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Plasmalogens, fatty acids and alkyl glyceryl ethers of marine and freshwater clams and mussels

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ABSTRACT

Shelled molluscs constitute an excellent source of protein, sugars and lipids, and the demand for various mollusks species is increasing. We analysed lipid composition of different bivalves, quite important in the diet of East Mediterranean inhabitants. Plasmamlogens, glyceryl ethers, and diacyl phospholipid forms as well as their fatty aldehydes, fatty alcohols, and fatty acid derivatives were examined. PE of clams and mussels, containing aldehydes C16 (variations from 4% to 31%), C18 (29–46%), C9–18:1(6–32%), C11–20:1 (3–19%), and several minor aldehydes, were detected. The major saturated 1-O-alkyl glycerol ethers C16 and C18. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in both PE and PS plasmalogens were dominated acids. The sum of these acids in PE varied from 33% to 43%, and in PS, from 45% to 66%. EPA levels in PE (30–37%) and PS (39–57%) of marine species were higher than those in freshwater species (PE, 13–16%; PS, 23–29%), and levels of DHA were higher in freshwater than in marine mollusks. A series of saturated fatty aldehydes C12–C24, with major C18:0 in all studied species (over 40%) and C16:0 (10–25%), as well as of unsaturated C16:1 (1–7%) and 18:1 (18–36%) species were isolated from neutral plasmalogens. Predominant fatty acids in neutral plasmalogens were found to be 16:0 (12–17%), 20:5*n*−3 (9–27%), and 22:6*n*−6 (9–18%). Distribution of plasmalogens, alkyl glyceryl ethers, and their fatty aldehydes and fatty alcohols in mollusks and other invertebrates is discussed.

1. Introduction

Marine and freshwater two-part shell mollusks, in which both valves are typically symmetrical along the hinge line, belong to the class Bivalves (they are also under names Pelecypoda, and/or Lamellibranchia). The words "clams, mussels, scallops and oysters" have no real taxonomic significance in biology, but in cookery these words have absolutely definite significance, and are associated with savoury food (Fernandez, Garcia, Asensio, Rodriguez, & Lobo, 2001). Edible bivalves, clams and mussels are mainly marine species with a few well-known freshwater representatives. They are widely used as nutriment around the world (Ackman, 2000), and they are present at food market for about 100 years.

It is well-known that smell and savour of food results from aldehydes, which are liberated from plasmalogens (both neutral and polar lipids) and/or free ones (usually rare) under cooking conditions of any animals and/or invertebrates (Le Cloirec, 2006). Plasmalogens of marine Bivalvia species have been well studied (Berdyshev, 1989; Dembitsky, 1979; Dembitsky & Vaskovsky, 1976; Kraffe, Soudant, & Marty, 2004, 2006), and a few papers also investigated plasmalogens isolated from freshwater (Dembitsky, Kashin, & Stefanov, 1992; Dembitsky, Rezanka, & Kashin, 1993a), as well as from brackish (Dembitsky, Rezanka, & Kashin, 1993b) Bivalvia.

Plasmalogen lipids are particular phospholipids characterised by the presence of vinyl ether bond at the C1 position of glycerol skeleton. Serving as structural component of mammalian and invertebrate cell membrane, plasmalogen is widely distributed in excitable tissues, like heart and brain. Plasmalogens mediate dynamics of cell membrane, they provide storage of polyunsaturated fatty acids and can contribute to endogenous antioxidant activity (Brosche, Brueckmann, Haase, Sieber, & Bertsch, 2007). Plasmalogen phospholipids are also suggested to be involved in signal transduction (Latorre, Collado, Fernandez, Aragones, & Catalan, 2003).

Taking into account the growing interest for food plasmalogens and an insufficient data concerning Bivalvia lipids, fatty aldehydes, and fatty acids, we characterised the plasmalogenic phospholipids, and other lipid profiles in edible Bivalvia species from the Mediterranean Sea, Red Sea, and Sea of Galilee.

The benefits of including omega-3 fatty acids in the diets of humans are well documented. Fatty acids play a major role in the functioning of the immune system and the maintenance of

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all hormonal systems of the organism. Marine and freshwater clams and mussels are an excellent source of both docosahexaenoic, and eicosapentaenoic acids. Bivalves are one of the most popular treats in East as well as in West Mediterranean couisine.

2. Materials and methods

2.1. Clams and mussels samples (class Bivalvia)

Eight clams and mussels were sampled. Marine species were collected from the Mediterranean Sea (Haifa bay, September 2006): *Donax trunculus* (*Linnaeus,* order *Veneroida,* family *Donacidae), Mactra corallina* (Linnaeus, order *Veneroida,* family *Apoidea*), and *Mytilus galloprovincialis* (Lamarck = Mytilus edulis, Mytilus trossulus, family *Mytilidae* Rafinesque), from the Red Sea (Gulf of Aqaba, October 2005): *Callista florida* (Lamarck, order *Veneroida,* family *Veneridae* Rafinesque), and *Pteria aegyptia* (order *Pterioida*, family *Pteriidae*) Freshwater species were collected from the Sea of Galilee at July–August 2004: *Corbicula fluminalis* (order *Veneroida*, family Corbiculidae), *Potomida littoralis semirugatus* (Lamarck, order *Unionoida*, family *Unionidae* and *Unio terminalis* (Lea, order *Unionoida*, family Unionidae).

