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Determination of Fatty Acids in the Tissues of Four Commercially Important Oyster Species Inhabiting Pakistan Coast

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Abstract. In order to determine the fatty acids composition by Gas Chromatography (GC) of four native oyster species, samples of candidate oyster species (*Ostrea nomades, Saccostrea cucullata, Crassostrea gryphoides* and *C. madrasensis*) were obtained during June 2008 from Buleji rocky Intertidal zone (24° 50′ N, 66° 48′ E) and deltaic zone of Hub river (24° 54′ N, 66° 43′ E) situated along the Sindh and Balochistan coast respectively. Present work unveils the variability in fatty acids components in four oyster species that have been investigated for the first time from Pakistan. A total of thirty nine (39) compounds, in aforementioned species were present comprised of saturated (SFA) mono-unsaturated (MUFA) poly-unsaturated fatty acids (PUFA) including dimethyl acetals (DMA) non-methylene interrupted acids (NMI) were isolated and among these poly-unsaturated fatty acids (PUFA) amounted larger proportion in all four species, about 50.02±0.15 mg/g in *O. nomades*, 49.07±1.63 in *C. gryphoides*, 47.93±4.82 mg/g in *C. madrasensis* and 45.51±3.34 mg/g in *S. cucullata*. Conspicuously 20:1n-9 and 22:1n-9 compounds were only present in *S. cucullata*, whereas these fatty acids were completely missing in other three species. Moreover, 20:3n-3 was found in trace amount in three species excluding *C. madrasensis*.

Key words: edible oysters, fatty acids, Hub river delta, Pakistan coast

Introduction

Oysters are not only a well known and commonly cultivated sea food commodity that has been considered delicacy in several parts of the world. Certainly, these could be a source of exploitable lipids for health benefits. Despite this resource is not utilized in best possible manners yet (Dagorn *et al*., 2016; Asha *et al*., 2014; Siddiqui, 2005). Studies on species diversity, reproduction, nutritional attributes, commercial importance, biochemical composition, mineral content of oyster meat, fatty and amino acid, protein and carbohydrate etc. have been done widely (Dagorn *et al*., 2016; Asha *et al*., 2014; Siddiqui, 2005; Siddiqui and Ahmed, 2002 a,b; Soudant *et al*., 1999). From Pakistan notable work has also been done on native oyster species diversity, their repro-ductive cycle, culture prospectus and pathology reported by (Afsar *et al*., 2014a; Siddiqui *et al*., 2008; Siddiqui, 2005; Siddiqui and Ahmed, 2002 a,b). Siddiqui and Ahmed (2002 a) also have described nine (9) native species of oysters belonging to three different genera which are Crassostrea, Saccostrea and Ostrea. Whereas reported species are namely *Crassostrea gryphoides, C. madrasensis, C. belcheri, C. glomerata, Saccostrea cucullata, S. echinata, Ostrea nomades, O. folium* and *O. cristagalli.* A report of amino acid composition of tissue protein in five species of oysters from Pakistan is also available (Aftab, 1988). Moreover, in contrary studies on nutritional aspect have been taken. Other than Houcke *et al*. (2016) reported the profile of biochemical and volatile organic compounds which they found in European flat oyster *Ostrea edulis* and Pacific cupped oyster *Crassostrea gigas* sampled from eastern Scheldt and lake Grevelingen of the Netherlands. Studies revealed the presence of main volatile organic compound in the European flat oyster that was 3-cyclohexene-1-ethanol whereas 1,5-octadien-3-ol was the main volatile organic compound in the Pacific cupped oyster.

Since fatty acids and in particular PUFA are very important dietary fats, because these are incredibly vital for human health, due to their use in certain medicinal products. Thus PUFA supply from aquaculture veracity has increased ultimately, to overcome global discrepancy in fishery supplies (Afsar *et al*., 2012; Tacon and Metian, 2008; Bergé and Barnathan, 2005). Oyster species are characterized by low fat as well as considered to be a good source of important PUFA compounds such as eicosapen-taenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) fatty acids which are interrelated in

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deterrence of human diseases. Hence, recommended for human consumption (Martino and Maria da Cruz, 2004; Sargent and Tacon, 1999; Sargent, 1997).

