International Journal of Advanced Engineering and Management Research

Vol. 4, No. 01; 2019

ISSN: 2456-3676

EFFECTS OF SEASON AND REGION ON THE BIOCHEMICAL AND FATTY ACID COMPOSITIONS OF THE SARDINA PILCHARDUS

Imen Zaghbib*[1], Soumaya Arafa[1], Sabrine El Adab[1] et Mnasser Hassouna[1]

[1]Research Unity "Bio-Preservation and Valorization of Agro-Food Products–UR 13 AGR02", High Graduate School of Food Industries of Tunisia, University of Carthage, 58 Avenue Alain Savary, Tunis El Khadra 1003, Tunisia
*Corresponding author: Tel: (+216) 92 72 32 21

Abstract
The effects of seasonal and regional variation on the proximate and fatty acid compositions of sardine (Sardina pilchardus) were determined. The levels of lipid displayed pronounced seasonal than regional fluctuations with the highest values in summer and the lowest values in winter for both areas (Kélibia and Téboulba, Tunisia). Negative correlations between fat and water contents were observed. The higher water contents were recorded during winter for sardine of Kélibia and Téboulba (78.5 ± 0.86% and 77 ± 0.5%, respectively). For both areas, the samples were not influenced by seasonality in terms of their total fatty acid ($p>0.05$). The main fatty acids of the total lipid were C16: 0, C14: 0, C18: 0, C18: 1, DHA C22: 6n3, EPA C20: 5n3 and DPA C22: 5n3. Sardina pilchard us muscle contained appreciable levels of omega-3 polyunsaturated fatty acids suggesting the use of this fish as a source of healthy diet for humans.

Keywords: Sardina pilchard us, Season, Region, Variation, Biochemical composition, Fatty acid composition.

Introduction

Marine food especially fish are an important part of the human diet across the world and particularly of the Mediterranean diet (GMA, 2004). Seafood lipids are the best natural source of highly unsaturated fatty acid and are the major contributor of omega-3-PUFA, particularly eicosapentaenoic acid (EPA, C20: 5n-3) and docosahexaenoic acid (DHA, C22: 6n-3) (Nisa et Asadullah, 2011). They are of particular interest because of their role in improving health and reducing the risk of chronic afflictions like cardiac diseases, autoimmune disorders, diabetes, even cancer (Simopoulos, 1991). Therefore, the fatty acid composition should be considered.

Throughout the year, the composition of several fish species varies from season to season due to its natural cycle, maturity stage, geographic location, etc (Bandarra, Batista, Nunes & Empis, 2001 ; Aro, Tahvonen, Mattila, Nurmi, Sivonen & Kallio, 2000). The spawning cycle and food supply are the main factors responsible for this variation (Love, 1980).

Sardine (*Sardina pilchardus*) is one of the most important commercial fish species in the Mediterranean sea and, in particular, in the Tunisian sea, as it is very abundant and for its low cost. Knowledge of biochemical composition of muscles of *Sardina pilchardus*, is of great help in evaluating not only its nutritive value but also helps in quality assessment and optimum utilization of this natural resource. Formulation, processing and preservation of fishery products needs correct information of the biochemical composition. This in turn can help technologist to

Vol. 4, No. 01; 2019

ISSN: 2456-3676

identify the best possible processing, storage conditions and prevent wastage or loss of constituents such as free amino acids, proteins and fats (Jan, Mustafa, Tahila & Showkat, 2012).
The purpose of this study was to clarify the regional and seasonal variation of the biochemical and fatty acid compositions in the Tunisian sardine (*Sardina pilchardus*) in the sea areas of Téboulba and Kélibia.

Materials and methods

Study area

In the present study two stations, the Sahel area particularly the port of Téboulba and Hammamet gulf area particularly the port of Kélibia, were selected (**figure 1**) (Ben Abdallah et Gaamour**,** 2004) according to the higher sardine abundances wich are mostly located in these two areas.

Sample collection and preparation

This study was carried out during one year period, except the months of February and March because of poor weather conditions. For the biochemical composition, fish samples were obtained monthly, and for the fatty acid composition, samples were obtained quarterly (Automn, winter, spring and summer) from Téboulba and Kélibia ports, Tunisia.
Fishes were rapidly transported in ice boxes to the laboratory for preparation to chemical analyses. The average length (13-15 cm) of fish was measured in order to select homogenous samples. Boneless fish fillets were used for the analysis.

