*ejpmr, 2016,3(11), 143-147*

SJIF Impact Factor 3.628

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# IDENTIFICATION OF OMEGA-3, 6 AND 9 FATTY ACIDS COMPOSITION AND LIPID CONTENT IN LIVER AND MUSCLE TISSUES OF *SALMO SALAR* IN THE SOUTH OF THE CASPIAN SEA

**Keivandokht Samiee*[1], Abdolhossein Rustaiyan[2] and Mehri Golchehreh[3]**

[1]Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran.
[2]Department of Chemistry, Science & Research Branch, Islamic Azad University, Tehran, Iran.
[3]Department of Marine Sciences and Technology, Science & Research Branch, Islamic Azad University, Tehran, Iran.

**Corresponding Author: Keivandokht Samiee**

Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran.

**Article Received on 08/09/2016** **Article Revised on 28/09/2016** **Article Accepted on 18/10/2016**

## ABSTRACT

Fish are a unique dietary source beneficial to human health. These valuable effects originate from Omega-3 polyunsaturated fatty acids, particularly EPA and DHA in the fish oil. These essential fatty acids keep the blood cholesterol level low and thus helps in controlling the cardiovascular diseases. In the present study, the liver and muscle tissues of *Salmo salar* from Chalus region in the south of the Caspian Sea, Iran in June 2015 were separately extracted for their lipid content especially Omega-3, 6 and 9 fatty acids composition using the method of Blight & Dyer. The compounds were determined by Gas Chromatography-Mass Spectrometry (GC- MS). The components detected in the liver and muscle tissues, including saturated fatty acid Palmitic acid, monounsaturated fatty acid Oleic acid, polyunsaturated fatty acids Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and Linoleic acid and two methyl esters of fatty acids including Octadecanoic acid, methyl ester and Hexadecanoic acid, methyl ester. The results showed that the dominant fatty acids in liver and muscle tissues of *Salmo salar* were Oleic acid (37.68- 45.46%), Palmitic acid (28.34- 31.93%), EPA (8.32- 9.82%), Linoleic acid (5.89- 6.02%) and Docosahexaenoic acid (5.63- 5.68 %).

**KEYWORDS**: Omega-3, Omega-6, Omega-9, fatty acids, liver, muscle, *Salmo salar*, Caspian Sea.

## INTRODUCTION

*Salmo salar* (Atlantic salmon) is a salmon in the family Salmonidae. It is found in the northern Atlantic Ocean, in rivers that flow into the north Atlantic and in the north Pacific.[10]. Most *Salmo salar* follow an anadromous fish migration pattern, in that they undergo their greatest feeding and growth in saltwater; however, adults return to spawn in native freshwater streams where the eggs hatch and juveniles grow.[11] through several distinct stages (Fig 1).

**Fig 1.*Salmo salar***

The Caspian Sea is the largest enclosed inland body of water on Earth by area, variously classed as the world's largest lake or a full-fledged sea. The sea is bordered by the countries of Azerbaijan, Russia, Kazakhstan, Turkmenistan and Iran.[23] Sea and freshwater fish, which constitute majority of water products, makes up an important part of animal food sources for humans. Fish is quite different from the other animal food sources, because they provide low energy and high-level proteins, which contain all essential amino acids. So they constitute beneficial nutritional sources.[1, 21]. Omega- 3 fatty acids are considered essential fatty acids.[2].They are essential to human health but cannot be manufactured by the body. For this reason, Omega-3 fatty acids must be obtained from food. There are three major types of omega- 3 fatty acids that are ingested in foods and used by the body: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[33]

Once eaten, the body converts ALA to EPA and DHA, the two types of Omega-3 fatty acids more readily used by the body.[26]. Fish, plant, and nut oils are the primary dietary source of Omega-3 fatty acids. EPA and DHA are found in cold water fish such as salmon, mackerel, halibut, sardines, tuna, and herring. Although fish is a dietary source of *n*−3 fatty acids, fish do not synthesize them; they obtain them from the algae in their diet. DHA

is recognized as a physiologically essential nutrient in the brain and retina for neural functioning and visual activity.[19].

In the body, essential fatty acids are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels.[7] the immune response.[17] heart health.[35],depression, inflammation.[14, 15] and the response to injury infection.[9].

The objective of this study was to identify of the lipid content especially Omega-3, 6 and 9 fatty acids composition of liver and muscle tissues of *Salmo salar* in the south of the Caspian Sea.

