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EFFECT OF SMOKING AND FRYING ON NUTRITIONAL QUALITY OF FOUR SPECIES OF FISH COMMONLY CONSUMED IN BENIN

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ABSTRACT

Fatty acids are vital for human health. The objective of the study was to evaluate the impact of processing and preservation processes on nutritional quality. Thus, 60 fish samples were used for fatty acids quantification through Gas Chromatography coupled with a Mass Spectrometer (GC-MS). It appears that total lipid contents were higher when fresh fish were fried in *Coptodon guineensis* and in *Sarotherodon melanotheron* than fresh, fried and smoked fish. The dominant saturated and monounsaturated fatty acids were respectively Palmitic and oleic acids. In *Trachurus trachurus*, *Coptodon guineensis* and *Sarotherodon melanotheron*, smoking significantly ($p<0.05$) reduced polyunsaturated fatty acid (PUFA) levels compared to fresh samples. Similarly, frying fresh fish lowered PUFA levels in brackish water species. All the fish had high linoleic acid levels after frying. DHA and EPA acids were higher in marine fish. DHA was higher in fresh *Coptodon guineensis*, *Trachurus trachurus* and *Scomber scombrus*. In all species DHA and EPA contents dropped after frying. Smoked marine fish showed a high level of DHA and EPA. The ratio H/H not exceeding 2 were obtained in smoked *Scomber scombrus* and fried *Scomber scombrus*. By contrast, the lowest atherogenicity index was obtained in fried *Trachurus trachurus* (0.35). Likewise, smoked *Trachurus trachurus* and *Scomber scombrus* had low thrombogenicity indices. *Coptodon guineensis* and *Trachurus trachurus* had higher Peroxidability Indices while all the fried fish had the lowest values. The control of variation parameters of essential fatty acids during the processes would guarantee a better nutritional quality to the consumer.

Keywords: Fatty Acids, DHA, EPA, Food safety, Fat quality, Food security

INTRODUCTION

Fish is an important animal source of protein playing globally a major role in food security, particularly among low-income and rural populations (FAO, 2017, 2024). In Benin, fish is usually consumed fresh or processed through techniques such as frying, smoking, refrigeration, and/or freezing (**Assogba and *al.*, 2018a, 2018b**). Fresh fish is an important means of acquiring vitamins and minerals, fatty acids, and animal proteins, and essential amino acids (**Samples, 2014; Al-Reza *et al.*, 2015; FAO, 2017**).

However, it is widely acknowledged that the majority of processing and preservation techniques result in biochemistry changes that can compromise the nutritional value of foodstuffs (**ANSES, 2011; Bassey *et al.*, 2014; Sampels, 2014; Al-Reza *et al.*, 2015; Saladoye *et al.*, 2015**). Cooking affects protein, carbohydrates, lipids, and vitamins (**Leduc, 2011; Nguyen, 2014**), whereas cold storage increases lipid oxidation (**Leygonie *et al.*, 2012; Aubourg *et al.*, 2013; Senturk and Alpas, 2013; Pazos *et al.*, 2015**). These changes worsen nutrient depletion and generate undesired molecules. In fact, nutritional value is affected in terms of lipid content and its bioactive components and mainly depends on the presence and amounts of long-chain polyunsaturated fatty acids, specifically EPA and DHA. These fatty acids are responsible for cardiovascular, neurological, and anti-inflammatory effects (**Fontagné-Dicharry *et al.*, 2010**). Research showed that consuming fish rich in essential fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can offer a number of significant health benefits. These include protection against many diseases (**Lorente-Cebrian *et al.*, 2013; Weintraub, 2013; Akintola *et al.*, 2014; Douny *et al.*, 2014; Tamma *et al.*, 2015; Tenyang *et al.*, 2017**). The advantages are also apparent in terms of cognitive function, neuronal activity and visual development.

Processing effects on the above fatty acids have been reported in various studies from Spain, India, Canada, the United States, Tunisia, Nigeria, Cameroon, and Brazil (**Bouriga *et al.*, 2012; Oparaku and Nwaka, 2013** ; **Bassey *et al.*, 2014; Neff *et al.*, 2014; Pazos *et al.*, 2015; Chandrani *et al.*, 2016; Flaskerud *et al.*, 2017; Lira *et al.*, 2017; Tenyang *et al.*, 2017; Okpanachi *et al.*, 2018**). In Benin, research has focused on the microbiological quality and processing practices and physico-chemical characteristics of fish processed (**Degnon *et al.*, 2012, 2013; Kpodekon *et al.*, 2014; Assogba *et al.*, 2019; Iko Afé *et al.*, 2020**). Apart from the study of **Assogba *et al.* (2025)** on the fatty acid profile of fresh fish flesh, only **Dossou Yovo *et al.*** (2016) had studied the effect of fermentation on fatty acids. There is a lack of information on the effects of prevailing methods (like smoking and frying) on the fatty acid profile in major local species.

The information gap will be addressed by this study since it aims to assess various impacts of smoking and frying on fatty acid composition in four economically important fish (**Youssao *et al.*, 2011; El Ayoubi and Failler, 2013; Djessouho, 2015; Amoussou *et al.*, 2016; Achoh *et al.*, 2018**) such as two brackish-water fish (*Coptodon guineensis* and *Sarotherodon melanotheron*) and two marine fish (*Scomber scombrus* and *Trachurus trachurus*) from Benin. The study will provide essential information regarding, fatty acid quality of processed fish and optimal processing techniques to improve the nutritional value of fish consumption.

MATERIAL AND METHODS

Study area

The work was done in the Littoral and Atlantic departments in Abomey Calavi and Cotonou, respectively. The fish samples were from Akassato market and banks of

Kpota in Abomey Calavi, while they were from the Xwlacodji smoking site in Cotonou.

The Xwlacodji neighborhood is one of the oldest in Cotonou, where fishermen settled, and is the center of post-harvest activities such as smoking in Benin. It is located at an altitude of 8 meters between latitude 6° 2' North and longitude 2° 26' East. It covers approximately 2 hectares and is located at a sandy angle between the sea and the Cotonou channel. It is 7 km from the fishing port of Cotonou. It is generally home to Xwlà and Xwédà women who carry out fish processing activities. The Xwlacodji smoking site is the reference point for the smoking of lagoon and sea fish products and imported fish.

The municipality of Abomey-Calavi, located in the Atlantic Department of Benin, covers 539 km^2 and had a population of 656,358 in 2013. It is located between 6°22' and 6°30' North latitude and 2°15' and 2°22' east longitude. It is bordered to the north by the municipality of Zè, to the South by the Atlantic Ocean, to the East by the municipalities of Cotonou and So-Ava, and to the West by the municipalities of Ouidah and Tori-Bossito. From the coastal plain to a plateau, its territory is relatively flat. The hydrographic network is dominated by Lake Nokoué and the Djonou lagoon.

The vegetation varies from mangroves to degraded savannahs. The population, mainly Aïzo and Fon, practices various religions. The main economic activities are agriculture, fishing, trade, and crafts. The markets of Kpota and Akassato serve as a platform for commercial exchange among the local populations.

Sampling and fish processing

The studied was carried out on four commonly consumed fish species namely *S. melanotheron*, *C. guineensis*, *T. trachurus* (horse mackerel), and *S. scombrus* (mackerel).

A total of 60 fish were collected for the four species and five fish per processing method (fresh fish, fried fish, smoked fish). Samples were taken at different sites in order to cover the diversity of local distribution and processing channels:

- Kpota market: fresh fish of *S. melanotheron* (5) and *C. guineensis* (5);
- Akassato market (for smoked and fried products): smoked specimens of *S. melanotheron* (5) and *C. guineensis* (5); fried fish of *S. melanotheron* (5) and *C. guineensis* (5); of *T. Trachurus* (5) and *S. scombrus* (5);
- Abomey-Calavi fishmongers' site: frozen fish imported fresh of *T. trachurus* (5) and *S. scombrus* (5);
- Wlacodji smoking site in Cotonou: smoked specimens of *T. trachurus* (5) and *S. scombrus* (5).

According to common practices, the fish were fried in groundnut oil and sold at the local market. The frying time was not experimentally standardized; it was left to the discretion of skilled processors, who determine the optimal cooking point using empirical criteria (color, texture). The smoking process was carried out according to traditional methods used at the sampling sites.

In accordance with the requirements of ISO 7218: 2007, all samples were immediately placed in a cooler at a stable temperature of +4°C and then transported to the Animal Biotechnology and Meat Technology Laboratory for processing. The fish were scaled, gutted, filleted, and rinsed before being analysed at the Fundamental and Applied Research for Animal and Health at the Foodstuffs Analysis Service of the Department of Sciences of the University of Liege.

