Original Research Paper

Nutritional and Fatty Acids Profile Analyses of Commonly Consumed Fresh Water Fish Species in Pakistan

[1,2]Ahsan Hameed, [1,2]Syed Ammar Hussain, [1]Muhammed Asim Shabbir [1]Imran Pasha and [2]Yuanda Song

[1]National Institute of Food Science and Technology, University of Agriculture Faisalabad, 38000, Pakistan
[2]Colin Ratledge Center for Microbial Lipids, School of Agriculture Engineering and Food Science,
Shandong University of Technology, Zibo 255049, Shandong, PR. China

Abstract: The aim of this study is to evaluate nutritional value of selected commonly consumed fresh water fish species of Pakistan. Proximate composition and fatty acids profile of mostly consumed fresh water fish species of Pakistan namely Balm or Baam (*Anguilla anguilla*), Sole (*Channa marulius*), Damra (*Labeo rohita*) and Malhi (*Wallago atto*) were analysed. Proximate composition data showed a significant difference ($p<0.05$) among the nutritional components except carbohydrate and gross energy contents. Protein and moisture contents found plenteous in the Baam (*Anguilla anguilla*) whereas ash and fat contents in Malhi (*Wallagoatto)* are found copious. Saturated Fatty Acids (SFAs) and Monounsaturated Fatty Acids (MUFAs) found to be principal Fatty Acids (FAs) with their ranges from (48.43 to 65.76%) and (25.72 to 31.37%) respectively. In SFAs and MUFAs, palmitic acid (32.4 to 47.71%) and oleic acid (10.97 to 21.26%) were identified as major FAs respectively in all species. The existence of short and medium chain SFAs is advantageous for designation of these fish species as alterative diet as these above mentioned FAs not only involved in metabolic regulation but also helpful to remove the metabolic disparities related to Coronary Heart Diseases (CHD), brain seizure, Alzheimer disease, glucose homeostasis, appetite and gastric acid secretion. Among the PUFAs, Omega-6 PUFAs are found to be more abundant than Omega-3 with Linoleic acid (LA, C18:2ω6) as predominant PUFA in *A. anguilla* (9.137%) and *L. rohita* (9.128%) and γ-linolenic acid (GLA, C18:3ω6) and cis-8, 11, 14 Eicosatrienoic (hGLA, C20:3 ω6) as most prevailing PUFAs in *C. marulius* (2.3%) and *W. atto* (1.61%) respectively. These species found to be a substantial source of Omega-6 PUFAs with ω3/ω6 ratio less than 1. The overall all summation of Eicosapentaenoic (EPA, C20:5 ω3) acid and Docosahexaenoic acid (DHA, C22:6 ω3) ranged from 0.38% in *C. marulius* to 1.93% in *L. rohita*.

Keywords: Bioactive Components, Fatty Acids Profiling, Nutrition of Fresh Water Fishspecies

Introduction

There are, in Pakistan, reportedly 531 species of fish out of which 233 are of fresh water fish species (Saify *et al*., 2000). A large variety of fresh water fish species are cultured commercially in Pakistan. The area which is used for the fish farming system is about 60,500 ha. The fresh water fishes that are mostly cultured and consumed in Pakistan are Damra (*Labeo Rohita)*, Sole (*Channamarulius*), Catla (*Catla Catla*), Baam or Balm (*Anguilla anguilla*), Brown trout (*Salmotrutta*), Rainbow trout (*Oncorhynchusmykiss*), Malhi (*Wallagoatto*), Marigal (*Cirrhinusmrigala*), Grass carp (*Ctenopharyngodonidella)* and Silver carp (*Hypophthalmichthysmolitrix)* etc. (Saify *et al*., 2000). Fish is also an important source of superior kind protein, essential minerals and vitamins. But the most important feature of fish carcass is its oil which is the origin of unparalleled and ample source of Omega-3 (ώ3) and Omega-6 (ώ6) long chain polyunsaturated fatty acids

(LC-PUFAs). These PUFAs have a lot of health significances, especially as, precursors of prostaglandins which govern many activities such as blood clotting, inflammation and preserving the wholeness of tissue layer of all living cells. The absorption of fat soluble vitamins K, E, A and D from the diet and metabolism of cholesterol of body is also regulated by the PUFAs (Connor, 2000). A lot of PUFAs are present in the carcasses of these fresh water fishes like docosahexaenoic (DHA, C22:6 $\acute{\omega}$3), eicosapentaenoic (EPA, C20:5 $\acute{\omega}$3) and arachidonic acid (C20:4 $\acute{\omega}$6), these PUFAs are not formed by the human body but their involvement in body metabolism is very crucial (Kolanowski and Laufenberg, 2006; Holub and Holub, 2004; Glogowski and Ciereszko, 2001). The composition of fresh water fish species and marine water fish species may vary due to their different feeding habits and ecological factors (Rahnan *et al.*, 1995). Fish consumption is very important for health as it also contain many essential amino acids in large quantity peculiarly Lysine which deficient in pulses and cereals (Glogowski and Ciereszko, 2001). Fish usage should be enhanced to accompaniment the pattern of amino acids and to increase the quality of intake protein (Holub and Holub, 2004). Some omega-3 and Omega-6 PUFAs are reputed as Essential FAs (EFAs). These EFAs have crucial role in the preventing and intervention of wide kind of disparities like breast cancer, inflammatory illnesses, osteoporosis, atherosclerosis, hypertriglyceridemia, autoimmune, hypertension, embolic phenomena, thrombosis and rheumatoid arthritis (Uauy and Valenzuela, 2000).

Pakistan has growing population with low availability of quality protein and handiness of per capita protein is much underneath the lower limit of daily necessities. The livestock sector alone does not have enough capacities to cope with the rising demands of population of human being (Chaudhry, 2008). Fish is a trashier and splendid source of animal protein, therefore increase in the consumption of fish oil and fish meat can help us to bring together the EFAs and protein gap and may bring the multifaceted economical benefits and nutritional significance (Waseem, 2007). Owing to the crucial dietetic and medical role of these fatty acids in human health, it is very important to have awareness of the composition profile of the vast fatty acids for mostly consumed fish species Baam (*Anguilla Anguilla)*, Sole (*Channamarulius*), Damra (*Labeo Rohita*) and Malhi (*Wallagoatto*). So the objective of this work is to look into visibility of fatty acids and composition in ordinarily ingested fish species, *A. anguilla, C. marulius, L .rohita* and *W. atto*, that were selected on the basis of consumption pattern, prime economic importance, fast growing nature, taste, savor, sturdy and resistant nature towards various ecological conditions. From the last

twenty years, there is an extension in the consciousness of people of getting health attains from the usage of fish oil (Lovegrove *et al.*, 1997), because this oil have revealed a richest source of Omega-3 and Omega-6 LCPUFAs peculiarly docosahexaenoic (DHA) and eicosapentaenoic (EPA). Since human beings are incapable of synthesizing these Fatty Acids (FAs) with double bond in $\acute{\omega}$6 and $\acute{\omega}$3 places so their involvement in the body is very crucial but fish can prepare these PUFAs by ingesting on microalga or bacteria (Simopoulos, 2002). The fat quantity and composition of fatty acids diverges according to various factors like reproductive cycle period, size, fishing season, sex and geographical location (Luzia *et al.*, 2003).

