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Abstract

Purpose: This study was conducted to provide occurrence on the various mycological strains associated with the spoilage of fish smoked with various kilns in the city of Douala.

Material and Methods: Three commonly most consumed fish species (*Ethmalosa fimbriata*, *Sphyraena afra* and *Gadus morhus*) were randomly collected from the Douala sea port market for this purpose. Ten samples of each species were smoked with a Half-barrel, Banda, Altona and an Improved Altona (FN23) kilns respectively and stored for 42 days at room temperature. Fungi isolation and purification were performed on the Chloramphenicol Sabouraud Agar. Identification of isolated strains was done using standard macroscopic and microscopic morphological characters. Data collected were analysed by one-way analysis of variance (ANOVA) at $p < 0.05$.

Findings: Results showed that the maximum number of moulds in the fish smoked with Half-barrel, Banda and Altona was 61.0±4.5, 56.6±5.2 and 45.6±1.4 respectively in *G. morhua*, *S. afra* and *E. fimbriata* on day 28. The most prevalent isolates were *Aspergillus* (40%), *Penicillium* (30%), *Fusarium* (10%), *Absidia* (10%) and *Cladosporium* (10%). For fish smoked with FN23, the maximum was reached on day 35 with 29.6±3.4 moulds in *S. afra*. With the lowest water activity ranging from 0.52±0.4 in *S. afra* to 0.57±0.6 in *G. morhua*, fish smoked with FN23 showed better resistance to fungal contamination with just 3 genera: *Aspergillus* ; *Penicillium* and *Fusarium* (with 2, 1 and 1 isolates respectively).

Unique Contribution to Theory, Practice and Policy: Results from this study revealed the significant impact of the type of kiln in the preservation of smoked fish. Proper awareness and regular monitoring of moulds in smoked fish have to be implemented in Douala.

Keywords*: Mycological identification, spoilage, smoked fish, kilns.*

JEL codes: *I10, I15, Q13, Q20, Q22*

1.0 INTRODUCTION

Fish is a foodstuff of high nutritional value and beneficial to health. It contributes to food security and the reduction of non-communicable diseases (Manz et al., 2020; Nchoutpouen et al., 2020). It also serves as income for a vast majority of the population, particularly riverine dwellers. However, a significant proportion of its production is subject to high post-harvest losses. These losses are estimated at 35% of world production (equivalent to 62.3 million tonnes per year) and 15% of national production in Cameroon. Because of its texture, fish is an extremely perishable food (FAO, 2022).). Deterioration of fish occurs as a result of complex enzymatic, microbial, chemical and physical changes (Aliyu et al., 2018).

One of the traditional means of fresh fish preservation in Cameroon is by smoking. Indeed, 80% of fish from continental and artisanal marine fisheries are distributed in the form of smoked products (ACP Fish II, 2013, Nsoga et al., 2021). To this end, several types of kiln are used to produce these smoked fish, including: the Half-barrel, the Banda, the Chorkor and the Altona (Tiwo, 2020). These methods affect the concentration of microorganisms differently in fish, in particular by the drop in their water contents, the reduction of microorganisms by heat or an inadequate smoking. Other factors can also influence the development of microorganisms: improper handling leading to off-odors and flavors, post-processing contamination (Exposure to dust, insects, and unsanitary handling in open markets) and improper Storage such as inadequate refrigeration or storage at ambient temperatures allows microbial activity and spoilage to accelerate. All this causes result in different types of micro-flora and different risks from spoilage organisms and pathogens (Hagos, 2021, Tahiluddin et al. 2022, Sissoko et al., 2025). So, proper control of time/temperature parameters during smoking, strict sanitation measures during fish handling, filleting, and processing are crucial to minimize contamination. Appropriate packaging and effective storage can also help to preserve the quality and to control the growth of spoilage microorganisms by extending the shelf life of hot-smoked products.