Fatty acids analysis

Fat extraction

For fatty acid analysis, 50 g of flesh from each fish was minced and homogenized. The Folch method (chloroform/methanol, 2:1 v/v) (**Folch, 1957)** was used to extract total lipids from a 2 g subsample. The extracted lipids were derivatized to fatty acid methyl esters (FAMEs), which were subsequently separated, detected, and quantified by gas chromatography-mass spectrometry (GC-MS) (**Assogba *et al*., 2025**).

Preparation of fatty acid methyl esters

The fatty acid profile was determined by analysing the fatty acid methyl esters using GC-MS according to Douny *et al*. (2015) as modified in **Assogba *et al.* (2025)**.

Fifty milligrams of the Folch-extracted lipid were dissolved in 5 mL of hexane. From this solution, a 10 µL sample was transferred to a reaction tube for saponification and methylation. Nonadecanoic acid (C19:0; 75 µL of a 0.05 mg/mL solution) was added as an internal standard, and the hexane was evaporated to dryness under nitrogen stream (**Assogba *et al*., 2025**). The residue was then treated with 1 mL of toluene and 5 mL of 2% (v/v, in methanol) sulfuric acid. The tube was sealed, heated at 100 °C in a water bath for 2 hours, and vigorously stirred using a magnetic stirrer. After cooling, 3 mL NaCl 5% solution were added. The fatty acid methyl esters (FAMEs) were extracted twice with 2 mL of hexane. The combined hexane extract was washed with 4 mL of 2% (w/v) potassium carbonate (K_2CO_3), and a portion was dried over anhydrous sodium sulfate (Na_2SO_4). The final extract was evaporated to dryness to completely eliminate the toluene. Finally, four hundred microliters of hexane were added and the tube was vortexed. The sample was transferred into an injection vial (**Assogba *et al*., 2025**).

GC–MS separation, detection and quantification of fatty acids

Fatty acid methyl esters (FAMEs) were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Thermo Fisher Scientific system comprising a Focus GC and a PolarisQ ion trap mass spectrometer (**Assogba *et al*., 2025**). Chromatographic separation was achieved on a CP-Sil 88 fused-silica capillary column (100 mm × 0.25 mm, 0.20 µm film thickness; Agilent Technologies). Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The injector was operated in splitless mode at 250 °C with an injection volume of 1 µL. The GC oven temperature was planned as follows: initial temperature 55 °C (hold 1 min), ramped at 5 °C min^{-1} to 180 °C, then at 10 °C min^{-1} to 200 °C (hold 15 min), and finally at 10 °C min^{-1} to 225 °C (hold 14 min), yielding a total analysis time of 59.5 minutes. FAME identification was based on comparison of both mass spectra and relative retention times with those of authentic reference standards (**Assogba *et al*., 2025**).

The Mass Spectrometer was operated under the following conditions: transfer line and ion source temperatures of 250 °C and 220 °C, respectively; collision energy of 35 eV; and positive ionization mode. Detection was carried out in selected ion monitoring (SIM) mode across five-time segments. Quantification ions were monitored for specific fatty acid classes: m/z 74 and 143 for saturated fatty acids (SFAs), and m/z 79 + 91 for monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). For each of the 23 target fatty acids, quantification was based on a six-point calibration curve generated from standard solutions using the internal standard method. The response factor (ratio of FAME peak area to internal standard peak area) was plotted against concentration, and data were fitted by linear regression. Finally, total SFA, MUFA, and PUFA contents were summed, and the n-6/n-3 and PUFA/SFA ratios were calculated (**Assogba *et al*., 2025**).

Nutritional quality index of lipids

Four key indices were applied to both fresh and processed samples of the four fish species for the nutritional quality assessment according to **Assogba *et al*., (2025)**. These included the thrombogenicity index and the atherogenicity index (**Ulbricht and Southgate, 1991**), the hypo/hypercholesterolemic fatty acid ratio (**Santos *et al*., 2002**), and the peroxidation index (**Arakawa and Sagai, 1987**). Each index was calculated according to established formulas, providing a comprehensive assessment of lipid health impact and oxidative stability.

- Atherogenicity index (AI) = [(C12 :0 + 4xC14: 0 + C16: 0)] / (ΣMUFA + Σn-6 + Σn-3)];
- Thrombogenicity index (TI) = (C14: 0 + C16: 0 + C18: 0) / [(0.5x ΣMUFA) + (0.5xΣn-6) + (3xΣn-3) + (Σn-3 / Σn -6)];
- Hypocholesterolaemic / hypercholesterolaemic ratio (H/H) = (C18: 1n-9 + C18: 2n-6 + C20: 4n-6 + C18: 3n-3 + C20: 5n-3 + C22: 5n-3 + C22: 6n -3) / (C14: 0 + C16: 0).
- Peroxidability Index (PI) = [(0.025 x ΣMUFA) + (C18: 2n-6 + C20: 2n-6) x1 + (C18: 3n-6 + C18: 3n-3) x 2 + (C18: 4n-3 + C20: 4n-6) x 4 + (C20: 5n-3 + C22: 5n-3) x 6 + (C22: 6n-3 x 8)].

Statistical analysis

Statistical analysis was performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). A generalized linear model (GLM) procedure was used for analysis of variance (ANOVA). The model included species (*C. guineensis*, *S. melanotheron*, *T. trachurus*, and *S. scombrus*), treatment (fresh, fried, smoked), and their interaction as fixed effects. The significance of each effect was determined using Fisher's test. Post-hoc pairwise comparisons of means were performed using Student's t-test.

RESULTS

Lipid content

Fresh, fried, and smoked fish's total lipid content and fatty acid composition (% total fatty acid) are shown in Table 1. The lowest total lipids percentages were obtained in fresh *C. guineensis* and *S. melanotheron* and the highest in fresh *T. trachurus* and *S. scombrus*. These levels were similar ($p>0.05$) between brackish water species and between marine water species. Compared to fried *S. scombrus* and *T. trachurus*, fried S. *melanotheron* and *C. guineensis* had a higher ($p<0.05$) total lipid content. The contents were elevated ($p<0.05$) when *S. scombrus* and *T. trachurus* were smoked rather than *S. melanotheron* and *C. guineensis*.

On the other hand, the total lipid contents increased when fresh *C. guineensis* and *S. melanotheron* were smoked and fried. The same trends were observed when fresh *T. trachurus* was smoked and fried. Fresh and smoked *T. trachurus* had similar total lipid contents ($p>0.05$). Similar contents ($p>0.05$) were also obtained

in fresh and fried *S. scombrus* but with an increase in the smoked ones. Fried fish had a higher total fat content than smoked fish in most cases.

Saturated fatty acids

Table 1 displays the total fat content and fatty acid composition (total fatty acid%) of fresh, fried, and smoked fish. The fresh flesh of *C. guineensis*, *S. melanotheron*, and *T. trachurus* did not have any differences in their saturated fat acids (SFA) levels ($p>0.05$). Contrarily, smoked *C. guineensis* and *S. melanotheron* had a higher ($p<0.05$) SFA content than smoked *T. trachurus* and *S. scombrus*. These contents were similar between smoked brackish water species and between smoked marine water species ($p>0.05$). Those of fried *S. melanotheron*, *C. guineensis* and *S. scombrus* were similar but different ($p<0.05$) from that of fried *T. trachurus*. However, the SFA content of fresh C. *guineensis* and *S. melanotheron* increased more when smoked than fried. By contrast, a decrease in SFA content was observed when fresh *T. trachurus* and *S. scombrus* were smoked and fried. The SFA content in smoked fish surpassed the SFA content in fried fish.

Palmitic acid (C16:0) constituted the largest proportion of saturated fatty acids (SFA). Fresh flesh of *T. trachurus* and *S. scombrus* contained a significantly proportion ($p<0.05$) of this fatty acid compared to fresh *C. guineensis* and *S. melanotheron*.

The proportions of SFA didn't differ significantly between fresh flesh of *C. guineensis* and *S. melanotheron* ($p>0.05$). In smoked and fried samples, however, *C. guineensis* and *S. melanotheron* exhibited higher SFA and palmitic acid (C16:0) levels, respectively, than *T. trachurus* and *S. scombrus* ($p<0.05$). Furthermore, smoking and frying increased the palmitic acid content in initially low-SFA species (*S. melanotheron* and *C. guineensis*) but decreased it in the initially high-SFA species (*T. trachurus* and *S. scombrus*).

