RESEARCH ON THE COMPOSITION OF LIPIDS, FATTY ACIDS, AND AMINO ACIDS FROM EGG AND BODY OF SEA URCHIN *TRIPNEUSTES GRATILLA*

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ABSTRACT

The Sea urchin *Tripneustes gratilla* (Linnaeus, 1758) is a famous species of invertebrate member of the phylum Echinodermata of marine animals living in shallow tropical water. In Viet Nam, it is usually called by the name of yellow sea urchin. In this research, we focused on determining the total lipid contents, the components of lipid classes and the compositions and contents of fatty acids, amino acids from the body and the egg of the yellow sea urchin *T. gratilla* (Linnaeus, 1758) collected in Hon Tam, Nha Trang, Khanh Hoa, Vietnam in 2016.

Although the yellow sea urchin *T. gratilla* has low percentage of total lipid in fresh weigh basis, it consists of essential lipid ingredients such as Wax and Hydrocabon (H + W), Triacylglycerol (TG), Monodiacylglycerol MDAG, Free Fatty Acid (FFA), sterol (ST), and Polar Lipid (PL). In both egg and body samples palmitic acid (16:0) was the most abundant fatty acid with the proportion of about 25 %. In addition, arachidonic acid (20:4n-6) is the dominant Omega-6 fatty acid in the Monounsaturated Fatty Acid group (MUFA). Furthermore, the research result also showed the very high percentage of omega-3 fatty acids in the egg and body of *T. gratilla*, with the total content of 13.97 % and 20.67 %, respectively, especially the presence of eicosapentaenoic fatty acid (C20:5n-3, EPA), a very valuable fatty acid, was also observed. By the HPLC method, the research also found 17 amino acids in the egg and body of *T. gratilla* with the existence of 7 essential amino acids.

Keywords: Tripneustes gratilla, lipid classes, yellow sea urchin, echinodermata, fatty acid content.

1. INTRODUCTION

Sea urchins are invertebrates of the phylum Echinodermata that are omnivorous animals and therefore eat both plant and animal matter. They mainly feed on algae on the coral and rocks, along with decomposing matter such as dead fish, mussels, sponges and barnacles. Up to now, about 800 species of sea urchin have been found. They are half-moon shaped, yellow-orange in colour, and chiefly composed of moisture, protein, carbohydrate and lipid [1]. This species is mentioned much about its economic and commerce properties. Recently, many researchers have been paying attention to sea urchin because of its positive effect on human health. In terms of medicine and nutrition, the egg of yellow sea urchin was rich in Polyunsaturated Fatty Acids (PUFAs) and essential amino acids. Many recent researches have demonstrated that sea urchins have many valuable features such as nutritional values and medicinal properties [2]. These values from chemical compositions of egg and body of sea urchin also are main components of echinoderms. They are steroids, saponins, and cerebrosides. Recently, in many countries, with distinctive aroma and good taste, the egg of sea urchins have become the healthy food because of their essential effects on human health arising from their polypeptides, polysaccharides, carotenoids, vitamins and mineral [1, 3]. The studies on the compositions of lipid classes, fatty acids, medicinal and nutritional aspects of sea urchin are inspiring many researchers due to their abundance of active lipid contents, especially the omega -3, -6, -9 and polyunsaturated fatty acids (PUFAs) [3]. They play an important role in curing the condition as well as the incidence of patients infected with heart diseases and controlling the pressure of blood, inflammation, arrhythmia and cancer [4]. This research illustrates results of lipid classes, fatty acid contents and amino acids of egg and body samples of *T. gratilla* collected in Hon Tam, Nha Trang, Khanh Hoa, Vietnam in 2016.

2. MATERIALS AND METHODS

2.1. Materials

The sea urchins *Tripneustes gratilla* (Linnaeus, 1758) were collected in Hon Tam, Nha Trang, Khanh Hoa, Viet Nam and their scientific names were examined by Dr. Nguyen An Khang, Nha Trang Institute of Oceanography, Vietnam Academy of Science and Technology. All samples were cleaned and stored under standard condition with the temperature of 4 ^{o}C. Voucher specimens were deposited at Institute of Natural Products Chemistry, VAST.

