 0.45 (HMSO, 1994).

The established n-3/n-6 ratios showed high levels in spring for both males and females with 11.2 and 11, respectively. In fact, according to Özogul *et al.,* (2007) the n-3/n-6 ratio for marine fish species varies from 5 to 10 or more, which is in accordance with our findings, which ranged from 5.4 to 11.2.

In the present data EPA + DHA concentration for males ranged from 77mg/100gww in autumn to 186 mg/100g ww in summer. For females EPA + DHA concentration ranged from 117 mg/100g ww in spring to 311 mg/100g ww in summer. We deduced that in summer we have the highest EPA+DHA concentrations for males and females. The obtained values are in accordance with the recommendations of the organizations listed below and are included in the range recommended by the International Society for the Study of Fatty Acids and Lipids (ISSFAL), which is 300 – 400 mg EPA+DHA / day for the general adult population, which corresponds to 2-3 servings/wk of fish. Moreover, the AFFSA organization France (2013) recommends 500 mg EPA+DHA /day with (250mg EPA + 250 mg DHA) for the general adult population. The NATO workshop on ω-3 and ω-6 fatty acids recommends 300-400 mg EPA+DHA/day for the general adult population, Simopolous (1989). The German society for nutrition and healthy start - young family network recommend 250 mg LCPUFA /day for primary prevention of CVD. In order to supply the recommended 200 mg/day of DHA, one should consume 2 servings/wk of marine fish, including at least one serving of fatty sea fish (such as mackerel, herring, sardine, salmon) for pregnant women.

4.3. Nutritional quality indexes

In our study the AI mean value for males and females was about 0.5. This value is lower than those found by Turan *et al*., (2011) in their study on *Crangon crangon* and *Raja clavata* from the Black sea which were about 1.35 and 2.37, respectively; and that of Valfre, *et al.,* (2003) in their study on anchovy, which was about 1.35. TI mean value for males and females was about 0.65. This value is close to that found by Valfre *et al.,* (2003) in their study on anchovy (0.45).

5. CONCLUSIONS

From the nutritional point of view, and whether it be male or female, *Scomber scombrus* fillets are characterized by high levels of fats and protein contents and by a high proportion of n-3 PUFAs, mainly DHA. Based on EPA + DHA concentrations, summer seems to be the most favorable period for its consumption, when these nutrients are at their optimal levels. The levels of these valuable components vary seasonally and depend on fish diet composition and environemental parameters. These variations are reflected in fish body composition, influencing the physiological state of the fish, spawning, reproduction, growth, recruitment and survival at younger stages.

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