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Chemical compositions and fatty acid profiles of three freshwater fish species

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ABSTRACT

This study investigated the chemical composition and fatty acid profiles of *Cyprinus*(*C*) *carpio, Labeo*(*L*) *rohita* and *Oreochromis*(*O*) *mossambicus* from the Indus River, Pakistan. Significant differences were observed for most chemical components and fatty acids ($P < 0.01$) in the examined fish species. *O. mossambicus, C. carpio* and *L. rohita* were high in saturated, mono-unsaturated and poly-unsaturated fatty acids, respectively. Palmitic acid was the most abundant fatty acid in all species ranging from 32% to 46%. Although these fish contained reasonable amounts of essential PUFA such as docosahexaenoic, eicosapentaenoic and arachidonic acids, *L. rohita* contained the highest amounts of PUFA and protein. These fish contained appreciable levels of Omega-6 PUFA suggesting that these fish especially *L. rohita* could be used as a source of healthy diet for humans. These findings may benefit the fishing industry, nutritionists and researchers who are striving to improve the nutritive value, processing and marketing of selected fish species.

1. Introduction

Fish are a source of high quality protein, vitamins and essential minerals but, above all, a virtually unique, rich source of omega-3 long-chain poly-unsaturated fatty acids (PUFA). The PUFA are important for maintaining the integrity of membranes of all living cells; for making prostaglandins which regulate many body processes such as inflammation and blood clotting. The fats are also needed in diets to absorb fat-soluble vitamins A, D, E and K from food; and for regulating body cholesterol metabolism (Connor, 2000; Kris-Etherton, Harris, & Appel, 2003). The fish fats contain essential PUFA like eicosapentaenoic (EPA, C20:5 *n*-3), docosahexaenoic (DHA, C22:6 *n*-3), and arachidonic (C20:4 *n*-6) acids which are not synthesised in the human body but their inclusion in human diets is essential (Alasalvar, Tylor, Zubcov, Shahidi, & Alexis, 2002; Glogowski & Ciereszko, 2001; Holub & Holub, 2004; Kolanowski & Laufenberg, 2006). Although PUFA composition may vary among different fish species of both fresh water and marine origins (Rahman, Teh, Osman, & Daud, 1995), it is important for human health, to increase the consumption of fish and its products (Burr, 1989; Sargent, 1997). Fish also contains significant amounts of essential amino acids, particularly lysine which is low in cereals. Fish protein can therefore be used to complement the amino acid pattern and the overall protein quality of a mixed diet (FAO, 2005).

Pakistan has a growing population (2.2 percent growth rate per annum in 2002–03) but limited sources of protein foods. The present per capita availability of protein is much below the minimum daily requirements and the livestock sector alone will not be able to meet the protein requirements of ever increasing human population (Chaudhry, 2008). Fish is an excellent and relatively a cheaper source of animal protein of high biological value. Therefore its use may help bridge the protein gap because of its multifarious economic advantages and nutritional significance (Waseem, 2007). The knowledge of the fatty acid composition for important fish species such as the carps and tilapia is desirable, due to the recent dietary and medical emphasis on the role of fatty acids in human health. Therefore, the aim of this study was to investigate the chemical and fatty acid profiles of three freshwater fish species (*Cyprinus*(*C*) *carpio, Labeo*(*L*) *rohita* and *Oreochromis*(*O*) *mossambicus*), which are commonly used by the local people around the Indus River in Mianwali district, Pakistan. Fish quality in the study area is important for rural communities since it contributes to their healthy diets and livelihoods. *C. carpio* and *O. mossambicus* were selected because of their hardy and tolerant nature towards various environmental conditions, taste and omnivorous feeding habit. *L. rohita* was selected because of its prime economic importance to the local inhabitants due to its fast growing nature and taste. *L. rohita* mostly feeds on periphytonic (phytoplankton) forms found attached to the submerged vegetation and other objects occupying the water column. Despite high demand, commercial

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value and wide availability of these fish species, there is a need to obtain precise data on their chemical composition because no such data is available for these fish in the study area where these fish are regularly consumed. This study is likely to open new areas of research as the knowledge of chemical and fatty acid compositions of fish species is of fundamental importance in the application of different technological processes in fish preservation, processing and product development of high added values.

