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ORIGINAL ARTICLE

Oil Soluble Vitamins and Fatty Acids Profile of Smoked European Eel Fillets

El OUDIANI Salma*, MISSAOUI Hechmi
Institut National Agronomique de Tunisie, 43 Av. Charles Nicole, 1082, Tunis, Tunisie
Email : salma.inat@yahoo.fr

ABSTRACT
The fat content and fatty acids composition of the total lipids from European eel were compared before and after hot smoking process. Smoking was made in an automatic kiln and carried out on different stages, the first one consist on drying and the second is about simultaneous heating and smoking; which have an effect on the quality of the final product. Total lipids were extracted from the meat according to the Soxhlet method; fatty acids methyl ester was carried out by gas chromatography (GC). Results showed that the content of eicosapentaenoic C20:5 (EPA) and docosahexaenoic acid C22:6 (DHA) decreases respectively at (2.59% and 1.95%); wile retinol and alpha tocopherol diminish slightly but not significantly. The observed losses are the result of the processing applied on the fish flesh. In the same time oxidation indexes as peroxide value: PV, free fatty acids: FFA and total volatile basic nitrogen (TVB-N) were calculated and indicated that frozen eels quality still acceptable to consummation.
Key words: *European eel, smoking process, fatty acids, retinol, alpha tocopherol, oxidation indexes.*

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INTRODUCTION

Now a day's, especially in food chemistry the determination of total lipids and the evaluation of long chain polyunsaturated fatty acids (LC PUFA) are very important for human health. Clinical studies showed that omega-3 PUFA may prevent some diseases as bronchial asthma and myocardial infarction [1], [2]. Considerate as a rich source of vitamin and PUFA as EPA and DHA, fish oil are very susceptible to oxidation; the main objective of this work is to determine hot smoking impact on α-tocopherol, retinol and on polyunsaturated fatty acids of yellow eels (*Anguilla anguilla*) flesh from the north east of Tunisia : Ghar El Melh lagoon.

MATERIALS AND METHODS

Sampling

The samples fish were collected in March 2007 to May 2007 from Ghar el Melh lagoon located in the northeast of Tunisia (Figure 1) which has an elliptical shape of 25.8 km^2 approximately; the latitude is 37° 08' north and 10°10' east of longitude, the water feature are as follows for salinity, temperature and pH (36.4‰, 19.2°C, 8.12) all species belong to the same size class (49cm ± 3) and the total of weight is about (207.8 g ± 5). Until arrival to the laboratory samples were separated in two lots: the first one was weighed, measured and stored at (-20°C) for analysis, the second lot was prepared for the smoking process.

Hot Smoking process

Smoking process was made in an automatic kiln, the smoke was produced from oak sawdust, the processing time was divided in two stages: the first one is about drying the samples during 45min at 15°C and the second is about the smoking and a partial cooking period at 75°C during 60min; Then the smoked product were packed in vacuum and taken to the laboratory for analysis.

Biochemical analysis

Proximate Composition Analysis

Crude protein (N×6.25) was determined by the Kjeldahl method (ISO 5983-1997). Moisture gravimetrically after drying for 4 h at 105°C (ISO 6496-1999) and ash after combustion for 5h at 550 °C (ISO 5984-2002). Total lipid was determined by Soxhlet extraction (AOCS, Ba 3-38) and fatty acid analyses were carried out by AOCS Official Method (Ce1b-89) and then identified by gas chromatography using an Agilent Technologies 6890N ^{R} model equipped with split injector, a capillary

column HP Innowax (Polyethylene Glycol) 30 X 0.25 mm of internal diameter, 25 µm film thicknesses. Signal integration was made by «Agilent Technologies ChemStation Family R» software, FID temperature detector is about 280°C.

Vitamins Analysis

Considered as biological antioxidant [3] vitamin E is composed about different tocopherol analogues (α, β, γ, δ) and their corresponding tocotrienols, in sea food product the principal and the most active form of vitamin E is α-tocopherol. Also vitamin A has an antioxidant activity; it promotes good vision and bones growth, generally fish species are able to transform easily carotenoids in to vitamin A [4]. Typical retinoids in fish are all-trans-retinol and all-trans-3,4-dehydroretinol which is the typical vitamin A compound [5].

