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ORIGINAL ARTICLE

Biochemical and microbiological characterization of "Soumbara" from African locust bean (*Parkia biglobosa)* seeds consumed in Abidjan (Côte d'Ivoire)

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Abstract

Background: "Soumbara" as well as other traditional foods of Côte d'Ivoire are produced in a traditional way. These foods may contain pathogenic and spoilage microorganisms. **Aims**: The aim of our work was to assess the sanitary quality of "Soumbara" sold in five communes of Abidjan (Côte d'Ivoire), in order to valorize it as a flavor enhancer. **Material and Methods:** A consumption survey as well as the analysis of some physico-chemical (pH, titratable acidity, Brix degree, moisture, and organic acids) and microbiological parameters (enumeration of MAG, *Clostridium*, *Bacillus*, *Staphylococci*, *Salmonella, E. coli*, and coliforms) were carried out in five (05) municipalities of Abidjan (Abobo, Yopougon, Port Bouet, Adjamé, and Treichville) on 75 samples. **Results:** The results of the survey showed that most of the respondents were familiar with "Soumbara" and often consumed it. These consumers stated that they did not experience any discomfort after consuming this food. Physicochemical analyses showed that the water content of the samples of "Soumbara" ranged from 16.50% to 19.28% and the pH varied from 6.32 ± 0.01 to 7.91± 0.02. "Soumbara" contained little follow-through which ranged from 0.10 ± 0.001 to 0.27 ± 0.05. "Soumbara" also contained phenolic compounds such as coumarins, hydroquinones, and caffeic acid. Microbiological analyses had revealed the presence of spoilage and pathogenic germs such as *Bacillus*, *Staphylococcus,* and *E. coli* at high loads exceeding the 2019/229/EC standard. **Conclusions**: However, due to the high load of spoilage and pathogenic microorganisms, this food eaten by sprinkling directly on the dish, without passing through a cooking process could expose the consumer to a risk of food poisoning.

Keywords: Food poisoning, "Soumbara", Pathogenic and Spoilage Microorganisms.

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1 Introduction

Two billion people in the world are suffering from what the United Nations calls hidden hunger, or malnutrition [1]. Hunger and malnutrition affect people in developing countries where the notion of food security remains a luxury [2]. In order to ensure food security for the population, it would be necessary to increase agricultural production and valorize local products.

Local products from the fermentation of oilseeds are culturally used in human food in various countries of West Africa and particularly in Côte d'Ivoire. They are the subject of many commercial transactions between different countries and are found in all local markets[3]. Among these condiments is "Soumbara" which is also called "Soumbala" in Burkina Faso and Mali, "Dawa-dawa" in Niger and Nigeria, "Nététu" in Benin and Senegal [4]. "Soumbara" produced in Côte d'Ivoire is a flavoring agent used to enhance the taste of sauces and dishes, and is an important source of protein, lipids, carbohydrates, vitamins, and trace elements [5]. In addition, "Soumbara" in general is believed to have therapeutic benefits such as regulating blood pressure, jaundice, and preventing intestinal obstruction [4]. According to Koura *et al.* [6], "Soumbara", a product resulting from the fermentation of African locust bean seeds, contains a microbial flora of biotechnological interest. However, other pathogenic microorganisms could cohabit with microorganisms of biotechnological interest. The consumption of such a contaminated product requires cooking to preserve the health of consumers. The implementation of a food quality policy in the country is a priority in terms of public health. In addition, "Soumbara" is a particularly favorable environment for the development of microorganisms, especially *salmonella*, likely to cause serious food poisoning [7]. Thus, to ensure the health of consumers and enhance the value of this local product, it is necessary to know its microbiological quality. The aim of our work was to assess the sanitary quality of "Soumbara" sold in five communes of Abidjan (Côte d'Ivoire), in order to valorize it as a flavor enhancer.

2 Material and Methods

2.1 Material

2.1.1 Survey form

A survey form was developed on the system of preference and the frequency of consumption of "Soumbara" by the population.