## MATERIAL AND METHODS

In this research, 30 *Salmo salar* samples were obtained of Chalus region in the south of Caspian Sea (Fig 2). Initially the liver and muscle tissues were weighed separately and mixed into a soft uniform mixture.


**Fig 2. Map of study area and location of sampling station in the south of the Caspian Sea**

Mixtures of chloroform and methanol were added as the lipid extract.[4].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 polar components. The lipid in the chloroform layer is 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.

## RESULTS

This study investigated on the fatty acid composition and lipid content in the liver and muscle tissues of *Salmo salar*. The results are shown in Tables 1 and 2. Chloroform phase is discussed in this research because the fat content of the muscle tissue is extracted with chloroform [4]. Table 2 shows the components identified by GC-MS analysis of the muscle samples from species. The present study indicates that compounds identified are common between liver and muscle tissue such as saturated fatty acids Palmitic acid (31.93% in liver and muscle 28.34%), Monounsaturated fatty acid Oleic acid (42.43% in liver and muscle 34.78%), polyunsaturated fatty acids Eicosapentaenoic acid (8.32% in liver and muscle 9.82%), Docosahexaenoic acid (5.68 % in liver and muscle 5.63%) and Linoleic acid (25.89% in liver and muscle 6.02%), two esters of fatty acid consist Palmitic acid– methylester (4.01% in liver and muscle 4.83%) and Stearic acid-methylester (1.28 % in liver and muscle 4.39 %) and alkanes including Heptadecane (0.54 % in liver and muscle 0.49%) and Octadecane (0.78% in liver and muscle in 1.52%).The amounts of alkanes are identified in liver and muscle tissues that they are environmental pollution

**Table1.The compound identified in the chloroform phase of liver tissue of *Salmo salar* from Chalus region in the south of the Caspian Sea**

| Compound | MF | KI | % of total |
|---|---|---|---|
| **Fatty acid**<br>**Saturated fatty acid**<br>Palmitic acid(Hexadecanoic acid) | $C_{16}H_{32}O_2$ | 1614 | 31.93 |
| **Monounsaturated fatty acid**<br>Oleic acid (9Z Octaecenoic Acid) | $C_{18}H_{34}O_2$ | 1679 | 42.43 |
| **Poly- unsaturated fatty acid**<br>Eicosapentaenoic acid (EPA)<br>Docosahexaenoic acid (DHA)<br>Linoleic acid | <br>$C_{22}H_{32}O_2$<br>$C_{20}H_{30}O_2$<br>$C_{18}H_{32}O_2$ | <br>1811<br>1819<br>1802 | <br>8.32<br>5.68<br>6.02 |
| **Ester**<br>Palmitic acid – methylester | $C_{17}H_{34}O_2$<br>$C_{19}H_{38}O_2$ | 1546<br>1619 | 4.01<br>1.28 |

| | | | |
|---|---|---|---|
| ( Hexadecanoic acid ,methyl ester)<br>Stearic acid-methylester (Octadecanoic acid, methyl ester) | | | |
| **Alkane**<br>Heptadecane<br>Octadecane | $C_{17}H_{36}$<br>$C_{18}H_{38}$ | 1823<br>1631 | 0.54<br>0.78 |

**MF: Molecular Formula KI: Kovats Index**

**Table2.The compound identified in the chloroform phase of muscle tissue of *Salmo salar* from Chalus region in the south of the Caspian Sea**.

| Compound | MF | KI | % of total |
|---|---|---|---|
| **Fatty acid**<br>**Saturated fatty acid**<br>Palmitic acid (Hexadecanoic acid) | $C_{16}H_{32}O_2$ | 1614 | 30.34 |
| **Monounsaturated fatty acid**<br>Oleic acid (9Z Octaecenoic Acid) | $C_{18}H_{34}O_2$ | 1679 | 38.80 |
| **Poly- unsaturated fatty acid**<br>Eicosapentaenoic acid (EPA)<br>Docosahexaenoic acid (DHA)<br>Linoleic acid | $C_{22}H_{32}O_2$<br>$C_{20}H_{30}O_2$<br>$C_{18}H_{32}O_2$ | 1811<br>1819<br>1802 | 9.12<br>5.63<br>5.89 |
| **Ester**<br>Palmitic acid – methylester ( Hexadecanoic acid ,methyl ester)<br>Stearic acid-methylester (Octadecanoic acid, methyl ester) | $C_{17}H_{34}O_2$<br>$C_{19}H_{38}O_2$ | 1546<br>1619 | 4.83<br>4.39 |
| **Alkane**<br>Heptadecane<br>Octadecane | $C_{17}H_{36}$<br>$C_{18}H_{38}$ | 1823<br>1631 | 0.49<br>1.52 |