2.2. Extraction and separation of lipids

Lipids were extracted according to the modified method of Bligh and Dyer (Dembitsky, Gorina, Fedorova, & Solovieva, 1989) using chloroform and methanol as solvents. Total lipids (TLs) were separated into neutral and polar lipid fractions using column chromatography on silica gel G, and chloroform and methanol as eluents. Neutral lipids (NLs) and phospholipids (PLs) were detected by thin layer chromatography, as described previously (Dembitsky et al., 1993a, 1993b). TLs in chloroform were separated into NLs and PLs by passing through a glass column (20 mm diameter × 30 cm) packed with slurry of activated silicic acid (70–230 mesh, Merck, Darmstadt, Germany) in chloroform (1:5, v/v). The eluting solvents for NLs, glycolipids (GLs) and PLs were chloroform, acetone and methanol, respectively. The solvents were evaporated by using a rotary evaporator, and the percentage of each fraction was determined gravimetrically. Residue was dissolved in chloroform and then stored at −20 °C as lipid classes. The fatty acid profile of two major individual classes was estimated by gas chromatography mass spectrometry (GC–MS). Polar lipids were separated into PC, PE, PS, PI, ceramide 2-aminoethylphosphonate (CAEP), and lyso-PC fractions using two dimensional (2D-TLC) on glass plates. Silica gel G plates (20 × 20 cm, Macherey-Nagel GmbH & Co., Düren, Germany) were prewashed in the solvent system, containing isooctane/isopropyl alcohol/acetic acid (95:5:1, v/v/v), air dried for 0.5 h and activated by heating for 1 h at 120 °C under reduced pressure (15 mm Hg). Before development, the plates were dried using a hand dryer on a cool setting for 5 min. The chromatography chamber was saturated with vapour from the solvent system for 30 min before the development of plates. The plates were allowed to develop until the solvent front was about 2 cm from the top, they were then removed and air-dried for half an hour and were visualised in iodine vapor. The solvent systems for 2D TLC were chloroform–methanol–25% aqueous ammonium (65:35:5, v/v/v, (first dimension), and chloroform–methanol–acetic acid–water (35:15:4:2, v/v/v/v, second dimension) (Dembitsky et al., 1993b).

2.3. Preparation of fatty acid methyl esters (FAMEs)

Neutral and polar lipid samples were transesterified (Dembitsky, Rozentsvet, & Pechenkina, 1990). A 10-mg sample was dissolved in 1 mL diethyl ether and 20 mL methyl acetate. Two hundred and fifty milliliters of 1 M sodium methoxide in methanol was added. The sample was worked up and left for 5 min at room temperature. Saturated oxalic acid solution (35 mL) was added, with brief agitation, to neutralise the solution. The solvent was removed under nitrogen, and an appropriate volume of hexane was added to bring the sample to the concentration required for analysis.

2.4. Preparation of fatty aldehyde dimetylacetales (DMAs)

The PE, PS and PC fractions, containing plasmalogen, alkyl-acyl-, and diacyl-forms were prepared from clams and mussels by preparative thin layer chromatography, as previously described (Dembitsky & Rozentsvet, 1989, 1996; Dembitsky et al., 1993b; Go, Řezanka, Srebnik, & Dembitsky, 2002). Pure saturated C14:0, C16:0, C18:0, C20:0, and unsaturated C9–18:1 and C11–20:1 species were prepared from the purified bisulphite compounds (Kodak Ltd.) as described by Dembitsky (1988) and Dembitsky et al. (1989). Purification was completed by preparative thin-layer chromatography. The 2 N methanolic HCl at 95 °C was used as methylating agent. Dimethylacetals and methyl esters were then separated as follows. The methyl esters were converted to their sodium salts by saponification with 0.5 N NaOH in 90% methanol at 85 °C under reflux for 2 h. Alternatively, samples were saponified overnight at 37 °C under nitrogen in glass-stoppered tubes. The saponification mixture was extracted three times with equal volumes of petroleum ether, and the combined upper phases were washed with the alkaline lower phase (water–ethanol–3 N NaOH, 40:10:1, v/v/v).

2.5. Quantification of plasmalogen, alkyl-acyl- and diacyl-forms of glycerophospholipids

Lipid extract (with a lipid concentration of 10 mg/mL) was spotted on Silica gel G plates (20 × 20 cm, Macherey-Nagel GmbH & Co., Düren, Germany). The plate was developed in the first direction with chloroform–methanol–28% ammonia solution (130:70:10, v/v), and after evaporating the solvents plasmalogens on the plate were hydrolysed with hydrochloric acid–methanol as described previously (Dembitsky, 1988; Dembitsky, Rezanka, & Kashin, 1993c; Dembitsky et al., 1989) was and then in the second direction with chloroform–acetone–methanol–acetic acid–water (100:40:20:20:10, v/v/v/v/v/v). After the distribution of lipids over the plate they were removed and burned on a block at 180 °C after treatment with 10% sulphuric acid in methanol. The amounts of PLs and their plasmalogen forms were determined by the phosphorus absorption method.

2.6. Preparation of isopropylidenes of α-alkyl glyceryl ethers

Individual phospholipid classes (PE, PS, PC) were separately hydrolysed with 1 N HCl/MeOH. Fatty aldehydes and lyso-phospholipids were separated by TLC on Silica Gel G plates (20 × 20 cm, Macherey-Nagel GmbH & Co., Düren, Germany) with hexane-ethyl ether-acetic acid (90:10:1, v/v/v). Lyso-phospholipids (1-alkyl-2-acyl glyceryl ethers of PS, PE and PC) and 1,2-diacyl PS, PE, and PC were saponified by 1 N NaOH/MeOH. Alkyl glyceryl ethers and fatty acids were separated by TLC on Silica Gel G plates (20 × 20 cm) with solvent system: petroleum ether (60/70 °C)-diethyl ether-acetic acid (90:10:1, v/v/v). Alkyl glyceryl ethers from PS, PE and/or PC, and 1-O-hexadecyl glycerol (chimyl alcohol), 1-O-octadecylglycerol (batyl alcohol) and 1-O-octadec-9-enyl glycerol (selachyl alcohol) as standards were converted to their isopropylidenes derivatives. Solutions of alkyl glyceryl ethers (10–20 mg) in dry acetone (2–4 mL) were converted with 95% yield to their isopropylidene derivatives by a rapid, room temperature

Table 1
Lipids and phospholipids of edible clams and mussels.

