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Nutritional and Technological Potential of Oils Extracted from the Most Consumed Fish in Abidjan Town (Côte d'Ivoire)

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Abstract: ***This study aimed to evaluate the nutritional and technological potential of oils extracted from the heads and headless parts of the most consumed fish in the Abidjan city (Ivory Coast). Two oil extraction techniques (i.e. Folch and enzymatic hydrolysis methods) were tested. Then, the oil extracted chemical characteristics such as iodine, acid, saponification and peroxide values were determined using standard fraction methods. In addition, the fatty acid compositions of these oils were evaluated by GC-MS. The results showed the better extraction yield with the Folch method than that of hydrolysis. The mackerel head contains more lipids (19.87%) compared to other fish. The monounsaturated fatty acid content (31.07-54.76%) of fish is higher than that of saturated fatty acids (19.79-42.03%), regardless of the fish species. For the ω3, DHA (C22:6 ω3) predominates with 17.28% in the heads and headless parts of fish, while EPA (C20:5 ω3) representing only 0.90-2.79% of the total fatty acids. The chemical propertie analysis shows that these oils have high iodine and saponification indices, as well as low acid and peroxide indices. Saturated fatty acids are in the majority whatever the fraction studied. The extracted oils can be used for the formulation of livestock feed or ingredient of lipoprotein complex rich in omega 3 extract for children.***

Keywords: fish oil, extraction method, nutritional potential, technological potential

1. Introduction

Fish products play an important role in people's diets to meet their nutritional needs **(FAO, 2014)**. Fishing can further contribute to the eradication of hunger, food insecurity and malnutrition. In Côte d'Ivoire, the aquaculture sector plays an important role in the national economy. Indeed, in 2001, the fishing and aquaculture sectors represented 3.1% of agricultural Gross Domestic Product (GDP) and 0.74% of total GDP **(Direction of Fisheries Productions, 2010).**

Nutritionally, fish is one of the most important sources of animal protein, accounting for 17% of intake globally, but more than 50% in many least developed countries. Fish proteins are of high biological quality, easily assimilated and contain all the essential amino acids **(Andeson *and al.*, 1993).**

In addition to protein, fish also provides other valuable nutrients such as long chain omega 3 fatty acids, DHA (Docosahexaenoic Acid) and EPA (Eicosapentaenoic Acid), essential for the neurological development of children and the improvement cardiovascular health **(Qawasmi *and al.*, 2013; Hurtado *and al.*, 2015)**. Reducing the risk of death from cardiovascular disease and improving the neurological development of infants and young children whose mothers consumed fish before and during pregnancy is evidence of the beneficial effects of fish consumption. On human health **(Gow and Hibbeln, 2014; Loomans *and al.*, 2014).**

Otherwise; Fish heads, skeletons and viscera are a source of protein hydrolysates, which are attracting increasing interest as they are a potential source of bioactive peptides (**INFOFISH International, 2002)**. Fish protein hydrolysates are still considered the most nutritious and digestible ingredients for humans and animals **(Hoyle and Merrit, 1994).** Fish, thanks to its valuable nutritional properties, can play an important role in correcting unbalanced diets or in the fight against malnutrition.

The use of fish-based nutrients seems like a promising way of recovery. This study focused on the extraction of nutrients with high nutritional potential rich in long-chain polyunsaturated fatty acids, more particularly those of the n-3 series, in protein and minerals. Thus, the objective of this study is to evaluate the nutritional and technological potential of oils extracted from the heads and headless parts of the most consumed fish in the city of Abidjan (Côte d'Ivoire). Specifically, the first step was to extract the protein and lipid fractions; then to characterize them in order to highlight their potential uses.

2. Material and Methods

2.1 Study material

The biological material was made up of heads and headless parts of jawfish (*Sciades couma*), mackerel (*Scomber scombrus*), tilapia (*Oreochromis niloticus*) and tuna (*Thunnus albacares*) from the "pêche et froid" company in Abidjan, Côte d'Ivoire **(Figure 1).**

Whole fish transported in a cooler were immediately conditioned in a freezer at -20°C. They were then headless after defrosting. Quantities of 1000 to 1500 g of heads or headless parts of each species of fish were cut and ground separately in a grinder of the Moulinex^{R} type (Tomado: 400W). The ground material was freeze-dried for the extraction of lipids. Fish heads and headless parts were the subject of our study for their richness in omega 3 polyunsaturated fatty acids

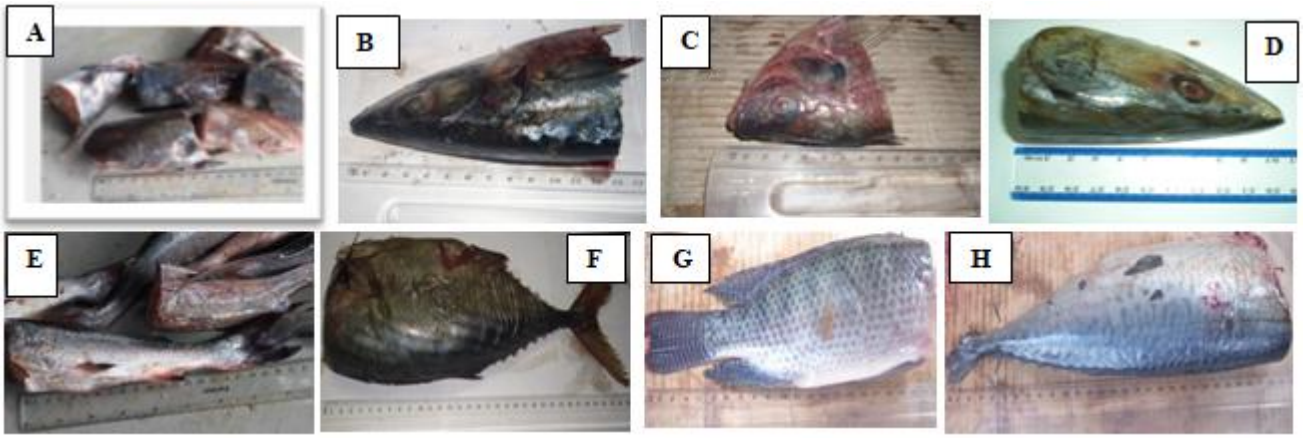

Figure 1: Different types of fish used as biological material
A: Jaw head (TEMAC); B: Mackerel head (TEMAQ); C: Tilapia head (TETIL); D: Tuna head (THETO); E: headless jawfish (MACET); F: headless mackerel (MAQET); G: headless tilapia (TILET); H: headless tuna (THOET).

