

Lipid class and fatty acid composition of meat and nonmeat components of selected seafoods

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ABSTRACT

Lipid extracts from meat, head and processing waste of selected Indian seafoods, viz., tuna, seerfish and shrimp were analyzed for lipid class distribution and fatty acid profile. Total lipid (TL) content was in the range of 18.8–30.1% in head, 10.34–25.23% in meat and 8.3–14.4% in waste, on dry weight basis. The highest TL was in seerfish head (30.10 %) and least in shrimp meat (25.23%). Neutral lipids (NL) were the dominant lipid class with highest being in head portions of the analysed samples (74.5–79%). Docosahexaenoic acid (DHA) was found in concentrations as high as 41.6% of total fatty acids in the phospholipids (PL) fraction of tuna meat. The present study highlights the utility value of marine by-products as a potential source of essential fatty acids like eicosapentaenoic acid (EPA) and DHA.

Keywords: DHA, Fatty acid composition, Lipid class, *Metapenaeus monoceros*, Seerfish, Tuna

Introduction

Global marine fish production currently stands at 141 million metric tonnes (mmt) (FAO, 2010) and India generates considerable foreign exchange by exporting seafoods in excess of 0.8 mmt (MPEDA, 2011). The major items of export include shrimps, molluscs and finfishes. Other than this, processed marine products like surimi, canned products and some readily eatable fishes are also exported. As per recent global estimates (FAO, 2010), the fish processing industry generates more than 63 mmt of processing waste which is rich in various biomolecules such as lipids, protein, chitin and carotenoid (Bhaskar *et al.*, 2010).

Consumption of marine products including fish is beneficial to the health and development of the human body. These natural products provide essential nutrients that are unavailable in terrestrial plants and animal products. In addition, fish lipids are valuable products, which have well documented health benefits (Calder, 2003; Vanschoonbeek, 2003). Marine fish oil preparations contain considerable amounts of unsaturated fatty acids of > 20 carbon atoms like eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6); while, most of the freshwater fish lipids contain fatty acids of < 20 carbon atoms (Zafar *et al.*, 2003). EPA and DHA contents of total fatty acids vary from 5 to 20% in most marine fish and from 3 to 5% in shellfish (Kamal-eldin and Yanishlieva, 2002; Amit *et al.*, 2011) which gives marine fish an edge

over freshwater fishes. It has been reported that highest concentrations of EPA and DHA are generally found in fish parts like viscera that are discarded. Lipids rich in PUFA are potential sources of antiaging, antithrombotic, antiinflammatory, anticholesterolemic and anticancer drugs to immunostimulant and immunosuppressant therapeutics (Sahena *et al.*, 2009).

There is an ever increasing demand for fish oil due to its various applications in healthcare and pharmaceutical products, besides being a valuable ingredient in specialty feeds used in aquaculture industries (Kim and Mendis, 2006; Turchini *et al.*, 2009). Apart from this, several other products with technical and cosmetic applications based on fish oil fatty acids have also been developed and produced commercially. Data on fatty acid composition aid food scientists and nutritionists in dietary formulation, processing and product development (Jadranka *et al.*, 2003). On account of its wide applications, researchers across the world have been seeking to augment fish oil production utilizing fish processing wastes as a source. The use and recycling of fish waste will also considerably reduce the cost of fish feed production and lessen pollution related problems (Turchini *et al.*, 2009). Against this background, the present work was carried out to assess qualitatively and quantitatively the lipids present in different body components (head, meat and waste) of selected Indian marine finfish and shellfish with special emphasis on fatty acid composition.

Materials and methods

The seerfish, *Scomberomorus commerson*, yellowfin tuna, *Thunnus albacares* and the shrimp, *Metapenaeus monoceros* were used in the study. Samples were collected from the local market and transported to the laboratory under frozen condition. Protein content was estimated by Kjeldahl method (AOAC, 2000).

