An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

A STUDY ON PROXIMATE CONTENT, FATTY ACID AND AMINO ACID PROFILE OF SEA CUCUMBER *STICHOPUS NOCTIVAGUS*

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ARTICLE INFO

Key Words

Amino acids, fatty acids, proximate, *Stichopusnoctivagus*

ABSTRACT

Stichopus noctivagus is one of the common sea cucumber species harvested in Indonesia waters. The objective of this study was to highlight the proximate content, fatty acid and amino acid profile of the sea cucumber *S. noctivagus* from West Nusa Tenggara waters, Indonesia. Proximate (moisture, ash, fat and protein) contents were determined by AOAC standard method. Fatty acid was determined by GC method and amino acid was determined by UPLC method. Results show that moisture (94.39%), ash (2.94%), fat (0.40%) and protein 1.78%) basis on the wet weight. Fourteen amino acids were identified in this study where glutamic acid was the major constituent in the species. Ten fatty acids were identified where palmitic acid was the major constituent in this species. The nutritional value of the sea cucumber *S. noctivagus* from West Nusa Tenggara waters Indonesia indicated that it will have the potential to be a supplement food for human in the future.

INTRODUCTION:

Indonesian coastal waters are suitable habitat for diversities of marine flora and fauna related to its tropical region. Sea cucumbers have been fishing all over the world and found abundant in the tropical region [1].[2] reported that the total annual catch all over the world is in the order of 100,000 tons of fresh sea cucumbers. Majority of fisheries survive in Malaysia, Madagascar, Philippines, Japan, China, Ecuador, New Caledonia, Republic of Korea, Indonesia and Australia [3]. Sea cucumbers species are commercially harvested in the fresh or dried form, meanly in China, Korea, Japan and Indonesia as functional foods due to their high protein content and their tonical, putative aphrodisiac and medicinal properties.

Nevertheless, some of them have been over exploited, which may result in a population collapse and also loss of significant potential source of anticancer drug for the future [4]. In spite of the fact that there are many studies have been reported about benefits of the sea cucumbers, but there is no scientific information yet related to *Stichopus noctivagus* from West Nusa Tenggara waters Indonesia in terms of their nutritional value. Based on above, the objective of the present study was to highlight the proximate content, fatty acid profile and amino acid profile of sea cucumber *S. noctivagus* in the particular location related to the quality and potency as an alternative source of food supplements for human in the future.

MATERIALS AND METHODS:

Sample collection and preparation

Sea cucumber *Stichopus noctivagus* was collected from West Nusa Tenggara waters, Indonesia in April 2017. Immediately after collected, the sea cucumbers were cleaned and washed with seawater to remove their internal organs. Only the body wall was kept in ice box and brought to the Marien Natural Product laboratory, Research Center for Oceanography, Jakarta for keeping at -2o^{0}C until further analysis.

Proximate analysis: Proximate analysis including moisture, ash, fat and protein contents was determined according to the standard method of AOAC. The moisture content (% wet weight) was determined by drying 2 g sea cucumber *S. noctivagus* in an oven at 105^{0}C for 3 hours. Immediately after being cooled in a desiccator, sample was reweighed [5]. Fat content (% wet weight) was determined by loosely wrapping 2 g sea cucumber *S. noctivagus* with a filter paper and put into the thimble which was fitted to a clean round bottom flask, dried and weighed. A 120 ml of petroleum ether was put into the flask. The sample was heated and allowed to reflux for 5 hours. The spent samples with the tumble kept and later weighed [5]. Protein content (% wet weight) was analysed by calculating the elemental N determination using the nitrogen-protein conversion factor of 6.25 [5].

