Results in Chemistry 7 (2024) 101515

Contents lists available at ScienceDirect

Results in Chemistry

journal homepage: www.sciencedirect.com/journal/results-in-chemistry

Comparative lipid composition and GC–MS fatty acid profiling of tropical African freshwater fishes

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ARTICLE INFO

Keywords:
Fish fatty acids
Afrotropical fish
Fatty acid composition
Omega-3 fatty acids
Multivariate data analysis

ABSTRACT

In dietary contexts, fish are renowned for the diverse array of unsaturated fatty acids they provide to the body. While data regarding the fatty acid composition of freshwater fish are readily available in most developed countries, research on African freshwater fish remains notably limited. In this study, we investigated the fatty acid composition of fifteen freshwater fish species from Africa, primarily Afrotropical regions. Lipids were extracted using a solvent mixture of methanol, water, and chloroform. Subsequently, total fatty acids were determined by saponification, and the fatty acid profile was analyzed via GC–MS following methylation in the presence of BF_3 and methanol. The results revealed lipid levels in fish fillets ranging from 1.99 % for *T. guineensis* to 6.41 % for *M. bananensis*. Total fatty acid percentages of lipids were found to be 81.40 ± 9.15 % for *S. nigripinnis*, 83.75 ± 6.12 % for *L. niloticus*, and 92.99 ± 11.30 % for *C. dageti*. The fatty acid profiles of 15 properly identified fish species have been determined, with the majority being identified for the first time. The composition of health-important omega-3 fatty acids EPA and total DHA ranged from 4.82 ± 0.2 % for *Cyprinus lepidotus* to 17.43 ± 2.50 % for *Mugil bananensis*. Unsupervised multivariate data analysis of fatty acid profiles of the different fish species showed significant differences. When the fish were grouped into 4 categories based on their similarities, 12 discriminant fatty acids characteristic of each group were identified. Among these fatty acids are omega-6 acids such as γ-linolenic acid and arachidonic acid, and omega-3 7,10,13,16,19-docosapentaenoic acid (DPA). This research opens avenues for consumers to make informed dietary choices aligned with their specific fatty acid needs and preferences regarding fish consumption.

Introduction

Fish are categorized into marine and freshwater groups based on their habitat, with water temperature and salinity being critical environmental factors. While the precise number of existing fish species remains uncertain, FishBase reported approximately 35,400 species in 2023 [1], yet experts speculate the actual count could be significantly higher. Freshwater fish currently account for nearly 13,000 species (and 2,513 genera), including those strictly found in freshwater habitats and those extending to brackish waters, totaling around 15,000 species. Biogeographically, freshwater species and genera distribution includes 4,035 species (705 genera) in the Neotropical region, 2,938 (390 genera) in the Afrotropical, 2,345 (440 genera) in the Oriental, 1,844 (380 genera) in the Palaearctic, 1,411 (298 genera) in the Nearctic, and 261 (94 genera) in the Australian region [2].

Fish have long been recognized as a crucial component of human diets, offering a rich source of protein, essential nutrients, and beneficial fatty acids [3]. The diversity of fish species across different habitats presents a vast array of nutritional profiles, with marine and freshwater fish each contributing distinct compositions of fatty acids and other bioactive compounds. While marine fish have been extensively studied for their nutritional value and health benefits, research on the fatty acid

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https://doi.org/10.1016/j.rechem.2024.101515
Received 7 February 2024; Accepted 20 April 2024
Available online 21 April 2024

composition of freshwater fish, particularly in African regions, remains relatively limited [4,5].

In rural sub-Saharan Africa, many children have insufficient dietary diversity, with 20 % relying solely on fish from nearby inland fisheries for protein [6]. In Togo, fish holds a significant place in the dietary habits, contributing approximately 13.8 % of the total animal protein intake. Current statistics indicate that the average annual per capita fish consumption in Togo stands at 14 kg, surpassing the continental African average of 10.1 kg [7]. Understanding the nutritional composition of these fish species is critical for assessing their role in providing essential nutrients and supporting overall health, particularly in regions where malnutrition and food insecurity are prevalent challenges. Despite the recognized importance of freshwater fish in African diets, comprehensive studies exploring their fatty acid profiles and nutritional attributes are scarce. The few available studies primarily focus on marine fish species, leaving a significant gap in our understanding of the nutritional value of African freshwater fish. including fishes from the important Nangbéto Lake in Togo (West Africa) [8,9]. Lake Nangbéto significantly contributes to Togo freshwater fish supply, covering an area of 180 km^2 at its maximum elevation (144 m) with a water volume of 1.7 billion m^3, and 41 km^2 at its minimum elevation (130 m) with a water volume of 250 million m^3. The reservoir has the capacity to support over thirty fish species, with more than 6,600 tons of fish captured in 2022 [10].

The significance of fats in human nutrition has heightened with increasing attention to human health. Fats play pivotal roles in hormone synthesis, cell membranes, signaling molecules, and energy sources [11]. Fatty acids are categorized by the presence and number of double bonds: saturated fatty acids lack double bonds, monounsaturated fatty acids feature one double bond, and polyunsaturated fatty acids (PUFA) have two or more double bonds. While the human body synthesizes some fatty acids, it relies on dietary intake for essential fatty acids (EFAs) like n-3 and n-6 PUFA. Linoleic acid (LA) from sources like soya and maize oil, and arachidonic acid (AA) primarily from meat, constitute n-6 fatty acids in modern diets. On the other hand, alpha-linolenic acid (ALA) from meat can be converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), crucial for human health and predominantly sourced from fish products [12].

Recent studies demonstrated the significance of n-3 fatty acids in human development and health, with positive impacts observed on maternal and fetal health during pregnancy and on newborns and children [13]. Fish consumption is pivotal for n-3 PUFA intake, yet the composition of n-3 fatty acids varies among species and individuals. The nutritional and fatty acid profiles of fish are influenced by diverse factors, such as species diversity and ecological conditions. Variation in fatty acid profiles among fish species is prominent, influenced by factors like fishing season, size, and reproductive status [14]. Given this variability, it is imperative to explore the nutritional composition of fish across species, regions, and climates. In this study, we aim to fill this gap by investigating the fatty acid composition of fifteen freshwater fish species native to Africa, with a specific focus on Afrotropical regions. In selecting the 15 freshwater fish species investigated in this study, we prioritized species endemic to Africa, with a focus on those known to be commonly consumed in the region. These species were chosen to represent a diverse array of fish commonly found in African freshwater habitats, with particular emphasis on those most relevant to the diets of local populations. By employing advanced analytical techniques, including lipid extraction and gas chromatography-mass spectrometry (GC–MS), we seek to elucidate the intricate composition of fatty acids in these fish species. Our research endeavors to contribute to the broader understanding of the nutritional significance of African freshwater fish and its implications for human health and well-being.

