CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences


# Screening of PAH concentrations and determination of the total fat content and fatty acid profile of traditionally smoking fish in Central region of Cameroon

MASTER'S THESIS

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**Declaration:**

"I Kateřina Šrámková hereby declare that this thesis entitled Screening of PAH concentrations in traditionally smoked fish products from Cambodian rural areas, is my own work and all the sources have been quoted and acknowledged by means of complete references."

In Prague ……..

(Signature of student)

Bc. Kateřina Šrámková

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**Abstract:**

Fish is the preferred food among the Cameroonian population, and with the advancement of aquaculture, traditionally smoked fish is going to play an increasingly large role in the Cameroonian diet. Smoked fish is an important source of nutrients and essential fatty acids; however, also a source of dangerous polycyclic aromatic hydrocarbon exposure. While PAHs are known to be produced during the traditional fish smoking process, the fatty acid and PAH content in Cameroonian smoked fish have rarely been investigated before. During field research and interviews with smoked fish producers within tree Yaoundé markets, 16 samples of 8 different fish species have been collected and further investigated for their fat content, FA profile, and PAH profile. Fat content was determined using a Soxhlet extractor and derivatizated for FA analysis via GC-MS. PAHs were extracted by a modified QuChERS method with EMR-Lipid and DLLME in triplicate, subsequently analyzed by GC-MS, and quantified using the internal standard (IS) method. The interval of fat content ranged between 3.22 % to 24.58 %, with significant differences among species and also within a single species. This was explained by a different origin, diet, age, and level of movement for sampled fish, and applicable to the FA profile as well. Moisture content varied greatly (7.04 % - 53.22 %) due to the non-uniform methods of smoking, in particular uncontrolled temperatures and time ranges between 1 hour and 1 week of smoking. FA profiles showed generally lower NQIs within samples than is reported on average, along with a total absence of EPA. However, the content of essential FAs (ALA and LA) was above average. *Labeo coubie* had the most positive NQI values, while *Parachanna obscura* displayed the highest total content of LC-PUFAs (including all-important DHA). The highest PAH maximum levels (MLs) for traditional smoked fish given by EC 1881/2006 were exceeded by 6 - 19 times for BaP (31.33 $\mu g.kg^{-1}$ to 94.81 $\mu g.kg^{-1}$) and 7 - 25 times for the sum of 4 PAHs (208.02 $\mu g.kg^{-1}$ to 745.74 $\mu g.kg^{-1}$). This could lead to developing carcinogenic and other diseases related to PAH exposure. Further research focused on PAH profile, FA profile, and traditional smoking methods in Cameroonian smoked fish was recommended.

**Key words:** smoked fish, fat content, fatty acid profile, NQI, PAH, Cameroon

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**List of the abbreviations used in the thesis:**

| | |
|---|---|
| **ALA** | Alpha-linolenic acid |
| **ANVISA** | National Sanitary Surveillance Agency |
| **BaP** | Benzo[a]pyrene |
| **DHA** | Docosahexaenoic acid |
| **DLLME** | Dispersive Liquid-Liquid Microextraction |
| **DPA** | Dipicolinic acid |
| **EFs** | Emission factors |
| **EFSA** | European Food Safety Authority |
| **EMA** | European Medicines Agency |
| **EMR-Lipid** | Efficient lipid/Matrix Removal |
| **EPA** | Eicosapentaenoic acid |
| **(US) EPA** | US Environmental Protection Agency |
| **FA** | Fatty acid |
| **FAME** | Fatty acid methyl ester |
| **FAO** | Food and Agriculture Organisation of the United Nations |
| **FDA** | U.S. Food & Drug Administration |
| **FID** | Flame ionization detector |
| **FTT** | FAO-Thiaroye fish processing technique |
| **GC** | Gas Chromatography |
| **HACCP** | Hazard Analysis and Critical Control Points |
| **HPLC** | High-Performance Liquid Chromatography |
| **IARC** | International Agency for Research on Cancer |
| **IFAD** | International Fund for Agricultural Development |
| **LA** | Linoleic acid |
| **LC-PUFAs** | Long-chain Polyunsaturated fatty acids |
| **MLs** | Maximum levels |
| **MS** | Mass Spectrometry |
| **MUFA** | Monounsaturated fatty acid |
| **NQI** | Nutritional quality index |
| **PAHs** | Polycyclic Aromatic Hydrocarbons |
| **PCBs** | Polychlorinated biphenyls |
| **PCDDs** | Polychlorinated dibenzo-p-dioxins |
| **PCDFs** | Polychlorinated dibenzofurans |
| **PUFA** | Polyunsaturated fatty acid |
| **QuEChERS** | Quick, Easy, Cheap, Effective, Rugged and Safe method |
| **SCF** | The European Union Scientific Committee on Food |
| **SFA** | Saturated fatty acid |
| **TEF** | Toxicity Equivalency Factor |
| **TEQ** | Carcinogenic toxic equivalent |
| **UFA** | Unsaturated fatty acid |
| **UV** | Ultraviolet |
| **WHO** | World Health Organisation |

# 1 Introduction

Cameroon is located in central Africa with a territory of 475,442 $km^2$ and a population of about 25.2 million inhabitants in 2018 (World Bank 2019a). In many developing countries aquaculture fisheries and fish are essential for food security of their population. The fisheries industry has over 65,000 employees in Cameroon. Inland fisheries alone are estimated to employ more than 31,000 workers, who are also involved in the production of dried and smoked fish (FAO 2019a). In Cameroon fish is the preferred protein source of the most underprivileged levels of society due to its low cost (1-6 $USD.kg^{-1}$). Additionally, it is available in conveniently small units that can be easily purchased by the poor, with small portions of smoked fish sold in markets (Kaktcham et al. 2015). National average fish consumption was 15.4 $kg.pp^{-1}$ in 2013 (FAO 2019a). The annual consumption of fish in Cameroon from 2010 to 2013 was estimated to be over 300,000 tons (FAOSTAT 2016). However, the fisheries in Cameroon only produced around 239,000 tons in 2015, with 75,000 tons coming from inland waters and 164,000 tons from marine waters, and small pelagic accounting for about 67 percent of the total marine catch (FAO 2019a). Cameroon is experiencing a steady decline in national production of capture fisheries which contributes less than 1 % to GDP and aquaculture less than 0.1 % (Temegne & Momo 2019).

Aquaculture production is still very low. The total production level in 2015 was estimated at 840 tons and only 6,000 people in both full and part-time employment were reported for aquaculture. However, annual aquaculture potential is about 20,000 tons (FAO 2019a). Therefore, to address the production deficit, according to OEC (2017) Cameroon spends close to 200 million USD each year for imports of fish. This makes up 3.2 % of the total Cameroonian import value and the numbers are rising. The African Development Bank (2019) extended a loan of 93.4 million USD to Cameroon to support livestock and fish production in 2018, a project that is co-funded by the Cameroonian government with 17 million USD. However, the demand for fish is increasing due to a growing population (2.8 % annually) which consequently causes a significant increase in fish prices and rapid

urbanization. Demand for fish is estimated to be over 400,000 tons from 2015 and beyond (Temegne & Momo 2019; FAOSTAT 2018), which will be increasingly costly to the Cameroonian government. Nonetheless, conditions for fish culture in Cameroon are good, with a good climate suitable for the rearing of many warm water species, appropriate soil for pond construction, natural inland waters covering over 40,000 square kilometers, and freshwater fish suitable for aquaculture (Ponzoni et al. 2008).

In response to the above mentioned situation the Cameroonian government, headed by the Minister of Livestock, Fisheries and Animal Industries, cooperates with development partners on aquaculture promotion and expansion of its production, such as the International Fund for Agricultural Development (IFAD), the Food and Agricultural Organization (FAO), and collaborates with the Ministry of Small and Medium-sized Enterprises, Handicraft and Social Economy (Cameroon Tribune 2018). Cameroon plans to produce around 100,000 tons of fish per annum by developing aquaculture, thanks to the construction of intensive production centers with modern ponds and the training of young fish farmers (Business in Cameroon 2014).

Therefore, the quality of fish product from local processing is going to be more important as a part of the common diet among the population. Moreover, with the spreading of aquaculture production, it will be possible to control various factors influencing the quality of local fish products over the course of the animal's life cycle.

In general, low living standards prevail in Cameroon. People face problems such as the absence of electricity, which according to the World Bank (2019b) only 60 % of the population has access. Because of this reality, the old traditional ways of processing are the most popular methods. Smoking is one of the oldest traditional methods of food preservation and it is quite common in Cameroon. Fresh farmed fish is also very popular. With higher demand than supply, fish are rarely processed but unsold fish tend to be smoked (FAO 2019a). Fish processing is mostly dominated by smoking and drying, which accounts for 75 to 80 % of the category and contributes about 16.8 thousand million to the country's economy (Nyebe et al. 2015). Smoking techniques have been used for centuries

as a method for preserving meat and fish. Smoking impregnates the high-protein food with aromatic components which lend flavor and color to the food, and also play a bacteriostatic and antioxidant role (FAO & WHO 2009).

During the smoke-curing process, fish accumulates naturally produced chemicals such as phenols, aldehydes, acetic acids, and a range of polycyclic aromatic hydrocarbons (PAHs) resulting from the thermal breakdown of wood (Basak et al. 2010). PAH compounds are a very well-known class of natural toxins that are harmful to human health, with some known to be carcinogenic (Vazquez Troche et al. 2000; Kishikawa et al. 2003; Janoszka et al. 2004; Yusty & Daviña 2005). The formation of PAHs is known to occur through pyrolysis of fat at temperatures above 200 °C. PAHs are lipophilic in nature and usually accumulate in the fatty tissues of organisms, and as such are produced from the fatty tissues of fish during smoking at higher temperatures. Fish with a higher content of fat are expected to show higher content of PAHs after smoking process (European Commission 2002).

Based on the above information and thanks to little general knowledge about this topic in Cameroon, this work examines the quality of smoked fish products and the process of smoking from the smallholders in the capital city Yaoundé. Particularly, fatty acids (FAs) and polycyclic aromatic hydrocarbons (PAHs) were targeted.

# 2 Literature review

## 2.1 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are the largest class of chemical compounds known to be cancer causing agents due to their mutagenicity and toxicity. Some of them which are not carcinogenic themselves may have a synergistic effect when combined. PAHs can be found in the whole environment - in water, air, soil, and therefore also in food. Most of them are produced from human activity, such as wood-burning and combustion of other biofuels such as dung or crop residues, and contribute more than half of annual global PAH emissions, particularly due to biofuel use in India and China (Šimko 2002; Ramesh et al. 2011; Zhao et al. 2013). They originate from heterogeneous sources and can be found in engine exhaust, petroleum distillates, and derived products of coal. They can be released during volcanic eruptions, from incineration of municipal refuse, and during cooking. PAHs are formed by the incomplete combustion or pyrolysis of organic matter, therefore they are generated wherever fossil fuels or vegetation are burned. PAHs are also one of several classes of carcinogenic chemicals present in tobacco smoke. Combustion sources are the primary source of PAH generation (Šimko 2002; FAO & WHO 2009; Farhadian et al. 2010; Ramesh et al. 2011). Food can be contaminated in literally any stage of processing and handling as well as due to the presence of these compounds in the environment. PAHs are often formed directly in food as a result of certain heat processes like charcoal grilling (Fretheim 1983; Pánek & Jehličková 1995; Mottier et al. 2000), roasting (De Kruijf et al. 1987; Jägerstad & Skog 2005), smoke drying (Afolabi et al. 1983), and smoking (Wu et al. 1997; Chen & Lin 1997; Phillips 1999; Mottier et al. 2000; Chen & Chen 2005). Another frequent way of contaminating food is through contaminated additives (Šimko 2002) and migration from contaminated packaging (Grob et al. 1991; Šimko et al. 1995).

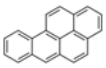
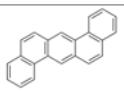
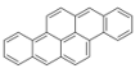
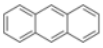
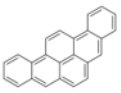
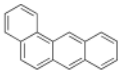
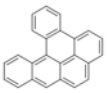
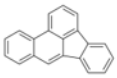
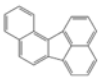
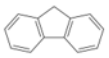
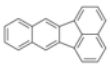
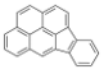
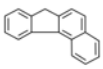
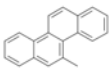
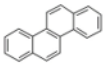
PAHs are a group of compounds composed of multiple fused aromatic rings (organic rings in which the electrons are delocalized) and by definition are composed of hydrogen and carbon with a strong lipophilic character (Douben 2003; Šimko 2005). PAHs which contain

up to four fused benzene rings are called light PAHs, and those which contain more than four benzene rings are known as heavy PAHs. Light PAHs are more volatile, water soluble, and less lipophilic than the heavy PAHs (Wild & Jones 1995; Ferrarese et al. 2008). Heavy PAHs are more stable and more toxic than light ones (Wenzl et al. 2006). As molecular weight increases the solubility of PAHs in water generally decreases, while their boiling and melting point increases correspondingly (Prabhukumar & Pagilla 2010). Aromatic hydrocarbons of four or five rings such as chrysene and benzo[a]pyrene are practically insoluble in water (Whittle et al. 1982).

Table 1 describes sixteen PAHs which are on the priority pollutants list of the US Environmental Protection Agency (EPA), which should be monitored in the environment. Those are heavy highly stable contaminants in air, water, soil, and food (Zhao et al. 2013) and are known as potentially genotoxic carcinogens to humans. Their carcinogenicity is caused by their metabolic conversion in mammalian cells to diolepoxides that bind covalently to cellular macromolecules, including DNA, causing errors in DNA replication and mutation (Phillips & Grover 1994). The European Union Scientific Committee on Food (SCF) has identified 15 PAH compounds as genotoxic carcinogens which contribute between 1 % to 20 % of total carcinogenic effects found in smoked products (Scientific Committee on Food 2002; Swastawati et al. 2007). The European Union (EU) has identified 16 PAHs as hazardous to health (European Commission 2011). Another marker of toxicity is the so-called “Sum of PAH4”, which has been regulated since September 2012 by the EU for maximum levels in food. It is the sum of the most “risky” PAHs: benz[a]anthracene, chrysene, benzo[b]fluorantene, and benzo[a]pyrene.

PAH derivatives are ubiquitous atmospheric pollutants with toxic, mutagenic, and carcinogenic properties. They include nitrated, oxygenated, and hydroxylated derivatives: NPAHs, OPAHs, and OHPAHs (Schuetzle 1983; Salmeen et al. 1984).

**Table 1** Names and structures of PAHs frequently monitored according to recommendations by the EU Scientific Committee for Food (SCF), the European Union (EU), and the US Environmental Protection Agency (EPA) (European Commission 2011)

| List | Common Name | Structure | List | Common name | Structure |
|---|---|---|---|---|---|
| EPA, SCF, EU | Benzo[a]pyrene |  | EPA,SCF,EU | Dibenz[a,h] anthracene |  |
| EPA | Acenaphthene |  | SCF, EU | Dibenzo[a,e] pyrene |  |
| EPA | Acenaphthylene |  | SCF, EU | Dibenzo[a,h] pyrene |  |
| EPA | Anthracene |  | SCF, EU | Dibenzo[a,i] pyrene |  |
| EPA,SCF,EU | Benz[a] anthracene |  | SFC, EU | Dibenzo[a,l] pyrene |  |
| EPA,SCF,EU | Benzo[b] fluoranthene |  | EPA | Fluoranthene |  |
| SCF,EU | Benzo[j] fluoranthene |  | EPA | Fluorene |  |
| EPA,SCF,EU | Benzo[k] fluoranthene |  | EPA, SCF, EU | Indeno[1,2,3-cd]pyrene |  |
| EU | Benzo[c]fluorene |  | SCF, EU | 5-Methyl chrysene |  |
| EPA,SCF,EU | Benzo[ghi] perylene |  | EPA | Naphthalene |  |
| EPA, SCF,EU | Chrysene |  | EPA | Phenanthrene |  |
| SCF, EU | Cyclopenta [cd]pyrene |  | EPA | Pyrene |  |

### 2.1.1 PAH extraction and determination

There are several methods of PAH extraction and determination which are generally accepted according to the guidelines of official organizations, such as the U.S. Food & Drug Administration (FDA), European Medicines Agency (EMA), and the National Sanitary Surveillance Agency (ANVISA) (Locatelli et al. 2014). For example, the FDA proposed method for analysis of PAHs in sea food is QuEChERS-Based Extraction and High-Performance Liquid Chromatography (HPLC) with Fluorescence Detection (Gratz et al. 2017). PAHs have a very

characteristic signature on the UV absorption spectrum. As each ring structure has a unique UV spectrum, each isomer exhibits a unique UV absorbance spectrum. This is especially useful in the identification of PAHs. Most PAHs are also fluorescent, emitting characteristic wavelengths of light when they are excited (Masih et al. 2010).

Extracts are measured by either HPLC or Gas Chromatography (GC) depending on the nature of the sample and its volatility with the detectors: mass spectrometer (MS), ultraviolet (UV), or fluorescence spectrophotometers (Li et al. 2003; EMA 2016).

For the extraction and determination of PAHs this thesis used a combination of the QuEChERS extraction method with a clean-up step by pre-concentration EMR-Lipid and DLLME techniques. For the final quantification GC-MS was used. In a very recent study experiment by Slámová (2020) this method was successful and provided purified samples at the same time as acceptable recoveries of PAHs in smoked fatty products.

### 2.1.2 Process of accumulation of PAHs in smoked fish

Raw fish material usually does not contain high levels of PAHs, as this level of contamination depends on the environmental background of fish and the handling process before the smoking process (Abdel-Shafy & Mansour 2016).

When it comes to the smoking process, the rate of PAH accumulation in smoked food depends upon the type of smoke generator, degree of smoking, temperature, smoking time, distance between product and smoke source, humidity, flow rate and density of the smoke, the water solubility and volatility of the particular compounds, as well as the properties of the surface of the fish (Foster 1957; Jägerstad & Skog 2005; Hokkanen et al. 2018). According to some studies smaller fish species contained higher levels of PAHs after the smoking process than larger fish species, which could be caused by the fact that small fish are more heavily smoked due to the smaller surface area-to-volume ratio (Lawrence & Weber 1984; Duedahl-Olesen et al. 2010). Cameroonian fish markets mainly offer small smoked fish because the bigger and therefore higher priced pieces are more difficult to sell and the

majority of fish production is sold fresh. Those small fish are mostly consumed with the skin, which contains more PAHs than the rest of the fish body. As the smoke reaches the surface of the fish many contaminants are caught in the skin, which acts as a barrier to penetration by smoke particles (Wretling et al. 2010; Pöhlmann et al. 2013). If the fish skin is removed before smoking, the accumulation of PAHs is greater as the PAHs can easily migrate into the underlying fatty tissue due to their fat solubility. The water activity and fat content have a significant role in the diffusion process (Fasano et al. 2016; Hokkanen et al. 2018).

