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Evaluation of the physicochemical and nutritional characteristics of carp fish (*Tilapia nilotica*) from Lake Fitri in Chad

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Abstract

The carp fish (*Tilapia nilotica*) from lake Fitri in Chad is of significant commercial and economic interest both nationally and sub-regionally. However, the nutritional and physicochemical characteristics of this fish are not sufficiently documented. Thus, the present study aimed to carry out a nutritional and physicochemical characterization of the carp fish of lake Fitri. For this purpose, 100 fresh fish samples and 100 dried fish samples were used for the analyses. The water content of the fish samples was determined by drying in an oven and then the dry matter was deducted. The total ash content was quantified using the AOAC method. Quantification of total proteins was done by the Kjeldahl method. The lipid content was determined by the Soxhlet method. In addition, eight fresh fish samples were used for analysis of the amino acid composition of fish proteins by high-performance liquid chromatography (HPLC). The results obtained during this study show that the dry matter contents of fresh and dried fish were on average 20.79±2.84% and 28.12±1.79% respectively. The lipid and ash contents, relative to dry matter, were similar for the two types of fish with respective averages of 22.15±2.79% and 11.44±0.55% for fresh fish, 21.45±2.69% and 10.48±1.73% for dried fish. However, the average protein content was relatively higher in fresh fish with an average of 53.74±0.65% compared to 50.65±4.67% for dried fish. This study also revealed the presence of seventeen (17) amino acids with varying proportions in the fresh fish analyzed, including most of the essential amino acids. Thus, carp fish from lake Fitri could be used as a suitable food to improve the nutrition and growth of children.

Keywords: *Tilapia nilotica* ; Characteristics physico-chemistry; Nutrition; Lake Fitri; Chad

1. Introduction

Global capture fisheries production has reached a record high of around 179 million tonnes today. Indeed, 156 million tonnes were used for human consumption, which is equivalent to an annual supply estimated at 20.5 kg per person. Fishing contributes to the survival of populations living near rivers and lakes by providing them with additional food and monetary income. It also creates a significant number of jobs in fish processing and marketing (Kien et al., 2018). The populations living around lake Fitri in Chad in the Sahelian zone practice this activity in parallel with agriculture and livestock breeding (Ahidjo, 2010 ; Lemoalle, 2014). The FAO estimates that fish constitutes for 22% of the protein

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diet in sub-Saharan Africa (FAO, 2020). It is also the most physically and financially accessible form of product for poor households in urban and peri-urban areas. Fish is a highly appreciated food commodity all over the world for its taste value and its nourishing qualities (Dergal, 2015). It is a food of high nutritional value, but also a valuable supplement in diets poor in proteins, vitamins and essential mineral salts (Oulaï et al., 2007) and does not suffer from socio-cultural prohibition (Akilimali Itongwa et al., 2019). Fish is one of the most important sources of animal protein in the diet of populations (Assogba et al., 2018). It is essentially rich in proteins of good biological value, minerals and essential fatty acids (Chabi et al., 2014). It is very digestible and contains essential amino acids such as: lysine, leucine, valine, arginine, methionine, tryptophan and histidine (Gómez-Requeni et al., 2003 ; ANSES, 2010). It also contains polyunsaturated fatty acids of the omega-3 series, precursors of prostanoids with an antithrombotic effect (Oladipo et Bankole, 2013). Its consumption is therefore beneficial for protection against cardiovascular diseases and other nutritional diseases (Brito et al., 2021). Tilapia is one of the most widely produced and consumed fish in the world (Lazard, 2009). It represents more than 30% of animal protein consumption in the majority of African countries including Chad (FAO, 2005). Like other fish, its nutrient content and composition varies with age, sexual cycle and environmental factors such as water temperature and salinity (Corraze and Kaushik, 1999). In addition, the drying process impacts overall nutritional quality. During drying, the surface of the fish dries more quickly and hardens, thereby locking moisture inside, which promotes protein degradation and fatty acid oxidation (Abdoullahi et al., 2018). Therefore, it is essential to regularly study the nutritional characteristics of fish in order to be able to assess their nutritional quality. So, this study was undertaken with the objective to contribute to the valorization of cap fish from lake Fitri through an evaluation of its physicochemical and nutritional characteristics.

