Seasonal variation of polyunsaturated fatty acids (n-3) composition in *Diplodus annularis* from the gulf of Tunis: nutritional benefits

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Résumé : Les sparidés sont des poissons appréciés par le consommateur tunisien malgré leur taille réduite. L'intérêt accru pour les acides gras polyinsaturés de la série (n-3) chez les poissons est dû à leur rôle préventif particulièrement comme les maladies cardiovasculaires. Dans le présent travail, nous avons suivi l'évolution saisonnière de la masse des acides gras polyinsaturés de la série (n-3) chez une espèce de sparidés immature (*Diplodus annularis*) du golfe de Tunis. L'analyse chromatographique sur colonne capillaire a révélé des variations significatives ($P < 0.01$) des acides gras totaux en fonction des saisons. Nous avons obtenu respectivement pour l'automne, l'hiver, le printemps et l'été: 0.49; 2.93; 1.55 et 0.77 mg/g de matière fraîche. Ces résultats révèlent une richesse en acides gras polyinsaturés (n-3) durant l'hiver chez *Diplodus annularis* du golfe de Tunis.

Abstract : The sparida species are much appreciated by Tunisian consumer's in spite of their small size. The great interest in polyunsaturated fatty acids (n-3) is due to the importance they have in the Mediterranean diet in addition to their ability to prevent and protect against a great number of diseases especially cardiovascular ones. In the present work, we have followed the polyunsaturated fatty acids (n-3) mass progress in an immature sparida species (*Diplodus annularis*) from the Gulf of Tunis according to seasons. The results obtained by gas chromatograph with polar capillary column reveal a significant variation ($P < 0.01$) of total fatty acids according to seasons: 0.49; 2.93; 1.55 and 0.77 mg/g wet weight for autumn, winter, spring and summer respectively. These results reveal better polyunsaturated fatty acids (n-3) values during winter in immature *Diplodus annularis* form the Gulf of Tunis.

1. Introduction

The common *Diplodus annularis*: sparaillon is a specie of fish living in sandy depths and rarely in rocky ones [1] up to 50 meters in depth [2]. This sparida species is highly appreciated by the Tunisian consumers in spite of their small size. It is one of 23 living species in the Mediterranean and black seas [1], thus it has a great importance in the Mediterranean diet representing an international reference in cardiovascular disease prevention [3-8]. In fact, many studies demonstrated the benefits of a rich diet in fish for the health [9-11].

Keywords: Fish, Polyunsaturated fatty acids, Season

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Generally eicosapentaenoïc acid C20: 5 (EPA) and docosahexaenoïc acid C22: 6 (DHA), two groups of polyunsaturated fatty acids (n-3) dominate the fish oil composition. Thus the epidemiologic, the clinic and the experimental studies have shown a higher risk of cardiovascular diseases, thrombotic events, dysfunction of hlood pressure and higher plasma level of lipids and lipoproteins. All these events are correlated to a low EPA concentration.

Moreover (n-3) PUFA has also been used as a therapeutic complement for hypertension [12], psoriasis [13], hyperlipidemia [14], ulcerative colitis [15] and some types of cancer [16].

In addition, many studies demonstrated that PUFA (n-3) consumption could increase HDL plasmatic level [8, 17] and could decrease the triacylglycerols one [17]. Other studies demonstrated that fish oil decreases the synthesis of mutagen toxics and the thrombogenic agents [18], inhihits thromboxane A_2 which is involved in platelet aggregation and vasoconstriction [19, 20].

But just few studies systematically evaluated the variations of fish oil composition [21-23] and the relative proportion of the different fatty acids in the mackerel scomber scombrus [24] along the year. Now we admit that the nutritional value of fish diet is directly related to how rich it is in PUFA (n-3). In fact, all the importance is to determine the seasonal variations of lipid composition and proportions of different fatty acids in immature *Diplodus annularis* from Tunis Gulf (Tunisia). For these reasons, it seems important for us to quantify for Tunisian consumers the seasonal, nutritional needs of fatty acids the most conform to nutritional standards (50% monounsaturated fatty acids, 30% saturated fatty acids and 20% polyunsaturated fatty acids) recommended by Richard et *al.*[25].

2. Methods and Materials

Our study concerns *Diplodus annularis* samples collected from the Gulf of Tunis during the different seasons (autumn, winter, spring and summer in the year 2000-2001). The muscle was removed from the left side between the two dorsal fins.

