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Research

# Profiling of bioactive lipids of the wild edible land snails of the genus Helix

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**CONFLICTS OF INTEREST**

There are no conflicts of interest for any of the authors.

**ABSTRACT:**

Pulmonate gastropod mollusks of the genus Helix are being used by human as food more than 10,000 years and they are quite important in the diet of many European countries. We investigated lipid composition of wild land snails sampled in France, Germany, Luxemburg,Norway, Switzerland, Sweden, and East Mediterranean inhabitants. Plasmalogens, glyceryl ethers, and diacyl phospholipid forms as well as their fatty aldehydes, alkyl ether glycerides, and fatty acid derivatives were studied. PE of snails, containing aldehydes C16 (variations from 17 to 40%), C18 (11-36%), C9-18:1 (22-31%), C11-20:1 (1-3%), and several minor aldehydes, were detected. The major saturated 1-O-alkyl glycerol ethers were C16:0 and C18:0. Eicosatetraenoic (ETA, 34%), a-linolenic*acid* (ALA, 14%), and eicosapentaenoic acid (EPA, 7%) in PE (all forms) were dominating fatty acids. ETA (49%), EPA (13%), ALA (7%), and *docosahexaenoic* (DHA, 6%) were major fatty acids in PS (all forms). In PC (all forms), major fatty acids were found oleic (20%), palmitic (15%), ETA (14%), and linoleic (10%). In neutral plasmalogens, the predominant fatty acids were palmitic acid (29%), oleic acid (13%), ALA (8%), and ETA (7.8%). Predominant fatty acids in neutral plasmalogens were found to be 16:0 (29%), C18:0 (13%), ALA (8%), and ETA (7.8%). Distribution of plasmalogens, alkyl glyceryl ethers, and their fatty aldehydes and fatty alcohols in gastropod species and other mollusks is also discussed.

**KEY WORDS:** Land snails, Helix, plasmalogens, fatty acids, glyceryl ethers, lipids, aldehydes

## INTRODUCTION

The genus *Helix* belongs to family Helicidae (class Gastropoda, phylum Mollusca) and represents large air-breathing land snails (pulmonate gastropods). This genus is native to Europe and to regions around the Mediterranean Sea [1]. These *snails* have been introduced throughout the world, and some of them, especially *H. aspersa*, have become garden pests. The best known species include *Helix aspersa* (common, or brown garden snail), and *Helix pomatia* (Burgundy snail, escargot, Roman snail, or edible snail). *Helix pomatia* is indigenous to Central and Southeast Europe, but has been moved by humans all over Europe, Asia, and the North and South America [2]. Land snails are a delicacy in Asian cuisine, Chinese and Japanese in particular. The French l'escargot de Bourgogne(*Helix pomatia*) as an appetizer and is consumed often as main meal. In African countries, such as Nigeria and South Africa, land snail called *'giant African snail'* is also a traditional food [3]. The French are the world's leading consumers of snails. In France snails come to market in a variety of ways. Estimated consumption of snails in France is around 40,000 tones/year. Escargot *Helix aspersa* ("petit gris") is sold at a price 5000 € pertonne.

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2,700 tones imported). Despite the large consumption, only 3% of snails in France come from farming. Italy is in second place in the world consumption of snails, and Spain and Germany are in the third and fourth place [4].

Lipids of snails, including the land snails, have been studied and partly reviewed [5-8]. Other studies included also investigation of fatty acids, proteins, mineral compounds and amino acids of the genus *Helix* also were reported [6-14]. In 1960's the first detail analysis of fatty acids of *Helix pomatia* was reported by German's physiologist Thielle [15,16].

In last decades, we have certain a growing interest for edible snail lipids, a particular for plasmalogens, fatty aldehydes, alkyl glyceryl ethers, and also essential fatty acids. In present work, we characterized the plasmalogenic neutral and phospholipids, and other lipid profiles in edible snails collected in a number of European countries (France, Germany, Luxemburg, Norway, Switzerland, and Sweden), as well as in Mediterranean area (Jordan and Israel).

## 2. MATERIALS AND METHODS

### *2.1 Land snail samples (class Gastropoda)*

*Helix engaddensis*was collected in Plio-Pleistocene canyon, Mount Carmel, and Golan Hights (Israel) in December 2014; *Helix texta* in the Banias Nature Reserve, October 2013. It is endemic to Israel. *Helix* sp. from southwestern Jordan (Aqaba) in January 2014; *Helix aspersa* in forest around of Bergen (Norway) in September 2012; *Helix pomatia* in Kungshamn-Morga nature reserve, Uppland (Sweden) in September 2012; *Helix pomatia*in forest around of Gex (France) in August 2013; *Helix pomatia* in forest around of Viuz-en-Sallaz (Swederland) in August 2013. *Helix pomatia* also was collected in August 2013 in Luxemburg, and around Saarbrucken (Germany).

### *2.2 Extraction and separation of lipids*

Lipids were extracted according to the modified method of Bligh and Dyer [17,18], using $CHCl_3$ and MeOH as solvents. Total lipids (TL) were separated into neutral and polar lipid fractions using column chromatography on silica gel G, and $CHCl_3$ and MeOH as eluents. Neutral lipids (NL) and phospholipids (PL) were detected by thin layer chromatography, as described previously [19-21]. TL in $CHCl_3$ were separated into NL and PL by passing through a glass column (20 mm diameter x 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 NL, glycolipids (GL) and PL were $CHCl_3$, acetone and MeOH, respectively. The solvents were evaporated by using a rotary evaporator, and the percentage of each fraction was determined gravimetrically. Residue was dissolved in $CHCl_3$ and then stored at -20°C. 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) fractions using two dimensional (2D-TLC) on glass plates. Silica gel G plates (20 x 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 vapor 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 visualized in iodine vapor. The solvent systems for 2D TLC were $CHCl_3$– MeOH–25% aqueous ammonium (65:35:5, v/v/v, (first dimension), and $CHCl_3$–MeOH–acetic acid–water (35:15:4:2, v/v/v/v, second dimension) [19-21].