2.2. Total lipid analysis

The total lipid contents of egg and body samples are extracted according to the Bligh & Dyer method [5]. Shortly, after homogenization for 1-2 mm 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 6 h. 30 ml of chloroform and 60 ml of water were further added to the sample mixtures and leaved for separation. When partitioned, the lower layer (containing lipid) was separated and the residue (upper layer) was extracted twice using sonication for 2 h. The combined lipid extract solution was dehydrated by anhydrous Na_2SO_4 and after that it was evaporated 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. Finally, the lipid extract was stored in pure $CHCl_3$ at 20 °C. The dilute lipid samples used for further analysis were prepared daily, by diluting them using a mixture of chloroform and methanol (v/v = 1:2).

2.3. Lipid class analysis

Lipid classes were identified on thin-layer chromatography (TLC) using the precoated silica gel plates (6 cm × 6 cm, Sorbfil, Krasnodar, Russia). Firstly, 5 µl, 10 µl, and 15 µl of the total lipid solution at the same concentration were loaded on to a NP_TLC (6 cm × 6 cm, Sorbfil, Krasnodar, Russia). The TLC was initially developed with a solvent system of hexane/diethyl ether/acetic acid (v/v/v = 85/15/1) and developed with chloroform/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 heated at 210 ºC for 20 min. The TLC was then scanned by Epson Perfection 2400 PHOTO (Nagano, Japan) scanner under standard condition with grayscale. The lipid content was further calculated by the image analysis software, Sorbfil TLC Videodensitometer (Krasnodar, Russia) (Hamoutene, 2008; Khotimchenco, 2000) [6, 7]. The experiment was repeated three times.

2.4. Fatty acids analysis

The compositions and contents of fatty acids present in the total lipid were determined by ISO/DIS 5590:1998 method and all fatty acids must be converted to methyl ester forms. Firstly, 10 mg total lipid was added with 25 µl CH_3ONa solution in methanol (2 M), shaken well for 1 min, then added 1ml of distilled water and centrifuged at 3000 rpm for 1 min. The non-reactive wax layer was removed. Following this, the mixture was complemented with 100 µl of HCl, shaken well and centrifuged at 3000 rpm again. After the removal of the non - reactive wax layer, the upper layer was dehydrated by Na_2SO_4 and centrifuged at 3000 rpm/min. By filtration process the methyl ester of the fatty acid (FAMEs) was achieved. FAMEs were analyzed by GCMS Shimadzu QP 2010 Ultra series, capillary column DBXLB, (30 m × 0.25 mm × 0.25 µm), GC temperature program: 200 ^oC for 10 min, from 200 ^oC to 230 ^oC in 5 min, 230 ^oC for 10 min, and He was used as carrier gas. The mass spectra of FAMEs were compared with the Mass Spectral Library: WILEY275.L and NIST 98 [8].

2.4. 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 *T. gratilla* were determined based on the proportion of lipid content in the fresh samples. Results are illustrated in Table 1.

Table 1. Total lipid contents in egg and body of sea urchin *T. Gratilla.*

No	Sample	Code	Total lipid (% weight of fresh weight)
1	Egg of *T. gratilla*	VE	4.41 ± 0.03
2	Body of *T. gratilla*	VB	1.32 ± 0.03

The lipids from egg and body of sea urchin *T. gratilla* were bright yellow and odorless. The total lipid content of the egg was much higher than that of the body sample from sea urchin *T. Gratilla*, about 3.35 times higher. Compared with the results about lipid of black sea urchin *Diadema setosum*, one of the most popular sea urchin species in Nha Trang, the proportion of lipid in egg of *T. gratilla* was higher than that in egg of *Diadema setosum* (3.18 %) but the lipid in body of both species was nearly the same, at about 1.33 % [9]. Many previous reseaches have indicated that the total lipid contents in egg of sea urchins are usually in the range of 2.37 - 6.1 % compared with fresh weight [10] and often reach the highest propotion in the spring and the lowest in the winter. Therefore, with these *T. gratilla* samples collected in autumn (8/2017), the lipid content at 4.41 ± 0.03 % was reasonable and similar to previous researches on some species of sea urchin [11].

