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Isolation and identification of phospholipid molecular species in α wild marine shrimp *Penaeus kerathurus* muscle and cephalothorax

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Abstract

The concentration of TL in *Penaeus kerathurus* muscle and cephalothorax was 1.03 ± 0.04 (75.9 ± 0.8% of which was PhL) and 2.36 ± 0.07% (45.5 ± 0.8% of which was PhL) of the wet tissue, respectively. The phosphatidylethanolamine represented 26.4 ± 0.6% (85.6% diacyl- and 14.4% alkyl-acyl- or alkenyl-acyl-analogues) of muscle and 24.7 ± 0.2% (90.7% diacyl- and 9.3% alkyl-acyl- or 1-alkenyl-acyl-analogues) of cephalothorax phospholipids while the phosphatidylcholine represented 57.1 ± 0.6% (86.9% diacyl- and 13.1% alkyl-acyl- or alkenyl-acyl-analogues) of muscle and 47.2 ± 0.4% (89.1% diacyl- and 10.9% alkyl-acyl- or 1-alkenyl-acyl-analogues) of cephalothorax phospholipids, respectively.

The main fatty acids of phosphatidylethanolamine were C16:0, C18:0, C18:1 ω − 9, C20:4 ω − 6, C20:5 ω − 3, C22:6 ω − 3 and of phosphatidylcholine were C16:0, C18:0, C18:1 ω − 9, C20:4 ω − 6, C20:5 ω − 3. Low percentages of 2-OH C14:0 and cyclo-17:0 fatty acids were also determined. Phosphatidylethanolamine were found to contain a significantly ($P < 0.05$) higher percentage of polyunsaturated fatty acids compared to phosphatidylcholine. The ω − 3/ω − 6 ratio in muscle phosphatidylethanolamine and phosphatidylcholine was significantly ($P < 0.05$) higher to the ones of cephalothorax.

Keywords: *P. kerathurus*; Phospholipids; Phosphatidylcholine; Phosphatidylethanolamine; Fatty acids

1. Introduction

P. kerathurus, the most popular shrimp among the species of the *Penaeidae* family, is consumed in larger quantities than other edible crustaceans (such as lobsters and crabs), comprising a substantial part of the Mediterranean diet. The geographical distribution of this crustacean expands from Portugal to Angola, all the Mediterranean Sea, while its habitat is the bottom muddy sand and the bathymetry is ranging from 0 to 75 m, rarely reaching 90 m (Gonides et al., 2001).

The importance of lipids in crustaceans is suggested by their percentage contribution to the fresh weight of an organism, muscle and cephalothorax.

PhL of the marine species (including, crustaceans, molluscs and fish) are valuable components that can be applied within diverse areas like nutrition, pharmacy, medicine as well as within basic research, as they contain high levels of ω − 3 fatty acids. The most important ω − 3 fatty acids found in crustaceans are EPA and DHA. Crustaceans ingest and accumulate ω − 3 fatty acids through the food chain from algae and phytoplankton, the primary producers of ω − 3 fatty acids.

The deficiency of ω − 3 fatty acids in the brain induces memory and learning impairment, as well as psychological disorders (Lovas, 2006). Marine PhL can greatly facilitate the transportation of ω − 3 fatty acids over the blood–brain barrier, and thus prohibit the potential problems of ω − 3 fatty acid deficiency in the brain (Lovas, 2006). Health problems such as asthma, arthritis, diabetes, multiple sclerosis, hypertension, headaches, cancer and some kidney diseases may also be

Abbreviations: CL, cardiolipine; DHA, docosahexaenoic acid; EI, electron ionization; EPA, eicosapentaenoic acid; FA, fatty acids; FID, flame ionization detector; GC/MS, gas chromatography/mass spectrometry; HPTLC, high performance thin layer chromatography; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; MAH, mild alkakine hydrolysis; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PhL, phospholipids; PL, polar lipids; PS, phosphatidylserine; PI, phosphatidylinositol; PnL, phosphonolipids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Shm, sphingomyelin; SPE, solid phase extraction; TFA, total fatty acids; TLC, thin layer chromatography; TL, total lipids.

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controlled or alleviated by ω − 3 fatty acids (Arts et al., 2001; Understanding Nutrition, 2005).

Information about fishery and biology of the shrimp *P. kerathurus* were reported, but data on its lipid class composition and fatty acids have not yet been presented (Konidis, 2001; Rodriguez, 1987).

The aim of the present work is the qualitative and quantitative determination of muscle and cephalothorax of *P. kerathurus* phospholipids (Cl, PE, PC, PS, PI, Shm, LPC and PnL). Since PUFA are highly important to human health, a detailed study of fatty acid composition of polar lipids, phosphatidylethanolamine and phosphatidylcholine was conducted.

2. Experimental procedures

2.1. Reagents and standards

The lipid standards used (purity > 98%) were cardiolipine, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, lysophosphatidylcholine, lysophosphatidylethanolamine and sphingomyelin and they were 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 purchased from Sigma Chemical Co.; Matreya Bacterial Acid Methyl Esters CP™ Mix, Catalog No: 1114; Supelco™ 37 Component FAME Mix, Catalog No: 47885-U (Bellefonte, PA, USA).

All solvents used for sample preparation were of analytical 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, St. Louis, MO).