Owing to uprising requirement for LC-PUFAs, it is worthy to personate the novel origins from disposed by-products of the fish industry not only to maximize the gainfulness of disposed by-products of the fishing products but also to discover new worthful origins of prime quality of LC-PUFAs oil and to avert the overhunting of common fish species (Jayasinghe *et al.*, 2003). The reason behind the prime importance and significance given to LC-PUFAs is that it is useful to prevent the cardiac disorders (Connor, 2000; Simopoulos, 2002; Kinsella, 1998; Mozaffarian *et al.*, 2005), disorders of brain growth and retina functioning (Crawford, 1993) and also lower the chances of multiple inflammation, psoriasis and scelerosis (Kinsella, 1998; Goodnight *et al.*, 1982).

Marine fish species contain impressive amount of LC-PUFAs particularly DHA (docosahexaenoic acid, C22:6 $\acute{\omega}$3) and EPA (eicosapentaenoic acid, C20:5 $\acute{\omega}$3) (Ackman, 1999). The dietitians commonly recommend that for infants the daily intake of 0.5g EPA/DHA whereas coronary heart patients and normal adults should take an average uptake of 1g/day (Kris-Etherton *et al.*, 2003). According to investigators fresh water fish species contain lower quantity of Omega-3 than the marine fish species (Rahnan *et al.*, 1995). Marine fish species possess a high proportion of total $\acute{\omega}$3/$\acute{\omega}$6 fatty acids deviating from 5 to 10 or more which is much more eminent for sea fish species than for freshwater fish species. According to work done by (Simopoulos, 2002) ratio of $\acute{\omega}$6/$\acute{\omega}$3less than 3/1 has restrictive effects on many diseases, including cancer cardiovascular, inflammatory and autoimmune diseases and the summation of $\acute{\omega}$-3 PUFA could forbid diseases and can ameliorate nutritionary value (Moreira *et al.*, 2001). But it is very hard to find out the optimal $\acute{\omega}$6/$\acute{\omega}$3 ratio as most of fatal and chronic diseases are multigenic and multifactorial (Simopoulos, 2002).

Up to best of our knowledge, no study has been published yet about the nutritional quality assessments of market available three fish species namely *W. atto, C. marulius and A. Anguilla*. So keeping in all above, it is

very crucial to investigate and explore the nutritional value of local most commonly consumed fish species of Pakistan. As food processing sector is among the top growing sector and in the food manufacturing sector, dairy and meat processing industry is the most flourishing industry. Therefore this work may provide useful information for its applications in pharmaceutical products healthcare, food, neutraceutical and as constituents in feed, aquaculture and agricultural industry.

Materials and Methods

Raw Material Procurement, Dissection and Preservation

The fish samples were collected from reliable fisherman in local Fish market namely Jhang Bazar Lyallpur. The samples of each fish species Balm (*A. anguilla*), Sole (*C. marulius*), Damra (*L. rohita*) and Malhi (*W. atto)* were gathered on ice. Every Fish was stored at -20°C in sterilized polythene bags after dissecting, skinning and filleting. All the solvents and chemicals used were of analytical grade.

Fish Composition

The moisture, Crude Protein, Crude Fat and Ash contents were determined following the Horwitz (2006) method. Whereas Total carbohydrates were estimated by deducting the summation of % Crude Protein (CP) % fat (F) and % ash contents (A) from 100 (Onyeike *et al.*, 2000) by using the following equation:

$$\% \, Total \; carbohydrates = 100 - (F + CP + A)$$

Estimation of Gross Energy Value (Caloric value)

The percentages of Fat (F) Crude Protein (CP) and Carbohyderate (C) were found and multiplied by their respective caloric energy values i.e., 9, 4 and 4 Kcal in 100g of Fish sample (Farhat and Shakoor, 2011) to find the caloric value of each species as shown below:

$$Caloric \; value = (4CP + 9F + 4C) / 100 \; g \; weight$$

Fatty Acid Analysis

Extraction of Fat

The fish fat was extracted from 2.5g small pieces of fish meat by few modifications in the method utilized by Bligh and Dyer (Sargent *et al.*, 1995; Citil *et al.*, 2014). Different parts of fish were individually minced and homogenized using a homogenizer. The meat samples were impregnated with 12.5 mL of chloroform/methanol/distilled water (2:2:1) comprising 0.01% butylated hydroxyl toluene. Organic liquid phase

was incurred after mixed-phase separation. The liquid was passed through Whatman No. 1 filter paper and dried with moderate anhydrous sodium sulfate, then concentrated by using an evaporation-condensation rotating apparatus in a 40°C water bath. Total lipid content was gravimetrically quantified.

Fatty Acid Derivatisation

Fatty acids were determined and derivatised from samples (fat of each fish species) based on the method of Farhat and Shakoor (2011) with slight modifications. About 40 mL of methanol were taken in 50 mL conical flask. It was placed on ice water and then 10 mL of sulphuric acid were added and this solution was saved for further use. Methylation of fatty acids in the oils was carried out according to the procedure described by (Cocks and Rede, 1966) with some modifications as follows. About 200 mg (0.21 mL) oil was taken in a 50 mL screw capped Pyrex glass tubes having 50 cm length and 1cm internal diameter. Then 2 mL of methanolic sulphuric acid was added into each tube and glass vials were put in a pre-heated oven at 80°C for 1 h and shake for15 min. The glass vials were taken out cooled and 2 mL of distilled water were added into each tube to stop the reaction. Then esterified fatty acids (Fatty Acid Methyl Esters, FAME) were extracted with 1 mL of petroleum ether (40-60°C) thrice. After that the ether content was evaporated and remaining oily surface was injected into gas chromatography for fatty acid profile analysis.

Gas Chromatography (GC)

The FAME samples of each fish species was examined by Gas Chromatography by using Helium as a carrier gas and SGE forte BPX 70 column (30 m×0.25mmID×0.25 μm film thicknesses). The initial temperature of the column was set and held at 50°C for 1 min. The temperature was then raised at 2°C/min 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. The extraneous combined FAME standard will be used to discover and identify the peaks. Fatty acids was measured by equating their peaks with the relevant peak areas of the corresponding standard fatty acids where each fatty acid was then stated as a percentage of the total fatty acids measured (Farhat and Shakoor, 2011).

Statistical Analysis

The data obtained for each parameter was subjected to statistical analysis using Minitab statistical package (Minitab Inc, 2012). The level of significance was determined by applying analysis of variance technique (one factor and two factor factorial CRD). Significant

ranges were further postulated using DMR mean comparison test (Steel *et al.*, 1997). Each experiment was performed triple times.

Results and Discussion

The present investigation was contrived to measure various physiochemical characteristics, composition and fatty acids mapping in commonly consumed selected fresh water fish species. Especially quality and quantity of fish oil extracted from these fishes was checked with special reference to Saturated Fatty Acids (SFAs), Monounsaturated Fatty Acids (MUFAs), Essential Fatty Acids and Polyunsaturated Fatty Acids (PUFAs) like Omega-3 and Omega-6 and their fraction in the samples.

Fish Composition

To find out the nutritional and compositional values of our selected fish species, we analyzed the moisture, fat, crude protein, ash and carbohydrate contents of these fish species. The results showed (Table 1) that the moisture contents of the fish differed from species to species significantly ($p < 0.05$). The highest moisture (78.233%) was observed in *A. anguilla* and the least amount of moisture (73.933%) was observed in the *C. marulius*. Micro flora is best flourished at higher moisture levels. Oxidation of meat products is another issue related with moisture contents. Lower moisture contents are required to achieve a sustained shelf life in these types of products. It is reported that the increase in fat level in fish species decrease moisture content (Ayo *et al.*, 2007). Similar findings were observed by (O'Brien, 2004) and (Ashraf *et al.*, 2011) in lake, pond water and marine shallow water fish species which exhibit 73.46 to79.6% moisture contents.

