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Determination and study the fatty acid contents and their seasonal variations by temperature of a dominant bivalve(*Callista umbonella*) of Haleh Creek

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Abstract

In this study fatty acid contents, because of the importance in human's life, and their seasonal variations of a dominant bivalve of Haleh Creek were determined for the first time. Fatty acid identification was done by GC-MS (Gas Chromatography–Mass Spectrometry) method. After collecting and dissecting the species from the shells, samples weighed and frozen for further experiments. All the samples esterified and made ready for injection to the GC-MS. Temperature as an important environmental parameter monitored monthly, variability of the fatty acid components studied in two seasons, and the effect of temperature changes on seasonal variations of the fatty acids were studied by statistical analysis. In conclusion sixteen fatty acids identified in *Callista umbonella*, including twelve saturated and four unsaturated, which the most important were oleic, palmitic, myristic, hexadecanoic and nonadecanoic acids while saturated fatty acids were dominated over unsaturated ones. The most abundant saturated fatty acid was palmitic acid and unsaturated one was oleic acid in this species. Although fatty acids' seasonal changes did not show any significant difference and also any significant correlation with temperature in the species in this area, but in general fatty acid amounts might be vary in different temperatures and it could be related to many environmental and biological factors in species.

Keywords: Fatty acids, Seasonal variations, GC-MS, Haleh Creek

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Introduction

Haleh Creek, which is located on the shore of Persian Gulf in the Nayband Bay of Chah-e Mobarak District of Asaluyeh region, SE of Bushehr Province, Iran, provides high rates of primary productivity and so a diverse food source for mollusks in this area (Vazirizadeh and Arebi, 2011).

Molluscs phylum is the second largest phylum of animals which have enormous diversity in this area. They exhibit a range of lipid and fatty acid components in both freshwater and marine species and therefore fatty acid contents in mollusks are studied in many habitats, because of their importance in human's life (Joseph, 1989; Dembitsky *et al*., 1993; Abad *et al*., 1995; Ackman *et al*., 2000 , Misra *et al*., 2002).

The pioneering studies on the fatty acids of mollusks, mostly bivalves and gastropods were carried out in the 1970s (Gardner and Riley, 1972; Ackman and Hooper, 1973; Ackman *et al*., 1974). Lipid and fatty acid compositions of different classes of Mollusks have been reviewed (Joseph, 2003),however studies on the effects of environmental factors on the fatty acids is insufficient, thus thermo tropic behavior of major phospholipids in marine invertebrates (Sanina and kostetsky, 2002) and seasonal variations of fatty acids in flat oysters were studied (Abad *et al*., 1995).

Fatty acids have been found differently for mollusk species and are influenced by taxonomic relations, environmental conditions, nutrient habits, food availability and also physiological conditions (Khan and Parrish, 2006, Sajjadi *et al*., 2009).

In this manner there is no previous report about fatty acid contents of mollusks in Hale Creek. As a dominant bivalve mollusc, *C. umbonella*, studied for the first time for identification of fatty acid compositions and their seasonal variations in this area.
Identification of fatty acids of *C. umbonella* species was done by GC-MS and the results were compared by statistical analysis methods.

Materials and methods

About 100 specimens of *C. umbonella* were collected in every sampling point, in three stations located between intertidal zone of creek, Nayband Bay and the mangrove forest at the distance of around 10 km, where the water depth was 1 – 1.5m. Samples were collected with the same size in two seasons.

The species were transferred to the laboratory immediately after sampling. Whole body tissue dissected from the shells, weighed and frozen to -18°C for further experiments. Fatty acids extracted from whole body blended tissues by Johns *et al*. (1979) method. The fatty acids esterified (Morrison and Smith, 1964) and the solution made ready for injection to the gas chromatograph. Each sample was treated and analyzed for triplicates.

Separation of the fatty acids methyl esters performed using a Gas Chromatograph (Agilent Technologies,

6890) with a mass selective detector (6973N). The capillary column used was HP-5 (5% diphenyl 95% dimethyl siloxane copolymer) with 30m column, 320 µm internal decimeter and 1 µm film thickness.

The carrier gas was helium (purity 99.999%). 0.5 µL of the extract containing the fatty acids methyl esters was injected into the injector using split mode with 50:1 split ratio. The injector temperature was 200 °C, the detector temperature was 280 °C and the oven temperature was programmed from 75 °C/min to 270 °C at 30 °C/min, while the final temperature was held for 7 min (Casado *et al*., 1998). To insure that all the components were detected, the final temperature was held for 20 minutes in the replicated test runs. The fatty acids methyl esters were separated and identified in composition with the chromatograms of commercial fatty acids standards prepared from Sigma-Aldrich saturated fatty acid methyl esters (C12-C24). Peaks < 0.2% of the total area was not included in the results of the profiles.