The saturated fatty acids (SFA) capric (C10:0), lauric (C12:0), and tridecylic (C13:0) were not quantifiable in fresh or smoked fish. However, capric acid was detectable in all fried samples, showing significant interspecific variation ($p<0.05$). Its concentration was highest in fried *S. scombrus* and *T. trachurus*, and lower in fried *C. guineensis* and *S. melanotheron*.

The long-chain saturated fatty acids arachidic (C20:0), docosanoic (C22:0), and lignoceric (C24:0) were below the quantification limit in fresh flesh and smoked samples. After frying, lignoceric acid levels were highest in *S. scombrus* ($p<0.05$). Docosanoic acid showed no significant difference between smoked *C. guineensis* and *S. melanotheron* ($p>0.05$), but was significantly higher in fried *T. trachurus* than in fried *S. scombrus* ($p<0.05$).

The content of myristic acid (C14: 0) in fresh *C. guineensis* and *S. melanotheron* was lower ($p<0.05$) than that obtained in fresh *S. scombrus* and *T. trachurus*. The lowest content was obtained in fresh *C. guineensis*. The lowest levels were obtained in fried fish. This content was similar ($p>0.05$) in fried *S. melanotheron*, *C. guineensis* and *T. trachurus*. Likewise, the C14: 0 proportions of smoked *C. guineensis* and *T. trachurus* were similar ($p>0.05$). Nevertheless, the C14: 0 proportion decreased when fresh *C. guineensis* and *S. melanotheron* were fried and this content increased in the same smoked species. Conversely, frying and smoking significantly reduced the myristic acid content in *S. scombrus* and *T. trachurus*. Overall, the smoking process preserved a higher concentration of this fatty acid across species compared to frying.

Furthermore, heptadecanoic acid (C17:0) content did not differ significantly among fresh *S. scombrus*, *C. guineensis*, and *S. melanotheron* ($p> 0.05$). In smoked fish, however, levels were significantly lower in *S. scombrus* and *T. trachurus* than in *C. guineensis* and *S. melanotheron* ($p<0.05$). Smoking and frying also significantly reduced the level of this fatty acid in *S. melanotheron* and *S. scombrus* compared to their fresh state.

Monounsaturated fatty acids (MUFA)

Table 1 presents the total lipid content and fatty acid profile of fresh, fried, and smoked fish. Notably, fresh *S. melanotheron* contained a significantly higher proportion of heptadecenoic acid compared to fresh *C. guineensis* ($p<0.05$). In fresh *T trachurus* and *S. scombrus*, this content was below quantification limit. On the contrary, these contents were higher in fried *T. trachurus* and *S. scombrus* than in fried *S. melanotheron* and *C. guineensis*. Similarly, smoked *T. trachurus* and *S. scombrus* contained significantly higher proportions of heptadecenoic acid (C17:1) than smoked *C. guineensis* and *S. melanotheron* ($p<0.05$). When processed, the level of C17:1 in *C. guineensis* decreased significantly after both frying and smoking, with no significant difference between the two processed forms ($p>0.05$). Conversely, frying significantly increased the C17:1 content in *S. melanotheron* ($p<0.05$).

The proportion of palmitoleic acid (C16:1) varied significantly among fresh fish species ($p<0.05$), with the highest levels found in *S. melanotheron* and *S. scombrus*. Following processing, fried *S. scombrus* contained significantly higher C16:1 ($p<0.05$) than the other three fried species. Conversely, in smoked samples, *C. guineensis* and *S. melanotheron* had higher proportions than *T. trachurus* and *S. scombrus*, with the latter two showing similar concentrations ($p>0.05$). The processing differentially affected C16:1 levels: frying significantly decreased them in *C. guineensis*, *S. melanotheron*, and *S. scombrus*, while smoking increased them in these same species ($p<0.05$). For *T. trachurus*, both frying and smoking led to an increase in C16:1, with no significant difference between its fresh and fried states.

The oleic acid (C18:1) was identified as the most prevalent type of monounsaturated fatty acid (MUFA) among the fish samples analysed. The acid proportions in fresh *S. melanotheron* and *C. guineensis* were lower than in fresh *T. trachurus* and *S. scombrus*.

On the contrary, similar contents were got ($p>0.05$) in fried *S. melanotheron*, *C. guineensis* and *T. trachurus*. The levels of these compounds were found to be higher ($p<0.05$) in the cooked *S. scombrus* samples than in the fried samples. In addition, smoked *T. trachurus* and *S. scombrus* exhibited a higher C18: 1 proportion ($p<0.05$) in comparison to smoked *S. melanotheron and C. guineensis*. Following the frying of *S. melanotheron, C. guineensis* and *T. trachurus*, oleic acid levels increased significantly ($p<0.05$).

The same increasing trends were obtained in the same species but smoked. Conversely, in *S. scombrus*, the content has been shown to decrease ($p<0.05$) when the fish are subjected to smoking. Fried fish has been shown to contain higher levels of oleic acid than smoked fish.

Category of polyunsaturated fatty acids

The fatty acid composition (as a percentage of total fatty acid) of fresh, fried and smoked fish is presented in Table 1. The highest proportion of total Polyunsaturated Fatty Acids (PUFA) was obtained in fresh *C. guineensis* (45.9%) and *T. trachurus* (44.65%). The observed proportions were close to the expected values at a significance level of $p>0.05$. Conversely, the levels of *T. trachurus* and *S. scombrus* were elevated in comparison to those found in *C. guineensis* and *S. melanotheron*. In addition, the total PUFA content in smoked *T. trachurus* and *S. scombrus* was found to be higher ($p<0.05$) compared to those obtained from smoked *C. guineensis* and *S. melanotheron*.

Furthermore, the total PUFA contents decreased ($p<0.05$) when fresh *C. guineensis*, *S. melanotheron T. Trachurus* and *S. scombrus* were fried and smoked. The total PUFA content decreased more in fried fish than in smoked fish. Two types of PUFA from the n-3 and n-6 series were obtained. The total amount of n-3 PUFA content in *T. trachurus* (44.36%) was higher ($p<0.05$) compared to the other types of fresh fish. The level in fresh *S. melanotheron* and *C. guineensis* were not found to be significantly different ($p>0.05$). The investigation revealed that the total n-3 series PUFA content was found to be low in fresh *S. scombrus* (31.83%). Fried and smoked *C. guineensis* and *S. melanotheron* were found to have lower values than fried and smoked *T. trachurus* and *S. scombrus*. After frying and smoking, the contents of *C. guineensis* and *S. melanotheron* were found to be similar ($p>0.05$). Additionally, it was discovered that the total n-3 series PUFA contents of smoked *S. scombrus* and *T. trachurus* were similar. Furthermore, they were lower when fresh fish were fried than when smoked.

It was observed that the total n-3 series PUFA content in samples of fried fish was lower than the content in samples of smoked fish. The total n-6 polyunsaturated fatty acid (PUFA) content was significantly higher ($p<0.05$) in fresh *C. guineensis* (8.6%) and *S. melanotheron* (7.4%) than in fresh *S. scombrus* (1.39%) and *T. trachurus* (0.3%). Conversely, after frying, the n-6 PUFA levels were significantly higher ($p<0.05$) in *T. trachurus* and *S. scombrus* than in fried *C. guineensis* and *S. melanotheron*.

On the contrary, this content was lower in smoked *S. scombrus* and *T. Trachurus* than in smoked *C. guineensis* and *S. melanotheron*. It was not different ($p>0.05$) between smoked *S. scombrus* and *T. Trachurus*.

Frying significantly increased ($p<0.05$) the n-6 PUFA content in all four fish species (*C. guineensis, S. melanotheron, T. trachurus, S. scombrus*). In contrast, smoking significantly reduced n-6 PUFA levels in *C. guineensis* and *S. melanotheron*, with no significant difference ($p>0.05$) observed between fresh and smoked *C. guineensis*. For *T. trachurus* and *S. scombrus*, smoking increased n-6 PUFA proportions; in *S. scombrus*, these levels remained similar to those in the fresh state. Overall, fried fish contained a higher total n-6 PUFA content than smoked fish.

n-3 polyunsaturated fatty acids (n-3 PUFAs)

Table 1 presents the total fat content and fatty acid composition (% of total fatty acids) for the fish in fresh, fried, and smoked states. Alpha-linolenic acid (C18:3 n-3) was found to be absent in fresh *T. trachurus*. In contrast, fresh *S. melanotheron* contained a significantly higher proportion ($p<0.05$) of this fatty acid than fresh *C. guineensis*. This trend was sustained post-frying, where *S. melanotheron* exhibited considerably elevated levels ($p<0.05$) in comparison to fried *C. guineensis* and *S. scombrus*. Subsequent to the smoking process, the proportions in *C. guineensis* and *S. melanotheron* exhibited a significant increase ($p<0.05$) in comparison to those observed in *S. scombrus* and *T. trachurus* following smoking.

