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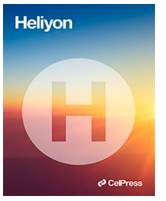

Research article

Assessment of cooking methods and freezing on the nutritional value and health risks of heavy metals in four fish species consumed in Douala, Cameroon

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ABSTRACT

The effects of smoking, boiling and freezing on the nutritional value and health risks of heavy metals in four fish species consumed in Douala was investigated. Fish samples from *Cyprinus carpio, Arius parkii, Ethmalosa fimbriata* and *Polydactilis quadrifilis* were collected at the Douala Fishing seaport, carried to the laboratory, washed with distilled water and processed. Proximate composition, mineral content, heavy metals and lipid quality were analyzed using AOAC standard methods. Estimated Daily Intake (EDI), Targeted Hazard Quotient (THQ), Hazard Index (HI) and Carcinogenic Risk (CR) were used to estimate the human health risk. Results showed that smoking and boiling increased significantly ($P < 0.05$) protein and ash levels. Lipid were reduced significantly ($P < 0.05$) with boiling and freezing compared to raw and smoked sample. Smoking increased significantly ($P < 0.05$) cadmium, lead, mercury and arsenic contents compared to boiling and freezing. EDI values of cadmium in all species of fish smoked were not acceptable for human consumption. THQ values of mercury in raw, smoked, boiled and frozen were not acceptable for human consumption. HI suggested a non potential carcinogenic effect for all fish while CR for cadmium and arsenic suggested a carcinogenic health risk for *Arius parkii* (smoked and boiled). All treatment decreased significantly ($P < 0.05$) iodine value and increased acid, peroxide, anisidine values, thiobarbituric acid reactive substances and total oxidation index compared to raw fish. Boiling was the best cooking method compared to smoking.

1. Introduction

Global malnutrition is a public health problem affecting a significant proportion of the world's population. Two types of manifestation can occur: undernutrition and overnutrition [1]. According to FAO estimates, the number of undernourished people worldwide rose to 735 million in 2022 from 613 million in 2019. It is projected that almost 600 million people will be chronically undernourished in 2030. Worldwide in 2022, an estimated 148.1 million children under five years of age were stunted, 45 million were wasted, and 37 million were overweight [2]. To combat this malnutrition, an important place is given to fish products. This is true given that 70% of the earth's surface is covered by water, and global fish production stands at 179 million tons, with world consumption estimated at 151.2 million tons, or 20.5 kg/year/in habitant [3]. As well as being a rich source of high-biological-value

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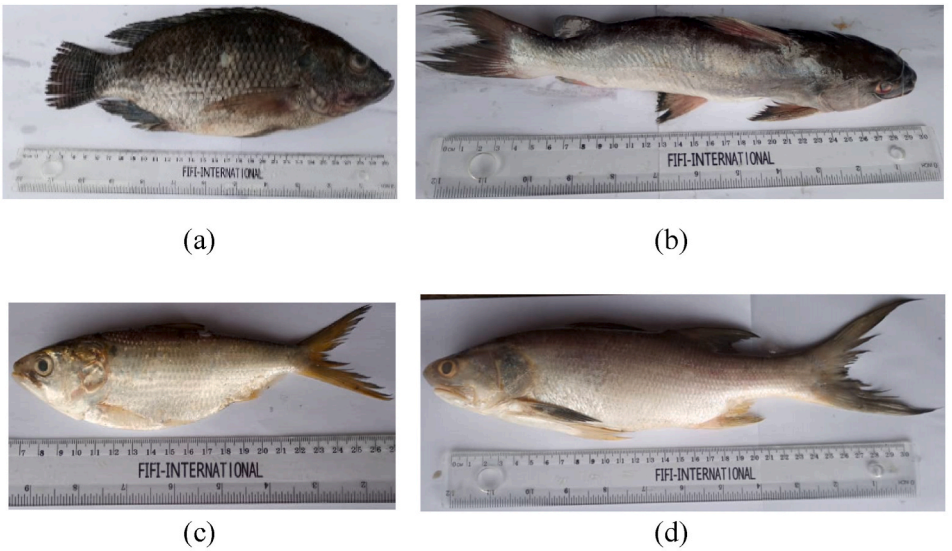
protein, fish contains vitamins, minerals and lipids. To determine the quality of fish oils, it is necessary to evaluate certain parameter such as iodine value (IV), acid index (AI), peroxide index (PI), anisidine index (IAn), thiobarbituric acid reactive substances (TBARS) and total oxidation index (TOTOX) [4]. Fish oils are rich in omega-3 polyunsaturated fatty acids, which help protect against cardiovascular diseases [5]. Minerals catalyze numerous metabolic reactions, maintain acid-base balance and are involved in the formation of bones, teeth, enzymes and hormones. Vitamins are involved in many biological processes and are precursors of coenzymes involved in metabolic pathways [6]. Cameroon, with a 402 km coastline on which artisanal fishing, industrial fishing and aquaculture are practiced, has an estimated annual fish production of 252,764 tons, with an average per capita consumption of 19.4 kg [7]. In Cameroon, fishing is a source of income and employment, and contributes over 46% of the population's animal protein requirements. Fishing provides the people of Cameroon with food security. As Douala is a coastal city, fish is the main source of animal protein and is consumed in several forms (boiled, roasted, fried and smoked). Fish is an integral part of the cultural rites and traditional ceremonies of certain Cameroonian ethnic groups, such as the Douala (Ngondo), Batanga (Mayi) and Bakoko (Mpo'oh). In this area, a variety of fishing products come from *Cyprinidae, Ariidae, Clupeidae* and *Polynemidae* families. *Cyprinids* is the largest family of freshwater fish, with only a few species venturing into the brackish found in the river estuaries. Most *cyprinids* are omnivorous with a herbivorous tendency, feeding mainly on invertebrates and benthic plants [8]. The *Ariidae* are demersal species, living in brackish or salt water in temperate and tropical regions. Species in this family are omnivorous, feeding on shrimp, parasites, plankton and numerous benthic invertebrates buried in the mud [9]. *Clupeidae* are pelagic species native to the coasts, beaches, lagoons and estuaries of West Africa. They are distributed along the tropical zone of the Atlantic Ocean. Some prefer shady waters. They feed on phytoplankton, benthic invertebrates, fish and fish eggs [10]. *Polynemidae* are demersal species, occurs in shallow coastal waters, over sandy and muddy bottoms, sometimes in brackish habitats. They are distributed along eastern Atlantic. Very large specimens are only found in marine water. This family is carnivorous and feeds on sea urchins, mollusks, crustaceans, crabs, amphipods and barnacles [11]. *Cyprinus carpio* (Linnaeus, 1758), *Arius parkii* (Günther, 1864), *Ethmalosa fimbriata* (Bowdich, 1825) and *Polydactylus quadrifilis* (Cuvier, 1829) are species of *Cyprinidae, Ariidae, Clupeidae and Polynemidae* families respectively targeted in this study. These fish species are easily accessible to all social strata and occupy an important place in the fishing catches and diet of Cameroonians. Eating fish can be an important source of exposure to numerous chemical contaminants such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr) and arsenic (As) found in the environment [12]. These contaminants can originate from volcanic eruptions, offshore oil discharges and leaks, domestic, industrial and agro-pastoral activities [13]. The biogeochemical cycles of these heavy metals are dominated by exchanges between the aquatic environment and the atmosphere. In aqueous environments, two essential chemical reactions are in competition: reduction and methylation. The first favours atmospheric recycling, while the second lead to bioaccumulation in sediments and aquatic species. This bioaccumulation process leads to cumulative effects on the occurrence of biomagnification through the food chain [14]. Sea organisms accumulate these contaminants primarily by uptake through the skin and gills via surface contacts with sediments, industrial effluent, wastewater, as well as via the food they consume [15]. Heavy metals are important aquatic pollutants with non-biodegradable and bioaccumulation properties, high toxicity, and long-time persistence. Numerous studies showed that contamination of the food chain by heavy metals can cause a number of health problems to humans such as erythrocyte and testicular damages, cancer, cardiovascular and skin disease, infertility and brain damage [15–18]. To limit the potential impact of these pollutants on human health, organizations such as the European Union (EU), the United States Environmental Protection Agency (USEPA)



(a) : *Cyprinus carpio* ; (b) : *Arius parkii* ; (c) : *Ethmalosa fimbriata* ; (d) : *Polydactilus quadrifilis*

Fig. 1. The experimental fish samples collected from Douala fishing seaport.

have established maximum limits for Pb, Cd, Hg, Cr and As in fish. It is therefore important to assess the health risks associated with consumption of fish potentially contaminated by these heavy metals, so that control measures can be taken. Fish is a highly perishable foodstuff and is rarely eaten fresh. It is processed according to the means available or the taste desired. The fisheries sector records significant losses, i.e. 30% of the fish caught, due to the lack of appropriate conservation infrastructures and inadequate climatic and environmental conditions [19]. In order to prolong its nutritional value, fish is processed using techniques in harmony with cultural values of consumers. There exist many different or closely related methods of preservation, all of which aim to eliminate free water, destroy microbes, or oppose certain chemical reactions that are potential causes of spoilage [20]. Fish processing and preservation techniques are known: smoking, freezing, salting, drying, brining, frying, boiling, microwaving, freeze-drying [21]. Smoking, boiling and freezing are the processing techniques evaluated in this study. These processing methods and preservation techniques, although intended to extend shelf life and improve the organoleptic quality of fish, are likely to affect the nutritional qualities of fish [22]. A number of studies have focused on fish cooking and preservation methods in Cameroon [1,22–25]. However, very few studies have explored the effect of its cooking methods and the health risks associated with heavy metals. This work evaluated the assessment of cooking methods and freezing on nutritional value and health risks of heavy metals in four fish species consumed in Douala.

2. Material and methods

2.1. Material

Twenty-four fishes of each specie (*Cyprinus carpio, Arius parkii, Ethmalosa fimbriata* and *Polydactilus quadrifilis*) were selected and purchased fresh on the boats as soon as they arrived in the Douala fishing seaport in November 2022. Selection was done to eliminate ultered or damaged fishes to avoid biases that could be due to enzymatic browning and rapid putrefaction. Identification of fish was done by the veterinary service of the Ministry of Livestock, Fisheries and Animal Industries of Cameroon, using FAO fish identification sheets. The fish samples (Fig. 1 (a): *Cyprinus carpio*, (b): *Arius parkii*, (c): *Ethmalosa fimbriata* and (d): *Polydactilus quadrifilis*) were put in icebox containing ice with a fish/ice ratio of 1:2, (w/w) and transported to the Laboratory [22]. The average weight and length of the fish used in this study were 513.45 ± 76.02 g and 32.61 ± 5.27 cm; 753 ± 64.29 g and 38.15 ± 4.73 cm; 312.53 ± 21.03 g and 21.10 ± 5.09 cm; 1125 ± 165.44 g and 56.1 ± 7.25 cm for *Cyprinus carpio, Arius parkii, Ethmalosa fimbriata* and *Polydactilus quadrifilis* respectively. After mensuration's, each specie was subdivided equitably in four groups. Fishes were used for raw, boiling, smoking and freezing.

2.2. Cooking and freezing treatments

2.2.1. Raw

Fishes were dissected with a cleaned stainless-steel knife. Heads and viscera were discarded. The edible part representing the parts consumed by the local population, was cut into small pieces and minced.

2.2.2. Smoking

Smoking was carried out using an improved Chorkor-type smoker from GIC LA COMPETENCE. Eviscerated fishes were placed on trays at a height of 1.3 m from the fire source. The smoking temperature generated was measured using an industrial thermometer (BIOBLOCK 76 MM IMM). The fuel was being continuously adjusted to maintain the required temperature in the kiln during smoking. Smoking procedure was conducted as followed:

- Pre-cooking: fishes are placed in a smokehouse for 2 h 30 min at 40 °C;
- Cooking: the fire was accentuated at a temperature varying between 80 °C and 90 °C for 8 h;
- Drying: the temperature was lowered to 50 °C–60 °C for 2 h [22].

2.2.3. Boiling

After rinsing with demineralized water, the fish were eviscerated. The fish fillets were introduced in a stainless-steel pot containing distilled water at a ratio of 1:1.5 (m/v). Boiling was conducted at 98 °C for 15 min [23].

2.2.4. Freezing

Fishes were stored in a −20 °C freezer (HISENSE CONGE 590 HS590) for three weeks. Following slow thawing, the fish were rinsed with distilled water, then eviscerated and filleted [22]. After these treatments, different portions of edible part (raw, smoked, boiled and frozen) were dried in an oven (Binder-78532) at 45 °C for 48 h and were homogenized thoroughly in a mixer (SWISS LINE SWITZERLAND UNIVERSAL MIXER SW-1354-P).