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2.1.2 Biological material

The biological material is "Soumbara" obtained from fermented almonds of *Parkia biglobosa* seeds collected from the markets of the five municipalities of Abidjan (Côte d'Ivoire).

consumers and vendors in the various districts concerned. The results of this pilot phase were not taken into account in the final result because most of these results were incorrect. The final questionnaire was submitted to the consumers and vendors at the different sites. At the sites, the questionnaire was explained point by point to the consumers. The questions were multiple choice

Table 1: Titratable acidity, pH, soluble dry extract and moisture of "Soumbara" sold in Abidjan

Physicochemical parameters	Municipalities					
	Abobo	Adjamé	Treichville	Port-Bouët	Yopougon	Means
pH	6.32 ± 0.01^{d}	6.86 ± 0 .01^{c}	7.71 ± 0.02^{b}	7.91± 0.02^{a}	6.32 ± 0.02^{d}	7,02
T.A (%)	0.35 ±0.005^{a}	0.29 ± 0.001^{b}	0.13 ± 0.004^{d}	0.26 ± 0.005^{c}	0.36 ± 0.004^{a}	0,27
ESS (°Brix)	0.27 ± 0.05^{b}	0.13 ± 0.03^{a}	0.10 ± 0.001^{a}	0.10 ± 0.003^{a}	0.10 ± 0.002^{a}	0,14
Moisture (%)	19.28 ± 0.01^{a}	18.60 ± 0.03^{a}	16.91 ± 0.15^{a}	16.50± 0.10^{a}	18.14 ± 0.08^{a}	17,90

For the same municipalities, on the same line, the mean values followed by different alphabetical letters are statistically different (p< 0.05) (DUNCAN multiple t-test), pH: Hydrogen Potential; TA: Titratable Acidity; ESS: Soluble Dry Extract.

2.2 Methods

2.2.1 Study site

This study was conducted in five communes of Abidjan District in Côte d'Ivoire, namely Abobo, Yopougon, Port Bouet, Adjamé, and Treichville, from July 22 to August 7, 2019. These five communes were chosen because of their different social levels and the high density of their populations. In addition, these five communes are home to the main supply markets for the Abidjanese population. For this study, 500 people were interviewed, 100 per municipality.

2.2.2 Size of individuals to be investigated

The sample size for this study was calculated using formula (1) as described by Kouamé *et al.* [8] for a non-exhaustive independent sample:

$$n = t^2.p.(1 - p)/e^2 \quad \ldots\ldots\ldots\ldots (1)$$

With n: the sample size, e: the margin of error, t: the margin coefficient deduced from the confidence rate, p: the population in the study area. The sample for each area was obtained using the probability method proportional to the size of households in each locality [9] based on data from the general population census of Côte d'Ivoire [10].

2.2.3 Data collection

Bibliographical research and a field survey on the different sites where "Soumbara" are sold and with the population in five municipalities of the District of Abidjan in Côte d'Ivoire, namely Abobo, Yopougon, Port bouet, Adjamé, and Treichville were carried out. This one-month phase made it possible to collect bibliographical data, observe consumers, establish relations with the consuming population, female vendors of "Soumbara", and design a survey questionnaire on the consumption and sale of "Soumbara". This questionnaire was tested on about 100

Table 2: Organic acid detected in "Soumbara"

Organic acid	Quantity (mg/kg)
Coumarin	0.0033±0.0032
Caffeic acid	0.0 1± 0.005
Hydroquinone	0.74 ± 0.54

questions with the possibility of 2 to 6 proposed answers or questions with yes or no and true or false answers. The final questionnaire was structured around three (3) main points:

- The profile of the respondent
- Knowledge and consumption of pork by the respondent
- Discomfort contracted by the consumer after consumption of the "Soumbara".

2.2.4 Sampling

"Soumbara" samples were collected from sales sites in five communes in the city of Abidjan (Abobo d'Adjamé, Treichville, Port-Bouët, and Yopougon). In each commune, five (5) "Soumbara" women vendors were selected. This amounts to a total of 25 women vendors for the five communes. Three samples of approximately 100 grams each were taken from each vendor. A total of 75 samples of "Soumbara" were taken in the 5 communes. The collected samples were put in a cooler containing carboglaces and transported to the laboratory within four hours after sampling for the microbiological analyses.

