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# A STUDY ON PROXIMATE COMPOSITION, AMINO ACID PROFILE, FATTY ACID PROFILE AND SOME MINERAL CONTENTS IN TWO SPECIES OF SEA CUCUMBER

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## ABSTRACT

Coastal waters of Pakistan have considerable biodiversity of sea cucumbers and to date 11 genera and fifteen species of sea cucumbers belonging to 3 families have been recorded. In the present study the biochemical composition and nutritional quality of two most common sea cucumbers (*Holothuria arenicola* and *Actinopyga mauritiana*) were determined. The study includes their proximate, amino acid, and fatty acid compositions. The protein content of *Actinopyga mauritiana* is ~ 67 % comparatively higher than that of *Holothuria arenicola*, in which it was ~ 45 %. The high value for ash content was found in *H. arenicola* i.e. ~45 % in comparison to ~32 % in *A. mauritiana*, The lipid content was not significantly different in the two species and attained values of 0.88 % and 0.76 % in *H. arenicola* and *A. mauritiana*, respectively. The total fatty acids were determined for both the species in which findings suggested that polyunsaturated fatty acid (PUFA) was more than saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA). Results also showed that eicosapentaenoic acid (EPA; 20:5ω3), arachidonic acid (AA; 20:4ω6) and docosahexaenoic acid (DHA; 22:6ω3) are dominant among all PUFA. All the essential amino acids were found in both the species in which Glycine was found as major amino acid. Profile also suggested that Lysine/Arginine ratio was in favorable range to produce Hypocholesterolemic and antiatherogenic effects. Both the species of sea cucumbers are potential source of macro and micro nutrients with having no toxic elements under studied. .

**Key words:** sea cucumber, biochemical composition, essential amino acid, total fatty acids.

## INTRODUCTION

The Holothuroidea, or sea cucumbers, are an abundant and diverse group of worm-like and usually soft-bodied echinoderms. They are found in nearly every marine environment. There are about 1400 living species of sea cucumber in a variety of forms. Some of these are about 20 cm in length, though adults of some diminutive species may not exceed a centimeter, while one large species can reach lengths of 5 m (*Synapta maculata*). (Kerr, 2000). At present 66 species of sea cucumbers are commercially exploited worldwide (Purcell, 2010).

Sea cucumbers are fished all over the world but abundant in the tropical region (Toral-Granda, 2008). The total annual global catch is in the order of 100,000 tones of live animals annually (Purcell, 2010). The major fisheries exist in China, Ecuador, Indonesia, Japan, Republic of Korea, Malaysia, Philippines, Madagascar, Australia and New Caledonia. However, the high value of some species, the ease with which such shallow-water forms can be collected and their top-heavy age structures all contribute to over-exploitation and collapse of the fisheries in some regions.

Sea cucumbers are considered as a culinary delicacy and as a traditional cure for many illnesses in many Asian countries. These are exploited for their potential nutritional and therapeutic properties. They play a vital role in the marine ecosystem by recycling the nutrients and sediment improvement.

Some species of sea cucumbers produce toxins that have medicinal value. Some compounds isolated to date exhibit antimicrobial and anti-inflammatory, (Ibrahim *et al*., 1992; Shaharah *et al*., 1998) antioxidant, (Hawa *et al*., 1999) anticoagulant activity and immune modulatory (Aminin *et al*., 2001). As a gourmet food item in the orient, they form the basis of a multimillion-dollar industry that processes the body wall for sale as beche-de-mer or trepang. Furthermore, the sticky Cuvierian tubules are placed over bleeding wounds as a bandage. Sea cucumbers have been incorporated into products which include juice, balm, liniment oil, cream, toothpaste, gel facial wash, body lotion, facial wash, soap gamat water and oil (Lovatelli *et al*., 2004).

