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Triacylglycerols and their fatty acid composition in edible Mediterranean molluscs and crustacean

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Abstract

The triacylglycerol (TAG) composition of edible Mediterranean molluscs (*Eledone moschata, Sepia officinalis, Todarodes sagittatus*) and crustacean (*Penaeus kerathurus*) was studied using a combination of preparative RP–HPLC and GC/MS. In *S. officinalis* and *T. sagittatus* mantle TAG, the main fatty acids were C16:0 and C18:0 while in *E. moschata* they were C18:1ω-9, C16:0, C20:5ω-3 and C22:6ω-3. In *P. kerathurus* muscle and cephalothorax TAG, the main fatty acids were C16:0, C18:0, C18:1ω-9, C20:4ω-6, C20:5ω-3, C22:6ω-3 and C16:0, C18:1ω-9, C20:4ω-6, C20:5ω-3, C22:6ω-3, respectively.

Thirteen TAG species were detected, the distribution of which was found to range according to the partition number from 34 to 48 for molluscs and from 36 to 50 for the crustacean. Over sixty TAG molecular structures were identified in the major TAG species. The most important in quantitative terms were long chain TAGs containing C14:0, C16:0, C18:0 as SFA, C16:1, C18:1 as MUFA and C18:2, C20:4, C20:5, C22:6 as PUFA.

Keywords: Mollusc; Crustacean; Fatty acids; Triacylglycerol; Neutral lipids

1. Introduction

The fatty acid compositions of triacylglycerols (TAG) of fish oils reflect their diet. These oils usually contain high concentrations of long chain ($C_{18,}$ C_{20} and C_{22}) monoenoic and polyenoic fatty acids, particularly of the ω-3 biosynthetic family. Fish oils consist of a large number of different fatty acids compared to animal fats and plant oils. The fatty acid composition of TAG can be easily modified by diet and is dependent on plankton composition (Shirai, Terayama, & Takeda, 2002). ω-3 PUFA are usually preserved in the form of TAG or ethyl ester. ω-3 PUFA are better absorbed in the human body as TAG than as ethyl esters (Lawson & Hughes, 1988). The presence of high levels of the long-chained ω-3 fatty acids, EPA and DHA, is identified as one of the major benefits of ingesting fish and shellfish species derived lipids (Mahaffey, 2004). TAGs, the main constituents of food lipids, are rather simple molecules formed by a glycerol moiety esterified with three FA. The number of possible FA combinations in a TAG molecule is very high. TAGs generally follow a unique and typical pattern in the glycerol molecule being characteristic in the different oils and fats. The advantage of using TAG profile comparing to FA profiles lies in the fact that the stereo specific distribution of FA on the glycerol molecule is genetically controlled (Aparicio & Aparicio-Rufz, 2000). Napolitano, Ratnayake, and Ackman (1988) reported that

Abbreviations: AA, arachidonic acid (C20:4ω-6); DHA, docosahexaenoic acid (C22:6ω-3); EeA, eicosaenoic acid (C20:1ω-9); EPA, eicosapentaenoic acid (C20:5ω-3); HA, heptadecanoic acid (C17:0); HeA, heptadecenoic acid (C17:1ω-7); LA, linoleic acid (C18:2ω-6); MA, myristic acid (C14:0-); MUFA, monounsaturated fatty acids; OA, oleic acid (C18:1ω-9); PA, palmitic acid (C16:0); PaA, palmitoleic acid (C16:1ω-7); PUFA, polyunsaturated fatty acids; SA, stearic acid (C18:0); SFA, saturated fatty acids; FA, fatty acid(s); TAG, triacylglycerol(s).

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one-third of bivalve larvae triacylglycerol fatty acids were polyunsaturated. Thus, it is suggested that this lipid fraction may act as a temporary reservoir of physiologically-important polyunsaturated fatty acids.

The aim of the present work is the determination and identification of the major triacylglycerol species and of their fatty acid composition, found in the mantle (edible part) of three cephalopod mollusc species (*Eledone moschata, Sepia officinalis, Todarodes sagittatus*) as well as in the muscle and cephalothorax of a shrimp (*Penaeus kerathurus*), which represent edible aquatic animals commonly consumed in Mediterranean countries.