### *2.3 Preparation of fatty acid methyl esters*

Neutral and polar lipid samples were transesterified [19-21]. 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 MeOH 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 neutralize 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*

The PE, PS and PC fractions, containing plasmalogen, alkyl-acyl-, and diacyl- forms were prepared from snails by preparative TLC, as previously described [18-20]. 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 earlier [17,22,23]. Purification was completed by preparative thin-layer chromatography. The 2 N methanolicHCl 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 hours. Alternatively, samples of PE, PS and PC fractions were saponified overnight at 37°C under nitrogen in glass-stoppered tubes. The saponification mixture was extracted 3 times with equal volumes of petroleum ether, and the combined upper phases were washed with the alkaline lower phase (water-ethanol-3 NNaOH, 40:10:1, v/v/v).

**2.5 Quantification of plasmalogen, alkyl-acyl- and diacylforms of glycerophospholipids**

Lipid extract (with a lipid concentration of 10 mg/mL) was spotted on Silica gel G plates (20 x 20 cm, Macherey-Nagel GmbH & Co., Düren, Germany). The plate was developed in the first direction with $CHCl_3$- MeOH-28% ammonia solution (130:70:10, v/v), and after evaporating the solvents plasmalogens on the plate were hydrolyzed with hydrochloric acid-methanol as described previously [17,22,23] was and then in the second direction with $CHCl_3$ – acetone – MeOH - acetic acid-water (100:40:20:20:10, 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 MeOH. The amounts of PLs and their plasmalogen forms were determined by the phosphorus absorption method [17,22,23].

***2.6 Preparation of isopropylidenes of alkyl glyceryl ethers***

Individual PL classes (PE, PS, PC) were separately hydrolyzed with 1 N HCl/MeOH. Fatty aldehydes and lyso-phospholipids were separated by TLC on Silica Gel G plates (20 x 20 cm, Macherey-Nagel GmbH & Co., Düren, Germany) with hexane-ethyl ether-acetic acid (90:10:1, v/v/v). Lyso-PL (1-alkyl-2-acyl glyceryl ethers of PS, PE and PC) and 1,2-diacyl PS, PE, and PC were saponificated by 1 N NaOH/MeOH. AGE and fatty acids were separated by TLC on Silica Gel G plates (20 x 20 cm) with solvent system: petroleum ether (60/70°C)-diethyl ether-acetic acid (90:10:1, v/v/v). AGE from PS, PE and/or PC, and 1-O-hexadecylglycerol (**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 AGE (10-20 mg) in dry acetone (2-4 mL) were converted with 95% yield to their isopropylidene derivatives by a rapid, room temperature acetonation in the presence of 0.01 M $HClO_4$, and to be later used for GC-MS analysis [17,22,23].

***2.7 Gas chromatography – mass spectrometry analysis***

Analysis of FAME from land snails was carried out using the Hewlett-Packard 5890 (series II) gas chromatograph (Palo Alto, CA) equipped with a 5971B mass selective detector. FAME were analyzed by GC-MS using an RTX-I 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 □-glyceryl etherswere described previously [17,18].

**3. RESULTS AND DISCUSSION**

***3.1. Total lipid composition***

Composition of the total lipids from the land snails is presented in Table 1. Total lipids fluctuated from 26.7 to 42.3 mg/g dry wt. Results of analysis by TLC showed that the main lipids in snails were TAG, FFA, ST, and PL. The contents of these compounds, relative to neutral lipid, were 45-60% for TAG, 4-13% for FFA, 9-22% for ST, and 14-30% for neutral plasmalogens. Furthermore, we analyzed PL using a 2D thin layer chromatography. We found that the PL was mainly composed of PE, PS, PC, CAEP and DPG. The latter PL is relatively abundant in some invertebrates, and it has been detected previously in many freshwater and marine mollusks [17-22,24-26]. The relative contents of the major PL classes were 23-33% for PE, 42-48% for PC, and 12-17% for PS (Table 1).

Previously, for the first time, we have reported that plasmalogenic PS was found in Gastropoda and Bivalvia mollusk species *only*, among all studied about 100 marine invertebrates, from phylumPorifera to phylum Urochordata [24]. Plasmalogens predominate in PE from mollusks, making up 80 to 88% of total PE, while in PC their contribution is quite low (18 to 28%). In PS, they vary from 74 to 80% (Table 1). These results confirm our previous findings [17-22,24] that any of the major phospholipids of marine and/or 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 marine gastropoda species [24-26]: *Acmaea pallida*(plasmolagen contribution to the total PE, PS, and PC being 61%, 39%, and 11%, respectively), as well as from *Collisellaheraldi* (73, 55, and 21%),

*Littorinakurila* (70, 68, and 10%), *L. brevicula* (75, 76, and 12%), *L. squalida* (74, 72, and 15%), *Nucellaheyseana* (70, 45, and 3%), *Tectonaticajanthostoma* (70, 58, and 8%), and *Tegularustica* (77, 65, and 0%).

Edible land snail eobania, *Helix vermiculata,* contains among neutral lipids (36% of the total): cholesterol, cholesterol esters, and triglycerides as NL major components (26, 29, and 25%, respectively). The NL contain also a significant amount of free alkyl glyceryl ethers (14%). Phospholipids were presented by cardiolipin (2.9%), PE 24.9% (19.8% of plasmalogen analog), PC 49.2% (45.6% glyceryl ether analog), ceramide amino-ethylphosphonate (7.5%), diglyceride-aminoethylphosphonate (6.3%), sphingoethanol-amine (1.7%), and PA. Unsaturated fatty acyl groups in PE and PC represent about 72.6 and 44.1%, respectively. The C16:0 alk-1-enyl chain was predominated (55.6%) in the side chains of PE plasmalogen. Batyl alcohol was the main AGE bound to choline phosphate (97.5%) [27]. Osborne and co-workers [28] reported that the nervous system of *Helix pomatia*contains PC (25%, including plasmalogen), PE (20%), Sph (10%), both PI and PS (43%), and traces of DPG.

