

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).

Materials and methods: The material consisted of *Hibiscus sabdariffa* and *Zingiber officinale* juices. A survey was conducted among the population and vendors. Several microbiological standards were used for the determination of mesophilic aerobic germs, *Escherichia coli*, *Staphylococcus* sp. and *Salmonella* sp. Physicochemical parameters such as: reducing sugar (g/100 mL), pH, Brix degree (°Brix), temperature (°Celsius), conductivity (µs/cm), calcium, phosphorus, zinc and iron (all three in mg/g) were determined in the juice.

Result: At the survey level, several sales locations were used by the vendors as a strategic environment to attract customers. 40% in small alleys, 19% at the market, 18% at the bus station, 14% near schools and (9%) near administrations. Regarding the microbiological results, there is a strong contamination of the mesophilic aerobic germs with a value of 1.82×10^5 and Total Coliforms (TC) with a value of 8.06×10^5 of the juices made from *Hibiscus sabdariffa* making the juices unfit for consumption.

Physicochemical analyses of *Hibiscus sabdariffa* and *Zingiber officinale* juices yielded the following highest values: pH (2.3 ± 0.05) (4.24 ± 0.31), temperature (15.65 ± 3.35) (17.7 ± 1 C°), conductivity (369 ± 88 µs/cm) (186.55 ± 12.05), calcium (0.88 and 0, 44 mg/g), phosphorus (2.4 and 1.26 mg/g), iron (0.37 and 0.53 mg/g), °Brix (20.5 ± 4.5) (20 ± 2) and reducing sugars (8.38 ± 1.4) (17.7 ± 0.68 g/100 mL).

Conclusion: The present study allowed to assess the microbiological and physicochemical quality of the drinks based on *Hibiscus sabdariffa* and *Zingiber officinale*. The microbiological results obtained, are worrying for the consumers. Also, the whole of the parameters determined in the various juices allow to have an idea of the risk 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

2.1. Sampling

The plant material was composed of two types of juice: *Hibiscus sabdariffa* juice and *Zingiber officinale* juice. The technical material is composed of the usual laboratory material of microbiology, physicochemistry and culture media

One sample consisted of juice contained in a 30 mL bottle. 27 samples of each juice were taken. A total of 54 samples of *Zingiber officinale* and *Hibiscus sabdariffa* juices were collected.

The survey of vendors and manufacturers was conducted using a questionnaire. It was based on the Kobocollect software. The questionnaire collected information on socioeconomic characteristics, cleanliness of production rooms, and practices and knowledge of street juice consumption. A total of 94 vendors and 45 manufacturers were recorded.

2.2. Microbiological analysis of juices

2.2.1. Preparation of the stock solution and decimal dilutions

The preparation of the stock solution and decimal dilution samples, was performed according to the international standard ISO 6887-1 (1999).

Twenty-five milliliters (25 mL) of *Zingiber officinale* and de *Hibiscus sabdariffa* was aseptically transferred to an Erlenmeyer flask containing 225 mL of sterile (BPW) (Buffered Peptone Water) medium to prepare the stock solution. After 15-20 min rest on the bench at room temperature, the stock solution was decimally diluted until up the 10^{-4} .

2.2.2. Enumeration of mesophilic aerobic germs

The enumeration of the mesophilic aerobic germs 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. Plates were incubated at 37°C for 24 hours and counted afterwards.

2.2.3. Total Coliforms (TC)

Thermotolerant coliforms were enumerated according to the international standard ISO 16649-2 (2001). Inoculation was done on VRBL agar and incubated at 44°C for 24 hours.

2.2.4. 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.

2.2.5. 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.

2.2.6. 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/mL) is performed according to the formula:

$$N = \frac{\sum c}{V(n1+0,1n2)dX F}$$

ΣC : Sum of characteristic colonies counted on all retained Petri dishes;

N1: Number of Petri dishes retained at the first dilution;

N2: Number of Petri dishes retained at the second dilution;

d: Dilution rate corresponding to the first dilution;

V: Inoculated volume (mL);

N: Number of microorganisms (CFU/mL).

