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PROTEINS, FATTY ACIDS AND NUTRITIONAL VALUE IN THE MUSCLE OF NINE MARINE SPECIES COMMONLY CONSUMED IN TUNISIA (CENTRAL MEDITERRANEAN)

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ABSTRACT

*Protein and fatty acid contents have been determined in nine marine species (*Sepia officinalis, Eledone cirrhosa, E. moschata, Loligo vulgaris, Trachurus trachurus, Scomber scombrus, Sardina pilchardus, Engraulis encrasicolus *and* Parapenaeus longirostris*) commonly consumed in Tunisia. Protein contents ranged from 15.8 % to 20.1 % and the total fatty acids varied between 2.2 % and 9.9 %. The values of the PUFA/SFA ratio were greater than 0.45 except for* S. pilchardus *which has a value of 0.36. The lower value of the ratio of n-3/n-6 was observed in* P. longirostris *(1.7) while the highest (11.7) has been related to L. vulgaris. Most of the species studied had revealed a high content in EPA+DHA, 500 mg / 100 g of muscle, which represent a high nutritional value for the human consumption.*

Keywords: proteins, total fatty acids, EPA+DHA, seafood products

PROTEINE, ACIDI GRASSI E VALORE NUTRIZIONALE NEL MUSCOLO DI NOVE SPECIE MARINE COMUNEMENTE CONSUMATE IN TUNISIA (MEDITERRANEO CENTRALE)

SINTESI

I contenuti di proteine e acidi grassi sono stati determinati in nove specie marine (Sepia officinalis, Eledone cirrhosa, E. moschata, Loligo vulgaris, Trachurus Trachurus, Scomber scombrus, Sardina pilchardus, Engraulis encrasicolus e Parapenaeus longirostris) *comunemente consumate in Tunisia. I contenuti proteici oscillavano tra il 15,8 % e il 20,1 %, mentre quelli degli acidi grassi totali tra il 2,2 % e il 9,9 %. I valori del rapporto PUFA/SFA erano superiori allo 0,45, eccetto che per* S. pilchardus, *con un valore di 0,36. Il valore più basso del rapporto n-3/n-6 è stato osservato in* P. longirostris *(1,7), mentre il più alto (11,7) è stato correlato a* L. vulgaris. *La maggior parte delle specie studiate ha rivelato un alto contenuto di EPA+DHA, 500 mg / 100 g di muscolo, che rappresentano un elevato valore nutrizionale per il consumo umano.*

Parole chiave: proteine, acidi grassi totali, EPA+DHA, prodotti ittici

INTRODUCTION

The nutritional qualities of seafood products such as fish, crustacean and cephalopods are resulting from the richness of their flesh in protein and fatty acids. Although the fish and meat (sheep, cattle, poultry, etc.) have equivalent levels of protein, the content of essential amino-acids of the fish is generally higher than that of the meat (Piclet, 1987; Médale *et al.*, 2003).

Especially fish, both marine and freshwater have beneficial effects on human health because of their lipid contents that are rich in fatty acids of n-3 (Steffens, 1997; Flaten *et al.*, 1997). As such, the fatty acids of marine seafood products are used in the prevention and treatment of arteriosclerosis, thrombosis and hyperglycemia and in the regulation of blood pressure (Barnerjee *et al.*, 1992; Brouwer *et al.*, 2006). To maintain a human health, (Sargent, 1997) recommends to increase the consumption of fish and its products, which are rich in n-3 PUFA and poor in n-6 PUFA series.

