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Nutritional value of some Egyptian sea cucumbers

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Functional food that contains biologically-active compounds is an important source for prevention, management and treatment of chronic diseases in the modern age. The present work showed the quality of some Egyptian sea cucumbers to encourage Egyptian natives using them as an alternative food. The present work investigated the morphometric parameters, the proximate chemical composition, the amino acids and fatty acid profiles of *Actinopyga mauritiana*, *Holothuria scarba*, *Bohadschia marmorata* and *Holothuria leucospilota*. The results showed that *Actinopyga mauritiana* had the highest length, width, weight, and body wall thickness. All the investigated sea cucumbers had high content of protein (43.23 to 48.27%), carbohydrates (44.62 to 48.56%) and very low content of fats (4.6 to 5.66%). Among the investigated specimens *A. mauritiana* showed the highest percentage of total protein (48.27%), *Holothuria leucospilota* showed the lowest level of total lipids (4.6%), while *Holothuria scarba* showed the highest percentage of carbohydrates (48.65%). Glycine was the most abundant amino acids in all studied sea cucumbers (18.38 to 19.172 g/100 g) and constituted 37 to 39% of the total amino acids. Lycine: argignin ratio was low in *H. scarba*, *H. leucospilota* and *B. marmorata* (0.410, 0.428 and 0.9, respectively) while was higher in *A. mauritiana* (3.56). Myristic acid was the most abundant saturated fatty acid (SFA) in all studied sea cucumbers (19.789 to 37.036 %) followed by palmitic acid (16.93 to 16.926%). Among the unsaturated fatty acid (UFA) oleic and linoelaidic acids were the most abundant acids in all investigated cucumbers. On the other side linoleic acid (omega 6) was abundant in *H. scarba* and constituted 26% of the UFA. In conclusion, all investigated sea cucumbers had high protein and low fat contents and the bioactive compounds in the sea cucumbers describe its efficacy in tissue regeneration and inflammatory diseases.

Key words: Egyptian sea cucumbers, nutritional values, protein, inflammatory disease.

INTRODUCTION

Sea cucumbers are marine animals which belong to Phylum echinodermata used in fresh or dried form in various cuisines. Sea cucumber and its food product are commonly known as bêche-de-mer in French, trepang in Indonesian, namako in Japanese, balatan in the Philippines and gamat in Malaysian (Lovatelli and Conand, 2004). Most cultures in far East and Southeast Asia regard sea cucumbers as a delicacy. The traditional ways of consuming or using sea cucumber varies from one place to another. The body wall is eaten raw in Japan, Samoaa and Micronesian, while grilled in Papua New Guinea (Preston, 1993).

The ability of the body wall of sea cucumber to regenerate after being cut up reinforced the people's confidence to its use in the traditional medicine. Consequently, it is used for wound healing especially after clinical surgery and caesarian operation (Fredalina et al., 1999). It is also credited to possess similar aphrodisiac powers as attributed to oysters (Singh, 1980). Nigrelli and Zahl (1952) and Yamonouchi (1955) revealed that sea cucumbers contain a biological active compound called Holothurin or saponins (triterpene glycosides). These com-

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pounds have a structure similar to the active constituents of ginseng, ganoderma, and other famous tonic herbs (Chen, 2003). Pharmacology studies indicate anti-inflammatory, anticancer and anti-arthritic properties of the sea cucumber saponins (Yamonouchi, 1955; Nigrelli, 1952; Idid et al., 2001). Also, it has been reported that holothurin is used in the treatment of asthma, gastric ulcer and high blood pressure (Ridzwan et al., 1990; 1995; Kaswandi et al., 1993; Hasan et al., 1996). Moreover, it has antiparasitic and a broad spectrum antifungal effect (Shimada, 1969; Kaswandi et al., 1999; Mona et al., 2012; Omran and Allam, 2012).