**MF: Molecular Formula KI: Kovats Index**

## 4. DISCUSSION

In the present research, the results indicate that the dominant fatty acids in liver and muscle tissues of *Salmo salar* are Oleic acid (37.68-45.46%), Palmitic acid (28.34- 31.93%), EPA (8.32- 9.82%), Linoleic acid (5.89 - 6.02%) and Docosahexaenoic acid (5.63- 5.68 %). Oleic acid is a monounsaturated Omega-9 fatty acid commonly found in vegetable oils and animal fat. While a diet high in saturated fat can increase cholesterol in your blood, eating monounsaturated fats in moderation can help reduce bad cholesterol (LDL) and lower your risk of heart disease and stroke, according to the American Heart Association [20]. Palmitic acid is the most common fatty acid (saturated) found in animals, plants and microorganisms. According to the World Health Organization, evidence is convincing that consumption of palmitic acid increases risk of developing cardiovascular diseases. Retinyl palmitate is an antioxidant and a source of vitamin A added to low fat milk to replace the vitamin content lost through the removal of milk fat. Palmitate is attached to the alcohol form of vitamin A and retinol to make vitamin A stable in milk[3]. Eicosapentaenoic acid (EPA) is an Omega-3 fatty acid. It is obtained in the human diet by eating oily fish or fish oil, e.g. cod liver, herring, mackerel, salmon, menhaden, sardine and various types of edible seaweed phytoplankton. It is also found in human breast milk. The human body converts alpha- linolenic acid (ALA) to EPA. EPA is also a precursor to docosahexaenoic acid (DHA), ensuring a sufficient level of EPA on a diet containing neither EPA nor DHA is harder both because of the extra metabolic work required to synthesize EPA and because of the use of EPA to metabolize into DHA. Medical conditions like diabetes or certain allergies may

significantly limit the human body's capacity for metabolization of EPA from ALA [37]. The US National Institute of Health's Medline Plus lists medical conditions for which EPA is known or thought to be an effective treatment. Most of these involve its ability to lower inflammation. Among Omega-3 fatty acids, it is thought that EPA in particular may possess some beneficial potential in mental conditions, such as schizophrenia [28]. Studies have suggested that EPA may be efficacious in treating depression [34]. A 2009 meta-analysis found that people taking Omega-3 supplements with a higher EPA: DHA ratio experienced fewer depressive symptoms. EPA has an inhibitory effect on CYP2C9 and CYP2C19 hepatic enzymes. At high dose, it may also inhibit the activity of CYP2D6 and CYP3A4, important enzymes involved in drug metabolism. Research suggests that EPA improves the response of patients to chemotherapy, possibly by modulating the production of eicosanoid.[25, 31]. Linoleic acid (LA) is a polyunsaturated Omega-6 fatty acid. LA is a polyunsaturated fatty acid used in the biosynthesis of arachidonic acid (AA) and thus some prostaglandins, leukotrienes (LTA, LTB, LTC), and thromboxane (TXA) Linoleic acid is an essential fatty acid that must be consumed for proper health. A diet only deficient in linoleate (the salt form of the acid) causes mild skin scaling, hair loss, and poor wound healing [27]. Docosahexaenoic acid is an Omega-3 fatty acid that is a primary structural component of the human brain [16, 30, 34], cerebral cortex, skin, sperm, testicles and retina [8, 32]. DHA was found to inhibit growth of human colon carcinoma cells, more than other Omega-3 PUFAs [22]. The cytotoxic effect of DHA was not caused by increased lipid peroxidation or any other oxidative damage [5, 18, 22] but rather a decrease in cell growth regulators [32] DHA may reduce the risk of coronary heart disease [35]. Docosahexaenoic acid may be the active biological components of these effects, research has shown that they decrease risk of arrhythmias [6] which can lead to sudden cardiac death, decrease triglyceride levels, decrease growth rate of atherosclerotic plaque and blood clots [12, 29, 36] each of which tends to clog arteries and reduce cholesterol (LDL) and triglyceride levels [24]. In the present study, our results suggest that *Salmo salar* can be a good source of Omega-3, 6 and 9 fatty acids. By virtue for these attributes, this fish can be successfully used in human health and preventing the cardiovascular diseases.

