ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Gas Chromatography- Mass Spectroscopy Analysis of Oil Extracted from Freshwater Edible Crab (*Barytelphusa Cunicularis*).

Mohammad Moaviyah Moghal[1], Vishal Ladniya[1], and Vidya Pradhan[2]*.

[1]Dr. Rafiq Zakaria Campus, Maulana Azad College, Aurangabad, Maharashtra, India.
[2]Dr. Rafiq Zakaria College for Women, Naukhanda, Aurangabad, Maharashtra, India.

ABSTRACT

Gas Chromatography Mass Spectrometry (GC-MS) is unique method for the analysis and measuring quantity of organic volatile and semi-volatile compounds. Gas chromatography is applied to separates mixtures into individual components employing a temperature-controlled capillary column. Mass spectrometry is applied to recognize a variety of components from their mass spectra. In the present study volatile/ semi-volatile compounds present in Oil extracted from Freshwater Edible Crab (*Barytelphusa Cunicularis*) are analyzed. Crab oil is extracted by Supercritical fluid extraction method and then analyzed by Gas Chromatography / Mass Spectrometry (GC/MS). A total of 60 volatile/ semi-volatile compounds are found and quantified in this study.

Keywords: GC-MS Analysis, Crab Oil, *Barytelphusa Cunicularis*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Freshwater Crab *(Barytelphusa Cunicularis)* is the main species of Marathwada region [1]. During the survey we found that Freshwater Crab *(Barytelphusa Cunicularis)* is available in large quantities in different parts of Marathwada region and this crab species is not only consumed by locals but these crabs are also used as a medicine in different treatments. The crab, *Barytelphusa cunicularis*, is the member of the family *parathelphusidae* of the suborder *Brachyura* appears to be abundant. *Barytelphusa cunicularis* dwells in small water bodies near Aurangabad and in the cultivated fields causing significant harm to the cultivated plans (crops) [2].

M. Miyagawa et al. studied Fatty Acid Composition of Oil in Snow Crab (*Chionoecetes opilio*) by Gas Chromatography/Mass Spectrometry. They found that hepatopancreatic fatty acid extract of the snow crab contains high percentage (26 %) of odd carbon numbered fatty acids and substantial quantity (29 %) of methyl branched fatty acids, as indicated by Gas Chromatography/Mass Spectrometry and gas liquid chromatography [3]. Taufik et al. employed Chromatography/Mass Spectrometry technique in order to identify and Determine the Fatty Acid Composition of Portunus pelagicus in Setiu Wetland Areas, Terengganu, Malaysia. They found 27 fatty acids in the fatty acid composition of P. pelagicus larvae. They reported that concentration of PUFA was highest in fatty acid composition of P. pelagicus compared with SAFA and MUFA. They came to conclusion that P. pelagicus is a basically omnivores crab specie with first choice of marine animal and with addition and supplementary fed plant stuff [4].

María Vilasoa Martínez et al. studied Fatty Acid Profile and Total Lipid Content of Chionoecetes opilio Shells by using Chromatography/Mass Spectrometry technique. They identified Twenty-one fatty acids, and they found high concentration of w-3 polyunsaturated fatty acids contributing 36 % of the total fatty acid content. They came to conclusion that snow crab shells may be taken as novel source of w-3 long chain polyunsaturated fatty acids for aquaculture feeding purposes [5]. MacPherson et al. determined Phospholipid composition of the granular amebocyte from the horseshoe crab, Limulus polyphemus by employing Chromatography/Mass Spectrometry method. Their study revealed high levels of 20-carbon polyunsaturated fatty acids (PUFA), particularly arachidonic (20:4n-6) and eicosapentaenoic (20:5n-3) acids. They found that approximately 20% of the total lipid profile was made up of dimethyl acetals of 16- to 20-carbon chain lengths [6].