The lipid contents and fatty acid profile of the fish are affected by various factors such as temperature, salinity, season, size, age, habitat of the species, the type and abundance food (Ackman, 1989). Research on the n-3 PUFAs have generally focused on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) obtained by endogenous synthesis from alpha-linolenic acid (ALA), by elongation and desaturation (Garcia-Alonso *et al.*, 2012). It is estimated that 5-10% of ALA intake can be converted to EPA and a rate of 2-5% can be further converted to DHA in healthy adults (Calder & Yaqoob, 2009). EPA is the most important essential fatty acid of n-3 series in the human diet because it is the precursor to the n-3 series eicosanoids (Chen *et al.*, 1995). Therefore, and from a nutritional point of view, it is acclaimed to determine the fat concentration and the relative proportions of various fatty acids in species usually consumed (Soriguer *et al.,* 1997). To fulfill the growing demand for marine seafood products and medical point of view, this study is carried out to analyze the biochemical composition; protein, fat and fatty acids of nine popular species; *Sepia officinalis, Eledone cirrhosa, Eledone moschata, loligo vulgaris,* horse mackerel (*Trachurus trachurus*), *Scomber scombrus, Sardina pilchardus, Engraulis encrasicolus* and pink shrimp (*Parapenaeus longirostris*) commonly consumed in Tunisia. The objective of this study is to provide consumers with information on the potential benefits that they can expect to find in these products.

MATERIALS AND METHODS

Sampling

Studied samples were provided from the production of the northern and eastern marine fishing areas of Tunisia (Fig. 1) carried out during the summer 2013.

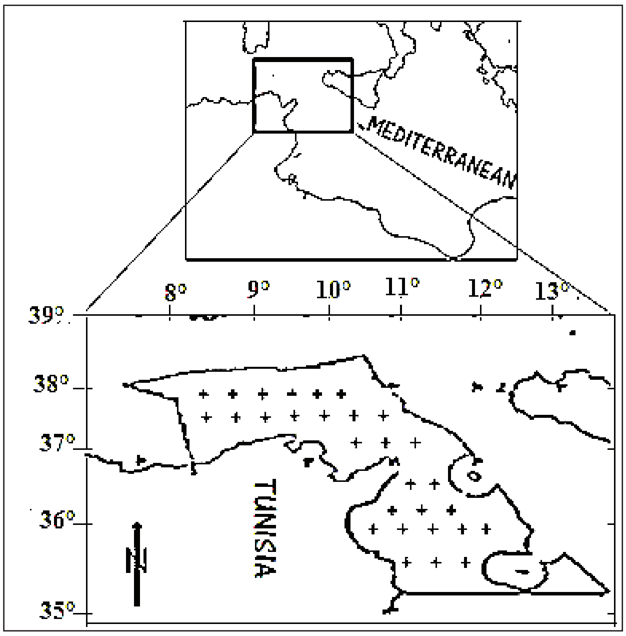

Fig. 1: Map showing the fishing area (+++) of the fish studied.
Sl. 1: Zemljevid ribolovnega območja (+++) obravnavanih vrst.

The collected individuals were kept in glass during the transfer to the laboratory of the University of Sciences of Tunis. Each individual was weighed to the nearest gram (WT). The fish total length (LT) and the mantle of cephalopod (MLT) were measured to the nearest cm. Biometric characteristic species are showing in Table 1.

Protein assay

The determination of the protein content was based on the method of Lowry *et al.* (1951) using as standard a solution of bovine serum albumin. Samples were incubated 10 mn at 50°C, then 20 mn at room temperature. The optical densities were measured at 540 nm.

Total lipid extraction and fatty acid analyses

All samples were fixed in boiling water to completely inactivate enzymatic activity, especially phospholipases (Shewfelt, 1981). The total lipids were extracted from the tissues according to the Folch *et al.* (1957) method, *i.e.*, chloroform-methanol (2:1, v/v). The total lipids fixed in a chloroform-methanol-BHT solution and stored in a freezer at -28°C. The fatty acids were converted to methyl esters according to the method Cecchi *et al.,* 1985).

A gas chromatograph type HP 6890 with a split/splitless injector with electronic pressure control and a flame ionization detector was used for the analysis. A aeparation was performed with a 30 m HP Innowax capillary column with an internal diameter of 250 μm and a 0.25 μmfilm thickness, the stationary polar phase of the column being polyethylene glycol.

Identification and quantification of fatty acids

The different fatty acids in the nine species were obtained by comparing the retention times of the fatty acids under study and those of a mixture of methyl esters (SUPELCO PUFA-3). The quantification of the fatty acids is based on an internal standard not present in our samples, methyl nonadecanoate or $C_{19:0}$ (Sigma Aldrich, Corporate Headquarters, St Louis, MO).

