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Bioavailability of Docosahexaenoic (DHA) and Eicosapentaenoic (EPA) Acids in the Oil Extracted from *Pellonula leonensis* from the Congo River and Nianga Lake

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Abstract: The objective of this work is to characterize and determine the fatty acid composition of *Pellonula leonensis* oil from the Congo River and Nianga Lake, in order to determine the bioavailability of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids. *Pellonula leonensis* fish were caught in the Congo River in southern Congo–Brazzaville in the localities of Kombé (Brazzaville) and Boko (Pool department) and in Nianga Lake (Pointe-Noire). The oil is extracted from the fish by the Soxhlet method using hexane as solvent. The fat content found is around 32% for all six samples from the Congo River (3 samples of kombe and 3 samples of Boko) and around 22% for the three samples from Nianga Lake. All samples have similar fatty acid profiles. These oils are characterized by the presence of palmitic acid C16:0, 27 to 28%; oleic acid: C18:1 cis, 23 to 24%; stearic acid: C18:0, 8 to 9%; palmitoleic acid: C16:1, about 6%; linoleic acid: C18:2 cis, about 5%; eicosapentaenoic acid (EPA): C20: 5 (n-3), 2-3%; arachidonic: C20:4 (n-6), 2-3%; margaric: C17:0, about 2%; myristic: C14:0, about 2%; linolenic: C18:3 (n-3), about 2%; docosahexaenoic (DHA): C22:6 (n-3), about 2%; docosapentaenoic (DPA): C22:5 (n-3), about 1%. The ratio of PUFAs to SFAs was approximately 0.50. Principal component analysis showed that docosahexaenoic acid (DHA) content is anti-correlated with eicosapentaenoic acid (EPA) content. *Pellonula leonensis* oils from the river, especially those from Boko, have the highest EPA content, whereas the oils from the lake have the highest DHA content. However, the presence of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the oils of *P. leonensis*, although low, proves its nutritional value in terms of lipids.

Keywords: *Pellonula leonensis*, Extraction, Bioavailability, Fatty Acid Composition, DHA, EPA

1. Introduction

The quality of the lipid fraction ingested by humans through food has significant impact on their nutritional status and health [1]. The lipids contained in animal tissues are very important sources of energy and essential fatty acids, long-chain polyunsaturated fatty acids (LCPUAs) of the n-3 and n-6 series and fat-soluble vitamins; thus, they have an important nutritional role [1, 2].

Omega 3 and 6 are polyunsaturated fatty acids. Three of them are essential, i.e. not synthesized by the body and essential to vital functions. They are linoleic acid (LA), belonging to the omega 6 family, alpha linolenic acid (ALA) and docosahexaenoic acid (DHA), both belonging to the omega 3 family. These essential fatty acids allow the production of essential fatty acids (EFA) of the omega 6 and omega 3 pathway: Arachidonic acid (ARA, omega 6), eicosapentaenoic acid (EPA, omega 3) [3].

Thus, omegas 3 and 6 have very important roles in the body, particularly in inflammation phenomena, cardiovascular and

cerebral functions and in tumor processes [4]. These Fatty Acids are all the more important in pregnant women, which is why it is fundamental to provide them in sufficient quantities (especially omega 3) and taking care not to be in excess (especially for omega 6 too present in our diet). [3]

As seen previously, DHA is an essential fatty acid because it is not synthesized by the body and blood levels depend on our diet. Fish oil supplementation has become a reliable source for a safe and pure way to obtain EPA and DHA.

In pregnant women, DHA is essential for the optimal development of the fetal brain, eyes, immune system and nervous system. Taking Omega-3 Fatty Acids before conception is important, but it is also important to maintain adequate levels throughout the pregnancy.

On the other hand, EPA and DHA prevent the synthesis of Triglycerides because they are poor substrates for the enzymes allowing this synthesis. In addition, they prevent the esterification of other Fatty Acids, a reaction allowing the transformation into Triglycerides [3]. The omega-3 fatty acids, EPA and DHA are essential for proper fetal development, and supplementation during pregnancy is linked to decreased immune responses in infants, including a decreased incidence of allergies in infants. [5]

Fish consumption, especially in the coastal areas of sub-Saharan Africa, is the main source of food. In Congo-Brazzaville, people are big consumers of fish in general. He is especially fond of fish from rivers in all its forms (fresh, smoked, etc.).

Indeed, the majority of the fish transported from the Congo River, Nianga Lake, the Sounda and Louémé rivers and regularly consumed by the urban communities of the cities of Pointe-Noire and Brazzaville and the rural communities consist mainly of fish called *Pellonula leonensis*. or "Nsangui".

Pellonula leonensis is the most widespread freshwater clupeid in West Africa. The species is found in lagoons, lakes as well as in the lower and upper reaches of Senegal up to the Cross. Outside the area considered, it has been found in the lower course of the coastal basins from Cameroon to DR Congo. The fish *Pellonula leonensis* consumed in Congo, comes in its great part from the Congo River and the Nianga Lake.

