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Full Length Research Paper

Fatty acid composition in two sea cucumber species, *Holothuria scabra* and *Holothuria leucospilata* from Qeshm Island (Persian Gulf)

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This study was carried out to extract total lipid of two local sea cucumber species, *Holothuria scabra* and *Holothuria leucospilata* from Qeshm Island, and the fatty acid composition was determined by capillary gas chromatography. The results show that all species were rich in palmitic acid (C16:0) and arachidonic acid (C20:4n6) of saturated (SFA) and polyunsaturated fatty acids (PUFA), respectively. However, the main monounsaturated fatty acids (MUFA) in *H. scabra* and *H. leucospilota* were gadoleic acid (C20:1) and cis-oleic acid (C18:1n9c), respectively. The results of this study showed the high content of fatty acids, specially (ω-3) series in *H. scabra* and *H. leucospilata.*

Key words: Sea cucumber, *Holothuria scabra, Holothuria leucospilata,* fatty acid profile, Qeshm Island, Iran.

INTRODUCTION

Although, there are a growing number of studies focusing on echinoderms and holothuroids worldwide, the Persian Gulf has not received much attention. At this time 1400 species of sea cucumber have been identified and reported in the seas of the whole world (Conand, 2006). Sea cucumbers are the aquatic creatures that have many important and useful properties known for human health (Mamelona et al., 2007). A lot of researches have been done on medicinal and therapeutic properties of different species (Murray et al., 2001). The rearing of sea cucumber with shrimp controls the environmental pollution results from extra enriched nutritious built on the pond bottom. These animals eat detritus and with devouring of organic materials on the surface, not only do they make the environment clean, but also they cause the fast growth of shrimp and themselves (James, 2001). Subsequently, the research interest in sea cucumbers as a source of pharmacological agents was initiated since *Stichopus variegatus*, Semper was widely utilized as a traditional remedy for, hypertension, asthma, sinus, rheumatism, cuts, and burns. This long standing practice needs to be analyzed scientifically. Similar to haruan, *Channa striatus*, or snakehead (a freshwater carnivorous air breather), the sea cucumber is also known to facilitate internal healing, especially after a clinical surgery, caesarian operation or injury. As a virtual cure for all, it is also credited to possess similar aphrodisiac powers (Singh, 1980).

For over many centuries, sea cucumbers have been a food medicines and delicacy for Asians. Primarily, sea cucumber has been collected for food but extensive research on sea cucumber has been explored as source of medical component. Sea cucumber has potential to be commercialized in the field of modern treatment and has good therapeutic value. They have been nominated as poly-anion reach food due to the presence of glycosaminoglycans that have influence in many physio-

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Abbreviations: SFA, Saturated fatty acid; **PUFA,** polyunsaturated fatty acid; **MUFA,** monounsaturated fatty acids.