On the other hand, when fresh *C. guineensis* and *S. melanotheron* were fried, alpha linolenic acid contents decreased. By contrast, the proportions increased after fresh *C. guineensis* smoking. The content didn't vary ($p>0.05$) when fresh *S. melanotheron* was smoked.

The octadecatetraenoic acid (C18: 4, n-3) contents were similar ($p>0.05$) in fresh *S. melanotheron*, *S. scombrus* and *T. trachurus*. The proportion was higher in smoked *S. scombrus* and *T. trachurus* than in smoked *S. melanotheron*. After

smoking of fresh *S. melanotheron*, *S. scombrus* and *T. trachurus*, C18: 4 (n-3) acid proportions increased. However, the values didn't vary between fresh and smoked *S. melanotheron* ($p>0.05$).

With regard to dihomo-γ-linolenic acid (C20:3 n-3), the highest content was observed in fresh *C. guineensis*, which was significantly higher ($p<0.05$) than in fresh *S. melanotheron* and *S. scombrus* (0.05%). However, it was below the quantification limit in fresh *T. trachurus*.

The proportion of acid was subject to alteration during the processing stage. The frying process resulted in a significant decrease in levels of *C. guineensis* and *S. melanotheron*, while it led to an increase in levels of *T. trachurus* and *S. scombrus*, with the latter showing the highest proportion among all fried samples. In addition, a decline in the acid content of *C. guineensis* and *S. melanotheron* was observed, whilst an increase was noted in *T. trachurus* and *S. scombrus*. Consequently, the levels of *C. guineensis* and *S. melanotheron* in their processed forms were found to exceed those of *T. trachurus* and *S. scombrus*.

The eicosapentaenoic acid (EPA, C20:5 n-3) content in fresh fish exhibited significant variation among species, with *T. trachurus* containing the highest proportion at 17.7%. This result was found to be significantly higher ($p<0.05$) in comparison to the levels recorded in *S. scombrus* (13.22%), *S. melanotheron* (2.91%), and *C. guineensis* (2.45%).

Following the completion of the requisite processing, this ranking was maintained. In both fried and smoked samples, the EPA content in *T. trachurus* and *S. scombrus* remained higher than in *S. melanotheron* and *C. guineensis*, between which levels were consistently similar ($p>0.05$).

Processing by smoking and frying significantly ($p<0.05$) lowered EPA proportions in *S. melanotheron*, *C. guineensis*, and *T. trachurus*, and by frying in *S. scombrus*. Conversely, smoking increased EPA levels in *S. scombrus* ($p<0.05$). In all direct comparisons, the level of eicosapentaenoic acid (EPA) of fried fish was lower than that of smoked fish.

The presence of octadecatetraenoic acid (C18:4 n-3) was observed in the other fresh species, with levels comparable to those found in fresh *S. scombrus*, fried *S. melanotheron*, and fried *T. trachurus* ($p>0.05$). However, this acid was absent in fresh *C. guineensis*. The results of the study demonstrated a significant increase in the proportions of C18:4 n-3 in *S. melanotheron*, *T. trachurus* and *S. scombrus* following smoking. Among the various types of smoked fish, *S. scombrus* exhibited the highest level of *S. scombrus* (0.04%), which was significantly higher ($p<0.05$) than that detected in smoked *T. trachurus* and *S. melanotheron*. In contrast, the application of frying resulted in a reduction of acidity below the quantification limit in *C. guineensis*, *S. melanotheron*, and T. trachurus.

Docosapentaenoic acid (DPA) content responded differently to processing in *S. scombrus* than in the other species. While frying and smoking significantly decreased DPA in *C. guineensis*, *S. melanotheron*, and *T. trachurus* (with frying producing the lowest levels), smoking *S. scombrus* resulted in a significant increase ($p<0.05$). Consequently, smoked *S. melanotheron* and *C. guineensis* contained the highest DPA proportions among processed fish, despite fried levels being statistically similar across all species. In their fresh state, *C. guineensis* and *S. melanotheron* had higher DPA than *T. trachurus* and *S. scombrus*.

Docosahexaenoic acid (DHA) levels varied significantly with species and processing method. Among fresh fish, *T. trachurus*, *S. scombrus*, and *C. guineensis* contained similar ($p>0.05$) proportions (17%), which were significantly higher ($p<0.05$) than the 10.67% found in *S. melanotheron*. While frying and smoking significantly reduced DHA content across all species, the degree of loss differed. Fried *S. scombrus* retained the highest DHA level after frying, and both smoked *T. trachurus* and *S. scombrus* maintained DHA levels comparable to their fresh state. Consequently, DHA proportions were consistently lowest in fried fish, higher in smoked fish, and highest overall in the fresh specimens.

Polyunsaturated acids (PUFA n-6)

The fatty acid composition, including arachidonic acid (C20:4 n-6; AA), is detailed in Table 1. In fresh fish, AA was highest ($p<0.05$) in *C. guineensis* and *S. melanotheron* than that in fresh *S. scombrus* and *T trachurus*. Processing differentially affected these profiles: AA content was significantly diminished by both frying and smoking in *C. guineensis* and *S. melanotheron* but was stable in *T. trachurus*. *S. scombrus* exhibited opposing trends, with AA increasing after frying and decreasing after smoking. These shifts reconfigured the relative rankings among processed fish; for example, fried *S. scombrus* contained the highest AA proportion of all fried samples, while its smoked counterpart ranked among the lowest.

Eicosadienoic acid (C20:2 n-6) was undetectable in most fresh fish but present in *C. guineensis* (0.31%). This pattern persisted after frying, where only *S. scombrus* contained a low level (0.1%). Smoking significantly altered the profile: the acid became detectable in all species, with *T. trachurus* (0.57%) containing the highest ($p<0.05$) proportion than *C. guineensis* (0.34%). Notably, the increase observed in smoked *C. guineensis* relative to its fresh state was not statistically significant.

Linoleic acid [C18:2(n-6)] was detected in fresh *S. melanotheron*, *C. guineensis*, and *S. scombrus* but was absent in fresh *T. trachurus*. Both frying and smoking increased levels across all species. After frying, *T. trachurus* showed the highest content ($p<0.05$), while after smoking, *C. guineensis* had the highest ($p<0.05$). Smoked *S. melanotheron*, *T. trachurus*, and *S. scombrus* exhibited similar levels. Overall, fried fish contained more linoleic acid than smoked fish.

Gamma-linolenic acid [GLA; C18:3(n-6)] was detected only in the fresh flesh *S. melanotheron* (0.68%) and *C. guineensis* (0.15%) and was absent in fresh *T. trachurus* and *S. scombrus*. The acid remained undetectable after frying in all species but became quantifiable after smoking in *S. melanotheron*, *C. guineensis*, and *S. scombrus*. Among smoked fish, *S. melanotheron* contained the highest GLA level, significantly higher ($p<0.05$) than that in *C. guineensis* and *S. scombrus*. While smoking increased GLA in *C. guineensis*, the rise in *S. melanotheron* from its fresh state was not statistically significant ($p>0.05$).

The ratio of polyunsaturated and saturated fatty acids (PUFA/SFA) to n-6 to n-3 fatty acids

Table 1 presents the total fat content and fatty acid composition (as a percentage of total fatty acids) for fresh, fried, and smoked fish. The n-6/n-3 ratio was similar among the different types of analyzed fresh fish.

This ratio varied from 0.02 to 0.22. In fried *C. guineensis, S. melanotheron* and *T. trachurus*, the ratio was equal between species and higher than in fried *S. scombrus*. By contrast, low ratios were obtained in smoked fish. Smoked *T. trachurus* and *S. scombrus* had very low ratios compared to smoked *C. guineensis* and *S. melanotheron*. These ratios increased when fish were fried and smoked. The increase was higher in fried fish but low in the smoked ones.

The PUFA/SFA ratio in fresh fish ranged from 0.93 to 1.19. Among fresh samples, the ratios for *C. guineensis* and *S. melanotheron* were not significantly different ($p>0.05$). The highest ratio was observed in fresh *T. trachurus*. In fried fish, *C. guineensis* and *S. melanotheron* exhibited lower PUFA/SFA ratios compared to *T. trachurus* and *S. scombrus*. This PUFA/SFA ratio was low in fried *S. melanotheron* (0.29) and high in fried *T. trachurus* (1.07). On the contrary, after smoking, the PUFA/SFA ratios varied from 0.48 to 1.27 and were similar paired between brackish water species and marine water species ($p>0.05$). Marine fish had the highest ratios. Besides, when fresh *C. guineensis* and *S. melanotheron* were fried and smoked, the ratios decreased significantly. A decrease in the PUFA/SFA ratio for *T. trachurus* and *S. scombrus* occurred only after frying. Conversely, smoking increased the ratio in both species, with the increase being statistically significant for *S. scombrus* when compared to its fresh state.

