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Chemical Characterization of Oil Extracted from Two Species of Fish (*Ilisha africana* and *Sardinella maderensis*) from the Cameroonian Coast

Jules Christophe Manzkoule[1], Mathieu Ndomou[1, *], Merlin Ngafon Nchoutpouen[1], Adelaide Mawamba Demasse[1], Charlotte Sabine Milong Melong[1], Georges Steve Fofou Bekwankoa[1], Fabrice Bruno Siewe[2], Marlène Tegueu Youogo[1], Romeo Auguste Dama[1], Rene Paul Njock Ndombol[3], Jean Valery François Nsoga[1], Christine Ngo Tang[1], Francois Tchoumbougnang[3], Inocent Gouado[1]

[1]Department of Biochemistry, Faculty of Science, University of Douala, Douala, Cameroon

[2]Department of Meat and Marine Science, Central Food Technological Research Institute, Mysore, India

[3]Department of Processing and Quality Control of Aquatic Products, Institute of Fisheries and Aquatic Sciences, University of Douala, Douala, Cameroon

Email address:

nmathieu2009@yahoo.fr (M. Ndomou)

[*]Corresponding author

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Abstract: Background: Fish is an important source of food for people. According to the FAO, It is man's most important single source of high-quality protein and dietary omega-three fatty acids. Objective: The study consisted of the chemical characterization of the oil extracted from *Ilisha africana* and *Sardinella maderensis* being fish species from the Cameroonian coast. Material and methods: The fish were collected at the fishing port of Douala, transported to the laboratory, washed with distilled water and filleted. Oil was extracted from the fillets using dichloromethane and methanol. The different indexes of the oil were determined using standard methods and the fatty acid profile by gas chromatography/mass spectrometry. Results: *Ilisha africana* and *Sardinella maderensis* are oily fish. The oil was of good quality various amounts of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Palmitic acid was the major SFA with 28.56% and 30.54% for *Ilisha africana* and *Sardinella maderensis* respectively. Palmitoleic acid and 15octadenoic were the major MUFAs in *Ilisha africana* and *Sardinella maderensis,* respectively. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) the main omega 3 PUFAs. In both species DHA was the predominant fatty acid among PUFAs. Arachidonic acid and linoleic acid were the major omega 6 PUFAs in *Ilisha africana* and *Sardinella maderensis.* Conclusion: These results show that *Ilisha africana* and *Sardinella maderensis* are a good source of EPA and DHA and the nutritional quality of lipids may beneficit for human health.

Keywords: *Ilisha africana*, *Sardinella maderensis*, Fatty Acid, EPA, DHA

1. Introduction

World fish production is estimated at 171 million tons with a consumption of 20.5kg/inhabitant/year [1]. Fish meat is the main protein sources for human and represent almost half of animal protein sources. It is more needed because of its richness in proteins, minerals, vitamins and lipids containing PUFA particulary omega 3 fatty acids. Omega 3 are involved

in healing of inflammatory deseases [2], hyperlipidemy [3], neurological diseases [4], and liver deseases [5]. Omega 3 fatty acids areal so involved in the prevention and treatment of cardiovascular deseases [6], diabetes [7] but can also act against free radical [8] and adipomégalie [9]. A set of studies had focused on fatty acid profile of fish oils [10-12]. Cameroon has a sea front of 402 km on which artisanal and industrial fishing are practiced. The annual fish production of Cameroon was estimated at 252,764 tons in 2016, with an average consumption of 19.4Kg/year/Inhabitant [13]. A variety of fishing products include *Ilisha africana* and *Sardinella maderensis* localy known as "*Munyanya*" and "*Strong Kanda*". These fish are easily available and hold important place either in fishing or in food of Cameroonians. Very few studies have focused on the Cameroon coast fish. In addition and to the extend of our knowledge, there is not report on the fatty acid profile of *Ilisha africana* and *Sardinella maderensis* oils. As part of actions taken to valuate fishery products of the Cameroonian coast, we intend to study the oil extracted from the two above species of fish.