As fish are high in fat content and PAHs are lipophilic there is a hypothesis that melted fat from the heated fish dripping on the hot wood or coals is pyrolyzed, giving rise to PAH generation which are then deposited on the fish surface as the smoke rises (Jägerstad & Skog 2005; Ezike & Ohen 2018). Generally, it is said that the more intense the heat, the more PAHs are present (Larsson 1986). It is not surprising that direct smoking generates higher concentrations of BaP and PAH4 than indirect smoking. The European Commission reported in 2004 an average BaP concentration of 5.3 $\mu g \cdot kg^{-1}$ for directly smoked fish, whereas for indirectly smoked fish the BaP concentrations ranged from 0.1 $\mu g \cdot kg^{-1}$ to 2.0 $\mu g \cdot kg^{-1}$ (EFSA 2004).

### 2.1.3 Toxicity of PAHs and related health risks for humans

The International Agency for Research on Cancer (IARC 2010) classifies some PAHs as known, possibly, or probably carcinogenic to humans (Group 1, 2A or 2B). Group one, which is proved to be carcinogenic for humans, includes only benzo[a]pyrene, while group 2A and 2B are PAHs possibly carcinogenic to humans (IARC 2010; Adeniji et al. 2017). Some PAHs are well known as carcinogens, mutagens, and teratogens and therefore pose a serious threat to the health and well-being of humans. The most significant health effect to be expected from inhalation exposure to PAHs is an increased risk of lung cancer (Kim et al. 2013).

Seven of the pyrogenic PAHs (with 4–7 rings) from the groups 1, 2A and 2B have been identified by the US EPA and the International Agency for Research on Cancer (IARC) as

being a great risk to humans (UNEP 2002; Dahle et al. 2006; IARC 2010; Hussein et al. 2016). These seven PAHs are synergistically genotoxic when combined with benzo[g,h,i]perylene, which on its own is not classified as carcinogenic to humans (Yan et al. 2004). For this group of PAHs, Toxicity Equivalency Factors (TEFs) were established to determine their level of toxicity. Benzo[a]pyrene is ten times as toxic as the second ranked PAH as is shown in Table 2 (EPA 1982; Nisbet & LaGoy 1992; Adeniji et al. 2017).

**Table 2** Toxicity Equivalency Factors (TEFs) of the carcinogenic PAHs (data source: Adeniji et al. 2017)

| **PAH congeners** | **TEF** |
|---|---|
| Benzo[a]pyrene | 1 |
| Benzo[a]anthracene | 0.1 |
| Benzo[b]fluoranthene | 0.1 |
| Benzo[k]fluoranthene | 0.1 |
| Dibenzo[a,h]anthracene | 0.1 |
| Indeno[1,2,3-cd]pyrene | 0.1 |
| Chrysene | 0.01 |

Carcinogenic toxic equivalents (TEQs) are used for risk characterization and health risk assessment and management purposes, such as prioritizing areas of clean-up and environmental health risks. TEQs report the toxicity-weighted masses of mixtures of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and PAHs. They are calculated from the TEF specific value, which is multiplied by the mass of each chemical in a mixture and is then summed with all other chemicals to report the total toxicity-weighted mass (EPA 2000). Exposure to health risk is primarily through the ingestion of animal products such as meat, dairy, fish, and human breast milk. The human diet accounts for over 95% of the total uptake of TEQs (WHO 1998).

Negative health effects from exposure to PAHs depend on how much has entered the body, how long the person has been exposed to PAHs, and how the body responds to PAHs. These effects may be either short-term or long-term, while it is not clear whether the short-term effects such as eye irritation, nausea, vomiting, diarrhea, and confusion are caused by PAHs or other compounds commonly found with PAHs (e.g. in smoke) (IDPH 2020; SA Health 2020).

Long-term health effects of exposure to PAHs may include cataracts, kidney and liver damage, and jaundice. Repeated skin contact to the PAH naphthalene can result in redness and inflammation of the skin. Breathing or swallowing large amounts of naphthalene can cause the breakdown of red blood cells.

Long term exposure to some PAHs including benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-c,d]pyrene have caused cancer in laboratory animals. Studies of workers exposed to mixtures of PAHs and other compounds have noted an increased risk of skin, lung, bladder, and gastrointestinal cancers. The information provided by these studies is limited because the workers were exposed to other potential cancer-causing chemicals beyond PAHs. Although animal studies have shown adverse reproductive and developmental effects from PAH exposure, these effects have generally not been seen in humans (ATSDR 1995; IDPH 2020; SA Health 2020).

### 2.1.4 Limits of PAH content in food

The European Union Scientific Committee on Food (SCF) has identified 15 PAH compounds in 2002 as genotoxic carcinogens as has been shown in Table 1 above. Namely they are benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, chrysene, cyclopenta[c,d]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indenol[1,2,3-cd]pyrene, and 5-methylchrysene. Benzo[a]pyrene has the highest carcinogenic value of all the PAH compounds (European Commission 2011; Tiwo

et al. 2019). Special attention in various studies was paid to benzo(a)pyrene, but in 2008 the European Food Safety Authority (EFSA) concluded that this alone is not a good enough indicator for the monitoring of occurence of PAHs in food. Therefore, the concept of PAH4 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) and PAH8 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) replaced the monitoring of benzo[a]pyrene, although PAH8 does not provide too much added value in comparison to PAH4 (EFSA 2008; Food Safety Authority of Ireland 2015). Commission Regulation (EC) No 1881/2006 is the framework EU legislation which sets maximum levels (MLs) for chemical contaminants in foodstuffs. MLs for PAHs have been set for the most affected food groups; for instance, smoked meat and smoked meat products, smoked fish and smoked fish products, oils, and fats. These MLs are set at a very low level (as low as reasonably achievable for the particular foodstuff in question) in order to reduce adverse effects on the health of consumers (The Commission of the European Communities 2006).

In accordance with actualized findings, the legislation was updated and separate maximum limits are now set for benzo[a]pyrene and PAH4. For smoked fish and smoked fishery products, maximum limits of benzo[a]pyrene up to 2.0 $\mu g.kg^{-1}$ and PAH4 up to 12.0 $\mu g.kg^{-1}$, are accepted as of 1$^{st}$ September 2014 (The European Commission 2011).

However, it has been shown that despite the application of good smoking practices the lower maximum levels coming into force in September 2014 were too strict in some cases. The lower levels for PAHs are not achievable in several EU Member States where the traditional smoking practices used for smoked meat and meat products and fish and fishery products cannot be changed without significantly changing the organoleptic characteristics of the foods. If the low MLs for PAHs were applied across the board, the traditionally smoked products would disappear from the market, resulting in the closure of many small and medium sized enterprises, which was considered as disproportionate to the low risk presented by the trace presence of PAHs in these foods. Therefore, the Commission granted

a three-year derogation for local and traditional production of smoked meat and smoked fish products by certain member states who followed the less strict regulations before September 2014 (The European Commission 2014; Food Safety Authority of Ireland 2015). The newest proposal after evaluation of MLs of PAHs in 2019 is considering establishing a higher limit with unlimited time for compliance for those exception countries (Ireland, Latvia, Romania, Finland, Sweden, and the United Kingdom for certain cases of traditionally smoked fish and smoked fishery products) and local and traditional foodstuffs. These exceptional MLs of PAHs for smoked fish and fishery products are 5.0 $\mu g.kg^{-1}$ for benzo[a]pyrene and 30.0 $\mu g.kg^{-1}$ for PAH4 (The European Commission 2011; The European Commision 2019).

## 2.2 Fatty acids

Fatty acids (FAs) are important components of lipids, characterized as fat-soluble components of living cells in plants, animals, and microorganisms. In biochemistry, a fatty acid with a carboxyl group makes it carboxylic acid, which contains a long aliphatic chain that is either saturated or unsaturated. If the carbon-to-carbon bonds are all single, the acid is saturated; if any of the bonds are double or triple, the acid is unsaturated and is more reactive. Unsaturated FAs are further divided into monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). Essential fatty acids, which cannot be made by the human body, include linoleic acid (C18:2 n-6) and alpha-linolenic acid (C18:3 n-3) and are required for normal growth and development as well as physiologic function of body systems (Moghadasian & Shahidi 2017). Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28 (Venkata Mohan et al. 2015). Fats and oils, which are nutritionally a major source of energy, are present naturally in many foods such as dairy products, meats, poultry, fish, and nuts, as well as baked goods, margarines, dressings, and sauces. Dietary Guidelines for Americans recommend a total fat intake between 20 % and 35 % of calories for adults to meet daily energy and nutritional needs. Intake of fat outside this range is not recommended for most individuals because of the potential adverse effects on achieving recommended nutrient intake levels and on risk

factors for chronic diseases (USDA 2015; Moghadasian & Shahidi 2017). Creation of fatty acids is made by so-called fatty acid synthesis from acetyl-CoA and NADPH through the action of enzymes called fatty acid synthases. This process takes place in the cytoplasm of the cell. Most of the acetyl-CoA which is converted into fatty acids is derived from carbohydrates via the glycolytic pathway (Dijkstra et al. 2008).

### 2.2.1 Saturated Fatty Acids

Saturated fatty acids are not essential and contain only single carbon–carbon bonds in the aliphatic chain while all other available bonds are taken up by hydrogen atoms. They are chemically low reactive and their melting point increases with chain length. Saturated FAs are predominantly found in butter, margarine, shortening, coconut and palm oils, as well as foods of animal origin, and their excessive intake can raise cholesterol levels. The American Heart Association's Nutrition Committee recommends the amount of saturated fat intake to be less than 7 % of total calorie intake (Krauss et al. 2000; Moghadasian & Shahidi 2017).

### 2.2.2 Unsaturated Fatty Acids

When the fatty acids contain one carbon–carbon double bond in the aliphatic chain they are called monounsaturated. In general, these fats are liquid at room temperature but start to solidify when refrigerated (Venkata Mohan et al. 2015; Moghadasian & Shahidi 2017). A major nutritionally important MUFA is oleic acid. MUFA sources are olive and canola oil, avocados, peanuts, nuts, and seeds. They have certain health benefits over saturated fatty acids and it is currently recommended that MUFA and PUFA sources are consumed more frequently than foods rich in saturated fat or trans fat, but within the restriction of 20–35 % of total calories from fat (Moghadasian & Shahidi 2017).

The major classes of PUFAs are the Omega-3 (n-3) and omega-6 (n-6) fatty acids. Omega-3 fatty acids are a type of PUFA containing more than two double bonds. They differ from other fatty acids because of the location of the first double bond in the aliphatic chain. The omega-3 fatty acids that are most important nutritionally are alpha-linolenic acid (ALA),

which is essential, as well as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA can be synthesized by the human body in very small amounts from ALA. Dietary sources of Omega-3 FAs are flaxseed oil, canola oil, soybean oil, walnuts, and sea foods, particularly fatty fish. The most common omega-6 essential fatty acid is linoleic acid, which is found in many vegetable oils, cereals, snack foods, and baked goods. Omega-6 FAs are recommended for lower intake than Omega-3 FAs (National Research Council (U.S.) 1989; Moghadasian & Shahidi 2017) but the ratio should not be too high, with the ideal between 4:1 and 1:4 (Simopoulos 2002), or 3:1 to 1:1 as present another author (Kim et al. 2007). However, more important is the absolute amount of omega-3 and omega-6 FAs.

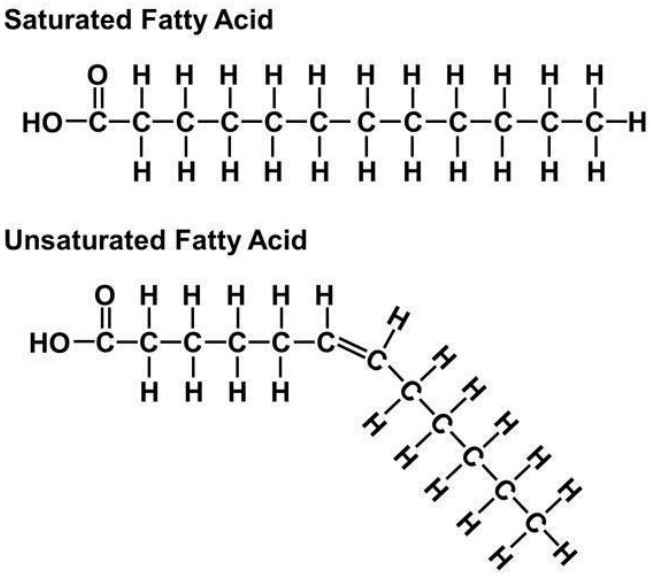



**Figure 1** Saturated and unsaturated fatty acids (Dallas County Community College District 2020)

### 2.2.3 Fatty acids and fat content in fish

Fish is one of the best sources of animal protein due to the composition of fish proteins compared to other animals. Fish contains a more favorable amino acid composition and significant amounts of free amino acids (Özogul et al. 2006; Toppe et al. 2007; Buchtová et al. 2010), as well as all essential amino acids the human body needs (Vladau et al. 2008). The vitamin and mineral content in meat of freshwater fish is very favorable (Özyurt et al. 2009). The lipid content differs depending on the type of fish, season, and what the fish is

fed with (Guler et al. 2008; Ćirković et al. 2011). Generally, the composition of saturated and unsaturated fatty acids in fish is 15-36 % saturated FAs (Ackman 1989; Buchtová et al. 2007; Zakes et al. 2010) and 58-85 % unsaturated FAs (Domaizon et al. 2000; Caballero et al. 2002). Fish and marine animal fat is an important source of PUFAs, which are important for human health. It has a unique nutritional value because it is the only source of n-3 long chain polyunsaturated fatty acids (LC-PUFA) like EPA, DHA, and dipicolinic acid (DPA) (Regulska-Ilow et al. 2013). However, essential EPA and DHA are originally synthesized by microalgae, not by the fish. When fish consume phytoplankton that consumed microalgae, they accumulate the omega-3s in their tissues (Coates et al. 2010). Beside the PUFAs, fish fats contain similar amounts of cholesterol (49–92 mg.100 $g^{-1}$) as pork or beef (45–84 mg.100 $g^{-1}$) and cholesterol content does not correlate with fat content (Piironen et al. 2002). Generally freshwater fish is lower in cholesterol compared to marine fish, though levels depend on the species. Therefore, freshwater fish in the diet is more favorable for human health (Moreira et al. 2001; Luzia et al. 2003; Ćirković et al. 2011).

According to a study by Kaya and teammates (2008), during the smoking process the temperature and the wood smoke components negatively affect the fatty acid composition, especially EPA, DHA, and some essential amino acids. On the other hand, a study from Rahimabadi et al. (2016) shows opposite results, with amounts of EPA and DHA increasing after smoking. However, this increasing trend of essential fatty acids is in conflict with other studies from Beltrán & Moral (1991) and Swastawati (2004).

The composition of a particular fish species often appears to vary from one fishing ground to another and from season to season, but the basic causes of change in composition are usually variations in the amount and quality of food that the fish eats as well as the amount of movement it makes (Burt & Murray 2001). Taking all species into account, the fat content of fish can vary much more widely than the moisture, protein, or mineral content. Whilst the ratio of the highest to the lowest value of protein or water content encountered is not more than three to one, the ratio between highest and lowest fat values is more than 300

to one (FAO 2001). Generally, fresh fish contains 0.1-22 % of fat in wet weight (Abraha et al. 2018).

Thermal treatment reduces the water and fat content in fish meat. During the smoking process fats and water drip from the fish resulting in the physical loss of lipids, protein, and micronutrients (Kiczorowska et al. 2019). However, the fat loss phenomenon was more intensive in boiled and solar dried fish than in smoked samples. Fat may exude with the moisture evaporation through extended heat treatment (Abraha et al. 2018).

### 2.2.4 Fatty acids extraction and determination

The main problem in determining accurate FA composition is the extraction of lipids, which is dependent on the binding of FAs to the matrix of the sample. For isolating lipids from the tissues there are different extraction methods using various solvents or mixtures of solvents. One of the most widely used methods for the extraction of lipids is that proposed by Soxhlet (Horwitz & AOAC International 2006; ISO 2016a, 2016b). This method is simple and efficient, but a disadvantage is its long duration and use of large amounts of solvent, which is usually petroleum ether. Other methods were proposed by Folch et al. (1956), Bligh & Dyer (1959), and Hara & Radin (1978), as well as other contemporary extraction methods like pressurized liquid extraction (PLE) and accelerated solvent extraction (ASE) (Schäfer 1998; Dodds et al. 2004). Some researchers have prepared *in situ* direct methylation of FAs without lipid extraction and purification steps (Carrapiso & García 2000; Meier et al. 2006; Polak et al. 2008; Ichihara & Fukubayashi 2010). In this research the improved Soxhlet extraction method was used.

FAs are generally analyzed after derivatization into fatty acid methyl esters (FAMEs). The classical method for FA determination after an extraction step is methanolysis, which produces FAMEs (Parrish et al. 2015). Lewis et al. (2000) suggested a direct transesterification method on freeze-dried microalgae (thraustochytrids), as well as its possible use for other animal tissues which are suitable for larger amounts of samples due to the lower time and cost needed. The esterification reaction involves the condensation of

the carboxyl group of an acid and the hydroxyl group of an alcohol. Esterification is best done in the presence of a catalyst (such as boron trichloride) and in this study esterification was performed with the use of petroleum ether and sodium hydride at room temperature. The last step in the of analysis of FAMEs is quantitative measuring by gas chromatography (GC). Options here are GC with a flame ionization detector (FID) (Zhang et al. 2015; Danish & Nizami 2019) or GC mass spectrometry (MS) (Jayasinghe & Dias 2013; Ren et al. 2013), which was used for this research.

## 2.3 Smoking technology and pre-treatment processes

Fresh fish are highly perishable and start to spoil as soon as they are caught. Spoilage occurs as the result of the action of enzymes (autolysis) and bacteria present in the fish as well as chemical oxidation of the fat, causing rancidity (Akinola et al. 2006). At the high temperatures prevalent in tropical countries bacterial and enzymatic actions are enhanced, and fish can become putrid within a few hours. With appropriate handling and processing it is possible to reduce post-harvest fishery losses, which negatively affect local food security (National Research Council (U.S.) 1988). Post-harvest losses of fish in some developing countries exceed those of any other commodity, often surpassing 50 percent of the landed catch. The losses are highest in the countries whose populations have the lowest protein intake. Reducing these losses could increase protein availability, improve nutritional status, and eliminate some of the need to import food (Diei-Ouadi & Mgawe 2011).

Smoking is a process of flavoring, browning, cooking, or preserving food by exposing it to smoke from burning or smoldering material, most often wood. It is a preservation method to prolong the shelf life of food due to components of the smoke inhibiting growth of some microorganisms. The most common products for smoking are meat, fish, and smoked tea (Fellows 2016).

Smoking technology can be divided into direct and indirect methods, and traditional or commercial means. Smoking can be generally done in four ways. There is cold smoking, warm smoking, hot smoking, and through the use of "liquid smoke". However, these

methods of imparting smoke only affect the food surface and are unable to fully preserve food, thus, smoking is paired with other microbial inhibitors such as chilling and packaging to extend food shelf-life (Fellows 2016). Fish are also often salted before they are smoked. Another and the most used traditional way - smoke drying - mentioned by Joardder and Hasan Masud (2019), involves temperatures between 45-85 °C where fish is firstly cooked and then dried. Smoke contains substances that kill bacteria, thus helping to preserve the product and the heat also dries the fish. In tropical countries, fish are generally heavily smoked at relatively high temperatures so that they are also cooked (National Research Council (U.S.) 1988). Samples for this research were hot-smoked and smoke-dried as is typical in Cameroon along with other tropical countries.