2.3. Proximate analysis

Moisture content was determined by **AOAC** [26] method. 10 g of sample were dried at 105 °C ± 2 °C for 24 h using oven (Binder-78532), until a constant weight was obtained. Difference between the fresh weight and the dry weight was calculated and expressed as a percentage relative to the sample. Crude protein content was determined using Kjeldahl's method by automatic micro Kjeldahl (BÛCHI Distillation Unit K-355) which determines the amount of nitrogen and uses the conversion factor 6.25 to infer the total

amount of protein.

Total lipid was determined by method described by **Bligh and Dyer** [27]. The solvent was then evaporated in an evaporator (BÜCHI ROTAVAPOR R-205). The lipid residue was weighed and the result is expressed as percentage of the sample. Ash content was obtained after combustion of sample for 20 h at 550 °C. The various samples dried were ground in a mixer to obtain fine particles that were easy to handle. Ash content was expressed as percentage of the sample. Total carbohydrate was determined using equation (1) [26].

$$\%\ \text{Carbohydrates} = 100 - \%\ \text{Moisture} - \%\ \text{Proteins} - \%\ \text{Fat} - \%\ \text{Ash} \quad (1)$$

2.4. Mineral analysis

Mineral content was determined according to **AOAC** [28]. The ashes obtained previously were transferred to 100 ml beakers in which 10 ml of concentrated nitric acid were introduced in microwave digestion system (Model Milestone, MLS-1200 mega, Germany) to digest the rest of the organic matter. The different beakers were placed in a boiling water bath for 30 min for optimal digestion. The solution was filtered through WHATMAN paper into a 100 ml volumetric flask and then the volume made up to the gauge mark. Calibration was performed using stock solutions. Calcium, potassium, magnesium, sodium, iron, zinc, copper and manganese were evaluated by atomic absorption spectrophotometry (PerkinElmer Atomic Absorption Spectrometer Pinnacle 900T, Perkin Elma, USA) Phosphorus content was determined colorimetry using spectrophotometry (PerkinElmer, Norwalk CT, USA). Mineral contents of the samples were determined from calibration curves of standards minerals. All samples were analyzed in triplicate. The concentration of a sample was obtained using a linear regression of the concentrations against the absorbance of the standards.

2.5. Heavy metal analysis

The filtrate resulting from digestion of the ashes was used for the evaluation of certain heavy metals. Standards were prepared from stock solutions of cadmium, lead, mercury and arsenic prepared at 1000 mg/l. The levels of cadmium, lead, mercury and arsenic in the digested sample solutions were obtained using Atomic Absorption Spectrophotometer (AAS ZEEnit-700P). All samples were analyzed in triplicate. The concentration of a sample was obtained using a linear regression of the concentrations against the absorbance of the standards. The concentrations were expressed in mg/kg of dry matter were established using the Excel software.

2.6. Health risk assessment

Assessment of potential health risks of heavy metals in fish as reported by **Huang et al.** [29] was evaluated from the mean concentrations of heavy metals for each sample. Estimated daily intake (EDI): health risk was assessed considering the average concentrations of all fish muscles and daily heavy metal intake (EDI) as shown in equation (2).

$$\text{EDI} = \frac{\text{EF} \times \text{ED} \times \text{FIR} \times \text{C}}{\text{BW} \times \text{AT}} \times 10^{-3} \quad (2)$$

EF = frequency of exposure) is 365 (days/year) [30];

ED = exposure duration (59.65 years which is the life expectation in Cameroon in 2022) [31]; FIR = food ingestion rate (53.11 g/person/day) [32];

C: concentration of heavy metals in the selected fish tissues (mg/kg, ww);

BW: average body weight (66.65 kg) [31];

AT: average exposure time 21787 days.

Non-carcinogenic health risk: The target hazard quotient (THQ) and hazard index (HI) are methods proposed by the US Environmental Protection Agency (USEPA) [30] for assessing the risk of heavy metals caused by food intake by the human body. If THQ <1, toxic effects are not expected to occur. If the THQ ≥1, there is a potential health hazard. THQ and HI were estimated using equations (3) and (4) respectively described by **USEPA** [30].

$$\text{THQ} = \frac{\text{EDI}}{\text{RED}} \times 10^{-3} \quad (3)$$

$$\text{HI} = \sum \text{THQ} \quad (4)$$

RFDs = reference dose of As, Hg, Cd, Cu, Zn, Pb are 0.0015, 0.00016, 0.001, 0.04, 0.3, 0.004 respectively [30].

Carcinogenic risk (CR): Not all trace metals have carcinogenic effects. However, As, Pb, Cd among the studied heavy metals are considered as carcinogens. For carcinogens, the individual risk assessment increases the probability of developing cancer due to exposure to potential carcinogens. Acceptable carcinogenic risk levels ranged from 10^{-6} to 10^{-4}. CR were evaluated using **USEPA** [30] equation (5).

$$\text{CR} = \frac{\text{EF} \times \text{ED} \times \text{FIR} \times \text{C} \times \text{CSFo}}{\text{BW} \times \text{AT}} \times 10^{-3} \quad (5)$$

CSFo (oral carcinogenic slope factor) was obtained from the database of the **USEPA** [30]. Available CSFo values (mg/kg/day) are:

1.5, 0.0085 and 6.3 for As, Pb and Cd respectively.

2.7. Quality control and quality assurance

Distilled water and all reagents used were of analytical quality in all preparations. Glassware was soaked and rinsed in 10% HNO3 for 24 h and distilled water respectively. Analyses of blanks and samples were performed in triplicate for quality assurance. The operation of the equipment was established using serial dilution to check its reliability. The precision and accuracy of the procedure were checked using certified reference materials 1570a collected from the National Institute of Standard and Technology (Gaithersburg, USA) approved by National Standard Agency of Cameroon (ANOR). The samples were treated in the same way as certified reference material during the standardisation of techniques. Percentage recovery (% R) of calcium, potassium, magnesium, sodium, iron, zinc, copper, manganese, cadmium, lead, mercury and arsenic was 92.65, 98.10, 97.35, 94.05, 93.05, 99.25, 93.20, 86.40, 96.30, 96.75, 94.10 and 97.90 respectively.

2.8. Chemical indexes analyses of fish oil

2.8.1. Iodine value

The iodine value (IV) was determined according the Wijs method, as described in the **AOAC** [26]**.** In a beaker of 100 mL, 0.3 g of oil, 15 ml of carbon tetrachloride solution, 25 ml of Wijs' reagent were added and were placed in a dark box for 1 h. After, 20 ml of aqueous solution of potassium iodide (10%), 15 ml of distilled water and 5 drops of 1% starch will be added to the beaker. Sodium thiosulfate solution (0.1 N) was used to titrate the solution in the beaker and the volume of sodium thiosulfate used noted against blank. The IV was expressed as g I_2/100 g of sample.

2.8.2. Acid index

Acid index (AI) was determined according to method described by **AFNOR** [33]**.** 1 g of fish oil sample was dissolved in 100 mL of ethanol and some drops of phenolphthalein were added as an indicator and swirled vigorously. Potassium hydroxide (0.1 M) was used to titrate the mixture contain in the beaker against the blank and the volume of KOH used is noted. The Acid index was expressed as mg KOH/g of oil.

2.8.3. Peroxide index

Peroxide index (PI) was evaluated by the method described by **Shantha and Decker** [34]**.** 40 mg of oil sample, 9.8 ml of a chloroform-methanol mixture (7:3 v/v) is added and vortexed for 4 s. Then 50 μl of a 30% aqueous solution of ammonium thiocyanate was added and the mixture vortexed for 4 s, followed by the addition of 50 μl of an aqueous solution of iron chloride II. The mixture is again vortexed for 4 s. After 5 min of incubation at room temperature, the absorbance is read at 500 nm using a spectrophotometer (PerkinElmer, Norwalk CT, USA) against a blank. The peroxide value was expressed as milliequivalents of O_2/kg of oil.

2.8.4. Anisidine value

Anisidine index (AnI) was determined according to the method described by **AOAC** [26]**.** 1 g of fish oil was dissolved to 25 mL of isooctane into a beaker. Absorbance of the solution was measured at 350 nm and using such as blank. After the measurement of absorbance, 5 ml of this solution and 5 ml of isooctane were distributed into two different tests tubes and 1 ml of 0.25% acetic acid solution of *para*-anisidine was added and vortexed. The absorbance of the solution was read after 10 min of incubation at room temperature at 350 nm using a spectrophotometer (PerkinElmer, Norwalk CT, USA) and compared to the blank.

2.8.5. Thiobarbituric acid number

Thiobarbituric acid number (TBA) was evaluated by method described by **Drapper and Hadley** [35]**.** 1 g of oil and an aqueous solution of 0.1% trichloroacetic acid were introduced in the tube and vigorously vortexed. Subsequently, 1 ml of a 0.375% thiobarbituric acid solution, 1 ml of a 15% trichloroacetic acid solution and 1 ml of a 0.25 N hydrochloric acid solution was added to this tube and incubated in a water bath at 95 °C for 30 min. The tubes were cooled to room temperature and the aqueous phase was used to measure the optical density measured at 532 nm using a spectrophotometer (PerkinElmer, Norwalk CT, USA) against a blank. The TBA was expressed as mg of malondialdehyde (MDA) per kg of oil.

2.8.6. The total oxidation number

The total oxidation (TOTOX) values of oil samples were obtained using the following equation (6) according to **Shahidi and Wanasundara** [36].

$$\text{TOTOX} = 2\,\text{PI} + \text{AnI} \tag{6}$$

where TOTOX: total oxidation number, PI: peroxide index and AnI: Anisidine index.

2.9. Statistical analysis

All measurements were done in triplicate. Data were expressed as mean ± standard deviation. One-way ANOVA was used to test

the differences between species and processing methods. Fisher's PLSD (Protected Least Significant Difference) (post hoc comparison test) was used to for comparisons between groups when the ANOVA p-value was significant. SPSS 16.0 for windows (SPSS, Chicago, IL, USA) was the software used for statistical analysis.

3. Results and discussion

3.1. Macronutrient content

Table 1 shows the various macronutrients of the fish species. This water content falls within the range defined by **Ude et al.** [37], who found a water content of 70–84% in fresh fish. Values varied significantly ($P < 0.05$) according to species and method of treatment. Results showed that water is the major constituent and explains the rapid deterioration of fresh fish. Different results were obtained by **Aiyeloja and Akinrotimi** [38] on *Ethmalosa fimbriata* (71.67%). This difference could be explained by environmental factors**.** Smoking and boiling decreased significantly ($P < 0.05$) water content of *C. carpio, Arius parkii* and *E. fimbriata* compared to fresh fish. Similar results were observed by **Tenyang et al.** [39] who showed that the water content of *Chrysichthys nigrodigitatus* decreased during smoking and boiling. According to **Nsoga et al.** [40] fish lose about 20% of their initial weight during heat treatments such as smoking. Water determines the quality of smoked fish. For smoked fish, a water content of 25% or more would not be sufficient to inhibit microbial growth, and a content of 15% would limit this growth [23]**.** During freezing and boiling, water content increased significantly ($P < 0.05$) in frozen *E. fimbriata* and *Polydactilus quadrifilis* compared with fresh respectively. Different results were obtained by **Aberoumand** [41] who showed that during freezing, water content decreased in some Iranian coastal fish species. **Malik et al.** [42] reported that during freezing at −18 °C, moisture content in *Lates niloticus* decreased significantly compared to that of raw fish. The addition of water during defrosting could explain the increase in water content of frozen fish [22]**.** Protein content varied from 13.53 to 18.26% and differed significantly ($P < 0.05$) between species studied in the fresh fish. These results are lower than those obtained by **Pateiro et al.** [43] on *Sparus aurata* (21.05%). The nature of the species, environmental factors and the fishing period could explain these results**.** Smoking and boiling increased significantly ($P < 0.05$) protein content compared to fresh and frozen samples. **Karimian-Khosroshahi et al.** [44] showed that boiling increased protein content of *Oncorhynchus mykiss* compared to raw fish. **Kiczorowska et al.** [45] showed that smoking increased protein content of *Abramis brama* and *Carassius carassius* compared to that of raw fish. Loss of water during smoking or presence of compounds such as benzo (a) pyrene on the surface of smoked could increase protein content [21]. *E. fimbriata* showed a significant decrease ($P < 0.05$) in protein content upon freezing compared to fresh. **Yagoub** [46] showed that protein content in *Oreochromis niloticus* decreased significantly ($P < 0.05$) of 20% after 21 days of freezing at −18 °C compared to that of raw sample. **Dama et al.** [22] showed that the protein content of *Pseudotolithus typus* decreased significantly ($P < 0.05$) after 21 days of freezing at −20 °C. During freezing, the formation of macrocrystals perforates cell membranes, promoting protein exudation and denaturation [47]. In the fresh fish, lipid content is higher significantly ($P < 0.05$) in *E. fimbriata* than in other species. According to the classification of **Omoruyi et al.** [48], *C. carpio* and *P. quadrifilis* are lean species while *A. parkii* and *E. fimbriata* are semi-fat species. Similar results were observed by **Manz et al.** [3] on these species. Smoking increased significantly ($P < 0.05$) lipid content in all three species. **Tenyang et al.** [39] showed that lipid content increases during smoking. Indeed, dehydration due to heat treatments such as smoking would increase lipid content**.** Lipid content decreases significantly ($P < 0.05$) in boiled *E. fimbriata* and *P. quadrifilis* compared to raw sample. **Djopnang et al.** [23] showed that during boiling, the amount of lipids in

Table 1
Proximate composition of the edible parts of different fish (%).

