J. Indian Chem. Soc.,
Vol. 97, June 2020, pp. 941-957

Frying and freezing effect on nutritional quality of major carps and potential contribution to human health from fatty acid signatures

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Manuscript received online 07 January 2020, revised and accepted 20 May 2020

Fish is a unique, beneficial and cheapest resource of healthy, nutritious food source all over the world. Most cultivable major carps in inland aquatic system, *Catla catla, Labeo rohita, Cirrhinus mrigala* are considered as good source of essential long chain n-3 polyunsaturated fatty acids with low fat high protein content. Though the effects of different cooking and preservation methods on many marine fish have been broadly studied, much less research has been done on the these carps which are more widely available and consumed. This study, therefore, recorded the changes in biochemical composition, fatty acid profiling during frying and freezing and analysed their potential contribution to human health from nutritional quality indices. Fried treatment had significantly ($p < 0.05$) higher n-6 polyunsaturated fatty acids content and due to uptake of culinary oil, reduced some advantageous parameters like essential n-3 fatty acids content, Σn-3: Σn-6 and *cis*:*trans* fatty acids ratio ($p < 0.05$). But during frying declining rate of index of atherogenicity, index of thrombogenicity along with rise in hypocholesteromic/ hypercholesteromic fatty acid ratio directly advocated for positive effect on health and prevention of cardiovascular diseases. However, during the most common and commercial preservation method, frozen storage directed to lipid deterioration that unfavourably affected the valuable nutritive food quality of fish. It was concluded that freezing affected nutritional quality of fish detrimentally but overall effect of frying is good for health. This study may initiate new line of research on implication of thermal perturbation in nutritional quality of food with their effect on human health.

Keywords: Major carps, nutrition, frying, freezing, fatty acids.

Introduction

There is a growing awareness all over the world regarding healthy and nutritional food. Fish is finding more acceptance as an excellent source of animal protein for providing means to tide over the nutritional difficulties of man[1]. Apart from being a significant source of protein and essential amino acids, it is also fundamental resource of vitamins, beneficial minerals, nutritionally valuable lipids and adequate amount of essential fatty acids (FAs)[2–5]. The polyunsaturated fatty acid (PUFA) is well-known to play fundamental roles not only in normal growth, development, and reproduction of all vertebrates[5] but also provide their efficacious activity to prevent inflammation, blood clotting. They regulate the body cholesterol metabolism for reducing the chances of atherosclerosis, hypertension and triacylglycerolemia, maintain a correlation between the dietary intake of omega-3 (n-3) long chain polyunsaturated fatty acid (LC-PUFA) with neurological disorders, diabetes and obesity[6–12]. Omega-3 and omega-6 (n-6) FAs are not synthesized in the human body, but their inclusion in the human diet is essential for their numerous nutritional benefit in the area ranging from fetal development to cancer prevention[6]. Recently, various scientific bodies recommend the dietary intake of essential n-3 FAs like eicosapentaenoic acid (20:5n3, EPA), docosahexaenoic acid (22:6n3, DHA), appropriate n-3:n-6 ratio in their nutritional guidelines for optimal health[10,13,14].

The major carps, *Catla catla, Labeo rohita* and *Cirrhinus mrigala* are the mainstay of freshwater aquaculture. These three major carps are highly preferred farmed fish all over the world because of their rapid growth rate and greater acceptability to consumers[5,15]. These carps are immense source of nutritionally enriched lipids, FAs and proteins to

enhance their economic importance to human nutrition. They contain a high amount of n-3 and n-6 LC-PUFAs[9,16]. However, the consumption of raw fish is rare all over the world. For preservation, frozen storage is considered to be an admirable process to prevent alteration in the product and to retain the food value for an extended time and reduce deterioration in product flavor, color, and texture by inhibiting microbial spoilage. The changes in FA profiling such as alteration of n-3:n-6 and the total amount of PUFA, protein and other biochemical components during the cooking and frozen storage may have an adverse effect towards human health[10,17]. LC-PUFAs are especially susceptible to oxidative breakdown and lead to production of volatiles associated with rancidity during different culinary treatment[14,18,19] and frozen storage[20–22]. However, a number of studies have revealed that FA compositions, EPA and DHA amount remain unaltered during different cooking and preservation methods[23,24].

Notably, most of the above mentioned studies were carried out to establish the effect of different types of cooking and preserving method on proximate composition, vitamins and minerals contents, lipid profiling of marine fish species. On the other hand, some studies have examined the variation in biochemical composition, FA profile among major carp species from different fish farms or diverse ecosystem. The literature study further reveals a dearth of data for the effect of different cooking and preservation processes on freshwater major carps involving various biochemical aspects and their implication towards human health. Therefore, in the present study, we have investigated the influential effect of freezing at –20°C, the most common domestic and commercial method for preservation[25] and the consequence of frying with mustard oil for both fresh and frozen conditions for the comparative study of proximate composition, FA profile of three Indian major carps along with multivariate analysis. In particular, the results were analyzed for highlighting the variation of different nutritional parameters and indices like index of atherogenicity (IA), index of thrombogenicity (IT), hypocholesteromic/hypercholesteromic FA ratio (HH) at different conditions. The importance lies in the fact that these factors have direct role in categorizing the nutritional importance of the food concerning human health.

Experimental

Sampling protocol:

Fish samples of 1±0.2 kg weight of the three Indian major carp species, i.e., *Catla catla, Labeo rohita* and *Cirrhinus mrigala* were collected in the fresh state from local markets of Durgapur, West Bengal, India. Three samples of each species (i.e., total 9 samples) were collected and kept in polyethylene bags and then transported to the laboratory facility immediately for further processing.

Sample preparation:

After measuring the biometric data (weight and length) of each specimen of fish, the head was cut off and was eviscerated, filleted and washed thoroughly with deionized water and processed to yield boneless, skinless fillets. Each fish sample was initially divided into two parts. First part was then analyzed in two conditions, i.e., fresh and fried. Frying was carried out in presence of 10 g mustard oil per 100 g sample at 180°C for 10 min. The second part was packed in polyethylene bags and stored in a deep freezer for 30 days at –20°C after which they were analyzed in two conditions, viz., frozen and frozen-fried. The raw edible part of the three samples of each fish species were homogenized together prior to analysis and the homogenate were taken for individual analytical tests. All the experiments were performed in triplicate to ensure the reproducibility of the data.

Chemicals and standards:

All the chemicals were of AR grade and purchased from MERCK. Standard of 37 components fatty acid methyl esters (FAME) were procured from Sigma Aldrich (St. Louis, MO, USA).

Proximate analysis:

Moisture content:

The moisture content of the fish samples for all condition were analyzed by using standard analytical methods of Association of Official Analytical Chemists (AOAC)[26]. 10±1 g of the wet weight of fish samples were taken and dried to constant weight at 105°C in a hot oven.

Ash content:

The ash content of all the samples was measured according to the methods of AOAC[26]. After determination of the moisture content, the samples were incinerated at high temperature (550°C) in a burning muffle for 5 h. After cooling at room temperature, the obtained ash was weighed.

Carbohydrate content:

The carbohydrate content of the samples were determined by the anthrone method[27]. 5±1 g of the sample was homogenized with 50 ml 5% TCA solution. Then, the mixture was centrifuged, and the supernatant was collected. Two percent anthrone reagent in sulfuric acid in ice-cold condition was added to each collected sample. Sample tubes were heated in a water bath, cooled and absorbance was measured at 625 nm using UV-Vis spectrophotometer.

Protein content:

The Kjeldahl method was performed according to method 981.10 of the AOAC[26]. The amount of total nitrogen in the samples was multiplied with the traditional conversion factor of 6.25[28] to estimate crude protein in the samples. The percentage of crude protein was calculated by the formula [% Protein = $(V_A - V_B)\times1.4007\times N\times6.25$/g sample].

Lipid content:

The lipid was extracted thrice from each sample with chloroform:methanol (2:1; v/v)[29]. The three extracts were combined into an acid wash followed by a salt wash (0.9% aqueous NaCl solution). Total lipid (TL) was determined gravimetrically by evaporating lipid containing organic solvent under reduced pressure using a rotary evaporator. Nitrogen was flushed into fish lipid samples and kept it at –20°C until analysis.

Caloric value:

The caloric value was calculated using the individual calorie factors, 4.0 kcal for protein, 9.0 kcal for fat and 4.0 kcal for carbohydrate[30].

Lipid class separation by column chromatography:

TL was subjected to column chromatography using silica (60–120 mesh) as described by Dreyfus *et al.*[31]. The column was conditioned with chloroform and neutral lipids (NL), glycolipids (GL) and phospholipids (PL) were eluted sequentially with 10, 30 and 10 column volumes of chloroform, acetone and methanol respectively. Solvents were evaporated by rotary evaporator and all types of lipid were estimated gravimetrically. All fractions were stored at –20°C for further analysis.

FA analysis:

The FAs from TL, NL, PL and GL were transmethylated to fatty acid methyl ester (FAME) with the sulphuric acid in methanol method[32]. FAME concentrations were quantified on an Agilent 7820 A GC with the following configuration: column = DB-23 (30 m×320 μm×0.25 μm), carrier gas = helium, flow rate of 1.7086 ml/min, injector = 250°C and detector = Flame ionization detector @ 280°C. Oven temperature = initially 50°C, 50–180°C at 15°C/min, 180°C for 5 min, 180–240°C at 8°C /min, 240°C for 15 min, sample volume = 1 μL. The detailed FAs composition of mustard oil before and after frying was analysed in similar way. Identification and quantification of the FAME in the samples was based on comparison of their retention times with a 37 component FAME standard (Supelco 37 component FAME Mix, Sigma-Aldrich). Results have been reported as percentage (%) contribution of each FAME to total quantified FAME.

