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Research on the component of lipid classes, fatty acid from egg and body of sea urchin *Diadema savignyi* (Audouin, 1809)

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Abstract
Sea urchin is an invertebrate member of the phylum Echinodermata of marine animals living on the ocean floor. It has become increasingly important because of its economic potential, nutritional value and medicinal properties. Recently, sea urchin has been considered as a golden food due to its positive effect on human health. In terms of medicine and nutrition, the gonad of sea urchin is a rich source of polyunsaturated fatty acids (PUFAs). In this research, we focused on determining the total lipid contents, the components of lipid classes and the compositions and contents of fatty acids from the body and egg of *D. savignyi* collected in Hon Tam, Nha Trang, Khanh Hoa, Viet Nam in 2016. Although total lipid contents in egg and body of *D. savignyi* are not high in fresh weight basis, they are full of essential lipid ingredients such as wax (H + W), triacylglycerol (TG), monodiacylglycerol MDAG, free fatty acid (FFA), sterol (ST), polar lipid (PL). Palmitic acid (16:0) accounted for the largest proportion of saturated fatty acids in both egg and body samples. Beside this, eicosenoic (20:1n-9) is dominent fatty acid in the monounsaturated fatty acid group (MUFA) group. In polyunsaturated fatty acid group (PUFA), arachidonic acid (20:4n-6) made up the highest proportion, accounting for over 50%. Furthermore, the research result also showed the existences of four omega-3 fatty acids in the egg and body of *D. savignyi*, with the total contents of 2.31% and 2.47% respectively, especially the presence of eicosapentaenoic fatty acid (C20:5n-3, EPA), a very valuable fatty acid. The PUFA/SFA ratio and the n3/n6 ratio present in the total lipid contents of the egg and the body of *D. savignyi* have been found to meet the WHO standards for healthy food.

Keywords: *Diadema savignyi*, lipid classes, black sea urchin, echinodermata, fatty acid conten

1. Introduction
Sea urchins are invertebrates of Echinodermata living on the ocean floor. Up to now, over 800 species of sea urchin have been found. They have become increasingly important because of their economic potential, nutritional values and medicinal properties (Ayyagari, 2016) [2].
Several studies of chemical compositions have shown that steroids, saponins, and cerebrosides are major chemical compositions of echinoderms. Recently, gonads of sea urchin have been considered as a golden food due to their positive effects on human health with the major nutritional components such as: polypeptides, polysaccharides, carotenoids, vitamins and minerals. In terms of medicinal and nutritional aspects, the genital glands of sea urchin are rich in polyunsaturated fatty acids (PUFAs) (Tolga, 2007) [20]. They are not only useful for improving intensively the condition as well as the incidence of patients infected with heart diseases but also can prevent high blood pressure, inflammation, arrhythmia and cancer (James, 2009) [12]. Studies on the compositions of lipid classes and fatty acid of sea urchin are attracting many research groups due to their abundance of active lipid content, especially the omega-3, -6, and -9 (Long, 2005) [15].

2. Materials and Methods
2.1. Material
The sea urchins *D. savignyi* were collected in Hon Tam, Nha Trang, Khanh Hoa, Viet Nam and their scientific names were identified by Dr. Nguyen An Khang, Nha Trang Institute of Oceanography. Voucher specimens were deposited at Institute of Natural Products Chemistry, VAST under standard conditions at 4^{o}C.

2.2. Total lipid analysis
The total lipid extracts of egg and body of *D. savignyi* are extracted using the Bligh & Dyer method (Bligh, 1957) [8]. Briefly, after homogenization for 2 min by a blender under cooled

condition, 90 mL of chloroform and methanol (v/v = 1/2) were added to the eggs and body (about 100 g) and then sonicated for 4 h. 30 mL of chloroform and 60 mL of water were further added to the sample mixtures and leaved for partition. When partitioned, the lower layer (containing lipid) was seperated and the residue (upper layer) was repeated the extraction twice again using sonication for 2 h. The combined lipid extract solution was dehydrated by anhydrous Na_2SO_4 and evaporatted to give a total lipid extract. The total lipid content was calculated as a percentage of lipid quantity compared to the original fresh sample weight. The lipid extract was stored in pure $CHCl_3$ at 20 °C for further studies.

