7. PRODUCTION OF FISH BODY OIL FROM *SARDINELLA LONGICEPS* EMPLOYING DIRECT STEAMING METHOD AND ITS QUANTITATIVE AND QUALITATIVE ASSESSMENT

7.1. Introduction

Production of fish oil is warranted as a demanding enterprise as there is a considerable and growing world market demand for high quality fish oil. Production of omega-3 enriched fish oil generated a boom and competitiveness in the fishery allied industry in recent days. By-products from different fish species such as tuna (Chantachum *et al*., 2000), herring, cod (Aidos *et al*., 2002), salmon (Wu and Bechtel, 2008) or walleye pollock (Wu and Bechtel, 2009) has been proposed as raw materials for fish oil production in European countries.

Besides the nutraceutical importance of fish oil, it is also appreciable in pharmaceutical and associated industries. Fish oil is different from other oil mainly because of the unique variety of fatty acids it contains including high levels of polyunsaturated fatty acids (Omega-3 and Omega-6) which are essentially required for metabolic activities. Quantum of oil extracted varies from species to species, so also other parameters that influences which includes age, gender, location, spawning and migration seasons coupled with environmental parameters such as sea surface temperature (sst), primary productivity etc. (Borgstrom, 1961; Huss, 1988). Similarly, the type of fatty acid present as free acid or as neutral lipids differs to a great extent between the species and environments (Dolve and Olcott, 1965; Mc Gill and Moffat, 1992).
Oil sardine fishery represented by *S. longiceps* Val. (Clupeidae), forms the mainstay of Indian marine fisheries, that contributing nearly one third of the total marine fish production in productive years. Oil sardine fishery in India is confined largely to the west coast from time immemorial though stray catches of this species are also available along the east coast in Tamil Nadu and Andhra Pradesh regions. In last two decades, there has been a tremendous increase in the landings of this species along the east coast especially on the Coromandal coast (Kasim *et al*., 2009). Over the decades, one of the major changes noticed in the fisheries along the southeast coast is the incursion and progression of the oil sardine population. The annual production on the west coast of India exhibits large fluctuations over the years, though it continues to be the most important and abundant pelagic fishery resource. A major management problem pertain to oil sardine fishery is its short term and long term fluctuations over the years.

Oil sardine (*S. longiceps*) fishery has been tremendously increased in the last few years in Tamil Nadu coast. During periods of bumper catch, the fish price will drastically fall down and a major chunk of these catch were dumped into fish drying yards from there it finds it way as poultry feed. Instead these fishes can be judiciously subjected for the extraction and production of fish oil.

From the results of the previous chapter it is proved that direct steaming method as the finest extraction process due to its winsome qualities such as higher yield, economic viability, less laborious and less time consumption etc. In this backdrop, this work was undertaken with the aim of extraction of fish body oil from *Sardinella longiceps* employing direct steaming method and also to analyse the quantitative and qualitative properties of the extracted crude fish oil.
7.2. Materials and Methods

7.2.1. Fish collection

*Sardinella longiceps* specimens were collected from fish landings of Muttom, Kanyakumari district, Tamil Nadu, southwest coast of India (lat. 8°0′7″ N; long. 77°19′E) for a period of one year (October 2011 - September 2012).

7.2.2. Extraction of body oil

Four size groups of *S. longiceps* were examined for the extraction of oil based on length. The size groups were arranged in range of 7.1-10 cm (size group I = S I), 10.1-13 cm (size group II = S II), 13.1-16 cm (size group III = S III) and 16.1-19 cm (size group IV = S IV). For the sake of convenience of data interpretation, the size groups were categorised as season wise, namely, winter, monsoon and summer.

Fishes were washed thoroughly in running fresh water so as to remove sand and debris. Scales, head, fins, spines, digestive system and excretory system were removed and only the tissue parts were subjected for the extraction of oil. The tissues were utilized for the extraction of oil by direct steaming methods as follows.

