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Assessment of Functional Lipid Constituents of Red (*Aristaeomorpha foliacea*) and Pink (*Parapenaeus longirostris*) Shrimps

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Abstract

Red shrimp is a unique source of nutraceuticals including omega-3 fatty acids and carotenoids. The presence of nutraceuticals depends on the quality and the consumption of phytoplankton and zooplankton. In the Mediterranean Sea, red shrimp (*Aristaeomorpha foliacea*) and pink shrimp (*Parapenaeus longirostris*) are two of the most prevalent species which are assessed in this study in respect to essential nutrients, fatty acids and carotenoids. Results indicated that the ω-3/ω-6 polyunsaturated fatty acids ratio in *A. foliacea* and *P. longirostris* muscle lipids exhibited considerable values (>2.9), indicating a healthy diet. Regarding carotenoids, astaxanthin was the most prevalent, already correlated with antioxidant support to nervous and musculoskeletal systems, followed by lutein, canthaxanthin, zeaxanthin, *α*- and *β*-cryptoxanthin. Phosphatidylcholine and polyunsaturated fatty acids predominated in shrimps' muscle lipids, while monounsaturated fatty acids in cephalothorax. Palmitic, oleic acids and the essential eicosapentaenoic (C20:5ω-3) and docosahexaenoic (C22:6ω-3) acids were also present fulfilling the high nutritional profile of both shrimps.

Keywords: Carotenoids; Cephalothorax; Fatty acids; Lipids; Muscle; Shrimp

Introduction

Shrimp represents one of the most widely consumed species of the Mediterranean and comprise an important source of nutrients in the human diet [1]. *Aristaeomorpha foliacea* (Risso 1827, commercial name: giant red shrimp) of the Aristeidae family is widely distributed in the eastern and western Atlantic, the western Pacific, the Indian Ocean and the Mediterranean Sea and located at depths ranging from 250 m to 1300 m with maximum abundance found between 500 m and 700 m [2]. *Parapenaeus longirostris* (Lucas 1846, deep seawater rose shrimp) of the Penaeidae family lives at depths ranging from 20 m to 700 m. *Parapenaeus longirostris* is commonly found in all over the Mediterranean and the Atlantic, as well as, in the West (from Massachusetts to French Guiana) and the East (from Portugal to Angola) [2]. *Aristaeomorpha foliacea* is distinguished by an intense uniform red coloring and it is considered large-sized, with ranging on average from 13 cm to 20 cm, while *P. longirostris* has a rose color and its length ranges from 8 to 16 cm [2]. The color of shrimp is due to its carotenoid content, which provides the typical red-orange tissue pigmentation and varies according to its native habitat [1]. The differences between the shrimp species seem to be highly dependent on the type and variability of the oceanography of the sea and the trophic characteristics in which these species spend most of their adult life [2].

Both types are active predators and scavengers. Furthermore, the above mentioned shrimps, are some of the most commercially important exploited crustaceans by trawlers in Mediterranean deep-water fisheries and the most economically and ecologically important crustacean resource in the Mediterranean basin [3]. They are available on the market fresh, refrigerated or frozen, while *P. longirostris* is also available through aquaculture. Their meat is considered to be highly nutritious and constitutes like many crustaceans, part of the Mediterranean diet, which is beneficial for different consumer groups [4], while for the Mediterranean countries, the cephalothorax (head and hepatopancreas) of shrimps is a distinctive delicacy, rich in substances essential for the human diet.

Shrimps are a widely distributed commercial catch in the Mediterranean Sea, comprising one of the main fishery food resources and a high proportion of fishery products consumed; therefore, it is of particular importance to evaluate their nutritional value. Moreover, the differentiation regarding the nutritive data of shrimp muscle (edible part) and cephalothorax (by-products) lipids has not been well-documented. An in-depth analysis was necessary in order to establish whether the above characteristics were reflected in proximate composition and lipid profile of shrimps, both qualitatively and quantitatively. Therefore, the aim of the present study was to assess the bioactive lipid constituents' profile of muscle and cephalothorax of the deep seawater giant red shrimp *Aristaeomorpha foliacea* and the rose shrimp *Parapenaeus longirostris*. The ultimate goal was to estimate the nutritional value of muscle as well as to attain the exploitation of cephalothorax, to produce bioactive compounds.

Materials and Methods

Chemicals, standards and solvents

The lipid standards used were: cholesteryloleate, cholesterol, tri-stearoyl-glycerol, lauric acid, oleic acid, linoleic acid, 1,2-distearoyl-glycerol, 1-monostearoyl-rac-glycerol, phosphatidylcholine, phospha-

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tidylethanolamine, lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and sphingomyelin standards of the Sigma Chemical Co (Sigma-Aldrich Company, Dorset, Great Britain and St. Louis, MO). Fatty acid methyl esters used as GC-FID standard mixtures were: Supelco TM 37 Component FAME Mix C4-C24, 100 mg and Supelco PUFA No.1, Marine Source, 100 mg. All-*trans* carotenoid standards (lycopene, β-carotene, lutein, zeaxanthin, canthaxanthin, α- and β-cryptoxanthin and astaxanthin) were purchased from Sigma Chemical Co (Sigma-Aldrich Company, St. Louis, MO, USA). All solvents used for sample preparation and extraction were of analytical grade and the solvents used for GC-FID, Iatroscan TLC-FID and HPLC-DAD analyses were of HPLC grade from Merck (Darmstadt, Germany). Double distilled water was used throughout this work. All reagents used were of analytical grade and they were purchased from Mallinckrodt Chemical Works (St. Louis, MO) and from Sigma Chemical Co (Sigma-Aldrich Company, UK).

