

Full Length Research Paper

Minerals content and fatty acids profile of Nile tilapia (*Oreochromis Niloticus*) fillet from lake Zeway: Effect of endogenous factors

Alemu Lema Abelti

Oromia Agricultural Research Institute, Zeway Fisheries Resources Research Center,
Oromia Regional State, P.O.Box 229, Ethiopia.
E-mail:alemulema@yahoo.com

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This study was aimed to investigate the influence of age and sex on minerals content and fatty acids profile of Nile Tilapia (*Oreochromis niloticus*) fillet. Specimens were collected from Lake Zeway during January 2011. Samples were cool transported to Food Science and Nutrition laboratory of Addis Ababa University. The concentrations of minerals were analyzed using Flame Atomic Absorption spectrophotometer and Fatty acid profile was determined using gas chromatography. Female fish contained significantly ($p < 0.05$) higher calcium and phosphorus as compared to male. Calcium and zinc significantly ($p < 0.05$) decreased as age of fish increased but phosphorus significantly ($p < 0.05$) increased with increase in age of the fish. Sex had no significant effect ($p > 0.05$) on Iron and Zinc content. Regarding to fatty acid composition, monounsaturated fatty acids constituted the largest proportion of total unsaturated fatty acids in both sexes. Oleic acid was relatively higher in female than male. Conversely, the polyunsaturated fatty acids were higher in male as compared to female.

Key words: Age, sex, minerals content, fatty acids profile, *Oreochromis niloticus*.

INTRODUCTION

Fish is an excellent source of most of the minerals which the body needs to develop properly and perform its functions. Calcium and phosphorus (without which proper development of bones and teeth is impossible) occur in fish fillets in about the same quantities as in beef round (FAO, 2009). Although fish contain less iron than the amount found in red meat, iron in white fish is well absorbed and so is a useful source of iron. The soft bones of small fish such as sardines and smelts and canned varieties like salmon are especially valuable sources of calcium. Fish is a rich source of protein, fatty acids, and essential vitamins and minerals such as vitamin A, calcium, iron, zinc, and iodine. The vitamin A, calcium and iron found in small fish species are particularly bio available that is, easily absorbed by the body (Watanabe *et al.*, 1997). Fish absorb minerals not only from their diets but also from the surrounding water (Huss, 1995). Exogenous factors that affect fish body composition include the diet of the fish (composition, frequency) and the environment in which it is found (salinity, temperature). The main exogenous factor

affecting proximate composition is diet. Various studies have examined the effects of temperature, light, salinity, pH and oxygen concentration on the proximate composition of fish but these factors would seem to have very limited effects. On the other hand, endogenous factors are genetic and linked to the life stage, age, size, sex and anatomical position in the fish (Huss, 1995). These endogenous factors govern the majority of principles that determines the composition of fish (Huss, 1995). A remarkably variation in the lipid and fatty acid contents of the herbivorous fish, *O. niloticus*, was found in the five ethiopian study lakes (Zenebe *et al.*, 1998b). This study supports findings from temperate lake studies which show that the variability of fatty acid and lipids can be great both within and between species. It has been suggested by Huss (1995) that genetic variation, size of (P) Iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) all of which have low concentrations in sea water.

Fish body composition is affected by both exogenous and via their gills and skin. Fish endogenous factors ethiopian study lakes (Zenebe. *et al.*, 1998b). This study

supports findings from temperate lake studies which show that the variability of fatty acid and lipids can be great both within and between species. It has been suggested by Huss (1995) that genetic variation, size of fish, season, water, temperature, abundance and composition of the diet are possible factors affecting the quality of lipids and fatty acids in fish composition. So it is important to consider the phytoplankton in detail, because it is present in the diet of the fish in study (Zenebe *et al.*, 1998b). Therefore, in view of this fact, the present research was carried out to determine minerals contents and fatty acids of Nile Tilapia fillet.

