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Fatty acids and amino acids contents in *Scomber scombrus* fillets from the South East of Tunisia

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Proximate composition, fats and amino acids of Atlantic Mackerel fillets (*Scomber scombrus*) from the South East of Tunisia in different seasons, were analyzed in order to assess nutritive characteristics of this species. Samples were collected monthly from Zarzis fishing port located in the South-East of Tunisia. Total fats and protein contents varied significantly (P<0.05) according to the season. The highest values were obtained in spring (11.53 and 24.1% DM, respectively). Gas chromatography analysis showed the existence of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids. Palmitic and stearic acids were the major fatty acids in SFA family. Palmitoleic and oleic acids were the predominant in MUFA family. Arachidonic acid was the dominant in n-6 PUFA family. While eicosapentanoic (EPA) and docosahexanoic (DHA) were the most abundant in n-3 PUFA series. We observed that high PUFAs percentages were related to those of n-3 PUFA family, mainly DHA which was present at a high level and varied significantly with season (P< 0.01) with the highest value in winter (40%). The n-6 PUFA series were present at low rates comparatively with those of n-3 PUFA series ranging between 4.5 and 5.7%. The highest level of n-6 PUFAs was observed for arachidonic acid in autumn 3.71%. The n-3/n-6 ratio exhibited the highest level in spring (11.02). The Atlantic Mackerel fillets were high in essential amino acids (34.59 g/100 g of proteins). The highest rates were noted for phenylalanine, valine, threonine, isoleucine, leucine and methionine. It was concluded that Atlantic Mackerel was high in interesting human feeding nutriments, mainly PUFA and essential amino Acids. Even when significant, differences between seasons were not drastic and *S. scombrus* could be consumed during all the year.

Key words: *Scomber scombrus*, lipids, fatty acids, amino acids, seasonal variation.

INTRODUCTION

Currently, a growing interest is given to marine resources, mainly fishes as high nutritive and dietetic value foods (Ackman et al., 2002). These nutritive qualities are related to the high quality of the nutriments they include and the high digestibility of their protein and fat. Fishes are known to have an advantageous composition of fatty acids particularly rich in polyunsaturated essential fatty acids (PUFAs).

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Unsaturated fatty acids may be synthesized by animals or humans only to a limited extent and must be supplemented by the diet (Steffens, 1997). These acids, mainly those from n-3 family interest consumers and scientists since they could have beneficial effects on human health by preventing some diseases such as asthma, arteriosclerosis, and joint inflammation as well as preventing cardiovascular diseases and delaying the development of some cancers (Kinsella, 1988; Sidhu, 2003; Chen et al., 2007; Calviello et al., 2007). Fish fats included also large amounts of saturated fatty acids mostly associated with triacylglycerols and minor's amounts of phospholipids (Shahidi and Wanasundara, 1998; Qari et al., 2014) and the HMSO (1994) recommend a ratio of PUFA/SFA less than 0.45 in consumed diets. The PUFAs are known by their different biological effects (James and Cleland, 1996) and include two main series consisting on n-3 and n-6 families. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, the most important PUFA from n-3 family, play the most important role in the prevention of cardiovascular diseases and the daily prophylactic dose of DHA and EPA for adults and patients with circulatory disorders is 1 to 4 g (Kris-Etherton et al., 2002). Fishes are also high in PUFA from the family of n-6, but in lower proportions than those of n-3 PUFA family. The n-6 family is mostly represented by arachidonic acid and could have an antagonist effect with n-3 PUFAs. That is why it is recommended to have a maximum n-6/n-3 ratio of 4. In addition, fishes are an advantageous source of amino acids which are considered as the major constituent of proteins content, known to play an important role in the synthesis of metabolic molecules, enzymes and hormones (Oluwaniyi et al., 2010).