2. Materials and methods

2.1. Reagents and standards

The triacylglycerol standards used (purity > 98%) were MMM, PPP, SSS, LLL, POP, EpEpEp and DhDhDh all being purchased from Sigma Chemical Co (Sigma–Aldrich Company, St. Louis, MO). Fatty acid methyl esters used as GC and GC/MS standard were: lauric acid M–E, L7272, cis-5,8,11,14,17-eicosapentaenoic acid M–E, E2012 and cis-4,7,10,13,16,19-docosahexaenoic acid M–E, D2659 (purity ⩾ 98%) also being purchased from Sigma Chemical Co; Matreya Bacterial Acid Methyl Esters CP^{TM} Mix, Catalog No:1114; Supelco^{TM} 37 Component FAME Mix, Catalog No:47885-U. All solvents used for sample preparation were of analytical grade and the solvents used for MS analyses were of HPLC grade from Merck (Darmstadt, Germany).

2.2. Experimental animals – Total lipid extraction and separation

Thirty adult specimens of the cephalopod molluscs *E. moschata,*, *S. officinalis* and *T. sagittatus* (three separate samples of composites, ten cephalopods per composite from Saronicos Bay, Greece, 2003) and ten adult specimens of the prawn *P. kerathurus* (North Aegean Sea, Greece, 2003) were, after being caught, brought to the laboratory alive, individually measured for weight (with an average of 245 g/octopus, 20 g/cuttlefish, 170 g/squid and 29 g/prawn) and immediately processed The mantle of each of the above molluscs as well as the muscle and cephalothorax parts of the prawn were separately homogenized in a blender and total lipids were extracted according to the Bligh and Dyer method (1959). The extracted lipids were weighed in order to determine the TL, then redissolved in chloroform/methanol (9:1, v/v) and finally stored at 0 °C until used.

Total lipids were, afterwards, separated as neutral and polar lipid constituents on pre-washed 500 mg silicic acid columns (Merck and Co., Kieselgel 60), by solid-phase extraction (SPE), using the modified method of Mastronicolis, German, and Smith (1996). Neutral lipid fractions were quantified by weight after being eluted from solid-phase extraction columns.

2.3. Isolation and purification of triacylglycerols

A neutral lipid fraction containing triacylglycerols was isolated by silicic acid preparative thin layer chromatography on precoated silica gel 60 G plate (20 × 20 cm, 0.50 mm, Merck, Darmstadt, Germany). After spotting the sample, the plates were developed using solvent systems consisting of petroleum ether/diethyl ether/glacial acetic acid (either 70:30:1, 80:20:1, or 90:10:1, by vol.). The individual spots were visualized by exposure to iodine vapors and then the triglyceride band on the plates was scrapped off and extracted from the silica gel using the solvent system of the Bligh–Dyer procedure, i.e. chloroform/methanol/water (2:2:1, by vol.). After phase separation, the chloroform extracts were evaporated until dryness. The residual triacylglycerol fractions were quantified by weight and used for further chromatographic analysis. The extracts of triacylglycerol fractions were rechromatographed on HPTLC for purity confirmation.

2.4. HPLC analysis of triacylglycerols

One gram of each one of the above mentioned triacylglycerol fractions were dissolved in 10 mL of acetone and separated by preparative non-aqueous RP–HPLC. Aliquots of 20 μL of this solution (10%w/v) were injected for HPLC analysis. Column: RP-Nova-Pack C18 60 Å (4 μm, 300 × 3.9 mm i.d.) Waters, Detector: Refractive index (R.I.) detector in temperature 42 °C (Waters 410, Diffential Refractometer). Mobile phase: acetone/acetonitrile (50:50 v/v) isocratic system. The flow-rate was 1.2 mL/min. The temperature of furnace was 45 °C. Acetone is the usual modifier used with refractive index detection. As indicated by HPLC analysis of triacylglycerols, the retention time is proportional to the polarity of triacylglycerols as well as to the polarity of the mobile phase. It was also observed that the lower the polarity of the mobile phase or the triacylglycerol molecule, the lower the retention time. Quantification was performed by an internal normalization method and identification by comparison of retention times with authentic standards.

The concept of the partition number (PN) has been used to rationalize the retention time of triacylglycerol. The PN is defined as the total number of carbon atoms (CN) in the fatty acid acyl chains minus twice the number of double bonds (N) per molecule, i.e. $PN = CN - 2N$. It is elsewhere documented that components with the same PN value, known as "critical pairs", are linked with certain problems in their separation when using RP–HPLC. In particular, they do not have the same retention times, since the presence of a second or third double bond does not have the same effect as that of the first bond. Nevertheless this concept of PN has been widely used to define regions of the chromatogram, in which these compounds are eluted.

