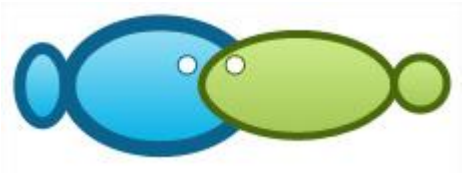

Evaluation of nutritional value of sea cucumber *Holothuria scabra* cultured in Bali, Indonesia

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Abstract. Sea cucumber *Holothuria scabra* has been successfully cultured by the Institute of Mariculture Research and Fisheries Extension in Bali, Indonesia since 2017. This study aimed to present the first report and evaluate the nutritional value of fresh *H. scabra*. *H. scabra* was collected from culture area of sea cucumber in Bali, Indonesia. Proximate composition showed that moisture, ash, protein and carbohydrate content was 87.12%, 6.82%, 5.10% and 0.96% of wet weight, respectively, while total fat was not identified in this study. About 0.48 mg 100 g^{-1} of vitamin E was contained in the sample, while vitamin A, B1, B2 and B12 were not identified. The order of mineral concentration found in the samples was Ca>Na>Mg>P>K>Fe. In fatty acid composition, the monounsaturated fatty acids (MUFAs) fraction was dominant (0.47%) followed by polyunsaturated fatty acids (PUFAs, 0.29%) and saturated fatty acids (SFAs, 0.28%). In amino acid profile, glycine was the major component (6,708.96 mg kg^{-1}) followed by glutamic acid (4,130.88 mg kg^{-1}), arginine (3,545.52 mg kg^{-1}) and proline (3,154.42 mg kg^{-1}). The results showed that *H. scabra* collected from culture area in Bali have potential to be used as a healthy food for human consumption.
Key Words: proximate composition, vitamin content, fatty acid, amino acid, wet weight.

Introduction. Sea cucumbers are marine invertebrates, belonging to the phylum Echinodermata and class Holothuroidea. They are distributed worldwide in marine habitats from shallow to deep sea area (Wen et al 2016; Rahman & Yusoff 2017; Gocer et al 2018). In the global market, sea cucumber species are known as *beche-de-mer, haisom* and *trepang* (Sicuro & Levine 2011). Although, high quantities of sea cucumber meat are consumed by many Asian countries, consumption of sea cucumber is becoming more popular around the world in recent years. Therefore the demand for them has increased dramatically every year (Aydin et al 2011; Liu et al 2017).

In general, the determination of biochemical content of sea cucumbers is focused on the nutritional value since they are regarded as potential functional food resources (Al Azad et al 2017). According to Drazen et al (2008), as one of the commercial products, the grade of sea cucumbers is related to some parameters such as the species, the thickness of the body wall, tastes, abundances, colours, appearances, textures, constituency, dryness and market demand.

In the last two decades, difficulties in obtaining sea cucumbers from wild stocks also occurred in Indonesia due to overfishing. Purcell (2014) reported that more than 70% of tropical sea cucumber fisheries are fully exploited, over-exploited or depleted.

The Japanese and Chinese were the first successful in developing hatchery technology for *Apostichopus japonicus* species (James 2004). In the other hand, *Holothuria scabra* was the first time successfully cultured in 1988 in India (James et al 1994). In recent years New Caledonia, Australia, Solomon Island, Australia, Vietnam, Maldives and Indonesia have also successfully cultured *H. scabra* using the same technology (James 2004).

Especially in the Institute of Mariculture Research and Fisheries Extension Bali, Indonesia *H. scabra* has been successfully cultured since 2017. However, there is no report about the nutritional value of this species collected from culture areas in Bali, Indonesia yet. In this study, we evaluated the nutritional value of *H. scabra* collected from culture areas in Bali and compare the data with the wild stocks. This data is very important to know the potency of cultured sea cucumber as a commercial healthy food product in the future.

Material and Method

Sample collection and preparation. About 5 kg fresh *H. scabra* was collected from cultured area in Bali, Indonesia. The sample was washed with running water to remove mud, sand as well as solid particles, and then dissected to remove its internal organs. After that, samples were packed in plastic bags and kept in icebox and transported to the Marine Natural Product Laboratory, Research Center for Oceanography, Indonesian Institute of Sciences in Jakarta. Thereafter, the samples were stored at -20^{O}C prior analysis.

