

EVALUATION OF MICROBIOLOGICAL AND PHYSICO-CHEMICAL PARAMETERS OF JUICES SOLD IN THE STREETS OF THE CITY OF MAN (CÔTE D'IVOIRE): CASE OF *ZINGIBER OFFICINALE* AND *HIBISCUS SABDARIFFA*.

ABSTRACT

Objective: To study the microbiological and physico-chemical quality of traditional drinks based on *Hibiscus sabdariffa* and *Zingiber officinale* sold in the streets of Man (Côte d'Ivoire).

Material and methods: the material consists of *Hibiscus sabdariffa* and *Zingiber officinale* juice. A survey was conducted on the population and on the vendors. Several microbiological standards were used for the determination of *Aerobius mesophilus*, *Escherichia coli*, *Staphylococcus* sp. and *Salmonella* sp. physicochemical parameters such as: reducing sugar, pH, Brix degree, temperature, conductivity, Calcium, Phosphorus, Zinc and Iron were determined in the juice.

Result: At the level of the survey, several sales locations were used by vendors as a strategic environment to attract customers. 38% in small alleys 17% at the bus station and 13% at the edge of schools (13%). In terms of microbiological results, there is a high contamination of microorganisms of alterations, specifically FMAT with a value of $1.82.105 \pm 4.56$ and Total Coliforms (TC) with a value of $8.06.105 \pm 2.44$ of the juices made from Guinea Husk. The physicochemical analyses gave values of pH (4.24 ± 0.31), Temperature (17.7 ± 1), Conductivity (369 ± 88), Calcium (8.84 ± 0.92), Phosphorus (19.85 ± 11.05), Iron (5.36 ± 0.08), °Brix (20.5 ± 4.5) and reducing sugars (17.7 ± 0.68).

Conclusion: The present study made it possible to appreciate the microbiological and physicochemical quality of the beverages based on guinea fowl and ginger. The microbiological results obtained, are worrying for the consumers. Thus, the whole of the parameters determined in the various juices makes it possible to evaluate the risks of contamination throughout the dies of manufacture and distribution incurred by the consumers.

Keywords: *Hibiscus sabdariffa*, *Zingiber officinale*, microbiological, physico-chemical

1. INTRODUCTION

In developing countries, drinks, meals and snacks marketed by street food vendors are widely consumed by millions of people [1]. Thus, they enable about 80% of the urban population to easily eat at low cost and outside the home [2].

Drinks being part of these street foods, occupy an important place in the daily life of man because they are used for refreshing, nutritious and curative purposes [3].

According to an estimate made by the international federation, the world consumption of juice and nectar reached 33 billion liters in 1998 and would have increased to 73 billion in the twenty years later [4].

However, the main concern with these foods is their microbiological safety [5]. Thus, the preparation and sale of street foods can cause major problems for consumer health [6]. Indeed, the preparation equipment, site cleanliness, personal hygiene and sanitation of the home are all risk factors for product contamination that can negatively influence the microbiological quality of the beverages sold [7]. They could be contaminated by pathogenic microorganisms and induce at the consumer level diarrheal diseases [5].

In France, out of the 250,000 to 750,000 food poisoning cases per year, 70,000 people were consulted in the emergency room, 15,000 people were hospitalized and 400 people died [8]. In Côte d'Ivoire, in January 2011, there was a resurgence of food-borne diseases and the emergence of certain diseases and a cholera epidemic. There were 933 cases including 22 deaths in 2011. Thus, the resurgence of these diseases is due to unsanitary conditions and difficulties in the management of food hygiene practices leading to health risks.

To this end, several food health inspection structures have been set up through ministries and laboratories equipped to analyze the quality of food submitted for consumption [9]. Thus, the control of food safety has become a concern for international organizations, public services, consumers, retailers and manufacturers [10].