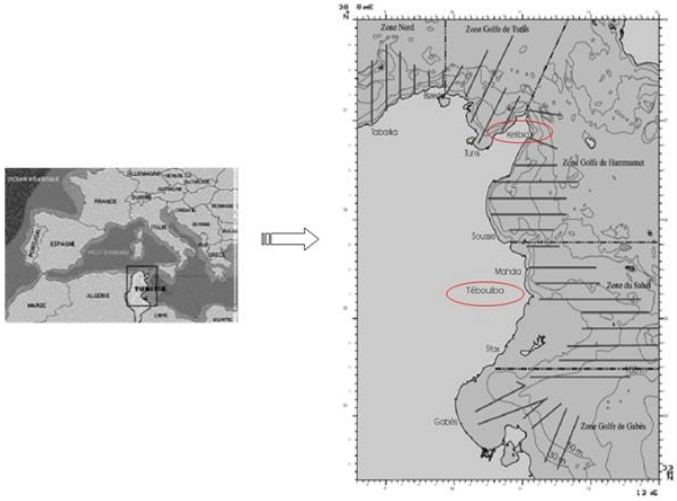

Figure 1. Map of the study area indicates the sampling locations

Proximate analysis

In proximate analysis four major constituents of edible portion of the fish; water, protein, fat and ash were analyzed. Determination of moisture content was conducted by AOAC method (2000). The protein quantity was carried out with the Kjeldhal method (NF V04-407, 2002) by determining the total organic nitrogen amount. The estimation of fat content was accomplished by Folch, Lees & Sloane method (1975). The ash content was determined according to the AOAC method (1995). Measurments were carried out three times.

Vol. 4, No. 01; 2019

ISSN: 2456-3676

Fatty acids analysis

The separation and quantification of the fatty acids were achieved throught gas chromatography (GC) after converting fats into fatty acid methyl esters (FAME) using BF3/methanol (Ackman, 1998).

1 µl of the sample was injected into a gas phase chromatograph with a FID detector (Périchrom 2000, France) fitted with a capillary column (25 m × 0.25 mm). The temperatures of the injector and the detector were set at 260°C. The oven temperature was 70°C, held for 2 min, raised to 180°C to remain at that temperature for 8 min and increased again to 220°C at a rate of 3°C/min. The vector gas used was helium with high purity (99.99%).

Peaks corresponding to FAME were identified by comparing the retention times of fatty acid methyl esters (FAME) with standard mixtures (Sigma Chemical Company).

Content of each fatty acid was expressed as percentage weight of total fatty acids (% wt).

Triplicate GC analyses were carried out and the results were expressed in GC area % as the mean value ± standard deviation (SD)

Statistical analysis

Statistical analysis were carried out with SPSS software® version 17.0 (SPSS, Inc, Chicago, IL). Values are expressed as mean ± standard deviation. A one-way ANOVA and a Duncan's multiple range tests were used to compare means at 0.05 probability level.

Results and discussion

Lipid variation

The present study indicated that there is substantial variation in lipid content of *S. pilchardus* collected from the Kélibia and Téboulba coasts ($p < 0.05$) and there were significant differences ($p < 0.05$) observed over the year (2013 – 2014). It was found that the lipid content varied between a maximum of 7.82 ± 0.18% and 7.14 ± 0.2% in the end of spring-summer, and a minimum of 1.67 ± 0.15% and 1.53 ± 0.06% in the winter in sardine of Kélibia and Téboulba, respectively (**table 1**).

Seasonal data showed that even though fat content is somewhat low in the present study but the variation trend was to some extent in agreement with the values of Zlatanos and Laskaridis (2007) for *S. pilchardus* who reported that Mediterranean sardine from northern Greece had a minimum lipid of 3.88% in February and a maximum of 11.86% in April. Shirai et al. (2002) also found a minimum lipid of 1.8% in February and a maximum of 7.2% in August in sardines (*Sardinops melanostictus*).

Low levels of lipid were observed in the reproductive season of the species. Thus it was found that fat content of muscle is less in the month of January for sardine of Kélibia (1.67 ± 0.15%) and Téboulba (1.53 ± 0.06%) during their spawning season.

Sexual maturation has been found to reduce lipid body stores in salmon because this species stops feeding during maturation and lipid stores are directed to gonad lipids or used for energy (Bell et al., 1998). Other studies have also shown that, although sea bream does not stop feeding,

Vol. 4, No. 01; 2019

ISSN: 2456-3676

reduced feeding level and sexual maturation contribute to reduced lipid levels during winter (Grikorakis et al., 2002).

Table 1. Monthly and regional variation of the proximate composition (%) of sardine (*Sardina pilchardus*)