**Table 1**. Lipids and phospholipids of land snails of the genus Helix

| Lipids | I | II | III | IV | V | VI | VII | VIII | IX | Σ |
|---|---|---|---|---|---|---|---|---|---|---|
| Total Lipids, mg/g dry wt | 40.4 | 39.2 | 36.7 | 38.9 | 41.4 | 42.3 | 28.9 | 26.7 | 34.5 | 36.5±4.4 |
| Neutral Lipids, of TL | 53.3 | 49.4 | 50.2 | 46.8 | 54.1 | 42.2 | 38.9 | 36.5 | 38.1 | 45.5±5.1 |
| FFA, % of NL | 11.8 | 5.8 | 13.2 | 7.1 | 4.2 | 5.9 | 6.1 | 5.6 | 7.3 | 7.4±1.9 |
| Steroids, both forms, free and esters, % of NL | 11.2 | 8.9 | 10.3 | 9.7 | 14.2 | 21.4 | 22.1 | 16.9 | 17.8 | 14.7±2.3 |
| TAG, % of NL | 45.9 | 56.4 | 49.2 | 56.9 | 51.0 | 54.0 | 57.3 | 45.5 | 60.0 | 52.9±5.4 |
| Neutral plasmalogens, % of NL | 31.1 | 28.9 | 27.3 | 26.3 | 30.6 | 18.7 | 14.5 | 13.7 | 14.9 | 22.9±2.6 |
| Glycolipids, % of TL | 19.8 | 22.8 | 26.1 | 25.9 | 29.2 | 18.9 | 17.6 | 18.3 | 23.7 | 22.5±1.6 |
| Phospholipids, % of TL | 26.9 | 27.8 | 23.7 | 27.3 | 16.7 | 38.9 | 43.5 | 45.2 | 38.2 | 32.0±4.2 |
| PC, all forms | 42.7 | 44.8 | 46.7 | 41.9 | 43.5 | 48.3 | 43.6 | 45.3 | 43.8 | 44.5±2.4 |
| PCP* | 26.9 | 22.6 | 28.1 | 19.8 | 22.4 | 21.8 | 23.7 | 18.6 | 19.3 | 22.6±2.7 |
| PCA** | 38.3 | 36.9 | 40.2 | 42.8 | 42.1 | 39.6 | 40.5 | 39.8 | 43.4 | 40.4±4.8 |
| PCD*** | 34.8 | 40.5 | 31.7 | 37.4 | 35.5 | 38.6 | 35.8 | 41.6 | 37.3 | 37.0±4.1 |
| PE, all forms | 28.1 | 22.9 | 25.8 | 24.6 | 27.3 | 25.8 | 32.4 | 31.5 | 33.1 | 27.9±3.6 |
| PEP* | 84.4 | 82.8 | 86.9 | 80.3 | 84.6 | 83.2 | 88.3 | 85.4 | 82.3 | 84.2±3.9 |
| PEA** | 8.9 | 7.8 | 4.3 | 9.5 | 5.3 | 8.2 | 4.2 | 6.6 | 8.4 | 7.0±1.8 |
| PED*** | 6.7 | 9.4 | 8.8 | 10.2 | 10.1 | 8.6 | 7.5 | 8.0 | 9.3 | 8.7±1.9 |
| PS, all forms | 16.6 | 16.9 | 15.2 | 15.7 | 15.7 | 16.3 | 12.8 | 13.2 | 12.2 | 15.0±1.8 |
| PSP* | 77.9 | 76.8 | 75.6 | 80.3 | 78.3 | 76.8 | 74.6 | 75.7 | 78.2 | 77.1±3.2 |
| PSA** | 11.6 | 13.4 | 14.9 | 15.2 | 14.6 | 13.8 | 14.2 | 15.1 | 13.4 | 14.0±1.7 |
| PSD*** | 10.5 | 9.8 | 9.5 | 4.5 | 7.1 | 9.4 | 11.2 | 9.2 | 8.4 | 8.8±1.2 |
| CAEP | 6.8 | 7.2 | 5.9 | 8.4 | 6.3 | 5.4 | 5.8 | 6.1 | 6.8 | 6.5±0.9 |
| Sph | 1.9 | 2.2 | 1.6 | 2.8 | 2.4 | 1.6 | 2.0 | 2.1 | 2.5 | 2.1±0.6 |
| DPG | 2.4 | 2.7 | 3.1 | 4.8 | 2.0 | 2.6 | 1.4 | 1.8 | 1.6 | 2.5±0.7 |
| PG | ND | 1.2 | 1.7 | ND | 1.4 | ND | 0.8 | ND | ND | |
| LPC | 1.2 | 0.8 | ND | 1.8 | 0.5 | ND | 1.2 | ND | ND | |
| LPE | 0.8 | 1.3 | ND | ND | ND | ND | ND | ND | ND | |
| PA | 0.5 | ND | ND | ND | 0.9 | ND | ND | ND | ND | |

ND, not detected; Σ, sum of main lipids, mean for the genus Helix. 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) CAEP, ceramideaminoethylphosphonate; Sph, sphingomyelin; DPG, diphosphatidylglycerol or cardiolipin; PG, phosphatidylglycerol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine, PA, phosphatidic acid. Footnote: The following

Gastropods were sampled: Burgundy Snail, Escargot, Roman Snail, or Edible Snail:I, *Helix pomatia,* Gex (France); II, *Helix pomatia,*Viuz-en-Sallaz (Swederland); III, *Helix pomatia* (Luxemburg); IV, *Helix pomatia*, Saarbrucken (Germany); and, V, *Helix pomatia,*Uppland (Sweden) Common, or Brown Garden Snail, VI, *Helix aspersa,* Bergen (Norway)

### *3.2 Composition of fatty aldehydes*

We further analyzed fatty aldehydes released from different plasmalogen subclasses (Table 2). In PE of snails, C16:0 aldehyde varied from 17 to 40%, for other fatty aldehydes similar variations were found: C18:0 - 11-33%, C9-18:1 – 22-30%; several minor aldehydes also were detected C18 family of fatty aldehydes also dominated in PS and PC plasmalogen forms. Molecular ions (*m/z*) 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*. [29].