Lipids	I	II	III	IV	V	VI	VII	VIII
Total lipids, mg/g dry wt	34.8	29.7	33.6	42.4	44.8	49.5	38.7	56.4
Neutral lipids, of TL	50.6	47.2	57.8	44.6	53.2	47.8	51.5	46.8
FFA,% of NL	9.8	ND	6.2	ND	3.7	4.1	6.8	ND
Steroids, both forms, free and esters, % of NL	0.5	12.2	19.7	9.0	21.5	42.5	44.0	47.3
TAG, % of NL	66.1	53.2	61.3	68.9	50.2	41.2	38.8	34.6
Neutral plasmalogens, % of NL	23.6	34.6	12.8	22.1	24.6	12.2	10.4	9.8
Glycolipids, % of TL	15.7	23.9	11.6	14.2	7.3	9.4	6.8	8.3
Phospholipids, % of TL	33.7	28.9	30.6	41.2	39.5	42.8	41.7	34.9
PC, all forms	**32.3**	**38.1**	**40.5**	**39.5**	**38.2**	**56.5**	**58.5**	**52.9**
PCP*	4.9	6.2	3.8	5.7	4.8	2.7	4.2	5.1
PCA**	34.7	38.8	29.7	30.5	27.8	31.2	34.3	28.6
PCD***	60.4	55.0	66.5	63.8	67.4	66.1	61.5	66.3
PE, all forms	**44.3**	**40.5**	**34.4**	**39.3**	**40.8**	**33.4**	**31.9**	**32.2**
PEP*	82.5	76.5	69.7	77.3	80.4	71.4	73.2	78.8
PEA**	6.6	8.8	15.8	11.3	7.8	10.3	9.4	6.9
PED***	10.9	14.7	14.5	11.4	11.8	18.3	17.4	14.3
PS, all forms	**11.7**	**10.4**	**8.3**	**5.9**	**7.2**	**3.1**	**3.6**	**3.5**
PSP*	66.8	55.9	54.4	60.2	57.1	46.6	49.2	45.6
PSA**	19.5	21.4	23.4	18.9	24.6	18.8	17.3	16.4
PSD***	13.7	22.7	22.2	20.9	18.3	34.6	33.5	38
CAEP	4.8	5.9	5.6	6.1	7.1	2.7	3.2	3.9
DPG	5.3	5.1	6.1	4.8	5.4	3.1	2.8	4.4
PI	1.6	ND	3.1	1.1	1.6	1.2	ND	ND
PA	ND	ND	0.8	1.5	ND	ND	ND	2.2
LPC	ND	ND	1.2	1.8	ND	ND	ND	0.9

ND, not detected.
Distribution of 1-O-alk-1′-enyl-2-acyl- (PEP*, PSP*, PCP*, plasmalogen form), 1-O-alkyl-2-acyl-(PEA**, PSA**, PCA**), and 1,2-diacyl-(PED***, PSD***, PCD***) forms in phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine, respectively (percentage of each individual lipid subclass).
Invertebrates were sampled in:
Mediterranean Sea: I. *Donax trunculus*; II. *Mactra corallina*; III. *Mytilus galloprovincialis.*
Red Sea: IV. *Callista florida*; V. *Pteria aegyptia.*
Sea of Galilee: VI. *Corbicula fluminalis*;VII. *Potanida littoralis semirugatus*; VIII. *Unio terminalis.*

acetonation in the presence of 0.01 M $HClO_4$, and to be later used for GC–MS analysis.

2.7. GC–MS analysis

Analysis of FAMEs from clams and mussels was carried out using the Hewlett-Packard 5890 (series II) gas chromatograph (Palo Alto, CA) equipped with a 5971B mass selective detector. FAMEs were analysed by GC–MS using an RTX-l capillary column: length, 60 m; internal diameter, 0.32 mm; and film thickness, 0.25 mm (Restek, Bellefonte, PA). The GC oven program had an initial temperature of 40 °C for 2 min, a 2 °C/min run to 300 °C and a final hold at 300 °C (20 min). The injector temperature was kept at 180 °C (splitless), and the carrier gas (helium) flow rate was 25 cm/s. The MS detector was operated at 194 °C, and the scan range was from 30 to 650 *m/z* at 0.9 scan/set scan rate. The solvent delay was 3 min. Other modification of analysis of methyl esters of fatty acids, DMA of fatty aldehydes, and isopropylidenes of α-glyceryl ethers were described previously (Dembitsky, Shkrob, & Dor, 1999).

3. Results and discussion

The general lipid compositions from the clams and mussels are given in Table 1. Total lipids fluctuated from 30 to 56 mg/g dry wt. Results of analysis by TLC showed that the main lipids in clams and mussels were triacylglycerides (TAG), free fatty acids (FFA), sterols (ST), and phospholipids (PL). The contents of these compounds, relative to neutral lipid, were 35–69% for TAG, 0–10% for FFA, 1–48% for ST, and 10–35% for neutral plasmalogens. Furthermore, we analysed PL using a thin layer chromatography. We found that the phospholipid was mainly composed of PE, PS, PC, DPG, and CAEP. The latter phosponolipid is relatively abundant in some invertebrates, and it has been detected previously in many freshwater and marine mollusks (Dembitsky et al., 1992; Dembitsky et al., 1993a, 1993b). The relative contents of the major PL classes were 32–44% for PE, 32–58% for PC, and 3–12% for PS (Table 1).