Some studies have been conducted on the proximate composition of sea cucumbers, (Prim *et al*., 1976; Chang-Lee *et al*., 1989; Wen *et al*., 2010; Salarzadeh *et al*., 2012) fatty acid analysis (Svetashev *et al*., 1991; Fredalina *et al*., 1999; Yahyavi *et al*., 2012) and amino acid composition (Wen *et al*., 2010). Sea cucumber is cholesterol-free. It is high in protein (55% of dry body weight) and contains 10-16% muco-polysaccharides (substances used for building cartilage) and saponins; it is helpful in reducing arthritic pain and arthralgia (due to the mucopolysaccharides content) and pharmacological studies indicate that the saponins of sea

cucumber have anti-inflammatory and anticancer properties (Dharmananda, undated). Scientists from the University of Malaya working on *S. hermanni* reported on the painkilling, anti-inflammatory and anti-itching properties of this sea cucumber (Awaluddin, 2001).

From Pakistan total 11 genera and 14 species of sea cucumbers have been recorded, belonging to the orders Aspidochirotida and Dendrochirotida to which most of the commercial species belong. There is no fisheries exist, since sea cucumbers are not consumed locally. No work has been done on assessing the resource potential and seasonal and spatial abundance of different species. The basic objective of the present study was to conduct the proximate and nutritional evaluation of the most abundant species from Pakistan coast, to explore its potential uses and to generate a base line data regarding its nutritional and biochemical properties for further research in the field.

## MATERIALS AND METHODS

**Chemicals and Reagents:** N-acetyl cysteine from E-Merck (Darmstadt-Germany); o-pthaldehyde (OPA), potassium hydroxide and hexane from Sigma-Aldrich (Austria); Ethanol (absolute) and methanol from PCSIR Chemica (Karachi, Pakistan); sodium hypochlorite 10% EP from International Laboratory (USA) and Reaction Solution A (sodium hypochlorite/borate buffer containing NaClO), Reaction Solution B (OPA, N-acetylcysteine/ borate buffer), buffer solutions A (MA), B (MB), C (MC) from Shimadzu (Japan); Amino acid standard A2908 from Aldrich; FAME (fatty acid methyl ester) standard from sigma; Sulphuric Acid from RDH (Germany).

**Sample Preparation:** Sea cucumbers were collected during low tide from Rocky shore of Buleji at random by hand picking during March, 2013. The visceral organs and body fluid of fresh sea cucumber specimens were taken out and the body wall (50-100g by weight and 10-15 cm in length) was washed with tap water. The body wall was cut into small pieces (1-1.5 cm), freezed at –80ºC for at least 12 h, and vacuum dried at 30ºC for 72 h. The dried sample was ground into powder and sieved with a ≤ 600 µm sieve. The powder was stored in a closed dark bottle at 2ºC before the initiation of experiments.

**Moisture, Ash and Fat analysis:** The muscle was homogenized by food processor and moisture content of 5 g of homogenized sample was determined by drying the sample in oven at 105˚C until a constant mass was obtained. Ash and Fat were determined by AOAC official method of analysis (Horwitz and Latimer, 2005 a, b).

**Fat extraction:** Total lipids of sea cucumber species were extracted (separately) according to the Bligh and Dyer (1951) Method. After phase equilibration, the lower Chloroform layer (TL) was removed and dried in a rotary vacuum evaporator at 32 ◦C. The extracted lipids were weighed in order to determine the TL, and then re-dissolved in chloroform/methanol (9:1, v/v) and finally stored at 0 ˚C until used.

**Fatty acid analysis:** Fatty acids were hydrolyzed and derivatized from total lipids according to the procedure described by O'Fallon *et al*., (2007). 50 µL of fish oil was taken in a pyrex screw cap tube. One mL of internal standard, 0.7 ml KOH solution and about 5.3 mL methanol were added, mixed and tubes were capped and pl*a*ced on a preheated water bath at 55°C for 1.5 hours. The tubes were then cooled under running tap water followed by $H_2SO_4$ was added to the sample. The tubes were then again immersed in water bath at 55°C for 1.5 hours. The tubes were then cooled down followed by 3 mL of hexane was added. Tubes were then placed into a vortex mixer for 5 minutes. Upper layer (hexane layer) of FAMEs was separated, filtered and then analyzed.