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

| DMA of Fatty Aldehydes | I# | II | III | IV | V | VI | VII | VIII | IX | Σ |
|---|---|---|---|---|---|---|---|---|---|---|
| PEPlasmalogen | | | | | | | | | | |
| 14:0, MW = 258 | ND | ND | 1.3 | 2.1 | 0.8 | ND | ND | 2.2 | 0.8 | |
| 16:0, MW = 286 | 17.6 | 26.3 | 24.6 | 18.8 | 24.9 | 28.7 | 34.4 | 39.5 | 38.2 | 28.1±3.4 |
| 17:0, MW = 300 | ND | ND | 0.9 | ND | ND | 0.7 | 0.8 | ND | ND | |
| 18:0, MW = 314 | 33.4 | 28.3 | 23.3 | 36.5 | 16.0 | 28.4 | 19.3 | 15.7 | 11.5 | 23.6±4.6 |
| 20:0, MW = 342 | 1.2 | ND | ND | ND | 2.4 | 1.6 | 2.3 | 3.1 | ND | |
| Unidentifiedbranchedsaturated | 3.6 | 5.8 | 9.4 | 5.8 | 8.5 | 2.3 | 3.4 | 2.8 | 6.6 | |
| Saturated | 55.8 | 60.4 | 58.6 | 53.2 | 52.6 | 61.7 | 60.2 | 63.3 | 57.1 | 58.1±4.3 |
| 7-16:1**, MW = 284 | 0.8 | 2.4 | 4.1 | 1.9 | 2.6 | ND | 1.1 | 1.6 | 1.4 | |
| 9-18:1**, MW = 312 | 28.8 | 24.5 | 29.4 | 30.8 | 25.7 | 29.1 | 28.3 | 22.6 | 23.8 | 27.0±2.8 |
| 11-20:1**, MW = 340 | 6.2 | 4.9 | 3.0 | 10.9 | 13.7 | ND | 3.6 | 3.3 | 12.6 | |
| Unidentifiedunsaturated | 8.4 | 7.5 | 4.9 | 3.2 | 5.4 | 9.2 | 6.8 | 9.2 | 5.1 | |
| Monoenoic | 44.2 | 39.6 | 41.4 | 46.8 | 47.4 | 38.3 | 39.8 | 36.7 | 42.9 | 37.4±4.2 |
| PSPlasmalgen | | | | | | | | | | |
| 16:0 | 25.6 | 29.7 | 22.8 | 25.9 | 27.9 | 30.3 | 31.2 | 27.5 | 24.6 | 27.3±3.9 |
| br-16:0*, MW = 300 | ND | ND | 3.8 | 5.1 | ND | ND | 2.0 | ND | ND | |
| 18:0 | 20.8 | 17.6 | 18.4 | 13.4 | 13.3 | 11.2 | 11.7 | 12.4 | 16.6 | 13.9±5.2 |
| br-18:0*, MW = 328 | ND | 1.8 | 1.1 | ND | ND | ND | ND | 1.6 | ND | |
| 20:0 | 1.2 | 1.0 | 1.4 | 0.9 | 2.2 | 2.1 | ND | 0.9 | 0.7 | |
| br-20:0*, MW = 356 | 2.2 | ND | ND | ND | 1.2 | 1.5 | ND | ND | ND | |
| Saturated | 49.8 | 50.1 | 47.5 | 45.3 | 44.6 | 45.1 | 44.9 | 42.4 | 41.9 | 45.7±2.8 |
| 16:1 | 12.4 | 13.6 | 10.9 | 15.2 | 17.4 | 12.9 | 16.4 | 14.4 | 13.9 | 13.0±3.2 |
| 18:1 | 34.0 | 30.6 | 35.4 | 37.1 | 31.6 | 33.2 | 33.0 | 35.7 | 31.8 | 33.5±2.3 |
| 20:1 | 3.8 | 5.7 | 6.2 | 2.4 | 6.4 | 8.8 | 5.7 | 7.5 | 12.4 | 6.5±4.4 |
| Monoenoic | 50.2 | 49.9 | 52.5 | 54.7 | 55.4 | 54.9 | 55.1 | 57.6 | 58.1 | 54.3±2.4 |
| PCPlasmalogen | | | | | | | | | | |
| 14:0 | 0.8 | ND | ND | 1.6 | ND | ND | ND | 0.6 | 1.9 | |
| 16:0 | 33.2 | 26.9 | 30.4 | 28.5 | 32.1 | 37.5 | 38.8 | 36.2 | 37.1 | 33.4±4.1 |
| 18:0 | 36.0 | 41.3 | 38.4 | 37.9 | 37.2 | 38.7 | 28.9 | 23.2 | 21.0 | 33.6±4.8 |
| 20:0 | ND | 1.2 | 1.8 | ND | ND | 3.8 | 2.3 | ND | 2.2 | |
| Saturated | 70.0 | 69.4 | 70.6 | 68.0 | 69.3 | 80.0 | 70.0 | 60.0 | 62.2 | 68.8±6.7 |
| 16:1 | ND | 1.9 | 0.8 | ND | ND | 0.9 | 1.2 | 2.1 | 1.5 | |
| 18:1 | 28.8 | 28.7 | 28.6 | 29.9 | 30.7 | 18.0 | 28.8 | 37.9 | 35.5 | 29.6±4.8 |
| 20:1 | 1.2 | ND | ND | 2.1 | ND | 1.1 | ND | ND | 0.8 | |
| Monoenoic | 30.0 | 30.6 | 29.4 | 32.0 | 30.7 | 20.0 | 30.0 | 40.0 | 37.8 | 31.2±5.1 |
| NeutralPlasmalogens | | | | | | | | | | |
| 14:0 | ND | ND | 1.2 | 0.8 | ND | ND | ND | 1.1 | ND | |
| 15:0 | ND | ND | 1.1 | 1.2 | 1.3 | ND | 0.8 | 0.7 | 0.6 | |
| 16:0 | 22.5 | 18.9 | 21.7 | 25.6 | 20.4 | 28.2 | 20.1 | 19.8 | 24.4 | 22.4±3.2 |
| 17:0 | ND | ND | ND | 1.1 | 1.2 | ND | NS | 0.8 | ND | |
| 18:0 | 46.3 | 46.6 | 43.6 | 39.9 | 44.0 | 36.1 | 39.2 | 42.8 | 45.1 | 42.6±4.4 |
| 20:0 | 3.4 | 2.3 | 1.5 | 1.8 | ND | ND | 2.3 | 0.6 | ND | |
| Saturated | 72.2 | 67.8 | 69.1 | 70.4 | 66.9 | 64.3 | 62.4 | 65.8 | 70.1 | 67.6±3.9 |
| 16:1 | 3.2 | 2.6 | 2.2 | 4.2 | 1.4 | 1.8 | 2.5 | 3.1 | 1.7 | 2.5±1.1 |
| 18:1 | 24.6 | 29.6 | 28.7 | 25.4 | 31.7 | 33.9 | 35.1 | 31.1 | 28.2 | 27.6±3.7 |
| Monoenoic | 27.8 | 32.2 | 30.9 | 29.6 | 33.1 | 35.7 | 37.6 | 34.2 | 29.9 | 32.3±4.6 |