Fish species are the excellent sources of superior quality protein and possess high amounts of essential and functional amino acids amino acids which regulate and take part in various metabolic pathways and with beneficial health effects on growth, survival, development, lactation and development of an organism (Wu, 2010). Proline, glycine, methionine, tyrosine, cystine, leucine, arginine, glutamic acid, aspartate, taurine and tryptophan, have been sorted out as functional amino acid in human nutrition. Furthermore, amino acids bring crucial functions in cell signaling and act as regulators of gene expression and protein phosphorylation cascade (Wu, 2013). The protein from the fish sources contains many kinds of essential and functional amino acids which are beneficial to human health. The range of protein level in the four species was from 49.61 to 53. 97%, with 49.61, 50.19, 53.61 and 53.97% in *C. marulius*, *W. atto*, *L. rohita* and *A. anguilla* respectively. These results are in accord with previous finding reported by (Maurizio *et al.*, 2010) according to which protein contents of most species of fresh nature contain protein from 42.4 to 51.5%. Luzia *et al.* (2003) also showed that protein contents also varied according to the location of fish caught.

Fat is a beneficial reservoir of vitality as it furnishes almost double energy (i.e., 9 kcal/gm) of the carbohydrates. Fat contents of four species (Table 1) differ significantly among four species. The fat contents are in the order of 11.83, 11.43, 10.6 and 10% for *W. atto*, *C. marulius*, *L. rohita* and *A. anguilla* respectively. It was observed that deep water fish species have high contents of fat than shallow water fish species due to availability of better quality and variety of feed. The variation in fat contents may arise due to Lipoxygenases which is major problem in post mortem fish species and enzymatic hydrolysis of lipid (Wedoud *et al.*, 2011). Wedoud *et al.* (2011) investigated the fat percentage in the marine fish species of Mauritius in different seasons and concluded that fish species caught in January and September possess high contents of fat (8.4 to 13%) which are the reproductive months of fish species. The ash contents from fish muscles are indicator of minerals contents as fish may also have many essential and trace minerals. Specie *W. atto* was found to be at top with highest ash contents (8.45%) followed by *C. marulius* (7.63%), *A. anguilla* (6.93%) and *L. rohita* (6.35%). These results were almost same as the findings of (Yesim and Fatih, 2007) who reported the ash content are in the range of 6.5 to 12.05% in the commercially important fresh water fish species of Turkey. Carbohydrates are the sources of energy with status "Ready" in the body and 1 gram consumption of carbohydrates supplies 4Kcal energy. The highest carbohydrate was found in *Channamarulius* (31.40%) and most dispirited amount of carbohydrates was found in *Anguilla anguilla* (26.44%). These results are in accord with the result of (Osman *et al.*, 2001) who found the carbohydrates in fresh water species fall between 17.8 to 37.4%. As fish is a rich source of protein, carbohydrates and fat, it is not only heath beneficial but a plenteous source of energy. So total energy from these nutritive components is the gross energy value which is rough estimation of energy we get from the consumption of fish. Fish species did not differ significantly with respect to gross energy value. It showed that energy values do not vary much to one another and only a negligible difference was found which ranges from 429.56 to 434.56%. Our findings are in accord with the results of (Zygmunt *et al.*, 2012) according to which the energy values from the consumption of fish varies from 428.7 to 437.5 Kcal/100g.

Table 1. Chemical composition of muscles from selected commonly consumed fresh water fish species of Pakistan

Name of analysis	Malhi (*Wallagoatto*)	Sole (*Channamarulius*)	Damra (*Labeorohita*)	Baam (*Anguilla anguilla*)
Total moisture (%)	75.03±3bc	73.93±3.69c	77.21±4.63ab	78.23±5.47a
Crude protein (%)	50.19±1.91b	49.61±2.93c	53.61±1.20a	53.97±0.46a
Crude fat (%)	11.83±0.47a	11.43±0.57ab	10.60±0.63bc	10.00±0.7c
Ash contents (%)	8.45±0.50a	7.63±1.04c	6.35±1.04c	6.93±1.66c
Total carbohydrates (%)	27.92±1.11b	31.40±1.57a	28.64±1.71ab	26.44±1.85b
Gross energy (Kcal/100g)	429.56±17.18a	432.75±21.63a	434.88±26.09a	430.74±30.51a

*Means sharing similar letters are statistically non-significant

Table 2. Fatty acids profile of fish species from selected commonly consumed fresh water fish species of Pakistan

FAMEs	Malh (*W. atto*)	Sole (*C marulius*)	Damra (*L. rohita*)	Baam (*A. anguila*)
C8:0, Caprylic acid	0.278_a	0.171_c	0.247_b	0.165_c
C10:0, Capric acid	0.011_a	0.005_b	0.000_c	0.000_c
C12:0, Lauric Acid	0.575_a	0.094_c	0.184_b	0.082_c
C13:0, Tridecanoate acid	0.854_a	0.043_c	0.549_b	0.500_b
C14:0, Myristic acid	3.487_b	2.766_c	3.781_a	2.568_c
C15:0, Pentadecanoic acid	1.867_a	1.750_{ab}	1.640_b	1.197_c
C16:0, Palmitic acid	35.050_b	47.710_a	32.410_b	32.400_b
C17:0, Heptadecanoic acid	4.435_a	2.585_c	3.038_b	1.572_d
C18:0, Stearic acid	10.487_a	9.403_b	7.977_c	8.416_c
C20:0, Arachidic acid	0.232_c	0.308_b	0.632_a	0.170_d
C22:0, Behenic acid	0.327_c	0.437_b	0.860_a	0.483_b
C23:0 Tricosanoic	0.61_a	0.18_a	0.72_a	0.86_a
C24:0 Lignoceric	0.64_a	0.31_a	0.18_a	0.12_a
∑ SFA	58.86	65.76	52.21	48.43
C14:1, Myristoleic acid	0.656_c	0.863_b	1.452_a	0.445_d
C15:1, cis-10 pentadeca acid	0.885_a	0.330_c	0.587_b	0.359_c
C16:1, Palmitoleic acid	6.787_{bc}	6.114_c	10.787_a	7.883_b
C17:1, cis-10 Heptadeca acid	0.920_a	0.597_c	0.743_b	0.685_b
C18:1, Elaidic acid	0.186_c	0.885_a	0.411_b	0.244_c
C18:1, Oleic acid	21.260_a	11.950_c	10.970_c	17.860_b
C20:1, cis-11 Eicosenoic acid	0.512_b	4.097_a	0.503_b	0.339_c
C22:1, Erucic Acid	0.164_d	0.961_a	0.274_b	0.228_c
∑MUFAs	31.37	25.79	25.727	28.048
C18:2, Linoleic acid (LA) (ω6)	0.213_b	0.166_b	9.128_a	9.137_a
C18:3, γ-linolenic acid (GLA) (ω6)	1.387_d	2.300_c	6.947_a	4.845_b
C18:3, α-linolenic acid (ω3)	1.187_c	2.08_a	0.573_d	1.772_b
C20:3 cis-8, 11, 14 Eicosatrienoic (hGLA) (ω6)	1.61_a	1.2_a	1.84_a	1.98_a
C20:3 cis-11,14,17 Eicosatrienoic (ω3)	0.93_a	0.51_a	0.61_a	1.23_a
C20:4, Arachidonic acid (ω6)	0.481_a	0.223_c	0.402_b	0.472_a
C20:5, Eicosapentaenoic acid (EPA) (ω3)	0.414_c	0.353_d	0.693_a	0.461_b
C22:5 Docosapentanoic (DPA) (ω3)	0.81_a	0.68_a	0.84_a	0.96_{ab}
C22:2 cis 13,16 Docosadienoic (ω6)	0.41_a	0.19_a	0.34_a	0.44_a
C22:6, Docosahexaenoic acid (DHA) (ω3)	0.37_b	0.110_c	1.242_a	1.294_a
∑PUFAS	7.81	7.812	22.615	22.591
ω3	3.71	3.73	3.96	5.72
ω6	4.1	4.08	18.66	16.87
ω3/ω6	0.9	0.92	0.21	0.34
ω6/ω3	1.11	1.09	4.71	2.95
EPA	0.39	0.31	0.693	0.461
DHA	0.37	0.07	1.242	1.294
EPA+DHA	0.76	0.38	1.935	1.755
EPA/DHA	1.05	4.43	0.56	0.36

