Omega-3 fatty acids composition and lipid content from liver and muscle tissues of *Rutilus rutilus caspicus* in the south of Caspian Sea in Iran

Keivandokht Samiee¹, Abdolhossein Rustaiyan², Fatemeh Ghulamali³

^{1.} Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran

^{2.} Department of Chemistry, Science & Research branch, Islamic Azad University, P.O. Box 14515-775, Tehran, Iran

^{3.} Faculty of Marine Sciences and Technology Science and Research Branch, Islamic Azad University, Tehran, Iran <u>k.samiee@yahoo.com</u>

Abstract: In the present study, the liver and muscle tissues of *Rutilus rutilus caspius* from the south of Caspian Sea, Iran in Feb 2013 were separately extracted for their lipid content and fatty acids composition using the method of Blight & Dyer (1959). The compounds were determined by Gas Chromatography-Mass Spectrometry (GC- MS). The components detected in the liver and muscle tissues, including saturated fatty acids Palmitic acid and Stearic acid, monounsaturated fatty acid Oleic acid, polyunsaturated fatty acids Docosahexaenoic acid (DHA) and (PUFA) Eicosapentaenoic acid (EPA), two methyl esters of fatty acids including Octadecanoic acid, methyl ester and Hexadecanoic acid, methyl ester, Vitamine E (α -Tocopherol) and Cholesterol (Cholest-5-en-3-ol(3 β). The results showed that the dominant fatty acids in liver and muscle tissues were Omega-3 fatty acids Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA).[Samiee K, Rustaiyan A, Ghulamali F. **Omega-3 fatty acids composition and lipid content from liver and muscle tissues of** *Rutilus rutilus caspicus* **in the south of Caspian Sea in Iran.** *Nat Sci* **2013; 11(5):1-5]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 1**

Keywords: Fatty acids, lipid content, liver, muscle, Rutilus rutilus caspius, Caspian Sea

1. Introduction

Rutilus is a genus of fishes in the family Cyprinidae, commonly called roach. *Rutilus rutilus caspius* is a fresh and brackish water fish (Froese & Daniel, 2007). It is one of the most important endemic species of fishes in the south of Caspian Sea (Figure 1). The Caspian roach (*Rutilus rutilus caspicus*) is regarded as one of the most important commercial species in the Caspian Sea. 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 (Khain & et al., 2007).



Figure 1. Rutilus rutilus Caspius

Omega-3 fatty acids are polyunsaturated fatty acids with a double bond starting after the third carbon atom from the end of the carbon chain. Omega-3 is called an essential fatty acid: It's essential to health, and because the human body doesn't produce it, it's essential in the diet. There are three

major types of omega- 3 fatty acids that are ingested in foods and used by the body: Alpha- linolenic acid Eicosapentaenoic acid (EPA), (ALA), and Docosahexaenoic acid (DHA) (Simopoulos, 2002). Fish, plant, and nut oils are the primary dietary source of omega-3 fatty acids. Omega-3 is used to regulate blood clotting, build cell membranes and support cell health. It's polyunsaturated, which is the relatively heart-healthy kind of fats that help reduce blood triglycerides (fats) and low-density lipoprotein (LDL), the so-called bad cholesterol (Chattipakorn et al.,2009).Omega-3 also curbs inflammation (David, et al., 2005). While inflammation is a normal part of the body's immune response (Harbige & Fischer, 2001), research indicates that it also underlies a host of serious illnesses, including cardiovascular diseases (Reiffel & McDonald, 2006; Allen & Harris, 2001), cancers and autoimmune diseases (Hardman, 2002; Simon et al., 2009). The objective of this study was to identify of the lipid content especially Omega-3 fatty acids of liver and muscle tissues of Rutilus rutilus caspius in the south of Caspian Sea.

2. Material and Methods

In this research, 30 *Rutilus rutilus caspius* samples were obtained from Gorgan Bay in the south of Caspian Sea (Figure 2). Initially the liver and muscle tissues were weighed separately and mixed into a soft uniform mixture.



Figure 2. Map of study area and location of sampling station in the South of Caspian Sea

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 polar components. The lipid in the chloroform layer is removed using a rotary evaporator under vacuum, at temperature of 40 $^{\circ}$ 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.

3. Results

This study investigated on the fatty acid composition and lipid content in the liver and muscle tissues of *Rutilus rutilus caspius*.

