FATTY ACID COMPOSITION AND NUTRITIONAL IMPUT OF POLYUNSATURATED FATTY ACIDS (n-3) AND (n-6) IN THE FLESH OF THE WILD SPECIES *PINCTADA RADIATA* TUNISIAN EAST SOUTHERN COAST

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ABSTRACT: The fatty acid composition of total lipids in the flesh of the pearl oyster *P. radiata* in Tunisian coast has been studied in order to assess and enhance this species. Analysis by gas chromatography revealed the presence of 25 fatty acids. Among these latter 5 predominate : C16 : 0 , C18 : 0, C20: 4 (n -6), C20: 5 (n -3) and C22: 6 (n -3) with respective contents of (33.91 ± 1.22 , 12.61 ± 1.74 , 4.86 ± 0.5, 5.77 ± 0.71, 16 ± 0.82 /100g). *P. radiata* displays a saturated fatty acid content of 214.06±21.23mg/100 g (wet weight), 36,32±7,33 mg/100g monounsaturated fatty acids and a content of 149.82±23.07mg/100g in polyunsaturated fatty acids. We also noted the presence of non-methylene-interrupted-dienoic fatty acid (NMID) (C22:2): C22:2i and C22:2j in small quantities respectively equal to 1.33 ± 0.65 mg/100g and 1.32 ± 0.73 mg/100g. Our results reveals also that the n-3/n-6 ratio in the flesh of *P. radiata* is three times higher in PUFA (n-3)than(n-6) mainly represented by the EPA (C20: 5n -3) + DHA (C22: 6n -3) complex whose content is (24.54mg +65.2mg) 89.75mg mean 21.77 % of Total Fatty Acids (TFA). Among the n-6 PUFA, arachidonic dominates with 20.15 ± 3.23 mg/100g content.

Key words: Poly-unsaturated fatty acids (n-3), poly-unsaturated fatty acids (n-6), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), *Pinctadaradiata.*

RESUME: La composition en acides gras des lipides totaux de la chair de l'huître perlière *P. radiata* de la côte tunisienne a été étudiée pour évaluer et valoriser cette espèce. L'analyse par chromatographie en phase gazeuse a révélé la présence de 25 acides gras. Parmi ces derniers 5 sont majoritaires : C16:0, C18:0, C20:4 (n-6), C20:5 (n-3) et C22:6 (n-3) avec des teneurs respectives égales à 33.91±1.22 ; 12.61±1.74; 4.86±0.5; 5.77±0.71; 16±0.82. Par ailleurs *P. radiata* révèle une teneur en acides gras saturés égale à 214.06±21.23mg/100g matière fraiche (MF) ;36.32±7.33mg/100g en acides gras monoinsaturés et une teneur de 149.82±23.07mg/100g en acides gras polyinsaturés. Nous avons également noté la présence des non-methylene-interrupted-dienoic (NMID) (C22:2) :C22:2i et C22:2jen quantités faibles de l'ordre de1.33 ± 0.65 mg/100g et 1.32 ± 0.73 mg/100g respectivement. En outre l'analyse du ratio n-3/n-6 révèle que la chair de *P. radiata*est 3 fois plus riche en AGPI (n-3) que ceux de (n-6) représentés essentiellement par le complexe EPA+DHA dont la teneur est de (24.54mg +65.2mg) 89,75mg soit 21,77% des acides gras totaux(AGT). Parmi les AGPI (n-6), l'acide arachidonique (AA) domine avec une teneur égale à 20.15 ± 3.23 mg/100g.

Mots clés :Acides gras polyinsaturés (n-3), acides gras polyinsaturés (n-6), acide eicosapentaénoïque (EPA), acide docosahexaénoïque(DHA), *Pinctada radiata*.

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1. INTRODUCTION

The fatty acids of fish in particular, have been reported in detail, with more recognition of beneficial uses of fish oils in human health. Studies related to lipids of marine organisms have shown that they can be unique sources of n-3 PUFA such as EPA (C20: 5 n-3) and DHA (C22: 6n-3). The fatty acid and sterol profile of the lipids in marine vertebrates and invertebrates reflect fatty acid and sterol profiles in zooplankton, dinoflagellates and algae[1-2].

Omega-3 PUFA from marine sources have been widely investigated in terms of their beneficial effect on reducing certain risk factors in human cardiovascular diseases such as decreasing platelets aggregation, reducing plasma triacylglycerol[3] as well as alleviating the symptoms of inflammatory conditions such as arthritis, psoriasis, ulcerative colitis[4-5].