Plasmalogens predominate in mollusks, PE, making up 70–82% of total PE, while in PC their contribution is quite low (3–6%). In PS, they vary from 45% to 67% (Table 1). These results confirm our previous findings (Dembitsky, 1979; Dembitsky & Vaskovsky, 1976; Dembitsky et al., 1993a, 1993b) that any of the major phospholides of marine and freshwater mollusks contain plasmologens, and that PE fractions, in some of the mollusks, may be composed predominantly of the plasmalogens. Thus, plasmalogen has been detected in PE, PS, and PC in different mollusks from Japan Sea (Dembitsky, 1979): from edible marine Bivalvia *Mercenaria simpsoni* (plasmolagen contribution to the total PE, PS, and PC being 69%, 48%, and 3%, respectively), as well as from *Modiolus difficilis* (81%, 75%, and 4%), *Peronidia venulosa* (63%, 50%, and 2%), *Patinopecten yessooensis* (67%, 29%, and 5%), *Spisula sachalinensis* (73%, 46%, and 10%), and *Swiftopecten swifti* (74%, 49%, and 10%).

We further analysed fatty aldehydes released from different plasmalogen subclasses (Table 2). In PE of clams and mussels, C16 aldehyde varied from 4% to 31%, for other fatty aldehydes similar variations were found: C18 – 29–46%, C9–18:1 – 6–32%, C11–20:1 – 3–19%; several minor aldehydes also were detected. C18 family of fatty aldehydes also dominated in PS and PC plasmalogen forms. Molecular ions (*m/e*) of the DMA generated from palmitaldehyde were 255 (weak peak) and 75 (strong peak), typical of C16:0 DMA, whereas corresponding values of the DMA generated from stearic aldehyde were 283 and 75, typical of C18:0 DMA. The mass spectrum yielded characteristic ions of $[M-31]^+$ (due to the loss of methoxy group from the parent ion) and *m/z* 75 (due to $[CH(OCH_3)_2]^+$) which usually appears in EI spectra of DMA derivatives. Mass spectra of isolated DMA fatty aldehydes from mollusks were published by Go et al. (2002).

Table 2
Composition of fatty aldehydes released from PE, PS, PC and neutral plasmalogens.

DMA of fatty aldehydes	I[c]	II	III	IV	V	VI	VII	VIII
PE plasmalogen								
10:0, MW = 216				0.6				
12:0, MW = 230		0.6		0.8	0.7			
13:0, MW = 244				0.5	0.5			
14:0, MW = 258	3.2	0.9		1.2	2.1	0.8		0.6
15:0, MW = 272				0.7	0.7			
16:0, MW = 286	22.5	17.6	31.6	8.9	22.1	6.8	4.4	5.9
17:0, MW = 300		0.5		0.8	0.6			
18:0, MW = 314	28.9	34.8	29.5	38.9	24.5	43.7	46.1	42.8
19:0, MW = 328				0.6				
20:0, MW = 342	2.1	0.6		0.9	2.2	1.2		1.6
Unidentified branched saturated	6.8	4.7	5.5	3.5	4.2		8.8	2.5
Saturated	**63.5**	**59.7**	**66.6**	**57.4**	**57.6**	**52.5**	**59.3**	**53.4**
14:1[a], MW = 256	1.1			1.4				
15:1[a], MW = 270				0.8				
7-16:1[b], MW = 284	6.6		2.2	4.8	2.1	3.4		1.9
9-18:1[b], MW = 312	10.2	24.1	17.8	6.3	17.5	22.6	18.4	32.2
11-20:1[b], MW = 340	13.4	12.3	13.2	19.6	19.5	14.8	16.9	3.1
Unidentified unsaturated	5.2	3.9		9.7	3.3	6.7	5.4	9.4
Monoenoic	**36.5**	**40.3**	**33.4**	**42.6**	**42.4**	**47.5**	**40.7**	**46.6**
PS plasmalgen								
16:0	11.2	12.4	7.6	18.9	22.5	28.8	27.3	31.4
br-16:0[a], MW = 300	0.9	1.2	6.4	4.5	2.3		0.9	
18:0	46.1	40.3	52.3	32.1	25.9	30.4	32.2	29.5
br-18:0[a], MW = 328	1.4	2.2	0.8	2.4	1.3			
20:0	2.6	1.1	3.1	4.7	3.7	3.3	2.1	1.3
br-20:0[a], MW = 356	3.3	4.8	0.7	1.9	0.8			
Saturated	**65.5**	**62.0**	**70.9**	**64.5**	**56.5**	**62.5**	**60.4**	**62.2**
16:1	1.4	4.4	1.1	4.8	2.4	6.4	7.7	8.2
18:1	27.9	26.8	12.2	27.0	36.5	26.7	26.7	25.7
20:1	5.2	6.8	15.8	3.7	4.6	4.4	5.2	3.9
Monoenoic	34.5	38.0	29.1	35.5	43.5	37.5	39.6	37.8
PC plasmalogen								
14:0		0.9		1.1				0.7
16:0	24.2	27.1	23.8	33.4	31.9	23.7	22.1	19.9
18:0	50.1	49.8	48.6	41.9	44.8	54.2	57.1	50.6
20:0	0.9	2.1	1.3	2.4	3.6	4.1	1.3	0.8
Saturated	**75.2**	**79.9**	**73.7**	**76.4**	**80.3**	**82.0**	**80.5**	**72.0**
14:1		0.8		2.1				
16:1	3.4	6.6	4.3	5.1	3.8	5.7	6.7	8.8
18:1	16.9	8.3	16.2	12.5	11.6	6.4	10.0	17.1
20:1	4.5	5.2	5.8	3.9	4.3	5.9	2.8	2.1
Monoenoic	**24.8**	**20.1**	**26.3**	**23.6**	**19.7**	**18.0**	**19.5**	**28.0**
Neutral plasmalogens								
12:0		0.9		1.3				
14:0	2.1	1.1	1.8	0.8			1.4	
15:0	0.8	2.1	0.4	0.4	1.1	0.9	0.6	1.6
16:0	11.8	10.2	14.9	15.1	13.5	14.8	21.4	26.4
17:0		0.9	0.7	0.6	1.3			
18:0	48.9	45.7	42.1	50.3	52.1	49.8	45.2	44.4
20:0	3.1	0.8		0.6	1.2	2.2	1.1	2.6
22:0	0.8		0.7		0.5			
24:0		1.1		2.3	3.2			
Saturated	**67.5**	**62.8**	**60.6**	**71.4**	**72.9**	**67.7**	**69.7**	**75.0**
16:1	1.4	2.2	3.4	0.8	0.9	5.8	7.2	6.9
18:1	31.1	35.0	36.0	27.8	26.2	26.5	23.1	18.1
Monoenoic	**32.5**	**37.2**	**39.4**	**28.6**	**27.1**	**32.3**	**30.3**	**25.0**

