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Nutritional Value and Heavy Metals Content of Sea Cucumber *Holothuria scabra* Commercially Harvested in Indonesia

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Abstract

Holothuria scabra is one of the commercial sea cucumber species harvested in Indonesia. The objective of this study is to analyze nutritional value and heavy metals content of *H. scabra* collected from 4 different locations, Bungin Island (West Nusa Tenggara), Belitung Island, Lampung, and Makassar. Proximate composition, mineral, and heavy metal were analyzed by the AOAC standard method. Amino acid profiles were analyzed by UPLC, fatty acid contents were determined by GC, and vitamin were detected by HPLC. The results show that all *H. scabra* from different locations have high protein and low fat content. Protein content in *H. scabra* from Bungin Island showed the highest percentage with 6.95%. Vitamins (A, B_1, and B_{12}) and heavy metals examined in this study were not detected, while vitamin B2 from Belitung, Makassar and Lampung were 0.06 mg/100 g, 0.06 mg/100 g, and 0.04 mg/100 g respectively and vitamin E from Bungin and Belitung were 0.18 mg/100g and 0.35 mg/100g. The calcium content was higher than other minerals followed by sodium. Total 18 amino acids were identified where glycine was the major constituent (5798.80–16789.18) mg/kg). In the case of fatty acids, omega-6 from Bungin Island and Makassar were the major constituent with 0.1834 % and 0.1377 %. Meanwhile, omega-9 from Belitung Island and Lampung were the major constituent with 0.2466 % and 0.1773 %. Therefore, it could be inferred that the sea cucumber *H. scabra* from all sampling locations is safely to consume and could be utilized as a source of functional food in the future.

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Introduction

Sea cucumber is one of the most potential export commodities from marine sectors in Indonesia. Sea cucumber commercially traded either in fresh or dehydrated form. It categorized based on economic value (low, medium or high) depends on several aspects such as species, appearance, abundance, color, odor, body wall thickness, market trends and needs.[1] It is well known as functional food due to its functional properties and have been consumed in Asian country for centuries.[2] However, this potential may lead to over exploited which may result in a population collapse.[3]

Sea cucumber are abundant in tropical region like Indonesia. Total catching of sea cucumbers in the world has reached 100,000 tons annually. According to the Food and Agriculture Organization's (FAO) global statistics reports in 2004, Indonesia has been the world's largest producer of sea cucumbers based on global reports of major importing countries, Hongkong and China. In addition, Indonesia still one of the major sea cucumber fisheries till now.[2,4,5]

One of the high-value sea cucumbers comes from *Holothurian* species with total 1200 species recorded all over the world (Mc Elroy, 1990). *H. nobilis* (black teatfish), *H. fuscogilva* (white teatfish), and *H. scabra* (sandfish) commonly trade in Indonesia. They are generally distributed in tropical waters in the Western Pacific and the Indian Ocean.[6,7] *Holothuroidea* are rich in functional materials, including saponin, chondroitin sulfate, collagen, amino acids, and phenols.[8] This bioactive material will lead to potential development not only in food industries but also biomedicine industries.[9]

Fig.1: Holothuria scabra from Bungin Island

Utilization of sea cucumbers in Indonesia as food compared with other fishery products are low and less popular, because sea cucumbers have a low aesthetic value seen from the physical form. However, sea cucumbers have a high nutritional content. The purpose of this research is to present the nutritional value and heavy metals content of sea cucumber *H. scabra* from 4 different locations that has potential source of functional food in the future.

Materials and Methods

Sample collection and preparation

H. scabra samples used in the present work were fresh sea cucumber collected from four different coastal areas in Indonesia including Bungin Island (West Nusa Tenggara) S 06°24.103’ E 116°04.707’ collected on 2017, Belitung Island S 02°32.239’ E 107°37.217’, Lampung S 05°33.706’ E 105°16.220’ and Makassar S 05°27.114’ E 119°23.822’ collected on 2018. The samples were instantly dissected to remove viscera, cleaned, packed with ice prior sending to the laboratory and kept in dark container at -80°C until analyze.

Proximate Analysis

Proximate compositions of *H. scabra* were analyzed as follows. The moisture, ash and protein were determined according to the standard AOAC method8. In brief, the samples were air-dried in an oven at 100°C for 18 hours then cooled in a desiccator and weighed. Weight lost was reported as moisture content. Ash content was analyzed by incineration in a muffle furnace at 550°C for 24 hours. Lipid content was measured by conventional soxhlet extraction using hexane solvent for 24 hours and carbohydrates were calculated by difference.

