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Study on lipid and fatty acid composition of *Thelenota ananas* collected from Bai Dong Island, Ha Long Bay, Vietnam

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ABSTRACT

The paper studies the lipid and fatty acid composition of the sea cucumber *Thelenota ananas* collected on Bai Dong Island, Ha Long Bay, Vietnam. The total lipid content of dry sample weight was relatively high (11.89%). The triacylglycerol TG has the highest concentration in total lipids with a ratio of up to 66.73%; the PL and PI classes do not have a significant difference with the proportions of 13.44 and 11.65% in total lipids. Two classes of sterol ST, hydrocarbon-wax HW and free fatty acid FFA account for a small proportion. The two main classes of phospholipids are PC and PE, with a ratio of 47.10% and 33.29% of total phospholipids. There are 36 fatty acids identified, of which total saturated fatty acids (SFA) account for 41.61%; Monounsaturated fatty acids (MUFA) account for 32.15%, and polyunsaturated fatty acids (PUFA) account for 25.63%; the main fatty acids are stearic acid (18:0) accounting for 13.15% of the total fatty acids; 16:1n-9 accounts for 14.87%, and 20:5n-3 EPA fatty acids account for 15.3% of the total fatty acids. Among the fatty acids, n-9 (omega 9) accounts for the highest proportion with 25.16%; n-3 fatty acids (omega 3) account for 18.43%; n-6 (omega 6), n-7 (omega 7) fatty acids account for 5.8 and 6.5%, respectively. In this study, the n3/n6 ratio of the sea cucumber sample *Thelenota ananas* was 3.18%, consistent with the evaluation standards of the World Health Organization (WHO) of n3/n6 ratio $\geq 0.1\%$. It is classified as a good foodstuff for human health; therefore, it can be considered a source of food that can be used to serve community health care needs.

Keywords: Lipid, fatty acids, *Thelenota ananas*, molecular species.

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INTRODUCTION

The sea cucumber *Thelenota ananas* belongs to the family Stichopodidae (Echinodermata) and it is distributed widely throughout the Indo-Pacific, India, Australia, Japan, China and Southeast Asia [1]. *Thelenota ananas* is one of the most famous sea cucumber species used as nutritional food for humans with high economic value [2]. Long Yu et al. found that *Thelenota ananas* has fucoidan, a polysaccharide found in brown algae, containing L-fucose and sulfate groups, which has many health benefits such as antioxidant, anti-inflammatory, anti-tumor, anticoagulant and anti-viral properties [3]. Based on these effects, *Thelenota ananas* has become a nutritious delicacy, making it of great commercial value. Sea cucumbers especially contain saponins, phospholipids rich in EPA that can regulate cholesterol metabolism, reduce blood fat and diabetes [4, 5]. Fatty acids are essential in the daily diet to provide energy for the body. *Thelenota ananas* contains two important fatty acids, including arachidonic acid (C20: 4n-6) with the content up to 7.54% and eicosapentaenoic acid (C20:5n-3) with the content up to 3.92% [6]. These two fatty acids positively affect on the heart and brain and reduce bad cholesterol [7, 8]. In Vietnam, the sea cucumber *Thelenota ananas* is mainly distributed in the coastal areas of Phu Yen, Khanh Hoa, Binh Thuan, Hoang Sa, Truong Sa, and Tho Chu (Dao Tan Ho, 2006) and it is widely caught and commercialized in the market.

Up-to-date, no detail study focuses on the lipid and fatty acid composition of the sea cucumber *Thelenota ananas* to evaluate the nutritional quality and impact of the living environment on this species. The detailed study of this species' chemical and biological composition might contribute to supplementing scientific information to guide the exploitation and use of their valuable bioactive compounds, creating medicinal products and increasing income for local people.

MATERIALS AND METHODS

Materials

The *Thelenota ananas* samples (Jaeger, 1833) were collected in May 2021 on Bai Dong Island, Ha Long Bay. Dr. Hoang Xuan Ben and colleagues identified the samples at the Institute of Oceanography in Nha Trang.



Figure 1. Sea cucumber samples *Thelenota ananas*

Methods

Total lipid extraction method

The total lipids of the sea cucumber samples were extracted according to the Folch J. F. method, using the solvent system CHCl_3 :MeOH at a ratio 2:1 by volume [9].