Sampling

Specimens of deep seawater shrimps, *A. foliacea* and *P. longirostris*, were studied. The shrimps were caught during spring time (March-May) from Ionian Sea area and were purchased from a commercial fish market, with two repetitions in 2014 and 2015. The specimens (batches of 30 kg, per species and per year) were transported to the laboratory where they were washed with cold distilled water; weighed (46.48 ± 5.55 g and 9.44 ± 3.37 g, respectively) and the average length was measured (18.95 ± 1.38 cm and 12.07 ± 1.31 cm, respectively). Then, the specimens were divided in three groups according to their weight, they were dissected; muscles and cephalothoraxes were weighed and then separately homogenized in a blender for the determination of proximate, lipids and fatty acid composition. The analytical parameters were determined at least in triplicate for each group (n=6 samples per species and tissue, for the two repetitions).

Proximate analysis

Moisture content was determined by oven drying at 105°C ± 2°C to reach constant weight, crude protein by Kjeldahl procedure and ash by heating in a muffle furnace at 550°C to constant weight [5]. Homogenized muscle and cephalothorax total lipids of shrimps were extracted (separately), according to the Bligh and Dyer method [6]. After phase equilibration, the lower chloroform layer (TL) was removed and dried in a rotary vacuum evaporator at 32°C. The extracted lipids were weighed in order to determine the TL content, then redissolved in chloroform/methanol (9:1, v/v) and finally stored at 0°C until further use. To prevent oxidation t-butyl-hydroquinone was added to all samples during preparation.

Iatroscan TLC–FID analysis of neutral and polar lipids

Lipid classes were separated on silicic acid-coated quartz rods, chromarods (Type SIII, 5 mm silica gel-coated quartz rod, Iatron Labs, Tokyo, Japan) and they were quantified using a thin layer chromatography–flame ionization detection system. Iatroscan TLC–FID analysis was performed by an Iatroscan thin-layer chromatograph (Model MK-6 TLC/FID – FPD Analyser Iatron Laboratories, Tokyo, Japan) equipped with a flame ionization detector. Individual lipid classes were quantified as described previously by Sinanoglou et al. [7].

Gas chromatography analysis of fatty acid methyl esters

Fatty acid methyl esters (FAME) of total lipids (TL) were prepared according to the procedure described by Sinanoglou et al. Both quantitative and qualitative analysis were performed on an Agilent 6890 Series Gas Chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector, as described by Sinanoglou et al. [7]. DB-23 capillary column (60 m × 0.25 mm i.d. 0.15 μm film) (50%-cyanopropyl-methylpolysiloxane) (Agilent Technologies, USA) was used. The individual FAME were identified by comparing their retention times with those of the authentic standard mixtures. The relative content of fatty acids in the sample was determined according to Sinanoglou et al. [7].

Indices calculations

The ratio between hypocholesterolaemic and hypercholesterolaemic fatty acids (h/H) was calculated according to the formulas suggested by Santos-Silva et al. [8]:

$$(C18:1\omega9 + C18:2\omega6 + C18:3\omega3 + C20:4\omega6 + C20:5\omega3 + C22:5\omega3 + C22:6\omega3) / (C14:0 + C16:0)$$

The atherogenic index (AI) and thrombogenic index (TI) were calculated according to the formulas proposed by Ulbricht and Southgate [9]:

$$AI = \left[12:0 + \left(4 \times 14:0\right) + 16:0\right] / (\omega-3PUFA + \omega-6PUFA + MUFA)$$

and

$$TI = \left(14:0 + 16:0 + 18:0\right) / (0.5MUFA + 0.5\omega-6PUFA + 3\omega-3PUFA + \omega-3PUFA / \omega-6\ PUFA)$$

The cholesterol index (CI) was calculated according to the Zilversmit [10] formula:

$$CI = 1.01(g\ of\ SFA/100\ g\ of\ fresh\ matter - 0.5 \times g\ of\ PUFA/100\ g\ of\ fresh\ matter) + (0.06 \times mg\ of\ cholesterol/100\ goffreshmatter$$

While the cholesterol-saturated fat index (CSI) was calculated according to the formula proposed by Connor et al. [11]:

$$CSI = \left(1.01 \times g\ of\ SFA/100\ g\ of\ fresh\ matter\right) + \left(0.05 \times mg\ of\ cholesterol/100\ g\ of\ fresh\ matter\right).$$

Carotenoid analysis

For the identification of the different carotenoids, total lipids from shrimp muscle and cephalothorax were further analyzed by HPLC-photodiode array detection. Before being injected, lipid samples were dried under nitrogen gas and dissolved in acetone:hexane (2:3, by volume). Afterwards, samples were filtered through a 0.45 μm membrane filter to remove particulate residues. Twenty microliters of solution were injected for the HPLC analysis. The identification and quantification of carotenoids was performed according to Strati et al. [12].