2.3 Lipid Classes analysis

Lipid classes were determinded on thin-layer chromatography (TLC) using the precoated silica gel plates (6cm × 6cm, Sorbfil, Krasnodar, Russia). Briefly, 5 µL, 10 µL, and 15 µL of the total lipid solution at the same concentration were loaded on to a NP_TLC (6cm × 6cm, Sorbfil, Krasnodar, Russia). The TLC was fisrt developed with a solvent system of *n*-Hexane/diethyl ether/acetic acid (v/v/v = 85/15/1) and second developed with cloroform/methanol/water (65:35:5, by volume) to 1/6 the height of TLC. The TLC was dried under room temperature and then sprayed with 10% of H_2SO_4 in MeOH before heating at 210 ºC for 20 min. The TLC was then scanned using Epson Perfection 2400 PHOTO (Nagano, Japan) scanner under standard condition with grayscale. The lipid content was further calculated by using the image analysis software, Sorbfil TLC Videodensitometer (Krasnodar, Russia) (Hamoutene, 2008; Khotimchenco, 2000) [11, 13]. The experiment was repeated three times.

2.4 Fatty acids analysis

The composition and content of fatty acids presented in the total lipid extract was determined based on the ISO/DIS 5590:1998 method. Firstly, 10 mg of total lipid extract was added with 25µl CH_3ONa solution in methanol (2M), shaked well for 1 min, then added 1mL of distilled water and centrifuged at 3000 rpm. The non-reactive wax layer was removed. The mixture was added with 100 µL of HCl and shaked well before centrifugation at 3000 rpm. The supernatant was collected and dehydrated by Na_2SO_4. Filtration of this solution before centrifugation at 3000 rpm yielded the methyl ester of the fatty acid (FAMEs). FAMEs were analyzed by GCMS (Shimadzu QP 2010 Ultra series, capilary column DBXLB: 30 m x 0.25 mm x 0.25 µm), GC temprature program: 200ºC for 10 minutes, from 200ºC to 230ºC in 5 min, 230ºC for 10 minnutes, carrier gas is He. The mass spectra of FAMEs were compared with the Mass Spectral Library: WILEY275.L and NIST 98 (The AOCS lipid library, 2014) [22].

2.5 Statistical analysis

The difference between mean values was analyzed by one-way analysis (ANOVA), using Excel 2010 software. The results were presented as: mean ± SD.

3. Results and Discussion

3.1. The total lipid content

The total lipid contents of the egg and body of sea urchin *D. savignyi* were calculated based on the percentage of lipid content in the fresh samples. The results are presented in Table 1.

Table 1: Total lipid contents in egg and body of sea urchin *D. savignyi*

No	Sample	Code	Total lipid *(% weight of fresh weight)*
1	Egg of *D. savignyi*	DE	3.18 ± 0.02
2	Body of *D. savignyi*	DB	1.33 ± 0.04

The total lipids were obtained as dark-yellow and odorless. The total lipid content in the egg was 2.5 times as high as that of the body of the sea urchin *D. savignyi*. The total lipid contents in egg of sea urchins are usually in the range of 2.37%-6.1% compared with fresh weight (Chandrika, 2002; Tolga, 2007) [4, 20] and often got the highest proportion in the spring and the lowest in the winter. In this study, the *D. savignyi* was collected in Autumn (August, 2017), therefor the total lipid content was obtained as low as 3.18 ± 0.02%, which was similar to previous reports (Ayyagari, 2016; Spiegel, 2003) [2, 19].

3.2. Composition and content of lipid classes in total lipid

The compositions of the lipid classes in the egg and body samples of *D. savignyi* were investigated and the result showed that TAG, FFA and ST were the major lipid classes in the non-polar lipid group of both the egg and body samples. This observation was in good agreement with previous publications (Cardin, 1953; Chandrika, 2002; Masatoshi, 1994) [1, 4, 16]. This is the first time that the lipid classes of the total lipid of the egg and body of *D. savignyi* have been reported.