3. Methods

3.1 Dosage of the total lipids of the fish used

Two (2) different methods were used to determine the total lipid content, namely the Soxhlet method and the enzymatic extraction method in order to compare their extraction yields.

3.1.1. Dosage of total lipids of fish used by the Folch

Oil Methodextraction was performed according to the method of **Folch *and al.* (1957)** modified by **Christie (1982)**, particularly well-suited to anhydrous products. The extraction was carried out on freeze-dried shredded mackerel head and headless part. To a 50 g sample, 200 mL of chloroform and 100 mL of methanol were added, then homogenized vigorously using a blender for 2 min. The mixture was filtered through a Bücher fitted with Whatman N^{o}.1 filter paper. The residues were redispersed in 300 mL of a chloroform-methanol mixture (2:1, v/v), homogenized for 3 min and filtered again. The remaining solids are washed with 60 mL of a chloroform-methanol mixture (2:1, v/v). All the filtrates are collected in separatory funnels. A volume of 0.2 mL of 0.7% NaCl solution is added to the filtrates. A separation into two phases is observed: a supernatant phase containing the non-lipidic compound and an organic phase which contains almost all of the lipids. The organic phase is recovered in a weighed flask, then the solvent is removed at 50°C on a rotary evaporator under vacuum. The lipid content is determined gravimetrically.

$$\%\,MG = \frac{P_1 - P_0}{P} \times 100 \qquad (1)$$

P: mass of the test sample (g);
P_0: mass of the dried balloon + pumice stone (g);
P_1: mass of the dried flask + pumice stone + residue (g).

3.1.2. Determination of the total lipids of the fish used by the enzymatic extraction method

The enzymatic reaction was carried out as described below. The oil extraction capacities with alcalase® 2.4 L (DX, 2.5 Au. A/g) were studied under their optimal activity conditions according to the technical data sheet from Novo Nordisk laboratories **(Table I)**.

Table I: Operating conditions for enzymatic hydrolysis using 2.4L alcalase for oil extraction.

	Alcalase® 2.4 L (DX, 2.5 Au.A/)
Reaction temperature	60°C
pH	8.00
Protein content (g / 100 g of wet product)	12.5
Volume of the reaction medium	1 liter
Agitation	450rpm
NaOH	4N
ratio E / S	5 g / 100 g

The oils were extracted in a 1000 mL Erlenmeyer flask placed on a heated plate with stirring where 500 g of ground fish were suspended in 500 mL of distilled water using the pH-sat technique **(Adler- Nissen, 1986)**. The hydrolysis conditions optimized by **Gbogouri (2005)** were strictly followed in this study. It consisted of keeping the pH constant by automatically adding 4N sodium hydroxide during the enzymatic reaction. The quantity of base consumed makes it possible to calculate the degree of hydrolysis. The reaction was carried out for two (02) hours under constant stirring at 450 rpm.

At the end of the hydrolysis, the enzymatic reaction is stopped by thermal inactivation of the reaction mixture. The hydrolyzate (oil, proteolysate and pellet) was heated in a water bath for 10 min at 95°C. The reaction mixture was subjected to centrifugation at 2000 x g for 05 min.

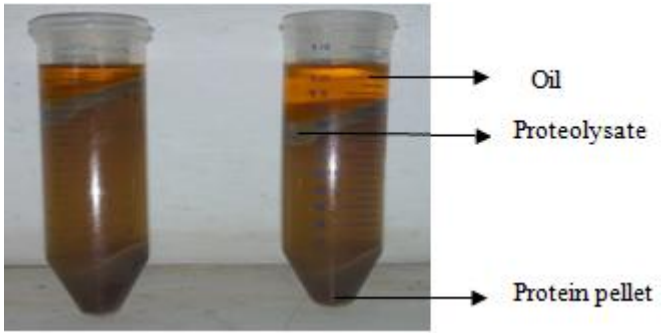

Figure 2: Different phases obtained from enzymatic hydrolysis after centrifugation

3.2 Determination of fatty acid composition by gas chromatography

3.2.1. Preparation of methyl esters

Fatty acid methyl esters were prepared by boron trifluoride reagent at 8% concentration in methanol (BF3/MetOH). One hundred (100) mg of sample was weighed into a 10 mL screw top test tube. 1.5 mL of hexane and 1.5 mL BF3/MetOH are added. The tube is hermetically sealed under nitrogen, shaken vigorously, then heated at 100° C. for 1 hour. After cooling to room temperature, 1 mL of hexane and 2 mL of distilled water are added thereto, before stirring under nitrogen. Two phases separate after standing. The upper phase was collected in another tube placed under nitrogen. The lower phase was extracted twice with 1 mL of hexane. All of the phases (methyl esters) collected are washed with 2 mL of distilled water, then dried over anhydrous sodium sulphate. The solvent is evaporated under nitrogen. Hexane was added for a concentration of methyl esters suitable for analysis by gas chromatography.

3.2.2. Analysis by gas chromatography

The analysis of methyl esters is carried out on a Péri 2000 chromatograph (Périchrom, Saulx-le-Chartreux, France) equipped with a flame ionization detector. They are separated on a capillary column (0.25 m in length, 250 µm in diameter, with a film thickness of 0.5 µm) packed with polyethylene glycol doped with terephthalic acid (Perichrom). The detector and injector temperatures are set at 260°C. The program used for the analysis is as follows: initially maintained at 70°C for 2 min, the column was brought to 180°C (39.9°C/min) and maintained for 8 min at this temperature, then underwent a second phase of heating up to 220°C (3°C/min) for 45 min. Cooling was carried out at 39.9°C/min. Peaks are identified using two fatty acid standards supplied by Supelco (Belle Fonte, USA): PUFA 1 (marine source) and PUFA 2 (animal source).