Nutritional quality indices (NQI):

The data from FA analysis of TL were used to calculate the nutritional impact of the lipid. In the present study, nutritional quality of the lipid was assessed by IA, IT and HH.

IA and IT was determined by using the following formula[33].

$$IA = \frac{aS' + bS'' + cS'''}{dP + eM + fM'} \quad (1)$$

S' = the percentage of C12:0, S'' = the percentage of C14:0, S''' = the percentage of C16:0, P = Σn-3 + n-6 PUFA, M = the percentage of C18:1, M' = the percentage of other monounsaturated fatty acids (MUFAs), a, b, c, d, e, f are constant term where b = 4 and a, c, d, e, f are unity

$$IT = \frac{mS}{nM + oM' + p(n-6) + q(n-3) + (n-3)/(n-6)} \quad (2)$$

S = the sum of the percentage of C14:0, C16:0 and C18:0, M = the percentage of C18:1, M' = the 218 percentage of other MUFAs, m, n, o, p, q are constant term where m = 1, n, o, p = 0.5 and q = 3.

IA and IT indicated a correlation between the sum of main saturated fatty acids (SFAs) and main classes of unsaturated fatty acids (UFAs). The SFAs were known as pro-atherogenic or pro-thrombogenetic while UFAs were defined as anti-atherogenic or anti-thrombogenetic for IA and IT respectively[34].

HH provided a corelationship between specific FAs on cholesterol metabolism of human health[34].

$$HH = \frac{(C18:1cn–9 + C18:2\ n–6 + C18:3\ n–3 + C20:5\ n–3 + C22:5\ n–3 + C22:6\ n–3)}{(C14:0 + C16:0)} \quad (3)$$

Statistical analysis:

All results have been expressed as mean ± standard deviation of triplicate analyses. A paired student's t test was applied to find out whether the changes were significant ($p < 0.05$) by using the software Graph Pad Prism version 5.01 (Graph Pad Software, Inc.). Multivariate analysis of variance (MANOVA) was carried out to assess the correlation between different nutritional importance dependent parameters with cooking (frying with mustard oil) for fresh and frozen preservation (–20°C). Then the Tukey's Honest Significant Difference (Tukey's HSD) test was performed to find out which specific condition's means were different compared with each other using IBM SPSS 20.

Results and discussion

Proximate composition:

The proximate compositions of fresh and frozen samples of three carps*, Catla catla*, *Labeo rohita* and *Cirrihinus mrigala* before and after frying have been presented in Table 1. The compositions of fresh samples were similar to findings of other research studies[5,35,36]. The proximate composition, caloric value varied with fish species. But, multivariate tests revealed that there was no significant difference in parameters between the three types of species since all belonged to carp family. However, there was significant difference in moisture, carbohydrate, protein, total lipid, caloric value between the treatments of the fish (Table 1).

Moisture content is an extremely important factor for the determination of the food value of products in terms of its shelf-life and sensory attributes[37]. The moisture content obtained for the fresh samples of three carps decreased non significantly ($p > 0.05$) during frozen storage (at –20°C for 30 days) due to water loss in chiller. During frying there was also an explicit tendency to enhance moisture loss due to evaporation in both fresh ($p < 0.05$) and frozen condition ($p > 0.05$).

The ash content of all the three carps were similar ($p > 0.05$) and it did not vary significantly among the studied conditions. This major component mainly attributes to nutritionally important minerals.

Table 1. Proximate composition of *Catla catla*, *Labeo rohita and Cirrhinus mrigala* in fresh, fried, frozen and frozen-fried condition

	Catla catla				*Labeo rohita*				*Cirrhinus mrigala*			
	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried
Moisture (%)	75.6520± 0.4397^{a,c,d}	72.3745± 0.5841^{b,d}	73.9379± 1.0166^{a,c,d}	71.5541± 0.0298^{a,b,c,d}	79.6591± 1.0187^{a,c,d}	72.1121± 0.9954^{b,d}	79.3322± 0.5614^{a,c,d}	74.0347± 1.2039^{a,b,c,d}	77.6520± 0.0564^{a,c,d}	71.2239± 0.0354^{b,d}	76.4402± 0.4320^{a,c,d}	73.0605± 0.0987^{a,b,c,d}
Ash (%)	1.6596± 0.0278^{a,b,c,d}	1.9903± 0.0198^{a,b,c,d}	1.1982± 0.0159^{a,b,c,d}	1.3616± 0.0923^{a,b,c,d}	1.1640± 0.0112^{a,b,c,d}	1.7719± 0.0224^{a,b,c,d}	0.9697± 0.0107^{a,b,c,d}	1.0257± 0.0112^{a,b,c,d}	1.5710± 0.0075^{a,b,c,d}	1.8798± 0.0024^{a,b,c,d}	1.4576± 0.0123^{a,b,c,d}	1.7790±. 0.0139^{a,b,c,d}
Carbohydrate (%)	0.2050± 0.0038^{a,b,c,d}	0.1767± 0.0051^{a,b,d}	0.2598± 0.0019^{a,c,d}	0.0873± 0.0024^{a,b,c,d}	0.2842± 0.0032^{a,b,c,d}	0.2124± 0.0029^{a,b,d}	0.4105± 0.0048^{a,c,d}	0.1051± 0.0017^{a,b,c,d}	0.1288± 0.0038^{a,b,c,d}	0.0586± 0.0073^{a,b,d}	0.3278± 0.0032^{a,c,d}	0.1912± 0.0062^{a,b,c,d}
Protein (%)	13.6323± 0.0045^{a,c}	5.3269± 0.0040^{b,c,d}	5.0111± 0.0023^{a,b,c,d}	1.6076± 0.0063^{d,b,c}	13.5330± 0.0040^{a,c}	8.4390± 0.0070^{b,c,d}	3.1885± 0.0034^{a,b,c,d}	1.0973± 0.0017^{d,b,c}	11.9600± 0.0050^{a,c}	4.6356± 0.0044^{b,c,d}	4.0200± 0.0023^{a,b,c,d}	1.4421± 0.0028^{d,b,c}
Lipid (%)	4.5200± 0.1377^{a,c}	10.8975± 0.2100^{b,d}	1.1137± 0.0092^{a,c}	8.1225± 0.1770^{d,b}	2.5640± 0.1754^{a,c}	10.2156± 0.2914$_{b,d}$	1.3294± 0.0651^{a,c}	9.8241± 0.1019^{d,b}	3.3762± 0.0263^{a,c}	10.7713± 0.1358^{b,d}	1.3214± 0.0154^{a,c}	6.5984± 0.0180^{d,b}
Calorific value (kcal/100 g)	96.0292± 1.2059^{a,d}	120.0919± 1.8536^{b}	31.1069± 0.0660^{c}	79.8821± 1.5582^{a,d}	78.3448± 1.5496^{a,d}	126.5460± 2.5828^{b}	26.3606± 0.6189^{c}	93.2265± 0.9035^{a,d}	78.7410± 0.2013^{a,d}	115.7185± 1.1754^{b}	29.2838± 0.1420^{c}	65.9188± 0.1258^{a,d}

Values are shown as mean ± standard error of triplicates. Superscripts indicate results of repeated measure MANOVA for comparison of each of the four treatments, where significant differences among pairs of treatments of each fish are indicated using different letters ($p < 0.05$). Significant pair wise comparisons were found using the Tukey's test.

The carbohydrate content was comparatively higher in *C. catla* and *L. rohita* than *C. mrigala*. However, in frozen condition the carbohydrate content most considerably increased for *C. mrigala* (60.7077%) compared to *L. rohita* (30.7673%) and *C. catla* (21.0931%). This might be due to accumulation of glycogen[38,39]. However, the carbohydrate content decreased slightly ($p > 0.05$) during frying for both fresh and frozen condition for all the species.

Protein is the most predominant in fish muscles than any other biochemical components. In the present investigation, the crude protein content was found almost similar and was notably high in both *C. catla* and *L. rohita* compared to *C. mrigala*. Fish protein provides a good amino acid profile, high degree of digestibility and high biological value to make it a potent nutrient towards the human health, thus playing a bigger role in preventing the protein-calorie malnutrition[37]. In the present investigation, the protein content was found to decrease 76.4390%, 63.2409% and 66.3879% for *C. catla, L. rohita and C. mrigala* respectively after 30 days of storage at –20°C. While assessing the effect of low temperature storage on biochemical changes in fish muscle, Kolodziejska *et al.*[40] reported a significant rate of denaturation and autolysis of fish protein. According to Xiong[41], denaturation of protein occurred due to textural deterioration during oxidizing environment, such as amino acid break down, peptide scission and formation of protein-lipid complexes. Therefore the most widely used preservation method could not prevent degradation of the essential food quality of cheapest animal protein source. The application of heat during frying abruptly decreased the protein content for both fresh and frozen samples of *C. catla, L. rohita and C. mrigala* (Table 1) and hence deteriorated the nutritional importance of food. These observed results were in high agreement with the literature[42]. Deman[43] and March[44] suggested that solubilization of protein during the cooking caused its loss from the final product. Although this result contradict with the findings of Gokoglu *et al.*[45] and Gall *et al.*[46], who accredited that due to the high amount of moisture loss at the time of frying inclined the high protein percentage in fried fish than raw ones. Deman[43] suggested that probability of generation of new product similar to protein while frying which could have influenced the quantification of protein content by Kjeldahl method.

