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Fatty Acid Composition of Oil Extracted from Freshwater Edible Crab (*Barytelphusa cunicularis*).

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ABSTRACT

The freshwater crab *Barytelphusa cunicularis* is a principal species in Marathwada region containing high nutritional values. The crabs wee collected from local market of Aurangabad (MS) India. The present study has been conducted in order to find out fatty acid composition of oil extracted from freshwater crab (*Barytelphusa Cunicularis*). Fatty acid composition of the oil was determined by Gas Chromatography. It was observed that the crabs are rich in Palmitic acid, Stearic acid, Oleic acid, Linoleic acid and Arachidonic acid.
Keywords: Freshwater crab, *Barytelphusa Cunicularis*, Fatty acid composition, Gas Chromatography

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INTRODUCTION

Lipids are generally classified into two groups namely: oils and fats, these are made up of several chemical compounds, such as monoglycerides, diglycerides, triglycerides, phosphatides, cerebrosides, sterols, terpenes, fatty alcohols, and fatty acids etc. Fatty acids make up the most important part of phospholipids, triglycerides, diglycerides, monoglycerides, and sterol esters. Fatty acids are composed of constituents, for instance carbon, hydrogen, and oxygen, which are set as a linear carbon chain frame of uneven length with a carboxyl group situated at one terminal. Fatty acids can be saturated (no double bond), monounsaturated (one double bond), or polyunsaturated (two or more double bonds), and are essential for energetic, metabolic, and structural activities [1].

The fatty acids are generally divided into two groups namely: Saturated fatty acids and unsaturated fatty acids. The saturated fatty acids do not hold double bonds on the other hand unsaturated fatty acids hold one or more double bonds. Saturated and unsaturated fatty acids are available in natural world. The fatty acids with one double bond are known as monounsaturated fatty acids (MUFA) whereas fatty acids with two or more double bonds are known as polyunsaturated fatty acids (PUFA) [2].

Fatty acids are carboxylic acids and contain a hydrocarbon side chain. These acids are the basic type of lipids. Fatty acids are generally available in natural world in the esterified form as components of lipids. They also occur as free (unesterified) fatty acids. Fatty acids present in fats and other lipids are of different varieties namely: Saturated fatty acids, unsaturated fatty acids, Hydroxy fatty acids, Dicarboxylic fatty acids and Cyclic fatty acids, etc [3-5].

Lipids are chemically dissimilar compounds; these lipids can be extracted from animals, plants and other sources by using different available techniques. There is no common explanation of the word lipid. Compounds which are insoluble in water and soluble in selected organic solvents such as chloroform, hexane, benzene. diethyl ether or methanol are generally known as lipids [6-7]. On the basis of their chemical composition, Fatty acids are divided into three categories namely: Simple Fats, Compound fats and derived fats [8-9].

Barytelphusa Cunicularis is the principal species of Marathwada region [10]**.** It is commonly found in freshwater environments such as rivers, ponds, lakes, wells, dams, running water streams, etc. It is plentifully available in freshwater bodies of Marathwada region and other parts of Maharashtra state [11].

MATERIAL AND METHOD

The crabs (*Barytelphusa Cunicularis*) are purchased from local market, at Aurangabad District (Maharashtra) India. The crab meat is dried in oven for 8 hours at 50 ^{o} C. After proper drying, the dried crab meat is subjected to supercritical fluid extraction process in order to obtain crab oil. Extraction is performed using SFC (L-tex, Japan) instrument. Carbon dioxide gas is used as supercritical fluid; Hexane is used as a modifier (co-solvent).

Extraction is performed at constant flow rate, Constant temperature and constant pressure. Extraction Conditions: flow rate of carbon dioxide = 1 ml/min, flow rate of hexane = 1 ml/min, temperature = 40^{0} C and pressure = 25 Mpa. Extracted oil from the freshwater crab *Barytelphusa Cunicularis* is used as a sample for fatty acid composition analysis.