*Position of double bond in monoenoic DMA was not confirmed by GC-MS
**Retention time DMA of natural fatty aldehydes was identical to that of the synthetic analogs
#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. A series of saturated fatty aldehydes C14:0-C20:0, with major C18:0 in all studied species (over 40%), and C16:0 (19-28%), and unsaturated C16:1 (1-4%) and C18:1 (24-35%) ones were isolated from land snails (Table 2).

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 PL to form both type of plasmalogens. Previous reports on a large number of marine invertebrates, including gastropoda species [25], revealed that practically all of the species studied contain a high percentage of plasmalogen in different PL subclasses. The distribution of fatty aldehydes and fatty acids of total lipids in mantle, foot and digestive gland of two prosobranch gastropod mollusks, *Bellamyabengalensis*and *Pila globosa*, from India were reported [18]. The major fatty aldehydes were 18:0, 16:0, and 16:1. The major fatty aldehydes from the plasmalogen of the abalone (*Haliotis discus hannai* and *Turbo cornutus*), seaweed feeders, were octadecanal, hexadecanal, and hexadecenal [30].

### *3.3Composition of alkyl glycerides*

Distribution of AGE in PE, PS and PC is presented in Table 3. The electron impact mass spectrum measured at 70 eV on the mixture of AGE isolated from mollusk 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 $[M\text{-}CH_3O]^+$ (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 (0.1-0.3%) indicative of the presence of C16:0 and C18:0 analogs.

While mass spectrometry of the intact AGE (direct insertion mode was used) indicated the presence of AGE with C16:0, C18:0 and C20:0 chains as major constituents, GC-MS of the isopropylidene derivatives led to identification of glycerol ethers with chain lengths from C16 to C20. GC-MS of the isopropylidene derivatives did not give molecular ion peaks, but it showed the $[M\text{-}15]^+$ ion and the characteristic base peak at *m/z* 101. The major saturated 1-O-alkyl glycerol ethers in decreasing order of abundance were C16:0 and C18:0 (Table 3).

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

| PE alkyl glycerides | I# | II | III | IV | V | VI | VII | VIII | IX | Σ |
|---|---|---|---|---|---|---|---|---|---|---|
| 16:0, MW = 356 | 34.7 | 30.6 | 32.1 | 27.3 | 25.9 | 34.8 | 36.7 | 35.1 | 29.8 | 31.9±4.8 |
| 18:0, MW = 384 | 34.3 | 42.0 | 34.8 | 40.8 | 50.8 | 31.8 | 37.7 | 35.0 | 49.2 | 39.6±6.3 |
| 20:0, MW = 412 | 3.2 | 1.8 | ND | ND | ND | 1.5 | 4.1 | ND | 2.2 | |
| *Unidentified branched saturated* | 5.6 | 4.8 | 6.8 | 7.3 | 3.5 | 11.2 | 2.8 | 5.5 | 1.9 | |
| *Saturated* | 77.8 | 79.2 | 73.7 | 75.4 | 80.2 | 79.3 | 81.3 | 75.6 | 83.1 | 78.4±4.6 |
| 16:1**, MW = 354 | 9.4 | 6.8 | 5.9 | 8.6 | 7.6 | 8.1 | 10.1 | 9.4 | 5.8 | 8.0±3.7 |
| 18:1**, MW = 382 | 12.8 | 15.0 | 20.4 | 16.0 | 12.2 | 12.6 | 8.6 | 14.8 | 11.1 | 13.7±4.4 |
| *Monoenoic* | 22.2 | 21.8 | 26.3 | 24.6 | 19.8 | 20.7 | 18.7 | 24.4 | 16.9 | 21.7±3.2 |
| PS alkyl glycerides | | | | | | | | | | |
| 16:0 | 22.4 | 18.5 | 16.8 | 24.1 | 15.5 | 20.3 | 27.3 | 26.2 | 19.4 | 21.2±3.8 |
| 18:0 | 35.9 | 25.8 | 28.5 | 25.4 | 33.6 | 31.2 | 19.3 | 19.1 | 18.6 | 26.4±4.6 |
| 20:0 | ND | ND | 3.4 | ND | ND | 4.3 | 1.8 | 2.2 | ND | |
| *Unidentified branched saturated* | ND | 5.8 | ND | ND | 3.2 | ND | ND | ND | 6.9 | |
| *Saturated* | 58.3 | 50.1 | 48.7 | 49.5 | 52.3 | 55.8 | 48.4 | 47.5 | 44.9 | 50.6±3.4 |
| 16:1** | 18.9 | 22.4 | 16.7 | 13.9 | 20.3 | 14.2 | 25.1 | 21.3 | 27.7 | 20.0±6.3 |
| 18:1** | 22.8 | 27.5 | 34.6 | 36.6 | 27.4 | 30.0 | 26.5 | 31.2 | 27.4 | 29.3±3.9 |
| *Monoenoic* | 41.7 | 49.9 | 51.3 | 50.5 | 47.7 | 44.2 | 51.6 | 52.5 | 55.1 | 49.4±4.1 |
| PC alkyl glycerides | | | | | | | | | | |
| 16:0 | 26.5 | 22.3 | 27.9 | 20.4 | 28.1 | 24.3 | 28.7 | 20.2 | 29.1 | 25.3±3.9 |
| 18:0 | 65.7 | 65.1 | 61.0 | 73.8 | 62.5 | 67.5 | 65.7 | 72.1 | 57.6 | 62.3±5.1 |
| *Saturated* | 92.2 | 87.4 | 88.9 | 94.2 | 90.6 | 91.8 | 94.4 | 92.3 | 86.7 | 90.9±3.3 |
| 16:1** | ND | 4.9 | ND | 2.3 | 3.2 | 1.9 | 1.6 | ND | 6.8 | |
| 18:1** | 7.8 | 7.7 | 11.1 | 3.5 | 6.2 | 6.3 | 4.0 | 7.7 | 5.5 | 6.6±2.3 |
| *Monoenoic* | 7.8 | 12.6 | 11.1 | 5.8 | 9.4 | 8.2 | 5.6 | 7.7 | 12.3 | 8.9±2.6 |