Lipid nutritional quality index

The lipid nutritional quality indices for fresh, smoked, and fried fish are summarized in Table 2. Analysis of the hypocholesterolemic/hypercholesterolemic (H/H) ratios revealed comparable values for fresh *C. guineensis* and *T. trachurus*, and separately, for fresh *S. melanotheron* and *S. scombrus*.

The H/H ratio exhibited distinct patterns depending on the species and processing method (Table 2). Among fresh fish, *C. guineensis* and *T. trachurus* had higher ratios than the other species. Frying generally increased the ratio in *C. guineensis*, *T. trachurus* and *S. scombrus*, with significant increases observed for *T. trachurus* and *S. scombrus* ($p<0.05$). Consequently, the ratios of fried *T. trachurus* and *C. guineensis* were higher than those of fried *S. melanotheron* and *S. scombrus*, and the ratios of fried *C. guineensis* and *S. scombrus* were similar. In contrast, frying decreased the ratio in *S. melanotheron*. Smoking had a different effect: it significantly decreased ($p<0.05$) the H/H ratio in *C. guineensis* and *S. melanotheron*, but increased it in *T. trachurus* and *S. scombrus*. Consequently, the ratios of smoked *T. trachurus* and *S. scombrus* were significantly higher ($p<0.05$) than those of smoked *C. guineensis* and *S. melanotheron*.

The atherogenicity index (AI) varied significantly between species and was strongly influenced by the processing method used (Table 2). Fresh *T. trachurus* and *S. scombrus* had a higher ($p<0.05$) AI than fresh *C. guineensis* and *S. melanotheron*. However, processing altered these initial values: smoking increased the AI in *C. guineensis* and *S. melanotheron*, while frying and smoking decreased it in *T. trachurus* and *S. scombrus*, with the lowest observed value of 0.35 being found in fried *T. trachurus*. Consequently, the rankings shifted. Among processed fish, smoked *S. melanotheron* and *C. guineensis* exhibited the highest AI values, while smoked *T. trachurus* (0.54) and *S. scombrus* had the lowest. The AI was similar ($p>0.05$) for *C. guineensis*, *S. melanotheron* and *S. scombrus* in fried fish, and lowest in *T. trachurus*. Overall, smoked fish consistently had a higher AI than fried fish across all species.

Furthermore, the Thrombogenicity Index (TI) was similar ($p>0.05$) in all the fresh fish. By contrast, this index was higher in fried *S. melanotheron* and *C. guineensis* than in fried *T. trachurus* and *S. scombrus*. The TI was similar ($p>0.05$) between fried *S. melanotheron* and *C. guineensis*. In smoked fish, the TI was higher in *C. guineensis* than in the other species. The lowest Thrombogenicity indices were obtained in smoked *T. trachurus* (0.21) and *Scomber scombrus* (0.24). Besides, when fresh *C. guineensis* and *S. melanotheron* were smoked and fried, the TI increased significantly. Likewise, the index increased when fresh *T. trachurus* and *S. scombrus* were fried, but decreased when they are smoked. Fried fish had higher IT than smoked fish.

The peroxidability index was higher in fresh *T. trachurus* and *C. guineensis* than in the other species. These indices were similar between fresh *T. trachurus* and *C.*

guineensis and between fresh *S. melanotheron* and *Scomber scombrus* (p>0.05). Fresh *S. scombrus* had the lowest PI of 228.76. In the fried state, the indices were similar between *C. guineensis*, *S. melanotheron* and *T. trachurus*. The highest index was observed in fried *S. scombrus*. By contrast, in smoked fish, high peroxidability indices were obtained in *T. trachurus* and *S. scombrus*. These indices were similar between smoked *C. guineensis* and *S. melanotheron*. However, when fresh *C. guineensis*, *S. melanotheron* and *T. trachurus* were fried and smoked, the indices decreased significantly except in *S. scombrus* where the indices increased during smoking. The decrease was larger in fried fish than in smoked fish.

Table 1 Lipid content and fatty acid profile (% total FA) by species and processing method (mean ± SD).

