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Processing and characterisation of low cost Balistid fish *Sufflamen capistratus* liver oil for edible purpose

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ABSTRACT

The physico-chemical properties of liver oil from low cost Balistid fish *Sufflamen capistratus* extracted by Soxhlet, Bligh and Dyer, direct steaming, solar extraction and Mc Gill and Moffat methods were assessed. The oil yield was high (70.3%) in Soxhlet method when compared with other methods. The physical properties such as solidification point, melting point, refractive index and moisture content of the extracted oil samples were differed between methods. The chemical properties like the acid value, free fatty acid (FFA) level and carotenoid content of the individual oil samples were showed extraction methods dependant variation. Fatty acid content was also showed variation between methods; polyunsaturated fatty acid (PUFA) content was high (23.07%) in Bligh and Dyer method. In all the oil samples, fat soluble vitamin K was more when compared with the other tested vitamins. Irrespective of methods of extraction, permissible level of heavy metals and minerals were recorded in the oil samples.

1. Introduction

Fish oil is one of the main fishery products, with pharmaceutical importance. The fish oils can be 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, Menenthira, Palavesam, & Peter Marian, 2002). The species like cod and shark show a considerable amount of oil accumulated in the liver (Kinsella, 1990; Navarro-Garcia, Pacheco-Aguilar, Bringas-Alvarado, & Ortega-Garcia, 2004). Generally shark liver and cod liver are the best source of high amount of oil and it can be extracted by different methods. The yield of oil from liver may vary from species to species and fishing areas (Vargheese, 2000). Fish oils are more complex than terrestrial animal and vegetable oils (Adebiyi & Bawa, 2006), having a complex nature of saturated, unsaturated and polyunsaturated fatty acids (Immanuel et al., 2002). In addition to these, fish oil contains free fatty acids, carotenoids, cholesterol and lipid-soluble vitamins A and D (Aidos, Vander Padt, Luter, & Boom, 2002). Carotene is stored in liver due to their liposoluble nature; hence it is extracted along with the oil (Metusalach, Brown, & Shahidi, 1996; Navarro-Garcia, Pacheco-Aguilar, Vallejo-Cordova, Ramirez-Suarez, & Bolanos, 2002). The level of carotenoid has to be assessed to determine the quality of the oil. Normally antioxidant capacity is present in carotenes and tocopherols (Clark, Faustman, Furr, & Riesen, 1996; Wanasundara, Shahidi, & Amarowicz, 1998). High content of the free fatty acids (FFAs) in the fish oil reduces the commercial value due to an established direct relationship between oil quality and FFA levels (FAO, 1986).

Various processing methods have been adopted for the extraction of fish oil from the liver and whole body. They are Soxhlet method, Bligh and Dyer method (Bligh & Dyer, 1959), direct steaming method, solar extraction method (Immanuel, 1996; Immanuel et al., 2002), Mc Gill and Moffat method (Mc Gill & Moffat, 1992), etc. Vargheese (Vargheese, 2000) extracted and characterised the liver oils of sharks caught in southwest coast of India through the above methods. Immanuel (1996) and Immanuel et al. (2002) have adopted different extraction methods for liver oil production from Balistid fish *Odonus niger* and also characterised the extracted oil for pharmaceutical purpose. Adebiyi and Bawa (2006), Navarro-Garcia et al.(2002, 2004), and Aidos et al. (2002) have extracted and characterised liver oil and body oil from several fish species like sharks, rays, mackerel, herring, etc. Despite these studies, a standard method to extract liver oil from low cost Balistid fish *Sufflamen capistratus* is still wanted. Therefore this work was undertaken to examine the possible methods for extraction of liver oil from *S. capistratus* and further to analyse the physico-chemical properties of the extracted oil for edible purpose or even for pharmaceutical purpose. In the southwest coast of India, this Balistid fish landing is high during trawling, and which is of uneconomic.

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However, this low value fish can be used as a main source of raw material for making aquaculture/poultry feeds (Immanuel et al., 2002).

2. Materials and methods

S. capistratus fish were collected from Chinnamuttam Fisheries harbour, Kanyakumari District, South India. The liver mass was aseptically separated from the fish by dissecting the abdomen, weighed and estimated the amount of liver (8.0 ± 1.25%) available from this species. The collected liver was chilled in ice and packed in airtight containers prior to arrival in the laboratory. They were in good condition and did not show any visual trace of spoilage. In the laboratory, the ice cubes were discarded and all the unwanted materials such as blood vessels and visceral materials were removed, cleaned well and stored at −60 °C in a deep freezer until further use.

2.1. Methods of oil extraction from the liver

Five different methods were adopted for extraction of oil from the liver mass, such as solar extraction method (crude method), direct steaming method (Immanuel et al., 2002), solvent extraction (Soxhlet) method, Bligh and Dyer method (Bligh & Dyer, 1959), and Mc Gill and Moffat method (Mc Gill & Moffat, 1992). Solar extraction method was standardised in our laboratory after being thawed the livers at ambient temperature. 100 g liver sample was taken in a glass bowl, exposed under hot sunlight (35 ± 2 °C). Within 15 min. the oil started melting and at every 30 min intervals the melted oil was collected in a well-cleaned glass beaker and within 4–5 h, the complete oil was separated.