2. Materials and methods

2.1. Fish sampling and measurements

The study was conducted in Mianwali, one of the north-western cities in the province of the Punjab, Pakistan, located around the Indus River, with an altitude of 211 metres. In Pakistan the inland fisheries are heavily dependant upon the River Indus. The fish samples were collected in September 2007 from two sites of the Indus River at Shehbaz khel (SK = upstream) and Chashma (CH = downstream). These sites were 40 km apart from each other and were known for commercial fish supply points in the city of Mianwali. Fishing was performed with the help of professional local fishermen. Twenty-seven samples of each fish species (*C. carpio*, *L. rohita* and *O. mossambicus*) of similar sizes by involving nine fish per net as replicates were collected on ice from each of these two sites. The size of these fishes was selected on the basis of fish size normally caught and sold in the study area. The fish samples were immediately transported to the laboratory where morphometric measurements by involving wet weight (WW), length, and width of each of these fish were carried out (Table 1).

2.2. Fish dissection and preservation

After morphometric measurements, each fish was dissected to collect different organs and tissues. These fishes were then skinned, filleted and transferred into marked sterilised polythene bags and stored in a freezer at −20 °C until further analysis.

2.3. Fish composition

2.3.1. Moisture content

Moisture content was determined in fish muscles in duplicate with a Lyo Lab G Freeze Dryer (Lyophilization Systems Inc., USA) at −50 °C for 72 h.

2.3.2. Determination of crude protein

Nitrogen (N) contents of fish muscle samples were determined by using the Dumas method on Leco (model FP-428, Leco corporation St. Joseph. MI, USA) nitrogen determinator (Sweeney & Rexroad, 1987). The N content was multiplied with 6.25 to estimate the crude protein (CP) of these samples.

2.3.3. Ash content

Ash was determined by burning the organic components from the known weight of the homogenised freeze-dried fish muscles by using a furnace (AAF1100, Carbolyte, Parson Lane Hope valley, England) at 550 °C.

2.3.4. Fat content

Total crude fat from freeze-dried muscle tissues was determined with the help of soxhlet apparatus using the non-polar organic solvent petroleum ether (Boiling point = 40–60 °C, Fisher Limited UK).

2.3.5. Total carbohydrates

Total carbohydrates were determined by subtracting the sum of % fat (F), % CP and % ash contents (A) from 100 (Onyeike, Ayoologu, & Ibegbulam, 2000) by using the following equation:

$$\%\text{Total carbohydrates} = 100 - (\text{F} + \text{CP} + \text{A}).$$

2.3.6. Estimation of gross energy value (caloric value)

Gross energy value of each sample was determined by multiplying the percentage CP, F and total Carbohydrate (C) contents with their respective energy values of 4, 9 and 4 kcal per 100 g of fish sample to obtain the caloric values of these samples by using the following equation:

$$\text{Caloric value} = (4\text{CP} + 9\text{F} + 4\text{C})\text{kcal}/100\text{ g weight}.$$

2.4. Fatty acid analysis

2.4.1. Fatty acid derivatisation

The fat of each fish sample was used for the analysis of fatty acids. Fatty acids were extracted and derivatised from samples by using a modified version of the method described by Sukhija and Palmquist (1988). This method has been tried and tested in our lab for its reproducibility and reliability for the recovery of maximum known fatty acids from various edible oils and fats.

Table 1
Morphometric variables and chemical composition as% dry matter (DM) of muscles of three freshwater fish species from selected locations (CH & SK) of the Indus River.

Variables	*Cyprinus carpio*		*Labeo rohita*		*Oreochromis mossambicus*		SEM and Significance		
	CH	SK	CH	SK	CH	SK	Location (L)	Species (S)	Lx S
Morphometric									
Length (cm)	34.97	33.44	41.49	35.56	19.81	20.15	0.74	0.91**	1.282
Width (cm)	11.09	11.35	11.85	10.33	7.96	7 .62	0.20	0.25**	0.354
Fish Weight (g)	633.3	568.3	950	500	206.7	142.7	122.81	150.44	212.72
Muscles as % of wet weight	65.25	62.91	64.74	60.87	60.34	61.25	1.13	1.38	1.953
Chemical composition									
Moisture % of wet weight	78.30	79.08	80.01	77.99	74.86	75.61	0.33	0.41***	0.58*
Fat	10.08	13.99	9.94	12.56	14.06	18.83	0.64***	0.78***	1.10
Crude Protein	45.53	53.59	50.19	57.31	50.09	39.84	1.19	1.19**	2.06***
Ash	7.14	7.91	6.37	6.42	12.05	10.82	0.40	0.40***	0.70
Total carbohydrates	37.25	24.51	33.50	23.71	23.80	30.51	1.35	1.35	2.34***
Energy value (kcal/100 g DM)	421.84	438.31	424.22	437.12	422.28	450.87	4.79	4.79	8.33

SEM = Standard error of means.
* Represent significance at $P < 0.05$.
** Represent significance at $P < 0.01$.
*** Represent significance at $P < 0.001$.

