



(RESEARCH ARTICLE)



## Evaluation of the microbiological and nutritional quality of dried frogs marketed in Man (Côte d'Ivoire)

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### Abstract

The frog represents an important food resource for the people of the city of Man (Cote d'Ivoire). For conservation purposes, frogs often go through a smoking-drying stage before being put on the market. The microbiological tests allowed the detection of the presence of Total Aerobic *Mesophilic flora*, *staphylococci*, *total coliforms*, *Escherichia coli* as well as *Salmonella sp.* at respective averages of 4.8.106 CFU/g, 5.105 CFU/g, 1.23.106 1.79.104 CFU/g, 4.97.104 CFU/g. The values obtained are not satisfactory, they exceed the required standards, and this is due to the poor storage conditions, and the lack of hygiene of the traders. The water content was determined by the method of A.O.A.C (2008), the content of total carbohydrates and lipids were determined respectively by the method of Munsen and Walker [1], and by the method of Folch, Lees and Stanley (1957). The average water content of the analyzed samples is 5.5%, the lipid content is 6.66 g/100 g and the carbohydrates are in trace amounts. Minerals were determined by x-ray fluorescence spectrometer. The results reveal the presence of Iron, Manganese, Potassium, Zinc, Calcium, and Phosphorus.

**Keywords:** Frog; Microbiological; Nutritional; Minerals

### 1. Introduction

In many countries, frogs have always been used locally in the diet [2]. Over the years, frog meat has become popular and highly prized [3]. The different parts such as the legs are sold in many markets, restaurants and even supermarkets. Frogs are part of the order Anurans, the largest order of Amphibians, grouping 5450 species into 48 families [4]. They have a very wide geographic distribution, and are found on all major continents except Antarctica and many oceanic islands.

According to the FAO, global aquaculture production reached a new record in 2018, with a figure of 131,300 tons for frogs [5]. Indeed, frogs are raised in countries such as Malaysia, China, Indonesia, Brazil and Mexico for consumption, while other countries, including the United States of America France, Canada, Belgium, Italy and Spain are the major importers of frog meat [6].

In Africa and particularly in West African countries such as Nigeria, Ghana, Senegal, Benin and Côte d'Ivoire, the most commonly caught and consumed edible species is the African tiger frog (*Hoplobatrachus occipitalis*) [3]. Frogs are prepared in different forms, dried, fried, prepared in soups [7], depending on the dish and preservation. In some parts

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of Asia (Indonesia) and Africa (Nigeria), the taste of frogs is compared to chicken [8] and fish because of its texture and aroma.

The consumption of frog meat is real in Côte d'Ivoire and is part of the dietary habits of some western peoples, notably the Yacouba, Guéré and Wobé. It is a source of animal protein highly valued, by these peoples [9]. Besides their food use, frogs are used in traditional medicine and also in cultural aspects [2].

The risks of food poisoning and contraction of diseases due to food sold in public are threats of which microbiological contamination is a major factor. As with many local products, the production to consumption chain of frogs is exclusively artisanal and informal; carried out in the absence of sanitary control of the commodity, which increases the risk of contamination of frogs and therefore the risk of contamination of consumers [10].

It has been noted that some families often consume dried frogs without cooking them first. The objective of this work is to evaluate the microbiological quality and nutritional potential of dried frogs.

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## 2. Material and methods

A total of 30 frog samples were collected at a rate of 10 samples per market. One sample consisted of 72 g of frogs. Two frog samples per market were collected per week in stomacher bags and transported to the laboratory in coolers for analysis.

### 2.1. Microbiological analyses

The microbiological analyses included the search for total aerobic *mesophilic flora* (FAMT), *Staphylococcus spp* (SPP), total coliforms (TC) and *faecal coliforms* (FC), and *Salmonella sp*.

#### 2.1.1. Preparation of stock solution and decimal dilutions

The solutions were prepared according to the NF V08-010 standard.