Fatty acid analysis: Analysis of fatty acid profile by using the gas chromatography (Perkin Elmer Clarus 580 GC). The apparatus condition: carrier gas (N_2); detector FID (24^{0}0C); column (Supelco SPTM 2560 100m 0.25 mm 0.2 µm); flow rate (18.0 cm/sec with column length 100 m); injector temperature (250C) and split (1:100). Sample preparation for fat extraction according to the standard method of AOAC [6]. A 5 g sea cucumber *S. noctivagus* was added with 4 ml isopropanol and shaken for 1 minute. The solution was added 6 ml n-hexane then centrifuged for 3 minutes at 9000 RPM. The upper solution was poured into a Hach tube and was dried at a water bath. About 0.03-0.04 g of the fat extract was added 1.5 ml KOH methanol 0.5 M. The solution was heated at a water bath at 100^{0}C for 20 minutes and added 1.5 ml BF_3 20% in methanol. The solution was heated at a water bath at 100^{0}C for 10 minutes and cooled down to 30^{0}C. A 3 ml saturated NaCl and 0.2 ml n-hexane was added. The mixture was allowed at room temperature for 10 minutes. The n-hexane methyl ester layer was poured into 10 ml volumetric flask, eluted with n-hexane and then injected into gas chromatography.

Amino acid analysis: Sample and standard solution preparation for amino acid analysis was done by literature method [7]. Amino acid was determined by using UPLC. Apparatus condition: column (AccQ Tag Ultra C18 1:7 µm Waters); flow rate (0.5 ml per minute); temperature (49^{0}C); mobile phase (mobile phase A = eluent A Concentrate AccQ Tag Ultra from Waters (Part No. 186003838); mobile phase B = 10% phase D; mobile phase C = aquabidest and mobile phase D = eluent B Accq Tag Ultra from Waters (Part No. 18600389); detector (PDA, wavelength 260 nm) and injection volume (1 µl). Sample preparation: A 0.1 g sea cucumber *Stichopus noctivagus* was added 5 ml HCl 6N. The mixture was hydrolised for 22 hours at 110^{0}C. After cooled down, the hydrolised mixture was poured into volumetric flask 50 ml and diluted to volume with aquadest. The solution was filtered by 0.45 µm filter. A 500 µl of filtrate was added 40 µl AABA and 460 µl aquabidest. A 10 µl solution was added 70 µl AcsQ Fluor Borate and 20 µl reagent fluor A. The solution was incubated for 10 minutes at 550C and then injected into UPLC system.

Standard solution preparation: A 40 µl standar solution was mixed of amino acid. A 40 µl internal standard AABA and 920 µl aquabidest were added. A 10 µl standard solution and 70 l AccQ Fluor were added and then homogenized. The solution was incubated for minutes at 550C and then injected into UPLC system.