Statistical analysis

The measurements of all the groups were carried out (at least) in triplicate; the values were averaged and reported along with their standard deviation (S.D). All data concerning proximate, lipid, fatty acid and carotenoid composition were analyzed with One-Way ANOVA Post Hoc Tests, and pairwise multiple comparisons were conducted with the Tukey's test. Probabilities lower than 0.05 were considered as statistically significant ($P<0.05$). All statistical calculations were performed with the SPSS package (IBM SPSS Statistics, version 19.0, Chicago, IL, USA) statistical software for Windows.

Results and Discussion

Proximate composition

The proximate chemical composition of red shrimp (*A. foliacea*)

and rose shrimp (*P. longirostris*) muscle and cephalothorax is shown in Table 1. Total lipid and ash contents of both shrimp cephalothorax were significantly (P<0.05) higher, while moisture content was significantly lower, than of respective muscle. Furthermore, total lipid content in the cephalothorax of *A. foliacea* was found to be significantly higher (P<0.05) than total lipid and ash content of the *P. longirostris* cephalothorax (Table 1). Protein was found to be the major constituent in the muscle of both shrimps, which is critical for muscle growth and normal function.

These results were significantly different compared to those of *Penaeus kerathurus* (pink shrimp) reported in an earlier study from this laboratory [13]. The total lipid content of *P. kerathurus* muscle and cephalothorax was 1.30% ± 0.06% and 2.40% ± 0.05%, respectively [13]. The results of this study, regarding fat content, were similar with those reported by Manjabhat et al. [14] for the edible part of *S. indica* and *A. alcocki* (0.94% and 2.7%, respectively) and for the cephalothorax (1.1% and 8.1%, respectively), by Turan et al. [15] for brown shrimp (*Crangon crangon*) muscle and by Li et al. [16] for 2 freshwater and 5 marine shrimps from China, higher than those reported by Rosa and Nunes [17] for red shrimp (*Aristeus antennatus*) and Norway lobster (*Nephrops norvegicus*) and lower than those reported by Sriket et al. [18] for *Penaeus monodon* and *Penaeus vannamei* muscle and by Yerlikaya et al. [4] for *Aristeus antennatus, Aristeomorpha foliacea, Plesionica martia, Parapenaeus longirostris*, and *Plesionica edwardsi* muscle from deep water; and *Metapenaeus monoceros, Penaeus semisulcatus, Penaeus kerathurus*, and *Penaeus japonicus* muscle from shallow water.

Other shrimp species reported, such as *Solonocera indica* and *Aristeus alcocki* from India, were found to have higher moisture content in muscle (83.6% and 81.0%, respectively) and in cephalothorax (80.4% and 77.2%, respectively) than the shrimp species studied, but lower or equal ash content (0.68% and 6.5% for *S. indica* muscle and cephalothorax respectively, and 0.65% and 4.0% for *A. alcocki* muscle and cephalothorax, respectively) [14]. Furthermore, our results regarding the moisture and ash content for the examined shrimp muscles were comparable with those reported by Sriket et al. [18] for black tiger shrimp (*Penaeus monodon*) (80.47% and 0.95% respectively), by Rosa and Nunes [17] for red shrimp (*Aristeus antennatus*) (73.6% to 74.5% and 2.0% respectively) and Norway lobster (*Nephrops norvegicus*) (74.7% to 75.2% and 2.0% to 2.1% respectively) and by Sriket et al. [18] for white shrimp (*Penaeus vannamei*) (77.21% and 1.47% respectively). Concerning protein content, *A. foliacea* and *P. longirostris* muscle had similar protein content with those reported by Sriket et al. [18] for *P. monodon* and *P. vannamei* and by Turan et al. [15] for brown shrimp (*Crangon crangon*) and lower than those reported by Rosa and Nunes [17] for red shrimp (*Aristeus antennatus*) and Norway lobster (*Nephrops norvegicus*).

Differences in proximate composition might result in differences in nutritional value, sensory qualities and shelf-life of the shrimps. Proximate compositions in shrimp muscles are governed by many factors, including species, growth stage, sex, feed and season [18].

Neutral and polar lipids

The lipid classes' profile of red shrimp (*A. foliacea*) and rose shrimp (*P. longirostris*) muscle and cephalothorax is presented in Table 2. Neutral lipids (NL) of the studied shrimp muscle mainly consisted of cholesterol, followed by triglycerides (TG) (Table 2), and while in the cephalothorax TG were the major NL as they constitute the main energy storage of the deep water shrimp species [19]. The detection of free fatty acids and diglycerides only in *A. foliacea*, muscle and cephalothorax suggested that lipid from red shrimp may be more susceptible to hydrolysis.