Statistical analysis

The results represent the mean values of a series of repetitions (n = 6). These results are considered significant if p < 0.05. the different values were analyzed according to the Duncan test. The statistical analyses were carried out with the SAS software program version 6.12.

RESULTS AND DISCUSSION

The protein percentage varies between 15.8 % for *S. pilchardus* and 16.8 % in *T. trachurus*. In cephalopods, it varies between 15.6 % in *E. cirrhosa* and 20.1 % in *L. vulgaris* (Tab. 1). Our results corroborate with the range of variation recorded for 540 fish species, which extends from 16 to 22 g / 100 g (Médale, 2008). Table 1 shows the levels of total fatty acids (TFA) expressed in grams per 100 g fresh weight (g / 100 g FW) different species. The lipid concentration in *P. longirostris* was 3 %. Limam *et al.* (2008) found in *Parapenaeus monoceros* and *Penaeus kerathurus*, lipid levels that did not exceed 2 %. Nunes *et al.* (2003) found a value of less than 5 % in *P. longirostris*. According to the classification of Ackman (1989) *P. longirostris* is a moderately species [2-4 %].

In cephalopod species, the lipid analysis showed quantitative variations ranging from 2.2 % (*E. muschata*) to 9.4 % (*S. officinalis*). Özoğül *et al.* (2008) observe in *S. officinalis*, *L. vulgaris* and *E. muschata* respective percentages of 1.29; 1.92 % and 0.68 % lower than those given in Table 2. Our results allow to classify *S. officinalis* as a fat species [> 8 %] and *L. vulgaris*, *E. moschata* and *E. cirrhosa* as a low fat species [2-4 %].

In fish, fat percentages are 9.4 % for *S. pilchardus*, 2.7 % in *S. scombrus*, 9.9 % in *T. trachurus* and 8.2 % in *E. encrasicolus*. Sirot *et al.* (2008) recorded a fat percentages of 5.7 % in *S. pilchardus*, 7 % for *S. scombrus* and 7.5 % for *E. encrasicolus*. Nunes *et al.* (2003) found in *T. trachurus* a percentage ranging from 5 % to 15 %. According Ackman (1989), *S. pilchardus*, *T. trachurus* and *E. encrasicolus* are fatty species [> 8 %] while *S. scombrus* is a poor lipid species [2-4 %].The percentages of saturated fatty acids (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) are presented in Table 2. In all these species, the lipid profile is SFA > PUFA > MUFA except in *E. cirrhosa* where PUFA> SFA> MUFA while in *E. moschata* SFA ~PUFA> MUFA.

According to Figure 2, there is an inverse relationship between the SFA and PUFA in different species. As shown in figure 2 and table 2, there are two subgroups. On the one hand the subgroup formed by *E. cirrhosa*, *E. moschata*, *S. officinalis* and *S. scombrus* with ratio PUFA / SFA of about 1 and a sub group of *S. pilchardus* with a

Tab. 1: Biometric characteristics, contents of TFA (g / 100 g) and proteins (%) of nine marine species (fish, cephalopods and pink shrimp) (mean ± SE; n=6; p<0.05).
Tab. 1: Biometrične značilnosti, vsebina TFA (g / 100 g) in delež beljakovin (%) pri devetih vrstah morskih organizmov (ribe, glavonožci in kozice) (povprečje ± SE, n = 6; p <0,05).

Species	Total length (cm)	Total weight (g)	Total fatty acids		Proteins %
			(g/100g)	%	
Fish					
S. pilchardus	14.8-18.0	22.4–36.5	9.4±0.4	9.4	15.8
S. scombrus	20.4–22.7	70.7–92.5	2.7±0.5	2.7	15.9
T. trachurus	16.0–22.8	32.7–97.8	9.9±2.4	9.9	16.8
E. encrasicolus	10.5–17.0	10.8–30.5	8.2±1.1	8.2	16.1
Cephalopods					
L. vulgaris	18.0–24.0	26.1–60.8	3.8±0.8	3.8	20.1
S. officinalis	3.5–10.8	61.0–197.0	9.4±0.4	9.4	18.2
E. moschata	25.5–31.5	67.6–89.3	2.2±0.4	2.2	16.2
E. cirrhosa	5.0–8.4	40.9–151.4	2.5±0.2	2.5	15.6
Crustacean					
P. longirostris	16.0–22.8	32.7–97.8	3.0±0.4	3.0	16.3