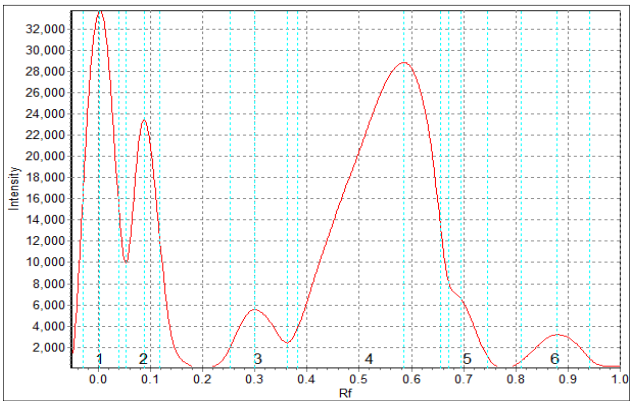

Fig 1: Lipid classes of the eggs of *D. savignyi* on Sorbfil TLC

In addition, TAG was the largest amount of lipid class in the total lipid of the egg sample (55.27%) and the second high amount (32.39%) after the polar lipid class in the body sample. However, the difference in content of TAG and the polar lipid class in the body sample was negligible (at about 1.55%) of TAG in the egg sample was significantly higher than that of the body. The polar lipid was also the major component of the total lipid in both the egg and the body samples with and 20.47% 33.94%, respectively. This result is similar to that reported by some reseacher group in the world when studying lipid profile in general and phospholipid in particular (Cardin, 1951; Chen, 2012) [1, 5].

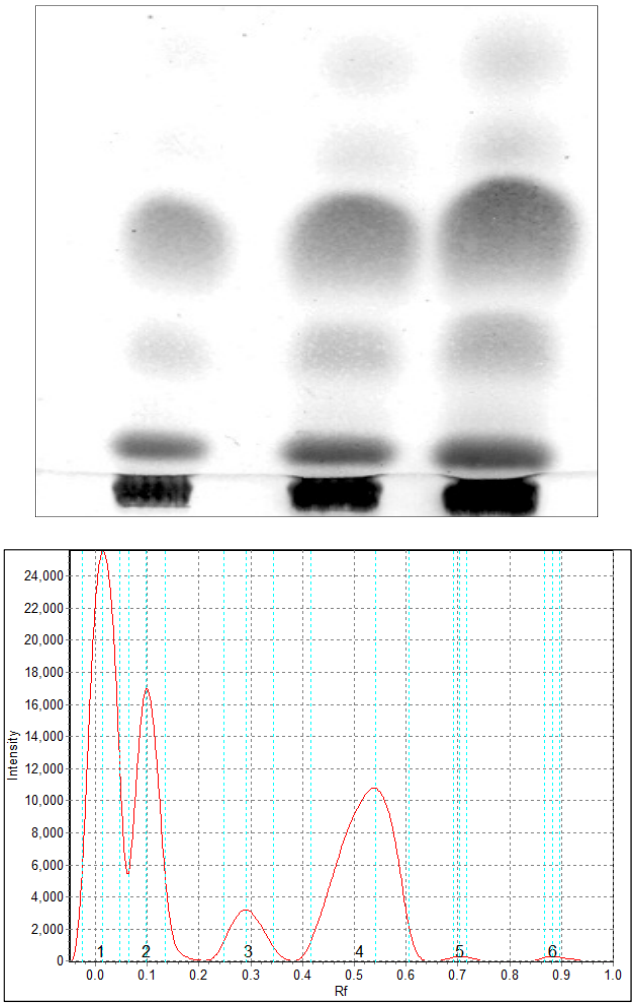

Fig 2: Lipid classes of *D. savignyi* body sample on Sorbfil TLC

Table 2. Composition and content of lipid classes of the egg and body of *D. savignyi*

No	Lipid class	Sample	
		Egg (DE)	Body (DB)
1	PL	20.47 ± 0.05	33.94 ± 0.60
2	ST	12.45 ± 0.01	19.57 ± 0.26
3	FFA	4.14 ± 0.05	9.28 ± 0.16
4	TG	55.27 ± 0.03	32.39 ± 0.30
5	MDAG	4.43 ± 0.04	2.50 ± 0.08
6	HW	3.24 ± 0.05	2.32 ± 0.07

