Studies on Lipid and Fatty Acid Compositions of Puffer Livers from Indian Coastal Waters with Seasonal Variation

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ABSTRACT: The puffer fishes *Chelonodon patoca, Sphaeroides oblongus, Lagocephalus lunaris,* and *L. inermis* of Indian coastal waters are wasted in huge quantity. The livers of these fishes were investigated for their lipid contents and fatty acid compositions in different seasons. It was found that monsoon season is the suitable time to obtain the maximal lipids (40.1–48.8%) from their livers, an amount similar to cod liver lipid content (39.5–55.0%). The fatty acids were mostly saturates and monoenes (60–70%). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations (7–12%) were high during monsoon season. Neutral lipids were the predominant lipid class (>80%) and comprised triglycerides (277–674 mg/g) and cholesterol (0.6–3.1 mg/g). Quality indices of puffer liver lipids, e.g., specific gravity, refractive index, acid value, iodine value, saponification value and unsaponifiable matter, were evaluated. Puffer liver lipids were quantitatively and qualitatively comparable to other commercially important marine fish oils. The overall study suggests the possibility of future commercial utilization of liver lipids from puffer, an unconventional, cheap, and easily available source.

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KEY WORDS: DHA, DPA, EPA, fatty acids, liver lipids, puffer fish, quality index, seasonal variation.

Over the last decade, a substantial number of experiments have indicated that consumption of fish oils rich in ω-3 polyunsaturated fatty acids (n-3 PUFA) has health benefits (1,2). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), n-3 PUFA of biomedical importance, are known to play a major role in modulating the biosynthesis of eicosanoids (3) and in controlling the levels of blood lipids (4). Moreover, DHA is found in membranes of important organs, possibly influencing membrane-lipid-dependent functions, especially in retina and brain (5). These findings have created a new market for fish oil as a food and dietary supplement (6). Several other products with technical and cosmetic applications based on fish oil fatty acids have also been developed and produced commercially (7). Particularly, partially hydrogenated fish oil is utilized for producing margarines, shortenings, and compound fats. In recent years, a rapid growth in aquaculture throughout the world has created a market for fish oil in fish feed (8,9). Thus, there is now a growing demand for marine fish oils, the main source of n-3 PUFA, for use in human foods, pharmaceuticals, and industrial intermediates. Therefore, this area of research is flourishing to meet the current need.

We investigated the liver oils of four puffer fishes from Indian coastal waters. The lipid content and essential fatty acids such as EPA and DHA were determined with seasonal variation. This study indicated that Indian puffers can be utilized as an unconventional commercial source for EPA and DHA.

EXPERIMENTAL PROCEDURES

Collection of puffer species. Puffer fishes were collected fresh during three seasons, premonsoon (March–June), monsoon (July–October) and postmonsoon (November–February), by drag-net fishing from coastal Bay of Bengal (latitude 87°30′E, longitude 21°40′N), India. A thorough survey, from March 1995 to March 1997, regarding availability and species diversity of puffers revealed that four species are predominant throughout the year. These are *Chelonodon patoca, Sphaeroides oblongus, Lagocephalus lunaris*, and *L. inermis*, all belonging to the family *Tetraodontideae*. After every collection, the fish were identified and body weights were noted. Then fishes were dissected immediately and their sexes and liver weights were recorded. The liver tissues were preserved at –20°C for further analysis.

Extraction of lipids from puffer livers. The lipids from pooled puffer fish livers in each season were extracted following the method of Bligh and Dyer (10). The chloroform methanol extracts were allowed to settle overnight at room temperature to separate the organic and aqueous layers completely. The chloroform layer containing the lipids was separated and dried over anhydrous sodium sulfate. The solvent was removed in a rotary evaporator under vacuum. The resulting lipids were stored in amber-colored bottles under nitrogen atmosphere.