During the smoking process PAHs are formed as a result of incomplete combustion of organic matter. Codex Alimentarius, the only international body responsible for the development of standards, codes of practice, guidelines, and recommendations for food smoking, has made PAHs a concern and published a specific code of practice in 2009. Codex Alimentarius found the variables that can lead to the formation of PAHs during the smoking and drying processes. These include type of fuel, smoking method (direct or indirect), smoke generation process, distance between the food and the heat source, position of the food in relation to the heat source, fat content of the food and what happens to it during processing, duration of smoking, temperature during smoking, cleanliness and maintenance of the equipment, design of smoking chamber, and how the equipment used for smoke influences the smoke density in the smoking chamber (FAO & WHO 2009).

Concern for quality of fish products should begin on board the vessel or at the first moment the fish is caught. Fish should be brought aboard alive and in good condition with hygienic handling and chilled as soon as possible. Although spoilage can never be prevented through chilling or cooling alone, the cooler the fish are the greater the reduction in bacterial and enzymatic degradation. For each 5 °C increase in storage temperature above 0 °C, there is a significant reduction in shelf life. Fish that can be stored for two weeks at 0 °C may only last a day or two at 10 °C (National Research Council (U.S.) 1988). Whole fish should first be

washed to remove loose scales and slime, then gutted and beheaded (if required). The belly cavity should be cleaned to remove traces of blood and any black belly wall lining removed (FAO & WHO 2009).

### 2.3.1 Salting

Salting is one of the important pre-treatments for flavor addition and preservation of the smoked product. Presence of sufficient quantities of common salt (sodium chloride) can significantly retard bacterial growth by lowering water activity. Salting has been used for thousands of years to preserve marine products. It has no adverse effect on the value of fish protein (Hall 1997; FAO & WHO 2009).

When fish is placed in a brine solution the salt penetrates the fish and water is extracted from the tissues by osmosis. At a salt concentration of 6-10 percent in the fish the activity of most bacteria that causes spoilage will be inhibited. Since fish contain 70-80 percent water, the strength of brine used must be adjusted accordingly (FAO & WHO 2009). The higher the salt concentration in the fish, the longer its storage life. A minimum concentration of 3 % has been found to be effective for hot smoked fish, particularly mackerel and trout (Bannerman 2001). Several methods of salting are commonly used: dry salting, kench salting, brine salting, and pickle salting (FAO & WHO 2009).

For tropical environments wet-salting methods such as brining and pickling are recommended, especially with fatty fish. Halophilic or salt-tolerant bacteria or molds may grow on incompletely dried salted fish or on dry salted fish that have become moist. However, pickle-cured fish are free of halophile growth because these organisms are aerobic and the brine of pickle-cured fish does not contain sufficient oxygen to support their growth. This oxygen-poor environment also reduces rancidity in fatty fish (FAO & WHO 2009).

### 2.3.2 Kilns and alternative dryers

Most traditional kilns used for smoke drying are very simple in design and construction. They range from the simplest type, which is an open fire where fish are laid on a grill above, to a mud or rush hut in which the fish are placed on racks above a fire. The main disadvantages for most traditional types of smoke drying kilns are a lack of control over the fire temperature and smoke production, inefficient use of fuel, and a low throughput of material (International Labour Office Geneva et al. 1982). A simple kiln design often used for smoking in some developing countries is a steel oil drum oven. Control of temperature in these types of ovens is challenging and eventually results in non-uniformly smoked food products. Moreover, these types of ovens are very sensitive to wind and rain (Joardder & Hasan Masud 2019).

**Figure 2** Barrel ovens in Guinea (FAO 1994)

Another common type is a traditional mud oven, popular due to the low cost of construction and easy availability of materials. The capacity of this traditional oven is small and there is high smoke loss (Joardder & Hasan Masud 2019).

A Chorkor smoker is an improvement on the traditional rectangular oven, where fish are placed inside on removable trays as well as on top of the oven. Advantages are a larger capacity and reduced fuel consumption, but initial costs are higher and it is less suitable for smoking fish of different sizes (FAO 1994). Chorkor ovens using acacia charcoal as fuel—compared to barrel kilns using either mango tree charcoal, acacia wood, or mango tree wood—were shown by Kpoclou et al. (2014) to be the only combination producing a product with PAH levels below current maximum European Union limits for safety.

**Figure 3** Traditional smoking kiln - Chorkor oven (Onyango et al. 2017)

A type of oven used often in West Africa is called a “banda”, which can be a simple open design or an improved closed version. An open banda is usually a table of chain-link wire fencing that is supported from the ground by a framework of mangrove or other available

wooden forked poles. The improved closed banda is made with mud blocks, with compacted clay or flattened metal barrel sheets used as walls around a rectangular fireplace (FAO 1994). Other popular improved kilns are the Altona-type kiln shown in Fig. 5 and cinder block kilns.

**Figure 4** Closed banda kiln - Flattened barrel banda (FAO 1994)

In 2017, The United Nations (FAO) engaged in a collaborative undertaking with the National Training Centre for Fish and Aquaculture Technicians in Senegal (CNFTPA) to design and validate the FAO-Thiaroye fish processing technique (FTT). This technique is not based on a new type of oven but simply small improvements on the advanced ovens, such as the Chorkor, banda, Altona, and cinder block types. The FTT was designed mainly for controlling PAHs and reduces fuel consumption, exposure to heat, burns, and smoke.

Alternatively, using solar dryers for preservation of fish confers certain advantages over the traditional drying and smoking kilns, such as protection from pests and no need for fuel (Akinola et al. 2006); however, solar drying is unable to provide the popular smoky flavor and this could be reflected negatively in the price compared to smoked products.

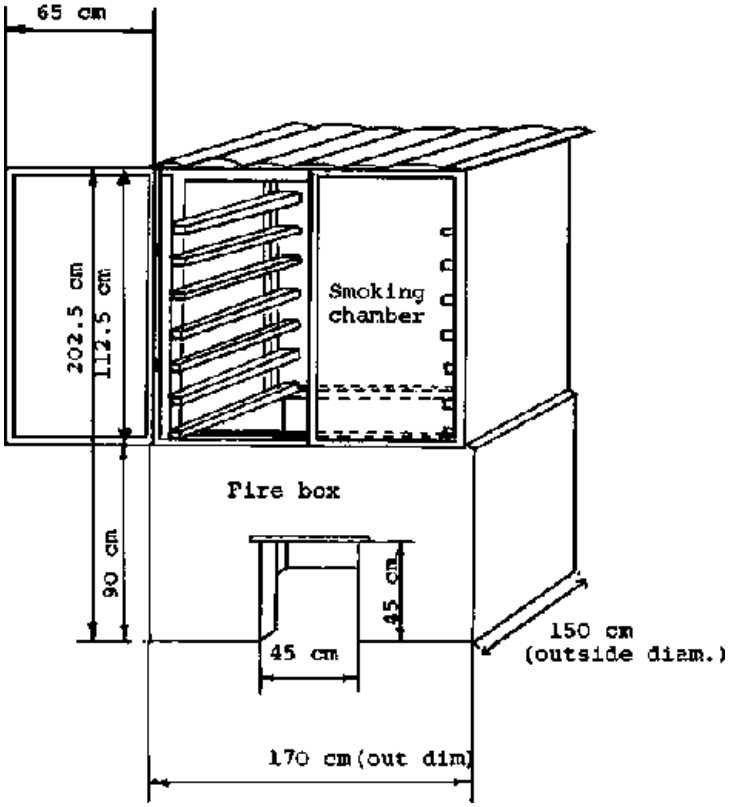



**Figure 5** Simple version of Altona-type oven with fire box built from clay (International Labour Office Geneva et al. 1982)

### 2.3.3 Fuel

Conditions of smoke generation can dramatically influence the level of PAHs in smoked foods (Tóth & Potthast 1984). PAH profiles generated during combustion are closely related to combustion fuel type. This is demonstrated in Table 3 with PAH profile differences between coal, gasoline, diesel, and wood fuels. Wood is used most often for smoking and there are differences in profiles between the types of wood as well (Khalili et al. 1995; Stumpe-Vīksna et al. 2008; Lee 2010; Forsberg et al. 2012). Suitability of individual species of wood and other plant materials for smoking processes should be evaluated in relation to PAH formation before use. Generally, it is recommended to use hardwood for smoking

(Šimko 2005; Essumang et al. 2013). Additionally, the wood to be used for smoking should preferably not be resinous (FAO & WHO 2009).

**Table 3** The emission factors (EFs) of PAHs from the different sources (Lee 2010)

| Emissions source | Emission Factor (mg/kg) | PAHs | References |
|---|---|---|---|
| Wood combustion | 16.4-1,282 | total PAHs | Schauer et al., 2001 |
| Rice burning | 9.29-23.6 | 16 PAHs | Lu et al., 2009 |
| Bean burning | 3.13-49.9 | 16 PAHs | - |
| Wood and root fuel | 5.3-13.2 | B[a]P | Gupta et al., 1998 |
| Two-stroke fuel-no catalyst | 0.021 | 6 PAHs | Gambino et al., 2000 |
| Two-stroke fuel-with catalyst | 0.014 | - | - |
| Oil-burner-boiler combination | 0.005 | B[a]P | IPCS, 1998 |
| Barbecue briquettes | 2.5-13 | total PAHs | - |
| Soot open fire | 3-240 | B[a]P | - |
| Boilers using heavy oil | 0.013 | total PAHs | Li et al., 1999 |
| Boilers using diesel | 0.3 | - | - |
| Feed-stock | 0.077-3.970 | total PAHs | Yang et al., 1998 |
| Feed-stock | 0.002-0.016 | B[a]P | - |
| Coal charged | 15 | Total PAHs | IPCS, 1998 |
| - | 0.02 | B[e]P | - |
| Aluminum production | 4.4 kg/tons | total PAHs | - |
| - | 0.11 kg/tons | B[a]P | - |
| Bituminous coal | 70.2 | total PAHs | Chen et al., 2005 |
| Scrap tire pyrolysis plant | 4 | - | Chen et al., 2007 |
| Honeycomb briquette | 56.94 | - | Oanh et al., 1999 |
| Open burning biomass fuels | 5-683 | 19 PAHs | Jenkins et al., 1996 |

Results obtained by Potthast (1979) show that the PAH concentration found in smoke coming from softwood (pine) and from hardwood (beech) are very similar. However, some softwoods like pines and firs hold significant quantities of resin which produce a harsh-tasting soot when burned. Furthermore, it has been reported that resinous and Euphorbia fuelwood species produced poisonous smoke while emitting resin acids and other organic

compounds, and therefore must not be used for fish smoking (Maddison et al. 1993; Berkel et al. 1995). They are also characterized by higher burning rates, resulting in very hot flames and a short, local drop of oxygen concentration during combustion. Softwood is often more resinous (Fine et al. 2002), while hardwoods are mostly made up of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are the basic material of the wood cells while lignin acts as a kind of cell-bonding glue, which produces fewer dangerous compounds during combustion.

Fuelwood around rural fishing communities in the tropics is often scarce, expensive, or found at great distance—sometimes over a hundred kilometers away. This problem can be caused by deforestation or overgrazing (Yerima & Ngulde 2015). Mangrove wood is the main resource used for fish smoking in most areas of Cameroon other than Kribi (Dongmo Keumo Jiazet 2019) despite the fact that globally, mangrove forests are the most threatened tropical ecosystem and are being degraded and depleted at alarming rates (Polidoro et al. 2010).

Fuels other than those mentioned above include: sawdust, dried cassava peels, wood shavings, bagasse (plant material from sugarcane), corn cobs, and coconut husk and shell (Akinola et al. 2006; FAO & WHO 2009).

### 2.3.4 Smoke

Smoke is made during combustion or pyrolysis, together with the quantity of air that is entrained or otherwise mixed into the mass. To produce smoke for smoking food, flames should be avoided by adjusting airflow. Smoke consists of liquid and solid particles suspended in a gaseous phase. These particles, generally a size of 0.2–0.4 µm (or as low as 0.05 to 1 µm), are estimated to constitute 90 % of its overall weight. The chemical composition of smoke is a complex mixture of more than 300 components (FAO & WHO 2009). The exact composition of smoke depends on various conditions, such as: the procedure and temperature of smoke generation, the origin and composition of the burning fuel, the water content in wood, and the conditions of combustion (Šimko 2005).

During fuel combustion many chemical contaminants in smoke are formed. For example: polycyclic aromatic hydrocarbons (PAHs), dioxins, formaldehyde, and nitrogen and sulphur oxides (e.g. relevant for formation of nitrosamines). Furthermore, heavy metals are also found in combustion gases. The types and amount of contaminants depend on the fuel used, the temperature, and other parameters (FAO & WHO 2009).

The method of smoke generation and the smoking process used (Cardinal et al. 2001) have a considerable influence on the sensory characteristics of smoked fish, particularly on smoke flavor perception (Cardinal et al. 2006). However, Cardinal et al. did not establish a relationship between sensory properties and the chemical composition of smoked fish, particularly the composition of odorant compounds provided by smoke.

### 2.3.5 Smoking

The art of fish smoking, said to be as old as civilization, is often divided into three groups depending on the temperatures used during the process. Cold smoking uses temperatures approximately between 18–25 °C and is used for some fish species (e.g. salmon) and salami-type sausages. Semi-warm smoking uses temperatures of approximately 30–40 °C and is used for some fish species, bacon, and pork loin. Finally, warm (or hot) smoking uses temperatures of approximately 70–90 °C and is used for some fish species, hams, and frankfurter type sausages (FAO & WHO 2009).

Hot smoking is generally comprised of the following three processes: First is cooking, since the smoking is done at temperatures above 70 °C. Bannerman (2001) describes hot smoking as curing fish by smoking at a temperature of 70-80 °C at some stage in the process in order to cook the flesh, and therefore hot smoked fish products do not require further cooking before consumption. The second process is drying, because the fire which produces the smoke also generates heat and in turn dries the fish. If the moisture content of fresh fish is reduced to around 25 %, bacteria cannot survive and autolytic activity will be greatly reduced; however, to prevent mold growth the moisture content must be reduced to 15 % (Akinola et al. 2006). The third process is the smoking itself. As the smoke is produced by

burning wood containing a number of compounds, some of which kill bacteria, the process has a preservative value.

Hot smoking usually takes 4-12 hours. This is long enough to eliminate non-sporulating spoilage bacteria and to deactivate enzymes in the guts and flesh. However, the spores of *Bacillus subtilis* and *Bacillus mesentericus* survive even with longer periods of smoking. The bactericidal action of the smoke is considerably increased by the presence of salt in the fish (FAO & WHO 2009). Factors which affect the rate of drying are heat, humidity, air velocity, air exchange, flesh characteristics, and flesh thickness. The rate that smoke deposits on fish depends on smoke density, air circulation, humidity, temperature, and nature of the surface (Hilderbrand 1992).

Last but not least, smoking processes are divided into direct and indirect methods. Direct smoking is a process where the smoke is accumulated in the same chamber in which the food is processed. Indirect smoking is a process where smoke generators are used and the smoke is in a chamber separate from where the food is smoked. The smoke may be cleaned in various ways, e.g. by use of a water filter or a tar condenser, before being fed into the smoke chamber (FAO & WHO 2009).

However, the traditional small-scale way of fish smoking generally differs from commercial methods thanks to the equipment available due to incomparable financial resources. The ability to monitor the smoking process, and therefore the quality of food product, differs greatly. Additionally, the HACCP and Codex Alimentarius are often not followed in small-scale traditional smoking. Still, there are financially feasible implementations and methods that have been researched for small-scale smoking to achieve high quality products (FAO 1994; FAO & WHO 2009).

## 2.4 Fisheries in Cameroon

Aquaculture production, which at large scale may be helpful for the Cameroonian economic and food security situation, is still negligible and underdeveloped. Fish farming is still poorly established and far from realizing its great potential (FAO 2019a).

Over fifteen years ago, small-scale fish farms and semi-extensive aquaculture systems were common in Cameroon. In the 80s and 90s many foreign-funded projects were established to improve aquaculture in Cameroon, but unfortunately without sustainable success (FAO 2004). Small-scale grow-out ponds were usually integrated with other farm activities. Most of the ponds were located in rural areas on land belonging to owners with or without formal property rights. Fish culture seldom constituted a major part of household income but was, in many cases, very important to the household diet. Production was low and discouraging for many farmers, who remain at the subsistence level. Farmers also had a lack of common knowledge on how to breed fish. Diagnostic surveys conducted in the Noun, Menoua, Meme, and Lekié divisions in the equatorial climatic zone of Cameroon found not a single farmer who was satisfied with fish output weighed against the effort involved in pond construction, seed stocking, and fish feeding and care (CIRAD 2004; Pouomogne et al. 2010; Ćirković et al. 2011).

According to Mindjimba et al. (2019) Cameroon saw a total fishery production of 226 thousand tons in 2015, of which 36 thousand tons—16 % of total fish production—were processed (dried, salted or smoked). Among processed products, smoking is the preponderant conservation method for fish products (75 %) in Cameroon, followed by drying and salting (Pouomogne et al. 1998).

According to Cho Achu (2009), the origin of smoked and dried fish from Yaoundé markets comes from two principal ponds—the river pond/dam (lakes) of Lagdo in the North of Cameroon, and Mappé in the western province of Cameroon. The chain of distribution of fresh fish is relatively short as compared to other products due to the conservation methods

and the nature of the product, which is highly perishable (Kleter 2004; Cho Achu 2009; FAO 2017).

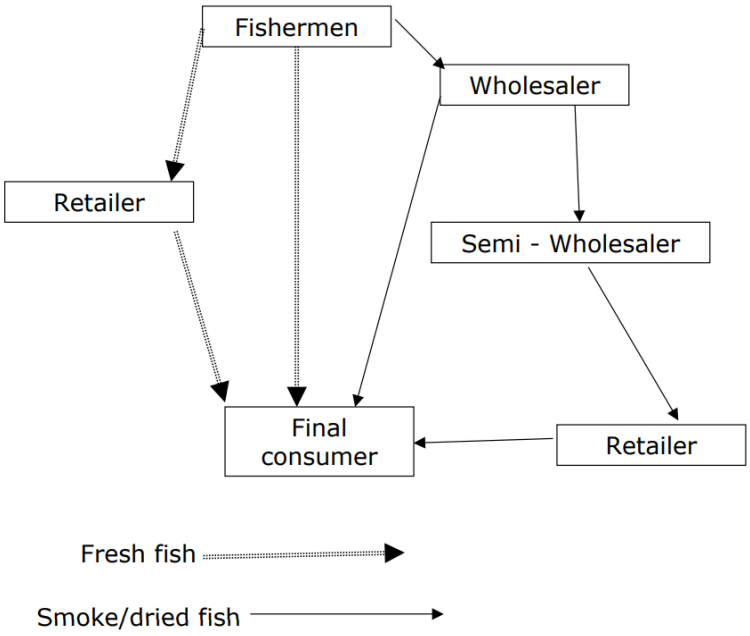



**Figure 6** Diagram showing smoked and fresh fish marketing channels in Cameroon (Cho Achu 2009)

According to FAO FishStat (2019), the capture of marine fish in Cameroon is increasing while the capture of inland fish is decreasing (Fig. 7). One of the fish species in this research is Atlantic cod (*Gadus Morhua*) even though it is not a native species, as the FAO confirms the presence of this fish in the dietary table of West Africa (FAO 2019b). It is becoming more and more attractive (along with other wild species) for aquaculture due to overfishing and decline of their availability in the wild (Van de Nieuwegiessen 2009). Most of the marine fish caught in Cameroon is processed by smoke-drying by fishermen's wives (FAO 1994).