The lipid composition of bivalves such as oysters (*Ostreidae*), scallops (*Pectinidae*) and mussels (*Mytilidae*) were studied in detail [6-7]. Few information is available about the biochemical compounds of other bivalve species, especially their lipid compositions.

The pearl oyster, *Pinctadaradiata* **(Leach, 1814)** is an Indo-Pacific origin sessile benthic species. In Tunisia, only one study on biological reproduction of this oyster harvested around the islands of Kerkennah was made until today[8-9].

In Tunisia *P. radiata* has no economic interest. Its flesh is disliked by tunisians (consumption is limited ~~to~~in the two islands Kerkennah and Djerba) and is often discharged into the sea in a fishing accident. Moreover, it does not produce pearls and even when present, they are of poor quality and very friable[10].

This species is abundant in the Gulf of Gabes, particularly at Kerkennah. It is scarce and with a presence that becomes sporadic in the Gulf of Tunis [11].

As many other marine organisms *P. radiata* consumes a diet rich in phytoplankton and algae rich on (n-3) PUFA (C18: 4 n-3, C20: 5n-3, C22: 6 n-3).

We propose to determine the fatty acid composition in the flesh of *P. radiata* as a potential source of (n-3) PUFA.

2. MATERIAL AND METHODS

2.1. Sampling

Samples of *P. radiata* are described in Table I. Samples were collected in February (2011) by benthic trawl at Maharès area (**Fig.1**).

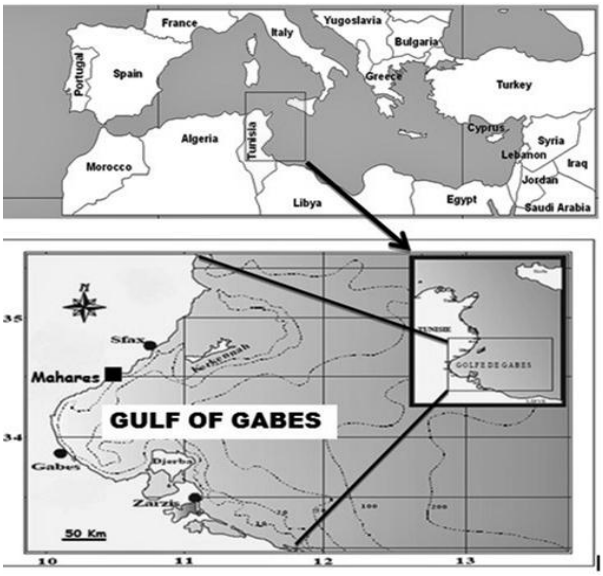

Figure1: Geographical location of the sampling site Maharès (■) at the Golf of Gabes

All samples were fixed in boiling water to inactivate enzymatic activity complexly, especially phospholipases. Samples along with the fixing liquid were stored in a freezer at -28 ° C. Samples of *P. radiata* are described in Table I.

Table I: Cultivation Locality and Biological Data of the Pearl Oyster *P. radiata*

Samples	Date	Locality		Depth (m)		Replicate animals	Lengh (cm)	Widh (cm)	Weight(g)
		beginning	End	beginning	End				
1	26th February 2011	34°25',818N 10°58',91E	34°26',661 N 11°03',080 E	19.6	21.2	6	8.7±0.57	7.83±0.42	106.25±7.26

2.2. Total lipid extraction

Lipids were extracted according to the Folch, Lees, and Sloane- Stanley (1957) [12] method with the solvent mixture chloroform–methanol (2:1, v/v) containing 0.01% butylhydroxytoluene (BHT) as an antioxidant.

2.3. Extraction, identification and quantification of total fatty acids (TFA).

A gas chromatograph type HP series 6890 with a split/splitless injector and a flame ionisation detector was used for the analysis. The device includes 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. The data of the chromatographic analysis figure in **table II**. The comparison of the retention times of fatty acids to those of standard methyl esters Supelco (PUFA-3) allowed to identify the different fatty acids. An internal standard, nonadecanoate methyl (C 19: 0) which does not exist in our sample served to quantify different fatty acids.