[a] Position of double bond in monoenoic DMA do not confirmed by GC–MS.
[b] Retention time DMA of natural fatty aldehydes were identical to synthetic analogs.
[c] See Footnote in Table 1.

Neutral plasmalogens (or 1-O-alk-1′-enyl-2,3-diacyl glycerols) contain a vinyl ether linkage, and are analogous to the neutral triacylglycerides. Composition of neutral plasmalogens (fatty aldehydes and fatty acids) is shown in Tables 2 and 4. A series of saturated fatty aldehydes C12–C24, with major C18:0 in all studied species (over 40%), and C16:0 (10–25%), and unsaturated C16:1 (1–7%) and C18:1 (18–36%) ones were isolated from different marine and freshwater Bivalvia species (Table 2). Composition of main fatty acids isolated from *sn*-2,3 positions of the neutral plasmalogens is shown in Table 4. Predominant fatty acids were C16:0 (23–46%), C18:0 (11–24%), C20:5*n*−3 (9–27%), and C22:6*n*−6 (9–18%). Neutral plasmalogens previously isolated from a clam *Chlamys tehuelcha* (San Roman, San Jose Gulf, Patagonia, Argentina) possessed, as major fatty acids, C18:4*n*−3 (10%), C20:5*n*−3 (13%) and C22:6*n*−6 (12%) (Pollero, Re, & Brenner, 1979). Joh and Hata (1979) detected plasmalogens in both neutral and polar lipid fractions in two bivalve mollusks *S. sachalinensis* and *Placopecten magellanicus*, with three major aldehydes released from plasmalogens, octadecanal, eicosamonoenal, and hexadecanal, and minor components C14:0, C14:1, and C15:1. Pollero, Brenner and Gros (1981)

found that plasmalogens in both neutral and phospholipid subclasses in the freshwater mollusk *Diplodom patagonicus* from lakes of the Patagonian Andes mountains (Argentina) were rich in the ω-6 fatty acids, linoleic and arachidonic (app. 25%), and poor in the ω-3 acids, C20:5 and C22:6 acids.

Long-chain fatty aldehydes are rarely found in free forms. They exist mainly as vinyl ethers (known as 1-O-alk-l′-enyls) integrated into *sn*-2,3-diacylglycerols and glycerophospholipids to form plasmalogens. Previous reports on a large number of marine invertebrates, including mollusks (Dembitsky, 1979), revealed that practically all of the spicies studied contain a high percentage of plasmalogen in different phospholipids subclasses. A series of saturated fatty aldehydes C14, C15, C16, C17, C18, and C18:1 were detected in Black Sea mussel *M. galloprovincialis* (Nechev, Stefanov, Nedelcheva, & Popov, 2007). Two marine bivalves, *Megangulus venulosus* and *Megangulus zyonoensis* from Coastal Waters of Hokkaido (Northern Japan), contain saturated C16, C17, C18 (major aldehyde type, 50–56%), C20, and unsaturated C18:1 and C20:1 (second major aldehyde, 25–34%); other fatty acids in the tissues were C16:0 (11.0–15.2%), C18:0 (4.1–8.6%), C20:5*n*−3 (13.3–25.6%) and C22:6*n*−3 (8.1–16.7%).The proportions of C20:5*n*−3 and C22:6*n*−3 were highest in muscle tissue lipids isolated from *M. venulosus* and *M. zyonoensis*: 33.1% and 36.2%, respectively (Kawashima & Ohnishi, 2003). Fatty aldehydes C16:0, C17:0, C18:0 and C20:1 were also isolated from lipid body of the Chilean scallop *Argopecten purpuratus* (Caers et al., 1999).