Sea cucumber fishery started in Egypt in a small scale since 1990s. Fishermen collected and processed sea cucumber and the products were sold to exporters for market in Hong Kong SAR (China) and Singapore (Lawrence et al., 2004). Hence, Egypt has become one of the most important suppliers of sea cucumber especially after the depletion seen in other areas (Lovatelli et al., 2004; Bruckner, 2006). However sea cucumber is unusual food in Egypt and people estranged its eating. For this, the present work aims to highlight the nutritional value of some local Egyptian sea cucumber inhabiting the red sea shore in order to evaluate their quality and their economic importance.

MATERIALS AND METHODS

Sample collection

Fresh samples of *Actinopyga mauritiana*, *Holothuria scarba*, *Bohadschia marmorata*, *Holothuria leucospilota* (three specimens each) were collected in April (2011) from the Egyptian coast of the Red Sea by SCUBA diving from deep water (35 m maximum depth) and by snorkeling and hand collection from shallow reef flat areas.

Preservation and morphological examination

Samples were relaxed first by using Magnesium chloride ($MgCl_2$) 7% (w/v) in seawater. The specimens were immersed in the solution for a few hours. The average of sample length, width, weight, body wall thickness and the color were determined. For estimating nutritional quality, fresh samples were stored in -4°C until their analysis. For taxonomic examination, specimens were preserved in 10% (v/v) formalin buffered with sea water. Identification of the sea cucumber was undertaken using morphological characters and types of spicules (Clark and Rowe, 1971; Cannon and Silver, 1986). The morphological characters include dorsal and ventral surface colors (were immediately recorded after collection), tentacle type, numbers of calcareous rings, respiratory trees and gonads.

Proximate chemical composition

Moisture content was determined according to AOAC (2000). Ash was determined according to AOAC (1986). Total carbohydrate was determined colorimetrically according to the study of Duboies et al. (1956). Total lipid was analyzed using Soxtherm; Gerhadt, laboratory instrument while total protein was analyzed by Kjeldatherm and Vapadest 50s; Gerhardt, laboratory instrument (AOAC, 1995).

Amino acid analysis

The dried ground samples (100 mg) were hydrolyzed with 6 N HCl (10 ml) in a sealed tube at 110°C for 24 h. The excess of HCl was then freed from 1 ml hydrolyzed under vacuum with occasionally addition of distilled water, then evaporated to dryness. The HCl free residue was dissolved in 2 ml of diluting buffer (0.2M, pH 2.2). Amino acid quantities were determined by using automatic amino acid analyzer AAA 400 INGOS Ltd (Block et al., 1958)

Fatty acid analysis

Lipid extracted from the tissue according to AOAC (2000) by using chloroform methanol (2:1 v/v). The associated non-lipids were removed by washing extract three times with CH_3OH: H_2O (1:1 v/v). The lipids in chloroform were dried over anhydrous sodium sulfate, and then the solvent was removed by heating at 60°C under vacuum.

The lipids were saponified over-night with ethanoic KOH (20%) at room temperature. The fatty acids were freed from their potassium salts by acidification with HCl (5N), followed by extraction with petroleum ether (40 to 60°C). The extract was washed three times with distilled water then dried over anhydrous sodium sulphate, and filtered (Vogel, 1975).

Fatty acids produced from lipid samples and standard fatty acids were dissolved in a little anhydrous methanol and ethereal solution of diazomethane was added in small portion until gas evolution ceased. Ether was evaporated under nitrogen stream at room temperature, then two drops of redistilled chloroform solution was added to dissolve the fatty acid methyl ester and 10 ml of this solution were injected into the gas chromatography.

A set of standard fatty acids of 10:0, 11:0, 12:0, 14:0, 15:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, and 22:0 with a stated purity of 99% by gas liquid chromatography was purchased from Nu-check Prop. Identification and determination of fatty acid was carried out using gas liquid chromatography according to the method of Farag et al. (1986). The methyl esters of fatty acids obtained from samples and standard were analyzed with a Pye Unicam Series 304 chromatograph equipped with dual flame ionization detector and dual channel recorder. The separation of fatty acid methyl esters was conducted using a coiled glass column (1.5 m × 4 mm) paced with Diatomite (100 to 120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8°C/min from 70°C to 190 C, then isothermally at 190°C for 25 min with nitrogen at ml/min. The unsaponifiables were also fractioned on a coiled glass column (2.8 m × 4 mm) packed with Diatomite (100 to 120 mesh) and coated with 3% OV-17. The oven temperature was programmed at 10°C/min from 70°C, then isothermally at 270°C for 25 min and nitrogen flow rate was 30 ml/min. Detector, injector temperatures and hydrogen, air flow rates were generally 300 and 280°C and 33 ml, 330 ml/min, respectively. Peak identification was performed by comparison the retention time (RT) of each compound with those of standard materials. Peak area was quantified and expressed as percentage of total fatty acids.

