Asian Journal of Fisheries and Aquatic Research

Volume 21, Issue 6, Page 44-57, 2023; Article no.AJFAR.98195
ISSN: 2582-3760

Fatty Acids Profiling, Lipid Quality Indices and Antioxidant Properties of Some Small Indigenous Fishes of Manipur, India under Indo-Burma Biodiversity Hotspot Region

Mayanglambam Shantosh [a*], Tongbram Seityamala [b] and Chungkham Sarojnalini [b]

[a] G.P. Women's College, Imphal, Manipur, India.
[b] Centre of Advance Study in Life Sciences, Fishery Laboratory, Manipur University, Canchipur, Manipur, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJFAR/2023/v21i6559

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/98195

Original Research Article

Received: 28/01/2023
Accepted: 30/03/2023
Published: 04/04/2023

ABSTRACT

Aims: The aim of the study is to access the nutritional qualities *viz*. fatty acids profile, lipid quality indices and antioxidant properties of fish oil. The present study will provide the information on the fulfillment of various nutritional qualities of small fishes.
Study Design: The study was carried out during the month of October, 2017 to February, 2018 in fishery laboratory, department of Life Sciences, Manipur University, India.
Methodology: The extraction and analysis of fatty acids were carried out following the method of Metcalfe et al. 1966. The fatty acids are identified searching in the NIST database using mass

**Corresponding author: Email: shantmay18@gmail.com;*

spectral search program. For antioxidant property the extract was analyzed using DPPH assay following the method described by Koleva et al. 2002. Data were analyzed using SPSS package (Version 17.0).

Results: The most abundant fatty acid (SFA) is palmitic acid (C16:0) which is followed by Stearic acid (C18:0) and undecylic acid (C11:0). The concentration of C16:0 was in the ranged of 14.67% to 27.96% which was highest in *H. myitkyinae* and lowest in *G. giuris*. Among the monounsaturated fatty acid (MUFA) Oleic acid (C18:1n9) was the most abundant fatty acid which is followed by Vaccenic acid (C18:1n11) and heptadecanoic acid (C17:1) respectively. Docosahexanoic acid (DHA) and Ecosapentanoic acid (EPA) are the predominant n3 fatty acids in all the fish studied. The index of atherogenicity (AI), index of thrombogenicity (TI), Hypocholesterolemic/ Hypercholesterolemic (HH) fatty acid ratio and Flesh lipid quality (FLQ) was in the ranged of 0.79% to 1.10%, 0.13% to 0.67%, 0.53%- 2.53% and 1.90% to 7.72% respectively. The highest antioxidant property was found in *P. chola* (8.69 µg/ml) and the lowest was observed *T. burmanicus* (46.83 µg/ml).

Conclusion: From the above analysis Small indigenous freshwater fishes (SIFFs) contained good lipid which provide enormous amount of PUFA and MUFA especially DHA and EPA contribute to various health related benefit. The scavenging activities of antioxidant properties of the SIFFs are also found to be considerable. Thus it can be indicates that these small fishes have good nutritional quality and can bring many benefits to human health.

Keywords: Antioxidant; DHA; EPA; fatty acid composition; high nutritive value; lipid quality index.

1. INTRODUCTION

"The Indo-Myanmar region is kaleidoscopes approximately expand over two million square kilometer of tropical Asia. It has amazing geographical diversity and is among the top 10 biodiversity hotspot in the world. It supports an enormous variety of habitat, area of endemism and plethora of biodiversity of the region due to their stunning variation of landform and climatic zone. Asia's mighty river such as Mekong, Chao Phyara, Irrawady, Salween, Chindwin, Shittaung, Red and Pearl are shaping the ecology and live of people of the region. Freshwater fishes support rural livelihoods in the region but they are among the most threatened organism in Indo-Myanmar region, due to unsustainable fishing practices, invasive species, and habitat alteration and loss" [1].

Small Indigenous Fish are those fish which grow to a maximum size of 25 cm or 9.8 inches in the mature or adult stage in their life cycle [2]. However, many of the Small indigenous freshwater fish species (SIFFs) are less than 10 cm or 3.9 inches in length and they are consumed as a whole. These fish have short life cycle and are highly prolific breeders and need little or no management to grow. They can easily propagate and live in backyard ponds, beels, wetland and can be grown in all types of inland water bodies.

National Bureau of Fish Genetic Resources (NBFGR) has recorded about 2,246 species of fin fish, out of these fish species 765 are freshwater fish. India has contributed 27.85% of native fish fauna, followed by China, Indonesia and Myanmar. Out of 765 fish species, about 450 species are classified as SIFFs. North East India owing to its topographical features provides an ideal habitat for various endemic small fish. As many as 216 species of small fish are recorded from North Eastern India. In Manipur, these small fish are abundant in river, beels, streams, canals and ponds.

"Fish and sea food are rich dietary sources of Docosahexaenoic acid [DHA, 22:6(n-3)] and eicosapentaenoic acid [EPA, 20:5(n-3)] but poultry and eggs provides lower. However, DHA and EPA are absent from vegetable oils, including nuts, grain and seed and also very low in ruminant fats including milk and dairy product" [3]. "DHA is the most abundant (n-3) fatty acid in the mammalian brain, and its level in brain membrane lipids is altered by the type and amount of fatty acids in the diet, and with life stages, increasing with development and decreasing with aging. Mammals obtain DHA either as DHA itself or the precursor α-linolenic acid [ALA, 18:3(n-3)], and intermediates between ALA and DHA, including EPA" [4].

"Dietary lipids have traditionally been considered as solely part of the exchangeable energy supply. Presently there is a growing interest in the quality of dietary lipid supply even in early childhood as a major determinant of growth,

infant development, and long-term health. Lipids are also the main sources of energy in the infant diet, since it plays an important role in normal growth and physical activity. The selection of dietary lipid supply in the first year of life is now considered to be critically important" [5]. Among the n–3 fatty acids EPA and DHA are increasingly being recognized as important modulators of multiple biological pathways that affect health and disease.

Moreover, fish are also known to be rich sources of polyunsaturated fatty acid (PUFA) especially n–3 and n–6 fatty acid. Fish oil is also a good source of n–3 fatty acid namely EPA (20:5n3) and DHA (20:6n3), which is known to play an important role in preventing various diseases like cardio vascular diseases, atherosclerosis, thrombosis, and arrhythmia. Some also play a role in reducing the cholesterol level [6], regulate prostaglandin synthesis and hence induce wound healing.

"Recent studies have shown that small fishes have a good antioxidant property. Antioxidants are able to protect the body against oxidative stress by neutralizing free radicals. The antioxidant activity of fish oil refers to its chemical property of neutralizing free radical damage present in the body. Ω-3s especially DHA and EPA are capable of reducing free radical levels although it is not considered as a super antioxidant" [7].