Problem Statement

Fungal contamination is a serious challenge faced by smoked fish processors and consumers in Cameroon where limited access to effective diagnosis and treatment exacerbates its impact. This infection may be caused as the initial deterioration agents or as a secondary contaminant due to mechanical damage (Walter et al. 2020, Etchutakang et al. 2023). Several moulds has being implicated in fish spoilage, which is also harmful to consumer's health because of their ability to produce mycotoxins. Among these we can cite yeasts and moulds, including *Aspergillus* and *Penicillium*, are common culprits for spoilage in processed fishery products (Hagos, 2021). Although consumers appreciate smoked fish for their aroma, taste and color, mycotoxins can pose serious threats such as food insecurity, shortage of essential nutrients and low income generation in developing countries (Andhikawati and Pratiwi, 2021). These toxins are relatively stable and their toxicity can persist for a long time even when the fungal elements are no longer viable. Harmful effects can be immediate, such as acute poisoning, or long-term, such as immune deficiency or cancer (Abdoullahi et al., 2019). Sometime these Food poisoning organisms can multiply profusely in foods without initially altering the appearance, taste or odor.

Despite the risks linked to the consumption of smoked fish, unfortunately, to the best of our knowledge, very little significant scientific data currently exists in Cameroon on the impact of the various smoking kilns on the growth of moulds on smoked fish (Ziem et al. 2024). This gap is important, because it gives an orientation on the kiln allowing the best to obtain and preserve the fungal quality of the smoked fish. Identification of this fungal flora is generally based on

observation of the cultural and morphological characteristics of the species (Mmandu and Esther, 2020; Ngo-Oum et al. 2021; Foba et al. 2023; Idris et al. 2024). Hense this study was designed to enumerate the mycological strains associated to the spoilage of three most consumed smoked fish species, namely *Ethmalosa fimbriata*, *Sphyraena afra* and *Gadus morhua* smoked with four different kilns.

Specifically, this study seeks to answer the following questions:

1. What are the most common moulds associated with contamination of smoked fish in the city of Douala?
2. Which kiln best preserves the fungal quality of smoked fish?

By addressing these questions, this study will contribute to the knowledge of common fungal strains that contaminate smoked fish in the city of Douala and to know the smokehouses that are more suitable for preserving the fungal quality of smoked fish. The results obtained will serve for the nutritional health of the population by encouraging processors to adopt the kiln model that has a better effect on public health.

2.0 MATERIAL AND METHODS

Sample Source

A total of 120 samples of fresh fish belonging to 3 of the most consumed species, namely: *Ethmalosa fimbriata*, *Sphyraena. afra* and *Gadus morhua* (Nsoga et al., 2021) were randomly purchased in the fishing port of the city of Douala. Douala is the economic capital of Cameroon, with a cosmopolitan population of around 5 million inhabitants. It lies on the Gulf of Guinea between 3°50' and 4°08' north latitude and 9°30' and 9°75' east longitude. After purchase, the fish were carefully packed into clean labelled polythene bags and kept inside a clean container and transported in coolers with an ice/fish ratio of 2:1 to 4 different processors using the Half-barrel, Banda, Altona and Improved Altona (FN23) respectively. The experimental fish samples used are presented in figure 1 bellow.

(a) *Ethmalosa fimbriata* (b) *Sphyraena afra* (c) *Gadus morhua*

Figure 1: The experimental fish samples collected from Douala fishing seaport

Description of the Smoking Kilns

Four smoking kilns were used: The Half-barrel, Banda, and Altona which are the most used in the city of Douala (Nsoga et al., 2021), and an Improved Altona kiln (FN23) designed by our research team. The Half-barrel is a 60 cm diameter and 90 cm length metal drum. It reduces heat loss but does not allow, due to the small surface of the rack, to smoke large quantities of fish. The Banda kiln is built from wooden posts on which rest large smoking racks. It can be used for large quantities of fish but the smoking room always completely opened leads to loss of heat and wood. Altona kiln consists of two structures: an exterior brickwork to improve energy efficiency and a metal structure to hold removable racks. The oven offers good possibilities for storing and re-smoking large amount of processed products without having to handle the fish. Improved Altona kiln (FN23) is an experimental smokehouse designed from the Altona model to improve the health quality of smoked fish. Its peculiarity is to allow the control of the heat that reaching the product. A stainless steel plate allows the fumes to be

channelled outwards, preventing continuous contact between the heat source and the products to be smoked. The aim was to limit the impact of the heat on the nutrients and reduce contaminations such as polycyclic aromatic hydrocarbons from wood combustion.