Macronutrients	Samples	*C. carpio*	*A. parkii*	*E. fimbriata*	*P. quadrifilis*
Moisture	Raw	$79.06 \pm 0.85^{c\beta}$	$82.68 \pm 0.39^{c\gamma}$	$75.52 \pm 0.52^{c\alpha}$	$77.52 \pm 1.72^{c\alpha\beta}$
	Smoked	$12.70 \pm 0.60^{a\alpha}$	$17.58 \pm 0.85^{a\beta}$	$16.62 \pm 0.21^{b\beta}$	$16.92 \pm 1.42^{a\beta}$
	Boiled	$75.43 \pm 2.5^{b\alpha\beta}$	$77.50 \pm 2.72^{b\beta}$	$72.66 \pm 0.12^{a\alpha}$	$82.08 \pm 1.14^{d\gamma}$
	Frozen	$79.88 \pm 1.12^{c\beta\gamma}$	$80.95 \pm 0.76^{c\gamma}$	$78.35 \pm 0.29^{d\beta}$	$74.63 \pm 0.22^{b\alpha}$
Protein	Raw	$14.83 \pm 0.22^{a\beta}$	$13.53 \pm 0.44^{a\alpha}$	$18.26 \pm 0.84^{b\delta}$	$16.06 \pm 0.06^{c\gamma}$
	Smoked	$67.18 \pm 0.64^{c\delta}$	$58.95 \pm 1.38^{c\beta}$	$65.22 \pm 0.30^{c\gamma}$	$54.85 \pm 0.24^{d\alpha}$
	Boiled	$17.06 \pm 1.85^{b\beta}$	$17.30 \pm 2.20^{b\beta\gamma}$	$20.19 \pm 1.12^{b\gamma}$	$13.01 \pm 0.17^{a\alpha}$
	Frozen	$15.49 \pm 0.85^{ab\beta}$	$13.65 \pm 0.77^{a\alpha}$	$14.89 \pm 0.65^{a\alpha\beta}$	$15.09 \pm 0.08^{b\beta}$
Lipid	Raw	$1.67 \pm 0.37^{a\beta}$	$2.22 \pm 0.27^{a\gamma}$	$2.79 \pm 0.13^{b\gamma}$	$0.80 \pm 0.03^{b\alpha}$
	Smoked	$9.53 \pm 0.36^{c\delta}$	$8.87 \pm 0.10^{c\gamma}$	$5.61 \pm 0.41^{c\beta}$	$2.03 \pm 0.15^{c\alpha}$
	Boiled	$1.23 \pm 0.10^{a\beta}$	$2.02 \pm 0.23^{a\gamma}$	$1.93 \pm 0.20^{a\gamma}$	$0.58 \pm 0.04^{a\alpha}$
	Frozen	$1.24 \pm 0.06^{a\beta}$	$2.17 \pm 0.17^{a\gamma}$	$2.28 \pm 0.05^{a\gamma}$	$0.62 \pm 0.03^{a\alpha}$
Ash	Raw	$1.12 \pm 0.21^{a\alpha}$	$0.86 \pm 0.12^{a\alpha}$	$1.09 \pm 0.00^{a\alpha}$	$5.47 \pm 0.21^{b\beta}$
	Smoked	$4.19 \pm 1.10^{d\alpha}$	$4.10 \pm 1.18^{c\alpha}$	$5.75 \pm 0.24^{c\beta}$	$25.59 \pm 0.32^{d\gamma}$
	Boiled	$3.91 \pm 0.17^{c\gamma}$	$2.49 \pm 0.64^{b\alpha}$	$4.51 \pm 0.33^{b\delta}$	$3.63 \pm 0.07^{a\beta}$
	Frozen	$2.69 \pm 0.40^{b\alpha}$	$2.18 \pm 0.54^{b\alpha}$	$4.33 \pm 0.27^{b\beta}$	$7.87 \pm 0.81^{c\delta}$
Carbohydrate	Raw	$3.40 \pm 0.59^{c\gamma}$	$0.69 \pm 0.11^{a\beta}$	$2.33 \pm 0.80^{c\gamma}$	$0.13 \pm 0.01^{a\alpha}$
	Smoked	$6.40 \pm 1.32^{d\beta}$	$10.50 \pm 1.74^{b\gamma}$	$6.80 \pm 1.03^{d\beta}$	$0.58 \pm 0.07^{b\alpha}$
	Boiled	$2.15 \pm 0.32^{b\beta}$	$0.66 \pm 0.54^{a\alpha}$	$0.69 \pm 0.03^{b\alpha}$	$0.68 \pm 0.08^{b\alpha}$
	Frozen	$0.68 \pm 0.15^{a\beta}$	$1.02 \pm 0.69^{a\gamma}$	$0.13 \pm 0.04^{a\alpha}$	$1.84 \pm 0.22^{c\gamma}$

The values are the mean ± standard deviation. Values in columns with different letters are significantly ($p < 0.05$) different. Values the same line with different symbols are significantly ($p < 0.05$) different. N = 3.

Chrysichthys nigrodigitatus decreased compared to fresh fish. The decrease in lipids is could be due to oil oxidation leading to the formation of other products such as peroxides, aldehydes, ketones which would result in a decrease in iodine index and an increase in weathering indices [1]. Freezing decreased significantly ($P < 0.05$) lipid content compared to fresh in *E. fimbriata*. Similar results were obtained by **Siddiqui et *al.*** [49] on three species of fish of the genus *Mystus* collected from the Khulna market in Bangladesh. Lipid hydrolysis by lipases and cold chain disruption could explain this decrease [24]. **Akinwumi** [50] reported different results by increase of lipid in *Clarias gariepinus* during smoking and freezing. Ash content varied significantly ($P < 0.05$) in *Polydactilus quadrifilis* compared with the other species studied. The results obtained by **Goswami and Kuntal** [51] on *Tenualosa ilisha* are more accurate to those of this study. The diet of the species studied and environmental factors could explain this result. Ash content provides information on the quantity of minerals present in the food. Heat treatments such as smoking and boiling increased significantly ($P < 0.05$) ash content compared to that of raw fish. Similar results were obtained by **Aiyeloja and Akinrotimi** [38], **Olopade and Dienye** [52] on *E. fimbriata* and *Sardinella eba* respectively fished in coastal Nigeria. However, **Goswami and Kuntal** [51] showed that boiling decreased the ash content of *Tenualosa ilisha*. The volatility of mineral elements during heat treatment could explain this decrease [22]. Freezing increased significantly ($P < 0.05$) ash content compared to fresh fish. Different results were obtained by **Aberoumand** [41] who showed that during freezing, ash content decreased on some fish from Iran. Storage time and water loss during freezing could account for this result [49]. *C. carpio* is the most carbohydrate-rich species in the fresh state. These results are higher than those found by **Manz et al.** [3] (0.98) on *C. carpio.* Environmental factors and the fishing period could influence the nutritional composition of fish. The presence of carbohydrates confers a sweet taste to fish flesh. Smoking increased significantly ($P < 0.05$) carbohydrate content in all species studied. **Djopnang et al.** [23] showed that smoking increased the carbohydrate content of *Chrysichthys nigrodigitatus*. This result could be explained by the concentration of carbohydrates during water loss during heat treatments [39]. Boiling decreased significantly ($P < 0.05$) carbohydrate content in *C. carpio* and *E. fimbriata*. **Dama et al.** [22] showed that cooking with water decreased the carbohydrate content of *Pseudotolithus typus* and *Pseudotolithus senegalensis*. During heat treatment, carbohydrates are combined with amino acids in the Maillard reaction to develop organoleptic qualities [20]. Decrease of carbohydrate content during freezing could be attributed to the activation of glycolytic enzymes that promote carbohydrate oxidation [41].

3.2. Mineral content

3.2.1. Macroelement content

Table 2 shows that *Cyprinus carpio* is the species with the highest fresh calcium content. This calcium content varies significantly ($P < 0.05$) in *C. carpio* compared with other species. Different results were obtained by **Manz et al.** [3] on *C. carpio* (1,232.98 mg/100 g) and *Ethmalosa fimbriata* (468.05 mg/100 g). This difference could be due to the fishing period and the environment. Smoking increased significantly ($P < 0.05$) calcium content compared to boiling, freezing and fresh in all species. **Djopnang et al.** [23] observed a decrease of calcium content in *Chrysichthys nigrodigitatus* during boiling. **Olopade and Dienye** [52] showed that calcium content in *Sardinella eba* increased during smoking. Different results were obtained by **Tenyang et al.** [1] who showed that smoking decreased significantly ($P < 0.05$) the calcium content of *Polypterus bichir bichir* compared to raw fish. **Yagoub** [46] reported a decrease of calcium content in *Oreochromis niloticus* during frozen storage at −18 °C. The nature of the species, the smoking technique and the loss water may explain this result [53]. The recommended daily allowance (RDA) for calcium is 800 mg. Consumption of 100 g of smoked *E. fimbriata* would cover 88.75% of the RDA in calcium. Calcium is involved in mineralization, skeletal structure, blood coagulation,

Table 2
Macroelement contents in the edible parts of different fish (%).

Macroelements	Samples	*C. carpio*	*A. parkii*	*E. fimbriata*	*P. quadrifilis*
Ca	Raw	$0.15 \pm 0.05^{a\beta}$	$0.04 \pm 0.01^{a\alpha}$	$0.04 \pm 0.02^{a\alpha}$	$0.04 \pm 0.01^{a\alpha}$
	Smoked	$0.21 \pm 0.03^{b\beta}$	$0.10 \pm 0.02^{b\alpha}$	$0.71 \pm 0.14^{b\delta}$	$0.31 \pm 0.03^{b\gamma}$
	Boiled	$0.07 \pm 0.03^{a\alpha}$	$0.04 \pm 0.01^{a\alpha}$	$0.04 \pm 0.03^{a\alpha}$	$0.03 \pm 0.00^{a\alpha}$
	Frozen	$0.09 \pm 0.03^{a\alpha}$	$0.04 \pm 0.02^{a\alpha}$	$0.04 \pm 0.01^{a\alpha}$	$0.05 \pm 0.02^{a\alpha}$
P	Raw	$0.79 \pm 0.17^{a\beta}$	$0.76 \pm 0.15^{a\beta}$	$1.12 \pm 0.27^{a\beta}$	$0.27 \pm 0.05^{b\alpha}$
	Smoked	$0.78 \pm 0.14^{a\alpha}$	$0.71 \pm 0.15^{a\alpha}$	$1.3 \pm 0.24^{a\beta}$	$1.08 \pm 0.09^{c\beta}$
	Boiled	$0.63 \pm 0.04^{a\beta}$	$0.61 \pm 0.13^{a\beta}$	$0.91 \pm 0.35^{a\beta}$	$0.12 \pm 0.03^{a\alpha}$
	Frozen	$0.73 \pm 0.21^{a\beta}$	$0.79 \pm 0.17^{a\beta}$	$1.11 \pm 0.22^{a\beta}$	$0.31 \pm 0.04^{b\alpha}$
K	Raw	$1.35 \pm 0.13^{b\beta}$	$1.52 \pm 0.09^{b\beta}$	$1.48 \pm 0.18^{a\beta}$	$0.23 \pm 0.01^{c\alpha}$
	Smoked	$1.27 \pm 0.24^{b\alpha}$	$1.09 \pm 0.1^{a\alpha}$	$1.49 \pm 0.4^{a\alpha}$	$0.94 \pm 0.11^{d\alpha}$
	Boiled	$0.94 \pm 0.08^{a\beta}$	$0.87 \pm 0.18^{a\beta}$	$1.1 \pm 0.15^{a\beta}$	$0.07 \pm 0.02^{a\alpha}$
	Frozen	$1.09 \pm 0.01^{a\beta}$	$1.37 \pm 0.38^{a\beta}$	$1.2 \pm 0.39^{a\beta}$	$0.19 \pm 0.01^{b\alpha}$
Mg	Raw	$0.11 \pm 0.01^{b\beta}$	$0.1 \pm 0.01^{a\beta}$	$0.11 \pm 0.01^{a\beta}$	$0.03 \pm 0.01^{b\alpha}$
	Smoked	$0.10 \pm 0.01^{ab\alpha\beta}$	$0.08 \pm 0.01^{a\alpha}$	$0.12 \pm 0.01^{a\beta}$	$0.12 \pm 0.02^{c\beta}$
	Boiled	$0.08 \pm 0.01^{a\beta}$	$0.61 \pm 0.47^{b\gamma}$	$0.10 \pm 0.01^{a\beta}$	$0.01 \pm 0.00^{a\alpha}$
	Frozen	$0.11 \pm 0.00^{b\beta}$	$0.1 \pm 0.02^{a\beta}$	$0.11 \pm 0.01^{a\beta}$	$0.04 \pm 0.01^{b\alpha}$
Na	Raw	$0.09 \pm 0.01^{b\alpha}$	$0.08 \pm 0.01^{ab\alpha}$	$0.08 \pm 0.00^{b\alpha}$	$0.45 \pm 0.04^{b\beta}$
	Smoked	$0.09 \pm 0.01^{b\alpha}$	$0.09 \pm 0.01^{b\alpha}$	$0.1 \pm 0.00^{c\alpha}$	$2.24 \pm 0.07^{c\beta}$
	Boiled	$0.06 \pm 0.01^{a\alpha}$	$0.06 \pm 0.01^{a\alpha}$	$0.06 \pm 0.01^{a\alpha}$	$0.17 \pm 0.01^{a\beta}$
	Frozen	$0.08 \pm 0.03^{ab\alpha}$	$0.09 \pm 0.00^{b\alpha}$	$0.08 \pm 0.00^{b\alpha}$	$0.42 \pm 0.02^{b\beta}$

