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Extraction of fish body oil from *Sardinella longiceps* by employing direct steaming method and its quantitative and qualitative assessment

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ABSTRACT

Objective: To analyze the quantitative and qualitative properties of the extracted fish oil from *Sardinella longiceps* (*S. longiceps*).
Methods: Four size groups of *S. longiceps* were examined for the extraction of fish oil based on length. The size groups included Group I (size range of 7.1–10.0 cm), Group II (size range of 10.1–13.0 cm), Group III (size range of 13.1–16.0 cm) and Group IV (size range of 16.1–19.0 cm). Fish oil was extracted from the tissues of *S. longiceps* by direct steaming method. The oil was then subjected to the determination of specific gravity, refractive index, moisture content, free fatty acids, iodine value, peroxide value, saponification value and observation of colour.
Results: The four groups showed different yield of fish oil that Group IV recorded the highest values of (165.00 ± 1.00) mL/kg followed by Group III [(145.66 ± 1.15) mL/kg] and Group II [(129.33 ± 0.58) mL/kg], whereas Group I recorded the lowest values of (78.33 ± 0.58) mL/kg in monsoon season, and the average yield was (180.0 ± 4.9) mL/kg fish tissues. These analytical values of the crude oil were well within the acceptable standard values for both fresh and stocked samples.
Conclusions: The information generated in the present study pertaining to the quantitative and qualitative analysis of fish oil will serve as a reference baseline for entrepreneurs and industrialists in future for the successful commercial production of fish oil by employing oil sardines.

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[1], herring, cod, salmon or walleye pollock, have been proposed as raw materials for fish oil production in European countries[2-4].

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 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, as also due to other parameters that influence age, gender, location, spawning and migration seasons coupled with environmental parameters such as sea surface temperature, and primary productivity *etc*.[5]. Similarly, the type of fatty acid present as free acid or as neutral lipids differs to a great extent between the species and environments[6].

Oil sardine fishery represented by *Sardinella longiceps* Val. (*S. longiceps*) (Clupeidae), forms the mainstay of Indian marine fisheries, which contributes 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[7]. Over the decades, one of the major changes noticed in the fisheries

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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 landings have 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 catches is dumped into fish drying yards, from there it finds its way as poultry feed. Instead, these fishes can be judiciously subjected to the extraction and production of fish oil.

It is proved that direct steaming method is the finest extraction process due to its winsome features such as higher yield, economic viability, less laborious and less time consumption *etc*. In this context, this work was conducted with the aim of extraction of fish body oil from *S. longiceps* by employing direct steaming method and also the analysis of quantitative and qualitative properties of the extracted crude fish oil.

2. Materials and methods

2.1. Fish collection

S. longiceps specimens were collected from fish landings of Muttom, Kanyakumari District, Tamil Nadu, southwest coast of India (8°7' N, 77°19' E) for a period of one year (October 2011 to September 2012).

2.2. Extraction of fish body oil

Four size groups of *S. longiceps* were examined for the extraction of oil based on length. The size groups included Group I (size range of 7.1–10.0 cm), Group II (size range of 10.1–13.0 cm), Group III (size range of 13.1–16.0 cm) and Group IV (size range of 16.1–19.0 cm). For the sake of convenience of data interpretation, the size groups were categorised with season, 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 to the extraction of oil. The tissues were utilized for the extraction of oil by direct steaming methods as follows.

2.3. Direct steaming method

About 1 000 g of homogenized fish tissues of each groups were taken separately in a muslin bag and kept in a steam boiler at 70–80 ˚C for 30 min. The boiled fish tissues were then pressed with the aid of fish oil extractor (designed in our laboratory and about to be patented), 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 2 000 r/min (REMI, C 24BL Cooling Centrifuge) for 15 min 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 was expressed as mL/kg.

2.4. Analytical properties of crude fish oil

The fresh and stocked fish oil which was stored in refrigerator at 0 ˚C for 30 day were subjected to the determination of specific gravity by the method outlined[8], refractive index by hollow prism method[9], moisture content by Indian Standard Institute methods[9], free fatty acid (FFA)[10], iodine value[11], peroxide value (PV)[10], saponification value (SV)[11], and observation of colour.

2.5 Data analysis

The data were subjected to Two-way ANOVA used for analysis of data, and difference was statistically significant at the level of P value < 0.05.

3. Results

3.1. Yield of fish oil according to size and collection season

Extraction of fish oil from the tissues of *S. longiceps* employing direct steaming method produced an average yield of (180.0 ± 4.9) mL/kg fish tissues. Totally 4 size groups were examined for oil extraction in relation to various seasons. Among all size groups, lowest yield of fish oil was recorded in Group I in all the seasons. In winter, among four groups, lowest yield of fish oil was in Group I [(102 ± 2) mL/kg]. Then the yield was gradually increased in Group II [(158.66 ± 2.08) mL/kg] as well as Group III [(187.33 ± 1.53) mL/kg], and declined in Group IV [(169 ± 2) mL/kg].