RESULTS AND DISCUSSION

Proximate compositions

In general, determination of proximate content was conducted focusing on the nutritional value of the sea cucumbers since they are deemed as functional food resources [8]. The sea cucumbers grade as the commercial product is related to the species, abundance, taste, colour, appearance, the thickness of the body wall, texture, constituency, dryness and market demand [10]. Inthis study, *Stichopus noctivagus* was evaluated whether their nutritional value have the potency to be a commercial importance product in the future.The proximate (moisture, ash, fat and protein) contents of the sea cucumber *S. noctivagus* from West Nusa Tenggara, Indonesia based on the wet weight was shown in table 1. Moisture content of the fresh sea cucumber *S. noctivagus* was 94.39%. Apparently, most of the fresh sea cucumbers have the moisture content in high value. In the previous study reported that the moisture content of several fresh sea cucumbers in high value, such as *Apostichopus japonicas* were in the range of 84% to 91% [9], *Isostichopus* sp. (83% to 86%) [10], *Holothuria polii* (81.24%), *H. tubulosa* (84.3%) and *H. mammata* (85.24%) [11], *Parastichopus* sp. (89% to 90%) [12], *Cucumaria frondosa* (87% to 90%) [13] and *Thelenot aananas* (87.83%) and *Acaudina molpadioide* (76.97%) [14], *H. edulis* (85.56%) [8], *H. scabra* (84.49% to 87.21%) [8, 15, 16], *Isostichopus* sp. (83.74% to 86.92%) [17], *A. japonicus*(89.05% to 91.10%) [18], *H. arenicola* (72.12%) and *Actinopyga mauritiana* (76.54%) [3], *A. mauritiana* (84.71%), *Bohadschia marmorata* (83.17%) and *H. leucospilota* (81.41%) [16], *H. parva* (67.92%) and *H. arenicola* (69.49%) [19], *H. leucospilota* (88.4%), *H. atra* (85.8%), *H. impatiens* (83.1%), *H. pardalis* (85.5%), *H. moebii* (86.8%), *Pearsonothuria graeffei* (89.3%), *B. argus* (91.4%), *S. chloronotus* (94.3%) and *Euapta godeffroyi* (84.2%) [20]. [12] was reported that moisture content organisms can be inflated by several factors, such as the collection time of year, geographical variations, feeding behaviour and environmental factors. Compare to the other fish and shellfish, in generally the fresh sea cucumbers contain higher moisture in the body wall [9]. Even the high moisture content of sea cucumber was deemed as a tonic food by fisheries [21]. The ash content examined in this study was 2.94%.The value obtainedin present study is lower than *Parastichopus* spp. (3.16% to 3.81%) [12], *H. polii* (7.85%), *H. tubulosa* (5.13% and *H. mammata* (5.13%) [11], *Isostichopus* sp. (3.16% to 3.81%) [17], *A. japonicas* (2.99% to 3.30%) [18] and *H. scabra* (3.59% to 11.06% [8, 15], but higher than *H. edulis* (1.27%) [8], *T. ananas* (1.6%) and *A. molpadioide* (0.99%) 14]. Apparently the ash content may be due to the mineral deposit and other inorganic matter in sae cucumbers [8]. The protein content of the fresh sea cucumber *S. noctivagus* examined in this study was 1.78%.The result was lower than other species reported in the previous study namely *H. polii* (8.66%), *H. tubulosa* (8.82%), *H. mammata* (7.88%) [11], *H. edulis* (7.48%) and *H. scabra* (5.45%) [8], *Isostichopus* sp (2.74% to 6.63%) [17], *Parastichopus* sp. (2.5% to 13.8%) [12], *Apostichopus japonicas* (1.13% to 3.99%)) [9], *T. pineapple* (16.64%) and *A. molpadioides* (12.94%) [14], *A. japonicas* (3.66 % to 5.40%) [18], *H. parva*(17.61%) and *H. arenicola* (24.37%) [19] and *H. scabra*

(5.78% to 9.53% [15]. [11] was reported that the seasonal variation could have influenced of the chemical composition of sea cucumbers. [15] also reported that the fluctuation of the protein content of sea cucumber may be influenced of the physiological characteristics, seasonal variation, the life cycle of the species and the geographical aspects. The fat content examined in this study was 0.40%. This result was lower than *S. japonicas* was 0.56% to 2.3% [9], *H. parva* (2.43%) and *H. arenicola* (2.88%) [19] but higher than *A. japonicas* (0.28% to 0.33%) [9], *T. ananas* (0.27%) and *A. molpadioides* (0.03%) [14], *H. tubulos, H. polii* and *H. mammata* were 0.09%, 0.15% and 0.18% respectively [11], *Isostichopus* sp. (0.07% to 24%) [17] *H. scabra*(0.17% to 0.37% [15]. The temperature has an effect due to the fat contents. The constant temperature was higher fat content than those under corresponding fluctuation temperature [22]. Apparently all sea cucumbers may have wide variation in the fat content and might be due to reproctive and the type of species, feed and feeding pattern as well as on environmental conditions [9].

Fatty acid profile: Sea cucumbers are the bottom sediment feeder where the microbial-enriched detritus as food source which are generally contain branched chain fatty acid, namely monounsaturated fatty acid, polyunsaturated fatty acid and saturated fatty acid [23]. The fatty acids profile of sea cucumber *Stichopus noctivagus* was shown in table 2.