In all the tested methods triplicate samples were analysed. The percentage oil yield in all the extraction methods was calculated as

$$\text{Yield of oil } (\%) = \frac{\text{Weight of the liver(g)}}{\text{Weight of oil extracted(g)}} \times 100$$

2.2. Analytical methods

After extraction, the oil samples were kept in individual containers in deep freezer (−20 °C) until further use. The following physico-chemical properties of oils extracted by various methods were performed. Physical properties such as solidification point as well as specific gravity of oil samples were analysed by the methods described by Immanuel et al. (2002). The refractive index of oil samples was analysed by Spectrometer-Hollow prism method. Moisture content was determined by ISI method, after drying of known weight of oil in an oven at 105 °C, the weight was measured. The weight difference was expressed as moisture content. The chemical properties like acid value and free fatty acid (FFA) were determined by AOCS (1998). The amount of FFA of the oil samples was determined by titration method. The percentage of FFA was calculated as oleic acid. The fatty acid profile of different extracted oils was analysed by gas chromatography (GC) by the method described by Miller and Berger (1985). Fatty acid methyl esters (FAMEs) of oil samples were prepared according to AOCS (1998) official method Ce lb-89 and analysed with regard to the amount of individual fatty acids. Total carotenoid and β-carotene contents of the oil samples were determined by UV–vis Spectrophotometer (Techcomp-8500, Taiwan). Fat soluble vitamins such as A, D, E and K were determined by HPLC (D-7000, Lochrom, Merck). Minerals such as copper, iron, sodium, potassium, magnesium, phosphorus, sulphur and zinc were determined using flame photometer (Elico-1205). Heavy metals such as mercury, arsenic, lead and cadmium in the extracted oil samples were determined by Atomic spectrum (Varien ASP 2050). All the above chemical properties of the oil samples were analysed using standard procedures described in AOCS (1998).

2.3. Statistical analysis

The data obtained in this study were expressed as mean ± SD and were analysed using one-way ANOVA test at 5% significant level. Further a multiple comparison test (SNK test) was conducted to compare the significant differences amongst the oil extraction methods using computer software Statistica 6.0 (Statsoft, UK).

3. Results and discussion

The results highlight the impact of different methods of oil extraction from *S. capistratus* liver mass and variation in physico-chemical properties of the extracted oil.

3.1. Oil yield

The percentage yield of oil from the liver mass of *S. capistratus* using different methods of oil extraction is given in Fig. 1. Amongst the five methods, Soxhlet method yielded the maximum percentage of oil (70.3 ± 1.0%) and it significantly ($P < 0.0001$) higher than the other extraction methods. Solar extraction method yielded lowest percentage (31.0 ± 1.0%). This was in agreement with the data on oil extraction from the liver mass of *O. niger* which are available along the south east and south west coast of India (Immanuel, 1996; Immanuel et al., 2002). The reason for the lowest yield of oil in the solar extraction method may be due to the unrupture of liver cells (Immanuel, 1996; Immanuel et al., 2002; Sunaraya, Michael Hole, & Antony Taylor, 1992). In the other methods, the liver tissues might be broken down either heating process or through the action of solvents used, so lipid content may easily come out.

3.2. Physico-chemical properties of extracted oil samples

The physico-chemical properties of the oil samples are important and to be considered on shelf life point of view during storage.

3.2.1. Physical properties

The solidification point of the oils extracted by various methods was similar, at 19.5 ± 0.5 °C all the oil samples solidified (Table 1). This result agrees with the solidification point temperature of *O. niger* liver oils (19.0 ± 0.50 °C) extracted by the above methods (Immanuel et al., 2002). But the solidification point of the different shark liver oil was varied from 27.83 to 29.5 °C in different extraction methods (Vargheese, 2000). The melting point of the oils extracted by direct steaming and solar extraction methods was higher (36 °C), whereas oil extracted by Soxhlet method showed very low melting point (25 °C). The specific gravity of the oils extracted by the five different methods was more or less similar (0.904–0.914), except direct steaming method (0.895). The specific gravity of solid fat (0.86) is to be lesser than that of the specific gravity of liquid fat (0.91–0.95) (Lehninger, 1984; Shanmugam, 1990). The specific gravity of *O. niger* liver oil extracted by the methods like Bligh and Dyer, Soxhlet, direct steaming and solar extraction had the same value of 0.95 (Immanuel et al., 2002), which described that the oils extracted from fish is liquid fat.