Material and methods

Materials and reagents

Methanol, chloroform, hexane, 14 % boron trifluoride (BF3), hydrochloric acid solution (HCl), butylated hydroxytoluene (BHT), and standards for fatty acids were acquired from Merck (Sigma-Aldrich, Milan, Italy), all of which were of analytical grades. The gas chromatograph (GC) utilized in this investigation was the Agilent Technologies 7890B system, while the mass spectrometer (MS) employed was the Agilent Technologies 5977B system (Agilent Technologies Inc., Santa Clara, CA).

Fish species sampling

For the fatty acid profile analysis of fish, sampling was conducted at Lake Nangbéto, located in Togo (West Africa). Lake Nangbéto is situated on the Mono River, which forms the border between Togo and Benin. Fifteen (15) freshwater fish species (Table 1), representing some of the most endemic and consumed species in Africa, were randomly collected directly from the lake with the assistance of local fishermen. Due to their abundance in the lake and appreciation by local population, *Lates niloticus* was selected at three different geographical points within the lake to study the influence of the fish presence zone on its fatty acid profile. The same approach was applied to *Chrysichthys nigrodigitatus*, which was selected from two extreme zones of the lake. A total of n = 59 fishes were sampled and analyzed in this study. On-site identification of the fish was performed by a fish specialist from the University of Kara, Togo (**Supplementary data 1**). Subsequently, the fish were transported on ice to the laboratory and stored at −80 °C until analysis, with a maximum storage duration of 48 h.

Table 1
Studied fish species with their most known local names and distribution (**Togo**).

N°	Scientific name	Author	Local name	Distribution [1]	N
1	*Tilapia zillii*	Rafinesque, 1810	Carpe rouge	Africa	4
2	*Sardinella rouxi*	Poll, 1953	Sardine	Sub-Saharan Africa	2
3	*Chrysichthys dageti*	Risch, 1992	Atikpomeblolo	Sub-Saharan Africa	4
4	*Auchenoglanis occidentalis*	Valenciennes, 1840	Sokli	Africa	3
5	*Lates niloticus*	Linneaus, 1758	Capitaine	Africa	9
6	*Tilapia rendalli*	Boulenger, 1897	Ofiko	Africa	2
7	*Mugil bananensis*	Pellegrin, 1928	Alovi	Africa	5
8	*Oreochromis niloticus*	Linnaeus, 1758	Akpavi	Africa	2
9	*Tilapia guineensis*	Bleeker, 1862	Azeguin	Africa	2
10	*Chrysichthys nigrodigitatus*	Lacépède, 1803	Blolovi	Africa	12
11	*Cyprinus lepidotus*	Geoffroy Saint-Hilaire, 1809	Gbogbovi	Africa	2
12	*Chromidotilapia guntheri*	(Sauvage, 1882)	Botovi	Africa	3
13	*Mormyrus macrophthalmus*	Günther, 1866	Godogon	Sub-Saharan Africa	2
14	*Sarotherodon nigripinnis*	Guichenot, 1861	Carpe blanche	Africa	4
15	*Schilbe uranoscopus*	Rüppel, 1832	Antassa	Africa	3

Sample extraction for lipidomic analysis

Fish fillets were acquired by precision cutting along the dorsal fin to extract the maximum amount of flesh while excluding bones. The weight of each fillet was recorded. To account for compositional variations across different parts of the fillets based on their locations, the fillets were diced into small portions (1 cm^3) and thoroughly mixed before sampling for analysis.

Lipid extraction

Fish lipids were extracted following the Bligh and Dyer method [15] with slight adjustments. Representative samples of fish fillets (30 g) were homogenized in a blend of methanol (60 mL) and chloroform (30 mL) using a Binatone blender (London, UK) for 2 min. Following homogenization, an additional 30 mL of chloroform was added, and after an additional 30 s of homogenization, distilled water (30 ml) was introduced. The homogenate was stirred and filtered through Whatman No. 1 filter paper using a Buchner funnel with slight suction. The filtrate was then transferred to a separating funnel. The clear lower phase was collected into a 250 ml round-bottom flask and concentrated using a rotary evaporator at 40 °C. The lipid extract was evaporated and adjusted to a final volume of 20 ml with chloroform. Aliquots of 4 ml each were evaporated in pre-weighed flasks under nitrogen until a constant weight was achieved to determine the lipid content. To the remaining lipid extract, 0.05 % concentration of Butylated Hydroxytoluene (BHT) relative to the lipid content was added, and the extract was stored at −40 °C for subsequent analysis.

Total fatty acid content

To ascertain the fatty acid composition of the extracted lipids, we focused on species highly favored by local populations and those most representative in our sampling. As a result, we conducted analyses on the total fatty acids present in *S. nigripinnis*, *L. niloticus*, and *C. dageti*. The total fatty acid content was determined by saponifying the lipids obtained from evaporating the 4 mL chloroform extracts. Saponification was conducted using 0.5 N alcoholic potassium hydroxide (7 mL) at 85 °C for 30 min. Following the addition of water (3 mL), non-saponifiable matter was extracted twice with hexane (3 mL × 2). The remaining soaps were acidified with hydrochloric acid (34 %) to achieve a pH between 1–2. Free fatty acids were then extracted twice with hexane (3 mL × 2). The two extracts from each sample were pooled, evaporated under nitrogen in pre-weighed flasks, and the masses of fatty acids were determined. The average results of replicate analyses are reported for each species (3 to 5 replicates).

Fatty acids methylation for GC–MS analysis

The total fatty acid content of each sample under investigation was obtained through saponification, as described in the preceding section, followed by methylation using 3 mL of 14 % boron trifluoride in methanol for 6 min at 85 °C. Subsequently, the methyl esters of fatty acids were extracted using 2 mL of hexane and diluted tenfold with hexane before injection into GC–MS.