Polar lipids (PL) were the predominant lipids (>87%) in both shrimp muscle. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were the major PL accounting for ~ 80% of total lipids and in a ratio of about 3:1 (w/w), whereas phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (SM) were detected as minors PL. Phosphatidylinositol (PI) detected in the muscles is a phospholipid signal molecule that may be important in marine organisms. It is thought that the precursors to prostaglandins in marine fish are eicosapentaenoic (20:5ω-3) and docosahexaenoic (22:6ω-3) acids derived from phosphatidylinositol [19]. The PL proportion in total lipids of both shrimp cephalothorax was significant lower (P<0.05) to that found in the muscle and PC was by far the largest component followed by PE.

On a weight basis, significant differences (P<0.05) in the neutral and polar lipids content were found between tissues and species (data calculated from Tables 1 and 2). Evaluated per 100 g of wet weight, the *A. foliacea*'s muscle contained 452.95 ± 34.18 mg of PC, 140.94 ± 9.94 mg of PE, 56.12 ± 3.86 mg of cholesterol, 24.35 ± 1.44 mg of TG and 24.05 ± 1.20 mg of SM, while the rest lipid classes were found in lower quantities. The principal lipid classes per 100 g of cephalothorax wet weight were 6112.35 ± 43.99 mg of TG, 1186.04 ± 48.29 mg of PC 193.46 ± 9.90 mg of PE and 119.53 ± 2.98 mg of cholesterol.

Parameters	*A. foliacea* (red shrimp)		*P. longirostris* (rose shrimp)	
	Muscle	Cephalothorax	Muscle	Cephalothorax
Total weight (%w/w wet tissue)	46.08 ± 0.44a	52.75 ± 0.88b	61.43 ± 3.17c	37.16 ± 2.69d
Moisture	78.97 ± 1.46a	72.80 ± 1.84b	76.98 ± 0.90a	71.70 ± 0.87b
Ash	1.13 ± 0.02a	2.77 ± 0.39b	2.39 ± 0.32b	5.44 ± 0.41c
Protein	17.75 ± 0.11a	9.69 ± 0.29b	18.72 ± 0.10c	5.26 ± 0.08d
Total lipids	0.74 ± 0.04a	7.82 ± 0.37b	1.04 ± 0.14c	4.66 ± 0.35d

Results represent means ± SD (n=6 separate samples).
Means in the same row bearing different letters differ significantly (P<0.05).

Table 1: Total weight and proximate chemical composition (% of wet tissue) of *Aristaeomorpha foliacea* and *Penaeus longirostris* muscle and cephalothorax.

	A. foliacea		*P. longirostis*	
Lipids	Muscle	Cephalothorax	Muscle	Cephalothorax
NL % f TL	12.58 ± 0.77a	80.68 ± 0.63b	11.85 ± 0.53a	63.06 ± 0.61c
Sterol esters	n.d	0.32 ± 0.03	n.d	n.d.
TG	3.30 ± 0.23a	77.82 ± 0.56b	3.35 ± 0.22a	60.10 ± 0.53c
FFA	0.84±0.04a	0.49 ± 0.04b	n.d.	n.d.
Cholesterol	7.6 ± 0.41a	1.53 ± 0.05b	8.50 ± 0.49a	2.96 ± 0.16c
DG	0.83 ± 0.03a	0.52 ± 0.03b	n.d	n.d
PL % of TL	87.42 ± 0.77a	19.32 ± 0.63b	88.15 ± 0.53a	36.94 ± 0.61c
PE	19.04 ± 0.45a	2.48 ± 0.23c	17.13 ± 0.35b	5.74 ± 0.15d
PS	2.39 ± 0.04a	0.39 ± 0.02b	2.94 ± 0.03a	0.43 ± 0.03b
PI	1.57 ± 0.03a	0.21 ± 0.02b	1.40 ± 0.05c	0.23 ± 0.02b
PC	61.16 ± 0.69a	15.17 ± 0.10b	63.17 ± 0.49c	29.43 ± 0.45d
SM	3.26 ± 0.13a	1.07 ± 0.05b	3.51 ± 0.15a	1.09 ± 0.03b

Results represent means ± SD (n=6 separate samples)
Means in the same row bearing different letters differ significantly (P<0.05).
TG: Triglycerides; FFA: Free Fatty Acids; DG: Diglycerides; MG: Monoglycerides; PE: Phospatidylethanolamine; PS: Phosphatidylserine; PC: Phosphatidylcholine; SM: Sphingomyelin.
n.d. = not detected

Table 2: Neutral and polar lipid classes % (w/w) of total lipids of shrimps' muscle and cephalothorax.