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

Table 1: Microbiological criteria for fruit juices

Parameters	UFC/ml
Mesophilic aerobic Total	10^3
Total Coliforms	10^1 - 10^2
Faecal coliforms	$1 \cdot 10^1$
<i>Salmonella</i>	Not detected in 10 ml
<i>Staphylococcus</i>	10^1 - 10^2

Sources: CODEX FAO/WHO and OTENG (1984); CODEX STAN

2.2. Physicochemical characterization of juices

2.2.1. Determination of the pH

The determination of the pH is carried out using a pH-meter (PHS38W). A quantity of 20 mL of the sample was used for this purpose. Three (3) measurements from one sample of 20 mL were performed.

2.2.2. Trace element content

Trace elements such as Zinc, Calcium, Phosphorus and Iron were determined by atomic absorption spectrophotometry or X-ray fluorescence at the central laboratory of the University of Man.

A juice sample was deposited in a film on the 0.07 mm diameter collimator of the apparatus. A beam of monochromatic radiation passes through the sample with high energy (50 Kev) ionizing the atoms of the sample to be analyzed.

2.2.3. Determination of Brix degree and amount of sugar in a juices

The degree of Brix was measured using a portable refractometer (ATAGO N-1 α) measuring up to 30 Brix. Three measurements were performed per sample. For the determination of the amount of sugar in the beverages, the volume of beverage contained in the bottle was spilled in a beaker and weighed. From Brix degrees and determined beverage masses, the amount of total sugars contained in a bottle of a juices was calculated according to the formula described by Monroe [11]:

$$\text{Quantity of sugar in the product (g)} = (\text{Brix degree of the product} / 100) \times \text{Amount of product (g)}.$$

3. RESULTS AND DISCUSSION

3.1. Survey results

3.1.1. Point of sale information

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 2).

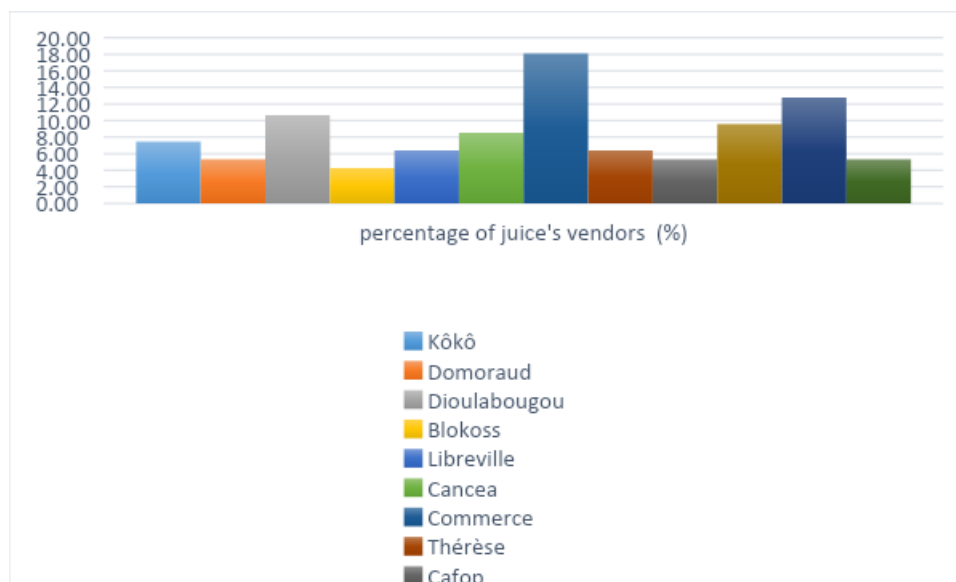


Figure 1: Information on the number of vendors by neighbourhood

Table 2: Vendor Location

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

3.1.2. Information on the hygiene status of vendors

Of the 94 vendors surveyed, 59% were judged to be unclean in terms of their hands, clothes, the cleanliness of the coolers and trays, and the location. Only 8% had good hygiene practices, while the rest (33%) were judged to be average (figure 2).