(a) Direct Steaming method

About 1000 g of homogenized fish tissues of each size groups were taken separately 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. The experiments were repeated for 5 times and the average yield was calculated.

Yield of oil was calculated between the size groups against various seasons and is represented as ml/Kg.

7.2.3. Analytical Properties of Purified fish oil

The oil was subjected for the determination of specific gravity by the method outlined by Immanuel et al. (2002), Refractive index by Hollow prism Method, moisture content by ISI methods, Free Fatty Acids (FFA) by of Cox and Pearson (1962), Iodine Value (IV) by Horowitx (1975), Peroxide Value (PV) by Cox and Pearson (1962), Saponification Value (SV) by Horowitx (1975), Observation of colour and the respective methodologies were detailed in previous chapter (chapter 1).

7.3. Results

7.3.1. Yield of fish oil: Size wise and Season wise

Fish oil extracted from the tissues of *S. longiceps* employing direct steaming method produced an average of 180 ± 4.9 ml/Kg for every 1000 g of fish tissues. Totally 4 size groups were examined for oil extraction in relation to various seasons. The length group were assigned as follows: 7.1-10 cm (S I), 10.1-13 cm (S II), 13.1-16 cm (S III) and 16.1-19 cm (SIV). The average yield of fish oil by different size groups in relation to different seasons are portrayed in Figs. 9-11.
Among all size groups, lowest yield of fish oil was recorded in S I length group in all the seasons. In winter, among all the size groups, lowest yield of fish oil was in S I length group (102± 2 ml/Kg). The yield was gradually increased in size groups S II (158.66 ± 2.081 ml/Kg), S III (187.33 ± 1.527 ml/Kg) and declined in S IV (169 ± 2 ml/Kg) respectively.

A similar pattern was recorded in the summer season, in which the lowest yield of 96.66 ± 0.577 ml/Kg was recorded in the smaller size fish (S I) followed by a gradual increase of 150.66 ± 1.154 ml/Kg and 176.33 ± 1.52 ml/Kg in size groups of 10.1-13 cm (S II) and 13.1-16 cm (S III) respectively; followed a slight decline in 16.1-19 cm (S IV) length groups were the yield was 158.33 ± 0.57 ml/Kg oil.

In monsoon season, the yield of fish oil among the four size groups was differed in such a way that S IV recorded higher values of 165 ± 1 ml/kg followed by, S III (145.66 ± 1.154 ml/kg), S II (129.33 ± 0.577 ml/kg), whereas S I recorded the lowest values (78.33 ± 0.577 ml/kg).

![Graph showing yield of fish body oil extracted from different size groups of S. longiceps during winter season](image)

**Fig. 9. Yield of fish body oil extracted from different size groups of *S. longiceps* during winter season**
Fig. 10. Yield of fish body oil extracted from different size groups of *S. longiceps* during summer season

Fig. 11. Yield of fish body oil extracted from different size groups of *S. longiceps* during monsoon season
Annual average yield of fish body oil evaluated for _S. longiceps_ collected from Muttom for the year (October 2011 - September 2012) is portrayed in Fig. 12. Between the size groups the maximum was recorded in S III followed by other groups which is expressed in the order of descend as follows:

S III > S IV > S II > S I.

In general, the season wise yield content was explained in the order of descend as:

Winter > Summer > Monsoon

Analysis of Variance (Two way) showed significant variation ($P<0.05$) between the seasons so also between different length groups ($P<0.05$) (Table 4).
Table 4. ANOVA (Two way) for oil extracted in relation to different size groups and seasons

<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 size groups</td>
<td>11221.33</td>
<td>3</td>
<td>3740.444</td>
<td>43.6486</td>
<td>0.00018</td>
<td>4.75706</td>
</tr>
<tr>
<td>Between seasons</td>
<td>1233.167</td>
<td>2</td>
<td>616.5833</td>
<td>7.19513</td>
<td>0.02547</td>
<td>5.14325</td>
</tr>
<tr>
<td>Total</td>
<td>12968.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