Fats have definite angles of refraction, variation from the normal value indicate mixture of fats. In this study, the refractive index value of oil samples recorded almost similar in all the five tested methods (1.428–1.469 μ). Immanuel et al. (2002) stated that

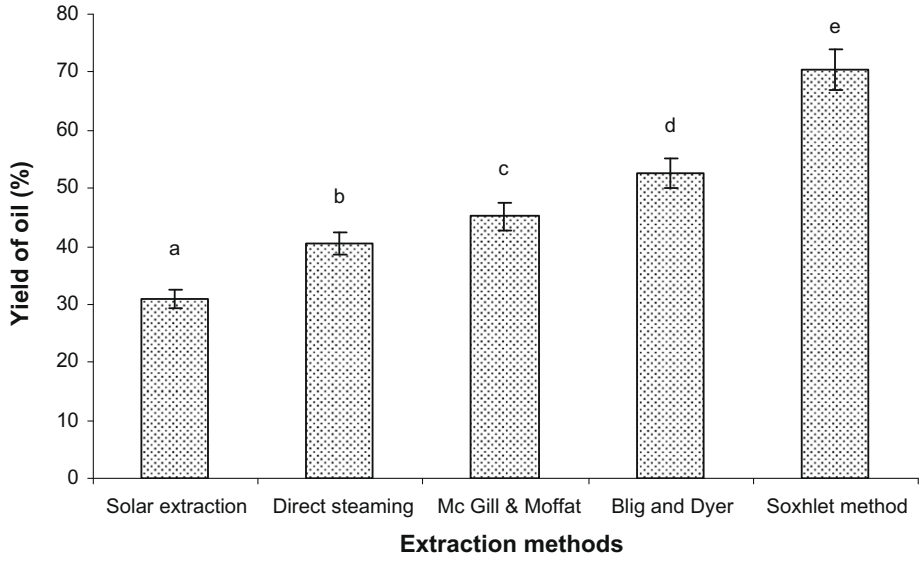

Fig. 1. Percentage (%) yield of *S. capistratus* liver oil samples extracted by different methods. Each value is a mean of three replicate samples; bars with different alphabets are statistically different from each other (one-way ANOVA test $P < 0.05$ and subsequent Post-hoc multiple comparison with SNK test).

Table 1
Physical property of *S. capistratus* liver oil samples extracted by different methods.

Extraction methods	Melting point (°C)	Solidification point (°C)	Specific gravity	Refractive index (μ)	Moisture content (%)
Solar extraction	36 ± 0.4^{a}	19.5 ± 0.5^{a}	0.905 ± 0.04^{a}	1.458^{a}	1.6 ± 0.02^{a}
Direct steaming	36 ± 0.47^{a}	19.5 ± 0.5^{a}	0.895 ± 0.01^{b}	1.469^{a}	6.4 ± 0.3^{b}
Mc Gill and Moffat	33 ± 0.47^{b}	19.5 ± 0.5^{a}	0.912 ± 0.05^{c}	1.46^{a}	0.6 ± 0.05^{c}
Bligh and Dyer	34 ± 0.84^{c}	19.5 ± 0.5^{a}	0.904 ± 0.20^{ad}	1.473^{a}	2.7 ± 0.04^{d}
Soxhlet method	25 ± 0.8^{d}	19.5 ± 0.5^{a}	0.914 ± 0.01^{ce}	1.428^{a}	0.7 ± 0.01^{ce}

Each value is a mean of three replicate samples; values in row different superscript alphabets is statistically significant (one-way ANOVA test $P < 0.05$ and subsequent Post-hoc multiple comparison with SNK test).

the refractive index of *O. niger* liver oil extracted by different methods (solar extraction, direct steaming, Bligh and Dyer and Soxhlet methods) are in between 1.42 and 1.48 μ at 29 °C. Like wise, the refractive index of the oils extracted by the above methods was also almost similar (1.454–1.462 μ at 28.5 °C) in the shark liver oil (Vargheese, 2000). The variations in all the specific parameters of the liver oil irrespective of extraction methods adopted may also be attributed to the temperature, solvents or both of these used in the respective methods, which may cause minor variation in the chemistry of the extracted oil.

Normally, fish oil has very low amount of moisture content, for example the herring oil sample has very low amount of moisture content (Aidos et al., 2002). In this study, amongst the five extraction methods, oil from direct steaming method showed maximum moisture content (6.4%), because the liver mass was boiled with water, whereas it was very low in Mc Gill and Moffat (0.6%) and Soxhlet (0.7%) extraction methods (Table 1). The reduction in moisture content in these oil samples may be due to the addition of anhydrous sodium sulphate during the extraction of oil samples from the liver mass. Usually certain chemicals like sodium sulphate can absorb water molecules (Immanuel et al., 2002).