Statistical analysis

All analyses were repeated three times. Results were expressed as mean values ± standard deviation (SD) and one-way analysis of variance (ANOVA) were carried out using a statistical analysis system (SPSS Version 17). Differences were considered to be significant when $P < 0.05$.

RESULTS

Morphometric analysis

Results in Table 1 (Figure 1) showed that the body length,

Table 1. Morphometric analysis of the investigated sea cucumbers.

Sea cucumber	Length (cm)	Width (cm)	Weight (g)	Thickness (cm)	colour
Actinopyga mauritiana	32	11	600	1	Black from the dorsal and white patch on the bottom
Holothuria scarba	26	7.5	300	0.6	Black
Bohadschia marmorata	18	8.5	300	0.7	Dark Brown from the dorsal and faint brown on the bottom
Holothuria leucospilota	22	5.5	200	0.5	Black

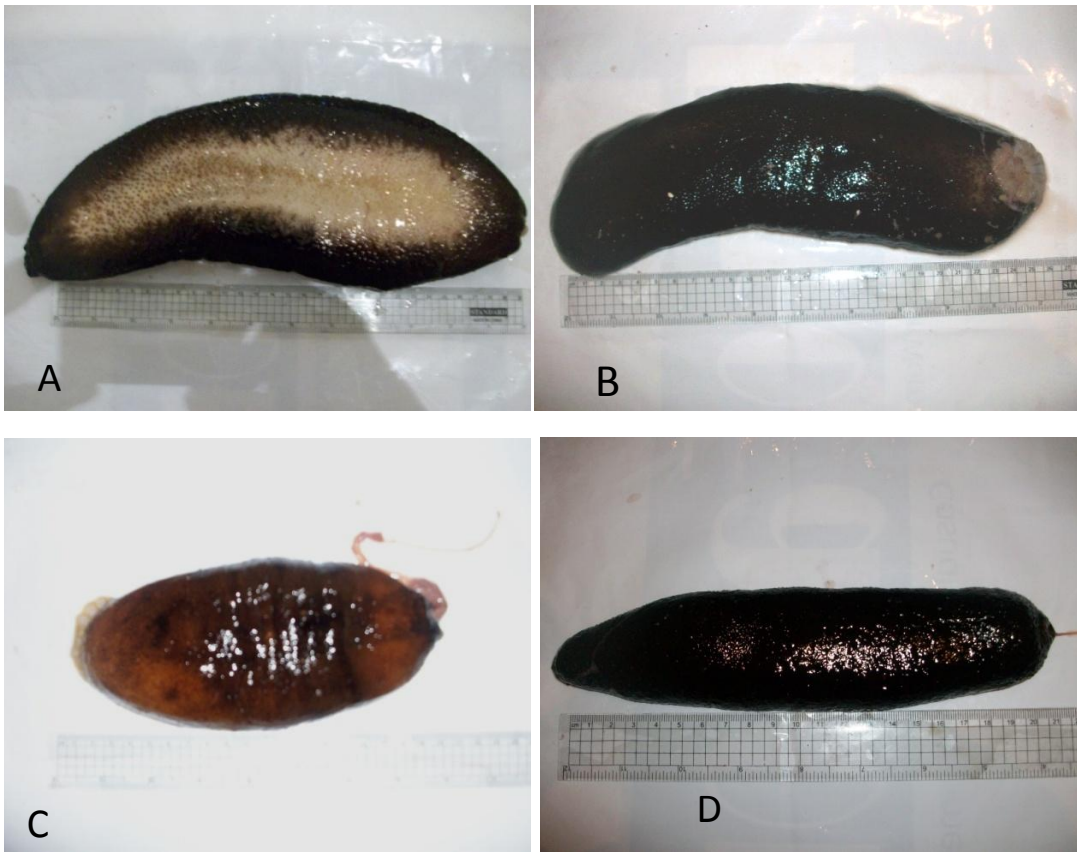

Figure 1. Photographs of *Actinopyga mauritiana* (A), *Holothuria scarba* (B), *Bohadschia marmorata* (C) and *Holothuria leucospilota* (D). All photos show the dorsal views except of A showed the ventral view.

width, weight and the body wall thickness of the investigated specimens ranged from 18 to 32 cm, 5.5 to 11 cm, 200 to 600 g, and 0.5 to 1 cm, respectively. Among the investigated specimens, *Actinopyga mauritiana* had the highest length, width, weight, and body wall thickness. *Bohadschia marmorata* followed *A. mauritiana* in the body wall thickness.

Proximate composition

