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*International Journal of Current Research*
*Vol. 8, Issue, 09, pp.38517-38525, September, 2016*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

**ISSN: 0975-833X**

## RESEARCH ARTICLE

# EXTRACTION AND EVALUATION OF FISH BODY OIL FROM THREE DIFFERENT LOW VALUE FISHES OF PARANGIPETTAI COAST, TAMIL NADU

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**ARTICLE INFO**

***Article History:***
Received 27th June, 2016
Received in revised form
18th July, 2016
Accepted 10th August, 2016
Published online 30th September, 2016

***Key words:***

Fatty acids,
Amino acids,
Proximate composition,
Fish body oil,
*Sardinella fimbriata*.

**ABSTRACT**

The fish body oil was extracted from the tissues of *Leiognathus* spp., *Trichiurus savala* and *Sardinella fimbriata* employing four different extraction methods, namely, Bligh and Dyer, Modified Bligh and Dyer, Mcgill and Moffat and Direct streaming (boiled tissues were then pressed using Fish Oil Extractor, designed in our laboratory). Fatty acid composition in different fishes using different methods, amino acid composition of crude oil by DS method and amino acid composition of crude oil extracted from *Sardinella fimbriata* in various methods were done and the results were explained in desk and in graphically. The result shows the superiority of the fishes in human health.



**Citation: Anandganesh, E., Nazar, A.R., Marichamy, G., Sunithadas, Gowtham, K. and Shanker, S. 2016.** "Impaction of maxillary first primary molar- A rare case report", *International Journal of Current Research*, 8, (09), 38517-38525.

## INTRODUCTION

Fish oil is an excellent dietary sources, rich in essential fatty acids, especially Polyunsaturated Fatty Acid (PUFA) in the form of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) (Kim *et al*., 2006, NLM, 2014; Pike and Jackson, 2010). Approximately one-third of the fatty acids present in fish oil are omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), and it is these fatty acids that have been linked to potential health benefits (Harris, 2004; Kidd, 2013; Rizliya and Mendis, 2014). There was increasing evidence for the many beneficial health effects connected with the consumption of the unique long-chain polyunsaturated ω-3 fatty acids (n-3 LC-PUFA) almost exclusively found in fish and other marine organisms (Bang, Dyerberg, and Nielsen, 1971; Herold and Kinsella, 1986; Tocher, 2015). Essential Fatty Acids (EFAs) are those which are not synthesized in human body, namely ω-3 (n-3) and ω-6 (n-6). Some fishes such as herring, mackerel, salmon, sardines and tuna have a fairly good quantity of these compounds (Harris, 2004).

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Because of its wide applications, the quality and stability of fish oil have gained more importance. The production of the fish oil deals with the separation of lipids from other constituents of the fish. Fish oil is produced by several methods, including physical fractionation (Hirata *et al*., 1993), low temperature solvent fractionation (Moffat *et al*., 1993) and supercritical fluid extraction (Dunford *et al*., 1997). However, the conventional process compromises of cooking, pressing and centrifuging to recover the oil from miscella, is the common method used to produce fish oil (Bimbo, 1990; Pigott and Tucker, 1990). Differences in lipid yield between various methods are often shown to correlate with the extraction efficiency of the polar lipids. This, in turn, is dependent on the polarity of the organic solvent used for extraction. Accordingly, mixtures of polar and non-polar solvents are generally used for efficient extraction of total lipids (De Boer, 1988; Phillips *et al.,* 1989). The conventional method of extraction is so far considered as the finest extraction process due to its winsome quality such as higher yield, economically viable, less time consumption etc. Moreover other methods involves more time consuming and are critical due to the off odor and flavor of solvents. Apart from fatty acids, amino acids are also present in fish oil, contributing to its beneficial

effect. Fish contains significant amounts of all essential amino acids, particularly lysine which is relatively poor in cereals (FAO, 2005). Even though amino acids are present in fish oil, they are not completely explored and there is no clear evidence up to date about the amino acid composition of fish oil. The fish oil contains reasonable quantities of amino acids and can be extracted along with the fatty acids during the process of extraction. Extensive studies have been carried about for the importance of fish oil in health point of view. Little work has been done pertain to the extraction of fish oil from trash fish and other low value fishes. In this backdrop the present study was planned to extract fish body oil from trash fishes and low value fishes such as *Leiognathus* spp. (Silver belly), *Trichiurus savala* (ribbon fish) and *Sardinella fimbriata* (lesser sardine) by four different extraction methods and to check the yield of fish oil between the methods and so as to exploit the fatty acid and amino acid composition present in these trash fishes.

## MATERIALS AND METHODS

The trash and low valued fishes such as *Leiognathus* spp., *Trichiurus savala* and *Sardinella fimbriata* were collected from fish landings of Muddasalodai, Parangipettai (lat.$11^0$29'N; long.$79^0$46'E). The fishes were identified with the help of FAO Species Identification Guide for Fishery Purposes (FAO, 1974).