2.2. Sampling, sample preparation and total lipids extraction

Twenty adult shrimps (*Penaeus kerathurus*), were caught in the North Aegean Sea (near Platamona Bay) in October 2004. Shrimps were brought to the laboratory alive and individually measured for weight and length (with an average of 28.5 g/shrimp and 16.7 cm/shrimp, respectively). Then samples of muscle and cephalothorax were taken; they were weighed, and afterwards separately homogenized. TL were extracted according to the Bligh and Dyer method (1959). 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, then redissolved in chloroform/methanol (9:1, v/v) and finally stored at 0 °C until used. Additionally, in order for that to be confirmed, aliquots are evaporated in preweighed vials to constant weight to determine the lipid content. To prevent oxidation *t*-butyl-hydroquinone was added to all samples during preparation.

2.3. Separation of polar from neutral lipids. Iatroscan analysis of polar lipids

Total lipids were separated into neutral and polar lipids on prewashed 500 mg silicic acid columns (Merck and Co., Kieselgel 60), by SPE, using the modified method of Mastronicolis et al. (1996). PL fractions were quantitated by weight after being eluted from SPE columns.

In order to determine the PL composition percentage, they were separated on silicic acid-coated quartz rods (Chromarods, Type SII) and they were quantitated by passing the rods through a hydrogen flame ionization detector (F.I.D.) operated with hydrogen flow-rate of 160 mL/min and air flow-rate of 2 L/min (Sinanoglou and Miniadis-Meimaroglou, 2000). Chromarods were developed with a solvent system consisting of chloroform/methanol/acetic acid/water (50:25:6:2, v/v/v/v) (solvent system A) to a height of 17 cm to detect the polar lipids. The rods were then scanned in an Iatroscan TH-10 Analyser; Mark II (Iatron Laboratories, Inc., Tokyo, Japan) and connected to an integrator.

2.4. High performance thin layer chromatography (HPTLC) of polar lipids and individual phospholipids

HPTLC was carried out on precoated 0.25 mm Silica gel 60-G chromatoplates (10 cm × 10 cm, E. Merck, Darmstadt, Germany). For separation and detection of PL a solvent system consisting of chloroform/methanol/glacial acetic acid/water (50:25:6:2, v/v/v/v, A) was used. Substances were visualised by exposure to iodine vapours, glycolipids, amino-groups, choline and PhL were detected by spraying with: a-naphtholsulfuric acid reagent (Jacin and Mishkin, 1965), ninhydrin reagent (solution 0.2% (w/v) of ninhydrin in ethanol), Dragendorff reagent (Kates, 1986) and phosphomolybdenum blue reagent (Dittmer and Lester, 1964), respectively. PnL were detected after spraying with phosphomolybdenum blue reagent combined with the Stillway and Harmon (1980) heating test.

2.5. Separation of polar lipids by preparative TLC

In order to determine the PhL composition percentage, PL were separated by preparative TLC as follows: the PL extract was applied to the head of a precoated silica gel 60 G chromatoplate (20 cm × 20 cm, 0.5 mm thick, E. Merck, Darmstadt, Germany) along with suitable PhL standards as a narrow band. The chromatogram was developed using solvent system A. Bands were visualised with iodine vapour, scraped off and then extracted from the silica gel using the solvent system of the Bligh–Dyer procedure, i.e. chloroform/methanol/water (2:2:1, v/v/v). After phase separation, chloroform extracts were evaporated and the residual PhL were rechromatographed on HPTLC for confirmation of purity.

2.6. Quantitative analysis of polar lipids and phospholipid components

Total phosphorus and phosphonate phosphorus were determined according to Kapoulas et al. (1984) method, esters

were determined by Snyder and Stephens (1959) method, glyceryl ethers by Hanahan and Watts (1961) method, and plasmalogens by Gottfried and Rapport (1963). MAH was performed with 0.1 mL NaOH (1.2 M) in a methanol solution (50%, v/v) and 45 °C for 20 min (Wells and Dittmer, 1966). Chloroform-soluble products of PhL components were analysed by one-dimensional HPTLC analysis carried out on precoated silica gel 60 G plates (10 cm × 10 cm, 0.25 mm thick, E. Merck, Darmstadt, Germany) using solvent system A. Visualisation of spots was effected by exposure to iodine vapours, by spraying with ninhydrin reagent and with phosphomolybdenum blue reagent (Dittmer and Lester reagent). Water-soluble products of PhL components were analysed by paper chromatographic analysis. The latter was carried out on Whatman paper No. 1 with an ascending (6–7 h) and descending (7–8 h) technique using solvent systems that consisted of phenol solution/water/ethanol/acetic acid (80:20:12:10, v/v/v/v) and phenol solution/ethanol/acetic acid (100:12:10, v/v/v), respectively. Spots were once sprayed with ninhydrin reagent, then with a Hanes–Isherwood reagent for PhL detection (Hanes and Isherwood, 1949) and finally with a Dragendorff reagent.

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

The fatty acids, of the PL that were received after the separation of TL by SPE—as previously described in Section 2.5 as well as those of PhL fractions received after the separation of PL by preparative TLC (Section 2.6) were converted to methyl esters for GC and GC–MS analysis. Fatty acids methyl esters were prepared by the procedure described by Sinanoglou and Miniadis-Meimaroglou (1998).

Quantitative and qualitative analysis were performed on a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector. The following capillary columns were used: column A, capillary SGE BPX-70, Model Number 054606 (70% cyanopropyl liquid phase; 25 m × 0.32 mm i.d. × 0.25 μm film thickness} and column B, capillary HP-23 *cis/trans* FAME Column, Model Number HP 19091 H-133 (30 m × 0.25 mm i.d. × 0.25 μm film thickness}. Helium was used as carrier and make-up gas at a pressure of 15 psi. The split less injector temperatures for column A and B were 220 and 250 °C and the detector temperatures were 275 and 300 °C, respectively. For column A, the temperature was programmed at 100 °C for 0 min, raised from 100 to 200 °C at a rate of 5 °C min^{-1} and held constant at 200 °C for 2 min, then raised from 200 to 230 °C at 10 °C min^{-1} and held constant at 230 °C for 5 min. The duration of the analysis was 30 min while the He flow-rate was 1.0 mL/min. For column B, the temperature was programmed at 100 °C for 0 min, raised from 100 to 200 °C at a rate of 4 °C min^{-1} and held constant at 200 °C for 3 min. The duration of the analysis was 28 min and the He flow-rate was 6.7 mL/min. The make-up gas was atmospheric air at a flow-rate of 400 and 450 mL/min, respectively. At the same time, hydrogen was supplied to the FID at a flow-rate of 40 mL/min.