GC–MS analysis of fatty acid methyl esters

One microliter of each derivatized sample underwent injection in splitless mode into a gas chromatograph coupled with a mass spectrometer. The injector temperature was set at 200 °C, maintaining a gas flow of 1 ml/min through the column. Utilizing a fused silica capillary column, specifically a DB5-MS column with a 0.25 μm thickness (30 m × 0.25 mm id.; J&W Scientific Inc., Folsom, CA), the following oven program was executed: an initial temperature of 40 °C for 4 min, followed by a 20 °C per minute ramp until reaching 100 °C, which was then isothermally maintained for 2 min. Subsequently, a 20 °C per minute ramp was applied until 150 °C, held isothermally for 5 min. Finally, a 3 °C per minute ramp was executed until reaching 265 °C, maintaining this temperature for 10 min. The transfer line and ion source temperatures were set at 280 °C and 180 °C, respectively. Ions were generated with an electron beam energy of 70 eV in electron impact ionization and recorded at a rate of 1.6 scans/s over a mass range of 50 to 550 *m/z*.

Metabolite identification involved comparing mass spectra with those in the NIST08 mass spectral database, utilizing a standard fatty acid methyl ester (FAME) mixture for retention time detection. Additional online databases were referenced as necessary to ascertain the structure of unknown fatty acid derivatives, or their retention indices were compared to those documented in the literature.

Deconvolution and integration of chromatograms

The GC–MS data underwent analysis using the "single job" of the XCMS R package (https://xcmsonline.scripps.edu/) [16]. The analysis employed the Centwave algorithm for peak selection, with peak width ranging from 5 to 10 s, a signal-to-noise (S/N) ratio exceeding 6, and a mass tolerance of 100 ppm. Subsequent steps included retention time alignment, normalization of peak areas (mean centering), application of log2 transformation (to address heteroscedasticity), and Pareto scaling. Each ion (mass) was considered a variable, resulting in a total of over 7500 features. The platform identified the abundance of each ion to generate a unique output data matrix encompassing all fish species.

Multivariate statistical analysis and data visualization

For multivariate data analysis of the obtained matrix, the ion intensities of fatty acids were normalized to 1000. Each feature in the matrix underwent standardization by subtracting the mean and dividing by the standard deviation across all samples. Multivariate analysis was performed to identify discriminative features among groups using SIMCA®18 software (Umetrics, Sweden). Initially, Principal Component Analysis (PCA) was utilized to verify the absence of outliers and ensure the accurate classification of quality control samples (QCs) post-normalization. Subsequently, Partial Least Squares Discriminant Analyses PLS-DA was conducted using the same software.

The quality of the PLS-DA model and the optimal number of principal components were determined based on cumulative R2Y (indicating classification ability) and Q2Y (assessing predictive ability during cross-validation) parameters, utilizing methods integrated into the SIMCA-18 program. Essential indicators extracted from the PLS-DA model included Variable Influence on Projection (VIP) scores and coefficients, which highlighted the influence of fatty acids, considering all validated components, in the separation of fish fatty acid profiles [17]. A VIP score > 1.5 was deemed an adequate and robust threshold for determining discriminant variables in the PLS-DA model.

Results and discussion

Total lipids contents

Fish are widely appreciated for their lipid composition, which plays a crucial role in their nutritional value. In this investigation, we examined the lipid content present in the fillets of 15 endemic fish species inhabiting the freshwater bodies of Sub-Saharan Africa. The comprehensive results are summarized in Table 2. The total lipid levels exhibited a considerable range, spanning from 1.99 % for T. *guineensis* to 6.41 % for *M. bananensis*, reflecting notable diversity among the fish species under study. Generally, lipid levels in fish, relative to the weight of fresh fish, vary significantly depending on the species and the fish body part [18]. Previous research on fish fillets has reported lipid percentages ranging from 0 % to 8 % in freshwater fish. The values presented in this study fall within this range, highlighting the potential nutritional significance of the lipids found in the examined fish species [19–21].

Table 2
Total lipid composition analysis of fish species fillets.

Fish species	Mean (%)	StdDev	95 %CI
Auchenoglanis occidentalis	3.93	1.622	(2.740; 5.119)
Lates niloticus	3.92	0.96	(2.736; 5.114)
Tilapia rendalli	6.68	2.31	(5.228; 8.141)
Oreochromis niloticus	3.56	2.62	(2.10; 5.02)
Tilapia guineensis	1.99	0.50	(0.804; 3.182)
Chrysichthys nigrodigitatus	4.25	0.50	(2.796; 5.709)
Mugil bananensis	6.41	1.79	(4.960; 7.873)
Sarotherodon nigripinnis	2.47	0.76	(1.441; 3.501)
Schilbe uranoscopus	1.96	0.34	(0.774; 3.152)
Tilapia zillii	2.01	1.46	(0.826; 3.205)
Chrysichthys dageti	2.37	0.99	(0.923; 3.836)
Sardinella rouxi	6.01	2.30	(4.559; 7.472)
Cyprinus lepidotus	4.13	0.70	(2.674; 5.586)
Chromidotilapia guntheri	2.23	0.39	(0.774; 3.686)
Mormyrus macrophthalmus	5.12	0.26	(3.664; 6.576)

Total fatty acid

Fish lipids encompass a diverse array of metabolites, including phospholipids, acylglycerols (predominantly triacylglycerols), cholesteryl esters, cholesterol, free fatty acids, wax esters, and a minor proportion of hydrocarbons. Total fatty acids comprise free fatty acids as well as those derived from phospholipids and acylglycerols following hydrolysis. In the present investigation, we quantified the total fatty acid contents in extracted lipids for *S. nigripinnis*, *L. niloticus*, and *C. dageti*, revealing proportions of 81.40 ± 9.15 %, 83.75 ± 6.12 %, and 92.99 ± 11.30 %, respectively. When a study examined the lipid content and fatty acid composition of 22 commercially important marine fish species from the Pearl River Estuary (PRE) in the South China Sea, total fatty acid levels ranging from 81.9 % to 96.3 % were observed [20]. These findings emphasize that the predominant portion of lipids in the fish fillets studied consists of fatty acid derivatives such as triglycerides and phospholipids. Additionally, they highlight the efficacy of the saponification method employed, which yielded free fatty acids utilized for subsequent characterizations in the study.