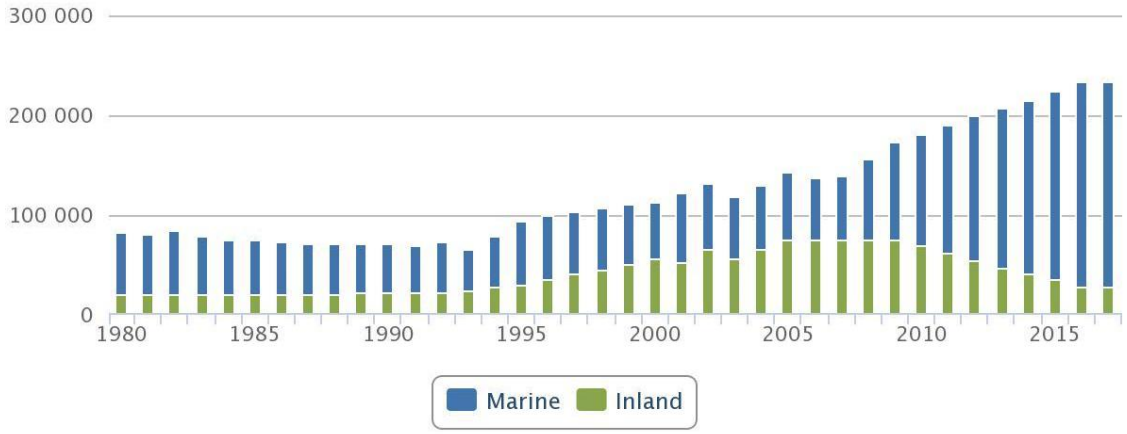



**Figure 7** Capture production by inland and marine waters for the Republic of Cameroon in tons (FAO 2019a)

As shown in Figure 8, the percentage share of general fish resources in Cameroon is mainly split between frozen fish from import and smoked fish from artisanal fisheries.

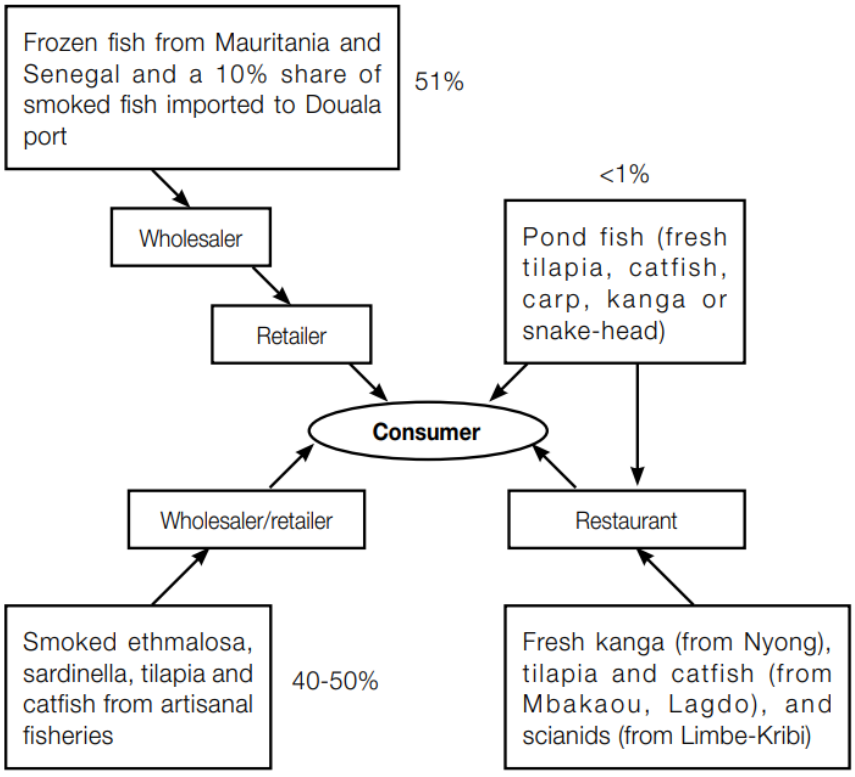



**Figure 8** Fish market chains with percentage share in Cameroon (WorldFish 2008)

According to Ćirković et al. (2011), fish farmers are usually adults aged 40-50 years, married, and with relatively large households. Though ponds are generally owned by men, all members of the household are involved in fish-farming activities. Most of the fish-farming businesses were established after observing neighbors or following directives from traditional authorities or older relatives. More than a third of fish farmers are involved in pond aquaculture primarily for generating income, which can explain the high rate of abandoned ponds (more than 55 %) given the poor outcomes (on average less than 2,000 kg of fish per hectare per year). Discouraging results arise from poor pond design, as farmers have insufficient technical support (WorldFish 2008). A study from the Ndian and Fako department, in the south-west region of Cameroon, had all women fish smokers in their study sample (Dongmo Keumo Jiazet 2019). Most surveyed women were married (88 %), and many (especially foreigners) had a husband who was a fisherman and who supplied them with fish. Fish smokers' ages ranged from 20 to over 60. Primary school was the highest education level for most respondents (42.4 %), while 26 % had completed secondary level. The majority of fish smokers were from Cameroon (47.3 %), followed by Ghana (23.45 %), Nigeria (22.63 %), and Benin (6.6 %). Ghanaians were more represented in Idenau than in all other areas. Most respondents had inherited work spaces, equipment, and necessary skills from their mothers and learned smoking since a young age. 60.5 % of fish smokers had at least 10 years of experience in smoking.

About 52 % of Cameroonians live in rural areas and depend on smoked fish as main source of protein. In urban areas, some local dishes are preferred with smoked fish. The price of smoked fish compared to fresh fish is much higher. In local areas where markets are held only once a week, smoked fish is preferred because it can be conserved for some time (El Gamal 2014). A number of studies, namely from Ajonina & Usongo (2001), CTA (2008), Magagi et al. (2010), and Nyebe et al. (2015) observed that fish smoking is practiced mostly by women in West Africa and Cameroon.

### 2.4.1 Traditional smoking technology in Cameroon

Smoke kilns in Cameroon are traditional ovens of different types found in plank huts. Small-holders use rectangular mud ovens or rectangular/square metal ovens or a simple open system where the grid is laid on bricks from the two sides, the so-called open banda ovens (FAO 1994; Adeyeye & Oyewole 2016). In a study from southwest Cameroon, 99.6 % of fish smokers used a traditional oven (banda). These were suspended iron bandas, suspended wooden bandas, or circular ovens.


**Figure 9** Oven types in study site. (a) Suspended iron banda. (b) Suspended wooden banda. (c) Circular oven. (d) Cinderblock oven (Dongmo Keumo Jiazet 2019)

The smoking process often consists of two phases. First, the pre-drying which lasts six to seven hours and reduces the weight of the fresh fish by one third. Second, the smoking itself which lasts two to three days. During these days the fish is constantly turned in order to avoid burning. The smoked fish is then stacked on trays or in raphia baskets for sale (Nkematabong 2017). However, different authors claim the traditional way of smoking has

a duration of 7 hours, with temperatures up to 80 °C and no less than 70 °C (Tenyang et al. 2018; Tiwo et al. 2019). Firewood is mostly used for smoking because of its availability and for better coloring of the fish product. In a study from southwest Cameroon the most common trees used for fish smoking were analyzed, highlighting the issue of overuse of Red mangrove wood for fish smoking and its deforestation (Dongmo Keumo Jiazet 2019). Ali et al. (2011) observed the smoking-drying process in terracotta smoking rooms at Lagdo Lake in the north of Cameroon, where fish were smoked for 2-3 hours at 70-80 °C followed by mild smoking (30-35 °C) for 24-48 hours. Although they admit that the technology is not standardized and most parameters are uncontrolled. Moreover, the hygienic conditions of fish handling are questionable.

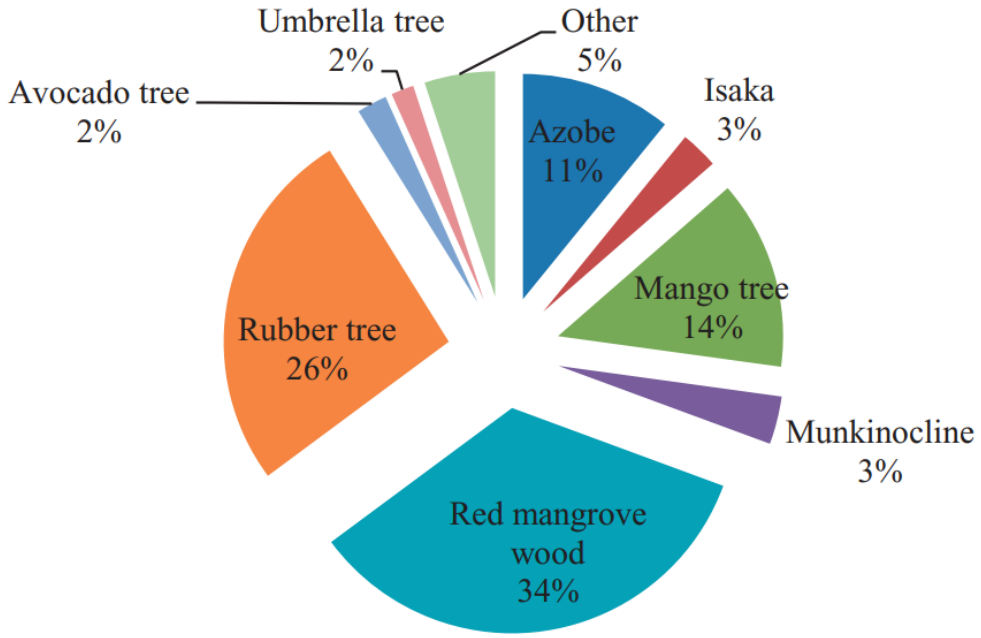



**Figure 10** Main tree species used for fish smoking in Southwest Cameroon (Dongmo Keumo Jiazet 2019)

**Figure 11** Open banda oven, for community use (Slámová 2017)

**Figure 12** Rectangular mud oven (Slámová 2017)

Air movement in a smokehouse is essential to the application of smoke and heat, as well as the removal of water from the product. Traditional smokehouses used natural (gravity) convection to circulate air (Hilderbrand 1992). Traditional methods of smoked food

preservation typically produced high salt and low moisture content products that are not desirable to most modern consumers.

As is seen frequently in fish markets, properly smoked fish products are dark brown in color and are nearly perfectly dried. This ensures that the shelf life is prolonged and the products get to the consumer in a relatively good state (Adeyeye & Oyewole 2016).

**Figure 13** Smokehouse (Slámová 2017)

### 2.4.2 Cameroonian Fish Species Used for Smoking

Many fish species are used in Cameroon for small-scale traditional smoking. The most typical Cameroonian smoked fish species are North African catfish (*Clarias gariepinus)*, Nile tilapia (*Oreochromis niloticus*), banded jewelfish (*Hemichromis fasciatus* and *Hemichromis elongatus*) (Ali et al. 2011; El Gamal 2014; Felix et al. 2018; FAO 2019a), Herring (*Clupea harengus*) (Cardinal et al. 2006), (Ali et al. 2011; Felix et al. 2018), Common carp (*Cyprinus*

*carpio*) (FAO 2019a; Tiwo et al. 2019), Kafue pike (*Hepsetus lineatus*), Smoothmouth sea catfish (*Carlarius heudelotii*) (Nyebe et al. 2015), Guinean sea catfish (*Arius parkii*) (Ali et al. 2011), and several fish species from genus *Pseudotolithus*.

This research examined six freshwater and two marine fish species. Due to fishery seasonality and time limitation for collecting samples, not all collected fish samples are typically offered during the year at the locality, and species are not represented in equal numbers. Studied samples are from *Clarias gariepinus, Alestes baremoze, Mugil cephalus, Labeo coubine, Parachanna obscura, Carlarius heudelotii, Brama dussumieri*, and *Gadus morhua*.

*Clarias gariepinus* is the most frequent fish species in this research and the most common smoke-treated species in Cameroon as well. It belongs to order Siluriforme and family Clariidae. It is a bony fish, characterized by a scaleless body and mandibular whiskers. Distribution of *Clarias gariepinus* is throughout Africa and the Middle East, living in freshwater lakes, rivers, and swamps, as well as human-made habitats such as oxidation ponds or even urban sewage systems. It is an endemic species and is used in polyculture with tilapia to reduce pond overloading (FAO 2019a).

**Figure 14** *Clarias gariepinus* (Smith et al. 1838)

*Gadus morhua* is a benthopelagic fish of family Gadidae. Its habitat is in the North Atlantic, so it is not an endemic species in Cameroon, but it was often mentioned in the interviews

as one of the main smoked fish species. Imports of Atlantic Cod increased in 2017 (FishBase 2020a; UN 2020).

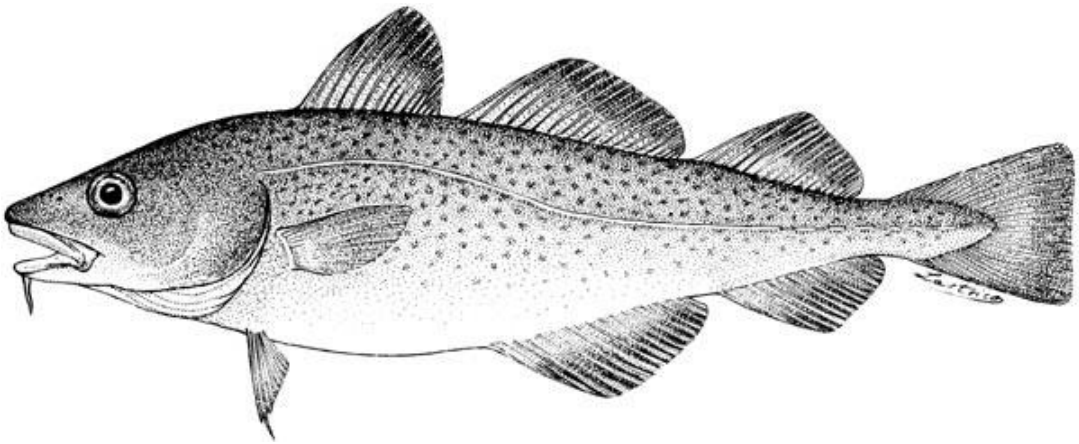


**Figure 15** *Gadus morhua* (FAO 2020)

*Alestes baremoze,* also known as Silversides, is another popular species for smoking. It is a characin fish from family alestidae and is distributed in the freshwater systems of northern and western Africa (FishBase 2020b).

**Figure 16** *Alestes baremoze* (FishBase 2020b)

*Parachanna obscura,* the African obscure snakehead from order anabantiformes and family channidae, is a medium-sized carnivorous fish found in central Africa along the western coastline from as far north as Senegal to as far south as Zaire and into central Africa into southwest Sudan (FishBase 2020b).

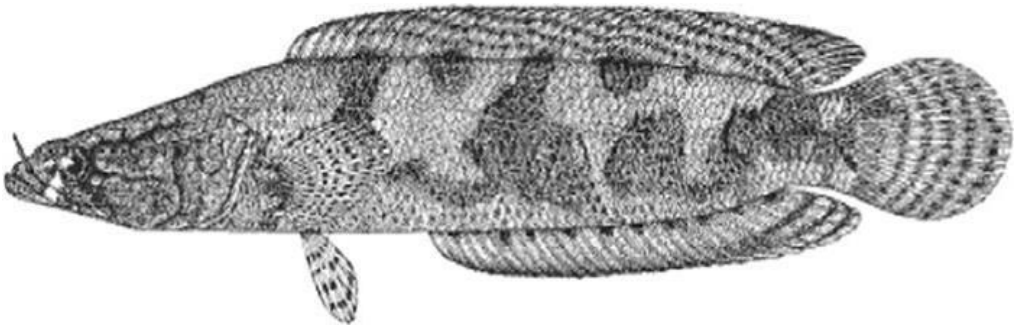


**Figure 17** *Parachanna obscura* (Wikipedia 2020)

*Brama dussumieri* is a species of Brama in the family Bramidae. This marine fish can be found in all tropical oceans (i.e. the Atlantic, Pacific, and Indo-Pacific) and associated seas (FishBase 2020b).

**Figure 18** *Brama dussumieri* (Slámová 2017)

*Mugil cephalus,* the Flathead grey mullet, is from family Mugilidae. It is cosmopolitan in habitat, living in coastal waters of the tropical, subtropical, and temperate zones of all seas (FishBase 2020b).

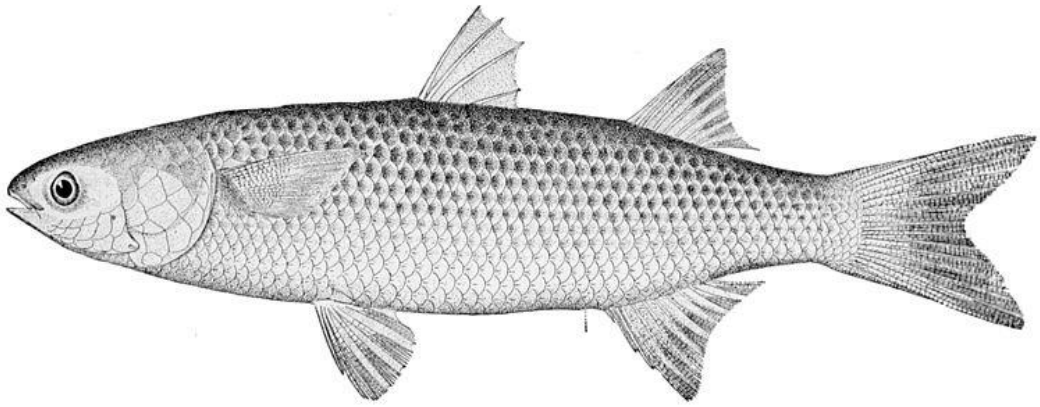


**Figure 19** *Mugil cephalus* (Brown Goode 1887)

*Carlarius heudelotii,* the Smoothmouth sea catfish from order Siluriformes and family Ariidae, occurs along the western coast of Africa from Mauritania to Gabon or Angola. It has also been reported from the Niger basin and the Benoué and Gambia Rivers (FishBase 2020b).