In view of the nutritional interest of lipids for human health, a study on the characterization of lipids of *Pellonula leonensis* is carried out in order to identify its nutritional value. Thus, this study is focused on the characterization and determination of the fatty acid composition of *Pellonula leonensis* oil from the Congo River and Nianga Lake, in order to determine the bioavailability of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids.

2. Materials and Methods

2.1. Material

Pellonula leonensis is known under the vernacular names of Nsangui in southern Congo in the departments of Brazzaville and Pool. *Pellonula leonensis* is a freshwater clupeid found in the Congo River and Nianga Lake (Figure 1).

Pellonula leonensis fish, were caught in the Congo River in southern Congo-Brazzaville in the localities of Kombé (Brazzaville) (4°13′44″ South and 15°7′52″ East), Boko (Pool department) (0°58'17" South and 15°32'19" East) and Lake Nianga (4°12'29" South and 11°47'45" East) in the Pointe-Noire department.

Figure 1. *Pellonula leonensis fish from the Congo River.*

The fishing was done during the period from August to October. The fish were dried in an oven at 70° C for 24 hours and ground using a manual mechanical grinder.

The sampling consisted of three (3) samples from Brazzaville (BV1, BV2 and BV3), three from Boko (BK1, BK2 and BK3) and three from Pointe-Noire (PN1, PN2 and PN3).

2.2. Methods

2.2.1. Oil Extraction Methods

To extract lipid materials from fish, the Soxhlet (S) method was used. A mass of 30 g of ground fish is put in the extraction cartridge and placed in the soxhlet. Using 200 mL of hexane contained in a 250 mL flask, the fat contained in the ground material was depleted by refluxing the solvent (approximately 80°C) for 6 hours. The extract is cooled and then dried with anhydrous sodium sulfate. The oil is obtained after evaporation of the solvent using a rotary evaporator at 40°C under vacuum at 200 mbar. The oil extraction rate is then determined in relation to the mass of crushed material.

2.2.2. Determination of the Physicochemical Characteristics of the Oils

The physicochemical characteristics determined are: the refractive index, density, acid index and peroxide value. These parameters were determined according to the classical methods of analysis described by the French Association of Standards [6].

2.2.3. Preparation of Methyl Esters and Fatty Acid (FA) Profile

The fatty acid methyl esters are obtained by basic transesterification: 3 drops of oil are introduced into the flask using a Pasteur pipette. Place 3 pumice stones in the flask and add 3 mL of sodium methylate. After placing the saponification rod on the flask, heat for 10 minutes at thermostat 1. Then add 3 mL of acetyl chloride until the phenolphthalein discolours, while heating for 10 minutes; and turn off the heating. After cooling, add 8 mL of hexane then 10

mL of water. A separation of the two phases (aqueous and hexane) is observed. Then 1 mL of the hexane phase is collected in a vial for GC analysis.

The analyzes were carried out on a Focus brand chromatograph fitted with an apolar column (50 m long, 0.25 mm internal diameter and 0.2 μm thick) and a flame ionization detector (FID) according to the following experimental conditions: vector gas, helium at constant flux: 1 mL/mm; the oven temperature is programmed from 50 to 280°C with a gradient of 5°C/min; injector temperature: 250°C; detector temperature: 280°C. The quantity injected is 1μL.

A mixture of known fatty acids in defined proportions is injected under the same conditions as the oil to be studied. The retention time and area of each control fatty acid are determined. The fatty acids of the oil studied are identified by comparison of retention times and they are dosed by their areas, referring to the area of an internal standard.

2.2.4. Statistical Treatments

The statistical processing of the various data from the oil extraction tests of the different samples and the physicochemical characterization, were carried out according to the classical statistical method. The calculation of averages and standard deviations, analysis of variance (ANOVA), principal component analysis (PCA), hierarchical ascending classification (HAC) were carried out with the software XLSTAT version 2016.02.28451 which is a macro command of Excel from Microsoft.

The one-way analysis of variance (ANOVA) was used to compare groups of fish oil samples, and to assess the presence of at least one group different from the others. For the fish samples studied, one group corresponds to the origin of the fish, namely Brazzaville, Boko and Pointe-Noire. When at least one group of samples was statistically different from the others, a post-hoc testing was performed for pairwise comparisons between groups. Tukey's HSD (Honest Significant Difference) test was chosen because it was considered conservative [7].