Total lipid extraction has been realized according to Folch's method [26] modified hy Bligh and Dyer [27]. The samples were preserved at -80°C inside a Vorbeck and Marinetti mixture.

The used methylation method was that of Metcalfe et *al.* [28] in which we utilized boron trifluoride (BF_3). To define the fatty acid mass, an authentic standard nonadecanoïc acid (C19:0) was used. The fatty acid analysis was realised hy gas chromatograph, type HP 5890 series II with a light ionization detector and a HP innovax type polar capillary column having 30 m of length and 0.32 mm of diameter. The film thickness was 0.5 μ. The temperature used for detector and injector were respectively 280 and 250°C with temperature program for the column from 180 to 250°C. Nitrogen was the employed as carried gas.

The average comparison was realized hy "t" student comparison test, a P less than 0.05 is considered as statistically significant.

3. Results

Tahle 1 represents the percentages of different fatty acid groups (saturated, mono and polyunsaturated). Tahle II indicates the composition in percentage of saturated fatty acids (C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0), monounsaturated (C14:1, C16:1, C18:1, C20:1, C22:1, C24:1), polyunsaturated (n-3) (C18:3 , C20:5, C22:5, C22:6) and polyunsaturated (n-6) (C18:2, C20:3, C20:4, C22:4).

For the first group of saturated fatty acids the results show a seasonal variation statistically significant ($P < 0.05$) throughout autumn, winter, and summer on the one hand and between winter and spring on the other hand. Those differences are related to the following fatty acid variations:

- ***Lauric acid*** C12:0, shows an increase of 128 % in spring compared to autumn and 72% in winter compared to spring, both are statistically significant ($P < 0.05$).
- ***Palmitic acid*** C16:0, shows a significant increase of 11% in spring and summer compared to autumn and 13% in summer and spring compared to winter, those differences are statistically significant ($P < 0.05$).
- ***Heptadecanoïc acid*** C17:0, we noted a significant difference between the seasons except between autumn-spring and winter-summer. These differences are statistically significant ($P < 0.05$).
- ***Arachidic acid*** C20:0, except for a decrease of 40% in autumn compared to winter corresponding to a statistically significant difference ($P < 0.05$), there are no significant differences between seasons.
- ***Docosanoïc acid*** C22:0, the results show that the lowest value is in winter and the highest one is during autumn and summer. There is a 56% decrease from autumn to winter, whereas there is a 98% increase from winter to spring and a 126% increase from winter to summer. Those differences are statistically significant ($P < 0.05$).
- ***Lignoceric acid*** C24:0, the results show a decrease of 47%, 38% and 38% respectively from autumn to winter, autumn to spring and autumn to summer. However, it increases by 19 % from winter to summer. Except for winter-spring and spring-summer differences, all the other differences are statistically significant ($P < 0.05$).

For the group of monounsaturated fatty acids (C14:1, C16:1, C18:1, C20:1, C22:1, C24:1), the results show different values between summer and spring, autumn and winter. Those differences which are statistically significant ($P < 0.05$) are related to seasonal variation of the following acids:

- ***Myristic acid*** C14:1, results show a decrease of 41%, 34% and 27% respectively between autumn and winter, autumn-summer and spring-summer. However an increase of 56% is ohserved from winter to spring. These differences are statistically significant ($P < 0.05$).
- ***Palmitic acid*** C16:1, results show a decrease of 45% from winter to summer this difference is statistically significant ($P<0.05$).
- ***Oleic acid*** C18: 1, our results show a decrease of 26%, 33% and 26% respectively from autumn to summer, winter to summer and spring to summer. These differences are statistically significant ($P<0.05$).
- ***Eicosenoïc acid*** C20:1, the results show insignificant levels lower than the unit.
- ***Erucic acid*** C22:1, results show a decrease of about 62% from autumn to winter, however these differences increase by 136% and 173% respectively from winter to spring and winter to summer. These differences are statistically significant ($P < 0.05$).
- ***Tetracosenoïc acid*** C24:1, results show a decrease of 93%, 90%, 94% and 91% respectively from autumn to spring, autumn to summer, winter to spring and winter to summer. These differences are statistically significant ($P < 0.05$).

For this group of polyunsaturated fatty acids, the results show an increase of 29% 22% and 14% respectively from autumn to summer, winter to summer and spring to summer. Those differences which are statistically significant ($P < 0.05$) are related to seasonal variations at species level of n-3 and n-6 groups and at the fatty acid levels of each group.