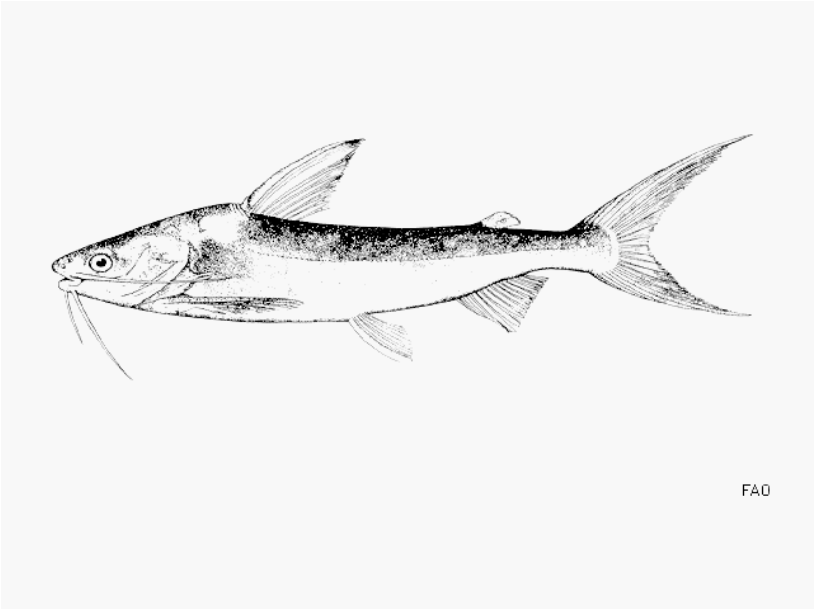



**Figure 20** *Carlarius heudelotii* (FAO 2019a)

*Labeo coubie,* the African carp, is a cyprinid fish widespread in Africa, where it occurs within the drainage basin of the Nile (Blue, White, Lake Albert) and in the Chad, Niger-Benue, Volta, Senegal, and Gambia Rivers, as well as the Cross River and Cameroon coastal rivers (FishBase 2020b).

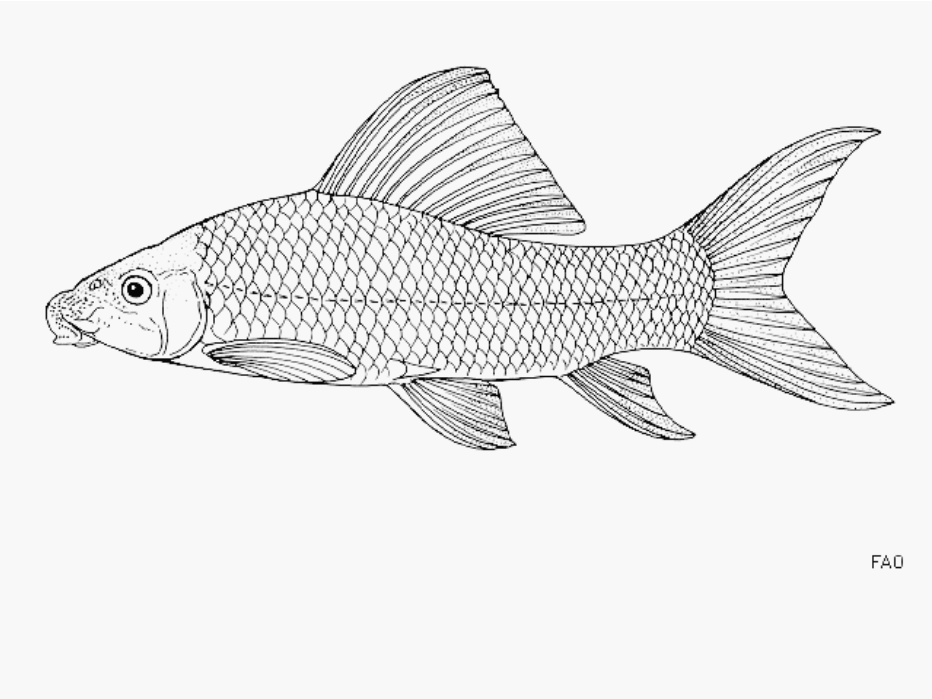



**Figure 21** *Labeo coubie* (FAO 2019a)

# 3 Aims of the thesis

Given that the quality of traditionally smoked fish in Cameroon, unlike in neighboring countries, has not been a much-studied topic in scientific literature until now, this work focuses on analysis of Cameroonian traditionally smoked fish. The objective of the thesis is to analyze PAH concentration, content of fish oil, and the fatty acid profile of selected smoked fish samples from the central region of Cameroon. The traditional smoking procedure of small-scale producers in the central region of Cameroon has been investigated as well.

# 4 Materials and Methods

This work is based mainly on laboratory analyses with support of previous field research, sample, and data collection by Ing. Tereza Slámová between December 2017 and February 2018.

## 4.1 Field research

### 4.1.1 Site area description

Samples and data were obtained from 3 different markets in Yaoundé, the capital city of Cameroon, namely: Nikol-Eton, Mfoundi, and Obili. Small producers targeted in these areas have fish selling as the main source of household income. Fish sources are variable, but consist mostly of freshwater from the Nyong river, and according to previous research in Yaoundé markets by Cho Achu (2009), river pond/dam (lakes) of Lagdo in the North of Cameroon and Mappé in the western province of Cameroon.

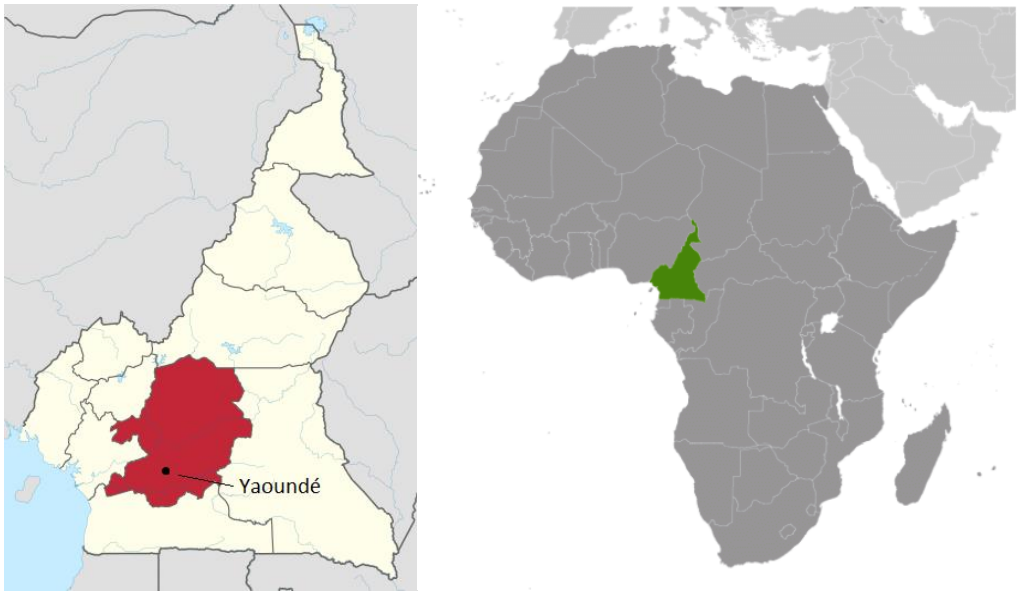



**Figure 22** left: Central province in Cameroon and Yaoundé location (Wikipedia 2019); right: Location of Cameroon within Africa (Driving Directions & Maps 2019)


**Figure 23** Site area of data and sample collection, Markets: Obili, Nikol-Eton, Mfoundi (Google maps 2020)

**Table 4** Responded producers overview

| Producer | Market | Samples | Full data from interviews |
|---|---|---|---|
| C1 | Nikol-Eton | 2 | yes |
| C2 | Nikol-Eton | 1 | yes |
| C3 | Mfoundi | 1 | yes |
| C4 | Mfoundi | 3 | no |
| C5 | Mfoundi | 4 | no |
| C6 | Mfoundi | 2 | yes |
| C7 | Mfoundi | 2 | no |
| C8 | Mfoundi | 0 | yes |
| C9 | Obili | 1 | yes |
| C10 | Obili | 0 | yes |
| **Total** | | **16** | **7** |

### 4.1.2 Data collection

Semi-structured personal interviews and observations were made among 9 small-scale producers of smoked fish product in Yaoundé's 3 markets. Full data were obtained from 7 producers, while another 2 responded partly. Questions in the interview were related to location of the producer, source of fish, fish species used for smoking, pre-treatment used before the smoking procedure, technique used for fish smoking, type of firewood used, period of smoking and main production, storage and selling practices, preparation of fish for eating, and smoke kiln description. All data were collected in local units and names, and all interviews were conducted in the French language. Gathered data were translated to English and scientific names and processed.

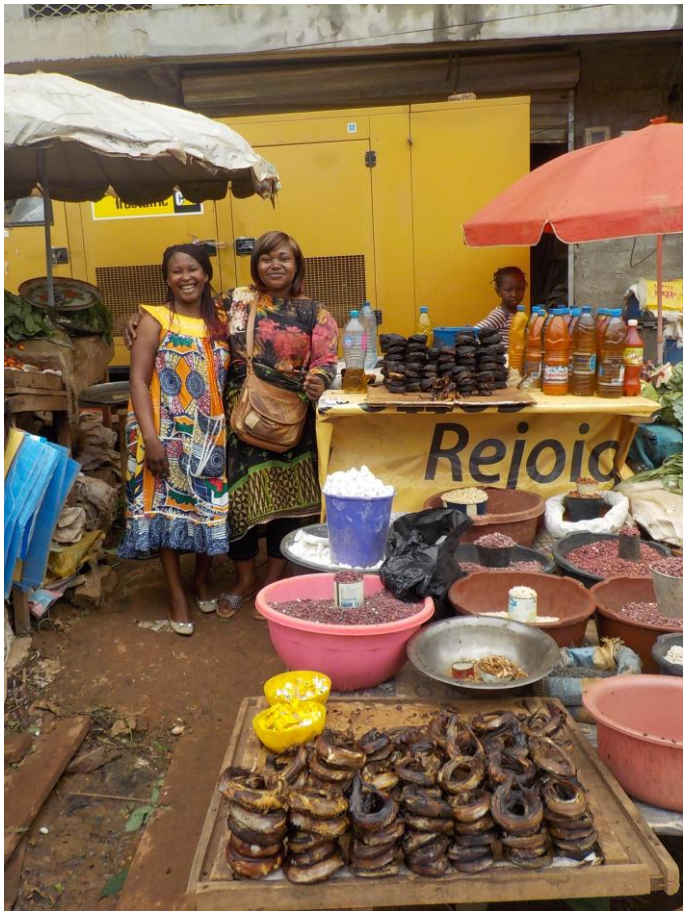



**Figure 24** Producer C1 at Nikon-Eton market, selling practices (Slámová 2017)

## 4.2 Determination of PAH and Fatty acids content

For PAH determination, the combination of QuEChERS extraction method with a clean-up step by EMR-Lipid and DLLME techniques as an extract pre-concentration was used. For the final quantification GC-MS was used. Fat content was determined by using a semi-automatic Soxhlet extractor. Extracted fat was derivatized to obtain fatty acid methyl esters, which were determined via GC-MS.

### 4.2.1 Sample collection

Considering the fact that the field research was made in the fishery offseason and the time for collecting samples and data was limited, there were a limited number of samples available at the markets. Also, the fish species were more variable than in the high season, as producers had less access to the popular varieties during this period. 16 samples of 8 fish species (see Tab. 5) from 8 small-scale smoked fish producers were collected during the field research of Slámová between December 2017 and February 2018. Obtained fish samples were placed in clean, labelled plastic bags that were then vacuum-packed. Immediately after sampling, the bags with samples were frozen in a freezer at -20 °C, and the samples were subsequently transported to Czech University of Life Sciences Prague for further laboratory investigation.

**Table 5** Overview of sampled species

| Scientific name | Common name | Local name | Number of samples |
|---|---|---|---|
| *Alestes baremoze* | Silversides | Queue rouge | 2 |
| *Brama dussumieri* | Brama brama | Poisson poulet | 1 |
| *Carlarius heudelotii* | Smoothmouth sea catfish | Machoiron | 2 |
| *Clarias gariepinus* | North African Catfish | Poisson-chat nord-africain | 4 |
| *Gadus morhua* | Atlantic cod | Morue | 3 |
| *Labeo coubie* | African carp | Ceale au Nez | 1 |
| *Mugil cephalus* | Flathead grey mullet | Mullet | 1 |
| *Parachanna obscura* | African obscure snakehead | Silur viper | 2 |

### 4.2.2 Sample preparation

#### 4.2.2.1 Homogenization

Prior to extraction, whole samples of fish (approximately 50 g per sample) were homogenized, by first cutting into small pieces and then mixed with a blender (IKA, Germany). Samples were thereafter held in plastic bottles and refrigerated at a temperature below -20 °C.

#### 4.2.2.2 PAH extraction

For PAH extraction, the modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method with EMR (Enhanced Matrix Removal) Lipid and Dispersive liquid–liquid microextraction (DMLLE) was used, which was recently verified by Slámová et al. (2020). 16 fish samples were extracted in 3 replications. Details of this method were described in schema in Figure 25.


**Figure 25** Schema of the sample preparation process; PP - polypropylene; centrifugation - 4 °C, 4.600 RPM, 15 min.

Chemicals were purchased from following suppliers: $MgSO_4$ and NaCl from Krakchemia SA, Krakow, Poland; ISs from Sigma-Aldrich, Saint Louis, Missouri, USA; Acetonitrile, chloroform, and hexane from Merck KGaA, Darmstadt, Germany; and EMR-Lipid material from Agilent Technologies, Santa Clara, California, USA.

#### 4.2.2.3 Soxhlet extraction

The improved Soxhlet extraction method (Randall extraction) was used for fat extraction from the sample. 16 fish samples were analyzed in 3 replications. Fully homogenized

samples were weighted in a thimble to 4 - 5 g each with an accuracy of 0.001 g and placed into a semi-automatic, six-position, Soxtec extractor, model VELP Scientifica SER 148 (Italy). Thimbles were immersed into approximately 50 ml of petroleum ether (p.a., PENTA, Czech Republic), placed in an extraction cup that was previously weighted (with boiling stone). The extraction had three phases: The first phase, immersion, took 120 minutes; the second phase, washing, took 60 minutes; and the third phase, recovery, took 20 minutes. All phases run at a hotplate temperature of 110 °C. After the whole program was finished, the extraction cups with fat were dried in an oven (Memmert, Germany) and subsequently placed into an exicator to aerate residual petroleum ether. The total amount of fat was ascertained from the weight difference in the extraction cup before and after extraction.


**Figure 26** Soxhlet extractor (author)

Total fat content (*w*) was calculated from the following equation:

$$w = \frac{m_2 - m_1}{m_0} * 100\ [\%]$$

Where:

$m_0$ weight of sample to dry matter content [g]

$m_1$ weight of dry extraction cup [g]

$m_2$ weight of extraction cup with extracted fat [g]

#### 4.2.2.4 Fatty acids derivatization

Approximately 100 µl of fish oil was transferred into a 10 ml volumetric flask. 1 ml of petroleum ether (Penta, CZ) and 1 ml of derivatization agent (methanolic NaH 0.4 M, delivered by Sigma Aldrich, CZ) was added. Samples were shaken and let stand for 20 minutes at room temperature. Approximately 10 ml of distilled water was added into the volumetric flask, shaken, and let stand overnight to separate the organic layer. Then 50 µl of the organic layer was transferred into a vial, diluted with 950 µl of hexane, and stored in a fridge at 6-8 °C before further analysis.

### 4.2.3 GC-MS and GC-FID analysis

#### 4.2.3.1 PAH identification

After an evaporation step, the samples were dissolved again in 0.2 ml of hexane (final conc. of sample 1 $g.ml^{-1}$), and then analyses were carried out on a gas chromatograph (Agilent 7890A, USA) with mass detector (Agilent 5975C USA). The auto-sampling injector in Splitless mode with a temperature of 280 °C injected all standards and samples with a volume of 1.0 µl. The GC oven was operated with the following temperature program: Initial temperature

of 50 °C (1 min) – 15 °C $min^{-1}$ – 150 °C, and when the temperature of 150 °C was achieved it continued at 150 °C (1 min) – 8 °C $min^{-1}$ – 310 °C (10 min). Total time of analysis was 37.7 minutes. Helium 5.0 (Linde Group, Munich, Germany) was used as the GC carrier gas at a flow rate of 1.0 mL $min^{-1}$. Column model HP-5MS (30 m x 250 µm x 0.25 µm; Agilent Technologies, Santa Clara, California, USA). The transfer line temperature was set at 280 °C, MS source temperature at 230 °C, and quadrupole temperature at 150 °C. PAHs were analyzed in the SIM regime. PAHs were quantified using the internal standard (IS) method, and deuterated PAH standards (Sigma, CZ) were used as IS. In addition, a standard containing 16 priority PAHs (Sigma, CZ) was used to prepare the calibration curve. The following internal standards were used: QTM PAH mix and standard, CLP Semivolatile Internal Std Mix containing 16 priority PAHs, and 6 deuterated PAH standards, purchased from Sigma Aldrich, USA.

**Figure 27** GS-MS (author)

#### 4.2.3.2 Fatty acid identification

A composition gas chromatograph (Agilent 7890A, USA) with mass detector (Agilent 5975C USA) was used for the identification of fatty acids. The GC was equipped with a Restek 2560 (USA) biscyanopropyl polysiloxane column (100 m × 250 µm × 0.2 µm). Injector temperature was 225 °C, the volume of the injected sample was 1 µl, and the split ratio was 1:50. A flow rate of 1.2 $ml.min^{-1}$ for the carrier gas (helium) was used. The initial temperature program of the analysis was at 70 °C, held for 2 minutes, then increased to 225 °C (rate 5 $°C.min^{-1}$), held for 9 minutes, then raised to 240 °C (rate 10 $°C.min^{-1}$) and held for 6.5 minutes. The total run time was 50 minutes. Mass acquisition parameters were set as follows: low mass 40.0, high mass 400.0, MS Source 230 °C with a maximum of 260° C, and MS Quad 150 °C with a maximum of 200 °C. Fatty acids were identified by comparing their retention times and spectra with the retention times and spectra of available standards (FAME mix, Sigma Aldrich, Czech Republic), and by comparing the data with the NIST database, version 2.0.

Gas chromatography (Agilent 7890A, USA) with flame ionization detector (FID) (Agilent 7890B, USA) was used for relative quantification of fatty acids. The chromatographic conditions of the analysis were the same as above. The FID detector was heated to 260 °C, hydrogen gas flow was set to 30 $ml.min^{-1}$, air flow at 400 $ml.min^{-1}$, and makeup flow was 30 $ml.min^{-1}$.

## 4.3 Dry matter identification

**Figure 28** Weighed samples prepared for drying (author)

Homogenized samples of approximately 4 - 4.5 g were weighed into weighing bowls with accuracy of 0.001 g. The samples in weighing bowls were placed into an oven (Memmert, Germany) and dried at 105 °C ± 2 °C to a constant weight for up to 24 hours. The sample bowls were then cooled in a desiccator and weighed.

Dry matter content was calculated by the following formula:

$$w = \frac{m_2 - m_3}{m_2 - m_1} * 100\ [\%]$$

Where:

$m_1$ weight of bowl before drying [g]

$m_2$ weight of bowl with sample before drying [g]

$m_3$ weight of bowl with sample after drying [g]

Dry matter [%] = 100 – water content (w) [%]

## 4.4 Data analysis

Obtained data from laboratory measurements were processed in Microsoft Office Excel 2016. All data, except fatty acid profiles with one replication within sample, are presented as means with SD. Statistical analysis was conducted in IBM SPSS 23 with one-way ANOVA and post-hoc Tukey test.