*Means sharing similar letters are statistically non-significant

Fatty Acids Profile

Thirty one fatty acids in *W. atto* and *C. marulius* and thirty fatty acids in *L. rohita* and *A. anguilla* were identified and evaluated in this study. Table 2 showed the percentages as mean value of fatty acids for four species. Relation of Individual lipid class with the total lipids contents was analyzed in this study. Unlike the

findings of (Zhang *et al.*, 2014) SFAs found to be have a positive relation with total Lipid contents and PUFAs have a reverse association with the total lipid contents. Our findings are in agreement with the work of (Belling *et al.*, 1997). Dominancy of SFAs was found over the MUFAs and PUFAs. The results showed that SFAs from C8:0 to C24:0 existed among the fish species. The detected range of SFAs in selected species was from 48.43% in *A. anguilla* to 65.76% in *C. marulius*. The key identified SFAs were palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0). A low amount of median chain FAs, ranged from 0.278 to 0.165% was found in these fishes, with caprylic acid (C8:0) as the major one which was plenteous in the *W. atto* (0.278%) and gloomiest in the *A. anguilla* (0.165%). The inclusion of caprylic acid in diet is crucial for lessen the amount of unacylatedgherlin, an orexigenic peptide hormone, as acylatedgherlin involves in regulation of growth hormones, gastric acid secretion, appetite, homeostasis of glucose and adiposity (Lemarié *et al.*, 2015). The *A. anguilla* and *L. rohita* were devoid of capric acid or decanoic acid (C10:0) while it was existed with trace amounts in *W. atto* (0.011%) followed by *C. marulius* (0.05%). Decanoic acid, an essential component of therapeutic diets, have recently showed an effectual control over brain seizure and delaying in picrotoxin-induced seizures in brain (Chang *et al.*, 2015) Lauric (C12:0) and trideanoate acid (C13:0) were also plenteous in the *W. atto* and deficient in *C. marulius*. Short chain and medium chain SFAs (C: 6C: 12) metabolized rapidly in body as compared to long chain SFAs. This ease of metabolism of short chain SFAs is due to free and rapid transportation of short SFAs across mitochondrial membranes in liver (Dayrit, 2015). Short chain SFAs also ascertained to minify the cholesterol degree of plasma and consequently letting down the peril of Coronary Heart Diseases (CHD) (Legrand *et al.*, 2010). Myristic acid (C14:0) showed different trend as it was more abundant in *L. rohita* (3.781%) and least amount was found in *A. anguilla* (2.568%) with 3.487 and 2.766% in *W. atto* and *C. marulius* respectively. A recent study conducted by (Noto *et al.*, 2015) amazingly revealed that C14 is involved in lowering of plasma level of HDL-C (High density lipoproteins carrying cholesterol to liver for metabolism) by trapping them in cell surface HSPG (heparan sulfate proteoglycan).

The amount of long chain SFAs from pentadecanoic acid (C15:0) tolignoceric (C24:0) were also significantly different among the four fresh water fish species. The results showed that highest amount of pentadecanoic acid (C15:0) was detected in *W. atto* (1.867%) and least amount of it found in *A. anguilla* (1.197%). Surplus palmitc acids (C16:0) was found in *C. marulius* (47.710%) followed by *W. atto* (35.050%) and almost equally distributed in *A. anguilla* and *L. Rohita* So it was

the most abundant fatty acid among SFAs. Heptadecanoic acid (C17:0) is copious in *W. atto* (4.435%) and *L. Rohita* (3.038%) followed by *C. marulius* (2.585%) and *A. anguilla* (1.572%). Like PUFAs, odd chain SFAs (specifically C15:0 and C17:0) are found to enhance the membrane fluidity and consequently decrease the risk of various type scleroses (Jenkins *et al.*, 2015). Fonteh *et al.* (2015) also concluded that Alzheimer disease stricken people have low tissue level of C15:0 and C17:0. These odd chain SFAs also showed reverse relation with carcinogenic influence and CHD occurrence (Jenkins *et al.*, 2015). Stearic acid (C18:00) was the second most abundant SFA found in the lipids of these fish species. *W. atto* is characterized with highest stearic acid quantity with 10.487% followed by 9.403, 8.416 and 7.977% in *C. marulius*, *A. anguilla* and *L. rohita* respectively. Like short chain SFAs, very long chains SFAs (C20-C24) were also detected in fractional quantity. The detected very long chain SFAs were arachidic acid (C20:0), behenic acid, (C22:0), tricosanoic acid (C23:0) and lignoceric acid (C24:0). So among the thirteen detected SFAs, palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acids were the major SFAs. These findings are in accord with the findings of Guil-Guerrero *et al.* (2011), High prevailing quantity of SFAs may be due to less efficiency of fish species in utilizing the SFAs as core energy source which resulted in the rise of SFAs (Nath and Banerjee, 2012). Regost *et al.* (2003) stated that De novo synthesis and diets are the two sources of fatty acids in muscles. If the fish feed mostly on the diet mainly insects and other aquatic then the saturated fatty acids contents rises and if they feed on the plant and algae sources then it contain mostly unsaturated fatty acids. So the fluctuation in the fatty acids is due to the continual recycling of Fatty acids in food web, habitat and feed.

Eight MUFAs were identified during analysis, ranged from 25.7% in *L. rohita* to 31.3% in *W. atto*, with aberrant association with total lipid contents. Results (Table 2) showed that myristoleic acid (C14:1), a promising anti-prostatic cancer therapeutic FA in future (Iguchi *et al.*, 2001), was chiefly detected in *L. rohita* (1.452%) whereas. Palmtoleic acid (C16:1), second key detected MUFA in all fish species, but predominantly detected in *L. rohita* (10.78%). Epidemiological reports disclosed that C16:1 involves in hemostasis, cholesterol metabolism and insulin sensitivity, lower risk of diabetes, lower levels of inflammation with net mixed effects on serum lipid and CVD (higher HDL, lower LDL, lower fibrinogen, but higher triglycerides and greater insulin resistance) (Bernstein *et al.*, 2014). Overall effects of Palmtoleic acid are still a parable and largely depend on its origin and area of action (De

Fabiani, 2011). The major abundant MUFA in all fish species was oleic acid (C18:1). *W. atto* (21.26%) contained higher amount of oleic acid than other selected fish species. Oleic acid comprised almost 60-80% of the collective MUFAs. Whereas eicosenoic acid (C20:1) and erucic acid (C22:1) was preponderantly found in *C. marulius*. These results are in accord with the results of Luzia *et al.* (2003), according to which the contents of MUFAs ranges from 23-33% in the fresh water fish species. All the spotted MUFAs were of "cis nature" and these cis MUFAs are regarded as with neutral effects on the LDL-cholesterol level in serum (Ros, 2013). Generally vegetable oil especially canola and virgin olive oil has been considered as MUFAs rich sources. Substitution of MUFAs in diet (rather than carbohydrates-CHO and SFAs) has unveiled even more betterment in insulin sensitivity, β-cells activity, glycemic regulation, HDL-Cholesterol level preservation, body weight maintenance and avoidance of CVD threats (Gillingham *et al.*, 2011). So one can accomplish the above revealed health attains by consuming the under study fish species as these species contained handsome and appropriate proportion of MUFAs.