Table I- *Season fatty acids composition (%) in Diplodus annularis.*

	Season			
	Autumn (n = 10)	**winter** (n = 9)	**Spring** (n = 10)	**Summer** (n = 9)
C10:0	0.09 ± 0.2	0.2 ± 0.09	0.1 ± 0.03	0.09 ± 0.01
C12:0	1.81 ± 0.36	2.39 ± 0.54	4.13 ± 0.60	5.44 ± 2.16
C14:0	2.08 ± 0.1	1.99 ± 0.15	2.54 ± 0.28	1.67 ± 0.21
C14:1	1.83 ± 0.23	1.07 ± 0.12	1.67 ± 0.16	1.21 ± 0.09
C16:0	18.54 ± 0.48	18.94 ± 0.40	16.51 ± 0.77	16.51 ± 0.56
C16:1	3.79 ± 0.57	4.62 ± 0.34	3.68 ± 0.48	2.54 ± 0.32
C17:0	1.40 ± 0.06	1.04 ± 0.09	1.30 ± 0.08	0.92 ± 0.07
C18:0	6.32 ± 0.15	6.07 ± 0.20	6.14 ± 0.48	5.47 ±0.36
C18:1	14.19 ± 0.47	15.55 ± 1.43	14.12 ± 3.63	10.44 ± 0.54
C18:2	2.13 ± 0.29	1.22 ± 0.06	1.57 ± 0.08	1.81 ± 0.15
C18:3	0.86 ± 0.1	0.66 ± 0.06	0.86 ± 0.10	1.01 ± 0.22
C20:0	0.91 ± 0.07	0.55 ± 0.12	0.80 ± 0.11	0.83 ± 0.09
C20:1	0.88 ± 0.11	0.53 ± 0.16	0.83 ± 0.10	1.04 ± 0.23
C20:2	1.11 ± 0.22	1.47 ± 0.40	1.05 ± 0.24	0.83 ± 0.08
C20:3	1.02 ± 0.18	0.50 ± 0.06	0.77 ± 0.09	0.69 ± 0.08
C20:4	5.14 ± 0.22	6.43 ± 0.32	6.76 ± 0.81	10.07 ± 0.68
C20:5	7.53 ± 0.28	6.68 ± 0.42	7.14 ± 0.53	6.43 ± 0.41
C22:0	1.30 ± 0.20	0.57 ± 0.07	1.13 ± 0.22	1.29 ± 0.19
C22:1	1.09 ± 0.13	0.41 ± 0.06	0.97± 0.17	1.12 ± 0.18
C22:4	2.60 ± 0.3	2.01 ± 0.19	2.98 ± 0.32	3.65 ± 0.34
C22:5	3.36 ± 0.24	3.23 ± 0.29	4.71 ± 0.57	4.16 ± 0.54
C22:6	14.89 ± 0.21	19.77 ± 1.64	18.97 ± 1.50	22.04 ± 0.77
C24:0	4.78 ± 0.19	2.51 ± 0.09	2.96 ± 0.32	2.98 ± 0.12
C24:1	2.72 ± 0.32	3.10 ± 0.78	0.17 ± 0.06	0.28 ± 0.10

Table II- *Saturated, Mono and Polyunsaturated fatty acid :seasonal variations (%) in Diplodus annularis.*

	Season			
	Autumn (n = 10)	**Winter** (n = 9)	**Spring** (n = 10)	**Summer** (n = 9)
Saturated fatty acids	35.0 ± 0.09	32.0 ± 1.0	34.0 ± 1.0	32.0 ± 1.0
Monounsaturated fatty acids	27.0 ± 1.0	27.0 ± 2.0	22.0 ± 2.0	17.0 ± 1.0
Polyunsaturated fatty acids	38.0 ± 1.0	40.0 ± 2.0	43.0 ± 3.0	49.0 ± 1.0
Polyunsaturated fatty acids (n-3)	27.53 ± 0.43	29.26 ± 2.06	29.31 ± 2.15	31.86 ± 0.97
Polyunsaturated fatty acids (n-6)	10.44 ± 0.47	11.55 ± 0.45	13.69 ± 0.64	17.14 ± 0.94

The PUFA's group (n-3) shows that only autumn to summer variation is statistically significant with an increase of 17.6%. This variation is due to the following fatty acids:

- ***Linolinic acid*** C18:3, the results reveal low and statistically insignificant seasonal differences
- ***Eicosapentaenoïc acid*** C20:5, the results show an increase of 15% from autumn to summer. This difference is statistically significant ($P < 0.05$).