Preparation of Methyl Esters (Method A):

500 mg sample is added to 100 mL boiling flask. 8 ml methanolic NaOH solution and boiling chip is added to the flask. Condenser is attached to the flask and refluxed until fat globules disappear (about 5–10 min). 9 ml BF solution is added through condenser and continued boiling for 2 min. Add 5 ml hexane is added through condenser and boiled for 1 more min. The boiling flask is removed and ca. 15 ml saturated NaCl solution is added. Stopper is placed on the flask and shaken vigorously for 15 s while solution is still tepid. Add additional saturated NaCl solution is added to float hexane solution into neck of flask. 1ml upper hexane solution is transferred into a small bottle and anhydrous Na_2SO_4 is added to remove H_2O.

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Injection of Standards and Samples into GC:

The syringe is rinsed three times with hexane, and three times with the reference standard mixture (25 mg of 20A GLC Reference Standard FAME dissolved in 10 ml hexane). 1 ml of standard solution is injected, syringe is removed from injection port, and then start button is pressed. The syringe is rinsed again three times with solvent. The chromatogram obtained is used as described below.

The syringe is rinsed three times with hexane, and three times with the sample solution prepared by Method A. 1 ml of sample solution is injected, syringe is removed from injection port, then start button is pressed. Syringe is rinsed again three times with solvent. The chromatogram obtained is used as described below.

Data and Calculations:

Retention times and relative peak areas are reported for the peaks in the chromatogram from the FAME reference standard mixture. This information is used to identify the peaks in the chromatogram [12].

RESULTS

The oil is tested for fifteen fatty acids out of which only five fatty acids are found in the crab oil namely: Palmitic acid, stearic acid which is saturated fatty acids, Oleic acid which is monounsaturated fatty acid, Linoleic acid and Arachidonic acid which are polyunsaturated fatty acids. The concentrations of saturated acids: Palmitic acid and stearic acid are found to be 8.60% and 6.97 % respectively. While the concentrations of unsaturated acids: Arachidonic acid Oleic acid and Linoleic acid are found to be 37.18%, 25.45% and 21.81% respectively.

Test Result Table:

Sr. No.	Parameter	RT (minute)	Width (minute)	Hight	Area	Area percentage
	Fatty acid Composition:					
	Methyl esters of					
1.	Caproic acid	0.00	0.00	0.00	0.00	0.00
2.	Caprillic acid	0.00	0.00	0.00	0.00	0.00
3.	Capric acid	0.00	0.00	0.00	0.00	0.00
4.	Lauric acid	0.00	0.00	0.00	0.00	0.00
5.	Myristic acid	0.00	0.00	0.00	0.00	0.00
6.	Palmitic acid	19.541	0.09	28.77	147.87	8.60
7.	Stearic acid	23.137	0.11	18.46	119.83	6.97
8.	Oleic acid	23.491	0.09	80.21	437.68	25.45
9.	Linoleic acid	24.011	0.08	75.94	375.81	21.81
10	Linolenic acid	0.00	0.00	0.00	0.00	0.00
11	Arachidonic acid	27.355	0.09	123.32	639.43	37.18
12	Behenic acid	0.00	0.00	0.00	0.00	0.00
13	Erucic acid	0.00	0.00	0.00	0.00	0.00
14	Lignoceric acid	0.00	0.00	0.00	0.00	0.00
15	Recenoleic acid	0.00	0.00	0.00	0.00	0.00