3. Results and Discussion

3.1. Physico-Chemical Characterization of Oils

The physico-chemical analysis of the oil of Pellonula leonensis gave the results shown in Table 1. The chemical properties of the oil are very important parameters that determine the quality of the oil. [8]

Table 1. *Physico-chemical characteristics of Pellonula leonensis oils.*

Physicochemical parameters	Samples from the Congo River						Samples from Nianga Lake		
	Samples from Brazzaville			Samples from Boko			Samples from Pointe-Noire		
	BV1	BV2	BV3	BK1	BK2	BK3	PN1	PN2	PN3
Refractive index	1.4655 (±0.0001)	1.4657 (±0.0003)	1.4655 (±0.0000)	1.4657 (±0.0004)	1.4655 (±0.0001)	1.4656 (±0.0002)	1.4657 (±0.0002)	1.4657 (±0,0003)	1.4656 (±0.0003)
Density at 28°C	0.897 (± 0.05)	0.898 (± 0.04)	0,897 (± 0.03)	0.896 (± 0.004)	0.895 (± 0.007)	0.897 (± 0.005)	0.897 (± 0.004)	0,897 (± 0.002)	0.899 (± 0.005)
Acid value (mg KOH/g oil)	7.37 (± 0.09)	7.53 (± 0.07)	7.61 (± 0.11)	7.45 (± 0.11)	7.45 (± 0.13)	7.45 (± 0.11)	6.75 (± 1.01)	7.12 (± 0.35)	6.43 (± 0.21)
Peroxide value (mg O_2 /g of oil)	4.25 (± 0.17)	4.13 (± 0.11)	4.19 (± 0.08)	4.05 (± 0.08)	4.13 (± 0.10)	4.09 (± 0.06)	3,49 (± 0.12)	3.57 (± 0.16)	4,01 (± 0.09)

Table 1 gives the values of the determined physico-chemical characteristics of *Pellonula leonensis* oils from the Congo River and Lake Nianga. The refractive indices and density of the different oil samples were determined and the treatment of the experimental data by analysis of variance reveals no significant difference ($p > 0.05$) according to the provenance of these samples. Indeed, regardless of the provenance of the fish, the extracted oils show refractive indices in the range of 1.4655 to 1.4557 and relative density values in the range of 0.895 to 0.899. The value of the refractive index lies in the fact that it allows the identification of fats; it also serves as a test of oil purity [9].

Acid and peroxide values are important quality parameters of edible oils. Examining the values of these indices of the different samples of *Pellonula leonensis* oil (Table 1), we note maximum values in the order of 7.61 mg KOH/g oil and 4.25 mg O_2 /g oil for the acid value and peroxide value respectively.

The maximum peroxide values noted are well below the maximum value allowed for virgin edible oils, i.e. 15 mg O_2 /g oil. [10]. It can therefore be clearly concluded that the oils extracted from Pellonula leonensis fish from the Congo River and Lake Nianga by the Soxhlet method using hexane as a solvent, have not undergone any oxidation that would impact the quality of these oils. It should be noted, however, that the acid number values found are slightly higher than the maximum value allowed, i.e. 4 mg KOH/g of oil [10].

Treatment of all these experimental data in Table I by one-factor analysis of variance (ANOVA), reveals no significant difference ($p > 0.05$) of *Pellonula leonensis* fish oil samples caught in the Congo River and Nianga Lake.

3.2. Oil Content and Fatty Acid Composition

The oils studied were obtained by Soxhlet extraction of dried *Pellonula leonensis* fish caught in the Congo River and in Lake Nianga. The oil content and the fatty acid profile of these oils were then analyzed by a gravimetric method and by gas chromatography as described previously. Figure 2 and Table 2 present the oil yields and the relative contents of the identified fatty acids obtained for each sample of fish.

Table 2. *Oil content and Fatty Acids composition (%) of Pellonula leonensis oils.*

