

Original Research Article

Nutritional quality and evaluation of some microbial flora of smoked fish sold in some public markets in the city of Abidjan (Côte d'Ivoire).

ABSTRACT

Objective : The general objective of this work is to evaluate the microbiological quality of some smoked fish sold in the markets of the city of Abidjan.

Methods : The collection of samples for analysis took place in the markets of the communes of Cocody II- Plateaux (Socody) and Cocody Center, Abobo, Adjamé Williamsville and the big market. Moisture content, lipid content, protein content and ash content were performed. A quantity of 10 samples composed of 5 smoked fish per sample were collected by market in sterile stomascher bags, then transported in a cooler containing ice to the laboratory to perform microbiological analysis. These are inoculation in the mass which took into account the Sabouraud media with chloramphenicol, VRBL, PCA, BEA VRBG and surface inoculation by spreading which took into account the E. coli Rapid 2 and Baird Parker media. . Also, the search for Salmonella was carried out in 4 stages. these are Pre-enrichment, enrichment, isolation and identification.

Results : After various tests, different microbial spoilage flora were found in smoked fish sold in the different markets of Abidjan. These include fungal flora (yeasts/molds), mesophilic aerobic germs and enterobacteria. All samples from the study markets were contaminated with these different microflora. The CFU load/g for mesophilic aerobic germs ranged from 38.106 ± 12 to 65.106 ± 12 CFU/g. For fungal flora, loads ranged from 103 ± 11 to 284 ± 14 CFU/g. Enterobacteria loads ranged from 183 ± 10 to 418 ± 11 CFU/g. These smoked fish contain potentially pathogenic bacterial species including *Escherichia coli* and *Staphylococcus aureus* with very high respective loads ranging from 51 ± 12 to 86 ± 12 CFU/g and from 125 ± 13 to 437 ± 13 CFU/g. These loads are not in conformity with the criteria set by the standard (10 CFU/g). The smoked fish samples studied contain several nutrients whose average levels vary from one sample to another. Thus, these smoked fish contain water, lipids, proteins and total ash with average values of $10.089 \pm 0.11\%$, 11.24% , 77.5% , 5.255 ± 0.0055 respectively.

Keywords: smoked fish, microbial quality, nutritional quality.

1. Introduction

Fish is an important source of food and livelihood in the world, providing accessible protein to the vast majority of populations, especially for African populations (FAO. (2016))[1]. It also plays an important role in the economy of these countries through trade and exports, particularly in the coastal states of West Africa, including Côte d'Ivoire.

In Côte d'Ivoire, it occupies an important place in the diet with a share of 50%, and represents between 15 and 16 kg/year of consumption per capita (Failler P. and al., (2014))[2]. Fish provides high quality protein that is easy to digest and also helps combat micronutrient deficiencies. In addition, a 150 g portion of fish covers 50-60% of an adult's daily protein requirement (FAO. (2018))[3]. Fish are rich in potassium and phosphorus and are a preferred source of water-soluble vitamins, notably B6 and B12, and fat-soluble vitamins, A, E and D. Along with milk and dairy products, they are the main dietary sources of iodine, contributing more than 8% of the average iodine intake of children (Girardet JP (2012))[4]. Therefore, this food is accessible to low-income households, especially in developing countries where the price of meat remains beyond the reach of the average consumer (FAO. (2009))[5].

Although fish is a vital resource. It remains a rapidly perishable commodity with a relatively high rate of spoilage due to its chemical-physical and microbiological characteristics (Assogba M. and al., (2018))[6]. However, to maintain the quality of fish over time, several preservation and processing techniques are used. These operations vary significantly depending on the country and dietary habits. The techniques generally used are freezing, drying, smoking, salting (FAO, (2018))[3]. In addition, the lack of hygiene in production and poor preservation considerably favor the microbial contamination of products. Thus, the contaminated fish obtained can be the cause of food poisoning (Abotchi K. (2010))[7].

