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Research Paper

ENZYMATIC EXTRACTION AND BIOCHEMICAL CHARACTERIZATION OF LIPIDS FROM THE MACKEREL (*Scomber scombrus*) AND JAWFISH (*Chrysichthys nigrodigitatus*) EATEN IN COTE D'IVOIRE

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Abstract

The objective of this study is the biochemical characterization of fish parts and lipids extracted from the heads of mackerels (*Scomber scombrus*) and jawfish (*Chrysichthys nigrodigitatus*) and from parts of the headless mackerel and jawfish. The analyses showed a similar protein content of 18.03 ± 2.23%, 16.62 ± 0.41%, 16.80 ± 0.01% and 15.90 ± 0.1 in the headless mackerel, mackerel head, in the headless jawfish and the head of the jawfish respectively. The ash contained most phosphorus (9.20 g / kg), calcium (3.20 ± 0.1 mg / Kg) and Magnesium (3.30 ± 0.1mg / Kg) in the headless mackerel, in the head of the jawfish and in the headless jawfish respectively. Enzymatic extraction of the lipids revealed 19.87% in the mackerel head. Lipid extraction (19.87%) from the mackerel head is higher than the other part of fishes. Two extraction methods (Folch and Enzymatic) of lipids were compared. The Folch method extraction is most efficient with a yield of 20.15% in the headless mackerel, 21.80% in the mackerel head, 09.64% in the headless jawfish and 10.51% in the head of the jawfish. These headless mackerel oils, mackerel heads, headless jawfish and jawfish heads were good chemical index, with high iodine values of 190.17 ± 2.01 for the headless mackerel, 194.74 ± 2.50 for the mackerel head, 180.32 for the headless jawfish and 193.65 for the jawfish head. All these properties showed that the headless and mackerel heads (*Scomber scombrus*) and jawfish (*Chrysichthys nigrodigitatus*) showed a great nutritional value and allowed us to predict their use in the food, pharmaceutical and cosmetic fields.

Key words: enzymatic extraction, mackerel, jawfish, polyunsaturated fatty acids, lipids.

INTRODUCTION

Oceans constitute a very diversified food wealth (algae, crustaceans, shellfish, molluscs, fish). According to the FAO (2008), more than 130 million tons of fish are currently caught or bred each year in the world, while 275,000 tons are fried in Côte d'Ivoire. Fish-based dishes such as: “attieke” with smoked or grilled mackerel and “attieke” with stung jawfish or supper are much used by the Ivorian public. Fish is the main source of animal protein for the Ivorian consumer with 11 and 14 kg / inhabitant / year (FAO, 2008). Fish such as mackerel, jawfish, tilapia and tuna are much consumed in Côte d’ivoire. Fish plays an important role in human nutrition owing to its nutritional qualities but also for the wide choice it offers to taste the texture or the form under which it is marketed: whole or in net, frozen, salted, smoked, dried or processed (preserves, prepared dishes, surimi). Fish also represent a source of lipids. which are the subject of much research, whose analytical results play an important role. These lipids are particularly composed of the long-chain polyunsaturated omega-3 (n-3) fatty acids family, mostly represented by eicosapentaenoic acid (EPA, C20: n-3) and docosahexaenoic acid (ADH, C22: 6 n-3), polyunsaturated fatty acids from the omega-6 (n-6) family and micronutrients such as vitamin A, vitamin D, calcium, magnesium, phosphorus (Liaset and Espe, 2000). This particular composition of fish oils gives them nutraceuticals in the prevention of cardiovascular diseases and arteriosclerosis (Bang and Dyerberg, 1972; Zyriax and Windler, 2000). The n-3 polyunsaturated fatty acids further promote the development of the central nervous system of the fetus (Bourre, 1996; Ackman, 1999). Some studies have shown the anti-inflammatory properties of fish oils in general (Kim *et al.,* 2006; Maroon and Bost, 2006). Fish oils fight against the development of atherosclerosis (Zampolli *et al.,* 2006). The use of food enzymes, especially proteases, has been a considerable success in modern techniques of protein valorization. It is a less expensive and relatively easy process to control, resulting in the production of products with functional and nutritional properties particularly interesting for the food, pharmaceutical and cosmetic industries (Kristinsson and Rasco 2000a, 2000b, Liaset *et al.,* 2003). In Côte d’Ivoire no research work has been carried on the enzymatic fish lipids extraction.