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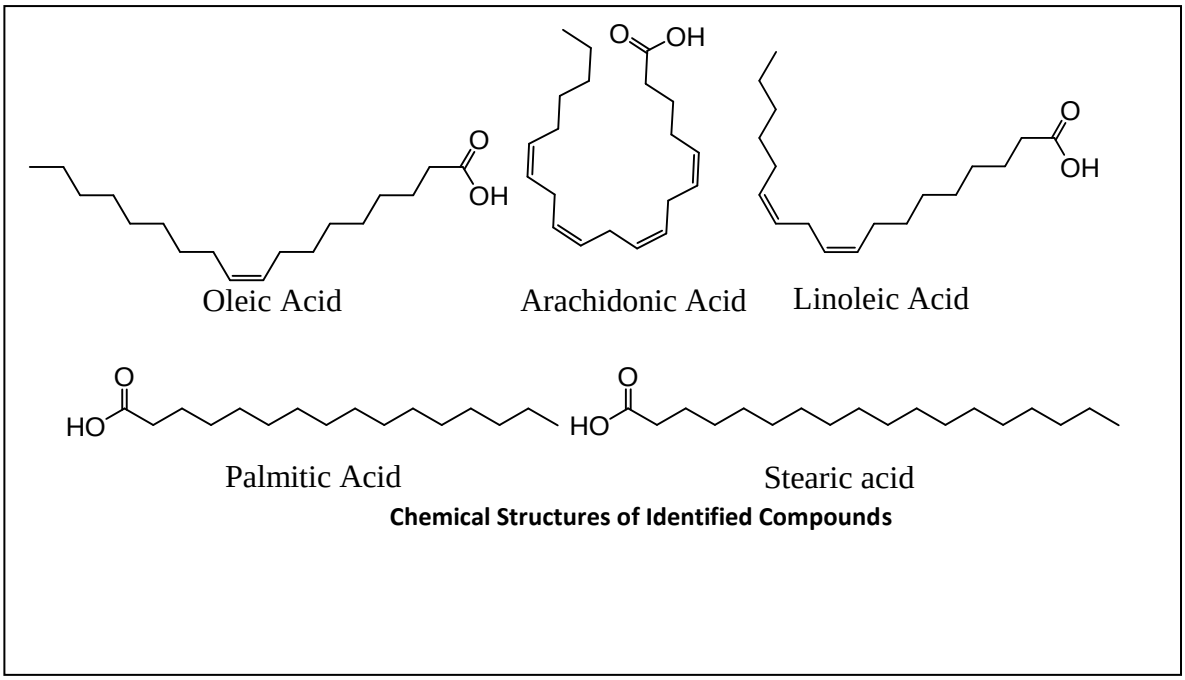
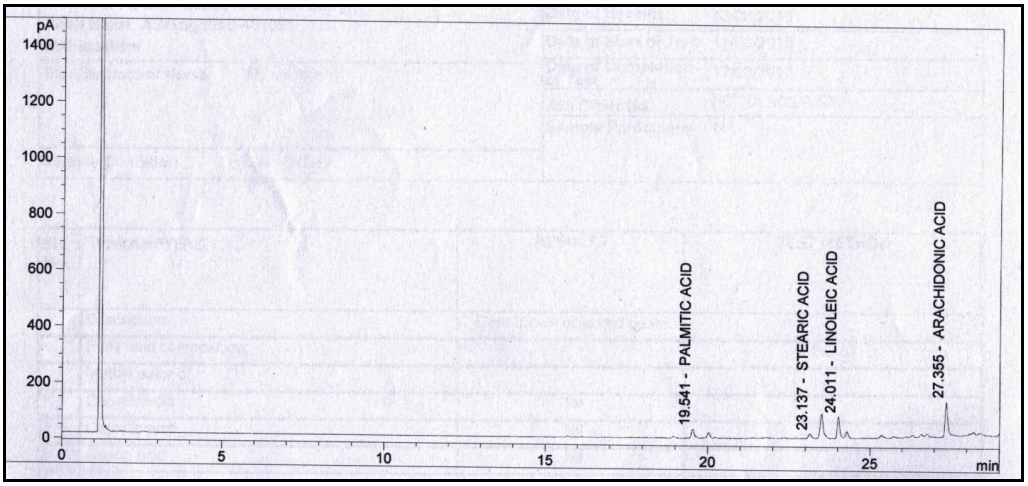