Study describes the fatty acid profiles of four (4) potential species from Pakistan belonging to three genera that are *Ostrea nomades, Saccostrea cucullata, Crassostrea gryphoides* and *C. madrasensis.* Afore-said species are commercially important edible oysters as well as these are also noteworthy in aquaculture standpoint. Studies on nutritional aspect have been taken for the first time from Pakistan.

Materials and Methods

Sample collection. Specimens of four commercially important edible oyster species were obtained during June 2008 from Buleji intertidal rocks (24° 50′ N, 66° 48′ E) and Hub delta (24° 54′ N, 66° 43′ E) localities situated along the Sindh and Balochistan coast. Collected species are *Ostrea nomades, Saccostrea cucullata, Crassostrea gryphoides* and *C. madrasensis*.

Animal tissue sample preparation. Fresh samples of pooled gonadal tissues (n=2 to n=3) of *Ostrea nomades, Saccostrea cucullata, Crassostrea gryphoides* and *C. madrasensis* were used to prepare a tissue sample. Subsequently samples were set aside in stoppered glass tubes and 20-40 mL 2:1 v/v chilled chloroform/ methanol (C:M) was added in each sample then stored in cold for further course of action.

Fatty acids extraction and GC-MS analysis. Extraction procedure was based on the method described by Folch *et al*. (1957) as already detailed previously (Afsar *et al*., 2015; Afsar *et al*., 2014a). Gonadal tissues of oysters were homogenized by using Ultra Turrax TM and 0.25 volumes of 0.88% (w/v). KCl added to homogenized samples then samples were centrifuged at 400 g ave (1500 rpm Jouan C 412 bench centrifuge) followed by top layer was removed through aspiration and bottom layer filtered with Whatman no. 1 filter paper. Solvent evapouration was done under a stream of oxygen free nitrogen (OFN) on a nitrogen evapourator and desiccated in vacuo overnight. Later total lipids were re-dissolved in C:M (2:1) and 0.01% (w/v) butylated hydroxy-toulene (BHT) used as an antioxidant, where BHT was added at a concentration of 10 mg/mL and subsequently stored under nitrogen in a freezer at -20±5 °C. After that 1 mg lipid aliquots with addition of heptadecanoic acid (17:0) as an internal standard at 10% of the total lipid mass evaporated under nitrogen and 2 mL of methylating reagent (1 mL conc. H_2SO_4 + 99 mL MeOH)1% H_2SO_4 in methanol was added then tubes kept under nitrogen for 16 h at 50 °C. As a final point fatty acid methyl esters (FAME) were extracted with iso-hexane: diethyl ether (1:1, v/v).

Methyl esters were purified by thin layer chromatography (TLC) on 20x20 cm plates (silica gel G 60, Merck) by using iso-hexane: diethyl ether: acetic acid (90:10:1) as solvent system. FAMES were determined with a Gas Chromatograph (GC) (Fisons MD800) equipped with a phenomenex ZB-WAX column (30 mt x 0.32 mm x 0.25) and cold on-column injection system, using helium as carrier gas at a flow rate of 2.0 mL/min. Primary oven temperature was set aside at 50 °C then elevated to 225 °C at a ramping 40 °C/min. to 150 °C then at 2 °C/min. to 225 °C and to end with hold for 5 min. at 225 °C. 1 mL of solution in iso-hexane was injected. Peaks were recorded and integrated in a personal computer using Chrom Card software (Fisons). FAMES were identified by comparison with fish oil standard known as "Marinol".