The gas chromatography was performed in GC-2010, Shimadzu corporation 07947 equipped with FID detector, split injector, and SP-2560 silica fused capillary column (100m, 0.25mm, 0.2µm) (Supelco) with the following operating program: injection volume 1 µL with temperature 250°C, detector temperature 260°C, column temperature 140°C for 5 minutes and then ramped to 240°C with 4°C per minute, remain stable for 15 minutes; helium was used as carrier gas with flow rate of 1.12 mL/min and linear velocity of 20 cm/s; split ratio 1:100. Results were expressed as FID response area relative percentages. The results were given as mean standard deviation.

**Amino Acid Analysis:** Normal hydrolysis was carried out by the method described in AOAC (2005c). Fish samples (100 mg) were taken in separate digestion tube with the addition of Hydrochloric acid-phenol solution (6N; 50ml) and kept for 18-24 hours at 110°C under vacuum. The hydrolyzed samples were then washed with water and evaporated to dryness on a rotary evaporator in vacuum, at 70°C. Final volume was made up at 25 ml with deionized water. Samples were filtered through syringe filter (0.22 micron) and diluted with buffer A solution in a sample vial, prior to injection (20 µL) into the amino acid analyzer.

Amino acid analysis was conducted on the Shimadzu Amino Acid Analyzer with Shim-Pack Amino-Na column (4.6mm, I.D x100mm) containing strong acidic cation exchanger resin (styrene divinyl benzene copolymer with sulphonic groups).

Sample was injected by the auto injector SIL-10ADVP.The mobile phase consisted of 0.2N sodium citrate pH 3.2(MA), 0.6 N sodium citrate and 0.2M boric acid pH 10(MB), and 0.2M NaOH (MC). A gradient program of 72 minutes was set for mobile phase A, B, C with the initial flow rate of 0.4ml/min at 100% MA

followed by MB 0-100% for 14-53 minutes; MC 100% for 53.01 to 58 minutes; MA 100% for 59-72 min. Ammonia trap column was used prior to column elution (Shim-pack ISC-30/SO504 Na). System controller was SCL-10A VP, while degasser used was DGU-14A. Reaction Solutions were kept at a flow rate of 2ml/min at 60°C. Flourescence detector RF-10A XL was adjusted at Ex=350nm, Em=450nm.The column oven CTO-10AV VP was set at 60°C. Flow rate of reaction solution was kept constant by peristolic pump (PRR-2A).

**Protein Determination:** The protein was determined by the macro-Kjeldhal method in AOAC (2005d). Sample (0.2g) was taken along with anhydrous copper sulphate (0.5g), potassium sulphate (5g), conc. $H_2SO_4$ (25ml) in a digestion flask and then digested in digestion unit (K-424, Buchi).The digested sample was then neutralized with NaOH (40%) ,washed and then distilled in a distillation unit (K-314, Buchi) . Nitrogen released was titrated with NaOH (0.1N). A blank was run along with the sample.

**Mineral analysis:** Estimation of macro (sodium, potassium, calcium and magnesium) and micro (nickel, manganese, copper and zinc) nutrients along with the toxic metals (lead, chromium and cadmium) was conducted by atomic absorption, official method of analysis for feed and food (European Standard, 2003). 0.1g dried samples were weighed and digested. Digestion was carried out with $HNO_3$ +$H_2O_2$ (7:1) by using microwave digester. When samples were completely oxidized and solutions became clear, the samples were made up to certain volume. Dilutions were made if required such as to keep the concentration of metal within the linear range of absorbance. The working solution to be analyzed was aspirated in to atomic absorption spectrophotometer (Hitachi, model Z-500) depending on the flame atomization mode. The instrumental conditions were set according to manufacturer instruction manual.