3.3 Methods of chemical analysis of extracted oils

3.3.1. Acid number

The acid number (Ia) was determined according to **AOAC (1997)**. It is the number of milligrams of KOH necessary to neutralize the free acidity of one gram of fat. It gives an estimate of the amount of free fatty acids. The free fatty acids are cold neutralized by an excess of alcoholic potash. 500 mg of fat from the enzymatic and chemical extraction are dissolved in an ethanol/diethyl ether mixture (1:1, v/v, 50 ml). 4 to 5 drops of phenolphthalein are added as a color indicator. The whole is titrated under magnetic stirring with a 0.1 N potassium hydroxide solution until the mixture turns pink. The acid number (Ia) is determined as follows:

$$Ia = \frac{(V \times N \times 56,1)}{\text{Masse de la matière grasse}} \quad (2)$$

V is the volume of KOH (mL) necessary for the neutralization of free fatty acids;
N is the normality of the KOH solution;
56.1 is the molar mass of KOH;
Acidity can be expressed as a percentage of oleic acid (molar mass: 282 g/mol).

(3)

3.3.2. Saponification index

The saponification index was calculated according to **AOAC (1997)**. The saponification index corresponds to the number of milligrams of KOH necessary to saponify 1 g of fat. It is an indicator of the amount of total fatty acids present in a fatty substance. The fat is dissolved in the potash. 5 g (±0.05 g) of the sample weighed in an Erlenmeyer flask, 50 ml of alcoholic potassium hydroxide solution (40 g of KOH / liter of ethanol) are added. The Erlenmeyer flask is adapted to a water cooler and the whole is boiled for 30 min. After cooling, 5 mL of distilled water is added. During this time, 50 mL of alcoholic potassium hydroxide were extracted with 0.5 N HCl in the presence of phenolphthalein (blank test). The sample is cooled in running water and titrated with 0.5 N HCl in the presence of phenolphthalein. The saponification index (Is) is expressed by:

$$Is = \frac{56,1 \times N \times (Vo - Ve)}{sample\ mass} \quad (4)$$

N: normality of HCl
V_0: volume of HCl required to titrate the blank;
V_e: volume of HCl required to titrate the sample.

3.3.3. Iodine value

The iodine value (Ii) was determined according to **AOAC (1997).** This index represents the number of grams of iodine fixed per 100 g of fat. It allows to know the overall degree of unsaturation of fats. The assay consists of causing a halogenating solution, the WIJS reagent, to act on the sample previously dissolved in carbon tetrachloride. A solution of potassium iodide (KI) is then added in order to extract the excess iodine in the aqueous phase and titrated in return with sodium thiosulfate. The addition reaction is accelerated by the use of a catalyst, mercuric acetate in acetic acid.

A dose of 0.6 g of oil is introduced into a 250 mL bottle. The oil was dissolved in 15 mL of chlorine and WIJS reagent. The bottle is protected from light for 2 hours. After this reaction time, 20 mL of potassium iodide (10%) and 150 mL of distilled water are added. The iodine released was assayed with 0.1N sodium thiosulfate in the presence of starch paste. A blank test is carried out under the same conditions. The iodine value is determined according to the following expression:

$$Ii = \frac{M \times M' \times (Vo - Ve)}{m} \quad (5)$$

Vo: volume of sodium thiosulfate (mL) required to titrate the blank;
Ve: volume of sodium thiosulfate (mL) required to titrate the assay;
M: Molarity of sodium thiosulfate solution (0.1 M);
M': Molar mass of iodine (126.9 g/mol);
m: mass of fat.

3.3.4. Peroxide value

The peroxide index (Ip) was calculated according to **AOAC (1997).** It corresponds to the quantity of active oxygen of the peroxide contained in 1 kg of fat, capable of being released under the conditions of the experiment. The principle consists of adding a solution of potassium iodide to the fat dissolved in chloroform, then titrating the iodine released in an acid medium with a solution of sodium thiosulfate.

To 5 g (±0.05g) of the sample in an Erlenmeyer flask, 10 mL of chloroform was added then stirred to dissolve the fat. Pure acetic acid (15 mL) and 1 mL of saturated KI solution were added, the bottle is stoppered and shaken for 1 min before allowing to stand in the dark. Distilled water (75 mL) and a few drops of starch paste which will serve as an indicator are also added. The released iodine is titrated under (vigorous) stirring with a solution of sodium thiosulfate (0.02 N). A blank test is carried out without fat. The peroxide value is determined as follows:

$$Ip = \frac{(Ve - V0) \times 1000 \times N}{m} \quad (6)$$

Ve: is the volume in ml of the thiosulfate solution;
V_0: is the volume in ml of the thiosulfate solution for the control;
N: is the exact normality of the thiosulfate solution;
m: mass of the test sample (g).

3.4 Statistical analysis of data

EXCEL and STATISCA version 8.0 software were the statistical tools used for the analysis of the results. The various results obtained were the mean of three repetitions and were expressed as mean ± standard deviation. The analysis of variance and the Duncan test at the 5% threshold were used to compare the means.