Batch Preparation

Ten (10) of the forty (40) fish of each species were used for each smoking method. The samples collected for the study were transported to the various processors in well-prepared sterile polythene bags. The fish were washed and smoked using methods specific to each type of smokehouse. After smoking, each processor stored the smoked fish in an aerated bamboo basket covered with sheets of paper, as is customary. This method of storing smoked fish is the same as that described by the processors. These storage containers are kept at room temperature under field conditions for 6 weeks according to the method of Watanabe et al., (2013). Every 7 days, the samples collected for the study were transported in well-prepared sterile polythene bags to the laboratory to check the water activity and the presence of fungi.

Water Activity

An Aqualab Aw-meter, model series 3 TE (Biotrace international, Villeneuve-la Garenne, France) was used to determine the water activity of the sample at room temperature. Its formula is: aw = p/ps (p: water vapour pressure above the surface of the product; Ps: water vapour pressure above the surface of pure water).

Preparation of the Culture Medium

A 65.5 g mass of commercially produced SCA (Sabouraud Chloramphenicol Agar) was dissolved in 1 litre of distilled water and allowed to homogenise in a water bath. The medium was then sterilised by autoclaving at 121°C for 15 minutes. The purpose of chloramphenicol is to inhibit the growth of any bacterial species likely to contaminate the isolate. This melted medium was poured into sterile Petri dishes (15 cm diameter Petri dishes) and cooled in a desiccator to solidify (Foba et al., 2023).

Preparation of Samples and Dilutions

Each sample was taken and crushed in a sterile mortar with pestle under laboratory condition then left to rest for 30 min. Tryptone Glucose Extract Agar (TGEA) was prepared according to manufactures by pouring 6 g into 250 ml of distilled water then autoclaved at 121° C for 15 minutes. The role of this agar is to revive moulds that have been stressed or altered and to restore their normal growth capacity, without necessarily promoting their growth. This medium was added to the various powders. In test tubes, 1 mg of each substrate obtained was added to 9 ml of distilled water to form the stock solution. The mixture was vortexed for 2 minutes. Then, using a micropipette, 1 ml of each stock solution was diluted in 9 ml of distilled water to obtain a 10^{-1} dilution. This procedure was used successively until the 10^{-3} dilution was reached (Foba et al., 2023).

Plating and Incubation

Near the Bunsen burner, 10 µL of each tube was taken and spread onto Petri dishes containing approximately 20 ml of SCA agar and sealed with cling film. After rotating gently, the plates were incubated at 27°C for 72 hours. Mould growth was observed (Idris et al. 2024).

Mould Counts

The number of moulds was counted directly on the Petri dishes for six consecutive weeks (Watanabe, 2002). A digital colony counter was used for this purpose.

Transplanting and Purification

Transplants were made by removing a mould fragment using a sterile pipette (avoiding contact with other neighbouring moulds) and placing it in the centre of a new agar plate (Mmandu and Esther, 2020). Representative colonies emerging from the plates were grouped according to their cultural characteristics, purified by repeated sub-culturing and were stored on Czapek Yeast Extrat Agar medium inclined in cryotubes at +4 °C (Flourage et al., 2024).

Macroscopic and Microscopic Identification

The fungal isolates were identified based on the macroscopic and microscopic characteristics, which include surface texture, topography and pigmentations as described by Anses (2020). Microscopic identification was carried out by placing a drop of 5% potassium manganese ($KMnO_4$) on a slide and a small portion of representative fungi mycelium was removed and teased onto the potassium manganese stain using a sterile needle to observe characteristics such as shape, size of hyphen, shape of sporangia, conidia, conidiophores and spores under the microscope using X10 and X40 magnification (Mmandu and Esther, 2020).

Data Analysis

Tables and figures were used to present the results. Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA) to estimate the effect of smoking kilns on the quality of smoked fish. Differences between the means were determined using Duncan's multiple range test. The statistical package used was SPSS 20.0 at a confidence level of 95% ($p < 0:05$).

3.0 FINDINGS

Evolution of Water Activity during Storage of Smoked Fish

The three fish species *E. fimbriata*, *S. afra* and *G. morhua* were smoked using the Half-barrel, Banda, Altona and FN23. Samples were taken to measure water activity. Table I shows the evolution of water activity in the different fish samples.