The qualitative analysis was performed on a HRGC Mega 2 Series 8560 MFC 800 (Fisons Instruments) gas chromatograph equipped with a mass spectrometer VG Trio-2000 Mass Spectrometer (Fisons Instruments) replacing the previously used FID; a fused silica capillary column of high polarity was used (Supelco SP 2340; 60 m × 0.32 mm i.d. × 0.2 μm film thickness; Supelco, Inc., Bellefonte, PA, USA). This polymeric stationary phase was a nonbonded poly-biscyanopropylsiloxane. Hydrogen was used as carrier gas at a pressure of 15 psi. The make-up gas was atmospheric air at a pressure of 15 psi. The temperature of the injector and detector was 350 and 250 °C, respectively.

The temperature was programmed at 150 °C for 15 min, raised from 150 to 170 °C at a rate of 2 °C min^{-1}, held constant at 170 °C for 10 min, then raised from 170 to 215 °C at 4 °C min^{-1} and held constant at 215 °C for 15 min. The duration of the analysis was in total 62 min (Sinanoglou and Miniadis-Meimaroglou, 1998). Electron ionization (E.I.) was produced by accelerating electrons from a hot filament through a potential difference at the standard value of 70 eV. In both GC and GC/MS methods, the fatty acid methyl esters peaks were identified by comparison of their retention times to those of the standard mixtures.

2.8. Schema of the experimental set up (procedure)

The experimental procedure of the isolation and identification of phospholipid molecular species in *P. kerathurus* muscle and cephalothorax described in the present study is depicted in Fig. 1.

2.9. Statistical analysis

All measurements were obtained (at least) in triplicate and values were averaged and reported along with the standard deviation (S.D). 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

3.1. Total and polar lipid content; polar lipid and their fatty acid composition of P. kerathurus muscle and cephalothorax

The TL content of the whole body of *P. kerathurus*, based on the weight determination of TL, represented 1.57 ± 0.05% of the wet tissue while in the muscle and cephalothorax it represented 1.03 ± 0.04 and 2.36 ± 0.07% of the wet tissue, respectively.

The PL proportion in muscle and cephalothorax TL was found to be 77.4 ± 0.2 and 48.3 ± 0.8%, respectively while PhL represented 98.1 ± 0.8 and 94.3 ± 0.5% of PL, respectively.

The results of the quantitative analysis of PhL and glucolipid classes based on the Iatroscan analysis are given in Table 1.

The ester, glyceryl ether and plasmalogen content of muscle and cephalothorax PL, were found to be 1.844 ± 0.010 and 1.922 ± 0.014 (mol/mol PL), 0.156 ± 0.002 and 0.078 ± 0.003 (mol/mol PL), 0.110 ± 0.003 and 0.072 ± 0.05 (mol/mol PL), respectively.