Extraction of lipids and separation of lipid classes

The different portions *viz.*, head (except in shrimp), meat and waste were analyzed separately for lipid content, lipid classes and neutral lipid composition. Lipid extraction was carried out according to the method of Bligh and Dyer (1959). The different portions were separately minced and homogenized using a homogenizer (Polytron PT3100, Kinematica AG, Switzerland) in a solvent mixture of chloroform: methanol (2:1), kept overnight and filtered. The lipid extract was dried over anhydrous sodium sulphate to remove traces of moisture for total lipid extract and evaporated to dryness using rotary flash evaporator (Superfit, Bangalore, India)

Lipid classes were separated by open column chromatography (OCC) on silica gel 60-120 mesh size (1:30 w/w of lipid) by successive elution with chloroform (1:100 w/v), acetone-methanol (9:1 v/v) mixture (1:150 w/v) and methanol (1:100 w/v) to get neutral lipids (NL), glycolipids (GL) and phospholipids (PL). The NL were further separated into different sub-classes namely hydrocarbons, sterol esters, triacyl glycerol (TAG), free fatty acids (FFA), diacyl glycerol (DAG), monoacyl glycerol (MAG) by eluting on silica gel column with hexane and diethyl ether in ratios varying from 1:99 to 15:85 v/v.

Fatty acid composition of lipid extract:

The total lipids and their classes (NL, GL and PL) were analysed for fatty acid composition. The lipids were transmethylated using 2M methanolic sodium hydroxide followed by 2M methanolic hydrochloric acid to obtain fatty acid methyl esters (FAME). FAMES were analysed by gas chromatography (Shimadzu GC 2014, Japan) for

identifying the individual fatty acids. FAME dissolved in hexane was analyzed using Omegawax™ 320 fused silica capillary column (30 m x 0.32 mm x 0.25 mm). The conditions used for GC analysis was injection temperature of 250 °C, detector (FID) temperature of 260 °C and column temperature of 200 °C for 60 min. The peaks were identified by comparing with standards. Peak concentrations above 1% of total were only considered for calculation of % composition of fatty acids and the results are presented as mean of two analyses.

Statistical analysis

All the determinations were done in triplicates. Yield of different portions and total lipid content in different portions were compared by ANOVA using the software STATISTICA (Statsoft, 1999) and significant differences if any were separated by Duncan's multiple range tests using the same software.

Results and discussion

The proximate composition of different parts (meat, head and waste) of the selected fishes and shrimp (meat and waste) is shown in Table 1. The lipid content (dry weight basis) of the seerfish and tuna were 30.10 and 18.8% in head, 25.23 and 10.34% in meat and 14.08 and 14.40% in waste, respectively (Table 1). Lipid content in these fishes is comparatively lower than that of freshwater fishes such as rohu and catla (Swapna *et al.*, 2010) and other marine fishes such as sardine and mackerel (Amit *et al.*, 2011). In an earlier study in freshwater fishes, Swapna *et al.* (2010), obtained higher lipid content in viscera than in head and meat. In shrimp, lipid content were 3.90% (meat) and 8.30% (waste), which were lower than in the marine fishes analyzed. Lipid content in marine wild shrimp has been reported to be 0.9 g per 100 g (Bragagnolo and Rodriguez-Amaya, 2001). In some related studies, lipid contents from marine catfish viscera (Sathivel, 2002), fishes of gadidae family (Krzynowek *et al.*, 2006) and salmon (Gbogouri *et al.*, 2006) have been reported to be in the range of 0.4–35 %. Moisture content of various portions of different fishes was in the range of 66.30-80.57%. Protein content

Table 1. Proximate composition (on dry weight basis) of different parts of seerfish, tuna and shrimp (n=6).

Fish	Parts	Moisture %	Lipid %	Protein %
Seerfish	Head	70.47 ± 3.9	30.10 ± 1.28	62.74 ± 3.8
	Meat	75.47 ± 2.1	9.23 ± 0.50	87.71 ± 4.9
	Waste	76.01 ± 3.5	14.08 ± 0.62	84.65 ± 5.1
Tuna	Head	66.30 ± 3.1	18.80 ± 0.71	53.98 ± 2.5
	Meat	70.58 ± 2.6	10.34 ± 0.41	69.43 ± 3.3
	Waste	71.76 ± 3.3	14.40 ± 0.63	45.90 ± 2.4
Shrimp	Meat	75.09 ± 3.1	3.90 ± 0.08	60.15 ± 3.1
	Waste	80.57 ± 4.4	8.30 ± 0.11	76.61 ± 6.1

Values expressed as mean ± standard deviation

in different body parts of fishes analyzed ranged from 45.9 to 87.71% on dry weight basis. Protein content was higher in meat portion of seerfish (87.71%) and tuna (69.43%) when compared to waste and head, but in shrimp, protein content was higher in waste (76.61%) than in the meat (60.15%).

