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Extraction and Analysis of Oil/Fat and Fatty Acids Content from Different Indigenous Fish of Lake Tana Source, Northwest Ethiopia

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Abstract: Fish/fish oil is generally considered as a useful component of the diet. It can be obtained from eating fish or by taking fish/fish oil and its supplements which is recognized as good sources of polyunsaturated fatty acids (PUFA) which are widely used for pharmaceutical purposes and as food supplements. This study was conducted to reveal the preliminary oil/fat and fatty acid content of indigenous fish species of Lake Tana source from September 2013 to June 2014. *In* this study, fish oil from different fish species of Lake Tana and various fishing sites was extracted by using a solvent system (chemical assay) and appropriate techniques. Fish oil/lipid content and fatty acids content was analyzed by using percentage yield calculations. *Results that:* The obtained results showed that the overall percentage yield of the oil (lipid content) of fish food samples in this study was 3.23±0.41 g/100g of wet tissue (edible portion or body muscle). Different factors including fish species, sex, season and sites of collection were considered for the differences in oil/lipid content in percentage yield calculations. *Labeobarbus* spp. showed the highest oil/lipid content (4.27±0.25%), followed by *Clarius gariepinus* (3.37±0.32%) and *Oreochromis niloticus* showed the least in oil content (2.93±0.35) which indicated that oil content in fish is statistically significant with species ($P<0.05$). The oil content difference with sex was not found statistically significant ($P>0.05$). Oil/lipid content was also found to be at higher levels in fish collected during the end of dry season than during dry season (4.42±0.31 vs 3.19±0.27, respectively) and the difference was statistically significant ($P<0.05$). Fish food samples collected from Fogera area were found to have the highest oil/lipid content (4.44±0.22), followed by Zegie area (3.47±0.24) and those coming from Gerima/Bata area were the least in oil content (3.47±0.24) which indicated statistically significant difference ($P<0.05$).The content of total fatty acids obtained from fish fat/oil (100gm of body oil) (% w/w) were also analysed and recorded for percentage yield calculations. The average yield of fatty acids from fish food samples (% w/w, from 100g of oil) was found to be 38.77±0.43. Fish species especially *Labeobarbus* spp. which most of them being endemic in Lake Tana were found to have good content of lipids which is an important component of human diet (especially being as a source of omega-fatty acids and their associated diverse physiological roles) and hence its better develop regular dietary inclusion of such fish dietary sources.

Key words: Fish Oil Content · Fatty Acids Content · Fish Species · Lake Tana

INTRODUCTION

Fish is generally considered as a useful component of diet and sometimes also known as 'brain food' besides its associated role for the treatment of various hyperlipidemia types. Many factors are found to be involved in causing plasma hyperlipidemia such as high fat diet, personality, trait and genetic background of individuals [1].

Fish oil can be obtained from eating fish or by taking fish/fish oil and its supplements. During the last two decades polyunsaturated fatty acids (PUFA) have

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attracted great interest among scientists for their medicinal and nutritional properties. Among the common sources of these PUFAs are fish/fish oils [2, 3]. PUF As are also called "good fats" and are found mostly in marine derived (sea foods) and flax seed products. Omega-3 and omega-6 fatty acids are essential fatty acids and are known for various physiological roles for humans which cannot synthesize them and hence must be supplied by diet. Omega-3 fatty acids are long-chain carbon compounds that include alpha-linolenic acid (ALA, C18: 3n-3), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) [4].

The functions and potential beneficial effects of these omega-3 fatty acids were recently reviewed as they are incorporated into phospholipids of cell membranes, influencing membrane fluidity, receptor-ligand interactions, cell-to-cell interactions, nutrient transport across membranes, neuronal transmission, modulator of gene expression and precursor for eicosanoids, preventive agent for cardiovascular disorders, autoim-mune disease and cancers, modulator of inflammation and thrombosis and it is important for brain development and visual acuity. Intervention studies have demonstrated that intake of these omega-3 fatty acids in the form of fish/fish oil increases HDL_2-cholesterol concentrations, reduces triglyceride concentrations, as well as postprandial lipaemia and chilomicron remnant concentrations, thus decreasing the risk of atherosclerosis and cardiovascular disease [1, 3, 5]. Some people use fish oil to lower blood pressure or triglyceride levels. The scientific evidence suggests that fish oil really does lower high triglycerides and it also seems to help prevent heart disease and stroke when taken in the recommended amounts [1, 5-7].