- ***Docosapentaenoïc acid*** C22:5, the results show an increase of 40% and 45% respectively from autumn to spring and from winter to spring. Both differences are statistically significant ($P < 0.05$).
- ***Docosahexaenoïc acid*** C22:6, results reveal an increase of 33%, 27%, 48% and 16% respectively from autumn to winter, autumn to spring then autumn to summer and from spring to summer. These differences are statistically significant ($P < 0.05$).

The group of PUFA (n-6) shows the following variations:

- ***Linoleic acid*** C18:2, our results show a decrease of 43% from autumn to winter and an increase of 29% and 48% respectively from winter to spring then from winter to summer. These differences are statistically significant ($P < 0.05$).
- *Dihomo-γ-linolenic acid* C20:3, our results show a decrease of 51% and an increase of 54% respectively from autumn to winter and from winter to spring. Both differences are statistically significant ($P < 0.05$).
- ***Arachidonic acid*** C20:4, our results show an increase of 25%, 96%, 57% and 49% respectively from autumn to winter, autumn to summer, winter to summer and spring to summer. These differences are statistically significant ($P < 0.05$).
- ***Docosatetraenoïc acid*** C22:4, our results show an increase of 40%, 48% and 82% respectively from autumn to summer, winter to spring and winter to summer. These differences are statistically significant ($P < 0.05$).

PUFA (n-3) analysis (C18:3, C20: 5, C22:6) expressed in mg/g dry weight (Fig 1) show that:

- ***Linolenic acid*** C18:3 exist in very low levels. The differences between seasonal values are statistically significant ($P < 0,05$) except for a decrease of 33 % in summer compared to spring.
- ***Eicosapentaenoïc acid*** (EPA) C20: 5, the results show that the variation level depends on seasons. In fact the lowest level is observed in autumn and the highest one is in winter. This increase reaches five times that of the autumnal value. These differences are statistically significant ($P < 0.05$).

On the other hand, the results obtained for docosahexaenoïc acid (DHA) C 22:6 show a winter value reaching more seven times than one observed in autumn. These seasonal differences are statistically significant ($P < 0.05$).

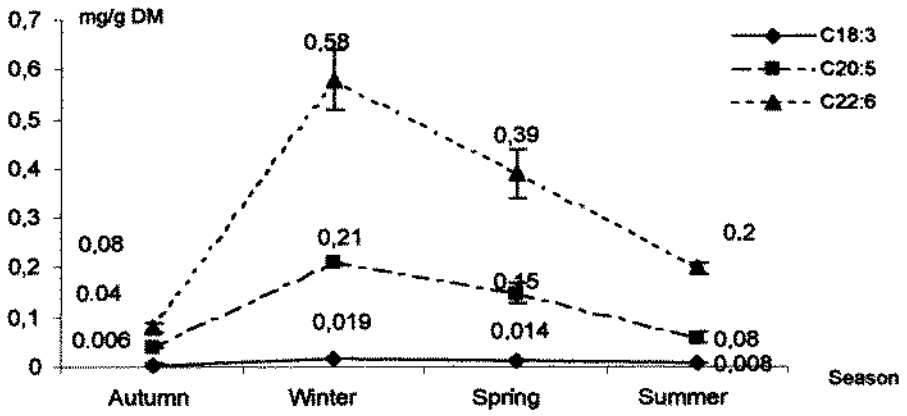

Fig. 1. Seasonal Fatty acid variations C18:3, C20:5, C22:6 (mg/g DM) in *Diplodus annularis*

The latter observation is confirmed by comparing the ratio of seasonal variation EPA/DHA (Fig 2). EPA/DHA values were 0.51, 0.35, 0.40, and 0.31 respectively for autumn, winter, spring and summer. So the value of EPA/DHA was in all the time less than the unit for the used *Diplodus annularis* species. This explains a seasonal accumulation of docosahexaenoïc acid (DHA) C22:6 during winter. These differences were statistically significant ($P < 0.05$) between EPA/DHA ratios of different seasons, except for those between winter and spring firstly and winter and summer secondly.