Tab. 2: Percentages of saturated fatty acids (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) in nine marine species (fish, cephalopods and pink shrimp).
Tab. 2: Odstotki nasičenih maščobnih kislin (SFA), enkrat nenasičene (MUFA) in večkrat nenasičene maščobne kisline (PUFA) v devetih vrstah morskih organizmov (ribe, glavonožci in kozice).

Species	SFA	MUFA	PUFA	PUFA/SFA
Fish				
S. pilchardus	60.37±3.07[a]	17.47±2.58[ab]	22.01±1.98[c]	0.36
S. scombrus	44.22±0.59[b]	14.16±0.51[b]	41.62±0.66[a]	0.94
T. trachurus	47.67±1.65[b]	23.98±1.73[a]	28.56±1.81[c]	0.59
E. encrasicolus	53.61±0.47[a]	13.07±0.62[b]	33.32±0.56[b]	0.62
Cephalopods				
L. vulgaris	53.46±2.81[a]	11.33±0.49[b]	36.37±3.36[b]	0.68
S. officinalis	45.18±0.78[b]	12.54±0.39[b]	42.27±0.89[a]	0.93
E. moschata	45.57±1.30[b]	11.31±1.06[b]	45.08±1.28[a]	0.98
E. cirrhosa	41.70±1.49[b]	11.15±0.27[b]	47.15±1.52[a]	1.13
Crustacean				
P. longirostris	43.46±1.49[b]	22.78±1.25[a]	33.90±0.52[b]	0.78

ratio of about 0.36. Referring to FAO /WHO (1994), the ratio's PUFA /SFA required for human is 0.45. Excepting *S. pilchardus*, we can state that species studied tended to accumulate PUFAs than SFA.

The percentages of the different families of PUFAs (n-3, n-6) and MUFAs (n-7 and n-9) were calculated for the nine seafood products studied (fish, cephalopods and pink shrimp); the results are displayed in Figure 3. Cephalopods are mainly distinguished by their high n-3 PUFA. Among fish, *T. trachurus* can be considered as a source of n-9 MUFA. While *S. pichardus* is characterized by almost equal concentrations of n-3 PUFA and n-9 MUFA in one hand, on the other hand, by its extreme poverty in n-6 PUFA. It has been shown that MUFA, often referred to as good fats, help reduce blood cholesterol levels and protect against heart disease (Shanmugam *et al.*, 2007).