Table 1: Evolution of Water Activity in Different Fish Samples

Fish species	Raw fish	Half-barrel	Banda	Altona	Improved Altona (FN23)
E. fimbriata	$0.96\pm0.01^{a\alpha}$	$0.71\pm0.07^{bc\alpha}$	$0.73\pm0.01^{b\alpha}$	$0.61\pm0.04^{c\alpha}$	$0.54\pm0.02^{d\alpha}$
S. afra	$0.92\pm0.04^{a\alpha}$	$0.77\pm0.05^{b\alpha}$	$0.75\pm0.03^{b\alpha}$	$0.63\pm0.05^{c\alpha}$	$0.52\pm0.04^{d\alpha}$
G. morhua	$0.95\pm0.02^{a\alpha}$	$0.72\pm0.04^{b\alpha}$	$0.71\pm0.03^{b\alpha}$	$0.64\pm0.08^{bc\alpha}$	$0.57\pm0.06^{c\alpha}$

Averages for rows with different superscript letters are significantly different at $p<0.05$.
Means for columns without the same superscript special characters are significantly different at $p<0.05$.

Mould Counts

Mould counts carried out every 7 days during 6 weeks of storage showed variations in the number of moulds in the different samples of smoked fish. Table II presents the results of these variations.

Table 2: Mould Counts (Number/Gram) During Storage of Smoked Fish

Kilns	Fish species	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Half-barrel	*E. fimbriata*	2.0±0.0^{a}	5.6±0.7^{b}	17.3±2.4^{c}	37.0±2.9^{d}	51.6±3.8^{e}	33.6±1.8^{d}	21.3±2.9^{c}
	S. afra	3.3±0.2^{a}	7.0±0.3^{b}	14.6±1.7^{c}	22.3±1.2^{d}	49.3±2.2^{e}	29.0±3.3^{f}	18.6±1.6^{g}
	G. morhua	4.3±0.3^{a}	5.0±0.1^{b}	13.0±2.0^{c}	19.0±2.3^{d}	61.0±4.5^{e}	43.6±4.7^{f}	30.0±1.6^{g}
Banda	*E. fimbriata*	2.0±0.6^{a}	6.6±1.3^{b}	16.0±1.0^{c}	25.6±1.8^{d}	54.6±2.9^{e}	41.6±3.8^{f}	27.6±2.8^{d}
	S. afra	4.0±0.7^{a}	8.0±0.6^{b}	17.6±2.8^{c}	28.3±2.6^{d}	56.6±5.2^{e}	48.0±5.0^{f}	31.6±1.4^{d}
	G. morhua	3.0±0.0^{a}	9.3±0.8^{b}	19.3±1.1^{c}	27.6±2.1^{d}	54.6±3.1^{e}	53.3±3.1^{e}	37.0±4.0^{f}
Altona	*E. fimbriata*	4.0±0.1^{a}	7.3±1.2^{b}	16.3±1.2^{c}	21.3±3.4^{d}	45.6±1.4^{e}	42.0±3.3^{e}	29.3±2.1^{f}
	S. afra	4.6±0.5^{a}	8.6±0.3^{b}	15.3±2.5^{c}	29.0±3.0^{d}	33.3±4.0^{de}	35.3±2.9^{e}	25.0±1.7^{d}
	G. morhua	3.0±0.3^{a}	6.3±0.9^{b}	16.0±1.9^{c}	20.6±1.9^{d}	26.0±3.3^{e}	29.6±3.9^{e}	19.3±1.7^{d}
Improved Altona (FN23)	*E. fimbriata*	2.0±0.3^{a}	4.0±1.2^{b}	10.6±2.0^{c}	16.3±0.7^{d}	21.6±1.7^{e}	22.0±1.0^{e}	16.3±0.9^{d}
	S. afra	1.0±0.0^{a}	3.6±0.2^{b}	7.0±1.3^{c}	13.0±1.0^{d}	26.3±2.6^{ef}	29.6±3.4^{e}	22.6±1.4^{f}
	G. morhua	1.0±0.0^{a}	4.6±0.9^{b}	8.6±0.6^{c}	14.6±2.1^{d}	21.0±2.8^{ef}	25.3±2.4^{e}	18.0±2.0^{f}

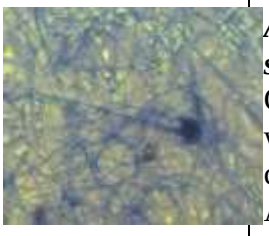
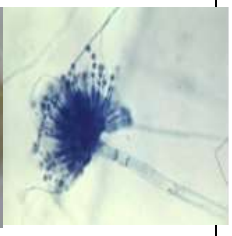
Means on lines not having the same superscript letters are significantly different at $p<0.05$.