3.2. Composition and content of lipid classes in total lipid

Lipid class compositions of the studied species were listed in Table 2. It can be seen from Figure 1 and Figure 2 that the lipid of the egg and body samples from sea urchin *T. gratilla* included seven classes. Many previous studies reported that the lipids of several species of sea urchin consisted of 6 classes, [12, 13] especially with the sea urchin *Diadema savignyi* collected at the same area with researched sea urchin *T. gratilla* [9]. The appearance of mono and diacyl glycerol was discovered. The lipid classes in the egg and body samples of *T. gratilla* were identified that the typical component was triglyceride, that was also reported from several previous publications. This is the first time the lipid classes of the total lipid of the egg and body of *T. gratilla* have been reported.

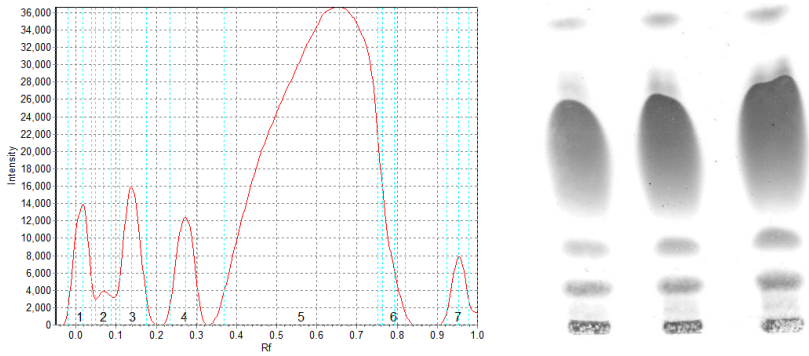

Figure 1. Lipid classes of the eggs of *T. gratilla* on Sorbfil TLC.

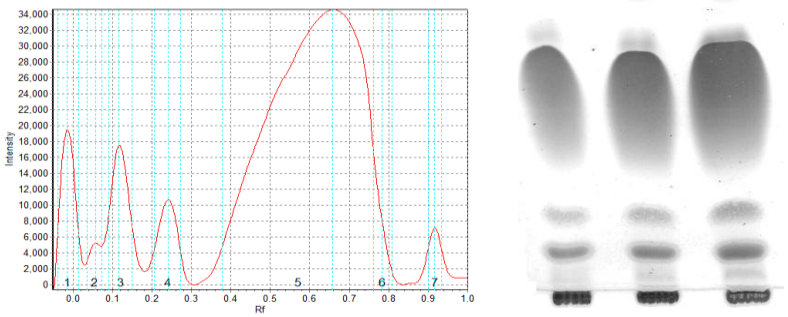

Figure 2. Lipid classes of the body of *T. gratilla* on Sorbfil TL.

Table 2. Results of composition and content of lipid classes of the egg and body of *T. gratilla*.

No	Lipid class	Sample	
		Egg of *T. gratilla*	Body of *T. gratilla*
1	Polar lipid	4.41 ± 0.05	6.36 ± 0.04
2	Mono and Diacyl Glycerol	1.11 ± 0.01	1.43 ± 0.01
3	Sterol	5.69 ± 0.05	6.63 ± 0.04
4	Fatty Acids	4.76 ± 0.03	4.49 ± 0.03
5	Triglyceride	78.37 ± 0.64	76.10 ± 0.57
6	Monoalkyl Diacylglycerol	3.31 ± 0.05	3.24 ± 0.05
7	Hydrocacbon + Wax	2.35± 0.04	1.75 ± 0.02

The results from Table 2 illustrated that triacylglycerol was the largest and most outstanding class in the total lipid of both egg sample and body sample, at 78.37 % and 76.10 %, respectively. In addition, the difference in content of triacylglycerol in these two researched samples was negligible (at about 2.27 %). Differently from previous studies about lipid profile of sea urchin, the polar lipid contents of egg and body from sea urchin *T. gratilla* were much lower, which were 4.41 % and 6.36%, respectively [14]. Sterol is a compound found in most marine organisms because of its biological importance. One of its popular roles is being a component of the membrane, which can be resistant to adverse environmental effects [15, 16, 17]. This study showed that the percentage of sterol was as low as that of polar lipid layer, with 5.69 % in egg sample and 6.63 % in the body sample. The remaining three groups (fatty acids, monoalkyl diacylglycerol, and hydrocarbon + wax) were low in contents, less than 5 % for both samples. Notably the difference between these contents in the body and the egg of the sea urchin *T. gratilla* was inappreciable.