Month	Region	Proximate composition (%)			
		Water content	Lipid content	Protein content	Ash content
January	Kélibia	77.06 ± 0,29[e]	1.67 ± 0.15[a]	15.45 ± 0.42[a]	2.76 ± 0.00[a]
	Téboulba	75.76 ± 0.36[f]	1.53 ± 0.06[a]	14.93 ± 0.23[a]	2.71 ± 0.01[b]
April	Kélibia	69.21 ± 0,24[b]	6.97 ± 0.08[e]	17.76 ± 0.17[c]	2.75 ± 0.01[a]
	Téboulba	68.73 ± 0.63[c]	6.36 ± 0.19[d]	17.66 ± 0.2[d]	2.68 ± 0.01[b]
May	Kélibia	68.36 ± 0,64[b]	7.24 ± 0.12[e]	19.2 ± 0.78[d]	2.75 ± 0.08[a]
	Téboulba	66.39 ± 0.37[b]	6.96 ± 0.14[e,f]	18.24 ± 0.13[e]	2.64 ± 0.06[b]
June	Kélibia	68.4 ± 0,55[a]	7.82 ± 0.18[f]	19.39 ± 0.3[d]	2.46 ± 0.1[a]
	Téboulba	67.66 ± 0.37[a]	7.14 ± 0.21[f]	18.94 ± 0.1[f]	2.34 ± 0.27[a]
July	Kélibia	68.46 ± 0,4[b]	7.09 ± 0.13[e]	18.63 ± 0.27[d]	2.59 ± 0.23[a]
	Téboulba	67.84 ± 0.58[b]	6.61 ± 0.16[d,e]	18.55 ± 0.06[e]	2.51 ± 0.1[a,b]
August	Kélibia	71.43 ± 0,71[c]	6.82 ± 0.08[e]	17.26 ± 0.32[b,c]	2.67 ± 0.1[a]
	Téboulba	71.12 ± 0.14[d]	6.45 ± 0.09[d]	17.7 ± 0.16[d]	2.62 ± 0.09[b]
September	Kélibia	74.79 ± 0,38[d]	6.03 ± 0.12[d]	17.15 ± 0.27[b,c]	2.7 ± 0.06[a]
	Téboulba	74.06 ± 0.41[e]	5.81 ± 0.13[c]	17.18 ± 0.14[c]	2.66 ± 0.04[b]
October	Kélibia	76.67 ± 0,58[e]	5.61 ± 0.34[c]	16.66 ± 0.21[b]	2.69 ± 0.25[a]
	Téboulba	73.67 ± 0.58[e]	5.67 ± 0.58[c]	16.29 ± 0.13[b]	2.7 ± 0.04[b]
November	Kélibia	77.33 ± 00,76[e]	2.34 ± 0.19[b]	16.5 ± 0.28[b]	2.64 ± 0.18[a]

Vol. 4, No. 01; 2019

ISSN: 2456-3676

	Téboulba	76.33 ± 0.29^{f,g}	2.98 ± 0.58^{b}	16.24 ± 0.13^{b}	2.67 ± 0.12^{b}
Décember	**Kélibia**	78.5 ± 0,86^{f}	2.08 ± 0.3^{b}	15.66 ± 0.26^{a}	2.78 ± 0.01^{a}
	Téboulba	77 ± 0.5^{g}	1.81 ± 0.07^{a}	15.44 ± 0.47^{a}	2.74 ± 0.01^{b}

* Values are means of triplicate determinations ± SD. Different letters in the same column indicate significant differences ($p < 0.05$).

Protein variation

The sardine of Kélibia and Téboulba contain on average 17.36% and 17.14% of proteins, respectively (**figure 2**). These figures thus enable sardine flesh to be rated among good nutritional quality food. Pelagic fish have a protein content varying from 15 to 20% (Hannachi et al., 2011). Protein fraction varies very little in relation to season ($p < 0.05$) and region ($p < 0.05$) and the variation trend is similar to that of lipid content. We recorded a variation from 15.45 ± 0.42 to 19.39 ± 0.3% for Kélibia's sardine and from 14.93 ± 0.23 to 18.94 ± 0.13% for Téboulba's sardine with a minimu noticed in winter and a maximum in summer (**table 1**) which is in good agreement with previously reported results by Jan et al. (2012) and Ahmed, Mustafa, Alam, Rubbi & Moslemuddin (1984).

The variation of the protein fraction may be due to the planktonic feed and to climatic changes in the year which influence the general biochmical composition of fish (Hannachi et al., 2011). In winter, food availability is less which resulted in poor growth as indicated in January. During spring and summer seasons, protein content raised as temperature and food availability changes. Intense feeding shown by the fish is because during spawning, fish had lost gonadal elements and recoups to compensate the expenditure through vigorous feeding activity (Jan et al., 2012).

According to Islam ET Joadder (2005), at the mature stage, muscle protein started declining gradually because most of the proteins might have been accumulated in the gonads at the time of spawning. The gonadal elements get released either as eggs or sperm carriyng the protein along with them. But immediately after spawning, as the gonad is in recovery stage and without any gonadal elements, the food that is consumed by the fish might have been used in the building up of the muscle. So, the protein cycle appears to be having a strong correlation with spawning.

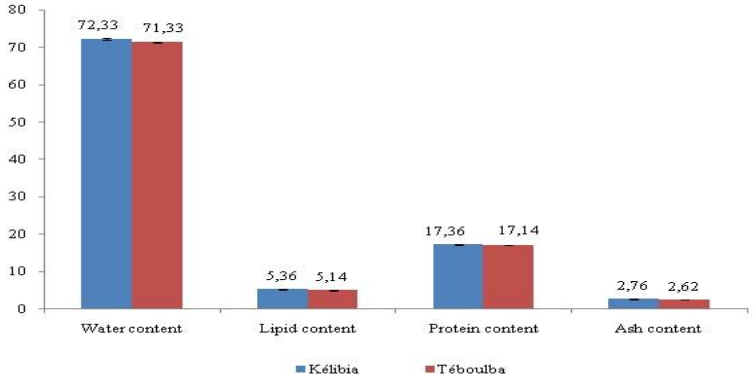

Figure 2. Average proximate composition (%) of sardine (*Sardina pilchardus*)

Vol. 4, No. 01; 2019

ISSN: 2456-3676

Water variation

Figure 2 shows that the average water content is 72.33% and 71.33% for sardine from Kélibia and Téboulba, respectively. From this result we can cofirm that the major component of fish fillet is water. It was found that the water content in different months varied from 78.5 ± 0.86% to 68.36 ± 0.64% in Kélibia's sardine ($p < 0.05$) and from 77 ± 0.5% to 66.39 ± 0.37% in Téboulba's sardine ($p < 0.05$).