Nutritional quality indices (NQIs) were calculated as follows:

The index of hypocholesterolemic/hypercholesterolemic fatty acids (HH) according to Santos-Silva et al. (2002):

$$HH = \frac{C18:1n9cis + C18:2n6 + C18:3n3 + C20:4n6 + C22:6n3}{C14:0 + C16:0}$$

The index of polyunsaturated/saturated fatty acids (PUFA/SFA):

$$\sum PUFA / \sum SFA = \frac{C18:2n6 + C18:3n3 + C20:4n6 + C22:6n3}{C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0}$$

The index of omega-6/omega-3 fatty acids (n-6/n-3):

$$\sum n\text{-}6 / \sum n\text{-}3 = \frac{C18:2n6 + C20:4n6}{C22:6n3 + C18:3n3}$$

# 5 Results and Discussion

## 5.2 Fish smoking practices

The results obtained from the personal semi-structured interviews are important to understand the local fish smoking practices in Yaoundé. However, due to the limited number of respondents the data are considered as complementary to the analysis of contents of PAHs and fatty acids.

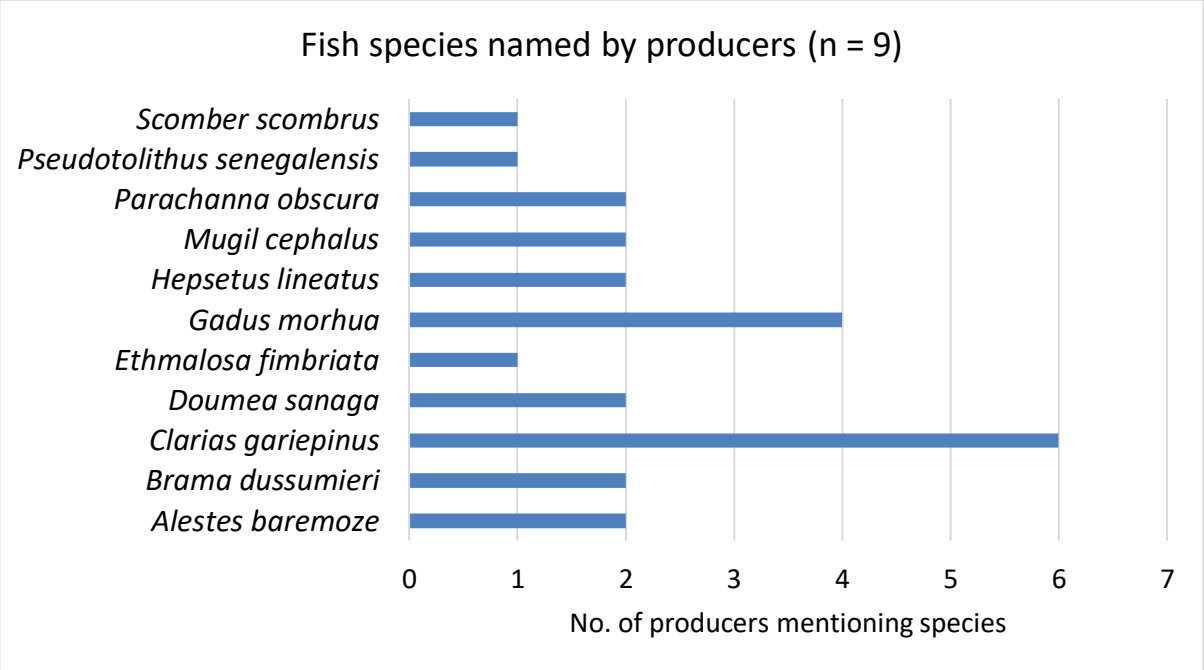



**Figure 29** Fish species usually used for smoking in targeted Yaoundé markets

According to interviews, the most common species for smoking are *Clarias gariepinus* and *Gadus morhua*. It is no wonder that *Clarias gariepinus*, which is often mentioned in literature (Ali et al. 2011; El Gamal 2014; Felix et al. 2018; FAO 2019b), is the most-often sampled fish species in this study as well. On the other hand, *Gadus morhua* has no records in literature as a common fish species for smoking in Cameroon, but in the targeted Yaoundé markets it was often mentioned as a local preference and samples were collected as well.

Out of nine respondents, 8 are buyers of fish, while only one is fishing and one combines both. Fish is purchased through middlemen or directly from fishermen. Mappé lake in the western province of Cameroon and the Nyong river were mentioned as localities where respondents buy fish. Both places are also fishing locations for self-sufficient producers. These fish sources for producers from Yaoundé's markets are in agreement with the Cho Achu study (2009).

Typically, the smoking procedure starts with pre-treatment. Five out of 7 respondents use a pre-treatment before the smoking process. This pre-treatment process always includes dry salting, and in some cases includes the addition of white pepper, while in other cases the fish is cut and salted inside. It is done next to the smoking kiln. Salting helps inhibit bacterial growth, prolongs the storage expiration, and last but not least adds a favorable flavor (Hall 1997; FAO & WHO 2009).

**Figure 30** Example of fuel wood (Slámová 2017)

All respondents use wood as a fuel. Most respondents reported using “white wood”, one uses “red wood”, and one uses any available wood. A study by Dongmo Keumo Jiazet (2019) dealing with the potential impact of fish smoking on mangrove resources in southwest Cameroon showed that red mangrove is the most commonly used fuel for smoking fish in that region, followed by the rubber tree. In this context, the “red wood” could potentially refer to the red mangrove and “white wood” could refer to the rubber tree.

Traditional ways of smoking lack any possibility of measuring temperature, as 100 % of respondents in this survey agreed with. This is mainly due to the simple traditional design of kilns. In addition, the length of time for fish smoking varied substantially—from 1 hour to 1 week. This time range is unusually large. Rather, it resembles the typical duration of the drying step, which can vary from 5 hours to 4 days as reported by a study from Benin (Assogba et al. 2019). According to Codex Alimentarius (FAO & WHO 2009) the smoking time should be as short as possible to minimize the exposure of the food surfaces to PAH-bearing smoke. However, in the case of hot smoking when the product is being cooked at the same time, sufficient time is needed for the product to be cooked thoroughly. In traditional smoke kilns where the hot smoke is the only heat source, the smoking chamber should be heated in advance. A carton to cover the fish products was used very often during smoking to improve the concentration of smoke. During the smoking process the fish are regularly turned. The smoking kilns in most of the cases were simply designed out of bricks or metal with no roof. The appropriate control of conditions during the smoking process is clearly not possible in these smoke kilns.

**Figure 31** Smoking fish with carton covering (Slámová 2017)

## 5.3 Fat and moisture content

Fat content within the fish species differs depending on the size of fish, age, fishing ground, season, variation in the amount and quality of food that the fish eats, and the amount of movement it makes (Burt & Murray 2001). In the case of smoked fish, fat loss during the process is another factor (Kiczorowska et al. 2019). Figure 32 and Table 7 show obtained values from analyses of fat content in dry matter of selected Cameroonian fish species. The fat content in this research ranged from 3.22 % (*Brama dussumieri*) to 24.58 % (*Parachanna obscura*). Fish species are classified according their fat content: high fat, low fat, and very low fat, although these classifications are for fresh unprocessed fish. Smoked fish loses moisture during processing (Tab.6), and therefore this classification cannot be applied and the results of fat content are expressed in dry matter. Because of this loss of moisture, the ratio between fat and sample weight is higher than reported in the literature.

**Table 6** Moisture content in smoked fish samples (%)

| Sampled species | Water content (%) | SD |
|---|---|---|
| *Brama dussumieri* | 53.22 | ± 0.48 |
| *Gadus morhua* | 49.07 | ± 0.43 |
| *Gadus morhua* | 45.65 | ± 0.19 |
| *Gadus morhua* | 44.14 | ± 0.27 |
| *Clarias gariepinus* | 16.97 | ± 0.11 |
| *Alestes Baremoze* | 13.19 | ± 0.13 |
| *Clarias gariepinus* | 13.00 | ± 0.48 |
| *Clarias gariepinus* | 12.99 | ± 0.12 |
| *Labeo Coubie* | 12.40 | ± 0.07 |
| *Parachanna obscura* | 11.71 | ± 0.29 |
| *Alestes Baremoze* | 10.56 | ± 0.01 |
| *Carlarius heudelotii* | 10.11 | ± 0.06 |
| *Mugil cephalus* | 9.17 | ± 0.05 |
| *Parachanna obscura* | 8.69 | ± 0.08 |
| *Clarias gariepinus* | 7.20 | ± 0.08 |
| *Carlarius heudelotii* | 7.04 | ± 0.07 |

As is obvious from the standard deviation and minimum and maximum fat content shown in Figure 32 and Table 7, the data are not uniform. *Alestes baremoze* had a significant difference in fat content (23.85 and 15.30) between the two samples, which could have been caused by the different origin of the sampled fish. *Parachanna obscura* showed the highest fat content (24.58 ± 1.86), along with an interesting observed organoleptic phenomenon after Soxhlet extraction where the fat had a considerably more liquid structure compared with the rest of the fish samples. This result value is also in conflict with the results of Fapohunda & Ogunkoya (2008) for smoke dried *P. obscura* (8.87 ± 0.03). Results from Fapohunda & Ogunkoya (2008) are not expressed in dry matter; however, after

recalculation they would show 9.48 % fat, which is still far from the result of this study. Notwithstanding this disparity, fat is the most variable macronutrient in fish. While the ratio of the highest to the lowest value of protein or water content encountered is not more than three to one, the ratio between highest and lowest fat values is more than 300 to one (FAO 2001).

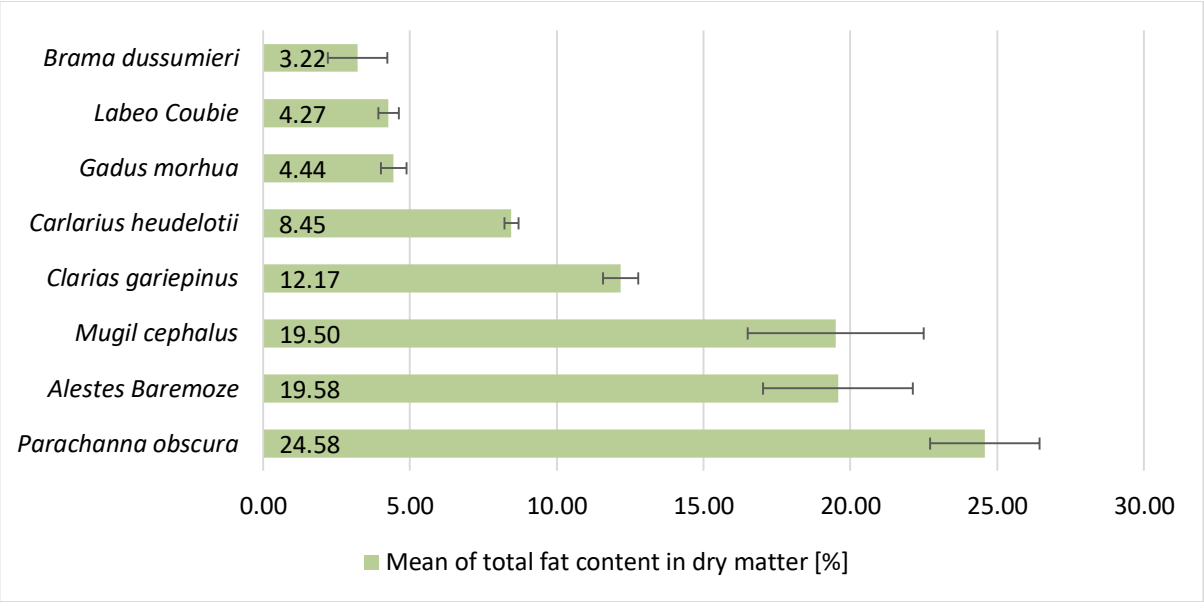



**Figure 32** Mean of species total fat content in dry matter [%] with SD (n =16)

As shown in Table 6, moisture content in samples varied greatly, from 7.04 ± 0.07 (*Carlarius heudelotii*) to 53.22 ± 0.48 (*Brama dussumieri*). An interesting fact is that *Brama dussumieri* and *Gadus morhua* showed the highest levels of moisture along with the lowest levels of fat in dry matter. According to Codex Alimentarius (FAO & WHO 2013), after the smoke-drying process the moisture content level should be 10 % or less to inhibit the growth of all foodborne pathogens and so that refrigeration is not required. This condition was achieved in only 4 samples out of 16.

**Table 7** Total fat content in dry matter (%)

| | Sample | n | Mean (%) | SD | Minimum (%) | Maximum (%) |
|---|---|---|---|---|---|---|
| *Gadus morhua* | 1 | 3 | 5.19 | 0.04 | 5.14 | 5.25 |
| | 3 | 3 | 4.84 | 0.25 | 4.49 | 5.04 |
| | 4 | 3 | 3.29 | 1.03 | 2.51 | 4.75 |
| *Clarias gariepinus* | 2 | 3 | 16.96 | 1.30 | 15.13 | 18.02 |
| | 10 | 3 | 9.02 | 0.37 | 8.5 | 9.31 |
| | 13 | 3 | 8.25 | 0.51 | 7.81 | 8.96 |
| | 15 | 3 | 14.46 | 0.19 | 14.24 | 14.71 |
| *Alestes Baremoze* | 5 | 3 | 23.85 | 4.79 | 19.83 | 30.59 |
| | 11 | 3 | 15.3 | 0.32 | 14.85 | 15.55 |
| *Mugil cephalus* | 6 | 3 | 19.5 | 2.99 | 16.22 | 23.46 |
| *Labeo Coubie* | 7 | 3 | 4.27 | 0.34 | 3.81 | 4.63 |
| *Parachanna obscura* | 8 | 3 | 22.98 | 2.02 | 20.3 | 25.17 |
| | 12 | 3 | 26.19 | 1.71 | 24.97 | 28.6 |
| *Carlarius heudelotii* | 9 | 3 | 2.17 | 0.11 | 2.02 | 2.3 |
| | 14 | 3 | 14.73 | 0.36 | 14.33 | 15.2 |
| *Brama dussumieri* | 16 | 3 | 3.22 | 1.01 | 2.28 | 4.63 |

## 5.4 Fatty acid profile

In total, 17 different fatty acids were identified within the samples of this research, with a maximum of 16 FAs (*Parachanna obscura*) and a minimum of 11 FAs (*Gadus morhua* and *Alestes Baremoze*) in one sample. A detailed overview of the FAs in individual samples is shown in Appendix II. A number of fatty acids in samples were not identified. The proportion of unidentified fatty acids varied between 4.80 % (*Alestes Baremoze*) and 35.46 % (*Clarias gariepinus*) in individual samples.

Generally, the composition of saturated and unsaturated fatty acids in fish is 15 - 36 % saturated FAs (Ackman 1989; Buchtová et al. 2007; Zakes et al. 2010), and 58 - 85 % unsaturated FAs (Domaizon et al. 2000; Caballero et al. 2002). Figure 33 illustrates the composition of SFAs, MUFAs and PUFAs within the various fish species analyzed in this research. These results presented different intervals from what is commonly reported in

literature. The SFA content ranged between 33 and 43 %, and in most cases somewhat exceeded the commonly reported SFA range in fish (15 - 36 %). Simultaneously, the range of unsaturated fatty acids (UFAs) was outside of commonly reported levels in all samples, reporting 38 - 51 % UFAs, with 21 - 39 % MUFAs and 5 - 29 % PUFAs respectively.

The highest level of SFAs was found in *Mugil cephalus* (43.18 %), which also contained the lowest level of PUFAs (5.38 %). However, two individual fish samples (Appendix II.) from other species (*Carlarius heudelotii* and *Clarias gariepinus*) achieved a higher SFA content (47.64 % and 47.82 %, respectively). Although such a high content of SFAs is not usual, it is in agreement with the findings of Deng et al. (1976), where SFAs ranged between 38.1 – 51.8 % in the *Mugil cephalus* species. When it comes to PUFAs, *Mugil cephalus* typically ranges between 13.7 % and 47.9 % (Deng et al. 1976; Argyropoulou et al. 1992; Sengör et al. 2003; Abd El-Ghafour et al. 2018), which is considerably higher than these results (5.38 %). For UFAs, the *Alestes Baremoze* species had the highest levels, with a proportion of 59.06 % (29.49 % MUFA and 29.57 % PUFA). *Alestes Baremoze* also had the highest proportion of PUFAs, which could be due to—among other things—having the lowest average percentage share of unidentified FAs (6.15 %). *Labeo coubie* featured the second highest level of PUFAs (29.27 %) and UFAs (50.68 %) generally; however, it still did not reach the typically reported

range for UFAs in fish (58 - 85 %). For instance, Ugoala & Ndukwe (2016) found 38 % PUFAs and 25 % MUFAs, for a total of 63 % UFAs in the *Labeo coubie* species.

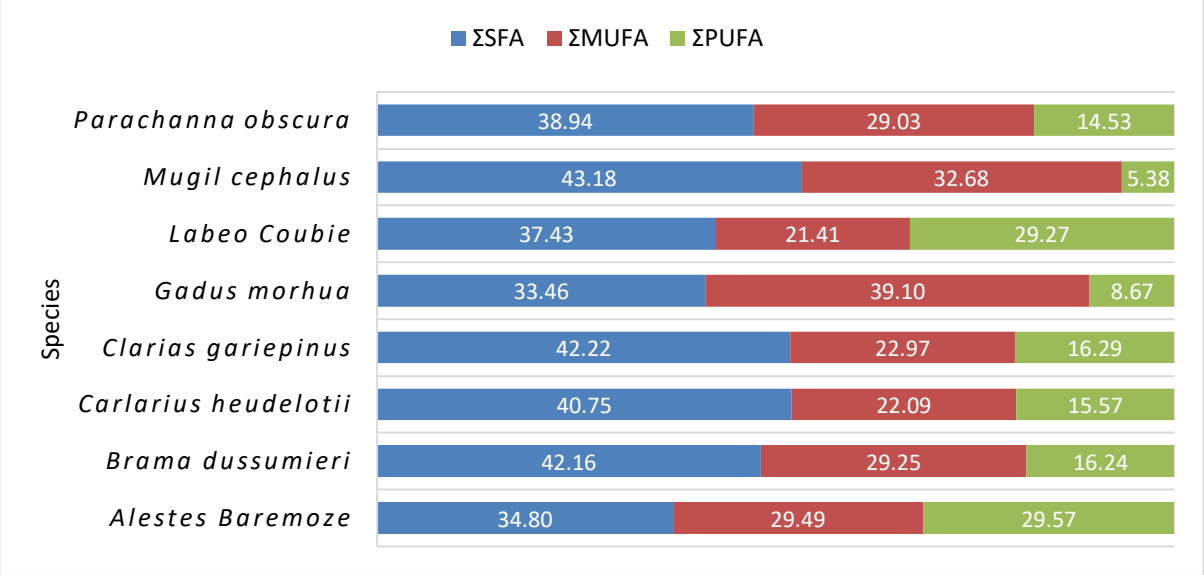



**Figure 33** Fish fat composition of saturated FAs by species – expressed in means; for table with SD see Appendix III

The PUFA/SFA ratio is considered a marker for cardiovascular health. SFAs are associated with an increase in total serum cholesterol and LDL-cholesterol, and a low PUFA/SFA ratio is linked to a higher prevalence of cardiovascular disease (Calder 2015). According to Wood et al. (2003) and Ospina-E et al. (2012), the risk of cancer and coronary heart disease is reduced when the PUFA/SFA ratio in the diet is greater than 0.4. The PUFA/SFA ratio in sampled species is shown in Table 8, ranging between 0.12 (*Mugil cephalus*) and 0.85 (*Alestes baremoze*), with only 2 reaching the nutritionally desirable minimum (*Labeo coubie* and *Alestes baremoze*). This low of a PUFA/SFA ratio within these fish species is in conflict with some of the scientific literature. For example, values of 1.30 have been reported for *Mugil cephalus* (Abd El-Ghafour et al. 2018), 1.02 for *Labeo coubie* (Ugoala & Ndukwe 2016), and 1.15 for *Clarias gariepinus* (Merdzhanova et al. 2018).