Table II: Data of the chromatographic analysis

Carrier Gas	Nitrogen
Gas Flow vector	1.52 ml per minute
Temperature inlet	250° C
Temperature detector	275° C
Program temperature column	- oven isotherm 150°C for 1minute, - 150 to 200° C for a rate of 15° C per minute, - 200 to 242° C for a rate of 2° C per minute,
Injected volume	0.5 µl
SFA:	C14:0 + C15:0 + C16:0 + C17:0 + C18: 0 + C22:0;
MUFA:	C14:1+ C15:1 +C16:1 + C18:1 + C20:1;
PUFA:	C18: 2n-6+ C20:2n-6 + C20: 4n-6 + C18: 3n-3 + C18: 4n-3 + C20: 4n-3 + C20: 5n-3 + C22: 5n-3+C22: 6n-3 + C16: 2n-4+ C16: 3n-4 + C18: 3n-4 + C22:2i+ C22:2j.

3. RESULTS AND DISCUSSION

Total fatty acids contained in the flesh of *Pinctada radiata* are listed in **table III.**

Table.III: Fatty acid contents in the flesh of *Pinctada radiata*
(Results are expressed as mg/100g, Mean ± SE, n=6).

FA	Mean±SE
C14:0	24.14±3.68
C15:0	1.05±0.42
C16:0	135.44±15.75
C17:0	0.15±0.06
C18:0	46.91±3.21
C22:0	6.35±0.94
C14:1	3.18±0.41
C15:1	1.12±0.33
C16:1	14.81±4.33
C18:1	11.24±1.90
C20:1	5.95±1.36
C18:2n-6	7.70±1.20
C20:2n-6	5.02±2.24
C20:4n-6	20.15±3.23
C18:3n-3	0.92±0.2
C18:4n-3	4.24±0.81
C20:4n-3	1.39±0.32
C20:5n-3	24.54±5.44
C22:5n-3	3.89±0.82
C22:6n-3	65.20±8.88
C16:2n-4	10.21±1.53
C16:3n-4	2.11±0.28
C18:3n-4	1.74±0.58
C22:2i	1.33±0.65
C22:2j	1.32±0.73
SFA	214.06±21.23
MUFA	36.32±7.33
PUFA	149.82±23.07
UFA	186.14±29.4
Σn-3	100.21±15.35
Σn-6	32.88±5.78
n-3/n-6	3.12±0.17
EPA+DHA	89.75±13.75

Twenty-five fatty acids were identified in the flesh of *P. radiata*. This latter present 1g/100g of TFA. Results revealed that the saturated fatty acids (SFA) are the majority group **(Fig.2)** in the flesh (214.06mg/100g) followed by polyunsaturated fatty acids (PUFA) with 149.82 mg/100g and monounsaturated fatty acids (MUFA) (36.32 mg/100g): the importance of SFA and MUFA was suggested that are indispensable in diets to fill gaps in energy expenditure and have by themselves physiological roles[13].

The analyzed profiles of FA showed that among SFA the stearic acid (C18:0) and the palmitic acid (C16:0) are predominant and respectively equal to 46.91mg/100g and 135.44mg/100g.
According to the results shown in **Table III**, we noticed that lipid content is richer in fatty acids with 18 carbon atoms as linoleic acid (C18:2n-6), stearidonic acid (C18:4n-3) and C18:3n-4. The present work shows also the fatty acid profile of total lipids in the flesh of pearl oyster *Pinctada radiata* is similar to that found in other bivalves as *Flexopecten glaber* (*Pectinidae*)[14], oyster *Crassostrea gigas*[15] and *Pinctada fucata martensii*[16].
Unsaturated fatty acids are the majority group in the flesh (186.14mg/100gof total fatty acids); these results confirm other studies on bivalves[17] and especially in oysters[16].
The predominance of PUFA was also observed in oysters and mussels from different sites of the mediterranean sea[18-15]. Unsaturated fatty acids levels are high because of the transformation of SFA to UFA (poly and mono) which is greater in the flesh where different metabolisms occur as reproduction [17].
Our results reveled also, the presence of non-methylene-interrupted-dienoicacids (NMID) (C22:2): C22:2i (1.33mg/100g) and C22:2j (1.32 mg/100g). According to several authors, these two fatty acids (C22:2i and C22:2j) are involved in the mechanisms of fluidity, integrity and structure of the membrane [19]. The presence of fatty acids C22:2i and C22:2j in bivalves confers protection against membrane alterations due to changes in the physicochemical environment. Gilles[19] reported the mollusk bivalve ability to synthesize polyunsaturated fatty acids called indeterminate C22:2i and C22:2j. In our study, these two fatty acids were recorded in the flesh with low quantities.
Concerning the monounsaturated fatty acids, the palmitoleic acid (C16:1) and oleic acid (C18:1) are respectively represented in the flesh with14.81mg/100gand 11.24 mg/100g**.** Levels of SFA recorded (535.15mg/100g) shown in **Table III** revealed that percentages of stearic acid (C18:0) and palmitic acid (C16:0) (46.91mg/100g and 135.44mg/100g respectively) were the highest, probably related to several parameters including richness of the environment on organic detritus source of C14:0, C15:0, C16:0 and C18:0 as mentioned by [7]. The SFA may not be considered as a whole because they differ in their structure, metabolism, cellular functions and even deleterious effects in case of excess [20].

