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Fatty acid composition of select sea anemones from Mandapam Coast, Tamil Nadu

S. Thangaraj[1*], S. Bragadeeswaran[2], & V. Gokula[1]

[1]PG & Research Department of Zoology, National College, Tiruchirappalli, Tamil Nadu, India

[2]Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences,Annamalai University, Parangipettai, Tamil Nadu, India

[*][E-mail: thangamjayam@gmail.com]

Sea anemones, such as *Heteractis magnifica*, *H. aurora*, *Stichodactyla haddoni*, and *S. gigantea*, were collected from Mandapam coast, south-east coast of India. The tissue samples were extracted and analyzed by gas chromatography–flame ionization detection using a one-step extraction–methylation method. Totally, 40 fatty acids were identified and confirmed. Among them, palmitic acid (C16:0) was found in all the sea anemones. It was higher in *H. aurora* (60.53%) than in *S. gigantea* (23.62%). The highest saturated fatty acid (SFA) content was observed in *S. gigantea* extract followed by *H. magnifica*, *H. aurora*, and *S. haddoni.* The predominant SFA C16:0N alcohol was present in *H. magnifica* (22.24%) and *S. haddoni* (27.79%). Monounsaturated fatty acid (MUFA) C16:1ω-7 was found in *H. magnifica* (3.88%) and *S. haddoni* (8.12%) and saturated fatty acid (SFA) C18:1ω-9 was found in *H. aurora* extract (3.87%). Polyunsaturated fatty acid (PUFA) C18:3ω-6 was found in *H. magnifica* (3.88%) and C20:4ω-6 was found in *S. haddoni* (9.49%), *H. aurora* (12.26%), and *S. gigantea* (3.47%). The percentage of total fatty acids and SFA was higher in *S. gigantea* extract, the MUFA was high in *H. magnifica*, and PUFA was higher in *H. aurora* than in other sea anemone species. It is presumed that the chief fatty acids present in a particular organ are related to the specific functions of the organ. In all the tissues that were analyzed, SFA levels were higher than MUFA and PUFA. To the best of our knowledge, this is the first report on the fatty acid composition in sea anemones from Indian waters.

[**Keywords**: Sea anemone; Palmitic acid; Saturated fatty acid; GC–flame ionization]

Introduction

In metabolism, lipids as one of the most elemental nutrients generate many bioactive lipid molecules, which are fundamental mediators of multiple signaling pathways. Any kind of changes in lipid metabolism can lead to the disruption of signaling networks and could be associated with some pathological states, such as cancer, cardiovascular and neurodegenerative disorder, metabolic diseases, and inflammatory complications.

Lipids consist of fatty acids that are classified mostly according to the presence or absence of double bonds as follows: (i) Saturated fatty acids (SFAs—without double bonds), (ii) Monounsaturated fatty acids (MUFAs—with one double bond), and (iii) Polyunsaturated fatty acids (PUFAs—with two or up to six double bonds).

They are also classified further as *cis* or *trans* based on the configuration of the double bonds and as *n*-3 or *n*-6 PUFAs depending on the position of the first double bond from the fatty acid methyl-end.

The study of total fatty acid composition, especially PUFAs and ω-3 in marine organism is of great interest due to their beneficial effects on coronary diseases[1,2]. The chemical composition of species may change depending on the species, location, diet, and seasonality. Hence, the chemical compositions of marine invertebrate species are of interest for scientists since some of them are known as sources of methylene-interrupted PUFA, in particular of the (*n*-3) and (*n*-6) series, and also their toxic levels are directly proportional to their protein levels[3,4].

Sea anemones as member of Phylum: Cnidaria are common organisms in many benthic marine communities. They are known as omnivorous marine organisms that consume everything they can catch and they can live by adhering to hard substrates, such as rocks, corals, other animals, or ship bottoms. Researches on the chemical composition are limited to a few species and regions[5,9]. Hence, an attempt was made to determine the fatty acid composition of four species of sea anemones, namely, *Heteractis magnifica*, *H. aurora*, *Stichodactyla haddoni*, and *S. gigantea*.

Materials and Methods

Sea anemones, *H. magnifica* (*n*=6), *S. haddoni* (*n*=8), *H. aurora* (*n*=10), and *S. gigantea* (*n*=10),

were collected from Mandapam coastal area, Ramanathapuram district, Tamil Nadu. The animals were isolated by removing the nematocysts and completely dried. The dried tissue materials were finely powdered for estimation of fatty acid analysis. The extraction was made following the Bligh and Dyer method[10]. The identification and quantification of fatty acids were done by using Agilent Technologies 6890 N, Network GC system.