2.5. Gas chromatography/mass spectrometry analysis of fatty acid methyl esters

Methyl esters of the fatty acids contained in total TAG fractions as well as in the fractions of the major TAG species (which were separated and isolated by preparative RP–HPLC) were prepared as follows: A sample containing 20–50 mg of lipids was dried before trans-esterification. The residue was redissolved in 0.75 mL *n*-hexane. Then 0.1 mL of potassium hydroxide 2 N in methanol was added and the solution was mixed for 2 min in a vortex mixer (Velp scientifica, Italy), dried over anhydrous sodium sulfate and left for 25 min. After phase separation the upper layer of *n*-hexane containing the fatty acid methyl esters was removed and immediately injected into the gas chromatograph (Sinanoglou & Miniadis-Meimaroglou, 1998).

Quantitative and qualitative analysis were performed on a HRGC Mega 2 Series 8560 MFC 800 (Fisons Instruments) gas chromatograph, equipped with a EL 980 CE Insruments FID (flame ionization detector) (Hellenic Labware). A fused silica capillary column of high polarity was used (Supelco SP 2340, 60 m × 0.32 mm i.d. and 0.2 μm film thickness; Supelco, Inc., Bellefonte, PA, USA). This polymeric stationary phase was a nonbonded poly(biscyanopropylsiloxane). The carrier gas was hydrogen at a pressure of 15 psi. The make-up gas was atmospheric air at a pressure of 15 psi and hydrogen was supplied to the FID at a pressure of 9 psi. The temperature of the injector was 230 °C and of the detector 250 °C. The temperature was programmed at 150 °C for 15 min, raised from 150 to 170 °C at a rate 2 °C min^{-1}, held constant at 170 °C for 10 min, raised from 170° to 215 °C at 4 °C min^{-1} and held at 215 °C for 15 min. The duration of the analysis was 62 min. In the GC/MS analysis system a mass spectrometer VG Trio-2000 Mass Spectrometer (Fisons Instruments) replaced the FID. Electron ionization (EI) was produced by accelerating electrons from a hot filament through a potential difference, usually of 70 eV. In both GC and GC/MS methods, the fatty acid methyl esters were identified by comparison to the standard mixture of fatty acid methyl esters.

2.6. Statistical analysis

All measurements were obtained (at least) in triplicate and values were averaged and reported along with the standard deviation (SD). Data were analyzed with one-way ANOVA post-hoc tests and pairwise multiple comparisons were conducted with the Tukey's honestly significant difference test. All data were analyzed with the SPSS 13.1 statistical software.

3. Results and discussion

3.1. General observations

Total lipids (TL) of the cephalopods *E. moschata* (octopus) *S. officinalis* (cuttlefish) and *T. sagittatus* (squid) mantle, constituted 2.2%, 1.3% and 1.8% of the wet tissue, respectively. Neutral lipid components of the mantles of the above mentioned organisms constituted 48.8 ± 0.7%, 34.5 ± 0.5% and 26.2 ± 0.9% of the total lipids, respectively while the triglyceride content was found to be 64.5 ± 1.1% (31.5% of TL), 30.2 ± 0.6% (10.4% of TL) and 6.2 ± 0.7% (1.6% of TL) of the neutral lipids, respectively. These results were similar to those reported in an earlier study from this laboratory (Sinanoglou & Miniadis-Meimaroglou, 1998). The total lipid content of *P. kerathurus* muscle and cephalothorax was 1.03% and 2.36%, respectively, the neutral lipids constituted 22.2 ± 0.9% and 51.7 ± 1.2% of total lipids, respectively while triglycerides constituted 36.1 ± 0.6% (8.0% of TL) and 44.2 ± 0.3% (22.9% of TL) of neutral lipids, respectively.

3.2. Triacylglycerol fatty acid composition

The fatty acid composition of total TAG fractions, after the isolation of the neutral lipids of the studied invertebrates by preparative TLC, was determined by GC and GC/MS (Table 1). The main fatty acids in *E. moschata* mantle TAG were found to be palmitic acid (C16:0), stearic acid (C18:0), octadecenoic acid (C18:1 ω-9, ω-7), eicosaenoic acid (C20:1 ω-9), eicosapentaenoic acid (C20:5 ω-3) and docosahexaenoic acid (C22:6 ω-3).

In *S. officinalis* and *T. sagittatus* mantle TAG, palmitic acid (C16:0) and stearic acid (C18:0) were identified as the major fatty acids (in proportions that ranged from 50.52 ± 0.65% to 56.13 ± 0.76% and from 19.84 ± 0.47% to 23.92 ± 0.38%), DHA and EPA were found in modest amounts. The ω-6 essential fatty acids linoleic (18:2 ω-6) and arachidonic acid (20:4 ω-6) were not detected. The predominant fatty acids of *P. kerathurus* muscle TAG were found to be palmitic acid (C16:0), stearic acid (C18:0), octadecanoic acid (C18:1 ω-9, ω-7), arachidonic acid (C20:4 ω-6) eicosapentaenoic acid (C20:5 ω-3) and docosahexaenoic acid (C22:6 ω-3). In the cephalothorax, TAG fatty acids were found to be the same as in the muscle, except for stearic acid, which was found to occur in smaller amounts (Table 1).