Variables	*C. guineensis*			*S. melanotheron*			*T. trachurus*			*S. scombrus*			Significance test
	Fresh	Fried	Smoked	Fresh	Fried	Smoked	Fresh	Fried	Smoked	Fresh	Fried	Smoked	
TF (%)	1.86±1.44^{f}	17.98±1.52^{b}	6.65±1.58^{e}	1.50±0.28^{f}	26.34±1.67^{a}	9.47±1.45^{de}	11.43±5.01^{cd}	14.18±5.27^{bc}	11.54±2.57^{cd}	11.08±4.64^{cd}	10.5±.2.27^{cd}	13.42±3.21^{c}	***
C10:0	<LQ	0.45±0.04^{b}	<LQ	<LQ	0.36±0.05^{c}	<LQ	<LQ	0.52±0.09^{b}	<LQ	<LQ	0.79±0.18^{a}	<LQ	***
C14:0	1.56±1^{d}	1.1±0.5^{d}	4.3b±1.44^{c}	4.8±1.94^{bc}	1.66±0.14^{d}	8.84±0.83^{a}	5.57±1.92^{b}	1.68±0.51^{d}	3.77±0.58^{c}	7.7±2.98^{a}	4.86±0.76^{bc}	5.7±1.07^{b}	***
C16:0	26.16±4.58^{de}	33.56±10.84^{abc}	37.99±1.67^{a}	24.74±3.47^{de}	37.65±0.95^{a}	34.05±1.5^{ab}	27.54±6.17^{cde}	18.49±1.66^{f}	21.85±1.27^{ef}	28.17±8.24^{bcd}	24.54±1.89^{def}	24.41±4.10^{def}	***
C17:0	0.62±0.6^{b}	<LQ	1.1±0.28^{a}	0.66±0.47^{b}	0.03±0.06^{d}	0.55±0.2^{bc}	<LQ	<LQ	0.08±0.17^{d}	0.72±0.44^{b}	0.6±0.11^{b}	0.22±0.21^{cd}	*
C18:0	12.18±1.74^{a}	4.81±0.33^{f}	10.3±1.7^{b}	9.08±1.58^{bc}	4.39±0.15^{f}	6.58±1.14^{de}	8.03±1.7^{cd}	5.92±0.36^{ef}	6.53±0.38^{de}	8.51±1.87^{c}	7.63±0.6^{cd}	5.74±1.47^{ef}	***
C20:0	<LQ	0.05±0.12^{b}	<LQ	<LQ	<LQ	<LQ	<LQ	0.27±0.01^{a}	<LQ	<LQ	<LQ	<LQ	***
C22:0	<LQ	0.36±0.81^{c}	0.05±0.13^{cd}	<LQ	<LQ	0.05±0.11^{d}	<LQ	1.67±0.12^{a}	<LQ	<LQ	0.94±0.13^{a}	<LQ	***
C24:0	<LQ	0.34±0.17^{b}	<LQ	<LQ	0.20±0.03^{c}	<LQ	<LQ	0.27±0.09^{a}	<LQ	<LQ	0.59±0.04^{a}	<LQ	***
∑SFA	40.52±5.08^{cd}	40.69±10.41^{cd}	53.76±2.49^{a}	39.28±5.53^{cde}	44.29±0.82^{bc}	50.08±2.07^{ab}	41.15±9.32^{cd}	29.23±2.32^{f}	32.22±1.63^{ef}	45.11±13.36^{bc}	39.98±3.22^{cde}	36.08±6.27^{def}	***
C16:1	3.99±2.96^{ef}	1.95±0.79^{f}	8.57±2.9^{b}	8.2±2.77^{bc}	2.6±0.14^{f}	16.3±1.81^{a}	1.74±2.78^{f}	2.39±0.48^{f}	5.25±0.38^{de}	6.55±2.59^{bcd}	5.13±0.71^{de}	5.86±1.16^{cde}	***
C17:1	0.91±0.49^{bc}	0.23±0.11^{d}	0.28±0.62^{d}	2.29±0.93^{a}	0.38±0.08^{cd}	<LQ	<LQ	0.48±0.13^{cd}	1.08±0.17^{b}	<LQ	1.02±0.17^{b}	1.86±0.76^{a}	***
C18:1	8.67±3.13^{ef}	39.04±1.12^{a}	11.06±4.01^{def}	7.57±0.64f	39.68±0.46^{a}	9.56±1.39^{ef}	12.45±8.04^{de}	36.71±2.49^{a}	20.36±1.40^{c}	15.1±5.34^{d}	27.36±2.45^{b}	12.5±1.19^{de}	***
∑MUFA	13.58±6.11^{e}	41.22±0.81^{a}	19.92±19.92^{d}	18.07±4.14^{de}	42.67±0.57^{a}	25.85±2.79^{c}	14.19±7.18^{e}	39.58±2.47^{a}	26.69±1.1^{c}	21.65±7.67^{cd}	33.52±2.34^{b}	20.21±2.19^{d}	***
C18:2 (n-6)	1.94±1.09^{de}	14.73±10.8^{b}	4.26±1.48^{d}	2.11±0.87^{de}	10.15±0.14^{c}	2.1±0.19^{de}	<LQ	24.9±2.21^{a}	1.38±0.26^{de}	0.44±0.61^{de}	11.23±2.57^{bc}	0.99±0.66^{de}	***
C18:3 (n-6)	0.15±0.21^{c}	<LQ	0.36±0.34^{b}	0.68±0.23^{a}	<LQ	0.78±0.07^{a}	<LQ	<LQ	<LQ	<LQ	<LQ	0.06±0.14^{c}	***
C20:2 (n-6)	0.31±0.19^{ab}	<LQ	0.34±0.2^{ab}	<LQ	<LQ	<LQ	<LQ	<LQ	0.03±0.07^{b}	<LQ	0.1±0.14^{ab}	0.57±1.28^{a}	***
C20:4(n-6)	6.24±3.37^{a}	0.57±0.27^{d}	3.63±0.97^{b}	4.63±1^{b}	0.29±0.06^{d}	2.15±0.45^{c}	0.29±0.4^{d}	0.17±0.12^{d}	0.6±0.09^{d}	0.95±0.64^{cd}	1.06±0.38^{cd}	0.55±0.33^{d}	***
∑PUFA w6	8.65±3.48^{cde}	15.3±10.63^{b}	8.62±2.47^{cde}	7.43±1.42^{de}	10.45±0.20^{cd}	5.04±0.49^{ef}	0.29±0.4^{g}	25.07±2.26^{a}	2.01±0.31^{fg}	1.39±1.15^{fg}	12.4±2.86^{bc}	2.17±0.47^{fg}	***
C18:3 (n-3)	0.53±0.5^{b}	0.05±0.11^{d}	1.13±0.3^{a}	1.32±0.46^{a}	0.17±0.1^{bc}	1.29±0.19^{a}	<LQ	<LQ	0.17±0.25^{bc}	<LQ	0.11±0.25^{c}	0.52±0.46^{b}	***
C18:4 (n-3)	<LQ	<LQ	<LQ	0.05±0.13^{a}	<LQ	0.15±0.14^{a}	0.17±0.23^{a}	<LQ	0.23±0.22^{b}	0.08±0.18^{a}	0.04±0.11^{a}	0.41±0.26^{a}	NS
C20:3 (n-3)	1.61±0.32^{a}	0.38±0.19b^{cd}	1.42±1.31^{ab}	1.29±0.31^{abc}	0.27±0.03^{cd}	1.27±0.72^{abc}	<LQ	0.39±0.14^{bcd}	0.45±0.27^{bcd}	0.05±0.11^{d}	0.82±0.66^{abcd}	1.19±2.31^{d}	***
C20:5 (n-3)	2.45±0.87^{d}	0.34±0.08^{e}	1.92±0.54^{d}	2.91±0.53^{cd}	0.31±00^{e}	2.34±0.12^{d}	17.7±3.34^{a}	2.52±0.75^{d}	14.18±0.42^{b}	13.22±5.53^{b}	5.73±2.05^{c}	18.19±5.12^{a}	***
C22:5 (n-3)	15.45±1.43^{b}	1.31±0.37^{f}	8.99±1.4^{cd}	18.93±2.84^{a}	1.35±0.15f	10.78±0.75^{c}	8.87±3.18^{cd}	1.33±0.39^{f}	8.29±1.12^{de}	2.05±3.16^{f}	2.1±0.48^{f}	6.50±1.61^{e}	***
C22:6 (n-3)	17.19±8.82^{a}	0.67±0.15^{a}	4.23±0.55^{a}	10.67±4.79^{a}	0.45±0.03^{a}	3.14±0.4^{a}	17.61±10.64^{a}	1.85±0.81^{a}	15.72±1.55^{a}	16.42±12.85^{a}	5.25±1.92^{a}	14.67±4.75^{a}	NS
∑PUFA w3	37.25±7.99^{ab}	2.77±0.8^{e}	17.71±2.54^{c}	35.2±7.03^{ab}	2.57±0.22^{e}	19.01±1.47^{c}	44.36±14.97^{a}	6.1±1.99^{de}	39.06±2.05^{ab}	31.83±19.91^{b}	14.08±4.98^{cd}	41.52±8.74^{ab}	**
∑PUFA	45.9±10.5^{a}	18.08±10.45^{ef}	26.32±3.66^{de}	42.63±6.81^{abc}	13.03±0.27^{f}	24.05±1.66^{def}	44.65±14.75^{ab}	31.18±3.42^{cd}	41.08±2.02^{abc}	33.23±20.99^{bcd}	26.49±4.94^{de}	43.69±8.33^{ab}	**
PUFA/SFA	1.17±0.39^{ab}	0.56±0.61^{cde}	0.49±0.08^{de}	1.11±0.30^{ab}	0.29±0.01^{d}	0.48±0.03^{de}	1.19±0.63^{a}	1.07±0.19^{abc}	1.27±0.12^{a}	0.93±0.83^{abcd}	0.67±0.16^{bcde}	1.26±0.39^{a}	*
n-6/n-3	0.22±0.07^{b}	5.96±4.56^{a}	0.49±0.13^{b}	0.21±0.06^{b}	4.08±0.36^{a}	0.26±0.02^{b}	0.02±0.01^{b}	4.58±1.85^{a}	0.05±0.01^{b}	0.04±0.01^{b}	0.97±0.37^{b}	0.05±0.03^{b}	*

***: p < 0.001, **: p < 0.01; , *: p < 0.05, ns: p>0.05; means of the same row followed by different letters are significantly different (p<0.05). TF (%): Percentage of total fat; capric acid (C10: 0), myristic acid (C14: 0), palmitic acid (C16: 0), heptadecanoic acid (C17: 0), stearic acid (C18: 0), arachidic acid (C20: 0), docosanoic acid (C22: 0), lignoceric acid (C24: 0). Palmitoleic acid (C16: 1), heptadecenoic acid (C17: 1), oleic acid (C18: 1); Linoleic acid C18: 2 (n-6), gamma α-linolenic acid C18: 3 (n-6), eicosadienoic acid C20: 2 (n-6), arachidonic acid C20: 4 (n-6); Alpha α-linolenic acid C18: 3 (n-3), octadecatetraenoic acid C18: 4 (n-3), di-homo-γ-α-linolenic acid C20: 3 (n-3), eicosapentaenoic acid (EPA) C20 : 5 (n-3), docosapentaenoic acid (DPA) C22: 5 (n-3), docosahexaenoic acid (DHA) C22: 6 (n-3; LQ : The proportion is below the limit of quantification.

Table 2: Lipid quality indices by species and processing method (mean ± SD)

Variables	*C. guineensis*			*S. melanotheron*			*T. trachurus*			*S. scombrus*			Significance test
	Fresh	Fried	Smoked	Fresh	Fried	Smoked	Fresh	Fried	Smoked	Fresh	Fried	Smoked	
HH	1.99±0.66^{bc}	2.1±1.8^{bc}	0.84±0.1^{de}	1.68±0.45^{bcd}	1.33±0.04^{cde}	0.73±0.02^{e}	1.84±0.66^{bc}	3.38±0.5^{a}	2.38±0.23^{b}	1.6±1.04^{bcde}	1.81±0.27^{bc}	1.86±0.58^{bc}	*
AI	0.55±0.18^{de}	0.67±0.27^{cd}	1.19±0.13^{ab}	0.74±0.21^{cd}	0.79±0.02^{cd}	1.39±0.08^{a}	0.89±0.36^{bc}	0.35±0.05^{e}	0.54±0.06^{de}	1.19±0.6^{ab}	0.73±0.12^{cd}	0.75±0.22^{cd}	***
TI	0.32±0.09^{ef}	1.11±0.39^{a}	0.76±0.1^{b}	0.32±0.09^{ef}	1.26±0.05^{a}	0.65±0.05^{bc}	0.3±0.12^{ef}	0.51±0.08^{cde}	0.21±0.01^{f}	0.37±0.25^{def}	0.58±0.16^{bcd}	0.24±0.11^{f}	***
PI	274.57±73.7^{a}	33.51±9.5^{c}	122.86±10.01^{bc}	242.40±53.99^{ab}	26.49±1.23^{c}	120.75±8.48^{bc}	303.24±109.45^{a}	64.30±14.23^{c}	266.58±13.65^{a}	228.76±146.01^{ab}	106.11±30.71^{bc}	273.12±77.14^{a}	*

*: The result is statistically significant. There is a less than 5% probability (p < 0.05); ***: Extremely Significant – The result is very highly significant. The probability it's due to chance is less than 0.1% (p<0.001) ***: p < 0.001, **: p < 0.01; , *: p < 0.05, ns: p>0.05; Means of the same row followed by different letters are significantly different (p<0.05); AI: Atherogenicity Index; TI: Thrombogenicity Index; HH: Hypocholesterolemia / Hypercholesterolemia ratio; PI: Peroxidability index; LQ : The proportion is below the limit of quantification.