### Extraction of Oil

The fishes were washed thoroughly in running water for the removal of sand and external debris; scales, head, fins, spines, digestive system and excretory system were removed and the tissues alone were taken for extraction of oil. The tissues were subjected for extraction of oil by different methods as follows (a) Bligh and Dyer method (Bligh and Dyer, 1959), (b) Modified Bligh and Dyer method **(**i.e 1:1.5 V/V methanol: chloroform**)**, (c) McGill and Moffat method (McGill and Moffat, 1992) and **(**d**)** Direct steaming method (boiled tissues were then pressed using Fish Oil Extractor, designed in our laboratory). The filtered oil was stored separately in opaque dark bottle and placed in deep freezer at -20°C.

### Estimation of fatty acids

Fatty acid in the samples were identified following the procedure outlined by Miller and Berger (1985) and quantified as methyl esters in NEON II Gas chromatography instrument (ASHMACO) equipped with Flame Ionization Detector (FID) and DEGS (10%) column. The oven temperature was set at 180°C and flashed with carrier gas ($N_2$) at rate of 3 ml/min

### Estimation of Amino acids

The fish oil was precipitated by heating and the residues was dissolved in known volume of 10% iso-propanol and centrifuged at 1000rpm for 30minutes. The supernatant was taken for amino acid estimation. The HPLC conditions were adapted from Herrera *et al.* (2005) and Cambra *et al.* (2009). Briefly, mobile phases were prepared by mixing ACN and water (1:5), both containing 5mM citric acid adjusted at pH 6.5 with sodium hydroxide. A gradient elution from 5 to 30 % ACN in 30 min, followed by an increase from 30 to 50% ACN in another 5min, was used. Detection was performed at 340nm. In all cases, 20µl was injected at a flow rate of 1ml/min. The HPLC used was Agilent 1100 series with UV-vis variable multiwave length detector, quaternary pump and Kromasil C18 column (250 mm × 4 mm, 5 µm particle size (thermostated column).

## RESULTS

The fish body oil was extracted from the tissues of *Leiognathus* spp., *Trichiurus savala* and *Sardinella fimbriata* employing four different extraction methods, namely, Bligh and Dyer, Modified Bligh and Dyer, Mcgill and Moffat and Direct streaming. The average yield of oil extracted using different extraction techniques, are presented in Table 1. Analysis of Variance (one way) showed significant variation between the fish species.Between fish species, there was significant difference in the yield of fish oil. Among the four methods, higher yield was obtained from *Sardinella fimbriata* followed by *Trichiurus savala* and *Leiognathus* spp.

**Table 1. Average yield of fish oil from *Leiognathus* spp., *Trichiurus savala* and *Sardinella fimbriata* employing different extraction methods**

| S.No | Fishes (1000 g of tissue) | B&D (ml) | B&D* (ml) | M&M (ml) | DS (ml) |
|---|---|---|---|---|---|
| 1 | *Leiognathus* spp. | 84 ± 2.0 | 86 ± 5.0 | 82 ± 3.5 | 86 ± 3.0 |
| 2 | *Trichiurus savala* | 92 ± 2.8 | 93 ± 4.0 | 89 ± 4.0 | 93 ± 3.5 |
| 3 | *Sardinella fimbriata* | 113 ±2.3 | 119 ± 4.6 | 105 ± 3.8 | 130 ± 4.5 |

**Table 2. ANOVA (Single Factor) for oil extracted using Bligh & Dyer method**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 1346 | 2 | 673 | 117.8634 | 1.53E-05 | 5.143253 |
| Within Groups | 34.26 | 6 | 5.71 | | | |
| Total | 1380.26 | 8 | | | | |

**Table 3. ANOVA (Single Factor) for oil extracted using Modified Bligh & Dyer method**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 1814 | 2 | 907 | 43.77413 | 0.000264 | 5.143253 |
| Within Groups | 124.32 | 6 | 20.72 | | | |
| Total | 1938.32 | 8 | | | | |

This disparity was largely due to the difference in proximate composition between fish species. 1000g of fish tissues of *Leiognathus* spp. produced 84 ± 2.0 ml, 86 ± 5.0 ml, 82 ± 3.5 ml and 86 ± 3.0 ml of crude fish oil by Bligh and Dyer (BandD), Modified Bligh and Dyer (BandD*), Mcgill and Moffat (MandM) and Direct Steaming (DS) methods respectively. An average of 1000g of *Trichiurus savala* fish tissues produced 92 ± 2.8 ml, 93 ± 4.0 ml, 89 ± 4.0 ml and 93 ± 3.5 ml of crude fish oil by B and D, B and D*, Mand M and DS methods respectively. An average of 1000g of *Sardinella fimbriata* fish tissues produced 113 ± 2.3 ml, 119 ± 4.6 ml, 105 ± 3.8 ml and 130 ± 4.5 ml of crude oil by BandD, BandD*, MandM and DS methods respectively. Analysis of Variance (one way) showed significant variation between the fish species and the results were shown in Table 2, 3, 4 and 5.

**Proximate composition of the fishes**

Lipid content extracted from the fish muscles varied between species (Table 1). The details of fatty acid composition of crude fish oil are arranged in Table 6.

Among all the four methods, from the three fish species saturated fatty acid (SFA) formed the maximum, ranging from 29.064 to 50.726 % (w/w) of total FA, followed by monounsaturated fatty acid (MUFA) ranging from 16.31 to 26.45 % (w/w) of total FA and polyunsaturated fatty acid (PUFA) contents ranging from 10.192 to 17.617 % (w/w) of total FA.