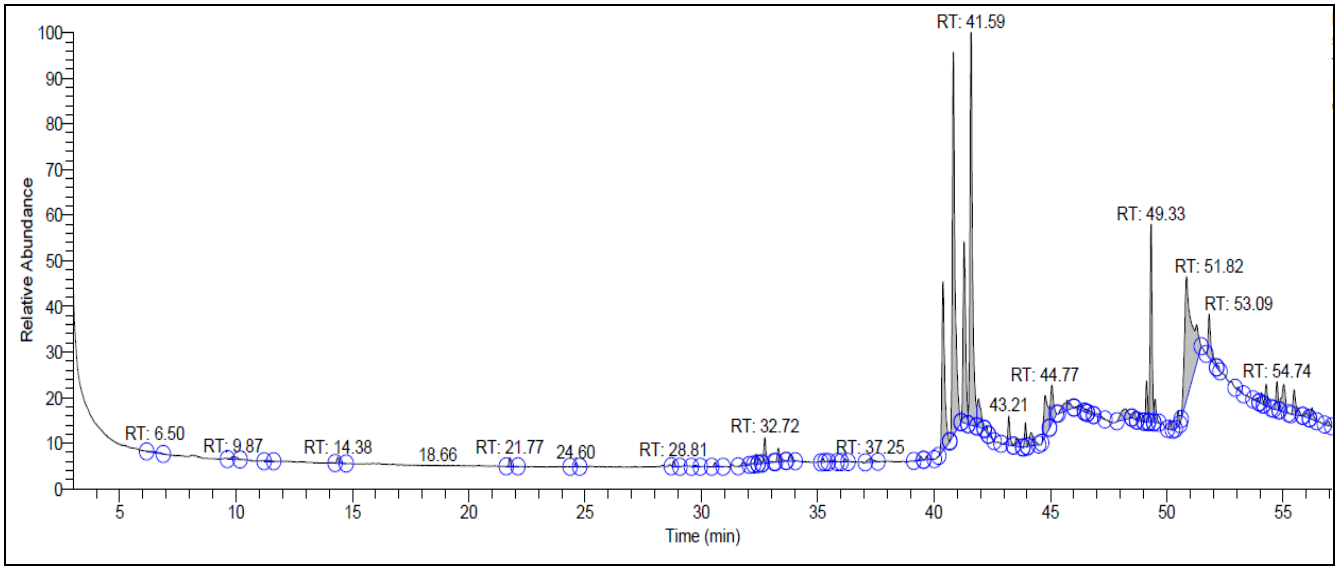
MATERIAL AND METHOD

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 a 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 gas chromatography/ Mass spectroscopy analysis. After extraction the crab oil is subjected to gas chromatography/ Mass spectroscopy analysis.

Table 1: Specification of GC/ MS

Conditions During gas chromatography/ Mass spectroscopy analysis	
Run Time(min):	54.09
Injection Volume(µl):	1.00
Scans:	6439
Low Mass(m/z):	40
High Mass(m/z):	400
Gas	Helium
Solvent	Hexane

ISSN: 0975-8585



GC/MS Analysis Spectrum of Crab oil

Table 2: Probable compounds present in crab oil

Sr. No.	Retention Time	Peak area %	Compound Names
1.	6.50	0.16	4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl] ethyl]amino-6-trichloromethylpyrimidine Pterin-6-carboxylic acid 1,8-Nonadien-3-ol
2.	9.87	0.27	1-Butanamine, 3-methyl-N-(3-methylbutylidene)- 1-Butanamine, 2-methyl-N-(2-methylbutylidene)- N-[Azirid-1-ylmethyl]piperidine
3.	11.33	0.11	Piperidine, 1-(2-methyl-1-propenyl)- 3-Amino-5-tert-butylpyrazole Pyrrolidine, 1-(2-methyl-1-butenyl)-
4.	14.38	0.30	Cyclopentasiloxane, decamethyl- Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester 3,4-Dihydroxymandelic acid, ethyl ester, tri-TMS
5.	21.77	0.28	Silane, dimethyl(dimethyl(dimethyl(2-isopropylphenoxy)sil yloxy) silyloxy)(2-isopropylphenoxy)- Cyclohexasiloxane, dodecamethyl- Acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-, trimethylsilyl ester
6.	24.60	0.15	Tetradecane Pentadecane Hexadecane
7.	28.81	0.19	Cycloheptasiloxane, tetradecamethyl- 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trim thylsiloxy) tetrasiloxane Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl
8.	29.74	0.11	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl- Octanedioic acid, 4-isopropyl-, dimethyl ester 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cyc loundec[1,2-b]oxiren-5(6H)-one, 7-(acetyloxy)decahydro-2,9,10-trihydroxy-3,6,8,8,10a-pentamethyl
9.	30.59	0.21	2,3-Dimethyl-1,4-dioxa-spiro[4.7]dodecane

