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Component and Content of Lipid Classes and Phospholipid Molecular Species of Eggs and Body of the Vietnamese Sea Urchin *Tripneustes gratilla*

Thi-Kim-Hoa Dinh [1,2,3,†], Phi-Hung Nguyen [1,2,*,†], Doan Lan Phuong [1,2], Thi-Phuong-Ly Dang [1], Pham Minh Quan [1,2], Thi-Kim-Dung Dao [1,2], Valeria P. Grigorchuk [4] and Pham Quoc Long [1,2,*]

1 Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi 122100, Vietnam
2 Department of Chemistry, Graduate University of Science and Technology, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Hanoi 122100, Vietnam
3 College of Agriculture and Forestry, Thai Nguyen University (TUAF), Quyet Thang, Thai Nguyen 24119, Vietnam
4 Federal Scientific Center of the East Asia Terrestrial Biodiversity (Institute of Biology and Soil Science), Far Eastern Branch, Russian Academy of Sciences, Pr-t 100-let Vladivostoka 159, 690022 Vladivostok, Russia
* Correspondence: nguyenphihung1002@gmail.com (P.-H.N.); phamquoclong1960@gmail.com (P.Q.L.)
† These authors contributed equally to this work.

Abstract: Sea urchins (*Tripneustes gratilla*) are among the most highly prized seafood products in Vietnam because of their nutritional value and medicinal properties. In this research, lipid classes and the phospholipid (PL) molecular species compositions from the body and eggs of *T. gratilla* collected in Hon Tam, Nha Trang, Khanh Hoa, Vietnam, were investigated. Hydrocarbon and wax (HW), triacylglycerol (TG), mono- and diacylglycerol (MDAG), free fatty acid (FFA), sterol (ST), polar lipid (PoL), and monoalkyl-diacylglycerol are the major lipid classes. In PL, five main glycerophospholipid classes have been identified, in which 137 PL molecular species were detected in the body and eggs of *T. gratilla*, including 20 inositol glycerophospholipids (PI), 11 serine glycerophospholipids (PS), 22 ethanolamine glycerophospholipids (PE), 11 phosphatidic acids (PA), and 73 choline glycerophospholipids (PC). PI 18:0/20:4, PS 20:1/20:1, PE 18:1e/20:4, PA 20:1/20:1, and PC 18:0e/20:4 are the most abundant species with the highest content values of 38.65–48.19%, 42.48–44.41%, 41.21–40.03%, 52.42–52.60%, and 7.77–7.18% in each class of the body–eggs, respectively. Interestingly, PL molecules predominant in the body sample were also found in the egg sample. The molecular species with the highest content account for more than 40% of the total species in each molecular class. However, in the PC class containing 73 molecular species, the highest content species amounted to only 7.77%. For both the body and egg TL samples of the sea urchin *T. gratilla*, a substantial portion of C20:4n polyunsaturated fatty acid was found in PI, PE, and PC, but C16, C18, C20, and C22 saturated fatty acids were reported at low levels. The most dominant polyunsaturated fatty acid in PI, PE, and PC was tetracosapolyenoic C20, while unsaturated fatty acid C20:1 was the most dominant in PS and PA. To our knowledge, this is the first time that the chemical properties of TL and phospholipid molecular species of the PoL of Vietnamese sea urchin (*T. gratilla*) have been studied.

Keywords: lipid class; phospholipids; *Tripneustes gratilla*; sea urchin; Cau gai vang

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1. Introduction

The study of marine invertebrates has recently increased, resulting in a rapid increase in the number of publications on lipidomics (fatty acids and lipid classes). However, studies on the molecular species of these invertebrates are limited, especially those within Echinodermata such as sea urchins [1]. Approximately 950 species of sea urchins have

been identified globally. Some are considered valuable food sources because their nutritional value and medicinal properties make them commercially, economically, and ecologically important [2].

Sea urchins are a rich source of metabolites, such as lipids, proteins, polypeptides, polysaccharides, carotenoids, vitamins, and minerals. Steroids, saponins, and cerebrosides are secondary but important metabolites of echinoderms. The medicinal and nutritional values of sea urchins are useful for improving the condition and incidence of heart disease. Other health benefits of sea urchins include the prevention of high blood pressure, inflammation, arrhythmia, and cancer [3]. The compositions of lipid classes and fatty acids in sea urchins are attracting many research groups because of their abundance of active lipids, especially omega-3, -6, and -9, in addition to polyunsaturated fatty acids (PUFAs) and essential amino acids [4].

Despite the diversity of data on fatty acids and lipid classes, the number of studies on the molecular species of these invertebrates is still limited, especially on *T. gratilla* [5]. For example, the chemical properties of TL (lipid classes, fatty acid content) and the PoL composition, particularly the glycerophospholipid molecular species in the eggs and body of the Vietnamese sea urchin (*T. gratilla*), are understudied.

2. Results and Discussion

2.1. Total Lipid, Lipid Classes, and Fatty Acids Composition of T. gratilla

The total lipid (TL) of eggs and body wet weight of *T. gratilla* was identified as 4.41 ± 0.03% and 1.32 ± 0.03%, respectively. The total lipid analysis of the eggs and body samples has resulted in the identification of hydrocarbon and wax (HW), monoalkyldiacylglycerol (MDAG), triglyceride (TG), fatty acids (FFA), sterol (ST), mono- and diacylglycerol (MADAG), and polar lipid (PoL). Of these, TG was the highest with 78.37 ± 0.64% and 76.10 ± 0.57%, respectively. FA was presented as 4.76 ± 0.03% in the eggs and 4.49 ± 0.03% in the body, while PoLs were 4.41 ± 0.05% and 6.36 ± 0.04%, respectively. The other non-polar lipid classes, including HW, MDAG, and MADAG were less abundant, ranging from 1.11% to 3.31% of the TL [6].

The composition and content of fatty acids (FAs) in the TL of the eggs and body of *T. gratilla* were also characterized (Table 1). As a result, twenty-five and twenty-four fatty acids were observed in the eggs and body samples, respectively, comprising carbon atoms from 12 to 22. It was found that the contents of SFA and MUFA in the eggs were higher than those in the body with 41.74% vs. 17.22% and 26.53% vs. 18.01%, respectively. However, the PUFA content of the eggs alone was 31.08%, about two times less than that of the body (60.50%). The contents of omega-3 (13.97%) and omega-6 (16.79%) in the eggs were lower than those in the body (consisting of 20.67% and 39.83%, respectively), while omega-9 content of the eggs (20.55%) was higher than those in the body (14.73%). The PUFA/SFA ratio of the eggs (0.74) was low compared to the body (3.51), while the n3/n6 ratio of both materials was nearly equal (0.83 vs. 0.52).

Pozharitskaya et al. reported that myristic (14:0) and palmitic (16:0) acids were two major saturated fatty acids (SFA) and eicosenoic acid (20:1ω-9) was a major monounsaturated fatty acid (MUFA). Eicosapentaenoic (20:5ω-3; EPA) acid appeared as the most abundant polyunsaturated fatty acid (PUFA) [7]. In our results, myristic (14:0) and palmitic (16:0) were also two major SFA with 14.50% and 25.10% in the eggs, and 3.59% and 11.74% in the body sample, respectively (Table 1). Eicosenoic acid (20:1ω-9) was not the major MUFA in *T. gratilla*, but (16:1ω-9) and (18:1ω-9) were two major components. Eicosapentaenoic acid (20:5ω-3) reached 6.42% and 13.39%, and (20:4ω-6) reached 10.95% and 30.96% in eggs and body samples, respectively. These were two major polyunsaturated fatty acids (PUFA) in *T. gratilla*. The amounts of MUFA and SFA in body sample were equal at 18.01% and 17.22%, respectively, but there was a significant difference between MUFA (26.53%) and SFA (41.74%) contents in the eggs sample. The total amount of MUFA and SFA was 68.27% in eggs and reached only 35.23% in the body sample.

Table 1. Composition and content of fatty acids in the eggs and body samples of the Vietnamese sea urchin *T. gratilla*.

No.	Fatty Acids	Eggs of *T. gratilla* (%)	Body of *T. gratilla* (%)	No.	Fatty Acids	Eggs of *T. gratilla* (%)	Body of *T. gratilla* (%)
1	12:0	0.08	-	15	18:0	1.39	1.57
2	14:0	14.50	3.59	16	20:0	0.23	0.12
3	14:1 (n-7)	2.03	0.33	17	20:3 (n-3)	0.32	0.68
4	15:0	0.44	0.20	18	20:2 (n-6)	0.67	-
5	16:1 (n-9)	8.66	3.59	19	20:1 (n-9)	2.50	6.25
6	16:2 (n-4)	0.32	-	20	20:4 (n-6)	10.95	30.96
7	16:1 (n-7)	3.08	2.04	21	20:5 (n-3)	6.42	13.39
8	16:0	25.10	11.74	22	20:3 (n-6)	2.46	5.55
9	18:4 (n-3)	3.67	2.64	23	20:4 (n-3)	1.15	1.46
10	18:2 (n-6)	1.86	1.81	24	22:6 (n-3)	0.22	0.31
11	18:1 (n-9)	8.87	4.62	25	22:1 (n-9)	0.55	0.27
12	18:1 (n-7)	0.87	0.91	26	22:6 (n-6)	-	0.30
13	18:3 (n-3)	2.19	2.19	27	22:4 (n-6)	-	0.57
14	18:3 (n-6)	0.85	0.64	28	others	0.65	4.27
SFA		41.74	17.22	Omega-6		16.79	39.83
MUFA		26.53	18.01	Omega-9		20.55	14.73
PUFA		31.08	60.50	PUFA/SFA		0.74	3.51
Omega-3		13.97	20.67	n3/n6		0.83	0.52

2.2. Polar Lipid Type and Phospholipid Classes

In marine invertebrates, the PoL usually consists of glycolipids (GLs) and glycerophospholipids (GPLs) with GPL as the largest PL class. In our study, molecular species of PL from the eggs and body of *T. gratilla* were detected following the previously described HRMS fragmentations of PL standards [8]. Five types of GPLs, including phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidic acids (PA), and phosphatidylcholine (PC), were identified (Figures 1 and 2). Their molecular structures and contents were analyzed using a Shimadzu LCMS-IT-TOF instrument with a Shimadzu LCMS Solution control and processing software (v.3.60.361, Shimadzu, Kyoto, Japan).