Water content of the sardine fillet, for both areas, was maximum in winter (December) and minimum in the end of spring-summer (May) (**table 1**) and showing an inverse relationship with fat content (**figure 3**). The results of fitting a linear model to describe the relationship between lipid and water contents for one year of analysis are shown in **figure 4**. Given the relationship between the two contents in question, a simple regression equation was developed based on the trend line in **figure 4** would allow the fat content to be estimated based on the water content. A very strong correlation between fat and water contents was observed. The coefficient of determination R^2 indicates, statistically, that the models as fitted explain 77% and 75% of the variability in fat content for samples of Kélibia and Téboulba, respectively. Pearson correlation coefficient reveled a negative correlation between the two fractions (r = -0,87 et -0,86 for samples of Kélibia and Téboulba, respectively). In fact, according to the regression line, it is observed that a decrease in fat content correspond to an increase in water content and conversely.

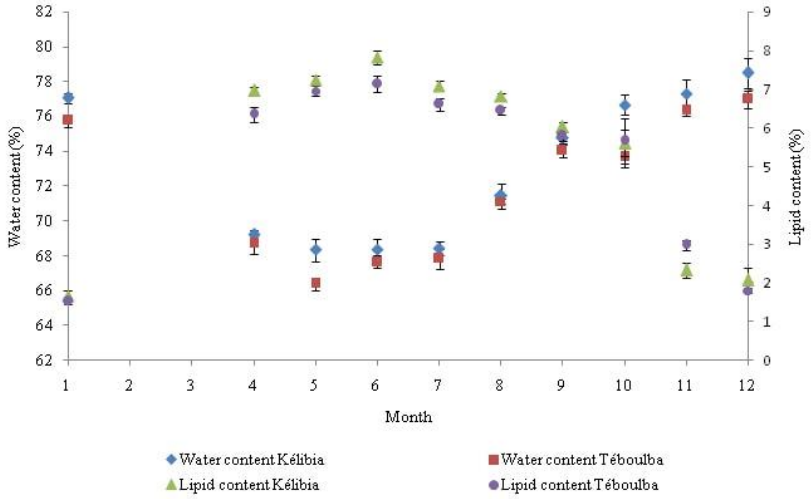

Figure 3. Functional relationship between lipid and water contents of sardine (*Sardina pilchardus*)

These results are in accordance with Hannachi et al. (2011) who found that water content of the Moroccan Mediterranean cost anchovy (*Engraulis encrasicolus*) varies according to the fatness of the fish. Huss (1995) has also noted that variations in percentage of fat should be reflected in the percentage of water, and the two normally constitute around 80% of the fillet.

Other studies have also shown inverse proportionality between fat and water contents in some medium-fatty and fatty fish species muscle (Love, 1997; Šimat and Bogdanović, 2012).

These variations may be attributed to the biologycal cycle of sardine and the environmental factors such as sainity. In facts, the minimal values of water contents, for both areas, were

Vol. 4, No. 01; 2019

ISSN: 2456-3676

registred in summer and the maximal ones were found in winter, a period characterised with high pluviometry which decrease water salinity and thus enhace muscle hydratation.
In this context, Pérez-Velazquez et al. (2014) reported a significant inverse relation between salinity and water content of fish. Liang et al. (2008) and Pérez-Velazquez et al. (2007) reported an increase in water content of juvenile shrimp *L. vannamei* when salinity decrease.

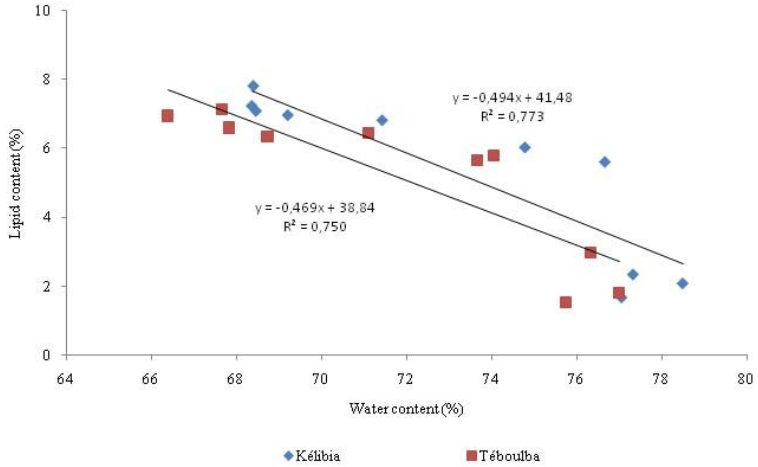

Figure 4. Seasonal changes of lipid content in fonction of water conetnt of sardine (*Sardina pilchardus*)

Ash variation

For the ash content, *Sardina pilchardus* muscle showed a non significant fluctuation according to season and region ($p > 0.05$) especially in December (2.78% and 2.74% for Kélibia and Téboulba, respectively) and Juin (2.46% and 2.34% for Kélibia and Téboulba, respectively) during the study period (**table 1**). The average annual ash content of the sardine muscle is about 2.76% for sardine of Kélibia and 2.62% for sardine of Téboulba (**figure 2**). Similar results were reported by Islam et Joadder (2005) indicating that there was no seasonal variation observed in the body muscle of freshwater Gobie (*Glossogobius giuris*).
Generally, the concentration of minerals in fish is influenced by a number of factors such as species, size, dark/white muscle, age, sex, sexual maturity, area of catch, food source, water chemistry, salinity, temperature and contaminants, etc. (Khitouni et al., 2011; Noël et al., 2011; Yildiz, 2008).