## REFERENCES


1. Ackman RG, 2005. Marin lipids and Omega-3 fatty acids. In: Akon, C.C. (ed.), Handbook of Functional Lipids, Taylor and Francis Group, New York, USA. pp: 311–24.
2. Allen KG and Harris MA, 2001. The role of n-3 fatty acids in gestation and parturition. Exp. Biol. Med.(Maywood) 226(6): 498-506.
3. Benoit SC, Kemp CJ, Elias CF, Abplanalp W, Herman JP, Migrenne S, Lefevre AL, Cruciani-Guglielmacci C, Magnan C, Yu F, Niswender K, Irani BG, Holland WL, Clegg DJ, 2009."Palmitic acid mediates hypothalamic insulin resistance by altering PKC-θ subcellular localization in rodents". Journal of Clinical Investigation 119(9): 2577–2587.
4. Blight EG and Dyer WJ, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.
5. Bousquet M, Saint-Pierre M, Julien C, Salem N, Cicchetti F and Calon F, 2008. Beneficial effects of dietary Omega-3 polyunsaturated fatty acid on toxin-induced neuronal degeneration in an animal model of Parkinson's disease. *The FASEB Journal*. 2008; 22: 1213-1225.
6. Calo L, Bianconi L, Colivicchi F, 2005. N-3 fatty acids for the prevention of atrial fibrillation after coronary artery bypass surgery. J AM Coll Cardio. 45: 1723-8.
7. Chattipakorn N,Settakorn J, 2009.Cardiac mortality is associated with low levels of Omega-3 and Omega-6 fatty acids in the heart of cadavers with a history of coronary heart disease. Nutr Res. 29(10); 696-704.
8. Cunnane SC, Plourde M, Pifferi F, Bégin M, Féart C and Barberger- Gateau P, 2009. Fish, docosahexaenoic acid and Alzheimer's disease. Progress in Lipid Research 48(5): 239-25.
9. David, L. Nelson, and Michael M. Cox, 2005. Principles of Biochemistry.(W.H.Freeman), New York.
10. Dempson CJ, Schwarz DG, Reddin MF, O'Connell CC, Bourgeois M, 2001. "Estimation of marine exploitation rates on Atlantic salmon (*Salmo salar* L.) Stocks in Newfoundland, Canada". ICES Journal of Marine Science: 331–341.
11. Douglas BS, Letcher BH, Cunjak RA, 2006."Influence of beaver activity on summer growth and condition of age-2 Atlantic salmon parr". Transactions of the American Fisheries Society 135 (4): 1068–1075.
12. Frenoux JR, Pros ED, Bellelle JL and Prost JL, 2001. A polyunsaturated fatty acid diet lowers blood pressure and improves antioxidant status in spontaneously hypertensive rate. Journal of Nutrition.131: 39-45.
13. Freyhof J and Kottelat M, 2008. *Coregonus peled*. The IUCN Red List of Threatened Species, version 2015, 2.
14. Gil A, 2002. Polyunsaturated fatty acids and inflammatory disease. Biomed. Pharmacother. 56: 388-396.
15. Grimm HK, Mayer P, Mayser P and Eigenbrodt E, 2002. Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. Br. J. Nutr. 87 Suppl 1: S59: 67.
16. Guesnet P, Alessandri JM, 2011. "Docosahexaenoic acid (DHA) and the developing central nervous system (CNS) - Implications for dietary recommendations". Biochimie 93(1): 7–12.