2. Materials and methods

2.1. Sampling site

Lake Fitri, located between 12°42'30" and 13°2'0" North latitude and between 17°26'0" and 17°57'30 East longitude, covering an area of 2088 km2, served as the sampling site for this study. It is located in the center of Chad, in the province of Batha, department of Fitri, which the capital is Yao. It is located approximately 300 km east of N'Djamena and is one of the two main water reserves in Chad (Magrin et al., 2009). Figure 1 shows the location of lake Fitri.

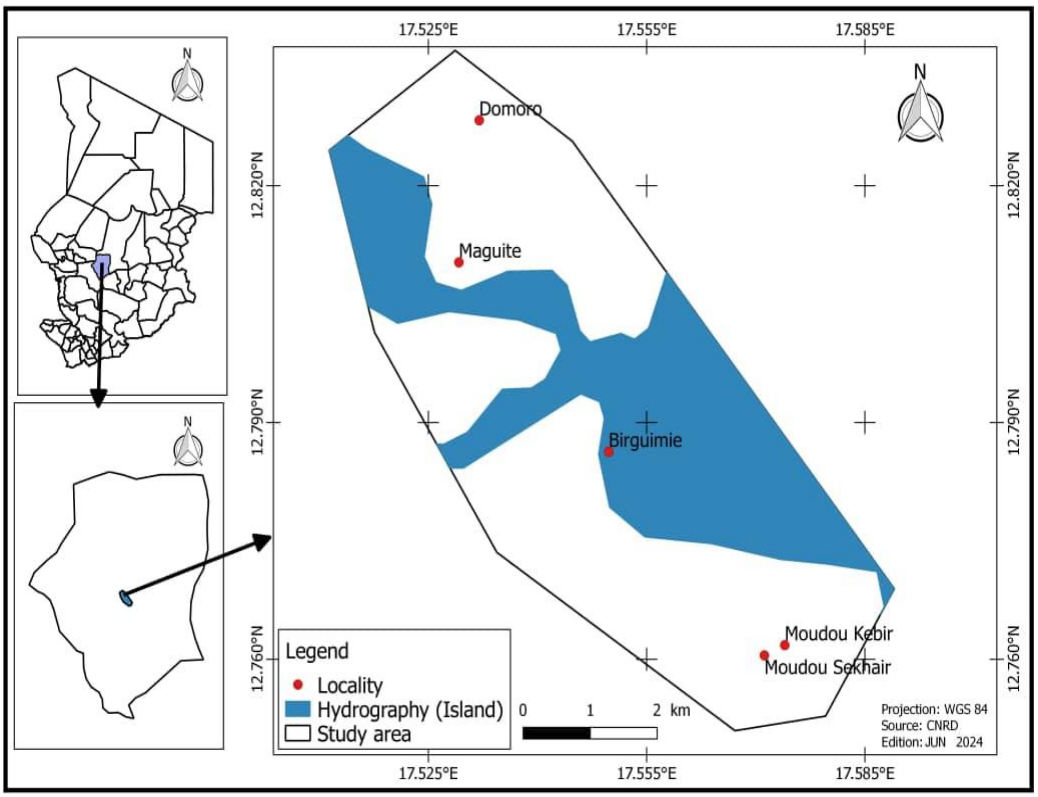

Figure 1 Location of the sampling site

2.2. Sampling

From their place of capture (lake Fitri) which is 300 km from the capital N'Djamena, the fish were collected, put in bags then in a cooler containing ice and transported to the laboratory. In the laboratory the fish were placed in a freezer at -20 for a brief period (at most one night). The fish thus conditioned was used for the various analyses. Samples of dried fish were also taken from residents of the lake. A total of 100 fresh fish samples and 100 dry fish samples were collected for the study.