The total saturated fatty acids in the crude fish oil extracted from *Leiognathus* spp., *Trichiurus savala* and *Sardinella fimbriata* employing four different methods namely BandD, BandD*, MandM and DS accounted to 37.623, 38.181, 37.374, 41.498; 31.419, 30.018, 29.064, 32.696; 42.515, 45.461, 49.11 and 50.726 % w/w of oil, respectively. The total monounsaturated fatty acids in the crude fish oil of all three fish species (*Leiognathus* spp., *Trichiurus savala* and *Sardinella fimbriata*) employing four different methods namely BandD, BandD*, MandM and DS accounted to 20.458, 21.021, 19.33, 23.15; 21.885, 16.31, 19.101, 19.537; 23.072, 26.45, 26.23 and 25.25 % w/w of oil, respectively. The total polyunsaturated fatty acids in crude fish oil obtained from

**Table 4. ANOVA (Single Factor) for oil extracted using Mcgill and Moffat method**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 1390 | 2 | 695 | 19.53619 | 0.000168 | 3.885294 |
| Within Groups | 426.9 | 12 | 35.575 | | | |
| Total | 1816.9 | 14 | | | | |

**Table 5. ANOVA (Single Factor) for oil extracted using Direct Steaming method**

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 3354 | 2 | 1677 | 121.2289 | 1.41E-05 | 5.143253 |
| Within Groups | 83 | 6 | 13.83333 | | | |
| Total | 3437 | 8 | | | | |

**Table 6. Fatty acid composition of fish oil from *Leiognathus* spp., *Trichiurus savala* and *Sardinella fimbriata* by different methods (w/w %)**

| Carbon chain | *Leiognathus* spp. | | | | *Trichiurus savala* | | | | *Sardinella fimbriata* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (B&D) | (B&D)* | (M&M) | (DS) | (B&D) | (B&D)* | (M&M) | (DS) | (B&D) | (B&D)* | (M&M) | (DS) |
| SFAs | | | | | | | | | | | | |
| C12:0 | 0.301 | 0.182 | - | 0.401 | 0.446 | 0.514 | - | 0.712 | 0.231 | 0.241 | 0.08 | 0.176 |
| C13:0 | 0.11 | 0.344 | 0.21 | 0.641 | 0.39 | 0.81 | 0.674 | 1.504 | - | - | - | - |
| C14:0 | 1.212 | 0.955 | 0.654 | 1.554 | 0.775 | 0.957 | 0.548 | 1.222 | 0.994 | 1.08 | 6.45 | 6.21 |
| C15:0 | 0.99 | 1.14 | 1.56 | 2.14 | 1.07 | 0.985 | 1.02 | 1.547 | 1.43 | 1.01 | 4.95 | 5.1 |
| C16:0 | 19.78 | 21.84 | 17.54 | 22.47 | 17.167 | 16.961 | 16.423 | 18.54 | 18.27 | 21.01 | 10.01 | 11.45 |
| C17:0 | 4.58 | 4.35 | 3.54 | 5.45 | 2.81 | 1.854 | 2.356 | 2.147 | 10.65 | 9.04 | 10.45 | 10.56 |
| C18:0 | 5.96 | 6.8 | 8.014 | 6.07 | 3.121 | 4.22 | 3.214 | 3.98 | 4.15 | 6.87 | 8.89 | 9.67 |
| C19:0 | 0.33 | 0.59 | 1.24 | 0.94 | 0.56 | 0.115 | - | 0.243 | - | - | - | - |
| C20:0 | 0.49 | 0.98 | 0.271 | 1.112 | 0.61 | 1.38 | - | 0.845 | 0.94 | 1.09 | 1.76 | - |
| C22:0 | 0.87 | 0.44 | 0.54 | - | 1.03 | - | 1.32 | - | 0.67 | 0.94 | 1.56 | 1.71 |
| C23:0 | 2.1 | 1.53 | 1.09 | 0.21 | 3.04 | 0.581 | 2.546 | 1.339 | 3.76 | 3.17 | 3.73 | 4.7 |
| C24:0 | 0.9 | 1.49 | 2.715 | 0.51 | 0.4 | 1.641 | 0.963 | 0.617 | 1.42 | 1.01 | 1.23 | 1.15 |
| Sum of SFAs | 37.623 | 38.181 | 37.374 | 41.498 | 31.419 | 30.018 | 29.064 | 32.696 | 42.515 | 45.461 | 49.11 | 50.726 |
| MUFAs | | | | | | | | | | | | |
| C14:1ω-5 | 0.04 | 0.14 | - | - | 0.07 | - | 0.09 | - | 0.13 | 0.14 | 0.14 | 0.11 |
| C14:1ω-4 | 0.321 | 0.481 | 0.74 | 0.61 | 0.6 | - | 0.691 | 3.887 | 4.3 | 5.01 | 5.89 | 5.56 |
| C16:1ω-7 | 7.787 | 8.541 | 8.05 | 8.97 | 9.565 | 7.07 | 8.21 | 4.11 | 1.952 | 2.01 | 2.17 | 1.50 |
| C18:1ω-7 | - | - | - | 0.12 | - | - | - | - | - | - | - | - |
| C18:1ω-9 | 12.31 | 11.89 | 10.54 | 13.45 | 11.65 | 9.24 | 10.11 | 11.54 | 16.69 | 19.29 | 18.03 | 18.08 |
| Sum of MUFAs | 20.458 | 21.021 | 19.33 | 23.15 | 21.885 | 16.31 | 19.101 | 19.537 | 23.072 | 26.45 | 26.23 | 25.25 |
| PUFAs | | | | | | | | | | | | |
| C18:2ω-3 | - | 0.04 | - | - | - | - | - | - | | | | |
| C18:2ω-6 | 1.721 | 2.04 | - | 1.09 | 1.756 | 2.18 | 1.12 | 1.74 | 1.857 | 1.556 | 1.821 | 1.845 |
| C18:3ω-3 | 2.785 | 2.218 | 4.006 | 2.04 | 2.712 | 3.157 | 2.54 | 4.21 | 3.96 | 3.966 | 2.947 | 3.659 |
| C20:4ω-6 | 0.97 | 1.31 | 0.54 | 0.94 | 1.34 | 0.653 | 0.65 | 0.547 | - | 0.73 | 1.76 | 1.67 |
| C20:5ω-3 | 1.955 | 2.05 | 1.655 | 1.965 | 2.435 | 1.331 | 2.54 | 2.004 | 2.564 | 1.795 | 1.654 | 1.803 |
| C22:5ω-3 | 1.01 | 0.806 | 1.007 | 1.21 | 0.98 | 0.45 | 1.54 | 2.148 | - | 1.60 | 2.75 | 2.75 |
| C22:6ω-3 | 3.675 | 4.2 | 2.984 | 3.05 | 2.07 | 2.712 | 1.954 | 0.985 | 3.545 | 3.896 | 5.97 | 5.99 |
| Sum of PUFAs | 12.106 | 12.664 | 10.192 | 10.295 | 11.293 | 10.483 | 10.344 | 11.634 | 12.226 | 14.043 | 16.902 | 17.617 |
| Unknown | 29.813 | 28.134 | 33.104 | 25.057 | 35.403 | 43.189 | 41.491 | 36.133 | 22.153 | 14.046 | 7.758 | 6.407 |