The proximate composition of the investigated specimens (Table 2) showed high percentage of moisture (81.41 to 85.17%), low percentage of ash (2.12 to 6.03%) and lipids (4.6 to 5.66%) in all investigated species. On the other hand, total protein (43.23 to 48.27%) and total carbohydrate (44.62 to 48.56%) were nearly the same. Among the investigated specimens *A. mauritiana* showed the highest percentage of total protein (48.27%), *Holothuria leucospilota* showed the lowest level of total lipids (4.6%), while *Holothuria scarba* showed the highest percentage of carbohydrates (48.65 %).

Amino acid composition

The amino acid profiles of the investigated sea cucumbers are shown in Table 3. Glycine is the most abundant

Table 2. Proximate composition (%) of the investigated sea cucumbers (mean values ± standard deviation).

Sea cucumber	Moisture (%)	Ash (%)	Total ptotein (%)	Total lipid (%)	Total carbohydrate (%)
A. mauritiana	84.71 ± 0.7[a]	2.12 ± 0.1[a]	48.27 ± 0.1[a]	4.99 ± 0.1[a]	44.62 ± 0.3[a]
H. scarba	85.76 ± 0.3[a]	2.26 ±0.15[a]	43.43 ± 0.2[b]	5.66 ± 0.09[a]	48.65 ± 0.2[b]
B. marmorata	83.17 ± 0.2[b]	6.03 ± 0.3[b]	43.23 ± 0.1[b]	4.83 ± 0.1[a]	45.91 ± 0.1[a]
H. leucospilota	81.41 ± 0.6[c]	4.3 ± 0.2[c]	45.71 ± 0.2[c]	4.60 ± 0.3[a]	44.96 ± 0.3[a]

Values in the same column bearing different letters are significantly different ($P < 0.05$).

Table 3. Amino acids analysis of the investigated sea cucumber body wall.