Frozen samples of fat were thawed overnight in the fridge (4 °C) and vortexed by using personal biovortex V-1 plus (peQLab, UK) before weighing 0.1 g of each sample into a soveril tube (washed with DeCon 90 and left to dry). About 1.7 ml of methanol: toluene (4:1) solution was added and then the contents were vortex mixed. In the fume cupboard Acetyl chloride (250 μl) was slowly added using a glass pipette. The samples were vortexed again for 30 s and the tubes were then placed in a heating block (Techne Dri-Block^R BD-3D) at 100 °C for one hour. Samples were then removed from the heating block and left to cool for 20 min before adding 5 ml of potassium chloride solution (5% KCl, in distilled water). These tubes were then gently shaken before centrifugation at 1000 g for 5 min. The supernatant was then removed from each tube using a Gilson pipette and transferred to a brown glass vial with a glass insert (Chromacol Ltd., Hertfordshire). These vials were refrigerated (4 °C) until the samples were analysed by using a gas chromatograph as described below:

2.4.2. Gas chromatography

The above mentioned samples were analysed on Shimadzu GC-2014 gas chromatograph by using helium as the carrier gas and a SGE forte BPX 70 column (30 m × 0.25mmID × 0.25 μm film thickness) (SGE Europe Ltd. Milton Keynes, UK). The peaks were identified by using an external 38 mixed FAME standard (FAME Mix C4-C24, Supelco; Sigma–Aldrich). The initial temperature of the column was set and held at 50 °C for 1 min. The temperature was then raised at 2 °C/minute to 188 °C which was held for 10 min followed by an increase at the same rate to 240 °C where it was held for 4 min, and then returned to the initial temperature. Fatty acids were quantified by comparing their peaks with the relevant peak areas of the corresponding standard fatty acids where each fatty acid was then expressed as a percentage of the total fatty acids quantified.

2.5. Statistical analysis

The data were statistically analysed by using the Minitab software to compare the effects of fish species (S), location (L) and S × L interaction on different nutrient and fatty acid components. Here S × L was studied to monitor if any potential variations between fish species for these components were linked to the variations between the two locations of the Indus River. These effects were declared significant if $P < 0.05$ and highly significant if $P < 0.01$. Tukey's test was used if there were more than two means to compare by using relevant standard errors of means (SEM). Means of each fatty acid for each fish together with their standard deviations (SD) were calculated to show variations from their relevant means. The fatty acids were also grouped as mean saturated, mono- and poly-unsaturated fatty acids for each fish species to show overall variations for these fatty acid groups among the fish of this study.

3. Results

Table 1 shows mean length, width, wet weight and chemical composition of three fish species from the Indus River. Significant differences were observed for length and width of fish ($P < 0.01$, Table 1) in the examined fish species. Fish wet weight and muscles as percentage of wet weight showed no difference for location, species, and the species × location interactions ($P > 0.05$; Table 1). *C. carpio* and *L. rohita* showed comparable moisture contents, but these differed significantly from *O. mossambicuss* ($P < 0.001$; Table 1). The mean fat contents differed significantly between locations and species ($P < 0.01$; Table 1). Here *L. rohita* was highest in moisture and crude protein contents but lowest in fat contents whereas, *O. mossambicus* was lowest in moisture and crude protein contents but highest in fat contents ($P < 0.05$; Table 1). Ash contents differed significantly among fish species ($P < 0.001$; Table 1). The mean ash contents were 7.5%, 6.4% and 11.4% muscle tissue for *C. carpio*, *L. rohita* and *O. mossambicus*, respectively (Table 1). Total carbohydrates ranged from 24.5% to 37.3%, 23.7% to 33.5% and 23.8% to 30.5% muscle tissue in *C. carpio*, *L. rohita* and *O. mossambicus,* respectively (Table 1) depending upon the location ($P < 0.01$; Table 1). There was no significant difference in energy values (kcal/100 g muscle tissue) among fish species ($P > 0.05$; Table 1).