Fatty acids variation

For many years, seafood such as fish, mollusks and crustaceans has often been the focus of attention in nutritional studies. Nutritionists (Médale et al., 2003) consider these products to be an important source of high-quality proteins, minerals, vitamin D and essential fatty acids (FAs) such as omega 3. Muscle is the main part of fish used for human consumption and when fish is suggested as means for improving health, fatty acids composition should be considered.
Analysis of fatty acids composition of samples in function of season and region showed a little variation in total fatty acids for both areas (**table 2**).

Vol. 4, No. 01; 2019

ISSN: 2456-3676

Table 2. Seasonal variation in the fatty acid composition (%) of total lipids from sardine (*Sardina pilchardus*)

Fatty acids	Season							
	Winter		Spring		Summer		Automn	
	Kélibia	Téboulba	Kélibia	Téboulba	Kélibia	Téboulba	Kélibia	Téboulba
C12:0	$0.57 \pm 0.02^{a.b}$	0.51 ± 0.08^{a}	0.22 ± 0.03^{a}	0.26 ± 0.02^{a}	$0.38 \pm 0.18^{a.b}$	0.4 ± 0.24^{a}	0.69 ± 0.30^{b}	0.65 ± 0.29^{a}
C13:0	0.8 ± 0.07^{a}	0.93 ± 0.09^{b}	0.75 ± 0.05^{a}	0.51 ± 0.08^{a}	0.74 ± 0.06^{a}	$0.63 \pm 0.22^{a.b}$	0.75 ± 0.11^{a}	$0.71 \pm 0.07^{a.b}$
C14:0	3.64 ± 0.79^{a}	4.25 ± 1.8^{a}	7.78 ± 0.15^{c}	7.38 ± 0.28^{b}	7.75 ± 0.16^{c}	7.25 ± 0.35^{b}	4.89 ± 0.62^{b}	$5.28 \pm 0.46^{a.b}$
C14:1	0.16 ± 0.02^{a}	0.19 ± 0.1^{a}	0.15 ± 0.01^{a}	0.17 ± 0.01^{a}	0.17 ± 0.03^{a}	0.18 ± 0.02^{a}	0.29 ± 0.01^{a}	0.22 ± 0.02^{a}
C15:0	1.47 ± 0.11^{b}	1.43 ± 0.35^{a}	0.8 ± 0.02^{a}	1.22 ± 0.06^{a}	0.83 ± 0.06^{a}	1.25 ± 0.07^{a}	1.28 ± 0.34^{b}	1.25 ± 0.12^{a}
C16:0	23.1 ± 0.26^{a}	$22.39 \pm 0.08^{b.c}$	22.39 ± 0.04^{a}	19.96 ± 0.37^{a}	23.15 ± 0.42^{a}	22.97 ± 0.62^{c}	22.13 ± 1.45^{a}	21.8 ± 0.24^{b}
C16:1	5.47 ± 0.18^{a}	5.28 ± 0.26^{a}	6.07 ± 0.7^{a}	5.8 ± 0.68^{a}	6.05 ± 0.7^{a}	5.72 ± 0.69^{a}	5.55 ± 0.84^{a}	5.3 ± 0.35^{a}
C16:2n4	0.89 ± 0.13^{a}	1.12 ± 0.29^{a}	0.9 ± 0.02^{a}	1.01 ± 0.08^{a}	0.87 ± 0.06^{a}	0.98 ± 0.12^{a}	1.36 ± 0.19^{b}	1.24 ± 0.05^{a}
C16:3n6	$1.04 \pm 0.01^{a.b}$	0.99 ± 0.01^{a}	0.88 ± 0.04^{a}	0.91 ± 0.03^{a}	0.89 ± 0.04^{a}	0.91 ± 0.03^{a}	1.1 ± 0.14^{b}	1.12 ± 0.22^{a}
C17:0	0.34 ± 0.03^{a}	0.37 ± 0.02^{a}	0.57 ± 0.06^{b}	0.52 ± 0.08^{a}	0.58 ± 0.06^{b}	0.55 ± 0.09^{a}	0.4 ± 0.01^{a}	0.39 ± 0.05^{a}
C18:0	3.64 ± 0.09^{a}	3.42 ± 0.19^{a}	4.02 ± 0.61^{a}	3.87 ± 0.41^{a}	4.35 ± 0.32^{a}	4.25 ± 0.23^{a}	5.35 ± 0.34^{b}	4.09 ± 0.43^{a}
C18:1	6.28 ± 0.12^{a}	5.75 ± 0.24^{a}	6.72 ± 0.51^{a}	6.64 ± 0.39^{b}	8.15 ± 0.13^{b}	8.09 ± 0.14^{c}	8.29 ± 0.09^{b}	7.65 ± 0.06^{c}
C18:2n6	$1.37 \pm 0.02^{b.c}$	$0.96 \pm 0.02^{a.b}$	$1.1 \pm 0.07^{a.b}$	0.67 ± 0.03^{a}	0.93 ± 0.32^{a}	0.62 ± 0.08^{a}	1.6 ± 0.03^{c}	1.33 ± 0.43^{b}