The objective of this study is to perform protease-assisted aqueous lipid extraction from the headless parts and heads of mackerel *(Scomber scombrus)* and jawfish (*Chrysichthys nigrodigitatus*). To carry out the study, some biochemical characteristics of the fish will be determined.

MATERIALS AND METHODS

Sampling

The biological material consists of heads and headless mackerel and jawfish parts from the "Pêche et Froid" company of Abidjan (Côte d’Ivoire).

After being unloaded at the port of Abidjan (Côte d'Ivoire), the fish is immediately frozen at -20°C before being transported to conservation and marketing companies. The products analyzed were taken from the "Pêche et Froid" company located in the port area of Vridi (Abidjan). This company was chosen for its compliance with hygiene guideleines, which confers to its products very good microbiological and organoleptic qualities. Mackerel (*Scomber scombrus*) and jawfish (*Chrysichthys nigrodigitatus*) were

randomly selected for our study. These fish is transported in a cooler to the laboratory of the Swiss Scientific Research Center where it is beheaded and frozen at 20°C.

Physico-chemical analysis of samples

The total nitrogen content of the fresh ground product is determined by the Kjeldahl method (AOAC, 1990). The ashes are determined in the muffle furnace at 540°C. in porcelain crucibles on 5g of sample until complete mineralization for 12 hours according to the AOAC, 1990. This determination is made by the so-called steaming method at moderately high temperature for 24 hours.

The calcium, potassium, magnesium, iron and sodium contents were determined according to the AOAC, 1999. Phosphorus was measured according to the method of Tausky and Shorr (1953)

Lipids extraction by the Folch method

The oil was also extracted according to the method of Folch *et al.,* (1957) modified by Christie (1982), which is particularly well suited to anhydrous products. The extraction was carried out on lyophilized crusts of heads of, and headless mackerel and jawfish. 50g sample was added to 200 ml of chloroform and 100 ml of methanol and then homogenized vigorously with a mixer for 2 minutes. The mixture was filtered through a Büchner filter fitted with Whatman filter paper. The residues were redispersed in a chloroform methanol mixture (2: 1, v / v, 300 ml), homogenized for 3 min and filtered again. The remaining solids were washed with chloroform-methanol (2: 1, v / v, 60 ml). All the filtrates were collected in separating funnels. Filtrates were added to 0.2 volume of 0.7% NaCl solution. The organic phase was recovered in a calibrated flask, and then the solvent was removed at 50°C. in a rotary evaporator under vacuum. The oil content was determined by gravimetry.

Extraction of lipids by enzymatic hydrolysis

500g of the initially ground sample was suspended in 500ml of distilled water. The pH-stat technique that was used has been developed by Adler-Nissen and Olsen (1979), Adler-Nissen (1982, 1986), Adler-Nissen (1983). It consists in maintaining the constant pH by addition of 4N of NaOH during the enzymatic reaction. The reaction was carried out for 2 hours under constant stirring at 450 rpm.

Hydrolysis degree calculation

The degree of hydrolysis is defined as the percentage of hydrolyzed peptide bonds on the number of total peptide bonds after evaporation of the solvent at 50°C.