Sterols are presented in most marine organisms and played biological importances such as membrane composition, a factor which resist to adverse environmental effects (Bernsdorff, 2003; Chen, 2012; David, 2013; Edidin, 2013) [3, 5, 6, 7]. In *D. savignyi*, sterol was the third abundant lipid class of the nonpolar-lipid with the percentage of 12.45% (in gg) and 19.57% (in body), respectively. FFA, HW, and MDAG displayed low contents, less than 10%, in both samples. Notably, the content of FFA in the body was twice as high as that in the egg. This raises the question of whether possibly lipase enzymes in the intestinal organs are involved in the hydrolysis of TAG into FFA. This was also recorded in the study on green sea urchin *Strongylocentrotus droebachiensi* by Chandrika in 2012 [4].

3.3. Composition and content of Fatty acids in total lipid of *D. savignyi* egg and body

The Fatty acids containing in the total lipid of the egg and body of *D. savignyi* were shown in the table 3 and they were very abundant. In this study, we found 27 fatty acids in the lipid sample of the egg and 30 fatty acids in the body sample with 14 to 24 carbon atoms. Most of them were 14:0, 16:0, 20:4n-6, 20:1n-9, 18:0 fatty acids with relatively high contents. In contrast, the ratios of residual Fatty acids were low. Fatty acids are really important because they affect to the flavor and the preservation of sea urchin egg (Ayyagari, 2016) [2]. For both the egg and body samples of *D. savignyi*, the saturated fatty acids (SFA) accounted for 60.08% and 52.26%, respectively, while the monounsaturated fatty acids (MUFAs) were 23.61% and 26.27% and the polyunsaturated fatty acids (PUFAs) were 15.96% and 20.94%, respectively. In the SFA component, palmitic acid (16:0) made up the highest ratio and exceeded other fatty acids. Myristic acid (14:0) and stearic acid (18:0) were followed. The remaining SFAs content was less than 1%. This was similar to the data reported by Chandrika *et al* (2002) [4], Mol *et al.* (2008) [17], and Ayyagari *et al.* (2016) [2]. In 1970s decade, these typical fatty acids were also found in some species of sea urchin such as *A. crassipina*, *S. pulcherrimus*, *S. franciscanus*, *S. intermedius* and *E. esculentus* and palmitic acid (16:0) was still the most dominant component of the SFA group and it was also followed by myristic acid (14:0) (Fujino, 1970) [9]. For fatty acid composition in MUFAs group, eicosenoic acid (20:1n-9) accounted for the largest proportion in both egg and body samples, at 9.90% and 12.90%, respectively. It was followed by oleic acid (18:1n-9) and vaccenic acid (18:1n-7) with no significant difference between the egg sample and the body specimen, fluctuating in about 3%. This result was significantly different from the observation reported by Guanqun Chen in 2010 when studying three species of sea urchin *Anthocidaris crassispina*, *Diadema setosum* and *Salmacis sphaeroides* collected in Hong Kong. His research indicated that in the compositions of MUFAs group, the concentration of vaccenic acid (18:1n-7) was highest ranging about 7.6% to 13% and eicosenoic acid (20:1n-9) ranged from 0 to 3% (Guanqun, 2010) [10]. Most of the remaining Fatty acids in the MUFAs group extracted from *Diadema savignyi* have a very low percentage, less than 1% of the total lipid.

For polyunsaturated fatty acids (PUFAs), arachidonic acid (20:4n-6) accounted for the highest proportion, at 8.58% in the total lipid of the egg, and at 11.34% in the total lipid of the body sample and held over 50% of the PUFAs component. Compared with the results of the study on the composition of PUFA found in the gland of *S. droebachensis*, these results were quite similar, although green sea urchin has been collected in 4 different seasons. In contrast to fatty acids 20:4n-6, other polyunsaturated fatty acids in both egg and body of *Diadema savignyi* had a very low content, most under 0.5% except for 18:2n-6 (at 1.29 %).