	A. mauritiana	***H. scarba***	***B. marmorata***	***H. leucospilota***
Essential amino acids (g/100g)				
Threonine	2.189 ± 0.09[a]	1.98 ± 0.02[a]	0.369± 0.03[b]	2.73 ± 0.02[c]
Valine	2.132 ± 0.1[a]	1.794 ± 0.2[a]	2.002 ± 0.02[a]	1.508 ± 0.01[a]
Methionine	0.416 ± 0.02[a]	0.29 ± 0.03[b]	0.4108 ± 0.02[a]	0.1924 ± 0.01[c]
Isoleucine	0.4316 ± 0.01[a]	0.58 ± 0.01[a]	0.582 ± 0.02[a]	0.494 ± 0.01[a]
Leucine	1.576 ± 0.02[a]	1.89 ± 0.02[b]	1.732 ± 0.03[b]	1.856 ± 0.01[b]
Phenylalanine	0.998 ± 0.02[a]	1.07 ± 0.01[a]	1.196 ± 0.01[a]	0.7488 ± 0.02[b]
Histidine	0.645 ± 0.03[a]	0.21 ± 0.02[b]	0.317 ± 0.02[a]	0.3588 ± 0.01[a]
Lysine	3.520 ± 0.012[a]	0.75 ± 0.01[b]	1.534 ± 0.01[a]	0.7332 ± 0.02[b]
Arginine	0.988 ± 0.012[a]	1.83 ± 0.05[b]	1.67 ± 0.011[b]	1.7108 ± 0.021[b]
TEAA	12.896 ± 0.01[a]	10.39 ±0.03[b]	9.813 ± 0.03[b]	10.33 ±0.02[b]
Nonessential amino acids (g/100g)				
Aspartic acid	4.4772 ± 0.7[a]	4.81± 0.8[a]	5.044 ± 0.7[a]	4.654 ± 0.2[a]
Serine	2.106 ± 0.1[a]	2.35 ± 0.01[a]	2.3088 ± 0.1[a]	2.3296 ± 0.3[a]
Glutamine	5.2468 ± 0.1[a]	4.971 ± 0.1[a]	5.16 ± 0.1[a]	5.642 ± 0.12[a]
Proline	0.239 ± 0.02[a]	0.229 ±0.01[a]	0.208 ± 0.01[a]	0.140 ± 0.01[a]
Glycine	18.798 ± 1.02[a]	18.38 ± 1.1[a]	18.80 ± 1.1[a]	19.172 ± 1.3[a]
Alanine	6.45 ± 1.02[a]	6.52 ± 1.05[a]	6.526 ± 1.07[a]	5.803 ± 1.03[a]
Tyrosine	0.333 ± 0.01[a]	0.614 ±0.01[b]	0.541 ± 0.02[b]	0.489 ± 0.01[b]
TNEAA	37.648 ± 0.012[a]	37.874±0.02[b]	38.595 ± 0.03[b]	38.231 ± 0.023[b]
TAA	50.544 ± 0.02[a]	48.26 ± 0.03[a]	48.407 ± 0.01[a]	48.561 ± 0.021[a]
TEAA/TNEAA	0.34 ± 0.02[a]	0.27 ± 0.01[b]	0.25 ± 0.01[b]	0.27 ± 0.01[b]
TEAA/ TAA	0.26 ± 0.01[a]	0.22 ±0.01[b]	0.20 ± 0.01[b]	0.21 ± 0.01[b]
LYS/ARG	3.56 ± 0.01[a]	0.410 ±0.02[b]	0.9 ± 0.01[c]	0.428 ± 0.02[b]

Values in the same raw bearing different letters are significantly different ($P < 0.05$). TEAA: total essential amino acids; TNEAA: total non-essential amino acids; TAA: total amino acids; LYS/ARG: lysine arginine ratio.

amino acids in all studied sea cucumbers (18.38 to 19.172 g/100g) which constituted 37 to 39% of the total amino acids, followed by alanine (5.803 to 6.526 g/100g), glutamic acid (4.971 to 5.642 g/100g) and aspartic acid (4.4772 to 5.044 g/100g). All sea cucumbers had nearly the same total amino acids (TAA) (50.544 to 48.27 g/100g). The content of essential amino acids (EAA) was lower than non essential amino acids (NEAA) and the ratio of EAA: NEAA ranged from 0.25 (*B. marmorata*) to 0.34 (*A. mauritiana*). Lycine: argignin ratio was low in *H. scarba*, *H. leucospilota* and *B. marmorata* (0.410, 0.428 and 0.9, respectively) while was higher in *A. mauritiana* (3.56).

Fatty acid composition

The fatty acid profiles of the investigated sea cucumbers are shown in Table 4. Myristic acid is the most abundant saturated fatty acid (SFA) in all studied sea cucumbers (19.789 to 37.036%) followed by palmitic acid (11.45 to 16.93). Among the unsaturated fatty acid (UFA) oleic and linoelaidic acids are the most abundant acids in the all investigated cucumbers. On the other side linoleic acid (omega 6) is abundant in *H. scarba* and constituted 26% of the UFA followed by *A. mauritiana* that constituted 18% of the UFA. The highest total fatty acids were in *H. leucospilota* (119.16 %) followed by *B. marmorata* (98.99

Table 4. Fatty acids analysis of the investigated sea cucumber body wall.