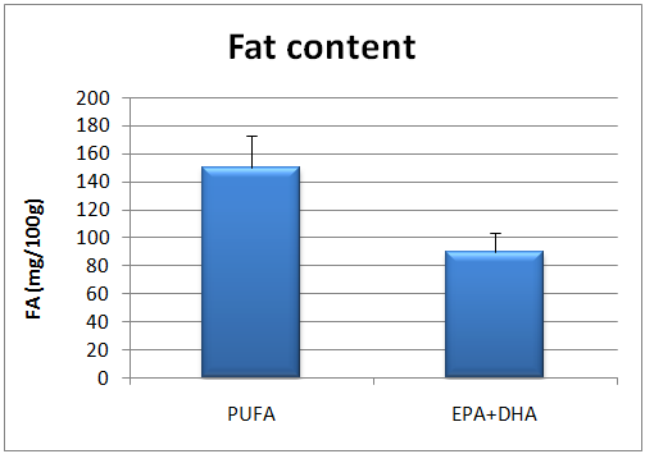

Figure 2. Fat contents in the flesh of *Pinctada radiata* (mg/100g FM)

The physiologically active fatty acids necessary for a normal development in vertebrates are the arachidonic acids (ARA) (C20:4n-6), the eicosapentaenoic acid (EPA) (C20:5n-3) and the docosahexaenoic acid (DHA) (C22:6n-3).ARA that later synthesis in the human body may be too low to meet the requirements[21].

Among the identified fatty acids, three contribute to the nutrition value in *P. radiata* EPA, DHA and ARA. A great deal of research demonstrated the health benefits of those three fatty acids on cardiovascular, and inflammatory diseases; and brain development and function [22].
Nowadays, the American Heart Association (AHA) recommends to patients with known coronary heart disease (CHD) consuming about 1 g/day of EPA + DHA. For those without known CHD, the AHA recommends at least two servings/week of fish (preferably oily) to reduce the risk of cardiovascular disease; this would equate to approximately 500 mg/day of EPA+DHA **[23].**Concerning the polyunsaturated fatty acids, EPA and DHA were predominant in the flesh (24.54 mg/100g and 65.2 mg/100g) **(Fig.2)**. Moreover, many PUFA are enough represented such as C18:3 (n-3),C20:4 (n-3), C16:3 (n-4) and C18:3 (n-4).
A daily intake of 250 mg of DHA +EPA was considered to be too low and not optimal to reduce Cardiovascular Disease risk, as beneficial effects have been observed with doses beyond 250 mg per day. Either an adequate intake (AI) around 500 mg or 200g (about 6 medium oysters of *P. radiata*) were suggested to prevent Cardiovascular Disease in healthy subjects [21].
According to our results **(Table III)**, the flesh is significantly rich in polyunsaturated fatty acids among the series (n-3) (100.21mg/100g). In fact, EPA and DHA were the highest (24.54 mg/100g and 65.2 mg/100g respectively) **(Fig.2)**. In the other hand, PUFA levels among the (n-6) series are 32.88 mg/100g.
Our result is explained by the predominance of ARA in the flesh (20.15mg/100g) while this later is richer with 18:2 (n-6) and C20:2 (n-6) (7.7mg/100g and 5.02 mg/100g respectively).
We recorded also an elevated amount of DHA and EPA in the flesh of *P. radiata* among total lipids (89.75mg/100g) when compared to those registered in *Crassostrea gigas*[15].
Meanwhile, compared to Coast Sea Foods Company[24]**,** oysters are loaded with Omega 3 fatty acids which are good for joints, skin, vision, brain, heart, and helps lower bad cholesterol (LDL) levels. 100g (about 6 medium oysters) provides 740mg PUFA Omega 3. The average recommended daily value is 1000mg for women and 600mg for men. In practice300g/day of shell of *P. radiata* (about 18medium oysters) provides 750mgof Omega 3 Fatty Acids.
Dietary fat has for many years been known to influence serum lipid levels. In general, saturated fats raise the level of serum cholesterol, while polyunsaturated fats lower serum cholesterol. Effects of marine oils on lowering serum cholesterol levels have been investigated both in experimental animals and in humans [25]. In addition, consumption of high level of fish may explain the low incidence of cardiovascular disorders in Japanese [26]. Besides the effects of n-3 PUFA on heart diseases. Some research suggests that n-3 fatty acids decrease the undesirable effects of inflammatory diseases, the incidence of vascular stroke, and breast cancer, and could alleviate certain skin diseases [27].
The n-3/n-6 ratio is 3.12 in the flesh **(Table III).** This is due to high (n-3)PUFA levels despite Alpha-Linolenic Acid (ALA) low level. This later acid is however to reduce the cardio-vascular risk diseases. The AI recommended for ALA could include n-3 Long Chain up to 10 % of the PUFA's value[21].
n-6PUFA, specifically linoleic acid (LA)were in low quantity. It was suggested that the proposed AI for LA is too low. In fact, on one hand the AI of LA, have a role in reducing the cardio-vascular risk disease, on the other hand it was suggested to set a LATolerable Upper Intake Level (UL), because its over-consumption can lead to adverse effects, as lipid peroxidation, increases in body fat and instability of atherosclerotic plaques as well as inhibition of desaturation of Alpha-Linolenic Acid (ALA)[21].
Flesh of *P. radiata* is a better (n-3) series source among PUFA. This result is similar to those found in other bivalve molluscs such as scallops, *Saint Jacques,Chlamys varia, Pecten jacobaeus***[28]** and in the Pectinids, *Flexopecten glaber*[14] from northern Tunisian coasts. The later species presents