Results and Discussion

Present work describes the fatty acids methyl ester profiles of the four (4) native species and variability of FAME components among all four species. Oyster species namely *Ostrea nomades, Saccostrea cucullata, Crassostrea gryphoides* and *C. madrasensis* were sampled and analyzed for fatty acids methyl ester (FAME) by Gas-chromatography mass spectrometric technique. A total of thirty nine (39) compounds were detected, comprised of saturated (SFA) mono-unsaturated (MUFA) poly-unsaturated fatty acids (PUFA) together with dimethyl acetals (DMA) non-methylene interrupted acids (NMI) as given in Table 1.

Noticeably 20:1n-9 and 22:1n-9 compounds were only present in *S. cucullata* whereas these fatty acids were completely missing in *Ostrea nomades, Crassostrea gryphoides* and *C. madrasensis.* Moreover, 20:3n-3 compound was not found in *C. madrasensis* whereas traces of 20:3n-3 has been detected in other three species.

Generally, in lipid content of *O. nomades* SFA contributed 32.98% of the total lipid, MUFA 16.95% and 50.06% PUFA found in the profile. Similarly in *S. cucullata* 36.49% (SFA) 18.00% (MUFA) 45.51% (PUFA) proportions contributed to the total lipid. Likewise, in *C. gryphoides* SFA, MUFA and PUFA percentages were 34.55%, 16.38% and 49.07%, where

as in *C. madrasensis* amounted 33.79%, 18.28% and 47.93% respectively (Table 1 and Fig. 1).

Overall highest proportion of saturated fatty acids (SFA) were found in *S. cucullata* (36.49±2.09 mg/g) and *C. gryphoides* (34.56±1.48) where as, 33.79±3.02 and 33.02±0.42 mg/g quantified in *C. madrasensis* and *O. nomades* respectively.

Amount of total mono-unsaturated fatty acids (MUFA) remained highest (18.28±1.80 mg/g) in *C. madrasensis* followed by 18.00±1.47 mg/g in *S. cucullata,* 16.97±0.27 mg/g in *O. nomades* and 16.38±0.39 in *C. gryphoides*.

Larger proportion of total poly-unsaturated fatty acids (PUFA) was determined in *O. nomades* (50.02±0.15 mg/g) followed by 49.07±1.63 mg/g in *C. gryphoides.* Where as, 47.93±4.82 and 45.51±3.34 mg/g fractioned in *C. madrasensis* and *S. cucullata*. Other PUFAs include 16:2, 16:3, 16:4, 18:0 DMA, 20:2 NMID, 22:2NMID, 22:2NMID and 22:3NMIT found in all four species contributing low proportion of total PUFAs.

The aim of the study was to unveil fatty acid profiles of native oyster species owing their nutritional significance. Fatty acids profiles of four species showed variable fatty acids components. For instance *O. nomades* profile showed 32.98% SFA make up where as 16.95% MUFA and 50.06% PUFA in the tissues. Similarly, in *S. cucullata* 36.49% SFA, 18.00% MUFA and 45.51% PUFA contributed within the total lipid proportion. In the same way SFA, MUFA and PUFA percentages

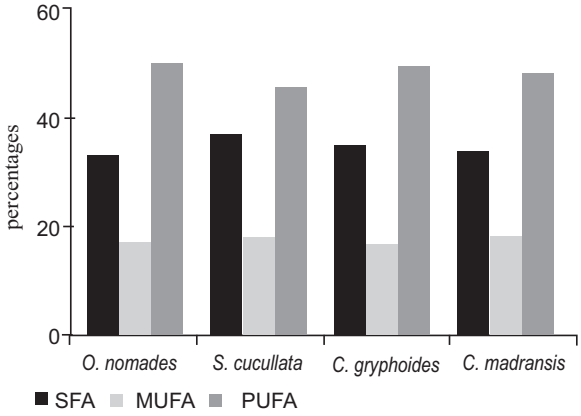

Fig. 1. Showing overall percentages of saturated fatty acids (SFA) monounsaturated fatty acids (MUFA) and polyunsaturated (PUFA) in total lipid (mg fatty acid/g tissue).

