6. EXTRACTION AND EVALUATION OF FISH BODY OIL FROM LESSER SARDINES EMPLOYING DIFFERENT EXTRACTION PROCEDURES

6.1. Introduction

Fish oil is an excellent dietary source, 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). Essential Fatty Acids (EFAs) are those which are not synthesized in the 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). Production of fish oil from low value fishes has gained increased momentum in recent past because of its wider application prospects.

Fish oil when compared to terrestrial animal and vegetable oil, is characterised by a complex nature of saturated, unsaturated and polyunsaturated fatty acids (Immanuel et al., 2002; Adebiyi and Bawa, 2006). Production of high and pure grade fish oil acquired greater importance as it is considered as one of the main natural repository of omega-3 polyunsaturated fatty acids (PUFAs); which provides tremendous benefits to human health (Chow, 2000).

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). Various processing methods have been adopted for the extraction of fish oil from the liver and whole body, such as
Extraction and Evaluation of Fish body oil from Lesser sardines employing different Extraction procedures


The lesser sardine fishery along Indian coast is represented by 10 species among which seven species formed the bulk of its fishery, the remaining occurs sporadically and in stray numbers at certain fish landing centres (Bennet, 1965). Among this group, *Sardinella fimbriata*, *Sardinella gibbosa* and *Sardinella albella* are arrived in significant proportions than that of other species of lesser sardines. Commercial level production of fish oil are largely confined to economically important fishes like cod fishes and little effort has been directed towards extraction of fish oil from low value fishes such as lesser sardines.

Extensive studies were carried on the importance of fish oil in health point of view. Little work has been done pertain to the extraction of fish oil from low value fishes. In this backdrop the present study was planned to extract fish body oil from low value fishes such as lesser sardines, namely, *Sardinella fimbriata*, *Sardinella gibbosa* and *Sardinella albella* employing four different extraction methods so as to check the yield of fish oil obtained between the species.

6.2. Materials and Methods

Lesser sardines such as *S. fimbriata*, *S. gibbosa* and *S. albella* were collected from fish landings of Muttom, Kanyakumari district, Tamil Nadu, southwest coast of India (lat. 8°7’ N; long. 77°19’E) for a period of one year (October 2011 - September 2012). Since
these species are landed only during certain corners of the year, specimens of uniform size (at maximum length) from all three species were alone taken into consideration for the production of fish oil.

The fishes were identified with the help of FAO (Fish and Agricultural Organisation) Species Identification Guide for Fishery Purposes.

6.2.3. 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 & Moffat, 1992) and (d) Direct steaming method.

(a) **Bligh and Dyer Method (Bligh and Dyer, 1959)**

100g of homogenised fish tissues were weighed into beaker (Capacity 1 litre) to this 10ml of distilled water was added and mixed. Methanol:chloroform was added at the ratio of 1:2V/V and the mixture was thoroughly homogenized. The mixture was centrifuged at 2000rpm for 20 minutes at room temperature. The resultant aqueous layer was removed with the help of separating funnel. The chloroform fraction was evaporated using Rotatory evaporator and finally the yield of obtained oil was recorded.
(b) Modified Bligh and Dyer Method

Bligh and Dyer Method was modified and carried out for oil extraction, i.e 1:1.5 V/V Methanol:chloroform was added, mixed and the above mentioned procedure was repeated and the yield was recorded.

(c) McGill and Moffat Method (McGill & Moffat, 1992)

30g of anhydrous sodium sulphate was added to 100g of homogenized fish tissues. The mixture was homogenized for 3 minutes and centrifuged at 2000rpm for 20 minutes at room temperature. The resulting oil was separated from the aqueous layer using separating funnel and the yield was recorded.