Twenty-five (25) grams of each sample taken were added to 225mL of Buffered Peptone Water (BPW) to obtain the stock solution. 1mL of this solution was added to 9 mL of BPW medium. The solution obtained is the  $10^{-1}$  dilution. This same action is repeated 3 times in a row to obtain the decimal dilutions  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  which were used to inoculate the plates containing the culture media.

#### 2.1.2. Enumeration of Total Aerobic Mesophilic flora (TAMF)

This enumeration is done according to ISO 4833: February 2003.

Plate Count Agar (PCA) is the agar used for the enumeration of this flora. 0.1 mL of the dilutions were aseptically taken and streaked in the agar mass poured in Petri dishes. The colonies formed were counted after 72h of incubation at 30°C.

#### 2.1.3. Enumeration of *staphylococcus sp*.

*Staphylococcus* were tested according to NF ISO 6888.

0.1 mL of each dilution was directly plated on Baird-Parker (BP) medium by streaking. The colonies of *Staphylococcus sp*. have a characteristic appearance after 24 hours of incubation at 37°C: blackish surrounded by a clear sural.

#### 2.1.4. Enumeration of total coliforms (TC) and *Escherichia coli*

The enumeration of total *coliforms* and *Escherichia coli* was carried out in accordance with the standards NF V 08-054, and NF V08-060 respectively for total and faecal coliforms.

Each dilution was plated on VRBL (Violet Red Bile Lactose) agar and incubated at 37°C for total coliforms and at 44°C for fecal coliforms for 24 hours. Typical colonies counted are purplish red with or without a halo of precipitate.

#### 2.1.5. The research of *Salmonella* in food involves essential steps:

Pre-enrichment, enrichment, isolation and confirmation.

### Enumeration of *Salmonella*

The enumeration of *Salmonella* is done according to ISO 6579/NF V 08 standards.

A pre-enrichment was performed by mixing 25 g of the sample in 225 mL of EPT then homogenized and incubated at 37 °C for 24h.

### Enrichment

0.1 mL of the solution from the pre-enrichment is added to 10 mL of Rappaport Vassiliadis broth. The mixture is then homogenized and incubated at 37 °C for 24 hours.

### Isolation

Isolation is done from cultures from the selective enrichment medium which have been streaked separately into sterile petri dishes in which SS medium has been poured. The plates were incubated for 24 h to 48 h at 37 °C. Typical *Salmonella* sp colonies are black with a transparent halo on SS agar and small in size (2 to 4 mm in diameter).

### Confirmation

Confirmation is done by purification of colonies in Petri dishes containing SS medium then incubated at 37°C for 24 hours.

$$N = \frac{\Sigma C}{V(n1 + 0,1n2)d}$$

## 2.2. Biochemical analysis

### 2.2.1. Water content

The water content was determined according to the dry matter content (DMC) following the A.O.A.C. method (2008).

Empty cups of mass  $m_0$  were weighed. For each sample, a quantity of ten grams (10g) is weighed and put in the oven at 105°C. After 24 hours, the cups containing the samples are removed and weighed again. The MSD (%), of each sample is obtained by the formula.

$$TMS = \frac{(m1 - m0)}{m} \times 100$$

### 2.2.2. Mineral content

The determination of the mineral composition was done by scanning the minerals with the x-ray fluorescence spectrometer.

A quantity of 3g of frog powder is put in the x-ray fluorescence spectrometer. Under the action of bombardment by photons emitted by x-rays, the minerals constituting the powder sample change from their ground state to the excited state. When they return to their steady state, they emit energy at wavelengths specific to each ion. The emitted wavelengths are read on the device in order to quantify the mineral content.

### 2.2.3. Determination of the carbohydrate content

The total carbohydrate content was determined by the method of Munson and Walker (1906)

A quantity of the sample powder is hydrolyzed with a 0.2N HCl solution. After heating and stirring, the mixture is neutralized with a NaOH solution. The reaction mixture is then transferred to preheated Fehling's liquor. The total carbohydrate content is determined by the mass of the CuO precipitate.