2. Material and Methods

2.1. Extraction and Determination of Chemical Indexes

The fresh fish was bought at the fishing port of Douala as soon as the fishermen returned from the sea. After purchase, the fish were transported to the laboratory in a cooler containing ice at a fish / ice ratio of 1/2. At the laboratory fish were rinsed with distilled water and effilated. Oil was extracted from the fish fillets according to the method of Bligh and Dyer [14]. Iodine number, saponification number, acid number, peroxide number, anisidine number and thiobarbiturate number were then determined using AOAC method [15]. The total oxidation index (I Totox) was deduced the following equation: I To = 2 IP + Ian where To = total oxidation number, IP = peroxide number and Ian = anisidine number

2.2. Fatty Acid Composition

Methyl fatty acid esters (FAME) extract was carried out by dissolving 100 mg of oil in 1.6 ml of hexane. Then 100 µl of the mixture were added to 0.2 ml of 1 M sodium methoxide (prepared in 30% methanol) and stirred with a vortex (GENIE 2 brand BOHEMIA, New York, USA) for 30 s. The resulting mixture was allowed to stand for 5 min tillappearance of a bilayer. The fatty acid profile of the extracted oils was determined by gas chromatography coupled to mass spectrometry (CPG-MS) according to Gómez-Estaca and *al* [16]. 1 µL of the transparent upper layer containing the FAME was injected into the PERKIN ELMER brand chromatograph. The injection mode was split with at aratio of 20/1. Helium was carrier gas used. The separation was carried out in a SUPELCO fused silica capillary column (30m×0.25mm×0.25µm film thickness). The oven temperature was set at 80°C, then rises 15°C / min to 120°C, rises at 8°C/min to 160°C, maintained 2.50 min, and finally rises from 5°C/min up to 230°C then is maintained for 3.83 min. The temperatures of the detector and the injector were set at 180°C and 220°C, respectively. The temperature of the transfer line between the chromatograph and the mass spectrometer was 200°C. The elution time of the solvent was 3 min. Fatty acids were identified and quantified by comparison with the retention times of commercial standards. Spectrometer detection of the molecules was carried out for m/z ratios between 40 - 400 Da. The results were expressed as a relative percentage in FAME.

2.3. Statistical Analysis

Results are expressed as mean ± standard error (M ± ES) of three replicates (n=3). In between groups comparison was made by the ANOVA test using Statgraphics Centurion Version 17.1.8 software.

3. Results

Lipids contents and chemical indexes of oils of *I.africana* and *S.maderensis* are shown table 1.

Table1. *Total lipid content and chemical indexes of the oils of the species studied.*

Chemical indexes	*I.africana*	*S.maderensis*
Total lipids(%DM)	13.46±0.11[a]	13.79±0.23[a]
Iodine number (gI_2/100gdeMG)	162.09±4.27[a]	180.45±2.42[b]
Acid number (mgKOH/gdeMG)	2.15±0.00[a]	1.10±0.01[b]
Saponification number (mgKOH/gdeMG)	190.26±7.49[a]	192.12±1.87[a]
Peroxyde number ($meqO_2$/KgdeMG)	6.03±0.01[a]	5.96±0.09[b]
Anisidin index	3.19±0.01[a]	3.55±0.10[b]
Thiobarbituric acid index (ppm)	4.67±0.01[a]	5.15±0.08[b]
Total oxidation index	15.07±0.00[a]	15.48±0.16[b]

DM: dry matter; n=3. Values in the same line with different subscript letters are significantly (p<0.05) different.

Table 1 shows that lipid contents do not vary significantly between *I.africana (*13.46%) and *S.maderensis* (13.79%). Oil degradation indexes such as total oxidation index, acid, peroxide, anisidine and thiobarbituric acid numbers vary significantly between different species.