(d) Direct Steaming method

About 1000 g of homogenized fish tissues was taken in a muslin bag and kept in steam boiler (Sakthi Instrument) at 70-80 °C for 30 minutes. The boiled fish tissues were then pressed with the aid of Fish Oil Extractor (designed in our laboratory and about to be patented) (Plate I), so as to remove the liquid content from the tissues (containing oil and water). Then the oil was separated from the water by centrifuging at 2000 rpm (REMI, C 24BL Cooling Centrifuge) for 15 minutes and further by using separating funnel. The filtered oil was stored separately in an opaque dark bottle and placed in deep freezer at -20 °C. These was repeated for 5 times and the average yield was calculated, which is expressed in percentage. The filtered oil was stored separately in opaque dark bottle and placed in deep freezer at -20°C.
6.2.4. Quality Assessment of Fish Oil

(a) Determination of moisture content

Moisture content was estimated following Indian Standard Institute (ISI) methodology (1974). Moisture content of the crude oil was estimated by calculating the loss in the mass of oil on heating at 105 °C under operating conditions specified.

5-10 g of fish crude oil was heated in an electric oven at 105 ± 1°C for 1 hour. Then, the sample was removed and subjected for cooling by placing in a desiccator containing Phosphorus pentoxide for 48 hours and then weighed. The process was repeated till the change in weight between two successive observations was not occurred and the moisture content was analysed, with the following formula:

\[
\text{Moisture} = \frac{W_1 \times 100}{W}
\]

Where,

\[W = \text{Initial weight of sample (g)}\]
\[W_1 = \text{Loss in weight of sample after desiccation (g)}\]

The results were expressed in percentage.

(b) Determination of Free Fatty Acid (FFA)

The FFA’s in the fish crude oil sample was calculated following the methodology of Cox and Pearson (1962).

Reagents used

(i) Ethyl alcohol

Freshly heated and neutralized 95% alcohol.
(ii) Phenolphthalein indicator solution

One gram of phenolphthalein dissolved in 100 ml of Ethyl alcohol.

(iii) 0.1 N Sodium hydroxide freshly prepared

10 g of crude oil sample was taken in a 250 ml conical flask. To this, 100 ml of freshly neutralized hot ethyl alcohol and 1 ml of phenolphthalein indicator solution were added and boiled for five minutes. This sample solution was titrated against 0.1 N sodium hydroxide till the end point. The procedure was repeated for 3 samples and the average reading was noted. From this the FFA was calculated using the formula given below:

\[
\text{FFA value (mg of NaOH/g)} = \frac{40VN}{W}
\]

Where,

\[V = \text{Average volume of sodium hydroxide used (ml)}\]

\[N = \text{Normality of the sodium hydroxide}\]

\[W = \text{Weight of the sample (g)}\]

(c) Estimation of iodine value

The iodine value of the fish crude oil sample was estimated using the methodology of Horowittx (1975).

Reagents used

(i) Hanus iodine solution

\[
13.6 \text{ g of iodine dissolved in 825 ml of heated and cooled glacial acetic acid. 25ml of this solution was titrated against 0.1 N sodium thiosulphate.}\]
3 ml of Bromine added in 200 ml of glacial acetic acid and 5 ml of this solution was taken and added with 10 ml of 15% potassium iodide solution and titrated against 0.1 N sodium thiosulphate.

The bromine solution required for doubling the halogen content of the remaining 800 ml of the above iodine solution was calculated as follows:

\[ X = \frac{B}{C} \]

Where,

- \( X \) – Volume of Bromine solution required doubling the halogen content,
- \( B \) – 800 × volume 0.1N sodium thiosulphate used for titrating iodine solution
- \( C \) – Volume 0.1N sodium thiosulphate used for titrating Bromine solution.

(ii) 15% Potassium iodide solution

(iii) 0.1% Sodium thiosulphate

(iv) 1% Starch

0.5 g of crude oil was weighed and taken in a conical flask. 10 ml of chloroform and 25 ml of Hanus iodine solution was added to the sample and mixed well. It is allowed to place in dark exactly for 30 minutes with anodic shaking. To this, 10 ml of 15% potassium iodide and 100 ml of distilled water was added and mixed thoroughly. The sample was then titrated against 0.1N sodium thiosulphate till the yellow solution became colourless. Few drops of starch solution (indicator) was added to this colourless solution and titrated again till the blue colour disappears. The same procedure was repeated for blank solution.
Calculation

\[
\text{Iodine Value (I}_2/100g) = \frac{(B-S) \times N \times 12.69}{\text{Weight of sample (g)}}
\]

Where,

- B – Volume of thiosulphate for blank (ml),
- S – Volume of thiosulphate for sample (ml) and
- N – Normality of thiosulphate solution

(d) Estimation of peroxide value

The peroxide value was estimated following the method of Cox and Pearson (1962).

