Iranica Journal of Energy & Environment

Journal Homepage: www.ijee.net

IJEE an official peer review journal of Babol Noshirvani University of Technology, ISSN:2079-2115

Modified Extraction Method for Determination of Fatty Acids from Tuna's Eye in Iranian Coasts of Persian Gulf and Oman Sea

Y. A. Asadpour-Ousalou

Iranian Artemia Research Center, Golmankhaneh Harbor, Urmia, Iran

P A P E R I N F O

Paper history:
Received 23 November 2014
Accepted in revised form 01 September 2014

Keywords:
Fatty Acids
Fish Oil
Tuna Fish
Saturated Fat
Unsaturated Fat
Persian Gulf

A B S T R A C T

Tuna fish belongs to *Scombridae* family which includes *Sardinae*, *Macerels*, *Tuna* and many other species. These fish are harvested in a 30 thousands kilometers area in Persian Gulf and are processed in conserve factories. About 20± 3 wet weight percent of *Tuna* fish are by product. These by product were studied to extract the high-value productions; marine oils rich of unsaturated multiple-band fatty acids, in which 100kg was obtained from Chabahar and transferred to Urmia for analysis. Extracting oil was made using the Dyer and Bligh standard method. The results showed that 80± 5 % of wet weight of eye wastes of *Tunas* fish was oil; profile of the extracted oils were made using gas chromatography. The results showed that eye wastes of *Tuna* fish oil had 21.47% saturated fatty acids, 25.70% single band unsaturated fatty acids, and the total value of the unsaturated multiple band was 40.99%. The fatty acid contents of arachidonic acid, linolenic acid, linoleic acid, eicosapentaenoic acid and docosahexaenoic acid were 1.78, 4.10, 6.20, 17.40 and 9.86% of the total fatty acids, respectively. Also, the results revealed that eye wastes of Tuna fish is considered for the first time as the new and rich source of Omega 3 and Omega 6.

***doi**: 10.5829/idosi.ijee.2015.06.01.12*

INTRODUCTION

The role of Omega-3 and Omega-6 series of unsaturated fatty acids in synthesis of the eicosanoid hormones and in cellular metabolism in human and aquatic animals has been reported [1]. These compounds, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are amongst the essential compounds act as a major role for the cell's membrane structure, osmosis setting and synthesis of the endocrine glands hormones and activation of the body's immune system in human and animals [2].

Use of marine oils in treating bone diseases and rheumatism has been common since early years. Around 1950's, the scholars learned the existence of EPA and DHA essential fatty acids, for which they used to the benefit of the sharks liver to extract the same [3]. Such fatty acids, together with arachidonic acid are considered as having anti-flammable and anti-aggregation of blood platelets affect on the human blood vessels. Omega-3 and omega-6 fatty acids decrease the body's tri-glycerides through dispersing VLDL in the liver and increasing the HDL level in the plasma [4].

Previous study indicated that the unsaturated long-chain essential fatty acids including but not limited to EPA, DHA and arachidonic acid are also quite important in different fishes' larva feeding [5]. Such fatty acids are incorporated in phospholipids which have sensitive structure and found in amongst the physiologic elements of the cell membrane of most of the tissues. It has been reported that such acids is involved in disorder of the body immunity system and growth.

The nutritional studies have indicated the importance of highly unsaturated fatty acids (HUFA) in the aquatic's metabolism [6]. In past decades, the importance of the EPA and DHA in the enrichments of the animal-husbandry aquatic larva feeding quotas to decrease the mortality and increase in the growth and survival percentage have been expressed in the aquatic-husbandry industry. In this regard, several investigations were locally and internationally conducted [6]. Hafeziyeh, *et al*. [7] studied on improvement in growth factors of the sturgeon fishes' larvae stages by

*Corresponding author: Youssef Ali Asadpour-Ousalou
E-mail: Asadnazlo@yahoo.com, Phone No: +984432573047,
Cell No: +989141402918

Please cite this article as: Y. A. Asadpour-Ousalou, 2015. Modified Extraction Method for Determination of Fatty Acids from Tuna's eye in Iranian Coasts of Persian Gulf and Oman Sea, Egypt. Iranica Journal of Energy and Environment, 6 (1): 72-76.

the Artemia enriching operations using EPA and DHA. They stated that such fatty acids can improve the growth, decrease mortality and increase survival ratio of the larva stage of such fishes.

Sistani, *et al*. [8] expressed the importance of EPA and DHA on the growth and survival of crustaceans and white-leg shrimp; while the long-chain unsaturated fatty acids considerably affected and improved the growth performance of the survival ratio of white-leg shrimp larvae.