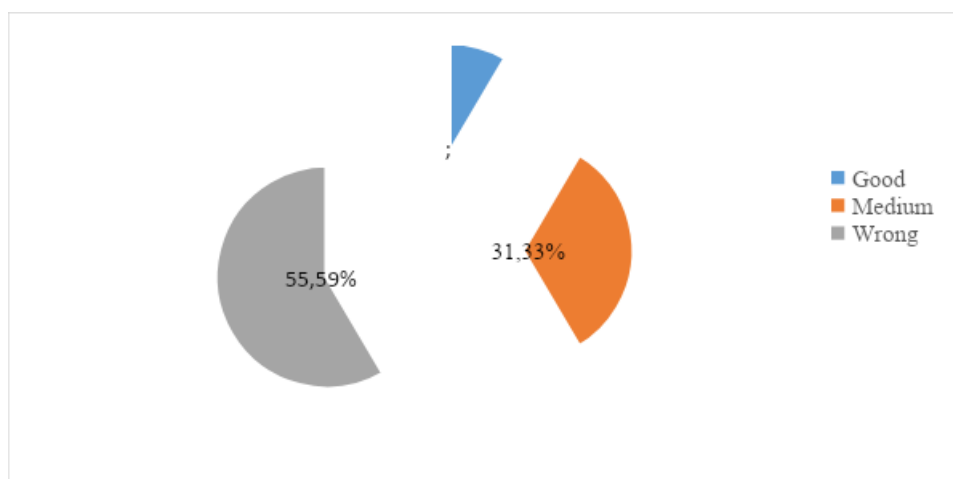


Figure 2: Information on the hygiene conditions of juice vendors. Information on the hygiene

Regarding the manufacturing facilities, 51% are considered to be in poor condition and not clean; 40% of the facilities are considered average and 9% of the facilities are considered good (Figure 3).

The quality of the water used for the manufacture of *Hibiscus sabdariffa* and *Zingiber officinale* juices is a determining 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 4).

