Fatty acid composition and Lipid content in Muscle Tissue of Ghost crab (*Ocypode rotundata*) in Bushehr Coastal Zone in Persian Gulf

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Abstract: This study was aimed to gain knowledge on fatty acid composition and lipid content in muscle tissues of *Ocypode rotundata* in the Bushehr Coastal Zone, Iran in Aug 2012 using the method of Blight & Dyer (1959).The compounds were determined by Gas Chromatography-Mass Spectrometry (GC- MS). The components detected in both male and female species, including saturated fatty acids (SFA) Palmitic acid and Stearic acid, monounsaturated fatty acid (MUFA) Oleic acid, polyunsaturated fatty acids (PUFA) alpha- Linoleic acid, two methyl esters of fatty acids including Octadecanoic acid, methyl ester and Hexadecanoic acid, methyl ester, Cholesterol (Cholest-5-en-3-ol (3β) and Alkane including Hexadecane, Heptadecane and Octadecane. The dominant fatty acid identified in both sexes was Omega-3 alpha- Linoleic acid (ALA).
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1. Introduction

Ghost crabs called *sand crabs* are crabs of the genus *Ocypode*, common shore crabs in all ocean, from the tropical and subtropical coasts, in the American Atlantic, through the Mediterranean and Red Sea, to the American Pacific and Indo-Pacific region. Characteristics of the genus include one claw being larger than the other, but this difference is not as marked as in male fiddler crabs (Grave et al., 2009) Ghost crabs dominate sandy shores in tropical and subtropical areas, replacing the sand hoppers that predominate in cooler areas (karleskint et al., 2009). They breathe through gills, which they periodically wet with seawater (Figure 1).

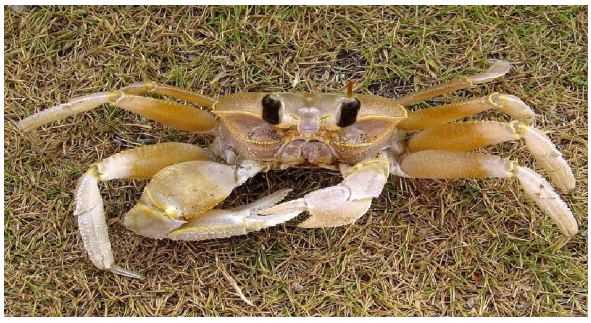

Figure 1. *Ocypode rotundata*

The scientific name *Ocypode* is derived from the Greek roots *ocy*- ("fast") and *podos*- ("foot") (Davey, 2010). There are 26 species in the genus Ocypode (Davie & Turkay, 2012). The Persian Gulf is located in Western Asia between Iran and the Arabian Peninsula. Persian Gulf has hosted some of the most magnificent marine fauna and flora (Ampf & Sadrinasab, 2006).
A fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated (monounsaturated or polyunsaturated) (Thomas, 2002).

Essential fatty acids (EFAs) are fatty acids that humans and other animals must ingest because the body requires them for good health (Harris et al., 2004) but cannot synthesize those (Reiffel et al., 2006). Essential fatty acids play a part in many biological processes (Anneken et al., 2006). Only two EFAs are known for humans: alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid (an omega-6 fatty acid). Some of the food sources of ω-3 and ω-6 fatty acids are fish ,shellfish, crab, flaxseed (linseed), hemp oil, soya oil, canola (rapeseed) oil, chia seeds, pumpkin seeds, sunflower seeds, leafy vegetables, and walnuts (Cornils & Lappe, 2006). The human body can convert α-linolenic acid (ALA) to ω-3 fatty acids eicosapentaenoic acid (EPA) and subsequently docosahexaenoic acid (DHA) (Rees et al., 2006). These omega-3 fatty acids (EPA& DHA) have been shown to prevent cancer (Hardman, 2002), and cardiovascular disease (Simopoulos, 2002), as well as being therapeutic for arthritis (Kremer, 2000), autoimmune disease (Harbige and Fischer, 2001), inflammatory effects (Grimm et al., 2002) and depression (Puri et al., 2001). DHA is also important during pregnancy for infant and brain development (Bousquet et al., 2008) and reduces the incidence of premature birth (Allen and Harris, 2002).