4. Results

1) Lipid content of the fish studied

Mackerel head contains a high fat content (19.87%) compared to headless mackerel (17.28%), tuna head (15.91 ± 0.10%), headless tuna (13.83 ± 0 .01%), tilapia head (11.83 ± 0.01%), headless tilapia (10.45%), jawfish headless (9.64%) and head jaw (8.79%) **(Table II) .**

Table II: Lipid content of fish

Fish	Lipids (g/100 g of dry matter
Headless mackerel	17.28 ± 0.00^{g}
Headless tuna	13.83 ± 0.01^{e}
Headless tilapia	10.45 ± 0.00^{c}
Headless jawfish	8.79 ± 0.00^{a}
Mackerel head	19.87 ± 0.00^{h}
Tuna head	15.91 ± 0.10^{f}
Tilapia head	11.83 ± 0.01^{d}
Jawfish head	9.64 ± 0.00^{b}

2) Fatty acid composition of total lipids extracted by chemical and enzymatic methods from the fish studied

The total fatty acid composition of mackerel, jawbone, tuna and tilapia heads and headless fish is given in **Table III**. In the left part of the columns of the mode of extraction appears the composition after extraction by the chemical method **(Folch *and al.*, 1957)**, while the right part we represented the content of fatty acids in the oil extracted by enzymatic treatment (Alcalase 2.4L). The contents (31.07-54.76%) of monounsaturated fatty acids (MUFA) are higher than those (19.79-42.03%) of saturated fatty acids (SFA) in the headed jawbone, headless tilapia, head of mackerel, jawfish head, tuna head and tilapia head regardless of the method of extraction. The polyunsaturated fatty acid (PUFA) contents of the heads are higher than those of the headless parts of mackerel, jawfish, tuna and tilapia fish, whether using the chemical or enzymatic method. These polyunsaturated fatty acid values vary for the heads (from 15.02-32.12%) and for the headless parts (from 13.03-29.85). The values found in the case of the determination of polyunsaturated fatty acids (PUFA) by the two (02) methods in each species of fish analyzed are close.

As for the fatty acids of the ω3 family, it is DHA (C22:6 ω3) which predominates up to 17.28% of the total fatty acids of the heads and headless parts of fish, the EPA (C20:5 ω3) representing only 0.90 to 2.79% of total fatty acids. Regarding the n-3 / n-6 ratio, the values obtained vary from 4.93 to 8.33; 3.42 to 3.69; 5.70 to 6.09; 3.01 to 3.49; 5.82 to 8.74; 2.68 to 3.20; 4.84 to 6.04 and 2.77 to 2.93; headless mackerel, headlessjawfish, headless tuna, headless tilapia, head of mackerel, head of jawfish, head of tuna and headless tilapia respectively.

Table III: Fatty acid composition (% of identified fatty acids) of total lipids extracted by methods using solvents (chemical) and enzymatic (with Alcalase® 2.4L) from heads and headless fish of Mackerel jawfish, Tuna and Tilapia.

fatty acids	Mackerel headless		Tuna headless		Tilapia headless		Jawfish headless		Mackerel head		Tuna head		Tilapia head		Jawfish head	
	Extraction method		Extraction method		Extraction method		Extraction method		Extraction method		Extraction method		Extraction method		Extraction method	
	Chem	Enzym	Chem	Enzym	Chem	Enzym	Chem	Enzym	Chem	Enzym	Chem	Enzym	Chem	Enzym	Chem	Enzym
C14 :0	02.72	02.18	01.87	01.15	01.10	0.88	02.36	01.89	01.34	01.12	02.85	02.21	01.50	01.20	03.86	03.30
C16 :0	21.53	23.00	14.41	15.40	21.06	22.5	21.48	22.95	22.37	23.19	13.90	14.85	20.84	32.95	20.22	21.6
C18 :0	10.00	08.3	06.10	04.42	07.63	05.93	06.7	04.90	06.51	04.00	05.39	02.73	09.40	07.88	03.79	01.35
∑SFA (%)	**34.25**	**33.48**	**22.38**	**20.97**	**29.79**	**29.31**	**30.54**	**29.74**	**30.22**	**28.31**	**22.14**	**19.79**	**31.74**	**42.03**	**27.87**	**26.25**
C16 :1n7	06.70	07.30	04.90	05.50	01.70	02.30	07.98	08.58	06.75	07.35	03.76	04.36	04.58	05.18	03.34	03.94
C18 :1n7	01.38	0.48	02.52	01.62	02.80	01.90	02.76	01.86	01.90	01.00	02.32	01.42	03.10	02.20	03.85	02.95

C18 :1n9	15.55	15.95	21.84	22.24	06.60	07.00	29.05	29.45	19.56	19.96	25.26	25.66	20.02	20.88	29.65	36.05
C20 :1n9	03.71	02.97	01.01	02.84	03.00	01.70	02.11	01.30	05.10	02.84	01.42	01.35	03.31	02.80	01.11	0.33
C22 :1n9	0.15	0.30	0.8	0.25	0.36	Trace	Trace	Trace	1.45	01.27	0.14	0.12	01.21	01.10	0.10	0.05
∑MUFA(%)	**27.49**	**27.00**	**31.07**	**32.45**	**14.46**	**12.90**	**41.90**	**41.19**	**34.76**	**32.42**	**32.90**	**32.91**	**32.22**	**32.16**	**38.05**	**43.32**
C18 :2 n-6	02.49	02.99	03.53	04.02	02.10	02.60	01.65	01.10	02.25	02.58	02.87	03.43	01.90	02.70	02.15	02.80
C20 :4 n-3	0.65	0.86	0.62	0.44	0.70	0.75	0.66	0.87	01.58	0.89	0.36	0.51	01.80	01.70	0.73	0.74
C20 :4 n-6	0.71	01.20	0.03	0.22	0.50	01.20	01.58	01.39	02.46	0.33	02.01	01.27	01.66	01.99	01.85	01.02
C20 :5 n-3	08.20	07.95	05.44	05.06	04.99	05.69	04.32	03.12	07.97	05.77	06.75	06.55	06.84	08.20	04.97	04.78
C22 :5 n-3 (EPA)	01.3	0.90	01.39	0.99	01.30	0.90	01.50	01.10	02.79	01.50	01.81	01.41	02.65	02.20	01.23	01.09
C22 :6 n-3 (DHA)	16.50	10.96	05.69	08.01	08.84	14.33	04.81	05.45	15.07	17.28	04.16	06.57	10.22	10.60	04.15	04.59
∑PUFA (%)	**29.85**	**24.86**	**16.70**	**18.74**	**18.43**	**25.47**	**14.52**	**13.03**	**32.12**	**28.35**	**17.96**	**19.74**	**25.07**	**27.39**	**15.08**	**15.02**
∑(n-3)	**26.65**	**20.67**	**13.14**	**14.50**	**15.83**	**21.67**	**11.29**	**13.54**	**27.41**	**25.44**	**13.08**	**15.04**	**21.51**	**22.70**	**11.08**	**11.20**
∑(n-6)	**03.20**	**04.19**	**03.56**	**04.24**	**02.60**	**03.80**	**03.23**	**04.49**	**04.71**	**02.91**	**04.88**	**04.70**	**03.56**	**04.69**	**04.00**	**03.82**
Ratio **(n-3/n-6)**	**08.33**	**04.93**	**03.69**	**03.42**	**06.09**	**05.70**	**03.49**	**03.01**	**05.82**	**08.74**	**02.68**	**03.20**	**06.04**	**04.84**	**02.77**	**02.93**

Chemical : Chim. ; Enzymatic : Enzym.