The lipid-lowering benefits of eating fish have been well known since epidemiologists noted that Greenland Eskimos had a low coronary mortality compared with Danes. Danes eat a high fat diet. Eskimos eat a high-fat, high-cholesterol diet but one rich in fish, especially those containing the omega-3 fatty acids EPA and DHA. These fatty acids lower plasma very low density lipoproteins (VLDL) and triglyceride concentrations by depressing synthesis of triglycerides in the liver [8-11].

Fish that are especially rich in the beneficial long-chain omega-3 fatty acids (EPA, DHA) include mackerel, tuna, salmon, sardines, herring, trout and menhaden [12]. The PUFA composition in fish oils are affected by several factors, such as geographical location, temperature and water salinity [13, 14]. A comparison between freshwater and saltwater fish clearly established that some fresh water fish are good sources of EPA and DHA [15].

Fish are the largest and the most varied group of aquatic vertebrates and there are various species of fresh water fish in Ethiopia including Lake Tana. The fish species in Lake Tana include Cichlidae, *Oreochromis niloticus* (Nile tilapia), which is the most widespread Tilapia species in Africa; the catfish family (Claridae) is also presented by one species, *Clarias gariepinus* (African catfish), which is the most common members of its genus; and the largest fish family in the lake is the Cyprinidea, represented by three genera: Barbus, Garra and Varicorhinus [16, 17].

Studies in Ethiopia with regard to the content of lipid/oil and fatty acids and their nutritional/health benefits from the different fish species (some of them endemic) are scarce though there is a high degree of fish consumption in many areas. Hence this study is designed to investigate the content of lipid/oil and fatty acids from different fish species and differences with other factors including fishing site, sex and collection/fishing season as well from Lake Tana.

MATERIALS AND METHODS

Study Area: This study was conducted in Bahir Dar where different fish species were collected from Lake Tana. Lake Tana is found in North West high lands of Ethiopia (as shown on figure 1 below), particularly in the Amhara National regional State (ANRS). It is situated with the altitude of 1830 meters above sea level and covers 3200 km^2. Lake Tana is the source of Blue Nile, the only river which drains Lake Tana from South east corner at Bahir Dar town. Lake Tana environment has a district seasonal pattern, particularly with respect to dry and wet periods. The water temperature fluctuates between 19°C during winter from January to March and to 24°C during May-June. Four large permanent rivers (Derma, Reb, Gumara and Gelgel Abay) as well as many short seasonal streams tribute to the Lake [17, 18].

Study Design

Collection of Fish Samples: In this experimental based study which was carried out from September 2013 to June 2104, the fishing/fish collection was carried out from Lake Tana area from different fishing sites as indicated on Figure 1 below. Fishing and collection of the species were done in different seasons in collaboration with the

