Squid (*Loligo loligo*): The new source to extract omega-3 and omega-6 rich marine oils

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Abstract

Squids belong to the cephalopod family, which composes more than 5% of the total annual fishing volume of southern Iran as by- products; however, these do not have significant usage in Iran. This product was studied to extract the high-value products, i.e. marine oils rich in unsaturated multiple-band fatty acids from 50kg squid (*Loligo loligo*) obtained from Chabahar fishing port and transferred to Urmia Research Center for further action. Extracting oil from squid was done by using the Dyer and Bligh standard method. The results indicated that 13±5% of wet weight of squid was oil; the percentage and profile of the extracted oils were made by using the G.C method. The results showed that squid oil had 29.40% saturated fatty acids and 23.70% single- band unsaturated fatty acids, and the total value of the unsaturated multiple-band fatty acids of the same was 40.20%. Also the contents of arachidonic acid was 2.78%, linolenic acid was 3.10%, linoleic acid was 5.20%, docosahexaenoic acid (DHA) was 15.40%, and eicosapentaenoic acid (EPA) was 9.60% of the total fatty acids. The results demonstrate that squid is considered for the first time as the new and rich source of Omega-3 and Omega-6.

Keywords: Squid, GC, Omega-3, Omega-6, EPA, DHA

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Introduction

The role of Omega-3 and Omega-6 series unsaturated fatty acids in synthesis of eicosanoid hormones and in cellular metabolism in human and aquatics has been reported earlier (Kanaza *et al*., 1979). These compounds, esp. Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), are from amongst the essential compounds playing a major role for the cell membrane structure, osmosis setting and synthesis of the endocrine glands hormones and activation of the body immunity system in the body of human and animals. Leger *et al*. (1987). Using marine oils in treating bone diseases and rheumatism has been common. Around the 1950s, the scholars learned the existence of EPA and DHA essential fatty acids, for which they used to take benefit from the liver of the sharks to extract the same (Terry, 2008). Such fatty acids, together with arachidonic acid are considered as having anti-flammable and anti-aggregation of plaquette effect in the human blood vessels. Omega-3 and omega-6 fatty acids decrease body tri-glycerides through dispersing very low-density lipoproteins (VLDL) in the liver and increasing the high-density lipoprotein (HDL) level in the plasma (Bruce, 2009).

The a previous study has 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' larvae feeding (Takeuchi and Yamagishi, 2007). Such fatty acids are of the phospho-lipids which have sensitive structure and from amongst the physiologic elements of the cell membrane of most tissues and it has been reported that such acid disorder the body immunity system and growth. (Horrobin, 1983).

The nutritional studies have indicated the importance of highly unsaturated fatty acids (HUFA) in the aquatic metabolism since the 80s (Wananabet and Kiron, 1994).

The importance and value of the EPA and DHA in enriching the animal-husbandry aquatic larvae feeding quotas to decrease mortality and increasing growth and survival percentage have been expressed in the aquatic-husbandry industry as of 1980 and several studies have been made locally and internationally in this regard. Hafezieh *et al.* (2010) studied the improvement in growth factors of the caviar fishes' larval stages by the *Artemia* enriching operations using EPA and DHA and stated that such fatty acids caused improved growth, decreased mortality and increased survival percentage of the larval stage of such fishes.

In 1980, Kanazawa *et al*. 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 affect the increased performance and growth and survival rate of white-leg shrimp larvae.

Today, several pharmaceutical compounds are traded globally as life DHA, ultra omega, super omega, etc as

the malnutrition treatment tablets with high prices (30 USD/ pack of 100 tablets "200mg each") (www.wholehealth.com) (www.imega.DHA.healthcare).
Generally speaking, DHA and EPA are found in marine sources and the livestock meat is poor in this regard. As reported by the FAO, FDA (USA) and AFSSA (France), each person shall consume at least 0.3- 0.05 gr/day of DHA and EPA to keep his health and prevent cardiovascular diseases.

Achieving new resources

This study has been completed according to the objectives of: (1) determining the percentage and structure of the squid fatty acid (2) whether the squid may be used as a new and rich source of long-chain unsaturated fatty acids, especially. DHA and EPA.

Materials and methods

In this study, 50Kg of squid was obtained from Chabahar and shipped to Artemia Research Center in Urmia. The required chemical solutions were obtained and used from laboratory facilities of Urmia University, Engineering laboratory of food stuff of Natural Resources Research Center and Livestock Affairs of Ministry of Agriculture of West Azerbaijan Province as well as the laboratory facilities of Artemia Research Center.