Fatty acids analysis

The extraction of fatty acid was carried out in a sonication bath for 20 min with HCl–MeOH (0.5 mL). Fatty acid methyl esters (FAMEs) were formed by heating at 90 °C for 2 h; HCl was dried and after cooling, FAMEs were extracted into hexane H_2O (2:0.5). The hexane layer was removed and 2 mL hexane was added just before chromatography analysis[11]. Gas chromatography (GC) was performed on a BD 23 column (60 mm × 0.25 mm i.d. × 0.25 mm film thickness) (Agilent Technologies, USA). Helium was used as the carrier gas. Samples (1 µL) were injected by a splitless injector at an oven temperature of 110 °C. After 3 min, the oven temperature was increased to 165 °C at 30 °C min^{-1}, then to 210 °C at 1 °C min^{-1}, and finally to 240 °C at 3 °C min^{-1} where it was held for 20 min. The GC-flame ionization analyses were performed on all samples using a gas chromatography distillation (GCD) 1800D gas chromatograph mass spectrometer (Hewlett-Packard, USA) with autosampler injection and using a GCD Plus ChemStation G1074B (version A.01.00) software. The fatty acids were identified by comparison with retention time of 37 FAMEs commercial standards (Supelco 47855-U), mass spectral data, and confirmed with the FAMEs mass spectrometry according to McLafferty and Turecek method[12]. The peak areas were used to attribute the relative proportions of fatty acids.

Results

In the present study, a total of 40 fatty acids were identified: 16 SFAs, 9 MUFAs, 3 PUFAs, 3 branched, and 9 unknown. Among the SFAs, C14:0, C16:0 N alcohol, C16:0, and C18:0 were the major acids (Table 1). The highest fatty acid content was observed in *S. gigantea* followed by *H. magnifica*, *H. aurora*, and *S. haddoni.* Among the fatty acids that were identified, the composition varied between different sea anemone species. In *S. gigantea*, C16:0 was the dominant fatty acid (Table 1). While SFAs are the most abundant in almost all the sea anemones, the composition was different between the species. In *H. magnifica*, C16:0 N alcohol (22.24%), C16:0 (12.50%), and C18:0 (21.50%) were the dominant SFAs; and C16:1ω-7 (3.88%), C18:1ω-7 (3.06%), C18:1ω-9 (1.93%), and C20:1ω-7 (2.38%) were the dominant MUFAs. In *H. aurora* extract, the following were recorded: SFAs C16:0 (23.62 %) and C14:0 (14.16%) and one PUFA [C20:4ω-6 (12.26%)]. In the case of *S. haddoni*, the SFAs C16:0 N alcohol (27.79%) and C16:0 (15.91%) were recorded, but PUFAs of C16:1ω-7 (8.12%), C18:3ω-6 (0.66%), and C20:4ω-6 (3.47%) were found in lower amount. *S. gigantea* extract showed the dominant SFAs of C14:0 (11.83%), C16:0 (60.53%), and C20:4ω-6 (9.49%), but MUFAs were not detectable in this extract. Palmitic acid (C16:0) was observed as the dominant SFA in all the sea anemone extracts.

Figure 1 shows the percentage of SFA, MUFA, and PUFA composition in the four sea anemone species. The total SFA contents of *H. magnifica*, *S. haddo ni*, *H. aurora*, and *S. gigantea* were observed as 66.31%, 54.17%, 59.6%, and 78.35%, respectively. The corresponding total MUFA contents were 10.23%, 10.74%, and 11.25%. The total amount of PUFA of *H. magnifica*, *S. haddoni*, *H. aurora* and *S. gigantea* were 5.86%, 4.13%, 14.47%, and 9.49, respectively. The SFA was higher (78.35%) in *S. gigantea* than in other species (Fig. 2).