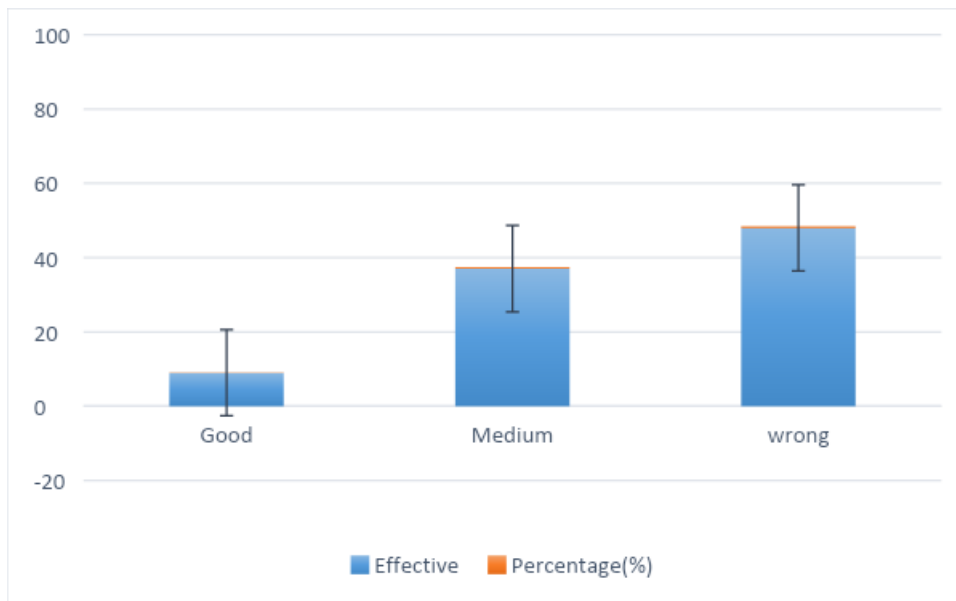


Figure 3: Information on the cleanliness of the production room

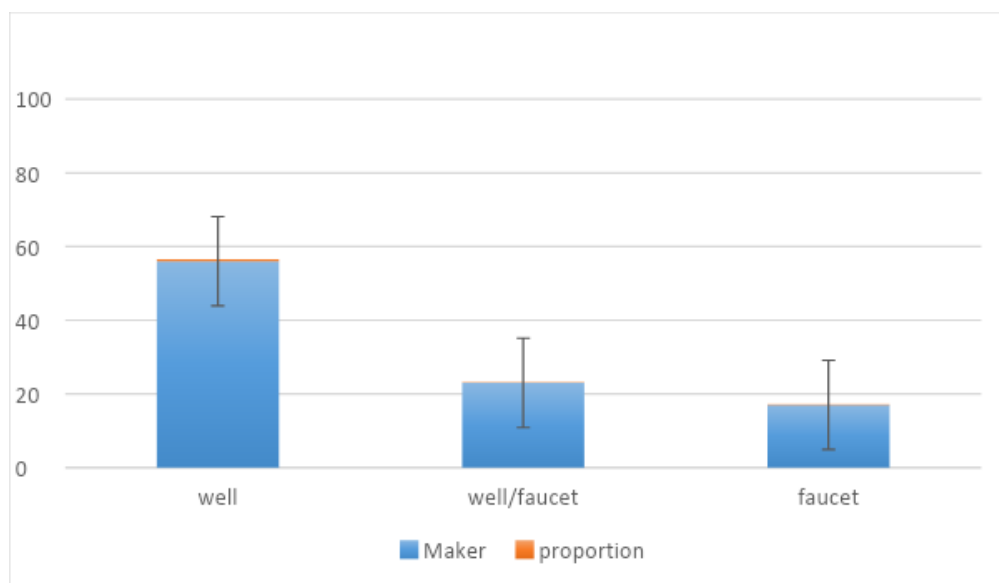


Figure 4: Information on water used for juice production

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

The physicochemical results show average pH values between 2.1 ± 0.11 and 2.3 ± 0.05 . The temperature is between 10.45 ± 3.35 and 15.65 ± 3.35 . The conductivity of these juices ranges from $231, 85 \pm 66.5$ to 369 ± 88 . This table also shows proportions of Ca; P, Fe and Zn in mg/g. The Brix degree varied from (17.5 ± 4.5) to (20.5 ± 4.5) . On the other hand, the reducing sugar content (g/100ml) ranged from (5.58 ± 1.3) to (8.38 ± 1.4) (Table 3).

Table 3: Results of analysis of the parameters of *Hibiscus sabdariffa* juice

Neighborhoods			
Parameters			
Jus	mB1	mB2	mB3
pH	$2,22 \pm 0,02$	$2,1 \pm 0,11$	$2,3 \pm 0,05$
Temperature (°celsius)	$12,7 \pm 2,7$	$15,65 \pm 3,35$	$10,45 \pm 3,35$
Conductivity (µs/cm)	$255,25 \pm 87,75$	369 ± 88	$231,85 \pm 66,5$
Ca (mg/g)	0,5	0,88	0,84
P (mg/g)	2,4	1,98	-
Fe (mg/g)	0,26	0,28	0,37
Zn (mg/g)	-	-	-
°Brix	$18,5 \pm 3,5$	$20,5 \pm 4,5$	$17,5 \pm 4,5$
Reducing sugar (g/100ml)	$5,9 \pm 1,03$	$8,38 \pm 1,4$	$5,58 \pm 1,3$

*BDO: Dioulabougou; *QMC: Commerce; *KLB: Blokoss; *P: Phosphorus; *Ca: Calcium; *Zn: Zinc; *mB1: average of *Hibiscus sabdariffa* juices sampled from the BOD; *mB2:

average of *Hibiscus sabdariffa* juices sampled from the RMC; *mB3: average of ginger juices sampled from the KLB.