Extraction method of marine fats

Squid fat was subject to Bligh and Dyer (2009) method, after reaching the frozen sample temperature in the laboratory to ambient temperature, 1kg of the samples were totally crushed and mashed by using electrical mill. Then 100mL petroleum benzene and 1 lit. methanol were added to the same in a big vessel and mixed for 15 minutes and the resulting mixture was again added with another 500mL of petroleum benzene. The solution volume shall be in a way that the samples are totally wet and a certain amount of the same was placed on the samples. In order to prevent solvent evaporation, the vessel was capped by using aluminum foil. Again the resulting solution is mixed for 10 minutes and then poured into a water bath and heated up to 50 °C for 20 minutes with frequent vibrations. Then the mixture sample was extracted from the fabric bag with the pressure of a manual juicer and then filtered by using Buckner cone containing filter paper. The resulting product was transferred from the filter to a decanter and after complete separation of 2 phases (water phase and oil-solvent phase), the oil and solvent solution was evaporated by using rotary machined equipped with vacuum pump by heating to 40 °C temperature and the resulting oil volume was finally measured in millimeters.

Analysis method of samples fatty acids and determination of their profiles and percentages

The fat extracted from the samples was soaped by adding 3mL methanol potassium hydroxide (2 molars) and then by adding 5mL methanol sulfuric acid (2 molar) it was converted into methyl ester (2). Fatty acids methyl ester was extracted in 1mL of normal heptane and in order to analyze the fatty acids profile, 1 micro-liter of heptane normal phase was injected into the gas chromatography machine. In order to identify each and every fatty acid, the fatty acids standard mixture made by Sigma Co. was used by comparing the stoppage times. The Agilent- 6890 GC machine made by Agilent Co. (USA) equipped with 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) together with FID. At 100 °C, the oven primary temperature was increased up by 20°C/min and remained at 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 at 250, 260 °C, respectively. Processing the machine data was made by using the Chemstation software in windows environment.

Results

From extracting the fat out of squid by using, Bligh and Dyer (2009) the performance was calculated as 13±3 % of the wet weight and was further analyzed to identify its profile and fatty acids contents, of which the results were obtained as per Table 1:

Table 1: Results of analysis of profile and percentage of fatty acids of the oil extracted from squid.

Sample of oil extracted from squid	% of the fatty acids out of the total fatty acids	Profile of fatty acid
Meristic acid	5.50±1	C14:0
Tetradecanoic acid 7	2.08±.5	C14:1n5
Palmitic acid	7.48±1	C16:0
Palmitolenic acid	7.76±1	C16:1n7
Steraic acid	4.55±1	C18:0
Oleic acid	13.86±1	C18:1n9
Linoleic acid	3.10±1	C18:2n6cis
Alpha- linoleic acid	3.37±1	C18:3n3
Arachidic acid	6.10±2	C20:0
Gamma linoleic	5±1	C18:3n6
Alpha- linoleic acid - 5- 9- 12- 14- 5	0.02±.6	C18:4n3
Docosaenoic acid	4.25±1	C22:0
Di hemo gamma linoleic acid	3.90±.5	C20:3n6
Eicosapentaenoic acid (EPA) 5-8-11	3.10±.5	C20:3n3
Arachidonic acid	2.78±.4	C20:4n6
EPA 5- 8- 11- 14- 17	6.50±1	C20:5n3
Docosahexaenoic acid (DHA) 4-7- 10- 13- 16	6.51±1	C22:5n6
DHA 7- 10- 13- 16- 19	4.50±1	C22:5n3
DHA 7- 10- 13- 16- 191	4.40±1	C22:6n3
Tetracosaenoic acid	1.54±.5	C24:0

The results of the 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, EPA, DHA of squid oil were obtained as per the Table 2:

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

Compound structure	% of fatty acid out of the total composing fatty acids
SFA	29.42±2
MUFA	23.70±3
PUFA	40.28±2
EPA	9.60±1
DHA	15.41±1
ARA	2.78±/5
LA	3.10±/5
ALA	5.20±/5

Discussion

Squids known by the southern Iran locals as Khesak, are of invertebrate group which compose more than 5% of the southern Iran fishing, i.e. hundreds of tons, which are considered as the subsidiary products of fishing.

Squid is not industrially used, while it has high content of marine fat (Approx. 13+/-3% of the body combination). 50 kg of the same animal was transferred to Urmia Research Center and then 6.5liters of premium quality oil rich in oega-3 and omega-6 was extracted from the sample. Therefore, it 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, they are highly used in pharmaceutics, biotechnology, cosmetics, health and medicine and have always been highly emphasized in the academic and research conferences for their role as well as for achieving new sources to extract the same, for which the 2009 International Conference in Japan and 2010 International Symposium in Australia are among a few to be mentioned.

The results indicated the total value of the unsaturated multiple-band fatty acids of the same was 40.20% and the contents of arachidonic acid 2.78% and linolenic acid 3.10% and linoleic acid 5.20% and DHA 15.40% and EPA 9.60% out of the total fatty acids of the same may be mentioned.

The results demonstrate that squid is considered for the new and rich source of Omega-3 and Omega-6.

The research report of Rocha Filho *et al*. (2011) and Towfighian *et al*. (2012) indicated that the DHA and EPA had anti-inflammable effects, decreased hypertension and cholesterol and tri-glycerides and prevented the formation of athero- eschlerotic plaque in the blood vessels and improved the skin problems such as eczema and psoriasis, while since ages ago shark liver oil had been used to treat the same problems .