Vitamin A and E determination was carried out by AOAC [6] [7]. For this purpose the experimental planning used for extraction consist on saponification of flesh fish by organic solvent, separation from matrix and then quantification by liquid chromatography. Chromatographic conditions for vitamin A were: isocratic system, injection volume, 20 µL; eluent A (980 hexane, 20 ml isopropanol); flow rate, 1 mL/min; fluorescence detector, excitation at 325 nm, emission at 480 nm and column (Maxsil 5 Silica 125*4.00 mm) the same conditions are used for vitamin E but the eluent A used was (970 hexane; 30 ml 1, 4 dioxane) whereas the excitation is at 293 nm and emission is at 326 nm.

Chemical Analysis

In this study the quality of the frozen raw material is evaluated using chemical parameters in fact, total volatile basic nitrogen (TVB-N) content of eel was determined according to Antonocopoulus method [8], Peroxide value (PV) expressed in miliequivalents of peroxide oxygen per kilogram of fat was determined according to AOAS [9] whereas Free fatty acid (FFA) analysis, expressed as percent of oleic acid determined by the official method of analysis [9].

Statistical Analysis

For data analysis, standard deviation and ANOVA were used. Significance of differences was defined at (p<0.05). Statistical comparison was based on (n=6) samples for fatty acids analysis and the oxidation indexes, and in triplicates for oil soluble vitamin determination.

RESULTS AND DISCUSSIONS

Composition of European eel fillets**.**

Proximate composition of *Anguilla anguila* caught in March 2007 to May 2007 from the Ghar el Melh lagoon is reported in (Table1). In comparison with other fish species European eel fillets possessed considerable higher fat and lower moisture content [10], [11], **[**12**]** it is also considered as a rich source of protein content. Results obtained are similar with those found by Murray [13] and Özogul [14]. Generally, lipid level varies within species and is affected by catching season [15], [16]. In our investigation we found that smoking process affect eels composition especially proteins which decreased significantly [17] and who reported that the observed loss may be explained by the effects of smoking temperature on some essential amino acids as lysine and tryptophan [18]. This decrease is also observed on moisture [19]; and on lipid content which is induced by heating process applied on flesh fish during hot smoking process; in smoked sea food products, composition depends on the state of fish, its fat content, and the smoking itself [20].

Fatty acids in hot smoked eels

The GC analysis of sample studied show that fatty acids profile (Table 2) contains a high proportion of saturated (40.59%) and monounsaturated fatty acids (49.02%) in comparison with long chain polyunsaturated fatty acid of n-6 and n-3 family; their proportion are respectively (21.69%) and (10.43%). SFA represented in majority by (myristic, C14:0; pentadecanoic, C15:0; palmitic, C16:0 and stearic, C18:0) acids; for this category of FA, palmitic acid is the most abundant (25.43%). For monounsaturated fatty acids the highest amount is represented by oleic (C18:1, n-9) (29.91%) and palmitoleic acid (C16:1, n-7) (16.31%). The n-6 PUFA is represented by a high level of linoleic acid (C18:2, 15.96%).The n-3 PUFA are docosapentaenoic (DPA) and docosahexaenoic (DHA) which proportion are respectively (4.85% and 2.66%). On the other hand, the level of linolinic acid (18:3 n-3) was low (0.69%). Results obtained are in concordance with those found by Soriguer [21]. During the smoking process these contents decreases significantly especially EPA and DHA ($p<0.05$) which proportion after the processing is about (2.59% and 1.95%) whereas the other fatty acid losses was not significant ($p<0.05$). These findings are in accordance with results obtained on hot smoking sardine fillets, which induce a statistical significant decreases in the contents of n-3 LC PUFA [22]; whereas, in

stored seasoned-dried Pacific Saury treated with liquid smoke there were no significant changes in the concentration of EPA and DHA [23]. In the investigation on smoked Atlantic mackerel the concentration of EPA decreased slightly; however, the loss was not statistically significant [24].

