

## Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Document heading

doi: 10.12980/JCLM.2.2014JCLM-2014-0072

© 2014 by the Journal of Coastal Life Medicine. All rights reserved.

# Determination of fatty acids percentages and profile extracted from cuttlefish of Iranian coasts of Persian Gulf and Oman Sea

Yosief Ali Asadpour\*

Iranian Artemia Research Center, Golmankhaneh harbor, Urmia, Iran

## PEER REVIEW

## Peer reviewer

Hugo Daniel Solana Goya, Lab. Biol. Cel. y Mol. FCV-UNCPBA-Argentina. Tel/Fax: 52-249-443-9850, E-mail: hsolana@vet.unicen.edu.ar  
Co-reviewer: Masoud Seidgar, Tehran, Iran.

## Comments

This is a valuable research work in which the author has demonstrated the utility of the squid. This study shows the true nutritional characteristics of this species as a source of essential fatty acids. The new research finding of this project is that the squid is introduced as a new and proper source rich in EPA, DHA to be used in a variety of industries.  
Details on Page 858

## ABSTRACT

**Objective:** To determine the fatty acid profile extracted from cuttlefish of Persian Gulf and Oman Sea, including *Ancistrocheirus*, Enoplateuthidae, Cranchiidae, Ommastrephidae and Loliginidae.

**Methods:** Oil was extracted by the Bligh and Dyer method. The fatty acid profile of the oil was determined by gas chromatography.

**Results:** The results showed that (13±5)% of wet weight of cuttlefish is oil. The results also showed that cuttlefish oil has 29.40% saturated fatty acids and 23.70% single-band unsaturated fatty acids, and the total value of the unsaturated multiple-band is 40.20%, the contents of arachidonic acid 2.78%, linolenic acid 3.10%, linoleic acid 5.20%, docosahexaenoic acid 15.40%, and eicosapentaenoic acid 9.60% out of the total fatty acids of the same may be mentioned.

**Conclusions:** The results of the current study described that cuttlefish is considered for the first time as the new and rich source of omega-3 and omega-6.

## KEYWORDS

Fatty acids, Cuttlefish, Persian Gulf, Oman Sea

## 1. Introduction

The role of omega-3 and omega-6 series unsaturated fatty acids in synthesis of the eicosanoid hormones and in cellular metabolism in human and aquatic has been reported by Kidd *et al*[1]. These compounds, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are from amongst the essential compounds playing a major role for the cell's membrane structure, osmosis setting and synthesis of the endocrine glands hormones and activation of the body's immunity system in the body of the human and animals. Léger *et al.* reported using marine oils in treating bone diseases and rheumatism has been common since years ago[2]. Around 1950s, the researchers learned to use EPA and DHA essential fatty acids, which they

extracted from the liver of the sharks for this purpose[3]. Such fatty acids, together with arachidonic acid (ARA) 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 the body's triglyceride through dispersing low-density lipoprotein in the liver and increasing the high density lipoprotein level in the plasma[4].

The previous study has indicated that the unsaturated long-chain essential fatty acids including but not limited to EPA, DHA and ARA are also quite important in different fishes' larva feeding[5]. Such fatty acids are of the phospholipids which have sensitive structure and amongst the physiologic elements of the cell membrane of most of the tissues and it has been reported that such acids disorder the

\*Corresponding author: Yosief Ali Asadpour, Assistant Professor, Iranian Artemia Research Center, Golmankhaneh harbor, Urmia, Iran.

Tel: +9141402918

Fax: +984414362728

E-mail: asadnazlo@yahoo.com

Foundation Project: Supported by the Iranian Fisheries Research Organization [Grants Commission (Project Ref. No. 2-79-12-89009)].

Article history:

Received 29 May 2014

Received in revised form 6 Jun, 2nd revised form 17 Jun, 3rd revised form 20 Jun 2014

Accepted 24 Jun 2014

Available online undetermined

body immunity system and growth.

The nutritional studies have indicated the importance of highly unsaturated fatty acids in the aquatic metabolism since the 80's[6].

The importance and value of the EPA and DHA in the enriching of the animal–husbandry aquatic larva feeding quotas to decrease the mortality and increase the 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.* studied on the improvement in growth factors of the caviar fishes' larva stages by the *Artemia* enriching operations using EPA and DHA and stated that such fatty acids cause improved growth, decreased mortality and increased survival percentage of the larva stage of such fishes[7].

Kanaza *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 if white–leg shrimp larva[8].

Today, several pharmaceutical compounds are traded globally such as life DHA, ultra omega, super omega, *etc.* as the malnutrition treatment tablets with high price (30 USD/pack of 100 tablets “200 mg each”)[9,10].