Proximate analysis. Determination of proximate content was conducted according to the AOAC standard method. The moisture content (%) was determined by weighing about 2 g of *H. scabra* and oven dried at 105^{O}C for 4 hours. Afterward, the sample was cooled in a desiccator and reweighed until constant mass (AOAC 1990). Ash content (%) was determined by heating about 2 g of sea cucumber *H. scabra* for 4 hours in a muffle furnace at 550^{O}C. After cooled in a desiccator the sample was reweighed (AOAC 1990). The fat content (%) was determined by soxhlet apparatus. About 2 g of sea cucumber *H. scabra* was wrapped with a filter paper, and then placed it into thimble. Afterward, the soxhletation was conducted for 5 hours with 120 mL of petroleum ether. The spent samples with the thimble were reweighed (AOAC 2000).

Determination of the protein content (%) was performed by calculating the elemental N using the nitrogen-protein conversion factor of 6.25 (AOAC 2000). While the carbohydrate content (%) was determined by difference (Sroyraya et al 2017).

Mineral analysis. Minerals (sodium, calcium, potassium, phosphor, iron and magnesium) content were determined by using the Inductivity Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method.

Vitamin analysis. Determination of vitamins A and E was performed by using analytical High Performance Liquid Chromatography (HPLC). Vitamins B1 and B2 were determined by using Ultra Performance Liquid Chromatography (UPLC). While vitamin B12 was determined by using Liquid Chromatography Mass Spectrometry (LC-MM/MS)

Fatty acid analysis. Determination of fatty acid content was done by using gas chromatography (Perkin Elmer Clarus 580 GC). The condition of apparatus was used as follow: Column (Superco SPTM 2560 100 m 0.25 mm 0.2 µm), flow rate (18.0 cm sec^{-1} with column length 100 m); detector FID (240^{O}C); injector temperature (220^{O}C); split (1:100) and carrier gas (N_2).

Sample preparation for fat extraction was conducted according to AOAC standard method (AOAC 2000). About 5 g of *H. scabra* was added to 6 mL n-hexane and 4 mL isopropanol and then shaken for 1 minute. The solution was subsequently centrifuged for 3 minutes at 9,000 RPM. The supernatant was moved into a Hach tube and dried in a water bath. About 0.02–0.04 g of the fat extract was added to 1.5 mL, 0.5 M KOH methanol and the heated in a water bath at 100^{O}C for 20 minutes. Subsequently, 1.5 mL, 20% BF_3 in methanol was added and heated in a water bath at 100^{O}C for 20 minutes. The solution was cooled down to 30^{O}C while shaken and then 3 mL of saturated NaCl and 0.2 mL of n-hexane were added. The n-hexane-methyl ester layer was transferred into a 10 mL volumetric flask diluted with n-hexane and then injected to gas chromatography system.

Amino acid analysis. Preparation of sample and standard solution was conducted according to literature methods (Waters 2012). Determination of amino acid content was done by using Ultra Performance Liquid Chromatography condition as follow: Column (AccQ Taq Ultra C18 1.7 µm (2.1 x 100 mm); flow rate (0.5 mL minute^{-1}); temperature (49^{O}C); detector (FDA, wave length 280 mm); injection volume (1 µL) and mobile phase (mobile phase A = Eluent A Concentrate AccQ Taq Ultra from Waters (Part No. 186003838), mobile phase B = 10% mobile phase D, mobile phase C = Aquabidest and mobile phase D = Eluent B AccQ Taq Ultra from Waters (Part No. 1860005859).

Sample preparation: About 0.1 g *H. scabra* was added to 5 mL HCl 6N and then hydrolysed for 22 hours at 110^{O}C. The hydrolysed mixture was cooled and transferred into volumetric flask 50 mL and diluted to volume with distilled water. The solution was filtered with a 0.45 µm filter. About 500 µL of filtrate was added 40 µL AABA and 460 µl aquabidest. About 10 µL of solution was added to 70 µL AccQ Fluor Borate and 20 µL reagent fluor A and then injected into UPLC system.