The physicochemical results of the *Zingiber officinale* juices showed mean pH values ranging from 3.15 ± 0.32 to 4.24 ± 0.31 . The temperature ranged from 10.85 ± 3.15 to 17.7 ± 1 . The conductivity of these juices ranged from 132.05 ± 22.95 to 186.55 ± 12.05 . The table also shows proportions of Ca; P, Fe and Zn. The Brix degree varied from 13.5 ± 0.5 to 20 ± 3 . The reducing sugar content (g/100ml), varies from 6.73 ± 0.75 to 17.7 ± 0.68 (Table 4).

Table 4: Results of the analysis of physico-chemical parameters of *Zingiber officinale* juice

Neighborhoods			
Parameter			
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 (µs/cm)	$132,05 \pm 22,95$	$186,55 \pm 12,05$	$143,7 \pm 14,1$
Ca (%)	0,44	0,43	0,26
P (%)	1,26	-	0,26
Fe (%)	0,34	0,53	0,35
Zn(%)	0,24	-	-
°Brix	20 ± 2	20 ± 3	$13,5 \pm 0,5$
Reducing sugar (g/100ml)	$6,73 \pm 0,75$	$17,7 \pm 0,68$	$6,84 \pm 0,49$

*BDO: Quartier Dioulabougou; QMC: *Quartier commerce *KLB: Quartier Blokoss; *P: Phosphorus; *Ca: Calcium; *Zn : *Zinc; *mG1: average of *Zingiber officinale* juice from BOD; *mG2: average of *Zingiber officinale* juice from RMC; *mG3: average of *Zingiber officinale* juice from KLB.

3.3. Results of microbiological analysis of *Hibiscus sabdariffa* and *Zingiber officinale* juice

The microbiological analysis of the *Hibiscus sabdariffa* and *Zingiber officinale* juice based shows that:

Mesophilic aerobic germs, total coliforms and Staphylococcus sp of both types of juice are above microbiological standards: The microbiological quality of *Hibiscus sabdariffa* and *Zingiber officinale* is unsatisfactory.

Table 5: Microbial analysis of *Hibiscus sabdariffa* and *Zingiber officinale* juice

Germ	Load (CFU/ml) (<i>Hibiscus sabdariffa</i> juice)	Load (CFU/ml) (Jus de <i>Zingiber</i> <i>officinale</i> juice)	Standard	Results
Mesophilic aerobic germs	$1,82 \cdot 10^5 \pm 4,56$	$1,66 \cdot 10^5 \pm 6,58$	$3 \cdot 10^3 - 3 \cdot 10^4$	UNSATISFACTORY
Total Coliform	$2,69 \cdot 10^5 \pm 1,62$	$8,06 \cdot 10^5 \pm 2,44$	$10^1 - 10^2$	UNSATISFACTORY
<i>Salmonella</i> sp.	<1	$1,32 \cdot 10^5 \pm 5,84$	ND/10 ml	UNSATISFACTORY

Staphylococcus sp.	4,51.10²± 2,00	2,57.10³±1,12	10¹ - 10²	UNSATISFACTORY
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4. DISCUSSION

The majority of the vendors in the different juices studied made their own products and most of them could not read or write. This study showed that among the many sales outlets, back alleys occupied an important place that was 40% compared to other sales outlets. This observation is confirmed by Randrianantoandro [12] in his study with a high percentage of vendors at the street side (90%).

In terms of cleanliness, it was found that of all the vendors interviewed, only 8% were in a clean hygienic state, compared to 55.5% who were in an unsatisfactory hygienic state. These results differ from those of Ratsimihaba (2021) [13] who worked on a street food and who found that 75% of the vendors had an average hygienic state.