*Leiognathus* spp., *Trichiurus savala* and *Sardinella fimbriata* employing four different methods namely, BandD, BandD*, MandM and DS; accounted to 12.106, 12.664, 10.192, 10.295; 11.293, 10.483, 10.344, 11.634; 12.226, 14.043, 16.902 and 17.617 % w/w of oil, respectively. The Palmitic acid was the predominant fatty acid in the saturated content of the fish oil, accounting for around 50% of all saturated fatty acids.

The predominant fatty acids in the monounsaturated and polyunsaturated content of the fish oil were oleic acid (C18:1ω-9) and α-linolenic acid (C18:3ω-3) and docosahexaenoic acid (C22:6ω-3) respectively. According to fatty acid composition of fish oil from all three fishes by different extraction methods; the fatty acid profile of the crude fish oil obtained from *Sardinella fimbriata* extracted by Direct

**Table 7. Amino acid composition of Crude fish oil**

| Amino acids | *Leiognathus* spp. (DS) | *Trichiurus chavala* (DS) | *Sardinella fimbriata* (DS) | *Sardinella fimbriata* (MM) | *Sardinella fimbriata* (B&D) | *Sardinella fimbriata* (B&D)* |
|---|---|---|---|---|---|---|
| Non Essential amino acids (in µ/g) | | | | | | |
| Alanine | 0.154 | 0.579 | 0.896 | Traces | Traces | 0.906 |
| Arginine | 0.121 | 0.145 | 0.958 | 0.565 | 0.675 | 0.9867 |
| Asparagine | - | 0.818 | - | - | - | - |
| Aspartic acid | 0.147 | - | 1.01 | 0.854 | 0.928 | 1.215 |
| Cysteine | 0.321 | Traces | - | Traces | traces | - |
| Glutamic acid | 0.845 | Traces | 2.98 | 2.02 | 1.38 | 3.016 |
| Glutamine | traces | - | 3.11 | 2.64 | 2.985 | 3.25 |
| Glycine | - | - | 1.14 | 0.964 | 1.007 | 1.305 |
| Proline | 0.654 | - | 1.12 | 0.514 | 0.471 | 1.215 |
| Serine | - | 0.654 | 1.09 | 0.424 | 0.321 | 1.218 |
| Tyrosine | 0.056 | 0.565 | 0.896 | 1.89 | 1.983 | 0.945 |
| Total Non EAA | 2.298 | 2.761 | 13.2 | 9.871 | 9.75 | 14.0567 |
| Essential amino acids (in µ/g) | | | | | | |
| Isoleucine | 0.145 | 0.212 | 1.156 | 1.565 | 1.056 | 1.234 |
| Histidine | 0.301 | 0.788 | 0.212 | 1.565 | 0.995 | 0.256 |
| Leucine | 0.787 | 0.435 | 0.990 | 1.787 | 1.447 | 1.05 |
| Methionine | - | 0.454 | Traces | 1.77 | - | - |
| Lysine | 0.801 | 1.045 | 0.998 | 1.889 | 1.898 | 1.121 |
| Phenylalanine | - | 0.067 | 1.056 | 1.567 | 1.897 | 1.165 |
| Tryptophan | .121 | 0.112 | 1.12 | 0.499 | 0.123 | 1.215 |
| Threonine | traces | 0.079 | 1.14 | 0.167 | 0.154 | 1.215 |
| Valine | 1.012 | 0.121 | 1.78 | 0.96 | 0.990 | 1.907 |
| Taurine | - | - | - | - | - | - |
| Total EAA | 3.167 | 3.313 | 8.452 | 11.769 | 8.56 | 9.163 |