** All isomers
#See Footnote in Table 1.

Thompson and Lee [31] proved that tissues of a number of mollusks, such as clam, *Protothaca stamina*,marine snail, *Thais lamellose*,chiton, *Katherinatunicate*,and octopus, *Octopus dofleini* (tentacles) are rich in AGE PL (9, 20, 25, and 40%, respectively). AGE were found to be present in significant amount (up to 27%) in the nonsaponifiable lipids of the abalone, *Haliotis discus hannai*; and the major alkyl groups were 16:0, 18:0, 18:1, and 20:0 [17]. AGE has also been observed in total lipids of a number of other gastropods: *Acmaea pallida, Chlorostomaargyrostomalischkei, Collisella*dorsuosa, *Collisella*sp., *Littorinabrevicula, Thais clavigera*, and *T. lamellosa* [24,32]. A marine opisthobranch gastropod mollusk, named as Sea hare, *Aplysia* sp. from Sagami Bay (Japan), contains in PE, 13% AGE, 53% plasmalogen form, and 34% diacyl form [34]. 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) [35], as well as in human milk, bone marrow, atherosclerotic aorta [36].

### *3.4 Composition of fatty acids*

Fatty acid composition of mollusks has been extensively studied in many mollusk species, including slugs and snails [37,38]. We have identified more than 90 fatty acids including saturated, monoenoic, dienoic and polyenoic acids. From 2 to 31% of them were identified as minor compounds and they are not shown in Table 4. Eicosatetraenoic (ETA, 34%), a-linolenic*acid*(ALA, 13.8%), and eicosapentaenoic (EPA, 7.4%) in PE (all forms) were dominated acids. ETA (48.7%), EPA (13%), ALA (7%), and DHA (6.6%) were major fatty acids in PS (all forms), but another ratio was found in PC (all forms), where major fatty acids were oleic (20%), palmitic (15%), ETA (14%), and linoleic (9.8%). In neutral plasmalogens, palmitic (29%), oleic (13%), ALA (8%), and ETA (7.8%) were found to be predominant.

Quite interesting finding concerned fatty acids in PS (Table 4). Several freshwater and marine species contain two rare acids, stearidonic (all-*cis*-6,9,12,15-octadecatetraenoic acid, 18:4n−3), and octadeca-pentaenoic (all-*cis*-3,6,9,12,15-18:5, 18:5n-3) acids, both at concentrations 1 to 4%. These fatty acids were found in total lipids of freshwater gastropods: *Limnaeafragilis*from Volga River [19,20], *Valvatabaicalensis* and *V.piligera*from Lake of Baikal [22], *Melanopsispraemorsum, Melanoidestuberculata, Pyrguliabarroisi*, and *Thedoxusneritoides* from Sea of Galilee (Lake Kinneret) [29]. Total lipids of marine gastropods: *Littorina scarab, Nassaserata, Nassariusalbescens, Nodilittorinasubnodosa*, and *Pianaxissulcata* from the Red Sea; *Gibula cineraria, Littorinaneritoides*, and *Monodontaturbinata* from the Mediterranean Sea [29] also contains these two fatty acids. Additional information for distribution of these acids in marine gastropods has been presented by Joseph [24].

The □-3 as well as □-6 fatty acids are beneficial to the heart by regulating rate, blood pressure [39] and dilation of blood vessels thereby facilitating blood flow throughout the body, especially the brain (nerve tissue formation, [40]). ALA and □-3 fatty acids help to lower cholesterol and TAG levels in human [41]. ALA and γ-linolenic acids showed the antihepatic cancer effects on a rabbit liver cancer model, VX-2 [42]. GLA has decrease in D-6-D enzyme leads to various diseases including arthritis, diabetes, hypertension, eczema, psoriasis in human, suggesting that supplementation with γ-linolenicacid may balance inflammatory and anti-inflammatory cytokines [43]. GLA also induced apoptosis of tumor cells without harming normal cells. The low neurotoxicity of GLA to normal brain neurons and selective activity against tumor cells suggests that it could be an effective anti-glioma agent [44]. It is to indicate mention that ALA and GLA (γ-linolenic acid) present in land snails, could be of plant origin.