Saturated fatty acid: The majority of fatty acid obtained in *Stichopus noctivagus* was saturated fatty acid (SFA) followed by monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). Palmitic acid was the major SFA followed by stearic acid, lauric acid, myristic acid and butyric acid. In the previous study reported that palmitic acid was the major SFA in *Actinopyga mauritiana* [16], *Thelenota ananas, S. herrmanni, T. anax, Holothuria fuscogilva, A. caerutea, H. fuscounctata* and *Bohadschia argus* [21], *H. leucospilota*, *H. impatiens*, *H. atra*, *H. pardalis*, *Pearsonothuri agraeffei*, *H. moebii*, *A. lecanora*, *Euapta godeffroyi* and *S. chloronotus* [20], *H. polii* [24], *H. edulis* and *H. Scabra* [8], *H. leucospilota , H. scabra* and *H. atra* [25] and *Parastichopus californicus* [26]. Different with the other species, stearic acid was the major SFA in *A. mauritiana* [21], *H. tibulosa* [24] and *Athyonidium chilensis* [27], Myristic acid was the major SFA in *H. scabra, B. marmorata* and *H. leucospilota* [16], *S. horrens* [23] and *H. arenicola* [3]. Capric acid was the major SFA in *A. mauritiana* [3]. Behenic acid was the major SFA in *H. scabra* and *H. leucospilota* [28].

Monounsaturated fatty acid: Palmitoleic acid was the major monounsaturated fatty acid (MUFA) examined in this study followed by oleic acid. Palmitoleic acid also was the major MUFA in *Parastichopus californicus* [26], *H. polii* and *Holothuria tubulosa* [24], *Stichopus japonicas* and *Pearsonothuria graeffei* [20], *Thelenota ananas, H. fuscounctata, T. anax, H. fuscogilva* and *Actinopyga caerutea* [21], *H. scabra* [8, 25], *S. horrens, H. leucospilota* and *H. atra* [25]. The present of palmitoleic acid in the fatty acid composition may be originated from marine microalgae such as diatom *Phaeodactylum tricornutum* which contain high value of palmitoleic acid [29].

Oleic acid was the major MUFA obtained in *H. sacbra, Bohadschia marmorata, A. mauritiana* and *H. leucospilota* [16]. Eicosenoic acid was the major MUFA in *S. herrmanni, A. mauritiana, Bohadschia argus. H. atra, H. impatiens, H. leucospilota, H. pardalis, H. moebii, A. lecanora, B. argus, S. chloronotus* and *Euapta godeffroyi* [20]. Pentadecenoic acid was the major MUFA obtained in *A. mauritiana* and *H. arenicola* [3]. Erucid acid was the major MUFA obtained in *H. scabra* and *H. leucospilota* [28]. Nervonic acid was the major MUFA in *Athyonidium chilensis* [27].

Polyunsaturated fatty acid: Arachidonic acid and linoleic acid were the major polyunsaturated fatty acid (PUFA) in *Stichopus noctivagus* followed by cis-5,8,11,14,17-eicosapentanoic acid. In the previous study reported that arachidonic acid was the major PUFA obtained in *H. fuscounctata*, *Actinopyga caerutea, S. herrmanni, Thelenota ananas, Holothuria fuscogilva*, *T. anax* and *A. mauritiana* [21], *H. leucospilota*, *H. atra*, *H. impatiens*, *H. pardalis*, *H. moebii*, *A. lecanora*, *S. chloronotus* and *Pearsonothuria graeffei* [20], *H. atra*, *H. scabra*, *H. leucopsliota* and *S. horrens* [25], *Bohadschia argus* [20, 21]and *H. edulis* [8]. The other PUFA, namely Cis-5,8,11,14,17-eicosapentanoic was the major constituent in *H. arenicola* and linoleic acid in *A. mauritiana* [3]. Eicosatrienoic acid was the major PUFA in *H. tubulosa* and *H. polii* [24].Linoelaidic acid was the major PUFA in *Bohadschia marmorata*, *A. mauritiana*, *H. scabra*and*H. Leucospilota* [16]. Eicosapentaenoic acid was the major PUFA in *Euaptagodeffroyi* and *S. japonicus* [20] and *Parastichopus californicus* [26]. Docosanexaenoic acid was the major PUFA in *H. sacbra* and *H. leucospilota* [28]. Eicosadienoic acid was the major PUFA in *Athyonidium chilensis* [27]. Apparently the fatty acid composition varies due to the species and habitat where the species survive [8]. In addition, food sources and ambient temperature of different regions could influenced of the composition of fatty acids in the sea cucumbers [30].