3) Chemical characteristics of the oils extracted from the fish studied

The chemical characteristics of the oils of headless mackerel, head of mackerel, headless Jawfish, head of Jawfish, headless tuna, head of tuna, headless tilapia and head of tilapia resulting from the various chemical and enzymatic methods are recorded in **Table IV.** Analysis of the chemical properties shows that these oils have high iodine and saponification indices, then low acid and peroxide indices. Statistical analysis of oils shows significantly similar indices at the 5% threshold regardless of the method used for extraction. The iodine indices obtained by chemical extraction of fish samples mackerel head (194.74±2.50 g of iodine/100 g of oil) and jawfish (193.65±0.00 g of iodine/100g oil), headless mackerel (190.17±2.01g iodine/100g oil), headless tuna (188.41±2.11g iodine/100g oil), tuna head (191.51 ± 2.0 g iodine /100 g oil) and tilapia head (189.43 ± 1.84 g iodine /100 g oil) are not significantly different at the 5% level. These values are higher than those of headless tilapia (186.32 ± 1.86 g of iodine / 100 g of oil) and head of mackerel (180.32 ± 0.00 g of iodine / 100 g of oil). With regard to enzymatic extraction, the iodine indices of fish parts tuna head (192.20 ± 2.69 g of iodine / 100 g of oil) and jawfish head (193.02 ± 0.0g iodine/100g oil), headless mackerel (189.47±2.0g iodine/100g oil) and tilapia head (190.5±0.72g d iodine/100 g of oil) are not significantly different at the 5% level. Concerning the saponification indices, the values obtained in the fish are not significantly different either for the chemical methods or for the enzymatic method. The peroxide indices of headless tuna (1.42 ± 0.03 meq O2 / kg of oil), headless tilapia (1.47 ± 0.02 meq O2 / kg of oil) and head of tilapia (1.47 ± 0.07 meq O2 / kg oil), headless mackerel (1.5 ± 0.07 meq O2 / kg oil) and tuna head (1.67 ± 0.02 meq O2 / kg oil), head jaw (0.20± 0.11 meq O2 / kg oil) and jaw head (0.40 ± 0.07 meq O2 / kg oil) obtained by chemical extraction methods are not different at the 5% level. The peroxide indices of headless tuna fish (1.42 ± 0.02 meq O2 / kg oil), headless tilapia (1.40 ± 0.07 meq O2 / kg oil), tuna head (1. 50 ±0.18 meq O2/kg oil) and tilapia head (1.11 ± 0.31 meq O2/kg oil) obtained by the enzymatic extraction method are not significantly different. The acid indices of the samples analyzed by the chemical extraction method are not significantly different.

Table IV: Chemical composition of oils extracted from fish by two methods of extraction

Clues	Iodine value (g of iodine /100 g of oil)		saponification Index (mg KOH / g of oil)		Peroxide Index (meq O_2 / kg of oil)		Acid Number (mg KOH / g of oil)		Acidity (% oleic acid).	
	Extraction method		Extraction method		Extraction method		Extraction method		Extraction method	
Oil obtained	Chemical extraction	Enzymatic extraction	Chemical extraction	Enzymatic extraction	Chemical extraction	Enzymatic extraction	Chemical extraction	Enzymatic extraction	Chemical extraction	Enzymatic extraction
Mackerel headless	190.17± 2.01^{ab}	189.47± 2.0^{abc}	197.72± 3.39^{a}	196.62± 4.20^{a}	01.5± 0.07^{ab}	0.75± 0.30^{acd}	0.94± 0.03^{bcd}	0.40± 0.07^{a}	0.50 ± 0.03^{c}	0.20± 0.03^{a}
Tuna headless	188.41± 2.11^{ab}	187.80± 2.72^{ac}	195.17± 2.12^{a}	196.9 ± 1.73^{a}	1.42 ± 0.03^{a}	1.42 ± 0.02^{ab}	1.5 ± 0.21^{f}	1.5 ± 0.10^{b}	0.8 ± 0.04^{b}	0.9 ± 0.03^{b}
Tilapia headless	186.32 ± 1.86^{b}	186.00 ± 2.18^{c}	193.84± 1.74^{a}	193.85 ± 1.07^{a}	1.47± 0.02^{a}	1.40 ± 0,07^{ab}	1.41 ± 0.11^{ef}	1.40± 0.12^{b}	0.7± 0.05^{b}	0.75 ± 0.04^{b}
Jawfish headless	180.32± 0.00^{c}	179.86± 0.00^{d}	196.64± 4.45^{a}	195.64± 5.60^{a}	0.20± 0.11^{c}	0.25 ± 0.10^{c}	0.70± 0.06^{ab}	0.91 ± 0.13^{b}	0.44 ± 0.03^{a}	0.46± 0.07^{b}
Mackerel head	194.74± 2.50^{a}	194.99± 0.01^{b}	199.97± 3.89^{a}	200.56± 2.80^{a}	1.73± 0.11^{b}	1.70 ± 0.40^{b}	0.80± 0.08^{abc}	0.84± 0.27^{ab}	0.40± 0.03^{ab}	0.42 ± 0.13^{ab}
Tuna head	191.51 ± 2.0^{ab}	192.20± 2.69^{ab}	198.41± 2.07^{a}	198.9 ± 0.49^{a}	1.67± 0.02^{ab}	1.50 ± 0.18^{ab}	1.1 ± 0.12^{cde}	1.13 ± 0.14^{b}	0.55 ± 0.03^{b}	0.50 ± 0.08^{b}
Tilapia head	189.43± 1.84^{ab}	190.5± 0.72^{abc}	196.38± 1.97^{a}	195.42 ± 2.93^{a}	1.47 ± 0.07^{a}	1.11 ± 0.31^{ab}	1.3 ± 0.13^{def}	1.2 ± 0.07^{b}	0.63 ± 0.05^{b}	0.6 ± 0.08^{b}
Jawfish head	193.65± 0.00^{a}	193.02± 0.0^{ab}	198.00± 1.48^{a}	199.70± 5.6^{a}	0.40± 0.07^{c}	0.45 ± 0.10^{cd}	0.53 ± 0.01^{a}	0.63± 0.13^{ab}	0.30± 0.07^{b}	0.32 ± 0.07^{ab}