**Statistical analysis:** The data were analyzed using SPSS 11.0 software. One way analysis of variance (ANOVA) was performed and was followed by Duncan's multiple range test. A value of $P<0.05$ was used to indicate significant differences.

## RESULTS

**Moisture, Ash and Fat analysis:** Two species of sea cucumbers *Holothuria arenicola* and *Actinopyga mauritiana* from the coastal area of Buleji, Karachi, Pakistan (Long and Lat) were studied for proximate and biochemical analysis. Proximate analysis was done for moisture, ash, protein and fat, values of which were found to be 72.12% (wet weight basis), 45.16%, 44.56%, 0.88% (dry weight basis) in *H. arenicola* and 76.54% (wet weight basis), 31.81%, 66.86%, 0.76 (dry weight basis) in *A. mauritiana* respectively (Table 1). The high protein value was observed in *A. mauritiana* in comparison to *H. arenicola*. Low fat values for both the species showed no significant difference, while ash contributed higher value in the case of *H. arenicola*.

**Table 1. Proximate analysis of sea cucumbers**

| Proximate | *Holothuria arenicola* g/100g samples | *Actinopyga mauritiana* g/100g sample |
|---|---|---|
| **Moisture** | 72.12±0.25[a] | 76.54±0.09[a] |
| **[*]Ash** | 45.16±0.22[a] | 31.81±0.34[a] |
| **[*]Fat** | 0.88±0.05[a] | 0.76±0.02[b] |
| **[*]Protein** | 44.56±0.04[a] | 66.86±0.06[a] |

The same superscripts in a row indicate means which do not differ significantly, _ANOVA, Duncan's multiple range test, P<0.05
Values (mean±SD) are the means of three determinations
[*] analyzed on dry weight basis

**Fatty acid composition:** The main fatty acid content in *A. mauritiana* is the capric acid (C: 10) which was found to be 25.07% while it was absent in *H. arenicola*. Cis-10-Pentadecanoic acid (15:1) was also dominant in both the species which were 17.8% and 14.44% for *H. arenicola* and *A. mauritiana* respectively. Oleic acid, arachidic acid, cis-8,11,14,17- Eicosatrienoic Acid, and Erucic Acid were found to be in almost same proportion in both the species in which *H. arenicola* contain 6.6%, 1.6%, 0.8% and 1.7% while *A. mauritiana* contain 5.55%, 1.7%, 0.89%, and 1.77% respectively (Table 2). The value of γ-Linolenic (C18:3n6) was varied in both the species in which *A. mauritiana* possessed higher value 13.05% than *H.arenicola* 10% while α-Linolenic was found to be same for both the species i.e 1.3% and 1.31% respectively. Another dominant fatty acid in *H. arenicola* was arachidonic acid 14.6%, comparatively higher than the value contained in *A. mauritiana* which was found 6.87% (Table 2).