Determining the content and composition of lipid classes

Quantitative analysis was performed using thin-layer chromatography and the image analysis program Sorbfil TLC Video-densitometer DV (Krasnodar, Russia) [10, 11].

Total lipids were analyzed on 1-dimensional silica gel thin plates (6 × 6 cm, Sorbfil, Krasnodar, Russia), using the solvent system n-hexane:Et₂O:CH₃COOH (90:10:1, v:v:v), visualized with 10% H₂SO₄/MeOH. Dry the thin plate at 200°C for 15 minutes, then scan on Epson Perfection 2400 PHOTO (Nagano, Japan), with standard resolution and size. The

percentage of total lipids classes was determined based on area and color intensity measurements in the image analysis program Sorbfil TLC Videodensitometer DV (Krasnodar, Russia).

Determining the content and composition of phospholipid classes

Qualitative analysis of phospholipid classes: Phospholipid classes were determined by 2-dimensional thin layer chromatography on Sorbfil thin plates (10 × 10 cm, Sorbfil, Krasnodar, Russia), using solvent system 1: CHCl₃:MeOH:C₆H₆:28% NH₄OH (70:30:5:1, by volume) and the second solvent system: CHCl₃:MeOH:(CH₃)₂CO:CH₃COOH:H₂O (70:30:5:5:2, by volume). After drying, visualize with 0.2% ninhydrin reagent in BuOH at 100°C and determine the classes of PE, PS, and CAEP. Next, use molybdate reagent to determine the PC and PI classes [12].

Quantitative analysis of phospholipid classes: Total lipids were analyzed on Sorbfil thin plates (10 × 10 cm, Krasnodar, Russia) using solvent systems CHCl₃:MeOH:C₆H₆:28% NH₄OH (70:30:5:1, by volume), visualized with 10% H₂SO₄/MeOH reagent at a temperature of 200°C. The thin plates were scanned on an Epson Perfection 2400 PHOTO machine (Nagano, Japan) with standard resolution and size. The percentage of phospholipid classes was determined based on area and color intensity measurements in the image analysis program Sorbfil TLC Videodensitometer DV, Krasnodar, Russia [13].

Determining the content and composition of fatty acids

Fatty acids are methylated to methyl ester form with 2% H₂SO₄/MeOH, at a temperature 80°C for 2 hours. The obtained mixture was dissolved in n-hexane, then purified methyl ester mixture of fatty acid on a thin plate prepared with the solvent system n-hexane:Et₂O (85:15, by volume). The fatty acid methyl ester mixture was dissolved in n-hexane and analyzed on a gas chromatograph (GC) and gas chromatography-mass spectrometry (GC-

MS), Shimadzu (Kyoto, Japan), the NIST standard spectrum library was used for comparison.

RESULTS AND DISCUSSION

Total lipid content

The total lipid content of the sea cucumber sample *Thelenota ananas* is shown in Figure 2.

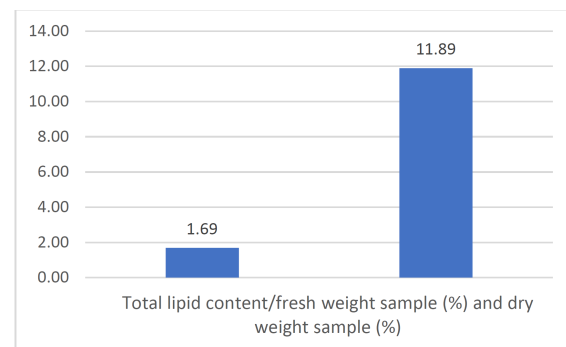


Figure 2. Total lipid content/fresh and dry weight samples of *Thelenota ananas*

Research results show that the total lipid content of *Thelenota ananas* samples collected on Bai Dong Island, Ha Long Bay, is 1.69% calculated on the fresh weight sample and 11.89% on the dry weight sample. These results are relatively high compared to the data on the total lipid of some other Vietnamese sea cucumber samples reported by T. T. T. Huong and colleagues in 2017 (0.29–0.43% calculated on fresh weight sample) [14]. The index of total lipid content can be influenced by species characteristics, possibly by the influence of many other conditions such as habitat depth or reproductive cycle, v.v.

Content and composition of lipid classes

Total lipid studies of the *Thelenota ananas* sample were carried out on TLC thin-layer chromatography and analyzed using the Sorbfil TLC Videodensitometer DV image analysis program. The TLC chromatogram image of the sample's total lipid is shown in Figure 3. The results on the content and composition of lipid

classes in the total lipid of the *Thelenota ananas* samples are shown in Figure 4.