Table 2 shows mean fatty acid profiles, SE and significance for the main effects of location, species and location × species interactions. Among 37 fatty acids analysed in this study, 27% showed highly significant ($P < 0.01$), 11% significant ($P < 0.05$) and 62% non-significant ($P > 0.05$) differences among three fish species (Table 2). Non-significant differences were observed for location and location × species interactions for most fatty acids ($P > 0.05$). In the present investigations, myristic, pentadecanoic, palmitic, heptadecanoic and stearic acid were the dominating fatty acids among saturated fatty acids in all fish species (Table 3). Palmitic acid was highest in percentage in all fish species. Overall mean saturated fatty acid composition was 55.7%, 50.5% and 63% for *C. carpio*, *L. rohita* and *O. mossambicus* respectively. 50% of saturated fatty acids showed non-significant differences ($P > 0.05$) for species (Table 3). Fish species from the Indus River showed non-significant differences for mono-unsaturated fatty acids except cis-10 pentadecanoic acid ($P > 0.05$; Table 3). Mono-unsaturated fatty acid composition of *C. carpio*, *L. rohita* and *O. mossambicus* was 32.9%, 27.2% and 24.8%, respectively, where palmitoleic and oleic acids were the dominating mono-unsaturated fatty acids (Table 3). Overall PUFA composition of *C. carpio*, *L. rohita* and *O. mossambicus* was 11.4%, 22.2% and 12.2%, respectively (Table 3). Among PUFA, Linoleic, γ-Linolenic and α-Linolenic acids were the highest in all three freshwater fish species (Table 3). Among PUFA, 50% PUFA showed non-significant differences among the three freshwater fish species from the Indus River ($P > 0.05$; Table 3). A higher ω3/ω6 ratio (0.27) was observed in *C. carpio* than both *L. rohita* and *O. mossambicus* which contained similar ω3/ω6 ratio of 0.23 (Table 3).

4. Discussion

This study investigated the nutritional quality of three freshwater fish species commonly consumed in the study area. The chemical analysis of freshwater fishes is important because it provides useful information to the nutritionists concerned with readily available sources of low-fat but high-protein foods and to the food scientists who are interested in developing them into high-protein foods while ensuring their finest quality, flavour, colour, odour, texture, and safety for the consumers. Overall the moisture contents in the fish of this study were within the ranges observed by other authors (Abii, Afieroho, & Nnamdi, 2007; Islam & Joadder, 2005). Fish are often classified on the basis of their fat contents into lean fish (fat less than 5%), medium fat fish (fat 5–10%) and fatty fish (fat more than 10% by weight) (Suriah et al., 1995). Based on this classification, fish species of this study were classified as fatty fish (Table 1). The considerable variations in the total fat contents of the fish muscles among species and within species at two locations, were in line with the findings of other authors (Grela & Dudek, 2007; Kolakowska, Szczygielski, Bienkiewicz, & Zienkowicz, 2000; Luczynska, Borejszo, & Luczynski, 2008). In the present investigations crude proteins of 39.8–57.3% dry muscle tissues (=10.6–11.3% wet weight) (Table 1) were much lower than the protein levels for Carp (16% wet weight; FAO, 2008) and Tilapia (50–55% dry muscle tissue; Onyeike et al., 2000) but these were within the range of 30 ± 3.2–54 ± 5.8 recorded for the Cichlidae

Table 2
Mean fatty acid profiles of three freshwater fish species (S) at two sampling locations (L = CH, SK) of the Indus River together with the standard error of the means (SEM) and significance.