The floodplain areas are the main source of small fish which are mostly occupied by the rural people. Thus, these small fishes have contributed as main sources of micronutrients in their daily diet. "However, with the present trends of decreasing floodplain fisheries and increasing aquaculture, a larger proportion of SIFFs as a diet will be substituted by mostly larger fish and overfishing and deterioration of natural habitat have results in a decline of SIFFs. This will have a negative impact on the nutritional contribution especially of vitamin A content, calcium and phosphorus which is much lesser in content in larger fish species than the SIFFs. To maintain and enhance SIFFs intake, sustainable management and restoration of floodplain fisheries must be given high priority" [8].

There are numbers of findings in the nutritional value of many fishes. However, there is lack of information on importance on nutritional aspect of these SIFFs. Therefore, it is necessary to know the chemical composition and nutritional status of these fishes. Thus, the aim of the current study is to investigate nutritional aspects and biochemical composition of these Small Indigenous Fishes to improve the nutritional information and to share the knowledge of nutritional value to the people.

2. MATERIALS AND METHODS

SIFFs studied were collected from different areas of Manipur. *Devario yuensis*, *Hypsibarbus myitkyinae* and *Tariqilabeo burmanicus* were collected from Moreh market in Chandel district. *Lepidochephalichthys guntea* and *Pangio pangia* were collected from Nambol Market in Bishnupur district. Whereas, *Glossogobius giuris* and *Puntius chola* were collected from Moirang Market in Bishnupur district and Khuwairamband Market (Ima Market) in Imphal west District respectively. *Syncrossus berdmorei* was collected from Sekmai River, Kakching of Thoubal district and the tributaries of Manipur River, Manipur (Fig. 1). The length and weights of the fishes are shown on Table 1.

2.1 Fatty Acid Analysis

Total lipid was extracted by following the modified method of Singh, et al. 1990 by using chloroform and methanol in the ratio of 2:1. Fatty acid methyl esters (FAME) were prepared from the lipid extract following the method of Metcalfe et al. [9]. Methanolic NaOH of 4 ml was added to about 150 mg of fish oil and refluxed until the fat globule become soluble. Then 5 ml of BF_3CH_3OH (Boron trifluoride methanol) was added and refluxed for another 5 min. Saturated sodium chloride of 16 ml was added to the mixture to separate the fatty acid methyl esters (FAMES) and it was transfer to separating funnel. The upper layer was collected and extracted by 20 ml of petroleum ether twice. The collected petroleum ether fraction was passed through anhydrous sodium sulphate and evaporated. The ester was dissolved in 5ml hexane and made ready for injection. The prepared sample was injected into Gas chromatogram for analysis and by injecting 10 µl of FAME into a Clarus 680 GC & Clarus 600C MS, Perkin Elmer, USA; Liquid Autosampler equipped with C18 column. The fatty acids were confirmed manually and by searching the NIST database using NIST mass spectral search program [10].

Table 1. The respective collection sites and length-weight

Species	Local name	Collection site	GPS Location	Total Length (cm)	Weight (gm)	IUCN Red list Status
Devario yuensis	Ching-nga	Moreh Market	24˚14'57.34"N 94˚18'07.50"E	6.73±0.25	2.81±0.54	Vulnerable
Glossogobius giuris	Nilon ngamu	Moirang Market	24˚30'05.03"N 93˚46'34.21"E	13.56±0.81	19.86±2.83	Least concern
Hypsibarbus myitkyinae	Heikak nga	Moreh Market	24˚14'57.34"N 94˚18'07.50"E	12.86±0.6	23.58±0.72	Least concern
Lepidocephalichthys guntea	Ngakijou	Nambol market	24˚43'01.49"N 94˚50'09.88"E	7.93±0.31	3.12±0.32	Least concern
Puntius chola	Phabou Nga	Khuwairamband Market, Imphal (Ima Market)	24°48'28.48"N 93°56'01.45"E	8.30±0.17	6.42±0.14	Least concern
Pangio pangia	Nganap	Nambol market	24˚43'01.49"N 94˚50'09.88"E	6.90±0.15	1.42±0.06	Least concern
Syncrossus berdmorei	Sareng Khoibi	Sekmai river, Kakching (A tributary of Imphal river)	24˚28'05.79"N 94˚59'44.54"E	13.76±0.14	17.92±0.61	Near threatened
Tariqilabeo burmanicus	Ngaroi	Moreh Market	24˚14'57.34"N 94˚18'07.50"E	10.86±0.40	8.76±0.39	Least concern

Values are mean of three replicates; Standard deviation (±SD) of the three replicates is shown