Considering that the seafood products present a

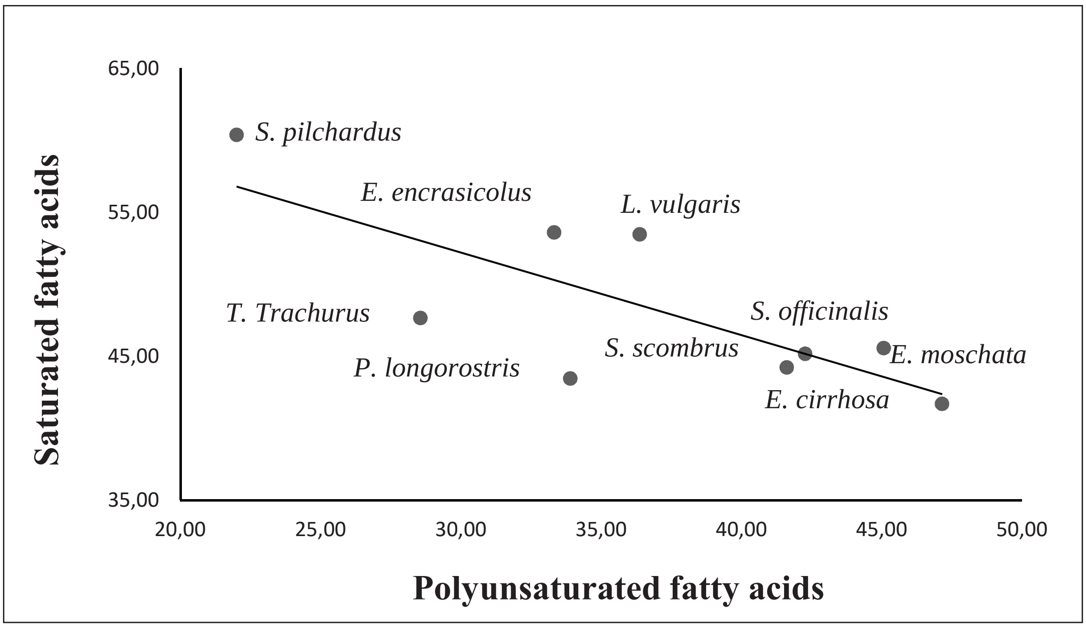

Fig. 2: Relationship between saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) (in % TFA) in nine marine fish (fish, cephalopods and pink shrimp).
Sl. 2: Razmerje med nasičenimi maščobnimi kislinami (SFA) in polinenasičenimi maščobnimi kislinami (PUFA) (v % TFA) v devetih vrstah morskih organizmov (ribe, glavonožci in kozice).

Tab. 3: Relative proportions of different fatty acids in nine marine species (fish, cephalopods and pink shrimp).
Tab. 3: Relativni deleži različnih maščobnih kislin v devetih vrstah morskih organizmov (ribe, glavonožci in kozice).

Species	PUFA (g/100g)	n-3 (g/100g)	n-6 (g/100g)	n-3/n-6	EPA+DHA (g/100g)
Fish					
S. pilchardus	2.07	1.74	0.30	5.8	1.27
S. scombrus	1.12	0.89	0.22	4.1	0.80
T. trachurus	2.80	2.25	0.45	5.0	1.85
E. encrasicolus	2.73	2.39	0.33	7.5	1.87
Cephalopods					
L. vulgaris	1.38	1.26	0.11	11.7	1.23
S. officinalis	3.98	3.59	0.33	11.3	2.96
E. moschata	0.99	0.85	0.13	6.3	0.73
E. cirrhosa	1.18	0.99	0.18	5.7	0.91
Crustacean					
P. longirostris	1.02	0.64	0.37	1.7	0.56

relation between the wealth of the fatty acids in omega 3 and their nutritional quality (Mori *et al.*, 1997), the relative proportions of different fatty acid groups were calculated for the nine species studied (Tab. 3).

To compare the quality of fat of different species, it is preferable to use the ratio n-3/n-6 than the ratio PUFA/SFA since the first one takes into account the families of fatty acids of the n-3 and n-6 separately (Sargent *et al.*, 1995). According to Table 3, (n-3/n-6) values ranged from 1.7 to 11.7. A part *P. longirostris*, cephalopod and fish species had a high nutritional value for human consumption (4.1 ≤ n-3 / n-6 ≤ 11.7). Fish and fishery products rich in n-3 fatty acids and low in n-6 fatty acids are considered beneficial for human health (Sargent, 1997). An increase in the n-3/n-6 ratio is essential to help the body use n-3 fatty acids. A low ratio indicates that the enzymes that convert fatty acids to their active forms are likely to be used by n-6 PUFAs (Hossain, 2011). The