an interesting nutritional values due to their relatively high levels of PUFA (n-3), especially EPA and DHA. These acids are important in human nutrition and prevent development and progression of several disease states including cardiovascular disease (platelet aggregation inhibiting properties and reduction in blood pressure) and cancer [29]. In addition, this species contain a smaller amount of (n-6)PUFA.

The ratio n-3/n-6 in the diet recommendations is 0.2 [30], howeverthis ratio in *P. radiata*is 3.12. This finding characterizes the high nutritional value of this species[31].

In fact, this ratio ranged from 4.28 – 10.81 in the bivalve mollusk *Chamelea gallina* from the central Adriatic Sea [32]. Other authors registered n-6/n-3 values ranging between 0.09and0.13 in*Mytilusgalloprovincialis* from spanish coast[33]. A healthy balance between n-3 and n-6 PUFA by consumption of seafood is recommended by current dietary guidelines [32].

In humans, studies of bone cross-sections have shown that diets rich in polyunsaturated fatty acids have a positive effect on the latter, the n-3/n-6 ratio, have an effect on the health and bone remodeling**.** In fact, it seems that the (n-3) levels should be higher than n-6 in bones.

Many studies revealed that the fatty acids of the (n-6) family were associated with an increased bone loss, while (n-3) are presumed to protect the bone [34]. Recent data highlighted the beneficial effect of (n-3)PUFA in bone health [35]. Some researchers investigated the role of PUFA (n-3)reducing the osteoarthritis severity[36]. Some other authors revealed the important PUFA action on reducing inflammatory mediators (eicosanoids: prostaglandins, thromboxans…) in human osteoblast-like cells [37].

4. CONCLUSION

In the present work, we highlighted the fatty acid profile of total lipid in the flesh of pearl oyster *P. radiata* of Tunisian coast especially in the rich season [38]. However, quantitative compositions were different. *P. radiata* could be used as nutrition complement because its richness in polyunsaturated fatty acids especially omega 3 and omega 6.In our study, we have presented the fatty acids composition in the Tunisian shell *P. radiata***.**100 g of *P. radiata* shell provides 0.22 g of EPA+DHA. This amount is superior compared to servingsfrom crab, lobster, mussels, clams or fishes as snapper or catfish.100g of *P. radiata* shell (about 6 medium oysters) provides 750mg of Omega 3 Fatty Acids per day.

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