

Nutritional components of the sea cucumber *Holothuria scabra*

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ABSTRACT

Background: *Holothuria scabra* is one of the most commercially important species found in the Pacific region. The sea cucumber extracts have been widely reported to have beneficial health effects. The aim of this study was to determine the nutritional compositions of *H. scabra*, and compare its important nutritional contents with that of other species.

Methods: The sea cucumbers were dissected, sliced into small pieces, and then freeze-dried. The nutritional compositions, including proximate composition, amino acids, fatty acids, collagen, GABA, Vitamin A, C, and E of the whole body and body wall of *H. scabra*, were analyzed.

Results: *H. scabra* contained a high quantity of protein (22.50% in whole body and 55.18% in body wall) and very low lipids (1.55% in whole body and 1.02% in body wall). The three most abundant amino acids found in both the whole body and body wall were glycine, glutamic acid, and proline. The main fatty acids found in the whole body were stearic acid and nervonic acid, and in the body wall were arachidonic acid and stearic acid. The whole body and body wall also contained high levels of essential amino acids, essential fatty acids, and collagen, in addition to moderate amounts of vitamin E and low amounts of GABA and vitamin C.

Conclusions: The sea cucumber, *H. scabra*, contained high quantity of protein and very low lipid. It contained high essential amino acids, essential fatty acids, nervonic and arachidonic acids, and collagen, which also contained GABA, vitamin C, and vitamin E.

Keywords: sea cucumber; *Holothuria scabra*; nutrition components; functional food

INTRODUCTION

In China, sea cucumbers are one of the more popular health foods, as they are delicious and contain high levels of nutritional components [1]. In China, the sea cucumber or *Apostichopus japonicus* is the sole species of scaled aquaculture that is the most popular in the market [2]. In particular, they have been reported to contain a significant amount of high protein and low amount of fat [3]. They have been used in traditional medication to relieve pain and to heal internal and external wounds [4]. Sea cucumber extracts from various species exhibit tissue repair and wound healing capabilities [5, 6]. Water-soluble extracts from the sea cucumber *Stichopus hermannii* incorporated into hydrogel enhances wound contraction and improves the histological reorganization of the regenerating tissue [6]. Moreover, sea cucumbers contain high amount of bioactive compounds such as peptides, polyunsaturated fatty acids [4], triterpene glycosides [7], and chondroitin sulfates [8]. Numerous studies have reported that sea cucumber extracts have the abilities to cure asthma [1], have cytotoxic property [7], and are also anticancer [7], anti-inflammatory [9], and antioxidant [10].

The sandfish, *Holothuria scabra*, is found mostly in the tropic areas of the Pacific region and is one of the most commercially important species [11]. However, there is little published nutritional information on this species. Thus, the purpose of our study was to determine the nutritional composition, including proximate composition, amino acid profile, fatty acid profile, collagen content, gamma aminobutyric acid (GABA) content, vitamin A, C, and E in the whole body and body wall; we then compared important nutritional contents found in this species to other species.

MATERIALS AND METHODS

Sample preparation

Sea cucumber, *H. scabra*, size 300 ± 50 g (n=4) were obtained from the Coastal Fisheries Research and Development Center in Prachuap Khirikhan Province, Thailand. The sea cucumbers were dissected and three samples collected, namely from the whole body (including, body wall, intestine, respiratory tree, nervous system, and gonad), body wall, and viscera (internal organs). All samples were sliced into small pieces and then freeze-dried before further preparations and analysis at the Central Laboratory Co., Ltd., Thailand.

Proximate composition

The proximate compositions of the whole body and body wall were analyzed for moisture content by oven-drying according to the AOAC method 950.46 [11]. In brief, the samples were air-dried in an oven at 100°–102°C for 16-18 h then cooled in a desiccator and weighed. Loss-in-weight was reported as moisture content.

Crude protein was measured by the Kjeldahl (block digestion) method followed by AOAC method 981.10 [11]. Each dried sea cucumber sample was ground and weighed. Nitrogen in the sample was digested into ammonium sulfate by H_2SO_4 , a catalyst tablet, and 30-35% H_2O_2 , in a block digester at 410°C for 45 min, until the solution was clear. Ammonium sulfate solution was then converted into ammonia by adding NaOH- $Na_2S_2O_3$ solution, followed by the distillation of ammonia in 25 mL H_3BO_3 . The amount of ammonia was quantified by titration with 0.2 M HCl until reaching an end point.