The fatty acid profiles of TAG exhibited some interesting and significant differences ($P < 0.05$) among the studied specimens. *E. moschata* mantle TAG as well as *P. kerathurus* cephalothorax and muscle TAG, were found to be more unsaturated since they contained a higher percentage of monounsaturated and polyunsaturated fatty acids (37.10%, 37.79%, 24.81% and 34.11%, 27.23%, 24.84%, respectively) compared to *S. officinalis* (13.59% and 3.14%, respectively) and *T. sagittatus* (13.78% and 3.00%, respectively) TAG, which were found to contain higher amounts of saturated fatty acids (83.27 and 83.22, respectively). There were no significant differences in SFA, MUFA and PUFA in TAG fractions of *S. officinalis* and *T. sagittatus* mantle. The contents of the essential fatty acids C20:4 ω-6 (AA), C20:5 ω-3 (EPA) and C22:6 ω-3 (DHA) were significantly ($P < 0.05$) higher in *E. moschata* and *P. kerathurus*

Table 1
Composition of the total triacylglycerol fraction fatty acids of mollusc mantles and shrimp muscle and cephalothorax

Fatty acids[a]	*E.moschata* mantle	*S. officinalis* mantle	*T. sagittatus* mantle	*P. kerathurus* muscle	*P. kerathurus* cephalothorax
C14:0	4.40 ± 0.15a	5.29 ± 0.21b	7.00 ± 0.11c	0.95 ± 0.04d	–
C15:0	–	1.53 ± 0.13a	1.78 ± 0.05b	1.00 ± 0.05c	–
C16:0	17.13 ± 0.32a	56.13 ± 0.76b	50.52 ± 0.65c	23.50 ± 0.34d	31.09 ± 0.98e
C16:1 ω-7, ω-9	5.08 ± 0.40a	1.44 ± 0.07b	1.50 ± 0.09b	–	3.17 ± 0.12c
C17:0	–	0.48 ± 0.04a	–	–	1.30 ± 0.08b
C17:1 ω-7	2.13 ± 0.06a	5.02 ± 0.08b	3.93 ± 0.24c	–	–
C18:0	7.26 ± 0.17a	19.84 ± 0.47b	23.92 ± 0.38c	23.90 ± 0.29c	6.59 ± 0.11d
C18:1 ω-9	16.84 ± 0.39a	3.42 ± 0.08b	4.97 ± 0.25c	17.52 ± 0.33a	27.88 ± 0.72d
C18:1 ω-7	6.89 ± 0.44a	3.71 ± 0.51b	3.38 ± 0.22b	6.35 ± 0.16a	4.74 ± 0.28a
C18:2 ω-6	1.80 ± 0.04a	–	–	0.89 ± 0.05b	2.06 ± 0.05c
C20:0	–	–	–	1.00 ± 0.03	–
C20:1 ω-9	6.16 ± 0.29a	–	–	0.94 ± 0.06b	–
C20:4 ω-6	5.59 ± 0.34a	–	–	9.24 ± 0.58b	10.32 ± 0.39c
C20:5 ω-3	14.32 ± 0.58a	1.10 ± 0.06b	1.76 ± 0.08b	6.93 ± 0.13c	7.15 ± 0.25c
C22:6 ω-3	12.40 ± 0.67a	2.04 ± 0.12b	1.24 ± 0.05b	7.78 ± 0.71c	4.70 ± 0.09d
Σω-0	28.79 ± 0.31a	83.27 ± 0.64b	83.22 ± 0.46b	50.35 ± 0.30c	34.98 ± 0.74d
Σω-1	37.10 ± 0.42a	13.59 ± 0.41b	13.78 ± 0.23b	24.81 ± 0.25c	37.79 ± 0.62a
Σω-n	34.11 ± 0.48a	3.14 ± 0.10b	3.00 ± 0.06b	24.84 ± 0.22c	27.23 ± 0.28d
Σω-3	26.72 ± 0.61a	3.14 ± 0.10b	3.00 ± 0.06b	14.71 ± 0.52c	14.85 ± 0.14c
Σω-6	7.39 ± 0.27a	0.00b	0.00b	10.13 ± 0.45c	12.38 ± 0.21d

Means in the same row bearing different letters differ significantly ($P < 0.05$).
[a] Fatty acid% (w/w) of total TAG fatty acid. Data are expressed as wt.% of total fatty acids and represent means ± standard deviation of three replicate determinations.