Analysis of lipid classes. Lipid classes, such as neutral and polar lipid, were separated by column chromatography. Silicic acid (100–200 mesh) was used as column material, and lipids were applied at 15 mg/g silicic acid to the column. Neutral lipid was eluted by chloroform (10 column vol), and polar

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lipid by methanol (10 column vol). Total lipid, neutral lipid, and polar lipid contents were determined gravimetrically. A small quantity of lipid was applied on thin-layer chromatography (TLC) plates coated with silica gel G (Kieselgel 60 G; Merck, Darmstadt, Germany) activated at 110°C for 1 h prior to development, and developed in the solvent mixture hexane/diethyl ether/acetic acid (70:30:1, by vol). Neutral lipid fractions such as cholesterol and triglycerides were identified, eluted by chloroform, and the solvent was then evaporated. An enzymatic assay method was adopted for the estimation of cholesterol (11) and triglycerides (12). Phospholipids were estimated spectrophotometrically (13).

Fatty acid analysis. About 0.2 g of lipid was hydrolyzed with 8% methanolic potassium hydroxide (50 mL) for 1 h at reflux temperature. After cooling, water (50 mL) was added and the basic solution was extracted twice with *n*-heptane/diethyl ether (1:1, vol/vol). The aqueous methanolic layer was acidified to pH 2; then fatty acid was extracted with *n*-heptane/diethyl ether (1:1, vol/vol, 3 × 25 mL), dried over anhydrous sodium sulphate, filtered, and the filtrate was concentrated to 2–3 mL. The fatty acids were esterified with BF_3-methanol (14).

Methyl esters of fatty acids were analyzed by gas–liquid chromatography with an HP 4890A instrument (Hewlett-Packard, Palo Alto, CA) equipped with flame-ionization detector (FID). A Hewlett-Packard capillary column (HP-5, 15 m × 0.53 mm i.d., film thickness 1.5 μm), was used for analysis. The column temperature was programmed for a linear increase of 2°C/min from 170 to 220°C and the injection and detector temperatures were 250°C. Nitrogen was used as carrier gas. Component fatty acids were identified by comparison of their chromatographic retention times with those of authentic standards obtained from Sigma Chemical Co. (St. Louis, MO) and/or with literature retention time values (15) and logarithmic plotting procedures (relative retention time vs. carbon number).

Quality assessment of liver lipids. The specific gravity (Method No. 920.213), refractive index (Method No. 921.08), acid value (Method No. 940.28), iodine value (Method No. 993.20), saponification value (Method No. 921.160), and unsaponifiable matter (Method No. 993.08) characterizing the quality of oils were determined following AOAC methods (16).

Statistical analysis of data. The results were expressed as mean ± SD and/or SEM.

RESULTS

Availability of puffer species. The four species of puffer fishes were abundantly available throughout the three seasons in the coastal regions of the Bay of Bengal. A 2-yr cumulative study from March 1995 to March 1997 revealed that the body weights of *S. oblongus, C. patoca, L. lunaris,* and *L. inermis* ranged between 300–500, 400–1200, 180-250, and 150–250 g, respectively. The hepatosomatic index (liver weight percentage with respect to body weight) was highest in *S. oblongus* (15.0%), *L. lunaris* (7.9%), and *L. inermis* (9.1%) in the monsoon (July–October), compared to premonsoon (March–June) and postmonsoon (November–February) seasons as shown in Figure 1. But the hepatosomatic index of *C. patoca* was highest (14.0%) in postmonsoon season. That might be due to the availability of the largest *C. patoca* in that season.