**Table 2. Fatty Acid Composition of dried seacucumbers**

| Fatty acid composition | | *Holothuria arenicola* g/100g total fatty acid | *Actinopyga mauritiana* g/100g total fatty acid |
|---|---|---|---|
| **Butyric Acid** | C4:0 | 0.0±0.00[a] | 2.92±0.03[a] |
| **Caproic Acid** | C6:0 | 0.4±0.03[a] | 0.84±0.01[a] |
| **Caprylic Acid** | C8:0 | 0.0±0.00[a] | 0.00±0.00[a] |
| **Capric Acid** | C10:0 | 0.0±0.00[a] | 25.07±0.04[a] |
| **Undecanoic Acid** | C11:0 | 0.0±0.00[a] | 0.00±0.00[a] |
| **Lauric Acid** | C12:0 | 0.0±0.00[a] | 0.00±0.00[a] |
| **Tridecanoic Acid** | C13:0 | 1.2±0.20[a] | 0.79±0.06[b] |
| **Myristic Acid** | C14:0 | 3.5±0.20[a] | 2.69±0.04[b] |
| **Myristoleic Acid** | C14:1 | 2.5±0.26[a] | 1.42±0.04[b] |
| **Pentadecanoic Acid** | C15:0 | 2.1±0.26[a] | 1.15±0.05[b] |
| **Cis-10-Pentadecenoic Acid** | C15:1 | 17.8±0.33[a] | 14.44±0.3[a] |
| **Palmitic Acid** | C16:0 | 1.0±0.20[a] | 0.00±0.00[b] |
| **Palmitoleic Acid** | C16:1 | 1.1±0.06[a] | 0.76±0.11[b] |
| **Heptadecanoic** | C17:0 | 3.9±0.10[a] | 2.49±0.26[b] |
| **cis-10Heptadecenoic** | C17:1 | 0.6±0.04[a] | 0.53±0.01[b] |
| **Stearic Acid** | C18:0 | 0.8±0.10[a] | 0.53±0.03[b] |
| **Oleic Acid** | C18:1n9c | 6.6±0.26[a] | 5.55±0.15[b] |
| **Elaidic Acid** | C18:1n9t | 0.3±0.10[a] | 0.67±0.01[b] |
| **Linoleic Acid** | C18:2n6c | 2.3±0.00[a] | 3.04±0.03[a] |
| **Linolelaidic Acid** | C18:2n6t | 2.6±0.05[a] | 1.64±0.01[a] |
| **γ-Linolenic Acid** | C18:3n6 | 10.0±0.06[a] | 13.05±0.05[a] |
| **α-Linolenic Acid** | C18:3n3 | 1.3±0.006[a] | 1.31±0.0[b] |
| **Arachidic Acid** | C20:0 | 1.6±0.04[a] | 1.70±0.05[b] |
| **cis-11, Eicosatrienoic Acid** | C20:1n9 | 0.6±0.07[a] | 0.45±0.05[b] |
| **cis-11,14 – Eicosatrienoic Acid** | C20:2 | 1.5±0.06[a] | 0.81±0.01[a] |
| **cis-8,11,14,17- Eicosatrienoic Acid** | C20:3n6 | 0.8±0.05[a] | 0.89±0.03[b] |
| **c-s-11,14,17- Eicosatrienoic Acid** | C20:3n3 | 0.4±0.1[a] | 0.00±0.00[b] |
| **cis-5,8,11,14,17 Eicosapentanoic acid** | C20:5n3 | 15.3±0.26[a] | 4.14±0.08[a] |
| **Arachidonic acid** | C20:4n6 | 14.6±0.36[a] | 6.86±0.03[a] |
| **Heneicosanoic Acid** | C21:0 | 0.7±0.06[a] | 0.57±0.03[b] |
| **Behenic Acid** | C22:0 | 0.3±0.04[a] | 0.87±0.02[a] |
| **Erucic Acid** | C22:1n9 | 1.7±0.025[a] | 1.77±0.01[b] |
| **cis-13,16-Docosadienoic Acid** | C22:2 | 0.4±0.00[a] | 0.39±0.02[b] |
| **cis-4,7,10,13,16,19- Docosahexaenoic Acid** | C22:6n3 | 1.8±0.05[a] | 0.00±0.00[a] |
| **Tricosanoic Acid** | C23:0 | 0.0±0.00[a] | 0.00±0.00[a] |
| **Lignoceric Acid** | C24:0 | 0.6±0.03[a] | 0.00±0.00[a] |
| **Nervonic Acid** | C24:1n9 | 1.9±0.02[a] | 2.69±0.02[a] |

The same superscripts in a row indicate means which do not differ significantly _ANOVA, Duncan's multiple range test, P<0.05 values (mean±SD) are the means of three determinations