Since *Hibiscus sabdariffa* and *Zingiber officinale* juices are part of these street foods, this study was conducted to investigate the physico-chemical and microbiological quality of these traditional beverages sold in the streets of Man in order to raise awareness among consumers.

2. MATERIAL AND METHODS

The plant material is composed of two types of juices: *Hibiscus sabdariffa* juice and *Zingiber officinale* juice. The technical material is composed of usual laboratory equipment of microbiological, physicochemical and culture medium.

A sample is composed of at least 30 cl of juice. 27 samples of each juice were taken. That is to say a total of 54 samples of juice containing ginger and guinea oiselle.

The survey of vendors and manufacturers was carried out by means of a questionnaire. It was established from the Kobocollect software. The latter made it possible to collect information on socio-economic characteristics and on the practices and knowledge of street drinks. A total of 94 vendors and 45 manufacturers were registered.

2.1. Microbiological analysis of juices

2.1.1. Preparation of the mother suspension and decimal dilutions

The preparation of the samples, the mother suspension (MS) and the decimal dilutions was performed according to the international standard ISO 6887-1 (1999).

A volume of 25 mL of collected sample was added to 225 mL of EPT (Buffered Peptone Water). The mixture was homogenized and left for 15-20 min for revival of the microorganisms. The solution obtained corresponds to the 10^{-1} stock suspension. From this stock suspension a series of successive decimal dilutions was performed until the 10^{-4} dilution.

2.1.2. Enumeration of aerobic mesophilic flora

The enumeration of the aerobic mesophilic flora was performed according to the international standard ISO 4833 (2003).

1 mL of each dilution was introduced into Petri dishes containing PCA medium. The depletion streak plating method was used for bacterial culture. The plates were incubated at 37°C for 24 hours. Characteristic colonies appeared whitish or yellowish.

2.1.3. Total Coliforms (TC) and Fecal Coliforms (*Escherichia coli*)

Total and thermotolerant coliforms were enumerated respectively according to the international standard ISO 4832 (2006) and ISO 16649-2 (2001). Inoculation was done on VRBL agar and incubated at 37°C for total coliforms and at 44°C for thermotolerant coliforms for 24 h.

2.1.4. Research and enumeration of *Staphylococcus* sp. (Standard ISO 6888-1: July 2003).

For the detection of suspected pathogenic *Staphylococcus*, 0.1 mL of the stock solution (MS) and each dilution were streaked in Petri dishes containing Baird Parker medium. Incubation of the seeded media was done at 37°C for 48 h. The characteristic shiny black colonies surrounded by clear halo were counted.

2.1.5. Research of *Salmonella* sp.

The search for *Salmonella* in food involves essential steps: Pre-enrichment, enrichment, isolation and confirmation.

- Pre-enrichment

25 mL of juice sample was mixed in 225 mL of EPT, homogenized and incubated at 37°C for 24 ± 2 h.

- Selective Enrichment

0.1 mL of the solution from the pre-enrichment was inoculated into a test tube containing 10 mL of Rappaport Vassiliadis broth. The mixture was homogenized and incubated at 37°C for 24 ± 2 h.

- Isolation

Isolation was done from the cultures from the selective enrichment medium. Petri dishes containing SS medium were streaked and incubated at 37°C for 24 h. Typical *Salmonella* sp. colonies are black with transparent halo on SS agar and small in size (2-4 mm in diameter).

- Confirmation

After incubation; biochemical tests are performed using leminor racks to purify similar colonies in Petri dishes that contain the already cast SS medium. Afterwards, they are incubated at 37°C for 24 hours to have pure strains.