Vitamins, Minerals and Heavy Metals Analysis

Vitamin A and E content were analyzed using HPLC Alliance Waters, Photo Diode Array (PDA) with LiChrospher 100 RP-18 (5um) 4 mm x 250 mm column while vitamin B_1 B_2, and B_{12} content were determined by using UPLC H Class Waters, Photo Diode Array (PDA) with ACQUITY UPLC BEH Amide 1.7 um 2.1 x 100 mm column. Minerals content including calcium (Ca), potassium (K), iron (Fe), and sodium (Na) were analyzed by the standard AOAC method[10] while the phosphorus (P) content was analyzed by spectrophotometric method. The heavy metals content including mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) were analyzed by the standard AOAC method.[11]

Amino Acid Composition

Amino acid profile was analyzed with UPLC apparatus with condition: Mobile phase (Gradient composition system); Flow rate (0.5 ml per minute); Injection volume (1 µL). Column used (AccQ.Tag Ultra C18 1.7 µm (2.1 x 100 mm), Waters); Detector (PDA, wavelength 260 nm); and Temperature (49°C).

Fatty acid Composition

Determination of fatty acid was analyzed by gas chromatography (GC) with condition: Flow rate (18.0 cm/sec with column length 100 m); Injector temperature (225°C), ;Column (Supelco SPTM 2560 100m 0.25 mm 0.2 µm); Carrier Gas (N_2), Detector FID (240°C); and Split (1:100).

Results

Nutrient composition of *H. scabra* collected from four different locations is depicted in Table 1. Table 2. shows the heavy metal contents of all *H. scabra* samples. The amino acid and fatty acid compositions were given in Table 3 and 4.

Table 1: Nutrient content of sea cucumber *Holothuria scabra*

Nutrient component	*H. scabra*			
	Bungin	**Belitung**	**Makassar**	**Lampung**
Moisture (%)	84.55_b (0.03)	87.84_c (0.21)	87.95_c (0.11)	83.40_a (0.02)
Ash (%)	7.38_c (0.07)	5.24_a (0.02)	6.57_b (0.01)	7.90_d (0.16)
Protein (%)	6.95_d (0.04)	5.22_b (0.12)	4.78_a (0.12)	5.73_c (0.01)
Lipid (%)	0.78_b (0.02)	1.22_d (0.04)	0.48_a (0.01)	0.90_c (0.01)
Carbohydrate (%)	0.34_{ab} (0.01)	0.48_b (0.04)	0.22_a (0.01)	2.07_c (0.14)
Vitamin A (µg /100 g)	nd	nd	nd	nd
Vitamin B1 (mg/Kg)	nd	nd	nd	nd
Vitamin B2 (mg/Kg)	nd	0.06_b (0.00)	0.06_b (0.01)	0.04_a (0.00)
Vitamin B12 (mg/Kg)	nd	nd	nd	nd
Vitamin E (mg/100 g)	0.18_a (0.00)	0.35_b (0.00)	nd	nd
Sodium (mg/100 g)	669.97_b (1.37)	380.79_a (2.21)	820.36_c (5.61)	829.89_d (2.78)
Calcium (mg/100 g)	4074.57_d (14.23)	1374.51_a (4.26)	3017.26_b (15.8)	3668.98_c (6.3)

Potassium (mg/100 g)	61.57_b (0.69)	30.08_a (0.08)	73.88_c (0.54)	73.16_c (0.3)
Phosphorus (mg/100 g)	96.34_b (5.23)	86.69_a (2.00)	131.99_d (1.18)	123.22_c (0.00)
Iron (mg/100 g)	15.95_c (0.28)	4.59_b (0.03)	22.78_d (0.38)	3.89_a (0.02)
Magnesium (mg/100g)	509.06_c (1.4)	240.88_a (2.88)	357.41_b (3.97)	502.23_c (6.84)

Note. Numbers in parentheses are standard deviations. Means sharing subscripts differ at $p < 0.05$ according to Duncan Significant Difference comparison. *nd=not detected

Table 2: Heavy metal content of sea cucumber *Holothuria scabra*

Parameter	*H. scabra*			
	Bungin	Belitung	Makassar	Lampung
Mercury	nd	nd	nd	nd
Lead	nd	nd	nd	nd
Cadmium	nd	nd	nd	nd
Arsenic	nd	nd	nd	nd