Generally speaking, DHA and EPA are found in the marine sources and the livestock meat is poor in this regard. As reported by the Food and Agriculture Organization, Food and Drug Administration (USA), and Agence Française de Sécurité Sanitaire des Aliments (France), each person shall consume at least 0.30–0.05 g per day of DHA and EPA to keep healthy and prevent cardiovascular diseases.

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

## 2. Materials and methods

In this study, 100 kg of squid was obtained from Chabahar (24°13' N, 57°60' E) and studied accordingly.

### 2.1. Extraction method of marine fats

Squid fat was subject to Bligh and Dyer method. In this method, after reaching the frozen sample temperature in the laboratory to ambient temperature, 1 kg of the samples were totally crushed and mashed by using electrical mill. Then 100 mL petroleum benzene and 1 L methanol were added to the same in a big vessel and mixed for 15 min and the mixture was again added with another 500 mL of petroleum benzene. The solution volume shall be in a way that the samples were totally wet and a certain amount of the same was placed on the samples. In order to prevent the solvent evaporation, the vessel was capped by using aluminum foil. Again the solution was mixed for 10 min and then poured into Ben Murray basin which had been heated up with 50 °C for 20 min with frequent vibrations. Then the mixture sample was extracted from the fabric bag with the pressure of manual juicer and then filtered by using Buckner cone containing filtering paper. The final product was transferred from the filter to a decanter machine

and after completing separation of two phase (water phase and oil–solvent phase), the oil and solvent solution was evaporated by using rotary machined equipped with vacuum pump through heating under 40 °C temperature and the ultimate oil volume was finally measured in millimeters.

### 2.2. Analysis method of sample's fatty acids and determination of their profiles and percentages

The fat extracted from the samples was soaped by adding 3 mL methanol potassium hydroxide (2 mol) and then by adding 5 mL methanol sulfuric acid (2 mol) it was converted into methyl ester. Fatty acids methyl ester was extracted from 1 mL of normal heptane and in order to analyze the fatty acids profile, 1 mL of normal heptane phase was injected into the gas chromatography machine. In order to identify fatty acids, 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) (30 m×0.25 mm) with polyethylene glycol static phase (thickness 0.25 µm) together with flame ionization detector. In 100 °C, the oven primary temperature increased up to 20 °C/min and remained in the same temperature for 12 min. 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 °C and 260 °C, respectively. Processing the machine data was made by using the Chemstation software in windows environment.

## 3. Results

From extracting the fat out of squid by using Bligh and Dyer method, the performance was calculated as (13±3)% of the wet weight and was further analyzed to identify its profile and fatty acids contents. The results are shown in Table 1.

**Table 1**

Analysis of profile and percentages of fatty acids of the oil extracted from cuttlefish.

Sample of oil extracted from cuttlefish	Percentages of the fatty acids out of the total fatty acids (%)	Profile of fatty acid
Meristic acid	5.50	C14:0
Tetradecanoic acid	2.08	C14:1n5
Palmitic acid	7.48	C16:0
Palmitolenic acid	7.76	C16:1n7
Steraic acid	4.55	C18:0
Oleic acid	13.86	C18:1n9
Linoleic acid	3.10	C18:2n6cis
ALA	3.37	C18:3n3
Arachidic acid	6.10	C20:0
Gamma linoleic	5.00	C18:3n6
ARA –5–9–2–14–5	0.02	C18:4n3
Docosanoic acid	4.25	C22:0
Di hemo gamma linoleic acid	3.90	C20:3n6
EPA 5–8–11	3.10	C20:3n3
ARA	2.78	C20:4n6
EPA 5–8–11–14–17	6.50	C20:5n3
DHA 4–7–10–13–16	6.51	C22:5n6
DHA 7–10–13–16–19	4.50	C22:5n3
DHA 7–10–13–16–191	4.40	C22:6n3
Tetracosanoic acid	1.54	C24:0

ALA: Alpha–linoleic acid.

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, ARA, ALA, linolenic acid (LA), EPA, DHA of squid oil were obtained (Table 2).

**Table 2**

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

Compound structure	Percentages of fatty acid out of the total composing fatty acids (%)
Saturated fatty acid	29.42
Monounsaturated fatty acid	23.70
Polyunsaturated fatty acid	40.28
EPA	9.60
DHA	15.41
ARA	2.78
LA	3.10
ALA	5.20

#### 4. Discussion

Cuttlefish known by the Southern Iran locals as Khesak, are of invertebrate group which composes more than 5% of the Southern Iran fishing, *i.e.* hundreds of tons, which are considered as the subsidiary products of fishing<sup>[11]</sup>.