Amino acid profile: Total 14 amino acids were identified in fresh sea cucumber *Stichopus noctivagus* where glutamic acid and glycine were the major component. The major essential amino acid was phenylalanine (1352.53 mg/kg) followed by threonine (771.28 mg/kg), leucine (641.93 mg/kg), lysine (528.9 mg/kg), valine (479.40 mg/kg), isoleucine (435.36 mg/kg) and methionine (118.07 mg/kg).The other essential amino acid namely threonine was the major essential amino acid in *Thelenota ananas, T. anax, Bohadschia argus, Holothuria fuscogilva*, *Stichopus herrmanni, H. fuscounctata* and *A. caerutea* [21], leucine in *B. argus* [21], *A. Mauritiana* [3], lycine in *A. mauritiana*, [16], histidine in *H. leucospilota* and *H. scabra* [16], valine in *B. marmorata* 16], arginine in *H. polii*and *H. tubulosa* [24], *Parastichopus californicus* [26], *H. arenicola* [3] and *Apostichopus japonicas* [9]. The major non-essential amino acid identified in this study was glutamic acid (2952.05 mg/kg) followed by glycine (2672.38 mg/kg), alanine (1443.7 mg/kg), aspartic acid (1324.07 mg/kg), proline (1118.77 mg/kg), serine (830.17 mg/kg) and tyrosine (413.73 mg/kg). Similar to previous study, glutamic acid was the major non-essential amino acid in *P. californicus* [26] and *A. japonicas* [9].

In the other species, glycine was the major non-essential amino acid in *H. fuscounctata*, *A. caerutea*,*S. hermanni*, *T. anaans*, *T. anax*, *H. fuscogilva* and *B. argus* [21], *B. marmorata*, *A. Mauritiana*, *H. scabra* and *H. Leucospilota* [16], *H. tubulosa*and *H. polii* [24], *A. mauritiana* and *H. arenicola* [3]. The omega-3/omega-6 ratio of sea cucumber *S. noctivagus* examined in this study was 0.25. This value was similar to reported by [21] for common sea cucumbers which range from 0.25 to 0.61. Based on recommendation of [31] that the omega-3/omega-6 ratio should be ranged from 1.125 to 0.4, therefore the result of this study may contribute to maintaining the recommended omega-3/omega-6 ration in the diet for human.

Table 1: Proximate composition of fresh sea cucumber *Stichopus noctivagus*

No	Parameter	Result (% wet weight)
1	Moisture	94.39
2	Ash	2.94
3	Fat	0.40
4	Protein	1.78

Table 2: Fatty acid composition of fresh sea cucumber *Stichopus noctivagus*

No	Fatty acid	Result (%)
1	C4:0 (Butyric acid)	0.02
2	C12:0 (Lauric acid)	0.04
3	C14:0 (Myristic acid)	0.03
4	C16:0 (Palmitic acid)	0.12
5	C16:1 (Palmitoleic acid)	0.03
6	C18:0 (Stearic acid)	0.04
7	C18:1 ω9C (Oleic acid)	0.02
8	C18:2 ω6C (Linoleic acid)	0.04
9	C20:4 ω6 (Aracidonic acid)	0.04
10	C20:5 ω3 (cis-5,8,11,14,17-Eicosapentanoic acid)	0.02
11	SFA (Saturated Fatty Acid)	0.25
12	MUFA (Monounsaturated Fatty Acid)	0.09
13	PUFA (Polyunsaturated Fatty Acid)	0.06

Table 3. Amino acid composition of fresh sea cucumber *Stichopus noctivagus*

No	Amino acid	Result (mg/kg)
	Essential amino acids	
1	Histidine	nd
2	Threonine	771.28
3	Valine	479.40
4	Isoleucine	435.36
5	Phenylalanine	1352.53
6	Leucine	641.93
7	Methionine	118.07
8	Cysteine	nd
9	Lysine	528.9
10	Tryptophan	nd
11	Arginine	nd
	Non-essential amino acids	
12	Alanine	1443.7
13	Glutamic acid	2952.05
14	Serine	830.17
15	Proline	1118.77
16	Tyrosine	413.73
17	Aspartic acid	1324.07
18	Glycine	2672.38

CONCLUSION

The protein content examined in this study was significantly and low-fat content was observed in the sea cucumber *Stichopus noctivagus* from West Nusa Tenggara water, Indonesia. Glutamic acid was the major component of amino acid, whereas palmitic acid was the major component of fatty acid. The nutritional value of the sea cucumber *S. noctivagus* indicated that it will have the potential to be a supplement food for human in the future.