Reagents used

(i) Solvent mixture

Glacial acetic acid was mixed with chloroform in 2:1 ratio.

(ii) 5% Potassium iodide solution

(iii) 1% Starch solution

(iv) N/500 Sodium thiosulphate solution

1 g of crude oil was taken in a clean test tube and to this 1 g of powdered potassium iodide and 20 ml of solvent mixture was added. The test tubes were boiled in a water bath for 1 minute. The content of the test tube was transferred quickly to a conical flask containing.

20 ml of 5% potassium iodide solution. The test tube was rinsed with 25 ml of distilled water and the same was poured into previous conical flask. The whole mixture was titrated against N/500 sodium thiosulphate till the yellow colour just disappeared. To this 0.5
ml of starch solution (indicator) was added and titrated till the blue colour just disappears. The same procedure was repeated for the blank solution.

**Calculation**

\[
\text{Peroxide Value (mEq/Kg)} = \frac{S \times N \times 1000}{g \text{ of sample}}
\]

Where,

\(S\) – Volume of thiosulphate for blank (ml)

\(N\) – Normality of thiosulphate solution

**(e) Determination of saponification value**

Saponification value was calculated using the method of Horowitx (1975).

**Reagents used**

(i) **0.5N Hydrochloric acid**

(ii) **Alcoholic potassium hydroxide**

40 g of potassium hydroxide dissolved in 1 litre of distilled alcohol. (Temperature was kept below 15 °C, when potassium hydroxide was dissolved).

(iii) **1% Phenolphthalein Indicator**

The crude oil sample was heated and filtered using a thin layer of non absorbent cotton so as to remove the impurities. About 4-5 g of filtered sample was added to a conical flask containing 50 ml of alcoholic potassium hydroxide. Blank was prepared separately by taking only 50 ml of alcoholic potassium hydroxide alone. An air condenser was connected to the flask and boiled for one hour. The flask was subjected to cool; 1 ml of indicator was added and titrated against 0.5N HCl until the pink colour just disappeared. The same was
repeated for the blank solution. The experiment is conducted in 3 replicates and the average titre values were recorded.

**Calculation**

\[
\text{Saponification value (mg/ KOH/g)} = \frac{28.5 \times (B - S)}{W}
\]

Where,

- \( B \) - Titre value of blank
- \( S \) - Titre value of sample and
- \( W \) - Weight of sample (g)

**Estimation of Specific Gravity (Immanuel et al., 2002)**

Specific gravity bottles were cleaned, dried and the initial weight of the empty bottles was noted (\( W_1 \)). These bottles were filled with distilled water at room temperature, closed with a stopper without allowing air bubble to enter in it. The weight of the bottle with water was recorded (\( W_2 \)). Followed to this, the water was renounced and the bottle was dried.

The bottle was filled with oil, closed and the weight was noted (\( W_3 \)). Then, the specific gravity of oil was calculated by the formula given below:

\[
\text{Specific Gravity of the oil sample} = \frac{W_3 - W_1}{W_2 - W_1}
\]

Where,

- \( W_1 \) = Weight of the empty specific gravity bottle
- \( W_2 \) = Weight of bottle + water
- \( W_3 \) = weight of bottle + oil
(g) Refractive Index of oil (Spectrometer – Hollow Prism Method)

To find the refractive index of the oil, the crude oil was transferred into a hollow prism, using minimum deviation method. The angle of minimum deviation was calculated using Spectrometer and Sodium vapour lamp, with wavelength of $5993\times10^{-10}$ m. The angle of prism was found to be 60° using the rotation.

$$\mu = \frac{\sin (A+D)/2}{\sin (A/2)}$$

Where,

- $A =$ Angle of prism
- $D =$ Angle of minimum deviation
- $\mu =$ refractive index at room temperature

(h) Observation of colour

The colour of the freshly prepared crude fish oil was observed by placing against luminescent light.