The flavour and aroma of cooked meat of mollusks appear to be produced by a variety of complex reactions between lipids, amino acids, and sugars [45]. Neutral lipids (mainly) and PL, 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, AGE, and fatty acids to the mixture of volatile components [46]. PL, especially plasmalogen PE, PS, and PC, have been also revealed as major contributors to the "warmed-over flavour" in cooked meat of mollusks [47,48]. For human health reasons, there is a desire to increase the PUFA and fatty aldehydes in mollusks and/or animals meat. Off-flavors can be developed, especially during cooking. The production of □,□-unsaturated aldehydes (□,□-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 due to catalysis of amino groups of meat constituents and increasing of □,□-UA levels. Free state C14-20 aldehydes are present in all the heated meats, and a relative content of these fatty aldehydes is similar to that of plasmalogen-bound aldehydes. The effects of heating at 132° on the fatty acids and fatty aldehydes of neutral lipids and

PL of meat were reported [49]. Heating causes hydrolysis of the plasmalogens, and varying amounts of the liberated fatty aldehydes are recovered in the neutral lipid fractions. PC and PE loose PUFA, while amount of free fatty aldehydes increases after that heating [49]. In addition, hydrogen sulfide, produced from cysteine upon heating, is an important contributor to the flavor of cookedmeat. During the later stages of the Maillardreaction hydrogen sulfide can also react with other volatile compounds to form additional flavor compounds. Aldehydes, formed *via* lipid oxidation, may react with hydrogen sulfide, to give cyclic sulfur-containing compounds, including 2-alkyl-3-thiazolines, 2-alkylthiazoles, 2-alkyl-(2H)-thiapyrans and 2-alkyl-thiophenes. All of these groups of compounds have been identified in cookedmeat [50].

**Table 4.** Composition of main fatty acids from PE, PS, PC and neutral plasmalogens (% of total FAs, over 1%)

| PE (all forms) | I# | II | III | IV | V | VI | VII | VIII | IX | Σ |
|---|---|---|---|---|---|---|---|---|---|---|
| 16:0 | 2.2 | 5.6 | 4.1 | 3.5 | 6.4 | 5.8 | 4.0 | 2.3 | 4.3 | 4.3±1.9 |
| 18:0 | 3.5 | 1.2 | 2.4 | | 1.3 | | | | 1.1 | |
| 16:1n-9 | 1.1 | | 1.6 | 1.3 | | | 2.2 | | | |
| 18:1n-9 | 3.4 | 5.9 | 6.1 | 4.4 | 6.6 | 4.8 | 5.3 | 7.1 | 5.2 | 5.4±2.7 |
| 18:2n-6 | 4.1 | 2.8 | 5.2 | 1.1 | 3.8 | 2.4 | 3.1 | 5.3 | 6.3 | 3.8±2.2 |
| 18:3n-6 | 6.5 | 5.5 | 3.9 | 4.6 | 4.8 | 5.6 | 3.4 | 2.9 | 3.7 | 4.7±2.5 |
| 18:3n-3 | 16.4 | 13.9 | 14.3 | 17.7 | 16.2 | 15.3 | 8.5 | 14.8 | 7.1 | 13.8±2.1 |
| 20:4n-6 | 23.8 | 32.4 | 34.6 | 32.5 | 35.7 | 29.7 | 42.0 | 38.2 | 37.3 | 34.0±5.8 |
| 20:5n-3 | 3.6 | 9.7 | 8.8 | 4.3 | 4.4 | 10.8 | 6.3 | 4.2 | 14.8 | 7.4±3.9 |
| 22:5n-3 | 2.8 | 2.2 | 1.6 | 3.8 | 4.1 | 6.3 | 5.7 | 2.2 | 2.9 | 3.6±2.1 |
| 22:6n-3 | 4.2 | 2.7 | 4.4 | 5.2 | 4.8 | 6.9 | 3.4 | 8.3 | 6.2 | 5.1±2.4 |
| Others | 28.4 | 18.1 | 13.0 | 21.6 | 11.9 | 12.4 | 16.1 | 14.7 | 18.2 | |
| PS (all forms) | | | | | | | | | | |
| 16:0 | 1.1 | 1.4 | | 1.3 | 1.4 | 2.2 | | 2.5 | 1.4 | |
| 18:0 | 1.2 | | | 2.2 | 1.8 | | 1.6 | 1.0 | 3.7 | |
| 18:3n-3 | 4.9 | 5.7 | 8.3 | 6.8 | 7.3 | 9.2 | 7.3 | 6.5 | 7.7 | 7.1±2.8 |
| 18:4n-3 | 1.3 | | 3.8 | | 2.3 | 2.7 | 3.5 | 4.8 | | |
| 18:5n-3 | 1.0 | | 2.2 | | | 1.0 | 1.1 | | | |
| 20:4n-6 | 55.5 | 53.9 | 47.1 | 43.2 | 40.6 | 54.1 | 39.3 | 44.4 | 60.4 | 48.7±5.2 |
| 20:5n-3 | 12.1 | 9.6 | 14.8 | 11.2 | 10.9 | 9.8 | 19.6 | 21.2 | 8.3 | 13.0±6.1 |
| 22:6n-3 | 7.4 | 6.3 | 3.8 | 4.2 | 8.9 | 11.2 | 8.3 | 4.3 | 5.1 | 6.6±3.6 |
| Others | 15.5 | 23.1 | 23.8 | 31.1 | 26.8 | 9.8 | 19.3 | 15.3 | 13.4 | |
| PC (all forms) | | | | | | | | | | |
| 14:0 | | 1.4 | | | 2.1 | | | 1.2 | 1.0 | |
| 16:0 | 10.6 | 12.5 | 18.3 | 14.8 | 15.3 | 13.4 | 19.1 | 14.4 | 15.3 | 14.8±5.2 |
| 18:0 | 3.3 | 1.2 | 1.3 | 3.8 | 2.3 | 1.1 | 3.1 | 3.9 | 1.6 | 2.4±1.6 |
| 16:1n-7 | 1.4 | | | 1.2 | 1.5 | | | 1.0 | | |
| 18:1n-9 | 22.8 | 27.6 | 17.4 | 20.3 | 21.0 | 19.6 | 14.6 | 12.5 | 23.3 | 19.9±5.5 |
| 18:1n-7 | | 1.3 | 1.7 | 1.8 | | | 2.1 | 1.2 | | |
| 20:1n-9 | | | 1.4 | 1.3 | 1.5 | 1.3 | | | | |
| 18:2n-6 | 15.2 | 13.2 | 12.7 | 4.9 | 8.3 | 6.3 | 16.5 | 6.2 | 5.1 | 9.8±6.3 |
| 18:3n-6 | 1.1 | | | 1.4 | 1.7 | | 2.2 | | 3.1 | |
| 18:3n-3 | 4.2 | | 1.3 | | 1.1 | | | 4.6 | 1.2 | |
| 20:3n-3 | | 1.2 | | 1.7 | | 1.1 | | | | |
| 20:4n-6 | 12.1 | 13.8 | 13.2 | 10.8 | 14.2 | 11.6 | 20.5 | 17.3 | 12.8 | 14.0±5.7 |
| 20:5n-3 | 3.3 | 2.5 | 1.9 | 2.8 | 3.6 | 2.4 | 2.3 | 2.1 | 1.6 | 2.5±1.3 |
| 22:4n-6 | | 1.2 | 1.8 | 1.7 | | 2.7 | 1.3 | | 1.9 | |
| 22:5n-3 | 1.8 | 2.3 | 1.4 | 1.8 | 1.0 | | 2.2 | 2.4 | 1.3 | |
| 22:6n-3 | 6.8 | 2.1 | 4.5 | 8.2 | 10.4 | 9.3 | 3.4 | 4.3 | 6.7 | 6.2±3.6 |
| Others | 17.4 | 19.7 | 23.1 | 23.5 | 16.0 | 31.2 | 12.7 | 28.9 | 25.1 | |
| Neutral Plasmalogens* | | | | | | | | | | |
| 16:0 | 32.1 | 28.6 | 29.2 | 30.1 | 27.5 | 31.8 | 26.8 | 30.5 | 27.8 | 29.4±3.9 |
| 18:0 | 11.3 | 10.8 | 12.3 | 11.6 | 9.8 | 7.9 | 12.1 | 7.4 | 11.5 | 10.5±4.3 |
| 16:1n-7 | | 1.1 | 1.8 | 2.5 | 4.1 | | 1.0 | | | |
| 18:1n-9 | 15.8 | 16.3 | 12.2 | 12.6 | 10.1 | 15.2 | 9.8 | 12.0 | 17.5 | 13.5±4.7 |
| 18:1n-7 | | 1.3 | | 1.4 | 2.1 | | | | | |
| 18:2n-6 | 3.8 | 4.6 | 6.1 | 4.8 | 7.9 | 6.9 | 5.7 | 10.4 | 11.3 | 6.8±5.6 |
| 18:3n-6 | | 2.1 | 1.3 | 2.4 | | | 1.1 | 1.7 | | |
| 18:3n-3 | 11.2 | 13.1 | 14.2 | 10.1 | 9.0 | 4.3 | 3.8 | 5.1 | 2.3 | 8.1±5.1 |
| 20:4n-6 | 3.8 | 4.6 | 7.7 | 13.2 | 5.8 | 11.4 | 6.5 | 12.3 | 4.8 | 7.8±5.8 |
| 20:5n-3 | 6.9 | 9.8 | 9.4 | 3.8 | 5.2 | 8.5 | 9.1 | 1.5 | 4.3 | 6.5±4.8 |
| 22:5n-6 | | | 1.2 | 1.7 | 1.2 | | | 2.1 | | |
| 22:6n-3 | 1.3 | 4.8 | 2.6 | 1.6 | 8.5 | 4.1 | 3.2 | 2.6 | 5.3 | 3.8±5.1 |
| Others | 13.8 | 7.5 | 2.0 | 4.2 | 8.8 | 10.0 | 20.9 | 14.4 | 15.2 | |