Distribution of alkyl glyceryl ethers (AGE) in PE, PS and PC is presented in Table 3. The electron impact mass spectrum measured at 70 eV on the mixture of alkyl glyceryl ethers isolated from Bivalvia species showed a useful range of [M+1] peak at *m/z* 317 (0.7–1.0%) and other significant peaks at *m/z* 285 $[M-CH_3O]^+$ (0.6–0.9%), 255 $[M-C_2H_5O_2]^+$ (0.8–1.0%) and 225 $[M-C_3H_7O_3]^+$ (2.8–3.2%) characteristic of chimyl alcohol, as the major component. The mass spectrum also showed additional weak [M+1] ion peaks at *m/z* 331 (0.2–04%) and 345 (0.1–.03%) indicative of the presence of C17 and C18 analogs. While mass spectrometry of the intact alkyl glyceryl, using direct insertion mode, ethers indicated the presence of glycerol ethers with 16:0, 17:0 and 18:0 hydrocarbon chains as major constituents, GC–MS of the isopropylidene derivatives led to identification of glycerol ethers with hydrocarbon chain lengths from C16 to C20. GC–MS of the isopropylidene derivatives did not give molecular ion peaks, but it showed the $[M-15]^+$ ion and the characteristic base peak at *m/z* 101. The major saturated 1-O-alkyl glycerol ethers in decreasing order of abundance were C16 and C18.

Thompson and Lee (1965) proved that tissues of a number of mollusks, such as clam, *Protothaca stamina*, marine snail, *Thais lamellose*, chiton, *Katherina tunicate,* and octopus, *Octopus dofleini* (tentacles) are rich in alkyl glyceryl ether phospholipids (9%, 20%, 25%, and 40%, respectively). Alkyl glyceryl ethers were also identified in total lipid fractions obtained from several Bivalvia species (as% of TL): *Anadara broughtoni* (8.4%), *Arca boucardi* (4%), *Crassostrea gigas* (3.4%), *Crenomytilus grayanus* (3.2%), *M. difficilis* (4.6%), *Glycymeris yessoensis* (2.3%), *Chlamys nipponensis* (3.6%), *S. swifti* (1.4%), *Patinopecten yessoensis* (4.2%), *Callista brevisiphonata* (2.6%), *S. sachalinensis* (4.8%), *Mactra sulctaria* (0.8%), *Mercenaria stimpsoni* (1.5%), *Mya japonica* (4.5%), *P. venulosa* (2.6%), and *Macoma* sp. (9.9%) (Isay, Makarchenko, & Vaskovsky, 1976). They were also found in Japanese prickly scallop *Chamys nipponensis*, with principal alkyl groups 18:0 (38%), 18:1 (21%), 16:0 and 20:0 (both 15%) (Hayashi & Yamada, 1973). 1-O-Hexadecylglycerol (chimyl alcohol), 1-O-heptadecylglycerol and 1-O-octadecyl-glycerol (batyl alcohol) have been identified as major native constituents in elasmobranch fish (sharks, skates and rays) (Le Nechet, Dubois, Gouygou, & Berge, 2007), as well as in human milk, bone marrow, atherosclerotic aorta (Urata & Takaishi, 1996).

Table 3
Composition of alkyl glyceryl ethers released from PE, PS, and PC.

	I[b]	II	III	IV	V	VI	VII	VIII
PE alkyl glycerides								
16:0, MW = 356	21.4	17.8	26.2	14.9	12.6	36.8	38.5	40.1
17:0, MW = 370		2.3		0.9	1.6			
18:0, MW = 384	55.3	54.8	48.9	51.8	60.2	48.9	50.6	52.3
19:0, MW = 398		1.6		1.1	2.4			
20:0, MW = 412		2.3		2.5	2.1	1.4	2.2	
Unidentified branched saturated	1.3	2.6	0.8	5.4	4.8		1.2	
Saturated	**78.0**	**81.4**	**75.9**	**76.6**	**83.7**	**87.1**	**92.5**	**92.4**
16:1[a], MW = 354	7.6	5.7	8.0	6.9	3.1	8.7	5.4	5.8
18:1[a], MW = 382	14.4	12.9	16.1	16.5	13.2	4.2	2.1	1.8
Monoenoic	**22.0**	**18.6**	**24.1**	**23.4**	**16.3**	**12.9**	**7.5**	**7.6**
PS alkyl glycerides								
16:0	2.2	0.9	1.2	3.1	0.7	44.8	46.2	50.1
17:0	1.1			0.9				
18:0	63.1	59.4	60.2	64.3	65.4	53.6	50.9	48.8
19:0	0.6			2.1				
20:0				1.4				
Unidentified branched saturated	8.7	9.1	5.7	11.2	9.7		1.3	
Saturated	**75.7**	**69.4**	**67.1**	**83.0**	**75.8**	**98.4**	**98.4**	**98.9**
16:1[a]	2.2	8.3	9.1	4.4	7.2	0.9	1.1	1.1
18:1[a]	22.1	22.3	23.8	12.6	17.0	0.7	0.5	
Monoenoic	24.3	30.6	32.9	17.0	24.2	1.6	1.6	1.1
PC alkyl glycerides								
16:0	18.9	20.1	11.6	9.4	10.5	52.3	56.7	53.1
18:0	77.8	78.1	85.2	86.2	88.7	47.0	41.7	46.9
Saturated	**96.7**	**98.2**	**96.8**	**95.6**	**99.2**	**99.3**	**98.4**	**100**
16:1[a]						0.7	1.6	
18:1[a]	3.3	1.8	3.2	4.4	0.8			
Monoenoic	**3.3**	**1.8**	**3.2**	**4.4**	**0.8**	**0.7**	**1.6**	

[a] All isomers.
[b] See Footnote in Table 1.

Table 4
Composition of main fatty acids from PE, PS, PC and neutral plasmalogens (% of total FAs, over 1.0%).