Fatty acid	*A. mauritiana*	*H. scarba*	*B. marmorata*	*H. leucospilota*
Saturated fatty acid				
Lauric acid	0.29 ± 0.001[a]	0.42± 0.002[b]	0.63 ± 0.001[c]	0.96± 0.003[d]
Myristic acid	19.789 ± 2.1[a]	25.45 ± 2.12[b]	25.097± 1.5[b]	37.086 ± 2.3[c]
Palmitic acid	16.93 ± 1.03[a]	11.45 ± 1.11[b]	13.66 ± 1.02[b]	11.64 ± 1.1[b]
Margaric acid	0.277± 0.01[a]	0.740 ± 0.02[b]	0.68 ± 0.002[c]	0.852 ± 0.01[d]
Stearic	4.575 ± 0.7[a]	7.419 ± 0.03[b]	6.807 ± 0.1[b]	6.430 ± 0.1[b]
Arachidic acid	0.661 ± 0.02[a]	5.342 ± 1.4[b]	5.241± 1.3[b]	5.219 ± 1.02[b]
Total saturated fatty acid	42.522 ± 0.64[a]	50.822 ± 0.78[b]	52.12 ± 0.65[b]	62.187± 0.75[c]
Unsaturated fatty acid				
Palmitelaidic acid	1.017± 0.02[a]	1.296 ± 0.01[a]	1.294± 0.01[a]	0.896± 0.02[a]
Oleic acid	32.813 ± 2.23[a]	12.52 ± 3.21[b]	18.32 ± 2.22[c]	6.761± 0.11[d]
Linoleic acid (Omega 6)	7.820 ± 0.3[a]	12.02 ± 0.21[b]	4.871 ± 0.13[c]	2.167± 0.21[d]
Linoelaidic acid	14.59 ± 1.1[a]	19.19 ± 1.1[b]	17.49 ± 1.01[b]	23.31± 1.33[c]
Linolenic acid (Omega 3)	0.55 ± 0.01[a]	1.55 ± 0.05[b]	1.716 ± 0.02[b]	0.947 ± 0.03[c]
Total unsaturated fatty acid	41.86 ± 0.73[a]	45.48 ± 0.9[b]	46.88 ± 0.68[b]	56.97 ± 0.34[c]
Total fatty acids	84.38 ± 0.68[a]	96.30 ± 0.79[b]	98.99 ± 0.66[b]	119.16 ± 0.56[c]

Values are in percentage (%). Values in the same raw bearing different letters are significantly different ($P < 0.05$).

%), *H. scarba* (96.302 %), while *A. mauritiana* showed the lowest value (84.38 %).

DISCUSSION

Protein content of all investigated sea cucumbers showed high values ranged from 43 to 48%. These levels are higher than those of widely used fishes such as raw tilapia fish *Oreochromis niloticus* (23.06%), raw cat fish Clarias *gariepinus* (20%) and raw salmon (40%) (Chukwu, 2009; Adeniyi et al., 2012). Also carbohydrates were much higher in sea cucumber than in tilapia (6.85%), cat fish (3.85%) and electric fish (*Malapterurus electricus*, 8.86%) (Adeniyi et al., 2012). On the other hand all studied sea cucumbers had very low lipids content ranged from 4.6 to 4.99%. These levels of lipids were lower than those of tilapia fish meat (12.85%), *Clarias gariepinus* (13.86%) and salmon (10%) (Chukwu, 2009; Adeniyi et al., 2012).

Among the investigated sea cucumbers *Actinopyga mauritiana* showed the highest value of protein and lowest value of ash. This result is in accordance with the study of Wen et al (2010) who stated that *A. mauritiana* has the highest protein and lower ash values when compared with *Stichopus herrmanni*, *Thelenota ananas*, *Thelenota anax*, *Holothuria fuscogilva*, *Holothuria fuscopunctata*, *Actinopyga caerulea* and *Bohadschia argus.*

The present study showed that the non essential amino acid glycine is dominant in all investigated sea cucumbers. It has been known that glycine is used to help create muscle tissue and convert glucose into energy.