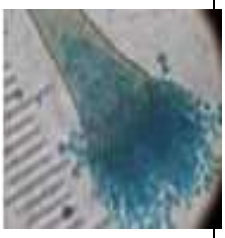
Macroscopic and Microscopic Identification of Fungal Isolates

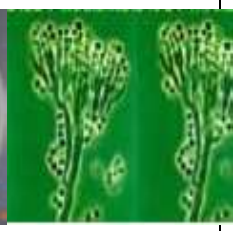
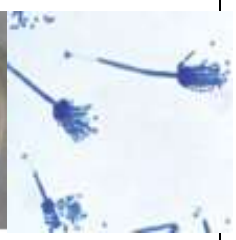
Macroscopic Identification

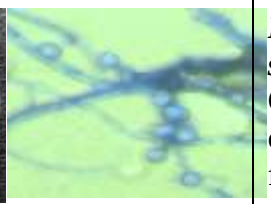
The macroscopic characteristics of the various isolates were studied on the SCA medium. Moulds isolated were subjected to microscopic identification by observation at X40 magnification. Identification was based essentially on observation of the mycelium and spores (shape, colour, wall texture). Table III shows the macroscopic and microscopic appearance of the ten fungal isolates identified and highlighted in the Petri dish.

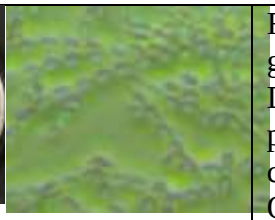
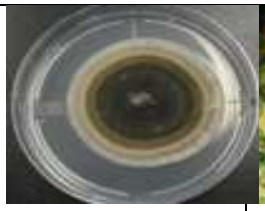
Table 3: Macroscopic characteristics of moulds isolated from smoked fish compared to reference pictures

Aspects of the isolates		Description	Reference Picture (ANSES, 2020)	
Macroscopic	**Microscopic**		**Macroscopic**	**Microscopic**
 (SCA)		*Aspergillus spp.* Rapid growth (2-3 days) on Sabouraud medium. white at first, then grey or yellowish depending on the medium. Hyphae: septate - Conidiospore: very long and not septate -Conidia : globular -Aspergillus head: biseriate, radiate	 **(MA)**	
 (SCA)		***Aspergillus spp.*** Colour: white with grey centre Appearance: velvety -Thallus with septate mycelium (erect conidiophores) -Uniseriate or biseriate	 **(PDA)**	

		conidial heads; -Radiant conidial mass, Conidia in unicellular chains		
 (SCA)		***Aspergillus spp.*** Colour: grey (Sabouraud) or green (PDA) with white centre. Appearance: cottony Reverse side: black - Conidiospore: long, not septate, -Conidia: globular, pale green -Aspergillus head: uniseriate, radiate	 **(PDA)**	
 (SCA)		***Aspergillus spp*** Colour: white or light green Appearance: velvety to powdery Reverse: no pigment Green and obscure conidial head, in a compact column of	 MA	

		uniform diameter.		
 (SCA)		***Penicillium spp.*** Colour: grey Appearance: cottony, invasive Reverse: black, no pigment Can form flaky colonies with a brush-like organisation. Thallus with smooth or granular conidiophores	 (PDA)	
 (SCA)		***Penicillium spp.*** Colour: grey Appearance: velvety to powdery surface exudate droplets Reverse side: yellowish The conidia are produced by phialides inserted at the dilated end of a wide, unpartitioned conidiospore	 **(SCA)**	

		(aspergillate head arrangement).		
 (SCA)		***Penicillium spp*** Colour: light brown Appearance: powdery, invasive -Hyphae: septate, hyaline, bearing conidiospores - Conidiospore : branched cylindrical, septate -Phialides in -Conidia : round	 **(PDA)**	
 (SCA)		***Fusarium spp.*** Culture: colonies, flaky, yellowish white or vinous. Reverse: yellow Conidia resulting from the budding of sporocysts. This budding gives rise to a string of conidia	 **(PDA)**	
		Cladosporium spp.		