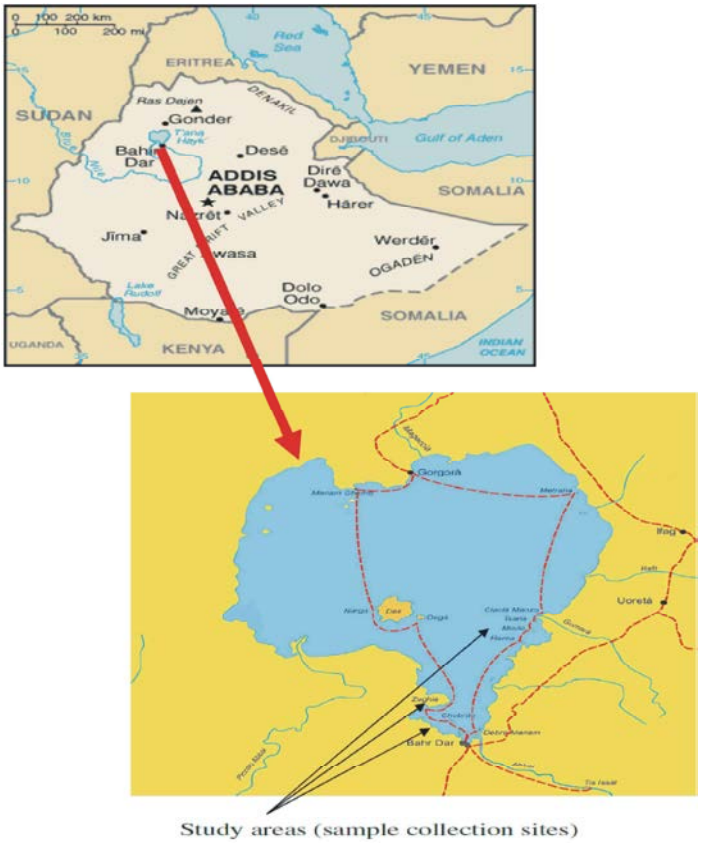

Fig 1: Map of Lake Tana and sample collection sites (Fogera area/long arrow, Zegie area/medium arrow and Gerima and Bata/Bahir Dar area/short arrow and) (Sourse: ANRS Tourism Bureau).

Fig 2: Picture showing fish characterization and filleting for fish food sample collection for the study.

native fishermen fishing associations and Bahir Dar Fishery and Aquatic Life Research Center. A stratified random sampling procedure was used as it was the most suitable method in database work [19]. To ensure representativeness different fishing sites on Lake Tana (Gerima/Bata area, Zegie area and Fogera area) were considered for fish sample collections. Besides, data on fish species (identification by using morphological features) and sex of the fish were made and recorded along with two fisheries experts at Bahir Dar Fisheries and Aquatic Life Research Center after fish collections from Lake Tana sites. The length and weights of fish were also measured. Then, the fish were dissected, gutted, washed and filleted as shown on Figure 2 below. These primary samples (body muscle or edible portion) were placed in sealed plastic bags. Fish food samples (mainly containing body muscles) from each species of fish were prepared and labeled and then it was transferred to the freezer (ice box) within an hour before it is transferred to the laboratory for analysis. Appropriate precautions were performed to sustain freshness of samples and minimize oxidation throughout the study by performing procedures in chillers' room (-4°C) and under minimal light exposures.

Sample Preparation and Analysis: The extraction of fat/oil from the body muscle (edible portion samples) were achieved at different stages, for all samples (38) as outlined below according to the recommended methods;

(a) (b)

Fig 3: Pictures showing oil/lipid extraction procedures from the collected fish food samples. (a) Shows the extracted polar portion and nonpolar layer of the fish food sample and (b) the extracted fish oil collected with test tubes for oil percentage yield calculations and fatty acid analysis.

I, Preparation of samples for analysis, ii, Extraction of fats/oils from samples and iii, Extraction of fatty acids from samples.

Preparation of Samples for Analysis: At first, the caught fish were dissected at ambient temperature (gutting) and their tails and heads were separated. Then the filleted fish food samples were kept on ice block in the icebox in order to maintain the cold chain (low temperature storage) to reduce spoilage [20]. The samples of organs/tissues were stored in the ice box with plastic containers (after labeling) and then were transported to the laboratory for processing in the next stage.

Extraction of Oil/Fat: solvent extraction method was undertaken for the fish lipids/oil extraction according to the method of Bligh and Dyer [21] with some modification by Kinsella *et al*. [22]. Representative samples of chopped fish fillets (40 g) were homogenized for 2 min with a mixture of methanol (80 ml) and chloroform (40 ml) with a blender. One volume of chloroform (40 ml) was added to the mixture and after blending for additional 30 seconds, distilled water (40 ml) was added. The homogenate was stirred with a glass rod and filtered through Whatman No. 1 filter paper on a Buchner funnel with vacuum suction. About 16 ml of chloroform was used to rinse the remainder and then the filtrate was allowed to settle to separate into the organic and aqueous layers as shown on Figure 3(a) below. The chloroform layer containing the lipids was transferred into another beaker and about 3 g of anhydrous sodium sulphate was added to remove any remaining water. The mixture was again filtered through a Whatman no. 1 filter paper and chloroform was used to rinse the remainder. Finally, a known amount of EDTA was added to the extracted oil as an anti-oxidant to minimize lipid peroxidation [20]. Then the oil solution in a tared 100 ml rund-bottom flask was concentrated with a rotary evaporator at 40°C leaving behind the crude fish oils at a constant weight. The oils were poured into sample storing bottles as shown on Figure 3 (b) below and then, the bottles were stored immediately in a refrigerator (at -20°C) rapped with aluminum foils to avoid light exposure and only being taken out from freezer just before further analysis. The determination of lipid content (g/100g of sample) as percentage yield calculations was done separately for each fish.