The polyunsaturated fatty acids are those fatty acids that contain more than one double bond in the carbon chain. In this study, total ten PUFAs were identified in the oil of four species. The analysis of variance showed that highly significant difference ($p < 0.05$) existed among the PUFAs except cis-8, 11, 14 Eicosatrienoic (C20:3), cis-11,14,17 Eicosatrienoicacid (C20:3) and cis 13,16 Docosadienoic (C22:2). Total PUFAs varied from 7.8% (*W. atto*) to 22.61% (*L. rohita*) with γ-linolenic acid (GLA, C18:3ω6), α-linolenic acid (ALA, C18:3ω3) and cis-8, 11, 14 Eicosatrienoic (hGLA, C20:3ω6)as prevalent FAs in *W. atto* and *C. marulius* and Linoleic acid (LA, C18:2ω6), γ-linolenic acid (GLA, C18:3ω6), cis-8, 11, 14 Eicosatrienoic (hGLA, C20:3ω6), Docosahexaenoic acid (DHA, C22:6ω3) in *L. rohita* and *A. anguilla* respectively. This divergence arose among these species due to various factors such as state of their reproductive cycle, organ source, habitat, size, season and sex (Regost *et al.*, 2003). Table 2, showed the amount of PUFAs present in the oil of four species. Equal number of omega-6 and omega 3 PUFAs had been traced but quantitatively the contribution of omega-6 was found to be more than the omega-3 which is characteristic of fresh water fish species. Among the ω6 PUFAs, Linoleic Acid (LA) (C18:2) and γ-linolenic acid (C18:3) (GLA) were principally detected respectively whereas cis 13, 16 Docosadienoic acid (C22:2) ascertained to be in tiniest amount.

Among theω3-PUFAs, ALA was the most amplefatty acid in all fish species followed by cis-11,14,17 Eicosatrienoic, DHA, DPA and EPA and among ω6 PUFAs, GLA was the most plentiful followed by hGLA and AA.LA and GLA followed the same trend as up to 9

and 4-7% LA and GLA were detected in *A. anguilla* and *L. rohita* respectively. ALA, which is believed to have healthy effects on prevention of cardiovascular disorder (Kinsella, 1998; Mozaffarian *et al.*, 2005; Goodnight *et al.*, 1982) chiefly presented in *C. marulius* (2.08%) and *A. anguilla* (1.77%). Conditional essential fatty acids arachidonic acid (C20:4) with its known positive effects on muscle growth, brain development and Alzheimer disease (Sanchez-Mejia and Mucke, 2010) mainly observed in *W. atto* (0.48%) and *L. Rohita* (0.4%). Well known neurological, inflammation and CVD protecting (Dyall, 2015) long chainω3 PUFAs Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid (EPA) and Docosapentanoic (DPA) were identified chiefly in *L. rohita* and *A. anguilla*. The summation of DHA and EPA in the four species were 0.76, 0.38. 1.93 and 1.75% in *W. atto*, *C. marulius*, *L. rohita* and *A. anguilla* respectively. The collective EPA+DHA role is very crucial to acquire the above remarked health welfares while DPA may act as reservoir for DHA and EPA (Kaur *et al.*, 2013). The dietary recommendation for EPA+DHA is 500mg/day which can be achieved by 2 serving size (each serving size 4oz = 112 gm.) of fish per week (Kris-Etherton and Innis, 2007). It was suggested that elevated level of DHA than EPA is linked with importance of cell membrane's enzymatic activity, physiological equilibrium (Watanabe, 1982) stronger immune defense system against pathogenesis, homeostatic regulation of osmotic pressure and communal conducts (Shulman and Love, 1999). The species *A. anguilla* supposed to have highest motility among the four species due to possessing highest DHA and MUFAs contents as (Bell and Sargent, 2003) deduced that breeding relocation and physical movement is allied to eminent level of DHA and MUFAs. The ratio of ω6/ω3 is another way of assessing the nutrition and health benefits of a diet. Most of western countries diets are lacking in omega-3 and have highω6/ω3 ratio. According to dietitians the recommended ω6/ω3 ratio is 1-5 and if ω6/ω3 ratio of any diet lies in this range then this food might be proved as a neutraceutical and can help body against the autoimmune diseases, cardiovascular disease, cancer and inflammatory disease. The ratios of ω6/ω3 detected in specimen fish species range from 1.09 to 4.71 which broaden the importance of consumption of these fish species. These findings are in accord with the findings of Yesim and Fatih (2007) according to which PUFAs are the dominating fatty acids after the saturated fatty acids and Pirini *et al.* (2000) according to which fresh water species is richer in the Omega-6 fatty acids. So these fresh water fish species are rich in the ω6 fatty acids as compared to Omega-3 fatty acids. These findings are also in agreement with the findings of Rasoarahona *et al.* (2005) Ward and Singh (2005) who stated that among the PUFAs, in Fresh water fish species, the most abundant fatty acids are γ-Linolenic Acid (GLA) (C18:3, ω6) and α-linolenic acid (C18:3, ω3) which ranges from 1.3 to 7.70 and 0.46 to 2.37% respectively. Sargent *et al.*

(1995) and Saito *et al.* (1999) reported that variation in the fatty acids is not only due to the cause of habitat but it also rely on the diet whether it is natural or artificial and nature of taking feed like whether the species are carnivores, herbivores or omnivores, ecological conditions like temperature and water, age, size and reproductive cycle.

In this era of curiosity, inventions and discoveries, it has, now, become very crucial to compare and characterize the fish species from different countries. Like many other developing countries Fish and other aquaculture foods are main source of superior quality protein and lipids in China, India and Turkey. A research study on fresh caught wild fish species from the Yangtz basin conducted by Zhang *et al.* (2014) found these low fat species deficient in short chain SFAs (C8:0, C10:0) and long chain SFAs (C22:0 and C24:0) as compared SFAs copious fresh water fish species of Pakistan. MUFAs and PUFAs are more dominant in Chinese fish species where their range is 21.83-50.53 and 19.43-45.6% respectively but in Pakistani fish species the ranges of MUFAs and PUFAs is 25.72-31.37 and 7.4-22.76% respectively. Among the PUFAs, Pakistani fish species lacked eicosadienoic acid (C20:2n6), docosatetraenoic acid (C22:4n6) and docosapentaenoic acid (C22:5n6). The ratios of Omega3/Omega6 in Chinese species ranges from 0.25 to 1.16 and most of Chinese species have Omega3/Omega6 >0.5. The summation of EPA and DHA in Chinese fish species is 2.77 to 14.86% and most of them have summation of EPA and DHA >9%. So we can say that Chinese fresh water fish species are rich source of PUFAs and Pakistani Fish species are rich in SFAs. In another study carried out by Jakhar *et al.* (2012) on four Indian fresh water fish species found these fish species contain low to medium fat with their protein contents are also low as compared to Pakistani fish species. Among the total fatty acids, SFAs are high in Pakistani Fish species; MUFAs are high in Indian fish species whereas PUFAs are comparable. Among total FAMEs of these Indian fish species lacked (C8:0), (C10:0), (C22:0), (C23:0) and (C24:0 (C14:1), (C15:1), (C17:1), (C22:1) and γ-Linolenic Acid (C18:3 ω6 GLA) (Jakhar *et al.*, 2012). Omega-3/Omega-6 ratios for Indian fish species range from 0.7 to 6.2 which indicated high quantity of Omega-3 but for Pakistani Fish species this ratio range from 0.34 to 0.94 which shows that Pakistani fish species contain more Omega-6 fatty acids as compared to Indian fish species.