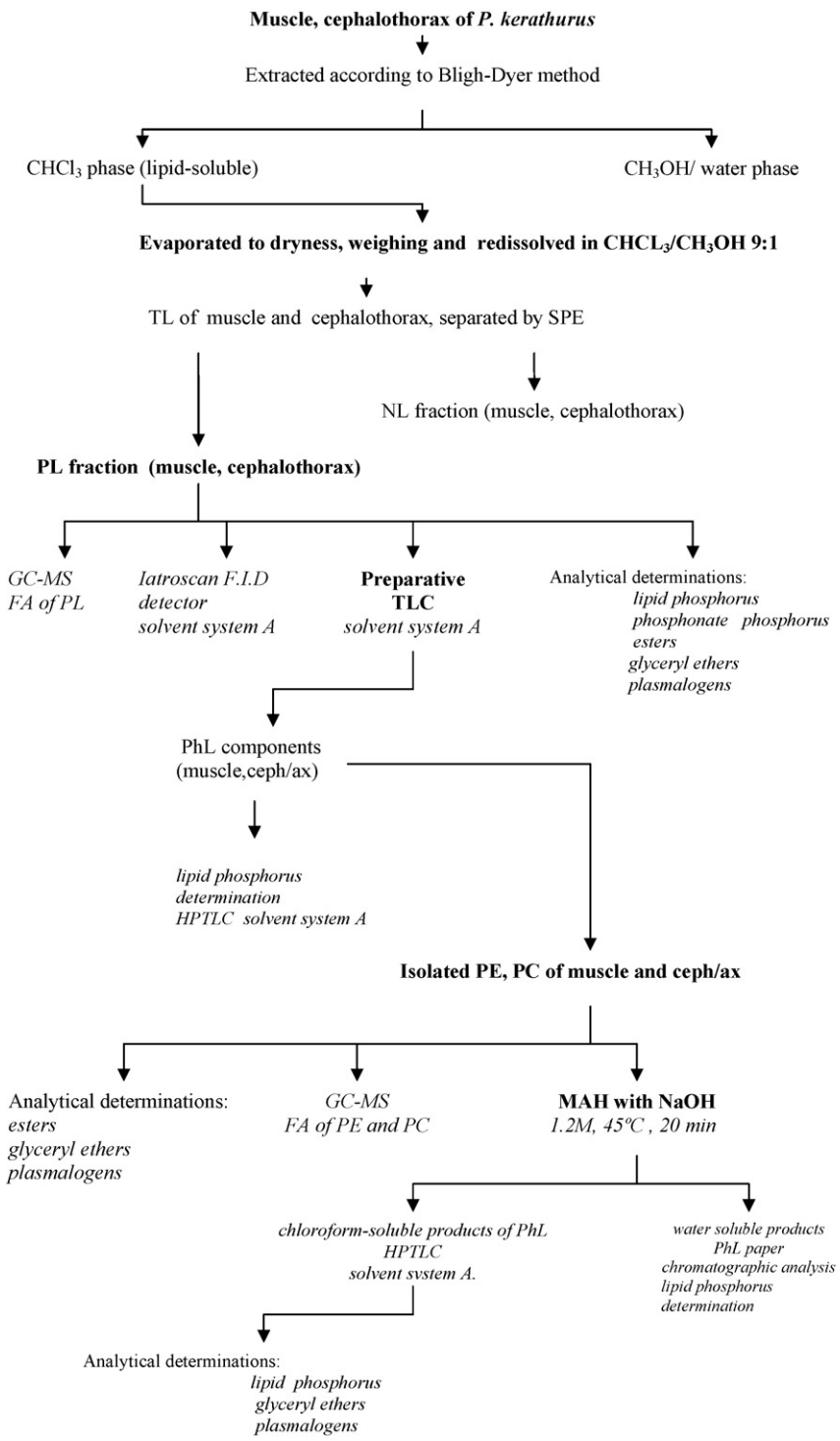

Fig. 1. Schema of the experimental set up (procedure) of the isolation and identification of phospholipid molecular species in α wild marine shrimp *Penaeus kerathurus* muscle and cephalothorax.

Table 1
Separation and composition (% of phospholipids) of muscle and cephalothorax of *P. kerathurus*, phospholipids

Sample	CL	PE	PS + PI	PnL	PC	Shm	LPC
Muscle	4.0 ± 0.2	26.4 ± 0.6	0.9 ± 0.2	4.3 ± 0.4	57.1 ± 0.6	6.3 ± 0.3	1.0 ± 0.3
Cephalothorax	12.3 ± 0.3	24.7 ± 0.2	1.9 ± 0.1	5.2 ± 0.1	47.2 ± 0.4	6.9 ± 0.2	1.8 ± 0.1

FID area percents were corrected to wt% according to (total weight (area lipid/area total lipid) × 100). Data are expressed as wt% of polar lipids and represent means ± standard deviation of four replicate determinations.

Table 2
Fatty acid composition (w/w, %) of polar lipid of the crustacean muscle and cephalothorax

Fatty acids	Muscle	Cephalothorax
C14:0	0.69 ± 0.02	0.68 ± 0.12
C15:0	1.41 ± 0.03	1.35 ± 0.18
C15:1	0.34 ± 0.01	0.36 ± 0.04
C16:0	14.01 ± 0.11	13.73 ± 1.22
C16:1 ω − 7	5.16 ± 0.01	4.43 ± 0.39
Iso-C17:0	0.91 ± 0.02	1.01 ± 0.06
C17:0	3.17 ± 0.04	2.96 ± 0.13
C17:0 cyclo	1.83 ± 0.02	1.85 ± 0.11
2-OH C14:0	0.70 ± 0.02	0.73 ± 0.02
C18:0	8.21 ± 0.17	7.89 ± 0.17
C18:1 ω − 9	10.53 ± 0.15	12.43 ± 0.33
C18:1 ω − 7	3.54 ± 0.02	3.74 ± 0.11
C18:2 ω − 6 *cis*	1.43 ± 0.03	1.17 ± 0.03
C18:2 ω − 6 *trans*	–	0.25 ± 0.10
C18:3 ω − 6	–	0.39 ± 0.05
C19:0 cyclo	–	0.11 ± 0.06
C18:3 ω − 3	0.45 ± 0.02	0.33 ± 0.00
2-OH C16:0	–	0.11 ± 0.15
C20:0	0.53 ± 0.02	0.79 ± 0.02
C20:1 ω − 9	1.01 ± 0.06	0.73 ± 0.01
C20:2 ω − 6	1.28 ± 0.01	1.43 ± 0.02
C20:3 ω − 6	–	0.15 ± 0.21
C20:4 ω − 6	10.78 ± 0.06	12.46 ± 0.30
C22:1 ω − 9	0.35 ± 0.00	0.10 ± 0.14
C20:5 ω − 3	15.09 ± 0.03	14.54 ± 0.26
C22:5 ω − 3	1.40 ± 0.08	0.44 ± 0.03
C24:0	2.34 ± 0.28	2.48 ± 0.22
C22:6 ω − 3	14.84 ± 0.34	13.37 ± 1.55

FID area percents were corrected to wt% according to (total weight (area lipid/area total lipid) × 100). Data are expressed as wt% of total fatty acids and represent means ± standard deviation of three replicate determinations.

Subsequently, the above results revealed that 84.4 ± 0.9% and 92.2 ± 0.6% of muscle and cephalothorax PL were glyceryl-ester analogues. The glyceryl-ether analogues represented the remaining 15.6 ± 0.2 and 7.8 ± 0.1% of total PL, 70.4 ± 0.8 and 92.2 ± 0.9% of which were vinyl-ether analogues, respectively.

The fatty acid profiles of PL of both tissues of *P. kerathurus* are shown in Table 2.