Values are expressed as mean values ± standard deviation. Values in the same column with different letters are significantly ($p < 0.05$) different. Values in the same line with different symbols are significantly ($p < 0.05$) different. N = 3.

hormone release and muscle contraction. It is also used as a cofactor in metabolic pathways [54]. Ingestion of high doses of calcium can cause abnormal drowsiness, lack of energy, kidney stones, bone pain or fractures, digestive problems, polydypsia and diuresis. If calcium intake is inadequate, bones can become brittle and develop osteoporosis. In children, calcium deficiency can lead to bone development problems and skeletal deformation [55]. Phosphorus content ranged from 0.76 to 1.12% and varies significantly ($P < 0.05$) in *Polydactilus quadrifilis* compared to other species studied. These results are higher than those obtained by **Pateiro et al.** [43] on *Sparus aurata* (256.38 mg/100 g) and lower than those obtained by **Manz et al.** [3] on *C. carpio* (4,767.49 mg/100 g) and *E. fimbriata* (1,569.43 mg/100 g) and *A. parkii* (2,324.27 mg/100 g). Smoking increased significantly ($P < 0.05$) phosphorus content in *P. quadrifilis* compared to raw, boiled and frozen. Boiling and freezing reduced phosphorus content non-significantly ($P > 0.05$) compared to fresh. Different results were obtained by **Dama et al.** [22], who showed that boiling and freezing decreased and increased phosphorus content respectively in *Pseudotolithus senegalensis*. **Kiczorowska et al.** [45] showed that smoking decreased phosphorus content of *Abramis brama* and *Carassius carassius* compared to raw samples. During smoking, **Tenyang et al.** [39] reported a decrease of phosphorus content in *Chrysichthys nigrodigitatus* from Lake Maga in Cameroon. Water losses during freezing and smoking allow nutrient concentration [41]. The daily phosphorus intake is 800 mg. Consumption of 71.42 g of smoked *E. fimbriata* would cover 100% of the RDA. Phosphorus is involved in membrane transport, energy storage and release, maintenance of osmotic pressure and acid-base balance. Phosphorus also facilitates calcium binding in bones and teeth [23]. Disturbances in the absorption of certain minerals, risks of kidney calcification and heart rhythm disorders could occur following ingestion of high doses of phosphorus. On the other hand, inadequate intake could be the cause of fatigue and muscular weakness, which can progress to serious forms such as epilepsy, coma or death [56]. Phosphorus levels being higher than those of calcium in the different species studied and depending on the treatment mode implies a Ca/P ratio of less than 1. This ratio shows that despite the small amount of calcium compared with phosphorus, the latter would enable better calcium fixation in bones and teeth. Magnesium content varies significantly ($P < 0.05$) in *C. carpio, A. parkii* and *E. fimbriata* compared to fresh *P. quadrifilis*. Results found by **Ozturan et al.** [54] on *Astacus leptodactylus* (178.60 mg/kg) are lower than in this study. Boiling reduced significantly ($P < 0.05$) magnesium content in *C. carpio* and *A. parkii*. Similar results were reported by **Ozturan et al.** [54] on *Astacus leptodactylus*. Smoking increased significantly ($P < 0.05$) magnesium content compared to fresh, frozen and boiled in *P. quadrifilis*. **Tenyang et al.** [39] showed that magnesium content increased significantly ($P < 0.05$) during smoking of *Chrysichthys nigrodigitatus*. **Djopnang et al.** [23] showed that magnesium content increased significantly ($P < 0.05$) during smoking and boiling of *Chrysichthys nigrodigitatus*. Different results were obtained by **Kiczorowska et al.** [45] who showed that smoking decreased magnesium content in *Abramis brama* compared to fresh fish. **Yagoub** [46] showed that magnesium content in *Oreochromis niloticus* increased significantly ($P < 0.05$) after 21 days of freezing at −18 °C compared to raw sample. The RDA for magnesium is 350 mg. Consumption of 57.37 g of boiled *A. parkii* would cover this RDA. Magnesium is involved in energy metabolism, fatigue reduction, proper functioning of the nervous system and psychological functions such as concentration, reasoning and memory [51]. In high quantities, magnesium can increase the risk of heart disease and stroke. An inadequate intake of magnesium can disrupt a number of important physiological functions, triggering fatigue, stress and muscular disorders, as well as dizziness, nausea or light-headedness, giving the sensation of feeling faint [57]. *P. quadrifilis* stands out as the species with the highest sodium content at 0.45%. This sodium content varies significantly ($P < 0.05$) in *P. quadrifilis* compared with other species. These results are higher than those found by **Njinkoue et al.** [58] on *Pseudotolithus typus* (0.27%) and *Pseudotolithus elongatus* (0.31%). This result could be explained by the nature of the species, environmental factors and the fishing period [59]. Smoking increased significantly ($P < 0.05$) sodium content compared to fresh, boiled and frozen in *E. fimbriata* and *P. quadrifilis* compared to A. *parkii* and *C. carpio*. **Kiczorowska et al.** [45], **Olopade and Dienye** [52] reported that sodium content increased during smoking in *Abramis brama* and *Sardinella eba* respectively compared to raw fish. Boiling decreased significantly ($P < 0.05$) sodium content in boiled *C. carpio, E. fimbriata* and *P. quadrifilis* compared to fresh. The decrease in sodium content was observed by **Tenyang et al.** [1] on boiled *P. bichir bichir* compared to fresh sample. Freezing reduced non significantly ($P > 0.05$) sodium content in all fish species studied compared to raw sample. Sodium content in *Oreochromis niloticus* increased significantly ($P < 0.05$) after 21 days of freezing at −18 °C compared to raw sample [46]. **Dama et al.** [22] showed that freezing at −20 °C for 3 weeks increased sodium content in *P. typus* and *P. elongatus*. The daily allowance of sodium is 500 mg, so consuming 22.32 g of smoked *P. quadrifilis* would cover 100% of the RDA in sodium. Sodium helps regulate blood pressure, maintains acid-base balance, and contributes to muscles and nerves functioning. It is essential for the transport of certain substances and water retention [3]. Sodium deficiency can lead to serious neurological problems, even coma, as well as heart, kidney, liver and hormonal disorders and water retention problems. The main health effect of a diet high in sodium is high blood pressure, which increases the risk of cardiovascular disease, gastric cancer, obesity, osteoporosis and kidney disease [60]. The results in Table 2 show that *A. parkii* (1.52%) and *P. quadrifilis* (0.23%) had the higher and lower content in potassium respectively. This content varies significantly ($p < 0.05$) between *P. quadrifilis* compared to other fish species studied. These results are lower than the results of **Tsegay et al.** [61] on *C. carpio* (17276.21) and higher than the data reported by **Goswami and Kuntal** [51] on *Tenualosa ilisha* (493.7). The minerals contained in fish flesh depend on the environment, physiological state and fishing period. Potassium is involved in electrolyte balance, nerve transmission and muscle contraction [58]. Boiling reduced significantly ($P < 0.05$) potassium content in *C. carpio, A. parkii* and *P. quadrifilis*. **Dama et al.** [22] obtained a decrease in potassium content during boiling in three fish species of the genus *Pseudotolithus*. The volatilization and solubilization of certain minerals during boiling could explain this result [51]. Smoking increased and decreased significantly ($P < 0.05$) potassium content in *P. quadrifilis* and *A. parkii* respectively. Similar results were obtained by **Tenyang et al.** [1] who showed that smoking increased potassium content in *P. bichir bichir*. Water losses during smoking allow the concentration of certain minerals [6]. Freezing reduced significantly ($P < 0.05$) potassium content in *C. carpio* and *A. parkii*. During defrosting, ice crystals perforate food membranes, leading to exudation of cellular contents [41]. Different results were obtained by **Malik et al.** [42], who showed that freezing (−18 °C) increased significantly ($P < 0.05$) potassium content in *Bagrus bayad*. **Akinwumi** [50] reported that freezing and smoking increased significantly ($P < 0.05$) potassium content in *Clarias gariepinus*. The RDA

for potassium is 800 mg, consumption of 53.69 g of smoked *E. fimbriata* would cover 100% of the RDA. Excess potassium in the blood can cause life-threatening changes in heart rhythm. Insufficient potassium intake can lead to muscle weakness, paralysis and respiratory failure [60]. The Na/K ratio is a good indicator for the prevention or treatment of hypertension and cardiovascular disease [59]. These results suggest that the Na/K ratio is less than 1 in *C. carpio, A. parkii* and *E. fimbriata.* These species could therefore be used for the management of hypertension and cardiovascular disease in humans. In *P. quadrifilis*, on the other hand, the ratio is greater than 1, making it suitable for people suffering from dehydration and burns.

3.2.2. Microelement content

Iron contents of fresh fishes varied significantly ($P < 0.05$) in *Polydactilus quadrifilis* and *Ethmalosa fimbriata* compared to *Cyprinus carpio* and *Arius parkii* with value ranging from 22.94 to 76.05 μg/g. These results are higher than those found by **Łuczyńska et al.** [62] on *Carassius carassius* (6.95 mg/kg). Environmental factors, species and the fishing period could account for these results. Iron is involved in haemoglobin structure and cell division. It is essential for several functions such as DNA repair, enzyme activity, mitochondrial function and neurotransmission [23]. Heat treatments (smoking and boiling) reduced significantly ($P < 0.05$) iron content in all species studied when compared to the fresh samples. **Tenyang et al.** [1] showed that smoking and cooking decreased the iron content of *Polypterus bichir bichir.* Frozen *A. parkii and P. quadrifilis* showed a significant ($P < 0.05$) decrease in iron content compared fresh fish. **Dama et al.** [22] observed a loss of iron in three fish species of the genus *Pseudotolithus* during freezing. Different results were obtained by **Akinwumi** [50] who reported that iron content in *Clarias gariepinus* increased significantly compared to raw sample during smoking and freezing. RDA for iron in pregnant women is 30 mg, consumption of 100 g of *P. quadrifilis* could cover 21.66% of the RDA. The quantities of iron contained in the various species studied (raw, smoked, boiled and frozen) are below the standard set by **FAO/WHO** [63] which is 186 mg/kg. Iron deficiency is the cause of cognitive problems, anaemia, muscle fatigue, paleness and shortness of breath. Excessive iron intake leads to liver dysfunction, hypogonadism, hypothyroidism, fatigue, heart failure, diarrhoea, hair loss and grey pigmentation of the skin [64]. *P. quadrifilis* is the species with the highest zinc content. Zinc content obtained by **Ayanda et al.** [65] on *Parachana obscura* muscle (6.78 mg/kg) is lower than in our study. These results are similar to those obtained by **Huang et al.** [29] on *Sebastiscus marmoratus* (27.15 mg/kg) and lower than those obtained by **Ozturan et al.** [54] on *Astacus leptodactylus* (426.40 mg/kg). According to **Singhato et al** [66], the chemical composition of fish varies with the environment, the fishing period and age. This mineral is involved in immune defense stimulation, protection against cellular aging, and its deficiency may induce thalassemia and erythrocyte fragility [3]. It is also involved in protein and fat metabolism, and in the production of prostaglandins. During boiling, we noted a significant decrease ($P < 0.05$) in zinc content in *C. carpio* and *P. quadrifilis*. In smoked *C. carpio* and *P. quadrifilis*, there was a decrease or increase significant ($P < 0.05$) in zinc content compared to fresh fish, respectively. **Tenyang et al.** [1] showed that zinc content in *P. bichir bichir* increased or decreased during smoking and boiling respectively. Freezing increased significantly ($P < 0.05$) zinc content in *E. fimbriata*. Similar results were obtained by **Dama et al.** [22] on three fish species of the genus *Pseudotolithus*. Indeed, the solubilization of certain minerals or loss water during boiling, smoking and freezing could explain this result. The levels obtained in *C. carpio* (raw), *A. parkii* (raw, boiled and frozen), *E. fimbriata* (raw, smoked, boiled and frozen) and *P. quadrifilis* (raw, smoked, boiled and frozen) are higher than the standard set by the **FAO/WHO** [63], which is 30 mg/kg of fish. Ingestion of high doses of zinc leads to copper deficiency, neurological and skin problems, immune deficiency and developmental disorders [67]. Consumption of 100 g of smoked *P. quadrifilis* can cover 69.09% of the RDA for zinc, which in humans is 11 mg. Table 3 shows that *E. fimbriata* is the species with the highest manganese concentration. Manganese content ranges from 3.69 to 6.31 μg/g. These results are lower than those found by **Ayanda et al.** [65] on the muscles of *Oreochromis niloticus* (579.56 mg/kg) and *Chrysichthys nigrodigitatus* (727.36 mg/kg). These results are higher to those obtained by **Łuczyńska et al.** [62] on *Carassius carassius* (0.12 mg/kg), **Adegbola et al.** [15] on *Sarotherodon melanotheron* (1.48 mg/kg). Manganese is an essential mineral involved in the

Table 3
Microelement contents in the edible parts of differents fish (μg/g).