Lipid component varies much more broadly than the other proximate parameters in case of fish depending on the species, size, habitat, seasonal/lifecycle variation, diet and the time of sampling[47]. Based on the low lipid percentage (less than 5%) of *C. catla, L. rohita* and *C. mrigala* in fresh condition, these three major carps are categorized as lean fish[5]. The present study confirmed an inverse relationship between the total lipid and moisture content because of water loss during cooking that increased the concentration of lipid in the final product as suggested by Gladyshev *et al.*[19]. Accordingly, as *C. catla* contained highest lipid and lowest moisture content, it had more white flesh color[5]. During frying due to the uptake of culinary oil, fried fish got enriched with significantly higher lipid content ($p < 0.005$) than the raw one in fresh and frozen condition. The interaction between culinary fat and fish fat during frying was reported to be influenced by the content and the composition of lipid of raw fish along with method, time and temperature of cooking[48]. Conversely, there was a huge decrease in total lipid content such as 75.3606% for *C. catla,* 48.1513% for *L. rohita*, and 60.8613% for *C. mrigala* during frozen storage. Oxidative deterioration of lipid, because of losses in triglyceride fraction during the prolonged storage, is considered as the main cause of degradation of nutritional quality of fish. The most common commercial preservation technique, freezing is mainly employed to preserve all the sensory and nutritional properties but even during this period many enzymatic and non-enzymatic rancidity occur deteriorating the shelf life of products caused by hydrolysis of fat, presence of unsaturated lipid and the prooxidant molecules in the muscles. Oxidative breakdown of lipid greatly affect the FA composition which is again interrelated with serum cholesterol level and heart diseases[7,17].

Fat content contributed most in the caloric value followed by protein and least by carbohydrate whose amount was meager in fish. Variation in these biochemical parameters was the major cause for the higher calorific value of *C. catla* in comparison with *L. rohita* and *C. mrigala*. After freezing, loss in both lipid and protein content caused lowering of energy values for all three carps. During frying, protein content reduced to lower values but due to absorption of cooking oil at different rate the lipid content increased rapidly for all the species. These opposing trends finally improved the energy values of fried samples over fresh and frozen ones. In the present study, *C. catla* showed the moderate calorific value while *L. rohita* provided the highest one.

Lipid classes:

Among the different classes of lipids the proportion of NL (expressed as % w/w of the total lipids) was predominant followed by PL and GL for all the three species in both fresh and frozen conditions (Fig. 1). Therefore NL had a leading role among all the lipid classes. After frying, due to absorption of cooking oil, the amount of NL increased further whereas polar lipid fraction i.e., PL ($p < 0.05$) and GL content considerably decreased ($p > 0.05$) for fresh and frozen storage condition. During the frozen storage the amount of NL classes increased 9.2082% for *L. rohita*, 11.8661% for *C. catla* and 12.8874% for *C. mrigala*. According to Undeland[49], during frozen preservation, the enzymatic decomposition of PL, the major cell membrane component, might occur at faster rate due to the endogenous lipolytic enzyme. Additionally, the NL content has been increased associatively due to production of free fatty acids from PL during storage[50]. Peroxidation detrimentally affects the PL due to attack by free radicals[37]. These might have caused further loss in PL during frying for all the species. The loss in NL, PL and GL components of all the three carps during freezing together contributed to the loss of TL leading to adverse effect on nutritional importance of food.

FA analysis:

The FA composition of TL, NL, PL and GL of all the carp species in fresh and frozen samples before and after frying were analyzed by gas chromatography and presented in Tables 2, 3, 4 and 5 respectively.

It was observed that TL of *C. catla* contained higher amount of SFA than both *L. rohita* and *C. mrigala* for fresh condition and the percentage of SFA was in agreement with freshwater fish samples studied by Jabeen and Chaudhry[9]. Not only in TL, SFA concentrations were substantially high in NL, PL and GL also. In all the lipid classes, SFAs were dominated by palmitic acid (C16:0) and stearic acid (C18:0) in three carps in all conditions. In NL and PL the presence of myristic acid (C14:0) was also very prominent. The high percentage of SFA in fish acts as energy reservoirs, temperature regulator of body and it is an essential component of cell biomembranes. The SFAs of chain length 2-16 have predominating nature for enhancing cholesterol level[51]. But the most predominant SFA, C16:0 abruptly decreased for all the

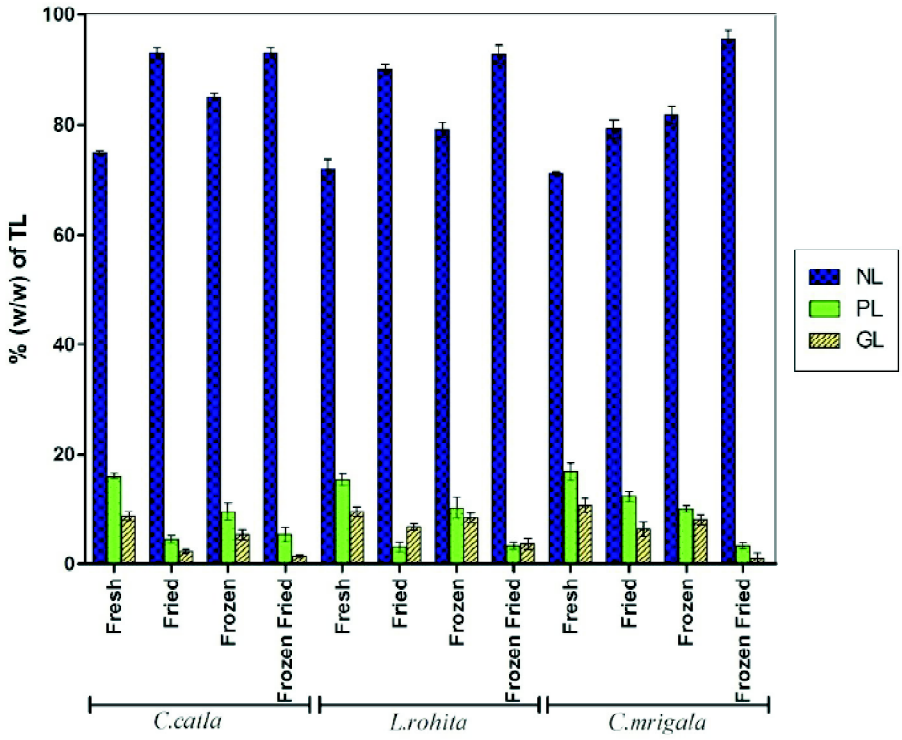

Fig. 1. Various classes of lipid compositions (% w/w of total lipid) of *Catla catla, Labeo rohita and Cirrhinus mrigala* in fresh, fried, frozen and frozen-fried condition.

Table 2. Fatty acid profiles (% total fatty acid) of total lipid (TL) of *Catla catla, Labeo rohita and Cirrhinus mrigala* in fresh, fried, frozen and frozen-fried condition

	Catla catla				*Labeo rohita*				*Cirrhinus mrigala*			
	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried
C12:0	0.79±0.07	0.31±0.03	0.69±0.07	0.29±0.11	1.22±0.06	0.88±0.10	1.19±0.19	0.93±0.13	1.85±0.04	0.98±0.05	1.90±0.02	0.67±0.04
C14:0	4.12±0.11	2.02±0.20	4.22±0.15	1.84±0.09	2.13±0.13	0.48±0.12	3.01±0.14	2.28±0.19	3.07±0.02	1.99±0.11	3.31±0.07	2.35±0.02
C15:0	0.64±0.06	0.13±0.04	0.71±0.08	0.17±0.07	–	–	0.96±0.15	0.25±0.13	2.10±0.10	1.79±0.03	2.13±0.02	1.92±0.07
C16:0	33.82±0.13	24.93±0.12	39.11±0.25	27.30±0.41	32.88±0.20	20.91±0.37	36.9±0.19	23.02±0.27	31.22±0.12	23.54±0.15	33.32±0.11	21.04±0.20
C17:0	0.78±0.09	0.45±0.07	1.01±0.10	0.49±0.03	3.60±0.08	1.40±0.18	4.16±0.12	3.40±0.13	0.98±0.03	0.91±0.06	0.88±0.04	0.31±0.02
C18:0	8.98±0.19	5.09±0.31	9.45±0.22	6.16±0.09	4.20±0.17	2.20±0.23	5.58±0.20	4.49±0.13	3.01±0.03	1.50±0.05	7.02±0.07	5.29±0.06
C20:0	–	2.04±0.07	0.19±0.06	1.37±0.10	1.28±0.09	1.95±0.15	1.41±0.12	1.73±0.11	0.89±0.09	2.16±0.02	1.01±0.05	3.83±0.14
ΣSFA	49.13±0.37	34.97±0.40	55.38±0.40	37.62±0.46	45.31±0.32	27.82±0.52	53.21±0.43	36.10±0.43	43.12±0.19	32.87±0.21	49.57±0.16	35.41±0.27
C16:1	4.25±0.14	3.33±0.15	4.77±0.11	3.99±0.23	4.82±0.21	2.47±0.22	6.01±0.30	3.29±0.18	3.71±0.15	3.09±0.18	3.92±0.13	2.77±0.12
C18:1c	16.01±0.24	20.07±0.37	12.91±0.29	19.01±0.22	19.35±0.29	23.68±0.22	14.87±0.33	20.03±0.26	17.76±0.08	19.70±0.21	15.71±0.09	19.12±0.17
C18:1t	–	1.89±0.34	–	1.02±0.15	–	1.78±0.34	–	1.11±0.31	0.71±0.05	2.12±0.11	0.33±0.08	1.69±0.30
C20:1	1.80±0.04	0.58±0.06	2.03±0.20	1.56±0.13	1.42±0.09	1.04±0.26	1.66±0.11	0.92±0.15	2.03±0.06	1.28±0.05	2.29±0.03	1.80±0.08
C24:1	1.64±0.02	1.03±0.08	0.30±0.13	0.09±0.08	0.42±0.1	0.13±0.10	0.21±0.11	0.07±0.20	1.06±0.03	0.83±0.02	0.76±0.10	0.45±0.02
ΣMUFA	23.70±0.28	26.90±0.53	20.01±0.39	25.67±0.38	26.01±0.38	29.10±0.54	22.75±0.47	25.42±0.51	25.27±0.19	27.02±0.30	23.01±0.21	25.83±0.37
C18:2c	10.01±0.34	26.88±0.20	9.79±0.19	27.37±0.26	13.07±0.16	30.10±0.14	10.88±0.12	27.98±0.15	11.12±0.11	26.34±0.13	12.01±0.22	29.04±0.11
C18:2t	–	2.13±0.16	–	3.22±0.35	–	3.23±0.25	–	2.33±0.26	–	3.91±0.10	–	2.60±0.15
C18:3	4.80±0.21	2.25±0.10	2.55±0.17	1.29±0.13	1.12±0.11	1.02±0.13	1.07±0.21	0.89±0.23	4.12±0.12	2.90±0.01	2.88±0.13	1.11±0.32
C20:4	2.58±0.05	1.16±0.10	1.98±0.04	0.42±0.11	1.19±0.19	0.82±0.31	0.81±0.29	0.16±0.09	5.11±0.05	3.02±0.04	3.05±0.08	0.62±0.11
C20:5	3.44±0.11	2.62±0.07	2.99±0.13	1.61±0.09	3.88±0.12	2.54±0.26	3.11±0.18	1.82±0.21	3.35±0.02	1.27±0.03	3.09±0.12	1.01±0.03
C22:6	5.79±0.08	2.98±0.09	4.51±0.06	2.15±0.17	8.29±0.14	5.22±0.12	5.23±0.15	3.55±0.17	6.84±0.04	2.14±0.05	5.92±0.20	2.42±0.05
ΣPUFA	26.62±0.43	38.02±0.31	21.82±0.30	36.06±0.51	27.55±0.33	42.93±0.53	21.10±0.44	36.73±0.47	30.54±0.18	39.58±0.18	26.95±0.36	36.80±0.39