4) Fatty acid composition of the total lipids of the pellet and the proteolysate after freeze-drying

The fatty acid composition (% of identified fatty acids) of the total lipids extracted from the pellet and the freeze-dried proteolysate of heads and headless fish of mackerel, jawbone, tuna and tilapia is recorded in **Table V**. Saturated fatty acids predominate when whatever the fraction studied: 22.93 - 60.46% for the oil of the proteolysate, 19.37-55.54% for the oil of the pellet while the fatty acids having a double bond within them represent respectively 24.40-42.15% and 22.50-48.32%. If the polyunsaturated fatty acids are in relatively high quantity and vary between 13.11-43.09% for the lipids of the proteolysate and between 14.41-38.51% for the lipids of the pellet, however, it remains lower than those of saturated fatty acid. With regard to fatty acids of the ω3 family, it is DHA (C22:6 ω3) which predominates with 15.98% of the total fatty acids of the pellet, EPA (C20:5 ω3) representing only 0.97 to 2.70% of total fatty acids. Concerning the n-3/n-6 ratio, the values obtained from the proteolysate vary from 2.21 to 10.17 and from 3.02 to 11.23 in the pellet.

Table V: Fatty acid composition (% of identified fatty acids) of the total lipids extracted after lyophilidation of the proteolysate and the pellet of the heads and headless fish of mackerel, Jawfish, tuna and tilapia

Fatty acid	Mackerel headless		Jawfish headless		tuna headless		Tilapia headless		Mackerel head		Jawfish head		tuna head		Tilapia head	
	Proteolysate	pellet	Proteolysate	pellet	Proteolysate	pellet	Proteolysate	pellet	Proteolysate	pellet	Proteolysate	pellet	Proteolysate	pellet	Proteolysate	pellet
C14 :0	02.06	02.14	02.48	01.18	03.10	0.80	01.98	01.83	03.30	01.10	02.81	02.25	01.80	02.20	02.86	01.97
C15 :0	0.50	0.30	Nd	Nd	0.60	0.40	0.25	0.17	23.10	24.34	Nd	Nd	01.10	01.40	0.39	0.28
C16 :0	20.90	23.10	20.95	15.48	25.50	22.30	20.64	22.13	23.39	23.10	16.30	14.84	30.20	32.91	22.91	21.33
C18 :0	10.60	08.80	05.89	03.57	07.60	11.10	06.99	04.78	10.67	07.00	03.82	02.28	10.15	10.85	04.15	04.21
∑SFA (%)	**34.06**	**34.34**	**29.32**	**20.23**	**36.60**	**34.60**	**29.86**	**28.91**	**60.46**	**55.54**	**22.93**	**19.37**	**43.25**	**47.36**	**30.31**	**27.79**
C16 :1n7	02.40	03.90	03.08	02.09	04.20	02.10	04.06	05.03	03.73	03.72	04.47	3.81	04.20	05.50	04.07	04.39
C18 :1n7	03.00	02.36	02.41	02.52	02.70	02.80	02.91	02.76	01.96	01.90	03.43	02.32	02.70	03.10	03.83	03.85
C18 :1n9	17.56	15.15	22.36	21.44	15.10	16.20	31.35	28.65	31.46	39.16	28.66	24.86	18.02	20.08	28.84	35.25
C20 :1n9	02.80	02.67	01.84	02.64	02.40	01.40	01.91	01.00	03.60	02.54	01.45	01.05	02.30	02.50	01.11	0.13
C22 :1n9	0.30	0.50	0.28	Trace	Trace	Trace	Trace	0.09	1.40	01.00	0.41	0.53	01.60	01.60	0.11	0.18
C24 :1n9	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
∑MUFA(%)	**26.06**	**24.58**	**29.97**	**28.69**	**24.40**	**22.50**	**40,23**	**37.53**	**42.15**	**48.32**	**38.42**	**32.57**	**28.82**	**32.78**	**37.96**	**43.80**
C18 :2 n-6	01.76	01.68	03.74	02.72	01.10	01.30	01.53	01.80	02.00	01.08	02.56	02.03	01.60	01.40	01.53	01.30
C18 :3 n-3	0.50	0.30	02.06	0.83	0.50	01.00	01.66	01.74	01.02	02.08	03.65	02.52	0.70	0.60	01.70	01.77
C20 :3 n-3	0.26	0.24	0.67	0.17	0.18	0.20	0.71	0.27	0.50	0.54	0.14	0.19	0.30	0.30	0.75	0.47
C18 :4 n-3	01.70	01.75	Nd	Nd	0.80	01.10	Nd	Nd	02.6	Nd	Nd	Nd	0.90	0.91	Nd	Nd
C20 :4 n-3	0.89	0.90	0.51	0.31	0.68	0.70	0.61	0.83	01.10	0.39	0.31	0.57	01.20	01.50	0.63	0.60
C20 :4 n-6	03.00	02.50	01.27	01.08	0.90	01.40	02.55	01.83	02.20	0.33	02.16	01.72	01.96	02.00	01.55	01.33
C20 :5 n-3	06.40	07.50	05.84	04.66	04.82	04.80	02.00	05.72	07.40	05.37	06.85	06.15	06.30	07.80	04.24	04.37
C22 :5 n-3 (EPA)	01.20	01.40	01.21	01.49	01.70	01.40	0.97	01.34	02.20	01.24	01.64	01.91	01.60	02.70	01.16	01.05
C22 :6 n-3 (DHA)	11.41	15.98	03.64	04.01	07.80	15.20	03.08	03.43	24.07	06.21	02.11	07.54	25.20	21.30	02.10	03.52
∑PUFA (%)	**27.12**	**32.25**	**18.94**	**15.27**	**18.48**	**27.10**	**13.11**	**15.96**	**43.09**	**17.24**	**19.42**	**22.63**	**39.76**	**38.51**	**13.66**	**14.41**
∑(n-3)	**22.26**	**28.07**	**13.93**	**11.47**	**16.48**	**24.40**	**09.03**	**13.33**	**38.89**	**15.83**	**14.70**	**18.88**	**36.20**	**35.11**	**10.58**	**11.78**
∑(n-6)	**04.76**	**04.18**	**05.01**	**03.80**	**02.00**	**02.70**	**04.08**	**03.63**	**04.20**	**01.41**	**04.72**	**03.75**	**03.56**	**03.40**	**03.08**	**02.63**
Ratio **(n-3/n-6)**	**04.70**	**06.72**	**02.78**	**03.02**	**08.24**	**09.04**	**02.21**	**03.67**	**09.26**	**11.23**	**03.11**	**05.03**	**10.17**	**10.33**	**03.44**	**04.48**