2.2.1. Determination of water and dry matter content

The water content was determined by the difference in the weight of each sample before and after passing it through an oven (brand DL53, model VWR. 12.11745) at 105°C until a constant weight was obtained according to the AOAC method (1990). For this, a test portion of 5g (PE) of each type of fish (dry and fresh) was used. The following formula was used for calculating the water content

$$\mathbf{WC}\% = \frac{\mathbf{Pe} - (\mathbf{Pf} - \mathbf{Po})}{\mathbf{PE}} \times \mathbf{100}$$

- WC: Water content (%)
- Po: Empty weight of the nacelles
- PE: Test portion
- Pf: Final weight (nacelle + test sample).
- The dry matter (DM) of the different samples was deduced from the following formula:
- % DM = 100 - % WC

2.2.2. Determination of total ash content

After determining the dry matter content, the fish samples were crushed in a porcelain mortar. Total ash was quantified according to the AOAC 923.03 (1997) method. Test portions of 5g of each sample were placed in porcelain crucibles then introduced into a muffle furnace brand L15/11/B180, Model Nebertherm, series 247900. These crucibles were heated to 550°C for 24 hours, removed using tongs and then cooled in a desiccator for approximately 1 hour before being weighed. The ash content (AC) was calculated according to the formula below

$$\boldsymbol{AC}\ (\%)\ = \frac{((\boldsymbol{P}\,\mathbf{3} - \ \boldsymbol{P}\mathbf{1})\ \boldsymbol{x}\ \mathbf{100})}{(\boldsymbol{P}\,\mathbf{2} - \ \boldsymbol{P}\mathbf{1})}$$

With

- AC (%): Ash content of fish samples
- P1: Weight of the empty crucible
- P2: Weight of the crucible and the sample
- P3: Weight of the crucible and ashes

2.3. Protein quantification

Quantification of total proteins in fish samples was done using the Kjeldahl method according to standard NF EN ISO 20483-(2014). Thus, for each sample, a test portion of 0.38 g of fish meal is combined with 9.7801g of concentrated sodium sulfate (Na_2SO4) and 0.6003g of copper sulfate ($CuSO_4$). Then, 20ml of Sulfuric Acid (H_2SO_4) 98% was added to the previous mixture for mineralization. The ammonia was released by the addition of a concentrated 32% sodium hydroxide solution. It was then distilled using an automatic distiller and then collected in a boric acid solution with a concentration of 40g/l. After this step, the ammonia was titrated directly using a sulfuric acid solution (0.1N) for the blank (control) and the test portions. Under these conditions, the % of protein in the sample was obtained by multiplying the % of nitrogen by a conversion factor and using the following formula

$$\boldsymbol{Protein}\ \% \ = \frac{\mathbf{14.007}\ (\ \mathbf{V1} - \mathbf{V0}\ )\ \mathbf{X}\ \ \mathbf{C}\ \mathbf{X}\ \mathbf{6.25}}{\boldsymbol{PE}}$$

- PE: mass of the test portion in grams
- C: Exact concentration in mol/l of the sulfuric acid solution
- V0: volume of acid used for the blank in milliliters
- V1: volume of acid used for the test portion in milliliter

- 6.25: conversation coefficient of total nitrogen in proteins

2.3.1. Determination of fat content

The lipid content was determined by the soxhlet method with a Bchr brand device, model SMA6, series 111373 according to the AOAC method (1990). The solid sample was crushed then dried in an oven for one hour at a temperature of 105°C. Then, a test portion of 5 g of each dried sample was weighed and then introduced into an extraction capsule. This capsule was connected to an extraction tube topped with a refrigerant connected to the running water tap. Approximately 120 ml of solvent (petroleum ether) was poured into a flask connected to the soxhlet extraction tube. The extraction device thus assembled was maintained at a temperature higher than the vaporization temperature of the solvent (105°C). The extraction was done for 8 hours. At the end of the extraction, the final mass of the flask was weighed. The total lipid content (TLC) expressed as a percentage relative to dry matter was determined according to the following formula

$$\mathbf{TLC}\% = \frac{(\mathbf{M\,2} - \mathbf{M1}) * \mathbf{100}}{\mathbf{PE}}$$