A similar pattern was recorded in the summer, in which the lowest yield of (96.66 ± 0.58) mL/kg was recorded also in Group I, and the yield gradually increased to (150.66 ± 1.15) mL/kg and (176.33 ± 1.52) mL/kg in Groups II and III, respectively; a slight decline of the yield was observed in Group IV [(158.33 ± 0.57) mL/kg]. In monsoon season, the yield of fish oil among the four size groups was different. Group IV showed the highest value of (165 ± 1) mL/kg followed by Group III [(145.66 ± 1.15) mL/kg] and

Group II [(129.33 ± 0.58) mL/kg], whereas the lowest value was recorded in Group I [(78.33 ± 0.58) mL/kg].

Annual average yield of fish body oil evaluated for *S. longiceps* collected from Muttom from October 2011 to September 2012 is portrayed in Figure 1. Among the size groups the maximum value was recorded in Group III followed by other groups which was expressed in the order of Group III > Group IV > Group II > Group I.

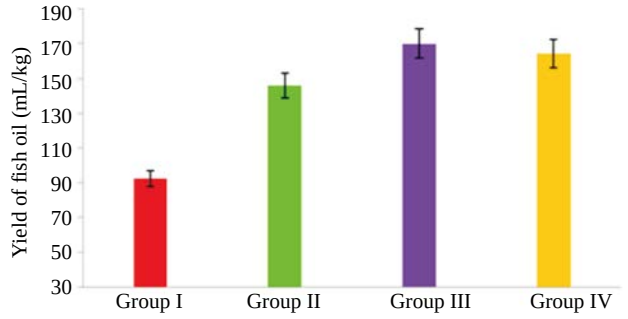

Figure 1. Average annual yield of fish body oil extracted from *S. longiceps* collected from Muttom (October 2011 to September 2012).

In general, the season-wise yield content was in the order of Winter > Summer > Monsoon.

Analysis of variance (Two way) showed a significant variation ($P < 0.05$) between the seasons as well as between size groups ($P < 0.05$) (Table 1).

Table 1
Two-way ANOVA for oil extracted in relation to different size groups and seasons.

Source of variation	SS	*df*	MS	*F*	*P*-value	*F*-crit
Between groups	11221.330	3	3740.444	43.6486	0.000018	4.757706
Between seasons	1233.167	2	616.583	7.1951	0.025547	5.143325
Total	12968.670					

3.2. Analytical properties of oil

Analytical properties of the crude fish oil were evaluated separately for freshly prepared samples and stocked samples which were stored in refrigerator at 0 °C for 30 days. It was observed that the crude oil exhibited moisture content in the range of 0.962% ± 0.380% and 0.98% ± 0.42%, FFA values at (3.71 ± 0.34) and (3.91 ± 0.48) mg KOH/g, iodine values at (192 ± 4) and (196 ± 7) I_2/100 g, PVs in the range of (2.780 ± 0.068) and (2.94 ± 0.71) mEq/kg, SVs in the range of (211.9 ± 2.4) and (213.42 ± 2.90) mg KOH/g, specific gravity in the range of 0.961 ± 0.360 and 0.972 ± 0.380, refractive index in the range of 1.470 ± 0.016 and 1.490 ± 0.019, for fresh and 30-days stocked samples, respectively. The colour of crude fresh and stocked fish oil was brownish yellow.

These analytical values of the crude oil fall within the acceptable standard values for both fresh and stocked samples. It was important to note that the values for both fresh and 30-day refrigerated samples did not exhibit much difference.

4. Discussion

Fish oil has gained immense importance in recent years because of its wider application prospects. Fish oil can be extracted from whole body (*e.g.* sardine and herring oils) and from liver (*e.g.* shark liver oil, cod liver oil, fish by-products, Balistid liver oil *etc.*)[8,10]. Fish oil when compared to terrestrial animal and vegetable oil, is characterised by a complex nature of saturated, unsaturated and polyunsaturated fatty acids[11]. 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 which provides tremendous benefits to human health[12]. Production of fish oil from low value fishes has gained increased momentum in recent past.

From the obtained results, it is proved that the direct steaming method is 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 which emphasise that direct steaming is a simple and economical technique that ensures viable results[13].

Fish oil produced from *S. longiceps* showed profound variation in yield 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 conditions such as sea surface temperature[14]. The lipid content varied between individuals of the same species[15]. These variations were attributed to factors such as the difference in geographical area where the fish were caught and also due to the biological factors such as age, sex and size.