% AG	Echantillons du fleuve Congo						Echantillons du lac Nianga		
	BK1	**BK2**	**BK3**	**BV1**	**BV2**	**BV3**	**PN1**	**PN2**	**PN3**
% Huile	**32.33 (± 0.81)**	**32.33 (± 0.65)**	**32.10 (± 0.46)**	**31.69 (± 0.38)**	**31.83 (± 0.21)**	**31.85 (± 0.46)**	**22.32 (± 0.34)**	**21.79 (± 1.41)**	**22.73 (± 0.25)**
C12:0	0.31	0.31	0.32	0.29	0.30	0,29	0.19	0.21	0.20
C13:0	0.08	0.07	0.07	0.07	0.08	0,07	0.11	0.12	0.12
C14:0	2.52	2.51	2.53	2.60	2.61	2.60	3.21	3.23	3.23
C14:1	0.81	0.79	0.79	0.89	0.95	0.93	0.53	0.47	0.44
C15:0	0.73	0.76	0.76	0.91	0.88	0.89	1.02	1.04	1.06
C16:0	27.38	27.4	27.41	28.14	28.11	28.12	28.89	28.90	28.91
C16:1	6.61	6.74	6.73	6.96	6.92	6.94	7.12	7.15	7.15
C16:2	1.18	1.18	1.17	1.18	1.21	1.19	0.64	0.66	0.65
C16:3	0.36	0.36	0.34	0.37	0.40	0.38	0.16	0.17	0.18
C17:0	2.81	2.80	2.81	2.95	2.98	2.98	2.71	2.65	2.63
C17:1	0.53	0.52	0.53	0.56	0.57	0.57	0.74	0.76	0.76
C18:0	9.47	9.47	9.45	9.23	9.25	9.20	10.51	10.53	10.55
C18:1 trans	0.36	0.35	0.37	0.41	0.39	0.40	0.40	0.41	0.41
C18:1	24.40	24.38	24.23	23.27	23.31	23.23	26.01	25.98	25.97
C18:2	5.61	5.61	5.55	5.69	5.81	5.74	3.25	3.30	3.27
C18:3 (n-6)	0.31	0.30	0.31	0.36	0.34	0.35	0.34	0.34	0.35
C18:3 (n-3)	2.51	2.50	2.52	2.54	2.55	2.54	2.69	2.71	2.71
C18:4 (n-3)	0.33	0.32	0.33	0.34	0.35	0.34	0.78	0.79	0.79
C20:0	0.45	0.51	0.52	0.47	0.44	0.45	0.38	0.39	0.41
C20:1	0.27	0.26	0.26	0.55	0.57	0.56	0.41	0.31	0.31
C20:2 (n-6)	0.22	0.21	0.23	0.36	0.32	0.34	0.33	0.34	0.35
C20:3 (n-6)	0.23	0.21	0.23	0.29	0.23	0.27	0.27	0.28	0.28
C20:4 (n-6)	2.89	2.89	2.90	2.59	2.61	2.60	1.95	1.97	1.96
C20:3 (n-3)	0.14	0.13	0.14	0.17	0.15	0.16	0.15	0.15	0.16
C20:4 (n-3)	0.32	0.30	0.32	0.33	0.34	0.33	0.61	0.62	0.62
EPA	3.61	3.64	3.64	2.87	2.74	2.85	1.81	1.82	1.84
C22:0	0.41	0.38	0.42	0.34	0.29	0.32	0.29	0.30	0.31
C22:4 (n-6)	0.27	0.27	0.26	0.26	0.28	0.27	0.00	0.02	0.00
C21:5 (n-3)	0.10	0.10	0.10	0.11	0.13	0.12	0.00	0.00	0.00
C22:5 (n-6)	0.39	0.39	0.37	0.51	0.53	0.52	0.49	0.47	0.46
C22:5 (n-3)	1.76	1.69	1.76	1.55	1.59	1.59	0.72	0.74	0.74
DHA	2.40	2.43	2.43	2.69	2.57	2.68	2.86	2.87	2.89
C24:0	0.23	0.22	0.20	0.15	0.20	0.18	0.37	0.30	0.28

The main purpose of the extraction of *Pellonula leonensis* oils is to study their fatty acid compositions in order to determine the contents of polyunsaturated conjugated fatty acids of interest, mainly docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids.

The oil contents (Table 2) are between 32.10 and 32.33% for fish from Boko, 31.69 and 31.85% for fish from Brazzaville and 21.79 and 22.73 for fish from Pointe-Noire. The very low values of the standard deviations of the oil content (Table 2) indicate a reproducible extraction on each sample, so the sampling seems homogeneous. These contents vary significantly ($p<0.05$) between the different fish samples. To determine which sample group was statistically different from the others, Tukey's HSD test was chosen to perform a pairwise multiple comparison (Figure 2).

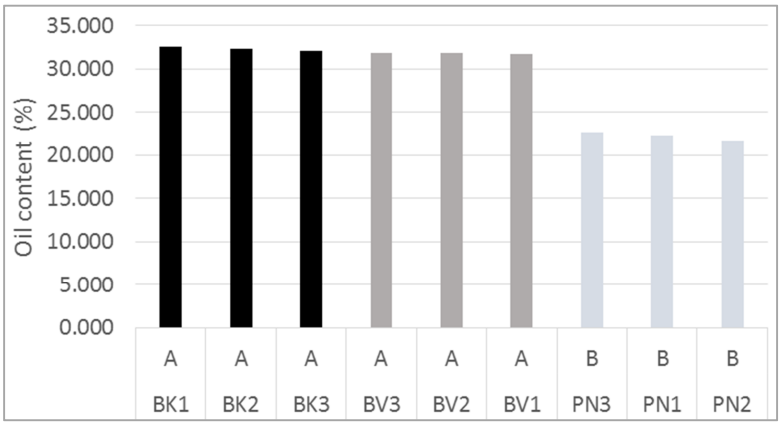

Figure 2. *Estimated Mean Oil Contents of Fish Samples (Multiple Pairwise Comparisons).*

Figure 2 shows that the three samples from Boko and the three from Brazzaville have the letter A in common and the samples from Pointe-Noire all have the letter B in common. Identical letters show the absence of significant difference, taking into account all the samples (Anova and Tukey, $p > 0.05$). The samples from Boko and Brazzaville are not significantly different, nor are the three samples from Pointe-Noire as significantly different. However, the samples from Boko and Brazzaville, which represent samples from the Congo River, have oil contents that are significantly different from those of the samples from Pointe-Noire, which are samples from Nianga Lake.