 (SCA)		Fairly slow growth Downy to powdery colony Olive brown to black in colour septate hyaline hyphae, conidiophores, phialides, macroconidia and microconidia observable under the microscope	 **(PDA)**	
 (SCA)		**Absidial spp** Colour: white Appearance: domed cottony Reverse: no pigment Branched sporocystophore ending in a columella projecting into the globular sporocyst	 **(MA)**	

SCA: Sabouraud Chloramphenicol Agar. MA: Malt Agar. PDA: Potato Dextrose Agar.

3.4 Composition of Fungal Isolates

Figure 2 shows the different fungal genera identified in the various samples of smoked fish.

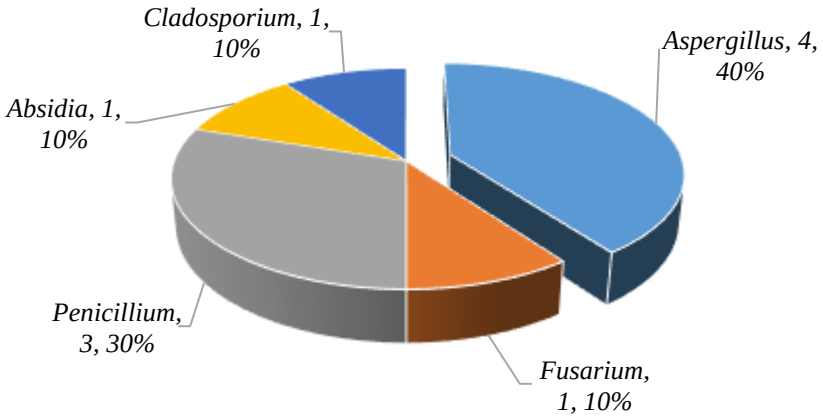


Figure 2: Frequency of Fungal Isolates during Storage of Smoked Fish Samples

3.5 Frequency of Isolates According to Smoking Method

During storage, smoked fish were grouped according to smoking method. Figure 3 shows the distribution of fungal genera according to smoking method.