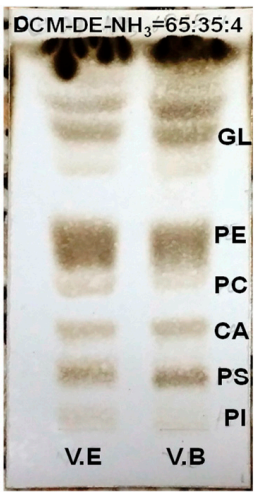


Figure 1. TLC profile analyzing the PoL classes of the eggs and body from *T. gratilla*.

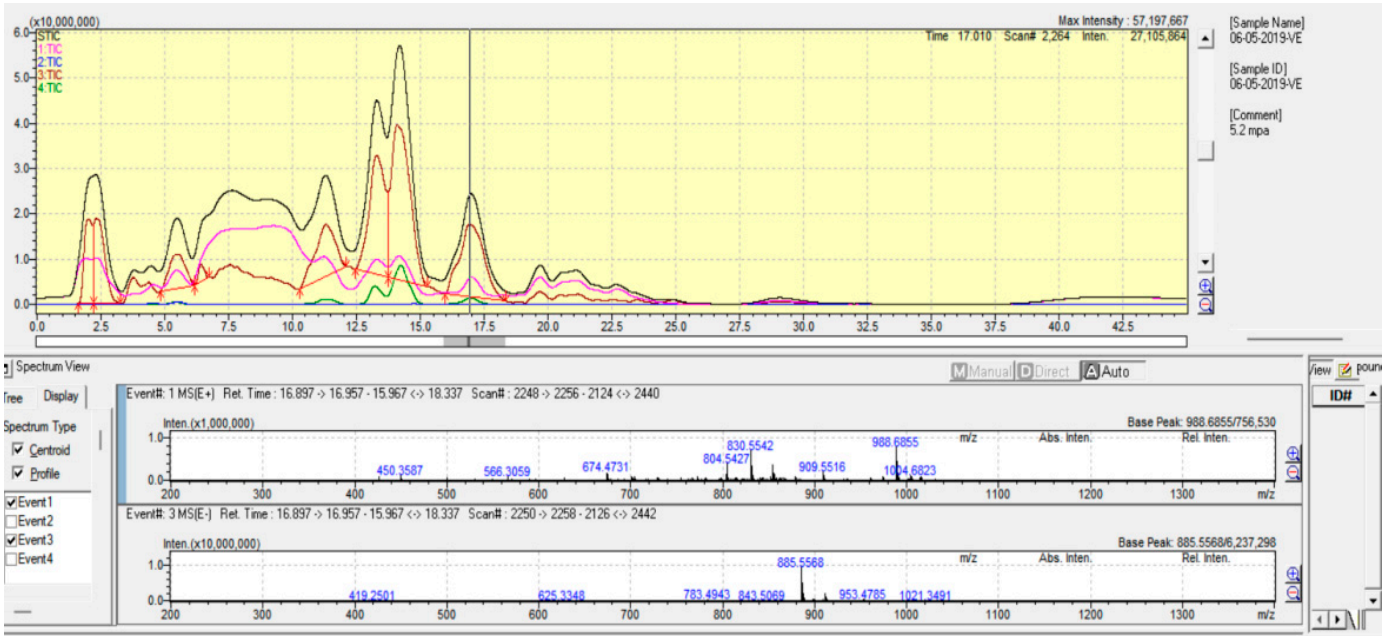


Figure 2. HPLC-HR/MS chromatography of the total PL from the eggs of *T. gratilla*.

The polar ends of the structures of the identified GPLs classes, inositol, serine, ethanolamine, and choline were major groups in the polar head contained in their molecules.

2.3. Molecular Species of Phosphatidylinositol (PI)

In total, 20 molecular species were found in the phosphatidylinositol (PI) class from the PoL in both samples of the sea urchin *T. gratilla* (Table 2). Among these, PI 18:0/20:4 (*m/z* [M − H]$^-$ 885.5562) was the highest peak with 38.65% (eggs) and 48.19% (body) content in the total PI species. PI 20:0/20:5 (*m/z* [M − H]$^-$ 911.5645, calc. for $C_{49}H_{85}O_{13}P$), PI 20:0/20:4 (*m/z* [M − H]$^-$ 913.5814, calc. for $C_{49}H_{87}O_{13}P$), and PI 18:0/20:5 (*m/z* [M − H]$^-$ 883.5401, calc. for $C_{47}H_{81}O_{13}P$) followed by 13.37%, 11.49%, and 9.36% content in the eggs and 12.64%, 9.89%, and 6.96% content in the body, respectively. PI 16:0/20:4, PI 18:0e/20:4, PI 18:0/20:3, and PI 20:1/20:5 were in the third highest group with the contents ranging from 2.17% to 4.82%. The other species presented low contents that were less than 1% (see Table 2 for detail).

The structure of PI can be characterized according to their MS$^-$ and MS/MS$^-$ data. Signals of negative quasi-molecular ions [M – H]$^-$ were observed in the HRMS spectra of all components of the formula species of PI (Figure 3A and Table 2).

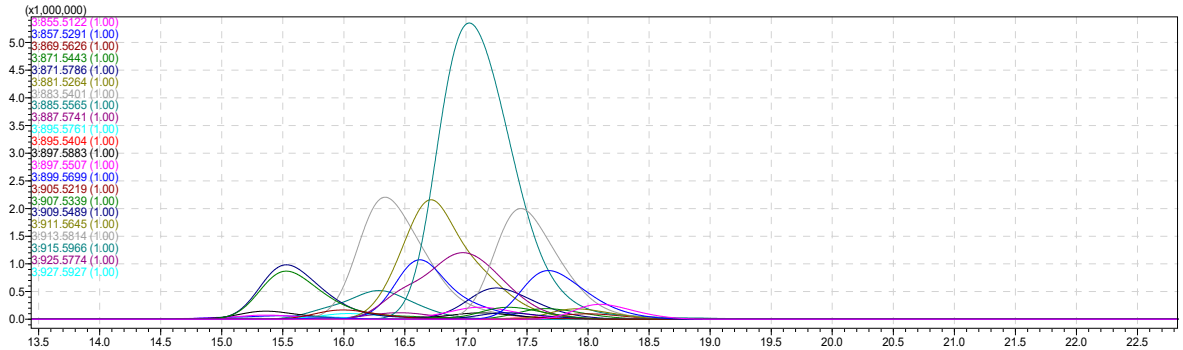
For example, a negative quasi-molecular ion [M – H]$^-$ at *m/z* 885.5562 for PI 18:0/20:4 was detected and assigned for a molecular formula of $C_{47}H_{83}O_{13}P$ with a calculated value of 885.5571 and a different value of 0.0009 (Figure 3B,C). This was the strongest signal (highest peak) in the HPLC–HR/MS of total molecular species of the PI class, with a retention time (Rt) of 17.939 min (Figure 3A,B). From the MS^{2-} spectrum of the ions [M – H]$^-$ of this PI 38:4 (Figure 3D), one signal corresponding to one carboxylate anion of 20:4 was detected at *m/z* 303.2308 (calc. for $C_{20}H_{32}O_2$).

Furthermore, the above observation was supported by a peak appearing at *m/z* 581.3085 which was calculated for $C_{27}H_{50}O_{11}P^-$ (Figure 4B). For the structure of PI, the fatty acid (FA) anion ([RCOO]–) was liberated from the sn^{-1} position due to the alkenyl linkages at the sn^{-2} position (Figure 4). Thus, the peak that appeared at *m/z* 283.2636 could be assigned for the loss of the fatty acid (C18:0) anion ($C_{18}H_{35}O_2^-$) at sn^{-1} in the molecular species of PI 38:4, and this observation was also supported by a signal corresponding to $C_{29}H_{46}O_{11}P^-$ at *m/z* 599.316 (Figures 3D and 4B).

Table 2. Molecular species of phosphatidylinositol (PI) identified from the PL of the egg and body of the Vietnamese sea urchin *T. gratilla*.