3.2.2. Chemical properties of oil samples

3.2.2.1. Acid and free fatty acids value. High content of free fatty acids (FFAs) in the fish oils reduce the commercial value due to an established direct relationship between oil quality and FFA levels (FAO, 1986). Young (1982) suggested the maximum acceptable value of FFA should be 4.0%. The FFA level of oil samples extracted from menhaden and herring fish was recorded as 2.9% and 5.6%, respectively (Young, 1982). The free fatty acid level of *S. capistratus* oil was more in Soxhlet (2.49 ± 0.07%) extraction and solar extraction (2.27 ± 0.03%) methods, but in the other methods, the FFA level ranged between 1.42 ± 0.06% and 1.93 ± 0.06% (Table 2). In these methods, the oil was extracted with temperature induction and it may be the reason for the variation in FFA. This result confirmed that the FFA level in these oil samples is below the acceptable level. Similarly, the acid value of *S. capistratus* oil samples varied from 3.0 ± 0.15 to 4.8 ± 0.18 mg/KoH. Comparatively the acid value of oil was higher in Soxhlet extraction method and lower in Mc Gill and Moffat method.

Table 2
Chemical properties of *S. capistratus* liver oil samples extracted by different methods.

Extraction methods	Acid value (mg/KoH)	Free fatty acid (%)	Total carotenoid (%)	β-Carotene (%)
Solar extraction	4.5 ± 0.01^{a}	2.27 ± 0.03^{a}	2.33 ± 0.04^{a}	1.37 ± 0.02^{a}
Direct steaming	3.8 ± 0.05^{b}	1.93 ± 0.06^{b}	2.6 ± 0.02^{b}	1.54 ± 0.02^{b}
Mc Gill and Moffat	3.0 ± 0.15^{c}	1.42 ± 0.06^{c}	2.8 ± 0.04^{c}	1.32 ± 0.03^{ac}
Bligh and Dyer	3.2 ± 0.2^{d}	1.42 ± 0.08^{c}	3.23 ± 0.04^{cd}	2.31 ± 0.02^{d}
Soxhlet method	4.8 ± 0.18^{ea}	2.49 ± 0.07^{d}	2.45 ± 0.04^{f}	1.12 ± 0.02^{e}

Each value is a mean of three replicate samples; values in row different superscript alphabets is statistically significant (one-way ANOVA test $P < 0.05$ and subsequent Post-hoc multiple comparison with SNK test).

3.2.2.2. Total carotenoid and β-carotene content. The fishes are not able to synthesise carotene; they need to get them from the diets (Negre-Sadargues, Castillo, & Segonzac, 2000). The carotene is stored in liver and due to their liposoluble nature (Metusalach et al., 1996); it's extracted with the oil (Navarro-Garcia et al., 2002). β-Carotene is known to be the precursor of vitamin A, which means, it is an essential part for the production process (Delia & Rodriqiuz, 2001). The presence of carotenoids in oils shows the coloration of the oil. Total carotenoid content of *S. capisturatus* liver oil samples was ranged from 2.33 ± 0.4% to 3.23 ± 0.4%, it was higher in Bligh and Dyer method, whereas, lower in solar extraction method. The β-carotene content of all the oil samples was more or less similar (1.12–1.54%) except the oil sample extracted by Bligh and Dyer method (2.31%) (Table 2). It may be attributed to the solubility of carotenoids in the solvent system used.

3.2.2.3. Fatty acid profile. Fatty acid profile of the oil is the major criteria to accept the quality of the same oils. In this study, the fatty acid profile showed that saturated FAMEs were 36.32%, 38.3%, 43.36%, 45.75% and 63.84% in Bligh and Dyer, Mc Gill and Moffat, direct steaming, Soxhlet extraction and solar extraction methods, respectively. According to Immanuel et al. (2002), the total saturated fatty acid content of *O. niger* liver oil samples extracted by different methods was varied from 47.05% to 59.54%. In this study, the most abundant saturated fatty acid was C16: 0 (palmitic acid) i.e., 29.78% in solar extraction, 16.46% in direct steaming, 18.73% in Soxhlet, 13.68% in Mc Gill and Moffat and 11.76% in Bligh and Dyer extraction methods. Immanuel et al. (2002) and Zafar et al. (2003) were also reported that, palmitic acid (C16.0) was high in liver oils of *O. niger* and wing head sand bar sharks, respectively.

The monounsaturated fatty acid percentage of *S. capistratus* liver oil was 40.90% in direct steaming, 40.61% in Bligh and Dyer, 39.4% in Mc Gill and Moffat, 36.3% in Soxhlet extraction and 23.76% in solar extraction methods. But in other fish species, the monounsaturated fatty acid level was varied much. For example, in *O. niger* liver oil, it ranged from 27% to 31% (Immanuel et al., 2002) and in shark liver oil, it ranged from 4.35% to 41.21% (Zafar et al., 2003). Likewise, in Madura anchovy fish body oil, it varied from 16.9% to 77.1% (Nair & Gopakumar, 1977). Lovern (1962) reported the highest level (77.1%) of monounsaturated fatty acids in cod liver oil. In this study, the most abundant monounsaturated fatty acid in the detected oil samples was oleic acid (C18: 1), it ranged from 13.22% to 24.48%. This is in agreement with the result of Zafar et al. (2003) in shark liver oil, which was ranged from 0.03% to 27.05%. At the same time, it was less in dogfish liver oil (Sunaraya et al., 1992).