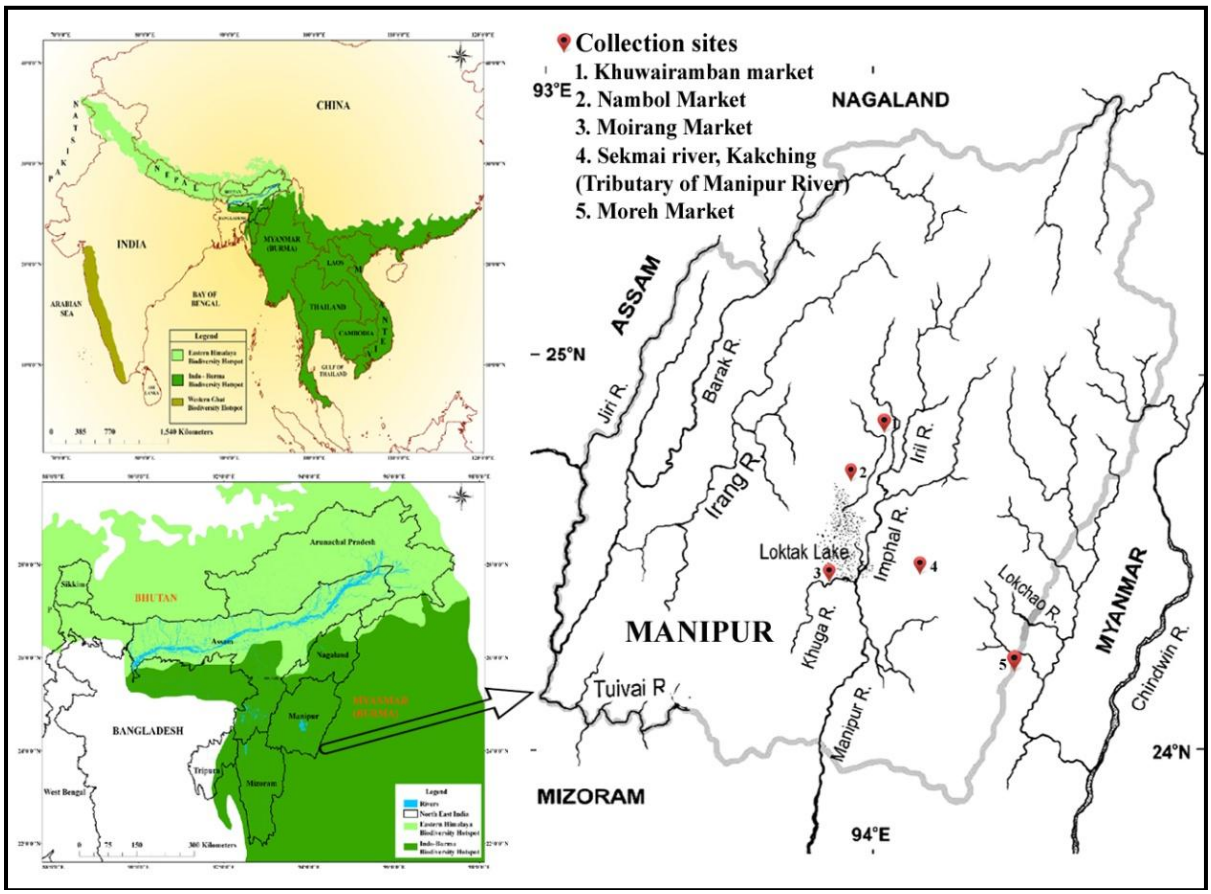

Fig. 1. Map showing Indo-Myanmar biodiversity hotspot and collection sites of Small indigenous fishes

2.2 Fatty Acids Quality

The Index of Atherogenicity (AI) and Index of Thrombogenicity (TI) were calculated following the method of Ulbricht and Southgate [11].

2.2.1 Index of atherogenicity (AI)

The AI indicates the relationship between the sum of the main saturated fatty acids with main classes of unsaturated fatty acids.

$$AI = [C12:0 + (4 \times C14:0) + C16:0]/(n\text{-}3PUFA + n\text{-}6PUFA + MUFA)$$

PUFA—polyunsaturated fatty acids, MUFA—monounsaturated fatty acids. C12:0—lauric acid, C14:0—myristic, C16:0—palmitic.

2.2.2 Index of thrombogenicity (TI)

The TI shows the tendency to form clots in the blood vessels. This is defined as the relationship between the prothrombogenetic (saturated) and the antithrombogenetic fatty acids (MUFA, n–3 PUFA and n–3 PUFA).

$$TI = [C14:0 + C16:0 + C18:0]/[(0.5 \times C18:1) + (0.5 \times \Sigma MUFA) + (0.5 \times n\text{-}6PUFA) + (3 \times n\text{-}3PUFA) + n\text{-}3PUFA/n\text{-}6PUFA)]$$

2.2.3 Hypocholesterolemic / hypercholesterolemic (HH)

It is a fatty acid ratio as described by Santos-Silva et al. [12].

$$HH = (C18:1n9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:6n3) / (C14:0 + C16:0)$$

2.2.4 Flesh-lipid quality (FLQ)

“The FLQ indicates the percentage correlation between the main n–3 PUFA (EPA + DHA) and the total lipids. The higher value of this index is an indicator of the higher quality of the dietary lipid source” [13].

$$FLQ = 100 \times [EPA + DHA]/[\%\ of\ total\ fatty\ acids]$$

Hypocholesterolemic fatty acids (OFA): ***(OFA)*** = *C12:0 + C14:0 + C16:0*

Hypercholesterolemic fatty acids (DFA): ***(DFA)*** = *C18:0 + UFA*

EPA—eicosapentaenoic acid (C20:5), DHA—docosahexaenoic (C22:6), UFA—unsaturated fatty acids (MUFA + PUFA), C18:0—stearic acid

2.3 Antioxidant Analysis from Fish Oil

Fish oil of 1g was extracted with 8 ml of hexane. Then 2 ml of methanol/water (60:40) was added and then the mixture was vortexes vigorously for 2 min. The extracted concentration was analyzed for DPPH assay following the method described by Koleva *et al*. 2002. Each concentration (0.02 – 4.00 mg/ml) of a test sample (10 µL) in methanol: water was added to 190µL of 150 µM DPPH in methanol. After vortex mixing, the mixture was incubated for 10 minutes at room temperature and the absorbance values were measured at 517 nm. The differences in absorbance between a test sample and Control (DPPH alone) was taken and the IC_{50} values were determined as the concentration of the sample that gave a 50% decrease in the absorbance from a blank test.

2.4 Statistical Analysis

The data were subjected to one way-ANOVA and the significant mean were compared by Duncan's multiple range tests ($P<0.05$). Relationship of correlation coefficients between fatty acids and length-weight was identified using Pearson's correlation coefficients. Differences and correlations were considered significance when $p<0.05$ and $p<0.01$ were obtained. Species were grouped and classified in a cluster by their similarities produce in the data. All the statistical analyses were performed using SPSS version 16.0.

3. RESULTS AND DISCUSSION

3.1 Fatty Acids

The fatty acid composition of the SIFFs is shown in Table 2. A total of 30 fatty acids of various chain and saturation level are presented in the fatty acid composition of the present study.

The most abundant fatty acid (SFA) is palmitic acid (C16:0) which is followed by Stearic acid (C18:0) and undecylic acid (C11:0). The concentration of C16:0 was in the ranged of 14.67% to 27.96% which show highest in *H. myitkyinae* and lowest in *G. giuris*. Other SFAs such as pelargonic acid (C9:0), lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), pentadecanoic acid (C15:0), heptadecanoic acid (C17:0), aracidic acid (C20:0), behenic acid (C22:0) and melissic acid (C30:0) were found in very low concentration. The predominance of palmitic acid, stearic acid and undecyclic acid is appeared to be characteristics of freshwater fishes. The presence of these fatty acids has been usually associated to planktonic or bacterial sources and they are commonly present in fish fat [14]. The findings are in agreement with many reports. Enrique *et al*. 2014 have reported that C16:0 is the most dominant SFA followed by C18:0 in four eastern central Pacific native fish species. Renata and Danuta [15] observed predominance of palmitic acid and stearic acid in Grass Carp, Bighead Carp, Siberian Sturgeon, and Wels Catfish.

Oleic acid (C18:1n9) is the most abundant Monounsaturated fatty acid in all the species studied. Prato and Biandolino [16] also reported oleic acid as the most abundant MUFAs in most marine fishes. The concentration of oleic acid of present study is comparable with the concentration reported by Gonzalez et al. [17] in farmed and wild yellow perch. The difference in the concentration may be due to dietary effect and saturation and elongation mechanism of the fatty acids [18]. In an investigation of Yaqoob et al. [19] shown that the consumption of MUFA diet for 2 months affect the NK cell activity by increasing immune function in middle aged men.

