Extraction and Identification of Natural Compounds in Muscle Tissue of Blue Swimming Crab (*Portunus pelagicus)* in Persian Gulf Coasts

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Abstract: *Portunus pelagicus* known as sand crab is important in commercial and recreational fisheries in the most coastal marine waters. In this study, natural compounds from muscle tissue of blue swimming crab (*Portunus pelagicus*) from the coastal waters of Bushehr, Iran in May 2012 was extracted using the method of Blight and Dyer. Compounds were identified using Gas Chromatography-Mass Spectrometry (GC- MS). Components were identified in both male and female species. Some of the compounds identified in both sexes, including saturated fatty acids including Palmitic acid and Stearic acid, monounsaturated fatty acid Oleic acid (9Z-Octaecenoic acid), Polyunsaturated fatty acid alpha- Linoleic acid, two methyl esters of fatty acids including Octadecanoic acid, methyl ester and Hexadecanoic acid, methyl ester, Vitamin E(α-Tocopherol) and Cholesterol (Cholest-5-en-3-ol(3β). The dominant fatty acids identified in both sexes were Omega-3 alpha- Linoleic acid (ALA), and Omega-9 Oleic acid.
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Keywords: blue swimming crab, natural compounds, muscle tissue, Persian Gulf, *Portunus pelagicus*

1. Introduction

Crustaceans form a very large group of arthropods, which includes such familiar animals as crabs, lobsters, crayfish, shrimp, krill and barnacles (Romano and Zeng, 2006) .Crabs are generally covered with a thick exoskeleton, and armed with a single pair of clawsand belong to decapods crustaceans(potter and Lestang, 2000). Crabs are found in all of the world's oceans (Guerra-Castro et al.,2007).The Persian Gulf is one of the unique ecosystems and includes marine sensitive ecological zones such as estuary, rivers, coral reef, mangrove forest, marsh and stone and mud coasts (Ampf and Sadrinasab, 2006). In this basin, fish, crustacean and molluscs have fisheries and economic values. Most of the marine crabs found along the Persian Gulf coasts belong to the family Portunidae (Radhakrishnan, 2005). In this study, the blue swimming crab (*Portunus pelagicus)* is investigated. It is an economically important crustacean in the region (Chaiyawat et al., 2009). Male species are bright blue in color with white spots and with characteristically long chelipeds (Figure 1), while the females have a duller greenish brown, with a more rounded carapace (Marshall et al., 2005). The carapace can be up to 20 centimeters wide (Chaiyawat et al., 2008).

Figure 1. Male blue swimming crab (*Portunus pelagicus*)

Seafood products are currently in high demand as they are considered healthy and nutritional (Skonberg and perkins, 2002). Fatty acids (FA) are acids produced when fats are broken down, and they can be used for energy by most types of cells (Thomas, 2002). They may be monounsaturated (with one double bond), polyunsaturated (with more than one double bond) or saturated (without double bonds) (Gil, 2002). Omega-3 and omega-6 are essential fatty acids for humans which cannot be synthesized and must be supplied by diet (Anneken et al., 2006). Omega-3 is called “good fats” and is found mostly in seafood and flaxseed products (Cornils and Lappe, 2006). Omega-3 fatty acids are polyunsaturated FA that includes Alpha-Linolenic Acid (ALA), Eicosapentaenoic Acid (EPA) and Docosahexaenoic (DHA). They are found in seafood such as fish, shrimp, crab, oysters and certain plants such as flaxseed, walnut and canola (Sulivan et al., 2001). Omega-3 free fatty acid is an energy source (Weintraub et al., 1998), a component of membranes (Connor, 2000), modulator of gene expression and precursor for eicosanoids (Calo et al., 2005), preventive agent for cardiovascular disorders (Mozaffarian et al., 2006), autoimmune disease, cancers (Aronson et al.,2001) and Diabetes Mellitus (Stirban et al., 2010), modulator of inflammation (Calder, 2004) and thrombosis (Baylin et al., 2003) and is also important for brain development (Simon et al., 2009) and preventive agent for Parkinson's disease (Bousquet et al ., 2008). The purpose of this study was to identify natural compounds, especially

fatty acids in muscle tissue of blue swimming crab found in Persian Gulf.