$$DH = \frac{B \times Nb}{MP \times \alpha \times htot} \times 100$$

B: volume of NaOH (ml)
NB **:** NaOH concentration
α **:** Mean degree of dissociation of alpha amino groups
MP **:** Mass of proteins (NT x 6.25) present in the reaction medium (g)
htot **:** Number of peptide bonds in the protein (7.5 meq / g)
The degree of dissociation is defined as follows:

$$\alpha = \frac{10^{pH-pK}}{1 + 10^{pH-pK}}$$

pK represents the mean pK of the α-mineral functions released during the hydrolysis:

$$pK = 7{,}8 + \frac{298 - T}{298 \times T} \times 2400$$

T is the temperature expressed in Kelvin

Lipids extraction by heating of water

The heat extraction was carried out in a pot in which 500 g of ground fish was dispersed in 500 ml of distilled water and brought to a boil. The mixture was stirred at 95°C for 30 minutes. The broth from the cooking was passed over a sieve and then the filtrate was centrifuged at 2000 × *g* for 15 min. The supernatant oil was separated from the mud (Gbogouri, 2005). After extraction, the oil was stored in a plastic jar before characterization.

Lipid analysis

The acid index was measured according to the method, AOAC, Standard 969.17, 1997

The saponification index was determined according to the method, AOAC, Standard 920.160, 1997. The iodine value was measured according to the method, AOAC, Standard 993.20, 1997. The peroxide value was measured according to the method, AOAC, 965.33, 1997

STATISTICAL RESULTS ANALYSIS

Fishes comparison versus variables was made using the 5% variance analysis according to the STATISCA version 8.0 software. Duncan comparison test of average two to two indicates the homogeneous groups.

RESULTS AND DISCUSSION

Characterization of the various substrates

Biochemical and morphological fish characteristics

The biochemical and morphological characteristics are presented in Table 1. The protein contents of the products are not significantly different from the 5% threshold (P<5%). Lipids and proteins make up dry fish mats.

Table 1. Biochemical and morphological fish characteristics

Samples	**Humidity** (%Fw)	**Proteins** (%Fw)	**Lipids** (%Fw)	**Ash** (%Fw)
	Moy ± ET	Moy ± ET	Moy ± ET	Moy ± ET
MAQET	73.03 ± 1.88[a]	18.03±2.23[a]	13.45±0.00[b]	1.69±0.24[a]
TEMAQ	77.04 ± 2.39[a]	16.62±0.41[a]	15.64 ±0.00[a]	2.00±0.45[a]
MACET	78.37 ± 1.46[a]	16.80±0.01[a]	10.51±0.00[c]	2.89±0.65[a]
TEMAC	75.09 ± 3.66[a]	15.90 ±0.1[a]	9.64±0.00[c]	5.60±3.05[a]
		Morphology		
	Weight (g)	**Average**	**Length (cm)**	**Average**
MAQET	400-1500	950	32.5-85.9	52.50
TEMAQ	260-343	277.34	10.2-12.9	11.89
MACET	150-200	175.7	nd	nd
TEMAC	150-200	157.1	nd	nd

Fw : (Fresh weight)
MAQET : Headless mackerel
TEMAQ : Mackerel's head
MACET : Headless jawfish
TEMAC : Jawfish's head
In the same column, the values assigned to the same letter are not significantly different at 5% threshold

Mineral composition

The minerals (potassium, phosphorus, sodium, calcium, magnesium and iron) constitute the smallest part of the dry matter and are predominantly represented by phosphorus whose values are for the mackerel head 9.20g/kg for mackerel head 2.61±1.19 g/kg, for the jawfish head 8.60 g/ kg and for the jawfish head 8.60 g/kg (Table 2).

Table 2. Mineral composition

Samples	K (g/kg)	P (g/kg)	Na (g/kg)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)
	Moy ± ET	Moy ± ET	Moy ± ET	Moy ± ET	Moy ± ET	Moy ± ET
MAQET	1.40± 0.00^{a}	9.20 ± 0.00^{a}	0.50±0.00^{c}	0.26±0.04^{a}	0.21±0.03^{a}	01.30±0.01^{c}
TEMAQ	1.60 ± 0.00^{a}	2.61 ± 1.19^{a}	0.20± 0.06^{a}	2.88±0.04^{b}	0.20±0.25^{a}	01.08±0.01^{b}
MACET	2.30 ±0.00^{a}	8.60 ±0.00^{b}	0.32± 0.00^{b}	0.39±0.01^{a}	3.30±0.03^{c}	0.80±0.00^{a}
TEMAC	2.01 ± 0.00^{a}	2.85 ±0.72^{b}	0.25± 0.00^{ab}	3.20±0.1^{c}	1.73±0.30^{b}	1.50±0.01^{d}

MAQET : Headless mackerel
TEMAQ : Mackerel's head
MACET : Headless jawfish
TEMAC : Jawfish's head
In the same column, the values assigned to the same letter are not significantly different at 5% threshold.