GC–MS analysis of total fatty acids of the fish

Considering that gas chromatography with flame ionization detector (GC-FID) analysis of fatty acid methyl esters is the standard method for quantifying fatty acids in a lipid extract [22], the adoption of a GC–MS platform was determined suitable for an untargeted characterization of the fatty acids. This technique enabled to obtain chromatographic profiles all fatty acids including the branched ones for the15 fish species under study (**Supplementary data 2**). For most of these fish, this marks the first reporting of their fatty acid profiles in fillets. Following analysis, it was possible to identify nearly all the fatty acids from the different chromatograms (**Supplementary data 3–17**). The greatest number of fatty acids was identified in *Tilapia rendalli*, with 51 fatty acids, while the lowest number was detected in *Lates niloticus*, with 26 fatty acids. It is noteworthy to mention the high levels of branched fatty acids found in almost all the fish (up to 5 %).

As fish are primarily valued for the health benefits of their omega-3 and omega-6 fatty acids, elevated levels of these acids in the various fish species were identified, particularly the most studied fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). They were then compared across different species (Table 3). All fish fillets contained a relatively high percentage of EPA plus DHA. These percentages ranged from 4.82 ± 0.20 (equivalent to 199 mg/100 g of wet fillets) for *C. lepidotus* to 17.43 ± 2.50 % (equivalent to 1117 mg/100 g of wet fillets) for *M. bananensis*. Generally, DHA levels were higher than EPA levels. The highest level of DHA was found in *M. bananensis* (13.61 ± 2.50 %), while the highest level of EPA was found in *Auchenoglanis occidentalis* (4.74 ± 1.00 %).

Table 3
Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) percentage composition of the studied fish oils.

Fish species	Local name	EPA (%)	DHA (%)	EPA + DHA (%)
Auchenoglanis occidentalis	Sokli	4.74 ± 1.00	2.00 ± 0.50	6.74 ± 1.82
Lates niloticus	Capitaine	1.34 ± 0.30	5.76 ± 1.20	7.10 ± 1.20
Tilapia rendalli	Ofiko	3.39 ± 0.80	4.80 ± 0.90	8.19 ± 0.90
Oreochromis niloticus	Akpavi	2.15 ± 0.50	4.15 ± 0.70	6.30 ± 0.70
Tilapia guineensis	Azeguin	1.83 ± 0.60	9.42 ± 1.80	11.25 ± 1.80
Chrysichthys nigrodigitatus	Blolovi	3.83 ± 0.70	5.17 ± 1.10	9.00 ± 1.10
Mugil bananensis	Alovi	3.82 ± 0.90	13.61 ± 2.50	17.43 ± 2.50
Sarotherodon nigripinnis	Carpe blanche	3.69 ± 0.70	11.74 ± 2.30	15.43 ± 2.30
Schilbe uranoscopus	Antassa	2.36 ± 0.60	5.73 ± 1.15	8.09 ± 1.15
Tilapia zillii	Carpe rouge	2.85 ± 0.80	8.19 ± 1.60	11.04 ± 1.60
Chrysichthys dageti	Atikpomeblolo	4.27 ± 0.90	9.95 ± 1.90	14.22 ± 1.90
Sardinella rouxi	Sardine	3.30 ± 0.60	7.19 ± 1.40	10.49 ± 1.40
Cyprinus lepidotus	Gbogbovi	4.09 ± 0.20	0.73 ± 0.20	4.82 ± 0.20
Chromidotilapia guntheri	Botovi	4.74 ± 1.00	2.00 ± 0.50	6.74 ± 1.82
Mormyrus macrophthalmus	Godogon	1.34 ± 0.30	5.76 ± 1.20	7.10 ± 1.20

Several studies have already explored the fatty acid profiles of lipids from various fish species, typically targeting between 20 and 40 fatty acids in fish oil investigations [23,24]. Here, an untargeted study allowed us to identify a greater number of fatty acids in certain fish species including branched fatty acids. Dietary intake or the synthesis of oil in specialized glands, similar to human sebaceous and meibomian glands, appears to be the mechanism for the accumulation of branched-chain and odd-chain fatty acids in fish [25]. In addition to consuming fish, which are known to be piscivorous, fish have a diverse diet that includes various aquatic organisms such as phytoplankton, zooplankton, macroalgae, invertebrates, and their larvae. Some phytoplankton species contain branched-chain and odd-chain fatty acids similar to those found in fish, albeit at higher concentrations ranging from 3 % to 6 %. Similarly, certain types of algae, mollusks, and shrimps also contain notable concentrations those fatty acids [26].

In their exploration of omega-3 fatty acids, Mohandy et al. investigated the composition of EPA and DHA, as well as fatty acid profiles, in 39 fish species native to India. Their findings revealed a broad spectrum, with EPA + DHA values ranging from 70 to 1472 mg per 100 g of wet fish fillets for *Xenentodon cancila* and *Sardinella longiceps*, respectively [19]. Notably, well-known fish such as mackerel and tuna exhibited values of 2500 mg and 400 mg, respectively [27]. Despite the high level of EPA + DHA observed in *Auchenoglanis occidentalis* in this study, to our knowledge, no research has investigated the fatty acid profile of this fish species.

Moreover, our observations revealed that the top three fish species exhibiting elevated levels of EPA + DHA omega-3 fatty acids were of omnivorous nature. Specifically, M. bananensis, S. nigripinnis, and C. dageti demonstrated significant concentrations of omega-3 fatty acids at 17.43 %, 15.43 %, and 14.22 %, respectively. Interestingly, a previous study suggested a contrasting trend, indicating that omega-3 fatty acids peak in carnivorous-benthivorous fish, followed by carnivorous–piscivorous fish, and are lowest in herbivorous-omnivorous fish. This discrepancy highlights the complex array of factors affecting

omega-3 PUFA levels in fish across diverse environments [28]. This study paves the way for international recognition in the nutritional value of this fish and others documented herein.

Multivariate analysis of fish samples

Unsupervised multivariate analysis of fatty acids profiles

One of the main objectives of this study was to verify the difference in fatty acid profiles among various freshwater fish species present in Tropical Africa. To achieve this, an initial principal component analysis (PCA) of all the studied fish was conducted. In the obtained projection, it was observed that some species had similar fatty acid profiles, while others were markedly different (Fig. 1). For instance, *A. occidentalis* was distinctly separated from other species in the Hotteling's space. *A. occidentalis, C. nigrodigitatus*, and *C. dageti*, which share similar physical appearances (**Supplementary data 2**), did not separate in the PCA projection, indicating similar fatty acid profiles. These species will be referred to as "brolo" for the remainder of the study. Similarly, *Sarotherodon nigripinnis*, *Tilapia zillii*, and *Oreochromis niloticus*, which also have similar physical appearances, were grouped closely together, demonstrating comparable fatty acid profiles. For the continuation of the study, these species will be combined and referred to as "carp". *Lates niloticus*, a highly esteemed fish among the population known as "captain", did not exhibit a specific fatty acid profile according to the PCA projection.