Microelements	Samples	*C. carpio*	*A. parkii*	*E. fimbriata*	*P. quadrifilis*
Fe	Raw	$22.94 \pm 5.1^{b\alpha}$	$28.98 \pm 0.92^{c\beta}$	$73.18 \pm 7.58^{b\gamma}$	$76.05 \pm 4.91^{b\gamma}$
	Smoked	$16.81 \pm 0.05^{a\alpha}$	$19.42 \pm 0.79^{b\beta}$	$59.51 \pm 14.93^{a\gamma}$	$65.10 \pm 7.53^{a\gamma}$
	Boiled	$22.31 \pm 1.9^{b\beta}$	$20.52 \pm 0.02^{b\alpha}$	$54.34 \pm 14.3^{a\gamma}$	$62.91 \pm 9.67^{a\gamma}$
	Frozen	$21.82 \pm 2.34^{b\beta}$	$17.60 \pm 0.85^{a\alpha}$	$71.79 \pm 2.32^{b\gamma}$	$69.41 \pm 2.04^{a\gamma}$
Zn	Raw	$31.77 \pm 5.9^{b\alpha}$	$39.43 \pm 13.4^{a\alpha}$	$33.18 \pm 4.56^{a\alpha}$	$64.34 \pm 4.90^{b\beta}$
	Smoked	$20.86 \pm 4.5^{a\alpha}$	$29.28 \pm 10.47^{a\alpha\beta}$	$35.6 \pm 4.3^{a\beta}$	$76.28 \pm 3.51^{c\gamma}$
	Boiled	$18.98 \pm 1.0^{a\alpha}$	$30.23 \pm 4.03^{a\beta}$	$31.35 \pm 3.39^{a\beta}$	$36.20 \pm 1.75^{a\beta}$
	Frozen	$28.81 \pm 9.57^{ab\alpha}$	$45.97 \pm 12.95^{a\alpha}$	$63.23 \pm 9.55^{b\beta}$	$71.08 \pm 5.74^{bc\beta}$
Cu	Raw	$1.66 \pm 0.57^{a\alpha}$	$1.83 \pm 0.29^{a\alpha}$	$3.97 \pm 0.02^{b\beta}$	$3,36 \pm 0,35^{a\beta}$
	Smoked	$8.07 \pm 3.14^{b\gamma}$	$1.81 \pm 0.01^{a\alpha}$	$2.65 \pm 0.57^{a\beta}$	$4,05 \pm 1,12^{a\beta}$
	Boiled	$1.33 \pm 0.58^{a\alpha}$	$2.00 \pm 0.01^{a\beta}$	$2.16 \pm 0.76^{a\alpha\beta}$	$10,15 \pm 3,57^{b\gamma}$
	Frozen	$1.66 \pm 0.57^{a\alpha}$	$2.15 \pm 0.76^{a\alpha}$	$2.65 \pm 0.58^{a\alpha}$	$4.47 \pm 0.76^{a\beta}$
Mn	Raw	$3.63 \pm 0.81^{ab\alpha}$	$3.96 \pm 0.74^{a\alpha}$	$6.31 \pm 1.2^{a\beta}$	$5.38 \pm 0.2^{a\beta}$
	Smoked	$2.65 \pm 0.57^{a\alpha}$	$11.81 \pm 3.4^{b\gamma}$	$13.72 \pm 3.31^{b\gamma}$	$6.3 \pm 2.1^{b\beta}$
	Boiled	$5.15 \pm 0.4^{c\alpha}$	$4.6 \pm 1.17^{a\alpha}$	$6.36 \pm 1.47^{a\alpha}$	$9.2 \pm 0.3^{c\beta}$
	Frozen	$4.12 \pm 0.73^{b\alpha}$	$5.15 \pm 0.71^{a\alpha}$	$9.27 \pm 2.3^{a\beta}$	$7.62 \pm 0.24^{b\beta}$

Values are expressed as mean values ± standard deviation. Values in the same column with different letters are significantly ($p < 0.05$) different. Values in the same line with different symbols are significantly ($p < 0.05$) different. $N = 3$.

prevention of protein-energy malnutrition, the fixation of minerals and the activation of pyruvate kinase [68]. From this table, we note an increase significant ($P < 0.05$) in manganese content in smoked *A. parkii, E. fimbriata* and *P. quadrifilis* compared to raw sample. Boiling increased significantly ($P < 0.05$) manganese content compared with fresh in *C. carpio* and *P. quadrifilis*. Compared to fresh, frozen *P. quadrifilis* has a higher significant ($P < 0.05$) manganese content. Some authors showed that heat treatment influences manganese content. Indeed, **Tenyang et al.** [39] showed that smoking increased significantly ($P < 0.05$) the manganese content of *Chrysichthys nigrodigitatus* caught in Lake Maga in the Far North of Cameroon. **Dama et al.** [22] showed that boiling and freezing reduced the manganese content of *P. typus* and *P. elongatus* purchased at the Youpwe market in Douala. Loss of water and exudation of certain minerals during heat treatment and rapid defrosting could account for this result [24]**.** The recommended daily allowance (RDA) for manganese is 2–5 mg. The values obtained for all the fish species studied (raw, smoked, boiled and frozen) are below the tolerable limit for manganese in fish (12.97 mg/kg) set by the **FAO/WHO** [63]. Manganese deficiency impairs insulin production, causes transient dermatitis, hypocholesterolemia, increased alkaline phosphatases and harmful effects on bone tissue formation. Excessive intake of manganese could lead to neurotoxicity, memory and muscular disorders [69]**.** Consumption of 100 g of boiled *E. fimriata* would cover 27.44% of this RDA. Copper is necessary for the proper functioning of the nervous and immune systems. It also acts as an antioxidant and anti-inflammatory agent [70]. Its content varied significantly ($P < 0.05$) in *E. fimriata* and *P. quadrifilis* compared to *C. carpio* and *A. parkii*. These values are lower than those obtained by **Ozturan et al.** [54] on *Astacus leptodactylus* (36.12 mg/kg), **Ahmed et al.** [71] on *Liza parsia* (9.50 mg/kg) and **Younis et al.** [72] on *Parastromateus niger* (18.90 μg/g) collected at the Red Sea fishing port in Jeddah, Saudi Arabia. In smoked *C. carpio* and *E. fimriata*, there was an increase and decrease significant ($P < 0.05$) in copper content compared with fresh respectively. **Tenyang et al.** [1] showed that smoking increased significantly ($P < 0.05$) copper content in *Polypterus bichir bichir* compared to fresh. During boiling, copper content decreased and increased significantly ($P < 0.05$) in *E. fimriata and P. quadrifilis* respectively compared to fresh fish. **Karimian-Khosroshahi et al.** [44] reported that in boiled *Oncorhynchus mykiss* and *T. ilisha* respectively, copper content decreased significantly ($P < 0.05$). Freezing decreased significantly ($P < 0.05$) copper content in *E. fimbriata*. Different results were obtained by **Dama et al.** [22] who showed that freezing increased non-significantly ($P > 0.05$) copper content in three *Scianidae* species. Consumption of 100 g of boiled *P. quadrifilis* can cover 35% of the copper RDA of 1.5–3 mg. Copper levels in all the species studied except *P. quadrifilis* boiled are below the tolerated limit in fish established by the **USEPA** [30] which is 10 mg/kg of fish. Excessive copper intake leads to liver and kidney damage, anaemia due to reduced production of red blood cells, overproduction of reactive oxygen species and, consequently, diabetes. On the other hand, a deficiency leads to vomiting, diarrhoea, enteropathy, mental deficiency, hypothermia, osteoporosis and retarded growth [73].

3.3. Heavy metal content

The heavy metal assessment of the studied fish species (Table 4) shows that cadmium content varied significantly ($P < 0.05$) in *Polydactilis quadrifilis* when compared to the other raw species. This difference could be attributed to diet, age and the environment in which these species were found**.** Indeed, *Polynemidae* are demersal species who feeds on sea urchins, mollusks, crustaceans, crabs, amphipods and barnacles. These halieutic products accumulate heavy metals via their diet and, as a result, *Polydactilis quadrifilis* bioaccumulates more heavy metals than the other species studied [11]. **Huang et al.** [29] showed that demersal species accumulate most heavy metal compared to Middle-upper species. These values were lower than those obtained by **Mendoza et al.** [18] on *Tilapia* spp (1.45 mg/kg) and higher than those obtained by **Huang et al.** [29] on nine commercial fish species from Dachen Fishing Ground, East China Sea. Our results are below the standards set by the **European Union (EU)** [74] which are 0.5 mg/kg. Ingestion of high doses of cadmium can cause high blood pressure, kidney, erythrocyte and testicular damages. Excess cadmium can also cause bone demineralization and lung cancers [17]**.** Smoking increased significantly ($P < 0.05$) cadmium content in all species. Boiling increased

Table 4
Heavy metal contents in the edible parts of different fish (mg/kg DW).

Heavy metal	Samples	*C. carpio*	*A. parkii*	*E. fimbriata*	*P. quadrifilis*
Cd	Raw	$0.14 \pm 0.03^{a\alpha}$	$0.22 \pm 0.03^{a\beta}$	$0.19 \pm 0.03^{a\alpha\beta}$	$0.38 \pm 0.03^{b\gamma}$
	Smoked	$0.53 \pm 0.02^{c\beta}$	$0.36 \pm 0.02^{b\alpha}$	$0.41 \pm 0.03^{c\alpha}$	$0.54 \pm 0.02^{c\beta}$
	Boiled	$0.33 \pm 0.05^{b\alpha}$	$0.52 \pm 0.01^{c\beta}$	$0.26 \pm 0.04^{a\alpha}$	$0.45 \pm 0.05^{b\beta}$
	Frozen	ND	$0.35 \pm 0.03^{b\beta}$	$0.21 \pm 0.02^{a\alpha}$	$0.18 \pm 0.02^{a\alpha}$
Pb	Raw	$0.13 \pm 0.02^{a\alpha}$	$0.24 \pm 0.03^{ab\beta\gamma}$	$0.18 \pm 0.02^{a\beta}$	$0.28 \pm 0.04^{a\gamma}$
	Smoked	$0.33 \pm 0.02^{c\alpha}$	$0.46 \pm 0.02^{c\beta}$	$0.41 \pm 0.03^{c\alpha\beta}$	$0.58 \pm 0.02^{b\gamma}$
	Boiled	$0.25 \pm 0.04^{b\alpha\beta}$	$0.31 \pm 0.05^{b\beta\gamma}$	$0.35 \pm 0.01^{b\gamma}$	$0.23 \pm 0.01^{a\alpha}$
	Frozen	$0.18 \pm 0.03^{a\alpha}$	$0.17 \pm 0.04^{a\alpha}$	$0.20 \pm 0.05^{a\alpha\beta}$	$0.27 \pm 0.03^{a\beta}$
Hg	Raw	$0.36 \pm 0.04^{a\alpha}$	$0.48 \pm 0.05^{b\beta}$	$0.54 \pm 0.05^{c\beta}$	$0.38 \pm 0.03^{b\alpha}$
	Smoked	$0.63 \pm 0.05^{c\beta}$	$0.58 \pm 0.03^{c\beta}$	$0.28 \pm 0.02^{a\alpha}$	$0.86 \pm 0.02^{d\gamma\gamma}$
	Boiled	$0.45 \pm 0.07^{ab\beta\gamma}$	$0.31 \pm 0.02^{a\alpha}$	$0.43 \pm 0.03^{b\beta}$	$0.54 \pm 0.05^{c\gamma}$
	Frozen	$0.53 \pm 0.04^{b\gamma}$	$0.35 \pm 0.06^{a\beta}$	$0.56 \pm 0.07^{c\gamma}$	$0.23 \pm 0.04^{a\alpha}$
As	Raw	ND	0.03 ± 0.01^{a}	ND	ND
	Smoked	$0.1 \pm 0.02^{b\beta}$	$0.13 \pm 0.01^{c\beta}$	$0.05 \pm 0.00^{a\alpha}$	ND
	Boiled	$0.04 \pm 0.00^{a\alpha}$	$0.09 \pm 0.01^{b\beta}$	$0.08 \pm 0.00^{b\beta}$	ND
	Frozen	ND	ND	ND	ND