Extraction of Fatty Acids: to separate free fatty acids, acid hydrolysis was performed and about 30 ml of HCl was added to the sample solution according to AOCS Official Method [23] and Nezhad *et al.* [7]. Then 50 ml of a mixture of petroleum ether and diethyl ether (containing butylated hydrohy tolune/BHT at 10 ppm as a stabilizer or anti-oxidant) at the same proportion were added and mixed for two minutes with the solution. At this stage, fatty acids were placed at the upper phase. The lower phase was thrown and the upper phase (fatty acids part) was poured at balloon by using a separating funnel and then the solvent was evaporated with a rotary evaporator at 40°C [7]. Then the weight of total fatty acids extracted was weighed and used for percentage yield calculations.

Data Collection: Data were collected during sample collection (fish species, sex, site of collection, body weight and length) and during laboratory analysis (oil/fatty acids extraction and analysis of content). Meanwhile the content of oil/fat and content of total fatty acids were measured from different fish species samples collected from different sites of Lake Tana.

Data Analysis: Data was recorded and entered in to Microsoft excel during the study before analysis. Descriptive statistics was used and quantitative data was presented and expressed in percent w/w (gm/100g body tissue) for oil/fat content and total fatty acids content and was presented as mean±SE. The respective content differences were compared and tested with independent t-test for sex and season and one-way analysis of variance for factors like species and collection site/source by using the statistical software SPSS 18. In all the statistical tests, a confidence level of 95% and $p< 0.05$ was considered significant.

RESULTS AND DISCUSSION

Fish Species Samples Collected from Lake Tana: About 38 fish samples were collected during the study period from different sites of Lake Tana (Fogera area, Gerima and Bata area and Zegie area). The different species and local names of the collected fish for this study were described below on Table 1.

Extraction and Determination of Lipid/Oil Content: The overall percentage yield of the oil (lipid content) of fish food samples in this study was 3.23±0.41 g/100g of wet tissue (edible portion or body muscle). This study indicated a higher oil/lipid content of fish body tissue when compared with other study results by Razak *et al.* [2] which reported oil/lipid content of the eels between 0.50 and 1.06 g/100 g wet tissue in Malaysia.

Different factors including fish species, sex, season and sites of collection were considered for comparing the differences in oil/lipid content with percentage yield calculations as shown on Table 2. *Labeobarbus* species showed the highest oil/lipid content (4.27±0.25%), followed by *Clarias gariepinus* (3.37±0.32%) and *Oreochromis niloticus* showed the least in oil content (2.93±0.35) which indicates that oil content in fish is statistically significant with species ($P<0.05$). The oil content was higher in female fish than males (4.07±0.29 vs 3.49±0.34, respectively) but the difference was not found statistically significant ($P>0.05$).

Oil/lipid content was also found to be at higher levels in fish collected during the end of dry season than during dry season (4.42±0.31 vs 3.19±0.27, respectively) and the difference was statistically significant ($P<0.05$). Fish food samples collected from Fogera area were found to have the highest oil/lipid content (4.44± 0.22), followed by Zegie area (3.47±0.24) and those collected from Gerima and Bata area were the least in oil content (3.47±0.24) and

Table 1: Different fish species collected from Lake Tana during the study period.

Scientific name	Common name	Local name	Number (n)	Length range (cm)	Weight range (g)
L. crassibarbis	Labeobarbus spp.	Nech Assa	4	12.7-38.5	350-1000
L. intermedius	Labeobarbus spp.	Nech Assa	4	12.3-13.8	156-350
L. gorguari	Labeobarbus spp.	Nech Assa	2	22.2-24.4	105.2-148.5
L. longissmus	Labeobarbus spp.	Nech Assa	3	30-31	600-650
L. megastoma	Labeobarbus spp.	Nech Assa	3	34-35	550-600
L. tsanensis	Labeobarbus spp.	Nech Assa	2	12.7-12.8	350-360
O. niloticus	Tilapia	Keresso	10	11.6-12.7	103-426
C. gariepinus	African cat fish	Key Assa (Ambaza)	10	30-51.3	319-850
Total			38		

Table 2: Factors considered/tested for fat/oil content from fish food samples collected in this study.