TAG fractions than in *S. officinalis* and *T. sagittatus* TAG fractions. In particular, ω-6 PUFA as well as the ω-3/ω-6 ratios, were indeed significantly ($P < 0.05$) higher in *E. moschata* and *P. kerathurus* TAG fractions than in *S. officinalis* and *T. sagittatus* TAG fractions, whereas ω-6 PUFA were not detected. The contents of palmitic (C16:0) and oleic acid (C18:1 ω-9) were significantly ($P < 0.05$) higher in *P. kerathurus* cephalothorax than in muscle. On the contrary, the contents of stearic (C18:0) and docosahexaenoic acid (C22:6 ω-3) were significantly ($P < 0.05$) higher in *P. kerathurus* muscle than in cephalothorax.

The ratios of saturated fatty acids as well as the ratios of long chain unsaturated fatty acids in *E. moschata, S. officinalis* and *T. sagittatus* mantle and *P. kerathurus* muscle TAG fractions were compared.

In saturated fatty acids, the 14:0/16:0 ratios were 0.26 ± 0.004, 0.09 ± 0.002, 0.14 ± 0.000 and 0.04 ± 0.001, respectively while the 16:0/18:0 ratios were 2.36 ± 0.011, 2.83 ± 0.029, 2.11 ± 0.006 and 0.98 ± 0.003, respectively. In monounsaturated fatty acids, the 18:1ω-9/18:1ω-7 ratios were 2.44 ± 0.10, 0.93 ± 0.11, 1.47 ± 0.02 and 2.76 ± 0.02, respectively. In polyunsaturated fatty acids, the EPA/DHA ratios were 1.15 ± 0.015, 0.54 ± 0.002, 1.42 ± 0.007 and 0.90 ± 0.099, respectively. All ratios differed significantly ($P < 0.05$). Ratios of 14:0/16:0 and EPA/DHA in *E. moschata* and *T. sagittatus* TAG fractions were higher than those of *P. kerathurus* muscle and *S. officinalis* TAG fractions. The 16:0/18:0 ratio was found to be the lowest in *P. kerathurus* and the highest in *S. officinalis.* The opposite was observed for the 18:1ω-9/18:1ω-7 ratio, as it was found to be the highest in *P. kerathurus* and the lowest in *S. officinalis*, respectively.

The results for the octopus and the shrimp TAG composition are in agreement with the results reported by Shirai, Higuchi, and Suzuki (2005) for the Japanese salmon and flyingfish roe TAG which mainly consisted of long-chain polyunsaturated (ω-3) fatty acids. The composition *of S. officinalis* and *T. sagittatus* mantle TAG is in agreement with the ones, reported by Yamashiro, Oku, Higa, Chinen, and Sakai (1999) for 15 cnidarians from Okinawa Japan, the TAG of which mainly consisted of saturated fatty acids containing low PUFA amounts. The relatively high content of PUFA in octopus mantle and shrimp muscle and cephalothorax TAG (one-third and one fourth of their total fatty acid content, respectively), as suggested by Napolitano et al. (1988), is persistent to meet future energy requirements and TAG (except for their role as depot lipids), may also act as a temporary reservoir of physiologically-important polyunsaturated fatty acids, which could be transferred to the structural lipids or directed to specific metabolic pathways. According to this concept *E. moschata* (octopus) and *P. kerathurus* (shrimp) TAG may be an excellent source of polyunsaturated ω-3 and ω-6 fatty acids for these invertebrates as well as a good source of MUFA and PUFA for the human diet.

3.3. Triacylglycerol species

The total TAG fractions were separated according to their PN values by preparative RP–HPLC. The TAG molecular species distribution in *E. moschata*, *S. officinalis* and *T. sagittatus* mantle and in *P. kerathurus* muscle and cephalothorax according to their CN and the degree of unsaturation (PN values), as well as their relative ratio, were

determined, leading to the identification and qualification of thirteen TAG species (Table 2). The TAG species with PN values of 36, 40, 42 and 40, 42, 44, 46, 48 were found in greater proportion in octapodo *E. moschata,* and in decapoda *S. officinalis* and *T. sagittatus*, respectively. In *P. kerathurus* muscle and cephalothorax, the highest percentage refers to TAG species with PN values of 38, 40, 42, 44, 46 as well as 42, 44, 46, 48, 50, respectively. The unusual TAG with PN 35, 37, 39 and 41, containing fatty acids with odd carbon numbers such as heptadecanoic (C17:0) and heptadecenoic (C17:1) acids, were also detected in *E. moschata* mantle (TAG with PN 35 and 37), *S. officinalis mantle* (TAG with PN 37, 39 and 41) and *P. kerathurus* cephalothorax (TAG with PN 37) in average amounts.