The results of Table 2 show that the fatty acid (FA) contents also varies significantly between species. *S. maderensis* had high level of saturated fatty acids (SFA) compared to *I. africana*. Among SFAs, palmitic acid (16:0) was predominant, ie 30.54% and 28.56% respectively for *S. maderensis* and*I. africana*. Myristic acid (14:0) wasthe second most abundant fatty acid and varied significantly (p <0.05) between the two species with respective values of 6.24% and 7.58% for *S. maderensis* and *I. africana*.

Regarding monounsaturated fatty acids (MUFA), 15-octadecenoic acid (18:1ω3) and palmitoleic acid (16:1ω7) were the most abundant in *S. maderensis* and *I. africana*.7-Hexadecenoic acid has an average percentage and does not vary significantly (p>0.05) between the two species. Its percentageswere 5.78% and 4.71% in *S. maderensis* and *I. africana* respectively. 7- methyl-10- hexadecenoic acid (7-

Me -16: 1ω10) which is a rare fatty acid was found in low contents 1.03% and 0.99% for *S. maderensis* and *I. africana* respectively.

As the major polyunsaturated fatty acids, docosahexaenoic acid (DHA) was present and did not vary significantly (P>0.05) among the two species. It was not the case for eicosapentaenoic acid (EPA). Linoleic acid and arachidonic acid were the major fatty acids for the omega 6 PUFA series in *S. maderensis* and *I. africana* respectively. The ω3 and ω6 content and the ω3/ω6 ratio were higher in *S. maderensis* compared to *I. africana*.

Table 2. *Fatty acid profile of oils extracted from I.africana and S.maderensis.*

Fatty acids (% of FAME)	*Ilisha africana*	*Sardinella maderensis*
12:0	1.62±0.04^{b}	0.50±0.01^{a}
14:0	7.58±0.05^{b}	6.24±0.23^{a}
15:0	3.28±0.08^{b}	2.53±0.01^{a}
16:0	28.56±0.35^{b}	30.54±0.35^{a}
15-Me-16:0	0.58±0.06^{b}	1.53±0.11^{a}
17:0	2.58±0.04^{b}	2.88±0.06^{a}
18:0	3.18±0.09^{b}	5.28±0.02^{a}
ΣSFA	47.38	49.5
12:1ω3	3.76±0.55^{a}	4.44±0.77^{a}
16:1ω7	19.44±0.22^{b}	4.63±0.24^{a}
16:1ω9	4.71±0.63^{b}	5.78±0.24^{a}
7-Me-16:1ω10	0.99±0.02^{a}	1.03±0.07^{a}
18:1ω9	3.24±0.01^{b}	4.64±0.09^{a}
18:1ω3	3.84±0.26^{b}	7.96±0.18^{a}
ΣMUFA	35.98	28.48
16:4ω3	ND	1.97±0.06
18:2ω6	0.84±0.01^{b}	2.68±0.04^{a}
18:3ω3	1.68±0.03^{b}	1.58±0.02^{a}
20:2ω6	0.93±0.01^{b}	2.23±0.10^{a}
20:3ω3	ND	2.32±0.06
20:4ω6	2.67±0.02^{b}	1.17±0.08^{a}
20:5ω3	3.94±0.10^{b}	4.28±0.07^{a}
22:6ω3	5.78±0.48^{a}	5.34±0.13^{a}
ΣPUFA	15.84	21.57
Σω6	4.44	6.08
Σω3	19	27.89
Σω3/Σω6	4.27	4.58

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ND: not determined; FAME: methyl fatty acids ester. The values are the means ± the standard deviation, n=3. The Values on the same line with different subscript letters are significantly ($p < 0.05$) different.

4. Discussion

Lipids contents of *l. africana* and *S. maderensis*were greater than 10. According to the Mohtadji classification *l. africana* and *S. maderensis* are fatty fish [17]. This resultdoes notconfirm either that of Adeyeye et *al* on the chemical and microbiological qualities of smoked *llisha africana* from the large markets of Ibadan in the state of Oyo in Nigeria or that of Bou M'handi et *al* on the effect of frozen storage on the quality of *Sardina pilchardus* produced in Morocco [18, 19]. Howerver, Aberoumand reports that the lipid content of fish varieswith fishing season, sex and environment [20].