Discussion

In the present investigation, the SFAs such as lauric acid (C12:0), myristic acid (C14:0), penta decyclic acid (C15:0), palmitic acid (C16:0), margaric acid (C17:0), and stearic acid (C18:0) were found in all the sea anemone extracts and collectively the SFAs were present in higher quantity than MUFAs and PUFAs. Among the SFAs, stearic acid occurred in major quantity among all the fatty acids. Hardwick *et al.*[13] reported that SFAs, such as capric, lauric, tridecylic, and myristic acids, were active in antisporulation. In recent years, lauric acid is used in the manufacture of soaps, shampoos, and other surface active agents, including special lubricants. Rakshit *et al.*[14] reported SFAs such as C16:0, C18:0, and C20:0; MUFAs such as C18:1ω9, C16:1ω7, and C20:1ω9; and PUFAs such as C22:5ω6, C20:5ω3, C18:2ω6, and C20:4ω6 acids as major constituents in different organs of *Telescopium telescopium*. The present study also reported SFAs, such as C12:0,

Table 1 — Retention time (in mins) and fatty acid profile (values in mean and SD) of the sea anemones, *Heteractis magnifica*, *Heteractis aurora*, *Stichodactyla haddoni*, and *Stichodactyla gigantea.*

Formula	Common name	Nomenclature name	Molecular weight*	Boiling point (°C)*	Retention time	*Heteractis magnifica*	*Heteractis aurora*	*Stichodactyla haddoni*	*Stichodactyla gigantea*
Saturated *Monocarboxylic fatty acids*									
C10:0	Capric, decylic	Decanoic	172.27	270^{760}	3.244	0.47 ± 0.01	0.11 ± 0.00	0.43 ±0.01	Nil
C12:0	Lauric, laurostearic	Dodecanoic	200.33	225^{100}	4.930	1.08 ± 0.01	0.74 ± 0.00	0.81 ±0.01	4.67 ± 0.00
C14:0	Myristic	Tetradecanoic	228.38	250.5^{100}	7.537	3.01 ± 0.01	14.16 ± 0.00	2.71 ±0.01	11.83 ± 0.00
C15:0	Penta decyclic acid	Pentadecanoic	242.41	257^{100}	9.113	0.64 ± 0.00	0.53 ± 0.00	Nil	Nil
C15:0 Anteiso	12-Methyltetradecanoic (sarcinic)	–	–	–	8.662	-	0.25 ± 0.00	0.74 ±0.01	Nil
C16:0 N alcohol		–	–	–	10.058	22.24 ± 0.01	3.28 ± 0.00	27.79 ±0.01	Nil
C16:0 ANTEISO	13-Methylpentadecanoic	–	–	–	10.353	Nil	0.29 ± 0.00	Nil	Nil
C16:0	Palmitic	Hexadecanoic	256.43	390^{760}	10.812	21.50 ± 0.06	23.62 ± 0.00	15.91 ± 0.01	60.53 ± 0.00
C16:0 10 methyl		–	–	–	11.570	Nil	Nil	Nil	1.32 ± 0.00
C17:0 cyclo		–	–	–	12.369	0.61 ± 0.01	4.15 ± 0.00	Nil	Nil
16:0 2OH		–	–	–	12.989	3.16 ± 0.01	0.87 ± 0.00	Nil	Nil
C17:0	Margaric	Heptadecanoic	270.46	227^{100}	12.564	Nil	0.88 ± 0.00	Nil	Nil
C17:0 10 methyl		–	–	–	13.295	Nil	0.10	Nil	Nil
C18:0	Stearic	Octadecanoic	284.50	360 d*	14.340	12.50 ± 0.06	9.03 ± 0.00	5.78 ±0.01	Nil
C19:0		Nonadecanoic	298.52	297^{100}	15.929	Nil	1.59 ± 0.00	Nil	Nil
C20:0	Arachidic, Heneicosanoic acid	Heneicosanoic	312.54	328 d*	17.841	1.10 ± 0.06	Nil	Nil	Nil
Sum						**66.31** ±0.07	**59.6** ± 0.05	**54.17** ±0.02	**78.35** ± 0.01
Monounsaturated									
C14:1ω-5		–	–	–	7.399	Nil	1.38 ± 0.00	Nil	Nil
C16:1ω-5		–	–	–	10.659	Nil	0.19 ± 0.00	Nil	Nil
C16:1ω-7		–	–	–	9.776	3.88 ± 0.01	0.75 ±0.00	8.12 ±0.01	Nil
C17:1 ω-5		–	–	–	11.635	Nil	0.18 ± 0.00	Nil	Nil
C17:1ω-8		–	–	–	12.196	Nil	0.30 ± 0.05	Nil	Nil
C18:1ω-5		–	–	–	14.204	Nil	0.26 ± 0.00	Nil	Nil
C18:1ω-7		–	–	–	14.030	3.06 ± 0.01	3.30 ± 0.05	Nil	Nil

(*Contd.*)

Table 1 — Retention time (in mins) and fatty acid profile (values in mean and SD) of the sea anemones, *Heteractis magnifica*, *Heteractis aurora*, *Stichodactyla haddoni*, and *Stichodactyla gigantea.* (*Contd.*)