3.3. Composition and content of fatty acids in total lipid of *T. gratilla* egg and body

The fatty acids composition analysis results from the body and the egg samples Table 3.

It is clear that the fatty acids in the total lipid of the egg and body of *T. gratilla* were abundant. In this study, twenty five and twenty four fatty acids were identified in the egg and the body sample with 12 to 22 carbon atoms, respectively. The difference between percentages of SFA in the egg and the body sample from *T. gratilla* was significant, at 41.74 % with the egg sample and 17.22 % with body sample. The 14:0, 16:1n-9, 16:0, 18:1n-9, 20:4n-6, 20:5n-3 fatty acids accounted for relatively high contents. In contrast, the ratios of residual fatty acids were low. Fatty acids are really important because they affect the flavor and preservation of sea urchin egg. For both egg samples and body samples of *T. gratilla* in this report, the percentages of fatty acids with one double bond in the structural formula (MUFAs) were 26.53 % and 18.01 % and these numbers of polyunsaturated fatty acids (PUFAs) were 31.08 % and 60.50 %, respectively.

Palmitic acid (C16:0) and myristic acid (14:0) were observed to be predominant SFAs in all samples, ranging from 3.59 % to 14.50 % (C14:0), and from 11.74 % to 25.10 % (C18:0), respectively in egg and body samples. This was agreed with previous studies [18]. On the other hand, most of remaining saturated fatty acids accounted for less than 1 %. This result is similar

to the publication about sea urchin *Diadema savignyi* [9], sea urchin *S. droebacheinsis* in 2002 by C. Liyana-Pathirana et al. [10] and about *P. lividus* by Mol S. Baygar et al. [19].

Table 3. Composition and content of fatty acids (%) in egg and body samples of *T. gratilla.*

No	Sample / Fatty acids	Egg of *T. gratilla*	Body of *T. gratilla*	No	Sample / Fatty acids	Egg 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	14:1n-7	2.03	0.33	17	20:3n-3	0.32	0.68
4	15:0	0.44	0.20	18	20:2n-6	0.67	-
5	16:1n-9	8.66	3.59	19	20:1n-9	2.50	6.25
6	16:2n-4	0.32	-	20	20:4n-6	10.95	30.96
7	16:1n-7	3.08	2.04	21	20:5n-3	6.42	13.39
8	16:0	25.10	11.74	22	20:3n-6	2.46	5.55
9	18:4n-3	3.67	2.64	23	20:4n-3	1.15	1.46
10	18:2n-6	1.86	1.81	24	22:6n-3	0.22	0.31
11	18:1n-9	8.87	4.62	25	22:1n-9	0.52	0.27
12	18:1n-7	0.87	0.91	26	22:6n-6	-	0.30
13	18:3n-3	2.19	2.19	27	22:4n-6	-	0.57
14	18:3n-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		74.46	3.51
Omega-3		13.97	20.67	n3/n6		0.83	0.52

(SFA: Saturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, PUFA: Polyunsaturated Fatty Acids)

The proportions of total MUFAs were about 26.53 % and 18.01 % for researched egg and body samples, respectively. In this group, the eicosenoic acid (20:1n-9) accounted for the largest proportion in the body sample, at 6.25 %, while 16:1n-9 and 18:1n-9 were the most abundant monounsaturated fatty acids in the egg sample, with the proportion of approximate 9 %. In addition, the other fatty acids in the MUFAs group extracted from *T. gratilla* have had a very low percentage, less than 1 % of the total lipid.