Lipids extracted were analyzed for lipid class composition, subclass of neutral lipid and fatty acid composition. Neutral lipids (NL) accounted to be the major class among the lipid classes, which ranged from 58.8 to 78.7% of the lipid content. the highest NL was found in head portion of tuna (78.7%) and seerfish (74.5%) as shown in Fig. 1A. This correlated well with previous studies on selected freshwater fishes (Swapna *et al.*, 2010) and marine fishes (Amit *et al.*, 2011). Considerable amount of glycolipid (GL) (19.2–34.8%) was found in all the species. - highest glycolipid (GL) was found in seerfish (22.4–34%) and lowest in shrimp (19.3–22.1%) (Fig. 1B). Low phospholipid (PL) was found among the lipid classes in all the fishes studied. PL was found to be high in shrimp compared to other marine fishes (Fig. 1C).

Analysis of total lipid and their classes (NL, GL and PL) for fatty acid composition revealed major fatty acids with chain lengths ranging from 14–22 carbons with 0–6 double bonds (Table 2, 3 and 4). The percentage composition of fatty acids in the lipid classes of marine fishes (Table 2–4) show higher amounts of unsaturated fatty acids. The USFA ranges from 46.3 to 63.1% in head, 47.6 to 66.7% in meat and 32 to 66.9% in waste of different fishes. The dominant fatty acids are palmitic acid (16:0) and stearic acid (18:0) among saturates and palmitic and oleic acids among the monounsaturated fatty acids. Of the two forms of oleic acid observed *i.e.*, 18:1n-9 and C18:1n-7, 18:1n-9 was dominant. Higher amount of palmitic acid in tuna meat has been reported earlier by Nimish *et al.* (2010).

The major n-3 polyunsaturated fatty acids (PUFA) observed in total lipids and their classes were eicosapentanoic acid (20:5n-3) and docosahexanoic acid (C22:6n-3) and linolenic acid (18:3n-3) in lower concentration (Table 2–4). Among n-6 fatty acids, arachidonic acid (AA) (20:4n-6) and linoleic acid (18:2n-6) were the major ones. AA and EPA are further responsible for future production of metabolites like eicosanoids, which play an important role in different reactions in vascular and immune systems. Presence of higher EPA and DHA content has been reported in various marine fishes, which thus differ from linolenic acid rich freshwater fishes (18:3n-3). Freshwater fishes (rohu, catla, tilapia, *etc.*) consume substantial amount of 18:3n-3 and 18:2n-6 derived from terrestrial and aquatic plants but marine fishes feed on marine plankton which contain more

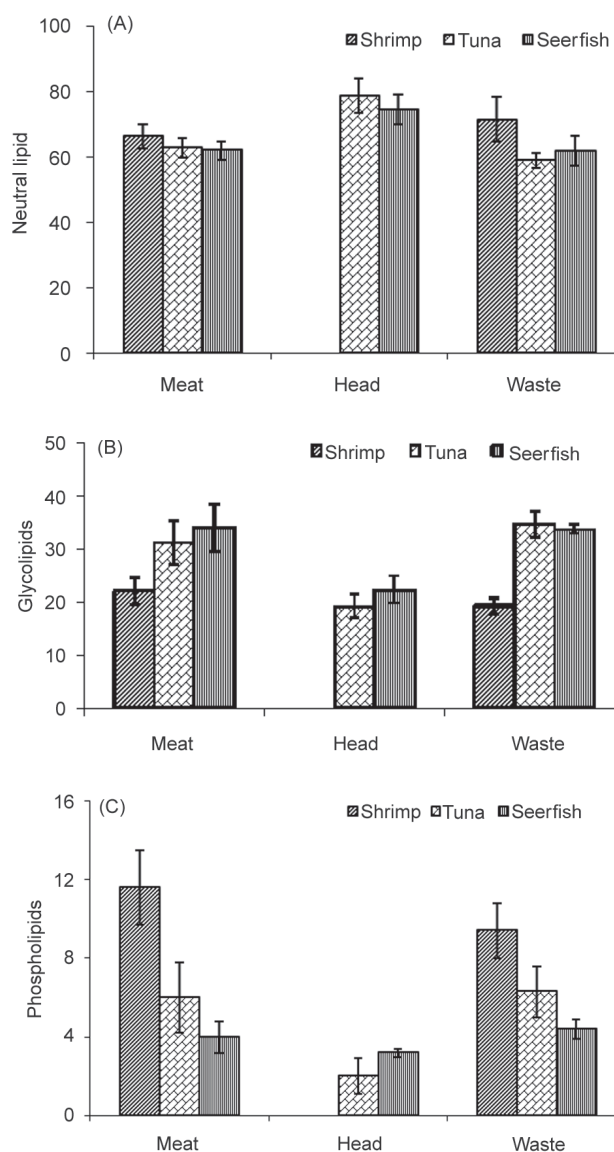


Fig 1. (A) Neutral lipid (%) (B) Glycolipid (%) and (C) Phospholipid (%) in different body components of seerfish, tuna and shrimp (n=3)