The lipid classes composition in the total lipid of *Thelenota ananas* sample was studied

with the presence of all basic lipid classes, polar lipids (PL), sterols (ST), free fatty acids (FFA), triacylglycerol (TG), monoalkyldiacylglycerol (MADG) and hydrocarbon-wax (HW).

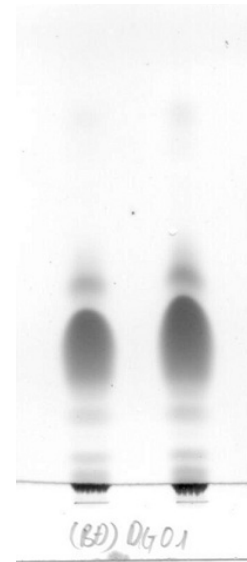
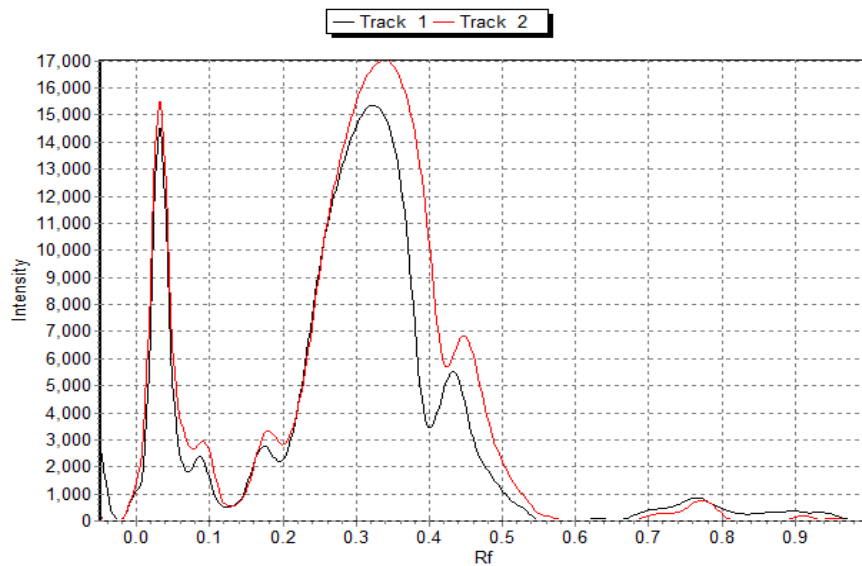


Figure 3. Sorfil TLC chromatogram of the *Thelenota ananas* sample

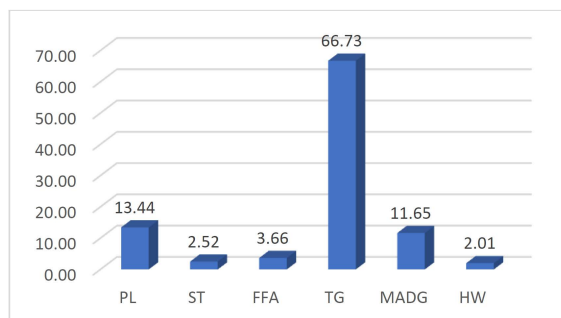


Figure 4. Content and composition of lipid classes in total lipid of the *Thelenota ananas* sample (Note: PL: polar lipid; ST: sterol; FFA: free fatty acids; TG: triacylglycerol; MADG: monoalkyldiacylglycerol; HW: hydrocarbon - wax)

The content between lipid classes of *Thelenota ananas* differs significantly, in which triacylglycerol TG has the highest concentration in total lipids, with a ratio of 66.73%. Between the two polar lipid classes PL and monoalkyldiacylglycerol class MADG, there is no significant difference with the ratio of 13.44 and 11.65% in total lipid, respectively. The low content of free fatty acids at 3.66% represents

a good quality lipid extract, ensuring further chemical research. The two classes of sterol ST and hydrocarbon-wax HW have negligible content in lipid composition, with a ratio of 2.52 and 2.01% in total lipid, respectively.

