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Evaluation of the Hygienic Quality of "Bissap" Juice (*Hibiscus sabdariffa*) Sold in the Markets of Primary and Secondary Schools in Korhogo (Côte d'Ivoire)

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Abstract Bissap juice, obtained by decoction of *Hibiscus sabdariffa* calyxes, is a non-alcoholic drink consumed by populations in sub-Saharan Africa in general, and particularly by students of primary and secondary schools in Korhogo city. This refreshing drink, produced under hygienic conditions beyond any official control, could constitute a public health problem. This study was carried out to assess the health quality of bissap juice consumed by students. Physicochemical analysis revealed that bissap juice is very acidic (pH varies between 2.52 ± 0.09 and 2.79 ± 0.58). This acidity would protect the bissap against the proliferation of certain pathogenic germs. Indeed, microbiological analysis revealed that these juices contain less than 1 CFU/mL of total coliforms, *Escherichia coli*, *Staphylococcus aureus*. For these sprouts, these juices would be of acceptable sanitary quality. However, this acidity of the juices promotes the proliferation of yeasts and molds with an average value which varies between 1.21×10^2 ± 0.22 CFU/mL and 2.91×10^2 ± 0.91 CFU/mL. The addition of sugar makes bissap a refreshing sweet drink with an average Brix degree value ranging between 10.15 ± 0.35 and 18.95 ± 2.47. This sugar constitutes a substrate which would promote the proliferation of yeasts and molds. These microorganisms could release secondary metabolites into bissap juice which could have an adverse effect on the health of consumers. It is therefore necessary to characterize and identify the different fungal flora as well as their possible pathogenic metabolites in perspective, in order to assess the health risk incurred by the consumer.

Keywords *Hibiscus sabdariffa*, Non-alcoholic drink, Hygienic quality, Korhogo

1. Introduction

Population growth in sub-Saharan African countries has increased significantly over the last two decades [1,2]. For example, the population of Côte d'Ivoire has increased from 16454660 inhabitants in 2000 to 26378275 in 2020 [3]. Also, the agglomerations are experiencing significant development and population growth.

Working people are often away from their homes at meal times during the day. They resort to street food. High consumer demand has led to the development of this activity [4,5]. Therefore, schoolchildren, unable to go home to eat or refresh themselves, resort to street food sold either around schools or the streets leading to them, or in markets within the schools.

Vendors usually offer snacks and soft drinks [6]. The latter usually consist of water, flavoured and sweetened juices. These juices are made from plants or synthetic flavours. For example, gnamankoudji juice is obtained from the rhizomes of *Zingiber officinale*, tomi juice from the fruit of the tamarind tree (*Tamarindus indica*), and bissap juice from the dried calyxes of *Hibiscus sabdariffa*.

Schoolchildren in Korhogo use this type of food during the school year. The sale of food in the school markets in Korhogo is mainly the activity of women. Bissap juice is sold by almost all the women vendors, making it the most popular soft drink among schoolchildren.

Hibiscus sabdariffa calyxes contain minerals, organic acids, antioxidants, proteins, lipids, carbohydrates, vitamin C [7-10]. Also, bissap juice would constitute a favourable environment for the microorganisms growth. The food offered to students is cooked in an informal setting and in hygienic conditions that escape to any official control.

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As these vendors have very little knowledge of catering, there is a risk of illness linked to the consumption of the food they offer to pupils. It seems necessary to evaluate the sanitary quality of these foods, mainly bissap juice, in order to preserve the health of the pupils.

2. Materials and Methods

2.1. Study Matrix

The study matrix is bissap juice, obtained from *Hibiscus sabdariffa* calyxes, sold in the markets of primary and secondary schools in the city of Korhogo (Figure 1).

2.2. Method of Making Bissap Juice

The process for obtaining bissap juice is summarized in (Figure 2) according to the results of the survey from women vendors.

2.3. Sampling

Bissap juice was randomly collected from vendors in 3 (three) secondary schools and 6 (six) primary schools across Korhogo. A total of 30 (thirty) women vendors were involved in this study. A sample was taken (purchased) from each of them by putting ourselves in the conditions of a student at each visit. A total of three visits was made to each vendor, at least one week apart, for a total of 90 (ninety) samples. The samples taken at each visit were transported aseptically in coolers containing ice condensers and immediately transported to the laboratory for analysis.