Lipids extracted enzymatically

Hydrolysis degree

Hydrolysis curves (Fig. 1) show that the degree of hydrolysis (DH) increases very rapidly in the first hour before stabilizing after 20 min. DH values ranged from 0-11.04, 0-14.75, 0-17.66, 0-13.30 for headless mackerel, mackerel head, headless jawfish and jawfish head respectively.

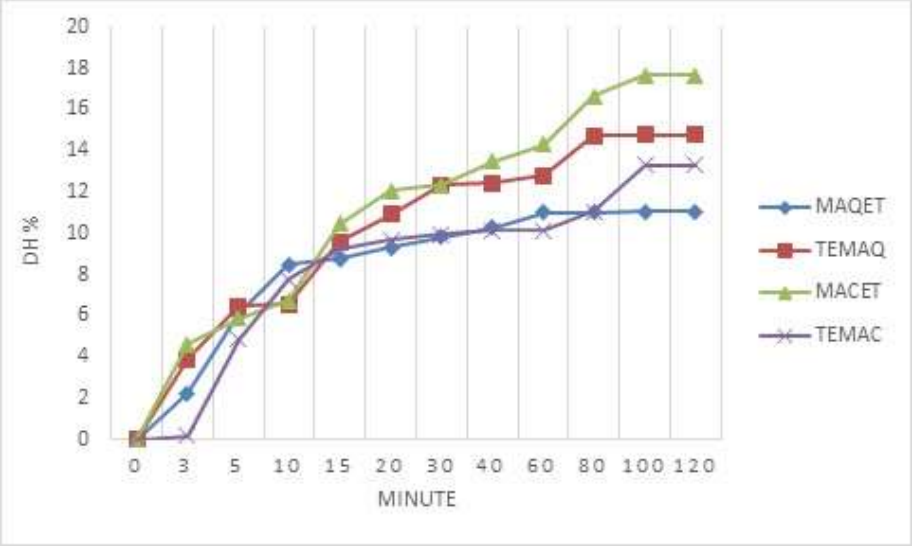

Figure 1. Hydrolysis Degree Evolution

MAQET : Headless mackerel TEMAQ : Mackerel's head
MACET : Headless jawfish TEMAC : Jawfish's head

Lipid extraction rate by different methods

The results of lipid extraction from the mackerel head with the various methods are shown in Figure 2. The graph analysis makes it possible to observe that the solvent extraction method (Folch, 1957) extracts a lipid level (21.80%) very close to that of the enzymatic extraction method (19.87%). These values are higher than those obtained with cooking extraction (14.18%).