EPA lowers blood cholesterol (Pal et al., 2002) and reduces blood clotting, allowing better blood circulation

(Heller et al., 2002).

The main objective of the present study was to identify of the lipid content especially fatty acids in muscle tissue of *Ocypode rotundata* as one the source of omega-3 fatty acids in Bushehr Coastal Zone in Persian Gulf.

2. Material and methods

In this study, 30 *Ocypode rotundata* samples were obtained of Bushehr Coastal Zone in Persian Gulf (Figure 2).

Figure 2. Map of study area and location of sampling station in the Persian Gulf

Initially the muscle tissue was weighed separately and mixed into a soft uniform mixture.

Mixtures of chloroform and methanol were added as the lipid extract (Blight & Dyer, 1959).This solvent system allows for extraction of both polar and non polar compounds. The lower chloroform layer includes the lipids and the top methanol-water layer generally contains the non- polar components. The lipid in the chloroform layer is isolated using a separating funnel and then the solvent removed using a rotary evaporator under vacuum, at temperature of 40 ° C. The weight of the lipid was determined.

The lipid extract obtained was injected into chromatograph equipment with a mass spectra detector (GC- MS).components were identified by comparison of the retention time and mass spectra of the unknowns with those of authentic samples and also comparative analysis of kovats index & using references of Eight peak. It should be noted that the extraction and identification was performed separately for both sexes.

3. Results

This study investigated on the lipid content and fatty acid composition in muscle tissue of *Ocypode rotundata.*

The results are reported in Tables 1 and 2. Chloroform phase is discussed in this research because the fat content of the muscle tissue is extracted with chloroform (Blight & Dyer, 1959). The components identified in GC-MS analysis of the samples from female species is shown the below table.

Table 1.The compound identified in the chloroform phase of muscle tissue from the female *Ocypode rotundata* in Bushehr Coastal Zone in Persian Gulf

Compound	MF	KI	% of total
Fatty acid Saturated fatty acid			
Palmitic acid (Hexadecanoic acid)	$C_{16}H_{32}O_2$	1839	11.32
Stearic acid (Octadecanoic Acid)	$C_{18}H_{36}O_2$	1996	8.67
Mono-unsaturated fatty acid Oleic acid (9Z Octaecenoic Acid)	$C_{18}H_{34}O_2$	2103	22.87
Poly- unsaturated fatty acid Alpha-Linoleic acid	$C_{18}H_{32}O_2$	1988	40.74
Ester			
Palmitic acid –methylester (Hexadecanoic acid , methyl ester)	$C_{17}H_{34}O_2$	1836	3.8
Stearic acid-methylester (Octadecanoic acid, methyl ester)	$C_{19}H_{38}O_2$	1989	2.7
Esterols			
Cholesterol (Cholesta-5en-3-ol (3.β)	$C_{27}H_{46}O_2$	1990	2.4
Alkane			
Hexadecane	$C_{16}H_{34}$	1991	2.7
Heptadecane	$C_{17}H_{36}$	1991	2.5
Octadecane	$C_{18}H_{38}$	1991	2.3

MF: Molecular Formula KI: Kovats Index
%: Percent of the sample mass

Table 2 shows the components identified in GC-MS analysis of the samples from male species

Ocypode rotundata in Bushehr Coastal Zone in Persian Gulf

MF: Molecular Formula KI: Kovats Index
%: Percent of the sample mass

Results of this research indicate that compounds identified are common between the two sexes such as saturated fatty acids Palmitic acid (11.32% in female and male 11.22%) and Stearic acid (8.67% in female and male 8.65%), monounsaturated fatty acid Oleic acid (22.87% in female and male 20.82%), polyunsaturated fattyacid alpha-linoleic acid (40.74% in female and male 40.71%). Two esters of fatty acid consist Palmitic acid –methylester (3.8% in female and male 3.7%), and Stearic acid-methylester (2.7% in female and male 2.6%), Cholesterol (2.4 % in female

and male 5.11%) and Alkane including Hexadecane (2.7% in female and male 2.6%), Heptadecane (2.5 % in female and male 2.4%) and Octadecane (2.3% in female and male 2.2 %).