The major TAG molecular species that were obtained by preparative RP–HPLC were received separately and their constituent fatty acids were determined by GC and GC/MS (Table 3). The fatty acid composition of major TAG species varied among specimens. Eicosapentaenoic (20:5 ω-3) and docosahexaenoic acid (22:6 ω-3) represented almost 40% of the total fatty acids in *E. moschata* mantle major TAG species that were analyzed, with PN values of 36, 40 and 42. The fatty acid composition of *E. moschata* mantle major TAG species was remarkably different from the one of *S. officinalis* and *T. sagittatus*, respectively, mainly due to a higher unsaturated acids profile. On the other hand, the *E. moschata* mantle major TAG species fatty acids resembled those of the *P. kerathurus* muscle and cephalothorax on the basis of their high content in arachidonic acid (20:4 ω-6). Another characteristic of these TAG species was their high content of oleic acid (18:1 ω-9). *S. officinalis* and *T. sagittatus* mantle major TAG species were characterized by a significant low degree of unsaturation with 16:0 and 18:0 being particularly high. The fatty acid components of the above TAG species were almost similar and showed a particularly low content of PUFA (below 7.00% of total fatty acids) (Table 3).

GC/MS analysis of major TAG molecular species fatty acid composition indicated the presence of more than three different fatty acids in the same chromatographic peak. This was a clear evidence that more than one TAG isomer with the same PN value had co-eluted. Therefore, more than one possible molecular structure of major TAG species was identified. These molecular structures do not give information concerning the binding position of each fatty acid, they simply indicate several distribution patterns and possible combinations of these fatty acids in TAG molecular species according to their composition and the related distribution theories (Table 4).

Myristic (14:0), palmitic (16:0) and palmitoleic (16:1) acids are preferentially esterified in positions *sn*-1 and *sn*-3. One-third of the saturated fatty acids were acylated in the *sn*-2 position (Christensen & Hoy, 1996). Oleic (18:1) and longer-chain monoenoic fatty acids are mainly located in the primary positions with a tendency for the *sn*-3 position as the chain-length increases (Christie, 1986). Christensen and Hoy (1996) reported that in fish oils 18:1, 20:1, and 22:1 were mainly esterified in the *sn* 1,3 positions as reduced levels were found in the *sn*-2 position. Polyunsaturated fatty acids are in greatest concentration in position *sn*-2 with substantial amounts also being found in position *sn*-3 (Christie, 1986). In fish TAG, DHA is generally esterified in the *sn*-2 position in preference to the *sn*-1 and *sn*-3 positions (Ando, Satake, & Takahashi, 2000). DHA and EPA were preferably esterified in the *sn*-2 position of the zooplankton (e.g., fish eggs, amphipods, copepods, isopods and annelids) TAG. DHA and EPA were located in the *sn*-2 position at concentrations higher than 11 mol%, whereas the contents of these fatty acids in the *sn*-1 and *sn*-3 positions were less than 2 mol% (Ando, Kobayashi, Sugimoto, & Takamaru, 2004; Brockerhoff, Yurkowski, Hoyle, & Ackman, 1964). Christensen and Hoy (1996) reported that in fish oils the 22:6ω-3 was concentrated in the *sn*-2 position of the TAG, whereas the 20:5ω-3 was more evenly distributed between the *sn*-2

Table 2
Distribution (mol%) of triacylglycerols species in mollusc mantles and shrimp muscle and cephalothorax according to retention time (RT) and PN value

PN	RT (min)	*E. moschata* mantle	*S. officinalis* mantle	*T. sagittatus* mantle	*P. kerathurus* muscle	*P. kerathurus* cephalothorax
34	7.10	6.1 ± 0.20aA	3.3 ± 0.08aB	2.9 ± 0.04aC	0.00	0.00
35	7.87	1.9 ± 0.05b	0.00	0.00	0.00	0.00
36	9.21	43.2 ± 0.56cA	3.4 ± 0.07aB	4.8 ± 0.16bC	0.00	4.2 ± 0.07aD
37	10.30	3.1 ± 0.04dA	4.1 ± 0.11bB	0.00	0.00	2.1 ± 0.04bC
38	11.02	6.3 ± 0.15aA	3.8 ± 0.05abB	1.5 ± 0.07cC	13.7 ± 0.21aD	5.2 ± 0.19cE
39	11.90	0.00	1.4 ± 0.03c	0.00	0.00	0.00
40	13.67	12.4 ± 0.12eA	12.2 ± 0.14dA	12.7 ± 0.23dA	20.5 ± 0.68bB	2.7 ± 0.08dC
41	15.65	0.00	1.2 ± 0.06c	0.00	0.00	0.00
42	17.18	18.1 ± 0.31fA	18.2 ± 0.23eA	17.6 ± 0.15eA	14.4 ± 0.18cB	15.5 ± 0.16eC
44	21.48	7.6 ± 0.11gA	13.7 ± 0.16fB	18.3 ± 0.27fC	25.8 ± 0.35dD	10.3 ± 0.24fE
46	26.21	4.7 ± 0.05hA	16.4 ± 0.18gB	19.4 ± 0.18gC	14.6 ± 0.16cD	32.2 ± 0.44gE
48	32.10	5.6 ± 0.13kA	22.3 ± 0.45kB	22.8 ± 0.32kB	7.3 ± 0.08eC	12.2 ± 0.25kD
50	38.75	0.00	0.00	0.00	3.7 ± 0.06fA	15.6 ± 0.14eB