Fatty Acids as % of total fatty acids	*Cyprinus carpio*		*Labeo rohita*		*Oreochromis mossambicus*		SEM and significance		
	CH	SK	CH	SK	CH	SK	L	S	L x S
C6:0, Caproic	0.950	0.620	0.024	0.030	0.435	0.269	0.17	0.21	0.30
C8:0, Caprylic	0.278	0.272	0.165	0.247	0.171	0.404	0.05	0.06	0.08
C10:0, Capric	0.011	0.027	0.00	0.00	0.005	0.018	0.01	0.01	0.01
C11:0, Undecanoate	0.065	0.040	0.013	0.00	0.00	0.008	0.01	0.02	0.02
C12:0, Lauric	0.575	0.440	0.082	0.184	0.094	0.138	0.07	0.08***	0.12
C13:0, Tridecanoate	0.854	0.359	0.500	0.549	0.043	0.050	0.11	0.13*	0.18
C14:0, Myristic	3.487	3.080	2.568	3.781	2.766	2.947	0.34	0.42	0.60
C14:0, Myristoleic	0.656	0.816	0.445	1.452	0.863	0.603	0.12	0.15	0.21*
C15:0, Pentadecanoic	1.867	1.443	1.197	1.640	1.750	1.404	0.16	0.20	0.28
C15:1, cis-10 pentadecanoic	0.885	0.746	0.359	0.587	0.330	0.197	0.07	0.08***	0.12
C16:0, Palmitic	35.050	30.87	32.40	32.41	47.71	44.11	1.89	2.31***	3.28
C16:1, Palmitoleic	6.787	5.374	7.883	10.787	6.114	5.164	0.86	1.06	1.50
C17:0, Heptadecanoic	4.435	2.244	1.572	3.038	2.585	2.095	0.47	0.58	0.82
C17:1, cis-10 Heptadecanoic	0.920	0.860	0.685	0.743	0.597	0.651	0.11	0.13	0.19
C18:0, Stearic	10.487	11.99	8.416	7.977	9.403	7.590	0.44	0.54***	0.77
C18:1, Elaidic	0.186	0.132	0.244	0.411	0.885	0.383	0.12	0.15	0.21
C18:1, Oleic	21.260	25.64	17.86	10.97	11.95	15.58	2.19	2.68*	3.80
C18:2, Linolelaidic	0.213	0.177	0.375	0.200	0.166	0.1131	0.05	0.07	0.10
C18:2, Linoleic (LA) (ω6)	4.969	7.861	9.137	9.128	7.314	6.573	1.78	2.18	3.09
C20:0, Arachidic	0.232	0.099	0.170	0.632	0.105	0.308	0.07	0.09	0.12
C18:3, γ-linolenic (GLA) (ω6)	1.387	0.679	6.947	4.845	2.300	0.433	0.92	1.13**	1.60
C20:1, cis-11 Eicosenoic	0.512	0.529	0.339	0.503	0.798	4.097	0.80	0.97	1.38
C18:3, α linolenic (ω3)	0.976	1.457	1.772	0.573	0.574	0.314	0.29	0.35	0.50
C21:0, Heneicosanoic	0.174	0.094	0.096	0.234	0.392	0.285	0.05	0.06	0.08
C20:2, cis-11,14 Eicosadienoic(ω6)	0.117	0.335	0.363	0.543	0.311	0.825	0.10	0.12	0.18
C22:0, Behenic	0.293	0.327	0.483	0.860	0.155	0.437	0.05***	0.06***	0.09
C20:3, cis-8,11,14 Eicosatrienoic (hGL)(ω6)	0.413	0.814	1.184	2.211	0.102	0.588	0.20*	0.24***	0.34
C22:1, Erucic	0.00	0.164	0.228	0.274	0.323	0.961	0.22	0.27	0.38
C20:3, cis-11,14,17 Eicosatrienoic (ω3)	0.252	0.340	0.334	0.416	0.595	0.926	0.11	0.13	0.19
C20:4, Arachidonic(ω6)	0.353	0.481	0.472	0.402	0.061	0.223	0.06	0.07**	0.10
C23:0, Tricosanoic	0.111	0.111	0.772	0.818	0.015	0.140	0.06	0.08***	0.11
C22:2, cis 13,16 Docosadienoic (ω6)	0.158	0.138	0.109	0.286	0.351	0.382	0.03	0.04***	0.06
C24:0, Lignoceric	0.213	0.250	0.111	0.132	0.096	0.081	0.04	0.04	0.06
C20:5, Eicosapentaenoic (EPA) (ω3)	0.257	0.414	0.461	0.693	0.353	0.496	0.06	0.07	0.11
C24:1, Nervonic	0.101	0.226	0.258	0.454	0.097	0.086	0.06	0.08	0.11
C22:5, Docosapentanoic (DPA) (ω3)	0.111	0.218	0.685	0.743	0.082	0.527	0.12	0.14*	0.20
C22:6, Docosahexaenoic (DHA) (ω3)	0.399	0.321	1.294	1.242	0.110	0.594	0.21	0.25*	0.36

* Represent significance at $P < 0.05$.
** Represent significance at $P < 0.01$.
*** Represent significance at $P < 0.001$.

family (Ukoha & Olatunde, 1988). Ash contents (6.4–12.1% dry weight muscle tissue) observed in this study was within the ranges found by Abii et al. (2007).