Content and composition of phospholipid classes

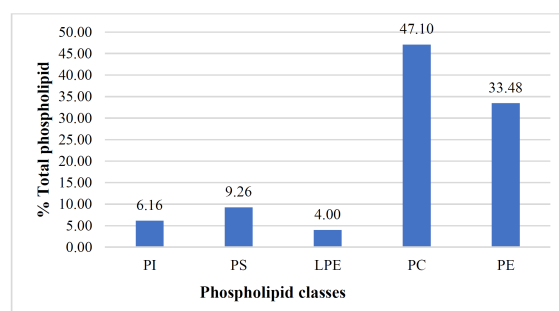


Figure 5. Content of phospholipid classes of *Thelenota ananas* sample

The qualitative analysis results determined that in the total lipid of *Thelenota ananas*, typical phospholipid classes are present,

including phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), and lyso phosphatidylethanolamine (LPE). Detailed results are shown in Figure 5.

In the phospholipid composition of the sample, the two classes with the highest concentrations are phosphatidylcholine (PC), 47.10%, and phosphatidylethanolamine (PE), 33.29%. These are the two main lipid classes in many other marine organisms such as corals, mollusks, v.v. The phosphatidylserine (PS) class recorded in *Thelenota ananas* is 9.26%. The remaining phospholipid classes are

phosphatidylinositol (PI) and lyso phosphatidylethanolamine (LPE), accounting for small concentrations of 6.16 and 4.0% of total phospholipids.

Content and composition of fatty acids in total lipids

The GC-MS chromatogram analyzing the fatty acid composition of *Thelenota ananas* is shown in Figure 6.

The content and composition of fatty acids in *Thelenota ananas* sample are presented in Table 1.

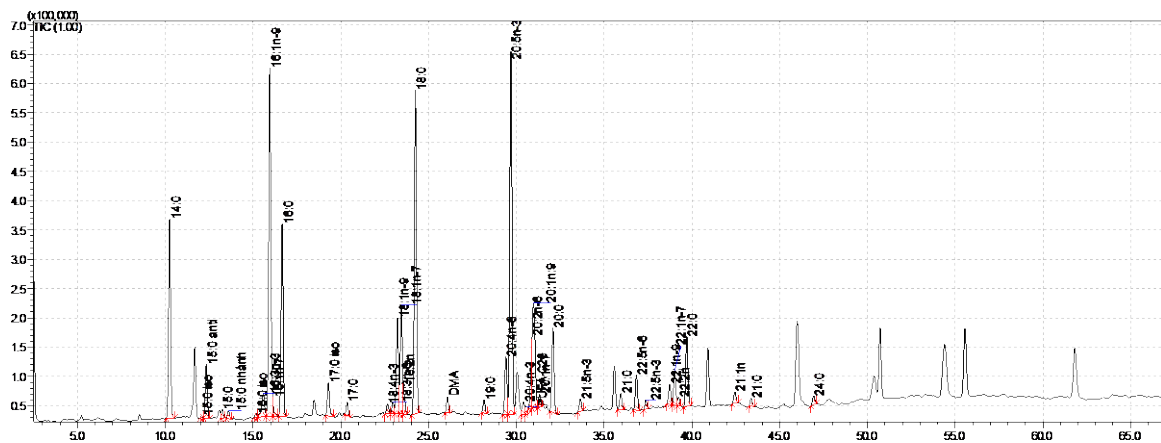


Figure 6. GC-MS chromatogram of fatty acid composition in *Thelenota ananas* sample

Fatty acids are an essential component because of their nutritional value and use. Compared to the fatty acid composition of other marine animals, sea cucumbers have a diverse fatty acid composition and the presence of long-chain fatty acids (C20–C24). For the sea cucumber sample *Thelenota ananas*, we determined the presence of 36 fatty acids, of which total saturated fatty acids (SFA) accounted for 41.61%; monounsaturated fatty acids (MUFA) accounted for 32.15% and polyunsaturated fatty acids (PUFA) account for 25.63%. In the composition of saturated fatty acids, stearic acid (18:0) accounts for a dominant proportion compared to other saturated fatty acids, with a content of 13.15% of total fatty acids; in addition, myristic fatty acids (14:0) and palmitic (16:0) has an equal ratio and is also relatively significant, 7.76% and