2.2.4. Determination of lipid content

The technique used was liquid-liquid extraction with chloroform and ethanol. Lipids were extracted from the sample with chloroform as described by Folch, Lees and Stanley (1957).

A quantity of the sample, chloroform, ethanol and water were homogenized in the magnetic stirrer for 30 minutes. The mixture is then filtered, decanted and the recovered chloroform phase is put in the oven. The lipid content is determined by the fat residues that appear after steaming.

3. Results

3.1. Microbiological analysis of frog samples

The analysis of the results reveals that the levels of contamination of the frogs vary according to the flora sought and the sampling locations (markets). Total Aerobic *Mesophilic flora* (TAMF) is the most prevalent with an overall average load of 4.80.10<sup>6</sup> CFU/g (Table I). The average load per market for TMAF is 4.10.10<sup>6</sup> CFU/g, 5.22.10<sup>6</sup> CFU/g and 5.07.10<sup>6</sup> CFU/g for the Doyagouiné, Cacasport and Grand-gbapleu markets respectively (figure1).

Table I Microbiological quality of all market frog samples

Searched germs	General averages (CFU/g)	Criteria (CFU/g)	Standards	Résultats	Average rate of conformity (%) of the samples
FAMT	4,80.10 <sup>6</sup>	5.10 <sup>4</sup> -5.10 <sup>5</sup>	ISO 4833	NS	13,34
SPP	4,98.10 <sup>5</sup>	10 <sup>2</sup> -10 <sup>3</sup>	Reg.2073/2005/CE	NS	10
CT	1,23.10 <sup>6</sup>	10 <sup>5</sup>	NF V08-054	NS	10
CF	1,79.10 <sup>4</sup>	Absence	NF V08-060	NS	10
Salmonella	4,96.10 <sup>4</sup>	Absence	NF V08-052	NS	80

FC: Faecal Coliforms; TC: Total Coliforms; SPP: Presumed Pathogenic *Staphylococci*; FAMT: Total Aerobic *Mesophilic flora*; NS: Not Satisfactory; Reg: Regulation