3.2. Fractionation of muscle and cephalothorax polar lipids by preparative TLC and determination of individual phospholipids and their bound fatty acid composition

The PhL of muscle and cephalothorax were separately fractionated by preparative TLC into seven PhL bands (CL, PE, PS + PI, PnL, PC, Shm and LPC). The lipid phosphorus determination in each one of the extracted lipid bands confirmed the proportion of individual PhL as shown in Table 1.

The data on fatty acid composition of the PE and PC of both tissues are shown in Table 3.

The proportion of the Σsaturated, Σmonounsaturated, Σpolyunsaturated fatty acids as well as the ω − 3/ω − 6 and EPA/DHA ratio of PL, PE and PC of *P. kerathurus* muscle and cephalothorax, are given in Table 4.

The isolated PE and PC of both tissues were further subjected to MAH.

Analysis (lipid phosphorus, esters, glycerylethers and plasmalogens) of muscle and cephalothorax PE and PC fractions before and after MAH, revealed that 85.6 and 90.7% of PE (muscle and cephalothorax, respectively) corresponded to the structure of 1,2 diacyl-glyceroethanolamine while the remaining percentage corresponded to the structure of 1-*O*-alkyl-2-acyl-glyceroethanolamine or 1-*O*-(1-alkenyl)-2-acyl-glyceroethanolamine, whereas 86.9 and 89.1% of PC (muscle and cephalothorax, respectively) corresponded to the structure of 1,2-diacyl-glycerocholine while the remaining percentage corresponded to the structure of 1-*O*-alkyl-2-acyl-glycerocholine or 1-*O*-(1-alkenyl)-2-acyl-glycerocholine.

4. Discussion

4.1. Lipid content

The present data based on the weight determination of TL of *P. kerathurus* whole body (1.57 ± 0.05% of the wet tissue) are comparable to those reported by Donnelly et al. (1993) for *Funchalia villosa* (1.5%, from Eastern Gulf of Mexico) and Krzynowek and Murphy (1987) for *Pandalous montagui* (1.64% males and 1.86% females from the estuary of River Crouch Essex, U.K.) and *P. aztecus* (1.32%, from Louisiana).

Bragagnolo and Rodriguez-Amaya1 (2001) obtained lower content (0.8–1.1 g/100 g of the wet tissue) in the whole body of various species of shrimp from five different countries.

Higher TL content was reported by Read and Caulton (1980) for *Penaeus indicus* (2%) from India, as well as by Clarke and Wickins (1980) for cultured *P. merguiensis* 1.99% (from Conwy, U.K.).

A significant difference was also seen in the TL content of *Penaeus japonicus* Bate (males: 2.80% and females: 2.75% of the wet tissue from the Sea of Japan) by Guary et al. (1974) and in the cultured prawn *Makrobrachium rosenbergii* (3.18% of the wet tissue) by Krzynowek and Murphy (1987).

The TL content of *P. kerathurus* muscle (1.03 ± 0.04% of the wet tissue) was comparable to the data reported by a number of other authors: Guary et al. (1974), Krzynowek and Murphy (1987), O'Leary and Matthews (1990), Bragagnolo and Rodriguez-Amaya1 (2001) and Striket et al. (2007).

The previous data on *P. kerathurus* muscle TL were lower to those reported by Krzynowek and Murphy (1987) (for TL content of the wet muscle tissue of Prawn banana 1.3%, Prawn King 1.5% from Sydney market and Royal red 1.5% also from Sydney market) and higher to the ones reported by Heu et al. (2003) concerning the TL content of northern pink shrimp (*Pandalus borealis*) and spotted shrimp (*Trachypena curvirostris*) 0.4 and 0.3% of the wet muscle tissue, respectively.

Furthermore, Karakoltsidis et al. (1995) mentioned that the TL of the edible part of the Aegean Sea prawn *Aristeus antennatus* ranged from 0.2 to 2.0% of the wet tissue in different seasons.

The results of *P. kerathurus* cephalothorax TL (2.36 ± 0.07% of the wet tissue) were slightly higher than those reported for the cephalothorax of wild prawn, *Penaeus monodon* Fabricius in which the average lipid content was 1.75% of the wet tissue, whereas in the farmed *P. monodon* Fabricius the cephalothorax

Table 3
Fatty acid composition (w/w, %) of *P. kerathurus* muscle and cephalothorax PE and PC as determined by GC/MS analysis