Lipid was determined by an acid hydrolysis method according to the AOAC method 922.06 [11]. Briefly, lipid was extracted from each sample using HCl at 70-80°C for 30-40 min and then transferred to a Mojonnier fat-extraction apparatus. Redistilled petroleum ether was added to the extraction apparatus, the mixture shaken vigorously, and then left to stand until the upper liquid was practically clear. Ether-fat solution was filtered, evaporated, and then the samples were dried and weighed.

Ash content was determined by mineralization of samples according to the AOAC 920.153 [11]. The organic matter of the sea cucumber sample was removed by heating overnight in a muffle furnace at 550°C. The residue of inorganic matter or ash was then weighed.

Carbohydrate was calculated by the following formula: Weight of carbohydrate = Total dry weight - (weight of moisture + weight of crude protein + weight of lipid + weight of ash)

Amino acid composition

The total and free amino acid compositions of the sea cucumber whole body and body wall were analyzed by reversed-phase HPLC. For the total amino acid analysis, sea cucumber samples were ground and passed through a 40 mesh sieve. The samples were hydrolyzed under nitrogen with 6 M HCl. To avoid the loss of sulphur containing amino acids, i.e. cysteine and methionine, each sample was oxidized by performic acid prior to HCl hydrolysis at 110 °C for 24 h. The amino acids were derivatized with phenylisothiocyanate into isothiocyanate derivatives and then analyzed by reversed-phase HPLC. Individual amino acids were identified and quantified by comparison with retention times and peak areas of standard amino acids.

For free amino acid determinations, sea cucumber samples were mixed with 0.5% trifluoroacetic acid in methanol and centrifuged at 3000 ×g for 5 min. Free amino acids in the samples were then converted into phenyl isothiocyanate derivatives, and analyzed by reversed-phase HPLC as described previously.

Fatty acid composition

Fatty acid compositions of the sea cucumber whole body and body wall were analyzed by gas chromatography with a flame ionization detector (GC-FID), according to the AOAC method 996.06 [12]. In brief, each sea cucumber sample was weighed, mixed with an internal standard (TAG 13:0 and TAG 11:0), and then hydrolyzed with HCl. The fat was extracted with ethyl ether and petroleum ether. The ether extract was filtered, dried, and methylated into fatty acid methyl ester (FAME). The FAME of each sample was determined by GC-FID and each fatty acid identified and quantified by comparison with retention times and peak areas of FAME against the internal standard.

Collagen content

The collagen of sea cucumber whole body and body wall were determined using a hydroxyproline assay, according to the AOAC method 990.26 [12]. Each sample was ground, weighed, and hydrolyzed in H₂SO₄ at 105°C for 16 h. The solution was diluted to 500 mL with distilled water. Part of the solution was filtered and then the filtrate was diluted to make a final concentration of hydroxyproline in range 0.5-2.4 µg/mL (5 mL filtrate + 95 mL distilled water). Hydroxyproline was oxidized to pyrrole, mixed with color reagent (4-dimethylaminobenzaldehyde 10 g in 35 mL perchloric acid and 65 mL 2-propanol), and incubated in a water bath at 60°C for 15 min. The optical density of the solution and blanks was measured at 558 nm with a spectrophotometer. The hydroxyproline standard with varying concentrations were used to plot a standard curve. The conversion factor for calculating the collagen contents from hydroxyproline was 8.

Gamma aminobutyric acid (GABA) content

The GABA contents of the sea cucumber whole body and body wall were determined by gas chromatography-mass spectrometry (GC-MS). The samples were prepared according to Iwaki and Kitada [13]. In short, the freeze-dried samples were ground into powder and homogenized separately with 25 mL of 75% ethanol in a homogenizer. Each homogenate was filtered through filter paper with pore size 5-10 µm. The residues were then extracted 3 times with 25 mL of 75% ethanol and the volume of filtrate was made up with 75% ethanol to 100 mL. The samples were analyzed by GC-MS and the GABA contents were determined and quantified by comparison with retention times and peak areas of an internal standard.

Vitamin A (retinol) and vitamin E (α-tocopherol)

Vitamin A and E contents in the sea cucumber whole body and body wall were analyzed, according to an in-house method based on a liquid chromatographic analysis of food and beverage [14]. Briefly, dried samples were ground, passed through a 40 mesh sieve, mixed homogeneously, and then weighed. Vitamin A standard solution (30 µg/mL retinol), vitamin E standard solution (350 µg/mL α-tocopheryl acetate), and approximately 3 g of samples were added separately to amber Erlenmeyer flasks equipped with a reflux condenser and a nitrogen inlet, after which 95% ethanol was added and the Erlenmeyer flask swept with nitrogen and heated to a reflux temperatures (~78.1 °C). Two milliliters of aqueous potassium peroxide at concentration 0.5 g/mL was added to each sample. The samples were then refluxed for 45 min and cooled to room temperature, after which potassium peroxide was neutralized with the addition of acetic acid in acetonitrile through swirling. The solutions were diluted to 100 mL with acetonitrile, and filtered through 0.45 µm filters. The samples and standards were determined by reversed-phase HPLC, and comparison of the peak areas of the samples versus the peak areas of the standards were used for quantifications of vitamin A and E.