Preparation of standard solution was performed as follow: about 40 µL of standard solution was mixed of amino acid. About 40 µL internal standard AABA and 920 µL aquabidest were added and then homogenized. About 10 µL of standard solution, 70 µL AccQ Fluor Borate and 20 µL of reagent fluor A was added and then vortexed for 1 minute. The solution was incubated for 10 minutes at 55^{O}C and then injected into UPLC system.

Results and Discussion

Proximate composition. The results of the proximate analysis, including moisture, ash, protein, fat and carbohydrate content of the *H. scabra* collected from culture area in Bali are shown in Figure 1. It was found that the moisture content of the sample was about 87.12% based on the wet weight. This result was same to be reported by Omran (2013), Ozer et al (2014) and Al Azad et al (2017) of the fresh *H. scabra* (84.49-87.21%) from wild stocks.

The high moisture content of sea cucumber was deemed as a tonic by fisherman before going to sea (Wen et al 2010). On the other hand, the fresh body walls of sea cucumbers contain higher moisture if compare to other shellfishes and fishes (Lee et al 2012). According to Chang-Lee et al (1989), there are several factors can be influenced the moisture content of organisms such as environmental, geographical variations, behaviour, feeding and the collection time of the year.

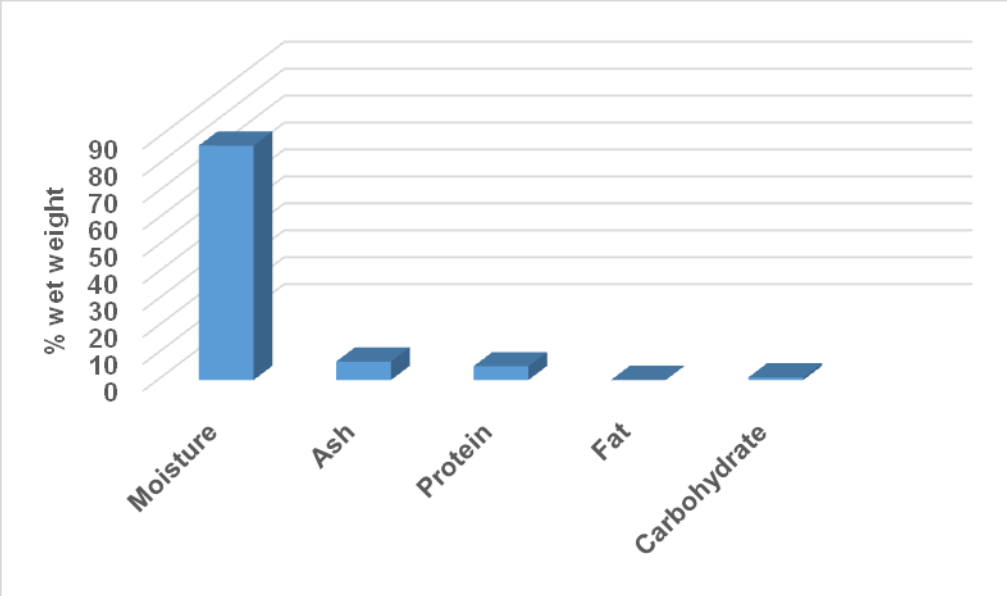

Figure 1. Proximate composition of *Holothuria scabra*.

High moisture content of fresh sea cucumber was also reported in previous studies of several fresh sea cucumbers, such as *H. mammata* (85.24%), *H. tubulosa* (84.3%) and *H. polii* (81.24%) (Aydin et al 2011), *H. arenicola* (72.12%) (Haider et al 2015), *H. edulis* (85.56%) (Al Azad et al 2017), *H. parva* (67.92%) and *H. arenicola* (68.49%) (Salarzadeh et al 2012), *H. leucospilota* (81.41 to 88.4%) (Omran 2013), *H. nobilis* (76.05%) (Oedjoe 2017), *A. japonicus* were in the range of 84 to 91% (Lee et al 2012; Vargara & Rodriguez 2016; Gao et al 2011), *Actinopyga mauritiana* (76.54 to 84.71%) (Omran 2013; Haider et al 2015) and *Bohadschia marmorata* (83.17%) (Omran 2013).