Fatty acids	Muscle PE	Muscle PC	Cephalothorax PE	Cephalothorax PC
C14:0	–	1.73 ± 0.07	0.38 ± 0.03	1.52 ± 0.05
C15:0	–	3.21 ± 0.12	–	2.82 ± 0.09
C15:1	–	0.68 ± 0.02	–	0.62 ± 0.03
C16:0	21.30 ± 0.51	27.37 ± 1.95	14.03 ± 0.60	25.10 ± 0.48
C16:1 ω − 7	4.63 ± 0.18	10.08 ± 0.76	3.64 ± 0.13	8.36 ± 0.44
Iso-C17:0	1.85 ± 0.16	1.54 ± 0.02	1.17 ± 0.06	1.52 ± 0.05
C17:0	5.19 ± 0.43	4.37 ± 0.12	4.70 ± 0.29	4.24 ± 0.06
Cyclo-C17:0	2.16 ± 0.10	3.00 ± 0.15	2.30 ± 0.12	2.88 ± 0.17
2-OH C14:0	1.59 ± 0.16	0.93 ± 0.03	1.22 ± 0.04	1.03 ± 0.09
C18:0	13.64 ± 2.91	8.93 ± 0.74	10.86 ± 0.65	8.59 ± 0.02
C18:1 ω − 9	11.39 ± 2.57	18.58 ± 2.25	14.65 ± 0.79	18.56 ± 0.23
C18:1 ω − 7	4.87 ± 0.17	5.53 ± 2.78	3.61 ± 0.18	5.69 ± 0.46
C18:2 ω − 6	–	–	1.82 ± 0.01	1.43 ± 0.07
C18:3 ω − 6	–	–	–	0.19 ± 0.07
C18:3 ω − 3	–	0.13 ± 0.33	1.27 ± 0.04	0.28 ± 0.12
C20:0	–	0.67 ± 0.04	1.02 ± 0.06	0.73 ± 0.01
C20:1 ω − 9	–	0.84 ± 0.17	1.02 ± 0.05	1.00 ± 0.01
C20:2 ω − 6	3.86 ± 1.33	0.76 ± 0.01	5.56 ± 0.20	0.78 ± 0.02
C20:3 ω − 6	–	–	–	1.25 ± 0.07
C20:4 ω − 6	8.69 ± 1.29	3.25 ± 0.15	11.28 ± 0.75	3.82 ± 0.09
C22:1 ω − 9	–	0.16 ± 0.41	–	0.54 ± 0.05
C20:5 ω − 3	10.90 ± 0.08	3.82 ± 0.37	12.39 ± 0.57	4.19 ± 0.08
C22:5 ω − 3	–	0.83 ± 0.57	1.64 ± 0.08	2.51 ± 0.15
C24:0	–	0.22 ± 0.57	1.38 ± 0.08	0.61 ± 0.02
C22:6 ω − 3	9.91 ± 0.81	2.30 ± 0.37	4.52 ± 0.05	1.19 ± 0.04

FID area percents were corrected to wt% according to (total weight (area lipid/area total lipid) × 100). Data are expressed as wt% of total fatty acids and represent means ± standard deviation of three replicate determinations.

TL ranged from 0.8 to 3.2% of the wet tissue (O'Leary and Matthews, 1990).

It should be noted that data concerning the crustacean cephalothorax TL are quite limited, especially those of the hepatopancreas, which were for *P. japonicus* Bate, 10.5% of the wet tissue (Guary et al., 1974).

The TL content of the whole body, muscles and cephalothorax of *P. kerathurus* was similar to the one of *Palinurus vulgaris* (spiny lobster, from Argolicos Bay), which was found 1.6, 1.0 and 2.4% of the wet tissue respectively, as reported by the same research group (Garofalaki et al., 2006).

4.2. Polar- and phospholipid content

The PhL percentage of TL in *P. kerathurus* muscle and cephalothorax vary greatly (75.9 ± 0.8 and 45.5 ± 0.8% of TL, respectively). These results are comparable to the ones reported for the muscle and head PhL percentage of wild *P. monodon* Fabricius (average 70.54 ± 5.89 and 56.70 ± 6.51% of TL, respectively) and different from the farmed *P. monodon* Fabricius (average 57.00 ± 10.18 and 39.23 ± 5.86% of TL, respectively) (O'Leary and Matthews, 1990).

The previous data on *P. kerathurus* muscle and cephalothorax PhL are different to the ones found in *P. japonicus* muscle and cephalothorax (from Gulf of Cadiz, south-west Spain): 68.4 ± 3.86 and 35.5 ± 1.93% of TL, respectively (Muriana et al., 1993) as well as in *P. japonicus* Bate muscle and hepatopancreas PhL were 22.1 and 7.7% of TL, respectively (Guary et al., 1974).

Similar high PhL percentage (72–74% dry weight) was also found in *P. monodon* (black tiger shrimp) meat and *Penaeus vannamei* (white shrimp) meat, quoted by Striket et al. (2007).

Table 4
Statistical analysis of total fatty acids (w/w, %)[a,b] of *P. kerathurus* muscle and cephalothorax PhL, PE and PC

Fatty acids	PL muscle	PL cep/rax	PE muscle	PE cep/rax	PC muscle	PC cep/rax
Σ−0	33.80 ± 0.10 A	33.67 ± 0.11 A	45.74 ± 1.24 B	38.10 ± 1.07 C	51.99 ± 0.66 D	49.24 ± 0.70 E
Σω − 1	20.93 ± 0.13 A	21.80 ± 0.17 A	20.89 ± 0.49 A	24.49 ± 0.38 B	35.71 ± 0.57 C	34.91 ± 0.45 C
Σω − *n*	45.27 ± 0.16 A	44.53 ± 0.21 B	33.37 ± 0.22 C	37.42 ± 0.25 D	12.15 ± 0.36 E	15.85 ± 0.32 F
Σω − 3	30.38 ± 0.14 A	28.68 ± 0.16 B	20.81 ± 0.25 C	19.82 ± 0.14 D	7.08 ± 0.35 E	8.17 ± 0.12 F
Σω − 6	12.21 ± 0.08 A	15.85 ± 0.11 B	12.55 ± 0.30 A	18.66 ± 0.65 C	4.01 ± 0.08 D	7.47 ± 0.12 E
ω − 3/ω − 6	2.49 ± 0.005 A	1.81 ± 0.003 B	1.66 ± 0.02 C	1.06 ± 0.03 D	1.76 ± 0.05 B	1.09 ± 0.002 D
EPA/DHA	1.02 ± 0.03 A	1.09 ± 0.02 B	1.10 ± 0.005 B	2.74 ± 0.01 C	1.66 ± 0.004 D	3.52 ± 0.002 E

[a] FID area percents were corrected to wt% according to (total weight (area lipid/area total lipid) × 100). Data are expressed as wt% of total fatty acids and represent means ± standard deviation of three replicate determinations.