2.2.5 Isolation and enumeration of bacteria

The stock solution and decimal dilutions were performed according to the methods of Bouatenin *et al.* [11]. For the analyses,

ten grams (10 g) of samples were crushed and taken under sterile conditions created by the flame of a Bunsen burner and mixed in a Stomacher bag with 90 mL of buffered peptone water (AES Laboratoire, Combourg France) previously sterilized and used as diluent. Mesophilic aerobic germs (MAG) were counted on PCA (Plate count Agar) agar (Oxoid LTD, Basingstore, Hamsphire, England) after two (2) days of incubation at 30°C according to AFNOR Standard NF V08-051,1999. The research and counting of *Staphylococcus aureus* were done on Baird Parker agar after one (1) day of incubation at 30°C using Capita *et al.* [12] method. The quantitative estimation of spores of *Bacillus cereus* was performed by a standard plate-counting method. Isolations were achieved from heat-treated dilutions by plating on mannitol egg yolk polymyxin B agar [8]. Presumptive colonies of *Bacillus cereus* were randomly selected based on characteristic colony feature, purified on the same medium, and identified by morphological, cultural, and biochemical characteristics according to the documented procedures [13]. Violet crystal and neutral red biliated lactose agar (VRBL agar) was used for coliform count, after one (1) day of incubation according to AFNOR Standard, NF ISO 4832 July 1991. The isolation and enumeration of *Salmonella* were carried out using Hendriksen [14] method in several steps. This was achieved by pre-enrichment in a non-selective medium, followed by enrichment in a selective medium and culture on selective agar. For enrichment in non-selective or pre-enrichment media, a quantity of Twenty-five grams (25) g of samples was homogenized with 225 mL of peptone water in a sterile jar, incubated at 37°C for 24 h. For selective recording, one milliliter (1 mL) of the pre-enriched culture was transferred using a sterile pipette into 10 mL of previously prepared sterile Rappaport Vassiliadis. The method of Kouamé *et al.* [8] was used. The tryptone sulphite neomycin (TSN) agar (Bio-Rad, Marnes-La Coquette, France) was used for the detection of *Clostridium perfringens* after a thermal shock of the dilutions (80°C for 15 min and immediately cooled). One milliliter of each appropriately treated dilution was used to inoculate the TSN agar (Bio-Rad) stored in surfusion at 45°C in assay tubes. After the agar had solidified, all inoculated media were incubated in an upright position for 24 h at 46°C. Tubes containing between 30 and 300 colonies were counted, and five colonies were picked for confirmation in the motility-nitrate medium. The medium used for the research and enumeration of *Escherichia coli* was RAPID' *E.coli* agar (Standard NF ISO 16140, 2003). Inoculation was done by spreading 0.1 ml of the stock solution or decimal dilutions on the surface of the agar previously poured and cooled in a Petri dish. Incubation was done at 37°C for 24 hours. Presumptive *Escherichia coli* colonies were purple to pink. Presumptive *Escherichia coli* colonies present in plates containing 15 to 150 colonies were counted.

2.2.6 Biochemical analysis

Titratable acidity and pH were determined according to the method described by Kouamé *et al.* [8]. The percentage of moisture was determined using the Official Methods of Analysis [15]. The total soluble solids, expressed as °Brix value was determined by using a hand refractometer (Atago N-20E, Japan). Organic acids of "Soumbara" samples were before extracted and then analyzed by high performance liquid chromatography using an ion exclusion ORH-801 column (300 mm×6.5 mm) (Interchrom, France) as achieved by [16]. Running conditions were: mobile phase H2SO4; 40 mmol L^{-1}; flow rate, 0.8 ml min^{-1}; wavelength, 210 nm; room temperature (25 °C). The separated components were detected with an UV spectrophotometric detector (SPD-6A, Shimadzu Corporation, Japan).