The most abundant n–3 polyunsaturated fatty acid (PUFA) was docosahexaenoic acid (DHA, C22:6n3) which is followed by eicosapentaenoic acid (EPA, C20:5n3) and α–linolenic acid (ALA, C18:3n3). The concentration of docosahexaenoic acid (DHA, C22:6n3) was in the range of 4.13% to 5.03% which shows highest concentration in *D. yuensis* and lowest in *P. chola*. The predominant DHA among the n–3 PUFA were reported in many previous studies. The predominance of DHA is reported in the native fish species of eastern central Pacific, reaching up to maximum of 19.65% in *C. caballus* [20]. The present DHA concentration obtained is in agreement with the finding of Manoharan et al. [21] in Indian freshwater fish *L. thermalis*. Other n–3 PUFA such as eicosapentanoic acid (EPA, C20:5n3), α-linolenic acid (ALA, C18:3n3),

mangiferic acid (C18:2n3) and eicosatrienoic acid (ETE, C20:3n3) are also found in considerable amount.

Arachidonic acid (AA, C20:4n6) is the most abundant n–6 PUFA which is followed by dihomo–gamma–linolenic acid (DGLA, C20:3n6). Highest concentration of arachidonic acid was found in *D. yuensis* (3.29%) and the lowest was found in *P. chola* (1.87%). Moreover, concentration of DGLA, C20:3n6 was highest in *D. yuensis* (3.14%) and lowest in *P. chola* (1.82%). The higher concentration of arachidonic acid in SIFFs studied could be attributed to the type of diet they are exposed in the wild such as insect larvae, freshwater algae, crustacean that are rich in linoleic and linolenic acid [18]. Hei and Sarojnalini, [22] reported in traditionally processed hill stream fish of Manipur that α-linonelic acid (C18:3n3) is the most abundant PUFA followed by Linoleic acid (C12:2n6), other PUFA such as DHA and EPA concentration are in agreement with their report. The concentration of n–6 PUFA of the present study was higher from the previous report of Strobel et al. [23] and Iwona et al. [24].

The difference in accumulation of fatty acids in fish muscle is influenced by several factors besides diet and genetics, such as sexual maturity, geographic location and the season of the catch. Moreover, the difference in characterization of fatty acid composition of fresh water fishes may be reflected to the composition of their dietary lipids [25].

In our study total SFA shows the highest percentage which is followed by total PUFA and total MUFA in all the fishes (SFA>PUFA>MUFA). The trend of the percentage was in agreement with the findings of Stancheva and Merdzhanova 2011 in grey mullet. However, Hadjinikolova (2005) presented the relative FA pattern as MUFA>SFA>PUFA.

The total fatty acid, ratio of n–3/n–6 and n–6/n–3 ratio was shown in Table 3. Among the fatty acids total saturated fatty acids were the most abundant among the fishes studied. However, good amount of PUFAs was also observed in the fishes studied. The n–3/n–6 ratio and n–6/n–3 ratio is frequently used to analyzed the nutritional quality of fish oil. The UK Department of Health has recommended that the value of n–6/n–3 should be lower than 4.0 [26]. In our finding the n-6/n-3 ratio has a value lower than the threshold limit, with a maximum of 1.15 placing all the SIFFs studied to be categorized as potentially healthy species. The lower ratio of n–6/n–3 is much more desirable in reducing the risk of many chronic diseases highly prevalent in western societies and developing countries [27]. It is also revealed from the study that the n–3/n–6 ratio have values ranging from 0.86 in *H. myitkyinae* to 1.52 in *G. giuris* which showed that the SIFFs studied can be considered as high-quality fish having potential health benefit when consumed.

The Pearson's correlation between the total fatty acid and weight–length of SIFFs studied was presented in Table 4. From the comparison it was revealed that the length and weight of the fishes is negatively correlated with total SFA (–0.224 and –0.025 respectively) and total n–6 PUFA (–0.172 and –0.026 respectively). Both length and weight are positively correlated with total MUFA (0.241 and 0.116 respectively). The total SFA showed negative correlation with total MUFA (–0.621), total n–3 PUFA (–0.679) and total n–6 PUFA (–0.099). However, total MUFA, total n–3 PUFA (0.455) and n–6 PUFA (0.036) were shown positive correlated among each other.

3.2 Lipid Quality Index

Another index used to evaluate the nutritional value of oil and fat in food are atherogenicity and thrombogenicity and the relation between hypocholesterolemic and hypercholesterolemic fatty acids [28]. Saturated fatty acids such as lauric acid (C12:0). myristic acid (C14:0) and palmitic acid (C16:0) are the only fatty acid considered as hypercholestesterolomic [11] whereas stearic acid (C18:0) is thought to be neutral with respect to atherogenicity but is instead considered to be thrombogenic [29]. Foods with lower concentration of these fatty acids have lower index of atherogenicity (AI) and index of thrombogenicity (TI) and therefore, have more nutritional benefit. Lower AI and TI values suggest a higher amount of anti-atherogenic fatty acids presence in a given oil or fat, and a higher potential for the prevention of coronary disease [30] and higher values of AI and TI (> 1.00) are detrimental to human health [31].

Lipid quality index viz. index of atherogenicity (AI), index of thrombogenicity (TI), flesh lipid quality (FLQ) and hypocholesterolemic / hypercholesterolemic (HH) property was depicted in Fig. 2. *H. myitkyinae* (0.67) showed highest TI whereas *D. yuensis* (0.53) shows lowest. Moreover, the highest HH was found in

Table 2. Fatty acids concentration (%) of small indigenous fish species (SIFFS)