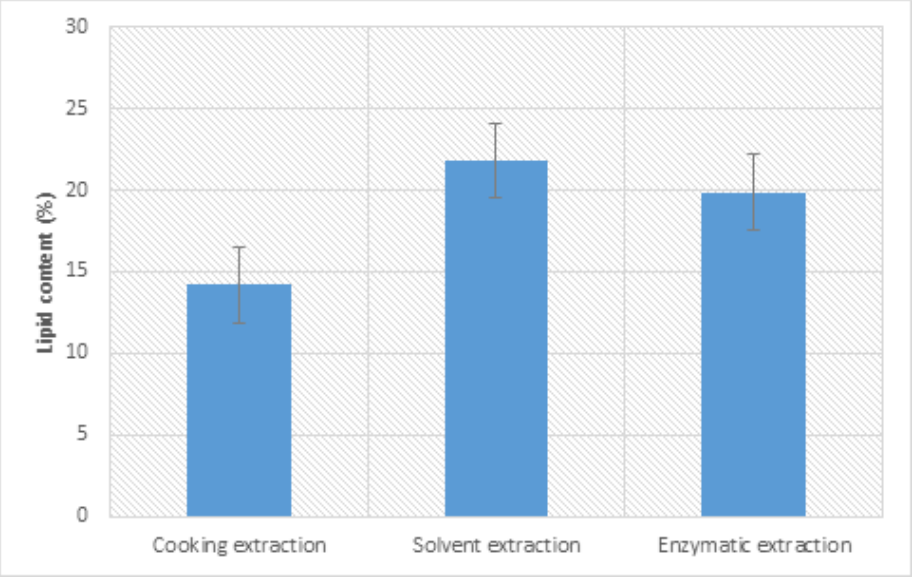

Figure 2. Extraction rate of total mackerel head lipids using various methods

Oils chemical characteristics extracted from fish

Oils chemical characteristics from headless mackerel, mackerel head, headless jawfish and jawfish head explain the two methods of extraction (Folch and Enzyme) reported in Table 3. The analysis of physical properties shows that these oils have iodine values and high saponification acid but the peroxide index is low. Oil analysis shows a difference between the chemical and enzymatic concerning the oil index.

Table 3. Oils chemical analysis extracted from fish by chemical (Folch, 1957) and enzymatic methods

Index Samples	**iodine Index** (g iode /100 g of oil)		**Saponification Index** (mg KOH / g oil)		**Peroxide Index** (méq O_2 / kg oil).		**Acid Index** (mg KOH / g oil)		**Acidity** (% oléique acid).	
	Oil EC	Oil EE	Oil EC	Oil EE	Oil EC	Oil EE	Oil EC	Oil EE	Oil EC	Oil EE
MAQET	190.17±2.01^{a}	189.47±2.00^{a}	197.72±3.39^{a}	196.62±4.20^{a}	1.51± 0.07^{b}	0.75 ± 0.30^{a}	0.94 ± 0.03^{b}	0.40± 0.07^{a}	0.50± 0.03^{c}	0.20±0.03^{a}
TEMAQ	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^{ab}	0.84±0.27^{ab}	0.40 ± 0.03^{ab}	0.42 ± 0.13^{ab}
MACET	180.32±0.00^{b}	179.90±0.00^{c}	196.64±4.45^{a}	195.64±5.60^{a}	0.20± 0.11^{a}	0.25 ± 0.10^{a}	0.70 ± 0.06^{a}	0.91 ± 0.13^{b}	0.44 ± 0.03^{a}	0.46± 0.07^{b}
TEMAC	193.65±0.00^{a}	193.02±0.0^{ab}	198.00±1.48^{a}	199.70±5.6^{a}	0.40± 0.07^{a}	0.45 ± 0.10^{a}	0.53 ± 0.01^{c}	0.63± 0.13^{ab}	0.30± 0.07^{b}	0.32 ± 0.07^{ab}

In the same column, values assigned to the same letter are not significantly different at 5% threshold
MAQET : Headless mackerel
TEMAQ : Mackerel's head
MACET : Headless jawfish
TEMAC : Jawfish's head
EC Oil : Oil obtained by chemical extraction
EE Oil : Oil obtained by enzymatic extraction.