Vitamin C (ascorbic acid)

Vitamin C contents in the sea cucumber whole body and body wall were determined according to compendium of methods for food analysis [15]. In short, freeze-dried samples were cut into

small pieces and then homogenized as finely as possible. The samples were weighed and 2.5 g dissolved with 3% meta-phosphoric acid in separate 100 mL volumetric flasks. The solutions were shaken vigorously for 2 min, sonicated for 5 min, and made up to 100 mL with 3% m-phosphoric acid. They were filtered through 0.45 μm membrane filters and analyzed for vitamin C by a reversed-phase HPLC. In each analysis, approximately 20 μl of ascorbic acid of a standard solution and 20 μl of each sample was injected into a HPLC column, with the quantification of vitamin C being determined by comparison of the peak with the retention time and peak area of the ascorbic acid standard.

RESULTS

Proximate composition

The proximate compositions of freeze-dried *H. scabra* whole body and body wall are shown in table 1. The whole body of *H. scabra*, sample contained 79.65% moisture, 22.50% protein, 1.55% lipid, 61.98% ash, and 7.71% carbohydrate (dry base). The body wall of *H. scabra* contained 12.13% moisture, 55.18% protein, 1.02% lipid, 27.97% ash, and 3.70% carbohydrate (dry base).

Table 1. Proximate composition of the sea cucumber, *Holothuria scabra*.

Component	Gram per 100 g tissue (dry weight)	
	Whole body	Body wall
Moisture	6.25	12.13
Protein	22.50	55.18
Lipid	1.55	1.02
Ash	61.98	27.97
Carbohydrate	7.71	3.70

Amino acid composition

The compositions of total amino acids of *H. scabra* whole body and body wall are shown in Figure 1A. Eighteen amino acids were detected, including 9 essential amino acids and 9 nonessential amino acids, and 2 major amino acids of collagen, namely hydroxyproline and hydroxylysine. In the following order, the three most abundant amino acids found in both whole body and body wall were glycine (34.11 mg/g and 79.65 mg/g dry weight respectively), glutamic acid (31.91 mg/g and 67.64 mg/g dry weight respectively), and proline (22.34 mg/g and 44.52 mg/g dry weight respectively). Free amino acids in *H. scabra* whole body and body wall are shown in Figure 1B. Three of the major free amino acids found in whole body and body wall included glutamic acid, arginine, and aspartic acid, of which glutamic acid levels were the highest at 1.92 mg/g and 1.22 mg/g dry weight respectively.