Now days, several pharmaceutical compounds are globally traded as life DHA, ultra Omega ,super Omega, etc as the malnutrition treatment tablets[2] marketed with high prices ($30 per pack of 100 tablets with 200mg of DHA and EPA). These products are found in marine sources and in livestock meat is poor in this regard. According to reported and recommend impact of DHA and EPA by FAO, USA-FDA and AFSSA France, each person shall consume at least 0.05-0.3 g/day of DHA and EPA to secure his health and prevent cardiovascular diseases.

Achieving new resources

The present study was conducted according to the following aims: (1) To determine composition and percentage of the fatty acids of Tuna eye-by products (2) Whether the eyes wastes of Tunas fish may be used as a new and rich source of long-chain unsaturated fatty acids, esp. DHA and EPA.

MATERIALS AND METHODS

In this study, 100 kg of eye wastes of Tuna fish obtained from Chabahar in 24 ,13 north longitude up to 5 0 latitude and processed.

Extraction of marine fats

Based on a modified version of the protocol described by Bligh and Dyer, eye wastes of Tunas fish was used for the fat extraction [9]. In this method, after reaching the frozen sample temperature in laboratory to ambient temperature, 1kg of the samples were totally crushed and mashed using electrical mill. Then 100mL of petroleum benzene and 1 L of methanol were added to the sample in a big vessel and then mixed for 15 minutes. The resulted mixture was again added with additional 500mL of petroleum benzene. The solution volume shall be in a way that the samples are totally wet and covered by solvents with a certain amount of the sample placed. In order to prevent the solvent evaporation, the vessel was covered by aluminum foil. Again the resulted solution was mixed for additional 10 minutes and next poured into Ben Murray basin. The mixture heated up to 50 °C for 20 minutes with frequent mixing. Then the mixture sample was extracted from the fabric bag with pressure of manual juicer and then filtered by Buckner cone contained a filter paper. The obtained product was transferred from the filter to a decanter machine, after complete separation of organic and aqueous phases including water phase and oil-solvent phase. The oil and solvent solution was evaporated by using rotary machine equipped with vacuum pump through heating under 40°C temperature. The resulted oil volume was finally determined.

Fatty acids analysis of samples and determination of oil composition

The fat extracted from the samples was soaped by addition of 3 mL methanol potassium hydroxide (2M solution) and then it was converted into methyl ester by addition of 5mL methanol sulfuric acid (2 molar). Fatty acids methyl ester was extracted in 1mL of normal heptanes. In order to analyze the fatty acids concentrations, 1 micro-liter of normal heptane phase was injected into the gas chromatograph (GC). In order to identify each and every fatty acids, the fatty acids standard mixture supplied by Sigmaba Company was used for comparison of the stoppage times. Agilent-6890 GC machine (Agilent Technology, USA) equipped with FID and capillary injection cap, capillary column specialized for decomposing fatty acids (DB- wax) (L:30m ID: 0.25mm) with polyethylene glycol static phase (THK: 0.25 micron). Temperature program was used for GC analysis with initial oven temperature set at 100 C, the oven temperature increased by a rate of 20 °C/min and remains in the same temperature for 12 minutes. Nitrogen gas was used as the carrier gas and arraying agent for 1 and 45 mL/min velocity, respectively. The injection and detector cap temperatures were set on 250 and 260 °C, respectively. Processing the machine data was made using the Chemstation software in windows environment.

RESULTS

From extracting the fat out of eye wastes of Tunas fish using modified Bligh and Dyer method. The performance was calculated as 80 ±5 % of the wet weight and was further analyzed to identify its concentration and fatty acids contents. The obtained results are summarized in Table 1.

The results of structure and combination percentage of the saturated fatty acids, unsaturated fatty acids with a double band, unsaturated fatty acids with some double bands, arachidonic acid, linoleic acid, linolenic acid, Eicosapentaenoic aicd, docosahexaenoic acid of eyes wastes of Tunas fish oil were obtained as the Figure 2.