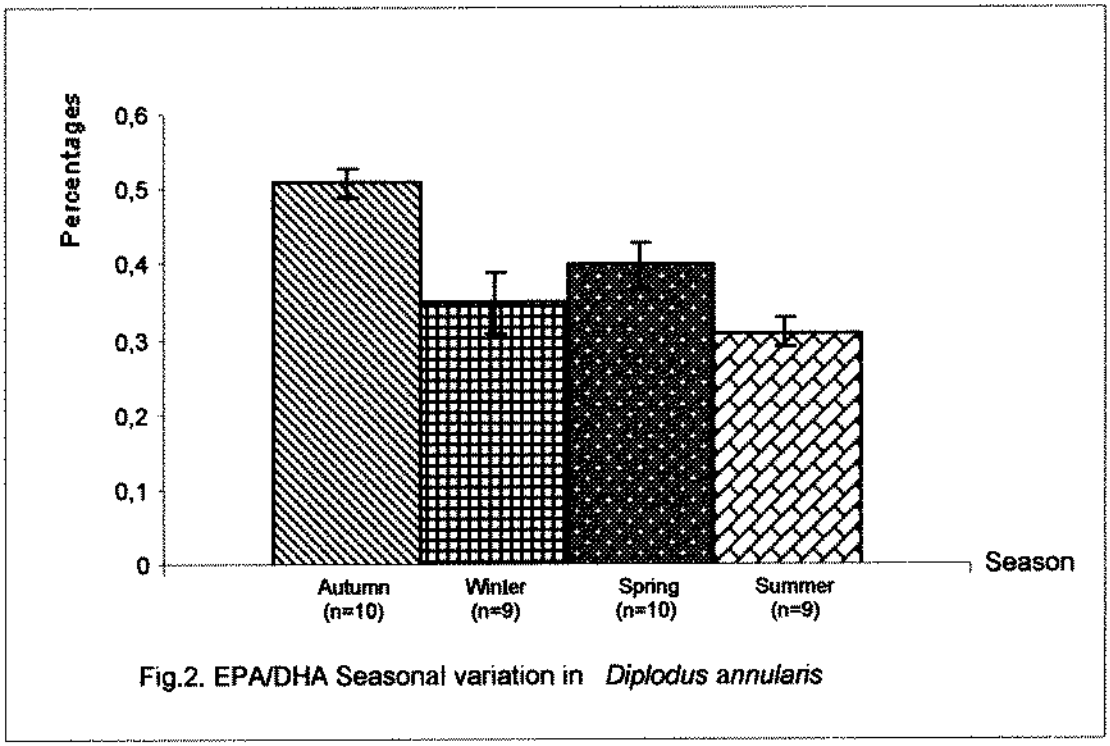

Fig.2. EPA/DHA Seasonal variation in *Diplodus annularis*

4. Discussion

Our results show a different seasonal variation of fatty acids in *Diplodus annularis* species. We noted that PUFA are the most abundant. Their quantities expressed in percentage of total fatty acids increase from autumn 38.0 ± 0.01 to reacb a maximal level (49.0 ± 0.01) in summer. Tbese results are identical to tbose obtained by Belling et *al.* [29] in eleven species from Queensland Australian fisb. These authors identified PUFA average of 42.3 ± 6.9 of total fatty acids. Our results are also identical to those described by Piclet [30] in salmo gairdnerii (40.1%), sardinops sagax (38.1%), mullet cabot (35.0%) and pink salmon (41.8%).

Tbe PUFA (n-3) ohtained in our study shows the same variation as the total polyunsaturated. In fact, the lowest observed level was in autumn 27.53 ± 0.43%, and then this level increases during seasons to reach its higbest level in summer 31.86 ± 0.97%. The total PUFA (n-3) obtained in our study is similar to fish oil composition of Brevoortia Tyranis (25.8%) and Japanese Sardine Sardinops melanosticta (25.9%) reported by Ackman [31].

Moreover, PUFA (n-6) has also a seasonal variation with its lowest level in autumn (10.44 ± 0.47%) increasing during seasons to reacb its highest value in summer 17.14 ± 0.94%. Similar results were found by Belling et *al.*[29] in Australian fish species. While Brown et *al.*[32] found middle value for PUFA (n-3) and PUFA (n-6) respectively 30.7 ± 10.1 % and 11.2 ± 5.9 %.

Our results reveal that the arachidonic acid C20:4 is the most frequent among PUFA (n-6) with the highest level observed in summer (10.07 ± 0.68%). This is similar to the results ohtained by other authors in other fish species [21, 29, 32, 33].

Arachidonic acid is followed hy docosatetraenoïc acid C22:4 which have the same seasonal variation with (2.60 ± 0.30%) in autumn and increasing during seasons to reach its highest level in summer (3.56 ± 0.34). Belling et *al.*[29] identified an average of 3.1 ± 1.3% in Australian fish species.