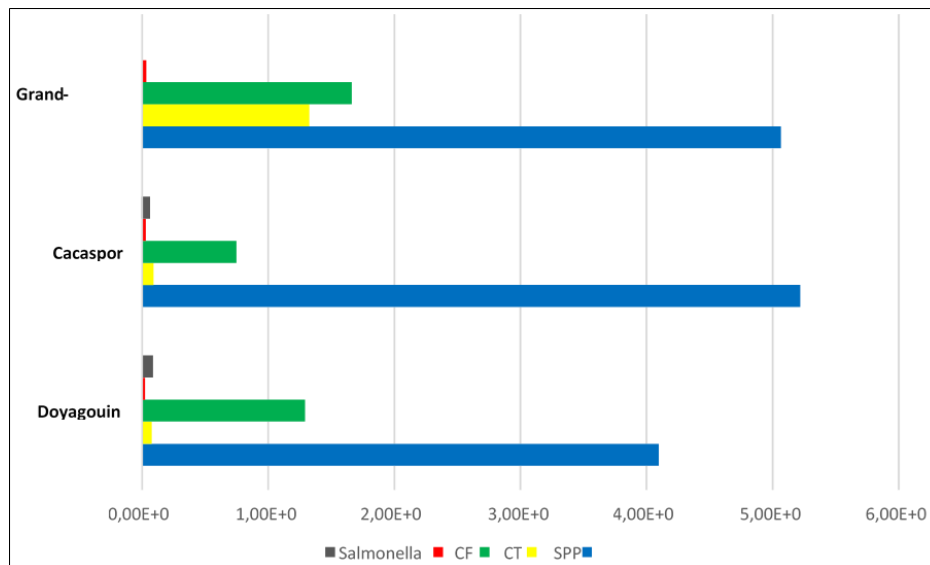
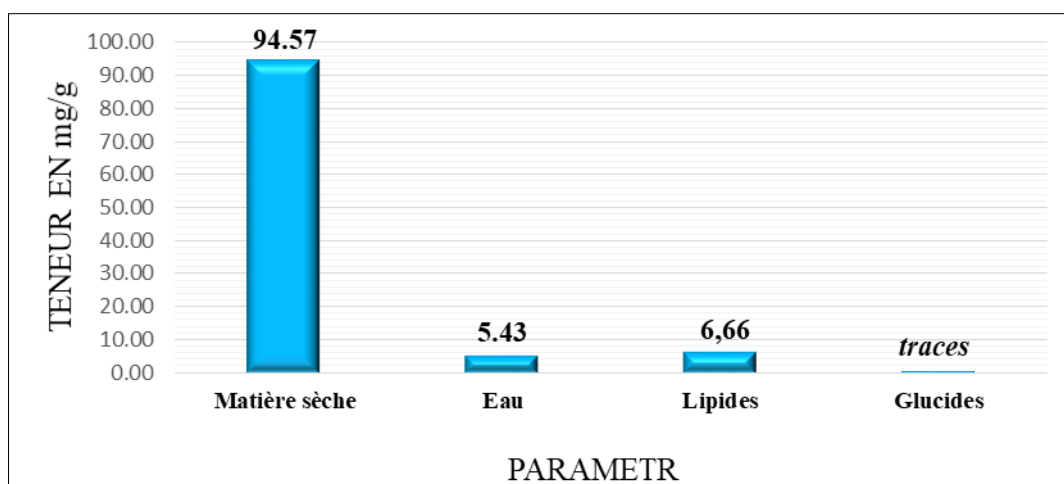


Figure 1 Average loads of germs tested by market FC: Faecal Coliforms; TC: Total *Coliforms*; SPP: Presumed Pathogenic *Staphylococci*; TAMF: Total Aerobic *Mesophilic flora*

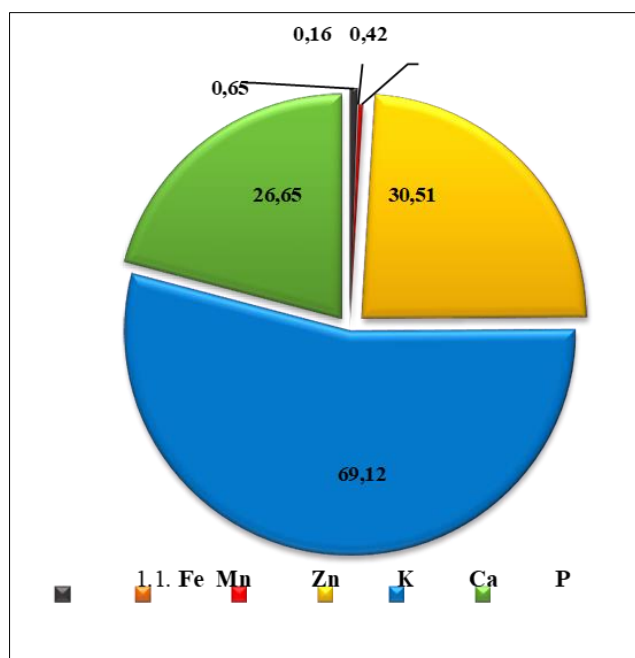
### 3.2. Biochemical characteristics

The analysis of the powdered frog samples allowed us to calculate the average dry matter content which is 94.56% with a water content of 5.5% and a lipid content of 6.6% (Figure 2).



**Figure 2** Dry matter, water, lipid and carbohydrate content of dried frog

After analysis of the minerals, we note the presence of microelements such as Iron (0.65 mg/100 g), Manganese (0.16mg/100g) and Zinc (0.42 mg/100 g) and macroelements such as Potassium (30.51mg/100g) Calcium (69.12 mg/100 g) and Phosphorus (26.65 mg/100 g) (figure 3).