Factors		n	Oil content (% in g/100g of edible food sample, Mean +SE)	P-value (F-and t-tests)
Species	Labeobarbus spp.	18	4.27±0.25	0 .023 *
	O. niloticus	10	2.93±0.35	
	C. gariepinus	10	3.37±0.32	
Sex	Male	20	3.49±0.34	0.224
	Females	18	4.07±0.29	
Season of collection	Dry season	20	3.19±0.27	0 .006*
	End of dry season	18	4.42±0.31	
Site of collection				0.004 *
	Fogera area	17	4.44±0.22	
	Gerima and Bata area	11	2.70±0.24	
	Zegie area	10	3.47±0.24	

*P<0.05 indicates statistically significant difference.

Table 3: Factors considered/tested for fatty acid content differences from fish food samples in this study.

Factors		n	Fatty acid content (% in g/100g body oil, Mean+SE)	P-value (F-and t-tests)
Species	Labeobarbus spp.	18	41.20±0.52	0.02*
	O. niloticus	10	30.32±0.29	
	C. gariepinus	10	36.13±0.32	
Sex	Male	20	34.51±0.32	0.25
	Females	18	36.33±0.28	
Season of collection	Dry season	20	36.76.6±0.11	0.17
	End of dry season	18	38.29± 2.31	
Site of collection	Fogera area	17	38.45±0.15	0.03*
	Gerima and Bata area	11	32.11± 0.48	
	Zegie area	10	36.45±0.42	

*P<0.05 indicates statistically significant difference.

site of collection for fish showed statistically significant (P<0.05) difference. Many reports before this study also indicated that there exists a difference in oil/lipid content of between species, season and collection sites which could be associated with genetic differences, nutrition and temperature/climate variations [2, 15].

Determination of Fatty Acids Content: The content of total fatty acids obtained from fish fat/oil (100gm of body oil) (% w/w) were recorded and used for percentage yield calculations. The average yield in % was found to be 38.77±0.43.The differences in percentage yield of fatty acids with different factors was also analyzed. Fish species and collection site differences showed statistically significant (P<0.05) differences in oil content where as sex and season of collection did not show statistically significant differences (P>0.05) in oil/lipid content as reported below on Table 3.

CONCLUSION

The overall percentage yield of the oil/lipid content of fish food samples in this study was high (3.23±0.41 g/100g of wet tissue/edible portion or body muscle). *Labeobarbus* species (most of them are endemic species to Lake Tana) showed the highest oil/lipid content as compared with others in this study. Similarly the content of fatty acids was also the highest in *Labeobarbus* species and since fish oil is known to be rich with PUFAs especially omega-3 and omega-6 fatty acids, the high content of fatty acids the fish species in this study would suggest the high benefit of these fish species of Lake Tana though further studies are indicated. Other factors like season and site of collection/fishing also indicated significant differences in oil/lipid and fatty acid content.

Based on these findings, the following recommendations were forwarded:

- Fish species (especially *Labeobarbus* species most of them being endemic) that are found in Lake Tana are found to have good sources of fish lipids which is an important component of human diet (especially being as a source of omega-fatty acids and their associated diverse physiological roles) and hence its better develop regular dietary inclusion of such fish dietary sources.
- The low oil/lipid and fatty acid content of fish especially during dry season and for those fish collected from Bahir Dar city vicinity area of Lake Tana might be associated to reduced levels of nutritional supply and environmental influences for fish and hence it seems to do much more to conserve and protect the Lake Tana and its territory which is harboring such potential fish recourse.
- Further research work in this issue especially for future industry based considerations for production of fish oil as nutritional supplements from Lake Tana source is highly recommended.