Fatty acid as% of fatty acid	*D. yuensis*	*G. giuris*	*H. myitkyinae*	*L. guntea*	*P. chola*	*P. pangia*	*S. berdmorie*	*T. burmanicus*
Saturated Fatty Acids (SFA)								
C9:0	0.11±0.06^{a}	0.26±0.78^{a}	0.13±0.07^{ab}	0.21±0.08^{a}	0.5±0.11^{a}	0.16±0.07^{ab}	0.28±0.03^{a}	0.89±0.08^{c}
C11:0	1.84±0.11^{b}	1.14±0.07^{a}	0.37±0.10^{a}	2.81±0.07^{c}	8.05±0.07^{e}	6.46±0.06^{d}	3.24±0.13^{c}	1.63±0.06^{b}
C12:0	0.1±0.05^{a}	0.1±0.06^{a}	0.25±0.09^{bc}	0.29±0.07^{c}	0.11±0.04^{a}	0.19±0.07^{abc}	0.14±0.01^{ab}	0.2±0.05^{abc}
C13:0	0.09±0.06^{a}	0.1±0.05^{a}	0.17±0.10^{ab}	0.29±0.07^{ab}	0.11±0.06^{a}	0.19±0.04^{ab}	0.14±0.01^{ab}	0.2±0.05^{ab}
C14:0	0.11±0.07^{a}	0.26±0.10^{a}	0.13±0.03^{a}	0.21±0.07^{a}	0.5±0.10^{b}	0.16±0.04^{a}	0.28±0.01^{a}	0.89±0.13^{c}
C15:0	0.09±0.00^{a}	0.1±0.06^{a}	0.17±0.11^{ab}	0.29±0.07^{b}	0.11±0.07^{a}	0.19±0.07^{ab}	0.14±0.01^{a}	0.2±0.06^{ab}
C16:0	19.74±0.36^{f}	14.67±0.16^{d}	27.96±0.12^{h}	25.2±0.07^{g}	17.86±0.05^{e}	9.66±0.12^{b}	7.67±0.12^{a}	12.36±0.05^{c}
C17:0	0.9±0.11^{a}	0.09±0.00^{a}	0.12±0.07^{a}	0.15±0.06^{a}	0.37±0.06^{b}	0.16±0.07^{a}	0.29±0.03^{b}	0.04±0.02^{a}
C18:0	10.84±0.27^{g}	10.14±0.10^{f}	0.23±0.12^{a}	2.81±0.09^{c}	8.05±0.06^{e}	6.46±0.05^{d}	I.25±0.02^{b}	10.63±0.15^{g}
C20:0	0.031±0.00^{s}	0.17±0.07^{sb}	0.15±0.08^{sb}	0.18±0.07^{sb}	0.14±0.06^{sb}	0.16±0.06^{sb}	1.87±5.61^{c}	0.46±0.06^{b}
C22:0	0.49±0.12^{c}	0.19±0.09^{ab}	0.1±0.06^{a}	0.28±0.09^{b}	0.87±0.06^{d}	0.2±0.07^{ab}	0.54±0.02^{c}	0.12±0.07^{a}
C30:0	0.08±0.24^{a}	0.08±0.00^{a}	0.13±0.08^{ab}	0.23±0.07^{b}	0.21±0.06^{b}	0.2±0.06^{b}	0.17±0.02^{ab}	0.15±0.06^{ab}
Monounsaturated Fatty Acids (MUFA)								
C14:1n5	0.029±0.01^{a}	0.08±0.00^{ab}	0.08±0.02^{ab}	0.39±0.07^{c}	0.23±0.07^{b}	0.42±0.06^{c}	8.19±0.18^{d}	0.16±0.06^{ab}
C16:1n9	0.1±0.05^{a}	0.25±0.07^{a}	0.1±0.06 ^{a}	0.1±0.08 ^{a}	0.14±0.07 ^{a}	0.17±0.06 ^{a}	0.22±0.03 ^{a}	0.06±0.03 ^{a}
C16:1n10	0.49±0.12^{c}	0.11±0.07 ^{a}	0.11±0.08 ^{a}	0.28±0.06^{b}	0.87±0.06^{d}	0.2±0.03 ^{ab}	0.54±0.03^{c}	0.22±0.08 ^{ab}
C17:1n7	0.03±0.00 ^{a}	0.27±0.07^{b}	0.13±0.07 ^{ab}	0.27±0.06^{b}	0.12±0.07 ^{ab}	0.19±0.07 ^{ab}	3.47±0.17^{c}	0.1±0.06 ^{ab}
C18:1n6	0.02±0.00 ^{a}	0.16±0.06 ^{a}	0.08±0.05 ^{a}	0.16±0.06 ^{a}	0.11±0.08 ^{a}	0.35±0.15^{b}	0.1±0.02 ^{a}	0.16±0.06 ^{a}
C18:1n9	2.53±0.22^{d}	3.4±0.2^{e}	5.22±0.07^{g}	0.3±0.06 ^{a}	3.94±0.07^{f}	6.4±0.07^{h}	2.23±0.17^{c}	1.34±0.05^{b}
C18:1n11	2.07±0.10^{c}	0.75±0.08 ^{b}	0.1±0.07 ^{a}	0.18±0.06 ^{a}	0.9±0.16^{b}	0.17±0.07 ^{a}	0.91±0.09^{b}	0.23±0.07 ^{a}
n–3 Polyunsaturated Fatty Acids (PUFA)								
C18:2n3	3.52±0.09^{e}	2.96±0.10^{b}	3.09±0.09^{bc}	3.97±0.08	2.51±0.07 ^{a}	3.35±0.13^{cd}	3.17±0.15^{c}	2.91±0.07^{b}
C18:3n3	3.11±0.11^{c}	3.09±0.07^{c}	1.1±0.07^{a}	4.1±0.08^{d}	2.16±0.08^{b}	4.27±0.05^{e}	3.22±0.08^{c}	2.13±0.07^{b}
C20:3n3	0.93±0.18^{ab}	1.18±0.07^{d}	0.77±0.10^{a}	1.05±0.05^{bc}	0.83±0.06^{a}	1.16±0.06^{d}	1.05±0.09^{ac}	1.13±0.07^{d}
C20:5n3	3.08±0.16^{c}	2.17±0.07^{b}	2.14±0.10^{b}	4.12±0.06^{e}	1.14±0.06^{a}	3.25±0.08^{c}	3.47±0.14^{d}	4.1±0.08^{e}
C22:6n3	5.03±0.28^{c}	4.69±0.12^{b}	4.25±0.06^{a}	6.29±0.08^{d}	4.13±0.06^{a}	5.19±0.06^{c}	6.14±0.15^{d}	5.2±0.07^{c}

Fatty acid as% of fatty acid	*D. yuensis*	*G. giuris*	*H. myitkyinae*	*L. guntea*	*P. chola*	*P. pangia*	*S. berdmorie*	*T. burmanicus*
n6–Polyunsaturated Fatty Acids (PUFA)								
C18:2n6	1.26±0.09^{cd}	0.72±0.07^{b}	0.81±0.07^{b}	1.1±0.08^{c}	0.51±0.07^{a}	1.17±0.06^{c}	0.88±0.12^{b}	1.36±0.05^{d}
C18:3n6	1.49±0.13^{e}	1.35±0.09^{de}	2.11±0.07^{g}	0.98±0.07^{ab}	0.87±0.07^{a}	1.75±0.08^{f}	1.22±0.12^{cd}	1.09±0.06^{bc}
C20:2n6	1.33±0.10^{c}	1.72±0.08^{d}	0.83±0.10^{ab}	1.03±0.05^{c}	1.91±0.07^{e}	2.13±0.06^{f}	1.57±0.12^{d}	0.67±0.07^{a}
C20:3n6	3.14±0.19^{d}	2.17±0.07^{b}	2.79±0.08^{c}	3.67±0.06^{e}	1.82±0.08^{a}	3.12±0.07^{d}	2.91±0.19^{c}	1.79±0.08^{a}
C20:4n6	3.29±0.21^{e}	2.75±0.07^{d}	3.12±0.07^{e}	1.81±0.07^{a}	1.87±0.07^{a}	4.53±0.08^{f}	2.55±0.11^{c}	2.32±0.06^{b}
C22:4n6	1.11±0.10^{bc}	0.54±0.08^{a}	1.18±0.07^{c}	1.37±0.09^{d}	1.16±0.07^{c}	0.96±0.07^{b}	1.1±0.14^{bc}	1.78±0.07^{e}