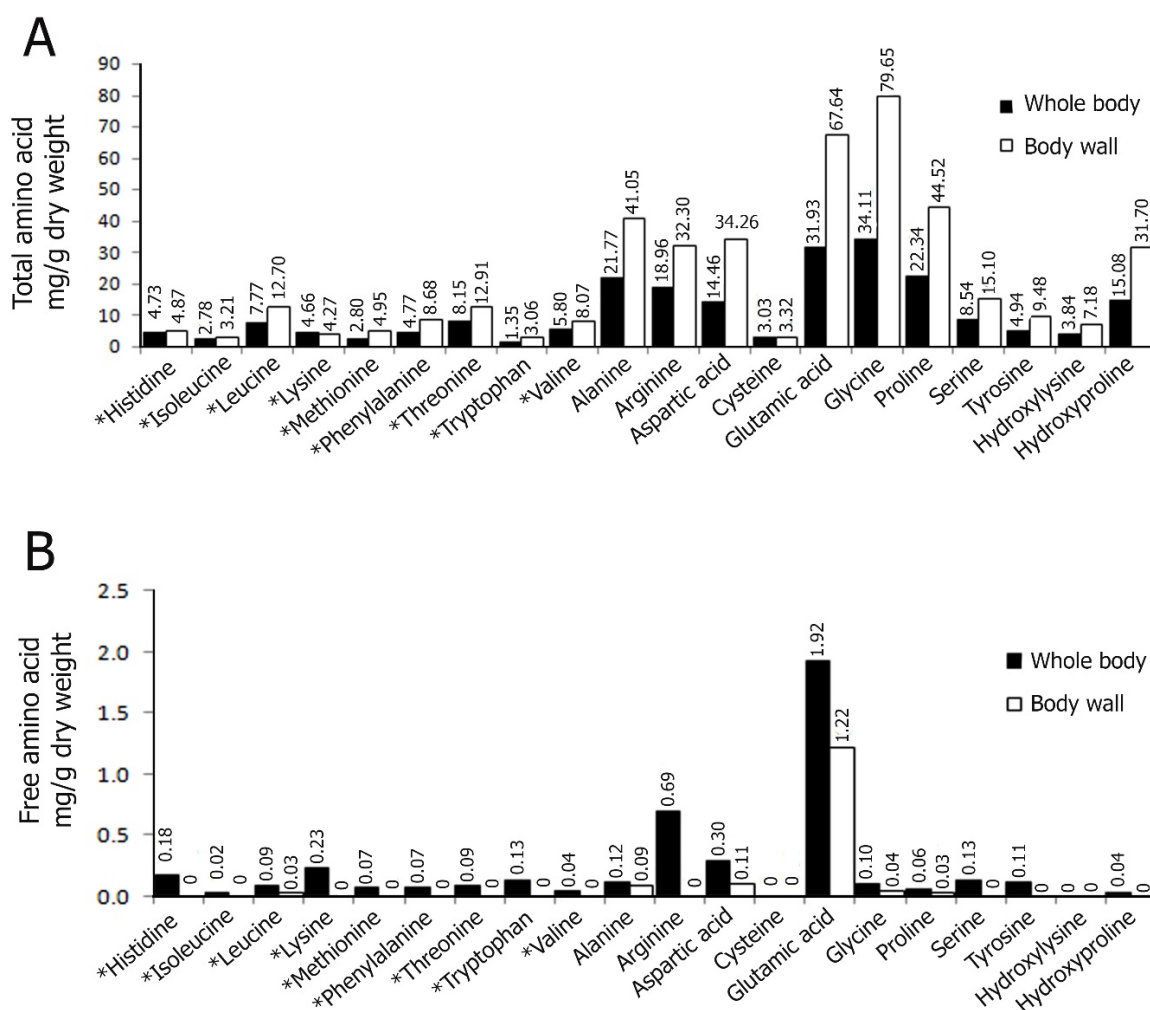


Figure 1. Total (A) and free amino acids (B) of freeze-dried *H. scabra* whole body and body wall (milligram per gram of dry weight). *, essential amino acids.

Fatty acid composition

The fatty acid compositions of the whole body and body wall of *H. scabra* are shown in Table 2. The main fatty acids found in the whole body were C18:0, C24:1, and C18:1, while those found in the body wall were C20:4, C18:0, and C24:1. High levels of stearic acid (C18:0), a direct precursor of the oleic acid, were found in the whole body at 2.12 mg/g dry weight and in the body wall at 1.50 mg/g dry weight. Other fatty acids important for the growth and development of humans were also found in *H. scabra*. Nervonic acid (NA, C24:1) is present at high levels with 1.71 mg/g in the whole body and 1.44 mg/g in the body wall. Linoleic acid (LA), an essential omega-6 fatty acid, was found in the whole body of the sea cucumber *H. scabra* at 5.46% and in the body wall at 3.37%. Alpha-linolenic acid (ALA), an essential omega-3 fatty acid, is a precursor of longer-chain unsaturated n-3 fatty acids, including EPA and DHA. ALA was detected in the whole body at 1.13% and body wall at 0.82%. Arachidonic acid (ARA), an omega-6 polyunsaturated fatty acid, was found in whole body at 3.63% and found at a very high level in the body wall at 28.42%. Eicosapentaenoic acid (EPA) was found in the whole body at 1.13% and in body wall at 6.57%.

Table 2. Fatty acid composition of freeze-dried *Holothuria scabra* whole body and body wall (milligram per gram of dry weight). Asterisks indicate the fatty acids that have been shown to be important for growth and development of humans. N/D; not detected.