- TLC%: Fat content
- M1: Mass of the empty flask
- M2: Flask mass containing the fat
- PE: Sample mass (test portion)

2.4. Determination of amino acids in proteins from fresh fish samples

High-performance liquid chromatography (HPLC) was used for the detection and determination of amino acids. The analysis of the amino acid profile of the 8 fresh fish samples was carried out according to the following steps (Bidlingmeyer et al., 1984 ; Reddy et al., 1986):

2.4.1. Hydrolysis

After delipidation, 0.4 g of each sample was weighed exactly, dried under vacuum and then hydrolyzed using 15 ml of 6 N hydrochloric acid. The hydrolyzed sample was then taken up with 50 ml of mili-Q water. The solution was then incubated at 105 °C for 24 hours, filtered with a 0.45 µm millipore filter and collected in tubes.

2.4.2. Preparation of eluent A

19.0 g of sodium acetate trihydrate were weighed and diluted with 1000 mL of ultrapure water in a volumetric flask then mixed with 0.5 mL of triethylamine (TEA). Then, the pH of the solution was adjusted to 6.4 using acetic acid. The solution was filtered with a 0.45 µm pore membrane and stored in the refrigerator.

2.4.3. Preparation of eluent B

710 mg of disodium hydrogen were weighed and introduced into a solution containing 600 ml of acetonitrile and 400 ml of ultra-pure water. The solution obtained is mixed with 10% phosphoric acid and the pH adjusted to 7.4. The mixture was filtered with a 0.45 µm pore membrane and stored in the refrigerator.

2.4.4. Drying, derivatization and injection

Using a micropipette, 10 µL of hydrolyzed sample and 05 µL of the standard amino acid solution were taken. Then, these samples are dried under vacuum for 15 minutes. After drying, 20µL of derivatization solution was added. The mixture was incubated at room temperature for 20 minutes and then dried under vacuum for 45 minutes. The injection was done with 4 µL of the solution obtained after diluting the dried mixture with 100 µL of HPLC diluent. The injector temperature was set at 38 °C and the injection rate was 1-1.5 mL/min. A Hewlett-Packard type chromatograph, model HP 1050, was used. The separation of the amino acids was carried out using two columns in series, of the Lichrocart 125-4 cartridge type containing a Lichrospher 100 RP18 column. The length of each of these columns is 10 cm and the particle diameter is 5 µm. A pre-column of the same type was placed at the start of each column. The o-phthaldialdehyde (OPA) "precolumn" derivatives of these substances were detected using a Jasco spectrofluorimeter, model 821-FP. The device is also equipped with a computerized system for data acquisition and calculation. Each analysis was done in duplicate. Tables 1 and 2 present, respectively the reaction mixtures used for the determination of amino acids and the composition of the standard amino acid solution.

Table 1 Reaction mixture used for the determination of amino acids

Drying solution	**For 1 sample (µl)**
Mili-Q water	4
Triethylamine (TEA)	4
Phenylisothiocyanate (PITC)	2
Derivatization solution	For 1 sample (µl)
Ethanol	14
Mili-Q water	2
Triethylamine (TEA)	2

An amino acid standard solution (Thermo Scientific Pierce Amino Acid Standard H) consisting of 17 amino acids at different concentrations (g/mol) was used during the analyses. The standard was diluted with milli-Q water (Table 2) to obtain the desired concentration.