Results

Fatty acid compositions of *C. umbonella* (GC-MS) are shown in Tables 1 and 2, including sixteen fatty acids, twelve saturated and four unsaturated, which the most important were oleic, palmitic, myristic, hexadecanoic and nonadecanoic acids which saturated were dominated over unsaturated and their maximum level were in summer (90.28%). Also due to the Table 4, the maximum percentage level of unsaturated was observed in winter (22.19%) reached to its minimum in summer (9.72%).

Fatty acid variations of *C. umbonella* in two seasons are shown in Fig.1 which palmitic acid, hexadecenoic (16:0); oleic acid, octadecenoic (18:1n-9) and nonadecanoic (19:0) acids have their maximum levels in winter and palmitic (16:0) and nonadecanoic (19:0) acids have their maximum levels in summer.

The major unsaturated one was oleic acid and palmitic acid was the major saturated and also the main fatty acid in this species.

In both seasons the minimum percentage (0.135% in summer and 0.163% in winter) of fatty acids were found in alpha linolenic, (18:3n-3).

Table 3 shows the temperature variation in a year which the highest is in the beginning of July 2013, and the minimum temperature was recorded in February 2013.

The fatty acids amounts had normal distribution due to Kolmogorov-Smirnov test (p>0.05), so parametric T-test did not show significant variation in two seasons (p>0.05) especially in the most important identified fatty acids such as palmitic and oleic acids.

Table1: GC-MS results of fatty acids in *Callista umbonella*(Summer 2013).

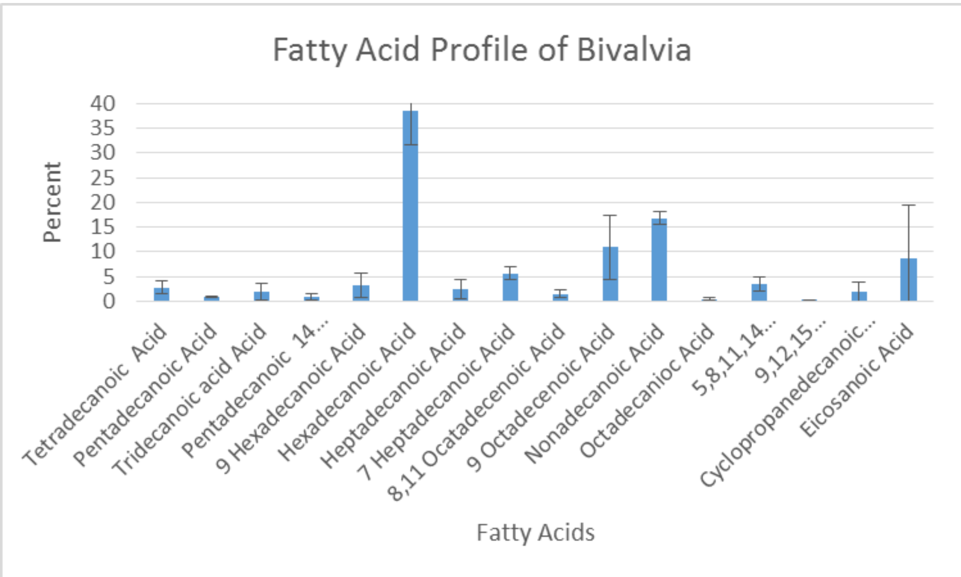
No	Retention time	Name	Percent report(%)
1	14.058	Tetradecanoic acid	1.852
2	14.602	Pentadecanoic acid	0.900
3	15.127	Tridecanoic acid acid	0.745
4	15.785	Pentadecanoic 14 methyl acid	0.365
5	15.967	9 Hexadecanoic acid	1.506
6	16.202	Hexadecanoic acid	33.651
7	16.785	Heptadecanoic acid	1.044
8	17.139	7 Heptadecanoic acid	4.769
9	17.796	8,11 Ocatadecenoic acid	2.009
10	17.859	9 Octadecenoic acid	15.518
11	18.094	Nonadecanoic acid	15.853
12	18.739	Octadecanioc acid	0.258
13	19.351	5,8,11,14 Eicosatetraenoic acid	4.503
14	19.825	9,12,15 Octadecatrienoic acid	0.163
15	20.437	Cyclopropanedecanoic acid	0.615
16	20.951	Eicosanoic acid	16.251

Table 2:GC-MS results of fatty acids in *Callista umbonella* (Winter 2013).

No	Retention time	Name	Percent report(%)
1	14.058	Tetradecanoic acid	3.687
2	14.602	Pentadecanoic acid	1.020
3	15.127	Tridecanoic acid acid	3.158
4	15.785	Pentadecanoic 14 methyl acid	1.265
5	15.967	9 Hexadecanoic acid	4.880
6	16.202	Hexadecanoic acid	43.249
7	16.785	Heptadecanoic acid	3.855
8	17.139	7 Heptadecanoic acid	6.538
9	17.796	8,11 Ocatadecenoic acid	0.938
10	17.859	9 Octadecenoic acid	6.246
11	18.094	Nonadecanoic acid	17.654
12	18.739	Octadecanioc acid	0.629
13	19.351	5,8,11,14 Eicosatetraenoic acid	2.401
14	19.825	9,12,15 Octadecatrienoic acid	0.135
15	20.437	Cyclopropanedecanoic acid	3.332
16	20.951	Eicosanoic acid	1.012

Table 3: Variations of temperature at Haleh Creek during 2013 year.