			Dodecanoic acid, but-3-enyl ester Heptadeca-5,8-dione
10.	31.80	0.26	Z-8-Methyl-9-tetradecenoic acid Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate E-8-Methyl-7-dodecen-1-ol acetate
11.	32.35	0.37	Cyclohexane, (1,1-dimethylpropyl)- 2-Decene, 8-methyl-, (Z)- 10-Methyldodecan-5-olide
12.	32.49	0.20	Hexadecane Pentadecane Pentadecane, 7-methyl-
13.	32.72	0.99	Cyclohexane, (1,1-dimethylpropyl)- 2-Undecena 2-Tridecenal, (E)-
14.	33.30	0.59	4-Nitrophenyl laurate Naphthalene, decahydro-1,6-dimethyl-4-(1-methylethyl)- Dodecanoic acid, ethenyl ester
15.	33.81	0.29	4-Nitrophenyl laurate Naphthalene, decahydro-1,6-dimethyl-4-(1-methylethyl)- 1,1,6,6-Tetramethylspiro[4.4]nonane
16.	35.24	0.12	Hexasiloxane, tetradecamethyl- Cyclooctasiloxane, hexadecamethyl- Silane, [[4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylen e]bis(oxy)]bis[trimethyl-
17.	35.57	0.18	1,3-Dioxolane, 4-methyl-2-pentadecyl- 1,3-Dioxane, 2-heptyl- 1,3-Dioxolane, 2-heptyl-4-methyl-
18.	36.18	0.15	2-Pentadecanone 2-Tridecanone 2-Nonadecanone
19.	37.25	0.34	1-Ethyl-3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclo hexan-1-ol Undecanoic acid, 10-bromo-2-Trifluoroacetoxypentadecane
20.	39.42	0.19	n-Tridecanoic acid, trimethylsilyl ester Silane, dimethyl(undec-2-enyloxy)propoxy- 10,12-Tricosadiynoic acid, trimethylsilyl ester
21.	39.69	0.17	Eicosane Heptadecane Heneicosane
22.	40.39	7.55	Cyclohexanol, 4-(1,1-dimethylethyl)-1-(2-propenyl)- 4-t-Butyl-1-(1-methylallyl)cyclohexanol 10-Undecenoic acid, 2-hydroxy-, methyl ester
23.	40.83	16.94	Cyclohexanol, 4-(1,1-dimethylethyl)-1-(2-propenyl)- 4-t-Butyl-1-(1-methylallyl)cyclohexanol Decanoic acid, 2-propenyl ester
24.	41.29	7.53	Cyclohexanol, 4-(1,1-dimethylethyl)-1-(2-propenyl)- 4-t-Butyl-1-(1-methylallyl)cyclohexanol Cyclohexanecarboxylic acid, 4-pentyl-, 4-propylcyclohexyl ester
25.	41.59	16.35	5-Tridecanol Cyclopropanecarboxylic acid,pentadecyl ester 5,6-Decanediol
26.	41.91	1.71	Cyclopropanecarboxylic acid,pentadecyl ester