Mean (±SD) followed the same latter are not significantly different (P≤0.05); Values are mean of three replicates

G. giuris (1.12) and lowest was in *H. myitkyinae* (0.59). The value of AI in all the fish studied were less than 1 except *H. myitkyinae* (1.10) and the value of TI was also less than 1 in all the fished studied. All studied species except *H. myitkyinae* (AI-1.10% which is greater than 1) are considered to potentially prevent diseases, since the AI and TI values were lower than one (<1), indicating that the influence of monounsaturated fatty acids and polyunsaturated fatty acids are higher than the influence of saturated fatty acids. From a nutritional perspective, H/H values >2.0 correspond to products with a desirable fatty acid composition and consequently reduce the risk of cardiovascular disease [32].

The FLQ value of the fishes are in the range of 1.9 to 4.81 which was comparable with the FLQ value (6.84) in the muscle tissue of carp [33], however the value was lower than the value reported by [34]. From the assessment of the nutritional quality of the lipids in SIFFs, it can be concluded that all the studied samples showed acceptable n-3/n-6, n-6/n-3, AI and TI. However, for some fishes HH indices showed lower value than the recommended values. Moreover, the n3 PUFA such as DHA and EPA which are highly beneficial in preventing various diseases are present in good concentration. Thus, the SIFFs can be recommended as dietary food fishes for human health.

3.3 Antioxidant in Fish Oil

The antioxidant property was expressed in IC_{50}% value and shown in Fig. 3. Higher the IC_{50}% value lowers the antioxidant activity of the fish. The highest antioxidant property was found in *H. myitkyinae* (10.89 µl/ml) which is followed by *G. giuris* (34.75 µl/ml) and the lowest was observed in *D. yuensis* (38.22 µl/ml). The scavenging activity of Vitamin C was 5.78 µl/ml. The scavenging activity of the present finding showed lower than the previous report of Sarjubala and Sarojnalini [35] in small indigenous fish species of the Eastern Himalayan and Nur and Anitha [36] in fish oils. However, it was found higher than the small indigenous species of Bangladesh [37]. The difference in the activity may be attributed to the difference in the availability of compound which have antioxidant properties and highly diversified in the fish. Some of the SIFFs studied have shown good antioxidant activity, it might be due to the availability of high amount of protein, branched chain amino acid such as valine, leucine and isoleucine, n–3 PUFA, Zinc, Vitamin A and E which are known to be having high antioxidant property [38]. Chen and Decker [39] described the peptides containing basic amino acids are electron receptor that takes electron from radicals formed during the oxidation of saturated fatty acids. Deficiency of dietary Zn caused increase susceptibility to oxidative damage of membrane fraction from some tissue [40].

Moreover, the rate of antioxidant is also greatly enhanced by iron and copper (Gordon 1990). The antioxidant activity of fish oil refers to its chemical property of neutralizing free radical damages present in the body. However, ω-3 fatty acids are capable of reducing free radical levels although it is not considered as a super antioxidant [7] From the results of the antioxidant properties of the SIFFs analyzed, it can be suggested that these small fish have good antioxidant property to scavenge the free radicals [41-45].

Table 3. Total fatty acid and ratio of n–3 and n–6 PUFA

	D. yuensis	*G. guiris*	*H. myitkyinae*	*L. guntea*	*P. chola*	*P. pangia*	*S. berdmorie*	*T. burmanicus*
ΣSFA	34.421	27.3	29.91	22.95	36.88	24.19	14.76	27.77
ΣMUFA	5.27	5.02	5.82	1.68	6.31	7.96	7.66	2.27
ΣPUFA	25.29	23.34	22.19	29.49	18.91	30.88	27.28	24.48
Σn-3–PUFA	13.67	14.09	9.35	19.53	10.77	17.22	17.05	15.47
Σn-6–PUFA	11.62	9.25	10.84	9.96	8.14	13.66	10.23	9.01
n–3/n–6 PUFA	1.17	1.52	0.86	1.96	1.32	1.26	1.66	1.71
n–6/n–3 ratio	0.85	0.65	1.15	0.50	0.75	0.79	0.60	0.58

Table 4. Pearson's correlation between total fatty acids and length–weight

	Length	Weight	ΣSFA	ΣMUFA	Σn-3–PUFA	Σn-6–PUFA
Length	1					
Weight	0.945**	1				
ΣSFA	–0.224	–0.025	1			
ΣMUFA	0.241	0.116	–0.621	1		
Σn-3–PUFA	0.310	0.135	–0.679	0.455	1	
Σn-6–PUFA	–0.172	–0.026	–0.099	0.034	–0.021	1

***Correlation is significant at the 0.01 level*

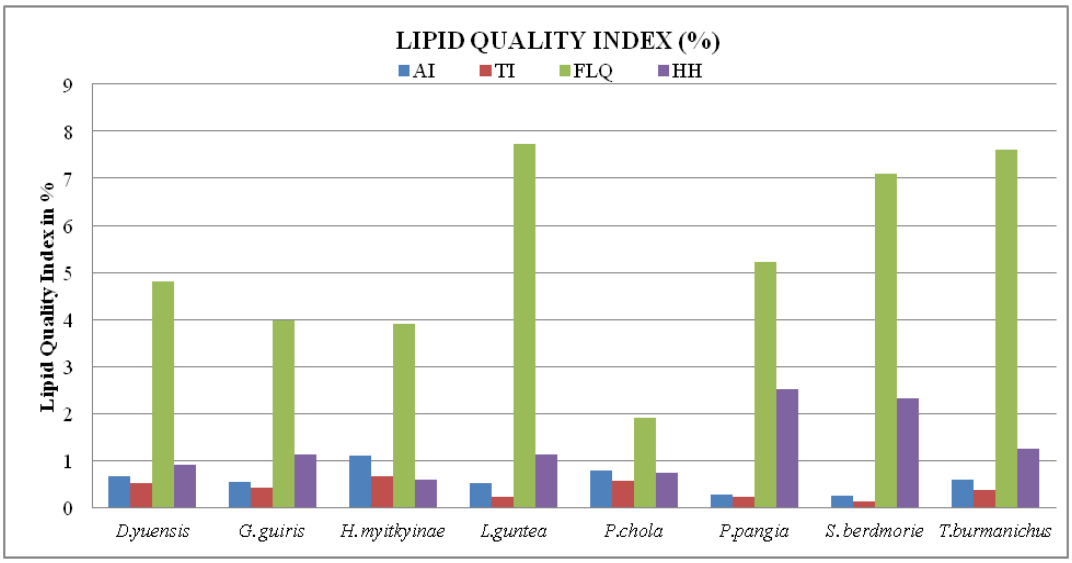

Fig. 2. Lipid quality index of Small indigenous fish species (SIFFs)
AI: index of atherogenicity; TI: index of thrombogenicity; FLQ: Flesh lipid quality; HH: Hypocholesterolemic/Hypercholesterolemic