In Tunisa, Atlantic Mackerel are produced in important quantities, during the last year's production reached an amount of 4725 tones (DGPA, 2014). This species, associated to some other blue ones such as *Sardina Pilichardus*, *Sardinelle aurita* and, *Trachurus trachurus* is considered as one of the most popular fish consumed by the population and largely included in several local gastronomical habits. *Scomber Scombrus* is known to be high in protein and fat and is characterized by its high content of long chain of PUFAs (Ackman, 1990). In Tunisia, some studies focused on Mackerel eco-biology investigations (Hattour, 1979; Hattour, 2000; Cherif et al., 2008), but there was few or not available details on their flesh biochemical composition as related to the variation factors.

The current study aimed to assess seasonal impact on proximate composition and fatty acid contents of *S. scombrus* fillets and to characterize its amino acid profile.

MATERIALS AND METHODS

Sample collection and preparation

The studied samples were collected monthly and randomly from Zarzis fishing port located in the south east of Tunisia during the period between June 2010 and May 2011. Samples were taken from the fishers at landing. A case of Mackerel samples (30 specimens) was transferred in ice, in polystyrene boxes and transported to the laboratory, where they were measured, beheaded, washed, filleted, bag packed and frozen at -20°C. Mean length and weight of analyzed samples were about 23.7 cm and 120.65 g, respectively.

Laboratory analysis

Chemical analysis

Moisture content of flesh fish samples was determined by oven drying (5 g, at 105°C until constant weight, ISO 6496-1999). Ash content was determined by calcinations of 5 g samples in a furnace at 550°C according to the AOAC (2000) and results were expressed as percentage of dry matter (DM). Crude protein (N×6.25) was determined by the Kjeldahl method (ISO 5983-1997) and results were expressed as percentage of DM. Total lipid quantification was carried out using the Soxhlet method. A sample of 5 g of flesh powder were added to 200 ml of petroleum ether and heated for 6 h (AOCS, Ba 3-38). The extracted oil was evaporated under vacuum at 65°C using a rotary evaporator, and then placed in an oven at 45°C for 1 h before being transferred into desiccators and reweighed. All the analysis were performed in 6 repetitions.

Amino acids analysis

Amino acids were analyzed according to the method described by Cohen and Michaud (1993). Flesh fish (5 g) was hydrolyzed in 6 M HCl for 22 h at 110°C and then the identification of amino acids (AAs) was made by Gas-Liquid Chromatography (L1100 Waters) equipped with a quaternary pump, a 20 µl injection valve, a diode array fluorescence detectors and a C18 column (internal diameterof: 250 × 4.6 mm) 5 µm. The mobile phase A was composed of 10% of acetonitrile/methanol/water (45:45:10; v/v/v) and the mobile phase B was composed of 90% of sodium phosphate buffer Na_2HPO_4 (pH= 6.5). The flow rate was constant (1 ml/min), and the column temperature was set at 25°C. The fluorescence excitation and emission wavelengths were 340 and 450 nm respectively. Amino acids were identified by comparison of their retention times with those of standards and quantified with the software EZChrom Elite™ CDS Chromatography. Analysis was made in duplicate and results were expressed in g of amino acid per 100 g of protein.

Fatty acids analysis

Methyl esters preparation was carried out using a direct transesterification according to the procedure described by Mosers (1991). A sample of 0.5 g of fish flesh was mixed with 2 ml of methanol/methylene chloride (3:1) and 50 µmol of 17:0 free fatty acid as an internal standard (in 50 µl of hexane) was added to the mixture. Under continuous vortexing, 200 µl of acetyl chloride was added and the mixture was incubated in the oven at 75°C for one hour. After cooling during 15 min at room temperature, 4 ml of 7% potassium carbonate was added, vortexed and then 2 ml of hexane was added. The mixture was vortexed for 60 s and then centrifuged for 5 min. The hexane layer was removed, dried under nitrogen to a final volume of 100µl approximately and 1 µl of the sample was used for gas chromatography analysis. Fatty acid identification was performed using a gas chromatograph (HP series 6890) with a split/splitless injector, and a flame ionization detector was used for the analysis. The device includes a 30 m long HP Innowax capillary

Table 1. Proximate composition of Atlantic Mackerel Fillets.