Recent study on Indian fresh water carp species conducted by (Dey *et al.*, 2015) revealed that short and medium chain fatty acids (C8:0, C10:0 and C12:0) are absent in the Indian fish species and long chain (more than C22:0) MUFAs are not in Pakistani fish species. Among PUFAs, (C22:5ω6) and (C20:4ω3) and (C21:5ω3) have not been detected in Pakistani species. But this study give us knowledge that Indian fresh water

fish species are plenteous in Omega-3 PUFAs and deficient in Omega-6 PUFAs which is not the typical characteristic of fresh water fish species. Their PUFA quantity is amazingly high which ranges from 6.9 to 18.3% (Dey *et al.*, 2015). Another study by Nath *et al.* (2014) has also confirmed these findings, But Cakmak *et al.* (2012) Identified 38 fatty acids from the wild fresh water fish species from Sugla Lake, Turkey. These Turkish fresh water fish species has less quantity of SFAs (21.43 to 21.36 to 26.33%) as compared to Pakistan fish species 48.5-65.7%). Among MUFAs the trend is not as flat as in SFAs. The maximum amount identified in Pakistani species is 31.3% but for Turkish fish species half of fish species have more than 31% MUFAs. Among the PUFAs, Pakistan fish species have 22.7% PUFAs maximally, however, Turkish fish species are rich in the PUFAs with maximum identified PUFAs up to 51.8% which is very enormous and amazing quantity. Pakistani Fish species contain less ω3PUFAs but the quantity of ω6 PUFAs is comparable to those in Turkish fish species. While comparing outcomes of Citil *at al.* (2014) and Ersoy and Şereflişan (2010) on other Turkish fresh water species with our findings, we presumed the homologous results as detailed above.

Conclusion

Characterization of four selected fresh water fish was carried out with special reference to nutrition and lipid profile. In conclusion, the data from nutritional values and fatty acids profile analyses suggested us that these high fat fish species adore with bounteous sum of nutritional fractions containing substantive and functional fatty acids and amino acids and generally these species are nutritious and healthy to consume. In the FA mapping, SFAs were detected as major lipid with positive association with total lipid contents. Among SFAs, short, medium chain and odd number FAs were detected, signifying the status of under study fish species as a remedial and therapeutic diet due to their curative strikes on various ailments related to regulation of growth and digestion hormones, metabolism (glucose and cholestrol), appetite, brain seizure, scleroses and Alzheimer disease. *C. marulius* noticed to bear most eminent amount of SFAs among four species. Most Substantial quantity of MUFAs was detected in *W. atto.* These MUFAs are quite helpful in maintenance of "good cholesterol" level and prevention of occurrence of prostate cancer, diabetes and obesity with neutral effects on the level of bad cholesterol. Concerning PUFAs, more prevalent quantity of PUFAs was found in *L. rohita* and *A. anguilla.* The understudy fish species ascertained to be rich source of Omega-6 LC-PUFAs with low fractions of DHA and EPA. But the consumption of these species according to endorsed per serving size can meet the dietetic sanctions of EPA and DHA. ω6/ω3 ratio of *L. rohita* was found to be most close to optimal (5/1). In comparison with neighboring

countries and Eurasian Turkeys' fresh water fish species, these understudy fish species were found to be rich in saturated lipids and short chain SFAs with low percentages of PUFAs. From this work, it is evident that all the selected fish species are the harbor of important fatty acids but the extents of their presence differ greatly from one to another. These species are the cheap source of PUFAs and other important fatty acids and by inclusion these species in food web, people can get the beneficial healthy attributes easily.

Acknowledgment

Author's Contributions

Ahsan Hameed: Carried out the experiment and drafted the manuscript.

Syed Ammar Hussain: Participated in FA identification and quantification.

Muhammed Asim Shabbir: Kindly guided in due analytical experimentations.

Imran Pasha and Yuanda Song: Design the experiment and critically reviewed the final manuscript.

Conflict of Interest

The authors declares that they have no conflicts of interests.

Compliance with Ethics Requirement

This article does not contain any clinical studies with human or animal subjects.

References

Ackman, R.G., 1989. Marine Biogenic Lipids, Fats and Oils. 1st Edn., CRC Press, ISBN-10: 0849348897, pp: 472.

Ashraf, M., A. Zafar, A. Rauf, S. Meshboob and N.A. Qureshi, 2011. Nutritional values of wild and cultivated silver carp (*Hypophthalmichthys molitrix*) and grass carp (*Ctenopharyngodon idella*). Int. J. Agric. Biol., 13: 210-214.

Ayo, J., J. Carballo, J. Serrano, B. Olmedilla-Alonso and C. Ruiz-Capillas *et al*., 2007. Effect of total replacement of pork backfat with walnut on the nutritional profile of frankfurters. Meat Sci., 77: 173-181. PMID: 22061588

Bell, J.G. and J.R. Sargent, 2003. Arachidonic acid in aquaculture feeds: Current status and future opportunities. Aquaculture, 218: 491-499. DOI: 10.1016/S0044-8486(02)00370-8

Belling, G., M. Abbey, J. Campbell and G. Campbell, 1997. Lipid content and fatty acid composition of 11 species of Queensland (Australia) fish. Lipids, 32: 621-625. DOI: 10.1007/s11745-997-0079-z

Bernstein, A.M., M.F. Roizen and L. Martinez, 2014. Purified palmitoleic acid for the reduction of high-sensitivity C-reactive protein and serum lipids: A double-blinded, randomized, placebo controlled study. J. Clin. Lipidol., 8: 612-617. DOI: 10.1016/j.jacl.2014.08.001

Cakmak, Y.S., G. Zengin, G.O. Guler, A. Aktumsek and H. Ozparlak, 2012. Fatty acid composition and ω3/ω6 ratios of the muscle lipids of six fish species in sugla lake, Turkey. Arch. Biol. Sci., Belgrade, 64: 471-477.

Chang, P., K. Augustin, K. Boddum, S. Williams and M. Sun *et al*., 2015. Seizure control by decanoic acid through direct AMPA receptor inhibition. Brain, 139: 431-443. PMID: 26608744

Chaudhry, A.S., 2008. Forage based animal production systems and sustainability, an invited keynote. Revista Brasileira de Zootecnia, 37: 78-84. DOI: 10.1590/S1516-35982008001300010

Citil, O.B., L. Kalyoncu and O. Kahraman, 2014. Fatty acid composition of the muscle lipids of five fish species in işikli and karacaören dam lake, Turkey. Vet. Med. Int. DOI: 10.1155/2014/936091

Cocks, L.V. and V.C. Rede, 1966. Laboratory Handbook for Oil and Fat Analysts. 1st Edn., Academic Press, London, pp: 419.