PE plasmalogen	I[b]	II	III	IV	V	VI	VII	VIII
14:0		1.1				1.3		
15:0		1.2						
16:0		2.3		1.2	1.6	2.1	3.4	3.9
18:0	1.2	1.4	1.1			1.3	1.1	1.5
20:1*n*−11	2.5	3.1	2.5	2.8	3.1	1.9	4.2	3.4
7,13-22:2NMI	3.6	3.8	4.1	4.7	3.9	1.9	2.3	2.7
7,15-22:2NMI	6.8	7.5	6.3	9.3	8.6	4.2	4.8	5.1
20:4*n*−6	2.1	1.8	2.6	1.5	1.9	3.2	2.8	2.6
20:5*n*−3	30.5	28.7	29.8	31.5	36.7	12.8	13.8	16.1
22:5*n*−3	2.4	1.9	3.5	4.1	2.2	3.1	3.9	2.4
22:6*n*−3	6.8	4.7	4.9	5.1	5.9	23.1	28.3	26.9
Others	44.1	42.5	45.2	39.8	36.1	45.1	35.4	35.4
PS plasmalogen								
16:0	1.5		1.8	1.4	2.1	2.7	2.4	3.1
18:0		3.3	4.1	3.6		1.2	1.8	2.2
18:4*n*−3			2.4	1.3	1.1			
18:5*n*−3		1.4	4.3	1.7	1.2			
20:4*n*−6	2.3	3.1	4.5	1.8	2.2	3.8	4.1	2.5
20:5*n*−3	42.2	44.8	39.2	53.2	56.8	29.1	22.8	26.3
22:6*n*−-3	8.6	5.2	6.9	7.7	9.4	36.2	32.4	38.9
Others	45.4	42.2	36.8	29.3				
PC plasmalogen								
14:0	1.2		2.2			1.3	1.8	
16:0	13.2	14.1	16.9	12.2	12.4	17.4	18.2	19.8
18:0	2.3	2.5	1.7	3.8	3.1	5.9	4.4	6.7
16:1*n*−7		2.3		1.6				
18:1*n*−9	2.2	1.4	2.5	2.1	3.2	3.3	2.8	1.5
18:1*n*−7			1.7	1.6				
20:1*n*−11	1.5				1.3			1.1
20:1*n*−9		1.0		1.2				
20:1*n*−7			1.8		1.4			
18:2*n*−6		3.2	2.6	1.5				
7,13-22:2NMI		1.3	2.1		1.1			
7,15-22:2NMI		1.7		2.0	1.4			
18:3*n*−3	2.1		1.7					
18:4*n*−3		2.4		1.1	3.5		1.2	
20:3*n*−3	1.0				1.3			
20:4*n*−6	3.9	2.6	4.8	6.1	5.2		2.2	1.3
20:5*n*−3	18.3	16.6	15.2	19.9	15.3	8.4	11.9	10.7
22:4*n*−6	1.3		2.3		1.4		1.0	
22:5*n*−3	2.3	1.7	2.2	4.5	1.1	1.3	1.6	2.6
22:6*n*−3	13.4	18.7	16.8	11.2	15.8	24.7	22.1	28.3
Others	37.3	30.5	25.5	31.2	32.5	37.7	32.8	28.0
Neutral plasmalogens[a]								
16:0	24.4	22.9	25.1	23.2	25.2	46.1	37.3	39.9
18:0	12.2	19.8	11.2	12.9	14.6	13.3	19.2	14.0
16:1*n*−7	1.3		2.2	1.7	1.9			
18:1*n*−9		2.1		1.1	3.2			
18:1*n*−7		1.1		1.7				
20:1*n*−11			1.4	1.5	2.1			
18:2*n*−6	2.4	1.8	1.3	2.3	2.4	3.4	4.1	1.9
18:3*n*−3	1.3		1.5		1.8	2.1		
18:4*n*−3		3.3	2.4	3.8	2.6	1.3		
18:5*n*−3			1.1	1.6				
20:4*n*−6	1.2	2.1	1.3		1.4	1.1	1.2	
20:5*n*−3	23.3	22.8	21.2	26.8	27.3	10.5	9.6	11.4
22:5*n*−6		3.1		4.1	4.9			
22:6*n*−3	9.6	11.2	8.7	9.3	12.2	13.1	15.4	17.8
Others	24.3	9.8	22.6	10.0	0.4	9.1	12.0	15.0

[a] From both *sn*-2,3 positions of neutral plasmalogens,% of total FA, over 1%).
[b] See Footnote in Table 1.

Fatty acid composition of mollusks has been extensively studied in many mollusk species, including clams and mussels (Ackman, 2000). We have identified more than 90 fatty acids including saturated, monoenoic, dienoic and polyenoic acids. From 29% to 45% of them were identified as minor compounds and they are not shown in Table 4.

Four non-methylene-interrupted (NMI, 5,11-C20:2, 5,13-C20:2, 7,13-C22:2 and 7,15-C22:2) acids have been identified among dienoic acids in PE plasmalogen form. Two acids, 5,11-C20:2 and 5,13-C20:2 have been found to contain not more than 1% of such acids, and two other 7,13-C22:2 and 7,15-C22:2 varied from 2% to 9% (Table 4). These NMI fatty acids have been also identified in PE isolated from the whole body of *C. gigas*, *M. edulis*, and *Ruditapes philippinarum* (Kraffe et al., 2004), in mussel *M. galloprovincialis* from Spain (Freites, Fernandez-Reiriz, & Labarta, 2002), in two New Zealand green lipped mussel (*Perna canaliculus*) and in the Tasmanian blue mussel (*M. edulis*) (Murphy, Mooney, Mann, Nichols, & Sinclair, 2002), in cultured mussels, *M. edulis*, grown in Notre Dame Bay (Newfoundland, Canada) (Alkanani, Parrish, Thompson, & McKenzie, 2007). All NMI acids have been found in both PS and PC plamalogens in trace amounts. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in both PE and PS plasmalogens were dominated acids. The sum of these acids in PE varies from 33% to 43%, and in PS, from 45% to 66% (Table 4). Level of EPA in PE (30–37%) and PS (39–57%) of marine species was higher than in freshwater species (PE, 13–16%; PS, 23–29%) and level of DHA was higher in freshwater than in marine ones.