In this study, the polyunsaturated fatty acids (PUFAs) in the FAME were minimum of 12.4% in solar extraction method and maximum of 23.07% in Bligh and Dyer method, followed by Mc Gill and Moffat method (22.3%), Soxhlet extraction method (17.95%) and direct steaming method (15.74%). This result is consistence with the earlier reports of Immanuel et al. (2002) in *O. niger* liver oil. Similar variation was noticed in dominant PUFA like C20: 5n-3 and C22: 5n-3 of oil samples of fish New foundland capelin (*Mallouts villosus*) (Ackman, Jangoard, Burgher, Huges, & Macallum, 1963) and in Atlantic herring and cod liver oils (New, 1987). Zafar et al. (2003) were also reported the variation in PUFA (from 1.08% to 7.38%) of liver oil samples of two shark species such as *Eusphura blochii* and *Carcharhinus bleekeri*.

Amongst the PUFA, linoleic acid (C18: 2n6) was predominantly seen in Mc Gill and Moffat (10.7%) and Bligh and Dyer (11.85%) methods, followed by arachidonic acid (C20: 4n6), which is more or less uniform (3.3–3.9%) in all the four methods except solar extraction method (1.32%). Similar to this result, Zafar et al. (2003) reported that the level of arachidonic acid in the liver oil samples of two shark species such as *E. blochii* and *C. bleekeri* was 3.87% each. However, this level was little lower than the values reported in *O. niger* liver oil (Immanuel et al., 2002). Like wise EPA (C20: 5n3) level was very low in solar extraction method (1.68%), than other tested methods (3.0–3.67%). But the DHA (C22: 6n3) level of Bligh and Dyer (2.11%) as well as Mc Gill and Moffat (2.24%) methods was much higher than the other methods (1.2–1.5%) (Table 3). In dogfish liver oil, C20: 4n-6 was absent and C20: 5n-3 was in between 4.4% and 4.48% (Sunaraya et al., 1992). In pollack liver oil, C20: 5n-3 was present in fairly good amount of 12% (Ackman et al., 1963). But in this study, C20: 4n6 and C20: 5n3 were recorded in the level of 1.32–3.9% and 1.68–3.6%, respectively. In C22 PUFA series, C22: 2, C22: 3 and C22: 4 were absent in the *O. niger* liver oil, but C22: 5n3 and C22: 6n3 were present with the level of 1.0–1.7% and 0.48–2.3%, respectively (Immanuel et al., 2002). In dogfish liver oil also these series were absent except C22: 5 (eicosapentaenoic acid) and C22: 6 (decosahexaenoic acid) with less amount (Sunaraya et al., 1992).

3.2.2.4. Vitamin content. The fat soluble vitamins (A, D, E and K) of oil samples were determined in this study. Vitamin A, which is of particular nutritional importance for the visual system in humans. Several carotenoids are precursors of vitamin A. Certain fish and fish oils are also noted to contain a high quantity of vitamin A. Cod liver oil is known to contain a high quantity of vitamin A. In the fish Burbot (*Lota lota*) liver oil, the vitamin A content was within the range of 500,000 μg, which is about 20 times higher than that reported in cod liver oil (Wong, 2008). In this study vitamin A content in the oil sample extracted by Bligh and Dyer method was high (775.75 mcg/100 g), but low (498.48 mcg/100 g) in direct steaming method (Table 4).

Table 3
Fatty acid composition (%) by weight of *S. capisratus* liver oil samples extracted by different methods.

Carbon No.	Solar extraction	Direct steaming	Soxhlet extraction	Bligh and Dyer	Mc Gill and Moffat
C9:0	nd*	0.03	nd*	0.28	0.19
C10:0	0.76	0.98	0.81	0.99	0.89
C11:0	2.74	1.23	1.42	1.24	1.16
C12:0	0.33	0.75	0.83	nd*	nd*
C13:0	1.41	0.65	0.99	0.32	0.34
C14:0	6.41	5.21	5.02	2.02	3.70
C15:0	2.22	2.19	1.92	1.96	1.90
C16:0	29.78	16.46	18.73	11.76	13.68
C17:0	3.02	1.66	1.48	1.03	0.96
C18:0	14.4	11.28	10.96	13.28	12.80
C19:0	nd*	nd*	nd*	nd*	nd*
C20:0	1.24	1.34	2.04	2.42	2.51
C21:0	0.89	0.19	nd*	0.15	0.12
C22:0	0.44	0.75	nd*	nd*	nd*
C23:0	0.1	0.31	0.35	0.6	0.40
C24:0	0.12	0.33	0.2	0.27	0.22
C14:1	0.11	1.28	0.4	0.57	0.48
C16:1	4.8	1.34	8.22	7.64	8.85
C18:1	16.5	13.22	24.40	24.48	22.36
C20:1	1.1	24	nd*	5.02	4.86
C22:1	1.25	1.06	3.28	2.9	2.2
C18:2n6	5.09	1.84	5.05	11.85	10.7
C18:3n3	2.01	3.6	3.6	0.68	0.72
C20:4n6	1.32	3.9	3.3	3.55	3.48
C20:5n3	1.68	3.4	3.0	3.45	3.67
C22:5n3	1.1	1.5	1.5	1.43	1.52
C22:6n3	1.2	1.5	1.5	2.11	2.24
ε SAFA	63.84	43.36	45.75	36.32	38.3
ε MUFA	23.76	40.90	36.3	40.61	39.4
ε PUFA	12.4	15.74	17.95	23.07	22.33
ε n3	5.99	10.00	9.60	7.67	8.15
ε n6	6.41	5.74	8.35	15.4	14.18