Lipid contents and lipid classes of puffer livers. It was found that the lipid content (%, wet weight of liver tissues) of all four species was greatest in the monsoon compared to other seasons (Fig. 2). Total lipids (TL) (% w/w) from puffer livers in the monsoon season were found to be 41.3%, 48.8, 42.0, and 40.1% for *S. oblongus*, *C. patoca*, *L. lunaris*, and *L. inermis,* respectively (Table 1). Neutral lipid fractions, 72.7–89.1% of TL, were the major lipid class in all four puffer liver lipids, and were most plentiful (89.1%) in *L. lunaris* liver. Polar lipid fraction was high in *C. patoca* (27.3%), whereas phospholipids were greatest in *L. inermis* (6.4%) and least in *L. lunaris* (1.5%). The concentration of free cholesterol was higher in *L. lunaris* (3.1 mg/g of TL) than in the other puffer liver lipids. Triglycerides, the main neutral lipid component, were found in maximal quantity (674.4 mg/g of TL) in *S. oblongus* liver lipids. It is clear from Table 1 that the chemical constitutions of the liver lipids from *S. oblongus*, *C. patoca*, *L. lunaris*, and *L. inermis* are different. No esterified sterols or wax esters were detected by TLC.

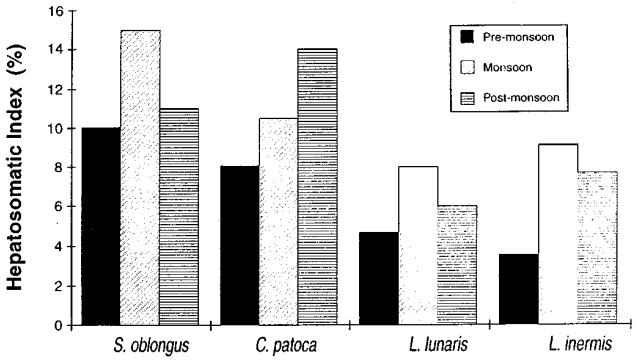

FIG. 1. Seasonal variation of hepatosomatic index (liver weight percentage with respect to body weight) of puffers of Indian coastal waters. Survey conducted March 1995–March 1997. For complete organism names see text.

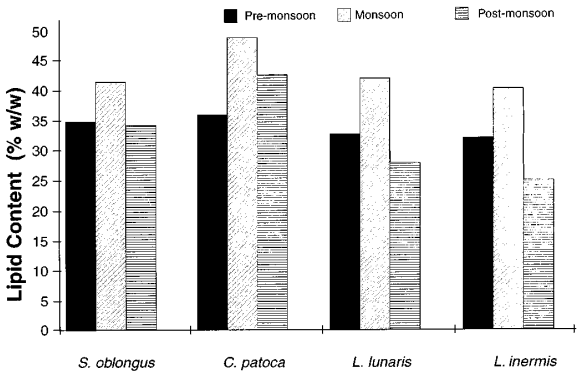

FIG. 2. Seasonal variation of lipid content from puffers of Indian coastal waters. Survey conducted March 1995–March 1997. For complete organism names see text.

TABLE 1
Lipid Compositions of Puffer Livers Collected in Monsoon Season

Lipid components[a]	*S. oblongus* ($n = 8$)[b]	*C. patoca* ($n = 8$)[b]	*L. lunaris* ($n = 10$)[b]	*L. inermis* ($n = 10$)[b]
Total lipid (TL) (% w/w)	41.3 ± 2.0	48.8 ± 2.7	42.0 ± 2.5	40.1 ± 2.2
Neutral lipid (% TL)	81.4 ± 1.2	72.7 ± 0.8	89.1 ± 0.2	82.4 ± 2.1
Cholesterol (mg/g TL)	0.7 ± 0.05	0.6 ± 0.03	3.1 ± 0.12	1.3 ± 0.01
Triglyceride (mg/g TL)	674.4 ± 0.01	277.1 ± 0.50	310.2 ± 0.01	560.9 ± 0.02
Polar lipid (% TL)	18.3 ± 0.5	27.3 ± 0.4	10.4 ± 1.2	17.6 ± 3.2
Phospholipid (% TL)	3.3 ± 0.5	2.0 ± 0.2	1.5 ± 0.4	6.4 ± 0.6

[a]Values are mean ± SD.
[b]Number of fish specimens taken in each determination. For complete organism names see text.