[2] www.wholehealth.com, www.imega.DHA.healthcare

TABLE 1. Analysis of profile and percentage of fatty acids of the oil extracted from eyes wastes of Tunas fish

Sample of oil extracted	The fatty acids out of the total fatty acids (%)	Profile of fatty acid
Meristic Acid	3.23	C14:0
Tetradecanoic Acid 7	0.08	C14:1n5
Palmitic Acid	11.48	C16:0
Palmitolenic Acid	6.53	C16:1n7
Stearic Acid	4.55	C18:0
Oleic Acid	18.86	C18:1n9
Linoleic Acid	4.11	C18:2n6cis
Alpha- linoleic Acid	1.85	C18:3n3
Arachidic Acid	1.43	C20:0
Gamma linoleic	4.87	C18:3n6
Alpha- linoleic Acid - 5- 9- 12- 14- 5	0.02	C18:4n3
Docosaenoic Acid	0.24	C22:0
Di hemo gamma linoleic Acid	0.49	C20:3n6
Eicosapentaenoic Acid (EPA) 5-8-11	2.21	C20:3n3
Arachidonic Acid	1.78	C20:4n6
Eicosapentaenoic Acid (EPA) 5- 8- 11- 14- 17	7.65	C20:5n3
Docosahexaenoic Acid (DHA) 4-7- 10- 13- 16	5.51	C22:5n6
Docosahexaenoic Acid (DHA) 7- 10- 13-16- 19	2.28	C22:5n3
Docosahexaenoic Acid (DHA) 7-10- 13-16-191	9.68	C22:6n3
Tetracosaenoic Acid	0.54	C24:0

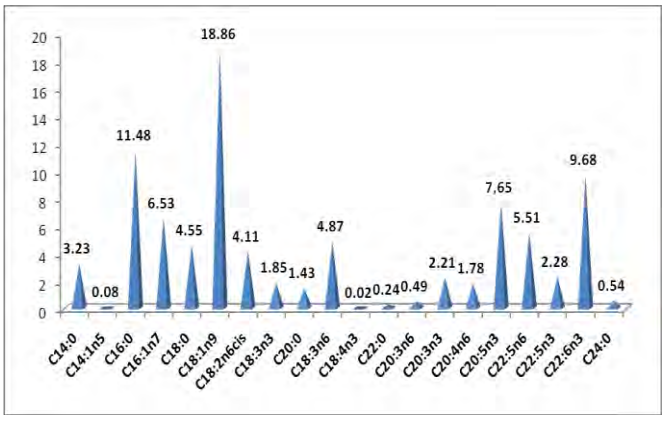

Figure 1. Analysis of profile and percentage of the oil fatty acids extracted from eyes wastes of Tunas fish

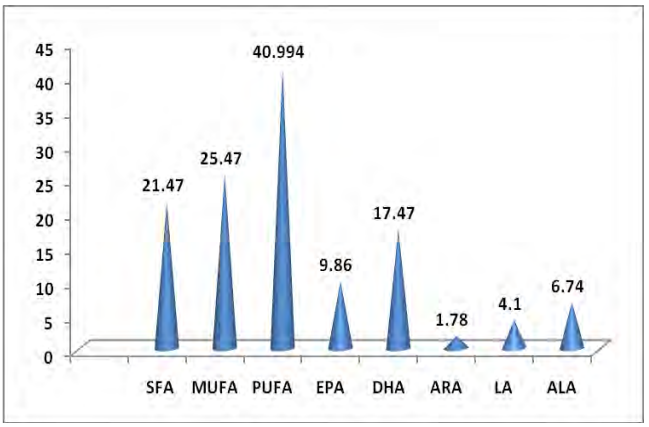

Figure 2. The results of structure and combination percentage of the saturated fatty acids

DISCUSSION AND CONCLUSION

Eye wastes of Tuna fish is not industrially used, while it has high content of marine fat (Approx. 80± 5% of the body combination). A 100 kg of eye wastes of Tuna fish was transferred to Urmia, Iranian Artemia Research Center and then was extracted from the sample using modified Dyer and Bligh method [9]. Therefore, the method is considered and introduced as a new source to extract marine oils.

As the marine oils contain high percentage of unsaturated fatty acid with multiple bands. The oily fats are highly used in pharmaceutics, biotechnology, cosmetics, health and medicine. The fish oils have always been highly emphasized in academic and research aspects on their role as well as on achieving new sources to extract the essential fatty acids. The multipurpose application of fish oil as nutrimental values and medicinal uses are internationally recommended. The results indicated that total value of the unsaturated multiple-band fatty acids was 40.99%. The fatty acids contents of arachidonic acid, linolenic acid, linoleic acid, DHA and EPA were 1.78, 4.10, 6.70, 17.40 and 9.860% of the total fatty acids, respectively. The results demonstrated that eye wastes of Tuna fish is considered for the first time as the new and rich source of Omega-3 and Omega-6. Today, several pharmaceutical compounds are globally marketing the life DHA, ultra meaga, super Omega, etc as the malnutrition treatment tablets with high prices ($30 per

pack of 100 tablets with dosage 200mg in each tablet). Therefore, a proper source to supply such needs may be used and operated by the oil extracted from the eye wastes of Tunas fish in the industrial scale of production and formulation of the aforementioned pharmaceutical compounds formulation.