7.61%, respectively. In the composition of monounsaturated fatty acids, 16:1n-9 fatty acids account for the highest content (14.87% of total fatty acids); Fatty acids 20:1n-9, 18:1n-7 and 18:1n-9 account for significant amounts (5.36%, 4.32% and 4.07% of total fatty acids, respectively). The mono unsaturated fatty acids mainly belong to the n-7 and n-9 families, in which the total content of n-9 fatty acids in the sample is relatively high, accounting for 25.16% of the total fatty acids. The total content of n-7 fatty acids is 6.5%. In the studied sample, 12 PUFA acids were identified, with the highest concentration of the fatty acid 20:5n-3 EPA, accounting for 15.3% of the total fatty acids. Arachidonic fatty acid 20:4n-6 (AA) presents a small concentration of 2.31%. These essential polyunsaturated fatty acids play an important role in living organisms but cannot be

synthesized independently and can only be supplemented into the body through food. In addition, in the *Thelenota ananas* sample, we also determined the presence of other essential fatty acids of the n-3 and n-6 families with a total fatty acid content of 18.43 and 5.8%, respectively. According to the World

Health Organization (WHO), the foodstuff with an n3/n6 ratio of $\geq 0.1\%$ is classified as very good for human health [15]. In this study, the n3/n6 ratio of the sea cucumber *Thelenota ananas* was 3.18%, so it could be considered a food source that can be used to serve public health.

Table 1. Content and composition of fatty acids in *Thelenota ananas*

No.	Fatty acids	Content	No.	Fatty acids	Content
1	14:0	7.76	23	20:2n-6	1.62
2	15:0 iso	0.07	24	20:1n-9	5.36
3	15:0 anti	2.08	25	20:1n-7	0.49
4	15:0	0.34	26	20:0	3.47
5	15:0 nhánh	0.32	27	21:5n-3	0.50
6	16:0 iso	0.13	28	21:1n	0.49
7	16:3n-3	1.26	29	21:0	1.00
8	16:1n-9	14.87	30	22:5n-6	1.41
9	16:1n-7	0.29	31	22:5n-3	0.34
10	16:0	7.61	32	22:2n	0.33
11	17:0 iso	1.39	33	22:1n-9	0.86
12	17:0	0.50	34	22:1n-7	1.41
13	18:3n-6	0.46	35	22:0	2.91
14	18:4n-3	0.42	36	24:0	0.36
15	18:2n-6	0.74	Total saturated fatty acids (SFA)		41.61
16	18:1n-9	4.07	Total mono unsaturated fatty acids (MUFA)		32.15
17	18:1n-7	4.31	Total polyunsaturated fatty acids (PUFA)		25.63
18	18:0	13.15	Total n-3 fatty acids (omega 3)		18.43
19	19:0	0.52	Total n-6 fatty acids (omega 6)		5.80
20	20:4n-6 (AA)	2.31	Total n-7 fatty acids (omega 7)		6.50
21	20:5n-3 (EPA)	15.30	Total n-9 fatty acids (omega 9)		25.16
22	20:4n-3	0.61	Ratio of n3/n6		3.18

CONCLUSION

This study conducted a lipid analysis on the sea cucumber *Thelenota ananas* collected on Bai Dong Island, Ha Long Bay, Vietnam. Data on total lipid content, composition, and content of lipid and phospholipid classes were obtained. As a result, the *Thelenota ananas* species has a relatively high total lipid content (11.89% of dry weight sample), with triacylglycerol TG being the main class with a high ratio of 66.73%, the two main phospholipid classes being PC and PE with a ratio of 47.10% and 33.29% of total phospholipids, respectively. The fatty acid composition is diverse, with 36 fatty acids

identified, of which total saturated fatty acids (SFA) account for 41.61%; monounsaturated fatty acids (MUFA) account for 32.15% and polyunsaturated fatty acids (PUFA) account for 25.63%, the main fatty acids are stearic acid (18:0) accounting for 13.15% of the total fatty acids; 16:1n-9 fatty acids account for 14.87% and 20:5n-3 EPA fatty acids account for 15.3% of the total fatty acids. Among the fatty acids identified, n-9 (omega 9) accounts for the highest proportion with 25.16%; n-3 fatty acids (omega 3) account for 18.43%; n-6 (omega 6) and n-7 (omega 7) fatty acids account for 5.8 and 6.5%, respectively. The n3/n6 ratio of the *Thelenota ananas* sample is 3.18%, according

to the evaluation standards of the World Health Organization (WHO), with the ratio $n_3/n_6 \geq 0.1\%$. Therefore, it is classified as a good foodstuff for human health.

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