Seasonal variation of fatty acids. The fatty acid compositions of total liver lipids from different puffer species were determined (Tables 2–5) with seasonal variations. The major constituents of total fatty acids of *C. patoca* liver lipid were saturates (e.g., 16:0, 18:0, 20:0) and monoenes (e.g., 18:1, 20:1) in the premonsoon (60.1%), monsoon (70.2%), and postmonsoon (73.0%) seasons, as shown in Table 2. Similar results were obtained for *S. oblongus* (Table 3), *L. lunaris* (Table 4), and *L. inermis* (Table 5). The majority of fatty acids (saturates and monoenes) showed gradual accumulation from premonsoon to postmonsoon seasons in *C. patoca* and *S. oblongus* liver lipids, whereas in *L. lunaris* the concentrations decreased and in *L. inermis* they remained almost unchanged. Moreover, concentrations of the predominant saturate, palmitic acid (16:0), gradually decreased from premonsoon to postmonsoon in *C. patoca* (Table 2), *L. lunaris* (Table 4), and *L. inermis* (Table 5) fatty acids, whereas, the predominant monoene oleic acid (18:1), gradually increased from premonsoon to postmonsoon. But *trans*-seasonal increases were observed in *S. oblongus* 16:0 and 18:1 fatty acids (Table 3), which constitute the majority of fatty acids. The concentrations of n-3 PUFA, EPA, and DHA are vital for their biomedical importance. EPA + DHA in *C. patoca* (7.2%), *S. oblongus* (7.6%), *L. inermis* (9.0%), and *L. lunaris* (12.2%) was greatest during the monsoon; DHA was found in higher concentrations than EPA in all fishes. n-3-Docosapentaenoic acid (DPA) was found in appreciable quantity in *L. inermis* (5.5%), *L. lunaris* (6.3%), *S. oblongus* (3.5%), and *C. patoca* (3.6%) during monsoon season. Higher quantities of n-3 DPA were found in the fatty acids of *L. inermis* (10.9%), *L. lunaris* (11.4%), and *C. patoca* (6.6%) in postmonsoon season.

TABLE 2
Fatty Acid Compositions of Liver Lipids from *C. patoca*

Fatty acid component	Percentage of total fatty acids[a]		
	Premonsoon	Monsoon	Postmonsoon
12:0	trace[b]	0.1 ± 0.1	0.2 ± 0.1
14:0	4.8 ± 0.4	3.0 ± 1.0	3.1 ± 0.4
14:1n-7	0.8 ± 1.3	2.1 ± 0.5	1.9 ± 0.2
16:0	24.0 ± 2.3	22.2 ± 1.7	12.8 ± 1.6
16:1n-7	13.6 ± 1.4	11.4 ± 0.9	9.9 ± 1.7
18:0	2.2 ± 2.4	10.7 ± 0.3	trace
18:1n-9	7.4 ± 0.9	12.1 ± 1.3	22.4 ± 1.11
18:2n-6	29.1 ± 0.7	2.3 ± 0.2	trace
18:3n-3	trace	2.8 ± 0.1	trace
20:0	0.6 ± 1.0	trace	13.6 ± 0.3
20:1n-9	5.4 ± 0.2	8.7 ± 0.7	trace
22:0	0.3 ± 0.9	trace	trace
22:1n-11	1.1 ± 0.8	trace	9.2 ± 1.2
20:4n-6	0.7 ± 0.8	7.2 ± 0.2	6.3 ± 0.1
20:5n-3	2.4 ± 0.1	2.8 ± 0.7	4.8 ± 0.5
22:4n-6	0.8 ± 0.9	2.1 ± 0.1	1.6 ± 0.1
22:5n-6	0.2 ± 0.6	0.8 ± 1.2	5.4 ± 0.1
22:5n-3	3.4 ± 0.6	3.6 ± 0.2	6.6 ± 0.7
22:6n-3	0.9 ± 0.1	4.4 ± 0.1	1.2 ± 0.3
Others	2.1 ± 2.6	3.4 ± 1.8	0.5 ± 1.2
Saturates	31.9 ± 1.2	36.0 ± 1.7	29.7 ± 2.3
Monoenes	28.3 ± 0.9	34.3 ± 0.7	43.4 ± 1.2
n-3/n-6	0.22	1.09	0.94
EPA + DHA	3.3 ± 0.3	7.2 ± 0.1	6.0 ± 0.4

[a]Values are mean ± SD.
[b]trace , <0.1%; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. For complete organism name see text.