long chain (n-3) PUFA than freshwater plankton. This is regarded as the major reason for the basic difference between marine and freshwater fishes.

Among the lipid classes studied for fatty acid composition, PL had the highest content of unsaturated fatty acids, which ranged from 62.8 to 63.1% in head, 54.6–66.9% in waste and 57–66.7% in meat. Among PUFA, higher amounts of EPA and DHA were detected in PL fractions in waste of different species studied. The fatty acid profile of phospholipids can be used for identification (taxonomic) purposes (Kolakowska *et al.*, 2003). However, the PUFA composition of the total phospholipid of various

Table 2. Fatty acid composition (%) of total lipid (TL) and its lipid classes (as % of TL) of heads of seerfish and tuna

Fatty acids	Seerfish			Tuna				
	TL	NL	GL	PL	TL	NL	GL	PL
14:0	8.8	8.4	ND	2.6	5.2	5.3	6.9	ND
15:0	0.8	0.8	10.0	ND	1.4	1.4	1.7	0.8
16:0	25.2	24.7	25.0	26.4	20.2	20.3	21.4	19.3
16:1	11.1	10.9	12.2	5.4	7.2	7.4	8.7	3.9
18:0	6.4	6.7	5.4	6.7	6.4	10.2	5.6	9.1
18:1n-9	9.9	ND	ND	14.0	9.8	2.0	11.4	21.5
18:1n-7	3.6	13.8	12.3	3.2	2.3	1.6	ND	ND
18:2n-6	1.3	1.3	1.3	2.5	1.6	1.4	1.7	1.5
18:3n-3	ND	ND	ND	ND	0.9	1.2	1.1	0.6
20:0	ND	0.7	0.6	ND	0.6	1.7	ND	ND
20:4n-6	2.4	2.3	2.2	5.1	1.8	5.7	1.8	4.3
20:5n-3	9.9	9.6	11.7	8.9	5.8	ND	7.1	4.4
22:5n-3	1.6	1.7	1.5	1.4	1.3	1.2	1.1	ND
22:6n-3	13.2	12.7	11.7	22.4	26.7	25.9	23.3	26.6
ΣSFA	41.2	41.3	41.0	35.7	33.8	38.9	35.5	29.2
ΣUSFA	52.9	52.3	53.1	63.1	57.3	46.3	56.3	62.8
UI	5.9	6.4	5.9	1.2	8.9	14.7	8.2	8.0

SFA- saturated fatty acid; USFA- unsaturated fatty acid; UI- unidentified;

ND- not detected; TL- total lipid; NL- neutral lipid; GL- glycolipid; PL- phospholipids

Table 3. Fatty acid composition (%) of total lipid (TL) and its lipid classes (as % of TL) from meat portion of seerfish, tuna and shrimp.