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

A lower PhL proportion in TL of the muscle tissue of four marine and one brackishwater species of Indian prawns was determined by Gopakumar and Nair (1975). Conclusively, the studied *P. kerathurus* muscle appeared to contain mostly PL, which seems to be reach in PhL, whereas the PL percentage in *P. kerathurus* cephalothorax was lower; the relative amounts of PhL, however, in different reported species, considerably varied depending on their sex and environmental parameters.

The PL of *P. kerathurus* muscle and cephalothorax (77.4 ± 0.2 and $48.3 \pm 0.8\%$ of TL, which mainly consisted of PhL as mentioned above) contained a high ratio of esters (1.844 ± 0.010 and 1.922 ± 0.014 mol/mol PL, respectively), which can only lead to the conclusion that PhL molecules are mainly glyceryl-ester analogues.

Since the glyceryl-ether and vinyl-ether proportions were found quite lower, the glyceryl-ether and vinyl-ether analogues of PhL represented a lower proportion.

4.3. Fatty acids

The fatty acids profile of *P. kerathurus* muscle and cephalothorax PL did not differ significantly. C16:0, C18:0, C16:1 $\omega-7$, C18:1 $\omega-9$, C20:4 $\omega-6$, C20:5 $\omega-3$ and C22:6 $\omega-3$ were the main fatty acids (Table 2). The mean fatty acids chain of muscle and cephalothorax *P. kerathurus* PL, was longer than 18 carbon atoms length and their content decreased according to the following order PUFA > SFA > MUFA.

The content of SFA and MUFA in *P. kerathurus* muscle and cephalothorax showed no significant differences ($P > 0.05$), while PUFA, $\omega-3$ and $\omega-6$ fatty acids in muscle and cephalothorax were significantly different. The contents of PUFA, the essential fatty acids C20:5 $\omega-3$ and C22:6 $\omega-3$ as well as the ratio $\omega-3/\omega-6$ were significantly ($P < 0.05$) higher in *P. kerathurus* muscle than cephalothorax (Table 4).

The percentage of unsaturation of the total PL fatty acids in both tissues of *P. kerathurus* fluctuated when observed for ΣPUFA of cold waters shrimp *Chorismus antarcticus* (Clarke, 1977) and for a series of warm water Indian prawns (Krzynowek and Murphy, 1987).

According to Bottino et al. (1980), cold water marine animals contained higher levels of monoenoic and polyenoic fatty acids and lower levels of SFA, since the total monoenoic and polyenoic fatty acids increased during the cold months and decreased during the warm season. On the other hand, the opposite behavior was exhibited by the total SFA.

Among published data, our results resemble those of O'Leary and Matthews (1990), in wild *P. monodon* Fabricius (muscle and head PhL), although farmed *P. monodon* Fabricius (muscle and head PhL) had a lower level of 16:1 (1.79 and 2.3%) and 20:4 $\omega-6$ (4.9 and 5.4%) and a higher level of 18:2 $\omega-6$ (9.8 and 9.6%). On the other hand, Gopakumar and Nair (1975) showed higher levels of EPA than DHA in various shrimp species PhL (*M. dobsoni*, *P. indicus*, *P. stylifera* and *M. monoceros*), and lower levels of EPA than DHA in muscle PhL of *M. affinis* as well as O'Leary and Matthews (1990) in muscle PhL of wild *P. monodon* Fabricius and Striket et al. (2007) in TL of black tiger shrimp (*P. monodon*) and white shrimp (*P. vannamei*) meat. O'Leary and Matthews (1990) also found a similar percentage of EPA and DHA in wild *P. monodon* Fabricius head PhL whereas a higher percentage of EPA than DHA was found in farmed shrimp *P. monodon* Fabricius (muscle and head tissue PhL).

The high percentage of polyenic acids and in particular that of EPA and DHA is characteristic of aquatic decapoda Crustacean. An increase in the level of $\omega-3$ fatty acids in these organisms was also effective in reducing the level of saturated fatty acids in the tissues, making them healthier for human consumption.

Phleger et al. (2002) reported that the EPA/DHA ratio might reflect a different dietary input since the ratio of EPA/DHA was lower in the total fatty acids of krill *E. frigida* – carnivorous – (0.7) and *E. tricantha* – omnivorous – (0.9) than in *E. superba* (>1.0), which is herbivorous in earlier life stages and omnivorous as an adult.

Furthermore, fatty acids 17:0-cyclo and 2OH-14 were found in small quantities in the muscle PhL (1.83 ± 0.02 and $0.70 \pm 0.02\%$, respectively) and cephalothorax PhL (1.85 ± 0.11 and $0.73 \pm 0.02\%$, respectively). These compounds probably derive from the nutrition.

2-OH C14:0 and cyclo-17:0 fatty acids were also identified in *P. vulgaris* (Garofalaki et al., 2006) muscle and cephalothorax though in low percentages. Furthermore, Dembitsky et al. (1994a,b,c) reported the presence of 17:0-cyclo in amphipod crustaceans of the Baical Lake.

4.4. Individual phospholipids

PE and PC, which are the major PhL components of *P. kerathurus* muscle and cephalothorax, have their percentages displayed in Table 1. The results were in agreement with Gopakumar and Nair (1975) who found that PE ranged from 23.1 to 30.8% and PC ranged from 44.5 to 52.5% in the muscle PhL. of five different Indian prawns (*Metapenaeus dobsoni*, *Metapenaeus affinis*, *P. indicus*, *Parapenaeus stylifera* (marine species) and *Metapeneus monoceros* (brackishwater species)). Currently O'Leary and Matthews (1990) reported that PE and PC were also the major PhL of wild *P. monodon* Fabricius muscle (26.43 and 44.84% of PhL, respectively) and head (27.05 and 52.0% of PhL, respectively), as well as in muscle and head of farmed *P. monodon* Fabricius ((29.56 and 47.71%) and (28.42 and 52.57%) of PhL, respectively).