Compared with MUFAs group, the contents of polyunsaturated fatty acids (PUFAs) were much higher, at 31.08 % with egg sample and 60.50 % with body sample. A remarkable characteristic was realized in the body of *T. gratilla*, the percentage of PUFAs was high notably, nearly twice as much as that of the egg. The arachidonic acid (20:4n-6) accounted for the highest proportion, at 10.95 % in the total lipid of the egg, and at 30.96 % in the total lipid of the body sample and held nearly 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. Following this trend,

20:3n-6, 20:4n-6 and 20:5n-3 had considerable proportions, especially 20:5n-3 held 13.39 % in the body. Furthermore, other polyunsaturated fatty acids in both egg and body of *T. gratilla* had relatively low contents, most under 3 % except 18:4n-3, 20:4n-6 and 20:3n-6.

From Table 3, the presence of omega-3, omega-6 and omega-9 fatty acid groups was determined. In the omega-3 group, the amount of eicosapentaenoic fatty acid (C20:5n-3 EPA) were 6.42 % in the egg and 13.39 % in the body sample and higher than those in previous publications. In addition, the valuable bioactivity of EPA has been published in many previous works. This can be concluded that the sea urchin *T. gratilla* promises to become a good source of healthy food.

Another noteworthy thing is that the PUFA/SFA ratio and the n3/n6 ratio found in the total lipid from the egg and the body of *T. gratilla* were (74.46 and 3.51) and (0.83 and 0.52) and these figures were high. Hence, according to the World Health Organization (WHO), the egg and lipid extract from *T. gratilla* used in the research were included in the top - quality food and very good for human health [(PUFA/SFA $\geq$ 0.4) and (n3 /n6 $\geq$ 0.1)].

3.4. Composition and content of amino acids in total lipid of *T. gratilla* egg and body

The amount of free amino acids of fresh egg sample and body sample from sea urchin *T. gratilla* were shown in Table 4.

Table 4. Content of free amino acids of egg and body from sea urchin *T. gratilla*.

No	Amino acid	The amount of free amino acids (mg/g)		No	Amino acid	The amount of free amino acids (mg/g)	
		Egg of *T. gratilla*	Body of *T. gratilla*			Egg of *T. gratilla*	Body of *T. gratilla*
1	Aspartate	-	-	10	Cysteine	-	-
2	Glutamate	-	-	11	Valine *	-	-
3	Serine	-	-	12	Methionine *	-	-
4	Histidine *	-	-	13	Phenylalanine *	-	-
5	Glycine	31.76	20.47	14	Isoleucine *	-	-
6	Threonine *	-	15.07	15	Leucine *	-	1.94
7	Arginine	4.45	5,22	16	Lysine *	-	-
8	Alanine	-	-	17	Proline	226.65	154.57
9	Tyrosine	-	-				
Amount of free amino acids		262.86	197.27				
Essential amino acids		0	17.01				
Common amino acids		262.86	180.26				

(-: trace; *: Essential amino acid).

The results from Table 4 illustrated that 17 amino acids were found in the egg and body samples of sea urchin *T. gratilla*, made by HPLC method. This result is similar to previous researches on other species of sea urchin. It was special that there was no essential amino acid which had high content in the egg sample. In contrast, from body sample the existences of threonine and leucine were revealed, at 15.07 and 1.94 mg/g, respectively. In the inessential amino acid group, proline was the most abundant compound, at 226.65 mg/g with egg sample and 154.57 mg/g with body sample and followed by the glycine and threonine. This was also demonstrated in the research of Lopez J. H. in 2000 about amino acid contents of *Paracentrotus lividus* [20].

4. CONCLUSIONS

The report revealed-information about the total contents and classes of lipid in the egg and body samples of sea urchin *Tripneustes gratilla* (Linnaeus, 1758). There were 7 lipid classes and the total lipid contents of both egg and body from sea urchin *T. gratilla* were much higher than that of other sea urchins investigated before. It has been found that the fatty acids of sea urchin *T. gratilla* were diversified with 25 fatty acids discovered. In addition, SFA and PUFA were the main components, ranging from 17.22 to 60.50 %. The lipid of egg and body from sea urchin *T. gratilla* fulfiled the requirement of healthy and healthful human body according to WHO recommendations for PUFA/SFA and n3/n6 index.

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