Values are shown as mean ± standard error of triplicates.

Table 3. Fatty acid profiles (% total fatty acid) of neutral lipid (NL) of *Catla catla, Labeo rohita and Cirrhinus mrigala* in fresh, fried, frozen and frozen-fried condition

	Catla catla				*Labeo rohita*				*Cirrhinus mrigala*			
	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried
C12:0	2.13±0.25	1.32±0.19	1.94±0.18	1.88±0.16	3.24±0.05	0.6±0.07	3.26±0.5	0.86±0.09	11.36±0.28	0.57±0.10	1.10±0.12	1.92±0.13
C14:0	3.79±0.18	2.34±0.11	4.13±0.14	1.71±0.15	3.91±0.14	0.33±0.11	5.61±0.28	0.94±0.15	8.08±0.13	1.56±0.05	6.73±0.09	1.55±0.19
C15:0	0.22±0.05	0.59±0.08	1.18±0.11	0.62±0.07	0.88±0.10	0.18±0.09	1.26±0.14	0.12±0.02	0.57±0.06	0.44±0.02	2.87±0.01	0.13±0.01
C16:0	27.35±0.44	20.01±0.35	33.53±0.55	20.94±0.28	34.71±0.45	4.90±0.01	42.91±0.55	5.91±0.29	23.57±0.52	9.44±0.31	35.63±0.16	7.96±0.43
C17:0	1.45±0.21	0.93±0.11	1.51±0.16	0.41±0.04	2.34±0.09	0.15±0.06	3.16±0.18	0.73±0.20	0.38±0.02	0.29±0.02	2.61±0.05	0.14±0.06
C18:0	8.33±0.32	3.77±0.36	8.54±0.05	4.84±0.29	5.13±0.16	1.88±0.13	3.48±0.07	1.25±0.16	5.43±0.09	1.94±0.19	7.16±0.05	0.32±0.07
C20:0	1.81±0.16	0.48±0.12	0.67±0.05	1.19±0.16	0.67±0.04	0.48±0.10	1.05±0.01	1.15±0.12	0.17±0.03	0.59±0.11	1.16±0.15	0.41±0.12
C22:0	1.12±0.20	0.70±0.00	1.06±0.18	1.06±0.12	–	–	–	–	–	–	–	–
C24:0	0.23±0.06	–	0.48±0.13	–	–	0.31±0.11	1.21±0.08	–	–	0.18±0.04	1.03±0.17	–
ΣSFA	46.43±0.71	30.14±0.57	53.04±0.66	32.65±0.50	50.88±0.51	8.83±0.26	61.94±0.83	10.96±0.44	49.56±0.61	15.01±0.39	58.29±0.32	12.43±0.51
C16:1	8.48±0.36	7.45±0.09	3.75±0.23	5.97±0.24	8.17±0.25	0.65±0.10	5.85±0.22	3.37±0.41	5.72±0.15	1.81±0.08	9.45±0.11	0.86±0.11
C17:1	0.43±0.09	1.33±0.16	0.78±0.08	0.35±0.02	0.97±0.18	0.15±0.08	0.8±0.01	0.2±0.01	0.34±0.03	0.29±0.07	2.3±0.17	0.11±0.02
C18:1c	23.01±0.28	26.18±0.19	23.23±0.29	21.37±0.22	12.78±0.33	29.97±0.27	9.05±0.10	19.67±0.31	20.36±0.29	12.13±1.00	9.21±0.09	26.56±0.34
C18:1t	–	–	–	–	2.16±0.18	10.17±0.19	2.18±0.11	14.24±0.20	0.71±0.16	20.34±0.07	1.21±0.31	18.23±0.12
C20:1	2.16±0.11	1.60±0.11	1.06±0.19	3.68±0.29	0.31±0.02	1.41±0.23	0.60±0.03	1.81±0.14	0.64±0.02	0.11±0.02	2.32±0.09	1.12±0.21
C24:1	0.27±0.18	0.54±0.16	1.45±0.29	1.99±0.09	0.51±0.12	0.19±0.09	1.02±0.03	–	0.41±0.01	0.07±0.00	1.1±0.01	–
ΣMUFA	34.35±0.51	37.10±0.32	30.27±0.51	33.36±0.44	24.9±0.50	42.54±0.43	19.50±0.26	39.29±0.56	28.18±0.36	34.75±1.00	25.59±0.39	46.88±0.43
C18:2c	5.22±0.22	9.78±0.41	3.96±0.31	9.62±0.48	11.89±0.26	20.72±0.42	8.73±0.23	20.64±0.30	10.3±0.31	22.21±0.10	5.48±0.13	19.03±0.17
C18:2t	1.16±0.18	5.46±0.34	1.25±0.03	7.03±0.29	2.11±0.09	15.32±0.38	2.70±0.02	12.44±0.19	0.58±0.01	15.00±0.14	0.55±0.02	12.92±0.22
C18:3	3.56±0.15	5.16±0.19	4.44±0.15	9.70±0.40	1.22±0.19	7.46±0.02	0.43±0.11	6.88±0.29	0.88±0.21	8.25±0.07	6.13±0.10	4.35±0.33
C20:3	1.02±0.09	0.26±0.20	0.53±0.06	0.35±0.05	–	–	–	–	–	–	–	–
C20:4	0.74±0.10	0.84±0.07	1.63±0.13	0.44±0.06	1.11±0.06	0.39±0.01	1.15±0.13	0.79±0.06	1.4±0.01	0.27±0.05	1.23±0.06	0.07±0.01
C20:5	2.70±0.19	9.21±0.21	1.11±0.26	5.59±0.38	4.88±0.25	3.31±0.18	1.58±0.12	7.88±0.09	3.17±0.09	3.44±0.22	1.22±0.18	3.41±0.03
C22:6	3.82±0.12	2.05±0.36	2.77±0.33	1.26±0.11	3.01±0.40	1.43±0.36	1.97±0.39	1.12±0.05	3.18±0.23	1.07±0.03	1.50±0.03	0.91±0.06
ΣPUFA	18.22±0.41	32.76±0.73	15.69±0.56	33.99±0.79	24.22±0.58	48.63±0.69	16.56±0.49	49.75±0.47	19.51±0.44	50.24±0.29	16.11±0.25	40.69±0.43

Values are shown as mean ± standard error of triplicates.