Fatty acid	Whole body		Body wall	
	% weight	mg/g dry weight	% weight	mg/g dry weight
C14:0	0.71	0.10	1.22	0.20
C15:0	0.44	0.06	0.86	0.14
C16:0	9.15	1.23	6.05	1.00
C17:0	1.24	0.17	1.29	0.21
C18:0*	15.80	2.12	9.01	1.50
C20:0	7.00	0.94	5.04	0.84
C21:0	6.26	0.84	4.37	0.73
C22:0	6.89	0.92	4.90	0.81
C23:0	2.70	0.36	2.00	0.33
C24:0	1.00	0.13	2.66	0.44
ΣSaturated fatty acid	51.19	6.87	37.39	6.20
C14:1	N/D	N/D	N/D	N/D
C15:1n-10	N/D	N/D	N/D	N/D
C16:1n-7	2.40	0.32	1.86	0.31
C17:1n-10	N/D	N/D	N/D	N/D
C18:1n-9t	0.86	0.11	0.48	0.08
C18:1n-9c*	11.93	1.60	6.32	1.05
C20:1n-11c	3.82	0.51	1.79	0.30
C22:1n-9	2.69	0.36	0.92	0.15
C24:1n-9*	12.76	1.71	8.69	1.44
ΣMonounsaturated fatty acid	34.46	4.61	20.06	3.33
C18:2n-6t	N/D	N/D	N/D	N/D
C18:2n-6c*	5.46	0.73	3.37	0.56
C18:3n-6	N/D	N/D	N/D	N/D
18:3n-3*	1.13	0.15	0.82	0.14
C20:2	3.01	0.40	2.46	0.41
C20:3n-6	N/D	N/D	N/D	N/D
C20:3n-3	N/D	N/D	N/D	N/D
C20:4n-6*	3.63	0.49	28.42	4.72
C22:2	N/D	N/D	N/D	N/D
C20:5n-3*	1.13	0.15	6.57	1.09
C22:6n-3	N/D	N/D	0.44	0.07
ΣPolyunsaturated fatty acid	14.36	1.92	42.56	7.07
ΣOmega-3	2.26	0.3	8.31	1.38
ΣOmega-6	9.09	1.22	31.78	5.28
ΣOmega-9	28.23	3.78	16.41	2.72

Collagen content

The collagen compositions of the whole body and body wall of *H. scabra* were 15.07 g/100g and 18.38 g/100g dry weight respectively. The yields of collagen from both the whole body and body wall of *H. scabra* are higher than that in cartilage of the blacktip shark, cartilage from brownbanded bamboo shark, and chicken feet (Figure 2).

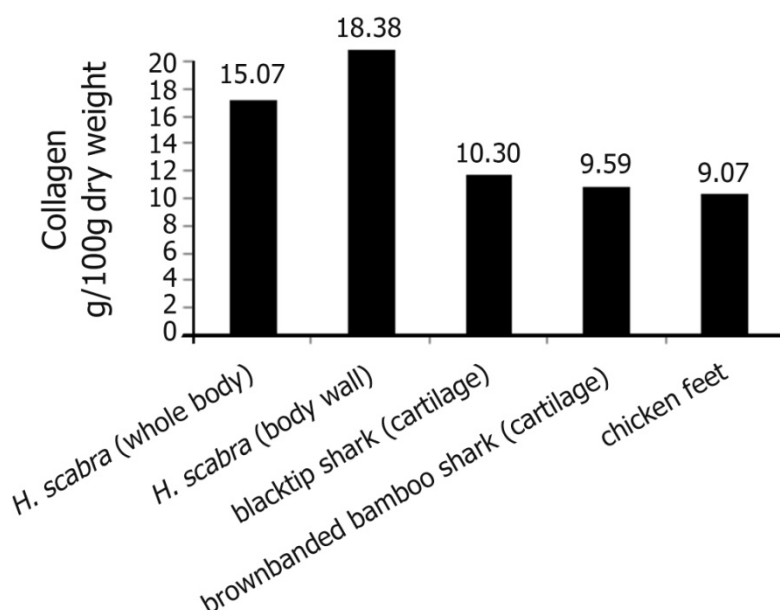


Figure 2. Comparison of collagen content from *H. scabra* whole body and body wall with cartilage of blacktip, and brownbanded bamboo sharks, and chicken feet.

GABA content

The GABA content in the *H. scabra* whole body was 4.67 mg/kg dry weight, which was not found in the body wall. The content of GABA in the sea cucumber was much lower than that in germ rice as shown in Figure 3.