No.	Molecular Species	Molecular Weight $[M-H]^-$	Molecular Formula (MF)	Retention Time (Rt, min)	Content in Total PI (%)	
					Egg	Body
1.	PI 16:0/20:5	855.5122	$C_{45}H_{77}O_{13}P$	18.308	0.77	0.07
2.	PI 16:0/20:4	857.5291	$C_{45}H_{79}O_{13}P$	17.893	3.36	1.44
3.	PI 18:0e/20:5	869.5626	$C_{47}H_{83}O_{12}P$	16.239	0.69	0.54
4.	PI 17:0/20:4	871.5443	$C_{46}H_{81}O_{13}P$	17.568	0.90	0.46
5.	PI 18:0e/20:4	871.5786	$C_{47}H_{85}O_{12}P$	15.748	4.82	4.71
6.	PI 18:1/20:5	881.5264	$C_{47}H_{79}O_{13}P$	18.156	0.57	0.17
7.	PI 18:0/20:5	883.5401	$C_{47}H_{81}O_{13}P$	17.661	9.36	6.96
8.	PI 18:0/20:4	885.5562	$C_{47}H_{83}O_{13}P$	17.212	38.65	48.19
9.	PI 18:0/20:3	887.5741	$C_{47}H_{85}O_{13}P$	16.938	2.17	2.19
10.	PI 20:1e/20:4	897.5883	$C_{49}H_{87}O_{12}P$	15.551	0.69	0.40
11.	PI 19:0/20:5	897.5507	$C_{48}H_{83}O_{13}P$	17.327	1.23	1.08
12.	PI 19:0/20:4	899.5699	$C_{48}H_{85}O_{13}P$	16.873	5.92	6.43
13.	PI 40:8	905.5219	$C_{49}H_{79}O_{13}P$	18.195	0.44	0.55
14.	PI 20:3/20:4	907.5339	$C_{49}H_{81}O_{13}P$	17.870	0.72	0.50
15.	PI 20:1/20:5	909.5489	$C_{49}H_{83}O_{13}P$	17.444	2.91	2.21
16.	PI 20:0/20:5	911.5645	$C_{49}H_{85}O_{13}P$	16.959	13.37	12.64
17.	PI 20:0/20:4	913.5814	$C_{49}H_{87}O_{13}P$	16.573	11.49	9.89
18.	PI 20:0/20:3	915.5966	$C_{49}H_{89}O_{13}P$	16.273	0.69	0.46
19.	PI 21:1/20:4	925.5774	$C_{50}H_{87}O_{13}P$	16.684	0.71	0.87
20.	PI 21:0/20:4	927.5927	$C_{50}H_{89}O_{13}P$	16.243	0.42	0.24

(**A**)



(**B**)

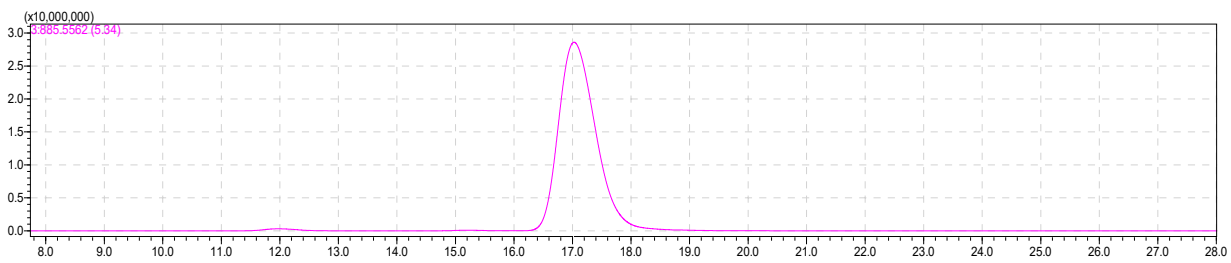
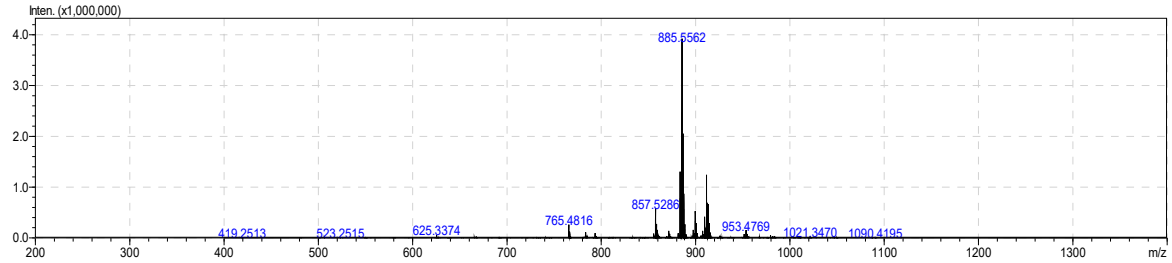


Figure 3. *Cont.*

(**C**)



(**D**)

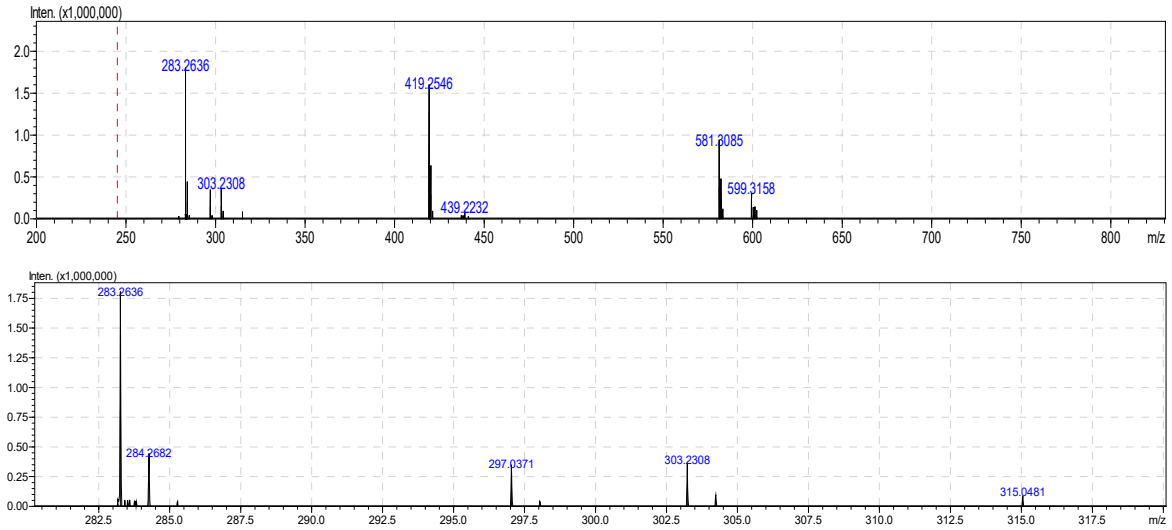
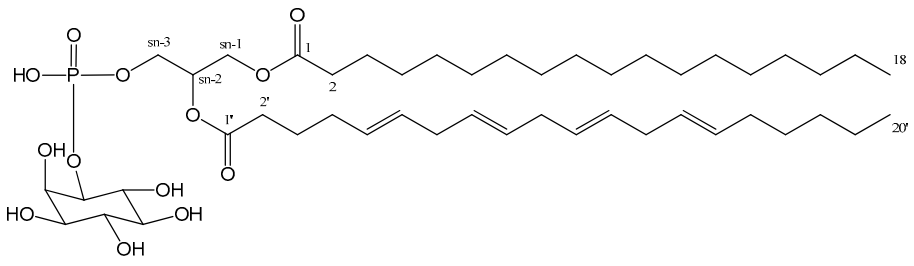


Figure 3. (**A**) The HPLC–HR/MS of total molecular species of the PI class. (**B**) HPLC–HR/MS of the molecular species PI at *m/z* 885.5662. (**C**) Fragmentation MS^{-} and (**D**) MS^{2-} of PI 18:0/20:4.

(**A**)



(**B**) (**C**)

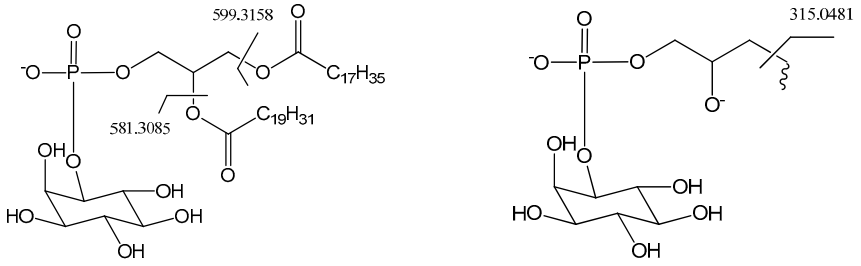


Figure 4. (**A**) Chemical structure of the identified molecular species PI 18:0/20:4. (**B**,**C**) Fragmentations of PI 38:4 with MS^{2-} assignment.

The ion peak at *m/z* 315.0481 corresponded to a fragmentation of a partial structure glycerol phosphatidylinositol unit (Figure 4C), which could mean the loss of two fatty acid units (C18:0) and (C20:4) at sn^{-1} and sn^{-2} in the molecular species PI 38:4. From

the observation above, the molecular species of PI 38:4 was therefore characterized as diacylglycerol phosphatidylinositol 18:0/20:4, and its structure is presented in Figure 4A.

2.4. Molecular Species of Phosphatidylserine (PS)

In the phosphatidylserine (PS) class from the PL of both samples of the Vietnamese sea urchin *T. Gratilla*, a total of 11 PS molecular species have been identified (Figure 5 and Table 3).