Means in the same row bearing different letters differ significantly ($P < 0.05$).Means in the same row bearing different capital letters differ significantly ($P < 0.05$).

Table 3
Distributions of fatty acids (% w/w of total fatty acids)[a] in the major TAG species in mollusc mantles and shrimp muscle and cephalothorax

Species	PN groups	Fatty acids								
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:4	C20:5	C22:6
E. moschata mantle	36	2.45 ± 0.05	10.72 ± 0.09	–	–	30.19 ± 0.25	-	9.75 ± 0.20	22.61 ± 0.39	24.30 ± 0.14
	40	5.93 ± 0.10	–	7.80 ± 0.13	10.58 ± 0.23	18.30 ± 0.33	2.79 ± 0.05	14.74±0.31	20.50±0.22	19.40±0.26
	42	3.50 ± 0.07	10.90 ± 0.21	9.25 ± 0.14	–	25.45 ± 0.25	3.67 ± 0.06	14.78 ± 0.16	18.24 ± 0.24	19.46 ± 0.17
S. officinalis mantle	40	13.43 ± 0.06	56.34 ± 0.53	10.17 ± 0.22	–	16.76 ± 0.19	-	–	1.35 ± 0.03	1.95 ± 0.08
	42	10.44 ±	67.32 ± 0.67	4.96 ± 0.05	–	13.39 ± 0.14	–	–	–	3.89 ± 0.13
	44	9.56 ± 0.09	63.36 ± 0.46	2.33 ± 0.03	16.78 ± 0.16	5.21 ± 0.04	–	–	–	2.76 ± 0.09
	46	12.45 ± 0.18	50.57 ± 0.37	3.98 ± 0.07	19.46 ± 0.27	13.54 ± 0.17				
	48	6.57 ± 0.11	54.74 ± 0.28	6.54 ± 0.17	27.89 ± 0.18	4.26 ± 0.08	–	–	–	–
T. sagittatus mantle	40	12.56 ± 0.24	60.78 ± 0.74	5.29 ± 0.23	–	14.38 ± 0.29	–	–	4.68 ± 0.10	2.31 ± 0.04
	42	8.56 ± 0.16	69.78 ± 0.46	4.17 ± 0.08	–	11.75 ± 0.09	–	–	5.74 ± 0.18	–
	44	7.51 ± 0.12	60.69 ± 0.56	3.65 ± 0.06	21.23 ± 0.34	4.78 ± 0.11	–	–	2.14 ± 0.04	–
	46	6.14 ± 0.23	58.92 ± 0.48	2.58 ± 0.08	23.59 ± 0.27	8.77 ± 0.29	–	–	–	–
	48	5.11 ± 0.15	58.63 ± 0.39	4.90 ± 0.14	25.37 ± 0.41	5.99 ± 0.08	–	–	–	–
P. kerathurus muscle	38	4.00 ± 0.06	39.10 ± 0.51	–	15.40 ± 0.09	25.90 ± 0.16	–	4.36 ± 0.05	5.20 ± 0.08	6.04 ± 0.08
	40	–	–	–	–	44.00 ± 0.22	–	20.00 ± 0.16	18.00 ± 0.24	18.00 ± 0.17
	42	–	35.11 ± 0.25.	–	14.35 ± 0.22	28.56 ± 0.27	2.21 ± 0.08	7.12 ± 0.20	5.23 ± 0.12	7.42 ± 0.26
	44	–	41.35. ± 0.22	–	17.23 ± 0.17	22.11 ± 0.13	3.76 ± 0.11	10.24 ± 0.28	2.10 ± 0.05	3.21 ± 0.06
	46	–	16.12 ± 0.30	–	41.24 ± 0.57	10.08 ± 0.10	–	8.24 ± 0.14	11.23 ± 0.27	13.09 ± 0.12
P. kerathurus cephalothorax	42	–	57.43 ± 0.63	16.21 ± 0.19	–	–	–	6.78 ± 0.19	10.24±0.18	9.34 ± 0.15
	44	–	45.26 ± 0.46	–	–	42.79 ±	–	11.95 ± 0.35	–	–
	46	–	–	–	8.21 ± 0.15	49.43 ± 0.64	7.23 ± 0.25	22.60 ± 0.31	12.53 ± 0.29	–
	48	–	43.57 ± 0.47	–	4.52 ± 0.17	47.12 ± 0.50	–	4.79 ± 0.09	–	–
	50	–	44.33 ± 0.51	–	13.40 ± 0.26	42.27 ± 0.38	–	–	–	–