Fattyacids	Seerfish				Tuna				Shrimp			
	TL	NL	GL	PL	TL	NL	GL	PL	TL	NL	GL	PL
14:0	9.2	10.7	9.9	1.9	4.9	5.2	5.5	1.0	1.8	1.0	ND	1.4
15:0	0.8	0.8	1.0	ND	1.3	1.4	1.4	ND	1.5	0.9	ND	1.9
16:0	25.4	23.9	23.8	21.9	20.7	21.7	19.8	16.3	19.2	13.9	25.2	22.0
16:1	11.7	10.6	12.3	3.3	5.5	5.9	19.8	2.0	8.8	6.3	12.4	8.7
18:0	6.5	6.8	5.4	8.8	9.1	10.2	1.6	8.5	11.4	13.8	8.6	8.8
18:1n-9	9.1	9.4	8.3	6.7	8.1	11.1	7.3	7.5	12.2	7.9	11.0	12.5
18:1n-7	3.6	3.5	3.3	3.0	2.6	ND	2.4	1.2	4.5	3.3	4.4	4.0
18:2n-6	1.2	1.6	1.3	0.9	1.4	1.5	1.3	1.0	2.0	2.2	2.6	2.1
18:3n-3	0.6	ND	ND	ND	1.5	1.7	ND	ND	0.7	ND	1.7	ND
20:0	0.5	ND	ND	ND	ND	ND	ND	ND	1.8	ND	2.7	1.2
20:4n-3	2.8	2.4	2.6	7.1	2.3	1.8	2.2	6.1	11.7	14.6	5.7	10.7
20:5n-3	9.9	8.1	11.8	10.2	5.0	4.5	4.8	6.2	9.6	14.1	5.0	9.6
22:5n-3	1.8	1.8	2.0	1.7	1.2	1.2	ND	1.1	ND	1.9	ND	1.6
22:6n-3	11.6	10.8	11.4	30.8	28.6	26.9	25.9	41.6	8.1	14.0	4.8	7.9
ΣSFA	42.5	42.2	40.1	32.6	36.0	38.5	28.3	25.9	35.7	29.6	36.4	35.3
ΣUSFA	52.2	48.2	53.0	63.7	56.3	54.7	63.7	66.7	57.7	64.3	47.6	57.1
UI	5.3	9.5	6.9	3.8	7.7	6.8	8.0	7.5	6.6	6.2	16.0	7.6

SFA- saturated fatty acid; USFA- unsaturated fatty acid; UI- unidentified; ND- not detected; TL- total lipid; NL- neutral lipid; GL- glycolipid; PL- phospholipid

species of fish and shellfish are not strikingly different (Kolakowska *et al.*, 2003), but the differences that do occur involve n-3 PUFA, particularly DHA.

Among TL of different fishes, DHA (22:6 n-3) was present in highest quantity in tuna meat (28.6%) followed by tuna head (26.7%). DHA content was found to be high

Table 4. Fatty acid composition (%) of total lipid (TL) and its lipid classes (as % of TL) from waste portion of seerfish, tuna and shrimp.

Fattyacids	Seerfish				Tuna				Shrimp			
	TL	NL	GL	PL	TL	NL	GL	PL	TL	NL	GL	PL
14:0	4.5	4.1	6.3	1.9	3.8	4.2	5.6	1.8	2.1	1.5	2.9	ND
15:0	0.7	0.6	0.9	0.6	2.7	1.4	1.6	1.1	1.7	0.9	1.7	ND
16:0	22.3	21.5	25.3	22.5	23.9	24.3	28.1	26.6	21.8	12.8	21.3	12.8
16:1	6.4	7.1	6.0	3.8	5.0	5.5	5.8	3.1	9.9	7.8	10.4	5.5
18:0	8.1	8.9	6.7	8.9	8.9	9.6	8.4	10.1	12.8	12.9	8.1	10.7
18:1n-9	7.4	8.3	9.0	9.9	8.7	9.4	10.1	6.7	13.7	8.3	9.8	9.9
18:1n-7	4.0	4.1	3.8	4.7	2.6	2.7	2.9	2.0	12.1	3.8	4.3	3.4
18:2n-6	1.1	1.2	ND	1.0	1.4	1.4	1.4	ND	2.4	2.5	1.4	1.8
18:3n-3	ND	ND	ND	ND	1.2	1.3	1.3	ND	0.7	ND	1.0	ND
20:0	ND	ND	ND	ND	ND	ND	ND	ND	1.5	1.5	3.7	ND
20:4n-6	6.4	7.9	3.3	11.5	3.0	2.9	2.5	5.8	12.3	12.6	5.8	15.9
20:5n-3	10.3	10.2	4.8	10.4	5.2	5.2	4.2	5.2	10.3	10.1	4.9	14.1
22:5n-3	3.2	3.8	2.1	2.1	1.4	1.3	ND	ND	2.1	1.5	0.9	2.1
22:6n-3	14.2	16.5	9.0	15.9	22.7	22.3	18.3	31.8	9.8	9.7	7.9	14.2
ΣSFA	35.6	35.2	39.2	34.1	39.3	39.4	43.6	39.5	39.9	29.7	37.8	23.5
ΣUSFA	53.0	59.1	38.0	59.3	51.3	51.9	46.5	54.6	63.5	56.3	39.3	66.9
UI	11.4	5.7	22.8	6.7	9.4	8.7	9.9	5.9	0.1	14.0	15.9	9.7