Table 2 Composition of standard amino acid solution

Amino acids	**Molar mass (g/mol)**	**Concentration after dilution (66 µL. 88.5p mol)**
Asp	132.11	0.002752
Glu	147.13	0.003065
Ser	105.09	0.002189
gly	75.07	0.001563
His	155.16	0.003232
Arg	174.2	0.003629
Thr	119.12	0.002481
Aln	89.10	0.001856
Pro	115.13	0.002398
Tyr	181.19	0.003774
Val	117.15	0.002440
Met	149.21	0.003108
Ile	131.17	0.002732
Leu	131.17	0.002732
Phe	165.19	0.003441
Lys	146.19	0.003045
cys	121.16	0.001262

2.5. Statistical analyzes

XLSTAT pro 2019.2.259614 software was used for the statistical analysis of certain data. The comparison of the means of the different parameters was made using the Tukey test at the significant threshold of p=0.05. Principal Component Analysis (PCA) was carried out on a data matrix consisting of the 8 fish samples used for the search for amino acids. The Pearson correlation matrix (n) between the fish sample variables was also determined.

3. Results and discussion

3.1. Characteristics of fresh fish

The physicochemical and nutritional characteristics obtained from the fresh fish samples are recorded in Table 3. For the 100 samples, a slight variation in dry matter content is observed with an average of 20.79±1.54%. The water and lipid contents seem more variable with respective averages of 79.84±2.84% and 22.15±2.79% (DM). For these same samples, the ash and protein contents varied very little with respective averages of 11.44±0.55% and 53.74±0.65%.

Table 3 Characteristics of fresh fish from lake Fitri

Variable	Minimum	Maximum	Average
Water content	72.00%	89.00%	79.84±2.84%
Dry matter	10.20%	27.99%	20.79±1.54%
Ahs (%DM)	10.01%	12.88%	11.44±0.55%
Protein (%DM)	52.53%	54.80%	53.74±0.65%
Lipid (%DM)	15.56%	25.77%	22.15±2.79%

3.2. Characteristics of dried fish

The physicochemical and nutritional characteristics of the dried fish samples used during this study are presented in Table 4. For these fish samples, the characteristics that varied the most between the different samples were the water content with an average of 71.08±4.48% and protein content with an average of 50.65±4.60%. The other characteristics varied less significantly. Thus, the average contents of dry matter, ash and lipids were respectively 28.12±1.79%, 10.48±1.73% and 21.45±2.69%.

Table 4 Characteristics of dried fish from lake Fitri

Variable	Minimum	Maximum	Average
Water content	60.00%	88.00%	71.08±4.98%
Dry matter	11.75%	39.88%	28.12±1.79%
Ash (%DM)	5.11%	12.88%	10.48±1.73%
Protein (%DM)	42.16%	54.80%	50.65±4.67%
Lipid (%DM)	15.56%	25.77%	21.45±2.69%

Variations in characteristics among different fish samples could be explained by the fact that the samples come from different locations in the lake where the fish are not treated in the same way. This observation has already been made by Latifou et al. (2019) who showed that the nutritional quality of fish depended on certain criteria including geographical location. The water contents of the fish used during this study are similar to those reported by Latifou et al. (2019) and by Brito et al. (2021) which ranged from 60 to 81.6% and 69.3 to 86.31% respectively for different fish species.

We also noted during this study, an average ash rate which indicates the abundance of mineral matter in the fish. The ash contents obtained during this study show that the fish samples are highly mineralized in comparison with the fish ash contents reported by and Brito et al. (2021) which were respectively from 1.13% to 3.93% for imported fish samples and from 1.34% to 3.76% for local fish.

The average water content of fresh fish is higher than that of dried fish. This is explained by the fact that drying leads to a significant loss of water and thus improves the conservation of the fish. The average ash and lipid contents are relatively similar for the two types of fish. This similarity in proportion is linked to the fact that these two parameters are expressed as a percentage relative to dry matter. However, the average protein content is slightly higher in fresh

fish, which could be explained by protein degradation during drying (Abdoullahi et al., 2018). Thus, fish being generally used as a source of protein, the fresh form should be consumed as much as possible.

3.3. Amino acid composition of fresh fish

The search for amino acids in fresh fish allowed to detect seventeen (17) amino acids at different concentrations. The results obtained (expressed in mg/100g DM) are presented in Table 5. In general, glutamic acid was the most abundant amino acid in fresh fish with average contents ranging from 1.65±0.28% to 3.75±0.30%. Cysteine was the least present amino acid with contents varying between 0.01±0.00% and 0.07±0.03%.