Table 3: Microbial load of "Soumbara" consumed in Abidjan

Germs (UFC/g)	Municipalities						
	Abobo	**Adjamé**	**Treichville**	**Port-Bouët**	**Yopougon**	**Means**	**Standards**
MAG	$(4.3\pm 0.2)10^{5b}$	$(2.8\pm0.4)10^{4b}$	$(1.8\pm0.2)10^{5b}$	$(1.7\pm0.6)10^{6a}$	$(1.8\pm0.5)10^{6a}$	$8.27.10^{5}$	10^{6}
E. coli	$(1\pm0.1)10^{4c}$	$(2.9\pm0.2)10^{4a}$	$(1.8\pm0.2)10^{4b}$	$(1.2\pm0.2)10^{4bc}$	$(1.5\pm0.7)10^{4bc}$	$1.52.10^{4}$	-
S. aureus	$(2.7\pm0.1)10^{4b}$	$(1.1\pm0.8)10^{7a}$	0	$(1.1\pm0.1)10^{6b}$	$(4.3\pm4.2)10^{5b}$	$2.51.10^{6}$	10^{2}
Bacillus cereus	$(1.8\pm0.5)10^{4cd}$	$(9.3\pm0.5)10^{4a}$	$(2.4\pm0.5)10^{4c}$	$(1.2\pm0.1)10^{4d}$	$(5.1\pm0.4)10^{4b}$	$3.96.10^{4}$	-
Coliforms T	$(1.2\pm0.1)10^{3a}$	$(4.4\pm0.5)10^{2d}$	0	$(9.2\pm0.1)10^{2b}$	$(7.06\pm1.3)10^{2c}$	$6.53.10^{2}$	10^{3}
Clostridium	<1	<1	<1	<1	<1	<1	
Salmonella	Absence	Absence	Absence	Absence	Absence	Absence	

For the same municipalities, on the same line, the mean values followed by different alphabetical letters are statistically different (P< 0.05) MAG: Mesophilic Aerobic Germs; *E. coli*: *Escherichia coli*; *S. aureus*: *Staphylococcus aureus*, Coliforms T : Total coliforms.

2.3 Statistical analysis

Statistical package R 3.0.1, ANOVA method with Duncan's test, with significance level 5% were used. The program was used to

calculate the means, standard deviation of microbiological, and physicochemical parameters. It was also used to compare the means of the microbiological and physicochemical parameters of the samples and to determine if the differences observed in the means of the microbiological parameters were significant at the 5% significance level. The survey data were processed with IBM SPSS software (Statistics 20).

Patients have been informed of the purpose of the study and are assured of the confidentiality of the data collected. Their consent has been given for the use of the information collected and the results obtained. The study has been carried out in accordance with the World Medical Association's Code of Ethics for experiments that involved humans. The aim of the study was clearly explained to all of the study participants and a written informed consent was obtained.

3 Results

3.1 Level of consumption and "Soumbara" type

Figure 1 illustrates the consumption rate of "Soumbara" in five municipalities of Abidjan. All respondents in the municipalities of Abobo, Adjamé, and Treichville consumed "Soumbara". The majority of the respondents consumed *néré's* "Soumbara" with rates of 99% for the municipalities of Abobo, Adjamé, and Yopougon and 100% for the municipalities of Port Bouet and Treichville.

3.2 Forms of "Soumbara" consumed

Among the two forms of "Soumbara" (powder or grains), the powder form was the most consumed by the populations. The consumption rates for this form were 66% in the municipalities of Abobo, 91% in Adjamé, 93% in Yopougon, 85% in Port Bouet, and 80% in Treichville (Figure 2).

3.3 Frequency of consumption of "Soumbara" by the population

Figure 3 shows the different rates of consumption of "Soumbara" in five municipalities. These results show that the percentages of respondents who consume at least once a day vary from 20 to 50%, with high proportions in the municipalities of Abobo (53%), Adjamé (37%), Port Bouet (25%), Treichville (26%), and lower proportions in the municipalities of Yopougon (16%). In addition, the rate of respondents who consumed "Soumbara" twice a day ranged from 20 to 25% in five municipalities respectively Abobo (23%), Adjamé (20%), Port Bouet (25%), and Yopougon (22%). The percentages of respondents who consumed "Soumbara" at least three times a day were very low in the communes of Adjamé (1%) and Abobo (7%) and very high in Port Bouet (24%). As for respondents who consume "Soumbara" at least once a month, we recorded nearly (17%) Abobo, (42%) Adjamé, (55%) Yopougon, (31%) Port Bouet, and (48%) Treichville.