MATERIALS AND METHODS

Description of the study area

Lake Zeway is the most northerly rift valley lakes of Ethiopia. The lake is located to 7° 52' to 8° 8' N latitude and 38° 40' to 38° 56' E longitude. It lies between East Shoa and Arsi Administrative zones of Oromia Region about 160 km due South of Addis Ababa, on the left side of Addis-Awassa highway. The altitude of Lake Zeway is 1636 meter above sea level. It is the shallowest of the rift valley lakes with maximum and mean depth 8.95 m and 2.5 m respectively. The lake has an open water area of 434 km² and shore line length of 137 km. It has a maximum length of 32 km and maximum width of 20 km. The lake contains five main Islands (Tullu guddo (4.8 km²), Tsedecha (2.1 km²), Debresina (0.3 km²), Funduro (0.4 km²) and Gelila (0.2 km²). The lake is fed by the two major rivers i.e. Meki and Katar and has one outflow in the south, the Bulbula river which flows into lake Abiyata. It is endowed with different kind of fish species like Nile Tilapia (*Oreochromis niloticus*) (Linnaeus 1758), African Catfish (*Clarias gariepinus*) (Burchell 1822), Crucian carp (*Carassius carassius*) (Linnaeus 1758), Ethiopia Barb *Labeobarbus intermidus* (Rüppell 1835), *Barbus paludinosus*, Zilli's cichlid *Tilapia zillii* (Gervais 1848) and *Gara* species.

Sample collection and preparation

Fresh fish was purchased from local fishermen at Bochessa, Korokonch and Menefesha landing sites of Lake Zeway. Live fish was transported to Zeway Fisheries Resources Research Center laboratory layered with flaked ice using ice box. Sex was identified by examining genital papilla located immediately behind the anus. In males the genital papilla has only one opening (the urinary pore of the ureter) through which both milk and urine pass (SRAC, 1999). In females the eggs exit through a separate oviduct and only urine passes through the urinary pore. Immediately after sex identification, length of fish was measured to the nearest 0.1 cm and

converted to age using Von Bertalanffy Growth Function (von Bertalanffy, 1938).

$$L_t = L_{\infty} [1 - e^{-k(t-t_0)}]$$

In the above Von Bertalanffy Growth Function equation constant parameters like L_{∞} , K and t_0 for female are 30.19cm, 0.25 per year and – 0.27 year respectively and for male are 30.81 cm, 0.27 per year and – 0.12 year respectively have been established (Demeke, 1998). To determine age, 19.08 cm and 22.10 cm for female 20.6 cm and 23 cm length for male were selected and inserted into the above Von Bertalanffy Growth Function. After sexes have identified and age was determined, composite of fish sample from the same age and sex was cleaned, descaled, eviscerated and filleted manually using sterile plastic knife. The fillet was oven dried at 60 °c for 72 hours then transferred into desiccators and cooled for 30 minutes. Dried fillet was grounded to 0.3 mm size using laboratory mill and the powder was stored in desiccators for further proximate composition analysis.

Minerals analysis (AOAC, 1998)

For minerals analysis, the ash was obtained from dry ashing of powdered fillet. The ash was wetted completely with 5 mL of 6 N HCl and dried on a low temperature hot plate. 7 mL of 3 N HCl was again added to the dried ash and heated on the hot plate until the solution just boils. The ash was cooled to room temperature in a hood and filtered into a 50 mL graduated flask using a 125 mm filter paper (Whatman 42). 5 mL of 3 N HCl was added to each crucible dishes and heated until the solution just boils, cooled, and filtered into the flask. The crucible dishes were again washed three times with deionized water; the washing was filtered into the flask. 2.5 mL 10% lanthanum chloride solution was added into each graduated flask. Then the solution was cooled and diluted to 50 mL with deionized water. A blank which contains 12 mL of 3N HCl and deionized water in 50 mL volumetric flask was prepared. Four series of working standard metal solutions were prepared by appropriate dilution of the metal stock solutions (nitrate of the metal) with deionized water containing 2.4 mL 3 N HCl in 10 mL volumetric flask. Calibration graph (concentration *versus* absorbance) for each element using the prepared standard solutions was prepared. The sample concentration was analyzed using Flame Atomic Absorption Spectrophotometer (Varian Spectra AA-20 Plus, Varian Australia Pty., Ltd, Australia). A single mineral hollow cathode lamp was used for each element. Series of working standards solutions for iron (0.00, 2.00, 6.00, 10.00, 12.00), for zinc (0.00, 0.60, 1.00, 1.40, 1.80) and calcium (0.00, 1.00, 1.50, 2.50, 3.00) concentrations of standard (µg/ mL) were prepared. Reading was taken from the graph, which illustrated the metal concentrations that correspond to the absorption values of the samples, and the blank. The metals contents were calculated by using

Table 1. Mean±SE minerals contents of Nile Tilapia (*O. niloticus*) fillet in mg / 100 g in fresh weight.