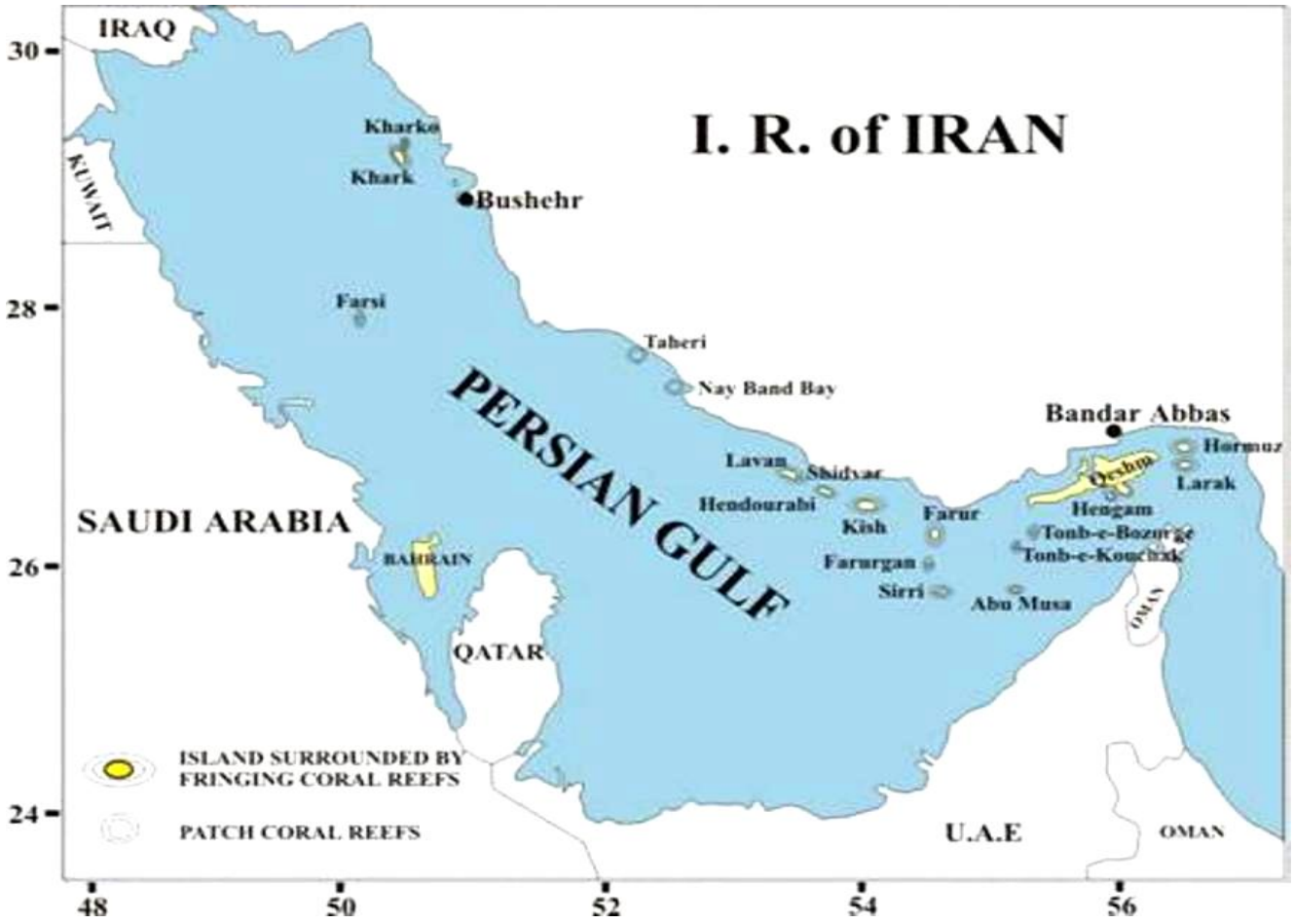

Figure 1. The map of site sampling area.

logically active function including wound healing activities (Liu et al., 2002).

For instance, most animals cannot synthesize longer chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA; 20:5ω3), arachidonic acid (AA; 20:4ω6) and docosahexaenoic acid (DHA; 22:6ω3). Instead, these are formed by phytoplankton and some bacteria and are transferred through the food web (Volkman et al., 1989; Brown et al., 1993).

Qeshm Island is the biggest Island in the Persian Gulf and located in the Strait of Hormuz. The fatty acid content of main species of sea cucumber, *Holothuria scabra* and *Holothuria leucospilata* particularly from the North coast of Persian Gulf has not been documented. This study was done to establish fatty acid composition of *H. scabra* and *H. lecospilata* collected from Qeshm Island waters.

MATERIALS AND METHODS

Sea cucumber samples

Fresh samples of *H. scabra* and *H. leucospilata* (10 specimens) weighing between 600 to 800 g were collected by SCUBA diving from North coast of Qeshm island (26°58' N 56°14' E) near Hamon jetty in 20 to 25 m from coast line where the water depth was 5 to 12 m, under the supervision of the Hormozgan fisheries Organization Board off the coastal areas of Qeshm Island (Figures 1 and 2). All samples were kept in plastic bags and stored at -80°C.

Lipid analyses

The animals were cleaned to remove the visceral organs and body fluid before homogenization. Lipids of sea cucumber species were extracted (separately) according to the Bligh and Dyer method (1959). After phase equilibration, the lower chloroform layer (TL) was removed and dried in a rotary vacuum evaporator at 32°C. The extracted lipids were redissolved in chloroform/methanol (9:1, v/v) and finally stored at 0°C until used.

Fatty acid methyl esters and gas chromatographic analyses

Separation of the methyl esters was done by gas chromatography, using a VARIAN Mod. 3300 gas chromatograph equipped with a flame ionization detector and a fused silica DB-WAX capillary column (30 m * 0.25 mm i.d.) (J and W Scientific, California, USA). The operation parameters were as follows: Detector temperature,

Figure 2. *H. scabra* (left) and H. *leucospilota* (right).