2.2. Expression of results (ISO 7218)

The counting of characteristic colonies was done for each box containing between 30 and 300 colonies. The calculation of the number of colony forming units (N) (CFU)/g is performed according to the formula:

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

The expression of the results was also done according to the microbiological criteria applied to fruit juices

Table I: Microbiological criteria for fruit juices

Parameters	UFC/ml
Aerobic Mesophilic Flora Total	10 ³
Coliformes Totaux	10 ¹ -10 ²
Faecal coliforms	1.10 ¹
<i>Salmonella</i>	Not detected in 10 ml
<i>Staphylococcus</i>	10 ¹ -10 ²

3. RESULTS

3.1. Survey results

The results show that there are more vendors of *Hibiscus sabdariffa* and *Zingiber officinale* juice in the commercial (18.09%) and administrative (12.77%) districts (Figure 1). Several sales locations are used

by these vendors as a strategic environment to attract customers. A percentage of 38% are in the small streets where they are dominant, followed by the bus station (17%) and the edge of the schools (13%). Also, 90% of these vendors claim not to have received any complaints from buyers about any contamination of these juices sold by pathogenic microorganisms (Table II).

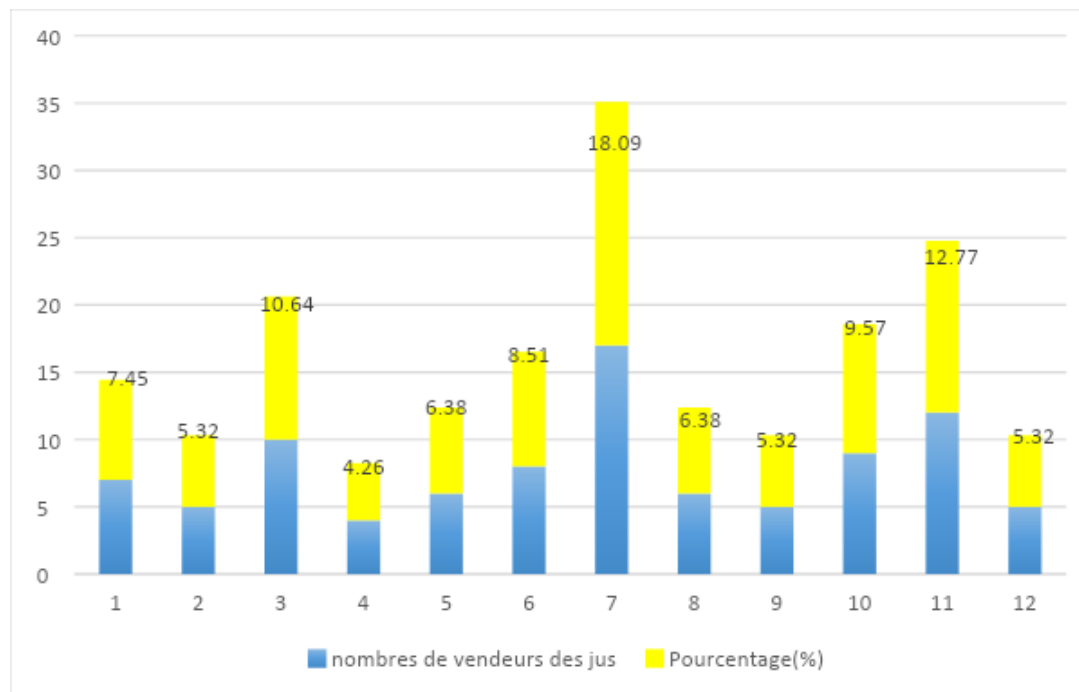


Figure 1: Information on the number of vendors by neighbourhood

Table II: Vendor Location

Place of Sale	Market	Bus station	Small alley	Edge of the Schools	Edge of the Schools
Vendors	18	17	38	13	8
Percentage (%)	19	18	40	14	9

In terms of manufacturing facilities, 51% are considered to be in poor condition and not clean; 40% of facilities are considered to be average and 9% of facilities are considered to be good (Figure 2).

The quality of the water used to make juices from guinea fowl and ginger is a decisive factor. The majority of manufacturers (60%) of these juices use well water in the manufacturing process. Only a minority (18%) use tap water and the rest (22%) use spring water (Figure 3).