5. Discussion

The interest of this study concerns the nutritional potential of oils extracted from the heads and headless parts of tuna, mackerel, jawfish and tilapia fish. The results show that the heads of the 4 fish contain higher lipid contents (9.64 - 19.87%) than those of the headless parts (8.79 -17.28%). However, the highest lipid content was observed in mackerel with 19.87%. In view of these results of this mackerel head, it is possible to affirm that the mackerel head represents a potential source of lipids and would therefore require particular attention for the formulation of feed.

The results concerning the fatty acid composition of the total lipids extracted by the methods using solvents and enzymatic are compared with the literature, in particular with the fatty acid content of the total lipids of rainbow trout **(Haliloglu *and al.*, 2003)** to those of salmon eggs **(Shirai *and al.*, 2006)**, as well as those contained in the polar lipids of salmon eggs. From the outset, we can observe that if the overall composition remains qualitatively identical according to the extraction methods, significant differences in proportions appear. This is particularly the case for total lipids for monounsaturated fatty acids (31.07-54.76%) in the present work and 22.41% according to **(Haliloglu *and al.*, 2003).**

A large variation appears for EPA (C20:5n-3) between the tissues of the fish studied. This content varies from 0.9 to 2.79%. The difference for DHA (C22:6n-3) is much more marked. It goes from simple to triple (4.15-17.28%). Overall, however, the total PUFA content does not show a very large difference (13.03-29.85%). These variations can obviously be explained by numerous parameters

(environment, season, sexual cycle), but they can be the consequence of faulty handling of the samples; it is necessary to remember the extreme sensitivity of PUFAs to oxidation, which makes them difficult to analyze. The fatty acid composition of the oils extracted by the chemical method and those extracted by the enzymatic method shows that saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) have very similar contents. On the other hand, it is important to note that mackerel lipids contain a large amount of DHA, which reached 17.28% in this study.

The chemical properties of the fat of the fish studied revealed a similarity between the indices of the oils extracted by the method using solvents (the chemical methods) and that extracted by enzymatic means. Analysis of these chemical properties of the fat extracted from the heads and headless parts of these fish revealed high iodine values (179.86-194.99 g iodine/100 g oil). These indices are higher than those of several other vegetable oils such as cottonseed oil (99 - 119 g iodine / 100 g oil) and soybean oil (120 - 143 g iodine / 100 g of oil) **(Geoffrey, 1990).** However, these values would reflect a good content of the oil in unsaturated fatty acids. These oils are likely to polymerize in the open air. It should be remembered that the iodine index is linked to the overall unsaturation in fat. Many studies have shown that the consumption of edible oils rich in saturated fatty acids causes cardiovascular and myocardial diseases caused by high blood cholesterol levels **(Mensink and Katan, 1990; Siguel and Lerman, 1993).** On the other hand, the consumption of oils rich in unsaturated fatty acids, in particular polyunsaturated ones, reduces this risk **(Connor, 2000; Dommels *and al.*, 2002; Toyoshima *and al.*, 2004).**

The acid number is a parameter which makes it possible to assess the degree of alteration of oils. It is low in the oils studied and is between 0.4 and 1.50 mg KOH/g oil. These low values could confirm that these oils do not contain enough free fatty acids. They are therefore very unlikely to rancidity. Indeed, free fatty acids, under the effect of atmospheric oxygen, cause oils to go rancid. These indices are lower than the limit value of 4 mg KOH/g of oil established for an edible oil by the **Codex Alimentarius (1992).** These low values predict good preservation of the oils extracted from fish.
The peroxide indexes of oils extracted from fish show good values (0.2-1.73 meq O2/kg) when it is known that these oils are crude and that the peroxide index of refined table oils can evolve up to reach the value 10. The quality of an oil varies inversely with the increase in the peroxide index. The degree of oxidation of the oil provides information on its stability or its aptitude for conservation, which is a determining factor for the subsequent storage of the products produced **(Olie, 1972)**. Indeed, the oxygen in the air reacts with the fatty acids in the oil. In this series of reactions, unsaturated fatty acids are the preferred target, because oxygen binds to the double bonds to form oxidic bonds characteristic of epoxides, which are toxic and cause cancer and cell aging.