C18:3	0.86 ± 0.04^{a}	0.79 ± 0.3^{a}	3.06 ± 0.04^{b}	3.11 ± 0.05^{b}	3.02 ± 0.08^{b}	3.05 ± 0.11^{b}	0.76 ± 0.05^{a}	0.75 ± 0.21^{a}
C18:4	1.12 ± 0.2^{b}	1.05 ± 0.63^{a}	0.88 ± 0.03^{a}	0.7 ± 0.08^{a}	0.84 ± 0.08^{a}	0.65 ± 0.11^{a}	1.48 ± 0.05^{c}	1.37 ± 0.05^{a}
C19:0	0.47 ± 0.08^{b}	0.44 ± 0.21^{a}	0.48 ± 0.07^{b}	0.54 ± 0.20^{a}	0.47 ± 0.08^{b}	0.53 ± 0.18^{a}	0.24 ± 0.02^{a}	0.34 ± 0.03^{a}
C20:0	0.25 ± 0.04^{a}	0.29 ± 0.08^{a}	0.36 ± 0.01^{a}	0.32 ± 0.03^{a}	0.53 ± 0.29^{a}	0.46 ± 0.24^{a}	0.41 ± 0.09^{a}	0.4 ± 0.1^{a}
C20:1	0.61 ± 0.06^{b}	0.45 ± 0.14^{a}	0.98 ± 0.04^{c}	1.05 ± 0.12^{c}	1.02 ± 0.07^{c}	1.1 ± 0.15^{c}	0.14 ± 0.01^{a}	0.71 ± 0.07^{b}
C20:5n3 EPA	5.61 ± 0.14^{a}	5.54 ± 0.07^{a}	5.28 ± 0.02^{a}	4.56 ± 0.39^{a}	5.19 ± 0.16^{a}	4.94 ± 0.76^{a}	7.29 ± 0.3^{b}	7.14 ± 0.13^{b}
C22:0	2.81 ± 0.68^{a}	2.87 ± 0.27^{a}	2.61 ± 0.03^{a}	2.79 ± 0.08^{a}	2.64 ± 0.06^{a}	2.83 ± 0.1^{a}	2.34 ± 0.1^{a}	2.38 ± 0.41^{a}
C22:1	0.6 ± 0.37^{a}	0.76 ± 0.15^{b}	0.61 ± 0.04^{a}	0.71 ± 0.03^{b}	0.6 ± 0.04^{a}	0.7 ± 0.04^{b}	0.29 ± 0.23^{a}	0.22 ± 0.11^{a}
C22:5n3 DPA	6.17 ± 0.02^{d}	4.31 ± 0.16^{b}	5.11 ± 0.18^{c}	3.8 ± 0.16^{b}	1.14 ± 0.14^{a}	1.2 ± 0.35^{a}	2.11 ± 0.84^{b}	1.96 ± 0.99^{a}
C22:6n3 DHA	24.56 ± 0.68^{b}	24.37 ± 0.37^{c}	23.52 ± 0.20^{b}	$23.4 \pm 0.03^{b.c}$	21.23 ± 0.54^{a}	20.93 ± 0.47^{a}	23.29 ± 0.75^{b}	22.58 ± 1.11^{b}
Σ SFA	37.1 ± 0.92^{a}	36.91 ± 1.52^{a}	$40 \pm 0.81^{b.c}$	37.41 ± 1.53^{a}	41.44 ± 0.71^{c}	41.1 ± 0.56^{b}	$38.52 \pm 1.76^{a.b}$	37.31 ± 0.75^{a}
Σ MUFA	13.14 ± 0.59^{a}	12.43 ± 0.41^{a}	$14.54 \pm 1.21^{a.b}$	14.38 ± 1.15^{b}	15.98 ± 0.72^{b}	15.94 ± 0.66^{b}	$14.47 \pm 0.64^{a.b}$	14.31 ± 0.34^{b}
Σ PUFA	41.63 ± 0.86^{c}	39.14 ± 1.35^{b}	40.74 ± 0.05^{c}	38.17 ± 0.43^{b}	34.09 ± 1.2^{a}	33.28 ± 0.74^{a}	39.01 ± 0.81^{b}	37.51 ± 1.54^{b}
EPA + DHA	30.17 ± 0.59^{c}	29.91 ± 0.42^{c}	28.8 ± 0.22^{b}	27.96 ± 0.42^{b}	26.41 ± 0.69^{a}	25.87 ± 0.72^{a}	30.58 ± 0.44^{c}	29.72 ± 0.98^{c}
Total	91.80 ± 0.64^{a}	88.5 ± 3.10^{a}	95.28 ± 1.96^{a}	89.96 ± 3.12^{a}	91.51 ± 2.18^{a}	90.33 ± 1.62^{a}	92.09 ± 0.68^{a}	89.13 ± 1.53^{a}

summer than in winter ($p < 0.05$). Thus, there was a variation from 13.14% to 15.98% and 12.43 to 15.94% in the sardines of Kélibia and Téboulba, respectively. The major fatty acid of this fraction is oleic acid (C18: 1), which plays a role in energy metabolism for fish spawning during gonad development (Huynh et al., 2004), which agrees with previous findings that the major

Vol. 4, No. 01; 2019

ISSN: 2456-3676

MUFA detected in marine lipids usually contains 18 carbon atoms (Zlatanos and Laskaridis, 2007).