Pellonula leonensis fish from the Congo River have oil contents that differ from those from Lake Nianga.

Gampoula et al. [11] described oil yields of 28 and 29% in *Pellonula leonensis* fish from Boko caught in the Congo River by Soxhlet (n-hexane) extraction. These yields are lower than those found in this study. *Pellonula leonensis* must therefore be classified among the fatty fish which are the species generally used to prepare edible oils. These are mainly herring, mackerel, sardines, salmon and anchovies. This category generally contains from 5 to 25% of fat. This little fish is very surprising. It could be classified as a lean fish because of its morphology. But it contains as much if not more oil than oily fish.

The lipid composition of *Pellonula leonensis* fish from the Congo River and Lake Nianga is given in table 2.

All samples have slightly differences in fatty acid profiles. These oils are characterized by the presence of palmitic acid C16:0, 27 to 28%; oleic acid: C18:1 cis, 23 to 24%; stearic acid: C18:0, 8 to 9%; palmitoleic acid: C16:1, about 6%; linoleic acid: C18:2 cis, about 5%; eicosapentaenoic acid (EPA): C20: 5 (n-3), 2-3%; arachidonic: C20:4 (n-6), 2-3%; margaric: C17:0, 2% approx.; myristic: C14:0, 2% approx.; linolenic: C18:3 (n-3), 2% approx.; docosahexaenoic (DHA): C22:6 (n-3), 2% approx.; docosapentaenoic (DPA): C22:5 (n-3), 1% approx. Whatever the origin of the fish, palmitic and oleic acids are clearly predominant and remain the major constituents of *P. leonensis* oil with a cumulative content of over 50%. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) present a cumulative content of more than 6% whatever the sample considered. These two acids EPA and DHA are precursors of several metabolites that are potential beneficial mediators in the prevention and treatment of many diseases [12, 13]. Studies by Parvarthy et al [14] revealed that long chain omega-3 PUFAs play an important role in the treatment of cardiovascular diseases, hypertension, diabetes, arthritis, depression, migraines, skin diseases such as psoriasis, eczema and other inflammatory and autoimmune diseases as well as cancer.

Fish is considered an inexpensive source of many essential nutrients, especially fats and proteins, and therefore has value in the human diet. It is highly recommended in the human diet because of its richness in the two main fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The main sources of omega-3 PUFA-rich oils are the flesh of fatty fish such as sardines and herring (1.5-2.4 g/100 g flesh), mackerel (1.3 - 2.0 g/100 g flesh), salmon (1.3-2. 2 g/100 g flesh), Tuna (0.3-1.3 g/100 g flesh), Halibut (0.7 - 1.3 g/100 g flesh), Shark (0.98 g/100 g flesh), Green/lip mussels (1.12 (g/100g flesh)...[15, 16].

According to Tsuchiya [17] the two main fatty acids of oily fish (herring, mackerel, sardines, salmon and anchovies) are EPA and DHA, which together represent from 20 to almost 40% of the total fatty acids. *Pellonulla leonensis* fish is therefore very low in DHA and EPA compared to the other fatty fish mentioned. Working on Sudan fishes *Polypterus senegalus*, *Clarias lazera* and *Lates niloticus*, Elagba et al. [18] have found that level of Eicosapentaenoic C20:5n3 was (0.36 ± 0.02 g/100g) in *P. senegalus*, (0.17 ± 0.02 g/100 g) in *C. lazera*. Eicosapentaenoic, EPA, and Docosahexaenoic C22:6n3, DHA, together formed 1.9% in *P. senegalus*, 10.9% in *C. lazera*, and 9.8% in *L. niloticus*. Omega-3s formed 1.74% 0.83%, 0.49% and 1.74% of TFA in *L. niloticus, P. senegalus* and *C. lazera*, respectively. Omega-6 formed 1.2% of TFA in L. niloticus and 1.13% in Polypterus, but not detected in *C. lazera*.

A principal component analysis was conducted with the fatty acid composition results described previously. It aims to explain the variations between individuals (the samples analysed) using numerical variables (the fatty acid composition of fish oils). Principal Component Analysis allows to visualize the link between variables and the similarity between individuals. The relationships between the fatty acids are presented in figure 3. The circles of correlations on the F1F2 plane (figure 3), representing 95.51% of information on the total variability, indicate a strong positive correlation between the palmitic acid contents (C16:0), DHA and palmitoleic acid (C16:1). These contents of these acids are in turn anti-correlated to the contents of the acids EPA, C20:0 and C20:4 (n-6), all three of which have positively correlated contents. We also observe a positive correlation between the contents of the acids C17:1, C18:3 (n-3), C124:0, C18:4 n-3), C20:4 (n-3) and C13:0. Another group of positive correlation of the contents is that of the following polyunsaturated acids: C22:5 (n-6), C20:3 (n-3), C18:3 (n-6), C20:2 (n-6), C20:3 (n-6). The oleic acid content is positively correlated with the contents of C24:0 and C18:0 acids and anti-correlated with the contents of C17:0 and C14:1 acids.