51% of the places where juice is made were not clean. In addition, 50% of the people used well water to make juice. This could lead to bacterial contamination of these juices that will make all consumers sick. These results were close to those of Baba-moussa et al. [14] who had worked on the possibility of contamination of street foods in Benin.

The physicochemical and microbiological quality of *Hibiscus sabdariffa* and *Zingiber officinale* juices depended on several parameters: processing, chemical preservatives, raw material, and pH. Thus, the physicochemical tests showed that the pH of the *Hibiscus sabdariffa* and *Zingiber officinale* juices was acidic. According to the standard CODEX-STAN 243-2003, the pH of fruit juices and beverages must be below 4.5. The pH of these juices is therefore satisfactory.

The sugar content was high, which represented a danger for the consumer. Indeed, according to the WHO, the recommended tolerable quantity is less than 50 g of sugar per day.

Moreover, the microbiological study of its different juices based on *Hibiscus sabdariffa* and *Zingiber officinale*, had shown not only a contamination in aerobic mesophilic germs but also in pathogenic germs such as salmonella and staphylococcus. Indeed, the Mesophilic germs is an indicator of the general level of hygiene of a product, it informs on factors such as, the environment, cross-contamination during handling, the environment, packaging and storage of the product [15]. The presence of *Staphylococcus* sp and *Salmonella* is a danger for the consumer. Indeed, *Staphylococcus* is a vector of skin contamination. These bacteria were the cause of food poisoning. The results obtained are similar to those of Noutais [16]. The presence of *Salmonella* could be due to cross-contamination. Overall, the processing could have an effect on the quality of its juices.

Indeed, *Hibiscus sabdariffa* and *Zingiber officinale* juices were often not manufactured under hygienic conditions. During the survey, most manufacturers reported that *Zingiber officinale* is crushed with the skin during the juice making process after a simple washing. Thus, the soil microorganisms had not been neutralized on this skin. In addition, the hygiene status of the vendors and grinders was not under control. Indeed, there are several products that were ground by its machines and their multiplicity could be considered a site of cross-contamination. Also, the preparation of *Hibiscus sabdariffa* was done by soaking the leaves

and not by boiling at 100°C, which would favor the multiplication of germs in this juice. It was also noticed that the beverages obtained for the two juices of *Hibiscus sabdariffa* and *Zingiber officinale* were always packaged in jars already used or recycled, which does not guarantee their cleanliness. In addition, the juice extractions would be done with bare hands. The hands would carry most of the contamination germs, when hygiene is not practiced.

The juices were sold in coolers and jars without ice to maintain the temperature at 4°C, moreover there is the cold chain that was not respected. Thus, the break of the cold chain could provoke the accelerated growth of certain micro-organisms.

Thus, according to WHO [17], the rate of bacteria in food should not exceed the standards, otherwise, the food becomes of unacceptable microbiological qualities. Therefore, these different juices based on *Hibiscus sabdariffa* and *Zingiber officinale* found in the different sites of sales sampling would be of unacceptable microbiological qualities.

4. CONCLUSION

The current situation of the sale of food and juices on the public road motivated us to make a study on the physicochemical and microbiological quality of juices based on *Hibiscus sabdariffa* and *Zingiber officinale* sold in the streets of the city of Man. Following this analysis, it appears that the juices based on *Hibiscus sabdariffa* and *Zingiber officinale* were contaminated by aerobic mesophilic germs, and pathogenic bacteria such as *Salmonella* and *staphylococcus*. Thus, the consumption of these juices represents a real danger for the health of the consumer. Adequate measures must be taken during the manufacturing of juices to avoid any kind of contamination. These measures can be at the level of packaging, the wearing of gloves and masks and the heat treatment.

It is also necessary to sensitize and train sellers and manufacturers on the rules of hygiene.

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