**Figure 3** Mineral content in mg/100 g of dried frog Fe: Iron, Mn: Manganese, Zn: Zinc, K: Potassium, Ca: Calcium, P: Phosphorous

## 4. Discussion

The presence of mesophilic aerobic germs, staphylococcus sp, total coliforms, fecal coliforms, and *salmonella* sp at considerable loads in the samples analyzed demonstrates the high level of contamination of dried frogs marketed in Man.

A high level of contamination by AFMT was obtained for all samples with a percentage of occurrence of 86.66% or only 13.34% of cases of compliance. According to Anihouvi *et al.* [11], the count of LAMF provides information on the general microbiological quality of a product, gives an indication of the state of spoilage of the product, and is therefore an index of sanitary quality. Such a figure obtained thus testifies to the importance of the risks incurred by the consumers. The abundance of these germs in the dried frogs sold is due to poor storage conditions and lack of respect for hygiene rules. Their presence in large numbers indicates the state of degradation of the product and may lead to economic losses due to spoilage. This level of contamination is lower than that observed by Gamane *et al.* [12] who found a higher contamination rate in fish (100%) with an equally high average (1.88.107 CFU/g) than those of the averages obtained (3.41.106 CFU/g).

The results obtained reveal that *staphylococcus* is present in 90% of the samples analyzed. The presence of this group of germs in the analyzed food samples indicates a skin and mucous contamination that could come from the handlers in a sales situation. Such a high level of these germs in the samples could be explained by the fact that the frogs are handled by shopkeepers and customers in an inappropriate manner. In addition, previously smoked frogs may be recontaminated because of the time spent before being purchased. Similar results obtained by Gamane *et al.* [12] are noted with a 100% contamination rate of presumed pathogenic staphylococci in dried fish.

The total *coliform* and *Escherichia coli* contamination levels of the frogs sold are above the French standards indicated. These germs are of faecal origin and therefore indicators of poor hygiene conditions during food handling.

Salmonella was detected in the frog samples. *Salmonella* are pathogens that cause typhoid fever, and their presence in the samples is a health concern for consumers. This contamination is probably due to the storage conditions of the frogs by the traders, and the poor storage conditions. These results are lower than those of Gamane *et al.* [12] (2018) who detected the presence of salmonella in 25 out of 30 samples during their work on the hygienic quality of smoked and dried fish.

The water content of the analyzed samples varies between 4.8% and 6%. These low water contents are due to the smoking and drying process. They indicate that the drying process was well done and that the frogs are adequate for eventual preservation. These results are largely different from those performed by Moustapha [13] who obtained 60.5% as results.

The analyzed samples contain a considerable amount of calcium and this would be due to the presence of the frog's bones. Indeed the sample was pulverized with the bones for the different analyses. The presence of the bones therefore increased the calcium content of the sample. These results are close to those of Blé *et al.* [9] who worked on the composition of the frog in Côte d'Ivoire.

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## 5. Conclusion

In order to preserve fresh frogs for commercial purposes, traders put their goods through a drying process that results in remarkable changes in the proportion of nutrients in the frog. Microbiological analysis showed a high presence of the desired germs in all samples. The abundance of AFM, Staphylococcus, and total coliforms is of concern both for the health of the consumers and for the shelf life of the food. The presence of Salmonella is alarming. This contamination is attributed to poor storage conditions, inadequate food handling, and unsanitary environment of the sales places.

The results obtained shed light on the nutritional potential of frogs, and their advantages in the dried state, but also alarm on the existence of a potential risk of microbiological contamination for consumers, and in particular for those who consume them without cooking. It is therefore necessary to provide recommendations to avoid diseases and economic losses related to the alteration of this food.

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## Compliance with ethical standards

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*Disclosure of conflict of interest*

The authors declare no conflict of interest, financial or otherwise

*Statement of ethical approval*

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

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