Table 4. Fatty acid profiles (% total fatty acid) of phospholipid (PL) of *Catla catla, Labeo rohita and Cirrhinus mrigala* in fresh, fried, frozen and frozen-fried condition

	Catla catla				*Labeo rohita*				*Cirrhinus mrigala*			
	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried
C12:0	–	–	–	–	0.25±0.07	0.22±0.11	0.23±0.05	0.26±0.07	1.00±0.14	–	0.37±0.04	–
C14:0	0.64±0.09	0.32±0.02	0.20±0.08	0.28±0.04	0.32±0.09	0.32±0.12	0.17±0.05	0.42±0.08	0.02±0.00	0.39±0.01	0.09±0.02	0.23±0.04
C15:0	3.91±0.25	2.18±0.11	5.16±0.16	4.92±0.18	0.25±0.07	0.19±0.06	11.22±0.19	7.67±0.16	–	–	–	–
C16:0	33.27±0.38	13.83±0.27	37.49±0.24	8.70±0.21	12.86±0.35	12.63±0.24	18.88±-.28	13.19±0.29	18.42±0.30	21.17±0.38	23.32±0.19	23.74±0.19
C17:0	1.99±0.09	1.66±0.09	0.76±0.07	1.14±0.11	1.05±0.15	0.96±0.09	1.80±0.10	2.01±0.15	1.31±0.16	2.00±0.14	0.88±0.09	2.00±0.13
C18:0	19.30±0.13	9.57±0.17	17.77±0.27	14.68±0.35	14.44±0.27	1.42±0.11	6.19±0.16	5.17±0.19	24.85±0.34	10.56±0.16	29.49±0.34	22.83±0.26
C20:0	0.67±0.07	0.48±0.02	1.81±0.10	1.19±0.09	0.57±0.08	0.39±0.04	0.77±0.014	0.47±0.09	0.98±0.09	0.48±0.04	0.11±0.03	0.5±0.11
C24:0	0.34±0.05	2.67±0.16	3.77±0.30	3.70±0.20	2.30±0.16	2.36±0.16	2.15±0.19	1.98±0.20	0.66±0.07	0.30±0.13	1.10±0.29	0.55±0.14
ΣSFA	60.12±0.49	30.71±0.38	66.96±0.51	34.61±0.51	32.04±0.51	18.49±0.36	41.41±0.45	31.17±0.47	47.24±0.51	34.90±0.45	55.36±0.49	49.85±0.39
C14:1	0.14±0.02	0.12±0.01	–	–	4.25±0.22	2.19±0.10	6.16±0.15	4.19±0.14	–	–	–	–
C15:1	–	–	–	–	1.00±0.10	2.74±0.23	1.84±0.11	1.01±0.20	–	–	–	–
C16:1	2.70±0.24	1.60±0.07	1.40±0.09	3.87±0.07	1.65±0.17	1.58±0.20	1.16±0.09	1.01±0.10	2.77±0.15	2.26±0.20	2.71±0.10	1.99±0.11
C17:1	1.04±0.11	0.42±0.01	0.16±0.01	0.16±0.02	0.24±0.09	0.24±0.07	2.11±0.14	2.01±0.13	1.55±0.20	0.71±0.07	0.12±0.01	0.31±0.07
C18:1c	8.26±0.23	14.58±0.25	7.38±0.22	8.01±0.30	15.01±0.30	17.13±0.22	4.88±0.11	6.69±0.20	13.64±0.25	19.41±0.20	12.12±0.14	15.93±0.27
C18:1t	7.56±0.16	10.72±0.30	3.86±0.19	5.45±0.28	2.41±0.16	11.25±0.26	2.31±0.09	12.12±0.27	2.76±0.14	10.13±0.19	1.88±0.10	0.77±0.30
C20:1	2.25±0.24	0.72±0.06	0.68±0.07	0.59±0.04	1.21±0.26	0.45±0.03	2.79±0.20	1.78±0.14	1.51±0.18	0.76±0.06	0.08±0.01	1.31±0.16
C24:1	0.46±0.03	2.76±0.14	4.35±0.32	3.44±0.18	3.35±0.18	3.30±0.41	2.00±0.12	2.13±0.24	1.21±0.17	0.62±0.08	1.11±0.11	0.73±0.06
ΣMUFA	22.41±0.45	30.92±0.42	17.83±0.44	21.52±0.45	29.12±0.55	38.88±0.62	23.25±0.37	30.94±0.52	23.44±0.45	33.89±0.36	18.02±0.22	21.04±0.45
C18:2c	1.12±0.09	3.56±0.12	2.46±0.19	4.33±0.24	4.28±0.29	5.24±0.18	1.02±0.08	3.91±0.16	4.12±0.16	6.70±0.25	9.75±0.24	13.02±0.23
C18:2t	1.78±0.10	2.48±0.10	1.60±0.14	3.21±0.16	0.11±0.04	2.57±0.20	1.77±0.12	5.77±0.14	4.80±0.18	15.22±0.26	3.11±0.16	6.78±0.34
C18:3	0.54±0.06	1.62±0.09	0.92±0.09	0.74±0.06	0.45±0.07	0.77±0.09	0.88±0.06	0.34±0.07	1.50±0.11	0.35±0.03	0.73±0.07	0.78±0.07
C20:2	0.51±0.12	0.67±0.04	0.59±0.05	0.42±0.07	1.34±0.18	0.98±0.06	1.11±0.21	0.89±0.10	0.98±0.09	0.51±0.05	1.42±0.12	0.38±0.08
C20:3	0.45±0.09	1.32±0.29	1.03±0.32	0.91±0.17	2.81±0.38	3.44±0.39	6.18±0.32	7.88±0.43	8.49±0.29	2.33±0.15	4.85±0.31	4.16±0.30
C20:4	1.49±0.14	9.16±0.30	2.99±0.23	10.27±0.34	7.69±0.30	7.41±0.23	1.15±0.16	1.95±0.18	2.06±0.15	1.23±0.11	1.41±0.20	1.07±0.12
C20:5	3.91±0.08	6.35±0.22	0.26±0.08	0.19±0.02	4.54±0.28	4.51±0.26	3.63±0.22	1.97±0.18	2.32±0.16	0.99±0.09	1.11±0.16	1.71±0.17
C22:6	7.67±0.21	13.21±0.28	5.36±0.38	21.88±0.40	17.62±0.38	17.71±0.36	19.60±0.30	15.18±0.32	5.05±0.24	3.73±0.10	2.36±0.23	0.21±0.02
ΣPUFA	17.47±0.31	38.37±0.57	15.21±0.60	41.95±0.62	38.84±0.70	42.63±0.63	35.34±0.49	37.89±0.55	29.32±0.47	31.06±0.42	24.74±0.50	28.11±0.51

Values are shown as mean ± standard error of triplicates.

Table 5. Fatty acid profiles (% total fatty acid) of glycolipid (GL) of *Catla catla, Labeo rohita and Cirrhinus mrigala* in fresh, fried, frozen and frozen-fried condition

	Catla catla				*Labeo rohita*				*Cirrhinus mrigala*			
	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried
C12:0	9.22±0.04	4.80±0.37	6.81±0.08	6.28±0.12	6.77±0.11	4.83±0.20	10.16±0.55	8.75±0.24	1.44±0.12	0.83±0.09	4.89±0.20	1.22±0.11
C14:0	9.85±0.08	5.12±0.06	2.31±0.16	8.45±0.05	6.86±0.29	3.69±0.34	14.34±0.33	8.61±0.33	2.05±0.14	0.44±0.04	2.19±0.16	0.61±0.06
C15:0	1.18±0.10	0.59±0.09	0.62±0.01	0.22±0.04	1.02±0.11	0.77±0.03	0.60±0.19	0.44±0.07	0.32±0.05	0.79±0.07	0.47±0.04	0.88±0.08
C16:0	26.75±0.22	15.77±0.24	30.83±0.13	14.62±0.51	20.09±0.30	16.56±0.28	23.59±0.44	15.07±0.52	18.21±0.29	9.56±0.28	22.52±0.36	13.01±0.27
C17:0	0.82±0.09	0.47±0.04	1.26±0.20	0.47±0.08	0.69±0.08	0.85±0.08	0.88±0.11	0.52±0.16	1.69±0.12	0.85±0.08	0.54±0.07	0.21±0.01
C18:0	8.85±0.14	7.60±0.20	18.38±0.09	8.88±0.36	18.20±0.39	12.53±0.48	13.24±0.52	8.06±0.18	17.12±0.30	12.55±0.26	19.76±0.25	10.08±0.29
C20:0	2.02±0.24	0.51±0.05	1.66±0.06	3.05±0.44	1.17±0.14	1.15±0.30	1.30±0.26	1.68±0.09	0.19±0.03	1.05±0.13	0.34±0.04	0.68±0.06
C24:0	1.19±0.10	1.03±0.18	2.11±0.27	0.29±0.18	1.06±0.15	1.09±0.29	1.43±0.19	1.28±0.26	0.66±0.09	0.79±0.06	1.03±0.16	0.28±0.02
ΣSFA	59.88±0.39	35.89±0.53	63.98±0.36	42.26±0.79	55.86±0.62	41.47±0.79	65.54±1.00	44.41±0.74	41.68±0.46	26.86±0.42	51.74±0.53	26.97±0.42
C16:1	6.36±0.18	2.89±0.14	4.54±0.17	4.79±0.27	3.93±0.27	3.33±0.15	4.85±0.38	4.25±024	2.13±0.16	1.33±0.10	1.23±0.12	1.52±0.14
C17:1	0.42±0.03	0.19±0.10	0.11±0.03	0.14±0.01	0.53±0.15	0.58±0.06	0.57±0.07	0.21±0.14	1.23±0.11	0.58±0.07	0.22±0.02	0.11±0.01
C18:1c	5.87±0.19	13.26±0.32	10.24±0.35	21.07±0.48	5.33±0.17	7.77±0.17	5.42±0.18	9.19±0.31	19.89±0.32	22.27±0.35	16.45±0.29	15.12±0.30
C18:1t	2.77±0.22	9.11±0.16	1.83±0.02	7.53±0.19	5.89±0.13	9.80±0.51	2.25±0.28	4.35±0.32	2.01±0.15	10.90±0.24	1.45±0.13	9.88±0.28
C20:1	4.17±0.16	2.39±0.13	2.04±0.13	1.00±0.02	2.97±0.18	2.34±0.46	2.30±0.40	2.79±0.06	0.97±0.09	1.43±0.15	0.96±0.09	1.99±0.20
C24:1	2.03±0.18	1.06±0.11	1.39±0.41	0.81±0.21	0.79±0.04	0.89±0.21	0.42±0.06	0.66±0.12	0.31±0.04	0.22±0.02	0.42±0.04	0.67±0.06
ΣMUFA	21.62±0.41	28.90±0.43	20.15±0.58	35.34±0.61	19.44±0.41	24.71±0.75	15.81±0.65	21.45±0.54	26.54±0.41	36.73±0.46	20.73±0.35	29.29±0.48
C18:2c	2.79±0.18	9.34±0.15	4.92±0.23	7.75±0.31	7.81±0.26	8.11±0.41	4.71±0.11	7.18±0.41	12.08±0.24	15.42±0.29	10.21±0.30	16.97±0.34
C18:2t	3.19±0.20	8.88±0.23	2.10±0.21	5.11±0.03	2.03±0.10	7.27±0.37	1.19±0.07	5.91±0.13	2.03±0.13	8.77±0.20	1.78±0.15	12.90±0.32
C18:3	1.34±0.11	2.88±0.01	1.57±0.07	3.11±0.17	3.82±0.57	6.27±0.45	2.10±0.40	8.02±0.33	2.80±0.23	4.76±0.31	2.55±0.27	6.14±0.37
C20:2	–	–	–	–	1.91±0.05	1.55±0.40	0.94±0.28	1.12±0.12	1.39±0.14	0.67±0.06	0.29±0.03	0.33±0.04
C20:3	1.49±0.07	0.41±0.07	3.61±0.18	1.22±0.18	1.58±0.07	3.29±0.13	3.25±0.37	4.01±0.22	4.10±0.32	2.46±0.18	5.19±0.31	4.66±0.34
C20:4	2.03±0.03	0.80±0.02	1.44±0.17	0.67±0.15	2.24±0.02	3.00±0.06	2.30±0.18	3.11±0.34	1.24±0.15	1.32±0.10	1.55±0.14	1.16±0.12
C20:5	3.31±0.12	11.33±0.41	0.59±0.20	4.14±0.32	2.09±0.18	2.12±0.17	1.35±0.40	2.77±0.17	1.01±0.10	0.88±0.08	0.99±0.10	0.56±0.06
C22:6	3.64±0.22	1.57±0.19	1.64±0.37	0.40±0.11	3.22±0.23	2.21±0.08	2.81±0.43	2.02±0.20	7.13±0.26	2.23±0.21	4.97±0.28	1.02±0
ΣPUFA	17.79±0.39	35.21±0.53	15.87±0.58	22.40±0.54	24.7±0.58	33.82±0.78	18.65±0.83	34.14±0.70	31.78±0.52	36.41±0.51	27.53±0.55	43.74±0.62