Parapenaeus longirostris' tissues showed similar results. Thus, 100 g of muscle contained 656.89 ± 7.08 mg of PC, 178.36 ± 6.65 mg of PE and 88.23 ± 4.39 mg of cholesterol, while the rest lipid classes were found in lower quantities. In the cephalothorax, TG were the most abundant lipid class with 2797.96 ± 24.68 mg per 100 g wet weight, followed by PC, PE and cholesterol (1373.06 ± 24.42, 267.61 ± 25.26 and 139.39 ± 27.13 mg per 100 g wet weight, respectively).

From a nutritional point of view, cholesterol content in *A. foliacea* and *P. longirostris* varied from 56.12 to 88.23 mg per 100 g in muscle respectively, which is contributory to a low cholesterol regime (300 mg/day for a man and 225 mg/day for a woman). Our values for cholesterol in shrimp muscles were similar to those reported by Rosa and Nunes [17] for red shrimp (*Aristeus antennatus*) and Norway lobster (*Nephrops norvegicus*) (57.8-72.4 mg per 100 g wet tissue) and much lower than those reported by Turan et al. [15] for brown shrimp (*Crangon crangon*) (173.56 mg per 100 g wet tissue) and by Bragagnolo and Rodriguez-Amaya [20] for the wild marine shrimps (*Penaeus brasiliensis, Penaeus schimitti, Xiphopenaeus kroyeri*) (114-134 mg per 100 g wet tissue).

Choline was established as an essential nutrient with recommended daily intake (RDI) of 550 mg for men and 450 mg for women [21]. By estimating that phosphatidylcholine consists of approximately 13% choline by weight, the muscle and cephalothorax of *A. foliacea* and *P. longirostris* were found as an excellent source of dietary choline, providing 59-85 mg and 154-178 mg of choline per 100 g wet tissue, respectively. Furthermore, the particularly high levels of PC in the cephalothorax of the studied shrimps make them suitable source for industrial manufacture of lecithin.

Fatty acids

The fatty acid (FA) pattern of total lipids (TL) isolated from muscle and cephalothorax of the studied shrimps is shown in Table 3. The carbon chain length of the forty (40) identified fatty acids ranged from 10°C to 24°C and the number of double bonds ranged from 0 to 6. The sums of FA for both shrimps muscles decreased according to the order PUFA>SFA>MUFA, while in the cephalothorax the order was MUFA>SFA>PUFA. The differentiation in individual FA proportions and sums of the examined shrimps was more pronounced in terms of the tissue (muscle and cephalothorax) than in terms of species. According to Ayas et al. (2013), the fatty acid composition of crustaceans is related to their life cycle and external factors, as temperature, water salinity, feed and season. The high level of unsaturated fatty acids for bathyal shrimp species may be explained due to the lower temperature of water at the deep seas [14].

Among the saturated fatty acids (SFA), palmitic (C16:0) and stearic (C18:0) acids were the most abundant in both shrimp muscle lipids, whereas palmitic (C16:0), stearic (C18:0) and arachidic (C20:0) acids predominated in cephalothorax lipids. Palmitic acid (C16:0) proportion was found to be more than the half of the total SFA proportion, in agreement to other researchers findings [4,15,16]. A significant difference ($P<0.05$) in C16:0 content was determined between *A. foliacea* and *P. longirostris* cephalothorax TL (1.33 instead of 0.70 g per 100 g wet tissue, respectively) (data calculated from Table 3).

Oleic acid (C18:1ω-9) was the principal MUFA in TL of tissues of both shrimps and its proportion was significantly higher in the cephalothorax than in the muscle TL. *P. longirostris* muscle TL had higher ($P<0.05$) C18:1ω-9 content (0.12 g per 100 g wet tissue) than *A. foliacea* muscle (0.10 g per 100 g wet tissue), although having lower proportion. Palmitoleic isomers (C16:1ω-7, ω-9) and vaccenic