The obtained ash content was 6.82% based on wet weight. This result is higher than reported in the some other species of fresh sea cucumber, such as *H. mammata* (5.13%) and *H. tubulosa* (5.13%) (Aydin et al 2011), *A. japonicus* (2.99 to 3.30%) (Gao et al 2011) *Isostichopus* sp. (3.16 to 3.81%) (Vergara & Rodriguez 2016), but lower than *H. polii* (7.85%) (Aydin et al 2011). Apparently, the differences in the levels of ash in fresh sea cucumbers are also influenced by the living environments of sea cucumber and the species. According to Al Azad et al (2017), the ash content may due to the mineral deposit and other organic matter in sea cucumber.

Determination of protein content showed that the *H. sabra* was found to be 5.10% based on wet weight. Ozer et al (2004) also reported that the protein content of *H. scabra* were in the range of 5.45 to 5.78%. The result was found in this study lower than other species namely *H. arenicola* (24.37%) and *H. parva* (17.61%) (Salarzadeh et al 2012), *H. polii* (7.37-8.66%), *H. mammata* (7.88-11.1%) and *H. tubulosa* (3.01-8.82%) (Aydin et al 2011; Gonzalez-Wanguemert et al 2018) and *H. edulis* (7.48%) (Al Azad et al 2017).

Aydin et al (2011) reported that the seasonal variation could have influenced of the chemical composition of sea cucumber. In the other hand, the fluctuation of the protein content in sea cucumbers may be influenced of environmental factors, the life cycle of species, the seasonal variation and the physiological characteristics (Ozer et al 2004.

Sea cucumbers are generally characterized by high amounts of protein and low fat contents (Bordbar et al 2011). In this study, the fat content of *H. scabra* was not detected. Previous studies also reported that *H. scabra* collected from the wild stock had a very low fat content (0.17-0.37%) (Ozer et al 2004). In the other species were reported very low fat contents, such as *A. japonicus* (0.28-0.33%) (Lee et al 2012), *H. mammata*, *H. Polii* and *H. tubulosa* 0.18-0.55%, 0.15-0.33% and 0.0-0.21% respectively (Aydin et al 2011; Gonzalez-Wanguemert et al 2018), *Isostichopus* sp. (0.07-2.4%) (Vergara & Rodriguez 2016).

Several factors that might influence the fats content of sea cucumbers, such as species, reproductive, feed and feeding pattern as well as on environmental conditions (Lee et al 2012). In the other hand, Dong et al (2006) reported that the temperature of their living habitat, in which, the constant temperature gives higher fat content than those under fluctuating temperature.

Carbohydrate is an essential part of the human diet and used primarily energy source but very limited references are available reporting the carbohydrate content in sea cucumber. The carbohydrate content evaluated in the present study was about 0.96%. In previous studies were also reported that the fresh sea cucumbers have very low carbohydrate content, namely *H. scabra*, *H. edulis*, *H. leucospilota*, *H. nobilis*, *H. atra*, *H. impatiens*, *A. lecanora* and *B. argus* showed 0.45%, 1.14%, 2.12%, 0.56%, 0.87%, 1.37%, 2.31% and 1.89% respectively (Oedjoe 2017), *Paracaudina australis* 0.86% (Widianingsih et al 2016), *A. japonicus* 0.2-0.6% (Gao et al 2011; Jiang et al 2013), *H. polii* 2.61% and *H. tubulosa* 0.88% (Gonzalez-Wanguemert et al 2018).

Mineral composition. Evaluation of minerals composition of *H. scabra* is presented in Figure 2. The results showed that calcium (1,812.32 mg 100 g^{-1}) was the major component in this study, followed by sodium, magnesium, phosphor, potassium and iron with 666.18 mg 100 g^{-1}, 304.61 mg 100 g^{-1}, 87.82 mg 100 g^{-1}, 61.42 mg 100 g^{-1} and 2.84 mg 100 g^{-1} respectively. Calcium was also reported as the major component in other species, as *Apostichopus californicus* (Bechtel et al 2013), *H. arenicola* (Haider et al

2015), *H. sanctori* (Gocer et al 2018), *H. polii* and *H. tubulosa* (Gonzalez-Wanguemert et al 2018).