To further the study, the fish groups were stratified to five: "Carp", "Brolo", *A. occidentalis* ("Alovi"), *Schilbe uranoscopus* ("Antassa"), and "Captain". A principal component analysis (PCA) was conducted with these five fish groups (Fig. 2). The projections reveal that the fatty acid profiles of Captain fish are relatively similar to those of Carp. Conversely, Alovi appear more analogous to Brolo concerning their fatty acids. The species Antassa exhibited a more dispersed distribution among the four previous species groups.

To validate the observed differences in fatty acid composition, pairwise comparisons were made between the different fish groups using multivariate unsupervised analysis. Initially, Brolo and Carp were compared (Fig. 3). The results demonstrated a clear separation between the two fish groups, showing the effectiveness of the model. Subsequently, Alovi and Brolo were assessed (Fig. 4). Despite appearing similar in the initial analysis, the two fish groups were distinctly segregated here, indicating a divergence in fatty acid composition between them. Finally, Captains and Brolo were scrutinized. Once again, a marked difference was discerned in the two principal components of the PCA. Consequently, this model can be effectively utilized to identify potential fish oil adulteration.

Another objective of this study was to evaluate how the presence zone of fish at the Nangbéto Dam affects their fatty acid profile. To explore this, PCA analyses were conducted separately for Brolo and Carp (Fig. 5). The results suggest that the variation in fatty acid profiles of fish from the Nangbéto Dam is not strongly influenced by the study area, as individuals appear to mix in the Hotelling's space. This implies that the fish feeding sources within the dam are similar, as well as the overall water composition.

The previous analyses demonstrate that most freshwater fish exhibit highly varied fatty acid profiles. These preceding studies have delineated 4 groups of fish based on the similarity of their fatty acid profiles: "Alovi" comprises *Mugil bananensis*; "Brolo" consists of *Auchenoglanis occidentalis* and *Chrysichthys nigrodigitatus*; "Capi" includes *Lates niloticus*; and "Carp" encompasses *Tilapia rendalli*, *Oreochromis niloticus*, *Tilapia guineensis*, *Sarotherodon nigripinnis*, and *Tilapia zillii*. Varying the consumption of fish from these different groups could enable consumers to diversify their intake of fatty acids. Furthermore, fish lipid capsules are increasingly prevalent in markets in developing countries, such as those in sub-Saharan Africa. Unfortunately, these products are often subject to food fraud, with cheaper lipids derived from animals or plants being substituted and eluding the scrutiny of competent authorities [29]. The unsupervised multivariate analysis models developed in this study could serve as robust tools for detecting such fraud. Lastly, this

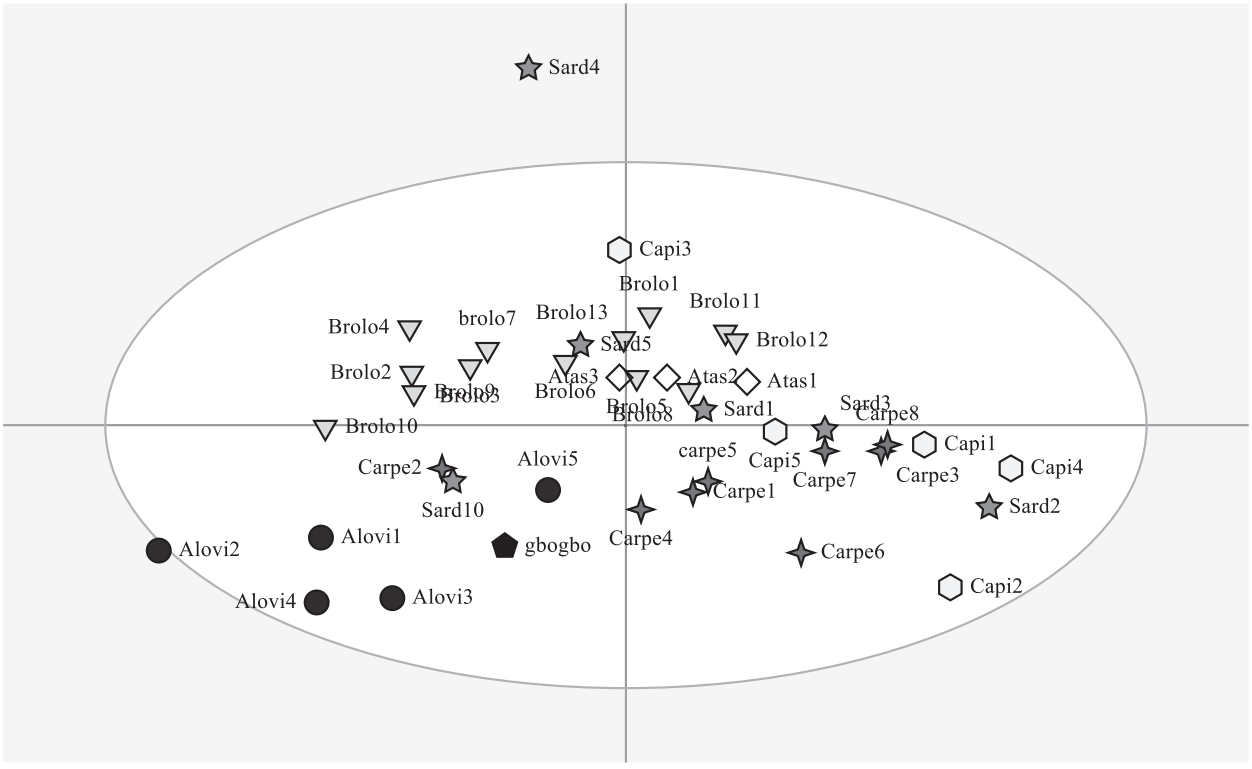

Fig. 1. Principal component analysis (PCA), a multi-dimensional statistical analysis method for unsupervised pattern recognition, is helpful to understand the total fatty acids changing among fish species. PCA showed a clear separation between some fish species identified here by their local names.