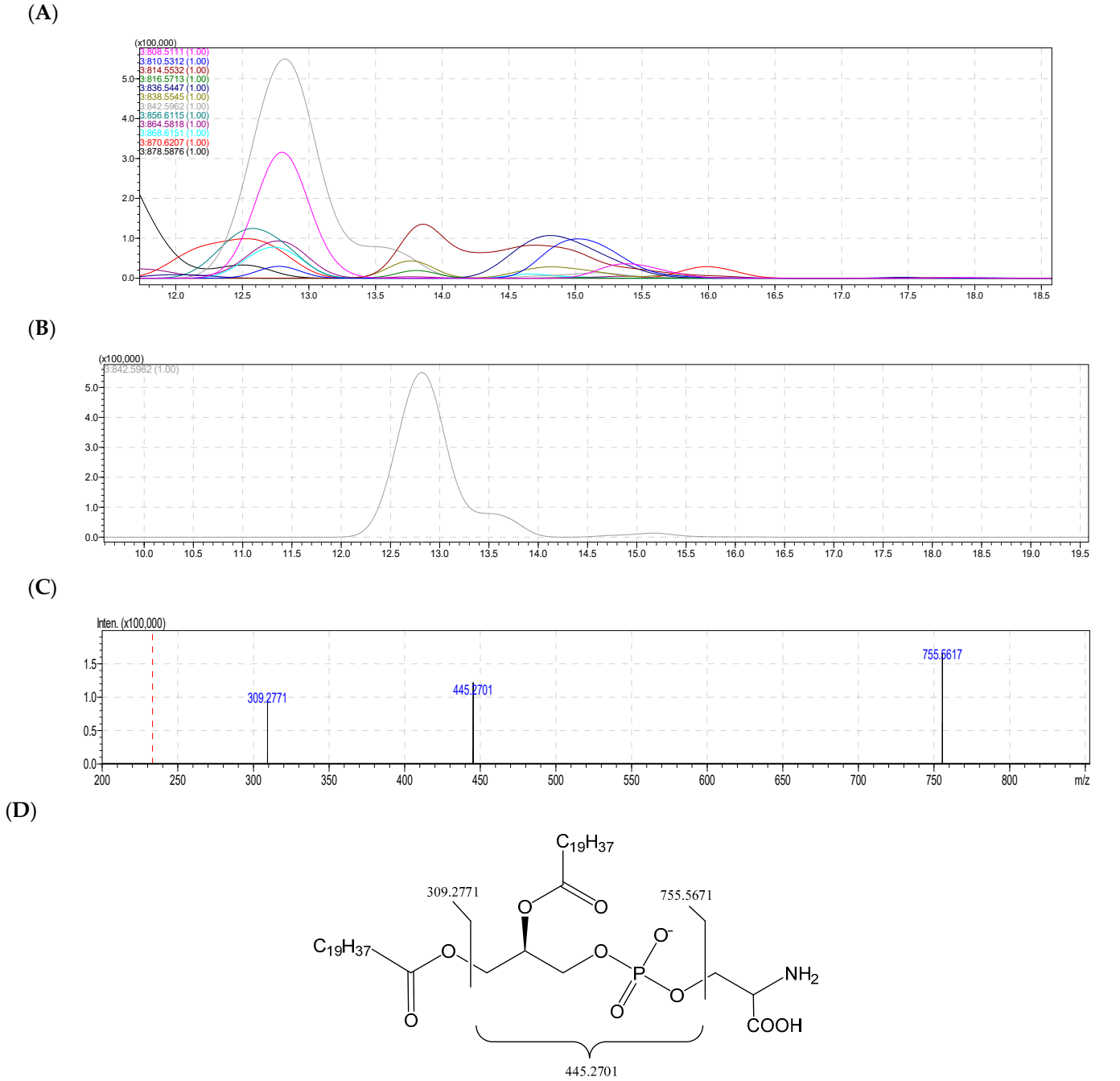


Figure 5. (**A**) The HPLC–HR/MS of total molecular species of PS class. (**B**) HPLC–HR/MS of the molecular species PS at *m*/*z* 842.5962. (**C**) Fragmentation MS^{2-} of PS 20:1/20:1. (**D**) Fragmentations of PS 40:2 (PS 20:1/20:1) with MS^{2-} assignment.

Table 3. Molecular species of phosphatidylserine (PS) identified from PoL of the egg and body of the Vietnamese sea urchin *T. gratilla*.

No.	Molecular Species	Molecular Weight [M – H]$^-$	Molecular Formula (MF)	Retention Time (Rt, min)	Content in Total PS (%)	
					Eggs	Body
1.	PS 38:5	808.5111	$C_{44}H_{76}NO_{10}P$	15.524	1.27	0.50
2.	PS 18:0/20:4	810.5312	$C_{44}H_{78}NO_{10}P$	15.067	5.47	3.88
3.	PS 20:1/18:1	814.5532	$C_{44}H_{82}NO_{10}P$	13.959	5.87	0.60
4.	PS 20:1/20:4	836.5447	$C_{46}H_{80}NO_{10}P$	14.747	9.67	5.30
5.	PS 40:4	838.5545	$C_{46}H_{82}NO_{10}P$	13.907	2.68	1.53
6.	PS 20:1/20:1	842.5962	$C_{46}H_{86}NO_{10}P$	12.900	42.48	44.41
7.	PS 20:1/21:1	856.6115	$C_{47}H_{88}NO_{10}P$	12.646	10.43	14.60
8.	PS 20:1/22:4	864.5818	$C_{48}H_{84}NO_{10}P$	12.874	7.71	6.03
9.	PS 20:1/22:2	868.6151	$C_{48}H_{88}NO_{10}P$	12.815	5.43	8.09
10.	PS 20:1/22:1	870.6207	$C_{48}H_{90}NO_{10}P$	12.338	7.31	11.32
11.	PS 21:1/22:4	878.5876	$C_{49}H_{86}NO_{10}P$	12.629	2.07	2.70

Of these, PS 20:1/20:1 with quasi-molecular ions [M – H]$^-$ at *m/z* 842.5962 (deduced for an MF $C_{46}H_{86}NO_{10}P$) was the highest with 42.48% content in the egg and 44.41% content in the body (Figure 5). PS 20:1/21:1 (*m/z* [M – H]$^-$ 856.6115, calc. for $C_{47}H_{88}NO_{10}P$) and PS 20:1/22:1 (*m/z* 870.6207, calc. for $C_{48}H_{90}NO_{10}P$) followed next with 10.43% and 7.31% in the eggs and 14.60% and 11.32% in the body, respectively. Except for PS 20:1/20:4 with a content of up to 9.67% in the eggs, the other PS molecular species presented the contents that were under 9%. There were two species, PS 38:5 and PS 20:1/18:1, which presented only 0.50% and 0.60% in body sample, respectively (see Table 3 for detail).

2.5. Molecular Species of Phosphatidylethanolamine (PE)

For phosphatidylethanolamine (PE), 22 molecular species were found (Table 4 and Figure 6A). Among these 22 components, 3 presented high content (over 10%) including PE 18:1e/20:5, PE 18:1e/20:4, and PE18:1e/20:2, accounting for 59.75% in the eggs and 60.31% in the body of total PE species. The remaining species presented low contents that were under 10%. There was no variation in the identified molecular species and little variation in the content of each composition between the two samples (eggs and body). In this study, the letter "e" denotes the head attached to the glycerol molecule with an ether bond.

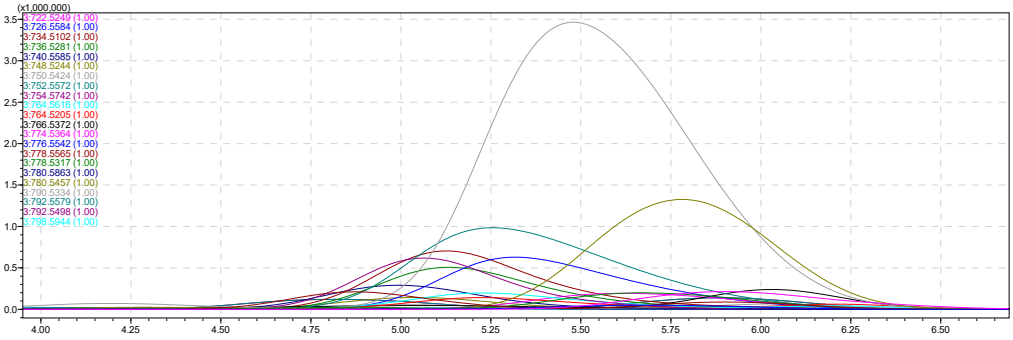
Table 4. Molecular species of phosphatidylethanolamine (PE) identified from PoL of the egg and body of the Vietnamese sea urchin *T. gratilla*.

No.	Molecular Species	Molecular Weight [M – H]$^-$	Molecular Formula (MF)	Retention Time (Rt, min)	Content in Total PE (%)	
					Egg	Body
1.	PE 16:1e/20:4	722.5249	$C_{41}H_{74}NO_7P$	5.931	2.83	3.58
2.	PE 36:3e	726.5584	$C_{41}H_{78}NO_7P$	5.619	1.15	1.80
3.	PE 37:6e	734.5102	$C_{42}H_{74}NO_7P$	5.986	0.62	0.41
4.	PE 17:1e/20:4	736.5281	$C_{42}H_{76}NO_7P$	5.724	2.11	2.47
5.	PE 37:3e	740.5585	$C_{42}H_{80}NO_7P$	5.250	0.42	0.93
6.	PE 18:1e/20:5	748.5244	$C_{43}H_{76}NO_7P$	5.811	10.56	6.60