Formula	Common name	Nomenclature name	Molecular weight*	Boiling point (°C)*	Retention time	*Heteractis magnifica*	*Heteractis aurora*	*Stichodactyla haddoni*	*Stichodactyla gigantea*
C18:1ω-9		–	–	–	13.933	1.93 ± 0.01	3.87 ± 0.00	2.62 ± 0.01	Nil
C20:1ω-7		–	–	–	17.551	2.38	Nil	Nil	Nil
Sum						**11.25** ± 0.01	**10.23** ± 0.06	**10.74** ± 0.01	**000**
Polyunsaturated									
C18:3ω-6		–	–	–	13.593	3.88 ± 0.01	Nil	0.66 ± 0.01	Nil
C20:2ω-6		–	–	–	17.380	1.98 ± 0.01	2.21 ± 0.00	Nil	Nil
C20:4ω-6		–	–	–	16.797	Nil	12.26 ± 0.00	3.47 ± 0.01	9.49 ±0.00
Sum						**5.86** ± 0.01	**14.47** ± 0.00	**4.13** ± 0.01	**9.49** ± 0.00
Σ of Branched									
C17:0 Iso	15-Methylhexadecanoic	–	–	–	11.918	Nil	0.22 ± 0.00	Nil	Nil
C18:0 Iso	16-Methylheptadecanoic (isostearic)	–	–	–	13.725	Nil	Nil	5.94 ± 0.01	4.19 ± 0.00
C20:0 Iso	18-Methylnonadecanoic	–	–	–	17.220	Nil	1.10 ± 0.00	Nil	Nil
Sum						**0.00**	**1.32** ± 0.01	**5.94** ± 0.01	**4.19** ± 0.00
Unknown									
Unknown 11.799		–	–	–	4.738	0.70 ± 0.00	Nil	Nil	Nil
Unknown14.263		–	–	–	7.943	0.47 ± 0.01	0.24 ± 0.00	3.83 ± 0.01	Nil
Unknown 14.502		–	–	–	8.333	Nil	0.23 ± 0.00	Nil	Nil
Unknown 15.669		–	–	–	10.230	0.98 ± 0.01	Nil	Nil	Nil
Unknown 16.582		–	–	–	11.818	7.73 ± 0.01	Nil	1.12 ±0.01	Nil
Sum in feature 2		–	–	–	9.959	Nil	1.67 ± 0.00	1.27 ±0.01	Nil
Sum in feature 3		–	–	–	10.507	4.90 ± 0.06	7.51 ± 0.00	8.28 ± 0.01	7.96 ± 0.00
Sum in feature 4		–	–	–	11.641	2.41 ± 0.01	Nil	Nil	Nil
Sum in feature 5		–	–	–	13.847	Nil	0.97 ± 0.00	3.54 ±0.01	Nil
Sum						**17.19** ±0.06	**10.62** ± 0.01	**18.04** ± 0.01	**7.96** ± 0.00
Total						**100.61** ±0.08	**96.24** ± 0.08	**93.02** ±0.04	**99.99** ± 0.02

*Molecular weight and boiling point source: Agilent Technologies 6890 N, Network GC system

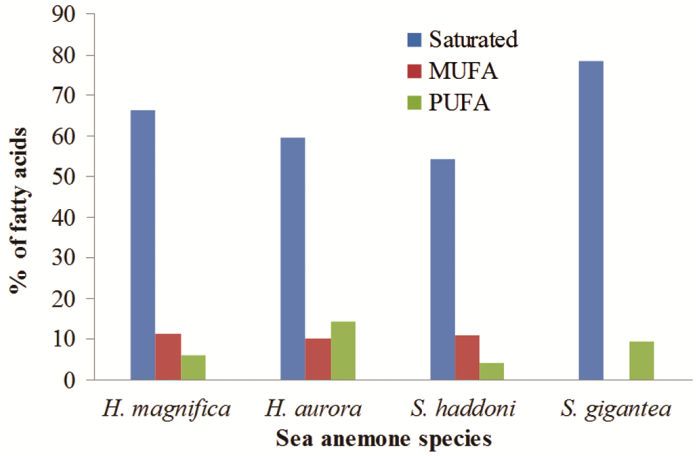

Fig. 1 — The percentage (%) of total fatty acids from sea anemones *Heteractis magnifica*, *Heteractis aurora, Stichodactyla haddoni*, and *Stichodactyla gigantea*