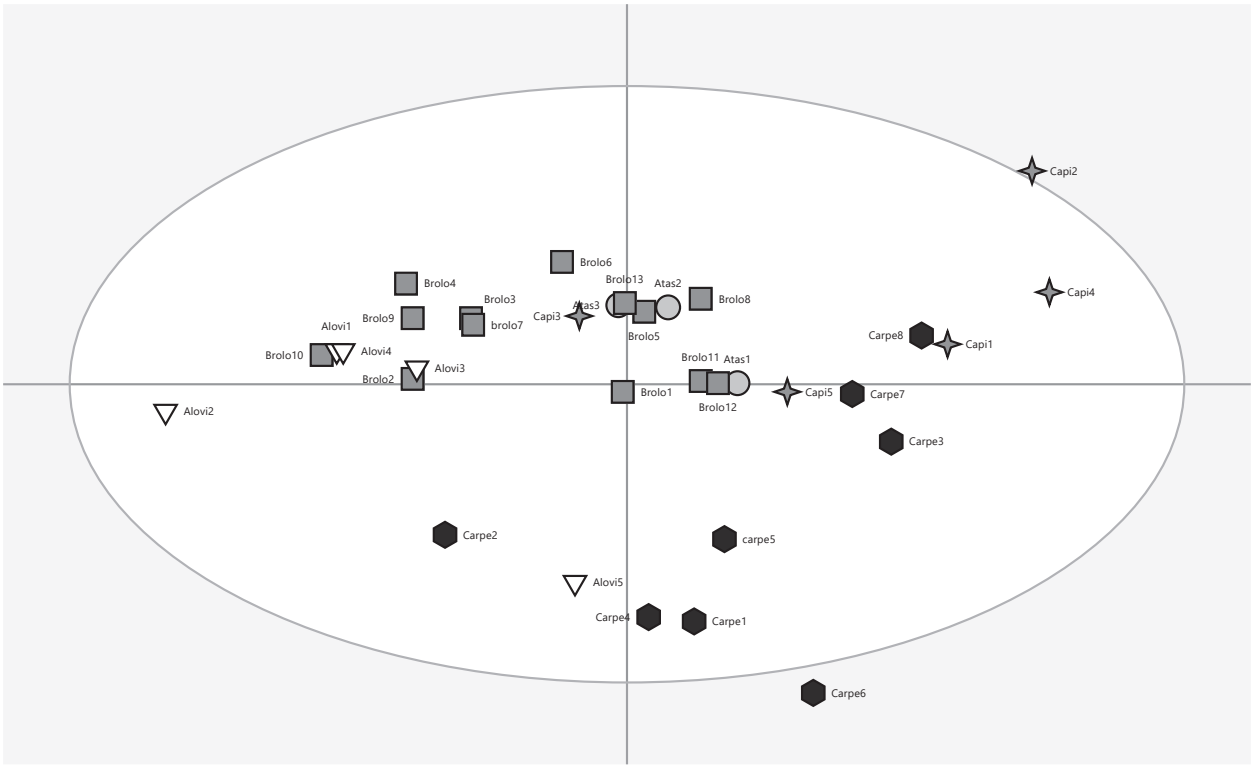

Fig. 2. Principal Component Analysis (PCA) of five fish species groups: Brolo, Alovi, Carpe, Antassa (Antas), and Capitaine (Capi). Spatial classification of fish groups based on fatty Acid profiles could be observed.

method could facilitate the monitoring of fatty acid profiles in freshwater fish from the region across different seasons.

Supervised multivariate analysis of fish fatty acid profiles

To identify the fatty acids contributing to the differences among various fish groups, a supervised PLS-DA analysis was conducted, considering four of the most consumed fish groups in Togo: Carp, Brolo, Capitaines, and Alovi. The projection distinctly separates Alovi from Brolo and Carp from Capitaines, consistent with previous PCA results (Fig. 6). To validate the model, permutation analyses comprising 100 permutations were performed, resulting in a negative Q2 prediction coefficient (Fig. 6**b**). Utilizing variables important for projection (VIP > 1.5) from different features, fatty acids that distinguish among the various fish groups consumed in the sub-region were identified. In total, 12 fatty acids were identified as characteristic of the different fish groups (Table 4). Among these, 7 are saturated, 1 is monounsaturated (MUFA), and 4 are polyunsaturated. Contrary to its EPA + DHA content, the Alovi group exhibits notably higher levels of saturated and branched fatty acids (Table 4), in addition to arachidonic acid (omega-6). The Carp group showed notably higher levels of 7,10,13,16,19-docosapentaenoic acid (DPA), a specific omega-3 fatty acid. Lastly, Brolo and Capitaines were characterized by elevated levels of stearic and oleic acids. It is also noteworthy that branched fatty acids were among the discriminants. These findings emphasize the need to be cautious about the saturated fatty acid composition, especially in Alovi, despite its richness in fatty acids.

The high percentage of saturated and branched fatty acids in freshwater fish may confer an advantage in the salting process [30]. The n-3 docosapentaenoic acid (n-3 DPA) is a less studied polyunsaturated fatty acid compared to its counterparts, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Current interest in DPA arises from its potential to increase EPA and DHA tissue levels and its specific or shared biological effects. Evidence suggests that DPA serves as a source of EPA and, to a lesser extent, DHA in major metabolic organs [31]. Moreover, DPA acts as a precursor to a wide array of lipid mediators (protectins, resolvins, maresins, isoprostanes), primarily involved in inflammation resolution with distinct effects compared to other PUFAs [32,33]. Recent findings highlight DPA involvement in improving cardiovascular and metabolic disease risk markers, including plasma lipid parameters, platelet aggregation, insulin sensitivity, and cellular plasticity [34]. Carps, which exhibit high levels of DPA, may be recommended for certain patients.

Reports indicate that branched-chain fatty acids levels in fish typically remain below 5 %, although they exhibit significant variation. For instance, levels range from as low as 0.3 % to 1.5 % in fish caught near Senegal, to an exceptionally high 40 % in flathead grey mullet (*Mugil cephalus*) found in a mangrove estuary. Chinese carp species raised for consumption showed branched-chain fatty acids levels ranging from 1.8 % to 4 %, while common carp (*Cyprinus carpio*) caught in Madagascar exhibited branched-chain fatty acids levels of 4 % to 5 %. Ongoing investigations highlight the active nature of branched-chain fatty acids, which demonstrated biological advantages including intestinal microbiota development [35] and antitumor properties [25,36].