Table 4. *Cont.*

No.	Molecular Species	Molecular Weight $[M-H]^-$	Molecular Formula (MF)	Retention Time (Rt, min)	Content in Total PE (%)	
					Egg	Body
7.	PE 18:1e/20:4	750.5424	$C_{43}H_{78}NO_7P$	5.531	41.21	40.03
8.	PE 18:0e/20:4	752.5572	$C_{43}H_{80}NO_7P$	5.365	6.88	5.28
9.	PE18:1e/20:2	754.5742	$C_{43}H_{82}NO_7P$	5.141	7.98	13.68
10.	PE 19:1e/20:4	764.5616	$C_{44}H_{80}NO_7P$	5.304	1.85	1.63
11.	PE 18:1/20:4	764.5205	$C_{43}H_{76}NO_8P$	6.382	1.41	2.95
12.	PE 18:0/20:4	766.5372	$C_{43}H_{78}NO_8P$	6.161	2.52	3.41
13.	PE 20:2e/20:5	774.5364	$C_{45}H_{78}NO_7P$	5.662	1.62	0.97
14.	PE 20:2e/20:4	776.5542	$C_{45}H_{80}NO_7P$	5.381	6.58	4.41
15.	PE 20:1e/20:4	778.5565	$C_{45}H_{82}NO_7P$	5.205	6.56	5.25
16.	PE 39:5	778.5317	$C_{44}H_{78}NO_8P$	6.057	0.31	0.14
17.	PE 20:2e/20:2	780.5863	$C_{45}H_{84}NO_7P$	5.075	2.25	2.34
18.	PE 39:4	780.5457	$C_{44}H_{80}NO_8P$	5.085	0.14	0.11
19.	PE 40:8e	790.5334	$C_{45}H_{78}NO_8P$	6.204	0.53	0.59
20.	PE 20:1/20:4	792.5579	$C_{45}H_{80}NO_8P$	4.945	1.07	1.12
21.	PE 20:1/20:4 isomer	792.5498	$C_{45}H_{80}NO_8P$	5.996	1.44	1.77
22.	PE 20:1/20:1	798.5944	$C_{45}H_{86}NO_8P$	5.237	0.27	0.54

(**A**)



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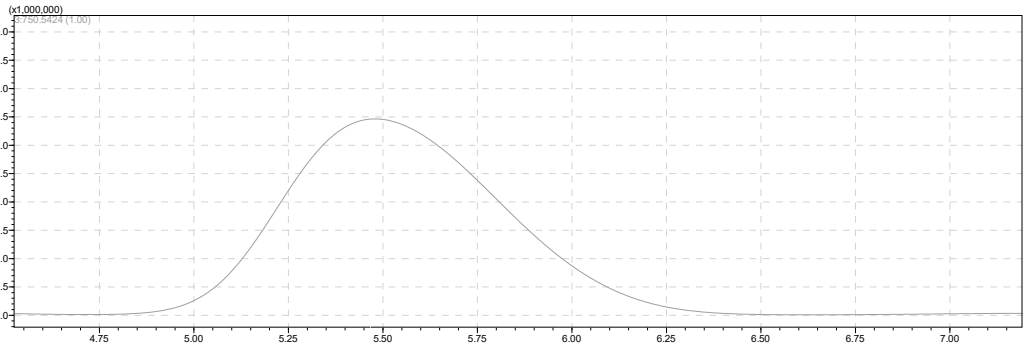
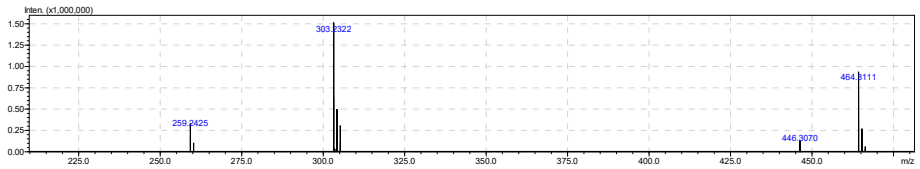


Figure 6. *Cont.*

(**C**)



(**D**)

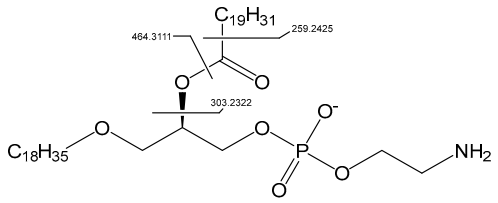


Figure 6. (**A**) The HPLC–HR/MS of total molecular species of PE class. (**B**) HPLC–HR/MS of a molecular species PE at *m*/*z* 750,5424. (**C**) Fragmentation MS^{2-} of PE 18:1e/20:4. (**D**) Fragmentations of PE 38:5e (PE 18:1e/20:4) with MS^{2-} assignment.

Among the received signals of the PE class, the negative ion signal $[M - H]^-$ had the highest intensity at *m/z* 750.5424 in both the eggs and body samples (41.21% and 40.03%), and the signal corresponding to the PE molecular species occupied the highest concentration in this class. The calculated molecular formula was $C_{43}H_{78}NO_7P$ which had seven oxygen atoms in the molecule, defining an acyl-alkyl PE. On the negative ion spectrum MS^{2-} of ion $[M - H]^-$ 750.5424, there were signals at *m/z* 303.2322 corresponding to the fatty acid anion $C_{20}H_{32}O_2$ ($C_{19}H_{31}COOH$, 20:4n); the signal at *m/z* 259.2425 corresponded to the anion $C_{19}H_{32}$; the signal at *m/z* 464.3111 corresponded to the molecular ion losing a neutral fragment $C_{20}H_{30}O$ ($C_{19}H_{31}COOH–H_2O$); and the signal at *m/z* 446.3070 corresponded to the molecular ion losing a neutral fragment, the fatty acid $C_{20}H_{32}O_2$ (Figure 6C,D).

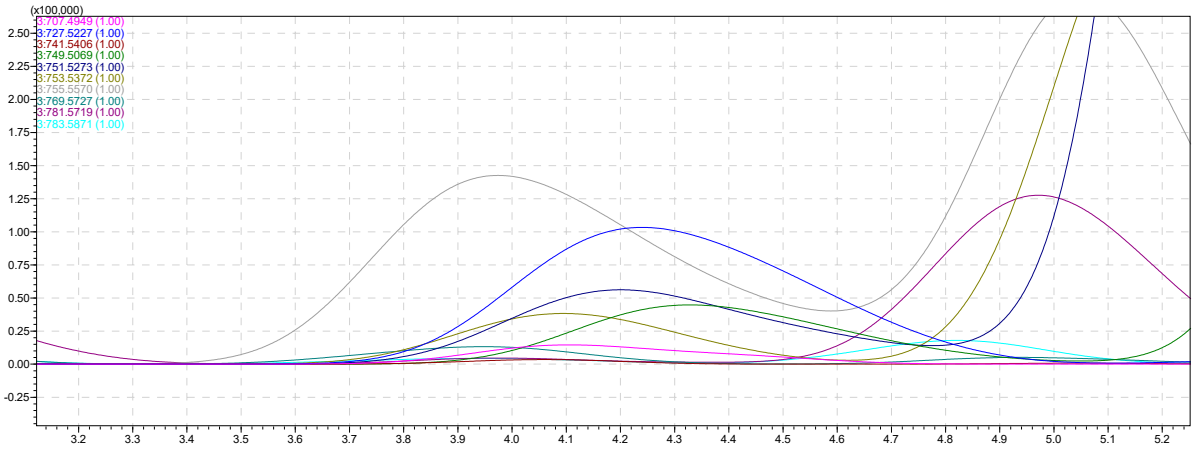
2.6. Molecular Species of Phosphatidic Acids (PA)

In the phosphatidic acid PA class, 11 molecular forms were identified (Table 5 and Figure 7). Among the received signals, the negative ion signal $[M - H]^-$ had the highest intensity at *m/z* 755.5570 in both the eggs and body TL samples of sea urchin *T. gratilla*, and the signal corresponding to the molecular species occupied the highest concentration in this PA class (Figure 7A,B).

Table 5. Molecular species of phosphatidic acids (PA) identified from PoL of the egg and body of the Vietnamese sea urchin *T. gratilla*.

No.	Molecular Species	Molecular Weight $[M - H]^-$	Molecular Formula (MF)	Retention Time (Rt, min)	Content in Total PA (%)	
					Egg	Body
1.	PA 18:1e/20:4	707.4949	$C_{41}H_{73}O_7P$	4.131	3.09	2.64
2.	PA 20:1/18:1	727.5227	$C_{41}H_{77}O_8P$	4.162	19.18	1.72
3.	PA 38:1	729.5479	$C_{41}H_{79}O_8P$	2.064	1.07	1.70
4.	PA 39:2	741.5406	$C_{42}H_{79}O_8P$	4.074	2.17	3.43
5.	PA 40:5	749.5069	$C_{43}H_{75}O_8P$	4.329	7.95	6.29
6.	PA 40:4	751.5273	$C_{43}H_{77}O_8P$	4.200	8.76	3.45
7.	PA 40:3	753.5372	$C_{43}H_{79}O_8P$	4.105	6.07	4.84
8.	PA 20:1/20:1	755.5570	$C_{43}H_{81}O_8P$	3.904	52.42	52.60
9.	PA 20:1/21:1	769.5727	$C_{44}H_{83}O_8P$	3.953	6.69	10.75
10.	PA 20:1/22:2	781.5719	$C_{45}H_{81}O_8P$	3.963	3.96	7.06
11.	PA 42:2	783.5871	$C_{45}H_{85}O_8P$	3.915	2.83	5.52

(**A**)