Results show that among PUFA (n-3) the most ahundant is the decosohexaenoïc acid C22:6 (DHA) with 14.89 ± 0.21% in autumn as the lowest level increasing during seasons to reach to 15.6 ± 6.3 % in summer. While Pascaud and Brouard [34] founded respectively 24.2 % and 21.7 % in saumon and Salmo gairdnerii.

Eicosapentaenoïc acid C22:5 (EPA) is present in our study in lower levels. We ohserved an average of 3.36 ± 0.24% and 3.23 ± 0.29% respectively in autumn and winter. This level increases to reach its highest level in spring with 4.71 ± 0.57%. Other studies demonstrated in saumon and salmo gairdnerii respectively a C22:5 level of 4.2 % and 3.0% of the total fatty acids [34]. In Australian fish species [29], the C22: 5 was found representing 3.5 ± 0.9% of total fatty acids.

Linolenic acid C18:3 is present in low proportions in *Diplodus annularis* specie with the highest level ohserved in summer (1.01 ± 0.22%). Similar results were described by Pascaud and Brouard [34] in Salmo gairdnerii.

The second most frequent fatty acids expressed in percentage are saturated fatty acids (SFA) with 30.0% of total fatty acids. The most elevated proportions of saturated fatty acids were palmitic acid C16:0 with 18.94±0.4% as the highest level ohserved in winter, stearic acid C18:0 with 6.32 ± 0.15% as the highest level ohserved in autumn, lignoceric acid C24:0 with 4.78 ± 0.19% as the highest level ohserved in autumn, myristic acid C14:0 with 2.54 ± 0.28% as The highest level ohserved in spring, heptadecanoïc acid C17:0 with 1.40 ± 0.06% as the lowest level ohserved in autumn, and arachidic acid C20:0 with 0.91 ± 0.07% as the highest level ohserved in autumn. Studies realized by Belling et *al.* [29] in eleven Australian fish species found 19.9 ± 2.5%, 8.2 ± 0.9% and 2.0 ± 1.1% for respectively C16:0, C18:0 and C14:0 whereas C15:0, C17:0 and C20:0 exist in very low levels.

Finally, our study revealed that monounsaturated fatty acids have seasonal variations. The lowest level was ohserved in summer (17.0 ± 1%) and the highest one in autumn and winter 27.0 ± 2%. Similar results were announced by Piclet [30] in alhacore thon (23.3%), sardinops sagax (27.6%) and mullet cabot (20.8%), whereas Belling et *al.* [29] found a mean proportion of 17.4 ± 4.3% of total fatty acids in Australian fish species.

The most ahundant monounsaturated fatty acids were oleic acid C18:1 and palmitic acid C16:1 with the highest level in winter and respectively 15.55 ± 1.43 % and 4.62 ± 0.34% expressed in percentage of total fatty acids. These results are identical to others found hy Belling et *al.* [29] in Australian fish species and where the mean levels were respectively 11.8 ± 2.6% and 4.3 ± 1.9%.

So, our results show that saturated fatty acids are the least affected by season's effects. In fact, because of their endogen origin, saturated fatty acids were less influenced hy the fatty acids alimentation type of fish [35, 36].

Finally, the PUFA seasonal variations observed in our study confirm the results obtained hy other autbors who claim that fatty acids composition variations are very likely to be related to the differences in fish nutritional habits [24, 37] .Lipids of marine animals differ from those of land animals especially hy their richness in very long chain fatty acids which derive from trophic chain due essentially to ahundance of algae and marine plankton [38, 39].

5. Conclusion

Our study results reveal fatty acid seasonal variations in sparida "*Diplodus annularis*" species from the Gulf of Tunis (Tunisia).

Our results show that the PUFA higbest level is observed in summer while the lowest level is ohtained in autumn and winter. However, unimportant seasonal variations were ohserved for saturated fatty acids. Because of their endogen origin, saturated fatty acids are less affected hy fish diet which depends on the period of the year.

According to our results, PUFA and monounsaturated fatty acids seem to be the most influenced hy seasons. In fact, an important accumulation of EPA and DHA is observed particularly in winter. This may be due to abundance of long cbain fatty acids deriving from trophic chain rich in algae and marine planktons.

Finally, the study of the nutritional results of fatty acids in *Diplodus annularis* compared to nutritionist recommendations (30% SFA, 50 % MUFA and 20% PUFA) reveals a better balance in favor of winter compared to other seasons.

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