In summary, our study elucidates the diverse fatty acid composition of freshwater fish species from Africa, highlighting their nutritional significance. Through rigorous lipid extraction and GC–MS analysis, we identified varying lipid levels and fatty acid profiles among the studied species, showing the importance of informed dietary choices. Multivariate analysis facilitated the categorization of fish species based on their fatty acid compositions, offering insights for dietary diversity. Additionally, our research sheds light on the less explored role of docosapentaenoic acid (DPA) and the biological significance of branched-chain fatty acids in freshwater fish. Overall, our findings contribute to understanding the nutritional value of African freshwater fish, emphasizing their potential role in addressing nutritional deficiencies and promoting public health.

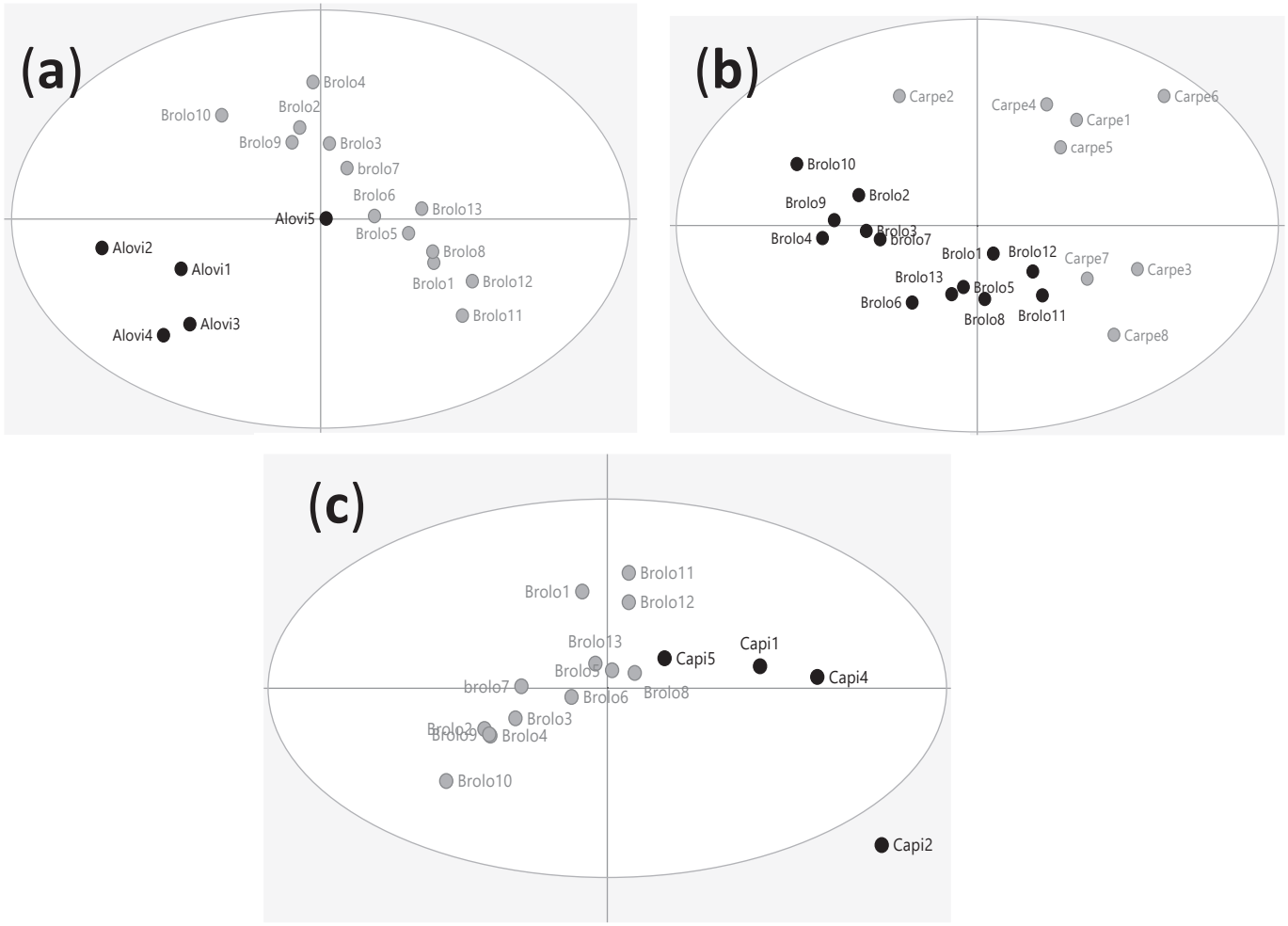

Fig. 3. PCA unsupervised classification two by two of the main groups of fish under study. Fish species are identified by their local names: Brolo, Alovi, Carpe and Capitaine (Capi). **(a)** Brolo vs Alovi, **(b)** Brolo vs Carpe and **(c)** Brolo vs Capi.

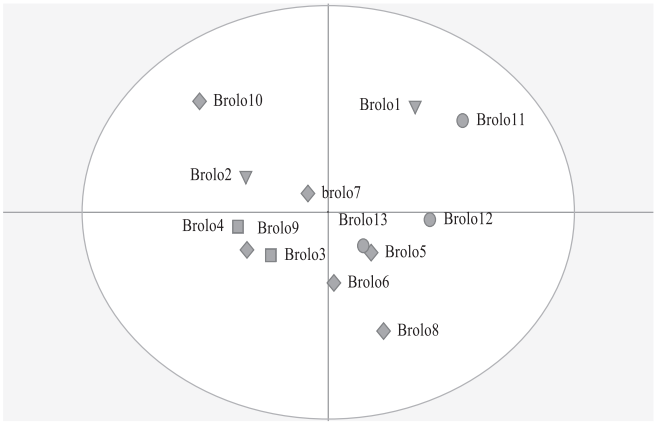

Fig. 4. PCA projection of same group of brolo for geographical fatty acid variation in the lake. The Brolo group was caught at three distinct locations within the lake: Zone 1 (Brolo 1–4), Zone 2 (Brolo 5–8), and Zone 3 (Brolo 9–12).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

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

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT-3.5 in order to enhance language expression. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

CRediT authorship contribution statement

Kodjo Eloh: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Affo Dermane:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Amakoé Adjanke:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Amana Adjaoute Tousso:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Oumbortime N'nanle:** Writing – review & editing, Validation, Supervision, Project administration, Investigation. **Gaston Kujoou Wolofer Tidiye:** Writing – original draft, Software, Investigation, Data curation.