280°C; injection port temperature, 250°C; column temperature, 170°C for 16 min, programmed to increase at 2°C/min up to 210°C with a final holding time of 25 min; carrier gas, hydrogen at 0.8 ml/min, linear velocity of 38 cm/s, with an oxygen filter coupled to the line; nitrogen was used as the makeup gas at 30 ml/min, hydrogen and synthetic air at 30 ml/min and 300 ml/min for the detector; split injection at 1:100 ratio. All the stages, from the transesterification to the final injection were accomplished under nitrogen. Retention times and peak area percentages were automatically computed by a Varian 4290 integrator. Fatty acid methyl esters used as GC standard were: lauric acid M-E, L7272, cis-5,8,11,14,17-eicosapentaenoic acid M-E, E2012 and cis-4,7,10,13,16,19-docosahexaenoic acid M-E, D2659 (purity ≥ 98%) and they were purchased from Sigma Chemical Co; Matreya Bacterial Acid Methyl Esters CPTM Mix, Catalog No: 1114; SupelcoTM 37 Component FAME Mix, Catalog No: 47885- U. All solvents used for sample preparation were of analytical grade and the solvents used for GC analysis were of HPLC grade from Merck (Darmstadt, Germany). Water used in this work were re-distilled. All reagents used were of analytical grade from Mallinckrodt Chemical Works (St. Louis, MO) and from Sigma Chemical Co (Sigma–Aldrich Company St. Louis, MO) (Afkhami et al., 2011).

Statistical analysis

At first, we checked normality distribution of our data by using Kolmogrov-Smirnoff test. Significant differences between two species were determined by using T-test about 5% probabilities. All statistical analyses were done by SPSS (ver. 19.5) software.

RESULTS

Table 1 shows the body wall fatty acid compositions of two species of sea cucumbers from the North coast of Persian Gulf, *Holothuria scabra* and *Holothuria leucospilota*. The main saturated (SFA) and poly-unsaturated fatty acids (PUFA) found in all analysed samples (body wall of *H. scabra* and *H. leucospilota*), were palmitic acid (C16:0) and arachidonic acid (C20:4n6), respectively. While the main monounsaturated fatty acids (MUFA) in *H. scabra* and *H. leucospilota* were gadoleic acid (C20:1) and cis-oleic acid (C18:1n9c), respectively. There was low concentrate of linolenic acid (ALA, C18:3n3) in all analysed samples (Table 1). All samples showed any level of some fatty acids, C11:0, C12:0, C13:0, C14:1, C17:1, C18:1n12c, C18:2n6t and C18:3n6. Moreover, C15:1, C18:1n9t, C18:3n3, C20:3n6, C20:3n3 and C22:2 were not distributing in *H. scabra*, and C10:0 was not distributing in *H. leucospilota* (Table 1).

H. scabra was found to contain significantly higher values of PUFA and SFA followed by MUFA, while it relatively contained higher levels of PUFA and SFA followed by MUFA in *H. leucospilota* (Table 2). Polyunsaturated fatty acids (PUFA) (36.84%) were significantly higher than monounsaturated fatty acids (MUFA) (25.43%) in *H. scabra* ($p < 0.05$). It was not significant for *H. leucospilota* (PUFA, 35.29% and MUFA, 30.14%) ($p > 0.05$) (Table 3).

Significant different was showed between the level of ∑MUFA in two species ($p<0.05$), while there were no significant different of ∑SFA and ∑PUFA between them ($p > 0.05$). Ratio of ∑PUFA/ ∑SFA was 0.97 mg/ml for *H. scabra* and 1.02 mg/ml for *H. leucospilota* (Table 2).

Results show ∑PUFA ω3 was 0.16 mg/ml in *H. scabra*, while it was higher for *H. leucospilota* (0.24 mg/ml). Level of ∑PUFA ω6 was equal in the two species (0.21 mg/ml). The amount of ω3/ω6 is an important factor to show the suitable fatty acids; results of this study showed the

Table 1. Fatty acid composition of *H. scabra* and *H. leucospilota* (%) (mean ± SD).