2. Material and Methods

In this study, 30 crab samples were obtained from Boushehr region of Persian Gulf (Figure 2).

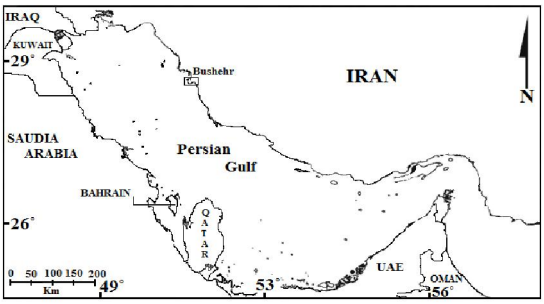

Figure 2. Map of study area and location of sampling station in the Persian Gulf

Initially the muscle tissue was weighed 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 lipids 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 a gas chromatograph t with a mass spectrometer as detector (GC-MS). Components were identified by comparison of the retention time and mass spectra of the unknowns with those of the authentic samples and also comparative analysis of kovats index. It should be noted that the extraction and identification was performed separately for male and female species.

3. Results

Results of this analysis, especially fatty acids present in the muscle tissue from the blue swimming crab and 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 and Dyer, 1959). The components identified in GC-MS analysis of the samples from female species are shown in Table 1.

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

Table 1. The compound identified in the chloroform phase of muscle tissue from female blue swimming crab (*Portunus pelagicus)* in Persian Gulf Coasts.

Compound	MF	KI	% of total
Fatty acid			
Saturated fatty acid Palmitic acid (Hexadecanoic acid)	$C_{16}H_{32}O_2$	1837	10.94
Stearic acid (Octadecanoic Acid)	$C_{18}H_{36}O_2$	1992	9.25
Monounsaturated fatty acid Oleic acid (9Z-Octaecenoic Acid)	$C_{18}H_{34}O_2$	2100	16.0
Polyunsaturated fatty acid Alpha-Linoleic acid Ester	$C_{18}H_{32}O_2$	1998	46.9
Ester			
Palmitic acid – methylester (Hexadecanoic acid ,methyl ester)	$C_{17}H_{34}O_2$	1839	2.4
Stearic acid-methylester (Octadecanoic acid, methyl ester) Terpenes	$C_{19}H_{38}O_2$	1994	1.6
Terpenes			
Vitamine E(2H-1-Benzopyran-6-ol,3,4-dihidro-2,5,7,8-tetramethyl-2-94,8,12-trimethyltridecyl) (α-Tocopherol) Esterols	$C_{29}H_{50}O_2$	2954	1.1
Esterols			
Cholesterol(Cholesta-5en-3-ol(3.β) Alkane	$C_{27}H_{46}O_2$	1992	2.6
Alkane			
n-Octane	C_8H_{18}	752	5.6
n-Decane	$C_{10}H_{22}$	1023	0.87
n-Dodecane	$C_{12}H_{26}$	1152	0.65
Hexadecane	$C_{16}H_{34}$	1580	0.35
Heptadecane	$C_{17}H_{36}$	1705	0.14
Octadecane	$C_{18}H_{38}$	1796	1.6

According to this study, most compounds identified are common between the two sexes such as polyunsaturated fatty acid alpha-linoleic acid (46.9% in female and male47.2 %), monounsaturated fatty acid Oleic acid(16.0% in female and male 16.2%), saturated fatty acids Palmitic acid(10.94 % in female and male11.12 %) and Stearic acid(9.25 % in female and male 9.95 %) ,two esters of fatty acid consist of Palmitic acid –methylester (2.4% in female and male 2.6%) and Stearic acid-methylester(1.6% in female and male 1.9%), Vitamin E(1.1 % in female and male 1.3 %) ,Cholesterol (2.6 % in female and male 2.9

%), n-Octane (5.6 % in female and male 4.7%), n-Decane (0.87% in female and male 0.76%), n-Dodecane(0.65% in female and male 0.34%), Hexadecane (0.35 % in female and male 0.25 %), Heptadecane(0.14 % in female and male 0.16 %) and Octadecane (1.6 % in female and male 0.62 %).

Table 2. The compound identified in the chloroform phase of muscle tissue from the male blue swimming crab (*Portunus pelagicus)* in Persian Gulf Coasts.