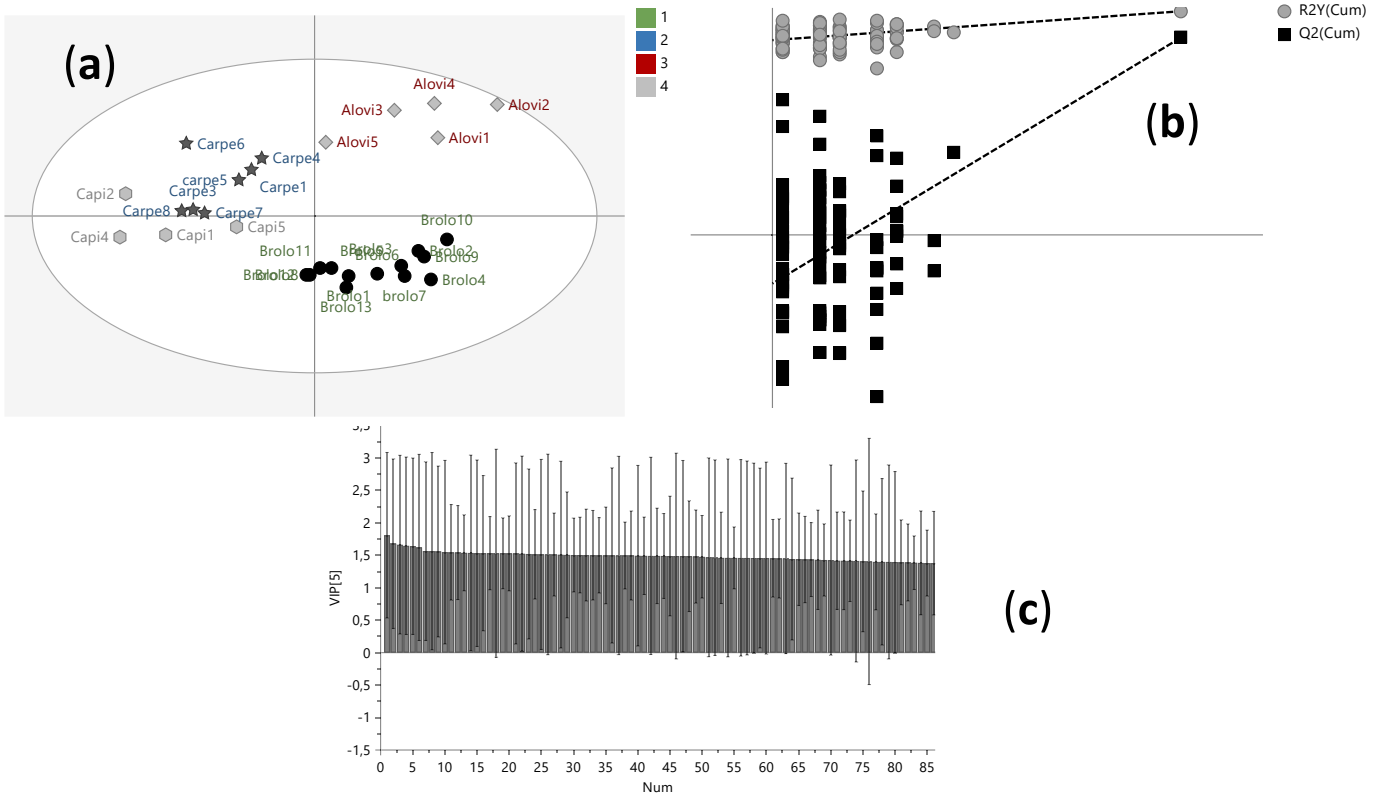

Fig. 5. (**a**) Partial Least Squares Discriminant Analysis (PLS-DA) depicting the primary four fish groups: Carp, Capitaine (Capi), and Brolo, illustrating distinct separation in the Hotelling's space. (**b**) Permutation validation of the model. (**c**) Plot showing Variables Important to the Projection with VIP > 1.5.

Table 4
Discriminant fatty acids between fish species groups. The fish species are grouped into 4 categories: "Alovi" comprises *Mugil bananensis*; "Blolo" consists of *Auchenoglanis occidentalis* and *Chrysichthys nigrodigitatus*; "Capi" includes *Lates niloticus*; and "Carpe" encompasses *Tilapia rendalli*, *Oreochromis niloticus*, *Tilapia guineensis*, *Sarotherodon nigripinnis,* and *Tilapia zillii*.

N°	RT	Name	Lipid number	Relative air percentage medium of the main features of fatty acids			
				Alovi	Blolo	Capi	Carpe
1	25.83	Pentadecanoic acid	C15:0	**78**	10	6	7
2	27.75	14-methylpentadecanoic acid		**77**	3	1	18
3	31.07	14-methylhexadecanoic acid		**89**	3	2	6
4	33.48	γ-linolenic acid	C18:3(n-6)	**51**	16	5	28
5	34.33	Oleic acid,	C18:1(n-9)	14	**38**	24	24
6	35.18	Stearic acid	C18:0	21	**30**	**30**	20
7	36.73	14-methyloctadecanoic acid		**81**	8	4	7
8	39.81	*cis*-11,14-Eicosadienoic acid	C20:2 (n-6)	23	**45**	12	20
9	40.67	Eicosanoic acid	C20:0	26	**43**	15	16
10	41.53	Arachidonic acid	C20:4(n-6)	**65**	12	4	19
11	44.34	7,10,13,16,19-docosapentaenoic acid (DPA)	C22:4(n-3)	16	22	20	**43**
12	45.91	Docosanoic acid	C22:0	**22**	47	14	17

Tchilabalo Abozou Kpanzou: Formal analysis, Visualization, Writing – review & editing. **Pierluigi Caboni:** Writing – review & editing, Methodology, Investigation, Data curation, 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.

Data availability

All relevant data are available upon request from the corresponding author.

Acknowledgements

The authors express their gratitude to Prof. Kokou TONA, Director of the "Centre d'Excellence Régional sur les Sciences Aviaires," for generously providing the laboratory equipment and reagents essential for this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2024.101515.

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