EAA – Essential Amino Acids, Non EAA – Non Essential Amino Acids.

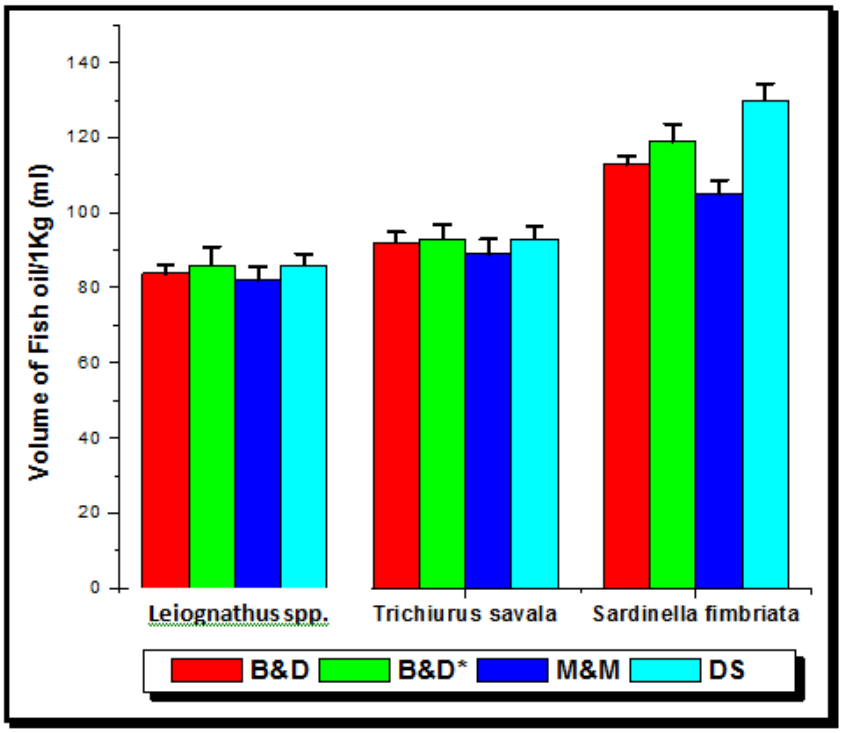


**Fig. 1. Extraction of fish oil by various methods**

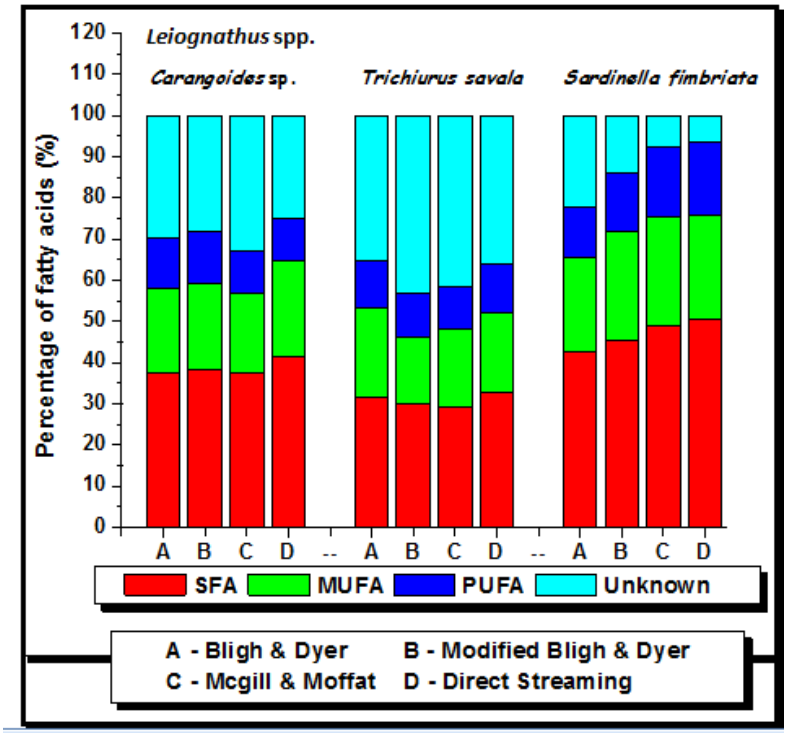


**Fig. 2. Fatty acid composition of fish oil from various fishes by various methods**

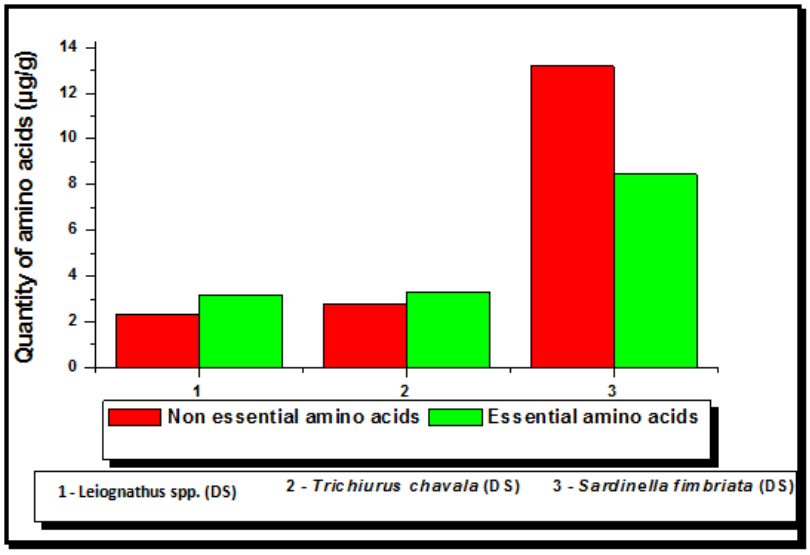


**Fig. 3. Amino acid composition of crude fish oil extracted by DS method**

Steaming showed the best results with higher concentration of unsaturated fatty acids, Docosa hexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) content was 5.99 and 1.803 % (w/w) respectively. At the same instance the saturated fatty acids also accounted for 50% of total fatty acids of the crude oil extracted from *Sardinella fimbriata* by direct steaming method.

**Amino acid composition of fish oil**

The amino acid composition of the crude fish oil from all three fish species extracted by direct steaming method and from *Sardinella fimbriata* by other three extraction methods was analyzed by high performance liquid chromatography. This forms a baseline work for the analysis of amino acid from fish oil. Except Asparagine, Cysteine and Methionine, all other non essential and essential amino acids were recorded in all the samples. The amino acid composition of the crude fish oil and refined oil was summarized in table 7. The maximum quantity of amino acid was recorded in the fish oil obtained from *Sardinella fimbriata* by Modified Bligh and Dyer method, with a total of 14.0567µg/g (of non essential amino acids) and 9.163µg/g (of essential amino acids). The amino acid profile of the sardine oil from all four extraction methods was more or less in similar quantity and composition i.e total non essential amino acids are 9.871, 9.75, 14.0567 and 13.2µg/g and