			Cyclopropanecarboxylic acid,tridecyl ester 5-Tridecanol
27.	42.26	0.18	7-Hexadecenoic acid, methyl ester, (Z)- Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis- Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-
28.	42.42	0.14	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester 7-Hexadecenoic acid, methyl ester, (Z)- 3-Trifluoroacetoxypentadecane
29.	43.21	1.12	9-Hexadecenoic acid, methyl ester, (Z)- Methyl hexadec-9-enoate 11-Hexadecenoic acid, methyl ester
30.	43.55	0.44	Geranyl isovalerate Curan-17-oic acid, 19,20-dihydroxy-, methyl ester,(19S)- 5-Ethenyl-5-(1-methyl-3-butenyl)-hexahydropyrimi dine-2,4,6-trione
31.	43.92	0.68	Hexadecanoic acid, methyl ester Methyl 3-methyl-pentadecanoate Methyl 13-methyltetradecanoate
32.	44.18	0.98	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- 1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl Ester Actinomycin C2
33.	44.77	2.72	Palmitoleic acid cis-9-Hexadecenoic acid Oxacycloheptadecan-2-one
34.	45.07	1.94	Phthalic acid, butyl dodecyl ester Phthalic acid, butyl undecyl ester Phthalic acid, butyl tetradecyl ester
35.	45.72	1.12	Palmitoleic acid 9-Hexadecenoic acid Methyl 16-hydroxy-hexadecanoate
36.	46.33	0.50	cis-10-Heptadecenoic acid, methyl ester Methyl 8-heptadecenoate cis-13-Eicosenoic acid
37.	46.54	0.11	Palmitoleic acid Methyl 16-hydroxy-hexadecanoate Hexadecenoic acid, Z-11-
38.	46.65	0.23	Palmitoleic acid Hexadecenoic acid, Z-11- Methyl 16-hydroxy-hexadecanoate
39.	47.11	0.31	Methyl 16-hydroxy-hexadecanoate Palmitoleic acid Estra-1,3,5(10)-trien-17á-ol
40.	48.19	1.30	l-(+)-Ascorbic acid 2,6-dihexadecanoate n-Hexadecanoic acid Methyl 16-hydroxy-hexadecanoate
41.	48.59	0.26	E-8-Methyl-9-tetradecen-1-ol acetate Estra-1,3,5(10)-trien-17á-ol Z-8-Methyl-9-tetradecenoic acid
42.	48.82	0.28	E-8-Methyl-9-tetradecen-1-ol acetate Palmitoleic acid Z-8-Methyl-9-tetradecenoic acid
43.	49.13	1.24	9,12-Octadecadienoic acid (Z,Z)-, methyl ester Methyl 9-cis,11-trans-octadecadienoate Methyl 10-trans,12-cis-octadecadienoate
44.	49.33	6.14	9-Octadecenoic acid (Z)-, methyl ester cis-13-Octadecenoic acid, methyl ester

			trans-13-Octadecenoic acid, methyl ester
45.	49.50	0.80	trans-13-Octadecenoic acid, methyl ester cis-13-Octadecenoic acid, methyl ester 9-Octadecenoic acid, methyl ester, (E)-
46.	50.14	0.28	Methyl 16-hydroxy-hexadecanoate Heptadecanoic acid, 9-methyl-, methyl ester Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl] methyl]cyclopropyl]methyl]-, methyl ester
47.	50.49	0.46	Methyl 16-hydroxy-hexadecanoate 15-Hydroxypentadecanoic acid Docosanedioic acid
48.	50.85	14.44	cis-13-Octadecenoic acid cis-Vaccenic acid trans-13-Octadecenoic acid
49.	51.82	1.97	Hexadecanamide Tetradecanamide Octadecanamide
50.	52.22	0.11	6-Octadecenoic acid trans-13-Octadecenoic acid cis-Vaccenic acid
51.	53.09	0.15	6-Octadecenoic acid Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate trans-13-Octadecenoic acid
52.	53.89	0.15	6-Octadecenoic acid Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate trans-13-Octadecenoic acid
53.	54.07	0.36	6-Octadecenoic acid Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate 12-Methyl-E,E-2,13-octadecadien-1-ol
54.	54.27	0.83	Dasycarpidan-1-methanol, acetate (ester) Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate 6-Octadecenoic acid
55.	54.74	1.01	6,9-Octadecadiynoic acid, methyl ester Heptanoic acid, docosyl ester Cyclohexanol, 4-[(trimethylsilyl)oxy]-, cis-
56.	55.03	1.71	Octanoic acid, 2-dimethylaminoethyl ester Fumaric acid, 2-dimethylaminoethyl dodecyl ester Hexadecanal, 2-methyl-
57.	55.48	1.32	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester Octadecanoic acid, 3-oxo-, methyl ester
58.	56.00	0.25	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate 9-Hexadecenoic acid cis-Vaccenic acid
59.	56.25	0.51	2-Pyrrolidinone, 1-(9-octadecenyl)- 7-Hexadecenoic acid, DMOX derivative 4-Hexadecenoic acid, pyrrolidide
60.	56.94	0.23	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate 6-Octadecenoic acid Z-8-Methyl-9-tetradecenoic acid