Parameter	Autumn	Winter	Spring	Summer	Significance
Moisture (%)	70.61 ± 0.41^{b}	71.09 ± 0.69^{b}	71.03 ± 0.73^{b}	72.5 ± 1.04^{a}	**
CP (%)	18.71±1.52^{a}	19.94 ± 0.90^{a}	24.1 ± 0.90^{b}	23.5 ± 2.52^{a}	**
Fat (%)	6.32 ± 0.91^{c}	7.55 ± 0.29^{b}	11.53 ±1.93^{a}	4.35 ± 0.11^{d}	***
Ash (%)	2.3 ± 0.75^{a}	1.97 ± 0.72^{b}	1.80 ± 0.28^{b}	1.95 ± 0.32^{b}	***

Means with the same letter in the raw are not significantly different.

column with an internal diameter of 250 µm and a 0.25 µm film. The stationary polar phase of the column was composed of polyethylene glycol. Comparison of the retention times of the studied fatty acids and those of standard fatty acid methyl esters (Supelco PUFA-3) allowed to identify the different fatty acids contained in mackerel oil extract. All the analysis were performed in 6 repetitions.

Statistical analysis

Statistical analysis was performed using analysis of variance according to GLM procedure (SAS software, version 9.1). The model included season effect and each month of sampling was considered as replication. Means were compared using Student Newman and Kull tests.

RESULTS

Chemical composition

S. scombrus proximate chemical composition is presented in Table 1. Moisture content varied significantly (P<0.01) with season, a maximum value was obtained in summer 72.5% DM and minimum one in autumn (70.61% DM). Ash contents varied significantly (P<0.01) with seasons, reaching a maximum value of 2.3% DM in autumn and minimum value in spring (1.80% DM). Fat seasonal variation was highly significant (P<0.001). The highest level was noted in spring (11.53% of DM), whereas the lowest one was observed in summer (4.35% of DM). Also, crude protein contents varied significantly with seasons (P<0.001) with highest content in spring (24.1% DM) and a lowest one in autumn (18.71%DM).

Fatty acids contents

Results relative to FAs composition are presented in Table 2. Mackerel lipid extract is composed mainly of PUFAs followed by SFAs and MUFAs. The SFAs group was represented by palmitic acid (C16:0) as the most important one, reaching in autumn 25.2%, followed by stearic acid (C18:0) with a maximum level in winter (11.52%) and the myristic acid (C14:0) showing a highest content in summer (2.24%). The season effect of the last FAs was significant (P<0.01). Total SFAs seasonal variation was highly significant (P<0.001) and rates ranged between 33 and 38%.

The MUFAs group was represented by palmitoleic (C16:1) n-7 and oleic (C18:1) n-9 acids, for these two FAs the highest levels were registered in autumn (3.01 and 11.20% respectively). The effect of season was significant (P<0.01).

The PUFAs represented the most important group of FAs in *S. scombrus* lipids. The seasonal variation for these FAs was significant (P<0.01). High PUFA levels are related to those of n-3 family, mostly represented by the EPA and the DHA. The highest levels were registered in spring (7.03%) for EPA and in winter for the DHA (40%). In the group of the n-6 PUFAs, the highest proportion was noted for the arachidonic fatty acid (C20:4). The values varied significantly with season (P<0.01) and reached the maximum level of 3.71% in winter. The highest n-3/n-6 ratio is obtained in spring 11. 02%.

Amino acid contents

Amino acids results are exhibited in Table 3. The highest values were noted for glutamic and aspartic acids 11.76 and 9.84 g/100 g CP, respectively. The highest EAA concentrations were observed for Lysine, followed by Threonine, Tyrosine, Isoleucine and then Leucine. The lowest concentration corresponded to Cystine (1 g/100 g CP). Total EAA was about (34.59 g/100 g of CP). The other identified amino acids are non-essentials (NEAA) and their total content was about 55.15 g/100 g of CP. The established TEAA/TNEAA ratio was 0.62.