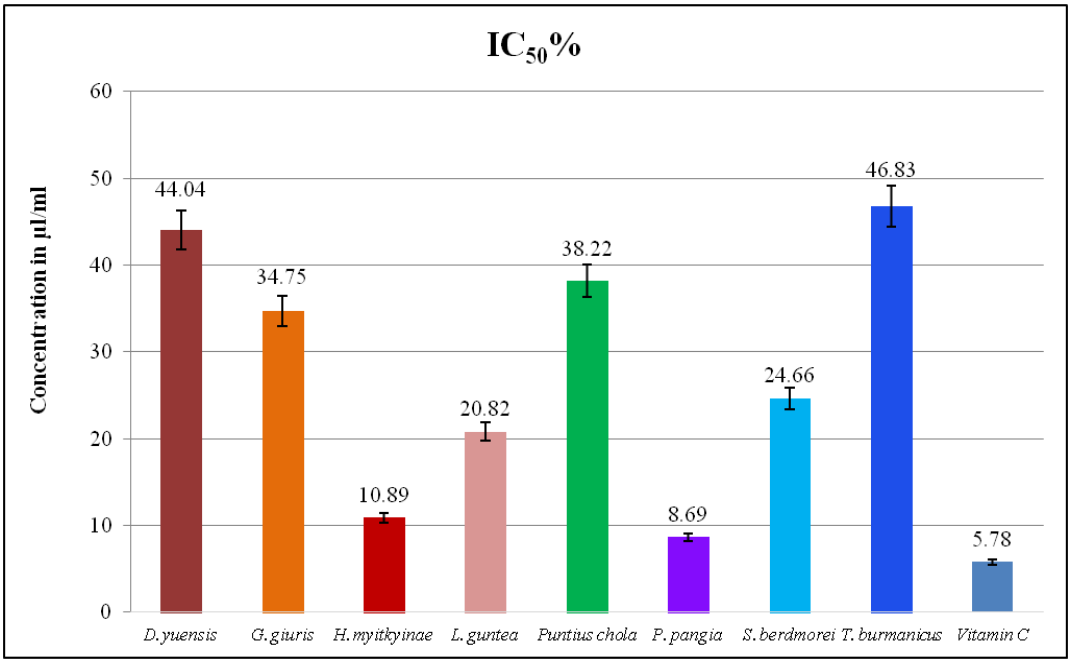

Fig. 3. Antioxidant properties of fish

4. CONCLUSION

Fish became one of the most demanded food items in the world due to it high-quality protein, high concentration of fatty acid especially EPA and DHA. Large fish are highly preferred and eaten but small fishes are ignored and have less market value due to their small size and bony nature. Many small fishes endemic to these regions are highly preferred among the people for their distinctive taste and flavor.

From the above analysis SIFFs contained good lipid which provide enormous amount of PUFA and MUFA especially DHA and EPA, which contribute to various health related benefits like lowering the risk of heart attack and stroke, developing eye and grey matter of brain and so on.

Moreover, n-3 PUFA especially DHA and EPA are high among the fishes studied. High amount of n3 fatty acid may result in a substantial decrease in health care costs by reducing the illness that accounts for the largest burden of diseases worldwide. The scavenging activities of antioxidant properties of the SIFFs are also found to be profound.

Thus, this finding may provide the knowledge about the importance of Small Indigenous Fishes for their high nutritive value and good food for monitoring many micro nutrients deficiencies, so consumption of Small Indigenous Fishes should be encouraged.

Moreover, further action is needed for conservation of small fishes. Many small fishes are in the verge of extinction due to ignorance of their nutritive value, destruction of habitat and feeding ground. Thus, it is necessary to enact a specific policy by the government and various research programs and other related agencies to co-ordinate their effort for the conservation of these fishes. These small fishes are highly nutritious so, further research is highly needed to propagate these fishes to increase their abundance and promote their distribution to the society.

ACKNOWLEDGEMENT

The authors are thankful to the Head of Department, Life Sciences, Manipur University, Director, Directorate of Environment, Government of Manipur for their kind help and extending the laboratory facilities and also to Director, IIT Biotech Park for providing lab facilities for the analysis of fatty acids.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anindya SB, Mamta S, Soibam KS, Himadri S, Yumlembam JS, Martina L, Pramod KP. DNA barcoding of freshwater fishes of Indo-Myanmar biodiversity hotspot. Scientific REPORTS. 2018; 8:8579, 1-12.
2. Felts RA, Rajts F, Akhteruzzaman M. Small indigenous culture in Bangladesh. IFADEP Sub-project-2. Development of Inland Fisheries. 1996;4.
3. Innis SM. Perinatal biochemistry and physiology of long chain polyunsaturated fatty acids. J. Pediatr. 2003;143:S1–8.
4. Innis SM. Dietary (n-3) Fatty acids and brain development. J Nutr. 2007;137: 855-859.
5. Uauy R, Castillo C. Lipid Requirements of Infants: Implications for Nutrient Composition of Fortified Complementary Foods. J Nutr. 2003;133:2962S-2972S.
6. Potter NN, Kiss H. Water finfish and marine finfish, shellfish and roes. In: Food Sciences (5ed.). Chapman and Hall N.Y. 1995;65-66.
7. Akmal NLHBI, Roy A. Free radical scavenging activity of fish oil - An *In-vitro* study. Int. J. Pharm. Sci. Res. 2017; 8(9):3872-3875
8. Roos N, Mohammed M, Islamy, Thilsted SH. Small indigenous fish species in Bangladesh: Contribution to vitamin A, Calcium and Iron Intakes. J Nutr. 2003; 133(11 Suppl 2):4021S-4026S.
9. Metcalfe LD, Schmitzj AA, Pelka R. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 1966;38(3):514-515.
10. Stein SE, Scott DR. Optimization and testing of mass spectral library search algorithms for compound identification. J. Am. Soc. Mass Spectr. 1994;5(9):859-866.
11. Ulbricht TL, Southgate DA. Coronary heart disease: Seven dietary factors. Lancet. 1991;338(8773):985-92.
12. Santos-Silva J, Bessa RJB, Santos-Silva F. Effects of genotype, feeding system and slaughter weigt on the quality of light