Fatty acid composition	*H. scabra*	*H. leucospilata*
C10:0	11.45 ± 0.62	-
C11:0	-	-
C12:0	-	13.14 ± 0.00
C13:0	-	-
C14:0	15.47 ± 1	15.5 ± 0.09
C14:1	-	-
C15:0	16.95 ± 0.83	16.97 ± 0.03
C15:1	17.97 ± 1.91	17.814 ± 0.02
C16:0	18.74 ± 0.07	18.76 ± 0.25
C16:1	20.3 ± 0.42	20.32 ± 0.03
C17:0	20.94 ± 0.08	20.96 ± 0.01
C17:1	22.86 ± 1.11	-
C18:0	23.76 ± 0.89	23.77 ± 0.06
C18:1n9t	25.35 ± 0.24	25.5 ± 0.02
C18:1n9c	25.7 ± 1.48	25.72 ± 0.00
C18:1n11c	25.95 ± 3.72	25.97 ± 0.76
C18:1n12c	-	-
C18:2n6t	-	-
C18:2n6c	28.28 ± 1.03	28.3 ± 0.27
C20:0	29.38 ± 0.62	29.39 ± 0.00
C18:3n6	-	-
C20:1	30.55 ± 0.39	30.55 ± 0.06
C18:3n3	-	30.74 ± 0.07
C21:0	31.65 ± 0.57	31.65 ± 0.01
C20:2	32.7 ± 0.93	32.71 ± 0.00
C20:3n6	-	32.79 ± 0.02
C22:0	33.78 ± 0.77	33.786 ± 0.02
C20:3n3	34.22 ± 1.04	34.2 ± 0.12
C22:1n9	34.75 ± 0.04	34.75 ± 0.01
C20:4n6	35.3 ± 0.36	35.3 ± 0.14
C23:0	-	-
C22:2	-	36.511 ± 0.61
C20:5n3	37.71 ± 0.62	37.71 ± 0.36
C24:0	-	-
C24:1	39.79 ± 0.083	39.81 ± 0.11
C22:6n3	44.1 ± 0.78	44.1 ± 0.05

significant higher ratio of ω3/ω6 in *H. leucospilota* (1.14mg/ml). The ω3/ω6 ratio was found to have the highest value in *H. leucospilota*, followed by *H. scabra*. Amount of EPA + DHA was more in *H. leucospilota* ($p < 0.05$) (Table 2). The ratio of omega fatty acids is shown in Table 4. Significant different was shown for ω3, ω6 and ω9 in the two species. The amount of ω3 and ω9 were more in *H. leucospilota*, while higher level of ω6 was in *H. scabra*.

DISCUSSION

Since many holothurians feed on bottom sediments, they should contain high levels of fatty acids (Graeme et al., 1988; Leo and Parker, 1966; Sargent et al., 1983; Phillips, 1984), so the fatty acid profile in *H. scabra* and *H. leucospilata* in this case will be of great interest. It is well known that sediments contain a high level of branched chain fatty acids (Leo and Parker, 1966;

Table 2. Fatty acid composition of *H. scabra* and *H. leucospilota* (mg/ml).

Fatty acid composition	*H. scabra*	*H. leucospilota*
∑ SFA[a]	0.43	0.47
∑ MUFA[b]	0.29	0.41
∑ PUFA[c]	0.42	0.48
∑ PUFA/ ∑SFA	0.97	1.02
∑ MUFA/ ∑SFA	0.67	0.87
∑ PUFA/ ∑MUFA	1.44	1.17
∑ Fatty Acid [d]	1.14	1.36
∑PUFA n3 [e]	0.16	0.24
∑PUFA n6 [f]	0.21	0.21
n3/n6	0.76	1.14
EPA[g]+DHA[h]	0.16	0.22

[a]∑SFA: 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; [b]∑MUFA: C14:1 + C15:1 + C16:1 + C17:1 + C18:1n9t + C18:1n9c + C18:1n11c + C18:1n12c + C20:1 + C22:1n9 + C24:1; [c]∑PUFA: C18:2n6t + C18:2n6c + C18:3n6 + C18:3n3 + C20:2 + C20:3n6 + C20:3n3 + C20:4n6 + C22:2 + C20:5n3 + C22:6n3; [d]∑Faty acid: ∑SFA + ∑MUFA +∑PUFA; [e]∑PUFA n3: C18:3n3 + C20:3n3 + C20:5n3 + C22:6n3; [f]∑PUFA n6: C18:2n6t + C18:2n6c+ C18:3n6+ C20:3n6+ C20:4n6; [g]EPA: eicosapentaenoic acid; [h]DHA: Docosahexaenoic acid. SFA, Saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 3. Amount of SFA, MUFA and PUFA in *H. scabra* and *H. leucospilota* (%).

