Total lipid contents and fatty acid composition from liver and muscle tissues of *Cynoglossus macrolepidotus* in the Persian Gulf

Abdolhossein Rustaiyan[1], Keivandokht Samiee[2], Maryam Mohsenifar [3]

[1.] Department of Chemistry, Science & Research branch, Islamic Azad University, P.O. Box 14515-775, Tehran, Iran
[2.] Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran
[3.] Department of Marine Sciences and Technology, North Tehran Branch, Islamic Azad University, Tehran, Iran
arustaiyan@yahoo.it

Abstract: In this research, the liver and muscle tissues of *Cynoglossus macrolepidotus* from Bushehr Coastal Zone in Persian Gulf, Iran in Jan 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 Myristic acid, Palmitic acid and Stearic acid, monounsaturated fatty acid Oleic acid, polyunsaturated fatty acids (PUFA) Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) 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 were Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and Palmitic acid.

[Rustaiyan A, Samiee K, Mohsenifar M. **Total lipid contents and fatty acid composition from liver and muscle tissues of *Cynoglossus macrolepidotus* in the Persian Gulf**. *Nat Sci* 2013; 11(4):114-117]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 19

Keywords: Lipid content, fatty acids, liver, muscle, *Cynoglossus macrolepidotus,* Persian Gulf

1. Introduction

Seafood is the major source of food of large number of people residing in the coastal areas. *Cynoglossus macrolepidotus,* commonly known as the Largescale tonguesole is a species of tonguefish and belongs to the family Cynoglossidae. It is commonly found in muddy and sandy bottoms of the Indo-West Pacific and Indian Ocean, from the Persian Gulf ,Sri Lanka and Indonesia and as far north as the south coast of Japan, down to depths of 125 meters (Froese et al.,2012). The eyed side of the fish is uniform brown, with a dark patch on the gill cover, and its blind side is white (Figure 1).

Figure 1. *Cynoglossus macrolepidotus*

Omega- 3 fatty acids are considered essential fatty acids. 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. Omega-3 fatty acids play a crucial role in brain function as well as normal growth and development (Allen &Harris, 2001). 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) (Simopoulos, 2002). Once eaten, the body converts ALA to EPA and DHA, the two types of omega-3 fatty acids more readily used by the body (Nami, *et al*., 2003). Fish, plant, and nut oils are the primary dietary source of omega-3 fatty acids. 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. 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 (Chattipakorn et al., 2009), the immune response (Harbige & Fischer, 2001, heart health, depression, and the inflammation response to injury infection (David, *et al*., 2005).

The objective of this study was to identify of the lipid content especially fatty acids of liver and muscle tissues of *Cynoglossus macrolepidotus* in Persian Gulf.

2. Material and Methods

In this research, 30 *Cynoglossus macrolepidotus* samples were obtained from Bushehr Coastal Zone in Persian Gulf (Figure 2).

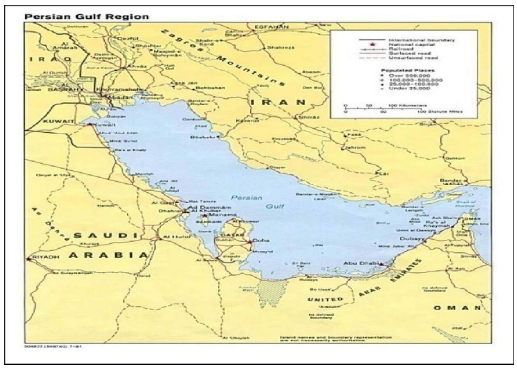

Figure2. Map of study area and location of sampling station in the Persian Gulf.

Initially the liver and muscle tissues were weighed separately 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 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.

3. Results

This study investigated on the fatty acid composition and lipid content in the liver and muscle tissues of *Cynoglossus macrolepidotus.*

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.

Table 1.The compound identified in the chloroform phase of liver tissue of *Cynoglossus macrolepidotus* from Bushehr Coastal Zone in Persian Gulf

Compound	MF	KI	% of total
Fatty acid Saturated fatty acid Myristic acid	$C_{14}H_{28}O_2$	1534	11.73
Palmitic acid (Hexadecanoic acid)	$C_{16}H_{32}O_2$	1617	14.33
Stearic acid	$C_{18}H_{36}O_2$	1623	13.25
Monounsaturated fatty acid Oleic acid (9Z Octaecenoic Acid)	$C_{18}H_{34}O_2$	1687	13.12
Poly- unsaturated fatty acid Eicosapentaenoic acid (EPA	$C_{22}H_{32}O_2$	1802	20.55
Docosahexaenoic acid (DHA)	$C_{20}H_{30}O_2$	1815	19.75
Ester Palmitic acid – methylester (Hexadecanoic acid, methyl ester)	$C_{17}H_{34}O_2$	1534	4.18
Stearic acid-methylester (Octadecanoic acid, methyl ester)	$C_{19}H_{38}O_2$	1610	2.19
Alkane Heptadecane	$C_{17}H_{36}$	1821	0.35
Octadecane	$C_{18}H_{38}$	1611	0.41