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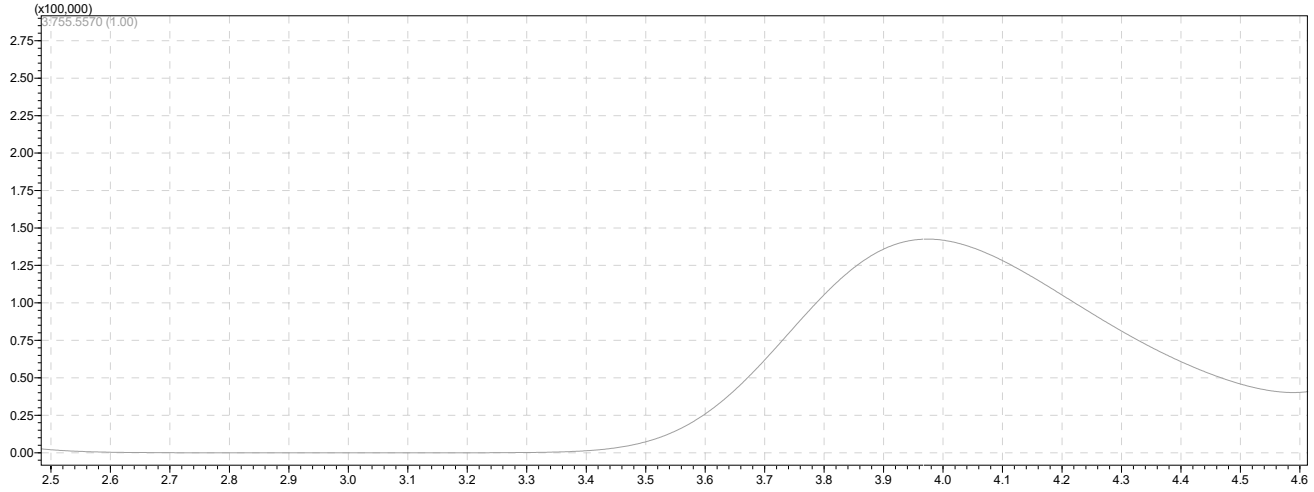
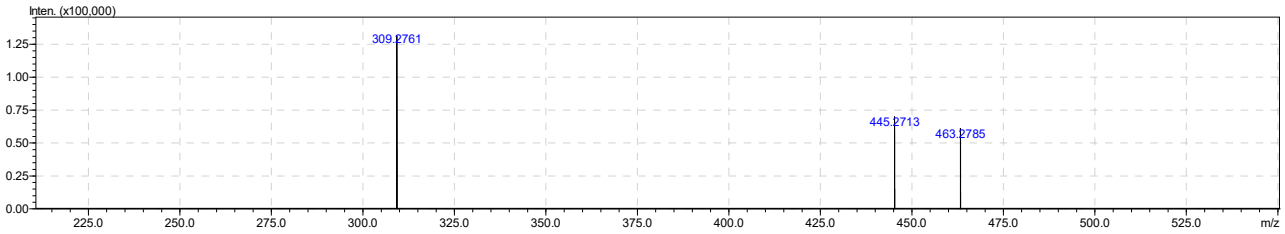


(**C**)



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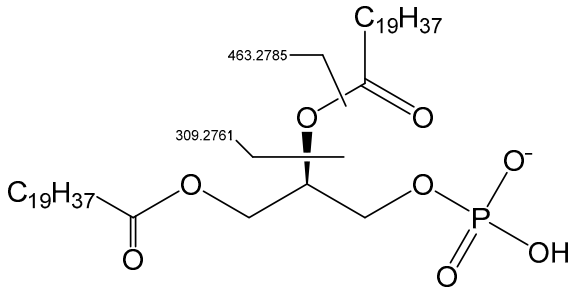


Figure 7. (**A**) The HPLC–HR/MS of total molecular species of PA class. (**B**) HPLC–HR/MS of the molecular species PA at *m/z* 755,5570. (**C**) Fragmentation MS^{2-} of PA 20:1/20:1. (**D**) Fragmentations of PA 40:2 (PA 20:1/20:1) with MS^{2-} assignment.

The calculated molecular formula was $C_{43}H_{81}O_8P$ which consisted of eight oxygen atoms in the molecule, defining a diacyl PA. On the negative ion spectrum MS^{2-} of ion $[M - H]^-$ 755.5570, it could be seen that signals at *m/z* 309.2761 belonged to the anion of fatty acid $C_{20}H_{38}O_2$ (20:1n); the signal at *m/z* 463.2785 corresponded to the molecular ion that lost a neutral fragment $C_{20}H_{36}O$ ($C_{19}H_{37}COOH–H_2O$); and the signal at *m/z* 445.2713 corresponds to the molecular ion losing a neutral fragment, the fatty acid $C_{20}H_{38}O_2$ (Figure 7C).

2.7. Molecular Species of Phosphatidylcholine (PC)

From the two samples (eggs and body) of sea urchin *T. gratilla*, 73 signals were identified in the composition of the phosphatidylcholine (PC) class (Table 6), corresponding to 73 molecular species (Table 5 and Figure 8A). Among them, the positive molecular ion signals $[M + H]^+$ at *m/z* 782.5703 had the highest intensity. The signals were observed simultaneously on the negative ion spectra of the $[M + CH_3COO]^-$ and $[M - CH_3]^-$ ions at *m/z* 826.5642 and 766.5371 (Figure 8B). The molecular formula was determined as $C_{44}H_{80}NO_8P$ which had eight oxygen atoms in the molecule structure, defining a diacyl PC.

On the negative ion spectrum -MS^2 of the ion $[M + CH_3COO]^-$ 826.5642, there was a signal at *m/z* 766.5360 corresponding to the anion $[M + CH_3COO - C_3H_6O_2]^-$ (Figure 8C).

The MS^2 spectrum of the ion with *m/z* 766.5371 showed fragment ions as follows: the ion with a signal at *m/z* 255.2389 corresponded to the fatty acid anion $C_{16}H_{32}O_2$ (16:0); the ion with a signal at *m/z* 303.2370 corresponded to the anion $C_{20}H_{32}O_2$ (20:4n); the ion with a signal at 480.3035 matched with a molecular ion losing a neutral fragment $C_{20}H_{30}O$ ($C_{19}H_{31}COO–H_2O$) (see Figure 8D). In addition, the chemical structure of the PC 16:0/20:4 was consistent with the other data. Therefore, the identified PC molecular species was identified as PC 16:0/20:4.

This study examined the lipid composition and content of sea urchins (*T. gratilla*) by comparing the extracts taken from eggs and body samples. Lipid classes were identified with the TLC method and the total lipid content was analyzed using Sorbfil TLC Video densitometer (Krasnodar, Russia) software. Furthermore, the non-polar lipid and polar lipid (PoL) were separated via chromatography. Fatty acid (FA) content and compositions were analyzed via GC-MS. This research advances the study of content and identification of polar lipid classes by using LC-MS/MS technique. As a result, five types of phospholipids were identified: PI, PS, PE, PA, and PC with 137 molecular species in total for each sample. Among those, PI 18:0/20:4; PS 20:1/20:1; PE 18:1e/20:4, and PC 16:0/20:4 were the most abundant phospholipid species with the highest contents of 38.65–48.19%, 42.48–44.41%, 41.21–40.03%, 52.42–52.60%, and 7.77–7.18% in each class of the body–eggs, respectively. Our data was the first to show phospholipid molecular species of PoL in the Vietnamese sea urchin (*T. gratilla*).

Imbs et al. reported that TG, WE, and DAGE were major classes of non-polar lipids in marine invertebrates [1], while the major polar lipid classes were phospholipids containing PC, PE, PS, and PI as glycerophospholipids (GPL). This study found that TG was the highest class with around 76–78%, but HW and MADAG were the two lowest classes with percentages between 1.1% and 2.3% for both eggs and body, respectively. Moreover, Kostetsky et al. reported the composition of PC and PE molecular species in a Japanese sea urchin *Strongylocentrotus intermedius* with alkylacyl PC and alkenylacyl PE were the dominant forms [5]. In detail, 29 PE and 26 PC molecular species were structurally identified. In addition, Imbs et al. also showed that the major PC molecular species were 18:1a/20:5, 16:0a/20:5, 18:0a/20:5, 18:1/20:5, 18:0/20:5, 20:1/20:5, and 16:0/20:5 and the major PE molecules were 18:0p/20:4, 18:0p/20:5, 18:0a/20:5, 18:0/20:4, 18:0/20:5, 18:1/20:5, and 20:1/20:5 in the muscle tissues of echinoderms. Our results showed the identification of five types of phospholipids including PI, PS, PE, PA, and PC with 137 molecular species in total for each sample with PI, PE, and PC being major classes. Among these, twenty PI molecular species, twenty-two PE molecular species, and seventy-three PC molecular species were structurally identified. It was also found that PI 18:0/20:4, PE 18:1e/20:4, and PC 16:0/20:4 were the most abundant phospholipid molecular species.

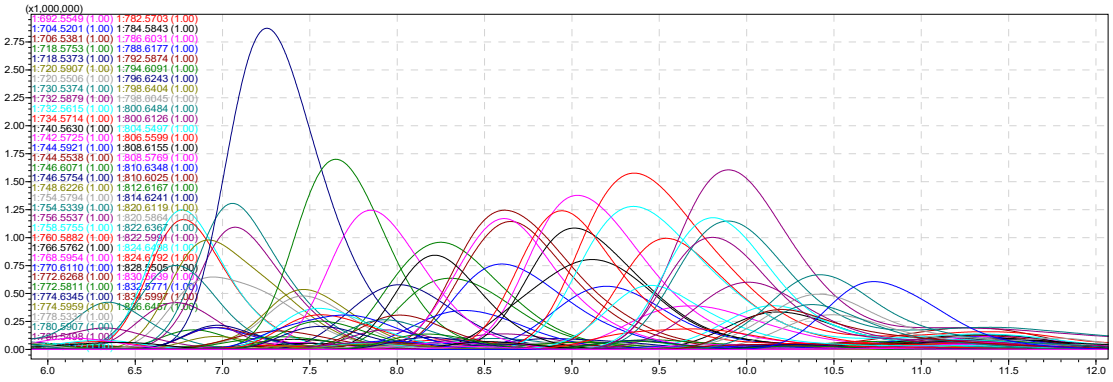
Table 6. Molecular species of phosphatidylcholine (PC) identified from PoL of the egg and body of the Vietnamese sea urchin *T. gratilla*.