[a] Data represent means ± standard deviation of three replicate determinations.

Table 4
TAG molecular species of individual PN groups

PN	*E. moschata* mantle	*S. officinalis* mantle	*T. sagittatus* mantle	*P. kerathurus* muscle	*P. kerathurus* cephalothorax
36	PEpDh LArEp MArDh OEpDh ArArAr	–	–	–	–
38	–	–	–	MArAr OArEp OArDh PArEp PArDh SEpDh	–
40	SArEp SArDh MOEp MLAr PaLAr OMEp OPaDh	PPaDh PPaEp MPDh MPEp MODh	PPaDh PPaEp MPDh MPEp MOEp	OArEp OArDh	–
42	PLAr MPAr POEp PODh OODh OOEp	MMM MMPa PPDh OODh	MMM MMPa PPEp OOEp	PLAr PODh POEp OODh OOEp SArAr	PaPaPa PPDh PPEp PPaAr
44	–	MPaO MMP MMO PSDh	MPaO MMP MMO PSEp	PSEp PSDh OOAr PPAr SLAr	OOAr PPAr
46	–	MPP PaOO MOO MpaS MMS	MPP PaOO MOO MpaS, MMS	SOAr SSEp SSDh SPAr	OOL SSEp SOAr
48	–	OOO PPP POP MPS PPaS	OOO PPP POP MPS PPaS	–	PPP OOO POP SSAr
50	–	–	–	–	SPP SOO

The order of the abbreviations (e.g. PPaS) does not mean the binding position of each fatty acid, but it just indicates several distribution patterns and possible combinations of these fatty acids in TAG molecular species, according to their composition.

and the *sn*-1,3 positions *sn*-1,3 positions of the TAG molecules.

Most of the carbon atom numbers in *E. moschata* major TAG were C58 and C60, which based on fatty acid proportions (Table 3) may correspond to the fatty acid carbon number distributions of 18:20:20–16:20:22 and 18:20:22–20:20:20, respectively. The acyl carbon number distribution in major TAG in *S. officinalis* and *T. sagittatus* was similar. Most of the carbon atom numbers in *S. officinalis* and *T. sagittatus* ranged from 46 to 52 with the following possible distributions of C14–C22 acyl-residues (14:16:16, 16:16:16, 14:16:18, 16:16:18, 14:16:20, 16:18:18, 16:16:20 and 14:16:22). Most of the carbon atom numbers in *P. kerathurus* muscle ranged from 54 to 60 having as possible distributions 14:20:20, 16:18:20, 16:16:22, 14:20:22, 16:18:22, 18:18:20, 18:20:20, 16:20:22, 18:20:22 and 20:20:20 while in *P. kerathurus* cephalothorax they ranged from 48 to 56, which might correspond 16:16:16, 16:18:18, 16:18:20, 18:18:18 and 18:18:20.

4. Conclusions

The edible Mediterranean molluscs (*E. moschata, S. officinalis, T. sagittatus*) and the crustacean (*P. kerathurus*) contain triacylglycerols (TAGs) of a large variety of different fatty acids including long-chain polyunsaturated components such as 20:5ω-3 and 22:6ω-3. This wide range of fatty acids results to a complex mixture of molecular species of triacylglycerols. The total TAG and ω-3 fatty acid intake (meal portion 250 g) from the mollusc mantle as well as from the crustacean muscle was statistically ($P < 0.05$) different and supplied 1.73, 0.34, 0.07 and 0.21 g of TAG and 0.463, 0.011, 0.002 and 0.030 g of ω-3 fatty acid, respectively. Human absorption of ω-3 PUFA is better as TAG than as ethyl ester (Lawson & Hughes, 1988). These results show that the consumption of these invertebrates may play an important role in the clarification of their nutritional value.

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