Note. *nd=not detected

Table 3: Amino acid composition of sea cucumber *Holothuria scabra*

Amino acid (mg/kg)	*H. scabra*			
	Bungin	Belitung	Makassar	Lampung
Threonine	3331.69_d (6.47)	2430.80_c (7.60)	1700.63_a (28.97)	2123.71_b (29.22)
Leucine	2517.73_d (2.98)	2181.22_c (8.89)	1270.90_a (21.76)	1831.75_b (38.16)
Lysine	1177.26_d (10.40)	1124.76_c (3.39)	698.38_a (16.34)	1023.71_b (13.12)
Arginine	4647.08_d (17.66)	4296.15_c (37.70)	2958.02_a (95.82)	3797.46_b (68.67)
Valine	2173.77_d (1.56)	1836.02_c (1.96)	1233.28_a (29.28)	1586.49_b (19.09)
Isoleucine	1309.11_d (0.32)	1164.94_c (2.31)	723.46_a (2.55)	1016.10_b (1.96)
Phenylalanine	2280.82_d (9.56)	1249.03_c (2.10)	842.69_a 17.21)	987.18_b (7.32)
Methionine	820.49_b (8.24)	nd	nd	126.09_a (2.81)
Histidine	nd	565.56_b (13.24)	670.23_c (22.22)	495.46_a (2.86)

Tryptophan	nd	192.61_a (4.41)	189.30_a (4.13)	199.22_a (1.22)
Total Non Essential	19598.42	15041.09	10243.7	13187.17
Proline	7020.93_c (23.98)	4383.33_b (13.75)	2730.21_a (75.80)	4416.44_b (61.66)
Tyrosine	1877.22_d (4.72)	1242.15_c (2.06)	929.33_a (25.58)	994.00b (9.61)
Aspartic acid	5482.08_d (1.51)	3324.28_b (10.88)	2150.23_a (50.76)	3628.53_c (56.31)
Glycine	16789.18_d (40.45)	8545.75_c (38.47)	5798.80_a (137.12)	8146.12_b (107.05)
Alanine	6812.11_d (32.07)	3851.15_b (13.99)	2273.59_a (56.67)	4081.66_c (49.56)
Glutamic acid	10272.28_c (11.52)	6158.75_b (24.99)	3609.83_a (107.49)	6324.15_b (82.27)
Serine	2563.33_c (4.49)	1960.72_b (14.91)	1831.58_a (34.92)	1956.37_b (5.77)
Cysteine	nd	nd	nd	nd
Total	50817.13	29471.13	19323.57	29547.27

Note. Numbers in parentheses are standard deviations. Means sharing subscripts differ at $p < 0.05$ according to Duncan Significant Difference comparison. *nd=not detected

Table 4:Fatty acid composition of sea cucumber *Holothuria scabra*

Fatty acid composition (%)	***H. scabra***			
	Bungin	**Belitung**	**Makassar**	**Lampung**
C8:0 (Caprylic acid)	0.0246 (0.0076)	nd	nd	nd
C12:0 (Lauric acid)	0.1264 (0.0052)	nd	nd	nd
C14:0 (Myristic acid)	0.0625_c (0.0014)	0.0232_b (0.0034)	nd	0.0102a (0.0011)
C16:0 (Palmitic acid)	0.1328_b (0.0021)	0.2990_d (0.0031)	0.0630_a (0.0016)	0.1929_c (0.0036)
C18:0 (Stearic acid)	0.0460_a (0.0035)	0.1185_c (0.0059)	0.0386_a (0.0028)	0.0660_b (0.0006)
Saturated fat	0.4411_c (0.0156)	0.4935_d (0.0061)	0.1370_a (0.0032)	0.3509_b (0.0110)
C16:1 (Palmitoleic acid)	0.0233_a (0.0030)	0.0504_b (0.0054)	0.0149_a (0.0001)	0.0193_a (0.0009)
C18:1 W9C (Oleic acid)	0.0748_b (0.0119)	0.2466_d (0.0093)	0.0211a (0.0165)	0.1731_c (0.0044)
C18:2 W6C (Linoleic acid)	0.1009_c (0.0160)	0.0650_b (0.0025)	0.0103_a (0.0013)	0.0495_b (0.0036)

C18:3 W3 (Alfa Linolenic acid)	0.0143_b (0.0034)	0.0057_a (0.0016)	nd	nd
C20:4 W6 (Aracidonic acid)	0.0825_a (0.0197)	0.1665_c (0.0085)	0.1274_b (0.0081)	0.1264_b (0.0040)
EPA	0.0429_a (0.0041)	0.0851_c (0.0061)	0.0683_b (0.0022)	0.0443_a (0.0052)
DHA	nd	0.0085_a (0.0006)	0.0108_b (0.0106)	nd
Unsaturated fat	0.3388_a (0.0025)	0.7348_c (0.0281)	0.3443_a (0.0062)	0.5502_b (0.0040)
Omega-3	0.0572_a (0.0076)	0.0994_c (0.0083)	0.0790_b (0.0033)	0.0543_a (0.0060)
Omega-6	0.1834_b (0.0037)	0.2314_c (0.0110)	0.1377_a (0.0093)	0.1760_b (0.0076)
Omega-9	0.0748_b (0.0119)	0.2466d (0.0093)	0.0326_a (0.0002)	0.1773_c (0.0040)
MUFA	0.0982_a (0.0087)	0.3848_c (0.0109)	0.1114_a (0.0032)	0.2934_b (0.0024)
PUFA	0.2406_a (0.0112)	0.3500_b (0.1732)	0.2329_a (0.0030)	0.2568_a (0.0016)