17. Harbige LS and Fischer BA, 2001. Dietary fatty acid modulation of mucosally-induced tolerogenic immune responses. Proc. Nutr. Soc. 60(4): 449-456.
18. Hardman WE, 2002. Omega-3 fatty acids to augment cancer therapy. J. Nutr. 132 (11 Suppl): 3508-3512.
19. Holub BJ, 2001. "Docosahexaenoic Acid in Human Health, Omega-3 Fatty Acids: Chemistry, Nutrition, and Health Effects". In: F. Shahidi and J. W. Finley, Eds., ACS Symposium Series 788, American Chemical Society, Washington DC, 2001, pp. 54-65.
20. John DB, Michael D, Curt DF, Ronald BG, Barbara VH, James HS and Joseph LWitztum, 2008. "Lipoprotein Management in Patients with Cardiometabolic Risk". J Am Coll Cardiol, 2008; 51:1512-1524.
21. Kandemir S and Polat N, 2007. Seasonal Variation of Total Lipid and Total Fatty Acid in Muscle and Liver of Rainbow Trout (*Oncorhynchus mykiss* W., 1792) Reared in Derbent Dam Lake. Turkish J. Fisheries Aquatic Sci., 7: 27–31.
22. Kato T, Hancock RL, Mohammadpour H, McGregor B, Manalo P, Khaiboullina S, Hall MR, Pardini L, Pardini RS, 2002. "Influence of Omega-3 fatty acids on the growth of human colon carcinoma in nude mice". *Cancer Lett.* 187 (1–2): 169–77.
23. Khain VE, Gadjiev AN, Kengerli TN, 2007. "Tectonic origin of the Apsheron Threshold in the Caspian Sea". Doklady Earth Sciences. 414: 552–556.
24. Kris-Etherton P, Eckel RH, Howard BV, Jeor S and Bazzare TL, 2001. AHA Science Advisory: Lyon Diet Heart Study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association Step I Dietary Pattern on Cardiovascular Disease. Nutritional Neuroscience 9(3): 98-113.
25. Martins JG, 2009. "EPA but not DHA appears to be responsible for the efficacy of Omega-3 long chain polyunsaturated fatty acid supplementation in depression: evidence from a meta-analysis of randomized controlled trials." Journal of the American College of Nutrition 28(5): 525–42.
26. Nami K, Yasuhiro A, Ryuchi M and Tohru M, 2003. Lipid profile of krill *Euphasia Pacifica* collected in the Pacific Ocean near Funka bay, Hokkaido, Japan. Journal of Oleo Science, 53: 1: 45-51.
27. Patwardhan AM, Scotland PE, Akopian AN, Hargreaves KM, 2009. "Activation of TRPV1 in the spinal cord by oxidized linoleic acid metabolites contributes to inflammatory hyperalgesia". Proceedings of the National Academy of Sciences of the United States of America 106(44): 18820–4.
28. Peet M, Brind J, Ramchand CN, Shah S, Vankar GK, 2001. "Two double-blind placebo-controlled pilot studies of eicosapentaenoic acid in the treatment of schizophrenia". Schizophrenia Research 49(3): 243–51.
29. Reiffel JA, McDonald A, 2006. "Antiarrhythmic effects of Omega-3 fatty acids". The American Journal of Cardiology 98 (4A): 50i–60i.
30. Rees D, Miles EA, Banerjee T, Wells SJ, Roynette CE, 2006. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. Am J Clin Nutr, 83:331-42.
31. Samiee K, Rustaiyan A, Nurtaj Z, 2014. Omega-3 fatty acids composition and lipid content from liver and muscle tissues of *Katsuwonus pelamis* in the Chabahar Port in the south west of Iran. International Journal of Agricultural Science ISSN 2167-0447 Vol. 5(1): 390-392.
32. Schonberg SA, Lundemo AG, Fladvad T, Holmgren K, Bremseth H, Nilsen A, Gederaas O, Tvedt KE, Egeberg KW, Krokan HE, 2006."Closely related colon cancer cell lines display different sensitivity to polyunsaturated fatty acids, accumulate different lipid classes and down regulate sterol regulatory element-binding protein 1". Cancer Lett. 273(12): 2749–65.
33. Simopoulos AP, 2002. The importance of the ratio of Omega-6/Omega-3 essential fatty acids. Biomed Pharmacother. 56(8): 365-79.
34. Song C, Zhao S, 2007. "Omega-3 fatty acid eicosapentaenoic acid. A new treatment for psychiatric and neurodegenerative diseases: a review of clinical investigations". Expert Opin Investig Drugs 16(10): 1627–38.
35. Stampfer MJ, Hu FB, Manson JE, Rimm EB and Willett WC, 2000. Primary prevention of coronary heart disease in women through diet and lifestyle. N. Engl. J. Med., 343:1:16-22.
36. Teres S, Barcelo- Coblijn G, Benet M, Alvarez R, Bressani R, 2008.Oleic acid content is responsible for the reduction blood pressure induced by olive oil. PNAS, 105(37); 13811-6.
37. Yao HT, Chang YW, Lan SJ, Chen CT, Hsu JT, Yeh TK , 2006. "The inhibitory effect of polyunsaturated fatty acids on human CYP enzymes". Life Sci. 79 (26): 2432–40.