DISCUSSION

Chemical composition

The fat contents in *S. scombrus* fillets varied significantly (P<0.01) with season. This finding is in agreement with those of Caponio et al. (2004), Ennouri et al. (2013), and Ben Rebah et al., (2014) in their studies on *Sardina pilchardus, Sarinella aurita, and Liza aurata*, from the Ionian Sea, the Gulf of Tunis and the Gulf of Gabès, respectively. However, according to Wallace (1991) mackerel fat fillets content values are ranging between 25 and 30% in December and are around 5% in May, when

Table 2. Atlantic Mackerel Fatty Acids (%TFAs).

Fatty acids	Autumn	Winter	Spring	Summer	Signifiance	SEM
C14:0	1.81±0.79[a]	1.20±0.37[a]	1.53±0.48[a]	2.24±0.20[b]	**	0.144
C16:0	25.25±4.78[a]	23.62±5.14[a]	21.48±0.77[a]	22.26±1.94[a]	ns	0.445
C18:0	10.94±2.22[a]	11.52±3.49[a]	9.99±1.43[a]	11.02±2.04[a]	ns	0.278
Total SFA	38 [a]	36,3[b]	33[b]	35[b]	***	0.648
C16:1	3.07±0.50[a]	2.45±0.74[a]	2.71±0.95[a]	2±0.31[b]	**	0.137
C18:1	11.20±1.6[a]	9±2.91[a]	9,62±2,28[a]	9 ± 0.25[a]	**	0.477
Total MUFA	13.8[b]	11.4[a]	12.33[a]	10.4[c]	***	0.566
C18:2 n-6	1.45±0.21[a]	1.31±0.14[a]	1.18±0.16[b]	1.42±0.15[a]	**	0.03
C18:3 n-6	0.20±0.08[a]	0.20±0.12[a]	0.16±0.05[a]	0.20±0.11[a]	ns	0.009
C20:2 n-6	0.34±0.47[a]	0.13±0.07[b]	0.29±0.13[a]	0.39±0.3[a]	**	0.025
C20:4 n-6	2.69±0.35[b]	3.71±0,55[a]	2.83±0,45[b]	3.7±0.49[a]	**	0.028
Total PUFA n-6	5.3[a]	5.3[a]	4.5[b]	5.7[a]	**	0.021
C18:3 n-3	0.37±0.11[a]	0.32±0.06[a]	0.54±0.13[a]	0.7±0.42[b]	***	0.084
C20:3 n-3	0.38±0.15[b]	0.29±0.17[a]	0.24±0.16[a]	0.44±0.17[b]	**	0.095
C20:5 n-3	5.04±0,78[a]	5.43±0.62[a]	7.03±0.85[b]	5.61±0.38[a]	**	0.185
C22:5 n-3	1.08±0.23[a]	1.15±0.25[a]	1.51±0.15[a]	1.13±0.05[a]	ns	0.043
C22:6 n-3	36±7.10[a]	40±10.23[b]	33±2.64[a]	36.01±3.36[a]	**	0.822
TotalPUFAn-3	42.7[b]	46.8[a]	50[a]	48[a]	**	0.831
PUFA/SFA	1.26a	1.43a	1.65a	1.53a	ns	0.042
n-3/n-6	8.08[a]	8.75[a]	11.02[a]	8.57[a]	ns	0.217

*, P < 0.05; **, P < 0.01; ***, P< 0.001; ns, not significant; SEM, standard error of the mean; SFA, Saturated fatty acids, MUFA,: monounsaturated, PUFA, polyunsaturated fatty acids.

Table 3. Mackerel flesh amino acid composition (g/100 g of crude proteins).