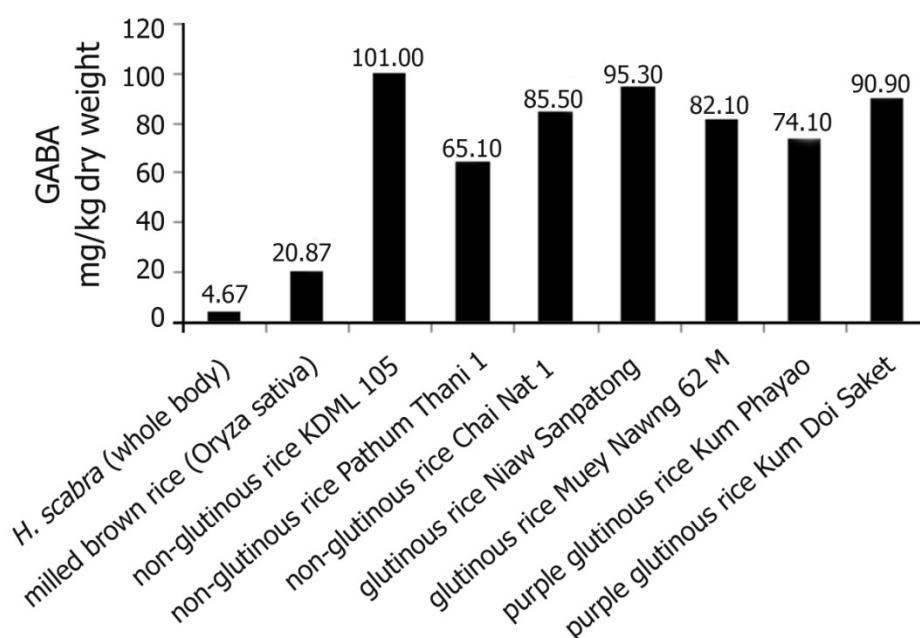


Figure 3. Comparison of GABA from *H. scabra* whole body with brown, non-glutinous, glutinous, and purple glutinous rice.

Vitamin A (retinol)

Vitamin A was not detected in the *H. scabra* whole body and body wall.

Vitamin C (ascorbic acid)

Vitamin C content in the *H. scabra* whole body was 3.19 mg/100 g, and in the body wall was 1.32 mg/100 g dry weight. The content of vitamin C in the *H. scabra* was much lower than in fruits as shown in Figure 4A.

Vitamin E (α -tocopherol)

Vitamin E content in the whole body was 2.82 mg/100g, and in the body wall was 4.94 mg/100 g dry weight. The content of vitamin E in the sea cucumber was lower than that in oil from seeds and fishes as shown in Figure 4B.