The iodine number is linked to the unsaturation degree of an oil. Oils are then classified as non-drying (II<100), semi-drying (100<II<130) and drying (II>130). Oils extracted from*Ilisha africana* and *S. maderensis* are therefore drying ones. Iodine numbers found are higher than those found in Bangladesh by Assielou and *al* in larvae oils of *Oryctes owariensis* (105.27 ± 0.15) and Molla and *al* in *Channa striata* oils (110.85 ± 0.18) [21, 22]. Acid number which measures the amount of free fatty acid in a foodwas lower than the standard (4 mg KOH / g) recommended by the Codex Alimentarius [23]. This could be explained by a low hydrolytic activity [24]. Peroxide number measures (amount of primary oxidation products in an oil) was lower than previously obtained by Manz and *al* on *Ilisha africana* (8.43 ± 0.11) [3]. The higher is peroxide number, the more oxidized is the fat. However, this parmeter is only an indicator of the onset of oxidation [25]. This is why the anisidine number (which measures the side products of oil oxidation and takes into account non-volatile aldehyde products) was also evaluated. Its value were low than recommended by the Codex Alimentarius (normal values less than 20) [23]. The thiobarbituric acid number quantifies the products of secondary oxidation of lipids. Our results are lower than those obtained by Bayong on *Polydactilus quadrifilis* (5.87 ± 1.64) of Wouri in Cameroon [26]. Increase in thiobarbituricnumber could be explained by the enhanced breakage of unstable primary oxidative compounds (hydroperoxides) into stable secondary derivatives [27]. Saponification number (SI) is a measure ment of the length of the hydrocarbon chains of fatty acids. SI of *I. africana* and *S. maderensis* oils were higher than that of *Channa striata* (146.94 ± 0.88) showing occurence of medium carbon chains fatty acids [22].

The total oxidation index is useful in assessing fat oxidation. Values obtained are lower than that recommended by Codex Alimentarius ≤ 26 oil for virgin fats and oils [23]. However high peroxide index can be obtained with fat without obvious signs of rancidity, such as the rancid odor, which is rather attributable to volatile aldehyde compounds. Conversely, a low peroxide number does not mean that a fat is not altered.

Results of the fatty acid profile analysis showed that palmitic acid (C16:0) was the most saturated fatty acids (SFA) in *S.maderensis* and *I.africana*. Similar results were obtained in Serbia by Pelic et *al* in *Acipenseridae* family [10]. According to Zhang et *al* palmitic acid is the major fatty acid in few commercial fish from the Pearl River Estuary in China [12]. Tenyang et *al* showed that palmitate was not the major saturated fatty acid of six fish species in Wouri river [28]. However fish fatty acids contains can vary with age, genetics or environmental factor like nutrition [29]. 15-octadecenoic acid (18:1ω3) and palmitoleic acid (16:1ω7) are the major MUFAs in *S.maderensis* and *I.africana*, respectively. Our results differ from those of Tenyang et *al* who found that oleic acid was the major MUFA in four fish species of Lake Maga in the Far North of Cameroon [30]. Lopez-Huerta et *al* showed that oleic acid was the major MUFA in *Domitator latifrons* from the El Quelele lagoon in Nayarit, Mexico [31]. Palmitoleic acid and-15-octadecenoic