essential amino acids 11.969, 8.56, 9.163 and 8.452µg/g for MandM, BandD, BandD* and DS extraction methods respectively. The data were statistically treated with the help of computer software packages such as MS-Excel (MS OFFICE 2007) and Origin (ver. 6.0).

evaluate the difference in the lipid levels (De Koning *et al.,* 1985; Kates, 1986; Randall *et al.,* 1991). Mcgill and Moffat method for extraction lipids and body oil triglycerides was also a popularly used technique for extraction of body oil (Mcgill and Moffat, 1992).

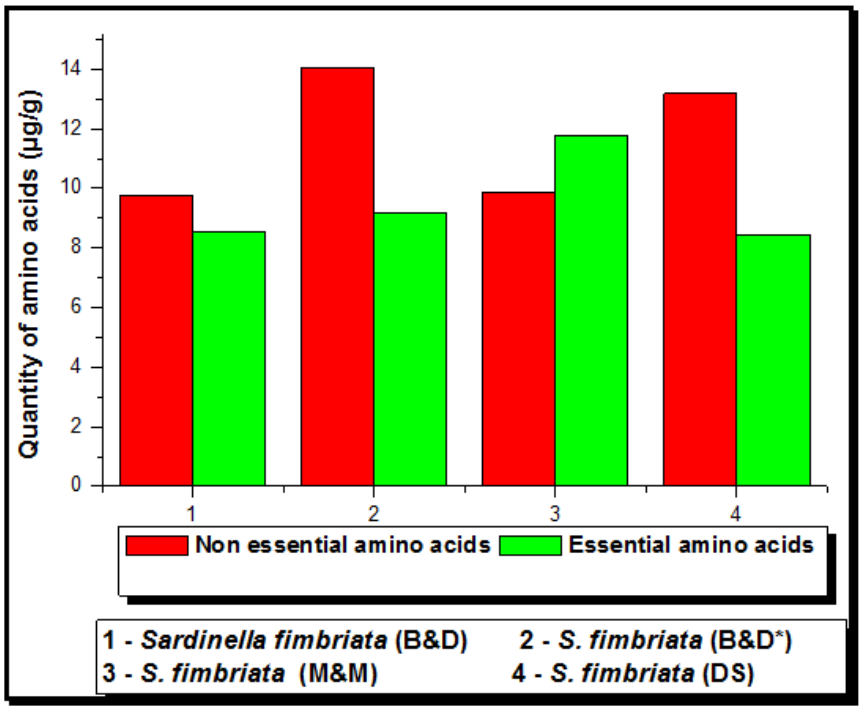


**Fig. 4. Amino acid composition of crude fish oil extracted from *Sardinella fimbriata* by various methods**

## DISCUSSION

During last few years, Fish oil is being approved for human consumption as food supplement and as an ingredient in food. Several hygienic and scientific measures were employed, so as to improve the quality of fish oil, in conventional meal plants and in other commercial processes, where fish oil is a byproduct. The fish species selected in this study showed profound variation in lipid content. Four extraction methods which are widely used were employed and comparisons were made. There was significant difference in the yield of oil extracted in such a way that *Sardinella fimbriata* samples gave higher yields from all four extraction methods than that of *Leiognathus* spp. and *Trichiurus savala* samples. This disparity was largely between the samples and was not high between the methods.