Compound	MF	KI	% of total
Fatty acid			
Saturated fatty acid Palmitic acid (Hexadecanoic acid)	$C_{16}H_{32}O_2$	1837	11.12
Stearic acid (Octadecanoic Acid)	$C_{18}H_{36}O_2$	1992	9.95
Monounsaturated fatty acid Oleic acid (9Z-Octaecenoic Acid)	$C_{18}H_{34}O_2$	2100	16.2
Polyunsaturated fatty acid Alpha-Linoleic acid Ester	$C_{18}H_{32}O_2$	1998	47.2
Ester			
Palmitic acid – methylester (Hexadecanoic acid ,methyl ester)	$C_{17}H_{34}O_2$	1839	2.6
Stearic acid-methylester (Octadecanoic acid, methyl ester) Terpenes	$C_{19}H_{38}O_2$	1994	1.9
Terpenes			
Vitamine E(2H-1-Benzopyran-6-ol,3,4-dihidro-2,5,7,8-tetramethyl-2-94,8,12-trimethyltridecyl)(α-Tocopherol) Esterols	$C_{29}H_{50}O_2$	2954	1.3
Esterols			
Cholesterol(Cholesta-5en-3-ol(3.β) Alkane	$C_{27}H_{46}O_2$	1992	2.9
Alkane			
n-Octane	C_8H_{18}	752	4.7
n-Decane	$C_{10}H_{22}$	1023	0.76
n-Dodecane	$C_{12}H_{26}$	1152	0.34
Hexadecane	$C_{16}H_{34}$	1580	0.25
Heptadecane	$C_{17}H_{36}$	1705	0.16
Octadecane	$C_{18}H_{38}$	1796	0.62

4. Discussions

In the present study, the dominant fatty acid was alpha-linoleic acid (46.9-47.2%). Alpha-linoleic acid is an essential fatty acid and is used in the biosynthesis of arachidonic acid (AA) and thus some prostaglandins (Anneken et al., 2006). It is in structure of the lipids of cell membrane and humans and animals cannot synthesize them and must be supplied by diet (Burdge and Calder, 2005). Comparison of the results of this study and similar studies by Chen et al (2007) on Chinese mitten crab (*Eriocheir sinensis)* ,Naczk et al (2004) on green crab (*Carcinus maenas*) and Sullivan et al (2001) on Australian blue swimming crab (*Portunus pelagicus*) indicated that the dominant fatty acid was alpha-linoleic acid. This supports the effectiveness of using seafoods such as crabs in health and possibly in prevention and treatment of coronary heart disease (Chattipakorn et al., 2009). In the present study, the next dominant fatty acid was Oleic acid (16.0-16.2%) that is classified as a mono-unsaturated omega-9 fatty acid. It is beneficial for cardiovascular disease and brings down HDL (bad cholesterol) blood levels (Teres, 2008). Also in industry, the dominant use of oleic acid is as its sodium salt, which is a major component of many kinds of soap. Small amounts of oleic acid are used as an excipient in pharmaceuticals; Oleic acid is used as an emulsifying or solubilizing agent in aerosol products (Cornils and Lappe, 2006). In similar studies, Sudhakar et al (2011) on *Podopthalmus vigil* crab, Chen et al (2007) on Chinese mitten crab (*Eriocheir sinensis),* Naczk et al (2004) on green crab (*Carcinus maenas),* Celik et al (2004) on the blue crab(*Callinectea sapidus*)and Sullivan et al (2001) on Australian blue swimming crab (*Portunus pelagicus*) found that the dominant monounsaturated fatty acid was Oleic acid, so using of crabs such as blue swimming crab in nutrition and processing industry is approved . The amounts of alkanes are identified in male and female species were 6.83% and 9.21% respectively. This is due to the fact that crabs are omnivorous absorbing more environmental pollution in the tidal areas of the seabed and are not able to digest the oil compounds.

Conclusions

Results of in this study showed that the muscle tissues of female and male blue swimming crab *(Portunus pelagicus)* are rich in fatty acids especially Omega-3 alpha- Linoleic acid (46.9-47.2%) and Omega-9 Oleic acid(16.0-16.2%), so these crabs are one of the healthiest seafood, and they are also suitable as a raw material in the processing industry.

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