Quite interesting were results concerning fatty acids in PS plasmalogen. Several marine species contain two rare acids, stearidonic (all-*cis*-6,9,12,15-octadecatetraenoic acid; C18:4n−3), and octadecapentaenoic (all-*cis*-3,6,9,12,15-18:5; C18:5*n*−3) acids, at concentrations 1–2% and 1–4%, respectively (Table 4). Analysis of published data showed that C18:4*n*−3 are present in PE, PS, and PC isolated from the whole body of *C. gigas*, *M. edulis*, and *R. philippinarum* (Kraffe et al., 2004), and in lipids of *Mytilus platensis* (De Moreno, Pollero, Moreno, & Brenner, 1980 *net ssylki*), and also are found in a freshwater mussel, *Unio tumidus*, from Bulgaria (Stefanov, Seizova, Brechany, & Christie, 1992). Both C18:4*n*−3 and C18:5*n*−3 acids, as well as NMI (5,11-C20:2, 5,13-C20:2, 7,13-C22:2, and 7,15-C22:2) were found in brackish mussel *Mytilaster lineatus* from Caspian Sea (Dembitsky et al., 1993b). Two deep hydrothermal vent mussels (13'N, Galapagos), and *Bathymodiolus themophitus* from East Pacific Rise (2600 m) contain a high level of C18:4*n*−3 (up to 12%) and C18:5*n*−3 (up to 4%), both these acids were also detected in *M. galloprovincialis* from Bay of Banyuls on the Mediterranean coast of France (Ben-Mlih, Marty, & Fiala-Medioni, 1992). Both C18:4*n*−3, and C18:5*n*−3, acids were also found in two New Zealand mussels *P. canaliculus* and *M. edulis* (Murphy et al., 2002). Three isomers of stearidonic acid, C18:4*n*−4, C18:4*n*−3, and C18:4*n*−2, as DMOX derivatives, were isolated from mussel *M. galloprovincialis* collected in Ria of Arosa coast (Pontevedra, Spain) (Garrido & Medina, 2002).

Composition of main fatty acids of PC plasmalogen form is shown in Table 4. Palmitic (12–17%), EPA (15–19%), and DHA (11–18%), are also predominant fatty acids, slightly varying in both marine and freshwater species, (17–20%), (8–12%) and (22–28%), respectively. High level EPA and DHA was detected in cultivated Newfoundland blue mussels (*M. edulis*) during September–October (Khan, Parrish, & Shahidi, 2006). A bivalve *Pelecyora trigona* was found to contain, as major components of total lipid fraction, palmitic (43.5%) and eicosapentaenoic (15.6%) acids (Chakraborty, Ghosh, & Bhattacharyya, 2002).

The flavour and aroma of cooked meat of clams and mussels appear to be produced by a variety of complex reactions between amino acids, lipids and sugars (Sekiwa, Kubota, & Kobayashi, 1997). In general, lipids are divided into two broad categories: neutral lipid, which is the stored fat and is mainly composed of TAG, and PL and cholesterol, which are building blocks of membranes. Neutral lipids (mainly) and phospholipids, in particular, seem to have an important effect on the nature of the volatiles from cooked meat, by contributing aliphatic and/or fatty aldehydes, alcohols, and fatty acids to the mixture of volatile components (Le Guen, Prost, & Demaimay, 2000). Phospholipids, especially plasmalogen PE, PS, and PC, have been also revealed as major contributors to

the "warmed-over flavour" in cooked meat of clams and mussels (Gokoglu, 2002; Le Guen, Prost, & Demaimay, 2001). For human health reasons, there is a desire to increase the ($n-3$) PUFA in mollusks and/or animals meat. Off-flavours can be developed, especially during cooking. The production of α,β-unsaturated aldehydes (αβ-UA) in heated meat has been shown to be due to a series of reactions, starting with hydrolysis of plasmalogen to free fatty aldehydes. The subsequent aldole condensation reaction of the formed free fatty aldehydes may occur by catalysis of amino groups of meat constituents and give α,β-UA. Free state C12-18 aldehydes are present in all the heated meats, and a relative content of these fatty aldehydes is similar to that of plasmalogen-bound aldehydes. Hexadecanal (47–65%) and octadecanal (14–30%) were the most predominant fatty aldehydes, and 2-tetradecyl-octadec-2-enal (35–46%) was the predominant α,β-UA in heated meat (Suyama, Arakawa, & Adachi, 1981).

Plasmalogens are not only components of plasma membrane and of lung surfactant, they serve as a reservoir for second messengers and may be also involved in membrane fusion, ion transport, and cholesterol efflux. Plasmalogens may also act as antioxidants, thus protecting cells from oxidative stress. Receptor-mediated degradation of plasmalogens by plasmalogen-selective phospholipases A2, C, and D results in the generation of arachidonic acid, eicosanoids, and platelet activating factor. Low levels of these metabolites have trophic effects, but at high concentration they are cytotoxic and may be involved in allergic response, inflammation, and trauma. Levels of plasmalogens are decreased in several neurological disorders including Alzheimer's disease, ischemia, and spinal cord trauma (Hartmann, Kuchenbecker, & Grimm, 2007).

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