Moreover, we found that polyunsaturated fatty acids (PUFA) represent the most important fraction of all the fatty acids of the sardine studied. Statistical analysis of the PUFA content showed that the season had a significant effect ($p < 0.05$) on the variation of this fraction. Thus, in Kélibia's sardine, the PUFA concentration varied from 34.09 to 41.63%, whereas in Teboulba's sardine, PUFA varied from 33.28 to 39.14%. The minimum value was recorded in summer, while the maximum value was reached in winter. Similar results were found by Som and Radhakrishnan (2013) in two species of sardine (*S. longiceps*) and (*Sardinella fimbriata*). Docosahexaenoic acid (C22: 6n3 DHA), eicosapentaenoic acid (C20: 5n3 EPA), and docosapentaenoic acid (C22: 5n3 DPA) are the major fatty acids of PUFA.

Comparison of the seasonal variation between lipid and fatty acid content shows a negative correlation between the lipid fraction and the n-3 fatty acid content, which means that the percentages of the n-3 fatty acid have been low during the months of high fat content. The negative correlation of the fat content with the percentages of n-3 fatty acids is probably a characteristic of the order Clupeiformes, to which sardine belongs, since Shirai et al. (2002) reported a similar correlation in sardine (*S. melanostictus*) and Zlatanos and Laskaridis (2007) also found the same correlation in sardine (*S. pilchardus*) and anchovy (*Engraulis encrasicholus*).

An inverse relationship was found for saturated fatty acids, which increased during the high-fat months. This observation suggests that the fatty acids in fish have different biological functions. SFA are probably used for energy storage. Consequently, their concentration increases during periods when feeding activity has been improved (Gökçe et al., 2002).

Conclusion

From this study, dedicated to specimens of *Sardina pilchardus*, caught from Kélibia and Téboulba areas (Tunisia), it can be concluded that the proximate and fatty acid compositions present seasonal and regional variations. Commonly, the chemical composition variations have been described and reported to be related to many factors (season, temperature, location, breeding cycle, diet, age, size, sex, etc.)

The lipid content is the most influenced by the seasonal effect than by zone, followed by the water content, fatty acid composition and protein content. Although differences in biochemical composition and fatty acids have been observed over a one-year cycle, this species is an excellent source of protein and is rich in unsaturated fatty acids, which represent 54.48 to 59.66% of total fatty acids.

References

Ackman, R.G. 1998. Remarks on official methods employing boron trifluoride in the preparation of methyl esters of fatty acids of fish oils. *J. Am. Oil Chem. Soc.*, 75: 541-546.

Ackman, R. G. 1990. Seafood lipids and fatty acids. *Food Rev. Int.*, 6(4): 617-646.

Vol. 4, No. 01; 2019

ISSN: 2456-3676

Ahmed, A. T. A., Mustafa, G., Alam, M. Z., Rubbi, S. F., & Moslemuddin, M. 1984. Biochemical composition of seven species of Gobi fish. *J. Asiatic Soc. Bangladesh*, 10: 107-111.

AOAC. 1995. Official Methods of Analysis. Association of Official Analytical Chemists. Washington, DC.

AOAC. 2000. Official Method of Analysis (17th Ed.). Association of Official Analytical Chemists. Maryland, USA.

Aro, T., Tahvonen, R., Mattila, T., Nurmi, J., Sivonen, T., & Kallio, H. 2000. Effects of Season and Processing on Oil Content and Fatty Acids of Baltic Herring (*Clupea harengus membras*). *J. Agric. Food Chem.*, 48: 6085-6093.

Bandarra, N. M., Batista, I., Nunes, M. L., & Empis, J. M. 2001. Seasonal variation in the chemical composition of horse mackerel (*Trachurus trachurus*). *Eur. Food Res. Technol.*, 212: 535-539.

Bell, J. G., McEvoy, J., Webster, J. L., McGhee, F., Millar, R. M., & Sargent, J. R. 1998. Flesh lipid and carotenoid composition of Scotish farmed Atlantic salmon (*Salmo salar*). *J. Agric. Food Chem.*, 46: 119-127.

Ben Abdallah, L., & Gaamour, A. 2004. Répartition géographique et estimation de la biomasse des petits pélagiques des côtes tunisiennes. *MedSudMed Tech. Doc.*, 5: 28-38.

Folch, J., Lees, M., & Sloane, S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.

GMA. Greek Ministery of Agriculture. 2004. The Mediterranean Nutrition.Athens, available from : http://www.minagric.gr/Greek/data.

Gökçe, M.A., Taşbozan, O., Celik, M., & Tabakoğlu, S. 2004. Seasonal variations in proximate and fatty acid compositions of female common sole (*Solea solea*). *Food Chem.*, 88: 419-423.