TABLE 3
Fatty Acid Compositions of Liver Lipids from *S. oblongus*

Fatty acid component	Percentage of total fatty acids[a]		
	Premonsoon	Monsoon	Postmonsoon
12:0	trace[b]	0.2 ± 0.1	trace
14:0	4.9 ± 1.1	4.8 ± 1.0	3.5 ± 0.1
14:1n-7	0.7 ± 1.0	1.1 ± 0.2	1.6 ± 0.2
16:0	22.4 ± 2.2	28.5 ± 1.7	34.5 ± 1.3
16:1n-7	12.9 ± 0.6	7.8 ± 0.3	trace
18:0	11.1 ± 0.1	13.3 ± 1.3	1.5 ± 1.0
18:1n-9	10.3 ± 2.3	11.7 ± 0.4	31.8 ± 0.9
18:2n-6	19.0 ± 0.2	3.9 ± 0.1	trace
18:3n-3	trace	1.5 ± 0.7	0.3 ± 0.9
20:0	0.7 ± 0.1	trace	6.5 ± 0.3
20:1n-9	4.7 ± 0.5	2.7 ± 0.7	trace
22:0	trace	trace	1.2 ± 0.1
22:1n-11	n.d.[b]	n.d.	n.d.
20:4n-6	1.9 ± 1.0	1.5 ± 0.3	2.0 ± 0.6
20:5n-3	2.1 ± 0.3	3.2 ± 0.1	0.1 ± 0.1
22:4n-6	1.1 ± 1.1	0.7 ± 1.7	trace
22:5n-6	0.5 ± 0.9	0.5 ± 1.3	0.8 ± 0.3
22:5n-3	2.9 ± 0.2	3.5 ± 0.6	1.7 ± 0.2
22:6n-3	2.4 ± 0.1	4.4 ± 0.4	1.2 ± 0.1
Others	2.1 ± 2.2	9.9 ± 0.9	12.9 ± 0.7
Saturates	39.1 ± 1.3	46.8 ± 2.3	47.2 ± 3.1
Monoenes	28.6 ± 1.7	23.3 ± 2.3	33.4 ± 3.1
n-3/n-6	0.32	1.93	1.19
EPA + DHA	4.5 ± 0.9	7.6 ± 0.3	1.3 ± 0.2

[a]Values are mean ± SD.
[b]n.d., not detected (<0.01%); for other abbreviations see Table 2. For complete organism name see text.