DISCUSSION

The focus of this study is on the biochemical, nutritional and chemical characteristics of fish and fish lipids. Protein content of these four (4) fish parts is substantially similar to that found by Wu Leung *et al.,* (1970), which is 18.8% protein in fresh fish. This high protein content makes fish an excellent source of protein and necessary for child growth (Schapira, 1981) and maintenance for adults. The ashes resulting from fish incineration made it possible to quantify some mineral elements in particular calcium, sodium, phosphorus, magnesium, iron and potassium. These minerals are indispensable. They play an important body strengthening role in the bones of adults, playing the role of bioactivator and osmotic balance in cellular metabolism. By comparing the biochemical and morphological characteristics of the mackerel's head with headless mackerel, jawfish head and headless jawfish, we noticed a difference in composition between the tissues. The mackerel head contained a higher lipid content (21.8%) than the headless mackerel (20.15%), than the jawfish head (09.58%) and headless jawfish (08.79%). The protein content of the mackerel head (16.62%) is comparable to that of the headless mackerel (18.03%). In view of the morphological parameters of the mackerel head, in particular the weight of about 0.3 kg, and by comparing the biochemical compositions of the head with those of the other fish parts, it can be stated that this by-product (head of mackerel) represents a potential source of lipids and proteins and requires special attention for the food formulation. Aitken (1984) reports the need for the industry to know these compositions before carrying out certain operations such as obtaining flours, drying and evaluating the nutritional properties for supplementation.

Enzymatic extraction of lipids is intended to improve the functional and nutritional qualities of the products resulting from this hydrolysis without developing a parasitic flavor or aroma such as bitterness. The organoleptic quality of the oligopeptides produced during hydrolysis is highly dependent on the specificity of the enzymes used (Adler-Nissen, 1986; Kristinsson and Rasco, 2000a). For example, Hoyle and Merritt (1994) observed that the cod protein hydrolyzate obtained with Alcalase is less bitter than that obtained with Papain, which is a priori unexpected because the degree of hydrolysis reached with the Alcalase is higher. In fact, these properties are obviously related to the degree of hydrolysis, which in turn depends on the nature of the proteins, the specificity of the enzyme used and the hydrolysis conditions.

The DH curves indicate a phase of rapid growth of the hydrolysis during which many peptide bonds are cleaved. These curves have been observed in most cases, by Quaglia and Orban (1997a, 1997b) on sardines, by Liceaga and Li-Chan (1999) on herring, by Kristinsson and Rasco (2000b, 2000c) on the salmon fillet and for tuna offal by Guerard *et al.,* (2001, 2002). During these experiments, different degrees of terminal hydrolysis depending on the experimental conditions were obtained after 120 min of reaction. After 20 min, the rate of hydrolysis decreases and the curves become asymptotic. The reduction of the hydrolysis rate is due, among other things, to the reduction of the concentration of available peptide bonds, to the inhibition by the product and to the desactivation of the enzyme (Moreno and Cuadrado, 1993). In the food industry, DH is a very important characteristic because it correlates with the functional characteristics of the hydrolysates resulting from the enzymatic fish hydrolysis (Shahidi *et al.,* 1995, Onodenalore and Shahidi, 1996, Periago *et al.,* 1998). For all samples, the highest DH (14.75%, 11.04%, 17.66% and 13.30%), respectively for mackerel's head, headless mackerel, headless jawfish and jawfish head obtained at 60 ° C and a pH of 8.0. These values are close to those observed by (Gbogouri, 2005) on hydrolysates with DH 11.5%

and DH 12.5% of the enzymatic hydrolysis of salmon heads which had emulsifying stabilities (87 to 88%) very close of that of sodium caseinate (90%). These values of degrees of hydrolysis could therefore indicate good functional properties of the hydrolysates resulting from these enzymatic hydrolyses in terms of emulsifying capacity and stability as well as the oil absorption capacity. Therefore the use of Alcalase can be a good alternative to the conversion of fish by-products into ingredients for industrial applications.