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REFERRENCES

1. Saify, Z.S., F. Ahmad, S. Akhtar, N. Mushtaq, M. Arif and S. Siddiqui, 2004. Effects of fish oil and lipid lowering drugs on serum triglycerides alone and in combination. Pakistan Journal of Pharmacology, 21(2): 31-37.
2. Razak, Z.K., M. Basri, K. Dzulkefly, C. Razak and A. Salleh, 2001. Extraction and Characterization of Fish Oil from Monopterus albus. Malaysian Journal of Analytical Sciences, 7(1): 217-220.
3. Wang, Y., L. Li, W. Jiang, Z. Yang and Z. Zhang, 2006. Synthesis and preliminary antitumor activity evaluation of a DHA and doxorubicin conjugate. Bioorg. Med. Chem. Lett., 16(11): 2974-2977.
4. Weintraub, M.S., R. Zechner and A. Brown, 1998. Dietary polyunsaturated fat of the omega-6 and omega-3 series reduces postprandial lipoprotein levels. J. Clin. Invest., 82: 1884-1893.
5. Gebauer, S.K., T.L. Psota, W.S. Harris and P.M. Kris-Etherton, 2006. "N-3 Fatty Acid Dietary Recommendations and Food Sources to Achieve Essentiality and Cardiovascular Benefits." American Journal of Clinical Nutrition, 83(6): 1526-35.
6. Robert, O., 2005. "Practical Applications of Fish Oil ([Omega]-3 Fatty Acids) in Primary Care." The Journal of the American Board of Family Practice, 18: 28-36.
7. Nezhad, Z., M. Khosravi, N. Baniasadi and Z. Daneshvar, 2008. Omega-3 Fatty Acid Content in Various Tissues of Different Persian Gulf Fish. Iranian Cardiovascular Research Journal, 2(1): 24-31.
8. Connor, W.E., 1997. Are fish oils beneficial in the prevention and treatment of coronary artery disease? Am. J Clin. Nutr., 66(Suppl): 1020-1031.
9. O'Keefe, J.H. and W.S. Harris, 2000. From Inuit to implementation: omega-3 fatty acids come of age. Mayo. Clin. Proc., 75: 607-614.
10. Rosenthal, R.L., 2000. Effectiveness of altering serum cholesterol levels without drugs. Proc. Bayl. Univ. Med. Cent., 13(4): 351-355.
11. National Institute of Health/ NIH, 2008. "Omega-3 Fatty Acids, Fish Oil, Alpha-Linolenic Acid." nlm.nih.gov. Medline Plus: Herbs and Supplements. 1 Mar. 2008. National Institutes of Health. <http://www.nlm.nih.gov/medlineplus/druginfo/natural/patient-fishoil.html>.
12. Kris-Etherton, P.M. and S. Innis, 2007. American Dietetic Association and Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J. Am. Diet. Assoc., 107(9): 1599-1611.
13. Green, D.A., 1990. Lipid Metabolism in Fish, in Fish Oils in Nutrition, edited by Stansby, M.E., Van Nostrand Reinhold, New York, pp: 226-241.
14. Sikorski, Z.E., A. Kolakowska and B. Pan, 1990. The Nutritive Composition of the Major Groups of Marine Food Organisms, in Seafood: Resources, Nutritional Composition and Preservation, edited by Zdzislaw E. Sikorski, CRC Press Inc, pp: 29-54.
15. Heran, T. and L. Sgoutas, 1987. Polyunsaturated fatty acid and fat in fish flesh for selecting species for health benefits. J. Food Sci., 52: 1209-1211.
16. Tesfaye, W., 1998. Biology and management of fish stocks in Bahir Dar Gulf, Lake Tana, Ethiopia. PhD Thesis, Wageningen.
17. Nagelkerke, L.A. and F.A. Sibbing, 1996. Reproductive Segregation among the Large Barbus (Barubs intermiduius complex) of Lake Tana Ethiopia. Fish Biology, 49: 1251.
18. Dereje, S., B. Temesgen and M. Bayabil, 2003. Evaluation of Performance and Prospects of Lake Tana Transport Enterprise (DRAFT). Study report, Bahir Dar, Ethiopia, pp: 10-15.
19. Greenfield, H. and D.T. Southgate, 2003. “Sampling,” in Book Food Composition Data: Production, Management and Use, Food and Agriculture Organization of United Nations, Rome, Italy.
20. Ghaly, A.E., D. Dave, S. Budge and M.S. Brooks, 2010. Fish Spoilage Mechanisms and Preservation Techniques: Review. American Journal of Applied Sciences, 7(7): 859-877.
21. Bligh, E.G. and W.J. Dyer, 1959. “A rapid method of total lipid extraction and purification, “Canadian Journal of Biochemistry and Physiology, 37(8): 911-917.
22. Kinsella, J.E., J.L. Shimp, J. Mai and J. Weihrauch, 1977. “Fatty acid content and composition of freshwater finfish,” Journal of the American Oil Chemists Society, 54(10): 424-429.
23. AOCS Official Method, 2011-2012. “Direct Methylation of Lipids in Foods for the Determination of Total Fat, Saturated, cis-Monounsaturated, cis-Polyunsaturated and trans Fatty Acids by Gas Chromatography”, AOCS Official Methods and Recommended Practices, 2011-2012 Method Additions and Revisions.