The dissimilarity in the yield between species was mainly due to variation in texture and proximate composition coupled with other factors such as gender and age, location; species origin characteristics such as spawning and migration seasons, seasonal variation in composition of plankton and also some environmental conditions such as temperature (Borgstrom, 1961; Leu *et al.,* 1981; Huss, 1988 and Shirai *et al*., 2002). The Bligh and Dyer method, using chloroform and methanol, is generally considered to be the best method for polar lipid extraction. A minor modification were employed in Bligh and Dyer by altering the ratio of the solvents and is termed as Modified Bligh and Dyer method, was also commonly used to

Direct steaming method is considered as a good old traditional and economic technique for extraction of oil. The present study was undertaken to analyse the proficiency in yield between the above said methods, proved that oil extracted by direct steaming method ensured higher yield than that of other methods. The present experiment supports the suggestions of Sunarya *et al*. (1991) that oil extraction by direct steaming method is easier, cheaper, quicker and is affordable to laymen and rural communities. It has been reported that solvent extraction methods are not employed for the preparation of vitamin A oil from fish, because the equipment itself is expensive and the recovery of the solvent is not satisfactory (Tanikawa, 1971). Hall (1992) emphasised that direct steaming method at 80-85°C is a simple and economical technique that ensures viable results.

Bligh and Dyer method was so far reported as the effective method for extraction of lipids (Nuraini *et al*., 2008) and in the present study, the yield from Bligh and Dyer method is 84 ± 2ml/Kg from *Leiognathus* spp., 92 ± 2.8ml/Kg from *Trichiurus savala* and 113 ± 2.3ml/Kg from *Sardinella fimbriata*, whereas Direct method gave better results than the other three methods with an yield of 86 ± 3ml/Kg from *Leiognathus* spp., 93 ± 3.5ml/Kg from *Trichiurus savala* and 130 ± 4.5ml/Kg from *Sardinella fimbriata*. In the present study the oil was prepared from the fish muscles, in which the breakdown of cell requires only less environmental shock which was easily afforded by direct steaming method. Steaming will coagulate the protein of fish, so that liquids and solids can be mechanically separated

and fat cells are also disrupted, releasing oil into the liquid phase (Bimbo, 1990). Bligh and Dyer method and other chemical extraction methods will definitely give higher yield than the conventional method, when fish liver was chosen, since rupture of the liver cells requires more shocks, where chemical method may be the better choice (Immanuel *et al*., 2002). There are evidences which supports our study proving that there also various methods which will yield in better lipid quantities other than Bligh and Dyer method like electrolyzed cathode water method (Toge and Miyashita, 2003). Bligh and Dyer method provides higher yield only in the abundant presence of polar lipids (De Boer, 1988 and Phillips *et al.,* 1989). Since the yield of BandD method is not high in the present study, which provides the information of the presence of low polar lipids in all the selected fishes. Rajion (1985) described a modified Folch *et al*. method (1957), using chloroform: methanol (2:1, v/v) solvent system, similarly Modified BandD method was carried out in triplicate by using methanol: chloroform (1:1.5, v/v) solvent system to extract lipids which resulted in better quantities of yield than the standard method due to the fact of presence of more non polar lipids. Mcgill and Moffat method resulted in lowest quantities of yield from all three fishes since it is highly specific for extraction of lipids from fish liver, especially for triglycerides extorting (Mcgill and Moffat, 1992). The oil extracted from *Sardinella fimbriata* was 10-13%, which was almost double the quantity of oil extracted from *Sardinella lemuru* (>6%) by Khoddami *et al*. (2009) in Malaysian waters.