TABLE 4
Fatty Acid Compositions of Liver Lipids from *L. lunaris*

Fatty acid component	Percentage of total fatty acids[a]		
	Premonsoon	Monsoon	Postmonsoon
12:0	0.1 ± 0.1	0.2 ± 0.2	trace
14:0	6.6 ± 1.7	5.6 ± 2.1	2.9 ± 0.7
14:1n-7	trace	1.1 ± 0.3	0.9 ± 1.2
16:0	34.6 ± 0.2	23.7 ± 0.6	21.9 ± 2.4
16:1n-7	11.5 ± 1.0	14.4 ± 1.3	10.3 ± 3.2
18:0	2.7 ± 2.3	5.4 ± 1.1	0.8 ± 0.9
18:1n-9	10.6 ± 1.7	12.0 ± 0.2	19.5 ± 0.8
18:2n-6	13.9 ± 1.3	1.6 ± 0.1	2.1 ± 0.3
18:3n-3	trace	2.6 ± 0.9	1.0 ± 0.3
20:0	0.5 ± 0.9	trace	2.8 ± 2.1
20:1n-9	1.9 ± 0.8	2.5 ± 0.4	trace
22:0	n.d.	n.d.	n.d.
22:1n-11	n.d.	trace	1.6 ± 1.4
20:4n-6	0.4 ± 1.7	2.7 ± 0.7	3.4 ± 1.0
20:5n-3	1.8 ± 1.3	3.6 ± 0.1	2.2 ± 0.1
22:4n-6	trace	0.8 ± 1.7	0.7 ± 0.7
22:5n-6	trace	1.1 ± 0.1	5.8 ± 1.4
22:5n-3	3.1 ± 0.6	6.3 ± 0.2	11.4 ± 0.6
22:6n-3	0.9 ± 0.3	8.6 ± 2.3	2.2 ± 1.8
Others	11.0 ± 0.9	7.4 ± 1.6	10.4 ± 1.3
Saturates	44.5 ± 2.1	34.9 ± 1.3	28.4 ± 3.1
Monoenes	24.0 ± 1.4	30.0 ± 1.1	32.3 ± 2.1
n-3/n-6	0.41	3.33	1.38
EPA + DHA	2.7 ± 0.4	12.2 ± 0.1	4.4 ± 0.7

[a]Values are mean ± SD. For abbreviations see Table 2. For complete organism name see text.

TABLE 5
Fatty Acid Compositions of Liver Lipids from *L. inermis*

Fatty acid component	Percentage of total fatty acids[a]		
	Premonsoon	Monsoon	Postmonsoon
12:0	trace	1.2 ± 0.1	trace
14:0	5.5 ± 0.9	5.3 ± 0.5	2.9 ± 1.4
14:1n-7	0.9 ± 0.1	0.4 ± 0.2	1.1 ± 0.1
16:0	35.1 ± 0.8	29.9 ± 2.1	18.6 ± 0.1
16:1n-7	10.1 ± 0.7	11.7 ± 1.3	14.6 ± 0.7
18:0	2.1 ± 1.1	7.4 ± 4.6	9.2 ± 0.4
18:1n-9	14.3 ± 2.3	14.5 ± 1.2	19.6 ± 0.3
18:2n-6	21.1 ± 0.4	0.7 ± 0.3	trace
18:3n-3	1.4 ± 0.1	0.8 ± 2.4	0.9 ± 1.2
20:0	0.7 ± 0.9	trace	2.9 ± 2.3
20:1n-9	1.1 ± 0.7	1.9 ± 0.3	0.5 ± 2.7
22:0	0.1 ± 0.6	trace	trace
20:2n-11	1.1 ± 0.6	0.8 ± 0.2	2.0 ± 3.1
20:4n-6	0.3 ± 0.1	2.1 ± 0.2	4.9 ± 5.6
20:5n-3	0.6 ± 1.7	2.5 ± 0.9	1.3 ± 0.4
22:4n-6	0.8 ± 1.3	1.0 ± 0.8	0.9 ± 0.1
22:5n-6	0.4 ± 0.4	0.9 ± 0.5	6.9 ± 0.1
22:5n-3	1.7 ± 0.1	5.5 ± 0.9	10.9 ± 0.7
22:6n-3	0.9 ± 0.7	6.5 ± 0.1	1.2 ± 1.03
Others	1.7 ± 0.5	6.7 ± 0.6	1.0 ± 1.3
Saturates	43.5 ± 2.4	43.8 ± 3.3	33.6 ± 1.7
Monoenes	27.5 ± 2.1	29.3 ± 2.7	37.8 ± 1.3
n-3/n-6	0.21	3.21	1.12
EPA + DHA	1.5 ± 0.3	9.0 ± 0.1	2.5 ± 1.4

[a]Values are mean ± SD. For abbreviations see Table 2. For complete organism name see text.