Oil soluble vitamins in hot smoked eels fillets

Oil-soluble vitamins determination of eel's muscle show that it is a source of retinol and vitamin E, which proportion are indicated on (Table 3). The results observed of hot smoking process on PUFA encourage us to determine the impact of this processing on (α-tocopherol) and retinol. [25] Reviewed Vitamins losses by the hot smoking process. In his study Junker [26] found that there is no destruction of vitamin A by the processing. In his investigation on *Scomber scombrus* Bhuiyan [27], reported that the decrease of vitamin A content is insignificant, this confirms that retinol is as stable as polyunsaturated fatty acids are labile and this is in accordance with our findings. As can be seen in (Table 3) α-tocopherol decrease is insignificant ($p<0.05$), as reported, if the loss of tocopherol is considerable the oxidation of fat contents is deep and this is due to the formation of the active fatty acids during the heat oxidation [28].

Oxidation of European eel oil during frozen storage

Eel fillets are rich source in polyunsaturated fatty acids which are susceptible to oxidation. Because of their high degree of insaturation, they are less resistant to oxidation than other animal oils [29]. Lipids oxidation is an autocatalytic chain reaction which occurs in four stages: initiation, propagation, chain branching and termination [30]. Lipid hydroperoxide are the primary products of lipid oxidation the breaking down of this compounds induce the formation of the secondary oxidation compound in charge of rancid flavor, taste and product nutritional value [31]; from the other hand enzymatic hydrolysis induce the lipid degradation, where phospholipids and triacylglycerols hydrolyze produce free fatty acids [32] which have a direct sensory impact [33]. The objective of this part of our investigation is to examine the effects of freezing storage at (-20°C) on changes of oxidation indexes (FFA, PV and TVB-N) in European eel oil. Results obtained are illustrated in (Table 4) we note that Free fatty acids (FFA) slightly increased ($p<0.05$) from the initial value 0.95% to 2.03% until six months of frozen storage. If (PV) value below (5meq/kg); the fat is considered fresh, if it is between (5 and 10 meq/kg), fat is commencing rancidity [34]. In this study the initial PV value is about (0.92 meq/kg) after 24 weeks of storage period it is about (16.85 meq/kg) this did not exceed the maximum recommended value for human consumption which is about (20 meq/kg); similar results were obtained in the study of frozen fillets of herring stored at (-30°C) [7]; whereas PV value increased to (80 meq/kg) for herring fillets stored at (-18°C) [35].

There were significant differences ($p<0.05$) in TVB-N value which fluctuated during storage period of smoked eels fillets. The initial TVB-N value was low (13.72 mg/100 g) and reached the maximum values at the 6th month (34.52 mg/100 g) which is below to the recommended limits of 35-40 mg TVB-N/100 g of flesh. These results are in concordance with those found by Pons [36] who reported a lower TVB-N content for fresh anchovy (7mg/100 g); lower TVB-N value for marinated sardine. Maximum TVB-N value (12.77 mg/100 g) was found for marinated tench after 150 days of storage period [37].