Table 2. The compound identified in the chloroform Phase of muscle tissue from the male

Compound	MF	KI	% of total
Fatty acid Saturated fatty acid			
Palmitic acid (Hexadecanoic acid)	$C_{16}H_{32}O_2$	1839	11.22
Stearic acid (Octadecanoic Acid)	$C_{18}H_{36}O_2$	1996	8.65
Mono-unsaturated fatty acid Oleic acid (9Z Octaecenoic Acid)	$C_{18}H_{34}O_2$	2103	20.82
Poly- unsaturated fatty acid Alpha-Linoleic acid	$C_{18}H_{32}O_2$	1988	40.71
Ester			
Palmitic acid –methylester (Hexadecanoic acid , methyl ester)	$C_{17}H_{34}O_2$	1836	3.7
Stearic acid-methylester (Octadecanoic acid, methyl ester)	$C_{19}H_{38}O_2$	1989	2.6
Esterols			
Cholesterol (Cholesta-5en-3-ol (3.β)	$C_{27}H_{46}O_2$	1990	5.11
Alkane			
Hexadecane	$C_{16}H_{34}$	1991	2.6
Heptadecane	$C_{17}H_{36}$	1991	2.4
Octadecane	$C_{18}H_{38}$	1991	2.2

4. Discussion

In conclusion, these results indicate that the dominant fatty acid was polyunsaturated fatty acid alpha-linoleic acid (40.71-40.74%). Alpha-linolenic acid, an *n*−3 fatty acid (Burdge &Calder, 2005), is a member of the group of essential fatty acids (EFAs) (Landmark et al., 2006), so called because they cannot be produced within the body and must be acquired through diet (Baylin et al., 2003). Research shows that omega-3 fatty acids reduce inflammation (Gil, 2002) and may help lower risk of chronic diseases (Lord & Bralley, 2002) such as heart disease (Reiffel & McDonald, 2006)) ,cancer (Simon et al., 2009), and arthritis(Calo et al., 2005). Omega-3 fatty acids are highly concentrated in the brain and appear to be important for cognitive (brain memory and performance) and behavioral function (Bousquet et al., 2008).

Comparison of this study results and similar studies by Sullivan et al (2001) on Australian blue swimming crab (*Portunus pelagicus*), Naczk et al (2004) on green crab (*Carcinus maenas*), Chen et al (2007) on Chinese mitten crab (*Eriocheir sinensis)* and Samiee et al(2012) on blue swimming crab (*Portunus pelagicus*) indicated that the dominant fatty acid was polyunsaturated fatty acid alpha-linoleic acid, so the effectiveness using of seafood such as crabs in health and possibly in preventing cardiovascular disease (including myocardial infarction and sudden cardiac death) (chattipakorn et al.,2009) acknowledges. In the present study, the next dominant fatty acid was Oleic acid (20.82-22.87%) that is classified as a monounsaturated omega-9 fatty acid. The dominant use of oleic acid is as its sodium salt, which a major component of many kinds of soap. Oleic acid may hinder the progression of adrenoleukodystrophy (ALD), a fatal disease that affects the brain and adrenal glands (Stirban et al., 2010). Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil (Teres et al., 2008). Adverse effects also have been documented, however, since both oleic and monounsaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer (Simon et al., 2009). Analysis of the lipids on Australian blue swimming crab (Sullivan et al., 2001), blue crab (*Callinectea sapidus*) (Celik et al., 2004), green crab (*Carcinus maenas)* (Naczk et al.,2004), Chinese mitten crab (*Eriocheir sinensis*) (Chen et al., 2007) *Podopthalmus vigil* crab(Sudhakar et al., 2011) and Samiee et al (2012) on blue swimming crab (*Portunus pelagicus*) indicated that the dominant monounsaturated fatty acid was oleic acid, so using of crabs such as *Ocypode rotundata* in nutrition and processing industry is approved .In the present study results showed that amount of alkane were identified in both male and female species .Since crabs are omnivorous absorb more environmental pollution in the tidal areas of the seabed and are not able to digest the oil compounds.

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