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 (Blight & Dyer, 1959). The components identified by GC-MS analysis of the chloroform phase of liver samples is shown the below table.

| Compound | MF | KI | % of total |
|--|-------------------|------|------------|
| Fatty acid | | | |
| Saturated fatty acid | $C_{16}H_{32}O_2$ | 1617 | 12.83 |
| Palmitic acid (Hexadecanoic acid) | | | |
| Stearic acid | $C_{18}H_{36}O_2$ | 1623 | 10.25 |
| Monounsaturated fatty acid | | | |
| Oleic acid (9Z Octaecenoic Acid) | $C_{18}H_{34}O_2$ | 1687 | 6.12 |
| Poly- unsaturated fatty acid | | | |
| Docosahexaenoic acid (DHA) | $C_{20}H_{30}O_2$ | 1815 | 24.75 |
| Eicosapentaenoic acid (EPA) | $C_{22}H_{32}O_2$ | 1802 | 22.53 |
| Ester | | | |
| Palmitic acid -methylester | | | |
| (Hexadecanoic acid, methyl ester) | $C_{17}H_{34}O_2$ | 1534 | 4.19 |
| Stearic acid-methylester | | | |
| (Octadecanoic acid, methyl ester) | $C_{19}H_{38}O_2$ | 1610 | 2.21 |
| Terpene | | | |
| Vitamine E | | | |
| (2H -1-Benzopyran-6-ol, 3, 4- dihidro-2, 5, 7, 8 tetramethyl-2-94, | $C_{29}H_{50}O_2$ | 2954 | 3.65 |
| 8,12 trimethyltridecy) (α-Tocopherol) | | | |
| Esterol | | | |
| Cholesterol (Cholesta-12.6, 5en-3-ol($3.\beta$) | $C_{27}H_{46}O_2$ | 1992 | 12.6 |

Table 1. The compound identified in the chloroform phase of liver tissue of *Rutilus rutilus caspius* from the south of Caspian Sea.

MF: Molecular Formula

KI: Kovats Index

%: Percent of the compound

Table 2 shows the components identified by GC-MS analysis of the muscle samples from species.

Table 2. The compound identified in the chloroform phase of muscle tissue of *Rutilus rutilus caspius* from the south of Caspian Sea.

| Compound | MF | KI | % of total |
|--|-------------------|------|------------|
| Fatty acid | | | |
| Saturated fatty acid | | | |
| Palmitic acid (Hexadecanoic acid) | $C_{16}H_{32}O_2$ | 1617 | 11.51 |
| Stearic acid | $C_{18}H_{36}O_2$ | 1623 | 9.98 |
| Mono unsaturated fatty acid | | | |
| Oleic acid (9Z Octaecenoic Acid) | $C_{18}H_{34}O_2$ | 1687 | 5.68 |
| Poly-unsaturated fatty acid | | | |
| Docosa hexaenoic acid (DHA) | $C_{20}H_{30}O_2$ | 1815 | 25.12 |
| Eicosapentaenoic acid (EPA) | $C_{22}H_{32}O_2$ | 1802 | 23.14 |
| Ester | | | |
| Palmitic acid –methylester | $C_{17}H_{34}O_2$ | 1534 | 4.68 |
| (Hexadecanoic acid, methyl ester) | | | |
| Stearic acid-methylester | | | |
| (Octadecanoic acid, methyl ester) | $C_{19}H_{38}O_2$ | 1610 | 3.98 |
| Terpene | | | |
| Vitamine E (2H-1-Benzopyran-6-ol,3,4- | | | |
| dihidro-2,5,7,8-tetramethyl-2-94,8,12- | $C_{29}H_{50}O_2$ | 2954 | 12.66 |
| trimethyltridecyl) (α-Tocopherol) | | | |
| Esterol | | | |
| Cholesterol (Cholesta- | $C_{27}H_{46}O_2$ | 1992 | 2.6 |
| $5 \text{en-3-ol}(3.\beta)$ | | | |

MF: Molecular Formula

KI: Kovats Index

%: Percent of the compound

The present study indicates that compounds identified are common between liver and muscle tissue such as saturated fatty acids Palmitic acid (12.83% in liver and muscle 11.51%) and Stearic acid(10.25% in liver and muscle 9.98%), Monounsaturated fatty acid Oleic acid (6.12% in liver and muscle 5.68%), polyunsaturated fatty acids Docosahexaenoic acid(24.75% in liver and muscle 25.12%) and Eicosapentaenoic acid(22.53% in liver and muscle 23.14%) and two esters of fatty acid consist Palmitic acid –methylester (4.19% in liver and muscle 4.68%) and Stearic acid-methylester (2.21% in liver and muscle 3.04%), Vitamine E(3.65% in liver and muscle 12.66%).

4. Discussions

In the present study, the results indicate that the dominant fatty acids in liver and muscle tissues of Rutilus rutilus caspius are Omega-3 fatty acids Docosahexaenoic acid (24.75-25.12%) and Eicosapentaenoic acid (20.55-26.36%). The biggest benefits from including omega-3 fatty acids in the diet relate to heart disease (Stampfer, et al., 2000; Reiffel & McDonald). Omega-3s protect the heart by decreasing arrhythmias, blood clot formation, blood triglycerides and growth rate of atherosclerotic buildup, blood pressure and inflammation, not to mention they may improve the function of artery cells (Grimm et al., 2002; Gil, 2002). Although both these fatty acids work together to benefit the body, there are some basic differences between the two (Frenoux et al., 2001). DHA is important for the structure, growth and development of the fetal central nervous system and retina Alzheimer's disease is the most common

cause of dementia in elderly adults. Research suggests that lower DHA levels are a risk factor for Alzheimer's, possibly because DHA may inhibit the progression of the disease (Guesnet & Alessandri, 2011). EPA fatty acid has beneficial potential, especially in schizophrenia (Rees et al., 2006; Schonberg et al., 2006; Cunnane et al., 2009). Numerous studies show a great reduction in symptom scales that are used to measure severity of schizophrenic symptoms (Simon et al., 2009).