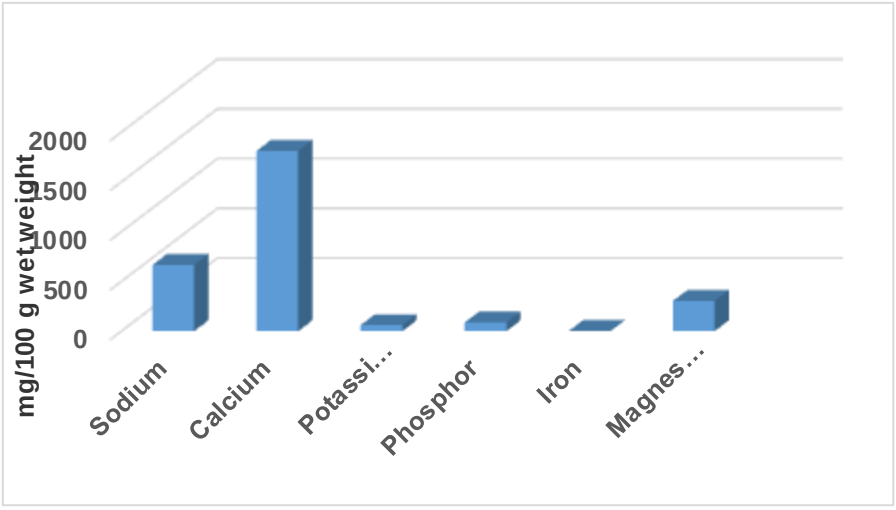

Figure 2. Mineral composition of *Holothuria scabra*.

Information about minerals content of sea cucumbers are still very limited. Minerals are important in terms of the nutritional point of view. Some minerals are essential for humans such as calcium, magnesium, sodium and phosphor (Gocer et al 2018).

Related to the result in this study and the results of previous studies showed that the difference in the major mineral content in sea cucumbers is influenced by species. According to Olgunoglu & Olgunoglu, (2017), the macro minerals levels of seafood may be influenced by season, age, maturity, water temperature, season, availability of food, type of diet and feeding system of organisms.

Barzkar et al (2017) reported that magnesium is the major component in the sea cucumber *H. arenicola,* while iron is the major component in *Stichopus horrens.* Sodium has been reported in high quantities in *A. mauritiana* sea cucumber (Haider et al 2015), *H. mammata* (Gonzalez-Wanguemert et al 2018), *A. californicus* (Bechtel et al 2013).

Vitamin composition. Sea cucumbers have an impressive profile of high-value nutrients such as vitamin A, vitamin B1 (thiamin), vitamin B2 (riboflavin) and vitamin B3 (niacin) (Bordbar et al 2011). In this study, we were examined several vitamins namely vitamin A, vitamin B1, vitamin B2, vitamin E and vitamin B12. The results showed that *H. scabra* contained vitamin E (0.48 mg kg^{-1} wet weight), while vitamin A, B1, B2 and B12 were not detected (Figure 3).

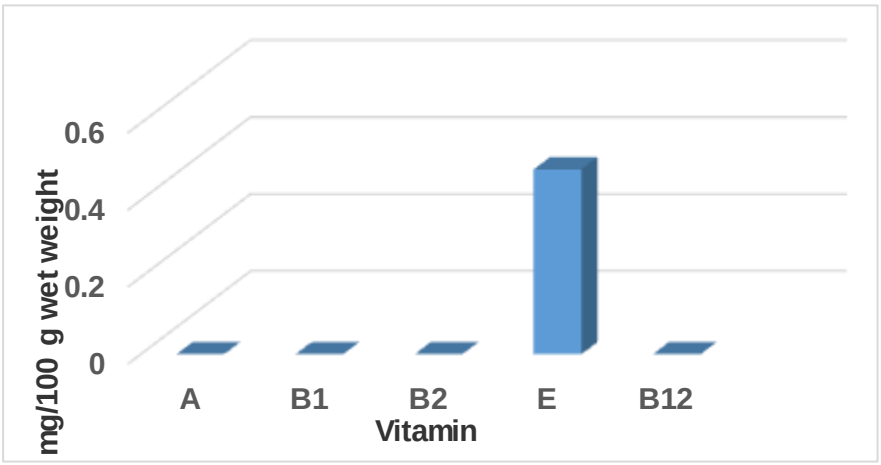

Figure 3. Vitamin composition of *Holothuria scabra*.