* Not detected; Fatty acid content is expressed as area for present FAME and % (By weight).

Table 4
Fat soluble vitamin content of *S. capistratus* liver oil samples extracted by different methods.

Extraction methods	Vitamin A (mcg/100 g)	Vitamin D (mcg/100 g)	Vitamin E (mcg/100 g)	Vitamin K (mcg/100 g)
Solar extraction	675.75	35.60	6.86	4890.0
Direct steaming	498.48	28.66	5.06	2670.0
Mc Gill and Moffat	501.81	38.06	7.03	10120.0
Bligh and Dyer	775.75	37.87	10.45	3560.0
Soxhlet method	637.87	37.12	6.89	8560.0

Vitamin D is essential for the maintenance of normal blood levels of calcium and phosphate (Trivedi, Doll, & Khaw, 2003). The presence of vitamin D in Burbot liver oil was compared with that of reported in cod liver oil by Wong (2008). It may be noted that in commercial preparation of cod liver oil and other fish oils for medicinal purposes or for the nutritional therapeutic market, it would be advantageous to retain the natural vitamin D (Wong, 2008). In this study vitamin D content of the oil samples was more or less uniform in Bligh and Dyer, Soxhlet and Mc Gill and Moffat methods (37.12–38.06 mcg/100 g), but it was 28.66 and 35.60 mcg/100 g in direct steaming and solar extraction methods, respectively.

Vitamin K plays an important role in blood clotting and bone metabolism pertaining to the prevention of osteoporosis and carotid artery elasticity (Cranenburg, Schurgers, & Vermeer, 2007). Wong (2008) reported that burbot liver oil contains a high amount of vitamin K (1.7 mg/100 g of oil). In this study also vitamin K content in different oil samples was much higher (2670–10,120 mcg/100 g), amongst these, maximum level of 10,120 mcg/100 g was recorded in Mc Gill and Moffat method and minimum level of 2670 mcg/100 g was recorded in direct steaming method. Apart from the above fat soluble vitamins, considerable amount of vitamin E also present in the fish oils. In this study, vitamin E content of the oil samples extracted by Bligh and Dyer method was high (10.45 mcg/100 g), whereas low (5.06 mcg/100 g) in direct steaming method. In other methods, it was almost similar (6.86–7.03 mcg/100 g).

3.2.2.5. Heavy metal content. The fishes that live in fresh and salt water are harmed when heavy metals accumulated in the tissues. They do not have any specific mechanism to prevent bioaccumulation. Hence it is necessary to know the accumulation of heavy metals in the tissues of fishes, especially they used for consumption. The need for assessment of heavy metals in the oils is obligatory, because of its usage for pharmaceutical purpose. The dietary intake data derived from 1995 national nutrition survey section 4.5 sets out estimated dietary intake of metals with maximum permitted level of mercury and zinc were 0.5 and 1.0 mg/kg, respectively (NSW health news – www.msucares.com). But the food standards of Australia and Newzealand-2003 specified that the limit of dietary heavy metals such as arsenic, cadmium, lead and mercury should be within the range from 0.002 to 0.01 mg/kg (www.food-standards.gov.au). Like wise the International Fish Oil Standards set forth by the Council for Responsible Nutrition (CRN) specified the heavy metal content of fish oil Nutra Sea has <0.1 mg/kg each of lead, cadmium, arsenic and mercury, respectively (www.1stvitality.co.uk). In this study, amongst the heavy metals analysed, mercury level in all the methods of extracted oil samples was similar (2 μg/100 g). Low levels of mercury in these species may be explained by their feeding habits and not by the effects of geographical location. The arsenic level of oil samples was 5 μg/100 g each in Bligh and Dyer, solar extraction and Mc Gill and Moffat methods, respectively. But it was 1.0 and 3.0 μg/100 g in direct steaming and Soxhlet extraction methods, respectively. The lead

Table 5
Heavy metals and mineral content of *S. capistratus* liver oil samples extracted by different methods.