Connor, W.E., 2000. Importance of n-3 fatty acids in health and disease. Am. J. Clin. Nutr., 71: 171S-175S. PMID: 10617967

Crawford, M.A., 1993. The role of essential fatty acids in neural development: implications for perinatal nutrition. Am. J. Clin. Nutr., 57: 703S-709S. PMID: 7682751

Dayrit, F.M., 2015. The properties of lauric acid and their significance in coconut oil. J. Am. Oil Chem. Soc., 92: 1-15. DOI: 10.1007/s11746-014-2562-7

De Fabiani, E., 2011. The true story of palmitoleic acid: Between myth and reality. Eur. J. Lipid Sci. Technol., 113: 809-811. DOI: 10.1002/ejlt.201100187

Dey, S., K.K. Misra and S. Homechoudhuri, 2015. Lipid classes and fatty acids of a freshwater Indian minor carp, Amblypharyngodon mola. Int. J. Res. Fisher. Aquac., 5: 5-18. DOI: 10.13140/RG.2.1.3695.7520

Dyall, S.C., 2015. Long-chain omega-3 fatty acids and the brain: A review of the independent and shared effects of EPA, DPA and DHA. Front Aging Neurosci., 21: 52-52. DOI: 10.3389/fnagi.2015.00052

Ersoy, B. and H. Şereflişan, 2010. The proximate composition and fatty acid profiles of edible parts of two freshwater mussels. Turkish J. Fisher. Aquatic Sci., 10: 71-74.

Farhat, J. and C. Shakoor, 2011. Chemical compositions and fatty acid profiles of three freshwater fish species. 125: 991-996. DOI: 10.1016/j.foodchem.2010.09.103

Fonteh, A.N., C. Ormseth, J. Chiang, M. Cipolla and X. Arakaki, 2015. Sphingolipid metabolism correlates with cerebrospinal fluid beta amyloid levels in alzheimer's disease. PLoS One, 10: e0125597-e0125597. DOI: 10.1371/journal.pone.0125597

Gillingham, L.G., S. Harris-Janz and P.J.H. Jones, 2011. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids, 46: 209-228. DOI: 10.1007/s11745-010-3524-y

Glogowski, J. and A. Ciereszko, 2001. Why we should increase food consumption, especially that of rainbow trout. Food Tech, 2: 95-102.

Goodnight, S.H.Jr, W.S. Harris, W.E. Connor and D.R. Illingworth, 1982. Polyunsaturated fatty acids, hyperlipidemia and thrombosis. Arteriosclerosis, 2: 87-113. PMID: 7039582

Guil-Guerrero, J.L., E. Venegas-Venegas, M.Á. Rincón-Cervera and M.D. Suárez, 2011. Fatty acid profiles of livers from selected marine fish species. J. Food Compos. Analysis, 24: 217-222. DOI: 10.1016/j.jfca.2010.07.011

Holub, D.J. and B.J. Holub, 2004. Omega-3 fatty acids from fish oils and cardiovascular disease. Mol. Cell Biochem., 263: 217-225. DOI: 10.1023/B:MCBI.0000041863.11248.8d

Horwitz, W., 2006. Official Methods of Analysis of Association of Official Analytical Chemists International. 1st Edn., The Association, Washington, DC.

Iguchi, K., N. Okumura, S. Usui, H. Sajiki and K. Hirota *et al.*, 2001. Myristoleic acid, a cytotoxic component in the extract from *Serenoa repens*, induces apoptosis and necrosis in human prostatic LNCaP cells. Prostate, 47: 59-65. DOI: 10.1002/pros.1047

Jakhar, J.K., A.K. Pal, A.D. Reddy, N.P. Sahu and G. Venkateshwarlu *et al.*, 2012. Fatty acids composition of someselected Indian fishes. Afr. J. Basic Applied Sci., 4: 155-160.

Jayasinghe, C., N. Gotoh and S. Wada, 2003. Variation in lipid classes and fatty acid composition of salmon shark (*Lamna ditropis*) liver with season and gender. Comparat. Biochem. Physiol. B Biochem. Mol. Biol., 134: 287-295. DOI: 10.1016/S1096-4959(02)00268-3

Jenkins, B., J.A. West and A. Koulman, 2015. A review of odd-chain fatty acid metabolism and the role of pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) in health and disease. Molecules, 20: 2425-2444. DOI: 10.3390/molecules20022425

Kaur, G., J.C. Molero, H.S. Weisinger and A.J. Sinclair, 2013. Orally administered [14C]DPA and [14C]DHA are metabolised differently to [14C]EPA in rats. Brit. J. Nutrit., 109: 441-448. DOI: 10.1017/S0007114512001419

Kinsella, J.E., 1998. Food lipids and fatty acids: Importance in food quality, nutrition and health. Food Tech., 42: 10-124.

Kolanowski, W. and G. Laufenberg, 2006. Enrichment of food products with polyunsaturated fatty acids by fish oil addition. Eur. Food Res. Technol., 222: 472-477. DOI: 10.1007/s00217-005-0089-8

Kris-Etherton, P.M. and S. Innis, 2007. Position of the American dietetic association and dietitians of Canada: Dietary fatty acids. J. Am. Diet. Assoc., 107: 1599-1611. PMID: 17936958

Kris-Etherton, P.M., W.S. Harris and L.J. Appel, 2003. Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. Arterioscler. Thromb. Vasc. Biol., 23: 20-30. DOI: 10.1161/01.ATV.0000038493.65177.94

Legrand, P., E. Beauchamp, D. Catheline, F. Pédrono and V. Rioux, 2010. Short chain saturated fatty acids decrease circulating cholesterol and increase tissue PUFA content in the rat. Lipids, 45: 975-986. DOI: 10.1007/s11745-010-3481-5

Lemarié, F., E. Beauchamp, S. Dayot, C. Duby and P. Legrand *et al.*, 2015. Dietary caprylic acid (C8:0) does not increase plasma acylated ghrelin but decreases plasma unacylated ghrelin in the rat. PLoS One, 10: e0133600- e0133600. DOI: 10.1371/journal.pone.0133600

Lovegrove, J.A., C.N. Brooks, M.C Murphy, B.J. Gould and C.M. Williams, 1997. Use of manufactured foods enriched with fish oils as a means of increasing long-chain $n-3$ polyunsaturated fatty acid intake. Brit. J. Nutr., 78: 223-236. DOI: 10.1079/BJN19970142

Luzia, L.A., G.R. Sampaio, C.M.N. Castellucci and E.A.F.S. Torres, 2003. The influence of season on the lipid profiles of five commercially important species of Brazilian fish. Food Chem., 83: 93-97. DOI: 10.1016/S0308-8146(03)00054-2

Maurizio, P., T. Silvia, V. Vittoria, P. Alessandra and B. Anna, 2010. Blue-back fish: Fatty acid profile in selected seasons and retention upon baking. Food Chem., 123: 306-314. DOI: 10.1016/j.foodchem.2010.04.036

Minitab Inc, 2012. Minitab Statistical Software Version 16.2.3. Minitab Inc., State College, PA. URL

Moreira, A.B., J.V. Visentainer, N.E. de Souza and M. Matsushita, 2001. Fatty acids profile and cholesterol contents of three Brazilian *Brycon* freshwater fishes. J. Food Composit. Analysisa, 14: 565-574. DOI: 10.1006/jfca.2001.1025

Mozaffarian, D., C.L. Bryson, R.N. Lemaitre, G.L. Burke and D.S. Siscovick, 2005. Fish intake and risk of incident heart failure. J. Am. Coll. Cardiol., 45: 2015-2021.
DOI: 10.1016/j.jacc.2005.03.038

Nath, A.K. and B. Banerjee, 2012. Comparative evaluation of body composition of hilsa, Tenualosa ilisha (Hamilton, 1822) in different size groups with special reference to fatty acid, in Hooghly estuarine system, West Bengal, India. Ind. J. Fish., 59: 141-146.