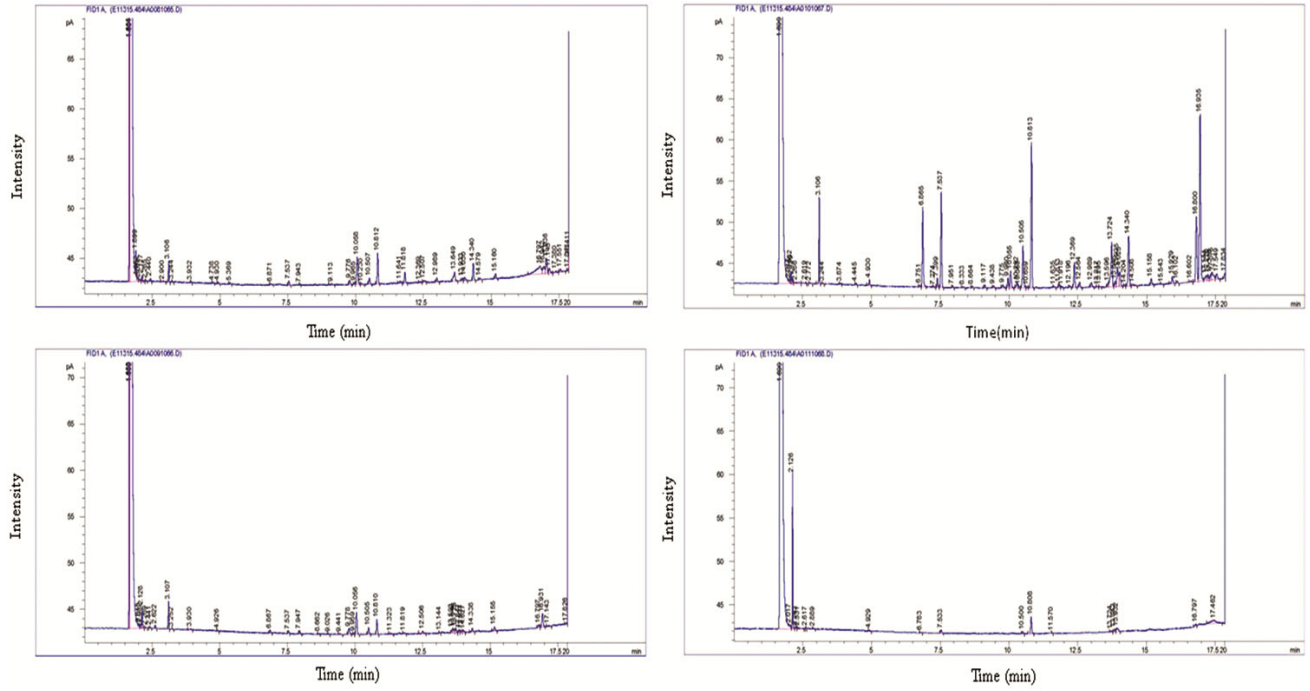

Fig. 2 — The amount of fatty acids in sea anemone, (A) *Heteractis magnifica*, (B) *Heteractis aurora*, (C) *Stichodactyla haddoni*, (D) *Stichodactyla gigantea* and retention times (min)

C14:0, C15:0, C16:0, C17:0, and C18:0 as the major acids from all anemone species.

The results from the present study coincided with the previous work by Gulgun *et al.*[15] in which they reported palmitic acid (C16:0) as the dominant SFA from flathead gray mullet fillet (20.3±1.2), caviar oil (5.9±0.3), and beeswaxed caviar oil (6.7±0.3). Pazos *et al.*[16] have studied the fatty acid composition from marine bivalves, such as *Pecten maximus*, *Crassostrea gigas*, *Tapes decussatus*, *Tapes philippinarum*, and *Scapharea inaequivalvis*. Both Palpandi *et al.*[17] and Shanmugam *et al.*[18] studied the fatty acid composition of different tissues of the marine neogastropod *Cymbium melo* and wedge clam *Donax cuneatus*, respectively. Okkes *et al.*[19] reported that the fatty acids in fish *Capoeta capoeta umbla* showed significant seasonality in genders.

In conclusion, this study has shown that the fatty acid composition of selected sea anemone species in Mandapam coastal waters differ significantly,

which may be due to the seasonal differences in food consumption and reproductive state of the animal. In all the species that were studied, the SFAs are found in higher concentration when compared to MUFA and PUFA. To our knowledge, this is the first report that studied the fatty acid profiles of sea anemones in India.

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