Fatty acids	*A. foliacea*		*P. longirostis*	
	Muscle	Cephalothorax	Muscle	Cephalothorax
C10:0	0.36 ± 0.00a	0.70 ± 0.00b	n.d.	0.67 ± 0.01c
C12:0	n.d.	0.13 ± 0.00a	n.d.	0.14 ± 0.00b
C14:0	1.18 ± 0.02a	1.75 ± 0.02b	1.25 ± 0.00c	1.79 ± 0.00cd
C14:1	0.07 ± 0.00a	0.23 ± 0.00b	0.16 ± 0.00c	0.22 ± 0.00d
C15:0	0.52 ± 0.00a	0.56 ± 0.00b	0.88 ± 0.00c	0.57 ± 0.00bd
C15:1ω-5	0.07 ± 0.00a	0.20 ± 0.00b	0.29 ± 0.00c	0.20 ± 0.00b
C16:0	17.67 ± 0.05a	20.23 ± 0.06b	18.46 ± 0.10c	18.65 ± 0.11d
iso-C16:0	0.41 ± 0.00a	0.83 ± 0.01b	0.37 ± 0.01c	0.78 ± 0.01d
C16:1ω-7, ω-9	4.03 ± 0.01a	9.27 ± 0.03b	4.13 ± 0.01c	8.79 ± 0.03d
iso-C17:0	0.81 ± 0.01a	0.51 ± 0.00b	1.26 ± 0.01c	0.47 ± 0.01d
cyclo-C17:0	0.18 ± 0.00a	0.20 ± 0.00b	0.10 ± 0.00c	0.47 ± 0.01d
C17:0	0.92 ± 0.01a	1.12 ± 0.01b	1.13 ± 0.01b	1.10 ± 0.01c
C17:1ω-7	0.28 ± 0.00a	0.47 ± 0.00b	0.50 ± 0.01c	0.30 ± 0.01d
C18:0	5.74 ± 0.06a	4.28 ± 0.05b	5.25 ± 0.06c	4.19 ± 0.04b
C18:1*trans*-9	0.20 ± 0.00a	0.18 ± 0.00b	0.36 ± 0.01c	0.33 ± 0.00d
C18:1ω-9	18.46 ± 0.18a	25.45 ± 0.25b	15.75 ± 0.16c	24.66 ± 0.25d
C18:1ω-7	3.42 ± 0.02a	7.73 ± 0.05b	5.03 ± 0.03c	7.39 ± 0.04d
C18:2*cis*-9*trans*-11	0.24 ± 0.00a	0.33 ± 0.00b	0.66 ± 0.01c	0.36 ± 0.01d
C18:2ω-6	1.31 ± 0.01a	1.10 ± 0.01b	1.36 ± 0.01c	1.09 ± 0.01b
C18:3ω-6	0.32 ± 0.00a	0.46 ± 0.01b	0.49 ± 0.01c	0.91 ± 0.01d
C18:3ω-3	0.49 ± 0.01a	0.92 ± 0.01b	0.39 ± 0.00c	0.55 ± 0.01d
C18:4ω-3	0.21 ± 0.00a	0.29 ± 0.00b	0.18 ± 0.00c	0.22 ± 0.00d
C19:0	0.17 ± 0.00a	0.50 ± 0.00b	0.69 ± 0.01c	1.11 ± 0.01d
C20:0	2.32 ± 0.03a	4.90 ± 0.07b	2.74 ± 0.04c	5.38 ± 0.08d
C20:1ω-9	0.34 ± 0.00a	1.02 ± 0.02b	0.65 ± 0.01c	1.17 ± 0.02d
C20:2ω-6	0.56 ± 0.01a	0.53 ± 0.01b	0.88 ± 0.01c	0.58 ± 0.01a
C20:3ω-6	0.08 ± 0.00a	0.78 ± 0.01b	0.17 ± 0.00c	0.17 ± 0.00c
C20:4ω-6	4.75 ± 0.09a	2.25 ± 0.04b	5.56 ± 0.10c	2.73 ± 0.05d
C20:3ω-3	0.26 ± 0.01a	0.45 ± 0.01b	0.18 ± 0.00c	0.28 ± 0.00d
C20:5ω-3	12.82 ± 0.18a	3.76 ± 0.05b	12.68 ± 0.18a	4.00 ± 0.06c
C22:0	0.25 ± 0.01a	0.13 ± 0.00b	0.61 ± 0.01c	0.16 ± 0.00d
C22:1ω-11	0.67 ± 0.01a	0.21 ± 0.00b	0.14 ± 0.00c	0.30 ± 0.01d
C22:1ω-9	0.23 ± 0.00a	0.19 ± 0.00b	0.09 ± 0.00c	0.23 ± 0.00d
C22:2ω-6	0.10 ± 0.00a	0.14 ± 0.00b	0.11 ± 0.00c	0.08 ± 0.00d
C22:4ω-6	n.d.	1.20 ± 0.01a	0.49 ± 0.00b	1.50 ± 0.01c
C22:5ω-6	0.32 ± 0.00a	0.56 ± 0.00b	0.64 ± 0.01c	0.63 ± 0.01c
C22:5ω-3	0.66 ± 0.00a	n.d.	0.55 ± 0.00b	0.63 ± 0.01c
C24:0	0.62 ± 0.01a	0.46 ± 0.01b	1.39 ± 0.03c	0.59 ± 0.01d
C22:6ω-3	18.62 ± 0.34a	5.71 ± 0.10b	14.28 ± 0.26c	6.28 ± 0.11d
C24:1ω-9	0.34 ± 0.01a	0.27 ± 0.01b	0.15 ± 0.00c	0.35 ± 0.01a
Σω:0 (SFA)	31.15 ± 0.18a	36.30 ± 0.22b	34.13 ± 0.22c	36.07 ± 0.22b
Σω:1 (MUFA)	28.11 ± 0.25a	45.22 ± 0.36b	27.25 ± 0.23c	43.92 ± 0.35d
Σω:n (PUFA)	40.74 ± 0.65a	18.48 ± 0.26b	38.62 ± 0.59c	20.00 ± 0.28d
Σω:3	33.06 ± 0.53a	11.13 ± 0.17b	28.25 ± 0.45c	11.95 ± 0.18d
Σω:6	7.44 ± 0.12a	7.03 ± 0.09b	9.71 ± 0.15c	7.69 ± 0.10d
ω-3/ω-6	4.44 ± 0.01a	1.58 ± 0.01b	2.91 ± 0.01c	1.55 ± 0.01b
MUFA/SFA	0.90 ± 0.01a	1.25 ± 0.01b	0.80 ± 0.01c	1.22 ± 0.01d
PUFA/SFA	1.31 ± 0.01a	0.51 ± 0.01b	1.13 ± 0.01c	0.55 ± 0.04b
TFA*	0.56	6.57	0.79	3.73
SFA*	0.17	2.38	0.27	1.35
MUFA*	0.16	2.97	0.22	1.63
PUFA*	0.23	1.22	0.30	0.75
Σω:3*	0.19	0.73	0.22	0.45
Σω:6*	0.04	0.46	0.08	0.29