Chemical Structures of Identified Compounds



Chromatogram of Fatty acid composition of Crab oil

DISCUSSION

G. Ramesh Kumar *et al* carried out investigational work in order to Compare Fatty Acid Profile in the Edible Crabs *Scylla serrata* and *Portunus pefagicus.* Oils obtained from fish are the most important source of Poly Unsaturated Fatty Acids. For this comparative study they collected two species of commercially important food crab *Scylla serrata and Portunus pelagicus* from in and around Parangipettai coastal waters. Their work showed that the *Scylla serrata and Portunus pefagicus* is a good substitute for the marine fin fisheries resources for the consumptions. Fatty acid profile showed that In *Scylla serrata* ovary eicosapentaenoic acid was 8.0 % and this acid is 4.82% in the chelate leg. In *Portunus pefagicus eicosapentaenoic acid* was more in the chelate leg (4.02%) as compared to ovary (3. 02%). In the *Scylla serrata,* particularly palmitoleic acid (MUFA) in chelate was 4% and in the ovary it was 7%. In the Portunus pefagicus, amount of palmitoleic acid (MUFA) in the chelate was 2.39% and amount of this acid in ovary was 0. 213% [13].

Ozogul *et al* studies and compared fatty acid, trace element and proximate compositions of male and female of blue crabs and swim crabs from mersin bay, Turkey. They found dissimilarities in protein and moisture content of both female crabs and male crabs' meat of these two crab species ($p<0.05$). 23.3%-24.8% Saturated fatty acid (SFA) content was found in blue crabs while in swim crabs amount of saturated fatty acid

was 24.7%-24.9%. They noticed that amount monounsaturated fatty acid (MUFA) in the body of blue crabs (26.6%-29.6%) was higher than that of swim crabs (24.1%-25.9%). Furthermore, they observed that amount of polyunsaturated fatty acid (PUFA) in swim crabs (43.8%-45.3%) was higher than that of blue crabs (39.2%-42.8%) ($p<0.05$). On the basis of the study they came to conclusion that crab meat is a rich source of trace element, particularly Copper, Zinc, and Iron [14].

Keivandokht *et al* studied Fatty acid composition and Lipid content in Muscle Tissue of Ghost crab (*Ocypode rotundata*) in Bushehr Coastal Zone in Persian Gulf by employing Blight & Dyer method (1959).They used Gas Chromatography-Mass Spectrometry (GC- MS) for the determination of compounds. They found monounsaturated fatty acid (MUFA) Oleic acid, saturated fatty acids (SFA) Palmitic acid and Stearic 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 in both male and female crab samples. They noticed that Omega-3 alpha- Linoleic acid (ALA) was dominant fatty acid in both male and female crabs [15].

Sullivan et al studied distribution of n-3 polyunsaturated fatty acids in different edible portions of the blue swimmer crab (*Portunus pelagicus*). They examined lipid content and n-3 PUFA and other fatty acids in muscle, gonad and hepatopancreas in blue swimmer crab (Portunidae: *Portunus pelagicus*). For lipid extraction they used chloroform: methanol mixture (2 volumes of chloroform: 1 volume of methanol) with 10 mg/L of butylated hydroxytoluene and 0.2 mg/mL of tricosanoic acid. They used standard methods for preparation of methyl ester of fatty acids. They used capillary gas liquid chromatography method for the separation of fatty acid methyl esters. In all three edible portions, they noticed that n-3 PUFA were considerably different ($P < 0.01$). Highest level of n-3 PUFA was observed in hepatopancrease and lowest level was observed in the muscle. In the three edible portions Total n-6 PUFA was not considerably different, but n-3 exhibited a noteworthy different among these three edible portion. The amount lipid content was higher in hepatopancreas while the amount lipid content was lower in muscle. They noticed higher ratio of n-3/n-6 (3.5) in the Muscle as compared with 1.8 for gonad and 1.3 for hepatopancreas [16].

CONCLUSION

Oleic acid is powerful antioxidant and free radical hunter [18]. Unsaturated fatty acids are necessary for appropriate wellness; they lower LDL cholesterol (Low density lipoproteins are referred to as bad cholesterol) but do not lower HDL cholesterol (High density lipoproteins are referred to as good cholesterol) [19]. Linoleic acid and Arachidonic acid are members of the omega 6 family of polyunsaturated fatty acids [20]. Omega 6 fatty acids have achieved significant interest in recent years. These fatty acids are necessary for good health. These fatty acids cannot be produced by human body. These fatty acids have to be consumed in diet [22].

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