7.3.2. Analytical properties of oil

Analytical properties of the crude fish oil were evaluated separately for freshly prepared samples and for 30 days old samples which were stored in refrigerator at 0°C. The analytical properties of the crude fish oil are explained in Table 5. It was observed that the crude oil exhibits Moisture content in the range of 0.962 ± 0.38 and 0.980 ± 0.42(%), Free fatty acid values at 3.71 ± 0.34 and 3.91 ± 0.48 (mg KOH/g), Iodine values at 192 ± 4 and 196 ± 7 (I₂/100g), Peroxide values in the range of 2.78 ± 0.068 and 2.94 ± 0.71 (mEq/Kg), Saponification values in the range of 211.9 ± 2.4 and 213.42 ± 2.9 mg/KOH/g, Specific gravity in the range of 0.961 ± 0.36 and 0.972 ± 0.38, Refractive index in the range of 1.47 ± 0.016 and 1.49 ± 0.019, for fresh and 30 days stocked samples respectively.

These analytical values of the crude oil are well within the acceptable standard values for both fresh and stocked samples. It is important to note that the values for both fresh and 30 days old refrigerated samples did not having much variation.
Table 5. Qualitative analysis of fresh and stock (refrigerated) samples of crude fish oil

<table>
<thead>
<tr>
<th>S. No</th>
<th>Analytical Parameters</th>
<th>Crude oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fresh (0 days)</td>
</tr>
<tr>
<td>1.</td>
<td>Moisture Content (%)</td>
<td>0.962 ± 0.38</td>
</tr>
<tr>
<td>2.</td>
<td>Free fatty acid (mg KOH/g)</td>
<td>3.71 ± 0.34</td>
</tr>
<tr>
<td>3.</td>
<td>Iodine Value (I₂/100g)</td>
<td>192 ± 4</td>
</tr>
<tr>
<td>4.</td>
<td>Peroxide Value (mEq/Kg)</td>
<td>2.78 ± 0.068</td>
</tr>
<tr>
<td>5.</td>
<td>Saponification Value (mg KOH/g)</td>
<td>211.9 ± 2.4</td>
</tr>
<tr>
<td>6.</td>
<td>Specific Gravity at RT</td>
<td>0.961 ± 0.36</td>
</tr>
<tr>
<td>7.</td>
<td>Refractive index</td>
<td>1.47 ± 0.016</td>
</tr>
<tr>
<td>8.</td>
<td>Colour</td>
<td>Slight Brownish yellow</td>
</tr>
</tbody>
</table>

7.4. Discussion

Fish oil has gained immense importance in recent years because of its wider application prospects. Fish oil is extracted from whole body (e.g., sardine and herring oils) and from liver (e.g., Shark liver oil, cod liver oil, Balistid liver oil etc.) (Immanuel et al., 2002). 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). Production of fish oil from low value fishes has gained increased momentum in recent past.
Production of Fish Body Oil from *Sardinella longiceps* employing Direct Steaming method and its Quantitative and Qualitative assessment

From the results of the previous chapter it is proved that the direct steaming method as the finest extraction process due to its winsome qualities such as higher yield, economic viability, less laborious and less time consumption etc. Yield of oil extracted from *S. longiceps* samples employing direct steaming method achieved significant results. The present experiment is in support of the suggestions of Sunarya *et al.* (1991) and Hall (1992) in which they emphasised that direct steaming is a simple and economical technique that ensures viable results.