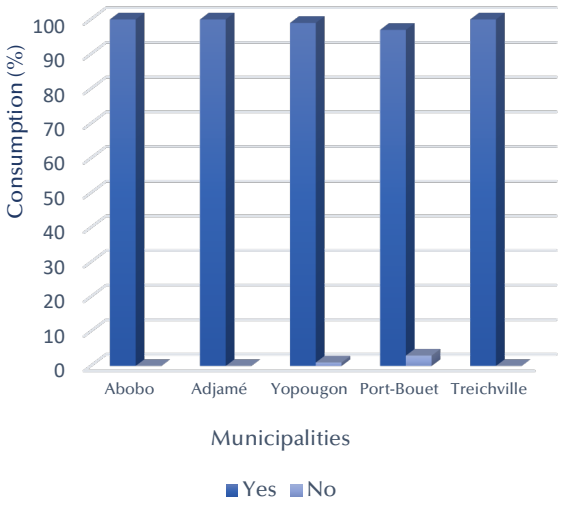

Figure 1: Level Level of "Soumbara" consumption by surveyed populations

3.4 Physico-chemical characteristics of "Soumbara"

Table 1 presents some physicochemical characteristics of the samples of "Soumbara" taken in the different municipalities of the study. The pH of "Soumbara" samples differs significantly (P<0,05) from one municipality to another. The lowest pH came from the municipalities of Abobo (6.32 ± 0.01) and the highest from the municipalities of Port-Bouët (7.91± 0.02). Acidity varied in the opposite direction of pH. Thus, the lowest acidity of "Soumbara" was recorded in Port-Bouët (0.26 ±

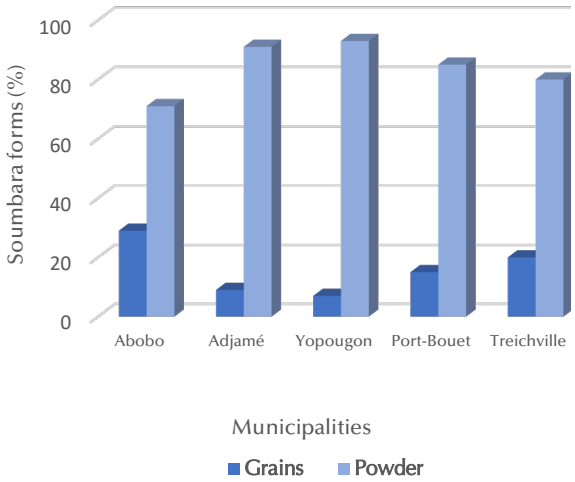

Figure 2: Form of "Soumbara" consumed by the surveyed population

0.005). The highest acidity level was recorded in Abobo with a value of (0.35 ± 0.005). Concerning the soluble dry extract, the value varied from 0.1 ± 0.001 (Treichville) to 0.27 ± 0.05 (Abobo). As for the moisture content, the results obtained showed that there was no significant difference (p≥0,05) from one commune to another. The moisture rate varied from 16.50 ± 0.1 (Port-Bouët) to 19.28 ± 0.01 (Abobo).

3.5 Organic acid detected in "Soumbara"

The organic acids found in "Soumbara" are coumarin, caffeic acid, and hydroquinone. The amount of hydroquinone (0.74 ± 0.54 mg/kg) was highest in "Soumbara". While coumarin (0.0033 ± 0.0032 mg/kg) and caffeic acid (0.01±0.005 mg/kg) were low in "Soumbara" (Table 2).

3.6 Microbial loads of "Soumbara" consumed in Abidjan

Salmonella and *Clostridium perfringens* were not detected in the "Soumbara" samples analyzed. However, they were heavily contaminated with mesophilic aerobic germs, *Escherichia coli, Staphylococcus aureus, Bacillus,* and total coliforms. The loads of these microorganisms were higher than the standard 2019/229/EC. Adjamé's samples were the most contaminated with *E. coli, Bacillus, and S. aureus* with loadings of (2.9 ± 0.2)10^4 CFU/g; (9.3 ± 0.5)10^4 CFU/g and (1.1 ± 0.8)10^7 CFU/g, respectively. Those from Abobo were more contaminated by total coliforms with a load of (1.2 ± 0.1)10^3 CFU/g. Yopougon samples were more contaminated with mesophilic aerobic germs with a load of (1.8 ± 0.5)10^6 CFU/g (Table 3).