6.3. RESULTS

Yield of fish oil extracted from *Sardinella fimbriata*, *Sardinella gibbosa* and *Sardinella albella* employing various extraction methods

The fish body oil was extracted from the tissues of *S. fimbriata*, *S. gibbosa* and *S. albella* employing four different extraction methods, namely Bligh & Dyer, Modified Bligh & Dyer, Mccall & Moffat and Direct Steaming.

Between fish species, there was significant difference in the yield of fish oil. Among the four methods, higher yield were obtained from samples of *S. fimbriata*,
followed by *S. gibbosa* and *S. albella*. This disparity was largely due to the difference in proximate composition between fish species. 1000g of fish tissues of *S. fimbriata* produced 91±3.7 ml, 94±3.2 ml, 80±2.5 ml and 116± 6.1 ml of crude fish oil by Bligh & Dyer (B&D), Modified Bligh & Dyer (MB&D), Mcgill & Moffat (M&M) and Direct Steaming (DS) methods respectively (Fig. 6). An average of 1000g of *S. gibbosa* fish tissues produced 78±4 ml, 75±2.1 ml, 67±1.5 ml and 93±4.3 ml of crude fish oil by B&D, MB&D, M&M and DS methods respectively (Fig. 7). An average of 1000g *S. albella* fish tissues produced 63±1.5 ml, 65±1.5 ml, 53±1.6 ml and 84±3.5 ml of crude fish oil by B&D, MB&D, M&M and DS methods respectively (Fig. 8).

![Graph showing yield of fish oil obtained from S. fimbriata employing different extraction methods.](image)

**Fig.6.** Yield of fish oil obtained from *S. fimbriata* employing different extraction methods.
Fig. 7. Yield of fish oil obtained from *S. gibbosa* employing different extraction methods

Fig. 8. Yield of fish oil obtained from *S. albella* employing different extraction methods

The average yield of oil extracted using different extraction techniques are presented in Table 1. Analysis of variance (two way) showed significant variation between the species and various methods (Table 2).
In general, the yields of fish oil obtained from tissues of different species of lesser sardines among all the methods are expressed in the following order of descend as follows:

*Sardinella fimbriata > Sardinella gibbosa > Sardinella albella*

Among the different extraction procedures, the highest yield was obtained in Direct Steaming in all the three species. In the case of *S. fimbriata* and *S. gibbosa* the yield was placed in the order of descend as follows:

Direct Steaming > Bligh & Dyer > Modified Bligh & Dyer > McGill & Moffat

Whereas, in the case of *S. albella* the yield was placed in the order of descend as:

Direct Steaming > Modified Bligh & Dyer > Bligh & Dyer > McGill & Moffat

Table 1. Average yield of fish oil produced by various methods in lesser sardines, *S. fimbriata, S. gibbosa* and *S. albella.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Bligh &amp; Dyer</th>
<th>Modified Bligh &amp; Dyer</th>
<th>McGill &amp; Moffet</th>
<th>Direct Steaming</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. fimbriata</em></td>
<td>91 ± 3.7</td>
<td>94 ± 3.2</td>
<td>80 ± 2.5</td>
<td>116 ± 6.1</td>
</tr>
<tr>
<td><em>S. gibbosa</em></td>
<td>78 ± 4</td>
<td>75 ± 2.1</td>
<td>67 ± 1.5</td>
<td>93 ± 4.3</td>
</tr>
<tr>
<td><em>S. albella</em></td>
<td>63 ± 1.5</td>
<td>65 ± 1.5</td>
<td>53 ± 1.6</td>
<td>84 ± 3.5</td>
</tr>
</tbody>
</table>
Table 2. Analysis of Variance (Two-Way ANOVA) in relation to oil extracted between species and different extraction methods