REFERENCES:

1. Torai-Granada, V., Lovatelli, A. and Vasconcellos, M. (2008). FAO Fisheries and Aquaculture Technical Paper No. 516. Food and Agriculture Organization of the United Nations, Rome, Italy: 257-282.
2. Purcell, S. W. (2010). Managing sea cucumber fisheries with and ecosystem approach. FAO Fisheries and Aquaculture Technical Paper No. 520 Food and Agriculture Organization of the United Nation, Rome, Italy: 9-26.
3. Haider, M. S., Sultana, R., Jamil, K., Lakht-e-Zehra, Tarar, O. M., Shirin, K. and Afzal, W. (2015). A study on proximate composition, amino acid profile, fatty acid profile, and some mineral contents in two species of sea cucumber. *The Journal of Animal and Plant Sciences.* 25(1): 168-175.
4. Perez-Espadas, A. R., Verde-Star, M. J. Rivas-Morales, C. Orandav-Cardenas, A., Morales-Rubro, M., Leon-Deniz, L. V., Canul-Canche, J. and Quijano, L. (2014). *In vitro* cytotoxic activity of *Isostichopus badionorus,* a sea cucumber from Yucatan Peninsula coast. *Journal of Pharmacy and Nutrition Sciences.* 4: 183-186.
5. AOAC. (1990). Official Methods of Analysis of the Association of Official Analytical Chemists. 15th Edition. Washington D.C.
6. AOAC. (2000). Official Methods of Analysis of the Association of Official Analysis Chemists. 17th Edition. Washington D.C.
7. Waters (2012). Acquity UPLC H-Class and H-Class Bio Amino Acid Analysis System Guide. USA.
8. Al Azad, S., Shaleh, S. R. M. and Siddiquee, S.(2017). Composition of fatty acid and proximate composition between *Holothuria edulis* and *Holothuria scabra* collected from coastal water of Sabah, Malaysia. *Advances in Biosciences and Biotechnology*.8: 91-103.
9. Lee, M. H., Kim, Y. K., Moon, H., Kim, K. D., Kim, G. G., Cho, H. A., Yoon, N., Sim, K., Park, H. Y., Lee, D. S., Lim, C. W., Yoon, H. D. and Han, H. N. (2012). Comparison on proximate composition and nutritional profile of red and black sea cucumber *(Apostichopus japonicas)* from Ulleungdo (Island) and Dokdo (Island), Korea. *Food Science and Biotechnology.* 21: 1285-1291.
10. Drazen, J., Phleger, C., Guest, M. and Nicholas, P. (2008). Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: Food Web Implications. *Comparative Biochemistry and Physiology.* Part B. 151: 79-87.
11. Aydin. M., Sevgili, H., Tufan, B., Emre, Y. and Kose, S. (2011). Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumber from Turkey. *International Journal of Food Science & Tecchnology*.46:500-508.00-508.

12. Chang-Lee, M.V., Price, R. J., and Lampila, L. E. (1989). Effect of processing on proximate composition and mineral content of sea cucumber *(Parastichopus* spp.*). Journal of Food Science.* 54: 567-568.

13. Zhong, Y., Khan, M. A. and Shahidi, F. (2007). Compositional characteristics and antioxidant properties of fresh and processed sea cucumber *(Cucumaria frondosa). Journal Agricultural and Food Chemistry*. 55: 1188-1192.

14. Chen, J.(2003). Overview of sea cucumber farming and sea ranching practices in China. *SPC Beche-de-mer Information Bulletin.* 18: 18-23.

15. Ozer, N. P. Mol, S. and Varlik, C. (2004). Effect on handling procedures on the chemical composition of sea cucumber. *Turkish Journal of Fisheries and Aquatic Sciences.* 4: 71-74.

16. Omran, N. E. E. (2013). Nutritional value of some Egyptian sea cucumber. *African Journal of Biotechnology*. 12(35): 5166-5472.