Under normal circumstances, fish obtain PUFAs from a diet mainly based on phytoplankton (Shamsudin & Salimon 2006). With regard to the origin of the samples (mostly bought from an unknown source), it is possible that they were raised via aquaculture with no access to

natural food such as phytoplankton to generate expected values of PUFAs. Furthermore, PUFAs are more unstable than SFAs, and thus more vulnerable to oxidation. The PUFA/SFA ratio could be reduced by oxidation of PUFAs contained in the fish tissue, where they are converted to products such as peroxides, aldehydes, ketones, and free fatty acids (Daramola et al. 2007).

Studies dealing with fish nutrient intake in relation to health are frequently carried out with data obtained from raw food. PUFA content in raw fish tissue may not provide explicit information on the nutritive value of the species after technological treatment. However, according to Sikorski & Kolakowska (2010), hot smoking only affects the levels of PUFAs and MUFAs without increasing the values of SFAs. This study also admitted the possibility of a small percentage loss of LC-PUFAs (EPA, DHA).

The best nutritional quality indices (NQIs) for evaluating fish are the n-6/n-3 ratio, EPA + DHA, and the hypocholesterolemic/hypercholesterolemic index (HH). The HH is related to the effect of specific fatty acids on cholesterol metabolism, where higher values are considered more desirable for human health (Hosseini et al. 2014). HH indices for this research are represented in Table 9. The highest HH value, with a significant lead among all analyzed fish species, was found for *Alestes baremoze* (2.56), followed by *Labeo coubie* (1.82). The fish with the lowest HH values were *Mugil cephalus* (1.13) and *Carlarius heudelotii* (1.25). These results agreed with recent studies of fish lipid and fatty acid content, which reported a HH ratio ranging from 0.65 to 2.93 (Fernandes et al. 2014; Rincón-Cervera et al. 2020; Zhang et al. 2020). Considering the PUFA/SFA ratio and the HH index, *Alestes baremoze* (0.85 and 2.56, respectively) and *Labeo coubie* (0.78 and 1.82, respectively) were the most favorable from a nutritional point of view.

According to a number of studies, the temperature and the wood smoke components during the smoking process negatively affect the fatty acid composition, especially EPA, DHA, and some essential amino acids (Beltrán & Moral 1991; Swastawati 2004; Kaya et al. 2008). Another study demonstrated that heat treatment (roasting) lowers the amount of n-6 and n-3 acids (n-3 especially), which caused a significant increase in the n-6/n-3 ratio (Čolović et

al. 2012). On the other hand, a study by Rahimabadi et al. (2016) reported the opposite results, with amounts of EPA and DHA (both n-3 acids) slightly increasing after the smoking process.

**Table 8** LC-PUFA percentage share in sample in dry matter

| Species | n-6/n-3 | n-3/sample | n-6/sample |
|---|---|---|---|
| *Alestes baremoze* | 4.05 | 1.15% | 4.64% |
| *Brama dussumieri* | 0.64 | 0.32% | 0.20% |
| *Carlarius heudelotii* | 1.73 | 0.48% | 0.83% |
| *Clarias gariepinus* | 1.47 | 0.85% | 1.25% |
| *Gadus morhua* | 0.79 | 0.22% | 0.18% |
| *Labeo coubie* | 1.18 | 0.57% | 0.68% |
| *Mugil cephalus* | 5.04 | 0.17% | 0.88% |
| *Parachanna obscura* | 1.09 | 1.71% | 1.86% |

Excessive intake of n-6 acids can have negative blood clotting and pro-inflammatory effects; however, this does not necessarily mean that decreasing the intake of n-6 acids is recommended, but that a higher intake of n-3 acids is needed (Doležal 2014). According to Simopoulos (2002), the ideal n-6/n-3 ratio is between 4:1 to 1:4, while another author proclaims the ideal to be 3:1 to 1:1 (Kim et al. 2007). Nevertheless, the absolute amount of omega-6 and omega-3 FAs is more important (Doležal 2014). Among the analyzed fish species, ratios which both authors agree with being in the ideal range were seen in *Carlarius heudelotii* (1.73), *Clarias gariepinus* (1.47), *Labeo coubie* (1.18), and *Parachanna obscura* (1.09) (Table 8 and Table 9). Moreover, Table 8 displays the percentage share of n-3 and n-6 FAs within the fish samples in dry matter, from which it is possible to conclude the total amount of LC-PUFAs. The highest total share of n-3 and n-6 FAs was found in *Parachanna obscura* (1.71 % and 1.86 %, respectively) and *Alestes baremoze* (1.15 % and 4.64 %, respectively). Taking into account the n-6/n-3 ratio, *Parachanna obscura* seems like the ideal dietary source of LC-PUFAs between the two.

Due to the NQIs from the results (Table 9), *Labeo coubie* proved to be the most nutritionally valuable species/sample of fish within this research, followed by *Parachanna obscura* and *Alestes baremoze*. The least nutritionally valuable species/sample was *Mugil cephalus*. Nevertheless, not all NQI values for the *L.coubie* sample achieved at least the average

reported in scientific literature (e.g. the PUFA/SFA ratio), and NQI values in the *M.cephalus* sample were rather low.

**Table 9** PUFA/SFA ratio, n-6/n-3 ratio, Σn-3, Σn-6, DHA share, and HH ratio of selected fish species

| Species | n | PUFA/SFA | n-6/n-3 | n-3 | n-6 | HH | DHA |
|---|---|---|---|---|---|---|---|
| *Alestes baremoze* | 2 | 0.85 | 4.05 | 5.85% | 23.71% | 2.56 | 1.06% |
| *Brama dussumieri* | 1 | 0.39 | 0.64 | 9.93% | 6.32% | 1.29 | 9.93% |
| *Carlarius heudelotii* | 2 | 0.38 | 1.73 | 5.71% | 9.86% | 1.25 | 2.88% |
| *Clarias gariepinus* | 4 | 0.39 | 1.47 | 7.02% | 10.30% | 1.49 | 2.94% |
| *Gadus morhua* | 3 | 0.26 | 0.79 | 5.04% | 3.98% | 1.31 | 5.04% |
| *Labeo coubie* | 1 | 0.78 | 1.18 | 13.43% | 15.84% | 1.82 | 3.07% |
| *Mugil cephalus* | 1 | 0.12 | 5.04 | 0.89% | 4.49% | 1.13 | 0.89% |
| *Parachanna obscura* | 2 | 0.37 | 1.09 | 6.96% | 7.56% | 1.54 | 3.31% |

The fatty acid profile of selected fish is shown in Figure 34. The dominant SFA in all examined fish was palmitic acid (C16:0), which was consistent with results obtained by other authors (Chow 1999; Zeng et al. 2010; Fernandes et al. 2014; Abd El-Ghafour et al. 2018). On the other hand, the absence of EPA, which occurred in all analyzed fish samples, was not consistent with any author. Although as mentioned above, studies conflict on whether EPA and DHA values could be lowered by the smoking process or not. For instance, Dr. Marit Espe, a senior scientist with Norway's National Institute of Nutrition and Seafood Research, claimed that the composition of omega-3 fatty acids does not change during the smoking process (Harvard University 2011). The percentage share of DHA (Table 9 and Figure 34) among the investigated fish species of this research ranged between 0.89 % (*Mugil cephalus*) and 9.93 % (*Brama dussumieri*). These results were in agreement with the usually reported DHA range of 2.8 to 14.6 % for smoked fish, except for *M.cephalus* (0.89 %) and *A.baremoze* (1.06 %). Conversely, the expected presence of EPA (0.7 – 9.0 %) was not met (Aminullah Bhulyan et al. 1986; Wang et al. 1990; Rørå et al. 2003; Stołyhwo et al. 2006; Regulska-Ilow et al. 2013). However, there is still the possibility that small amounts of LC-PUFAs within the analyzed samples, including EPA, could be located among the unidentified FAs which comprised a significant part of the profile of fish samples (4.80 – 35.46 %).

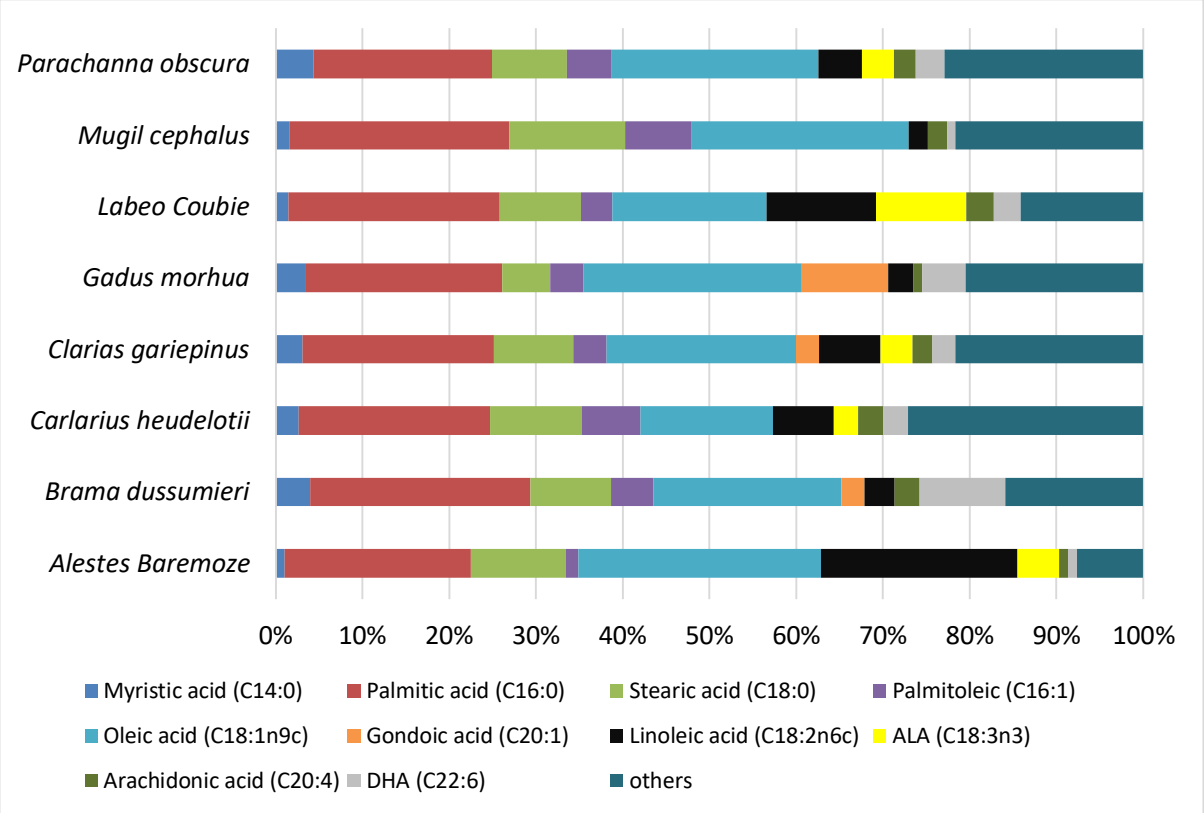



**Figure 34** Relative proportional ratios of selected FAs in fish fat, by species.

Essential ALA (alpha-linolenic acid C18:3n3) was found in *Labeo coubie* (10.36 %) in a considerably higher percentage compared with the other fish samples. The scientific literature reports a percentage range of ALA between 0.8 to 14.4 %, with most results under 5 % (Aminullah Bhulyan et al. 1986; Wang et al. 1990; Rørå et al. 2003; Stołyhwo et al. 2006; Regulska-Ilow et al. 2013). The result of *L.coubie* was therefore above average, but within the range of findings by other research. Despite the expectations supported by literature, the samples of *M.cephalus* and *B.dussumieri* did not show any presence of ALA; however, ALA could be among the unidentified FAs. The other essential FA – linoleic acid (C18:2n6c) – was found in *Alestes baremoze* with the highest percentage value (22.67 %). It is important to note that large differences were found among the FA values of individual fish samples within the species (Appendix II.). According to literature the range of linoleic acid is between 1.0 to 45.4 % for fish in general, while most of the study results were under 12 % (Aminullah Bhulyan et al. 1986; Wang et al. 1990; Stołyhwo et al. 2006; Regulska-Ilow et al. 2013), with only Rørå et al. (2003) reporting a value as high as 45.4 %. This may suggest that the share

of essential FAs (ALA and LA) in some of the fish samples within this research were higher than expected, based on the results from other scientific literature. Essential FAs in food are important, as ALA contributes to the maintenance of normal blood cholesterol levels, while LA supports hearth health with some evidence of also improving insulin sensitivity and blood pressure (European parliament 2015).

## 5.5 PAH content and profile

All determined values of the four priority PAHs in all samples in this research (Table 10) highly exceeded the maximum levels for PAH contamination in foodstuffs set by Commission regulation (EC) No 1881/2006. Maximum levels are currently set to 2 $\mu g.kg^{-1}$ for benzo[a]pyrene and 12 $\mu g.kg^{-1}$ for PAH4. When considering exceptions for some EU countries for traditionally smoked fish products, where maximum limits are set to 5 $\mu g.kg^{-1}$ for benzo[a]pyrene and 30 $\mu g.kg^{-1}$ for PAH4, these results were on average 13 times higher for BaP and 15 times higher for PAH4 than those MLs (The European Commission 2011; The European Commission 2019). Although the commission regulations reported these MLs in wet weight, which makes them lower compared to dry weight, the gap between these MLs and the results of this study remains enormous. Such high values of PAHs have rarely been reported for smoked fish within previous scientific literature. However, a study from Sri Lanka reported a very wide range of PAHs in the dry weight of smoked fish. Results reported a BaP presence from 35.3 to 1,489.6 $\mu g.kg^{-1}$ (Malika et al. 2017), and even after considering the possibility of a mistake in decimal point, the values were still higher than those reported in this study. Furthermore, in a study of traditionally smoked fish from Cambodia, Slámová (2017) reported an average BaP presence of 53.85 $\mu g.kg^{-1}$ which corresponds with the value in this research of 60.72 $\mu g.kg^{-1}$. More adequate comparisons would be with the studies targeting Cameroon due to the shared environmental conditions, traditional smoking process, and fish species; however, there have not been many relevant studies within this country published yet. One very recent study from Nigeria has reported BaP content from 3.0 to 38.0 $\mu g.kg^{-1}$ in dry weight in commercially smoked fish from markets (Akpambang et al. 2009), which is considerably lower than the results of this work (from 31.33 to 94.81

μg.kg$^{-1}$). However, these results corresponded with an expectation of high values of PAHs after the smoking process in uncontrolled “wild” conditions typical for households in developing countries (Šimko 2002). Moreover, small-sized fish, which are so common in Cameroonian markets and were also used in this research, are more heavily smoked than bigger fish due to the smaller surface area-to-volume ratio (Lawrence & Weber 1984; Duedahl-Olesen et al. 2010).

**Table 10** Determined values (μg.kg$^{-1}$) of four priority PAHs (each sample n=3)

| Sample No. | Producer No. | Species | Benz[a]anthracene | Chrysene | Benzo[b]fluoranthene | Benzo(a)pyrene | ∑PAH4 |
|---|---|---|---|---|---|---|---|
| 1 | 1 | *Gadus morhua* | 181.02$^{acd}$ (±7.55) | 36.16$^{ab}$ (±3.35) | 33.10$^{abcdegk}$ (±2.96) | 56.34$^{abcdij}$ (±6.38) | 306.62$^{abd}$ (±20.23) |
| 2 | 1 | *Clarias gariepinus* | 146.50$^{bce}$ (±3.52) | 59.24$^{abc}$ (±4.74) | 37.10$^{abcdegk}$ (±3.44) | 52.47$^{abcdijl}$ (±1.44) | 295.32$^{abd}$ (±13.14) |
| 3 | 2 | *Gadus morhua* | 196.03$^{acd}$ (±2.52) | 14.91$^{ab}$ (±1.54) | 37.45$^{abcdegk}$ (±2.50) | 57.80$^{abcdfij}$ (±1.08) | 306.19$^{abd}$ (±7.64) |
| 4 | 3 | *Gadus morhua* | 194.53$^{acd}$ (±8.17) | 1.10$^{*}$ | 27.06$^{abcegk}$ (±3.83) | 60.55$^{abcdefi}$ (±1.35) | 283.24$^{abd}$ (±13.35) |
| 5 | 4 | *Alestes Baremoze* | 168.18$^{abce}$ (±10.74) | 105.02$^{bcdf}$ (±14.84) | 49.35$^{abcdeghi}$ (±4.85) | 56.06$^{abcdij}$ (±3.28) | 378.61$^{abch}$ (±33.71) |
| 6 | 10 | *Mugil cephalus* | 206.24$^{adg}$ (±10.18) | 113.22$^{cdf}$ (±10.39) | 42.26$^{abcdegk}$ (±6.43) | 73.41$^{defik}$ (±8.26) | 435.12$^{bch}$ (±35.26) |
| 7 | 4 | *Labeo Coubie* | 193.87$^{acd**}$ (±11.05) | 92.57$^{bcdf**}$ (±6.75) | 65.52$^{acdfghi**}$ (±5.31) | 73.03$^{cdefik**}$ (±2.89) | 425.00$^{bch}$ (±26.00) |
| 8 | 6 | *Parachanna obscura* | 133.51$^{bce**}$ (±3.38) | 26.72$^{ab**}$ (±0.11) | 16.46$^{abcek**}$ (±0.53) | 31.33$^{gjl**}$ (±1.05) | 208.02$^{ad}$ (±5.07) |
| 9 | 5 | *Carlarius heudelotii* | 260.50$^{fg}$ (±9.56) | 176.92$^{eg}$ (±14.31) | 86.65$^{dfhij}$ (±3.12) | 90.89$^{h}$ (±6.05) | 614.97$^{efg}$ (±33.05) |
| 10 | 5 | *Clarias gariepinus* | 196.34$^{acd}$ (±3.87) | 125.33$^{cdfg}$ (±1.99) | 51.67$^{abcdghi}$ (±4.63) | 60.89$^{abcdefi}$ (±5.03) | 434.23$^{bch}$ (±15.53) |
| 11 | 5 | *Alestes Baremoze* | 255.44$^{fg}$ (±3.01) | 213.21$^{eg}$ (±3.80) | 78.24$^{cdfghij}$ (±1.87) | 94.81$^{h}$ (±2.67) | 641.70$^{efgi}$ (±11.35) |
| 12 | 5 | *Parachanna obscura* | 235.98$^{dfg}$ (±12.90) | 169.28$^{efg}$ (±15.75) | 78.20$^{cdfghij}$ (±16.58) | 66.54$^{abcdefik}$ (±3.33) | 550.00$^{efgh}$ (±48.56) |
| 13 | 6 | *Clarias gariepinus* | 233.77$^{dfg}$ (±9.58) | 183.01$^{eg}$ (±14.57) | 74.80$^{cdfghi}$ (±13.36) | 93.50$^{h}$ (±1.68) | 585.07$^{efg}$ (±38.18) |
| 14 | 7 | *Carlarius heudelotii* | 194.50$^{acd}$ (±9.57) | 179.50$^{eg}$ (±17.81) | 54.34$^{abcdghi}$ (±6.11) | 44.66$^{abcgjl}$ (±2.62) | 472.99$^{bcgh}$ (±36.12) |
| 15 | 7 | *Clarias gariepinus* | 247.59$^{fg}$ (±13.34) | 315.44 (±25.53) | 107.02$^{fhj}$ (±17.93) | 75.68$^{efik}$ (±2.76) | 745.74$^{fi}$ (±59.57) |
| 16 | 9 | *Brama dussumieri* | 167.37$^{abce}$ (±2.64) | n.d. | 17.11$^{abek}$ (±2.16) | 38.48$^{bgjl}$ (±1.90) | 222.96$^{ad}$ (±6.70) |
| | | Median | 195.28 | 113.22 | 50.51 | 60.72 | 429.61 |
| | | Average (±SD) | 200.71 (±36.5) | 113.23 (±83.95) | 53.52 (±25.34) | 64.15 (±18.14) | 431.61 (±154.93) |

n.d. not detected; $^{*}$ n=1; $^{**}$ n=2 ; $^{a-l}$ Values for each sample with the same superscript letters in the same column are not significantly different at P<0.05 (one-way ANOVA and Tukey post-hoc test)

There is no doubt that the smoking technique has an influence on the level of PAHs in fish (Foster 1957; Jägerstad & Skog 2005; Hokkanen et al. 2018). In the case of traditional smoking within simple kilns, the temperature cannot be controlled. The traditional smoking kiln also does not offer the possibility of controlling wood smoke, as do the modern smokehouses with an external generator where smoke is filtered. In the study by Stołyhwo & Sikorski (2005), traditional kilns raised the BaP level by about 50 $\mu g.kg^{-1}$ in comparison to smoke-controlling smokehouses (0.1 $\mu g.kg^{-1}$). As agreed by many authors (Foster 1957; Jägerstad & Skog 2005; Šimko 2005; Abdel-Shafy & Mansour 2016; Hokkanen et al. 2018), levels of contamination by carcinogenic PAHs can be significantly lowered with controlled conditions, good hygiene, proper handling and maintenance practices, and adequate equipment.