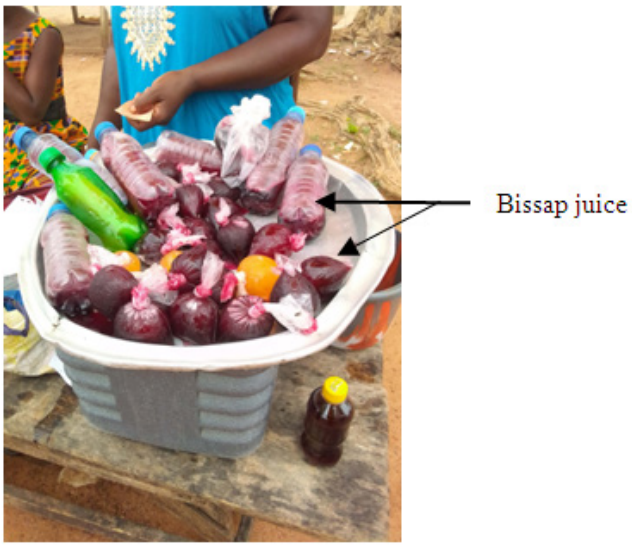

Figure 1. Bissap juice packaged in plastic bottle and sachet

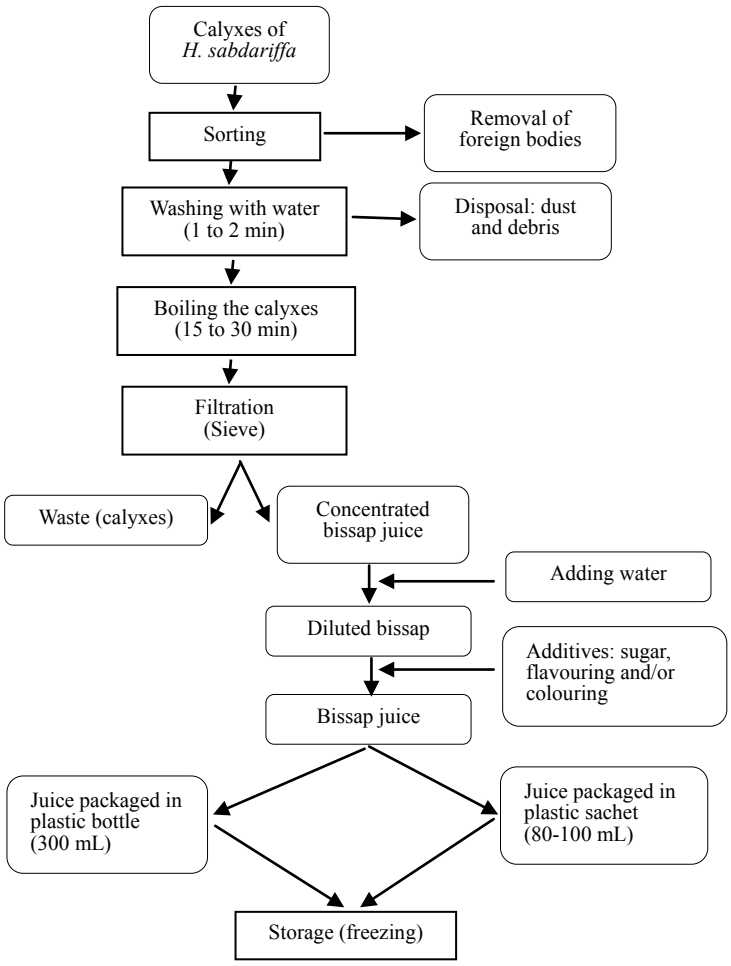

Figure 2. Empirical diagram of the manufacture of bissap juice by decoction (field survey)

2.4. Physicochemical Analysis

2.4.1. pH Determination

The samples pH was determined using a Hanna Instruments HI9124 digital pH meter. The measurements were carried out according to the manufacturer's recommendations.

2.4.2. Titratable Acidity

The bissap juice titratable acidity was determined according to the ISO 750: 1998 standard.

2.4.3. Brix Degree

The Brix degree was determined using an ATAGO digital refractometer, PAL-α (AT-3840). After calibration, using a pipette, a few drops of the sample were applied to the measuring well and then the start key was pressed to read the Brix value, according to the manufacturer's instructions.

2.5. Microbiological Analysis

The microbiological analysis were preceded by a successive decimal dilution of each sample taken from each vendor in accordance with NF V08-010 standard. The enumeration of the total mesophilic aerobic flora was carried out on Plate Count Agar (PCA), at 30°C for 72 hours according to the NF/ISO 4833: 2003 standard. Total coliforms were isolated and enumerated on Lactose Bile Agar with Cristal Violet and Neutral Red (VRBL) at 30°C for 24 hours according to NF/ISO 4832: 2006 standard. Rapid'*E. coli* 2 agar was used to isolate and enumerate *Escherichia coli* at 44 °C for 24 to 48 hours according to the NF/ISO16140: 2013 standard. Baird-Parker agar with potassium tellurite egg yolk and 0.2% Sulfamethazine was used to isolate and enumerate *Staphylococcus aureus* at 37 °C for 48 hours according to NF/ISO 6888: 2004 standard. Yeasts and moulds were isolated and enumerated on Sabouraud Chloramphenicol agar at 25°C for 5 days according to the NF/ISO 16212: 2011 standard. All of these media were prepared according to the manufacturer's instructions. Each decimal dilution of each sample was inoculated in duplicate.