The patterns of fatty acid profiles of different fish species of this study compared well with those observed by Osman, Jaswir, Khaza'ai, and Hashim (2007). The results of this study confirmed that our method of fatty acid analysis was able to recover almost all 37 fatty acids which were targeted in this study. However, the degree of their appearance did vary which were perhaps partly attributed to the procedures that were used to prepare fat samples for their fatty acid analysis. Oleic acid was the dominating mono-unsaturated fatty acid in all freshwater fish species with 71.3%, 53% and 55.5% in *C. carpio*, *L. rohita* and *O. mossambicus*, respectively (Table 3). This fatty acid has exogenous origin and usually reflects the type of diet of the fish (Ackman, 1980; Ackman, 1989). The other abundant mono-unsaturated fatty acid was palmitoleic acid. Likewise, Aggelousis and Lazos (1991) showed that in freshwater fish from Greece, the most abundant fatty acids were palmitic (C16:0), palmitoleic (C16:1), oleic (C18:1), eicosapentaenoic (C20:5 *n*-3), and docosahexaenoic (C22:6 *n*-3). According to Andrade, Rubira, Matsushita, and Souza (1995), the most dominating saturated fatty acids in freshwater fish from south Brazil were palmitic (C16:0) and stearic acid (C18:0), whereas palmitoleic (C16:1) and oleic (C18:1) acids were common among mono-unsaturated fatty acids which, are in good agreement with the present findings (Table 3). Among saturated and mono-unsaturated fatty acids of this study the highest palmitic acid (C16:0) followed by oleic acid (C18:1) and stearic acid (C18:0) was comparable with the findings of Luczynska et al. (2008). All fish species contained arachidonic acid (C20:4), which is a precursor for prostaglandin and thromboxane biosynthesis (Pompeia, Freitas, Kimj, Zyngier, & Curi, 2002). Arachidonic acid (C20:4) can facilitate the blood clotting process and attach to endothelial cells during wound healing (Rahman et al., 1995). Although the level of arachidonic acid was very low in these selected fish species, the inclusion of these fish in human diets might help in the wound healing process of the consumers. The presence of docosahexaenoic acids (DHA) in all fish species from the Indus River suggests that these fish species can have a healing effect to alleviate muscle pain and inflammation. Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) have been reported to have preventive effects on human coronary artery disease (Leaf & Webber, 1988). Therefore, fish have been suggested as a key component for a healthy diet in humans (Rahman et al., 1995). Significant levels of EPA and DHA in fish species of this study indicated that these species can be used to supplement essential fatty acids in the human diet. Although the EPA and DHA percentages in the examined fish species muscle total fatty acids are low (Table 2 and 3), they were found in significant levels in these fish species muscles, due to the large percentage of fat in the analysed fish species. It appeared from this study that *L. rohita*

Table 3
Mean (± SD) Fatty Acid profiles of three freshwater fish species from the Indus River.