**Fatty acid composition of crude fish oil from three fishes by different methods**

Estimation of fatty acid profile is one of the major criteria to assess the quality of the oil produced. In the present study, fatty acid profile of the crude fish oil produced from all three fish species by four extraction methods were analysed. The fatty acid profiles showed that saturated fatty acid of the crude fish oil from *Leiognathus* spp. were 37.623, 38.181, 37.374 and 41.498 %, those from *Trichiurus savala* were 31.419, 30.018, 29.064 and 32.696%, and those from *Sardinella fimbriata* were 42.515, 45.461, 49.11 and 50.726% for the samples extracted by Bligh and Dyer, Modified Bligh and Dyer, Mcgill and Moffat and Direct methods respectively. Sunarya *et al*. (1991) also explained that the level of saturated fatty acid varies depending on the extraction method adopted. Sathivel *et al*. (2003) extracted oil from various fishes like sardines, herring and catfish and analysed the fatty acid profile. They made a comparative account on saturated fatty acids in commercially available standard menhaden oil (33.3%) and cod liver (24.6%) with that of oil produced from sardines, herrings and cat fish, which were to the tune of 31.3%, 22.8% and 32.9%, respectively. Comparatively, the result of the present investigation yield more saturated fatty acids by all four extraction methods for oil obtained from *Sardinella fimbriata* and *Leiognathus* spp.; whereas for oil produced from *Trichiurus savala*, the saturated fatty acid were almost equal to that of standard Menhaden oil. The FA composition of fish lipids was highly dependent on a number of factors, among which the diets of the fish play a substantial role in health benefits. (Chanmugam *et al.,* 1986 and Morris *et al*., 1995, Rizliya and Mendis, 2014). The predominant saturated fatty acids in all the samples were palmitic acid (C16:0) followed by margaric acid (C17:0), stearic acid (C18:0) and tricosanic acid (C23:0). The highest level of C16:0 (22.47 %) was recorded in *Leiognathus* spp oil produced by Direct Steaming; whereas the highest levels of C17:0 (10.65 %), C18:0 (9.67%) and C23:0 (4.7%) were recorded in sardine oil obtained from BandD and DS methods. The results of the present investigation about the predominant SFAs in the oil samples were supported by the works of Chantachum *et al*. (2000), Sathivel *et al*. (2003), Barakat *et al*. (2007), Nuraini *et al*. (2008), and Khoddami *et al*. (2009). The monounsaturated fatty acid levels in the sardine oil ranged from 23.072 to 26.45% between the extraction methods. Similar levels of monounsaturated fatty acids was reported in sardine fish oil by Khoddami *et al*. (2009), in which the estimated range was 26.22 to 32.30%. The dominant MUFAs in all samples were oleic acid (C18:1ω-9) and palmitoleic acid (C16:1ω-7), in which the highest levels of oleic acid (C18:1ω-9) was 19.29%, obtained from *Sardinella fimbriata* by MandM method and for palmitoleic acid (C16:1ω-7) was 9.565% obtained from *Trichiurus savala* by BandD method. MUFA seems to be proficiently extracted by methods other than direct steaming method. The abundance of oleic acid (C18:1ω-9) and palmitoleic acid (C16:1ω-7) in the fish oil samples were also reported Chantachum *et al*. (2000), Sathivel *et al*. (2003), Zuraini *et al*. (2006), Barakat *et al*. (2007), Nuraini *et al*. (2008) and Khoddami *et al*. (2009).

According to Ackman *et al*. (1963) oil extracted from marine fishes, polyunsaturated fatty acid was dominated. In the present study, the level of total polyunsaturated fatty acids ranged from 10.192 to 17.717 % in all three fish oil samples by all four extraction methods. Khoddami *et al*. (2009) reported higher levels of polyunsaturated fatty acids ranging from 22.67 to 26.39% from liver samples of *Sardinella lemuru*. Among Polyunsaturated fatty acids, docosahexaenoic acid content (5.97%) being higher, followed by α-linolenic acid (4.21%), docosapentaenoic acid (2.75) and EPA (2.564%) in all the extracts. The composition of docosahexaenoic acid content in the present study was comparatively higher than that of eicosapentaenoic acid in all the samples which is in line with works of Agren and Hanninen, (1993), Colin *et al*. (1993), Zuraini *et al*. (2006), Nuraini *et al*. (2008) and Khoddami *et al*. (2009). The EPA: DHA ratio has been suggested as a useful indicator for comparing relative nutritional values of fish oils. It was suggested that a ratio of EPA: DHA is 1:1–1.5 would constitute better for healthy human diet (Osman *et al*. 2001). The oil obtained from the three fish spp. had the EPA: DHA ratio within the recommended ratio. The distinctive difference of the SFAs, MUFAs and PUFAs content in sardine and other fish lipids might be attributed to proximate composition of species coupled with other factors such as seasonal changes and the changes in composition of plankton (Bandarra *et al*., 1997 and Meza *et al*., 1999).

**Amino acid composition of fish oil**

The amino acid composition of the fish oil was illustrated separately Table 7. The present study is a baseline work for the analysis of amino acid from sardine oil. Herrera *et al*. (2010) classified the vegetable oil according to their botanical origin using amino acid profiles by HPLC with UV-Vis detection, which is a pioneering study about amino acids for classifying

their origin. A study of this kind will throw light on the need to research on amino acid profile in fishes so as to compile a database to classify the origin of fishes. The presence of amino acids though in low levels might be attributed to the occurrence of glycoprotein associated with fish oil. According to Venolia *et al*. (1957) brownish colouration in oil was obtained not only due to oxidation of PUFA and polymerisation of oxidation products into macromolecular compounds, but also due to the interaction of oxidizing lipids and amine groups of protein. Therefore, the brownish yellow colour of crude sardine oil was also due to the presence of amino acids, accounting 21.652µg/g whereas for the refined oil it was only 12.598µg/g. Major amino acid present was valine (2.07µg/g) and threonine (1.26µg/g).

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