Amino acids	Concentration (g/100 g of crude proteins)
Aspartic acid	9.84 ± 0.7
Glutamic acid	11.76 ± 0.9
Lysine [a]	5.90 ± 1
Histidine	4.56 ± 0.67
Arginine	5.86 ± 0.24
Threonine [a]	5.00 ± 0.36
serine	4.88 ± 0.2
Proline	6.02 ± 0.59
Glycine	5.30 ± 0.04
Alanine	6.93 ± 0.95
Cystine [a]	1.00 ± 0.01
Valine [a]	3.94 ± 0.50
Methionine [a]	3.20 ± 0.08
Isoleucine [a]	4.02 ± 0.39
Leucine [a]	3.89 ± 0.48
Tyrosine [a]	5.00 ± 0.78
Phenylalanine [a]	2.64 ± 0.11
Total amino acids (TAA)	96.67
Total essential amino acids (TEAA)	34.59
Total non-essential amino acids (TNEAA)	55.15
(TEAA)/(TNEAA)	0.62

[a]Essential amino acids according to FAO/WHO(1975); Values are expressed as means ±SD with (n=2).

fish spawns. These variations are the result of the impact of changes of some factors such as temperature, salinity, food availability and fish life cycle (Zaboukas et al., 2006; Pirini et al., 2010).

Crude protein content varied significantly with season ($P<0.01$) with a maximum value in spring 24.1% and a minimum value in autumn 18.71%.This finding is in line with those in other marine species as reported by Kacem et al. (2011) in their study on *Sardinella aurita*, *Sarpa salpa*, *Sepia officinalis* from Tunisia and by Orban et al. (2011) in their study on horse mackerel from the Southern Adriatic coast of Italy. This variation is the result of the impact of environmental parameters such as temperature, fluctuation in food availability and essentially fish life cycle. In fact, during the spawning period, lipids and proteins contents are mobilized from mussels and transferred to the gonads (Love, 1997).

Fatty acids composition

Fatty acid analysis indicated the presence of different categories of fatty acids, mainly SFAs, MUFAs and PUFAs. It was noted that the predominant fatty acid in SFA family was Palmitic acid followed by the stearic acid. However, Myristic acid (C14:0) exhibited the lowest proportion. This result is in line with those of Rioux and Legrand (2001) who claimed that the lowest proportion in the animal body is represented by Myristic acid (ranging between 0.5 and 2% of TFAs). Palmitic acid was the most represented SFA but the season effect was not significant (averaged 35.5%). Similar trend was found by Bouriga et al. (2010) on *Atherina boyeri, Atherina lagunae, Atherina* sp. and and by Ben Smida et al. (2010) on *Xiphias gladius* respectively.

Palmitoleic and oleic acids were the two main MUFAs identified in Mackerel lipid extract. The MUFAs highest proportions were those of oleic acid. This result is in accordance with those of Ben Smida et al. (2010) in *X. gladius* red and white mussels; those of Ben Rebah et al. (2014) in the males of *liza aurata* from the Tunisian coasts, and those of Kacem et al. (2011) in *Sardinella aurita* and *Sarpa salpa* from the Gabès Gulf. However, Soriguer et al. (1997) registered high MUFAs level in winter in Atlantic mackerel from Spain, this may be explained by the impact of environmental parameters essentially temperature. The Oleic acid is the characteristic of fish tissue (Steffens 1997) and is actively synthesized by cells (Legrand, 2007). Under the action of ACAT (acyl CoA-cholesterol acyltransferase), oleic acid binds to cholesterol (Legrand, 2007).The formed Cholesterol esters represents the form of the transport of cholesterol in lipoproteins (Steffens, 1997). As reported by Dalsgaard et al. (2003), high MUFAs level is an indicator of high degree of carnivory of this species. Indeed, according to the classification of fishes in functional groups based on their TROPH relation, *S. scombrus* is a pelagic and carnivorous fish species, fed only on the bases of animal species, mainly fish such as *Sardina pilchardus* and Crustaceans and Gasteropods (Stergiou and Karpouzi, 2002). This was confirmed by our observation on digestive tract content of *S. scombrus* (unpublished).