Sroyraya et al (2017) reported that *H. scabra* from wild stock contained vitamin E and vitamin C. Vitamin E was also detected in other species, namely *A. japonicus* (Wang et al 2013).

Fatty acid composition. Fatty acids profile of *H. scabra* is presented in Figure 4. Monounsaturated fatty acids (MUFAs) fraction were foundin the highest quantity followed by polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs). Fatty acid composition varied due to species and habitat of sea cucumbers (Al Azad et al 2017). In the other hand, food sources and ambient temperature of different regions could influence the fatty acid composition in the sea cucumber (Taboada et al 2003).

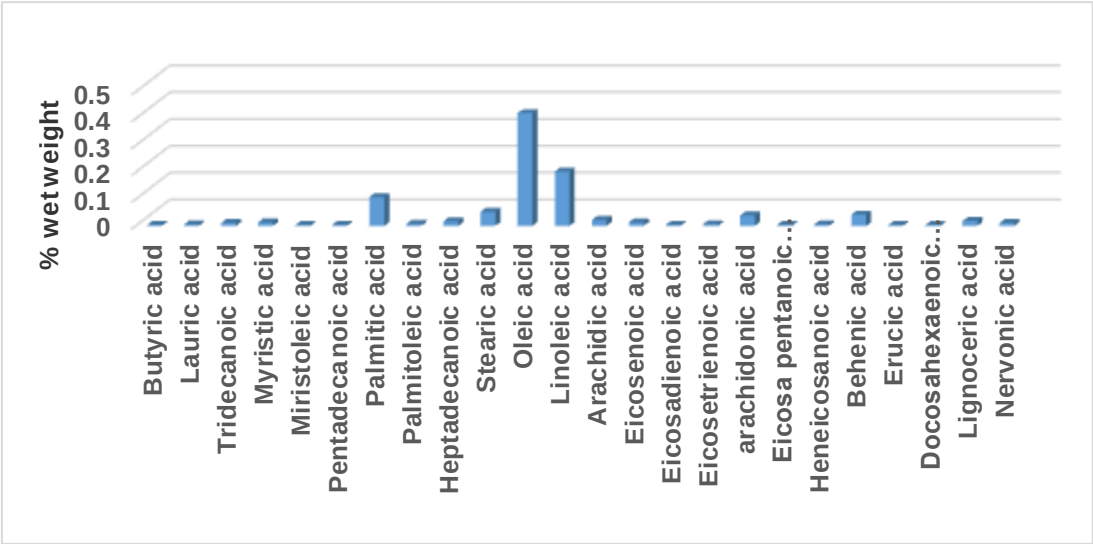

Figure 4. Fatty acids profile of *Holothuria scabra*.

Monounsaturated fatty acids. Analysis of monounsaturated fatty acids showed that oleic acid was the major component, followed by heptadecanoic acid, nervonic acid, eicosenoic acid, palmitoleic acid, erucic acid and miristoleic acid respectively. Similar to the previous study reported by Ozer et al (2004) that oleic acid was the major MUFA measured in *H. scabra*, *H. leucospilota*, *B. marmorata* and *A. mauritiana*, whereas Ridzwan et al (2014) and Al Azad et al (2017) reported that Palmitoleic acid was the major component in *H. scabra.*

In other species, *H. polii* and *H. tubulosa* (Sicuro et al 2012), *H. fuscounctata*, *H. fuscogilva*, *Actinopyga caerulea*, *Thelenota anax* and *T. ananas* (Wen et al 2010), *H. atra*, *H. leucospilota*, *S. horrens* (Ridzwan et al 2014) and *A. californicus* (Bechtel et al 2013) palmitoleic acid was the major MUFA. Related to the high value content of palmitoleic acid in marine organisms, it may be influenced by marine microalgae such as *Phaeodactylum tricornutum* (Frigon et al 2014). Nervonic acid was the major MUFA obtained in *Acthyonidium chliensis* (Careaga et al 2013), whereas erucic acid was the major MUFA obtained in *H. leucospilota* (Yahyavi et al 2012).