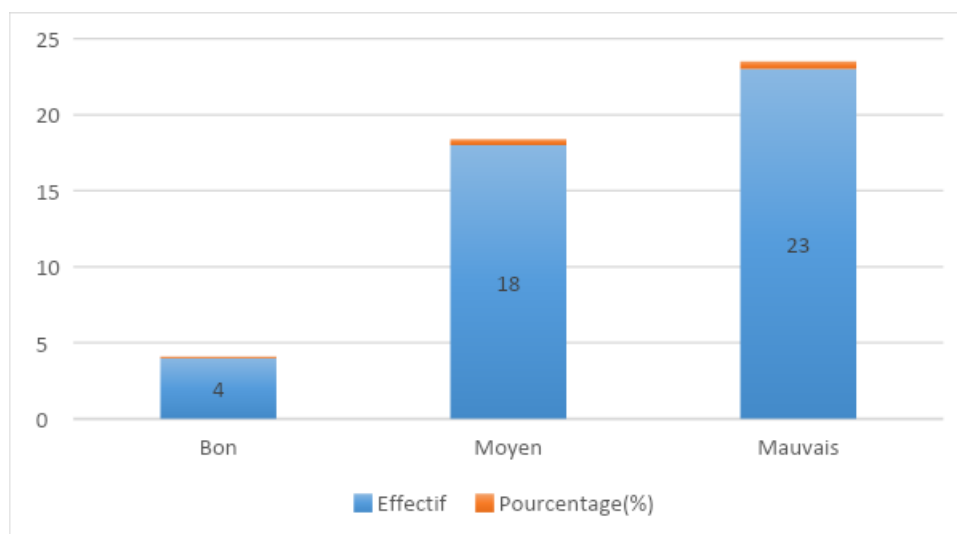


Figure 2: Assessment of the cleanliness of the production room

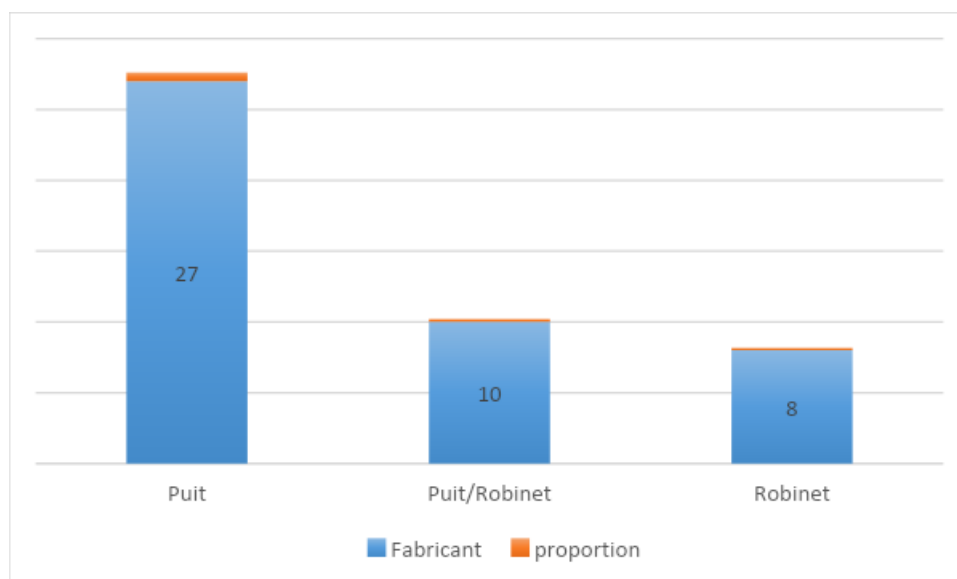


Figure 3: Presentation of the water used for manufacturing

3.2. Results of analysis of physico-chemical parameters of juices

The physico-chemical results show average pH values between 2.1 ± 0.11 (mB2) and 2.3 ± 0.05 (mB3). As for the temperature, it has an average value ranging from 10.45 ± 3.35 (mB3) to 15.65 ± 3.35 (mB2).