The saponification indexes of oils extracted from fish (193.84-200.56 mg KOH/g of oil) are higher than that of sunflower oil (189 mg KOH/g of oil) which is a very rich in oleic and linoleic acids (unsaturated fatty acids with 18 carbon atoms). In view of this comparison, we can say that these oils are made up of long-chain fatty acids. Their use in the soap industry could be recommended like certain animal fats such as herring oil **(FAO, 1975).**

The purpose of enzymatically extracting lipids is to improve the nutritional and technological qualities of the products resulting from this hydrolysis, without developing a parasitic taste or aroma such as bitterness.

Regarding the comparison of extraction methods, the results showed that the best fat extractions were obtained with the methods of extraction by organic solvents. These results are in agreement with those obtained by **Gunnlaugsdottir and Akman. (1993) and Undeland *and al.* (1998)**. Indeed, according to these authors, the mixture of apolar organic solvents such as hexane, petroleum ether, chloroform and polar solvents such as alcohols (methanol, ethanol or isopropanol), makes it possible both to extract neutral lipids and complex lipids. The most effective of these methods is that using cold solvents, i.e. Folch, since it extracts 20.76 and 18.51% of the total lipids respectively in the tuna head and in the headed tuna. In addition, **Manirakiza *and al.* (2001)** suggest that to better extract polar lipids it is advisable to use mixtures with alcohols such as ethanol, methanol and isopropanol. The method of **Folch *and al.* 1957** made it possible to extract a higher lipid level in the tuna head than in the headed tuna. These results confirm the classification made by **Sébédio (1992) and Corraze and Kaushik (1999),** classifying tuna in the category of "intermediate" fish that have in their muscles more than 1%. Furthermore, the difference between the contents of total lipids extracted by the method using solvents (20.76 and 18.51%) and the enzymatic extraction (18.91 and 15.83%) in the head of tuna and headless tuna does not exceed 2%. These results are in agreement with the results of **Linder*and al.* (2002)** who report that the difference between enzymatic extraction and solvent extraction does not exceed 2%.

The fatty acid compositions of the lipids of the two soluble (extracted oil) and insoluble (bottom lipid) fractions generated after 2 hours of hydrolysis of the heads and headless parts of the fish were established. The saturated fatty acids are in high proportion in the fractions studied: 22.9 - 60.46% for the oil of the proteolysate, 19.37 -55.54% for the pellet while the monounsaturated fatty acids represent respectively 24.40 at 42.15% and 22.50 to 48.32%. Polyunsaturated fatty acids still account for 13.11-43.09% of supernatant oil lipids and 14.4-38.51% of pellet lipids. Palmitic acid (C16:0) is the main fatty acid in the 2 fractions and represents nearly twice the quantified fatty acids. Oleic acid (C18: ω9) is the second most abundant fatty acid. As far as fatty acids of the ω3 family are concerned, it is DHA (C22:6 ω3) which predominates to even reach 15.98% of the total fatty acids of the pellet, EPA (C20:5 ω3) does not representing only 0.97 to 2.70% of total fatty acids. It is for this family (ω3) that the most notable differences are observed with a higher rate in the soluble fraction (38.89% compared to 35.40%) and this mainly because of the EPA and DHA which are very different. These two fatty acids have been widely studied for their biological function. Thus,

EPA helps to lower cholesterol levels and has a protective role against cardiovascular disease, while DHA plays an important role in the development of nervous tissue in the brain. The high levels of DHA found here were expected because fish orbital lipids are known to be an excellent source **(Yazawa *and al.*, 1991; Chantachum *and al.*, 2000)**. Several authors **(Stansby *and al.*, 1990; Ando *and al.*, 1996; Shimada *and al.*, 1997)** have even found that the DHA content in the oils of tuna species is often considerably higher than that of most species. Other species and that the DHA content greatly exceeded that of EPA. The hierarchy of palmitic acid, oleic acid, DHA is also consistent for this type of biological material **(Chantachum *and al.*, 2000; Al-Sayed Mahmoud *and al.*, 2008).** The relation between unsaturated/saturated fatty acids (PUFA/SFA) also testifies to the excellent quality of the fat of the heads and the headless parts of the fish in terms of balance.

The n-3/n-6 ratio for a healthy diet should be between 1 and 5 according to **FAO/WHO (2008).** The values obtained in the oil of the proteolysate (2.21 to 10.17) and in the pellet (3.02 to 11.23) make the fish analyzed food with enormous nutritional potential. According to **Henderson and Tocher (1987)**, the n-3 / n-6 ratio can be used to define the origin of fish: freshwater fish have an n-3 / n-6 ratio of 1.08 to 3.3, while in those of sea water, the ratio ranges from 8.3 to 11.4. This n-3 / n-6 ratio is higher in protein pellet oils where it reaches the value of 11.23, thus presaging good nutritional aptitude.

6. Conclusion

In this study, the objective was to evaluate the nutritional and technological potential of oils extracted from the heads and headless parts of the most consumed fish in the city of Abidjan (Côte d'Ivoire). The fish studied represent a real source of lipids. A high iodine number could be an indicator of a large number of unsaturation levels in the chain of these fatty acids. The low levels of peroxide index and free acidity show a possible resistance to oxidation of these fats. The comparison of the fatty acid contents according to the extraction methods did not show the difference between the values found for each species of fish.

A comparison of lipid extraction methods revealed that the Folch method is the most efficient in terms of oil extraction yield. However, the enzymatic extraction method made it possible to extract a high content of lipids from the analyzed samples. These fish therefore constitute a potential that can be qualified as lipids. The lipid fractions have a high content of unsaturated fatty acids, in particular DHA and EPA. Of the eight samples studied here, mackerel head is the most 'promising' substrate in terms of potential valuation.

Thus, the hydrolysis of fish is a method of choice for the recovery of lipids, particularly mackerel heads. Thus 2 hours of enzymatic reaction by alcalase makes it possible to obtain a soluble fraction that can be used in aquaculture feed and human nutrition, while the insoluble fraction can be incorporated into feed. This work therefore leads to encouraging prospects for fish processing plants, the formulation of livestock, poultry or children's feeds with the lipoprotein complex rich in omega 3 extract as an ingredient.

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