Polyunsaturated fatty acids. Linoleic acid was the major PUFA examined in this study followed by arachidonic acid, eicosapentanoic acid, eicosatrienoic acid and docosahexaenoic acid respectively. Linoleic acid was the major PUFA in *A. mauritiana* (Haider et al 2015). Arachidonic acid was the major PUFA in other sea cucumbers, such as *H. edulis* (Al Azad et al 2017), *H. scabra*, *H. atra*, *H. leucospilota*, *S. horrens* (Ridzwan et al 2014), *Bohadschia argus*, *H. fuscogilva*, *A. caerulea*, *Stichopus herrmanni*, *A. mauritiana*, *H. fuscopunctata*, *T. ananas* and *T. anax* (Wen et al 2010).

Eicosapentanoic acid was the major PUFA in *A. californicus* (Bechtel et al 2013) and *H. arenicola* (Haider et al 2015). Eicosatrienoic acid was the major PUFA in *H. tubulosa* and *H. polii* (Sicuro et al 2012). Docosahexaenoic acid was the major PUFA in *H.*

scabra and *H. leucospilota* (Yahyavi et al 2012), whereas eicosadienoic acid was the major PUFA reported in *Athyonidium chilensis* (Carega et al 2012).

Saturated fatty acids. Saturated fatty acid fractions examined in the present study showed that palmitic acid was the dominant SFA, followed by stearic acid, behenic acid, arachidic acid, ligniceric acid, myristic acid, tridecanoic acid, lauric acid, henecosanoic acid, butyric acid and pentadecanoic acid respectively. Similar to previous studies where palmitic acid was the major SFA in *H. scabra* (Al Azad et al 2017; Ridzwan et al 2014), *H. atra* and *H. leucospilota* (Ridzwan et al 2014), *H. polii* (Sicuro et al 2012), *H. edulis* (Al Azad et al 2017), *Actinopyga mauritiana* (Omran 2013), *A. californicus* (Bechtel et al 2013), *T. anax*, *T. ananas*, *S. herrmanni*, *Bohadschia argus*, *H. fuscopunctata*, *Actinopyga caerutes* and *H. fuscogilva* (Wen et al 2010).

In other species, stearic acid was the major SFA, such as *A. chilensis* (Careaga et al 2013), *H. tubulosa* (Sicuro et al 2012), *H. arenicola* (Haider et al 2015) and *A. mauritiana* (Wen et al 2010). Yahyavi et al (2012) reported that behenic acid was the major SFA in *H. scabra* and *H. leucospilota,* whereas myristic acid was the major SFA in *H. scabra*, *H. leucospilota* and *B. marmorata* (Omran 2013).

Amino acid composition. Analysis of the amino acids composition showed that 16 amino acids were detected in fresh *H. scabra*, where threonine was found to be the major essential amino acid, followed by leucine, phenylalanine, lysine, valine, isoleucine, histidine and tryptophan respectively (Figure 5). Wen et al (2010) reported similar finding that threonine was the major essential amino acid in *H. fuscogilva*, *H. fuscounctata*, *A. caerulea*, *S. herrmanni*, *T. anax* and *T. ananas.*

Leucine was the major essential amino acid reported in *B. argus* (Wen et al 2010) and *A. mauritiana* (Haider et al 2015), whereas lysine was the major essential amino acid in *A. mauritiana* (Omran 2013). Valine was reported to be the major essential amino acid in *B. marmorata* (Omran 2013). Histidine was the major essential amino acid reported in *H. scabra* and *H. leucospilota* (Omran 2013).