Extraction methods	Mercury (μg/100 g)	Arsenic (μg/100 g)	Lead (μg/100 g)	Cadmium (μg/100 g)	Copper (μg/100 g)	Iron (μg/100 g)	Sodium (μg/100 g)	Potassium (μg/100 g)	Magnesium (μg/100 g)	Phosphorus (μg/100 g)	Sulphur (μg/100 g)	Zinc (μg/100 g)
Solar extraction	2	5	<10	<1	**	6.45	50.67	114.6	*	350.8	*	0.787
Direct steaming	2	1	10	2	**	3.67	55.78	145.67	*	320.6	**	0.887
Mc Gill and Moffat	2	5	<10	1	0.054	7.89	78.6	186.78	**	320.6	*	3.56
Bligh and Dyer	2	5	<10	<1	0.123	7.89	45.56	105.5	*	343.3	**	1.565
Soxhlet method	2	3	<10	<2	0.012	5.89	60.78	185.7	**	330.5	**	1.78

* Not detected.
** Below detectable level.

content of all the oil samples was <10 μg/100 g except direct steaming method (10 μg/100 g). Cadmium content was 2.0 and 1.0 μg/100 g in direct steaming and Mc Gill and Moffat methods, respectively, whereas it was <1.0 g/100 g each in Bligh and Dyer and solar extraction methods, respectively (Table 5). The detected level of all these heavy metals is within the permissible limit, hence these oil samples can be used for edible or any pharmaceutical purposes.

3.2.2.6. Mineral content. The mineral contents recorded in the liver oil samples of *S. capistratus* also showed much variation and it was found to be influenced by the extraction methods adopted. The phosphorus content of liver oil was comparatively more (320.60–350.80 μg/100 g) in all the oil samples. Followed by, potassium content was ranged from 105.5 to 186.78 μg/100 g. Sodium content in all the oil samples was ranged between 45.56 and 78.6 μg/100 g with maximum level in Mc Gill and Moffat method. Similarly, the iron content of the oil samples was from 3.67 to 7.89 μg/100 g with maximum in Bligh and Dyer and Mc Gill and Moffat methods, respectively. Zinc content of all the oil samples was very low and it ranged from 0.787 to 3.56 μg/100 g with maximum at Mc Gill and Moffat method. Copper content recorded in all the oil samples was comparatively very low (0.012–0.123 μg/100 g), but in solar extraction and direct steaming methods, it recorded with below detectable level. Sulphur content of Bligh and Dyer, direct steaming and Soxhlet methods showed a below detectable level, whereas it was not identified in the other two methods such as solar extraction and Mc Gill and Moffat methods (Table 5). The above results are unique as for as fish liver oil is concerned. Despite the paucity of information on this line, Aidos et al. (2002) reported the presence of iron (0.8 ± 0.1 mg/kg wet sample) and copper (<0.1 mg/kg wet sample) in the herring oil sample. Young (1986) also reported the presence of iron (1.5 mg/kg) and copper (0.2 mg/kg) in marine fish oils.

4. Conclusion

The physico-chemical properties of liver oil of *S. capistratus* were found to be influenced by the isolation methods adopted. It revealed the presence of higher amount of PUFA and vitamins (vitamins A and K) in oil extracted by Bligh and Dyer method when compared with other extraction methods. It is evident from this study that Bligh and Dyer method is the suitable method for the extraction of liver oil from marine fishes without much loss of nutrients.

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References

Ackman, R. G., Jangoard, P. M., Burgher, R. D., Huges, M. L., & Macallum, W. A. (1963). An account of fatty acid components of New foundland capelin (*Mallouts villosus*). *Journal of American Oil Chemist Society, 40*, 564.

Adebiyi, O. D., & Bawa, A. A. (2006). Mackeral (*Scomber scrombrus*) oil extraction and evaluation as raw materials for industrial utilization. *Leonardo Journal of Science, 8*, 33–42.

Aidos, I., Vander Padt, A., Luter, J. B., & Boom, R. M. (2002). Seasonal changes in crude and lipid composition of herring fillets, byproducts and respective produced oils. *Journal of Agriculture Food Chemistry, 50*, 4589–4599.

AOCS (1998). *Official methods and recommended practices of the American oil chemist's Society* (5th ed.). Champaign, Illinois: AOCS.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Clark, T. H., Faustman, C. W., Furr, H. C., & Riesen, J. W. (1996). *Canathaxanthin as an antioxidant in a liposome model system and in supplemented rainbow trout* (*Oncorhynchus mykissy*) (p. 163). 1996 IFT Annual Meeting, Book of Abstracts.

Cranenburg, E. C. M., Schurgers, L. J., & Vermeer, C. (2007). Vitamin K: The coagulation vitamin that became omnipotent. *Thrombosis and Haemostasis, 98*, 120–125.

Delia, B., & Rodriqiuz, A. (2001). *A guide to carotenoid analysis in foods*. 1-57881-072-8. Washington, DC: ILS Press.

FAO (1986). The production of fishmeal and fish oil: Fishery industry division. *Fisheries Technology, 142*, 63.

Immanuel, G. (1996). *Studies on the resource, processing and utilization of marine fishery wastes as feed for shrimp*. Ph.D. thesis submitted to M.S. University, Tirunelveli, India.