Parameter	*H. scabra*	*H. leucospilota*
SFA	37.71	34.55
MUFA	25.43	30.14
PUFA	36.84	35.29

SFA, Saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 4. Amount of ω3, ω6 and ω9 in *H. scabra* and *H. leucospilota* (%).

Parameter	*H. scabra*	*H. leucospilota*
ω3	14.03	17.64
ω6	18.42	15.44
ω9	6.14	11.76

ω3, Omega-3-fatty acid; ω6, omega-6-fatty acid; ω9, omega-9-fatty acid.

Sargent et al., 1983; Phillips, 1984) which are believed to be of bacterial origin (Graeme et al., 1988). The main source of plant material in the diet of demersal animals from shallow (<100 m) coastal waters would most likely to be the macro algae. The macro-algae are grazed directly by the herbivorous and omnivorous species (Graeme et al., 1988). In many studies, macro algae have been found to be rich in arachidonic acid (AA) and EPA (Jamieson and Reid, 1972; Wood, 1974; Johns et al., 1979). The high content of EPA could well be associated with the ability of *S. chloronotus* to initiate tissue repair since EPA is believed to be the active component in fish oil and exerts its action through prostaglandin in inhibition and anti-thrombotic activity (Croft et al., 1987). In this study, this particular fatty acid was 37.71% in two surveyed species.

Marine organisms are believed to contain more fatty acids of the (ω-3) series, than fresh-water ones (Ackman, 1967). Results of this study showed the high content of fatty acids, specially (ω-3) series in *H. scabra* and *H.*

leucospilata.

Polyunsaturated fatty acids are important fatty acids in human feed, which the high level of PUFA found in all samples. Furthermore, our results are in line with previous studies (Lewis, 1967; Allen, 1968; Svetashe et al., 1990; Romashina, 1983). The high level of arachidonic acid (AA), which is known to be responsible in blood clotting (Mat Jais et al., 1994) is found in samples. Arachidonic acid content is remarkably higher in tropical holothurians, being the major acid in almost of all the species studied, except *Euapta godeffroyi*, which mainly contains eicosapentaenoic acid (Svetashev et al., 1991). However, arachidonic acid was less of palmitic acid in this investigates. For instance most animals cannot synthesize longer chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA; 20:5ω3), arachidonic acid (AA; 20:4ω6) and docosahexaenoic acid (DHA; 22:6ω3). Instead, these are formed by phytoplankton and some bacteria and are transferred through the food web (Volkman et al., 1989; Brown et al., 1993) such that high levels of these fatty acids are suggestive of herbivory. Graeme et al. (1988) showed the principal fatty acids of holothurians in Australia waters are as follows: Arachidonic acid, gadoleic acid, EPA, palmitoleic acid and stearic acid. They reported two saturated fatty acids (16:0 > 18:0), two monounsaturates (18:1 > 16: 1) and four polyunsaturates (DHA > EPA > AA > 22:5 n-3) are the main fatty acids in three groups of marine organisms, the bony fish (class: teleostomi; phylum: chordata), the cartilaginous fish (class: chondrichthyes; phylum: chordata) and the cephalopods (class: cephalopoda: phylum: mollusca).

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 (Simopoulos, 2002). The ω-3/ω-6 ratio is an important index of the fatty acid role in human health. The appropriate balance for ω-3/ω-6 ratio as recommended by Simopoulos (2002) varies from 1.1 to 1.4 depending on the disease under consideration. The ω-3/´-6 ratio differences may be explained by the large variability of the oil level in the fish muscle, which depends on the species, period of the year, age, size, reproduction period, the specific species as well as the fatty acid composition of the diet (Kromhout, 2001). Eicosapentaenoic acid is characteristic of invertebrates. It is represented in almost all the studied types and classes of invertebrates. The highest level was observed in echinoderms, in two species of holothurians: *Cucumaria jraudatrix* and *C. japonica* (Isay and Busarova, 1984). Palmitic acid (16:0) was the largest fatty acid component of Okinawan corals (Yamashiro et al., 1999).

Our study indicates that this species were particularly rich in PUFA and arachidonic acid (AA) and they are able to compete with more commercially sea cucumber species in terms of nutritional value. Consequently, more study is required in detailing with the origin and distribution of arachidonic acid in holothurians species.

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