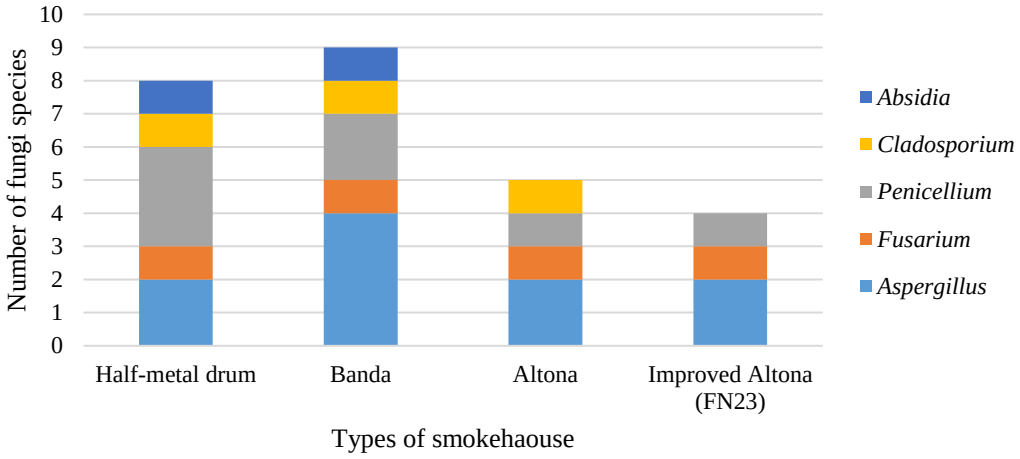


Figure 3: Distribution of Fungal Genera According to Smoking Method

4.0 FINDINGS

4.1 Evolution of Water Activity (aw) in Smoked Fish Samples during Storage

Water activity indicates the quantity of water that is biologically available to microorganisms. Of all the parameters that influence the growth of microorganisms, it is the most relevant (FAO, 1989). Smoking contributes not only to lowering aw, but also to preserving the quality of the finished product (Ndrianaivo et al., 2016). The water activities (aω) obtained for smoked fish range from 0.52±0.04 to 0.77±0.05. Low aw foods such as smoked fish keep well at room temperature and for long periods. Ndrianaivo et al (2016) obtained aw values between 0.492±0.009 and 0.495±0.029. This difference would be linked to a sharp drop in water activity after smoking. There were significant differences ($p<0.05$) between fish smoked in the FN23 with aw below 0.58 and those smoked in the other smokehouses, all of which had aw above 0.6. These differences are thought to be linked to a better reduction in free water in fish smoked

in the FN23. In addition, these low water activity values (aω<0.60) in fish smoked with FN23 could inhibit mould growth during storage (FAO, 1989). However, there was no significant difference (p>0.05) between fish smoked in the same smokehouse. This could be explained by the fact that, since these species are of the same size, they undergo the same amount of heat during smoking.

4.2 Mould Counts

At the start of storage, mould concentrations are low, mainly because of the quality of the fresh fish used. In addition, low water activity coupled with the action of the phenol groups contained in the smoke would inhibit mould growth. Table I shows significant differences (p<0.05) in the number of moulds, which increased gradually to reach a maximum in the fourth week (day 28). After this date, there was a decrease in the number of moulds in all samples. This is because smoked fish gradually reabsorb water when kept in a high-humidity environment (Ndrianaivo et al., 2016). Relative humidity in excess of 60% favours the proliferation of moulds, which would be the case in this study. In the city of Douala, air humidity can reach or exceed 90% (Tiwo, 2020). The environment then becomes particularly conducive to all types of rotting. Ndrianaivo et al. (2016) noted an increase in mould concentration up to the 45th day and then a decrease between the 45th and 60th day during storage of *Oreochromis niloticus* in Madagascar. According to these authors, this variation is linked to the quality of the smoking process and in particular to the effect of temperature, pH and water activity during storage. These parameters could justify the variations obtained in this study. Indeed, the maximum of moulds/g obtained were: 4±0.66; 9±0.33; 19±1.33; 37±3; 56±5; 53±3.33 and 31±0.66 on days 0, 7, 14, 21, 28, 35 and 42 respectively. Frequency of occurrence of fungi isolate on smoke dry fish samples were 25, 21, 26 and 19 from four urban markets in Cameroon (Ngo-Oum et al. 2021). This difference is thought to be linked to the quality of the smoking process, the free water content in the fish and the storage method. Contamination can be explained by the great capacity of yeasts and moulds to develop on substrates with low water activity down to 0.6 (Ndrianaivo et al., 2016). Mould spores can also be carried by insects infesting stored fish, thereby increasing their numbers (FAO, 1989). Fungal flora mainly cause spoilage but can also lead to the production of mycotoxins responsible for food poisoning in humans (Ziem et al. 2024). The presence of mouldy fish in the batch during storage can affect the whole batch. This would explain the presence of five genera in the batch smoked with Half-barrel and Banda compared with the batches smoked with Altona and FN23 (Figure 3).

4.3 Macroscopic and Microscopic Identification

Isolation from samples of smoked fish using the four smoking methods yielded 10 fungal strains belonging to 5 genera: *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium* and *Absidia* with 4, 3, 1, 1 and 1 isolates respectively (Figure 2). The genus *Aspergillus* showed a high frequency of occurrence (40 %) in the majority of samples analysed. These results are in agreement with the work of Ngo-Oum et al. (2021) who obtained globally *Aspergillus* (83,51 %), *Penicellium* (6,59 %), *Fusarium* (3,25 %), *Mucor* (4,39 %) and *Rhisopusstolonifer* (2,19 %) in smoked dried fish. Ikeh et al. (2024) cited *Aspergillus* (31,40 %), *Penicillium* (14,87 %), *Fusarium* (17,35 %), *Mucor* (19,83 %) and *Rhizopus* (16,52 %) among the strains usually isolated from smoked and dry fish. This composition can be influenced by organic matter content, environment, texture, pH, humidity, water activity, temperature, aeration and other factors (Smith et al., 2000). Similarly, Akwobu et al (2019) found 5 strains of *Aspergillus* (28.6%), *Mucor* (20.8%), *Penicillium* (18.2%), *Absidia* (9.1%) and *Candida* (7.8%) in smoked fish sold in Makurdi markets in Nigeria. These results could explained the predominance of *Aspergillus* in the soil and air through its spores. The fish were smoked and stored in a high-