$14.4 \pm 0.5\%$ of PE molecular species in muscle and $9.3 \pm 0.3\%$ in cephalothorax as well as, $13.1 \pm 0.5\%$ of PC molecular species in muscle and $10.9 \pm 0.3\%$ in cephalothorax of *P. kerathurus* were identified as glyceryl-ether analogues and vinyl-ether analogues.

The above levels obtained were lower than those reported by the same research group whereas in *P. vulgaris* muscle 25.0% of PE molecular species and 28.0% of PC molecular species were identified as glyceryl-ether analogues and vinyl-ether analogues (Garofalaki et al., 2006).

From all the above results in the PE and PC fractions, the relation to the percentage of alkali-stable products after MAH of intact percentage of glycerylether found in PE and PC of both tissues, could suggest the presence of vinyl-ether analogues

(plasmalogens) of PE in a proportion of 7.4 ± 0.4% PE (muscle) and 3.3 ± 0.3% PE (cephalothorax), as well as PC in a proportion of 6.2 ± 0.3% PC (muscle) and 2.6 ± 0.2% PC (cephalothorax), which agree with the results from the plasmalogen determination of total PhL.

The fatty acid distribution in the PC and PE of the muscle and cephalothorax lipids showed great differences, the latter containing significantly ($P < 0.05$) more PUFA (Table 4). The muscle and cephalothorax PC contained markedly more C16:0, C16:1 ω − 7 and C18:1 ω − 9 than the corresponding PE, while the PE contained markedly more C20:4 ω − 6, C20:5 ω − 3 and C22:6 ω − 3. Furthermore, cyclo-17:0 and 2-OH-14:0 fatty acids were determined in both tissues PE and PC.

In both muscle and cephalothorax, ω − 3 and ω − 6 fatty acids were found in significantly ($P < 0.05$) higher proportion in PE than in PC. The content of total saturated fatty acids were significantly ($P < 0.05$) higher in the PC compared to the PE. The ω − 3/ω − 6 ratio in muscle PL, PE and PC fractions were significantly ($P < 0.05$) higher than in cephalothorax, the opposite was observed with EPA/DHA ratios.

In this case there is a mutual increase both in the ratio of ω − 3/ω − 6 PUFA and of ω − 3 PUFA, which are beneficial for human health (Dyerberg, 1986; Celik et al., 2004).

Higher ratios of EPA/DHA were presented in PC of both tissues of *P. kerathurus* compared to PE while in *P. vulgaris* (spiny lobster, from Argolicos Bay), as reported by the same research group (Garofalaki et al., 2006), higher ratios of EPA/DHA were presented in PE compared to PC.

Furthermore, PS, PI, CL, Shm, LPC and PnL, were found in smaller amounts in both tissues PhL (Table 1).

PS + PI was found in muscle and cephalothorax PhL of *P. kerathurus* (Table 1) in a significantly lower percentage, compared to the one found in wild *P. monodon* Fabricius muscle and head PhL ((9.16 ± 0.73 + 3.9 ± 0.71%) and (7.87 ± 0.56 + 2.88 ± 0.47%), respectively), in farmed *P. monodon* Fabricius muscle and head PhL ((8.23 ± 1.74 + 3.0 ± 0.81%) and (8.16 ± 1.53 + 3.34 ± 0.53%), respectively) (O'Leary and Matthews, 1990) as well as in five different Indian prawns muscle PhL (9.5–12.0%) (Gopakumar and Nair, 1975).

Shm was found in *P. kerathurus* PhL (Table 1) in lower percentages than the one found in wild and farmed *P. monodon* Fabricius muscle (15.0 ± 0.5 and 9.03 ± 1.16%) and head PhL (11.39 ± 2.82 and 7.36 ± 2.56%), respectively (O'Leary and Matthews, 1990), whereas a higher percentage was found in five Indian prawns muscle PhL (1.9–7.4% of PhL) (Gopakumar and Nair, 1975).

LPC was found in muscle and cephalothorax PhL of *P. kerathurus* (Table 1) in quite similar values, compared to the one found in wild *P. monodon* Fabricius muscle and head PhL (0.9–3.0 and 1.5–2.8%, respectively) and farmed muscle and head PhL (0–1.3 and 0.6–1.3%, respectively) (O'Leary and Matthews, 1990), whereas the percentage in five Indian prawns muscle PhL ranged from 0.3 to 5.0% (Gopakumar and Nair, 1975).

The muscle tissue PhL of *P. kerathurus* (Table 1) was found to contain a higher percentage of CL than the one in muscle tissue PhL of five different Indian prawns (ranged 1.5–6.0% of PhL) (Gopakumar and Nair, 1975).

4.5. Conclusion

This article describes the separation of intact PhL classes focusing on their constituent fatty acids, as well as all PE and PC glyceryl-ester, glyceryl-ether and vinyl-ether analogues usually found in invertebrate tissues. PC and PE, the major PhL components, contained a high ratio of esters, meaning that PhL molecules mainly consisted of glyceryl-ester analogues. In addition, *P. kerathurus* muscle and cephalothorax were very rich in PhL and PUFA. ω − 3 and ω − 6 fatty acids were found in both muscle and cephalothorax in significantly ($P < 0.05$) higher proportion in PE than in PC. These results may be helpful to evaluate the nutritional significance of the studied shrimp and indicate that the lipid derived from the shrimp may be a useful food source for maintaining human health.

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