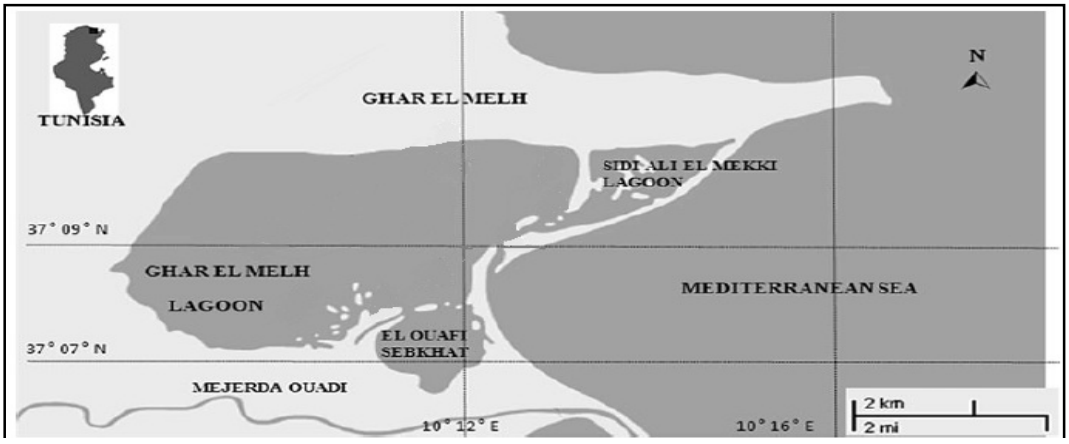

Figure1: Geographical location of the study site: Ghar el Melh lagoon

Table 1: Proximate composition of *Anguilla anguilla.*

	Anguilla anguilla	
	No Smoked (*n*=6)	**Smoked (*n*=6)**
Moisture (%)	56.03 ± 5.61	54.2 ± 6.44
Protein (N×6.25) (%)	17.51 ± 1.95	14.93 ± 1.91
Lipid (%)	24.98 ± 6.51	22.93 ± 3.71
Ash (%)	1.1 ± 0.54	1.04 ± 0.2

The values are expressed as mean±SD.

Table 2: The Fatty Acids present in the lipids extracted from the meet of eels before and after smoking process:

Fatty acid	**% of the respective Fatty Acids**	
	No Smoked	**Smoked**
C14:0	4.91 a ± 0.96	3.30 b ± 1.02
C15:0	0.91 a ± 0.12	0.71 a ± 0.04
C16:0	25.43 a ± 4.73	17.24 b ± 5.09
C18:0	10.25 a ± 1.96	8.02 a ± 2.22
Σ SFA	40.59	29.27
C16:1 *n*-7	16.31 a ± 3.69	7.06 b ± 4.06
C18:1 *n*-9	29.91 a ± 13.33	29.54 a ± 17.19
C18:1 *n*-7	2.8 a ± 0.09	0.17 b ±0.07
Σ MUFA	49.02	36.77
C18:2 *n*-6	15.96 a ± 0.89	6.05 b ± 5.27
C18:3 *n*-6	0.31 a ± 0.09	0.25 a ± 0.09
C20:2 *n*-6	0.31 a ± 0.13	0.28 a ± 0.1
C20:3 *n*-6	0.83 a ± 0.30	0.51 a ± 0.19
C20:4 *n*-6	4.28 a ± 0.94	2.45 b ± 1.03
ΣPUFA *n*-6	21.69	9.54
C18:3 *n*-3	0.69 a ± 0.41	0.33 b ± 0.22
C20:5 *n*-3 EPA	4.85 a ± 1.58	2.59 b ± 1.09
C22:5 *n*-3	2.46 a ± 0.67	1.27 b ± 0.32
C22:6 *n*-3 DHA	2.66 a ± 0.21	1.95 b ± 0.24
Σ PUFA *n*-3	10.43	6.14
DHA/ EPA	0.54	0.75

Mean values of three independent measurements and standard deviation are given. Values in the same row followed by different letters are significantly different ($P< 0.05$).

Table 3: Oil- soluble vitamin

Vitamins	Non Smoked	Smoked
Vitamin A (Retinol)	468µg/100g a ± 0.18	429µg/100g a ± 0.09
Vitamin E (α-tocopherol)	4.32µg/100g a ± 0.35	3.98µg/100g a ± 0.21

The values are expressed as mean±SD.

Table4: Changes in the levels of total volatile basic nitrogen (TVB-N), peroxide value (POV), free fatty acid (FFA), during storage of smoked European eel.

Storage time (month)	TVB-N (mg/100 g)	POV (meqO_2/kg)	FFA (oleic acid %)
0	13.72±0.36	0.92±0.02	0.95±0.1
2	23.35±0.11	5.12 ±0.16	1.14 ±0.09
4	29.87±0.21	7.75±0.01	1.85±0.03
6	34.52±0.59	16.85±0.04	2.03±0.07

The values are expressed as mean±SD.

CONCLUSION

From nutritional point of view European eel fillets are considered to be o source of vitamins and LC PUFA of n-3 family. Hot smoking process has an impact on the proximate composition of eels muscle and this is due to processing conditions. It is well known that lipids are susceptible to oxidation. Nevertheless, the parameters observed in this study were small and did not affect properties of frozen eel, they are acceptable, and eel fillets can be stored for many weeks at (-20°C).

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