DISCUSSION

Effects of processes on total lipid contents

The lipid contents obtained in the fresh *C. guineensis* and *S. melanotheron* allow their classification among lean fish of which lipid content is lower than 5% **(Faucouneau, 2004).** On the contrary, following the classification of **Barnathan (2007)**, the total lipid contents of *S. scombrus* and *T. trachurus* found in this study are higher than 10% and allow us to affirm that *S. scombrus* and *T. trachurus* are fat fish.

Frying resulted in a higher total fat content in the brackish water species (*C. guineensis* and *S. melanotheron*) compared to the marine species (*S. scombrus* and *T. trachurus*). In their study on the effects of food processing on chemical composition, **Bassey *et al.* (2014)** reported an increase in total lipids in three Nigerian fish species following frying. Likewise, the increase in total lipids content when fresh fish was smoked was obtained by **Bouriga *et al.* (2012)** studying smoking effect on *Oreochromis niloticus* chemical composition in El Hama in Tunisia. The total lipids high contents in fish flesh are due to frying effect, smoking technique and to the lipid's absorption capacity of muscle fibers which may differ from one species to another. During frying, lipids are brought through the groundnut oil that is used; hydrolysis reactions and partial water losses lead to free fatty acids formation and also increase the total lipids content of fish flesh. The same observations were made by **Aberoumand and Saeed (2015)** while studying cooking effect on marine fish **(*Platycephalus indicus*)** in Iran. During smoking, water loss concentrates lipids in the flesh and then increases lipids content. The similar contents between fresh and smoked *T. trachurus* prove that these fish were smoked the same way. Smoking technique, temperature and smoking time did not induce a difference in lipid content in smoked *T. trachurus*. On the other hand, applying groundnut oil directly on fish after smoking to make them more attractive may also increase total lipid contents in fish. However, the total lipid contents can also vary according to species, muscle, animal (sex, breed, age) and fish farming method **(Lebret and Picard, 2015)**.

Influence of processing methods on the contents of saturated and monounsaturated fatty acids

Fatty acid profile varies according to species and process. All fresh fish have similar total saturated fatty acid (SFA) content, except for *S. scombrus.* A similar content was got in fresh *Clupea harengus* (40.29%) by **Tenyang *et al*. (2017)** in Cameroon. Higher contents were obtained by **Ghaeni *et al*. (2013)** in fresh *Upeneus Sulphureus* (52.4%) and *Leiognathus bindus* (50%) caught in spring and autumn in Iran. The difference in SFA content obtained between fresh *S. scombrus* and the other species is because this species is naturally fatter **(Grégoire *et al*., 1994; Alinasabhematabadi, 2015)**.

This study's analysis of the saturated fatty acid (SFA) profile showed that fish flesh is characterized by the predominance of palmitic (C16:0) and stearic (C18:0) acids. Palmitic acid contents increased when fresh *C. guineensis* and *S. melanotheron* were fried and smoked, and decreased in smoked and fried *S. scombrus* and T. *trachurus*. The proportion of palmitic acid has increased as in this study while smoking fresh *Cyprinus carpio* and *Oncorhynchus mykiss* **(Cieślik *et al*., 2017)** and while frying fresh *Oncorhynchus tshawytscha*, *Cyprinus carpio*, *Salvelinus namaycush*, *Sander vitreus* and *Catostomus commersonii* **(Neff *et al*., 2014).**

The increase in palmitic acid during frying is due to frying oil (groundnut oil) absorption by flesh fish according to muscle tissue absorption capacity. This oil generally contains 20% saturated fatty acids and 10% palmitic acid **(Lambert, 2005)**. Additionally, hydrolysis reactions occur during frying, producing free fatty acids, mono- and diglycerides, and glycerol from fish flesh lipids when they come into contact with water vapour. These reactions increase saturated fatty acids content through **(Bentayeb, 2011; Brühl, 2014; Chahbi, 2016)**. According to **Bouriga *et al*. (2012),** lipase and phospholipase activity during smoking significantly increases saturated fatty acid content. In contrast, the decrease in palmitic acid observed in smoked and fried *S. scombrus* and *T. trachurus* is attributed to the high temperatures of these processing methods. Both species being fatty fish, during frying and smoking, the high temperature melts the fat and causes fatty acids loss. The lipids provided by the frying oil during fish flesh saturation with lipids are no more absorbed. Furthermore, the frying and smoking processes differed between the brackish water fish (*C. guineensis* and *S. melanotheron)* and the marine fish (*S. scombrus* and *T. trachurus*), including the temperature and cooking time. The cooking temperature, often uncontrolled, varies according to processor and process and this explains the variations in the decreases of fatty acid contents observed during smoking and frying.

The influence of temperature was less pronounced for monounsaturated fatty acids, especially oleic acid (C18:1), in fried fish. Fried *S. melanotheron*, *C. guineensis*, *S. scombrus* and *T. trachurus* have contents of C18: 1 very higher than those of fresh fish. Similar results on the effect of cooking (smoking, frying, boiling) and temperature (160°C and 180°C) on anchovy (*Engraulis encrasicolus*) nutritional quality were reported by **Uran and Gokuglu (2014).**

The increase in oleic acid is due to the C18: 1 brought by the groundnut oil used for fish frying in this study. According to **Lambert (2005)**, groundnut oil is a monounsaturated fatty acid that contains 50% oleic acid. This proportion, combined with the fatty acids released from fish flesh via hydrolysis during frying, increases the C18:1 content.

Influence of processing on polyunsaturated fatty acid content

The n-3 and n-6 polyunsaturated fatty acids (PUFAs) are known to have beneficial effects on human health, including protection against cardiovascular and other chronic diseases (**Abeynayake and Mendis 2014; Hamed *et al*., 2015; Pires *et al*., 2015, 2018).** Freshwater fish (*S. melanotheron* and *C. guineensis*) contained higher proportions of n-6 PUFA compared to the marine species (*T. trachurus* and *S. scombrus*). Conversely, the marine fish were characterized by high n-3 and very low n-6 PUFA contents. This pattern aligns with the general observation that marine species typically exhibit higher n-3 and lower n-6 PUFA levels than freshwater fish (**Simon *et al*., 2012; Łuczyńska *et al*., 2014**). It is also consistent with the findings of **Schneedorferová *et al*. (2015)**, who reported higher n-6 PUFA in freshwater species (*Esox lucius* and *Cyprinus carpio*) than in marine fish (*Gadus morhua* and *Clupea harengus*), the latter of which showed a complete absence of α-linolenic acid (C18:3 n-6). Furthermore, the n-3 and n-6 PUFA profile in fresh fish is influenced by three key factors: the breeding environment, dietary intake, and the species-specific metabolic capacity for fatty acid synthesis. Fish in natural environment feed phytoplankton, zooplankton and unicellular algae. Polyunsaturated fatty acids in fish come from these food sources.

The phytoplankton, zooplankton, and algae in brackish water have been found to be rich in α-linolenic (C18:3 n-3) and linoleic (C18:2 n-6) acids, whereas the marine counterparts have been found to be richer in eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (**Barnathan, 2007; Lavialle and Layé, 2010; Abouel-yazeed, 2013; Łuczyńska *et al*., 2014**). This dietary disparity elucidates the fatty acid profiles observed in the fish analyzed in this study. The brackish water species *S. melanotheron* and *C. guineensis* were found to contain both linoleic and α-linolenic acids, with a predominance of linoleic acid (C18:2 n-6). Conversely, these acids were either absent or present in low proportions in the marine species *T. trachurus* and *S. scombrus*.