In several studies (Nardini *et al.*, 1995; Suzakawa *et al.*, 1996; Terry, 2008), it has been reported that in pharmaceutics, the shark liver oil is used in the production of lipstick, cosmetic soap, hydration creams, skin treatment creams and burns, while it is used medically to alleviate muscle pains. The squid extracted oil may be a proper alternative for the production and formulation of such medicines.

Existence of DHA and EPA in the diet of pregnant women gets rid of babies' genetic disorders and prevent them from weight loss which indicate the importance of such compounds in social nutrition security and health. Towfighian *et al*. (2012) 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, squid oil can be considered as a new source rich in such fatty acids which shall highly be used in this field of industry.

Nutritional studies during the past decades on the importance of HUFA fatty acids expressed the role of the same in improvement of the nutrition metabolism of aquatics for which the studies of Watanabe *et al*. (1994) may be mentioned. Therefore, squid may be a 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 feeding quotas to decrease mortality and increase growth and survival percentage have been expressed in the aquatic-husbandry industry as of 1980 and several studies have been done 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 Globe supply ready to use enriching emulsion, for which INVE Co (European-American) may be mentioned which produces and sells a range of certain products branded as Selco, Super Selco, A1 Selco, DC DHA Selco, Easy with expensive commercial prices (Han *et al*., 2001). However, their percentage of HUFA content as reported by Sorgeloos *et al*. (2001) as well as the report in www.artemia-international.com/default.asp?contentID is around. Such equality of results indicates that the squid oil may be a proper alternative for such emulsions.

It is concluded that the squid is introduced as a new source rich in omega-3 and omega-6 and that the oil of this aquatic may be a proper alternative to be used in aquatic husbandry industries, pharmaceutics, production of cosmetics, and balancing diets.

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

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References

Bligh, EG. and Dyer, WY., 2009. A rapid method of total lipid extraction and purification. *Canadian Journal Biochemistry and Physiology*, 37, 911-917.

Bruce, J., 2009. Omega 3 levels in fish: Data quality, quantity and future, 4th national forum on fish, *Journal of nutrition*, Canada. pp.45-63.

Hafezieh, M., Mohammadd Salah Kamarudin, S., Che Rose Bin, S., Mostafa Kamal, A.S., Agh, N., Valinassab, T., Sharifian, M. and Hosseinpour, H., 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.

Han, K., Geurden, I. and Sorgeloos, P., 2001. Fatty acid changes in enriched and subsequently starved *Artemia franciscana* nauplii enriched with different essential fatty acids. *Aquaculture*, 199, 93F105.

Horrobin, D.F., 1983. The role of essential fatty acids and prostaglandins in the premenstrual syndrome. *Journal of Reproductive Medicine*, 28(**7**), 465-8.

Kanazawa, A., Teshima, S., Tokiwa, S., Kayama, M. and Hirata, M., 1980. Essential fatty acids in the diet of prawn. II. Effect of docosahexaenoic acid on growth. *Bulletin of the Japanese Society of Scientific Fisheries*, 45, 115l-1153.

Leger, P., Bengtson, D.A., Sorgeloos, P., Simpson, K.L. and Beck, A.D., 1987. The nutritional value of *Artemia*: a review. In: Sogeloos, P., Bengtson, D.A., Decleir, W., Jaspers, E. (Eds.), Artemia Research and its Applications. pp. 356-372.

Nardini, M., D'Aquino, M., Gentili, V. and Di Felice, M., 1995. Dietary fish oil enhances plasma and LDL oxidation modification in rats. *Journal of Nutritional Biochemistry*, 69, 474–80.

Rocha Filho, E.A., Lima, J.C., Pinho Neto, J.S. and Montarroyos, U., 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. Jan 17; 8, 2.

Sorgeloos, P., Leger, P. and Bengtson, D.A., 2001. Lake Urmia cooperation project-contract item A, report on the determination and identification of biological characteristics of *Artemia urmiana* for application in aquaculture, Faculty of agriculture and applied biological science laboratory of aquaculture and Artemia reference center, Universiteit Ghent, Belgium.

Suzakawa, M., Abbey, M., Clifton P.N. and Nestel, P., 1996. Enhanced capacity of ω–3 fatty acid enriched mocrophages to oxidize low density lipoprotein mechanisms and effects of antioxidant vitamin. *Atherosclerosis*, 124(**2**), 157-69.

Takeuchi, M. and Yamagishi, S., 2007. Possible involvement of advanced glycationend-products (AGEs) in the pathogenesis of Alzheimer's disease. *Current*

Pharmaceutical Design, 14, 973-978.

Terry, J., 2008. EPA and DHA and the whole stinking story .4th national forum on fish. Canada.

Towfighian, T., Koushki, A. and Rakhshani, M., 2012. Studying the effect of omega fatty acids on the pre-menstruation signs, 15th series, Zanan va Zayeman magazine, vol 32.

Watanabe, T. and Kiron, Y., 1994. Prospects in larval fish dietetics. *Aquaculture*, 124, 223-251.