Squid is not industrially used, while it has high content of marine fat [Approx. (13±3)% of the body combination]. A total of 50 kg of the same animal was transferred to Urmia Research Center and then 6.5 L of premium quality oil rich in omega-3 and omega-6 was extracted from the sample by using the Dyer and Bligh method. 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 pharmaceuticals, biotechnology, cosmetics, health and medicine and have always been highly emphasized in the academic and research conferences on their role as well as on achieving new sources to extract the same, for which the 2009 International Conference in Japan and 2010 International Symposium in Australia are a few to be mentioned<sup>[11]</sup>.

The results indicated the total value of the unsaturated multiple-band fatty acids of the same is 40.20%, and the contents of ARA 2.78%, LA 3.10%, linoleic acid 5.20%, 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 first time as the new and rich source of omega-3 and omega-6.

Today, several pharmaceutical compounds are traded globally such as life DHA, ultra omega, super omega, *etc.* as the malnutrition treatment tablets with high prices (30 USD/ pack of 100 tablets "200 mg each")<sup>[9,10]</sup>. Therefore a proper source to supply such needs may be used and operated by the oil extracted from the squid in the industry of production and formulation of the aforementioned pharmaceutical compounds formulation.

The research reports of Rocha Filho *et al.* and Towfighian *et al.* indicated that the DHA and EPA have had anti-inflammatory effects<sup>[12,13]</sup>, decreased hypertension and cholesterol and tri-glycerides and prevented the formation of atherosclerosis plaque in the blood vessels and improved the skin problems such as eczema and psoriasis, while

since years ago the shark liver oil has been used to treat the same symptoms and the results of this study indicates that squid oil is a proper alternative to treat these syndromes.

In several study sources<sup>[3,14,15]</sup>, it has been reported that in pharmaceuticals, the shark liver oil is used in production of lipstick, cosmetic soap, hydration creams, skin treatment creams for burned skin, while it is used medically to alleviate the muscle pains. The squid extracted oil may be a proper alternative for the production and formulation of such medicines.

Existence of DHA and EPA nutritious effects in the pregnant ladies food diets remove the babies' genetic disorders and prevent them from losing weight which indicate the importance of such compounds in social nutrition security and health. Towfighian *et al.* 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<sup>[13]</sup>. Therefore, the squid oil is a new source rich of such fatty acids which shall highly be used in this field of industry.

Nutrition studies during the past decades on the importance of highly unsaturated fatty acids express the same role in improvement of the nutrition metabolism of aquatic in which the studies of Watanabe *et al.* may be mentioned<sup>[6]</sup>. According to the studies as well as the reports of such scholars, squid 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 larva feeding quotas to decrease the mortality and increase the 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 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<sup>[16,17]</sup>. However, their percentages of monounsaturated fatty acid content as reported by Sorgeloos *et al.*<sup>[18]</sup>, as well as the report in Artemia International LLC<sup>[17]</sup> is equal to the same extracted level out of squid. Such equality of results indicates that the squid oil may be a proper alternative for such emulsions.

It 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 husbanding industries, pharmaceuticals, producing cosmetics, and balancing diets.

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

#### Conflict of interest statement

I declare that I have no conflict of interest.

#### Acknowledgements

The author is sincerely acknowledging the financial

assistance provided by Iranian Fisheries Research Organization [Grants Commission (Project Ref. No. 2–79–12–89009)].

## Comments

### Background

Squids known by the southern Iran are of invertebrate group which composes more than 5% of the Southern Iran fishing, *i.e.* hundreds of tons, which are considered as the subsidiary products of fishing. The omega–3 and omega–6 series unsaturated fatty acids are the essential compounds which play a major role for the cell's membrane structure, osmosis setting and synthesis of the endocrine glands hormones and activation of the body's immunity system in the body of the human and animals.

### Research frontiers

Today, several pharmaceutical compounds are traded globally such as life DHA, ultra omega, super omega, *etc.* as the malnutrition treatment tablets with high prices. The results in this work demonstrate that squid is considered for the first time as the new and rich source of omega–3 and omega–6.

### Related reports

In several study sources it has been reported that in pharmaceuticals, the shark liver oil is used in production of lipstick, cosmetic soap, hydration creams, skin treatment creams for burned skin, while it is used medically to alleviate the muscle pains. Nutrition studies during the past decades on the importance of fatty acids express the role of the same in improvement of the nutrition metabolism of aquatic in which the studies of Watanabe *et al.* may be mentioned. According to the studies as well as the reports of such scholars, squid may be roper alternative to enrich the emulsions used in this regard.

### Innovations and breakthroughs

The extraction method of marine fats is an interesting and economic method although its performance is not optimal.