Research also suggests that EPA can improve patients' response to chemotherapy (Bousquet et al., 2008). EPA can also reduce the probability of developing particular kinds of cancer. which includes multiple myeloma. Recent studies have shown that it can reduce depression and suicidal behavior. Omega-3 fatty acids such as EPA help increase levels of calcium in the body, deposit calcium in the bones, and improve bone strength. Studies show that intake of EPA and DHA fatty acids can benefit health conditions like heart disease (Stampfer, et al., 2000; Kris-Etherton et al., 2001) Reiffel & McDonald, 2006; Allen & Harris, 2001), high cholesterol(LDL) and triglyceride levels (Hardman, 2002; Kato et al., 2002; Bousquet et al., 2008), high blood pressure (Teres et al., 2008; Calo et al., 2005), depression levels(Song& Zhao, 2007; Bousquet et al., 2008; Kris-Etherton, et al., 2001), rheumatoid arthritis, inflammation (Grimm et al., 2002; Gil, 2002) autoimmune disease, cancers (Aronson et al., 2001; Kato et al., 2002) and Diabetes Mellitus (Stirban et al., 2010).

Corresponding Author:

Dr. Keivandokht Samiee Department of Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran E-mail: k.samiee@yaho

References

- Allen K. G. and M. A. Harris. 2001. The role of n-3 fatty acids in gestation and parturition. Exp. Biol. Med. (Maywood) 226(6):498-506.
- Blight, E.G., and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.
- 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.
- 4. Calo L., Bianconi L., Colivicchi F., et al. N-3 fatty acids for the prevention of atrial fibrilliation

after coronary artery bypass surgery. J AM Coll Cardio.2005; 45:1723-8.

- Chattipakorn N., Settakorn, J. et al. 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. 2009; 29(10); 696-704.
- Cunnane, S.C., 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.
- David, L. Nelson, and Michael M. Cox, 2005. Principles of Biochemistry. (W.H.Freeman), New York.
- 8. Frenoux, J. R., Pros, E. D., Bellelle, J. L. and Prost, J. L. 2001. A polyunsaturated fatty acid diet lowers blood pressure and improves antioxidant status in spontaneously hypertensive rate. Journal of Nutrition 131: 39-45.
- 9. Froese, Rainer, and Daniel Pauly, eds. (2012). Species of *Cynoglossus* in FishBase. October 2012 version.
- Grimm H., K. Mayer, P. Mayser, and E. Eigenbrodt. 2002. Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. Br. J. Nutr. 87 Suppl 1:S59:67.
- Guesnet, P., Alessandri, J.M. (2011). "Docosahexaenoic acid (DHA) and the developing central nervous system (CNS) -Implications for dietary recommendations". *Biochimie* 93 (1): 7–12.
- 12. Harbige L. S. and Fischer B. A., 2001. Dietary fatty acid modulation of mucosally-induced tolerogenic immune responses. Proc. Nutr. Soc. 60(4): 449-456.
- 13. Hardman W. E. 2002. Omega-3 fatty acids to augment cancer therapy. J. Nutr. 132 (11 Suppl):3508S-3512S).
- 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.
- Khain V. E. Gadjiev A. N. Kengerli T. N (2007). "Tectonic origin of the Apsheron Threshold in the Caspian Sea". *Doklady Earth Sciences* 414: 552– 556.
- Kris-Etherton, P., R.H. Eckel, B.V. Howard, St. Jeor S, and T.L. Bazzare, 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.

- Reiffel, J.A., McDonald, A. (August 2006). "Antiarrhythmic effects of Omega-3 fatty acids". *The American Journal of Cardiology* 98 (4A): 50i–60i.doi:10.1016/j.amjcard.2005.12.027. PMID 16919517.
- Rees, D., Miles, E.A., Banerjee, T., Wells, S.J., Roynette, CE, et al., 2006. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparision of young and older men. A.M. J. Clin. Nutr., 83:331-42, 2006.
- Schonberg S.A., Lundemo A.G., Fladvad T, Holmgren K, Bremseth H, Nilsen A, Gederaas O., Tvedt K.E., Egeberg K.W., Krokan H.E. (2006). "Closely related colon cancer cell lines display different sensitivity to polyunsaturated fatty acids, accumulate different lipid classes and downregulate sterol regulatory element-binding protein 1". *Cancer Lett.* 273 (12): 2749–65.

3/7/2013

- 20. Simopoulos A. P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 56(8):365-79.
- Song, C., Zhao S. (Oct 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.
- 22. Stampfer M.J., F.B. Hu, J.E. Manson, E.B. Rimm, and W.C. Willett, 2000. Primary prevention of coronary heart disease in women through diet and lifestyle. N. Engl. J. Med., 343:1:16-22.
- 23. Teres, S., Barcelo-Coblijn G., Benet, M., Alvarez R.,Bressani R. et al.,2008. Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. PNAS 2008, 105(37); 13811-6.