Table 1. Fatty acid composition (mg/g tissue) in gonadal tissues of four (4) oyster species. n-number of individuals

Fatty acids	*Ostrea nomads* n=2	*Saccostr eacucullata* n=3	*Crassostrea gryphoides* n=3	*C. madransis* n=2
14:0	4.93±0.11	2.80±0.27	3.51±0.29	3.23±0.50
15:0	0.53±0.00	0.56±0.06	0.82±0.09	0.85±0.13
16:0	20.55±0.43	24.02±2.00	22.95±0.83	23.29±1.50
18:0	6.27±0.00	8.31±0.26	6.68±0.41	6.00±0.78
20:0	0.33±0.02	0.37±0.03	0.27±0.04	0.21±0.03
22:0	0.21±0.04	0.20±0.01	0.14±0.01	0.14±0.01
24:0	0.20±0.04	0.24±0.02	0.18±0.03	0.07±0.10
16:1n-9	0.13±0.01	0.10±0.09	0.10±0.09	0.16±0.01
16:1n-7	6.03±0.07	3.77±0.10	3.50±0.27	3.42±0.48
18:1n-9	3.30±0.01	4.41±0.79	3.56±0.11	3.02±0.04
18:1n-7	2.89±0.14	3.39±0.32	3.25±0.03	3.99±0.39
20:1n-11	2.08±0.00	1.80±0.29	2.78±0.27	4.04±0.39
20:1n-9	0.00±0.00	0.51±0.55	0.00±0.00	0.00±0.00
20:1n-7	2.00±0.03	3.01±0.38	2.60±0.11	3.05±0.37
22:1n-11	0.28±0.00	0.68±0.40	0.38±0.04	0.42±0.04
22:1n-9	0.00±0.00	0.04±0.07	0.00±0.00	0.00±0.00
24:1n-9	0.26±0.07	0.28±0.08	0.21±0.06	0.19±0.07
18:2n-6	1.98±0.02	2.63±0.13	2.00±0.22	1.63±0.04
18:3n-6	0.42±0.01	0.35±0.05	0.37±0.02	0.37±0.04
20:2n-6	0.32±0.00	0.36±0.04	0.32±0.02	0.27±0.04
20:3n-6	0.29±0.02	0.42±0.12	0.28±0.05	0.28±0.02
20:4n-6	3.62±0.06	3.69±0.49	3.93±0.33	4.53±0.47
22:4n-6	0.73±0.04	0.51±0.08	0.52±0.04	0.69±0.02
22:5n-6	0.64±0.01	0.60±0.13	0.73±0.08	0.97±0.08
18:3n-3	1.74±0.13	2.77±0.15	2.11±0,21	1.61±0.14
18:4n-3	2.95±0.18	2.29±0.38	1.88±0.24	1.52±0.16
20:3n-3	0.04±0.05	0.03±0.05	0.03±0.06	0.00±0.00
20:4n-3	0.44±0.01	0.44±0.03	0.40±0.03	0.36±0.05
20:5n-3	12.11±0.23	12.23±1.08	11.38±0.54	10.81±1.25
22:4n-3	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
22:5n-3	1.19±0.08	1.15±0.18	1.08±0.05	1.45±0.12
22:6n-3	12.76±0.48	10.09±1.63	12.27±1.26	7.03±8.77
16:2	0.45±0.01	0.24±0.01	0.21±0.03	0.22±0.05
16:3	0.35±0.03	0.12±0.02	0.12±0.01	0.19±0.03
16:4	0.14±0.01	3.10±0.18	5.67±1.15	8.88±0.51
18:0 DMA	5.40±0.13	0.03±0.05	0.21±0.07	0.37±0.10
20:2NMID	0.20±0.00	0.48±0.05	0.47±0.01	0.47±0.04
22:2NMID	0.81±0.02	0.68±0.02	1.07±0.14	1.23±0.10
22:2NMID	2.42±0.14	2.38±0.27	3.06±0.27	4.03±0.57
22:3NMIT	1.02±0.00	0.92±0.10	0.96±0.03	1.03±0.10
Total saturated	33.02±0.42	36.49±2.09	34.56±1.48	33.79±3.02
Total Mono-unsaturated	16.97±0.27	18.00±1.47	16.38±0.39	18.28±1.80
Total PUFA	50.02±0.15	45.51±3.34	49.07±1.63	47.93±4.82