Fish oil produced from *S. longiceps* showed profound variation in lipid content between seasons and among various size groups. The dissimilarity in the yield within the species was mainly due to variation in texture, proximate composition coupled with other factors such as gender, age, location, species origin characteristics such as spawning and migration seasons, seasonal variation in composition of dietary plankton and also some environmental condition such as sea surface temperature (Borgstrom, 1961; Leu *et al.*, 1981; Huss, 1988; Shirai *et al.*, 2002). Ssali (1988) has been reported that the lipid content varied between individuals of the same species. These variations were attributed to factors such as the difference in geographical area from which the fish were caught so as to due to the biological factors such as age, sex and size.

The yield of extracted oil from the *S. longiceps* may vary with respect to seasonal abundance of plankton and spawning activity. Polyunsaturated Fatty Acids (PUFA’s) like Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) that are present in considerable quantities are not synthesised by the fish. These fatty acids (EPA and DHA) are produced by phytoplankton. In case of planktivorous fishes, they get accumulated by consuming microalgae that produces these fatty acids and through the food chain it reached
to next higher order fishes (Weber et al., 1986; Insel et al., 2003). According to Kumar and Balasubrahmanyan (1989) the major diet of *S. longiceps* consisted of 14 plankton groups. *Coscinodisus* sp. which was always dominant in the gut that ranged from 17.26% to 44.82% irrespective of size groups; followed by *Pleurosiga* spp. and *Biddulphia* spp. in the next order of abundance. Copepods, crustacean pieces, tintinnids, bivalve larvae, *Lucifer*, *Evadne* spp. and zoea were also occurred. An adult sardine, more than 10 cm in body length, is a typical plankton feeder which feeds mainly on phytoplankton through filtration with the aid of its structural gill rakers. Among 101-105 mm size groups of *S. longiceps*, copepods formed 28.66% of gut content but in 146-150 mm size group, the copepods contributed only 12.44% which is again lesser in higher size groups (Kumar and Balasubrahmanyan, 1989).

*S. longiceps* is a prolific breeder that continuously breeds throughout the year with major peaks achieved during certain corners of the year. Along the west coast, the length composition of catches ranges between 50 - 220mm. Virgin spawners (140-160 mm) enter the fishery on the Malabar coast during June/July; whereas on the Karnataka coast, spawners enter the fishery during July-September, while new recruits (100-120 mm) and juveniles (120-140 mm) dominate the fishery during August to October and October to February respectively. From the present results of fish oil extraction in three different seasons, the average higher yield of 187.33 ± 1.527 ml/Kg was occurred in winter in S III. The lipids content exhibits its peak values in June, August and December for *S. longiceps* of Japanese waters (Hayashi and Takagi, 1977). The lowest yield of oil 77 ± 3.05 ml/Kg (7-10 cm) was in monsoon season might be as a result of depletion of lipid contents which was utilised for spawning activities during monsoon. Generally, difference in lipid content might probably be due to the availability of differential diets distributed between different regions (Ahlgren
et al., 1996), or environmental factors such as temperature, pH and salinity (De Torrengo and Brenner, 1976; Farkas, 1984; Henderson and Tocher, 1987). The yield of extracted oil from the *S. longiceps* may vary with respect to seasonal abundance of plankton and spawning activity.

The oil extracted in the present study was from *S. longiceps* which was almost double the quantity of oil extracted from *S. lemuru* by Khoddami et al. (2009) in Malaysian waters. Anand (2010) extracted oil from sardines of Parangipettai coastal waters which was also fall well below the yield achieved in the present study.

The quality of fish oil gains momentum globally in recent past as it is intended for human consumption. The standard value for qualitative assessment of fish oil has been established by FAO (2005). In the present study, certain properties of crude fish oil was evaluated for fresh and refrigerated 30 days old fish oil so as to determine the deterioration level or stability of oil during storage. The analytical values of the crude oil are well within the acceptable standard values for both the fresh and stocked samples. It is important to that the values for both fresh and 30 day’s old refrigerated samples did not exhibit much variation. Undeland et al. (1998) indicated that unsaturated character of lipids and strong pro-oxidative systems naturally present in fish tissue could cause susceptibility of lipids to oxidize during processing and storing. Results of the present study agreed with the findings of Summers et al. (1991) in which the minor presence of oxidizing peroxides may be advantageous to the quality of the oil during long term storage. The results of free fatty acids, iodine value, peroxide value and saponification value were slightly increased from fresh to 30 days old samples; which were line in with the findings of Boran et al. (2006).
Production of Fish Body Oil from *Sardinella longiceps* employing Direct Steaming method and its Quantitative and Qualitative assessment