It is also essential to maintain central nervous and digestive systems healthy, and has recently been shown to provide protection via antioxidants from some types of cancer (Rose et al., 1999a; b). Moreover, ingestion of glycine before bedtime seems to produce subjective and objective improvement of the sleep quality (Yamadera et al., 2007).

It has been found also that glycine reduced serum total cholesterol level (Aljawad et al., 1991; Ikeda et al., 1993). The present result agrees with that of Wen et al. (2010) who showed that glycine was abundant in *Stichopus herrmanni*, *Thelenota ananas*, *Thelenota anax*, *Holothuria fuscogilva*, *Holothuria fuscopunctata*, *Actinopyga caerulea* and *Bohadschia argus.*

On the other side lysine: arginine ratio was low in *H. scarba*, *H. leucospilota* and *B. marmorata.* This ratio is lower than that of many fishery products such as *Channa striatus* (1.64, Zuraini et al., 2006), *Pampus punctatissimus* (1.49; Zhao et al., 2010) *Clarias anguillaris* (1.05; Adeyeye, 2009). Worthily, it has been reported that this low ratio reduced concentrations of cholesterol in the serum and aorta and has hypocholesterolemic effects (Sugano et al., 1984; Rajamohan et al., 1997).

The present study showed that the content of essential amino acids (EAA) was lowers than the non-essential amino acids (NEAA), although *A. mauritiana* had the highest value of EAA and the highest values of lysine, threonine, and valine. Lysine is important for proper growth, and it plays an essential role in the production of carnitine, a nutrient responsible for converting fatty acids into energy and helping to lower cholesterol. Some studies have found that taking lysine on a regular basis may help prevent outbreaks of cold sores and genital herpes (Beauman, 2005; Gaby, 2006). Lysine appears to help the body absorb calcium, and it plays an important role in the formation of collagen, a substance important for bones and connective tissues including skin, tendon,

and cartilage (Fini et al., 2001). This may interpret the usage of sea cucumbers as an anti-arthritic agent (Idid et al., 2001; Yamonouchi, 1955).

The present study showed that Myristic and Palmitic acids are the two dominant saturated fatty acids in all investigated sea cucumbers. Myristic acid has the ability to acylate proteins, in a reaction which is called N-terminal myristoylation (Beauchamp et al., 2009). It also plays an essential role in the activation of cellular functions such as signal transduction and constitutive proteins. Palmitic acid has antioxidant and anti-atherosclerosis properties.

Among the unsaturated fatty acids, both Oleic and Linoelaidic acids were dominant in all studied sea cucumbers. It has been reported that oleic acid is an anti-inflammatory fatty acid playing a role in the activation of different pathways of immune competent cells (Carrillo et al., 2012). Several studies showed that sea cucumber was used as an anti-inflammatory agent in rheumatoid arthritis and asthma (Idid et al., 2001; Yamonouchi, 1955); this capability may be attributed to the abundance of the oleic acid.

The present findings showed that Linoleic acid (an unsaturated omega-6 fatty acid) was found in *H. scarba* and *A*. *mauritiana.* Linoleic acid is an essential fatty acid that must be consumed for proper health. A diet only deficient in linoleate causes mild skin scaling, hair loss (Cunnane and Anderson, 1997) and poor wound healing in rats (Ruthig and Meckling-Gill, 1999). This explains the usage of sea cucumber extracts for wound healing especially after clinical surgery and caesarian operation (Fredalina et al., 1999).

In conclusion, the investigated Egyptian sea cucumbers have high protein content, high carbohydrates and very low content of fats, and it is suggested to be a source of food in Egypt especially *A*. *mauritiana* that contained high protein content and *H. scarba* rich in omega-6. Also, the bioactive compounds in the sea cucumbers describe its efficacy in tissue regeneration, inflammatory diseases, and suggested to be a functional food for people with hyperlipidemia.

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