Values are shown as mean ± standard error of triplicates.

three carps during frying with mustard oil where most important reduction was found in *L. rohita* (36.4051% for fresh and 37.4254% for frozen condition). Consequently, the total amount of SFA decreased during frying for all of the species. On the contrary, the increase of SFA contents were 11.2856%, 14.8468% and 13.0119% for *C. catla, L. rohita and C. mrigala* respectively during freezing, mainly due to increase in C16:0. This is also in agreement with the findings on *Scomberomours commersoni* and *Carcharhinus dussumieri*[20]. The contrasting effect of frying and freezing on the SFA content was also found to be similar in NL, PL and GL. The nutritional consequence of such alteration in SFA amount has been discussed later.

The MUFA content was highest for *L. rohita* (26.01±0.38%) and lowest for *C. catla* (23.7±0.28%) in fresh condition in TL. Oleic acid (C18:1c), palmitoleic acid (C16:1) were the major contributors for all the carps in all condition for all lipid classes. In TL 74.3944% of the total MUFA content was oleic acid for *L. rohita* while it was 67.5527% and 70.2809% for *C. catla* and *C. mrigala* respectively and similar high percentage of oleic acid was also found for NL, PL and GL in fresh condition. The existence of C18:1c in the fish samples was significantly high compared to other products containing similar composition[5]. Alteration in C18:1c and C18:1t between fresh and fried samples for both conditions was greater due to the high absorption of mustard oil during the frying procedure. This was evident from conversion of *cis* C18:1 and C18:2 FAs to *trans* isomer in the cooking medium during frying (Fig. 2). This pattern of increase in concentration of MUFA also suggested by the result of Neff *et al.*[14]. In frozen condition, the amount of MUFA reduced effectively, probably due to its susceptibility to oxidation[37]. This decreasing trend has been very much compatible with the result of Nazemroaya *et al.*[20]. The variation in FAs composition of TL during frying and freezing has been found to be similar in NL, PL and GL also.

Overall PUFA content in TL was highest for *C. mrigala* compared to *L. rohita* and *C. catla* respectively. Among PUFAs, EPA (C20:5, n-3), DHA (C22:6, n-3), arachidonic acid (AA) (C20:4, n-6), α-linolenic acid (C18:3, n-3) and linoleic acid (C18:2, n-6) were in high proportion in all three freshwater carps in both conditions for all lipid classes. In fresh TL, n-3 content was found to be comparably higher in *C. catla*

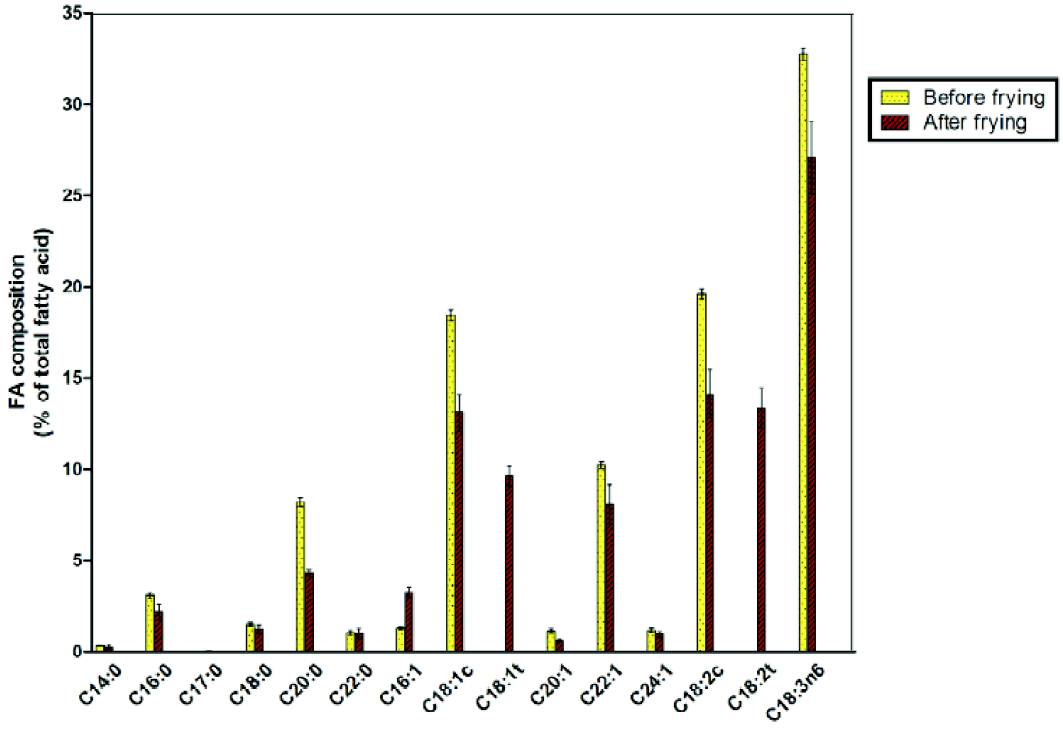

Fig. 2. Fatty acids compositions (% of total fatty acid) of used mustard oil before and after frying.

and *C. mrigala* than *L. rohita*, while the n-6 PUFA content was highest in *C. mrigala* and lowest in *C. catla* (Table 6). In fresh condition the presence of AA, the major n-6 FA, was maximum for *C. mrigala* (16.7321% of total PUFA) and lowest for *L. rohita* (4.3194% of total PUFA). Though the FA distribution for these species followed the trend SFA > MUFA > PUFA, they were notably good source of LC-PUFAs. The high content of n-3 PUFA in fish lipids help to reduce the level of cholesterol, triglycerides and amounts of low density lipoprotein cholesterol in blood serum thereby decrease the chance of cardiovascular disease, the risk of atherosclerosis, and arterial hypertension[52]. n-3 and n-6 PUFAs have contrasting physiological functions and their balance is important for normal growth and development. Fish samples, fried in mustard oil showed an exceptionally higher amount of linoleic acid (C18:2c) and linolelaidic acid (C18:2t) which could be attributed solely to the presence of linoleic acid in mustard oil (Fig. 2) and suggested a possible migration of cooking oil FAs to fish fillets. The differences in PUFA concentration between fresh and fried ones were quite less consistence across the species in both conditions but n-6 PUFA content effectively increased during frying due to high accumulation of *cis* C18:2n-6. The FAs of mustard oil mainly comprised of C20:0, C18:1c, C22:1, C18:2c and C18:3n6. After frying it was drastically modified causing production of unhealthy *trans* FAs (Fig. 2). In fried fish AA, DHA and EPA content markedly reduced by 55.0387%, 23.8372% and 48.5319% for *C. catla* and 31.0924%, 34.5360% and 37.0325% for *L. rohita* and 40.9002%, 62.0895% and 68.7134% for *C. mrigala*, respectively compared to fresh ones. The PUFA composition of NL, PL and GL during frying also varied in the similar pattern. Such reduction in the content of FA with high degree of unsaturation might have occurred due to high susceptibility of oxidation during heat treatment. In frozen storage the amount of PUFA decreased in contrast to SFA because of development of progressive lipid hydrolysis[20] which induced a considerable loss of nutritive value[24]. In TL, the deterioration of PUFA content was highest for *L. rohita* (23.4119%) compared with *C. catla* (18.0315%) and *C. mrigala* (11.7550%). Both n-6 and n-3 content correlatively decreased along with the total PUFA content. Production of hydroperoxide by the reaction of UFAs with oxygen might have decreased the amount of PUFA causing adverse effects on human health[37,53,54]. The declining rate in the percentage of AA, DHA and EPA during frozen preservation was also noteworthy for FA profile of NL, PL and GL.