The research reported by Rocha Filho, *et al.*[10] and Towfighian, *et al*. [11] indicated that Docosahexaenoic acid and Eicosapentanoic acid had anti-inflammable effects; also decreased hypertension, cholesterol and tri-glycerides of patients. DHAand EPA are also able to prevent the formation of atherosclerotic plaque in human blood vessels and improve the skin problems such as eczema and psoriasis. While, previous era the shark liver oil has been used to treat the same and the results of this study indicates that squid oil is a proper alternative to treat these syndromes.

In several research reports, it has been stated that in pharmaceutics, the shark liver oil is used in production of lipstick, cosmetic soap, hydration creams, skin treatment creams and burns, while it is used medically to alleviate the muscle pains. The eye wastes of Tunas fish extracted oil may be a proper alternative for the production and formulation of such medicines [3, 12, 13].

According to the nutritious effects of DHA and EPA in the pregnant women, dietary HUFA prevent the babies' genetic disorders and protect them against weight losing which indicate the importance of such compounds in social nutrition security and health. Towfighian, *et al*. [11] performed extensive studies on the treatment effects of omega-3 fatty acid on the menstruation signs and before the same and reported the relevant treatment effects. Therefore, the eye wastes of Tuna fish oil is a new source rich of such fatty acids which shall highly be used in this field of industry.

Nutritional studies in past decades on the importance of HUFA fatty acids expressed the role of the same in improvement of the nutrition metabolism of aquatic for which was investigated by Hellsten, *et al*. [14] could be mentioned. According to the same as well as the reports of such scholars, eye wastes of Tunas fish may be proper alternative to enrich the emulsions used in this regard.

The importance and value of the EPA and DHA in enriching the animal-husbandry aquatic larvae diets to decrease the mortality and increasing the growth and survival ratio have been expressed in the aquatic-husbandry industry. Several studies have been domestically and internationally conducted in this regard for which several enriching compounds are produced and marketed by different companies. The importance of using such compounds has caused that big research companies across the global supply are ready to use enriching emulsion, for which INVE Company (European-American) may be worth mentioned which produces and sells a range of certain products branded as Selco، Super Selco ،A1 Selco ،DC DHA Selco and Easy Selco with expensive commercial prices [15]. However, their percentage of MUFA content as reported by Narciso, *et al*. [16] as well as the marketed report is equal to the same extracted level out of Tuna eyes wastes. Such equality of results indicates that the eye wastes of Tunas fish oil may be a proper alternative for such emulsions.

It was concluded that the eye wastes of Tunas fish is introduced as a new rich source of omega-3 and omega-6 and that the oil of this aquatic may be a proper alternative to be used in the aquatic husbandry ndustries, pharmaceutics, cosmetic productions and supplementary diets.

The new research finding of this project is that the eye wastes of Tunas fish is introduced as a new and proper source rich in EPA, DHA to be used in a variety of industries.

ACKNOWLEDGMENTS

The foundational project was financially supported by the Iranian Fisheries Research Organization by the grants commissions, defined as the project Ref. No. 2-79-12-89009. We should acknowledge all the personnel of the research centers, including State Artemia Research Center, State Fishery Sciences Research Institute, Analytical Chemistry Laboratory of Urmia University, Livestock Affairs of Ministry of Agriculture of West Azerbaijan Province, and Chabahar Remote Waters Research Center.

REFERENCES

1. Kanazawa, A., S.-i. Teshima, S. Tokiwa, M. Kayama and M. Hirata, 1979. Essential fatty acids in the diet of prawn, 2: Effect of docosahexaenoic acid on growth. Bulletin of the Japanese Society of Scientific Fisheries.
2. Leger, P., D. Bengtson, P. Sorgeloos, K. Simpson and A. Beck, 1987. Nutritional value of Artemia: A review.
3. Terry, J., 2008. EPA and DHA and the whole stinking story. 4th national forum on fish, Canada.
4. Bruce, J., 2009. Omega 3 levels in fish: Data quality, quantity and future. 4th national forum on fish, Journal of nutrition, Canada: 45-63.
5. Agh, N., F. Noori, A. Irani, G. Stappen and P. Sorgeloos, 2013. Fine tuning of feeding practices for hatchery produced Persian sturgeon, Acipenser persicus and Beluga sturgeon, Huso huso. Aquaculture Research, 44(3): 335-344.
6. Watanabe, T. and V. Kiron, 1994. Prospects in larval fish dietetics. Aquaculture, 124(1): 223-251.
7. Hosseinpour, H., M. Hafezieh, M.S. Kamarudin, C.R.B. Saad, M.K.A. Sattar, N. Agh, T. Valinassab and M. Sharifian, 2010. Effects of enriched Artemia urmiana with HUFA on growth, survival, and fatty acids composition of the Persian sturgeon larvae (Acipenser persicus). Iranian Journal of Fisheries Sciences, 9(1): 61-72.