Grikorakis, K., Alexis, M. N., Taylor, K. D. A., Hole, M. 2002. Comparison wild and cultured gilthead sea bream (*Sparus aurata*); composition, appearance and seasonal variations. *Int. J. Food Sci. Technol.*, 37: 477-484.

Hannachi, O., Bouakka, M., Melhaoui, M., & Hakkou, A. 2011. Seasonal evolution of the biochemical composition of the Moroccan Mediterranean Cost Anchovy (*Engraulis encrasicholus*). *Adv. Environ. Biol.*, 5(7): 1787-1793.

Vol. 4, No. 01; 2019

ISSN: 2456-3676

Huss, H. H. 1995. Quality and quality changes in fresh fish, FAO Fisheries Technical Paper No. 348. FAO, Rome, Italy, pp. 20-92.

Huynh, M. D., Kitts, D. D., Hu, C. & Trites, A.W. 2004. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, Clupea harengus pallasii. Comp. *Biochem. Physiol. B: Biochem. Mol. Biol.*, 146(4): 504-511.

Islam, M. N., & Joadder, M. A. R. 2005. Seasonal variation of the proximate composition of freshwater Gobi *glossogobius giuris* (Hamilton) from the river Padma. *Pak. J. Biol. Sci.*, 8(4): 532-536.

Jan, U., Mustafa, S., Tahila, M., & Showkat, A.G. 2012. Seasonal and monthly variations of protein content in the muscle of fish Schizothorax esocinus. *Rec. Res. Sci. Technol.*, 4(7): 05-07.

Khitouni, I. K., Mihoubi, N. B., Abdelmouleh, & Bouain, A. 2011. Global chemical composition of the Mediterranean Horse Mackerel *Trachurus mediterraneus*: variations according to muscle type and fish sex. *J. Chem. Soc. Tun.*, 13: 117-122.

Liang, M., Wang, S., Wang, J., Chang, Q., & Mai, K., 2008. Comparison of flavor components in shrimp Litopenaeus vannamei cultured in sea water and low salinity water. *Fish. Sci.*, 74: 1173-1179.

Love, R.M. 1980. The Chemical Biology of Fishes. (Vol. 2). New York: Academic Press.

Love, R. M. 1997. Biochemical dynamics and the quality of fresh and frozen fish. In: Fish Processing Technology. Hall, G. M. (Ed.). Blackie Academic & Professional, London, UK. Pp. 1-26.

Médale, F., Lefèvre, F., & Corraze, G., 2003. Qualité nutritionnelle et diététique des poissons, constituants de la chair et facteurs de variations. *Cah. Nutr. Diét.*, 38 (1): 37-44.

NF V04-407. 2002. Détermination des protéines par la méthode de kjeldahl.

Nisa, K., & Asadullah, K. 2011. Seasonal variation in chemical composition of the Indian mackerel (*Rastrelliger kanagurta*) from Karachi Coast. *Iranian J. Fish. Sci.*, 10(1): 67-74.

Noël, L, Chafey, C, Testu, C, Pinte, J, Velge, P, & Guerin, T. 2011. Contamination levels of lead, cadmium and mercury in imported and domestic obsters and large crab species consumed in France: differences between white and brown meat. *J. Food Compos. Anal.*, 24: 368-375.

Vol. 4, No. 01; 2019

ISSN: 2456-3676

Pérez-Velazquez, M., Gonzáléz-Félix, M. L., Jaimes-Bustamente, F., Martínez-Córdova, L. R., Trujillo-Villalba, D. A., & Davis, D. A. 2007. Investigation of the effects of salinity and dietary protein level on growth and survival of pacific white shrimp *Litopenaeus vannamei*. *J. World Aquacult. Soc.*, 38: 475-485.

Pérez-Velazquez, M., Urquidez-Bejarano, P., González-Félix, M. L., & Minjarez-Osorio, C. 2014. Evidence of Euryhalinity of the Gulf Corvina (*Cynoscion othonopterus*). *J. Mod. Physiol. Res.*, 63: 659-666.

Shirai, N., Terayama, M., & Takeda, H. 2002. Effect of season on the fatty acid composition and free amino acid content of the sardine *Sardinops melanostictus*. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.*, 131: 387-393.

Šimat, V., & Bogdanović, T. 2012. Seasonal changes in the proximate composition of anchovy (*Engraulis encrasicholus*) from the central Adriatic. *Acta Adriat.*, 53(1): 125-132.

Simopoulos, A. P. 1991. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.*, 54: 438-463.

Som, C. R. S., & Radhakrishnan, C. K. 2013. Seasonal variation in the fatty acid composition of *Sardinelle longiceps* and *Sardinella fimbriata* : Implications for nutrition and pharmaceutical industry. *Indian J. Geo-Marine Sci.*, 42(2): 206-210.

Yildiz, M. 2008. Mineral composition in fillets of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*): a comparison of cultured and wild fish. *J. App. Ichthyol.*, 24(5): 589-594.

Zlatanos, S., & Laskaridis, K. 2007. Seasonal variation in the fatty acid composition of three Mediterranean fish-sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*). *Food Chem.*, 103: 725-728.