The yield of extracted oil from the *S. longiceps* may vary due to seasonal abundance of plankton and spawning activity. Polyunsaturated fatty acids like eicosapentaenoic acid and docosahexaenoic acid that are present in considerable quantities, are not synthesised by the fish. These fatty acids are produced by phytoplankton. In case of planktivorous fishes, they accumulated by consumed microalgae which produces these fatty acids and through the food chain they reached to next higher order fishes[16]. The major diet of *S. longiceps* consisted of 14 plankton groups. *Coscinodisus* sp. was always dominant in the gut that ranged from 17.26% to 44.82% irrespective of size groups, followed by *Pleurosigma* spp. and *Biddulphia* spp.[17]. Copepods, crustacean pieces, tintinnids, bivalve larvae, *Lucifer*, *Evadne* spp. and zoea were also included in diet of *S. longiceps*. 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 group 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 lesser in larger size groups[17].

S. longiceps is a prolific breeder that continuously breeds throughout the year with major peaks achieved during certain periods of the year. Along the west coast, the length of catches ranges between 50 and 220 mm. Virgin spawners (140–160 mm) enter the fishery on the Malabar Coast during June and July; whereas on the Karnataka Coast, spawners enter the fishery during July to 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 ± 1.53) mL/kg occurred in winter in Group III. The lipids content achieved its peak values in June, August and December for *S. longiceps* of Japanese waters[18]. The lowest yield of oil [(78.33 ± 0.58) mL/kg] (Group I) observed in monsoon season might be a result of depletion of lipid contents for spawning activities during monsoon. Generally, difference in lipid content might be probably due to the availability of differential diets distributed between different regions[19], or environmental factors such as temperature, pH and salinity[20].

The oil extracted in the present study was from *S. longiceps* which had almost double quantity of oil extracted from *Sardinella lemuru* in Malaysian waters[21]. The oil from sardines of Parangipettai coastal waters was also fall well below the yield achieved in the present study[15,22].

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[23]. In the present study, certain properties of crude fish oil were evaluated for fresh and 30-day refrigerated fish oil so as to determine the deterioration level or stability of oil during storage. The analytical values of the crude oil were well within the acceptable standard values for both the fresh and stocked samples. It is important to note that the values for both fresh and 30-day refrigerated samples did not exhibit much difference. The unsaturated character of lipids and strong pro-oxidative systems naturally present in fish tissue could cause susceptibility of lipids to oxidation during processing and storing[24]. Results of the present study agreed with the findings of another study, in which the minor presence of oxidizing peroxides may be advantageous to the quality of the oil during long term storage[23]. The results of FFA, iodine value, PV and SV were slightly increased from fresh to 30-day stocked samples, which were in line with the findings of other researchers[24,25].

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[26]. The FFA value is one of the most important factors to check the quality of lipid. In the present study, FFA value was found to be (3.71 ± 0.34) and (3.91 ± 0.48) mg KOH/g in fresh and stocked fish oil, respectively. 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%[25,27], and in the present results, FFA values were fall well below to this limit. The PVs of crude fish oil ranged between 3 and 20 mEq/kg[28].

In our study, the PV was found to be (2.780 ± 0.068) and (2.94 ± 0.71) mEq/kg in fresh and stocked fish oil, respectively, which was well below the acceptable limit of 20 mEq/kg oil. This indicates that the fish oil extracted is having low lipid oxidation rate as emphasised[25,29]. The iodine value of the refined oil reduced to (192 ± 4) and (196 ± 7) I_2/100 g for fresh and stocked oil, respectively, which implies that few of the double bonds in the oil hve been saturated as suggested[10]. Saponification is the process of breaking down neutral fat into glycerol and fatty acids through alkali treatment. The SVs of *S. longiceps* fish oil obtained in our study was higher [(211.9 ± 2.4) and (213.42 ± 2.90) mg KOH/g] than the earlier reported value for fish oil (180–200 mg KOH/g)[30]. The crude oil contains minor amount of non-triglyceride substances[31]. Thus, it is possible that high SV is mainly due to the impurities present in crude fish oil. The specific gravity of the crude fish oil was found to be 0.961 ± 0.360 and 0.972 ± 0.380 for fresh and stock samples, respectively, which is close to that of the commercially available standard menhaden oil with specific gravity of 0.90 to 0.91[32]. The refractive index of the crude fish oil was 1.470 ± 0.016 and 1.490 ± 0.019 for fresh and stocked oil, respectively, which falls between the standard values of 1.460 and 1.495. The colour of crude fish oil was brownish yellow, which might be due to the prolonged heating period during steaming, which often oxidizes the product (*i.e.* the oil) and thus imparts a brownish yellow colour[14].

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-dienal, during the reaction that imparts brownish color[33-35]. Crude oil contains levels of degradation products (*e.g.* aldehydes) which undergo 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 for the need for bulk level commercial production. The information generated in the present study pertaining to the quantitative and qualitative analysis of fish oil will stand as a reference baseline for entrepreneurs and industrialists in future for the successful commercial production of fish oil employing oil sardines.

Conflict of interest statement

We declare that we have no conflict of interest.

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