In figure 4, the individuals (oils) have been represented in such a way as to maximize the inter-individual variation, and according to components which are the linear sum of initial numerical variables. The results make it possible to identify the fatty acids of the fish oils explaining the most the differences between the samples, that is to say the contents with the greatest variability between the origins of the samples (oils). Thus figure 3 shows the distribution of oils on the F1F2 plane according to their fatty acid composition.

In relation to this space occupation (Figure 4), the oils are distributed according to the origin of the fish samples, i.e. Boko, Brazzaville and Pointe-Noire. This shows that the fatty acid composition is influenced by the environment of origin of the fish.

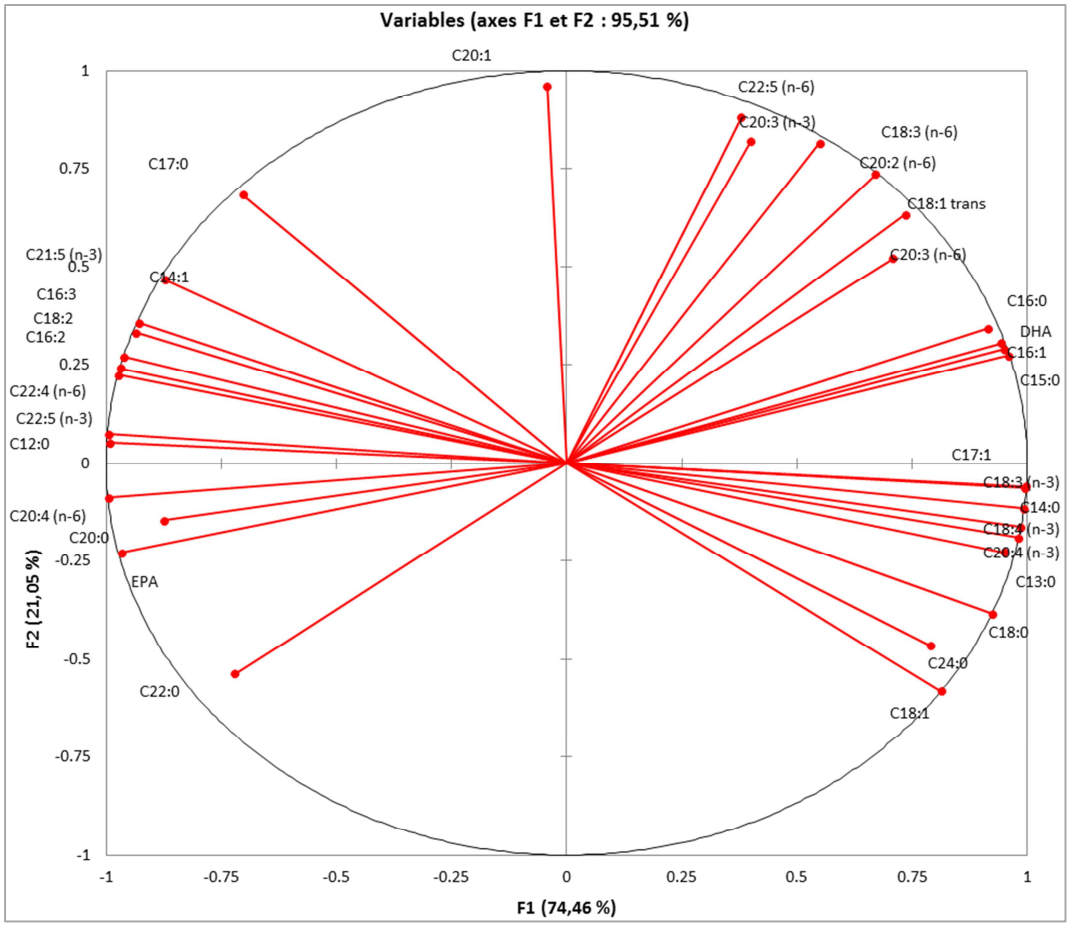

Figure 3. *Correlation circles in Principal Component Analysis of the FA composition of P. leonensis oils.*