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 *Cynoglossus macrolepidotus* from Bushehr Coastal Zone in Persian Gulf

Compound	MF	KI	% of total
Fatty acid Saturated fatty acid Myristic acid	$C_{14}H_{28}O_2$	1534	0.47
Palmitic acid (Hexadecanoic acid)	$C_{16}H_{32}O_2$	1617	14.45
Stearic acid	$C_{18}H_{36}O_2$	1623	13.21
Monounsaturated fatty acid Oleic acid (9Z Octaecenoic Acid)	$C_{18}H_{34}O_2$	1687	12.12
Poly- unsaturated fatty acid Eicosapentaenoic acid (EPA)	$C_{22}H_{32}O_2$	1802	26.36
Docosahexaenoic acid (DHA)	$C_{20}H_{30}O_2$	1815	24.17
Ester Palmitic acid – methylester (Hexadecanoic acid, methyl ester)	$C_{17}H_{34}O_2$	1534	4.76
Stearic acid-methylester (Octadecanoic acid, methyl ester)	$C_{19}H_{38}O_2$	1610	2.58

Alkane			
Heptadecane	$C_{17}H_{36}$	1821	0.18
Octadecane	$C_{18}H_{38}$	1611	1.32

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 Myristic acid (11.73% in liver and muscle 0.47%), Palmitic acid (14.33% in liver and muscle 14.15%) and Stearic acid (13.25% in liver and muscle 13.21%), Monounsaturated fatty acid Oleic acid (13.12% in liver and muscle 12.12%), polyunsaturated fatty acids Eicosapentaenoic acid (20.55% in liver and muscle 26.36%) and Docosahexaenoic acid (19,75 % in liver and muscle 24.17%), two esters of fatty acid consist Palmitic acid–methylester (4.18% in liver and muscle 4.76%) and Stearic acid-methylester (2.19% in liver and muscle 2.58%) and Alkane including Heptadecane (0.35 % in liver and muscle 0.18%) and Octadecane (0.41% in liver and muscle in 1.32%). The amounts of alkanes are identified in liver and muscle tissues that they are environmental pollution.

4. Discussions

In the present research, the results indicate that the dominant fatty acids in liver and muscle tissues of *Cynoglossus macrolepidotus* are Eicosapentaenoic acid (EPA) (20.55-26.36%), Docosahexaenoic acid (DHA)(19.75-24.17 %) and Palmitic acid (14.15-14.33%). EPA and DHA and can be synthesized from alpha-linolenic acid or obtained directly from the diet (Guesnet & Alessandri, 2011). EPA has positive effects on coronary heart disease (Reiffel & McDonald, 2006; Allen & Harris, 2001), high triglycerides (fats in the blood), high blood pressure (Teres et al., 2008; Calo et al., 2005), and inflammation (Grimm et al., 2002; Gil, 2002). Other studies suggest it may be EPA that has the positive effect on depression (Song& Zhao, 2007; Bousquet et al., 2008). Omega-3 fatty acids such as EPA help increase levels of calcium in the body, deposit calcium in the bones, and improve bone strength. Docosahexaenoic acid is an omega-3 fatty acid that is a primary structural component of the human brain (Rees et al., 2006), cerebral cortex, skin, sperm, testicles and retina (Schonberg et al., 2006; Cunnane et al., 2009). DHA was found to inhibit growth of human colon carcinoma cells, more than other omega-3 PUFAs (Kato et al., 2002). The cytotoxic effect of DHA was not caused by increased lipid peroxidation or any other oxidative damage (Hardman, 2002; Kato et al., 2002; Bousquet et al., 2008) but rather a decrease in cell growth regulators (Schonberg et al., 2006). EPA and DHA ω−3 fatty acids may reduce the risk of coronary heart disease (Stampfer, *et al.,* 2000). Eicosapentaenoic acid and Docosahexaenoic acid may be the active biological components of these effects, research has shown that they decrease risk of arrhythmias, which can lead to sudden cardiac death, decrease triglyceride levels, decrease growth rate of atherosclerotic plaque and blood clots (Frenoux et al., 2001), each of which tends to clog arteries and reduce low-density lipoprotein (LDL or "bad") cholesterol and triglyceride levels (Kris-Etherton, *et al.,* 2001).
Palmitic acid or hexadecanoic acid is the most common fatty acid found in animals, plants and microorganisms. Palmitic acid has been shown to alter aspects of the central nervous system responsible for the secretion of insulin and to suppress the body's natural appetite-suppressing signals from leptin and insulin-the key hormones involved in weight regulation (Anneken et al., 2006). In the present investigation, we got the positive result for the presence of Omega-3 fatty acids EPA and DHA in the muscle and liver tissues of *Cynoglossus macrolepidotus i*n Persian Gulf.

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

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3/7/2013