However, processing and preservation methods (frying and smoking) altered the fatty acid composition of the fish. Specifically, the content of linoleic acid (C18:2 n-6) increased after both frying and smoking, with frying resulting in the highest levels. In contrast, the proportion of α-linolenic acid (C18:3 n-3) decreased after frying but increased after smoking. The increase of the content is due to fatty acids hydrolysis caused by lipase and phospholipase during smoking. The difference between fried and smoked fish is because groundnut oil provides 30% linoleic acid **(Lambert, 2005)** supplementing then flesh fish content during cooking. However, the decrease in linolenic acid proportion is due to the thermo-oxidation which drastically destroys this acid when frying.

Furthermore, the contents of DHA, EPA, and DPA decreased significantly more in fried fish than in smoked fish. Indeed, levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased in smoked *S. scombrus*. The degradation of long-chain PUFAs, such as EPA, DHA, and DPA, during the frying process is attributed to thermal peroxidation. Similar reductions in EPA and DHA from various cooking methods (frying, smoking, braising, boiling) have been reported in multiple species (**Guizani *et al*., 2011; Zotos *et al*., 2011; Nurjanah *et al*., 2015; Purwaningsih *et al*., 2015; Chandrani *et al*., 2016; Cieślik *et al*., 2017; Flaskerud *et al*., 2017**).

Long-chain polyunsaturated fatty acids (LC-PUFAs) are highly labile and oxidize rapidly at high temperatures in the presence of oxygen and light, a process that increases their degree of unsaturation **(Saldanha & Bragagnolo, 2010; Zhang *et al*., 2013; El-Reffaei *et al*., 2014; Sebastien *et al*., 2014; Hosseini *et al*., 2014; Mesías *et al*., 2015; Nieva-Echevarría *et al*., 2016; Nurjanah *et al*., 2016)**. This inherent sensitivity explains the marked reduction in EPA and DHA observed during frying across all species. The extent of lipid peroxidation was higher in frying than in smoking, with brackish water species exhibiting higher thermo-oxidative sensitivity than marine fish. These differences in fatty acid content between the two processes are attributable to variations in cooking temperature and duration in this study, as well as the initial fatty acid composition of the fresh fish flesh.

Impact of processing methods on the n-6/n-3 Fatty acid ratio in fish

As an important indicator of nutritional quality, the n-6/n-3 polyunsaturated fatty acid (PUFA) ratio is determined by the relative proportions of these fatty acid classes; a higher ratio reflects a higher total n-6 PUFA content compared to n-3 PUFAs (**Gladyshev *et al*., 2014; Souza *et al*., 2017**). This ratio increased when fish were fried and smoked. The increase was higher in fried fish but low in the smoked one. Similar high ratio after frying were got in studies by **Gladyshev *et al*. (2014)** on pikeperch (*Sander lucioperca*) fried in sunflower oil and by **Chandrani *et al*. (2016)** on several species (*Leiognathus Dussumieri*; *Gazza minuta*) fried in coconut oil. The n-6/n-3 ratios exhibited variation between fresh and processed fish, attributable to several factors. These include the processing method itself, the high thermo-oxidative sensitivity of n-3 LC-PUFAs during the frying process, and the elevated n-6 PUFA content imparted by frying in vegetable oil. Consequently, smoked fish exhibited lower ratios than fried fish. This is attributable to the fact that smoking resulted in a total n-6 PUFA content that remained lower than that of n-3 PUFAs, in contrast to the shift observed with frying.

A high n-6/n-3 ratio, indicative of a relative decline in n-3 PUFA levels, is associated with increased risk of cardiovascular disease, cancer, and inflammatory diseases. Conversely, a high proportion of n-3 PUFAs and a low n-6/n-3 ratio mitigate these pathogenic effects (Simopoulos, 2006). Consequently, maintaining an adequate ratio, ideally not exceeding 4, helps prevent various diseases (**HMSO, 1994; Lira *et al*., 2014**; **Valencak *et al*., 2015**). In this study, smoked fish had a ratio below 4, while fried fish exceeded this threshold. Thus, based on this metric, the smoked fish demonstrate a more favourable nutritional profile.

For the PUFA/SFA ratio, the minimum value recommended by United Kingdom Department of Health is 0.45 (HMSO, 1994). All fish in this study both smoked and fried met this standard, with the exception of fried *S. melanotheron*, which had a ratio of 0.29.

Nutritional lipid quality index

The ratio of hypocholesterolemia and hypercholesterolemia (H/H) was high when fresh fish were fried and decreased when fresh fish were smoked. The Atherogenicity Index (AI) increased in smoked fish than in fried fish. On the contrary, fried fish had higher Thrombogenicity indices (TI) than those smoked. **Cieślik *et al*. (2017)** got in their study similar results in several species (*Oncorhynchus mykiss*, *Cyprinus carpio*, *Esox lucius*) where after smoking, HH index decreased and thrombogenicity and atherogenicity indices increased. **Tonial *et al*. (2014)** reported in their study that the atherogenic (AI), thrombogenic (TI), and hypocholesterolemic/hypercholesterolemic (HH) indices increased in *O. niloticus* juveniles after frying. These variations are influenced by the processing method, the quality of the frying oil, and the relative proportions of atherogenic (C12:0, C14:0, C16:0) and thrombogenic (C14:0, C16:0, C18:0) fatty acids, which directly determine these index values.

Low atherogenicity (AI) and thrombogenicity (TI) indices are desirable, as they indicate a dietary lipid profile associated with a lower risk of cardiovascular disease (**Simon *et al*., 2012; Oliveira *et al*., 2018**). An increase in these indices

signifies a deterioration in the nutritional quality of the fatty acids. In this study, the low thrombogenicity index in smoked *T. trachurus* and *S. scombrus*, and the low atherogenicity index in fried *T. trachurus*, reflect a lower content of pro-atherogenic and pro-thrombogenic fatty acids in these fish. The consumption of fish with favorable atherogenicity (AI) and thrombogenicity (TI) indices is associated with a lower incidence of thrombosis and atherosclerosis. According to **Łuczyńska *et al*. (2017),** AI and TI values higher than 1 are considered detrimental to human health. Conversely, a high hypocholesterolemic/hypercholesterolemic (H/H) ratio approximately 2 or above is beneficial (**Bentes *et al*., 2009; Rodrigues *et al*., 2017**). In the present study, both smoked and fried *S. scombrus* had an H/H ratio of 1.86, meeting this beneficial standard.

The Peroxidability Index (PI) reflects a lipid's susceptibility to oxidation, with a higher index indicating higher sensitivity of fatty acids to peroxidation (**Testi *et al*., 2006; Hosseini *et al*., 2014**). In this study, the fresh fish *C. guineensis* and *T. trachurus* exhibited higher PI values than the processed fish, making them more prone to oxidation. According to **Hosseini *et al*. (2014)**, a high PI is also indicative of a high total n-3 PUFA content. This is in accordance with our study results of 37.25% total n-3 PUFA contents in fresh *C. guineensis* and of 44.36% in fresh *T. trachurus*. On the other hand, smoked *S. scombrus* and *T. trachurus* had high PI levels and can then thermo-oxidize during new smoking or cooking. Fresh or fried fish with low PI have a low proportion of n-3 PUFA.

CONCLUSION

This study assessed how smoking and frying influence the nutritional quality, specifically the fatty acid profiles, of four fish species from South Benin: the brackish water species *C. guineensis* and *S. melanotheron*, and the marine species *S. scombrus and T. trachurus*. The analysis focused on changes in saturated, monounsaturated, and polyunsaturated fatty acids, including the nutritionally critical n-6 and n-3 series. It has been demonstrated that the frying in groundnut oil increases the total lipid content in the flesh of brackish-water fish. Frying and smoking increase the levels of SFA in brackish water fish, while they decrease in marine water fish. Although frying contributes to the supply of linoleic acid (C18:2 n-6) through groundnut oil into fish flesh, it also degrades EPA and DHA, particularly in brackish water fish. On the other hand, smoking better preserves these beneficial n-3 fatty acids.

EPA, DPA and DHA losses during frying with groundnut oil are a public health concern. On the contrary, *T. trachurus* fried in groundnut oil contains high quantities of anti-atherogenic fatty acids while smoked *T. trachurus* and *S. scombrus* contain high quantities of anti-thrombogenic fats acids. As for smoking, it gives a better nutritional quality to marine water fish flesh than that of smoked and fried brackish water fish. Smoked fish offers a nutritional advantage for consumers, as this processing method effectively preserves n-3 PUFAs.

Furthermore, enhancing the diet of farmed brackish water fish with α-linolenic and linoleic acids could improve the levels of beneficial EPA and DHA in their flesh. Finally, using frying oils rich in n-3 and n-6 PUFAs, rather than conventional oils, would improve the nutritional quality of prepared fish.

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