Infectious foodborne diseases in Côte d'Ivoire are thought to be related to the presence of microorganisms in food. In addition, they constitute a public health problem that is widespread throughout the world and generate a social and economic problem that represents a threat for the population (Ndoye F., Moity M. & Broutin C. (2002))[8]. It is therefore important to find solutions to the risks of fish contamination. It is in this context that this work is registered. The general objective of this work is to evaluate the microbiological quality of some smoked fish sold in the markets of the city of Abidjan.

2. Material and methods

2.1. Material

2.1.1. Sampling

The collection of samples for the analyses took place in the city of Abidjan. All samples were collected by purchase in some public markets of the city of Abidjan. These were the markets of Il- Plateaux Sococe, Cocody Center, the big market of Abobo, the market of Williamsville and the big market of Adjamé. The fish were collected in sterile stomascher bags. A quantity of 10 samples composed of 5 smoked fish per sample were collected per market. They were transported in a cooler containing ice to the laboratory for microbiological analysis.

2.2. Methods

2.2.1. Determination of moisture, lipid, protein and ash content

The water content and the ash content are determined according to the method described by (AOAC, (1980))[29]. The fat content of the cocoa powder is extracted by the (A.O.A.C, (1995)) [28] method using SOXHLET. The crude proteins are determined from the determination total nitrogen according to the Kjeldhal method (B.i.p.e.a., (1976))[27] It includes a mineralization phase, followed by a distillation phase and a titration phase with sulfuric acid.

2.2.2. Microbiological analysis

A 25g sample of smoked fish is weighed around the Bunsen burner on a balance (KERN). A volume of 225 mL of sterile Buffered Peptone Water (BPW) is added. The whole is carefully mixed for 5 minutes. The resulting solution is left to stand for one hour. Approximately 1 mL of the stock solution is withdrawn near the Bunsen burner flame using a sterile graduated pipette and transferred to a test tube containing 9 mL of sterile distilled water. Five (05) successive dilutions ranging from 10^{-1} to 10^{-5} were performed (NF EN ISO 6887-1, (2017)) [9]

2.2.3. Enumeration of the different microbial flora

The selected dilutions were plated. This is the plating in the mass which took into account the Sabouraud media with chloramphenicol, VRBL, PCA, BEA VRBG. One milliliter of each dilution obtained was introduced into the Petri dishes. A quantity of 20 mL of previously prepared medium is poured into the Petri dish. The whole is well homogenized. The plates are left on the bench for the solidification of the agar. Surface plating by spreading that considered E. coli Rapid 2 and Baird Parker media. A quantity of 0.1 mL of each decimal dilution concerned is placed in a Petri dish containing 20 mL of previously prepared and poured agar. The 0.1 mL is then spread on the agar surface using a sterile spreader. The solidified plates are incubated at 25°C for 7 days for yeasts and molds, at 30°C for 24 hours for total coliforms, at 30°C for 72 hours for aerobic mesophilic germs, at 37°C for 24 hours for Enterobacteriaceae and at 37°C for 24-48 hours for Streptococcus. Similarly, at 45°C 0 for 24 h for the detection and enumeration of E. coli and 37°C for 24 to 48 h for the detection and enumeration of Staphylococcus aureus. The enumeration is significant when the number of germs found per plate is between 30 and 300 colonies for GAM, 15 and 150 colonies for streptococci, enterobacteria, yeasts and molds, coliforms, Staphylococcus aureus and E. coli (NF V08-050, (2009) [10]; NF V08-060, (2009) [11]; NF V08-051, (1999) [12]; NF ISO,16649-2, (2001) [13]; NF ISO 7899-2, (2000) [14]; NF V08-054, (2009) [15]; NF ISO 6881-1, (1999)[16]).

2.2.4. Search for Salmonella

The search for Salmonella is carried out in four steps which are (ISO 6579-1,(2017) [17]:

Step 1: Pre-enrichment which consists in diluting 25 g of sample to be analyzed in 225 mL of EPT. The suspension obtained is left for about 30 minutes on the bench and then incubated at 37°C/24h.

Step 2: Enrichment which consists in putting 0.1 mL of suspension after 24h of incubation in 10 mL of sterile Rappaport de Vassiliadis broth previously prepared and poured in tube. The seeded tube is incubated at 44°C/18 to 24h.