In recent years, n-3 PUFA have been acclaimed for greater potency in the amelioration of heart disorder than n-6 PUFA. Therefore, the n-3/n-6 ratio is a marker of biomedical significance for fish oils. In puffers, the overall n-3/n-6 values reach maximum in *C. patoca* (1.1), *S. oblongus* (1.9), *L. inermis* (3.2), and *L. lunaris* (3.3) in monsoon compared to other seasons.

Quality assessment of liver lipids. The physical parameters identifying the quality of puffer fish lipid are shown in Table 6. Specific gravity of *S. oblongus* liver lipid is 0.9653, which is the minimum among puffer liver lipid, whereas refractive indices among the four species are similar. Acid values of lipids from *L. lunaris* (16.9), *S. oblongus* (15.7), and *C. patoca* (12.3) are higher than that of *L. inermis* (7.1). It is interesting to note (Table 6) that the iodine and saponification values of liver lipids from the four puffer species maintain an inverse relationship. For instance, in liver lipids of *S. oblongus*, iodine value (109.0) is maximum and saponification value (230.6) is minimum, whereas in *C. patoca* iodine value (76.4) is minimum and saponification value (458.5) is maximum. Unsaponifiable matter is greatest (2.2) in the lipids of *L. lunaris* among the four species of puffers (Table 6).

DISCUSSION

Several marine fishes have been investigated for fatty acid and lipid compositions since the biomedical implication of n-3 PUFA was established (17). Generally, fish oils for commercial purposes are obtained from the livers of fishes such as cod, shark, and various types of jack, even though other marine fishes such as anchovy, bass, menhaden, sardine, mackerel, and salmon are utilized in their entirety. Cod, being the most popular raw material for the production of oil, is going to be increasingly rare due to overexploitation, thus causing a hike in the market price. Hence, with a view to identifying other readily available commercial sources for fish oil,

TABLE 6
Quality Indices of Puffer Liver Lipids in Monsoon Season

Fish species[a]	Specific gravity at 25°C (mean ± SD)	Refractive index at 25°C (mean ± SD)	Acid value (mean ± SD)	Iodine value (per g of oil) (mean ± SD)	Saponification value (per g of oil) (mean ± SD)	Unsaponifiable matter (%) (mean ± SD)
S. oblongus	0.9653 ± 0.00	1.4742 ± 0.00	15.7 ± 0.03	109.0 ± 0.00	230.6 ± 1.12	0.6 ± 0.01
C. patoca	0.9985 ± 0.01	1.4739 ± 0.00	12.3 ± 0.01	76.4 ± 0.00	458.5 ± 0.50	0.1 ± 0.04
L. lunaris	0.9936 ± 0.00	1.4722 ± 0.00	16.9 ± 0.01	92.3 ± 0.01	360.2 ± 0.32	2.2 ± 0.15
L. inermis	0.9878 ± 0.00	1.4717 ± 0.00	7.1 ± 0.02	105.9 ± 0.00	332.8 ± 0.08	0.2 ± 0.09

[a]For complete organism names see text.

the puffers, which are wasted in huge quantity, were studied for liver lipids.

Puffer fish, like flounder, have a low muscle-to-liver lipid ratio, indicating that lipid is accumulated in their livers, not in their muscles (18). A 2-yr survey indicated that the hepatosomatic indices in different puffer species range from 7.2 to 15.0% (Fig. 1) and are highest in the monsoon season. A quite similar hepatosomatic index was found in cod, 9.5% (19) and in some 40 specimens of deep sea fish, *Laemonema longipes*, 10.3% (20). In shark it was much higher, 23.3% (21).

The lipid content as well as fatty acids in marine fishes varied remarkably with season, fishing ground, and sex (22,23). As lipid was mainly accumulated in the liver of puffers (18,24), a seasonal variation of lipid content was evaluated (Fig. 2) in four species of puffer from coastal Bay of Bengal, India. Lipid contents of puffer livers were found to be highest (40.1–48.8%) during monsoon season (Table 1), comparable to that of cod liver (39.5–55.0%) (19,22). Shark livers (80.5–95.3%) (21) and *L. longipes* (74%) (20) were the richest lipid sources.