NQI:

The three Indian major carps were found to be good source of LC-PUFAs (AA, EPA, DHA). The potential nutritive value and dietetic importance of fish depend mainly on the concentration of these essential FAs, ratio of Σn-3:Σn-6 and PUFA:SFA. Many research studies and health organizations worldwide recommend that healthy human diet should have n-3:n-6 ratio ≥ 1 but not <0.2; PUFA:SFA ratio > 0.4, and a daily ingestion of at least 250–450 mg of EPA+DHA[14,55]. In general, an average daily intake of 500 mg/day EPA+DHA for primary prevention of cardiovascular disease[14]. The NQI of *C. catla, L. rohita and C. mrigala* under all experimental conditions have been illustrated in Table 6. The present study indicated that these three carps had a high Σn-3:Σn-6 ratio in fresh condition due to presence high amount of n-3. In frozen condition, the decrease in this ratio was within the recommended limit attesting the suitability of the method for preserving these nutritional parameters. Frying of fish enhanced the amount of linoleic acid (C18:2c) and linolelaidic acid (C18:2t) as n-6 PUFA which affected Σn-3:Σn-6 ratio adversely in both fresh and frozen samples. High consumption of n-6 FA help to increase the plasma concentration of bioactive product like prostaglandins, thromboxanes, leukotrienes, hydroxyl fatty acid and lipoxins from AA. These eicosanoid metabolic products increase the thrombi and atheromas in blood vessels, contribute to the development of allergic and inflammatory disorders and excessive cell proliferation[10]. On the other hand, n-3 FA counteract these antagonistic effects of n-6 FA by declining the production rate of thromboxane A_2, leukotriene B_4 and prostaglandin E_2 contrastingly, increasing the concentration of prostaglandin I_3. In addition, n-3 FA suppress interleukin (IL) 1β, tumour necrosis factor α and IL6 for strong anti-inflammatory effects[10]. Furthermore, many research studies on animal model consistently showed the promoting effect of mammary tumorigenesis by high intake of n-6 against which n-3 FA exert a protective role[56]. The n-3 FA are known as "essential" due to its necessity from conception through pregnancy and infancy and undoubtedly throughout the lifespan from food[7] because inability of body to synthesize them. Fish and fish oil are mainly characterized by elevated levels of EPA, DHA and

Table 6. Nutritional quality indices (NQI) of *Catla catla, Labeo rohita and Cirrhinus mrigala* in fresh, fried, frozen and frozen-fried condition

	Catla catla				*Labeo rohita*				*Cirrhinus mrigala*			
	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried	Fresh	Fried	Frozen	Frozen-fried
EPA+DHA	9.23± 0.19	5.6± 0.16	7.5± 0.19	3.76± 0.26	12.17± 0.26	7.76± 0.38	8.34± 0.33	5.37± 0.38	10.19± 0.06	3.41± 0.08	9.01± 0.32	3.43± 0.08
Σn-3	14.03± 0.40	7.85± 0.26	10.05± 0.36	5.05± 0.39	13.29± 0.37	8.78± 0.51	9.41± 0.54	6.26± 0.61	14.31± 0.18	6.31± 0.09	11.89± 0.45	4.54± 0.40
Σn-6	12.59± 0.39	30.17± 0.46	11.77± 0.23	31.01± 0.72	14.26± 0.35	34.15± 0.70	11.69± 0.41	30.47± 0.50	16.23± 0.16	33.27± 0.27	15.06± 0.30	32.26± 0.37
Σn-3:Σn-6	1.1143± 0.0027	0.2601± 0.0046	0.8538± 0.0139	0.1628± 0.0088	0.9319± 0.0030	0.2571± 0.0096	0.8049± 0.0180	0.2054± 0.0166	0.8817± 0.0024	0.1896± 0.0012	0.7895± 0.0141	0.1407± 0.0107
PUFA:SFA	0.5418± 0.0089	1.0872± 0.0055	0.3940± 0.0040	0.9585± 0.0066	0.6080± 0.0061	1.5431± 0.0203	0.3965± 0.0096	1.0174± 0.00005	0.7082± 0.0008	1.2041± 0.0062	0.5436± 0.0109	1.0392± 0.0056
cis:trans	26.02± 0.58	11.6791± 1.3348	22.70± 0.48	10.9386± 1.1373	32.42± 0.45	10.7345± 1.2118	25.75± 0.45	13.9564± 2.2655	40.6760± 2.6120	7.6351± 0.2098	84.00± 20.8382	11.2261± 1.1267
EPA+DHA/ C16:0	0.2729± 0.0045	0.2246± 0.0053	0.1917± 0.0036	0.1377± 0.0074	0.3701± 0.0056	0.3711± 0.0116	0.2260± 0.0078	0.2332± 0.0137	0.3263± 0.0006	0.1448± 0.0025	0.2704± 0.0087	0.1630± 0.0022
IA	1.0153± 0.0121	0.5132± 0.0010	1.3550± 0.0208	0.5661± 0.0028	0.7957± 0.0064	0.3291± 0.0024	1.1432± 0.0255	0.5321± 0.0002	0.8125± 0.0060	0.4876± 0.0028	0.9699± 0.0147	0.4967± 0.0064
IT	0.7648± 0.0131	0.6120± 0.0057	1.1255± 0.0246	0.8086± 0.0219	0.6434± 0.0090	0.4051± 0.0047	0.9834± 0.0368	0.6347± 0.0233	0.5777± 0.0045	0.5486± 0.0014	0.7865± 0.0200	0.6700± 0.0207
HH	1.0556± 0.0191	2.0333± 0.0066	0.7558± 0.0124	1.7649± 0.0002	1.3056± 0.0111	2.9274± 0.0263	0.8809± 0.0175	2.1450± 0.0013	1.2595± 0.0056	2.0505± 0.0040	1.0813± 0.0154	2.2531± 0.0079

Values are shown as mean ± standard error of triplicates.

trace amount of α-linolenic acid as n-3 source. Due to the presence of high amount of EPA+DHA in all the three fish species in fresh condition, these can be used as key component for the supplement of essential FAs in the human diet. Bera *et al*.[57] experimentally showed that EPA/DHA enriched PUFA also played protective role against mercury poising and were highly preventive against diabetes[57]. The decline in the amount of EPA+DHA was more after frying than frozen storage for *C. catla* (39.3282% vs 18.7432%), *L. rohita* (36.2366% vs 31.4708%) and *C. mrigala* (66.5358% vs 11.5799%). This might cause adverse effect of frying on the nutritive value of fish. Though frying with mustard oil showed a deleterious effect on Σn-3:Σn-6 ratio but due to uptake of PUFAs enriched culinary fat and synergistic reduction in SFA, PUFA:SFA ratio of final product became greater than the raw ones in both fresh and frozen condition. However, during the frozen storage due to high susceptibility to lipid peroxidation, decrease of PUFA in contrast with SFA lead to an overall decrease in this ratio. In the present study, the result also implicated that the adverse consequences of frying and frozen storage (–20°C for 30 days) on the food value due to increase of *trans* FA and proportionately decrease the *cis*:*trans* ratio. *Trans* FA has the proficiency to increase the concentration of low density lipoprotein (LDL) in plasma and proportionately decrease the concentration of high density lipoprotein (HDL) in contrasting approach. The deleterious effect of *trans* FA is nearly two-fold compared to saturated fat and that directly lead to the risk of coronary artery disease[58]. The changes in EPA+DHA, Σn-3, Σn-6, Σn-446 3:Σn-6, PUFA:SFA, *cis*:*trans* for different experimental conditions with respect to fresh fish samples of different carps have been presented in Fig. 3. There were small changes in Σn-3:Σn-6 ratio and PUFA:SFA ratio with all species while other factors showed greater differences among treatments. The changes were strongly positive for Σn-6 after frying and very much negative for EPA+DHA and Σn-3 after frying and freezing.