SFA- saturated fatty acid; USFA- unsaturated fatty acid; UI- unidentified; ND- not detected; TL- total lipid; NL- neutral lipid; GL- glycolipid; PL- phospholipid

in phospholipids fraction among the lipid classes (41.6% in tuna meat). DHA has well documented health benefits as it is known to reduce the risk of cardiovascular disease, hypertension, autoimmune and inflammatory diseases (Rustad., 2003; Kim and Mendis, 2006; Bhaskar *et al.*, 2006). DHA also plays an important role in adaptive processes (temperature, salinity and oxygen adaptation) as well as in motoric and social behavior of fish (Shulman and Love, 1999). Fishes higher in the marine food chain incorporates n-3 PUFA and further elongate them to 20 and 22 carbon atom fatty acids containing four, five and six double bonds by the action of specific desaturases.

NL from different body components of marine fish/shellfish analyzed in this study was further separated into

sub-classes and the result is presented in Table 5. Hydrocarbon and triglyceride formed the major constituents of NL in all the body components, ranging from 28.5 to 89.3% and 5.2 to 46.7%, respectively, depending on the body components and species. Further, hydrocarbon content was significantly higher ($p < 0.05$) in shrimp than in the two fishes analyzed. Free fatty acids, DAG/cholesterols and MAG ranged from 2.4 to 17.4 %, 1.0 to 8.9 % and 1.5 to 8.0 %, respectively. Several factors like seasonal variation, geographic differences, feeding habits, depth of habitat, water temperature *etc.* contribute to the variations in different body portions of these fishes (Hori and Itasaka., 1978; Falch *et al.*, 2006). Thus, the occurrence of high amounts of unsaturates like DHA clearly shows the

Table 5. Subclasses of neutral lipids of different components of seerfish, tuna and shrimp (n=3)

Fishes	Parts	HC	SE/FAME	TG/C	FFA/FA	DAG/Chol	MAG
Seerfish	M	36.8±1.3	0.7±0.1	45.6±4.5	17.3±2.8	8.0±1.1	2.1±0.3
	H	34.8±0.9	2.2 ± 0.5	46.7 ± 4.2	17.4±2.2	7.2±0.7	2.2 ± 0.3
	W	39.5 ± 2.8	1.8 ± 0.6	29.3 ± 1.9	12.6±2.3	8.9 ± 0.6	8.0 ± 0.5
Tuna	M	30.3±1.0	5.1 ± 0.4	46.1± 4.2	10.4±1.4	3.2 ± 0.9	4.4 ± 0.7
	H	28.5 ± 1.5	15.2 ± 1.2	36.2±3.7	12.2±1.9	3.5 ± 0.7	4.3 ± 0.5
	W	34.2 ± 1.1	11.6 ± 1.6	41.0 ± 4.9	12.2±1.5	5.8 ± 0.3	4.9 ± 1.9
Shrimp	M	89.3 ± 7.7	0.5 ± 0.1	5.2 ± 0.7	2.4 ± 0.3	1.0 ± 0.1	1.5 ± 0.2
	W	68.8 ± 5.1	0.9 ± 0.1	14.4 ± 1.8	7.7 ± 0.9	5.4 ± 1.2	7.9 ± 0.3

SE – sterolesters; FAME – fatty acid methyl esters; TG – triglycerides; FFA – free fatty acids; DAG – diacylglyceride ; MAG – monoacylglyceride
All the values are mean±standard deviation

importance of marine fish processing wastes as an important source of PUFA rich lipids. The PUFA rich oil and different fractions recovered can be used as dietary supplement of marine oil in pure form, oil enriched food as nutritional products or capsules.

Nowadays, ω -3 fatty acid has been the subject of intense international research because of its medical importance. As the study indicates the presence of relatively high content of EPA and DHA in head and viscera, efforts should be made to recover the lipids from these tissues for commercial utilization. Thus, byproducts and wastes generated from marine fish processing can be used as sources of fish oil rich in unsaturated fatty acids. These can be used as valuable ingredients in specialty feed in aquaculture industry. With increasing demand for fish oil, recovery of fish oil from processing wastes should help augment the supply, apart from benefiting the industry by minimizing problems associated with disposal and environmental pollution.

Acknowledgements

This work was partially supported by funding from the Department of Biotechnology (DBT), Govt. of India through grant #BT/PR 9474/AAQ/03/345/2007. Authors thank the Director, CFTRI, for encouragement and permission to publish the work.

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