Apart from total lipids, the lipid composition, fatty acids, and cholesterol content have been investigated in many fish lipids (25). By examining the lipid class composition, puffer lipids were discriminated from other marine fish oils. He *et al.* (26) observed a clear discrimination in liver lipids of a Chinese variety of puffer, *Fugu vermicularis*. Puffer liver lipids of the four species under investigation showed the same consequences (Table 1). Fish stored fat mainly in the liver, which accounts for the accumulation of neutral lipids and, consequently, high triglycerides. This fact was supported with high neutral lipids (>80%) as well as triglycerides (277–674 mg/g) in puffers (Table 1). In *L. lunaris* liver lipid, the neutral lipid is exceptionally high, indicating large accumulation of fat in liver cells. The cholesterol concentration in fish lipids is important owing to its vital role in metabolism and nutrition. An appreciable quantity of cholesterol is found in the unsaponified matter of puffer liver lipid (27). Cod liver oil contains 570 mg/100 g of total lipid (17), whereas in puffer liver lipid the value is much lower, between 58 and 310 mg/100 g. The phospholipids are contributed mainly by liver cell membranes, since phospholipids have long been recognized as the building bricks of cell membrane. Increases in neutral storage lipids result in reduction in the proportion of polar lipids, particularly phospolipids (28). In puffers an inverse relationship between polar lipids and neutral lipids is in accordance with the above observation (Table 1).

The fatty acid composition of marine fish lipids is complex. Factors including season, water temperature, nature of fats in their diets, maturity, and gender affect the makeup of the distribution patterns. As a result, the composition and content of fatty acids may vary not only from species to species but also to an even greater extent from specimen to specimen of the same species. The fatty acid composition of puffer lipid shows distinct variation with season and also within the species in a particular season. The majority of fatty acids consists of saturates and monoenes, 60–70% (Tables 3–6). Palmitic acid (16:0) and oleic acid (18:1) are the predominant saturate and monoene, respectively. A similar observation was reported by McGill and Moffat (29) in liver lipids of cod, saithe, monkfish, and other fish oils marketed commercially. In striped sea bass (*Morone saxatilis*), palmitic acid and oleic acid constitute the majority (62%) of total fatty acids in every season (30). The concentrations of n-3 PUFA, EPA and DHA, signifying the biomedical importance of fish oil, are of vital consideration. In puffers EPA + DHA is at its highest quantity, 7–12%, in monsoon season (Tables 2–5)—low in comparision to cod liver oil (14.5–20.9%). DPA of the n-3 form was found in appreciable quantity in all puffer fatty acids, similarly to those in other marine fishes (31). Recent emphasis on n-3 PUFA over n-6 PUFA suggests that n-3/n-6 ratio could be an index of biomedical application (4,32). Fatty acids in puffer liver lipids have an n-3/n-6 ratio between 1 and 3 in monsoon season, which is quite good.

Quality assessment of fish oil indicates the stability of these products in technological processing and during storage. Therefore, the quality indices were determined for puffer liver lipids in monsoon season (Table 6). Fish liver lipids are very prone to enzyme activity accounting for high free fatty acids, as in cod livers (33). Higher concentrations of free fatty acids are not desired for fish oils used in nutritional applications. In puffer liver lipids, free fatty acid contents of *C. patoca* (12.3), *S. oblongus* (15.7), and *L. lunaris* (16.9) are comparable to that of cod liver oil (12.6). Puffer liver lipid has moderate iodine value (Table 6) compared to other Indian marine fishes (34). The specific gravity of puffer liver lipids is higher than other fish oils, whereas refractive index is the same. Saponification value is comparatively high, suggesting the high concentration of saturated short-chain fatty acids, such as palmitic acid (16:0), which is found in maximum quantity in the fatty acids of puffers (Tables 2–5). The raw puffer liver lipids have very little unsaponifiable matter. The overall quality of puffer liver lipids has significant similarity to commercially important marine fishes.

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