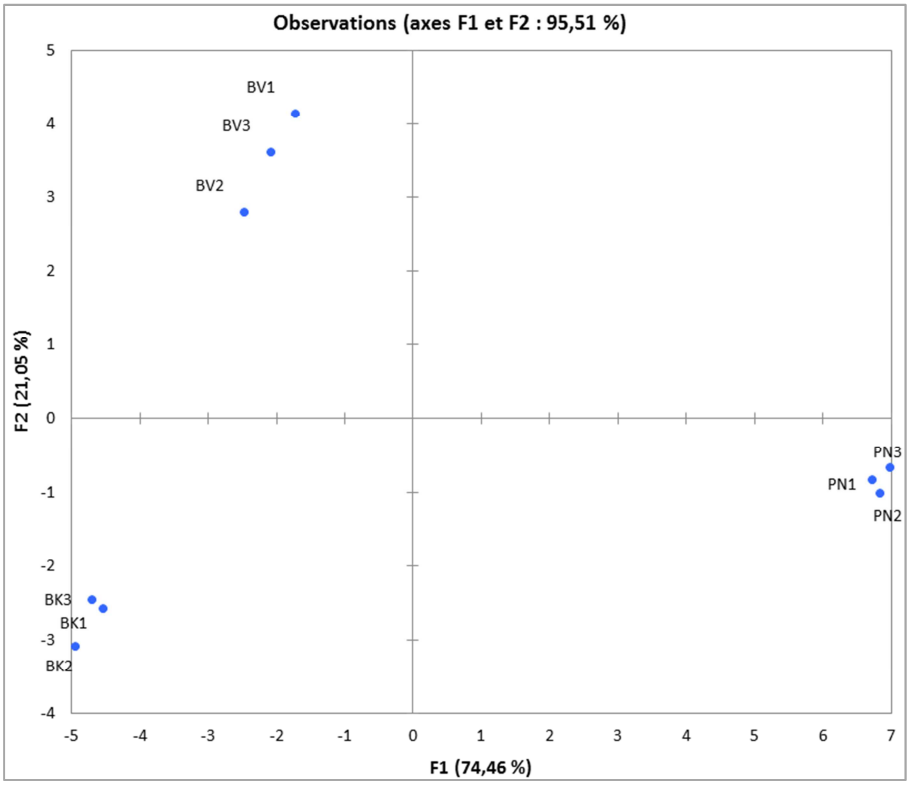

Figure 4. *Distribution of oil samples in Principal Component Analysis of the FA composition of Pellonula leonensis oils.*

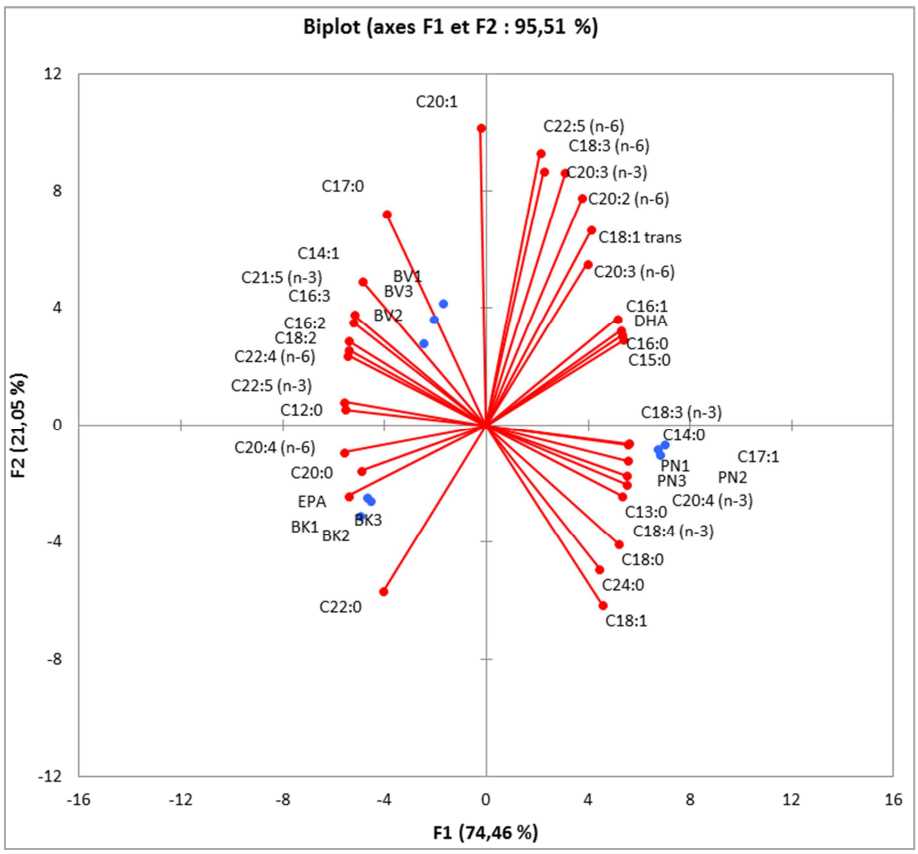

Figure 5. *Effect of fish origin on the fatty acid composition of their oils.*

Figure 5 gives the representation on the two principal components F1 and F2 of the analyzed samples of oils. The right segments represent the observed trends in fatty acid contents.

The first two components F1 and F2 represent respectively 74.46 and 21.05% of the variance between the samples, for a total of 95.51%. The provenance explains the variations between the samples only on the main component F1 ($p<0.05$) which is therefore relevant to analyze.

Oil samples from Boko fish show the highest contents of eicosapentaenoic acid (EPA), C20:4 (n-6) and C20:0; those of Brazzaville show intermediate levels, and those of Pointe-Noire give the lowest levels. Therefore, the provenance of the fish is distributed, in decreasing order of eicosapentaenoic acid (EPA) and C20:4 (n-6) content, from the Congo River to Lake Nianga with a p-value less than 0.05. Docosahexaenoic acid (DHA) levels vary inversely.