ISSN: 0975-8585

DISCUSSION

We observed that the oil contains cyclohexanol, 4-(1, 1 dimethyl ethyl)-4-t-butyl -1- (1-methylallyl) cyclohexanol decanoic acid, 2- propenyl ester as main compounds. The other main dominant compounds are 5-Tridecanol Cyclopropanecarboxylic acid, pentadecyl ester 5,6-Decanediol and cis-13-Octadecenoic acid, cis-Vaccenic acid, trans-13-Octadecenoic acid.

Ameshkumar *et al* used Gas chromatography method equipped with identification detector so as to compare Fatty Acid Profile in the Edible Crabs *Scylla serrata* and *Portunus pefagicus*. 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 % [7].

Ozogul *et al* employed Gas chromatography technique so as to study the fatty acid profile of the fat extracted from samples and to compare 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 [8].

Keivandokht *et al* used gas chromatography and Mass spectroscopy instrument in order to study Fatty acid composition of the fat extracted from muscle Tissue of Ghost crab (*Ocypode rotundata)*. They also studied 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 [9].

Sullivan *et al* employed gas liquid chromatography method in order to study 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 [10].

ISSN: 0975-8585

CONCLUSION

Gas chromatography / Mass spectroscopy analysis of crab oil reveals that the oil contains 60 different compounds. When we see the analysis table we find that among all compounds, compound with retention time 40.83 shows highest concentration (16.94 %) followed by compound with retention time 41.59 (16.35 %), compound with retention time 50.85 (14.44%), compound with retention time 40.39 (7.55 %), compound with retention time 41.29 (7.53%), compound with retention time 49.33 (6.14%) .

ACKNOWLEDGEMENT

I am Very thankful to Dr. Mazahar Farooqui, Head Department of Chemistry, Dr. Rafiq Zakaria College for Women, Jublee Park, Aurangabad (Maharashtra, India) for his constant encouragement, Support and innovative ideas.

REFERENCES

[1] Atul R. Chourpagar and G.K.Kulkarni. Recent Research in Science and Technology 2011; 3(3): 01-05
[2] Diwan, A., & Jain, K. Indian fresh water crab: *Barytelphusa cunicularis*. Mumbai, Central Institute of Fisheries Education, 2000. Pp. 01
[3] Miyagawa, M., Miwa, T., & Spencer, G. Journal of the American Oil Chemists' Society J Amer Oil Chem Soc 1979; 56(9): 834-836.
[4] Taufik, Zainuddin Bachok, Mohamad N. Azra and Mhd Ikhwanuddin. Middle-East Journal of Scientific Research; 2014; 21(10):1908-1915
[5] María Vilasoa Martínez, Ana Rodríguez-Bernaldo de Quirós, Julia López Hernández and M. Asución Lage Yusty. The Open Food Science Journal 2009; 3, 93-97
[6] MacPherson JC, Pavlovich JG, Jacobs RS. Lipids 1998; 33(9): 931-40.
[7] G. Rameshkumar, S. Ravichandran, Kumar Chandan and T. T. Ajithkumar. Global Journal of Environmental Research 2009; 3(1): 42-45.
[8] Y. Ozogul, D. Ayas, F. Ozogul, G. Ozyurt, E. B. Kuley and H. Yazgan. Rapp. Comm. Int Mer Médit 2010; 39: 618.
[9] Keivandokht Samiee, Abdolhossein Rustaiyan, Soheila Shahbazi. Nature and Science 2012; 10(12): 205-208.
[10] M Sullivan, Xq Su, D Li. Asia Pacific Journal of Clinical Nutrition 2001; 10: 42.