Values are expressed as mean values ± standard deviation. Values in the same column with different letters are significantly ($p < 0.05$) different. Values in the same line with different symbols are significantly ($p < 0.05$) different. DW: dry weight; ND: non determined. N = 3.

significantly ($P < 0.05$) cadmium content in *Cyprinus carpio* and *Arius parkii* compared to raw. **Cieślik et al.** [75] showed that smoking increased and decreased cadmium content in *Oncorhynchus mykkis* and *Cyprinus carpio* respectively. **Gheisari et al.** [76] showed that cadmium content increased during boiling in shrimp. The increase in cadmium during boiling and smoking could be due to water loss, smoking time and the materials (wood and grill) used for smoking which could themselves be contaminated with heavy metals [77]. Smoking wood is a source of heavy metals that have accumulated during the tree's growth as a result of atropic pollution and natural phenomena. The grill used for smoking is usually oxidisable and covered with a layer of paint (a source of heavy metals) which could react with heat and increase heavy metal levels [53]. It would therefore be advisable to control the wood used for smoking and to design stainless steel grills to reduce this contamination. Freezing increased and decreased significantly ($P < 0.05$) cadmium content in *Arius parkii* and *Polydactilis quadrifilis* respectively. **Nora et al.** [78] showed that cadmium content decreased in *Oreochromis niloticus* during freezing. The exudation of heavy metals via their low affinity with the macrocrystals formed during freezing could explain this decrease [79]. The large specific surface area and great capacity to establish bonds and adsorb heavy metals by microcrystals via colloidal substances would justify the increase in cadmium [80]. The results in Table 5 show that *P. quadrifilis* accumulates more lead than the other species studied. This result shows the degree of pollution of the *P. quadrifilis* habitat linked to human activities. Similar results were obtained by **Nakweti et al.** [81] on *Clarias gariapinus* (0.219 mg/kg) and *Oreochromis niloticus* (0.249 mg/kg) caught at the Kingabwa fishing station in the Democratic Republic of Congo. Our results are lower than those obtained by **Ayanda et al.** [65] on *O. niloticus* (9.73 mg/kg) and the standard set by **EU** [74], which is 0.3 mg/kg of fresh fish. These results are lower than those obtained by **Sabouang et al.** [82] on *E. fimbriata* (0.9 μg/g of wet weight) caught in Limbe, Cameroon and similar to those obtained by **Ngo-Massou et al.** [83] on *Cardisoma armatum* (0.19 mg/kg) collected in the mangrove of Kribi, Cameroon. Lead affects the central and peripheral nervous systems, causes brain damage, cardiovascular disease, adverse effects on the developmental stages of the fetus [17]. Lead content increased significantly ($P < 0.05$) during smoking compared to fresh, boiled and frozen in all species studied. **Jolaoso et al.** [84] showed that smoking respectively increases or decreases lead content compared to fresh in *Monoductylus sebae* and *Pamadasys jubelini*. Boiling increased significantly ($P < 0.05$) lead content in *C. carpio and E. fimbriata* compared with fresh and frozen. According to **Gheisari et al.** [76], boiling decreases lead content in shrimp and lobster. Water loss, evaporation or conversion of heavy metals to other compounds during heat treatments could account for these results [85]. Freezing did not affect significantly ($P > 0.05$) lead content in all species compared to fresh. Different results were obtained by **Nora et al.** [78] who showed that lead content decreased in *Oreochromis niloticus* during freezing. Table 4 shows that mercury content ranged from 0.36 to 0.54 mg/kg with *E. fimbriata* being the species containing the most mercury content. These results are higher than those obtained by **Rajeshkumar and Xiaoyu** [86] on *C. carpio* (0.066 μg/g). High-dose mercury ingestion is thought to cause fertility problems, brain and kidney damage [18]. Smoking increased significantly ($P < 0.05$) mercury content in all species studied, except for *E. fimbriata*, where a significant decrease was observed. During boiling, there was an increase significant ($P < 0.05$) in mercury content in *P. quadrifilis* and a decrease significant ($P < 0.05$) in *A. parkii* and *E. fimbriata*. **Igwegbe et al.** [87] showed that smoking increased mercury content in *Tilapia nilotica, Synodontis guntheri, Heterotis niloticus* and *Clarias anguillaris* from three locations in Borno State of Nigeria. According to **Monney** [85], mercury content decreased during smoking in *Euthynnus alletteratus, Sardinella maderensis* and *Scomber scombrus* collected in the Abobo station and Siporex markets in Abidjan (Ivory Coast). Mercury is the only metal that remains in liquid form at room temperature, can easily change into a gaseous or vaporous state. Thus, during the smoking or boiling process, water and volatile matter evaporate along with the mercury. *Arius parkii* stands out as the only specie to contain arsenic in its fresh state. The results obtained by **Adegbola et al.** [15] on *Clarias gariepinus* (0.130 mg/kg) were higher than those found in this study. This difference shows the degree of pollution in these collection areas, characterized by high levels of industrial activity and intense land use. Arsenic was not determined in the species *E. fimbriata, P. quadrifilis* and *C. carpio* because the levels are below the detection limit. In fish, arsenic causes neoplastic alterations and bizarre morphological alterations in the early life stages. In the short term, ingestion of high levels of arsenic could induce vomiting, diarrhoea, anaemia, liver damage and death. Long-term effects include high blood pressure, diabetes, skin

Table 5
Estimated daily intake (mg/kg/day).

Espèces	Transformation	Cd	Pb	Hg	As	Zn	Cu
C. carpio	Raw	0.11×10^{-3}	0.10×10^{-3}	0.28×10^{-3}	ND	25.29×10^{-3}	1.32×10^{-3}
	Smoked	0.42×10^{-3}	0.26×10^{-3}	0.50×10^{-3}	0.79×10^{-6}	16.61×10^{-3}	6.42×10^{-3}
	Boiled	0.26×10^{-3}	0.19×10^{-3}	0.35×10^{-3}	0.31×10^{-6}	15.11×10^{-3}	1.05×10^{-3}
	Frozen	ND	0.14×10^{-3}	0.42×10^{-3}	ND	22.94×10^{-3}	1.32×10^{-3}
A. parkii	Raw	0.17×10^{-3}	0.19×10^{-3}	0.38×10^{-3}	0.23×10^{-6}	31.39×10^{-3}	1.45×10^{-3}
	Smoked	0.28×10^{-3}	0.36×10^{-3}	0.46×10^{-3}	103.52×10^{-6}	23.31×10^{-3}	1.44×10^{-3}
	Boiled	0.41×10^{-3}	0.24×10^{-3}	0.24×10^{-3}	0.71×10^{-6}	24.07×10^{-3}	1.59×10^{-3}
	Frozen	0.27×10^{-3}	0.13×10^{-3}	0.27×10^{-3}	ND	36.60×10^{-3}	1.71×10^{-3}
E. fimbriata	Raw	0.15×10^{-3}	0.14×10^{-3}	0.43×10^{-3}	ND	26.42×10^{-3}	3.16×10^{-3}
	Smoked	0.32×10^{-3}	0.32×10^{-3}	0.22×10^{-3}	0.39×10^{-6}	28.34×10^{-3}	2.11×10^{-3}
	Boiled	0.20×10^{-3}	0.27×10^{-3}	0.34×10^{-3}	0.63×10^{-8}	24.96×10^{-3}	1.72×10^{-3}
	Frozen	0.16×10^{-3}	0.15×10^{-3}	0.44×10^{-3}	ND	50.35×10^{-3}	2.11×10^{-3}
P. quadrifilis	Raw	0.30×10^{-3}	0.22×10^{-3}	0.30×10^{-3}	ND	51.23×10^{-3}	2.67×10^{-3}
	Smoked	0.43×10^{-3}	0.46×10^{-3}	0.68×10^{-3}	ND	60.74×10^{-3}	3.22×10^{-3}
	Boiled	0.35×10^{-3}	0.18×10^{-3}	0.43×10^{-3}	ND	28.82×10^{-3}	8.08×10^{-3}
	Frozen	0.14×10^{-3}	0.21×10^{-3}	0.18×10^{-3}	ND	56.60×10^{-3}	3.55×10^{-3}

ND: non determined.

disease and cancer [17]. Smoking and boiling increased arsenic content significantly ($P < 0.05$) compared to fresh fish. During heat treatment, arsenic derivatives such as arsenobetaine, trimethylarsine oxide, tetramethylarsonium, dimethylarsinic acid and monomethylarsonic acid are converted into arsenic, leading to an increase in arsenic content [88]. In frozen species, arsenic was not detected, as the sensitivity limit was not reached. According to the results obtained in this study, boiling was the best cooking method because, smoking increased significantly heavy metal content in fish studied compared to boiling. These results could serve as a wake-up call to the public authorities on how to manage the pollution of Cameroon's marine ecosystems by heavy metals.

3.4. Health risk assessment

Estimated daily intake (EDI) values for the six metals calculated are presented in Table 5. The EDIs for lead, mercury, arsenic and copper are below the standards defined by **USEPA** [30], which are 2.5×10^{-3}, 1.0×10^{-3}, 0.2×10^{-3} and 10×10^{-3} mg/kg/day respectively. Similar results were obtained by **Dwipayanti et al.** [89] on *Oreochromis mossanbicus* and *Oreochromis niloticus*, **Adegbola et al.** [15] on *Sarotherodon melanotheron*. EDI reflects daily exposure to the heavy metal and is used to prevent any harmful effects on human health. EDI values below the maximum tolerable limit suggest a possible lower risk of these heavy metals to consumer health. However, it would be unwise to take these values as a permanent measure to reach a definitive conclusion. EDI values for cadmium in *Cyprinus carpio* (smoked and boiled), *Arius parkii* (smoked, boiled and frozen), *Ethmalosa fimbriata* (smoked) and *Polydactilus quadrifilis* (fresh, smoked and boiled) are above the standard defined by **USEPA** [30], which is 0.2×10^{-3} mg/kg/day. For zinc, the EDI values obtained on *A. parkii* (fresh and frozen), *E. fimbriata* (frozen) and *P. quadrifilis* (fresh, smoked and frozen) exceed the USEPA standard of 30×10^{-3} mg/kg/day. These results suggest a high health risk for consumers. Table 6 presents the targeted hazard quotient (THQ), hazard index (HI) and carcinogenic risk (CR) values for the different fish species studied. THQ values for the various metals studied are below 1, with the exception of mercury, whose value is above 1. HI values were above 1 for all species. **Magna et al.** [16] obtained THQ and HI values below 1 in *O. niloticus* from the Volta Bassin of Ghana. THQ values lower than 1 and HI values higher than 1 were obtained by **Dwipayanti et al.** [89] on *O. mossanbicus* and *O. niloticus*. **Adegbola et al.** [15] reported TQH and HI values greater than 1 on *Sarotherodon melanotheron*. Indeed, for THQ and HI values below 1, there is no potential non-carcinogenic effect for the consumer following ingestion of these species [18]. Given the multitude of heavy metals, their bioaccumulation and non-degradability in human systems, it is imperative to monitor levels of these metals as in the long-term humans could suffer considerably [12]. THQ and HI values greater than 1 could indicate non-carcinogenic risks for consumers. CR values below 10^{-6} indicate negligible risk to human health. Values between 10^{-6} - 10^{-4} are in the acceptable range. Values above 10^{-4} suggest exposure to carcinogenic risk [90]. CR values for lead in all species studied are in the range 10^{-6} - 10^{-4}. This result shows that lead could not induce carcinogenic risks in consumers. On the other hand, CR values for cadmium in all species and arsenic in *C. carpio* (smoked) and *A. parkii* (smoked and boiled) are greater than 10^{-4}. Thus, cadmium will induce carcinogenic effects in humans following consumption of these fish species. Different results were obtained by **Adegbola et al.** [15], **Huang et al**. [29] who showed that cadmium CRs did not expose to carcinogenic risk. For this reason, the carcinogenic risk should be the subject of greater vigilance due to the ingestion of aquatic products, particularly those from the fishing port of Douala. These results challenge the public authorities and the general public regarding the quality of Cameroon's marine waters and fish smoking, boiling and freezing. In order to reduce the risks associated with consumption of these fish, it is advisable to control the kind of wood and also the grill used during smoking, to organize health education, promotion and public awareness campaigns on the risks associated with abusive consumption of these fish. According to the results obtained, boiling was the best cooking method because smoking increased Health risk assessment.