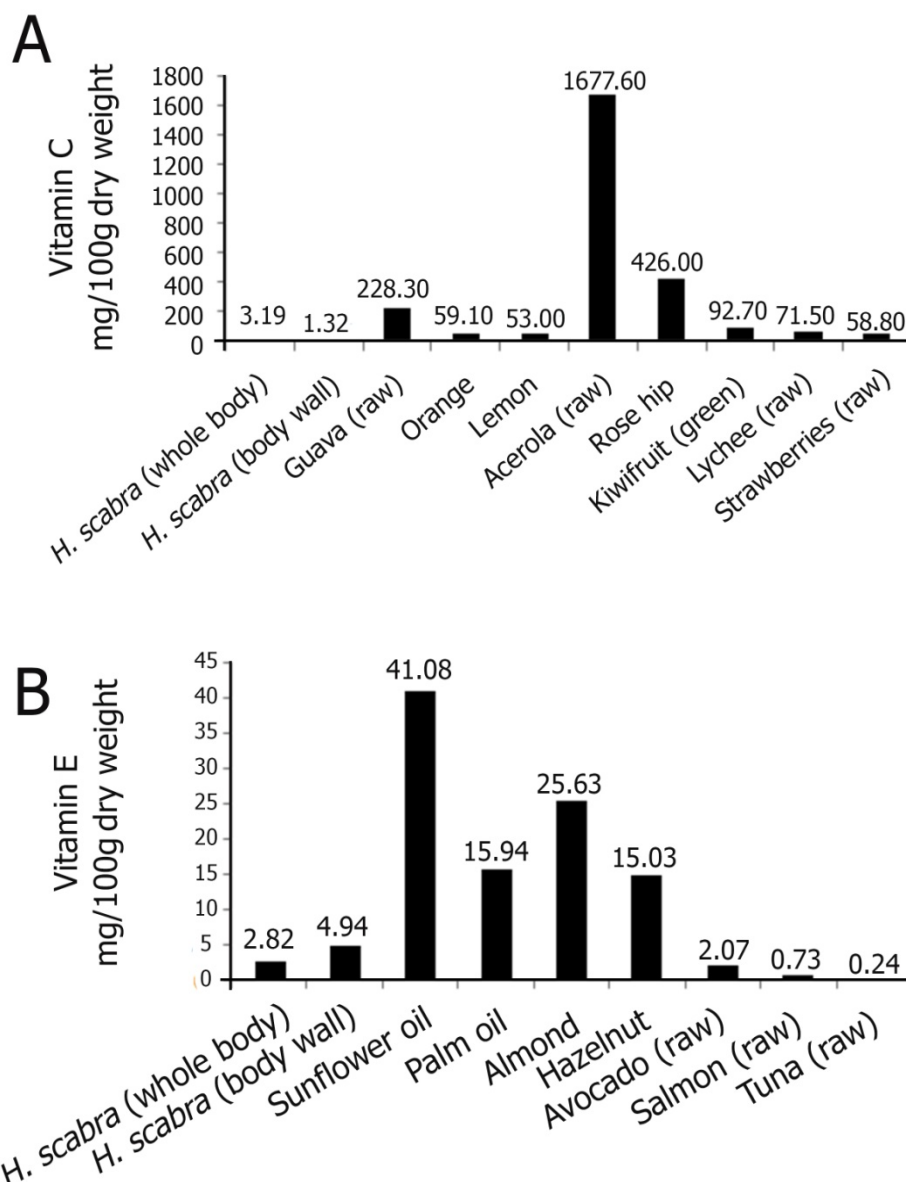


Figure 4. (A) Comparison of vitamin C from *H. scabra* whole body and body wall with fruits. (B) Comparison of vitamin E from *H. scabra* whole body and body wall with sunflower oil, palm oil, almond seed, hazelnut seed, avocado flesh, salmon flesh, and tuna.

DISCUSSION

The major contents of *H. scabra* are similar to those reported in dried *S. herrmanni*, *Thelenota ananas*, *H. fuscogilva*, *H. fuscopunctata*, *Actinopyga mauritiana*, and *Bohadschia argus* [2].

The main characteristics are the high quantity of protein, very low amounts of lipid, and the high quantity of ash which may be the result from the minerals deposit in the body wall of a sea cucumber.

According to amino acid composition, we expected high levels of glycine and proline, and the presence of hydroxyproline and hydroxylysine, as they reflected the high collagen content in the sea cucumber. Glycine and glutamic acid were also dominant amino acids in other sea cucumbers, including *S. herrmanni*, *Thelenota ananas*, *H. fuscogilva*, *H. fuscopunctata*, *Actinopyga mauritiana*, and *Bohadschia argus* [3], *Cucumaria frondosa* [8], and *A. japonicus* [2]. A high amount of proline was found only in *H. scabra*, particularly within the body wall. The essential amino acids, leucine, threonine, phenylalanine, and valine, were present at levels higher than others in the total amino acid analysis but not in the free amino acid analysis. This may reflect specific incorporation into structural proteins. Additionally, semi-essential amino acids, such as arginine and tyrosine, required for human infant and child growth [23], were detected at moderate levels in both whole body and body wall. Moreover, several studies have reported that a low lysine to arginine ratio could reduce levels of plasma cholesterol [24, 25]. In *H. scabra*, the ratio of lysine to arginine in the whole body was 0.25 and in the body wall was 0.13; moreover, these were consistent with those of other sea cucumbers, including *S. herrmanni* (0.27), *T. ananas* (0.39), *T. anax* (0.33), *H. fuscogilva* (0.13), *H. fuscopunctata* (0.15), *A. mauritiana* (0.25), *A. caerulea* (0.21), *B. argus* (0.36) [3], and *A. japonicus* (0.62) [2]. Interestingly, the ratios of lysine: arginine in *H. scabra* are lower than that in varieties of fish, including European seabass *Dicentrarchus labrax* (0.85), gilthead seabream *Sparus aurata* (0.86), and turbot *Psetta maxima* (0.95) [19]. Therefore, the results of the present study and previous reports indicated that a sea cucumber is a healthy food that can help to reduce hypercholesterolemia.

Amino acids, especially free amino acids, are the main contributors of the taste and flavor of foods. For example, free amino acids can give sweet, bitter, sour, sulfurous, and umami flavors [20]. Glutamic acid, particularly L-glutamic acid, plays important roles in the taste and palatability of foods [21], so the high level of glutamic acid in the sea cucumber probably enhances its taste. Furthermore, glutamate acts as an excitatory neurotransmitter in the central and peripheral nervous systems of mammals [22], which is the precursor in the syntheses of proteins, peptides, and glutathione, a natural antioxidant molecule [23]. Moreover, the neurotransmitter glutamate plays an important role in learning and memory in hippocampus by action through N-methyl-D-Aspartate receptors (NMDAR) [24].