Parameter		Phosphorus (P)	Iron (Fe)	Zinc (Zn)	Calcium (Ca)
Sex	Male	16.5 ± 0.06 ^b	1.61 ± 0.02 ^a	0.5 ± 0.01 ^a	30.5 ± 0.10 ^b
	Female	17.2 ± 0.07 ^a	1.62 ± 0.02 ^a	0.5 ± 0.01 ^a	35.0 ± 0.10 ^a
Age	Four	16.4 ± 0.07 ^y	1.63 ± 0.02 ^x	0.6 ± 0.01 ^x	38.2 ± 0.10 ^x
	Five	17.1 ± 0.07 ^x	1.60 ± 0.02 ^x	0.5 ± 0.01 ^y	27.3 ± 0.10 ^y
Sex*Age		Sig.	Sig.	Non Sig.	Sig.

At each level of parameters, values with different superscripts in the same column are significantly different ($p < 0.05$). NS: Not significant, Sig: significant.

the following formula:

$$\text{Metal content (mg/100 g)} = \frac{(A - B) \times V}{10W}$$

Where:

W= Weight of the sample (g)

V=Volume of the extract (mL)

A= Concentration ($\mu\text{g} / \text{mL}$) of sample solution

B= concentration ($\mu\text{g} / \text{mL}$) of blank solution

Phosphorous was determined by the colorimetric method using ammonium molybdate. Phosphorous was converted to phosphomolybdate, which was reduced to a blue molybdenum compound by amino naphtholsulphonic acid to give a blue molybdenum compound. A sample solution was obtained from mineral analysis (Fe, Zn and Ca). 1 mL of the clear extract was taken from the sample solution and diluted to 100 mL with deionized water in a 100 mL volumetric flask. A 5 mL (duplicates) of the sample dilution was added into test tubes. A 0.5 mL of molybdate and a 0.20 mL amino naphtholsulphonic acid was added into the test tube (sample solution) and mixed thoroughly step by step. A 0.20 mL amino naphtholsulphonic acid was added into the test repeatedly each time until the solution becomes clear. The solution was allowed to stand for 10 minute. The absorbance (reading A) of the solution was measured at 660 nm against distilled water. Simultaneously with sample phosphorous, standard and blank analysis were carried out. Standard and blank solutions were prepared as above but 5 mL of working standard and 5 mL of deionized water (reading B) in place of the sample dilution were used respectively. A standard curve was made from absorbance versus concentration. Phosphorous was calculated using the following formula:

$$\text{P mg 100 g} = \frac{(A-B) \times 50 \times 100}{\text{Slope} \times W \times 10}$$

Where: A=Reading of the sample solution

B= Reading of the blank solution

W=Weight of fresh sample

Fatty acids profile of Nile Tilapia fillet

Lipid Extraction

The procedure of Folch *et al* (1957) was used for lipid extraction. For each category, 20 g of dried sample was mixed with 250 mL of chloroform–methanol- distilled water in the ratio of (8:4:3). The mixture was filtered through Whatman No.1 filter paper on Erlenmeyer flask and the filtrate was collected, shaken and transferred to a separatory funnel. 15 mL of physiological saline solution (0.58 % NaCl) mixture was added to separate methanol phase from chloroform layer (containing lipid) and allowed to stand for phase separation (biphasic system formation). The two phases were collected separately. The upper phase containing high proportions of methanol and water was discarded. The chloroform phase was drained off into Erlenmeyer flask. The chloroform was evaporated at 55 °C using rotavapor (Heinz Eich GmbH model) from the mixture. The pure oil was transferred into small vial from bottom rounded flask using Pasteur pipette.