Table 6
Targeted hazard quotient, hazard index and carcinogenic risk in the edible parts of different fish.

		THQ (mg/kg/day)						HI (mg/kg/day)	CR		
		Cd	Pb	Hg	As	Zn	Cu		Cd	Pb	As
C. carpio	Raw	0.11	0.02	1.79	ND	0.08	0.03	2.04	7.02×10^{-4}	0.87×10^{-6}	ND
	Smoked	0.42	0.06	3.13	0.005	0.05	0.16	3.84	26.58×10^{-4}	2.23×10^{-6}	1.19×10^{-4}
	Boiled	0.26	0.04	2.23	0.002	0.05	0.02	2.63	16.55×10^{-4}	1.69×10^{-6}	0.47×10^{-4}
	Frozen	ND	0.03	2.63	ND	0.07	0.03	2.78	ND	1.21×10^{-6}	ND
A. parkii	Raw	0.17	0.04	2.38	0.001	0.10	0.03	2.75	11.03×10^{-4}	1.62×10^{-6}	0.35×10^{-4}
	Smoked	0.28	0.09	2.88	0.006	0.07	0.03	3.38	18.06×10^{-4}	3.11×10^{-6}	1.55×10^{-4}
	Boiled	0.41	0.06	1.54	0.004	0.08	0.03	2.14	26.08×10^{-4}	2.09×10^{-6}	1.07×10^{-4}
	Frozen	0.27	0.03	1.74	ND	0.12	0.04	2.21	17.55×10^{-4}	1.15×10^{-6}	ND
E. fimbriata	Raw	0.15	0.03	2.68	ND	0.08	0.07	3.04	9.53×10^{-4}	1.21×10^{-6}	ND
	Smoked	0.32	0.08	1.39	0.002	0.09	0.05	1.95	20.56×10^{-4}	2.77×10^{-6}	0.59×10^{-4}
	Boiled	0.20	0.06	2.14	0.004	0.08	0.04	2.54	13.04×10^{-4}	2.36×10^{-6}	0.95×10^{-4}
	Frozen	0.16	0.03	2.78	ND	0.16	0.05	3.21	10.53×10^{-4}	1.35×10^{-6}	ND
P. quadrifilis	Raw	0.30	0.05	1.89	ND	0.17	0.06	2.48	19.06×10^{-4}	1.89×10^{-6}	ND
	Smoked	0.43	0.11	4.28	ND	0.20	0.08	5.10	27.09×10^{-4}	3.92×10^{-6}	ND
	Boiled	0.35	0.04	2.68	ND	0.09	0.20	3.38	22.57×10^{-4}	1.55×10^{-6}	ND
	Frozen	0.14	0.05	1.14	ND	0.18	0.08	1.61	9.03×10^{-4}	1.82×10^{-6}	ND

3.5. Chemical indexes of extracted oil

3.5.1. Iodine value

Table 7 shows that the iodine value (IV) varies significantly ($P < 0.05$) between the different species in the fresh state, with *Ethmalosa fimbriata* having the highest value and *Polydactilis quadrifilis* the lowest. IV reflects the degree of unsaturation and the oil's susceptibility to oxidation. According to this index, oils are classified as non-siccative (IV < 100), semi-siccative (100 < IV < 130) and siccative (IV > 130). According to these results, *Cyprinus carpio* and *P. quadrifilis* oils are non-siccative, while *E. fimbriata* and *Arius parkii* oils are semi-drying. The high iodine value may suggest that these oils are rich in polyunsaturated fatty acids such as arachidonic eicosapentaenoic and docosahexaenoic acids. These have specific therapeutic virtues such as anti-inflammatory, antioxidant, anti-diabetic, anti-depressant, *anti*-hyperlipidemic and anticancerous, development of cognitive function [91]. These results are lower than those found by **Manz et al.** [92] on *Ilisha africana* (171.66), **Saher et al.** [93] on *Labeo rohita* (204). These results are superior to those obtained by **Noutsa et al.** [94] on *Fontitrygon margarita* liver oil (106.65) who extracted the oil by exudation and cooking pressing. Different results were obtained by **Mgbechidinma et al.** [95] on the waste (heads, viscera and bones) of *Scomberomorus sinensis* and *Carassius auratus* who extracted oil by conventional and milder extraction methods. These differences could be due to the extraction method, the nature of the species, the tissue sampled and the diet of the species studied [96]. *Polynemidae* are carnivorous and their diet includes sea urchins, molluscs, crustaceans, crabs, amphipods and barnacles. *Cyprinidae* feed on small benthic animals, *Ariidae* on shrimps, parasites and plankton, while *Clupeidae* feed on phytoplankton [9]. The IV decreased significantly ($P < 0.05$) with smoking and boiling in all species. **Tenyang et al.** [1] obtained similar results in *Polypterus bichir bichir*. This decrease could be explained by the oxidation of double bonds by lipoxygenase, the breaking of double bonds and the conversion of double bonds into epoxides during heat treatment. These epoxides are harmful to human health, causing respiratory problems, burns, dermatitis and cancer [97]. During freezing, *E. fimbriata* and *P. quadrifilis* oil showed a decrease significant ($P < 0.05$) in iodine value compared to raw sample **Ndidiamaka and Ifeanyi** [98] noted a significative decrease of iodine value after freezing of *Clarias gariepinus* purchased from the local market in Abraka, Delta state, Nigeria. Membrane lysis during freezing and increased activity of certain endogenous enzymes could account for this result [25].

3.5.2. Acid value

The acid number measures the amount of free fatty acid present in a fat. Table 7 shows that the acid number does not vary significantly ($P > 0.05$) between the different raw species. Similar results were obtained by **Pradhan et al.** [99] on *Opisthopterus tardoore* oil (1.14). These results are lower than the standard set (3 mg KOH/g of oil) by **Codex alimentatius** [100] and those obtained by **Manz et al.** [101] on *Ilisha africana* (2.15 mg KOH/g of oil). This result could be explained by a low hydrolytic activity of triglyceride lipases. Different results were obtained by **Minh et al.** [102] who showed that acid values of the *Pangasianodon hypophthalmus* oil extracted with isopropanol:hexane at three ratios (3:2, 1:1, and 2:3) were 4.94, 3.65, and 2.62 mg KOH/g respectively,

Table 7
Chemical indexes of the oil of the species studied.

Indexes	Samples	*C. carpio*	*A. parkii*	*E. fimbriata*	*P. quadrifilis*	Tolerable limited
IV	Raw	$93.18 \pm 5.31^{c\beta}$	$104.21 \pm 3.04^{b\gamma}$	$114.65 \pm 4.42^{d\delta}$	$72,06 \pm 0,18^{d\alpha}$	
(g I_2/100g of oil)	Smoked	$75.53 \pm 3.68^{a\beta}$	$83.47 \pm 3.13^{a\gamma}$	$85.15 \pm 2.40^{a\gamma}$	$54,81 \pm 0,11^{a\alpha}$	
	Boiled	$82.09 \pm 1.37^{b\beta}$	$91.32 \pm 4.14^{a\gamma}$	$94.82 \pm 3.26^{b\gamma}$	$59,79 \pm 1,03^{b\alpha}$	
	Frozen	$89.76 \pm 3.04^{c\beta}$	$102.18 \pm 5.02^{b\gamma}$	$102.76 \pm 3.08^{c\gamma}$	$64,81 \pm 0,11^{c\alpha}$	
AI	Raw	$1.18 \pm 0.06^{a\alpha}$	$1.12 \pm 0.10^{a\alpha}$	$1.31 \pm 0.23^{a\alpha}$	$1,84 \pm 0,73^{a\alpha}$	
(mg KOH/g of oil)	Smoked	$7.41 \pm 0.37^{d\beta\gamma}$	$5.06 \pm 0.63^{c\alpha}$	$8.17 \pm 0.76^{d\gamma}$	$7,06 \pm 0,11^{c\beta}$	3*
	Boiled	$5.53 \pm 0.28^{c\beta}$	$6.73 \pm 0.28^{d\gamma}$	$4.07 \pm 0.63^{c\alpha}$	$6,89 \pm 0,27^{c\gamma}$	
	Frozen	$1.86 \pm 0.29^{b\alpha}$	$1.87 \pm 0.36^{b\alpha}$	$2.05 \pm 0.34^{b\alpha}$	$2,84 \pm 0,13^{b\beta}$	
PI	Raw	$1.58 \pm 0.65^{a\alpha}$	$1.32 \pm 0.12^{a\alpha}$	$1.61 \pm 0.48^{a\alpha}$	$2,97 \pm 0,33^{a\beta}$	
(meq O_2/kg of oil)	Smoked	$8.46 \pm 0.52^{d\gamma}$	$4.41 \pm 0.46^{c\alpha}$	$6.73 \pm 0.62^{b\beta}$	$5,55 \pm 1,22^{c\alpha\beta}$	5*
	Boiled	$4.51 \pm 0.42^{c\alpha}$	$5.93 \pm 0.37^{d\beta}$	$5.71 \pm 1.07^{b\alpha\beta}$	$6,01 \pm 2,33^{b c\alpha\beta}$	
	Frozen	$2.83 \pm 0.51^{b\gamma}$	$1.72 \pm 0.16^{b\alpha}$	$2.08 \pm 0.18^{a\beta}$	$3,51 \pm 0,16^{b\gamma}$	
AnI	Raw	$0.14 \pm 0.04^{a\alpha}$	$0.21 \pm 0.08^{a\alpha}$	$0.41 \pm 0.06^{a\beta}$	$0,33 \pm 0,04^{a\alpha}$	
	Smoked	$2.02 \pm 0.15^{d\alpha}$	$3.51 \pm 0.76^{c\beta}$	$2.74 \pm 0.63^{c\alpha\beta}$	$2,07 \pm 0,36^{c\alpha}$	20*
	Boiled	$1.73 \pm 0.22^{c\alpha}$	$1.51 \pm 0.13^{b\alpha}$	$2.04 \pm 0.71^{c\alpha\beta}$	$2,11 \pm 0,09^{c\beta}$	
	Frozen	$1.13 \pm 0.05^{b\alpha}$	$1.23 \pm 0.49^{b\alpha}$	$1.34 \pm 0.25^{b\alpha}$	$1,47 \pm 0,36^{b\alpha}$	
TBA	Raw	$0.32 \pm 0.06^{a\alpha}$	$0.22 \pm 0.04^{a\alpha}$	$0.24 \pm 0.08^{a\alpha}$	$1,07 \pm 0,24^{a\beta}$	
(mg MDA/kg of oil)	Smoked	$3.08 \pm 0.05^{c\beta}$	$2.87 \pm 0.24^{d\alpha\beta}$	$2.61 \pm 0.18^{d\alpha}$	$3,13 \pm 1,08^{b\alpha\beta}$	10*
	Boiled	$3.53 \pm 0.16^{d\beta}$	$1.73 \pm 0.18^{c\alpha}$	$3.17 \pm 0.24^{c\beta}$	$3,51 \pm 0,19^{b\beta}$	
	Frozen	$1.09 \pm 0.13^{b\beta}$	$0.86 \pm 0.03^{b\beta}$	$0.63 \pm 0.06^{b\alpha}$	$0,85 \pm 0,04^{a\beta}$	
TOTOX	Raw	$3.30 \pm 0.23^{a\beta}$	$2.85 \pm 0.08^{a\alpha}$	$3.63 \pm 0.12^{a\beta}$	$6.27 \pm 0.13^{a\gamma}$	
	Smoked	$18.94 \pm 0.41^{d\gamma}$	$12.33 \pm 0.31^{c\alpha}$	$16.20 \pm 0.51^{d\beta}$	$13.16 \pm 0.67^{c\alpha}$	26*
	Boiled	$10.75 \pm 0.18^{c\alpha}$	$13.37 \pm 0.21^{d\beta}$	$13.46 \pm 1.01^{c\beta}$	$14.13 \pm 1.19^{c\beta}$	
	Frozen	$8.69 \pm 0.20^{b\gamma}$	$4.67 \pm 0.27^{b\alpha}$	$5.50 \pm 0.12^{b\beta}$	$8.49 \pm 0.25^{b\gamma}$	