4 Discussion

"Soumbara" production is a female activity and the transfer of know-how is carried out at the family level [17]. The survey showed a high consumption rate in the five communes of Abidjan. This food is therefore ingrained in the populations' eating habits. It is appreciated for its nutritional and therapeutic values and its low cost [18]. As for the absence of discomfort during consumption, it could be explained by the mode of consumption. Indeed, "Soumbara" is consumed after cooking. According to Yaméogo et Konkobo [19], they are added a lot in to sauces, rice sauces as well as in fatty rice. At these cooking temperatures, pathogenic microorganisms are destroyed. Concerning the results of physicochemical analysis, the pH values of the different samples of "Soumbara" from the five municipalities vary from 6.32 to 7.91. These results are consistent with the results of a study conducted by Compaoré *et al.* [20] on the controlled fermentation of African locust bean seeds and those of Parkouda *et al.* [21] during a study on the nutritional properties of fermented African locust beans seeds. These variations would be due to the differences observed in the production method of "Soumbara" especially at the stages of "Soumbara" fermentation. Indeed, the duration of fermentation and the variety of African locust beans varying from one producer to another, would also be the reason for the observed variations [4]. In addition, the conditions of purchase and conservation of the product could also be responsible for this pH variation. The moisture content determined in this study varies from 16.50% to 19.28%. These moisture levels recorded for these "Soumbara" *are* higher than those determined by kouamé *et al.* [4], which are between 6% and 8%. The high moisture content of the products would be explained by the fact that drying is done at room temperature empirically. This may also be due to the good water absorption and retention capacity of the néré seeds during the cooking and soaking phase. The moisture content of "Soumbara" allows us to assess the quality of drying and to predict its shelf life. The moisture content of "Soumbara" can promote the proliferation of microorganisms that can lead to its deterioration at the end of fermentation during packaging [4]. The Brix degree showed that there was practically no sugar in "Soumbara" this absence would be due to the method of production of "Soumbara". Its low sugar content would be beneficial for a diabetic diet. Concerning organic acids, two (2) were detected in the analyzed "Soumbara". These were coumarin and caffeic acid. In addition to these two organic acids, hydroquinone was also present in "Soumbara" at a high concentration of 0.74 ± 0.54%. In fact, during the production of "Soumbara" a condensation reaction takes place. In this reaction, activated phenols and ethyl acetoacetate give polyphenols such as coumarins, hydroquinone, caffeic acid and by-products such as ethanol and water [22, 23] etc. Polyphenols can have beneficial effects on consumer health especially coumarin. Indeed, coumarins constitute a large class of heterocycles. Most of them are endowed with varied biological activities. Among them, we can cite the anticoagulant activity which is promoted by warfarin, the anticancer and hepatotropic activity whose leader is the hymecromone, the antibiotic activity, the analgesic and the anti-inflammatory activity or the anti-HIV and photosensitizing activity [24]. However, at high doses, phenolic derivatives with anticoagulant properties are toxic by ingestion and can decree cases of acute cytolytic hepatitis [25]. Similarly, these acids heated to decomposition can release toxic fumes of carbon monoxide and dioxide, especially hydroquinone. Thus, its consumption could lead to nervous disorders, eye damage, skin allergies, chest pain, cough, headaches, dizziness, salivation, convulsions, nausea, and vomiting [26]. However, "Soumbara" containing small amounts of phenolic compounds is believed to play an important role in human nutrition.