Fatty acids as % of total fatty acids	*Cyprinus carpio*	*Labeo rohita*	*Oreochromis mossambicus*
Saturated fatty acids			
C6:0 Caproic	0.79 ± 0.32[a]	0.03 ± 0.01[b]	0.35 ± 0.23[ab]
C8:0 Caprylic	0.27 ± 0.04[a]	0.20 ± 0.05	0.29 ± 0.08[a]
C10:0 Capric	0.02 ± 0.01	0.00 ± 0.00	0.012 ± 0.001[a]
C11:0 Undecanoate	0.05 ± 0.03	0.007 ± 0.001	0.003 ± 0.00[b]
C12:0 Lauric	0.51 ± 0.12	0.13 ± 0.04[b]	0.12 ± 0.04[b]
C13:0 Tridecanoate	0.60 ± 0.16	0.52 ± 0.17	0.04 ± 0.01[b]
C14:0 Myristic	3.28 ± 0.55	3.17 ± 0.38[a]	2.86 ± 0.25[a]
C15:0 Pentadecanoic	1.65 ± 0.20[a]	1.42 ± 0.22[a]	1.58 ± 0.17[a]
C16:0 Palmitic	32.96 ± 1.6[a]	32.41 ± 1.11[a]	45.91 ± 3.24[b]
C17:0 Heptadecanoic	3.34 ± 1.0[a]	2.30 ± 0.37[a]	2.34 ± 0.16[a]
C18:0 Stearic	11.24 ± 0.7[a]	8.19 ± 0.40[b]	8.49 ± 0.59[b]
C20:0 Arachidic	0.17 ± 0.06[a]	0.40 ± 0.12[a]	0.21 ± 0.12[a]
C21:0 Heneicosanoic	0.13 ± 0.05[a]	0.17 ± 0.04[a]	0.34 ± 0.07[a]
C22:0 Behenic	0.31 ± 0.04[a]	0.67 ± 0.11[b]	0.29 ± 0.08[a]
C23:0 Tricosanoic	0.11 ± 0.01[a]	0.80 ± 0.12[b]	0.08 ± 0.04[a]
C24:0 Lignoceric	0.23 ± 0.06[a]	0.12 ± 0.03[a]	0.09 ± 0.02[a]
Total	55.66	50.54	63.00
Mono-unsaturated fatty acids			
C14:1 Myristoleic	0.74 ± 0.16[a]	0.95 ± 0.26[a]	0.73 ± 0.11[a]
C15:1 cis-10 pentadecanoic	0.82 ± 0.08[a]	0.47 ± 0.11[b]	0.26 ± 0.05[b]
C16:1 Palmitoleic	6.08 ± 1.06[ab]	9.33 ± 1.08[b]	5.64 ± 0.98[a]
C17:1 cis-10 Heptadecanoic	0.89 ± 0.15[a]	0.71 ± 0.10 [a]	0.62 ± 0.09[a]
C18:1 Elaidic	0.16 ± 0.07[a]	0.33 ± 0.09 [a]	0.63 ± 0.23 [a]
C18:1 Oleic	23.45 ± 3.17[a]	14.42 ± 3.04 [a]	13.77 ± 1.4 [a]
C20:1 cis-11 Eicosenoic	0.52 ± 0.11[a]	0.42 ± 0.05 [a]	2.45 ± 1.68 [b]
C22:1 Erucic	0.08 ± 0.08[a]	0.25 ± 0.09 [b]	0.64 ± 0.23 [b]
C24:1 Nervonic	0.16 ± 0.04[a]	0.35 ± 0.12 [b]	0.09 ± 0.02 [a]
Total	32.90	27.23	24.83
Poly-unsaturated fatty acids			
C18:2 Linolelaidic	0.19 ± 0.03[a]	0.29 ± 0.10[a]	0.14 ± 0.03[a]
C18:2 Linoleic (LA) ω6	6.41 ± 2.01[a]	9.13 ± 0.48[a]	6.94 ± 1.75[a]
C18:3 γ-linolenic (GLA) ω6	1.03 ± 0.63[a]	5.89 ± 1.62[b]	1.37 ± 0.69[a]
C18:3 α -linolenic ω3	1.22 ± 0.27[a]	1.17 ± 0.55[a]	0.44 ± 0.09[a]
C20:2 cis-11,14 Eicosadienoic ω6	0.23 ± 0.09[a]	0.45 ± 0.11[a]	0.57 ± 0.19[a]
C20:3 cis-8,11,14 Eicosatrienoic (hGLA) ω6	0.61 ± 0.24[a]	1.70 ± 0.35[b]	0.34 ± 0.18[a]
C20:3 cis-11,14,17 Eicosatrienoic ω3	0.30 ± 0.14[a]	0.37 ± 0.05[a]	0.76 ± 0.17[a]
C20:4 Arachidonic ω6	0.42 ± 0.10[a]	0.44 ± 0.03[a]	0.14 ± 0.05[b]
C22:2 cis 13,16 Docosadienoic ω6	0.15 ± 0.03[a]	0.20 ± 0.06[a]	0.37 ± 0.03[b]
C20:5 Eicosapentaenoic (EPA) ω3	0.34 ± 0.08[a]	0.58 ± 0.09[a]	0.42 ± 0.06[a]
C22:5 Docosapentanoic (DPA) ω3	0.16 ± 0.05[a]	0.71 ± 0.18[b]	0.30 ± 0.15[ab]
C22:6 Docosahexaenoic (DHA) ω6	0.36 ± 0.15[a]	1.27 ± 0.34[b]	0.35 ± 0.17[a]
Total	11.42	22.21	12.16
Total ω3/ω6 ratio	0.27	0.23	0.23