The PUFAs group was the most abundant in Mackerel flesh lipids. The content ranged from 54.5% in spring to 48% in autumn. The highest observed PUFAs levels are linked to the high content of n-3 FA series, mainly represented by EPA and DHA. The DHA represented the highest proportion in winter (40 %). The n-6 family high values are related to those of arachidonic acid, reaching 3.71% in winter. This result is in line with the findings of Özogul et al. (2007) on Mackerel from Turkey. Fishes are generally rich in n-3 FAs and low in n-6 fatty acids. These groups of FAs are known to have beneficial effects for human health (Pigott and Tucker, 1990). An increase in n-3/n-6 ratio is essential to help the body use of fatty acids since n-6 FAs could have an antagonist effect with n-3 FAs (Polak-Juszczak and Komar-Szymczak, 2009). The proportions of EPA and DHA are responsible of variation in n-3/n-6 ratio (Hossain, 2011). In our study the n-3/n-6 ratio was the highest in spring (11.02). This result is in concordance with those of Özogul et al. (2007). The PUFAs /SFAs ratio reached the highest level in spring 1.65. This value is higher than the minimum recommended value (0.45) as claimed by HMSO (1994) for human nutrition. Seasonal variation in PUFAs may be explained by genetic factors, fishing period, sexual maturity stage, reproduction activity, and nutritional factors. Moreover, water temperature is the most important factor influencing PUFA synthesis in fishes. Temperature variations influence desaturase and synthesize enzyme activities with a direct effect, or by long term adaptative process- leading to the synthesis of omega-3 fatty acids (De Torrengo and Brenner, 1976; Farkas and Csengeri, 1976; Caponio et al., 2004).

Amino acids contents

Results showed that Scomber scombrus was high in TAA (96.67 g/100 g protein). This result is in line with which of Oluwaniyi et al. (2010) found in Atlantic Mackerel from Nigeria. It was found that Glutamic acid had the highest concentration (11.76 g/100 g CP). This finding is similar to which of Selmi et al. (2009) study on *Sardina Pilchardus*. The established TEAA/ TNEAA ratio was about 0.62 which is close to the finding of Kaya et al. (2014) in *Sardina melonosticta* (0.69).

Amino acids are the basis of all life processes, as they are necessary for all metabolic processes. Their main task is to ensure optimal transport and to optimize the storage of all nutrients. Their composition in fish and in human tissues is similar. The essential amino acids cannot be synthesized in human body, so they are

required from fish consumption as reported by Osibona et al. (2009). In our study, we did not consider AA variation according to the season. It seems that the type and the amount of amino acids are related to fishing season, locality, feeding habit and fish life cycle (Wesselinova, 2000; Kaya et al., 2014). This aspect needs further investigation in *S. scombrus*.

Conclusions

It was concluded that *S. scombrus* flesh from the region of Zarzis (Tunisia) is rich in unsaturated fatty acids, mainly from the n-3 family, especially DHA. The studied samples had high n-3/n-6 ratio and the PUFAs/SFAs coefficient exceeded the recommended minimum value by HMSO. This represents an advantageous impact when consumed by human. Associated to high level of protein and essential amino acid, our results indicate that the studied species is of a high nutritive value and could be healthy compound in human diets. Even when significant, differences between seasons were not drastic and *S. scombrus* could be consumed beneficially during all the year.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations

AA, Amino acids; **TAA,** total amino acids; **TEAA,** total essential amino acids; **TNEAA,** total non-essential amino acids; **FA,** fatty acid; **SFAs,** saturated fatty acids; **MUFAs,** monounsaturated fatty acids; **PUFAs,** polyunsaturated fatty acids; **EPA,** eicosapentanoic acid; **DHA,** docosahexanoic acid; **ACAT,** acyl CoA-cholesterol acyltransferase.

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