Microorganisms such as *Bacillus, E. coli, Staphylococcus*, mesophilic anaerobic germs were found in the samples with the exception of some coliforms, and *Salmonella*. The presence of these germs in "Soumbara" would be due to the hygienic conditions of production of this food. The presence of *Staphylococcus* at high levels exceeding the standard 2019/229/EC could be due to the contamination of ingredients by the hands of the producers during production but also by domestic animals such as chicken, cattle because of the drying is done in the open air. Concerning the presence of *Bacillus* in "Soumbara" it could come from two origins. On the on hand "Soumbara" could on the one hand be contaminated by microorganisms from the environment, utensils, environment, personnel and by the raw material used for production. On the

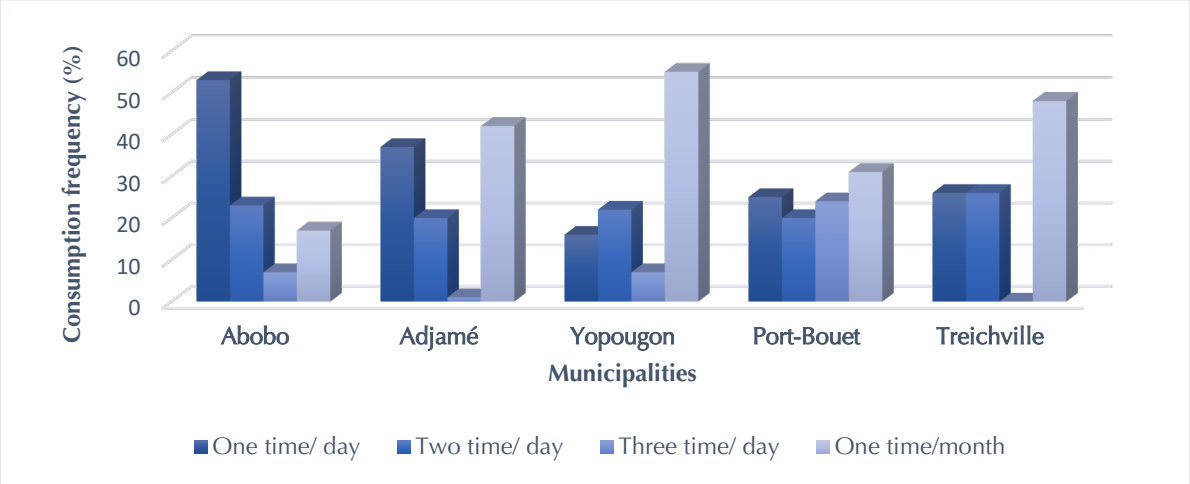

Figure 3: Frequency of consumption of "Soumbara" by the population

other hand, *Bacillus* found in the finished product could be due to those who participated in the fermentation. Indeed, *Bacillus* is the main strain responsible for the fermentation of "Soumbara" [27]. Authors such as Compaoré *et al.;* Ouoba *et al.* [20, 28] during fermentation of African locust bean seeds for "Soumbara" production have isolated and identified several *Bacillus* species. The presence of these strains could be due to spore survival during the seed drying and cooking stage and their ability to grow rapidly on cold cooked seeds that would allow the two main strains to dominate fermentation. The absence of coliforms and *salmonella* in the "Soumbara" of some municipalities would be due to the fermentation that makes the environment hostile to their growth[29].

5 Conclusions

The aim of our work was to assess the sanitary quality of "Soumbara" sold in five communes of Abidjan (Côte d'Ivoire), in order to valorize it as a flavor enhancer. Almost all of the people interviewed in this study knew, consumed "Soumbara" and had not experienced any discomfort following consumption. "Soumbara" contained organic acids such as coumarin, which have beneficial properties for the human body. "Soumbara" had a neutral pH and a high dry matter content. This food had a low sugar content and could also be recommended for diabetics. Microbiologically, "Soumbara" as produced contained spoilage and pathogenic microorganisms. The loads of these microorganisms exceeded the standard 2019/229/EC. This food eaten by direct sprinkling on the dish, without passing through a cooking process could expose the consumer to a risk of food poisoning.

Prospects: This study should cover the entire national territory.

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Author contribution: The work was carried out in collaboration among all authors. D.K.M. designed the study. K.K.A. performed the statistical analysis, drafted the protocol, and wrote the first draft of the manuscript. K.M J-P B. managed the analyses of the study, and W. H. managed the literature searches.

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