8. Sistani, M.A., Effect of enriched artemia with C vitamin and unsaturated fatty acids on growth and prevention of Vanami shrimp larva (Litopenaeus vannamei) M.Sc. degree thesis, Islamic Azad University, Bandar Abbas Branch. 2000.
9. Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 37(8): 911-917.
10. Rocha Filho, E.A., J.C. Lima, J.S. Pinho Neto and U. Montarroyos, 2011. Essential fatty acids for premenstrual syndrome and their effect on prolactin and total cholesterol levels: a randomized, double blind, placebo-controlled study. Reprod Health, 8(2).
11. Towfighian, T., A. Koushki and M. Rakhshani, 2012. Studying the effect of omega fatty acids on the pre-menstruation signs. Women and Accouchement Journal, 15(32): 23-42.
12. Suzukawa, M., M. Abbey, P. Clifton and P.J. Nestel, 1996. Enhanced capacity of n-3 fatty acid-enriched macrophages to oxidize low density lipoprotein mechanisms and effects of antioxidant vitamins. Atherosclerosis, 124(2): 157-169.
13. Nardini, M., M. D'Aquino, G. Tomassi, V. Gentili, M. Di Felice and C. Scaccini, 1995. Dietary fish oil enhances plasma and LDL oxidative modification in rats. The Journal of Nutritional Biochemistry, 6(9): 474-480.
14. Hellsten, R., M. Johansson, A. Dahlman, O. Sterner and A. Bjartell, 2011. Galiellalactone inhibits stem cell-like ALDH-positive prostate cancer cells. PLoS One, 6(7): e22118.
15. Han, K., I. Geurden and P. Sorgeloos, 2001. Fatty acid changes in enriched and subsequently starved *Artemia franciscana* nauplii enriched with different essential fatty acids. Aquaculture, 199(1): 93-105.
16. Narciso, L. and S. Morais, 2001. Fatty acid profile of Palaemon serratus (Palaemonidae) eggs and larvae during embryonic and larval development using different live diets. Journal of Crustacean Biology, 21(3): 566-574.

Persian Abstract

***DOI**: 10.5829/idosi.ijee.2015.06.01.12*

چکیده

ماهی تونا متعلق به خانواده سوکم برایده که شامل ساردین، مکارل ها، تونا و سایر گونه های ماهی می باشند. این نوع ماهی ها در منطقه حلیج فارس به مساحت ۳۰ هزار کیلومتر مربع بهره برداری شده و در کار خانه های کنسروسازی مصرف می شوند. حدود ۳±۲۰ در صد وزنی ماهی تونا فرآورده های جانبی با ارزش استحصال می گردد. روغن ماهی غنی از اسیدهای چرب غیر اشباع با پیوندهای چندگانه جهت انالیز از چابهار به ارومیه منتقل گردید. استخراج اسیدهای چرب با استفاده از خشک کن و روش استاندارد بیلاق انجام گردید. نتایج نشان داده است که ۵ ±۸۵ درصد وزنی ضایعات چشم ماهی تونا حاوی روغن تون می باشد. روغن استخراج شده بروش گروماتوگرافی گازی آنالیز گردید. نتایج بدست آمده نشان داده است که روغن تون ماهی حاوی ۲۱/۴۷ درصد اسید چرب اشباع و ۲۵/۷ اسید چرب غیر اشباع با یک پیوند دوگانه و ۴۰/۹۹ در صد اسید چرب غیر اشباع با چندین پیوند می باشد. اسیدهای چرب غیر اشباع نظیر اسید ارشیدونیک، لینولنیک، لینولئیک، ایکوساپنتائنویک و دوکوهگزانئویک به ترتیب ۱/۷۸، ۴/۱۰، ۶/۲۰، ۱۷/۴۰ و ۹/۸۶ در صد از کل اسیدهای چرب می باشد. همچنین نتایج حاصل از ضایعات چشم ماهی تون برای اولین بار بعنوان منبع جدید حاوی روغن امگا-۳ و امگا-۶ مورد توجه قرار گرفته است.