remained 34.55%, 16.38% and 49.07% for *C. gryphoides* where as in *C. madra-sensis* SFA, MUFA and PUFA amounted 33.79%, 18.28% and 47.93% respectively, while specimens procured during June 2008 in summer season. Dagorn *et al*. (2016) studied almost same pattern of exploitable lipids and fatty acids in the invasive oyster *Crassostrea gigas* from the French Atlantic coast during summer where SFA, MUFA and PUFA levels measured 40.1±0.1, 19.5±0.05 and 35.2±0.3 mg/g respectively. In addition, 3.39±0.05 mg/g percent fatty aldehyde dimethylacetals (DMAs) were found in *C. gigas*. Where as, 18:0 DMA found in all four species analyzed from Buleji and Hub area of Pakistan coast. However, only trace amount of DMA was found in *S. cucullata* (0.03±0.05 mg/g) where as, in *O. nomades* it was originated 5.40±0.13 mg/g and in *C. gryphoides* and *C. madrasensis* amounted 0.21±0.07 and 0.37± 0.10 mg/g. Other than, that non-methylene interrupted trienoic fatty acids (NMIT) and non-methylene interrupted dienoic fatty acids (NMID) were also present in minor quantities in all four species.

Collectively among all fatty acids poly-unsaturated fatty acids (PUFA) amounted larger proportion in all four species, about 50.02±0.15 mg/g in *O. nomades* followed by 49.07±1.63 mg/g in *C. gryphoides*, 47.93±4.82 mg/g in *C. madrasensis* and 45.51±3.34 mg/g PUFA content contributed in *S. cucullata*. Asha *et al*. (2014) also described the highest levels of poly-unsaturated fatty acids (PUFA) in cultured *C. madra-sensis* from India, where the eicosapentaenoic acid, docosahexaenoic acid and linoleic acid were found to be prominent fatty acids among PUFA. Linehan *et al*. (1999) concluded that lipid content may vary only slightly throughout the year, from 7.1% (winter) to 8.6% (spring). However, present study revealed the slight variation which differed from species to species in a same season even though in species which co-exist in the same habitat such as *C. gryphoides* and *C. madrasensis* they are found at Hub delta and *S. cucullata* and *O. nomades* at Buleji. Although *O. nomades* has smaller size range and flesh weight as compare to *C. gryphoides* and *C. madrasensis* (Afsar *et al*., 2014b) but results have shown highest PUFA enrichment (50.02±0.15 mg/g) in *O. nomades* whereas least quantity of PUFA was found in *S. cucullata* (45.51%). Whereas, *C. gryphoides* and *C. madrasensis* have also shown considerable amount of PUFA in the analyzed samples. The highest amount of linoleic acid (18:2n-6) was found in *S. cucullata*. Comparatively eicosapentaenoic acid (EPA) (20:5n-3) also found in high amount in *S. cucullata* (12.23± 1.08 mg/g) and *O. nomades* (12.11±0.23 mg/g) respectively when compared with *C. gryphoides* and *C. madrasensis*. In the same way Chakarburti *et al*. (2016) also presented the nutritional composition of *C. madrasensis* in wild and cultured population samples from southwest coast of India and studies were taken over 4 years during 2008-2011 within pre-monsoon season. Higher proportions of total poly-unsaturated fatty acids, eicosapentaenoic and docosahexaenoic acids in the samples collected from wild habitats correlated with chlorophyll-a concentration that is primarily connected to phytoplankton's being their specific diet.

This study is the first ever report presenting lipid profile of four native species of Pakistan with a potential to be utilized as alternative food recourse in the country.

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