The highest C18:3 (n-3) contents are obtained with samples from Pointe-Noire and therefore from Nianga Lake.

The ascending hierarchical classification confirms, from the point of view of the acid composition of the oils, the distinction between the three groups of *Pellonula leonensis* fish samples studied (Figure 6). The two samples from the Congo River are closer and both differ from the samples from Nianga Lake.

The "radar-plot" representation constructed on the cumulative contents of saturated, monounsaturated, polyunsaturated, omega 6, omega 3 and trans fatty acids of the oils of the three provenances shows the similarities and differences (figure 7) of these results of the fatty acid composition of the *Pellonula leonensis* fish oils of the Congo River and Nianga Lake.

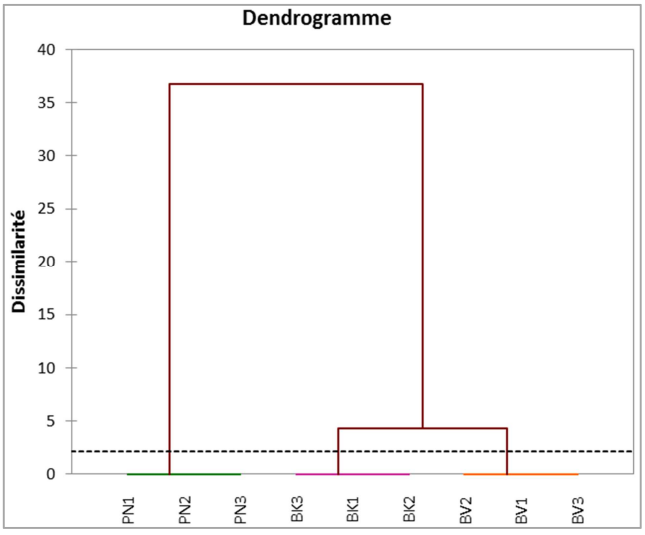

Figure 6*. Ascending Hierarchical Classification of oils from different samples of Pellonula leonensis.*

Figure 7 presents three superimposable geometric shapes, of which the two figures presenting the oils of the river fish show a perfect superposition. This reflects the similarity of the fatty acid composition of the two groups of samples from the

Congo River, namely those from Boko and Brazzaville. This figure 7 best represents the oils of *Pellonula leonensis* fish from the river and the lake, which differ essentially by the presence in significant quantities of polyunsaturated fatty acids, in particular omega 6.

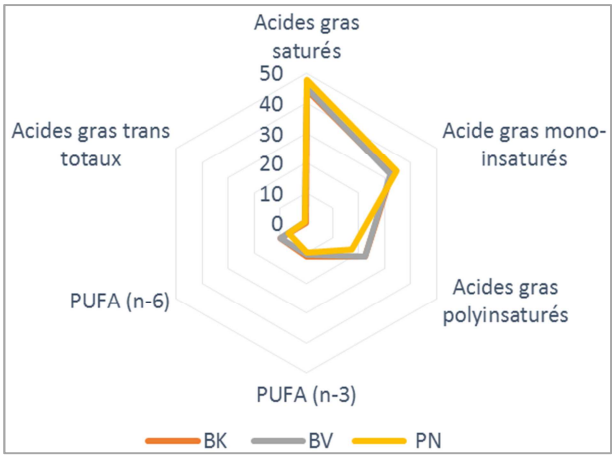

Figure 7. *Radar plot of fish oils from Boko, Brazzaville and Pointe-Noire.*

4. Conclusion

Freshwater fish occupy a prominent place in the feeding habits of the inhabitants of the congolese populations. This study focused on the physicochemical characterization and fatty acid composition of oils from *Pellonula leonensis* fish caught in the Congo River and Nianga Lake. In terms of analysis and physicochemical characterization, the treatment of all experimental data by one-factor analysis of variance (ANOVA), revealed no significant differences ($p > 0.05$) of the studied fish oil samples. All the studied oils had almost similar physicochemical characteristics. The peroxide values of all the studied oils are in accordance with the standards for edible oils on the other hand the acid values are slightly higher than the standards.

Whatever the origin of the fish, palmitic and oleic acids show a clear predominance and remain the major constituents of *P. leonensis* oil with a cumulative content of more than 50%. Docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids present a cumulative content of about 6% whatever the sample considered. By its composition in fatty acids and its content in DHA and EPA, it is thus demonstrated that the fish Pellonula leonensis from the Congo River and Lake Nianga is a source of omega 3 polyunsaturated fatty acids. Because of the dietary interest in DHA and EPA containing these oils, we must continue our investigations by studying the position of these fatty acids on glycerol. Rich in lipids, *Pellonula leonensis* could ensure the coverage of nutritional energy needs and their consumption could also prevent certain cardiovascular diseases.

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