*Fatty acids from both *sn*-2,3 positions of neutral plasmalogens #See Footnote in Table 1.

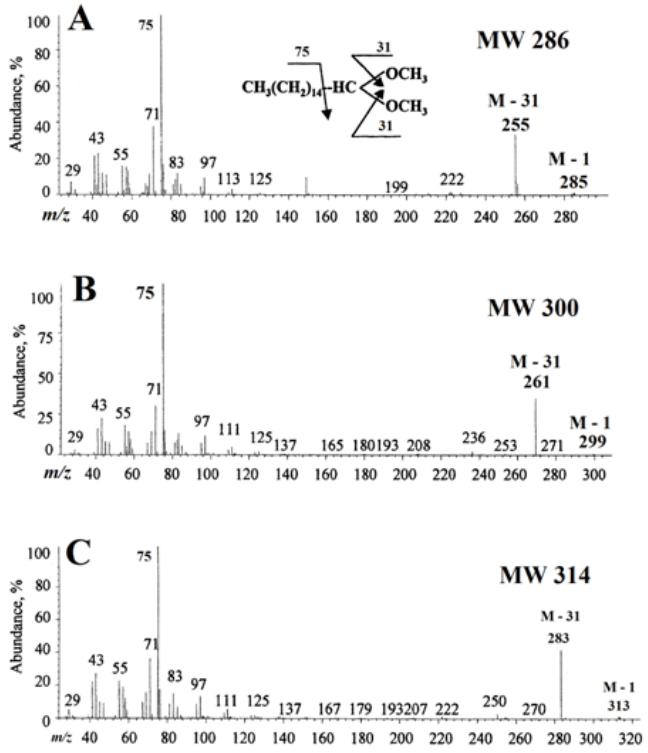


**Fig. 1.** Mass spectrum of DMA derivatives of fatty aldehydes isolated from theplasmalogen species of PE,PS and PC; A, C16:0, B, C17:0 and C, C18:0

## 4 CONCLUDING REMARKS

The benefits of including plasmalogens and their fatty aldehydes, alkyl glyceryl ethers, and □-6 and □-3 fatty acids in the diets of humans are well documented. Plasmalogens, alkyl glyceryl ethers, and fatty acids play a major role in the functioning of the immune system and the maintenance of all hormonal systems of the organism. Land snails belonging to the genus *Helix* are an excellent source of several fatty acids such as oleic (18:1n-9), eicosatetraenoic (20:4n-6), eicosapentaenoic (20:5n-3) and a-linolenic*acids*. Gastropods of the genus *Helix* are one of the most popular treats in European Countries and in East as well as in West Mediterranean areas.

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