Step 3: Isolation which consists of streaking on Hektoen medium previously prepared and poured on Petri dish at a rate of 20 mL is carried out from Vassiliadis Rappaport broth. The seeded plates are incubated at 37°C/24h.

Step 4: This last step consists of reading and identification. Colonies with black centers are taken into account for further work.

3. Results

3.1. Nutritional quality of smoked fish

The analysis of the results of the table shows that, the smoked fish samples studied contain several nutrients whose average contents vary from one sample to another. Regarding the moisture content, the results show that the studied fish samples have an average value of $10.089 \pm 0.11\%$. This shows that, despite their dry appearance, smoked fish contain traces of water during their conservation. Concerning the lipid content, the average value of the samples is 11.24%. These results show that the smoked fish studied have a significant amount of lipids. In terms of protein content, an average value of 77.5% is observed. These results show that the protein content of these fish is high. As for the total ash content, an average of 5.255 ± 0.0055 is observed (Table I).

Table I: Nutritional value per 100 g dry matter of smoked fish samples of smoked fish

Smoked fish samples	Composition (%)			
	Moisture content	Ash content	Lipid content	Protein content
E1	$10,20 \pm 0,02$	$5,85 \pm 0,0035$	12	76,56
E2	$8,20 \pm 0,11$	$3,18 \pm 0,0081$	13	77,5
E3	$11,12 \pm 0,12$	$6,48 \pm 0,0045$	9,32	79,125
E4	$9,29 \pm 0,17$	$4,75 \pm 0,0065$	11	76,86
E5	$7,91 \pm 0,10$	$5,25 \pm 0,0041$	10	77,115
E6	$11,32 \pm 0,18$	$6,58 \pm 0,0065$	12,20	79,152
E7	$12,02 \pm 0,16$	$3,28 \pm 0,0075$	10,5	75,56
E8	$8,70 \pm 0,13$	$5,15 \pm 0,0025$	13,2	77,7
E9	$11,81 \pm 0,08$	$5,25 \pm 0,0041$	9,70	78,5
E10	$10,32 \pm 0,03$	$6,78 \pm 0,0075$	11,5	76,96
Average	$10,089 \pm 0,11$	$5,255 \pm 0,0055$	11,24	77,5

3.2. Microbiological quality of smoked fish

3.2.1. Tainting and contamination flora

Different microbial flora of alteration were found in the smoked fish sold in the different markets of Abidjan. These are fungal flora (yeasts/molds), mesophilic aerobic germs and enterobacteria. All the samples from the study markets were contaminated by these different microflora. However, except for yeasts and molds, the other parameters analyzed did not comply with the criteria set by the standard in force. The CFU load/g for mesophilic aerobic germs varies from $38.10^6 \pm 12$ to $65.10^6 \pm 12$ CFU/g. For the fungal flora, the loads vary from 103 ± 11 to 284 ± 14 CFU/g. As for the enterobacteria, the loads oscillate from 183 ± 10 to 418 ± 11 CFU/g (Table II).

Table II: Average loads of spoilage and contamination flora in smoked fish

Average loads of microbiological parameters (cfu/g)			
Samples	Mesophilic Aerobic Germs (MAG)	Yeast and Moulds	Enterobacteriaceae
E1	41.10 ⁶ ± 10	284± 14	232 ± 11
E2	50.10 ⁶ ± 11	107 ± 13	183± 10
E3	39.10 ⁶ ± 10	196 ± 13	311 ± 11
E4	38.10 ⁶ ± 12	103 ± 11	264 ± 10
E5	56.10 ⁶ ± 13	142± 11	312± 12
E6	42.10 ⁶ ± 14	128± 11	418 ± 11
E7	65.10 ⁶ ± 12	189 ± 11	367 ± 10
E8	58.10 ⁶ ± 10	156± 12	309± 10
E9	45.10 ⁶ ±13	168 ± 13	464± 12
E10	57.10 ⁶ ±12	165± 16	548± 11
Microbiological criteria	10 ⁶ CFU/g	10 ⁵ CFU/g	10 FC/g

3.2.2. Fecal contamination flora

The average loads of the smoked fish analyzed vary from one sample to another. All loads were above the microbiological quality standard criteria for fecal streptococci and fecal coliforms. Fecal coliform loads ranged from 214 ± 11 to 405 ± 10 CFU/g, while fecal streptococci loads ranged from 123 ± 11 to 196 ± 12 CFU/g. The standard called for a total absence of germs in the fecal streptococci (Table III).