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F-crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between species</td>
<td>1698.667</td>
<td>2</td>
<td>849.3333</td>
<td>136.5</td>
<td>9.9458</td>
<td>5.1432</td>
</tr>
<tr>
<td>Between methods</td>
<td>1502.917</td>
<td>3</td>
<td>500.9722</td>
<td>80.5133</td>
<td>3.0866</td>
<td>4.7570</td>
</tr>
<tr>
<td>Total</td>
<td>3238.917</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Since higher yield of fish oil was obtained in *S. fimbriata* than that of other species; the qualitative analysis of *S. fimbriata* was alone recorded here so as to avoid redundancy (Table 3). All the analytical values are well within the acceptable standard values for all the methods. The moisture content values (0.831 ± 0.12) and free fatty acid value (1.56 ± 0.16) in modified Bligh and Dyer method were found to be superior to that of other methods. It is important to note that the results of various analytical parameters did not exhibit profound variation between the methods.
Table 3. Quality analysis of fish oil produced from *S. fimbriata* employing different extraction procedures

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters analysed</th>
<th>B &amp; D</th>
<th>MB &amp; D</th>
<th>M &amp; M</th>
<th>DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Moisture content (%)</td>
<td>0.94± 0.21</td>
<td>0.831± 0.12</td>
<td>0.962± 0.045</td>
<td>0.951± 0.34</td>
</tr>
<tr>
<td>2.</td>
<td>Free fatty acid (mg KOH/g)</td>
<td>3.2 ± 0.32</td>
<td>1.56 ± 0.16</td>
<td>2.8 ± 0.27</td>
<td>3.6 ± 0.13</td>
</tr>
<tr>
<td>3.</td>
<td>Iodine Value I$_2$/100g)</td>
<td>178 ± 4</td>
<td>184 ± 3</td>
<td>192 ± 2</td>
<td>187 ± 3</td>
</tr>
<tr>
<td>4.</td>
<td>Peroxide Value (mEq/Kg)</td>
<td>2.82 ± 0.13</td>
<td>2.35 ± 0.27</td>
<td>2.13 ± 0.11</td>
<td>2.73 ± 0.32</td>
</tr>
<tr>
<td>5.</td>
<td>Saponification Value (mg KOH/g)</td>
<td>210 ± 2.2</td>
<td>205 ± 1.3</td>
<td>201 ± 1.6</td>
<td>202 ± 1.7</td>
</tr>
<tr>
<td>6.</td>
<td>Specific Gravity at RT</td>
<td>0.81 ± 0.0005</td>
<td>0.93 ± 0.0003</td>
<td>0.95 ± 0.0002</td>
<td>0.91 ± 0.0004</td>
</tr>
<tr>
<td>7.</td>
<td>Refractive index</td>
<td>1.523 ± 0.004</td>
<td>1.731 ± 0.004</td>
<td>1.481 ± 0.003</td>
<td>1.92 ± 0.002</td>
</tr>
<tr>
<td>8.</td>
<td>Colour</td>
<td>Slight Brownish Yellow</td>
<td>Slight Brownish Yellow</td>
<td>Slight Brownish Yellow</td>
<td>Slight Brownish Yellow</td>
</tr>
</tbody>
</table>

6.4. DISCUSSION

During the last two decades polyunsaturated fatty acids (PUFA) imposed greater interest among scientists due to its winsome medicinal and nutritional properties. Fish oil is being approved for human consumption as an important nutraceutical and chief ingredient in human diet. This warrants a great demand for fish oil round the world. Marine fishes, especially Clupeoid fishes serves as the major repository of fish oil. The yield of oil recovered from body and liver may vary from species to species and also in different fishing areas (Vargheese, 2000).
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. Great improvements has been achieved and are being made in maintaining the condition of raw materials and primary products so that fish oil does not have any major oxidation problems prior to extraction and refining, ensures safe for human and animal consumption. Although both extraction and refining technology is advancing rapidly, most of the fish oil being produced around the world is a byproduct of the conventional fish meal process, a wet rendering technique. Other processes of fish oil extraction are hydrolysis, silage production, solvent extraction, critical extraction and ion exchange.