No.	Molecular Species	Molecular Weight $[M + H]^+$	Molecular Formula (MF)	Retention Time (Rt, min)	Content in Total PC (%)	
					Egg	Body
1.	PC 30:0e	692.5549	$C_{38}H_{78}NO_7P$	8.461	0.61	0.85
2.	PC 14:0/16:1	704.5201	$C_{38}H_{74}NO_8P$	10.767	1.05	0.69
3.	PC 31:0e	706.5763	$C_{39}H_{80}NO_7P$	4.094	0.21	0.27
4.	PC 14:0/16:0	706.5381	$C_{38}H_{76}NO_8P$	10.214	1.33	1.95
5.	PC 16:0e/16:1	718.5753	$C_{40}H_{80}NO_7P$	8.261	0.67	1.38
6.	PC 31:1	718.5373	$C_{39}H_{76}NO_8P/4$	10.245	0.12	0.15
7.	PC 32:0e	720.5907	$C_{40}H_{82}NO_7P$	7.923	0.69	1.27
8.	PC 31:0	720.5506	$C_{39}H_{78}NO_8P$	9.700	0.20	0.28
9.	PC 32:2	730.5374	$C_{40}H_{76}NO_8P$	10.409	0.78	0.68
10.	PC 33:1e	732.5879	$C_{41}H_{82}NO_7P/3$	7.898	0.22	0.41
11.	PC 16:0/16:1	732.5615	$C_{40}H_{78}NO_8P$	9.851	3.08	3.16
12.	PC 33:0e	734.6039	$C_{41}H_{84}NO_7P/2$	7.650	0.35	0.21
13.	PC 16:0/16:0	734.5714	$C_{40}H_{80}NO_8P$	9.651	1.46	2.25
14.	PC 34:4e	740.563	$C_{42}H_{78}NO_7P$	8.625	0.17	0.28
15.	PC 34:3e	742.5725	$C_{42}H_{80}NO_7P$	8.365	0.18	0.26
16.	PC 34:2e	744.5921	$C_{42}H_{82}NO_7P$	8.022	0.23	0.31
17.	PC 33:2	744.5538	$C_{41}H_{78}NO_8P$	10.034	0.08	0.15
18.	PC 16:0e/18:1	746.6071	$C_{42}H_{84}NO_7P$	7.637	0.87	1.20
19.	PC 17:0/16:1	746.5754	$C_{41}H_{80}NO_8P$	9.389	0.26	0.46
20.	PC 34:0e	748.6226	$C_{42}H_{86}NO_7P$	7.420	0.41	0.41
21.	PC 33:0	748.5921	$C_{41}H_{82}NO_8P/3$	9.003	0.27	0.18
22.	PC 35:4e	754.5794	$C_{43}H_{80}NO_7P$	8.230	0.08	0.11
23.	PC 34:4	754.5339	$C_{42}H_{76}NO_8P$	10.397	1.62	1.33
24.	PC 34:3	756.5537	$C_{42}H_{78}NO_8P$	10.019	1.66	1.37
25.	PC 16:1/18:1	758.5755	$C_{42}H_{80}NO_8P$	9.515	1.55	1.33
26.	PC 16:0/18:1	760.5882	$C_{42}H_{82}NO_8P$	9.020	3.59	3.37
27.	PC 34:0	762.6052	$C_{42}H_{84}NO_8P$	8.757	0.86	0.55
28.	PC 16:0e/20:5	766.5762	$C_{44}H_{80}NO_7P$	8.282	2.06	2.17
29.	PC 16:0e/20:4	768.5954	$C_{44}H_{82}NO_7P$	7.904	3.60	4.36
30.	PC 16:0e/20:3	770.6110	$C_{44}H_{84}NO_7P$	7.759	0.77	1.05
31.	PC 36:2e	772.6268	$C_{44}H_{80}NO_7P$	7.416	0.50	0.63
32.	PC 35:2	772.5811	$C_{43}H_{82}NO_8P$	9.076	0.20	0.28
33.	PC 36:1e	774.6345	$C_{44}H_{88}NO_7P$	7.076	0.49	0.52
34.	PC 35:1	774.5959	$C_{43}H_{84}NO_8P$	8.695	0.19	0.33
35.	PC 36:6	778.5337	$C_{44}H_{76}NO_8P$	10.421	0.66	0.50
36.	PC 37:5e	780.5907	$C_{45}H_{82}NO_7P$	7.992	0.62	0.53
37.	PC 16:0/20:5	780.5498	$C_{44}H_{78}NO_8P$	9.905	3.46	2.77

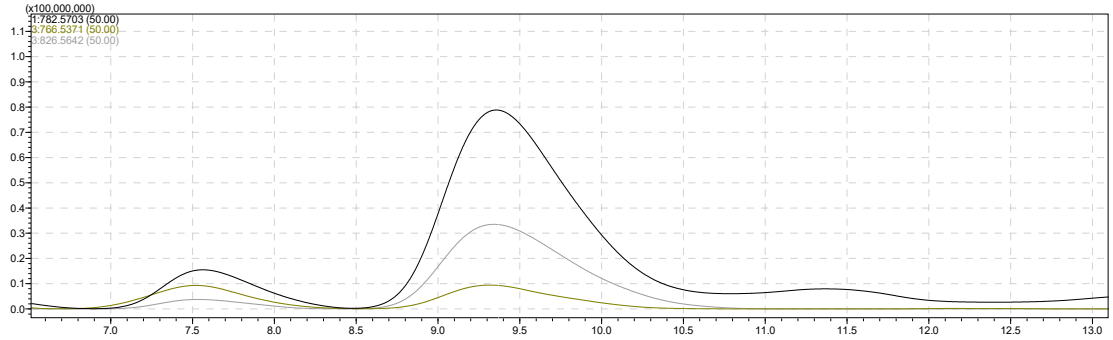
Table 6. *Cont.*

No.	Molecular Species	Molecular Weight $[M + H]^+$	Molecular Formula (MF)	Retention Time (Rt, min)	Content in Total PC (%)	
					Egg	Body
38.	PC 17:0e/20:4	782.6112	$C_{45}H_{84}NO_7P$	7.605	0.98	1.01
39.	PC 16:0/20:4	782.5703	$C_{44}H_{80}NO_8P$	9.409	4.76	4.21
40.	PC 16:0/20:3	784.5843	$C_{44}H_{82}NO_8P$	9.175	2.63	2.66
41.	PC 16:0/20:2	786.6031	$C_{44}H_{84}NO_8P$	8.702	3.60	3.42
42.	PC 16:0/20:1	788.6177	$C_{44}H_{86}NO_8P$	8.446	1.12	1.66
43.	PC 38:6e	792.5874	$C_{46}H_{82}NO_7P$	8.067	0.70	0.62
44.	PC 18:0e/20:5	794.6091	$C_{46}H_{84}NO_7P$	7.697	4.26	3.85
45.	PC 18:0e/20:4	796.6243	$C_{46}H_{86}NO_7P$	7.320	7.77	7.18
46.	PC 18:0e/20:3	798.6404	$C_{46}H_{88}NO_7P$	7.009	2.01	2.55
47.	PC 37:3	798.6045	$C_{45}H_{84}NO_8P/6$	8.783	0.17	0.16
48.	PC 18:0e/20:2	800.6484	$C_{46}H_{90}NO_7P$	6.837	1.32	1.37
49.	PC 37:2	800.6126	$C_{45}H_{86}NO_8P/5$	8.352	0.21	0.24
50.	PC 38:7	804.5497	$C_{46}H_{78}NO_8P$	10.103	0.87	0.62
51.	PC 38:6	806.5599	$C_{46}H_{80}NO_8P$	9.579	2.48	1.82
52.	PC 39:5e	808.6155	$C_{47}H_{86}NO_7P$	7.474	0.21	0.21
53.	PC 18:1/20:4	808.5769	$C_{46}H_{82}NO_8P$	9.086	4.00	3.05
54.	PC 39:4e	810.6348	$C_{47}H_{88}NO_7P$	7.047	0.34	0.31
55.	PC 18:0/20:4	810.6025	$C_{46}H_{84}NO_8P$	8.690	3.88	3.60
56.	PC 38:3	812.6167	$C_{46}H_{86}NO_8P$	8.377	1.69	1.55
57.	PC 18:1/20:1	814.6241	$C_{46}H_{88}NO_8P$	8.101	1.54	0.86
58.	PC 40:6e	820.6119	$C_{48}H_{86}NO_7P$	7.522	1.35	1.18
59.	PC 39:6	820.5864	$C_{47}H_{82}NO_8P$	9.069	0.19	0.17
60.	PC 20:1e/20:4	822.6367	$C_{48}H_{88}NO_7P$	7.138	3.11	3.03
61.	PC 39:5	822.5991	$C_{47}H_{84}NO_8P$	8.680	0.39	0.38
62	PC 40:4e	824.6498	$C_{48}H_{90}NO_7P$	6.862	1.98	1.82
63.	PC 39:4	824.6192	$C_{47}H_{86}NO_8P$	8.355	0.21	0.21
64.	PC 40:9	828.5505	$C_{48}H_{78}NO_8P$	10.116	0.84	0.91
65.	PC 40:8	830.5639	$C_{48}H_{80}NO_8P$	9.650	1.28	1.37
66.	PC 40:7	832.5771	$C_{48}H_{82}NO_8P$	9.214	1.66	1.69
67.	PC 20:1/20:5	834.5997	$C_{48}H_{84}NO_8P$	8.722	3.38	3.15
68.	PC 41:5e	836.6457	$C_{49}H_{90}NO_7P$	6.924	0.30	0.42
69.	PC 20:1/20:4	836.6159	$C_{48}H_{86}NO_8P$	8.320	3.74	3.23
70.	PC 20:2/20:2	838.6321	$C_{48}H_{88}NO_8P$	8.023	1.95	1.82
71.	PC 40:3	840.6399	$C_{48}H_{90}NO_8P$	7.753	0.78	0.83
72.	PC 40:5e	850.6595	$C_{50}H_{92}NO_7P/7$	6.748	0.45	0.61
73.	PC 41:5	850.6241	$C_{49}H_{88}NO_8P$	8.121	0.19	0.22