Note. Numbers in parentheses are standard deviations. Means sharing subscripts differ at $p < 0.05$ according to Duncan Significant Difference comparison. *nd=not detected

Discussion

It has been reported that sea cucumbers are also a source of vitamins and minerals. In our work, all *H. scabra* samples were checked for their vitamin contents including vitamin A, B_1, B_2, B_{12} and E. Similarly to previous research, vitamin E was detected in the *H. scabra* yet vitamin A was not detected.[14] Meanwhile, sodium (Na) was the second largest mineral content followed by magnesium (Mg), potassium (K), phosphorus (P), iron (Fe). According to Diniz *et al.,* (2012), the chemical composition of marine organisms, in general, may be influenced by a number of factors such as physiological characteristics, environmental conditions, habitat and life cycle.[18]

All heavy metals were analyzed in this study (Table 2) including Hg, Pb, Cd and As were not detected. Total 18 amino acids were identified (Table 3), 7 of them including Threonine, Leucine, Lysine, Valine, Isoleucine, Phenylalanine, and Methionine are essential amino acid needed for human. Glycine, proline and glutamic acid were the most abundant constituent in the sea cucumber H. scabra. In the case of fatty acids (Table 4), omega-6 from Bungin Island and Makassar were the major constituent in this species with 0.1834 % and 0.1377%. Meanwhile, omega-9 from Belitung Island and Lampung were the major constituent in this species with 0.2466 % and 0.1773 %. Eicosapentanoic acid (EPA) detected in all sampling locations but Docosahexaenoic acid (DHA) only found in Belitung Island and Makassar.

The heavy metals content in sea cucumber is specific, depend on sampling location and body compartment.[19] All the non-essential metals in the body wall of this *H. scabra* was below the allowed limits for human consumption as recommended by Indonesia, the US; Australia; the UK and European.[20-23] It means that *H. scabra* safely to consume.

Glycine, proline and glutamic acid were the most abundant constituent in the sea cucumber H. scabra. It is in line with another journal that said a high amount of proline was found in *H. scabra* only.[14] Arginine and tyrosine as semi-essential amino acid also detected nevertheless Histidine, Cysteine, Tryptophan were not detected.

Previous study conclude that a sea cucumber is a healthy food for human to reduce hypercholesterolemia due to ratio of lysine to arginine. According to this result the composition of lysine to arginine was good compared with another sea cucumber species such as *A. japanicus* (0.62).[24]

Omega-6 fatty acids have the potential to influence a number of chronic disease, such as cardiovascular disease and atherosclerosis.[25] On the other hand omega-3 found in this result was 0.0572 %. Omega-3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found naturally in fish oil and have the potential to be anti-inflammatory nutrient. It has been studying that it took role for the treatment of allergic diseases, arthritic pain, neuropatic pain, hypertriglyceridemia and cardiovascular prevention.[26-29] EPA and DHA are synthesized by marine plants both unicellular and multicellular such as phytoplankton and algae. They are eventually transferred through the food web and are incorporated into lipids of aquatic species.[30] The present results find that EPA detected in all sampling locations but DHA only found in Belitung Island and Makassar. It might be because of as a result of diet factors.

Previous study by Ridzwan *et al.,*[15] reported that saturated fatty acids in *H. scabra* were found dominated compared with unsaturated fatty acids. In this study, it happened only in Bungin Island. Another locations showed that unsaturated fatty acids were higher than saturated fatty acids. Main fatty acid found in the whole body were Palmitic acid, Oleic acid, Linoleic acid (LA), and Arachidonat acid (AA). There are also Alfa Linolenic acid (ALA), precursor fatty acid of the omega-3 fatty acids and Arachidonic acid (AA) as essential fatty acid for human, found in *H. scabra* in Bungin Island and Belitung Island. ALA is the precursor for longer chain unsaturated omega-3 to a limited extent desaturated to EPA and DHA. Through enzymatic metabolism of LA and ALA also generates arachidonic acid.

Conclusion

The high protein value and low fat were found in the sea cucumber Holothuria scabra from all sampling locations. The major mineral content were Calcium, Sodium, and Magnesium. Glycine was the major component of amino acid. Omega-6 and omega-9 were the major component of fatty acid. All heavy metals analyzed in this study were not detected. It can be inferred that the sea cucumber *H. scabra* is safely to consume and can be used as functional food in the future.

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Conflict of Interest

The authors have no potential competing interests.

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