Principal Component Analysis (PCA) was carried out on a data matrix consisting of 8 fish individuals used for amino acid analysis. The result obtained is presented in Figure 2. The analysis noted that the two axes F1 and F2 report 98.99% of the information on the samples analyzed including 97.61% for the F1 axis and 1.38% for the F2 axis.

The Pearson correlation matrix between fresh fish variables is represented by Table 6. Examination of the correlation matrix between variables reveals the presence of a first set of variables, made up of descriptors correlated with each other (moderately significant correlations). The two-way ANOVA carried out on the amino acid contents of fresh fish revealed through the Pearson test a significant difference (at P<0.001) between the different samples.

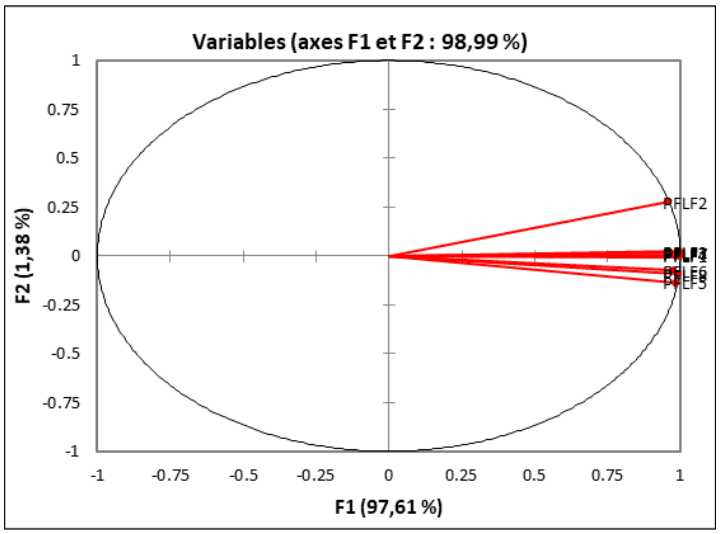

Figure 2 Principal component analysis of individual variabilities of fresh fish samples

Table 5 Amino acid contents of fresh fish

AA	PFLF1	PFLF2	PFLF3	PFLF4	PFLF5	PFLF6	PFLF7	PFLF8
Asp	1.64±0.17%	0.83±0.13%	1.52±0.17%	1.23±0.09%	2.07±0.10%	1.35±0.09%	1.32±0.07%	1.41±0.03%
Glu	3.28±0.36%	1.65±0.28%	2.92±0.31%	2.26±0.14%	3.75±0.30%	2.63±0.17%	2.58±0.13%	2.64±0.08%
Ser	0.63±0.067%	0.34±0.06%	0.60±0.07%	0.49±0.03%	0.78±0.06%	0.52±0.02%	0.49±0.03%	0.53±0.01%
Gly	1.08±0.11%	0.88±0.15%	1.03±0.12%	0.84±0.06%	0.89±0.06%	0.89±0.03%	0.94±0.05%	0.75±0.00%
His	0.42±0.03%	0.19±0.04%	0.37±0.04%	0.29±0.02%	0.50±0.04%	0.30±0.03%	0.31±0.01%	0.30±0.03%
Arg	1.00±0.08%	0.53±0.09%	0.96±0.10%	0.77±0.04%	1.23±0.06%	0.88±0.04%	0.81±0.04%	0.84±0.03%
Thr	0.88±00.09%	0.38±0.08%	0.79±0.10%	0.65±0.05%	1.09±0.09%	0.69±0.04%	0.67±0.04%	0.73±0.01%
Ala	1.34±0.14%	0.88±0.15%	1.21±0.14%	0.97±0.07%	1.40±0.10%	1.07±0.05%	1.05±0.05%	1.02±0.04%
Pro	1.07±0.11%	0.76±0.13%	0.99±0.11%	0.79±0.05%	1.48±0.11%	1.26±0.07%	0.88±0.05%	0.82±0.02%
Tyr	0.64±0.06%	0.28±0.07%	0.56±0.08%	0.49±0.03%	0.81±0.08%	0.52±0.03%	0.46±0.03%	0.53±0.01%
Val	1.02±0.09%	0.55±0.11%	0.89±0.13%	0.72±0.05%	1.12±0.13%	0.80±0.04%	0.71±0.04%	0.76±0.02%
Met	0.71±0.07%	0.32±0.08%	0.61±0.11%	0.52±0.04%	0.81±0.12%	0.59±0.03%	0.48±0.07%	0.51±0.07%
Cys	0.04±0.00%	0.03±0.02%	0.03±0.01%	0.03±0.00%	0.07±0.03%	0.05±0.00%	0.01±0.00%	0.02±0.00%
Ile	0.79±0.12%	0.42±0.07%	0.69±0.04%	0.57±0.05%	0.91±0.05%	0.63±0.07%	0.57±0.01%	0.63±0.00%
Leu	1.41±0.12%	0.77±0.015%	1.25±0.19%	1.02±0.06%	1.72±0.18%	1.13±0.02%	1.04±0.07%	1.14±0.05%
Phe	0.79±0.09%	0.44±0.08%	0.67±0.08%	0.56±0.04%	0.88±0.07%	0.63±0.03%	0.59±0.04%	0.65±0.02%
Lys	1.42±0.06%	0.69±0.12%	1.28±0.15%	1.01±0.08%	1.93±0.05%	1.19±0.06%	1.09±0.07%	1.19±0.03%