Results represent means ± SD (n=6 separate samples)
Means in the same row bearing different letters differ significantly (P<0.05).
*Expressed as g per 100 g wet tissue.
SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.
n.d. = not detected.

Table 3: Fatty acid composition % (w/w) of TFA in total lipids of *A. foliacea* and *P. longirostris* muscle and cephalothorax.

(C18:1ω-7) acids were the second most abundant MUFA in muscle and cephalothorax TL of the studied shrimps and their proportions were found almost double in cephalothorax than in muscle TL.

Eicosapentaenoic (C20:5ω-3, EPA) and docosahexaenoic (C22:6ω-3, DHA) acids were the dominant PUFA in lipid of both shrimps muscle and cephalothorax, followed by arachidonic acid (C20:4ω-6). The contents of both shrimp muscle and cephalothorax TL in DHA were almost 1.3-fold higher than those of EPA (data calculated from Table 3).

All the principal FA mentioned above have also been reported as main FA in several shrimp species [13,15,18,22,23]. It is important to point out the identification of the C18:1*trans*-9 (elaidic acid) as well as of the conjugated linoleic acid (CLA) isomer, C18:2cis-9*trans*-11(rumenic acid).

The ω-3/ω-6 ratio was found significantly higher in both shrimp muscle TL than the respective in cephalothorax TL, presenting the highest value in *A. foliacea* muscle (Table 3). The PUFA/SFA ratios are beneficial, as their values were higher to the recommended value of 0.45 [24] in all samples. Furthermore, the PUFA/SFA ratio of both shrimps muscle TL was 2-2.6 times higher than the respective one of the cephalothorax.

According to Dayal et al. [25], dieticians and consumers are sceptical about considering shrimp as a healthy food due to its relatively high cholesterol content. Therefore, lipid quality indices' calculation is of value, to pinpoint the cholesterolaemic effect of shrimp lipids. According to the results of this study (Table 4), the atherogenic (AI) and the thrombogenic (TI) indices of shrimp muscle TL were found 0.33-0.36 and 0.20-0.24, respectively, corresponding in much lower values compared to foods of animal origin [24,26]. The high hypocholesterolaemic/hypercholesterolaemic (h/H) ratio and the low values of cholesterol (CI) and cholesterol-saturated fat (CSI) indices of shrimp muscle TL indicate their protective nature against heart disease risk.

The amounts of PUFA provided by 100 g of cephalothorax are higher than those given by the same quantity of muscle, despite their lower relative percentages, related to cephalothorax higher total lipid content (Table 3). Thus, 100 g of *A. foliacea* and *P. longirostris* cephalothorax provide 1.22 and 0.75 g PUFA as well as 0.73 and 0.45 ω-3 fatty acids, respectively, encouraging the full exploitation of this by-product as an exceptional source of essential fatty acids.

Carotenoid composition

HPLC-DAD analysis of carotenoids revealed the presence of seven carotenoid molecular species (Table 5). The comparison among the muscle and cephalothorax carotenoids in both shrimps showed that free astaxanthin predominated in both tissues, followed by lutein. Furthermore, lesser amounts of zeaxanthin, canthaxanthin, *α*- and *β*-cryptoxanthin and unidentified astaxanthin esters were determined in all tissues. The last-mentioned compounds showed similar absorption spectra with *trans*-astaxanthin, demonstrating astaxanthin-derived compounds like astaxanthin esters [12]. Crustaceans, such as shrimps, contain astaxanthin as their main pigment, which is mainly formed from beta-carotene or zeaxanthin via the oxidative conversion [27,28].