Another factor that could lead to high PAH levels is smoking time. According the responses of the Yaoundé producers, about half of them smoke fish for a time of 24 hours up to 1 week (Table 11). This is in contrast to usually reported smoking times, which range between 2 - 12 hours (Bannerman 2001; Stołyhwo and Sikorski 2005; Essumang et al. 2013). This is a critical factor to consider during the smoking process because a prolonged smoking time increases the PAH exposure of the product, and it should be kept as short as possible while taking into account food safety and product shelf life (FAO & WHO 2009; Purcaro et al. 2013; Essumang et al. 2013). However, the opposite effect was reported in research from Hokkanen (2018), where shorter smoking times generated unexpectedly higher PAH concentrations than longer smoking times, although this was accompanied by wide differences among temperatures (fish smoked under higher temperatures reported higher values of PAHs). This may suggest that temperature is the key factor in controlling levels of PAHs in smoked foodstuffs, which also agrees with the findings of Stołyhwo & Sikorski (2005).

Another factor affecting the level of PAHs in smoked products is the distance between the product and the smoke source. In general, the greater the distance from the smoke source the lower the PAH levels found in food; therefore indirect smoking is a safer option (FAO &

WHO 2013; Hokkanen et al. 2018). The length of the smoking tube can vary between 0.2 and 23 meters (Hokkanen et al. 2018). Naturally, all the samples smoked with the direct technique were placed in the same chamber as the smoke source, and the distance was therefore always under five meters and in most cases only around 1 meter (Table 11).

The type of fuel can also influence the level of PAH concentration in smoked products. When it comes to wood, it is generally not recommended to use resinous wood (FAO & WHO 2009), which is mostly softwood types. For this reason, hardwood is more suitable for smoking (Šimko 2005; Essumang et al. 2013). Producers from this research stated that they have used "white", "red", or available wood (Table 11); therefore, it is not possible to clearly conclude which type of wood was used. However, Red mangrove wood (which could refer to the "red" type of wood) has been reported as the main fuelwood used for fish smoking in Cameroon, as shown in Figure 10 (Dongmo Keumo Jiazet 2019). It is an endangered tree species *inter alia* due to the role of fish smoking practices and it is a hardwood type with enormous water resistant characteristics. Most producers also used the "white" type of wood, possibly referring to the rubber tree (*Hevea brasiliensis*), which has a high content of gums and resins. Such resinous wood could contribute to the high PAH levels.

**Table 11** Smoking parameters reported by producers

| Producer | Time of smoking | Max. distance of fish from smoke source* | Type of fuelwood |
|---|---|---|---|
| 1 | 1 h | n.d. | red |
| 2 | 4 - 24 h | 0.95 m | white |
| 3 | 4 h | 1 m | n.d. |
| 6 | 2 days | 1 m | available |
| 8 | 7 days | n.d. | white |
| 9 | 1.5 h | 1.76 m | white |
| 10 | 2 h | 5 m | white |

* height of the smoke house/kiln; n.d. - not detected

Due to the small number of matches with interview respondents and fish samples it was not possible to prove clear correlations of PAH levels and smoking practices within this study; however, the examples of practices in this research corresponded to the scientific literature reporting the factors for occurrence of high PAH levels.

# 6 Conclusion

This research had the aim of analyzing some of the quality properties of traditionally smoked Cameroonian fish, such as fat content, fatty acid profiles, and PAH contents, along with a complementary investigation of the local traditional smoking method by small-scale producers.

Preferred local species for smoking were *Clarias gariepinus* and *Gadus morhua.* Fat content found for all species ranged from 3.22 % to 24.58 %, with large differences observed among individual samples of the same species. Fat content differs depending on the size of fish, age, fishing ground, season, variation in the amount and quality of food that the fish eats, and the amount of movement it makes (Burt & Murray 2001). These variables along with the findings of this research suggest that it is important to evaluate fat content and fatty acid profiles in fish samples on an individual basis. The moisture content in samples also varied greatly, from 7.04 % ± 0.07 to 53.22 % ± 0.48, which may be a result of, among other factors, the wide range of smoking times (1 hour to 1 week). The Codex Alimentarius (FAO & WHO 2013) recommends a moisture content of 10 % or less in order to inhibit the growth of all foodborne pathogens; therefore, most of these samples were at high risk of spoilage.

The majority of the samples showed unfavorable values of NQIs, with a higher SFA and lower UFA share and a lower PUFA/SFA ratio than is typically reported. In addition, essential FAs (ALA and LA) in some of the fish samples within this research were considerably higher than the reported average in scientific literature. With the absence of the fatty acid EPA in all samples and lower levels of DHA (yet within the reported ranges), the total content of LC-PUFAs was also lower than the reported average of other studies. On the other hand, HH indices met the generally reported values and the ideal n-6/n-3 ratio was achieved in more or less 50 % of the samples, although the absolute amount of omega-3 and omega-6 FAs is more important. Among examined samples, *Labeo coubie* had the most positive NQI values; however, *Parachanna obscura* seemed like the ideal dietary source of LC-PUFAs due to its overall high fat content and therefore higher total content of omega-3 and omega-6 FAs.

All determined values of the four priority PAHs in all samples in this research highly exceeded the maximum levels for PAH contamination of foodstuffs set by Commission regulation (EC) No 1881/2006. On average, they were 13 times higher for BaP and 15 times higher for PAH4 than the MLs for traditionally smoked products. These high values were rarely reported in previous literature and could lead to the development of carcinogenic and other diseases related to PAH exposure. However, these results corresponded with an expectation of high values for PAHs after the smoking process in uncontrolled "wild" conditions typical for households in developing countries (Šimko 2002). Fuelwoods were not clearly identified, although the most commonly used "white" wood could refer to the resinous rubber tree, which negatively influences the PAH content in smoked products. Traditional smoking kilns are designed simply, made only for direct smoking and without the possibility of controlling the temperature, which seemed to be the main factor for high PAH values. Small species of fish used for smoking in Cameroon tend to be subject to heavier smoking, which can also support higher PAH values. Although the appropriate control of conditions during the smoking process in traditional smoking kilns is not possible, practicable improvements such as the FAO-Thiaroye fish processing technique do exist (FAO 2017). Contamination by carcinogenic PAHs can be significantly lowered with controlled conditions, good hygiene, proper handling and maintenance practices, and adequate equipment. Better education in smoking techniques for small-scaled Cameroonian producers could be helpful in these circumstances. So far, there are a lack of studies targeting the PAH and FA values in smoked fish and traditional smoking methods in Cameroon. Therefore, further research focused on the PAH profile, fatty acid profile, and traditional smoking methods in Cameroonian smoked fish is recommended.

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## Appendices

### List of appendices

**Appendix I** Questionnaire (semi-structured interview) for producers of smoked fish in Cameroon

**Questionnaire for Producers of Smoked Fish Cameroon, 2018**

**A. Introduction part of traditional smoking process**

1. Location of the producer of traditional smoked fish. (GPS)
2. Do you buy the fish for smoking or you fishing them?
3. Location of fish collection.
4. Name mainly smoked fish species.
5. Way of pre-treatment's before smoking of fish (use of spices, salt, drying, brine, etc.)
6. How do you sell the smoked fish?
   a. By yourself on the local market
   b. Customers coming individually to your house
   c. To the middle-man
   d. Directly to some bigger company or supermarket

**B. Technical part of traditional smoking process**

1. What material do you use for smoking? CHARCOAL/WOOD
2. If wood what kind?
3. Do you know how much of fire wood or charcoal you use per day or per batch in kg or in $m^3$?
4. How long is the fish in smoke house?
5. How long does the whole process take including pre-treatment and fire preparation?
6. Do you use trays?
   a. If yes, do you change them regularly?
   b. Do you change trays in some order?
7. How looks the fire during fish smoking?
8. How do you prepare the fire before smoking?
9. Do you measure the right temperature?
10. How you estimate or recognize that the fish is ready (already smoked)?
11. Do you use any additional technique? (Usage of carton, covering,…)

**C. Marketing and selling practices of traditional smoking products**

12. What is the amount of production per day or per batch (how big is the batch)?
13. In which period of the year is main smoking season?
14. How often do you smoke? (How many days, mths per year?)
15. Do you use any packaging of marketed smoked fish?
16. If you store the fish, where you store them and how?
17. For how many days you store your smoked fish before selling usually?

**D. Consumption habits**

1. How do you eat/prepare the smoking fish most often?

**E. Smoke house description**

1. Dimension in cm:
   a. Length
   b. Width
   c. Height
   d. No. of trays
   e. Distance of the trays
   f. Structure
   g. Roof YES/NO
   h. Ventilation YES/NO
   **i. Picture OWNER/SMOKEHOUSE**

**Appendix II** Fatty acids profiles of fish samples

| Fatty acid (%) | *Alestes baremoze* Sample No 5 | *Alestes baremoze* Sample No 11 | *Carlarius heudelotii* Sample No 9 | *Carlarius heudelotii* Sample No 14 | *Clarias gariepinus* Sample No 2 | *Clarias gariepinus* Sample No 10 | *Clarias gariepinus* Sample No 13 | *Clarias gariepinus* Sample No 15 |
|---|---|---|---|---|---|---|---|---|
| **SFA** | | | | | | | | |
| **C11:0** | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| **C12:0** | n.d. | n.d. | 1.25 | 0.30 | n.d. | 0.68 | 0.76 | 0.54 |
| **C13:0** | n.d. | n.d. | 0.24 | n.d. | n.d. | 0.41 | 0.46 | 0.38 |
| **C14:0** | 1.68 | 0.46 | 2.12 | 3.12 | 2.03 | 3.49 | 5.90 | 2.13 |
| **C15:0** | 0.57 | 0.23 | 1.04 | 1.64 | 0.68 | 1.39 | 1.60 | 1.40 |
| **C16:0** | 26.42 | 16.47 | 16.51 | 27.74 | 24.89 | 23.76 | 25.71 | 22.86 |
| **C17:0** | 0.95 | 0.53 | 2.71 | 2.76 | 1.48 | 1.81 | 1.88 | 2.19 |
| **C18:0** | 12.48 | 9.47 | 9.47 | 11.72 | 9.74 | 10.56 | 10.90 | 9.08 |
| **C20:0** | n.d. | 0.34 | n.d. | n.d. | 0.64 | n.d. | 0.61 | n.d. |
| **C22:0** | n.d. | n.d. | 0.51 | 0.36 | 0.45 | 0.46 | n.d. | n.d. |
| **ΣSFA** | 42.10 | 27.50 | 33.85 | 47.64 | 39.91 | 42.56 | 47.82 | 38.58 |
| **MUFA** | | | | | | | | |
| **C16:1** | 2.35 | 0.55 | 8.09 | 5.50 | 3.12 | 4.70 | 3.30 | 5.76 |
| **C18:1n9c** | 31.35 | 24.72 | 13.10 | 17.48 | 33.11 | 18.45 | 20.54 | n.d. |
| **C20:1** | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 2.88 | n.d. |
| **ΣMUFA** | 33.70 | 25.27 | 21.19 | 22.98 | 36.23 | 23.15 | 26.72 | 5.76 |
| **PUFA** | | | | | | | | |
| **C18:2n6c** | 11.39 | 33.95 | 9.74 | 4.24 | 6.51 | 7.32 | 4.75 | 12.64 |
| **C18:3n3** | 3.26 | 6.32 | 2.15 | 3.50 | 4.09 | 4.72 | n.d. | 3.44 |
| **C20:4n6** | 1.07 | 1.01 | 3.39 | 2.35 | 1.79 | 3.18 | 2.63 | 2.36 |
| **C22:2n6** | 1.00 | 1.13 | 1.85 | 3.91 | 1.52 | 2.82 | 5.63 | 1.76 |
| **ΣPUFA** | 16.72 | 42.41 | 17.13 | 14.00 | 13.91 | 18.04 | 13.01 | 20.20 |
| **Unidentified** | 7.49 | 4.80 | 27.81 | 15.39 | 9.94 | 16.24 | 12.44 | 35.46 |

n.d. – not detected

**Appendix II continuing** Fatty acids profiles of fish samples

| Fatty acid (%) | *Brama dussumieri* Sample No 16 | *Gadus morhua* Sample No 1 | *Gadus morhua* Sample No 3 | *Gadus morhua* Sample No 4 | *Labeo Coubie* Sample No 7 | *Mugil cephalus* Sample No 6 | *Parachanna obscura* Sample No 8 | *Parachanna obscura* Sample No 12 |
|---|---|---|---|---|---|---|---|---|
| **SFA** | | | | | | | | |
| **C11:0** | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.57 | 0.08 |
| **C12:0** | n.d. | n.d. | n.d. | n.d. | n.d. | 0.57 | 1.12 | 0.30 |
| **C13:0** | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.95 | 0.25 |
| **C14:0** | 4.01 | 4.37 | 3.75 | 2.06 | 1.44 | 1.61 | 5.51 | 3.13 |
| **C15:0** | 1.32 | 0.42 | 0.48 | 0.68 | 0.51 | 0.36 | 2.02 | 1.12 |
| **C16:0** | 25.35 | 24.60 | 17.61 | 26.24 | 24.39 | 25.30 | 20.05 | 21.16 |
| **C17:0** | 1.65 | 0.89 | 0.76 | 1.25 | 1.18 | 1.70 | 1.99 | 1.40 |
| **C18:0** | 9.30 | 4.86 | 3.15 | 8.73 | 9.36 | 13.35 | 8.46 | 8.85 |
| **C20:0** | 0.53 | 0.33 | 0.20 | n.d. | 0.55 | 0.29 | 0.42 | 0.37 |
| **C22:0** | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.13 |
| **ΣSFA** | 42.16 | 35.47 | 25.95 | 38.96 | 37.43 | 43.18 | 41.09 | 36.79 |
| **MUFA** | | | | | | | | |
| **C16:1** | 4.86 | 3.57 | 5.00 | 2.92 | 3.61 | 7.67 | 5.81 | 4.50 |
| **C18:1n9c** | 21.72 | 22.98 | 19.34 | 33.64 | 17.80 | 25.01 | 20.14 | 27.61 |
| **C20:1** | 2.67 | 14.35 | 10.62 | 4.87 | n.d. | n.d. | n.d. | n.d. |
| **ΣMUFA** | 29.25 | 40.90 | 34.96 | 41.43 | 21.41 | 32.68 | 25.95 | 32.11 |
| **PUFA** | | | | | | | | |
| **C18:2n6c** | 3.47 | 2.01 | 1.11 | 5.74 | 12.62 | 2.23 | 5.46 | 4.60 |
| **C18:3n3** | n.d. | n.d. | n.d. | n.d. | 10.36 | n.d. | 3.57 | 3.74 |
| **C20:4n6** | 2.84 | n.d. | 0.62 | 1.42 | 3.22 | 2.26 | 3.00 | 2.07 |
| **C22:2n6** | 9.93 | 6.61 | 6.33 | 2.18 | 3.07 | 0.89 | 3.56 | 3.06 |
| **ΣPUFA** | 16.24 | 8.62 | 8.06 | 9.34 | 29.27 | 5.38 | 15.59 | 13.47 |
| **Unidentified** | 12.35 | 15.00 | 31.03 | 10.29 | 11.88 | 18.75 | 17.36 | 17.66 |

n.d. – not detected

**Appendix III** Fish fat composition of saturated FAs by species – expressed in means and SD

| **Species** | **ΣSFA** | **SD** | **ΣMUFA** | **SD** | **ΣPUFA** | **SD** |
|---|---|---|---|---|---|---|
| *Alestes Baremoze* (n=2) | 34.80 | ±7.30 | 29.49 | ±4.22 | 29.57 | ±12.85 |
| *Brama dussumieri* (n=1) | 42.16 | n.d. | 29.25 | n.d. | 16.24 | n.d. |
| *Carlarius heudelotii* (n=2) | 40.75 | ±6.89 | 22.09 | ±0.90 | 15.57 | ±1.57 |
| *Clarias gariepinus* (n=4) | 42.22 | ±3.54 | 22.97 | ±11.02 | 16.29 | ±2.95 |
| *Gadus morhua* (n=3) | 33.46 | ±5.50 | 39.10 | ±2.93 | 8.67 | ±0.52 |
| *Labeo Coubie* (n=1) | 37.43 | n.d. | 21.41 | n.d. | 29.27 | n.d. |
| *Mugil cephalus* (n=1) | 43.18 | n.d. | 32.68 | n.d. | 5.38 | n.d. |
| *Parachanna obscura* (n=2) | 38.94 | ±2.15 | 29.03 | ±3.08 | 14.53 | ±1.06 |

n.d. not detected; Table is addition to Figure 33