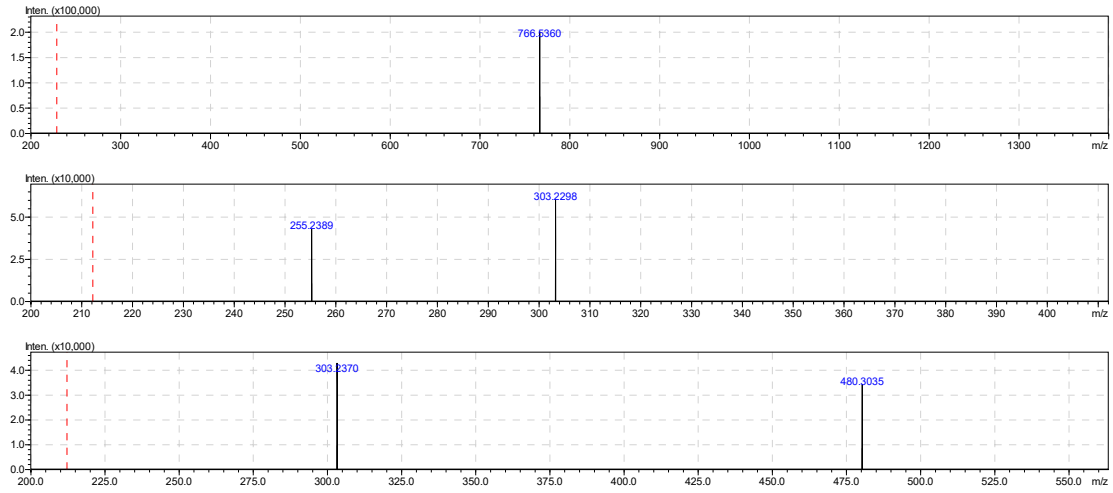
(**A**)



(**B**)



(**C**)



(**D**)

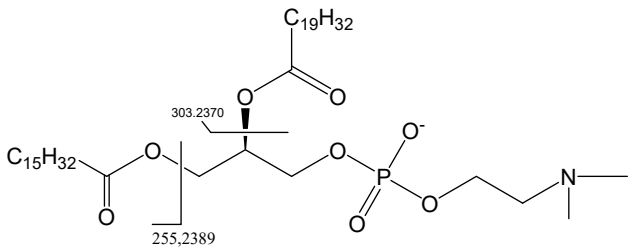


Figure 8. (**A**) The HPLC–HR/MS of total molecular species of PS class. (**B**) HPLC–HR/MS of the molecular species PC at *m/z* 782.5703. (**C**) Fragmentation MS^{2-} of PC 16:0/20:4. (**D**) Fragmentations of PC 36:4 (PC 16:0/20:4) with MS^{2-} assignment.

3. Materials and Methods

3.1. Material

A wild-caught sample of Cau gai vang *T. gratilla* (Linnaeus, 1758) was collected by divers in shallow water using specialized tools from the intertidal zone to a depth of about 70 m, on 17 November 2016, from Hon Tam Island, Nha Trang, Khanh Hoa, Vietnam (12°10′33.3″ N, 109°14′34.8″ E). Its scientific name was examined by Dr. Nguyen An Khang, Nha Trang Institute of Oceanography, Vietnam Academy of Science and Technology. After collection, the samples were stored in an insulated container and kept at a temperature of 0 to 4 °C while being transported by airplane to the laboratory in Hanoi. The total time of shipment was 2.5 h. A voucher specimen was deposited at the Institute of Natural Products Chemistry, VAST.

3.2. Extraction of Total Lipid

The sea urchins were cut in half and washed with distilled water. Eggs were separated from the body using a spoon. The eggs (TG-E) and body (TG-B) materials were immediately homogenized with a blender under cool conditions for 2 min before extracting the total lipid by using the Bligh and Dyer method [9]. Briefly, the eggs and body (each 300 g) materials were extracted with 900 mL of $CHCl_3$–MeOH (v/v = 1/2), the solid:liquid ratio being 1:3 (g/mL), and then sonicated for 2 h. Afterward, 300 mL of $CHCl_3$ and 600 mL of distilled water were added to the mixture for partitioning it. When partitioned, the lower layer (containing lipid) was separated, and the residue (upper layer) was extracted twice using continuous sonication for 2 h. Thus, the total extraction time was 6 h for one sample and the procedure was repeated three times. The combined lipid extract solution was dehydrated via anhydrous Na_2SO_4 and was evaporated to give a total lipid extract. The total lipid content was calculated as a percentage of lipid quantity compared to the fresh sample weight. Finally, the lipid extract was stored in pure $CHCl_3$ at −20 °C. Lipid samples used for further analyses were prepared daily by diluting them in a mixture of $CHCl_3$ and MeOH (v/v = 1:2).

3.3. Analysis of Polar Lipid Classes

Polar lipid classes composition was first analyzed via thin-layer chromatography (TLC) using silica gel plates (Sorbfil, Krasnodar, Russia) and a solvent system of dichloromethane/diethyl ether/NH_3 (65:35:4, $v/v/v$). Then, the TLC plate was sprayed with 10% H_2SO_4 in MeOH and heated at 240 °C for 10 min after air-drying. The PL classes were detected by comparing their Rf values with each standard sample. Polar lipid standards PC (16:0–20:4), PE (16:0–20:4), PS (16:0–20:4), PI (18:0–20:4), C18(Plasm)-20:4 PC, and C18(Plasm)-20:4 PE were purchased from Avanti Polar Lipids Co. (Alabaster, AL, USA).

3.4. Analysis of Molecular Species of Phospholipids

The molecular species of polar lipids (phospholipids) from eggs and body materials were detected using previously described HRMS fragmentations of PL standards [10,11]. The high-performance liquid chromatography/high-resolution tandem ion trap–time of the flight mass spectrometry with a Shimadzu LCMS-IT-TOF instrument (Shimadzu, Kyoto, Japan) was used to analyze the molecular species of PL. The LCMS-IT-TOF equipped with two LC-20AD pump units, a high-pressure gradient forming module, 9CCTO-20A column oven, SIL-20A auto sampler, CBM-20A communications bus module, DGU-20A3 degasser, and a Shim-Pack diol column (50 mm × 4.6 mm ID, 5 μm particle size) was operated both at positive and negative ion modes during each analysis at electrospray ionization (ESI) conditions. The ion source temperature was 200 °C, the range of detection was m/z 200–1600, and the potential in the ion source was −3.5 and 4.5 kV for negative and positive modes, respectively. The drying gas (N2) pressure was 200 kPa. The nebulizer gas (N2) flow was 1.5 L/min. The mobile phase condition for separation of PoL was performed using a binary gradient consisting of solvent mixture A: *n*-hexane/2-propanol/acid formic/$(C_2H_5)_3N$ (82:17:1:0.08, $v/v/v/v$) and mixture B: 2-propanol/H_2O/acid formic/$(C_2H_5)_3N$ (85:14:1:0.08, $v/v/v/v$).

The gradient started at 5% of mixture B, and its percentage increased to 80% over 25 min. This composition was maintained for 1 min before being returned to 5% of mixture B for more than 10 min and maintained at 5% for another 4 min (total run time of 40 min). The flow rate was 0.2 mL/min. Polar lipids were detected via high-resolution mass spectrometry (HRMS) and compared against authentic standards using a Shimadzu LCMS Solution control and processing software (v.3.60.361, Shimadzu, Kyoto, Japan). Individual molecular species within each PL class was measured by calculating the peak areas on extracted ion chromatograms [12].

4. Conclusions

Through experimentation to determine the total lipid and lipid classes of both eggs and body material from sea urchin *T. Gratilla*, this study found that the total lipid of eggs was much higher than that of the body sample (4.41% vs. 1.32% on wet weight base, respectively). The seven lipid classes of total lipid were hydrocarbon and wax (HW), triacylglycerol (TAG), free fatty acids (FFA), sterol (ST), polar lipid (PoL), and monoalkyl-diacylglycerol (MADAG). Among these, the proportions of TAG accounted for the highest amount from approximately 76% to 78%. The two lowest classes were HW and MADAG with percentages of 1.1% and 2.3% of the total lipid for eggs and body materials, respectively.

To our knowledge, this is the first study to determine the total lipid, lipid classes, fatty acid compositions, and phospholipid molecular species of sea urchins in general and *T. gratilla* in particular. The five types of phospholipids were identified as PI, PS, PE, PA, and PC with 137 molecular species in total for each sample. PI 18:0/20:4, PS 20:1/20:1, PE 18:1e/20:4, and PC 16:0/20:4 were the most abundant phospholipid species with the highest contents of 38.65–48.19%, 42.48–44.41%, 41.21–40.03%, 52.42–52.60% and 7.77–7.18% in each class of the body–eggs, respectively.

In both body and eggs, PoLs of the sea urchin *T. gratilla*, C20:4n was the most abundant polyunsaturated fatty acid in PI, PE, and PC classes, while C16, C18, C20, and C22 saturated fatty acids were less common. The most dominant polyunsaturated fatty acid in PI, PE, and PC was tetracosapolyenoic C20, while unsaturated fatty acid C20:1 was the most dominant in PS and PA classes. To our knowledge, this is the first time that the chemical properties of TL, especially the phospholipid molecular species of the PoL in the Vietnamese sea urchin (*T. gratilla*), have been studied.

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Sample Availability: All materials regarding the sea urchin can be supported by the authors.

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