lambs. II. Fatty acid composition of meat. Livest Prod Sci. 2002;77 (2/3): 187–194.
13. Senso L, Suárez MD, Ruiz-Cara T, Garcia-Gallego M. On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (*Sparus aurata*). Food Chem. 2007;101:298–307.
14. Ould Ahmed Louly AW, Gaydou EM, Ould El, Kebir MV. Muscle lipids and fatty acid profiles of three edible fish from the Mauritanian coast: *Epinephelus aeneus, Cephalopholis taeniops and Serranus scriba.* Food Chem. 2011;124:24–28.
15. Renata PA, Danuta KP. Fatty acid profile of fat of grass carp, bighead carp, Siberian sturgeon, and Wels catfish. J. Food Qual. 2017;2017, Article ID 5718125,6 pages.
16. Prato E, Biandolino F. Total lipid content and fatty acid composition of commercially important fish species from the Mediterranean Mar Grande Sea. Food Chem. 2012;131:1233–1239.
17. Gonzalez S, Flick GJ, O'Keefe SF, Duncan SE, McLean E, Craig SR. Composition of farmed and wild yellow perch (*Perca flavescens*). J Food Compost Anal. 2006; 19:720-726.
18. Steffens W. Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. Aquaculture. 1997;151:97–119.
19. Yaqoob P, Knapper JA, Webb DH, Williams CM, Newsholme EA, Calder PC. The effect of olive oil consumption on immune functions in middle-aged men. Am. J. Clin. Nutr. 1998;67:129-135.
20. Enrique M, Rao KS, Durant AA. The lipid content and fatty acid composition of four eastern central Pacific native fish species. J Food Compost Anal. 2014; 33:1–5.
21. Manoharan S, Kuppu R, Uthandakalaipandian R. Comparative assessment of nutritional composition in raw and cooked Indian freshwater fish *Lepidocephalus thermalis* (V.). Proc. Zool. Soc. 2019;72(2):154–159.
22. Hei A, Sarojnalini C. Nutritional assessment of some traditionally processed hill-stream fishes of Manipur with special reference to n-3 fatty acids. J. Aquat. Food Prod. Technol. 2015;24(8): 832-839.
23. Strobel C, Jahreis G, Kuhnt K. Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products. Lipids Health Dis. 2012;11:144.
24. Iwona C, Władysław M, Kinga T, Barbara M, Ewa C. Changes of amino acid and fatty acid profile in freshwater fish after smoking. J Food Process Pres. 2018;42: 1-8.
25. Karapanagiotidis IT, Yakupitiyage A, Little DC, Bell MV, Mente E. The nutritional value of lipids in various tropical aquatic animals from rice–fish farming systems in northeast Thailand. J Food Compost Anal. 2010;23(1):1-8.
26. HMSO U.K. Nutritional aspects of cardiovascular disease. Report on health and social subjects, 46. HMSO, London; 1994.
27. Simopoulos AP. Ω--3 fatty acids in inflammation an autoimmune disease. J Am Coll Nutr. 2002;21:495–505.
28. Gualda IP, Santos VJ, Figueiredo IL, Petenuci ME, Visentainer JV. Centesimal composition, fatty acids profile and the nutritional quality index of four seafood species from the southern region of Brazil. Acta Sci. Technol. 2018;40:39351.
29. Laudadio V, Tufarelli V. Growth performance and carcass and meat quality of broiler chickens fed diets containing micronized-dehulled peas (*Pisum sativum* cv. Spirale) as a substitute of soybean meal. Poult Sci. 2010;89:1537–43.
30. Novello D, Franceschini P, Quintiliano DA. The importance of ω-3 and ω-6 fatty acids for disease prevention and human health. Salus Magazine. 2008;2;77–87.
31. Ouraji H, Shabanpur B, Abediankenari A, Shabani A, Nezami A, Sudagar M, Faghani S. Total lipid, fatty acid composition and lipid oxidation of Indian white shrimp (*Fenne ropenaeus indicus)* fed diets containing different lipid sources. J. Sci. Food Agric. 2009;89: 993-997.
32. Bentes AS, Souza HAL, Simões MG, Mendonça XMFD. Physical and chemical characterization and lipid profile of three Amazonian fish species. Brazilian Journal of Agroindustrial Technology. 2009;3(2): 97–108.
33. Stancheva M, Merdzhanova A, Dobreva DA, Makedonski L. Common carp (*Cyprinus carpio*) and European catfish (*Silurus glanis*) from Danube River as sources of fat-soluble vitamins and fatty acids. Czech J Food Sci. 2014;32: 16–24.

34. Joanna L, Beata P, Joanna N, Marek JL. Mercury, Fatty acids content and lipid quality indexes in muscles of freshwater and marine fish on the Polish market risk assessment of fish consumption. Int. J. Environ. Res. Public Health. 2017; 14(10):1120.
35. Sarjubala W, Sarojnalini C. Effect of cooking on the polyunsaturated fatty acid and antioxidant properties of small indigenous fish species of the Eastern Himalayas. Int J Eng Res Appl. 2014; 4(7):146-151.
36. Nur LHBIA, Anitha R. Free radical scavenging activity of fish oil - An *In-vitro* study. Int. J. Pharm. Sci. Res. 2017;8(9):3872-3875.
37. Sunuram R, Iqbal AM, Muslima KM, Ashkar BMS, Sai SM, Golam SM. Antioxidant potential and nutrient content of selected small indigenous species of fish. Pharmacologyonline. 2014;2:48-53.
38. Klotz LO, Kroncke KD, Buchczyk DP, Sies H. Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. J Nutr. 2003;133(5 Suppl 1):1448S-51S.
39. Cheng KM, Decker EM. Endogenous skeleton muscle antioxidant. Critical Crit Rev Food Sci Nutr. 1994;34:403-426.
40. Sing RB, Niaz MA, Rastogi, SS, Bajaj S, Gaoli Z, Shoumin Z. Current zinc intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations of North India. J Am Coll Nutr. 1998; 17(6):564-70.
41. Geiger E, Borgstrom G. Fish protein: Nutritional aspects. Fish as Food. Vol. II. In: Nutritional, Sanitation and Utilization. Academic Press. 2013;29-91.
42. Hadjinikolova L. The influence of nutritive lipid sources on the grow and chemical composition of carp (*Cyprinus carpio L*.). Arch. Polish Fish. 2004;12:111-119.
43. Koleva II, Van Beek TA, Linssen JPH, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 2002; 13:8-17.
44. Shantosh M, Sarojnalini C. Nutritional quality of three cobitid fishes of Manipur, India: With special reference to essential mineral elements. Int. J. Sci. Res. Biol. Sci. 2018;5(2):24-33.
45. Singh MB, Sarojnalini C, Vishwanath W. Nutritive values of sundried *Esomus danricus* and smoked *Lepidocephalus guntea*. Food Chem. 1990;36:89-96.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/98195