Table III: Average loads of fecal contamination flora in smoked fish

Average loads of microbiological parameters (CFU/g)		
Samples	Fecal coliforms	Fecal Streptococci
E1	234 ± 12	108± 11
E2	299 ± 11	169 ± 11
E3	253 ± 12	154 ± 12
E4	214 ± 11	123± 11
E5	333 ± 11	162± 11
E6	352± 11	136 ± 12
E7	405 ± 10	142± 12
E8	333± 13	142± 12
E9	340± 11	178± 11
E10	352± 58	196± 12

Microbiological criteria	10 ² CFU/g	Absence
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3.3. Potentially pathogenic species

Smoked fish sold in the different public markets contain potentially pathogenic bacterial species, notably *Escherichia coli* and *Staphylococcus aureus*. These two bacterial species are found in all samples with very diverse loads. The loads of *Escherichia coli* are very high and vary from 51 ± 12 to 86 ± 12 CFU/g whereas the standard only provides for 10 CFU/g. The loads of *S. aureus* vary between 125 ± 13 and 437 ± 13 CFU/g. These loads do not comply with the criteria set by the standard (Table IV).

Table IV: Average loads of potentially pathogenic species in smoked fish

Average loads of microbiological parameters (cfu/g)		
Samples	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
E1	69± 12	252± 13
E2	70± 15	125± 13
E3	62± 14	301 ± 13
E4	51± 12	216 ± 13
E5	61 ± 13	209 ± 11
E6	75 ± 14	354 ± 12
E7	66 ± 12	328± 13
E8	71± 14	437 ± 13
E9	83 ± 12	347± 12
E10	76 ± 13	284± 14
Microbiological criteria	10 CFU/g	10 ² CFU/g

3.4. Pathogenic species: Salmonella

The genus *Salmonella* was present in the majority of the samples and in all the public markets in Abidjan that were used for the study. However, it should be noted that the genus *Salmonella* was absent from samples 1, 2 and 6 from the II-plateaux market and the Grand marché d'Abobo respectively (Table V).

Table V: Investigation of Salmonella genus in fresh and smoked fish

Sites sampled	Samples analyzed	Smoked fish
II-PLATEAUX	E1	-
	E2	-
COCODY CENTER	E3	+
	E4	+
BIG MARKET OF ABOBO	E5	+
	E6	-
WILLIAMSVILLE	E7	+

BIG MARKET OF ADJAME	E8	+
	E9	+
	E10	+

Presence : + , Absence : -

4. Discussion

The present study allows to assess the microbiological quality of smoked fish sold in some public markets of the city of Abidjan. The microbiological analyses of the fish revealed the presence of various microorganisms. These are the flora of alteration and contamination, flora of faecal origin, potentially pathogenic bacterial species and pathogenic species.

Regarding the flora of alteration and contamination, we count the aerobic mesophilic germs (AMG) both in smoked fish and fresh fish with loads that exceed the microbiological standards. These loads are respectively evaluated between 38.10^6 and 65.10^6 CFU/g for smoked fish. This would be related to the conditions under which the fish are sold. Indeed, in these markets, smoked fish are sold in the open air and sometimes near garbage heaps and toilets with the remarkable presence of flies around the fish. These results are similar to those of a recent study conducted by [Mouokeu and al., \(2018\) \[18\]](#) in Côte d'Ivoire and by [Abeid et al., \(2015\) \[19\]](#) on smoked fish sold in the markets of Abomey Calavi.