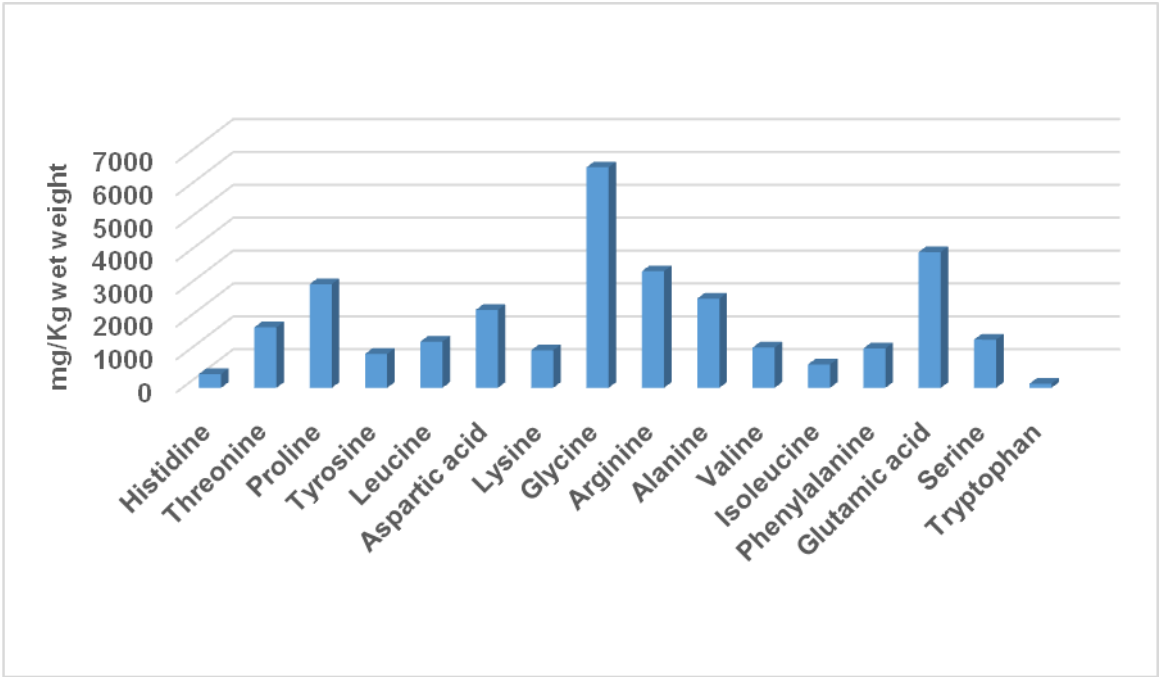

Figure 5. Amino acids profile of *Holothuria scabra*.

Glycine was the major non-essential amino acid examined in the present study, followed by glutamic acid, arginine, proline, alanine, aspartic acid, serine and tyrosine respectively. Omran (2013) also reported that glycine was the major non-essential amino acid in *H. scabra*, *H. leucospilota*, *B. marmorata* and *A. mauritiana*, whici is similar to

other species, namely *H. tubulosa* and *H. polii* (Sicuro et al 2012), *A. mauritiana* and *H. arenicola* (Haider et al 2015), *H. fuscogilva*, *H. fuscopunctata*, *T. anax*, *T. ananas*, *S. herrmanni*, *B. argus* and *A. caerulea* (Wen et al 2010). Glutamic acid was the major non-essential amino acid reported in *A. californicus* (Bechtel et al 2013), *A. japonicus* (Lee et al 2012), whereas arginine in *H. polii* and *H. tubulosa* (Sicuro et al 2012) and *H. arenicola* (Haider et al 2015).

Prospect of the cultured H. scabra from Bali. The success of the Institute of Mariculture Research and Fisheries Extension Bali, Indonesian culturing *H. scabra* will have an impact on the development and supply of sea cucumber raw materials in Indonesia which so far have relied on wild stocks. Moreover, this type has a very high price on the global market.

Based on the above results were showed that *H. scabra* cultured by the Institute of Mariculture Research and Fisheries Extension Bali, Indonesia has the same nutritional content as those from wild stocks. Utilization of *H. scabra* as a healthy food is very possible because it has high levels of protein without fat, contains vitamin E which can act as an antioxidant, contains minerals that are very important in high quantities, especially calcium and magnesium. It also contains omega-3, omega-6 and omega-9, and 16 types of amino acids.

Conclusions. *H. scabra* cultured by the Institute of Mariculture Research and Fisheries Extension in Bali, Indonesia has significant protein and no fat content, contains vitamin E, contains important minerals in large quantities mainly calcium and magnesium. The samples also contained omega-3, omega-6, omega-9 and 16 types of amino acids. Therefore, *H. scabra* from culture area of Bali could have potential nutritional value to be used as a healthy food for humans in the future.

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