The sum of all saturated fatty acids (∑SFA) in *H.arenicola* was found lower than that of *A. mauritiana* i.e. 15.91% < 39.62%. As far as ∑PUFA (polyunsaturated fatty acid), and ∑MUFA (monounsaturated fatty acid) are concerned, significant variation was observed in both the species (Table 5). *H. arenicola* possessed higher values which were 50.92% and 33.17% > 32.1% and 28.27% compared with *A. mauritiana*. ω3/ ω6 ration which is an important factor of fatty acids to function against diseases was found distinct figure of 0.63 in *H. arenicola* and 0.21 in *A. mauritiana*. The summation of EPA: eicosapentaenoic acid (C20:5n3) and DHA: Docosahexaenoic acid (C22:6n3) was also taken into account and found to be 17.0% and 4.14% in *H. arenicola* and *A. mauritiana* respectively (Table 5).

**Amino acid analysis:** Amino acid profile of both the species was evaluated and observed from Table 3 that all the essential amino acids were found in both the species. The values of all the amino acids were almost same in both the species except aspartic acid and glycine which exhibited the major differences in their values marked as 15.71%, 17.33% and 10.83%, 21.70% in *H. arenicola*

and *A. mauritiana* respectively. The least value of amino acid was observed in the case of methionine, the values of which found to be 0.43% and 0.07% in *H.arenicola* and *A. mauritiana* respectively while Cystein and tryptophan were not detected (Table 3).

**Table 3. Amino Acid Composition of dried seacucumbers.**

| Amino acids | *Holothuria arenicola* g/100g total fatty acid | *Actinopyga mauritiana* g/100g total fatty acid |
|---|---|---|
| **Aspartic acid** | 15.71±0.53[a] | 10.83±0.15[a] |
| ***Threonine** | 4.59±0.07[a] | 4.80±0.06[b] |
| **Serine** | 4.54±0.11[a] | 4.83±0.06[b] |
| **Glutamic acid** | 11.77±0.02[a] | 11.63±0.06[b] |
| **Proline** | 7.56±0.17[a] | 8.01±0.05[b] |
| **Glycine** | 17.33±0.02[a] | 21.70±0.21[a] |
| **Alanine** | 11.72±0.04[a] | 10.03±0.07[a] |
| **Cysteine** | 0.00±0.00 | 0.00±0.00 |
| ***Valine** | 2.94±0.03[a] | 2.86±0.03[b] |
| ***Methionine** | 0.43±0.07[a] | 0.07±0.01[b] |
| ***Isoleucine** | 3.37±0.24[a] | 2.52±0.11[b] |
| ***Leucine** | 5.19±0.06[a] | 6.40±0.17[a] |
| **Tyrosine** | 2.45±0.2[a] | 3.89±0.2[b] |
| ***Phenylalanine** | 2.80±0.26[a] | 3.83±0.14[b] |
| ***Histidine** | 1.41±0.02[a] | 1.30±0.04[b] |
| ***Tryptophan** | 0.00±0.00 | 0.00±0.00 |
| ***Lysine** | 2.06±0.06[a] | 2.23±0.06[b] |
| **Arginine** | 6.12±0.06[a] | 5.05±0.05[a] |

The same superscripts in a row indicate means which do not differ significantly _ANOVA, Duncan's multiple range test, P<0.05
Values (mean±SD) are the means of three determinations
*Essential amino acids

**Mineral contents:** Micro nutrients Chromium (Cr), Nickel (Ni), Manganese (Mn), Copper (Cu), Zinc (Zn) and toxic metals Lead (Pb), Cadmium (Cd) were estimated in this study; Cr, Cd and Pb were not detected in both the species while *A. mauritiana* showed markedly higher value of Cu i.e 5.11 mg/100g than the *H. Arenicola* which contained the values of 0.95 mg/100g. As far as macro nutrients are concerned, sodium, potassium, calcium, and magnesium were determined in which the values were found to be 4750 mg/100g, 520 mg/100g, 5700 mg/100g, 4750 mg/100g and 6220 mg/100g, 620 mg/100g, 2610, 1870 mg/100g in *H. arenicola and A. mauritiana*, respectively (Table 4).