As for yeasts and molds, they are present in smoked fish at very low levels compared to the microbiological standard. Their loads are between $1.03.10^2$ CFU/g and $2.84.10^2$ CFU/g. The results of the study are consistent with those of [Mouokeu and al., \(2018\) \[18\]](#) who obtained low loads of Yeast and Molds in smoked fish.

The presence of enterobacteria in smoked fish would explain that the samples have undergone too much handling. This would be related to the insalubrity of the immediate environment.

As for the flora of fecal origin, all the loads are higher than the criteria set by the microbiological quality standard. The loads of fecal coliforms for smoked fish range from 214 ± 11 to 405 ± 10 CFU/g, while those of fecal streptococci vary from 123 ± 11 to 196 ± 12 CFU/g. The standard foresaw a total absence of germs at the level of fecal streptococci. The high presence of these flora could be explained by the fecal contamination of humans and warm-blooded animals. These data are in agreement with those of the works [Agbabiaka and al., \(2016\) \[20\]](#) which counted germs of fecal origin, in particular fecal coliforms in smoked fish.

The different analyses performed on the smoked fish samples revealed the absence of salmonella in some samples. This could be explained by the fishing in unpolluted waters, the high temperature of smoking and the low water content in the smoked fish. These results are consistent with those obtained by [Oulai and al., \(2007\) \[21\]](#) who also showed the absence of these germs in some smoked fish.

Other samples, however, showed the presence of Salmonella. Our results are in agreement with those of [Thiam, \(1993\) \[22\]](#) who found the presence of these germs at 75% in fish samples. This compliance could be explained by poor handling of the fish.

For the potentially pathogenic species, the smoked fish samples analyzed are contaminated with *Staphylococcus aureus* and *E. coli*. In fact, smoked fish contains more *Staphylococcus aureus* germs with a load between 125 ± 13 and 437 ± 13 CFU/g. These results are not in conformity with the standard (10^2 CFU/g). Our results are similar to those of [Loir and Gautier, \(2009\) \[23\]](#) who have shown by recent studies that the presence of *Staphylococcus aureus* could be due to human contamination of foodstuffs, given that this bacterium is commensal of the skin and mucous membranes of humans. This would show the non-compliance with good hygienic practices and the ineffectiveness of the product in these different markets. The results of the study are also different from those found by [Dégnon and al., \(2012\) \[24\]](#), which revealed the absence of these germs in samples of smoked fish. This difference could be due to the fact that the sources of supply, and the smoking conditions are different.

The results of the microbiological analysis of *Escherichia coli* revealed the presence of these germs in all the samples of the different markets studied. Moreover, these results do not meet the microbiological criteria (10 CFU/g). The presence of *Escherichia coli* in all the samples attests to a contamination of fecal origin. The results of this study do not agree with those of [Degnon and al., \(2013\) \[25\]](#). This would explain why the fish are not sold under the right conditions. Also, the lack of application of good hygiene practices accelerates bacterial proliferation and compromises the quality of fish sold in the different markets of the city of Abidjan. Moreover,

these results are also consistent with those of previous work carried out by *Fujioka and al., (1988) [26]* who had detected the presence of these germs in smoked fish. This conformity could be due to the lack of respect for good hygiene practices and the unsanitary environment, as well as the stagnant water around the markets. This is therefore at the origin of the microbial contamination of the samples by *Escherichia coli*. In addition, smoked fish are kept in basins or baskets previously lined with paper or cardboard that has already been used for packaging. This could also be a source of contamination of the product by these pathogens.

5. Conclusion

The present study on smoked fish revealed the health risks associated with their consumption. The non-observance of good hygiene practices and the lack of hygiene at the smoking sites in the city of Abidjan are contrary to the required hygienic standards. The analyses identified germs that reflect a lack of good hygiene practices such as spoilage and contamination flora, flora of fecal origin, in the smoked fish samples. As for the potentially pathogenic species, *Escherichia coli* is present in smoked fish. The pathogenic species, *Salmonella* was detected in almost all samples of our analysis. The high presence of these germs would explain a lack of good hygiene practices in the different markets during the smoking process, which would represent a danger for consumers. Thus, fish in the different markets of Abidjan studied must be well boiled before consumption.

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