The major fatty acid found in *H. scabra* was stearic acid. Unlike other saturated fatty acids, stearic acid does not raise blood total cholesterol and low density lipoprotein (LDL) cholesterol levels. In humans, a diet containing high stearic acid lowers LDL cholesterol and triglyceride while increasing HDL cholesterol more than a diet containing high trans fatty acid [25]. The second most abundant fatty acid found in whole body of *H. scabra* was NA. NA is found in sphingolipids, such as sphingomyelin in the myelin sheath of nerve fibers and in the white matter of animal brains [26]. NA was also found in other sea cucumbers at a much lower amount than in *H. scabra*, and was not detected in fishes [27, 28]. NA supplementation is thought to improve neurological function [29] and enhance myelination in patients with demyelinating disease [30]. Martinez and Mougan [30] revealed that the predominant myelin monounsaturated fatty acid in human brain is NA, which increases very rapidly after birth until children are eight years old. Therefore, a low intake and decrease in NA could affect myelination. Our novel finding of the high level of NA in *H. scabra* indicated a major

neurological benefit of eating sea cucumbers. LA, ALA, ARA, and EPA levels in *H. scabra* were compared with other sea cucumbers and fishes. LA has also been detected at high levels in other sea cucumber, *S. chloronotus* (12.87%), and the Nile tilapia, *Oreochromis niloticus* (20.90%) [27]. The levels of ALA found in whole body and body wall of *H. scabra* were low and found very low in other sea cucumbers at 0 - 1.88% [3, 5], and fishes at 0 - 1.86% [27, 28]. ARA found in *H. scabra* was detected at the highest level among sea cucumbers and fishes. ARA is derived as an intermediate from diacylglycerol by the action of phospholipase-A₂ and then transformed to eicosanoids, including prostaglandins and leukotrienes, which are key anti-inflammatory factors [30]. EPA in *H. scabra* is much lower than that found in other sea cucumbers, including *C. frondosa* (52.00%) [8] and *S. chloronotus* [5]. EPA is also found in fishes at 1.88% - 7.08% [28]. Docosahexaenoic acid (DHA) was not found in the whole body and body wall of *H. scabra*, but was found in other sea cucumbers at 1.20%- 57.55% [5, 8] and in fishes at 1.07% - 28.30% [27, 28]. The wide range of EPA and DHA in various animals may be due to the different methods of extractions, or they have different contents as a result of diet and endogenous factors. Significantly, we found that *H. scabra* contains the highest levels of ARA and NA when compared with other sea cucumbers and fishes.

The yields of collagen from both the whole body and body wall of *H. scabra* are higher than that in the cartilage of the blacktip shark, cartilage from brownbanded bamboo shark, and chicken feet [32-34]. Zhao *et al.* [33] reported that gelatin from collagen of the sea cucumber, *Acaudina molpadioidea*, had the ability to inhibit angiotensin-I-converting enzyme (ACE), which plays an important physiological role in regulating blood pressure. The collagen from the body wall of sea cucumber was used as a nutrient supplement in anemic patients for enhancing hemotogenesis and arthritis patients for reducing pain [1].

The content of GABA in the sea cucumber was much lower than that in germ rice [13, 35] as shown in Figure 3. Nevertheless, the small amount of GABA was consistent with the anatomical structure of the sea cucumber that has small radial nerves, which contain GABA.

Vitamin A was not detected in the *H. scabra*. The content of vitamin C in the *H. scabra* was much lower than in fruits, including guava, orange, lemon, acerola, rose hip, kiwifruit, lychee, and strawberries [36]. The content of vitamin E in the sea cucumber was lower than that in sunflower oil, palm oil, almonds, and hazelnuts but higher than in avocado, salmon, and tuna [36].

CONCLUSIONS

In conclusion, the sea cucumber, *H. scabra*, contained high quantities of protein, especially collagen and very low lipid. It had high levels of essential amino acids, essential fatty acids especially NA and ARA, which also contained GABA, vitamin C, and vitamin E. Thus, this sea cucumber has the potential to be an effective functional food, due to its high nutritional values.

List of Abbreviation: GABA - gamma aminobutyric acid; AOAC - Association of Official Analytical Chemists; NA - Nervonic acid; LA - Linoleic acid; ALA - Alpha-linolenic acid; ARA - Arachidonic acid; EPA - Eicosapentaenoic acid; DHA - Docosahexaenoic acid

Competing Interests: All authors have no competing of interest.

Author's Contributions: All authors contributed to this study.

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