**Table 4. Mineral content of dried seacucumbers by atomic absorption method**

| Minerals | *Holothuria arenicola* mg/100g dried sample | *Actinopyga mauritiana* mg/100g dried sample | Minerals |
|---|---|---|---|
| **chromium** | Nd | Nd | **chromium** |
| **nickel** | 0.19±0.02[a] | 0.25±0.03[b] | **nickel** |
| **manganese** | 5.23±0.04[a] | 5.85±0.07[b] | **manganese** |
| **copper** | 0.95±0.01[a] | 5.11±0.1[a] | **copper** |
| **lead** | Nd | Nd | **lead** |
| **cadmium** | Nd | Nd | **cadmium** |
| **zinc** | 4.28±0.06[a] | 5.23±0.04[a] | **zinc** |
| **sodium** | 4750±12.5[a] | 6220±9.1[a] | **sodium** |
| **potassium** | 520±3.54[a] | 620±9.0[a] | **potassium** |
| **calcium** | 5700±7.07[a] | 2610±8.54[a] | **calcium** |
| **magnesium** | 4750±2.86[a] | 1870±11.36[a] | **magnesium** |

The same superscripts in a row indicate means which do not differ significantly _ANOVA, Duncan's multiple range test, P<0.05
Values (mean±SD) are the means of three determinations
Nd=not detected

## DISCUSSION

**Moisture, Ash and Fat analysis:** The chemical composition and nutritional quality of two most common sea cucumbers of Pakistan (*Holothuria arenicola and Actinopyga mauritiana*) were determined. The study includes their proximate, amino acids, fatty acids and mineral compositions. The chemical composition and proximate analysis in sea cucumber may differ from species to species and also differ among the same species of different region (Zhao *et al*., 2008; Wang *et al.,* 2009). Sea cucumbers generally contain a higher moisture and lower protein content than marine fish and shellfish (Wen *et al*., 2010). From table 1, it is obvious that sea cucumber being a tonic food contain high protein and low fat content, the result of which reflects in many articles (Chen, 2003; Wen *et al*., 2010). Lower values of fat in both the species of sea cucumbers were found similar as observed in the case of another study (Ridzwan *et al.,* 2014). The percent ash content in *H. arenicola* and *A. mauritiana* were at higher side as compared to other species undertaken for studies (Fangguo, 1997). This might be due to region specific as discussed earlier.

**Fatty Acid Composition:** The total fatty acids were determined for both the species categorized as saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acids (PUFA).

In our study, PUFA was of great interest as most of the long chain fatty acid cannot be synthesized easily by human body like eicosapentaenoic acid (EPA; 20:5ω3), arachidonic acid (AA; 20:4ω6) and docosahexaenoic acid (DHA; 22:6ω3). The distinct values of EPA and DHA were found in both the species studied here. The result supported the fact that the marine organisms are believed to contain higher EPA and DHA, compared to fresh water organism (Ridzwan *et al*., 2014).

One of the major fatty acid found in our study was arachidonic acid. It is revealed from the literature (Wen *et al*., 2010) that the arachidonic acid is one of the dominant fatty acid in almost all species of sea cucumber. In a study (Careaga, 2013), the main polyunsaturated fatty acids were found C20: 2ω-6, arachidonic (C20: 4ω-6) and eicosapentaenoic (C20: 5ω-3) acids. The high level of arachidonic acid is known to be responsible in blood clotting (Mat *et al*., 1994).