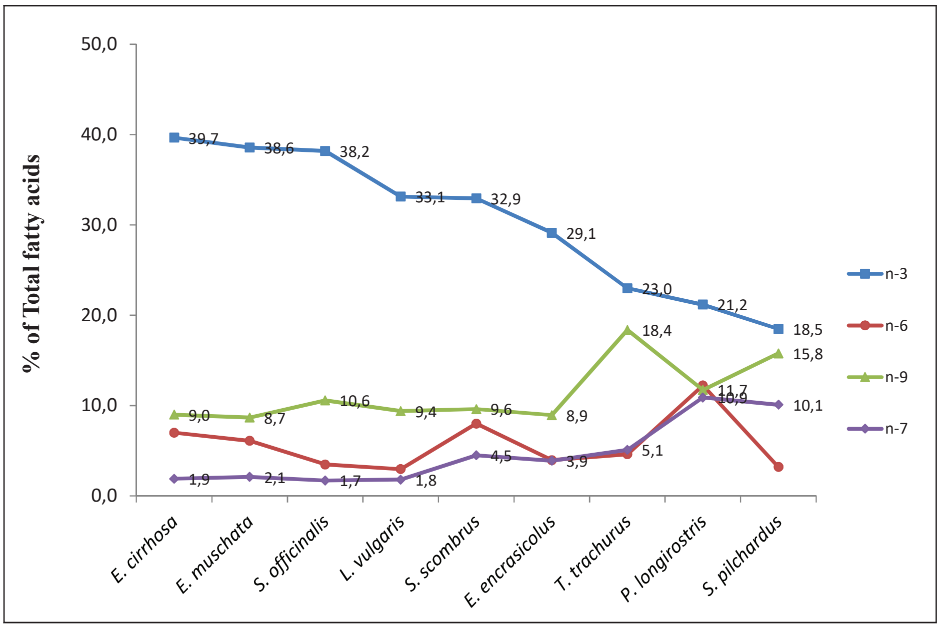

Fig. 3: Percentages of PUFAs (n-3, n-6) and MUFAs (n-7 and n-9) fatty acids per nine marine species (fish, cephalopods and pink shrimp).
Sl. 3: Delež PUFA (n-3, n-6) in MUFAs (n-7 in n-9) maščobnih kislin v vzorcih morskih organizmov (ribe, glavonožci in kozice).

percentage of EPA+DHA is responsible for variations in the n-3/n-6 ratios (Hossain, 2011).

The research has shown that there are significant health benefits of a diet rich in EPA+ DHA. A number of countries including Canada and the United Kingdom, and organizations such as the World Health Organization (WHO) and North Atlantic Treaty Organization have advocated dietary recommendations for n-3 PUFAs. These recommendations are 0.300 to 0.500 mg / day EPA+DHA (Kris-Etherton *et al*., 2003). Based on the results in table 4, these recommendations can easily be met by consuming 100 g fresh seafood product.

CONCLUSIONS

It can be argued that most species had a ratio PUFA / SFA greater than 0.45. The n-3/n-6 ratio is a very conclusive criterion to compare the nutritive value of marine fish fat. Fish or fishery products rich in fatty acids type n-3 and poor in fatty acids type n-6 are considered to be beneficial for human health. The ratio derived from our analysis, varying from 4 (*S. scombrus*) to 11.7 (*L. vulgaris*), is highly beneficial and desirable for human daily intake.

HRANILNA VREDNOST IN VSEBNOST PROTEINOV IN MAŠČOBNIH KISLIN V MIŠIČNINI MORSKIH VRST V TUNIZIJI (OSREDNJE SREDOZEMSKO MORJE)

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POVZETEK

*Določili smo vsebnost beljakovin in maščobnih kislin v devetih vrstah morskih organizmov (*Sepia officinalis, Eledone cirrhosa, E. moschata, Loligo vulgaris, Trachurus trachurus, Scomber scombrus, Sardina pilchardus, Engraulis encrasicolus *in* Parapenaeus longirostris*), ki se uporabljajo v prehrani v Tuniziji. Delež beljakovin je znašal od 15,8 % do 20,1 %, delež celokupne vsebnosti maščobnih kislin pa od 2,2 % do 9,9 %. Vrednosti razmerja PUFA/SFA so bile večje od 0,45 razen pri vrsti* S. pilchardus, pri kateri smo zabeležili vrednost 0,36. Nižja vrednost razmerja n-3/n-6 je bila ugotovljena pri vrsti P. longirostris *(1.7), najvišja pa pri* L. vulgaris *(11.7). Vrednosti EPA+DHA so pri večini obravnavanih vrst znašale nad 500 mg / 100 g mišičnine, zato ti morski organizmi predstavljajo visoko hranilno vrednost za človeško prehrano.*

Ključne besede: beljakovine, skupne maščobne kisline, EPA+DHA, morski proizvodi

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