Legend: AA: amino-acid; Asp: Aspartic acid; His: Histidine ; Pro : Proline ; Cys : Cystine ; Lys : Lysine ; Glu : Acid glutamique ; Arg : Arginine ; Tyr : Tyrosine ; Ile : Isoleucine ; Ser: Serine ; Thr : Threonine ; Val : Valine ; Leu : Leucine ; Gly : Glycine ; Ala : Alanine Met : Methionine ; Phe : Phenylalanine ; T.M.E : Total Moyenne standard deviation

Table 6 Correlation matrix between fish variables Tilapia nilotica from lake Fitri

Variables	PFLF1	PFLF2	PFLF3	PFLF4	PFLF5	PFLF6	PFLF7	PFLF8
PFLF1	1	0.954*	0.998**	0.997*	0.983*	0.959*	0.995*	0.993*
PFLF2	0.954*	1	0.962*	0.957*	0.910*	0.920*	0.961*	0.925*
PFLF3	0.998*	0.962*	1	0.998*	0.982*	0.959*	0.997*	0.991*
PFLF4	0.997*	0.957*	0.998*	1	0.982*	0.963*	0.998*	0.994*
PFLF5	0.983*	0.910*	0.982*	0.982*	1	0.961*	0.977*	0.990*
PFLF6	0.959*	0.920*	0.959*	0.963*	0.961*	1	0.969*	0.964*
PFLF7	0.995*	0.961*	0.997*	0.998*	0.977*	0.969*	1	0.992*
PFLF8	0.993*	0.925*	0.991*	0.994*	0.990*	0.964*	0.992*	1

Star values (*) are significantly different from 0 at a significance level of p=0.05; PFLF: Fresh fish from Lake Fit

Figures 3 and 4 respectively show the chromatogram of the amino acids of the standard solution and that of the amino acids of one of the fresh fish samples.