The extracted oil from the sardines are rich in lipid content, therefore, it will be typically accompanied by moisture content in the oil or vice versa (Ihekoronye and Ngoddy, 1985). The free fatty acid (FFA) value is one of the most important factors that act as a rider to check the quality of lipid. In the present study, free fatty acid value was found to be 3.71 ± 0.34 and 3.91 ± 0.48 (mg KOH/g). The lower FFA content ensures higher grade quality with fewer changes for further oxidation. The maximum limit for FFA content is reported to be 7% (Bimbo, 1998) and it in the present results, free fatty acid values were fall well below to this limit. Young et al. (1993) reported that peroxide values of crude fish oil ranged between 3 and 20 mEq/kg.

In our study, the peroxide value was found to be 2.78 ± 0.068 and 2.94 ± 0.71 (mEq/Kg), which is well below the acceptable limit of 20 mEq O₂/kg oil. This indicated that the fish oil extracted is having low lipid oxidation rate as emphasised by Pike (1998). The iodine value of the refined oil reduced to 192 ± 4 and 196 ± 7 (for fresh and 30 days old) I₂/100g which implies that few of the double bonds in the oil has been saturated as suggested by Adeniyyi and Bawa (2006). Saponification is the process of breaking down of neutral fat into glycerol and fatty acids through alkali treatment. The saponification values of *S. longiceps* fish oil obtained in our study was higher (211.9 ± 2.4 and 213.42 ± 2.9 mg/KOH/g) than the standard value for fish oil (180-200 mg KOH/g) given by AOCS (1992). Bimbo and Crowther (1991) reported that crude oil contains minor amount of non-triglyceride substances. Thus, it is possible that high saponification value was mainly due to the impurities present in crude fish oil. The specific gravity of the crude fish oil was found to be 0.961 ± 0.36 and 0.972 ± 0.38 for fresh and stock samples respectively, which is close to that of the commercially available standard Menhaden oil of 0.900 to 0.910 (Joseph,
Production of Fish Body Oil from *Sardinella longiceps* employing Direct Steaming method and its Quantitative and Qualitative assessment

1985). The refractive index of the crude fish oil was 1.47 ± 0.016 and 1.49 ± 0.019 (for fresh and stocked respectively) which falls between the standard values of 1.460 and 1.495. The colouration of crude fish oil was brownish yellow, which might be due to the prolonged heating period during steaming, that often oxidizes the product (i.e. the oil) and thus imparts a brownish yellow colour (Hall, 1992).

The aldehydes in autoxidized fish oil, such as 2-hexenal and acetaldehyde, appear to react by aldol condensation and dehydration to form crotonaldehyde and 2-(1-butenyl)-octa-2, 4-die-nal, during the reaction that imparts browning (Fujimoto and Kaneda, 1973). Since crude oil contains levels of degradation products (e.g. aldehydes) and these undergoes for browning reaction. As a result, a darker colour was obtained for the crude fish oil.

The present results will give useful information about extraction of fish body oil from *S. longiceps* in relation to different size groups against different seasons from the Muttom waters. The oil extracted from *S. longiceps* ensures higher yield through direct steaming underlines the need for it bulk level commercial production. The information generated in the present study pertain to the quantitative and qualitative analysis of fish oil will stand as a baseline line reference for entrepreneurs and industrialists in future for the successful commercial production of fish oil employing oil sardines.