Nath, A.K., A. Patra, B. Sen, D. Dey and I. Das *et al.*, 2014. Fatty acid compositions of four edible fishes of Hooghly Estuary, West Bengal, India. Int. J. Curr. Microbiol. App. Sci., 3: 208-218.

Noto, D., F. Fayer, A.B. Cefalù, I. Altieri and O Palesano *et al.*, 2015. Myristic acid is associated to low plasma HDL cholesterol levels in a Mediterranean population and increases HDL catabolism by enhancing HDL particles trapping to cell surface proteoglycans in a liver hepatoma cell model. Atherosclerosis, 246: 50-56.
DOI: 10.1016/j.atherosclerosis.2015.12.036

O'Brien, R.D.O., 2004. Fats and Oils: Formulating and Processing for Applications. 2nd Edn., CRC Press, New York, ISBN-10: 0203483669, pp: 616.

Onyeike, E.N., E.O. Ayalogu and C.O. Ibegbulem, 2000. Evaluation of the nutritional value of some crude oil polluted freshwater fishes. Global J. Pure Applied Sci., 6: 227-233. DOI: 10.4314/gjpas.v6i2.16112

Osman, H., A.R. Suriah and E.C. Law, 2001. Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. Food Chem., 73: 55-60. DOI: 10.1016/S0308-8146(00)00277-6

Pirini, M., P.P. Gatta, S. Testi, G. Trigari and P.G. Monetti *et al.*, 2000. Effect of refrigerated storage on muscle lipid quality of sea bass (*Dicentrarchus labrax*) fed on diets containing different levels of vitamin E. Food Chem., 68: 289-293.
DOI: 10.1016/S0308-8146(99)00190-9

Rahnan, S.A., T.S. Huah, O. Nassan and N.M. Daud, 1995. Fatty acid composition of some Malaysian freshwater fish. Food Chem., 54: 45-49.
DOI: 10.1016/0308-8146(95)92660-C

Rasoarahona, J.R.E., G. Barnathan, J.P. Bianchini and E.M. Gaydou, 2005. Influence of season on the lipid content and fatty acid profiles of three tilapia species (*Oreochromis niloticus*, *O. macrochir* and *Tilapia rendalli*) from Madagascar. Food Chem., 91: 683-694. DOI: 10.1016/j.foodchem.2004.07.001

Regost, C., J. Arzel, J. Robin, G. Rosenlund and S.J. Kaushik, 2003. Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (Psetta maxima): 1. Growth performance, flesh fatty acid profile and lipid metabolism. Aquaculture, 217: 465-482.
DOI: 10.1016/S0044-8486(02)00259-4

Ros, E., 2003. Dietary cis-monounsaturated fatty acids and metabolic control in type 2 diabetes. Am. J. Clin. Nutr., 78: 617S-625S. PMID: 12936956

Saify, Z.S., S. Akhtar, S. Hassan, M. Arif and F. Ahmed *et al.*, 2000. A study on fatty acid composition of fish oil from two marine fish, *Eusphyra blochii* and *Carcharhinus bleekeri*. Pak. J. Pharm. Sci., 13: 5-12. PMID: 16414840

Simopoulos, A.P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother., 56: 365-379.
DOI: 10.1016/S0753-3322(02)00253-6

Saito, H., R. Yamashiro, C. Alasalvar and T. Konno, 1999. Influence of diet on fatty acids of three subtropical fish, subfamily caesioninae (*Caesio diagramma* and *C. tile*) and family siganidae (*Siganus canaliculatus*). Lipids, 34: 1073-1082.
DOI: 10.1007/s11745-999-0459-4

Sanchez-Mejia, R.O. and L. Mucke, 2010. Phospholipase A_2 and arachidonic acid in Alzheimer's disease. Biochim. Biophys. Acta, 1801: 784-790.
DOI: 10.1016/j.bbalip.2010.05.013

Sargent, J.R., J.G. Bell, M.V. Bell, R.J. Henderson and D.R. Tocher, 1995. Requirement criteria for essential fatty acids. J. Applied Ichthyol., 11: 183-198.
DOI: 10.1111/j.1439-0426.1995.tb00018.x

Shulman, G.E. and R.M. Love, 1999. The Biochemical Ecology of Marine Fishes. In: Advances in Marine Biology, Southward, A.J., P.A. Tyler and C.M. Young (Eds.), Elsevier, London, ISBN-10: 012026143X, pp: 351-351.

Steel, R.G.D., J.H. Torrie and D. Dickey, 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Edn., McGraw-Hill, New York, ISBN-10: 0070610282, pp: 336-352.

Uauy, R. and A. Valenzuela, 2000. Marine oils: The health benefits of n-3 fatty acids. Nutrition, 16: 680-684. DOI: 10.1016/S0899-9007(00)00326-9

Ward, O.P. and A. Singh, 2005. Omega-3/6 fatty acids: Alternative sources of production. Process Biochem., 40: 3627-3652. DOI: 10.1016/j.procbio.2005.02.020

Waseem, M.P., 2007. Issues, growth and instability of inland fish production in Sindh (Pakistan) spatial–temporal analysis. Pak. Econom. Social Rev., 45: 203-230.

Watanabe, T., 1982. Lipid nutrition in fish. Comparat. Biochem. Physiol. Part B: Comparat. Biochem., 73: 3-15. DOI: 10.1016/0305-0491(82)90196-1

Wedoud, O.L., M.G. Emile and V.O.K. Mohamed, 2011. Muscle lipids and fatty acid profiles of three edible fish from the Mauritanian coast: *Epinephelus aeneus*, *Cephalopholis taeniops* and *Serranus scriba*. Food Chem., 124: 24-28.
DOI: 10.1016/j.foodchem.2010.05.097

Wu, G., 2010. Functional amino acids in growth, reproduction and health. Adv. Nutrit., 1: 31-37. DOI: 10.3945/an.110.1008

Wu, G., 2013. Functional amino acids in nutrition and health. Amino Acids, 45: 407-411.
DOI: 10.1007/s00726-013-1500-6

Yesim, Z. and Z. Fatih. 2007. Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black Seas. Food Chem., 100: 1634-1638.

Zhang, Z., L. Liu, C. Xie, D. Li and J. Xu *et al.*, 2014. Lipid contents, fatty acid profiles and nutritional quality of nine wild caught freshwater fish species of the Yangtze Basin, China. J. Food Nutrit. Res., 2: 388-394. DOI: 10.12691/jfnr-2-7-10

Zygmunt, U., S.R. Joanna and A. Maria, 2012. Variations in proximate composition and fatty acid profiles of Baltic sprat (*Sprattus sprattus balticus*). Food Chem., 130: 97-103.
DOI: 10.1016/j.foodchem.2011.07.003