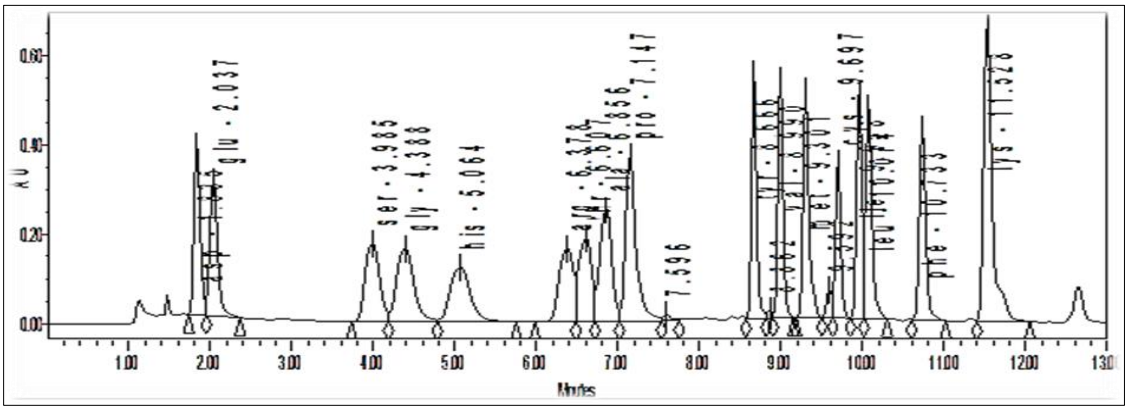

Figure 3 Chromatograms of the amino acids of the standard solution

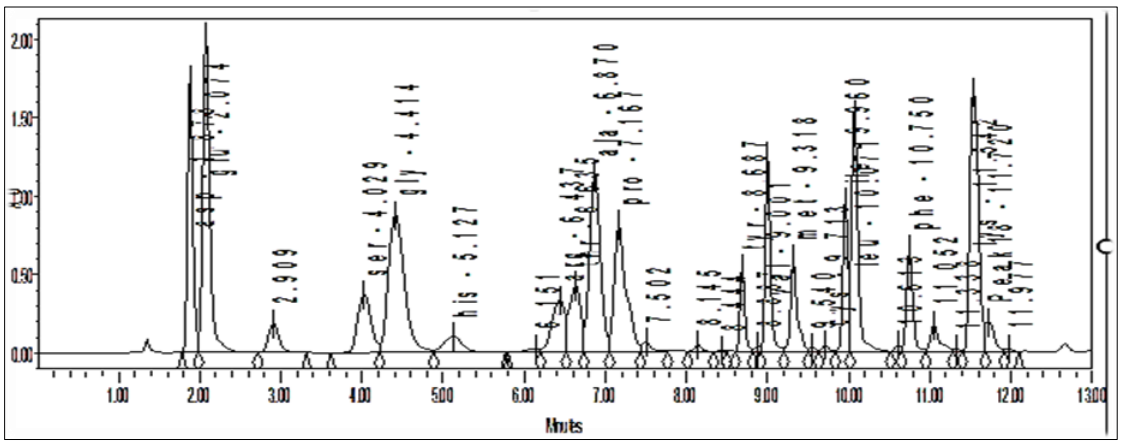

Figure 4 Amino acid chromatograms of a fresh fish sample

The results obtained during this study showed a significant difference in the amino acid content of different samples of fresh fish (Table 5). The results obtained show that fresh fish proteins are rich in amino acids and can largely cover a person's needs for essential amino acids (lysine, histidine, threonine, tyrosine, leucine, isoleucine, phenylalanine and

valine). However, the fresh fish studied showed low cysteine concentrations. The presence of most essential amino acids in fish has already been demonstrated by the study of Latifou et al. (2019). Among the essential amino acids, only tryptophan has not been identified in fresh fish. However, as the standard solution used does not contain this amino acid, the result obtained does not allow us to conclude that the fish do not contain tryptophan. The presence of lysine in fresh fish shows that this fish can be a particularly important supplement in Sahelian countries whose diets mainly consist of cereals poor in lysine.

4. Conclusion

This study revealed that fresh and dried fish from lake Fitri have different physicochemical characteristics. However, their nutritional characteristics are quite similar even if fresh fish has a relatively higher protein content. In addition, fresh fish contains at least seventeen amino acids including most essential amino acids. It therefore emerges from this study that fish from lake Fitri constitute an important source of quality proteins and lipids. They could thus ensure that the nutritional, energy, construction and protection need of children are covered. Their consumption could also prevent certain diseases linked to protein deficiency.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest related to this proposal.

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