In the present study, profound variation was recorded in the yield of fish oil produced from three different species, namely, *S. fimbriata*, *S. gibbosa* and *S. albella* employing four different extraction methods, namely Bligh & Dyer, Modified Bligh & Dyer, Mcgill & Moffat and Direct Steaming. There was significant difference in the yield of oil extracted in such a way that *S. fimbriata* samples produced higher yields from all four extraction methods than that of *S. gibbosa* and *S. albella* samples. 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 & Shirai *et al.*, 2002). Vargheese (2000) emphasised that the yield of oil
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recovered from body and liver may vary from species to species and also in different fishing areas.

The Bligh and Dyer method, using chloroform & 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 evaluate the difference in the lipid level (De Koning et al., 1985; Kates, 1986; Randall et al., 1991). Mcgill and Moffat method for extraction lipids and triglycerides is also a popularly used technique for extraction of body oil (Mcgill & Moffat, 1992). Direct steaming is considered as a good old traditional and economic technique for extraction of fish oil. The present study was undertaken to analyse the proficiency in yield between the above said methods, proved that oil extracted by Direct Steaming ensured higher yield than that of other methods. The present experiment supports the suggestions of Sunarya and Taylor (1991) that oil extraction by steaming 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 oil from fish, because the equipment itself is expensive and the recovery of the solvent is not satisfactory (Tanikawa, 1971). Hall (1992) emphasized that direct steaming at 80-85ºC is a simple and economical technique that ensures viable results. From the results of the present study it is concluded that the conventional method of extraction (direct steaming method) is considered as the finest extraction process due to its winsome qualities such as higher yield, economic viability, less laborious and less time consumption etc. Other methods of extraction are
comparatively more time consuming, laborious, costly and are critical due to the off odour and flavour of solvents.

Bligh & 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 91±3.7 ml/Kg from *S. fimbriata*, 78±4 ml/Kg from *S. gibbosa* and 63±1.5 ml/Kg from *S. albella*. Whereas, Direct Steaming method gave better results than the other three methods with an yield of 116±6.1 ml/Kg from *S. fimbriata*, 93±4.3 ml/Kg from *S. gibbosa* and 84±3.5 ml/Kg from *S. albella*. In the present study the oil was prepared from the fish muscles, in which the breakdown of cell wall requires only less environmental shock which was easily afforded by direct steaming. 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 methods, when fish liver was chosen, since rupture of the liver cells requires more shocks, where chemical methods 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 electrolysed cathode water method (Toge and Miyashita, 2003). Bligh and Dyer method provides higher yields only in the abundant presence of polar lipids (De Boer, 1988 & Phillips et al., 1989). Since the yield of Bligh and Dyer method is not high in the present study, which provides the information of the presence of low polar lipids in all the selected species of fishes. Rajion (1985) described a modified Folch et al. method (1956), using
chloroform: methanol (2:1,v/v) solvent system, similarly Modified Bligh and Dyer method was carried out in triplicate by using methanol: chloroform(1:1.5,v/v) solvent system to extract lipids that 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 the three fishes since it is highly specific for extraction of lipids from fish liver, especially for triglycerides (Mcgill & Moffat, 1992). The oil extracted from *S. fimbriata* in the present study, which was almost double the quantity of oil extracted from *S. lemure* by Khoddami *et al.* (2009) in Malaysian waters. The body oil extracted from *S. fimbriata* gave higher yield than the other two lesser sardine species.

The present study concludes with the fact that among lesser sardine of Kanyakumari waters, *S. fimbriata* produced higher yield of fish oil than *S. gibbosa* and *S. albella*. The results unravel the fact that Direct Steaming proved to be an efficient method than that of other methods. The results stand as baseline reference about extraction of fish body oil from lesser sardines employing different extraction methods.