IV: iodine index; AI: acid index; PI: peroxide index; AnI: Anisidin index; TBA: thiobarbituric acid number; TOTOX: Total oxidation index; MDA: malondialdehyde; meq: milli-equivalent. The values are the mean ± standard deviation. Values in columns with different letters are significantly ($p < 0.05$) different. Values the same line with different symbols are significantly ($p < 0.05$) different. (*): Maximum tolerable limit cited by Codex Alimentarius (2017). N = 3.

whereas those extracted with ethanol:hexane were 4.26, 3.22, and 2.35 mgKOH/g respectively. The oil composition, extraction method and freshness of the raw material affect the acid value of fish oil [103]. Acid index (AI) increased significantly ($P < 0.05$) with boiling and smoking in fish oil of the four species studied compared to raw fish. **Djopnang et al.** [23] showed that acid value increased significantly during boiling and smoking compared to raw *Chrysichthys nigrodigitatus* collected from Nkam River in Cameroon. **Yang et al.** [104] showed that acid value increased significantly during smoking and boiling in *Procambarus clarkii*. Increased in acid index during boiling may be linked to heating water that catalyzes the hydrolysis of ester bonds of triglycerides and phospholipids by triglyceride lipase and releases free fatty acids in oil [105]. High content of free fatty acid (FFA) in oil leads to the formation of off-flavor as a result of rancidity of oil [1]. Freezing increased significantly ($P < 0.05$) acid value in fish oil of four species studied compared to raw fish oil. **Tenyang et al.** [24] showed that acid value of *Cyprinus carpio* oil increased significantly after nine months (from 1.35 to 8.06) of frozen storage at −18 °C. **Fan et al.** [106] showed that after four weeks, the acid number increased significantly in *Eriocheir sinensis* oil frozen at −20 °C compared with oils stored at −40 °C and −80 °C. During freezing, cell membranes are lysed, releasing phospholipids and triglycerides, which are hydrolyzed by endogenous enzymes (phospholipases and triglyceride lipases) to FFAs [92]. These results can be explained by the very low water content of the oils who limits hydrolysis reactions and makes acidity not a critical parameter during storage of filtered oils [107].

3.5.3. Peroxide index

The peroxide value varies significantly ($P < 0.05$) in *Polydactilis quadrifilis* compared with the other species in the fresh state (Table 7). These values are below the standard defined (5 meqO2/kg of oil) by the **Codex alimentarius** [100]. These results are lower than those obtained by **Manz et al.** [92] on *Ilisha africana* (8.43) using soxhlet oil extraction. This difference could be due to the extraction method and the nature of the species. The peroxide value is generally used to measure the primary oxidation products (hydroperoxides) in an oil. This index is used to assess the degree of oxidation of the unsaturated fatty acids in the fat (rancidity). Boiling and smoking increased significantly ($P < 0.05$) peroxide value in all fish oil studied compared to raw fish oil. **Tenyang et al.** [1] showed that boiling and smoking increased peroxide value on *Polypterus bichir bichir*. During smoking, peroxide value of *Chrysichthys nigrodigitatus* oil increased significantly compared to raw fish oil [39]. During heat treatment, heat and water hydrolyse lipid compounds. Free fatty acids are oxidized via initiation, propagation and termination reactions, leading to the formation of hydroperoxides and peroxides [108]. The latter are implicated in the development of oxidative stress, cancer, skin irritation, eye damage and respiratory disorders [109]. Freezing increased significantly ($P < 0.05$) peroxide value in *Cyprinus carpio, Arius parkii* and *Polydactilus quadrifilis* oil compared to *Ethmalosa fimbriata* oil. **Minh et al.** [102] showed that during refrigerator storage (4 °C), the peroxide value of *Pangasianodon hypophthalmus* oil increased significantly after 15 days (from 1 to 3 mEq/kg). **Tenyang et al.** [24] showed that peroxide value increased during freezing of *Cyprinus carpio after nine months* (from 3.77 to 18.62 $mEqO_2$/kg). The development of rancidity during frozen storage was indicated by an increase in peroxide value in frozen fish as compared to fresh fish. One of the main impacts of lipid oxidation is the development of off-flavor, at a later stage of lipid peroxidation, changes in colour and nutritional value are seen as well [110]. Different results were obtained by **Tenyang et al**. [111] who showed that the peroxide value of *Clupea harengus* oil increased during the first six days of refrigeration and then decreased on the ninth day. The preservation technique used and the reduced half-life of the peroxides could explain this result. The higher the peroxide value, the more the fat is oxidized. However, the peroxide value is only an indicator of the start of oxidation, which increases to reach a peak and then decreases with the advanced state of oxidation [112].

3.5.4. Anisidin index

Anisidine index measures the secondary oxidation products of the oil and takes into account non-volatile aldehyde compounds. Table 7 showed that the anisidine number ranged from 0.14 to 41 and varied significantly ($P < 0.05$) in raw *Ethmalosa fimbriata* oil compared to other species. This may suggest an advanced state of oxidation of fresh *Ethmalosa fimbriata* oil compared to oils extracted from *Cyprinus carpio*, *Arius parkii* and *Polydactilus quadrifilis*. These results are lower than those obtained by **Manz et al.** [3] on *Cyprinus carpio* (0.66), *Arius parkii* (0.73) and *Ethmalosa fimbriata* (0.76) and the standard set (10) by the **Codex alimentarius** [100]. Boiling and smoking increased significantly ($P < 0.05$) anisidin value in all fish oil studied compared to raw fish oil. Similar results were obtained by **Noutsa et al.** [94] on *Fontitrygon margarita* liver oil extracted by exudation (3.32) and cooking-pressing (2.85). **Tenyang et al.** [111] observed an increase of anisidine value on smoked *Liza falcipins* (16.20) and *Oreochromis niloticus* (6.43) oil compared to raw fish oil (not detected). Increase in this index would reflect a conversion of hydroperoxides and peroxides into secondary oxidation products especially nonvolatile carbonyls (2-alkenal and 2,4-alkadienal) in the later stages of lipid oxidation stimulated by high temperatures [39]. Anisidin value increased significantly ($P < 0.05$) during freezing in all fish oil studied compared to raw fish oil. **Aydin and Gokoglu** [113] showed that during freezing at −20 °C and storage at −18 °C in *Engraulis encrasicholus* oil for six months, anisidine value increased compared to raw fish oil. The anisidine index increased significantly during freezing of *Eriocheir sinensis* at −20 °C compared to −40 °C and −80 °C after four weeks [106]. This could be explained by the fact that rapid freezing prevents the formation of microcrystals that can perforate cell membranes and release cell contents that can oxidise and the activity of endogenous enzymes [6]. As shown by **Shahidi and Zhong** [114], a desirable anisdine value of oil is lower than 4 with an upper limit of 6.0. The low anisdine value indicates the best quality of oil. Based on the results obtained, boiling was the best cooking method compared to smoking.

3.5.5. Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) measures oxidation products, particularly malondialdehyde (MDA). MDA is an indicator of lipid peroxidation in tissues and oils. High levels of MDA are thought to play a role in oxidative stress and the pathogenicity

of atherosclerosis [115]. TBARS varies significantly ($P < 0.05$) in raw *Cyprinus carpio, Arius parkii,* and *Ethmalosa fimbriata* oil compared to raw *Polydactilus quadrifilis oil* different species (Table 7). *P. quadrifilis* and *A. parkii* show the highest and lowest TBARS respectively. Similar results were reported by **El-Lahamy et al.** [116] in *Oreochromis niloticus* (0.55) and *Mugil cephalous* oil (0.95), **Manz et al.** [3] in *Cyprinus carpio* oil (0.87). Smoking and boiling increased significantly ($P < 0.05$) TBARS in all fish oil studied compared to raw fish oil. **Tenyang et al.** [25] showed that TBARS increased in smoked *Oreochromis niloticus* oil compared to raw fish oil. Smoking and boiling increased significantly TBARS in *Polypterus bichir bichir* oil [1]. The enzymatic hydrolysis and/or oxidation of lipids and proteins at high temperatures, which converts hydroperoxides and peroxides into MDA, could explain this result [117]. Freezing increased significantly ($P < 0.05$) TBARS in all fish oil studied compared to raw fish oil. **Bao et al.** [4] showed that TBARS increased in filet of *Micropterus salmoides* oil during freezing at −18 °C and storage at −18 °C. **Prabha and Manjulatha** [118] reported that TBARS increased in *Pampus argentus* oil during frozen storage at −20 °C for 180 days. Ghribi **et al.** [118] showed that freezing increased significantly TBARS in *Portunus segnis* during storage at −30 °C for a period of 120 days. Very low temperatures cause oxidation of lipids and proteins, leading to the synthesis of MDAs and a consequent increase in TBARS [119].

3.5.6. Total oxidation index

To estimate the quality of oil, the total oxidation (TOTOX) value may be used and measures oil oxidation in all its forms. TOTOX reflect the initial and later stages of the oil oxidation. The values of TOTOX in raw and treated fish oil were consigned in Table 7. TOTOX varies significantly ($P < 0.05$) between raw *Polydactilus quadrifilis* oil and the other species fish oil studied. *A. parkii* (2.85) and *P. quadrifilis* (6.27) oil had the lower and higher value in TOTOX. These results are below the standards set by the **Codex Alimentarius** [100] which recommends a value below 26 for virgin fats and oils. These results are similar than those obtained by **Manz et al.** [3] on *S. maderensis* (4.52) and *A. parkii* oil (3.59). The lower value of total oxidation indicates a higher quality of the oil. Smoking, boiling and freezing increased significantly TOTOX value in all fish oil studied compared to raw sample. **Tenyang et al.** [39] showed that TOTOX increased in smoked *C. nigrodigitatus* oil compared to raw oil sample. **Tenyang et al.** [111] reported that TOTOX value increased significantly in *Liza falcipins* and *Oreochromis niloticus* oil compared to raw fish oil during smoking. **Aydin and Gokoglu** [113] showed that during freezing at −20 °C and storage at −18 °C in *Engraulis encrasicholus* oil, TOTOX value increased compared to raw fish oil. The increase in these values during smoking and boiling could be due to the hydrolysis of the ester bonds releasing the fatty acids which oxidise to give primary and secondary oxidation products which alter the quality of the oil [115]. The increase in these indices of alteration during freezing could be due to the breaking of double bonds by endogenous enzymes [25]. According to ours results, the TOTOX value of all fish samples used were in acceptable limits and suggest the good quality of boiled, frozen and smoked fish oil.

3.5.7. Limitations of the study

However, this study has a few limitations.

1. The observations in this study does not provide information on others cooking methods (frying, steaming, microwaving and baking) and their effect on polycyclic aromatic hydrocarbons, heavy metals, fatty acid and amino acid profiles
2. Also, only One-Time sampling was done for collection of the different species of fish. Normally, collection of fishes species would have been done in seasonal sampling
3. Lastly, there is need to carry out a comparative study of the chemical composition and heavy metal content of the fish species studied at the various fish collection sites in the city of Douala.

4. Conclusion

This study showed that *Cyprinus carpio, Arius parkii, Ethmalosa fimbriata* and *Polydactilus quadrifilis* are good sources of protein and ash, the nutrients contents vary from one fish to another. Smoking increased protein, lipid, ash, carbohydrate and particular mineral such as Ca, P, K, Na and Zn in different fish studied compared boiling. Smoking increased significantly heavy metal (Cd, Pb, Hg and As) and health risk assessment (EDI, HI and CR) in fish studied compared boiling and freezing. It would be important to inform the populations about the risks of toxicity linked to the long-term overconsumption of these fishes. Consequently, health education campaigns and appropriate public awareness campaigns need to be carried out, and the necessary government environmental health management agencies need to set up regulatory systems. Make fish smokers aware of the quality of the wood used for smoking and grilling, which could be a source of contamination. Smoking, boiling and freezing affected negatively quality of fish oil by increase of acid, peroxide, anisidine, TBARS and TOTOX value while iodine value decreased. Boiling was the best cooking method compared to smoking and can be used by the population. Future studies will evaluate the effect of cooking methods (frying, steaming, microwaving and baking) on polycyclic aromatic hydrocarbons, heavy metals, fatty acid and amino acid profiles.

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Ethics declarations

This study was reviewed and approved by University of Douala Institutional Ethics Committee because it involved animals (fishes) and the approval number: N° 2714 CEI-Udo/06/2021/M.

Data availability statement

No data associated with this study has been deposited into a publicly available repository. Data will be made available on request.

CRediT authorship contribution statement

Manz Koule Jules Christophe: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Youogo Tegueu Marlène:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Nsoga Valery Jean François:** Writing – review & editing, Writing – original draft, Resources, Formal analysis. **Nchoutpouen Ngafon Merlin:** Writing – review & editing, Writing – original draft, Resources, Data curation. **Gouado Inocent:** Writing – review & editing, Writing – original draft, Conceptualization. **Ndomou Mathieu:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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