Immanuel, G., Menenthira, V., Palavesam, A., & Peter Marian, M. (2002). Physico-chemical properties and fatty acid profile of *Odonus niger* liver oil. *Indian Journal of Fisheries, 49*(2), 147–153.

Kinsella, J. E. (1990). Sources of omega-3 fatty acids in human diets. In R. S. Lees & M. Karel (Eds.), *Omega-3 fatty acids in health and disease* (pp. 157–200). New York, USA: Marcel Dekker Inc..

Lehninger, A. L. (1984). *Biochemistry* (4th ed.). Ludhiana, India: Kalyani Publisher.

Lovern, J. A. (1962). In E. Heen & R. Kreuzer (Eds.), *Fish nutrition*. London: Fishing News Ltd..

Mc Gill, A. S., & Moffat, G. F. (1992). A study of the compositions of fish liver and body oil triglycerides. *Lipids, 27*, 360–370.

Metusalach, S. J., Brown, J., & Shahidi, F. (1996). Deposition and metabolism of dietary canthaxanthin in different organs of arctic charr (*Salvelimis aplinusl*). *Aquaculture, 142*, 99–106.

Miller, L., & Berger, T. (1985). *Bacteria identification by gas chromatography of whole cell fatty acids. Gas chromatography, application note, 228–241*(pp. 1–8). Hewlelt–Packard, 3000 Hanover, St. Palo Alto, CA 94304–1181.

Nair, R. K. G., & Gopakumar, K. (1977). Fatty acid composition of marine fish body fat. *Journal of Food Science and Technology, 14*, 263–270.

Navarro-Garcia, G., Pacheco-Aguilar, R., Bringas-Alvarado, L., & Ortega-Garcia, J. (2004). Characterization of the lipid composition and natural antioxidants in the liver oil of *Dasyatis brevis* and *Gymnura marmorata* rays. *Food Chemistry, 87*, 89–96.

Navarro-Garcia, G., Pacheco-Aguilar, R., Vallejo-Cordova, B., Ramirez-Suarez, J. C., & Bolanos, A. (2002). Lipid composition of the liver oil of shark species from the Caribbean and Gulf of California waters. *Journal of Food Composition Analysis, 13*, 791–798.

Negre-Sadargues, G., Castillo, R., & Segonzac, M. (2000). Carotenoid pigments and tropic behavior of deep-sea shrimps (Crustacean, Decapoda, Alvinocarididae) from a hydrothermal area of the Mid-Atlantic Ridge. *Comparative Biochemistry and Physiology A, 127*, 293–300.

New, M. B. (1987). *Feed and feeding of fish and shrimp. A manual on the preparation and presentation of compound feeds for shrimp and fish in Aquaculture development and co-ordination programme*. Rome, FAO, ADCP /87/26.

Shanmugam, A. (1990). *Fundamentals of biochemistry for medical students*. Published by author, Madras, India.

Sunaraya, S., Michael Hole, J., & Antony Taylor, K. D. (1992). Extraction and composition of Dog fish liver oil. *FAO Fisheries report*, 470(Suppl.).

Trivedi, D. P., Doll, R., & Khaw, K. T. (2003). Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: Randomised double blind controlled trial. *British Medical Journal, 326*, 469–473.

Vargheese, S. (2000). *Studies on the resource, morphometric features and biotechnological approach on extraction and characterization of liver oil from chosen species of shark*. M. Phil. thesis, M.S. University, Tirunelveli, India.

Wanasundara, U. N., Shahidi, F., & Amarowicz, R. (1998). Effect of processing on constituents and oxidative stability of marine oils. *Journal of Food Lipids, 5*, 29–41.

Wong, A. (2008). Lipidic profiles of tissue and liver oil of Burbot, *Lota lota* (L). *Acta Ithyologia Et Piscatoria, 38*(1), 55–61.

www.foodstandards.gov.au (2003). Food standards Australia and Newzealand, 20th Australian total diet survey. Appendix 5: Definition and glossary of terms, a supplementary information.

www.health.nsw.gov.au (2001). *NSW health heavy metal contamination of fish species* (pp. 26–27). No. 7. Recommendations.

Young, F. V. K. (1982). The production and use of fish oils. In S. M. Barlow & M. E. Stansby (Eds.), *Nutritional evaluation long-chain fatty acids in fish oil* (pp. 1–24). London, UK: Academic Press Inc..

Young, F. V. K. (1986). The chemical and physical properties of crude fish oils for refiners and hydrogenators. International Association of fish meal manufactures. *Fish Oil Bulletin, 18*, 1–19.

Zafar, S. S., Shamim, A., Kalid Mohamed, K., Shanaz, P., Syed Abdul Majid, A., Sohail, H. M., et al. (2003). A study on the fatty acid composition of fish liver oil from two marine fish, *Eusphura blochii* and *Carcharhinus bleekeri*. *Turkish Journal of Chemical Tubitak*, 251–258.