### Applications

It 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 husbanding industries, pharmaceuticals, production of cosmetics, and balancing diets.

### Peer review

This is a valuable research work in which the author has demonstrated the utility of the squid. This study shows the true nutritional characteristics of this species as a source of essential fatty acids. The new research finding of this project is that the squid is introduced as a new and proper source rich in EPA, DHA to be used in a variety of industries.

## References

- [1] Kidd PM. Omega–3 DHA and EPA for cognition, behavior, and mood: clinical findings and structural functional synergies with cell membrane phospholipids. *Altern Med Rev* 2010; **12**(3): 207–227.
- [2] Léger P, Bengtson DA, Sorgeloos P, Simpson KL, Beck AD. The nutritional value of *Artemia*: a review. In: Sorgeloos P, Bengtson DA, Declair W, Jaspers E, editors. *Artemia research and its applications: 3. ecology, culturing, use in aquaculture*. Wetteren: Universa Press; 1987, p. 357–372.
- [3] Lemerond T. Fish oil, omega 3 fatty acids, EPA and DHA and the whole stinking story. 2008. [Online] Available from: <http://www.europharmausa.com/DBFiles/Documents/50.pdf> [Accessed on 22nd June, 2014]
- [4] Holub BJ. Omega–3 levels in fish: data quality, quantity and future. 2009 National Forum on Contaminants in Fish; 2009 Nov 4; Portland, USA.
- [5] Turchini GM, Mentasti T, Frøyland L, Orban E, Caprino F, Moretti VM, et al. Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta* L.). *Aquaculture* 2003; **225**(1–4): 251–267.
- [6] Watanabe T, Kiron V. Prospects in larval fish dietetics. *Aquaculture* 2011; **124**(1–4): 223–251.
- [7] Hafezieh M, Kamarudin SMS, Saad CRB, Mostafa Kamal AS, Agh N, Valinassab T, et al. Effects of enriched *Artemia urmiana* with HUFA on growth, survival, and fatty acids composition of the Persian sturgeon larvae (*Acipenser persicus*). *Iran J Fish Sci* 2010; **9**(1): 61–72.
- [8] Kanaza N, Boucaud–Camou E, Noel B. Effect of enriched natural diet ansurvival and growth of juvenile cuttlefish *Sepia officinalis* L. *Aquaculture* 2002; **203**(3–4): 293–310.
- [9] Rogers PJ, Appleton KM, Kessler D, Peters TJ, Gunell D, Hayward RC, et al. No effect of n–3 long chain Polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomized controlled trial. *Br J Nutr* 2008; **99**(2): 421–431.
- [10] Akbarzadeh Pasha H, Akbarzadeh Pasha A. [Writing instruction of gynecology, obstetrics and gynecology]. Tehran: Golban; 2013, p. 633–666. Persian.
- [11] Azgoli Gh, Selselei E, Alavi Majd H. The intensity of premenstrual syndrome and associated factors in students of beheshti University of Medical Sciences. *J Shahid Beheshti Univ Med Sci* 2010; **20**(68): 1–5.
- [12] Rocha Filho EA, Lima JC, Pinho Neto JS, Montarroyos U. Essential fatty acids for premenstrual syndrome and their effect on prolactin and total cholesterol levels: a randomized, double blind, placebo–controlled study. *Reprod Health* 2011; **8**: 2.
- [13] Towfighian T, Koushki A, Rakhshani M. [Studying the effect of omega fatty acids on the pre–menstruation signs, 15th series]. *Zanan va Zayeman Magazine* 2012; **32**: 75–83. Persian.
- [14] Suzakawa M, Abbey M, Clifton P, Nestel PJ. Enhanced capacity of n–3 fatty acid enriched mocrphages to oxidize low density lipoprotein mechanisms and effects of antioxidant vitamins. *Atherosclerosis* 1996; **124**(2): 157–169.
- [15] Nardini M, D'Aquino M, Tomassi G, Gentili V, Di Felice M, Scaccini C. Dietary fish oil enhances plasma and LDL oxidation modification in rats. *J Nutr Biochem* 1995; **6**: 474–480.
- [16] Han K, Geurden I, Sorgeloos P. Fatty acid changes in enriched and subsequently starved *Artemia franciscana* nauplii enriched with different essential fatty acids. *Aquaculture* 2001; **199**: 93–105.
- [17] Artemia International LLC. Purveyors of quality artemia cysts. Fairview: Artemia International LLC. [Online] Available from: <http://www.artemia–international.com/default.asp> [Accessed on 22nd June, 2014]
- [18] Sorgeloos P, Dhert P, Candreva P. Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture* 2001; **200**(1–2): 147–159.