17. Vergara, W and Rodriguez, A. (2016). Nutritional composition of sea cucumber *Isostichopu s*sp. *Natural Resources.* 7: 130-137.

18. Gao, Y., Li, Z., Qi, Y., Guo, Z., Lin, Y., Li, W., HU, Y. and Zhao, Q. (2016). Proximate composition and nutritional quality of deep sea growth sea cucumbers (*Stichopus japonicas*) from different regions. *Journal of the Science of Food and Agriculture*. 96(7); 2378-2383.

19. Salarzadeh, A. R., Alkhami, M., Bastami, K. D., Ehsanpour, M., Khazaali, A. and Mokhleci, A. (2012). Proximate composition of two sea cucumber species *Holothuria pavra* and *Holothuria arenicola* in Persian Gulf. *Animal of Biological Research.* 3(3): 1305-1311.

20. Svelashev, V. L., Levin, V. Lam, C. and Nga, D. (1991). Lipid and fatty acid composition of holothurians from tropical and temperate waters. *Comparative Biochemistry and Physiology.* Part B. 98: 489-494.

21. Wen, J., Hu, C. and Fan, S. (2010). Chemical composition and nutritional quality of sea cucumbers. *Journal of Science of Food and Agriculture.* 90: 2469-2474.

22. Dong, Y., Dong, S., Tian, X., Wang, F. and Zhang, M. (2006). Effects of dial temperature fluctuation on growth oxygen consumption and proximate body composition in the sea cucumber *Apostichopus japonicas* (Selenka). *Aquaculture.* 255:514-521

23. Fradelina, B. D., Ridzwan, B. H., Abidin, A. A. Z., Kaswandi, M. A., Zaiton, H., Zali, I., Kittakoop, P. and Jais, A. M. M. (1999). Fatty acid composition in local sea cucumber *Stichopus chloronotus* for wound healing. *Gen. Pharmacology*. 33(4): 337-340.

24. Sicuro, B., Piccinno, M., Gai,F., Abete, M. C., Danieli, A., Dapra, F. Mioletti, M. and Vilella, S. (2012). Food quality and safety of Mediterranian sea cucumber *Holothuria tubulosa* and *Holothuria polii* in Southern Adriatic sea. *Asian Journal of Animal and Veterinary Advances*: 1-9.

25. Ridzwan, B. H., Hanita, M. H., Nurzafirah, M., Norshuhadaa, M. P. S. and Hanis, Z. F. (2014). Free fatty acids composition in lipid extract of several sea cucumbers species from Malaysia. *International Journal of Biosciences, Biochemistry and Bioinformatics.* 4(3): 204-207.

26. Bechtel, P.J., Olievera, A. C. M., Demir, N. and Smiley, S. (2013). Chemical composition of giant sea cucumber *Parastichopus californicus*, commercially harvested in Alaska. *Food Sciences & Nutrition.* 1(1): 63-73.

27. Careaga, P.V., Muniainb, C. and Maiera, M. S. (2012). Fatty acid composition of the edible sea cucumber *Athyonidium chilensis. Natural Product Research*: 1-9.

28. Yahyavi, M., Afkhami, M., Javadi, A., Ehsanpour, M., Khazaali, A., Khoshnood, R. and Mokhlesi, A. (2012). Fatty acid composition in two sea cucumber species, *Holothuria scabra* and *Holothuria leucospilota* from Qeshm Island (Persian Gulf). *African Journal of Biotechnology*. 11(12): 2662-2668.

29. Frigon, J.C., Abdou, R. H., McGinn, P. J., O'Leary, S. J. andGurot, S. R. (2014). Fate of palmitic, palmitoleic and eicosapentaenoic acids during anaerobic digestion of *Phaeodaetylum tricormitun* at varying lipid concentration. *Algal Research.* 6:46-51.

30. Taboada, M. C., Gonzalez, M. and Rodriguez, E. (2003). Value and effects on digestive enzymes and serum lipids of the marine invertebrate *Holothuria forskali. Nutrition Research.* 23: 661-670.

31. FAO/WHO. (2003). Diet nutrition and the prevention of chronic disease. *Technical Report Series* 916. *Food and Agriculture Organization/World Health Organization*, Geneva. 149 pp.