Preparation of fatty acid methyl esters

Most of the fatty acids found in food lipids are covalently bound to an alcohol (glycerol) via ester bonds. Therefore, the complex lipids must be split off and converted into derivatives with lower boiling points such as alcoholic esters to be analyzed by Gas chromatography. Methyl ester of fatty acids was prepared for subsequent use in Gas Chromatography. Fatty acid methyl ester (FAME) was prepared according to alkaline based transesterification. 0.5 g of extracted lipid was saponified by 6.6 mL of 2 M Potassium methoxide (5.6 g KOH in 0.05 L methanol) followed by addition of 40 mL n-hexane. Fatty acid methylester was extracted in n-hexane and preserved at -18 °C prior to analysis. Layer containing

Table 2. Percentage fatty acid composition of Nile Tilapia (*O. niloticus*) fillet from Lake Zeway.

		14:0	16:0	18:0	21:0	ΣSAFA	18:1ω9	ΣMUFA	18:2ω6	18:3ω3	ΣPUFA	Σunid.
Sex	Age											
Male	4	0.6	26.1	3.7	14.9	45	13.9	13.9	5.2	11	16.2	24.9
	5	2.5	24.2	5.8	7.1	39.6	23.9	23.9	5.6	8.9	14.5	22
Female	4	2.5	25.5	4.1	10.5	42.6	34.9	34.9	6.5	8.3	14.8	7.7
	5	3.7	27.1	5.5	5.4	41.7	24.1	24.1	5.4	9.3	14.4	19.5

Note 14:0= Myristic acid
 16:0= Palmitic acid
 18:0 = Stearic acid
 21:0= Heneicosanoic acid
 SAFA=Saturated fatty acids
 18:1ω9=Oleic acid
 18:2ω6=Linoleic acid
 18:3ω3=Linolenic acid
 SAFA= Saturated fatty acids
 MUFA=Monounsaturated fatty acid
 PUFA=Polyunsaturated fatty acids

non-methylated lipids was discarded. The tube was shaken for 5 minutes then 2 g of Calcium chloride (CaCl₂) was added to dehydrate FAME if any water may exist. n-hexane was removed from FAME using rotavapor.

Determination of fatty acids composition

The fatty acid methyl esters were analyzed by Dani Italia (GC- 1000) gas chromatography with a flame ionization detector (FID) and capillary column (30 m length X 0.32 mm internal diameter). The oven temperature was 50 °C, held 2 min, raised to 250 °C at a rate of 4 °C / min and detector temperature was 260 °C. The carrier gas used was nitrogen. The injected sample volume was 0.3 μL. The fatty acids methyl esters were identified by comparing the retention time of the samples and appropriate fatty acids methyl esters standards (Ackman, 1969). Retention time and peak areas were recorded with the help of clarity chromatography soft ware. Fatty acid content was analyzed on once hence reported as percentage of total fatty acids.

RESULTS AND DISCUSSIONS

Phosphorus and Calcium contents

From [Table 1](#), age, sex and the interaction effect of age and sex significantly ($p < 0.05$) affected phosphorus and calcium contents. Phosphorus significantly ($p < 0.05$) increased from 16.4 mg / 100 g to 17.1 mg / 100 g and calcium decreased significantly ($p < 0.05$) from 38.2 mg / 100 g to 27.3 mg / 100 g with the increase of fish age from four to five years. Female fish contains higher

phosphorus (17.2 mg / 100 g) and calcium (35.0 mg / 100 g) than male phosphorus (16.5 mg / 100 g) and calcium (30.5 mg / 100 g). Arannilewa *et al.* (2005) reported the average phosphorus content of Nile Tilapia as 15.32 mg / 100 g which is very similar with the present study and higher calcium contents (61.67 mg / 100 g). Amer *et al.* (1991) reported that there is higher phosphorus (0.09 %) in female than male (0.086 %).

Iron content

The Iron content of fish muscles was not affected ($p > 0.05$) by both age and sex but the interaction effects of age and sex significantly ($p < 0.05$) affected iron content. Iron content was slightly higher in female (1.62 mg / 100 g) than in male (1.61 mg / 100 g). Iron content was decreased with increase of age. Petenuci *et al.* (2008) reported 1.3 mg/100 g of iron in Nile Tilapia. The major factors influencing iron absorption in fish are the proportion of organic and inorganic components of the diet, the amount of feed ingested and the conditions of the digestive tract of fish (Watanabe *et al.*, 1997).