Concerning the comparison of the extraction methods, the results showed that the best extractions of fat were obtained by using organic solvents. These results are consistent with those obtained by Gunnlaugsdottir and Akman (1993) and Undeland (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 the neutral lipids and complex lipids. The most effective of these methods is the use of cold solvents called Folch, since it extracts 21.80%, 20.15%, 10.51%, 09.64% of the total lipids respectively in the mackerel's head, headless mackerel, head of a jawfish and headless jawfish. In addition, Manirakiza (2001) suggests that to better extract the polar lipids it is appropriate to use mixtures with alcohols such as ethanol, methanol and isopropanol. The method of Folch (1957) resulted in a higher lipid content in the mackerel head than in the headless mackerel than in the head of jawfish than in the headed jawfish. These results confirm the classification made by Sebedio (1992) and Corraze and Kaushik (1999), classifying mackerel in the category of "fatty" fish that have more than 10% in their muscles. Moreover, the difference between the total lipid contents extracted by the method using the solvents (20.15%, 21.80%, 09.64%, 10.51%) and the enzymatic extraction (18.37 %, 19.87%, 08.79%, 09.58%) in headless mackerel, mackerel head, headless jawfish and jawfish head does not exceed 2%. These results are consistent with the results of Linder (2002) report that the difference between enzymatic extraction and solvent extraction does not exceed 2%.

The chemical properties of the fat of the fish studied revealed a similarity between the chemical index of oils extracted by the solvent-based method and those extracted enzymatically. Analysis of these chemical properties of the fat extracted from the heads and headless of these fish revealed high iodine numbers (180.32-194.74 g iodine / 100 g oil). These indexes are superior to those of several other vegetable oils such as cotton seed oil (99 - 119 g iodine / 100 g of oil) and soybean oil (120-143 g iodine / 100 g of oil) (Geoffrey, 1990). However, these values would reflect a fairly good oil content of polyunsaturated fatty acids. These oils would be drying and likely to polymerize in the open air. It should be recalled that the iodine value is related to the overall unsaturation in the fat. Many studies have shown that consuming dietary 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 the polyunsaturates, leads to a reduction of this risk (Connor, 2000, Dommels 2002, Toyoshima 2004). The acid index is a parameter which makes it possible to appreciate the degree of alteration of the oils. It is low in the studied oils and between 0.4 and 0.94 mg KOH / g of oil. These low values could indicate that these oils do not contain enough free fatty acids. They are therefore very unlikely to be rancid. Indeed, the free fatty acids, under the effect of the atmospheric oxygen cause the rancidity of the oils. These indexes are lower than the limit value established for a food by the Codex alimentarius (1992) which is 4. These low values presage a good conservation of the products.The peroxide values of the oils

are fairly good when it is known that these oils are crude and that the peroxide value of refined oils can change to a value of 10. Oil quality varies inversely with the increase in the peroxide value. Oil oxidation degree indicates its stability or shelf-life which is a determining factor for the subsequent storage of the products manufactured (Olie, 1972). Indeed, air oxygen reacts with the fatty acids of oil. In this series of reactions, unsaturated fatty acids are the preferred target because oxygen binds to the double bonds to form oidic bonds characteristic of epoxies which are toxic and which are the cause of cancers and cellular aging.

The oil saponification index is superior to those of sunflower oil which is an oil rich in oleic and linoleic acid, both unsaturated fatty acids with 18 carbon atoms. In view of this comparison, it can be said that these oils consist of long chain fatty acids. Their use in the soap industry could be recommended in the same way as certain animal fats such as herring oil (FAO, 1975).

CONCLUSION

in this study, the objective was to implement protease-assisted aqueous lipid extraction from the headless and heads of mackerel *(Scomber scombrus*) and jawfish (*Chrysichthys nigrodigitatus*) in order to promote their use in food as supplement. The studied fishes represent a true source of proteins and minerals elements, making them an essential food for the growth of children and maintenance for adults. A comparison of the lipid extraction methods revealed that the Folch method is the most efficient in terms of oil extraction yield. However, the enzymatic extraction method allowed us to extract a high content of lipid from headless mackerel, mackerel heads, headless jawfish and jawfish heads compared to the Folch method. These fishes are therefore an unavoidable potential of lipids. The chemical analysis of these oils has shown that they contain a high level of iodine index that could be indicative of a large number of degrees of unsaturation in the chain of these fatty acids. The low levels of peroxide index and free fatty acid would favor a possible resistance to the oxidation of these fats. All these properties make it possible to predict the possible use of the lipids of these fishes in terms of food, pharmaceuticals and cosmetics.

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