The conductivity of these juices ranges from 231, 85± 66.5 (mB3) to 369± 88 (mB2). This table also shows proportions of Ca; P, Fe and Zn. The Brix degree result for mB3 (17.5± 4.5) is lower than that of mB1 (18.5± 3.5) and mB2 (20.5± 4.5). On the other hand, the reducing sugar level (g/100ml) of mB3 (5.58±1.3) is lower than mB1 (5.9±1.03) and mB2 (8.38±1.4) (Table III).

Table III: Results of analysis of the parameters of the juice based on the guinea fowl.

Neighborhoods BOD Parç		RMC	KLB
Jus	mB1	mB2	mB3
pH	2,22 ± 0,02	2,1 ± 0,11	2,3 ± 0,05
Temperature (°celsius)	12,7 ± 2,7	15,65 ± 3,35	10,45± 3,35
Conductivity (µs/cm)	255,25 ± 87,75	369 ± 88	231,85 ± 66,5
Ca (%)	5,01 ± 0,45	8,84 ± 0,92	8,42 ± 2,50
P (%)	24,05 ± 15,82	19,85 ± 11,05	-
Fe (%)	2,63 ± 0,71	2,82 ± 0,63	3,74±0,87
Zn(%)	-	-	-
°Brix	18,5 ± 3,5	20,5 ± 4,5	17,5 ± 4,5
Reducing sugar (g/100ml)	5,9 ±1,03	8,38 ±1,4	5,58 ±1,3

QMC: trade; *BDO: Dioulabougou; *KLB: Blokoss; *P: Phosphorus; *Ca: Calcium; *Zn: Zinc; *mB1: average of ginger-based juices sampled from the BOD; *mB2: average of ginger-based juices sampled from the RMC; *mB3: average of ginger-based juices sampled from the KLB.

The physicochemical results of the ginger-based juices show average pH values ranging from 3.15 ± 0.32 (mG2) to 4.24 ± 0.31 (mG3). As for the temperature, its average value varies from 10.85 ± 3.15 (mG1) to 17.7 ± 1 (mG2). The conductivity of these juices averages between 132.05 ± 22.95 (mG1) and 186.55 ± 12.05 (mG2). The table also shows proportions of Ca; P, Fe and Zn. The average Brix degree result for mG3 (13.5 ± 0.5) is lower than that of mG2 (20 ± 3) and mG1 (20 ± 2). For reducing sugar (g/100ml), the average mG1 value (6.73 ± 0.75) is lower than mG3 (6.84 ± 0.49) and mG2 (17.7 ± 0.68) (Table IV).

Table IV: Results of the analysis of physico-chemical parameters of ginger-based juices

Parameter \ Neighborhoods	BOD	RMCC	KLB
	mG1	mG2	mG3
Temperature (°celsius)	mG1	mG2	mG3
pH	$3,35 \pm 0,41$	$3,15 \pm 0,32$	$4,24 \pm 0,31$
Temperature (°celsius)	$10,85 \pm 3,15$	$17,7 \pm 1$	$14,45 \pm 1,85$
Conductivity (qs/cm)	$132,05 \pm 22,95$	$186,55 \pm 12,05$	$143,7 \pm 14,1$
Ca (%)	$4,41 \pm 0,74$	$4,36 \pm 0,42$	$2,65 \pm 0,35$
P (%)	$12,67 \pm 8,80$	-	$2,64 \pm 0,35$
Fe (%)	$3,46 \pm 0,52$	$5,36 \pm 0,08$	$3,55 \pm 0,66$
Zn (%)	$2,47 \pm 0,46$	-	-
°Brix	20 ± 2	20 ± 3	$13,5 \pm 0,5$
Reducing sugar (g/100ml)	$6,73 \pm 0,75$	$17,7 \pm 0,68$	$6,84 \pm 0,49$