SD = standard deviation; Means within the same row with the same letters did not differ significantly ($P > 0.05$).

was the richest sources of protein, ω6 fatty acids and Linoleic (C18:2), γ-Linolenic (C18:3), Eicosatrienoic (C20:3) and docosahexaenoic acids (22:6). This can be related to the feeding habit of fish which consumes phytoplanktons that are usually rich in essential fatty acids. The ω-3:ω-6 fatty acids ratio has been suggested to be a useful indicator for comparing relative nutritional values of fish oils. The fish oils in the present study were characterised by low levels of Omega-3 PUFA with low Omega-3: Omega-6 ratio which was in line with the findings of Ugoala Ndukwe and Audu (2009a). In present investigations all fish species were of comparable nutritional value as no significant difference was observed in ω-3: ω-6 fatty acids ratio and caloric value. The results shown in Table 3 indicated that all fish species analysed were characterised by high levels of Omega-6 fatty acids than Omega-3 fatty acids. These results agree with those obtained in other studies where freshwater fish were mainly characterised by the elevated levels of Omega-6 PUFA especially linoleic (18:2) and arachidonic (22:4) acids (Aras, Haliloğlu, Yetim, & Ayik, 2003; Cowey & Sargent, 1972; Ugoala Ndukwe and Audu, 2009b), as well as substantial concentrations of eicosapentaenoic and docosahexaenoic acids. All fish species were rich in C18:2, *n*-6 which is essential in human nutrition as these fatty acids are not synthesised in the body but these are required for the tissue development. In general, saturated fatty acids in all fish species from the Indus River were higher than mono- and poly-unsaturated fatty acids. The high percentage of branched and saturated fatty acid in freshwater fish gives them an advantage in curing illnesses to improve the health processes. The trend of fatty acids of fin fish from seawater is slightly different when compared to the freshwater fish, where the concentrations of mono-unsaturated fatty acids were higher than the saturated and poly-unsaturated fatty acids (Suriah et al., 1995). Other researchers have also shown that freshwater fish had lower PUFA (Vlieg & Body, 1988) which is in line with the present findings. The differences can be due to the fact that freshwater fish feed mainly on vegetation and plant materials while marine fish feed on zooplanktons, which are rich in PUFA (Osman, Jaswir, Khaza'ai, & Hashim, 2007). PUFA play a vital role in alleviating cardiovascular disease, type-2 diabetes, inflammatory ailments and autoimmune disorders (Hooper et al., 2004; Montori, Farmer, Wollan, & Dinneen, 2000; Simopoulos, 2002). There are many other studies that report

the beneficial effects of omega 3 fatty acids on rheumatoid arthritis (Berbert, Kondo, Almendra, Matsuo, & Dichi, 2005) and more recently on mental health, including schizophrenia and bipolar disorders (Horrobin & Bennett, 1999). DHA is essential for the development of the foetal brain and the eye retina (Birch, Garfield, Hoffman, Uauy, & Birch, 2000; San Giovanni & Chew, 2005). Depending upon all the above facts it is quite evident that PUFA are crucial in human physiology and must be included in human diet perhaps in the form of fish as a rich source of these fatty acids. Our present findings suggest that the analysed freshwater fish from the Indus River are beneficial for human health but the extent of this effect could vary with the type of a fish species that could be available in this area. Therefore, it would help if such information could be applied to promote the growth and development of most important species in terms of their chemical components especially the most desirable fatty acid profiles of target fish species in similar areas of vital importance in Pakistan and beyond.

5. Conclusion

The fish especially *L. rohita* from the study area found to be a good source of protein and fatty acids. All examined fish species contained essential fatty acids, particularly DHA and EPA which are good for health. It appears that no authentic data on the chemical compositions and fatty acid profiles of local fish species in the study area are available. Therefore the data on chemical and fatty acid composition of this study will form the basis for further research in this field of fish chemistry for the benefits of human beings.

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