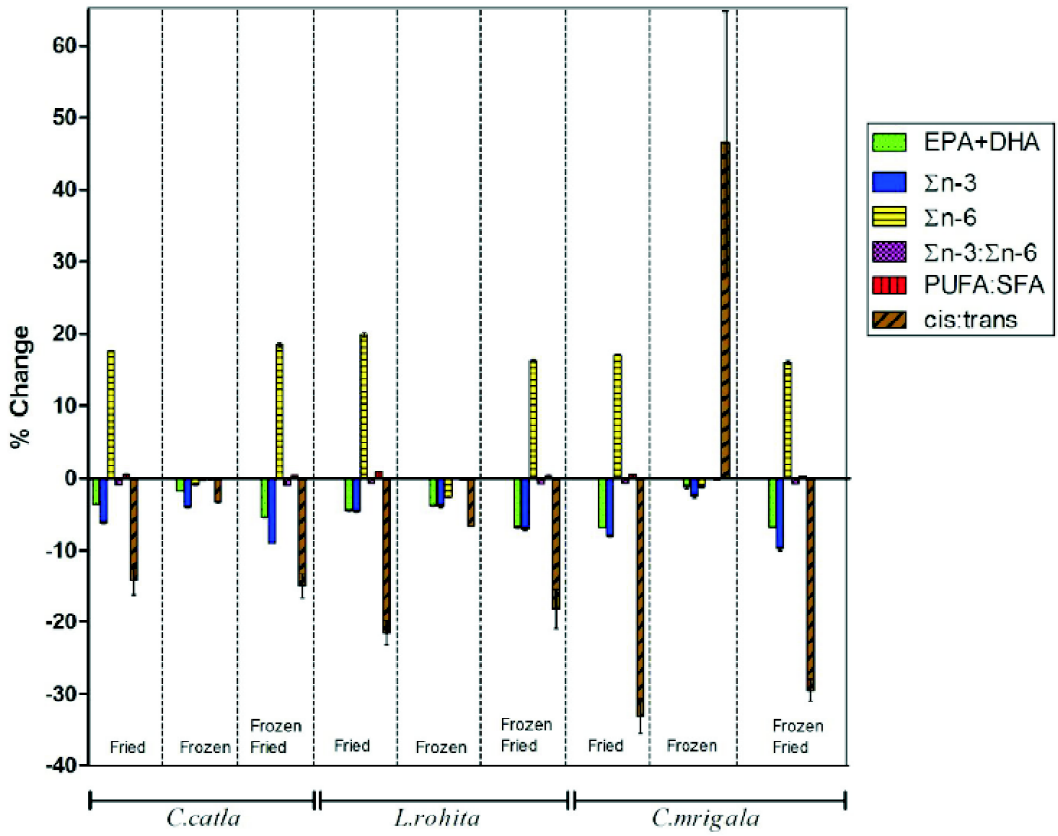

Fig. 3. Percent change in nutritional quality indices (NQI) of *Catla catla, Labeo rohita* and *Cirrhinus mrigala* for EPA+DHA, Σn-3, Σn-6, Σn-3:Σn-6, PUFA:SFA and *cis*:*trans* for fried, frozen and frozen-fried condition with respect to fresh one.

The difference in *cis*:*trans* ratio of fried, frozen and frozen-fried fish samples with untreated ones generally depressed for all species except *C. mrigala* in frozen condition.

EPA+DHA/C16:0 ratio has been recommended as an good index to evaluate lipid oxidation[20]. The ratio has been depleted for both fried and frozen storage condition for *C. catla* and *C. mrigala*. The negative correlation between this ratio and frying and frozen condition concluded that oxidation mechanisms were active for both cases. In comparison with *C. catla*, *C. mrigala* contributed more to the accelerated oxidative degradation of lipid during frying with mustard oil for both fresh and frozen conditions. IA has been used as one the best and novel biomarkers for cardiovascular disease (CVD) and obesity[59]. IA and IT are considered as representative of dietic quality of lipids and lower values for both the indices are favourable to represent better nutritional quality and correspondingly decrease the cardiovascular risk[34]. The positive association between IA with CVD and obesity here revealed that the adverse effect of frozen storage was more detrimental than the frying with mustard oil. The increased value of IT during frozen storage also might escalate the tendency for formation of clot in blood vessel. HH correlates the specific effect of FA on cholesterol metabolism. Nutritionally higher HH values are recommended as more beneficial due to lowering of adverse effect of SFA on human health[34]. HH values increased considerably during frying whereas the result showed an inverse relation in frozen condition. That result also suggested that not the total amount of lipid but FA composition of food was more influential for the quantification of some relatable health issues. With respect to fresh carp samples the changes in EPA+DHA/C16:0, IA, IT and HH for fried, frozen and frozen-fried condition has been depicted in Fig. 4. The trend of change in IA, IT and HH after frying completely contradicted with the frozen state except for IT of *C. catla* in frozen-fried condition. The negative change in IA, IT and positive change in HH

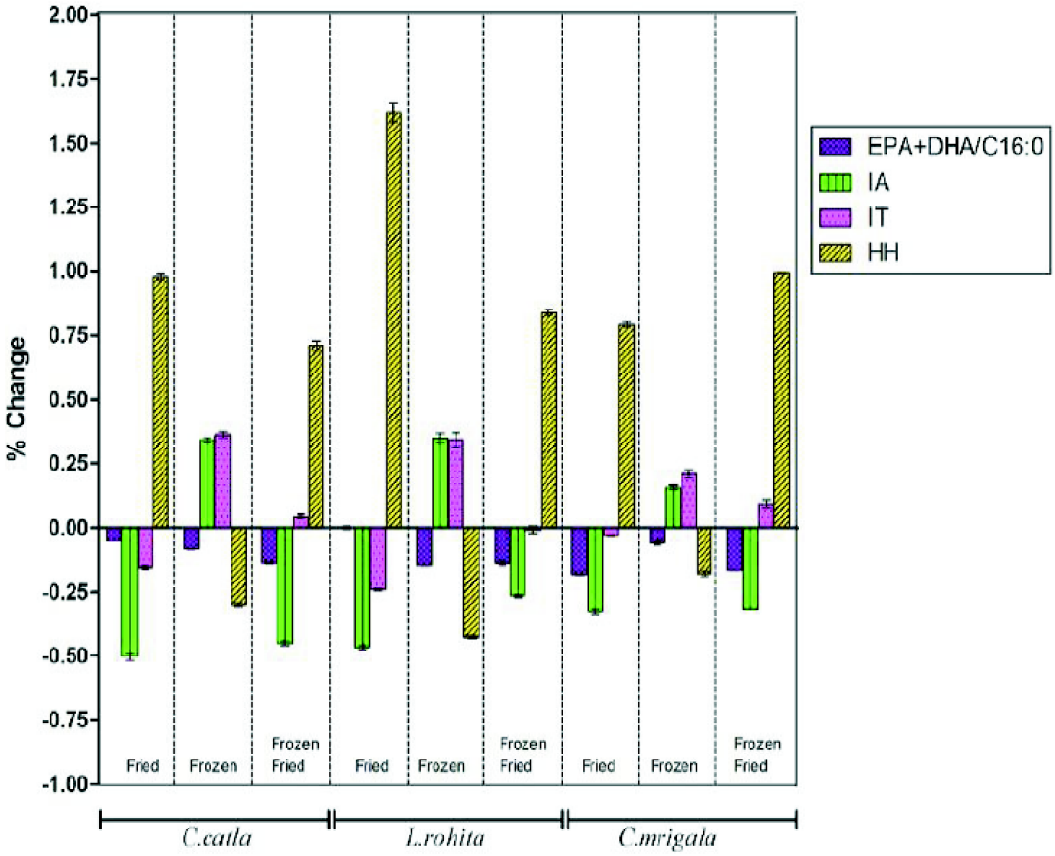

Fig. 4. Percent change in nutritional quality indices (NQI) of *Catla catla, Labeo rohita* and *Cirrhinus mrigala* for EPA+DHA/C16:0, IA, IT and HH for fried, frozen and frozen-fried condition with respect to fresh one.

implied improvement of nutritional quality which was achieved after frying. MANOVA of NQI showed that there was significant difference among the fish species as well as among the experimental conditions (fresh, fried, frozen and frozen-fried). Also there was a strong interaction between the fish and conditions.

The finding of this study would probably also help to develop a new area of research as the knowledge of dietary composition of fish species is of fundamental interest in the application of different technological processes in fish processing, preservation and product development maintaining nutritional quality.

Conclusions

In light of IA, CVD, IT and HH, this study confirmed that *Catla catla, Labeo rohita* and *Cirrhinus mrigala* were good source of nutrients. Though the biomedical components of all the carps were similar, their nutritional quality varied significantly across the species. Frozen storage has been most commonly and commercially employed to retain the nutritional quality of fish for long time. This study exhibited the deleterious effect of frozen carps towards human health. The results were attributed to the depletion in protein and lipid content, detrimental changes as well as hydrolysis of UFAs, and inclusion of *trans* FAs from braising medium which adversely affected the IA, IT, HH ratio. Whether fresh or frozen, fish is not generally consumed in raw condition. Frying is the most common first step, even often the only step for cooking fish. Different parameters like Σn-3, EPA+DHA, Σn-3:Σn-6, *cis*:*trans* ratio, considered as beneficial for human health, decreased on frying while the disadvantageous ones like Σn-6 increased. The extent of these individual changes apparently designated frying as an unsuitable process. But insignificant changes in EPA+DHA/C16:0 ratio indicated minimal oxidative damage during frying contradicting previous inferences. Further, decrease in IA, IT values along with rise in HH advocated for improved beneficial effect of fried fish on health than raw one. Frying was found to be more advantageous from various nutritional quality indices view point than the freezing. Nutritional changes after such thermal alteration reported here may pave way to study of similar alteration for different edibles.

Acknowledgements

This work was financially supported by the Department of Higher Education, Science & Technology and Biotechnology, Govt. of West Bengal, India [Sanction Order No. 839(Sanc.)/ST/P/S&T/1G-28/2016]. Infrastructural facility by DST-FIST (Grant No. SR/FST/CSI-267/2015) is also acknowledged.

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