Temperature (c̊)	
27.04±1.35	March
30.79±1.54	April
32.74±1.64	May
33.00±1.65	June
33.07±1.65	July
31.20±1.56	August
27.62±1.38	September
26.00±1.30	October
24.94±1.25	November
23.67±1.18	December
22.73±1.14	January
21±1.05	February
30,19±1.51	Spring
32,42±1.62	Summer
26,18±1.31	Fall
22,46±1.12	Winter
27,81±1.39	Annual average



Winter ▬ , Summer T

Figure 1: Variations of fatty acids in *Callista umbonella* (GC-MS) in two seasons.

Table 4: Total percents of saturated, total unsaturated and polyunsaturated fatty acids in *Callista umbonella*

Fatty acids (%)	Summer	Winter
Saturated fatty acids	68.906	67.934
Total unsaturated fatty acids	31.094	32.066
Poly unsaturated fatty acids	2.536	4.666

Discussion

The major fatty acids in the species were palmitic and oleic acids which coordinates with previous reports (Ackman, 2000; Feuntes *et al*., 2009) introduces these acids the main fatty acids in mollusks especially in all trophic levels.

Also this result is coordinates with other results of fatty acid components of mollusks in Oman sea (Chabahar Bay), which locates in the semi-similar region (Sajjadi and Eghtesadi , 2009).

In *C. umbonella* saturated fatty acids were dominated over unsaturated ones, which could not be compared with results of other researches (Fuentes *et. al*, 2009) which found mollusks as potential sources of polyunsaturated fatty acids (Monroig *et al*., 2013), but there are similar to the results of other mollusks in Chabahar Bay (Sajjadi *et al*., 2009). These findings could lead to the result which in similar habitats and food supply; species of a class could have similar dietary, metabolism and behaviors.

The maximum level of unsaturated fatty acids in *C. umbonella* was observed in winter and its minimum was in summer, and coordinates with other findings which had reported an inverse relationship between temperature and the amount of polyunsaturated fatty acids in adipose tissue of invertebrates, due to the adaptive regulation of melting point of cellular lipids (Chu *et al*., 1991; Pazos *et al*., 1996) which unsaturated have higher melting points (Yano *et al*., 1998).

Also it could be related to variations of food source supplies, which could be studied further, in two seasons in bivalves which a few unsaturated fatty acids must be gained from the mollusc's habitat and could not be biosynthesized in their body (Zhukova, 2014).

The maximum level of oleic acid availability level accrued in winter also, might be due to phytoplanktonic sources and chlorophyll A and increasing unsaturated fatty acids (Langdon and Waldock, 1981).

Polyunsaturated fatty acids were lower in the total percentage of the fatty acid contents of this species than the saturated and monounsaturated ones, which could be related to bivalves dietary and coordinates with other findings (Galap *et al*., 1999), whom indicates that the mollusk could have a food source of detritus and bacteria rather than phytoplankton which could be related to their filter feeding behavior.

The lipid composition of the mollusks can be affected by external (exogenous) factors, such as fluctuations in the environmental conditions (temperature and food availability), or by internal (endogenous) factors, such as metabolic and physiological activities (Ekin *et al*., 2011). One of the most important environmental factors altering tissue lipid levels of invertebrates is temperature, which may be contributed to seasonal variations in fatty acid compositions of these species. According to this purpose Pearson analysis was done for fatty acids of this species in relation to temperature.

Although the statistical results did not show any correlation with temperature, but with considering the comparison between two seasons which the percentage level of unsaturated fatty acids was higher in winter than the summer; it coordinates with other references which implies the inverse effect of temperature and unsaturated fatty acids which increase with decreasing temperature and fatty acid denaturizes are responsible for maintaining the appropriate fluidity of membranes under this condition because many invertebrates respond to low temperature by adjusting capacities of enzymes from energy metabolism, restructuring membrane phospholipids and modulating membrane fluidity (LOS and Murata, 1999; Crockett, 2000) .

In general, it can be concluded that, fatty acid contents of *C. umbonella* are similar and the seasonal variations of these common fatty acids could be the same. The major fatty acid was palmitic acid and polyunsaturated fatty acids percent were low.

Temperature did not show any relation with fatty acids, and in general it could be concluded it depends on many factors such as internal metabolism, food dietary and reproduction cycle, which are not studied in this research.

Since published reports are limited on lipids of gastropods of Haleh Creek area, this research would be baseline for other researchers who are interested in this field.

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