Figure 4: microbial comparison of the highest averages

4. DISCUSSION

The majority of sellers of the different juices studied make their own products and most of them cannot read and write. The marketing of these juices is dominated by women (96.81%). This result is close to that of Baba-moussa et al. [11] who worked on the study of the possibilities of contamination of street foods in Benin. The largest consumers of these juices are mainly young people (44%). The packaging (bags and cans) of the products is purchased with other traders. Two categories of vendors were identified: fixed (41.49%) and mobile (51.58%). Of all the vendors interviewed, only 8% had clean hygiene, compared to 55.5%. These results differ from those of Ratsimihaba [12] who worked on a street food with an average hygienic state of the vendors (75%). This study showed that the vendors were found more in the alleys (40%). This study is confirmed and demonstrated by (Randrianantoandro [13] with 9% of vendors at the edges of the streets. In the surveys (100%) of vendors use their hands without protection to serve juice to customers or buyers and this can lead to bacterial contamination inherent in the cleanliness of the hands. According to Adouane [14] hands are the most important anatomy of food handlers and therefore the main culprits of cross contamination.

Microbiological analysis is a quantitative study of the microbial flora (total flora, total coliforms, *staphylococcus* sp., *Salmonella* sp.) and this microflora reveals the sanitary quality and marketability of the product [15]. After observation of the different results, the samples of beverages based on guinea fowl and ginger have in common the presence of FMAT germs and total coliforms which have an unsatisfactory microbiological quality (INS). The significant presence of total coliforms is confirmed by a study by Lewis et al. [16]. Regarding the high presence of FMAT in these foods, it is demonstrated by Rakotondrazaka [17] who obtains a high presence of FMAT in samples from the city of Anktso and Ambohimangakely.

The quality in fecal contamination germs is satisfactory. In fact, the irregular presence of these hygiene indicator germs is attributed to the poor personal hygiene and clothing of the vendors as well as to the unsanitary conditions of the floor plan, the washing water and the working materials (knife, sponge, wrapping paper) [18].

For the case of *Salmonella* sp, the samples of ginger-based beverages present an unsatisfactory microbiological quality this could be explained by cross-contamination. These results are in line with those of Abisso et al. [19], who found germs in the majority in analyzed samples of different categories of juice.

The microbiological quality of guinea fowl juices in *staphylococcus* sp germs obtained at all study sites is unsatisfactory. This result is contrary to that of Komagbe [2]. where the author explains such absence in all his samples by the fact that they underwent heat treatment during their manufactures.

According to WHO [20], the level of bacteria in food should not exceed the standards, otherwise the food returns unacceptable microbiological qualities. Therefore, these juices found in the various sales sampling sites would be of unacceptable microbiological qualities.

The Hydrogen Potential (pH) results reveal that the ginger-based juice is the most acidic. These results differ from Olorunjuwon et al. [21] where the pH values of their fruit juice are higher.

This study compared the proportion of trace elements (Iron, Ca, K and P) in the different juices. It was found that the average proportion of calcium (Ca) and phosphorus (P) in the juices from Guinea goose was higher than in the juices from ginger. These average values of juices are on the whole not in accordance with those found by Mahfouf and Ihadadene [22] who found higher °Brix. The variation in these parameters is due to the richness of these beverages in nutrients especially sugars.

According to Brummer and Steve [23], carbohydrates are one of the most important components in foods.

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4. CONCLUSION

The sale of street foods has highlighted the importance of studying the microbiological and physicochemical quality of *Hibiscus sabdariffa* and *Zingiber officinale* juices sold at the roadside. These analyses showed unsatisfactory microbiological quality; mainly by spoilage germs (FAMT) and pathogens such as total coliforms, *Salmonella* sp. and *Staphylococcus* sp.

Vendors and manufacturers should be made aware of the hygiene rules and laws governing street vending.

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