Zinc content

Age significantly ($p < 0.05$) affected zinc contents where as sex and the interaction effect of age and sex has no significant ($p > 0.05$) effect on zinc content. Zinc content has decreased from 0.6 mg / 100 g to 0.5 mg / 100 g with the increase of fish age. The absorption and amounts of zinc in fish may be affected by the chemical form of Zinc in the diet, the source of protein and the presence of other dietary components such as Calcium, Phosphorus and Phytic acid (Watanabe *et al.*, 1997).

Fatty acids profiles

From **Table 2**, seven different fatty acids were identified. There were several unidentifiable peaks which were sum up and marked as Σ unidentified fatty acids for which standard is not available. Total fatty acid was calculated from the total integrated areas of all fatty acids in the chromatograms. Irrespective of age and sex, fatty acids identified from Nile Tilapia fillet were Myristic, Palmitic, Stearic, Oleic, Linoleic, Linolenic and Heneicosanoic. The fatty acids composition of fish tissue can be affected by diet, size, age, reproductive cycle, salinity, temperature, season and geographical location (Zenebe, 1998a; Zenebe, 2010).

Saturated fatty acids

From **Table 2**, the percentage of saturated fatty acids was 45 % and 39.6 % in four and five year male fish and 42.6 % and 41.7 % in four and five year female fish. The most abundant saturated fatty acids in animal and plant tissues are straight-chain compounds with 14, 16 and 18 carbon atoms. Myristic acid (14:0) is a ubiquitous component of lipids in most living organisms. Palmitic acid (16:0) is usually considered the most abundant saturated fatty acid in nature, and it is found in appreciable amounts in the lipids of animals, plants and lower organisms. It comprises 20-30 % of the lipids in most animal tissues, and it is present in amounts that vary from 10 to 40 % in seed oils (The AOCS Lipid library, 2011). As reported by Ackman (1969), palmitic acid as key metabolite not influenced by diet in fish, was the saturated fatty acids found in higher concentration (Satue and Lopez, 1996). Stearic acid (18:0) is the second most abundant saturated fatty acid in nature, and again it is found in the lipid of most living organisms (The AOCS Lipid library, 2011). Akpinar *et al.* (2009) reported that the major saturated fatty acids in male and female salmon trutta was palmitic acids. Study conducted by Suloma *et al.* (2008) shown that the predominant saturated fatty acids in Nile Tilapia was palmitic.

Monounsaturated fatty acid

The percentage of monounsaturated fatty acid was 13.9 % and 23.9 % in four and five year male fish and 34.9 % and 24.1 % in four and five year female fish. Oleic acid is the main monounsaturated fatty acids common to both sexes with higher concentration in female than male. Satue and Lopez (1996) reported oleic acid was higher in female rainbow trout as compared to male. Monounsaturated fatty acids constituted the largest proportion of total unsaturated fatty acids in both sexes.

Polyunsaturated fatty acids

The percentage of polyunsaturated fatty acids were 16.2 % and 14.5 % in four and five year male and 14.8 % and 14.4 % in four and five years female fish. Relatively similar average results were reported by various renowned researchers (Justia *et al.*, 2003; Petenuci *et al.*, 2008; Stevanato *et al.*, 2008; Suloma *et al.*, 2008; Zenebe, 2010; Jabeen and Chaudhry, 2011). The Polyunsaturated fatty acids was higher in male rainbow trout as compared to female for the similar age but the vice versa is true for monounsaturated fatty acids (Satue and Lopez, 1996) was reported. The lower polyunsaturated fatty acids in female may related to egg formation (Satue and Lopez, 1996). Fatty acids contents of lipid in fish are generally reported to vary considerably, both within and between species (Ackman, 1989; Ahlgren *et al.*, 1994; Zenebe, 1998a). A pronounced difference in the fatty acid composition of *O.niloticus* fillet is due to differences in maturation processes that exist between the two sexes (Biró *et al.*, 2009).

CONCLUSION

From the present study it can be concluded that, there is variation between age and sex of Nile Tilapia with regard to minerals contents and fatty acid profile. Eating fish with high contents of polyunsaturated fatty acids is believed to be important for human health in reducing the risk of cardiovascular disease and diabetes. In this case male Nile Tilapia is good source of polyunsaturated fatty acid. Female fish contains higher monounsaturated fatty acids than males.

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