ω-3/ω-6 ratio is the appropriate indicator for relative comparison of nutritional value of fish fat (Tokur *et al*., 2006). Generally, amount of ω-6 among freshwater fishes is more than ω-3 (Tokur *et al*., 2006). The ω-3/ω-6 ratio is an important index of the fatty acid which plays an important role in human health. The appropriate balance for ω-3/ω-6 ratio as recommended in an article (Simopoulos, 2002) varies from 1.1 to 1.4 depending on the disease under consideration. From table 5, results suggested that the content of ω-6 is higher than the ω-3, but the value did not mean too high to promote inflammatory diseases. As ω-6 is also act as pro-inflammatory; inflammation is essential for our survival. It helps to protect our bodies from infection and injury. In our study, the arachidonic acid a beneficent fatty acid as discussed earlier, was the dominant among all ω-6 fatty acid.

**Table 5. Comparative assessment on Saturated Fatty acids, Monounsaturated Fatty Acids, Polyunsaturated Fatty Acids, ω3 Fatty Acids and ω3 to ω6 ratio between the sea cucumbers.**

| Fatty acid composition | *H. arenicola* | *A. mauritiana* |
|---|---|---|
| **∑SFA** | 15.91% | 39.62% |
| **∑MUFA** | 33.17% | 28.27% |
| **∑PUFA** | 50.92% | 32.12% |
| **ω3/ ω6** | 0.63 | 0.21 |
| **EPA+DHA** | 17.0% | 4.14% |

ΣSFA: C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0; ΣMUFA: C14:1 + C15:1 + C16:1 + C17:1 + C18:1n9t + C18:1n9c + C18:1n11c + C18:1n12c + C20:1 + C22:1n9 + C24:1; ΣPUFA: C18:2n6t + C18:2n6c + C18:3n6 + C18:3n3 + C20:2 + C20:3n6 + C20:3n3 + C20:4n6 + C2
2:2 + C20:5n3 + C22:6n3; Σ ω3: C18:3n3 + C20:3n3 + C20:5n3 + C22:6n3; Σ ω6: C18:2n6t + C18:2n6c+ C18:3n6+ C20:3n6+ C20:4n6; EPA: eicosapentaenoic acid; DHA: Docosahexaenoic acid. SFA, Saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

**Amino acid profile:** Among all the amino acids analyzed in our samples, glycine was found the major amino acid which is quite evident from other literatures (Wen *et al*., 2010; Bordbar *et al*., 2011). The results of other amino acid contents were within the range found in other species likewise Glutamic acid (4.69–7.31 g/100 g wet weight), aspartic acid (3.48–5.06 g/100 g wet weight), alanine (2.95–5.77 g/100 g wet weight) and arginine (2.71–4.95 g/100 g wet weight) which were prominent among other amino acids (Wen *et al*., 2010). The combination or proportion of amino acid is of great importance, lower Lysine/ arginine ration has been documented as Hypocholesterolemic and antiatherogenic effects (Rajamohan and Kurup, 1990). Result obtained in our study showed that both species of sea cucumber contain lower Lysine/Arginine ratio. Similar results were

also observed in a study in which all the eight species of sea cucumber exhibited low lysine: arginine ratio and higher essential amino acid (wen *et al*., 2010).

**Mineral contents:** Both the species are rich source of calcium, magnesium, sodium, and potassium. More or less similar values were found in both the species with the exception of copper which was significantly high in *A. mauritiana*. Higher values of minerals (calcium, magnesium, iron and zinc) were also reported in a study (Bordbar *et al*., 2011). In our study, both the species of sea cucumber especially *Actinopyga mauritiana* were proved to be the excellent source of copper favorable for our daily requirement as the DRI (Dietary Reference Intake) report by the Food and Nutrition Board of the National Academy of Sciences (Institute of medicine, 2001) established a Tolerable Upper Intake Level (UL) of 10 mg per day for adult men and women. Among the heavy metals, the toxic elements like lead, cadmium and chromium were not detected which revealed the fact that these sea cucumbers are safe for human consumption from metallic toxicity viewpoint. As far as other toxic compounds are concerned, further studies need to be conducted. In the local region, never this kind of study for mineral contents was conducted in sea cucumbers; however macro and micro nutrients were determined in fin fish and shell fish from Karachi coastal waters in a study (Nisa *et al*., 1995).

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