From a nutritional point of view, the amount of total carotenoids in *A. foliacea* muscle and cephalothorax TL was found almost double than the respective amount in *P. longirostis* tissues TL (Table 5), possibly related to their coloration. Moreover, it is estimated that 100 g of *A. foliacea* muscle provides 1.56 mg of astaxanthin and 1.07 mg of lutein instead of 0.73 mg of astaxanthin and 0.47 mg of lutein for *P. longirostis* muscle TL (data calculated from Table 5). High levels of astaxanthin in cephalothorax make them an excellent natural source of this carotenoid. Astaxanthin obtained from shrimp waste can be used as coloring additive in foods or aquaculture feed formulations, in medical and biomedical applications or as a natural supplement in human nutrition [29]. The demand for natural sources of astaxanthin is growing because it is stable, harmless for human health as it doesn't cause allergic reactions in contrast to its synthetic form, so it is suitable for cosmetic applications [30]. Therefore, many studies have been conducted to extract astaxanthin from shrimp waste using various methods [29]. Astaxanthin and its esterified forms, canthaxanthin, free and esterified form of lutein, zeaxanthin and b-carotene are reported as the major pigments in various shrimp species (*Crangon crangon*, *Solonocera indica*, *Aristeus alcocki*, *Penaeus monodon*, *Penaeus indicus*, *Metapenaeus dobsonii* and *Parapenaeopsis stylifera*) [14,27]. As reported by Manjabhat et al. [14] and Sachindra et al. [27], the presence and the percentage of carotenoids in shrimp components are closely linked to their natural environment or provided food, because shrimps obtain carotenoids from their food, not being able to synthesize them *de novo*. The geographical position is also an important factor for the quantitative and qualitative profile of shrimp's carotenoids [31,32]. For instance, deep-shrimp species, similar to the the ones studied, contain higher amounts of total carotenoids than those in shallow waters, so they exhibit more intense color [19].

Indices	*A. foliacea*		*P. longirostis*	
	Muscle	Cephalothorax	Muscle	Cephalothorax
AI	0.33 ± 0.01a	0.43 ± 0.02b	0.36 ± 0.01a	0.41 ± 0.02b
TI	0.20 ± 0.00a	0.43 ± 0.02b	0.24 ± 0.01c	0.39 ± 0.01d
h	57.11 ± 0.36a	39.19 ± 0.42b	50.57 ± 0.57c	39.94 ± 0.48b
H	18.85 ± 0.17a	21.98 ± 0.26b	19.71 ± 0.19c	20.44 ± 0.24d
h/H	3.03 ± 0.02a	1.78 ± 0.03b	2.57 ± 0.02c	1.95 ± 0.01d
CI	3.42 ± 0.07a	8.96 ± 0.09b	5.42 ± 0.14c	9.35 ± 0.21d
CSI	2.98 ± 0.05a	8.38 ± 0.16b	4.68 ± 0.10c	8.33 ± 0.11b
Results represent means ± SD (n=6 separate samples). Means in the same row bearing different letters differ significantly (P<0.05).				

Table 4: Atherogenic (AI), thrombogenic (TI), hypocholesterolaemic and hypercholesterolaemic fatty acids (h/H), cholesterol (CI) and cholesterol-saturated fat (CSI) indices in total lipids of *A. foliacea* and *P. longirostris* muscle and cephalothorax.

Carotenoids	*A. foliacea*		*P. longirostis*	
	Muscle	Cephalothorax	Muscle	Cephalothorax
Astaxanthin	34.73 ± 0.87a	37.55 ± 0.64b	34.32 ± 0.58a	49.08 ± 0.82c
Zeaxanthin	9.55 ± 0.12a	16.48 ± 0.26b	10.39 ± 0.32c	11.82 ± 0.27d
Canthaxanthin	10.34 ± 0.29a	8.23 ± 0.16b	12.31 ± 0.43c	7.68 ± 0.26d
Lutein	23.84 ± 0.54a	29.59 ± 0.69b	22.12 ± 0.42c	18.64 ± 0.37d
Unidentified Astaxanthin esters	16.24 ± 0.39a	7.15 ± 0.22b	12.25 ± 0.17c	9.58 ± 0.24d
α-cryptoxanthin	3.20 ± 0.11a	0.65 ± 0.06b	4.53 ± 0.14c	1.67 ± 0.08d
β-cryptoxanthin	2.10 ± 0.05a	0.35 ± 0.04b	4.08 ± 0.12c	1.53 ± 0.09d
Total carotenoids mg/100 g wet tissue	4.49 ± 0.09a	28.24 ± 0.67b	2.12 ± 0.06c	10.08 ± 0.23d
Results represent means ± SD (n=6 separate samples). Means in the same row bearing different letters differ significantly (P<0.05).				

Table 5: Carotenoid composition (% (w/w) of total carotenoids) of shrimps' muscle and cephalothorax.

Conclusion

The shrimps *Aristaeomorpha foliacea* and *Parapenaeus longirostris* are typically and commercially exploited fisheries of the Mediterranean Sea. The nutritional value of shrimp muscles proved to be highly important due to the presence of bioactive lipid constituents, namely choline, astaxanthin and ω-3 fatty acids, beneficial to human health. Results from the present study confirm why red shrimps occupy a prominent position in the Mediterranean diet. Moreover, the recovered bioactive lipid molecules from shrimp cephalothorax, currently available in massive amounts as industrial bio-waste, can be used to produce functional supplements.

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