acid appear as side product of desaturation of palmitate and stearate respectively. Palmitoleic acid reduces hyperglycemia and hypertriglyceridemia by improving insulin resistance and by regulating the storage of triglycerides leading to weightloss [32]. This fatty acid has anti-inflammatory properties by deactivativating C reactive protein (CRP) which appears in inflammatory reactions and cellular stress. It also helps in wounds healing of and has an antiseptic action on the skin as a component of sebum [33]. Oleic acid which was found in an average amount of 3.94% of FAME is involved in bile secretions, absorption and digestion of lipids and in the regulation of blood pressure [34]. The lipids profile show that PUFAs is mainly consisted of omega3. The 4,7,10,13-hexadecatetraenoic acid (16:4ω3) and acid-11,14,17-eicosatrienoic (20:3ω3) may result from elongation and desaturation reactions of lauroleic and linolenic acids respectively. Our results corroborate that of Torrecillas et *al* who showed that these two fatty acids weref ound in low proportions in the dermal mucus of *Sparus aurata* from Denmark [35]. Infact, 11,14,17-eicosatrienoic acid has anti-inflammatory properties and prevents synthesis of nitrogenoxide (NO) responsible for cellular oxidative stress via inhibition of nitric oxide synthase [36]. Eicosapentaenoic Acid (20:5ω3) (EPA) and Docosahexaenoic Acid (22:6ω3) (DHA) were the most abundant n-3 PUFAs. Similar results were obtained by Tenyang et *al* showed that EPA and DHA were the most abundant among n-3 PUFAs in four fresh water fish from Lake Maga in the Far North region of Cameroon [30]. Matos et *al* showed that EPA and DHA were the most abundant n-3 PUFAs in five species of farmed fresh water fish from the western region of Santa Catarina in Brazil [34]. Jaya-Ram et *al* showed that EPA and DHA PUFA n-3 accumulate in the muscles of some fresh water fish from the Bukit Merah reservoir in the Perak region of Malaysia [37]. This result could be explained by the fact that these fish are mainly feed on phytoplankton which lipids are generally rich in n-3 PUFAs (EPA and DHA) and poor in n-6 PUFAs such as linoleic acid and arachidonic acid [28]. The presence of these fatty acids in our fish suggests that they could have beneficial effects in the body. Indeed, EPA and DHAhave anti-inflammatory properties via their conversion in to resolving which activate synthesis of protectins and maresines [38]. EPA and DHA are said to have preventive properties against human coronary heart disease, type 2 diabetes mellitus and fatty liver disease. They also have neuroprotective, antioxidant and hypotensive properties [11]. EPApreserves carbohydrate homeostasis and inhibits the expansion of fatty tissue [9]. DHA is essential in development of fetal brain and eye retina [39]. 11,14-eicosadienoic acid (20:2ω6) was the main n-6 PUFAs. This fatty acid is thought to result from an elongation reaction of linoleic acid. Linoleic acid and arachidonic acid are precursors of long – chain n-6 PUFAs found in cardiovascular disorders such as thrombosis and arteriosclerosis. Arachidonic acid is the precursor of serie 2 autocoids. It enhances blood clotting, and binds to endothelial cells during wound healing [30]. Theω-3/ω-6 ratio is a good indicator to compare the relative nutritional value of fish oils. A ratio greater than 1 being more effective in preventing cardiovascular disease associated with plasma lipid levels [33]. The studied fish have high ω-3/ω-6 ratios in *S.maderensis* (4.27) and *I.africana* (4.58) and are therefore considered as well balanced species for human nutrition with a ratio of 1:1±1:5 [40]. Similar results were obtained by Tenyang et *al* on freshwater fish from Lake Maga in the far north region of Cameroon and by Alemu on *Oreochromis niloticus* nets from Lake Zeway in Ethiopia [30, 41]. The result also corroborate that of Zhang et *al* on some commercial fish from the Pearl River estuary in China [12]. A high ratio of ω-3/ω-6 may be due to the high level of omega 3 present in the diet of these species.

5. Conclusion

This study on chemical characterization of oil extracts from *Ilisha Africana* and *Sardinella maderensis* from the Cameroonian coast shows that their chemical indices vary significantly between the two species. *Sardinella maderensis* is an oily fish. The oils are of good quality and rich in PUFAs especially omega 3 which have several benefits for human health. They could be therefore used for nutritional, pharmaceutical and industrial applications.

Conflicts of Interest

The authors declare that they have no competing interests.

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