There were the presences of all four omega-3 fatty acids with a total content of 2.31% and 2.47%, especially eicosapentaenoic fatty acid (C20:5n-3 EPA) always had the highest rate, at about 1.75%. EPA is a valuable bioactive fatty acid, this has been published in many previous works (Khotimchenko, 2000; Khotimchenko, 2009; Nalin, 2012; Thanh, 2013) [13, 14, 18, 21].

In addition, the PUFA/SFA ratio and the n3/n6 ratio found in the total lipid of the egg and the body of *D. savignyi* were 0.66/0.90 and 0.17/0.14, respectively. According to WHO, the total lipid extracts from the egg and the body of *D. savignyi* used in the study were included in the top-quality food and very good for human health with the ratios of PUFA/SFA ≥ 0.4 and n3 /n6 ≥ 0.1.

Table 3: Composition and content of fatty acids (%) in egg and body samples of *Diadema savignyi*

No	Sample / Fatty Acids	Egg (DE)	Body (DB)
1	14:0	17.35±0.77	9.38±0.27
2	15:0	1.35±0.04	1.59±0.03
3	16:1n-9	4.41±0.13	2.72±0.04
4	16:2n-6	0.37±0.01	0.15±0.00
5	16:0	0.15±0.00	-
6	16:0	31.40±0.15	27.49±0.21
7	17:0	1.77±0.04	1.69±0.06
8	18:4n-3	0.24±0.01	0.29±0.01
9	18:2n-6	1.29±0.05	1.01±0.02
10	18:1n-9	3.42±0.06	2.93±0.11
11	18:1n-7	2.78±0.07	2.75±0.09
12	18:3n-6	0.51±0.02	0.35±0.01
13	19:0	-	0.27±0.00
14	18:0	5.74±0.14	9.71±0.04
15	19:0	0.41±0.02	1.16±0.02
16	19:1n-9	0.96±0.01	1.77±0.00
17	20:0	0.69±0.02	0.46±0.01
18	20:1n-7	0.50±0.02	0.63±0.04
19	20:1n-9	9.90±0.36	12.90±0.25
20	20:4n-6	8.58±0.09	11.34±0.41
21	20:5n-3	1.76±0.05	1.75±0.01
22	20:2n-6	2.07±0.06	4.48±0.10
23	21:1n-9	0.64±0.00	1.23±0.03
24	21:0	0.18±0.00	0.26±0.00
25	22:4n-6	0.18±0.00	0.20±0.00
26	22:4n-3	0.13±0.01	0.30±0.01
18	22:6n-3	0.18±0.00	0.13±0.00
19	22:1n-9	0.93±0.04	0.96±0.04
20	22:1n-7	0.07±0.00	0.18±0.01
21	22:2n-6	-	0.14±0.00
22	22:0	0.12±0.01	0.26±0.01
23	23:1n	-	0.22±0.00
24	23:0	0.24±0.01	0.17±0.01
25	26:2n-6	0.28±0.01	0.26±0.00
26	24:0	0.09±0.00	0.11±0.00
27	24:1n-7	0.17±0.01	0.17±0.01
28	24:1n-9	0.20±0.01	0.35±0.01
29	other	0.13±0.00	0.51±0.02
SFA		60.08	52.26
MUFA		23.61	26.27
PUFA		15.96	20.94
Omega-3		2.31	2.47
Omega-6		13.28	17.67
Omega-9		20.46	22.86
PUFA/SFA		0.66	0.90
	n3/n6	0.17	0.14

SFA: saturated fatty acids, USFA: unsaturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

4. Conclusion

This report showed that according to the experience of the people living near the sea, this black sea urchin *D. savignyi* is not considered as the main source for harvesting food as other sea urchin species in Nha Trang, Vietnam but in fact it contains the polar lipids, sterols with quite high percentages, and it is also rich in fatty acids. In addition, the ratios of PUFA/SFA, n3/n6 fully match WHO's standard for healthy food. This result opens new avenues for more efficient exploitation and ultilization of black sea urchin *Diadema savignyi*-A species of marine organism currently considered to have no value.

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