humidity environment, which could explain the high presence of these moulds, as the conditions were favourable for their growth. To this could be added the lack of good manufacturing and hygiene practices of the processors. These fungal genera are present in the majority of poorly preserved or insufficiently dried foods. Similar studies carried out in Ivory-Coast and Nigeria have reported the predominance of strains belonging to the genera *Aspergillus* (Foba et al., 2023); Idris et al. 2024). Water activity and pH determine the start of fungal growth. The high adaptability of *Aspergillus* to these parameters may explain their high frequency compared with other moulds. According to Elia et al. (2016), biochemical and physical changes such as oxidation, reabsorption of water by smoked fish and microbiological modifications occur during the storage of smoked fish. Certain strains of *Aspergillus*, *Fusarium, Penicillium* and *Absidia* are associated to production of Aflatoxin B1, Ochratoxin A and fumonisin B in smoked fish. Imane and Mouhamed (2012) had isolated fungal strains identical to those in this study, which produce Type B1, B2, G1 and G2 aflatoxins that can cause liver cancer. These moulds could therefore be the cause of the appearance of toxins in smoked fish.

4.4 Composition of Fungal Isolates According to Smoking Method

During storage, the fish were grouped according to smoking method. Figure 3 shows 5 fungal genera present in smoked fish from the Half-barrel and Banda smokehouses, with 8 and 9 isolates respectively. Smoked fish from the Altona and FN23 smokehouses obtained 5 isolates belonging to 4 genera and 4 isolates belonging to 3 fungal genera respectively. These results may be explained by their low water activity values. Indeed, fish from the Altona and FN23 kilns obtained low water activity, $a\omega < 0.64\pm0.08$ and $a\omega < 0.57\pm0.06$ respectively. The presence of certain fungal genera such as *Aspergillus* (2 isolates), *Fusarium* (1 isolate) and *Penicillium* (1 isolate) can be explained by their ability to grow at low aω and pH values (Ndrianaivo et al., 2016).

4.0 CONCLUSION AND RECOMMENDATIONS

Macroscopic and microscopic identification showed a clear predominance of moulds of the genera *Aspergillus* and *Penicillium*. The presence of these fungal strains in smoked fish points to a lack of good hygiene and manufacturing practices. Overall, smoked fish from the Altona and FN23 showed a better resistance to fungal flora during storage than those smoked with the Half-metal drum and Banda. The health risk associated with the presence of mycotoxins in smoked fish during storage is an important factor for implementing improvements in smoking techniques. The FN23 presented an advantage in terms of good preservation against fungal spoilage of smoked fish compared with the other three smoking methods.

Recommendations

Given the risk of mycotoxins in smoked fish, we recommend that future researches are required to confirm by molecular identification methods the moulds isolated from different smoked fish species. With the results evidence in this studies, the food safety regulations must take clean shades to control the mushroom levels in smoked fish. It would also be important to determine the relationship between storage condition of smoked fish and presence of toxins so, proper handling of smoked fish should be employed.

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Conflict of interest

The authors declare that they have no competing interests related to this article.

Credit Authorship Contribution Statement

Nsoga J. V. F.: Design the research work, performed smoking and storage methods, Mycological identification, Formal analysis software, Writing-original draft, review. Ekwala N. M. R. J.: Mycological identification, Formal analysis software, review. Nchoutpouen N. M.: Design the research work, Formal analysis software, review. Tuem S. R.: Performed smoking methods, Formal analysis software, Wr iting-original draft. Manz K. J. C.: Performed smoking and storage methods, formal analysis software, and review. Ndomou M.: Design the research work, supervision. All authors have read and approved the final manuscript.

Ethical Approval

This study did not involve any human or animal testing.

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