2.6. Enumeration of Microorganisms

Germ count calculations were performed according to ISO-7218 (2007) which determines the number N of colonies according to the following formula:

$$N = \frac{\sum C}{(n_1 + 0.1n_2)d} \times \frac{1}{V} \quad (1)$$

N: is the number of microorganisms in CFU/mL,

∑C: is the sum of the colonies counted on the two dishes retained from two successive dilutions,

n_1: is the number of dishes retained at the first dilution,

n_2: is the number of dishes retained at the second dilution,

d: is the dilution corresponding to the first dilution retained,

V: in the volume of inoculum placed in each dish, in mL.

2.7. Statistical Analysis

All data were analysed using one-way analysis of variance (ANOVA) with significance level set at $P < 0.05$.

3. Results and Discussion

3.1. Physicochemical Characteristics

The analysis carried out on the different bissap samples revealed that the average pH values vary between 2.52 ± 0.09 and 2.79 ± 0.58. No significant difference ($p < 0.05$) is statistically observed between samples from the different schools (Table 1). These values are in line with the Tanzanian standard (TZS 585: 2011) for non-carbonated soft drinks (pH: 2.5 – 4.5). These results are close to those obtained by [11] and [12], respectively 2.5 ± 0.1 and between 2.67 and 2.77.

On the other hand, the mean values of titratable acidity vary between 0.17 ± 0.06% citric acid and 0.26 ± 0.01% citric acid. A significant difference ($p < 0.05$) was statistically observed for the samples from College Moderne, Lycée Moderne, Lycée Houphouet-Boigny and Groupe Scolaire Notre Dame de l'Assomption (Table 1). These values are higher than those recorded by [12] in Nigeria (0.02 – 0.08% citric acid).

The acidity of bissap juice is thought to be related to the chemical composition of the calyxes of *H. sabdariffa*; rich in organic acids (succinic, oxalic, tartaric, citric and malic acid) [7,13]. The decoction of the calyxes (Figure 1) would facilitate the diffusion of these compounds in the bissap juice.

As for the Brix degree, the average values obtained vary between 10.15 ± 0.35 °B and 18.95 ± 2.47 °B. Furthermore, there is a significant difference ($p < 0.05$) between the different bissap samples from:

- Groupe Scolaire Residentiel 3, Soba and Collège Moderne, Lycée Houphouet-Boigny, Groupe Scolaire Haoussabougou, Groupe Scolaire Jean Delafosse.
- Lycée Moderne, Groupe Scolaire Notre Dame de l'Assomption and Groupe Scolaire Jean Delafosse.
- Groupe Scolaire Haoussabougou and Groupe Scolaire Notre Dame de l'Assomption (Table 1).

These average values do not comply with the Tanzanian standard (TZS 585: 2011) for soft and non-carbonated drinks (≤ 10 °B). These values are close to those from the work of [14] on the "gnamakoudji" (rhizome-based juice from *Zingiber officinale*) which recorded a variation from 16.93 ± 1.38 °B to 19.73 ± 4.4 °B. The addition of sugar would reduce the astringency and improve the organoleptic quality of bissap juice. This sugar could constitute a substrate for the growth of possible microorganisms present in the bissap juice.

3.2. Microbiological Caracteristics

Table 2 shows that all bissap samples analysed contained less than 1 CFU/mL of total coliforms, *Escherichia coli* and *Staphylococcus aureus*. For these microorganisms, the consumption of these juices would not constitute a health risk for the consumer. These results are different from those observed by [12] whose work revealed the presence of *S. aureus* and *E. coli* in the juices. The results herein could be explained by the heat treatment of the calyxes of *H. sabdariffa* (figure 2), but also by the low pH (2.52 ± 0.09 and 2.79 ± 0.58) which is a growth inhibiting factor for most of the main aerobic mesophilic germs found in food [15]. Indeed, most pathogenic bacteria are neutrophiles (pH between 5.4 and 8.0) [16].

Moreover, the average values of the Aerobic Mesophilic Flora of the samples vary between $10.21\times10^2 \pm 3.6$ CFU/mL and $23\times10^2 \pm 2.87$ CFU/mL. Also, a significant difference ($p < 0.05$) is observed between the samples from:

- Collège Moderne, Lycée Moderne, Lycée Houphouet-Boigny, Groupe Scolaire Soba and Groupes Scolaires Notre Dame de l'Assomption, Nanguin, Résidentiel 3;
- Collège Moderne and Lycée Houphouet-Boigny, Groupe Scolaire Nanguin;
- Groupe Scolaire Jean Delafosse and Groupes Scolaires Nanguin, Résidentiel 3;
- Groupe Scolaire Haoussabougou and Groupe Scolaire Nanguin (Figure 3).

These average loads do not comply with the Tanzanian standard (TZS 585: 2011) which recommends a value of 10^2 CFU/mL. But they comply with the Luxembourg standard (10^3 CFU/mL, F-054 Rev05) and the French one ($<10^5$ CFU/mL, NF V08-059). The presence of these germs would be a cross-contamination of the bissap juice linked to the manufacturing process (addition of water, sugar, cooling, and packaging). This presence could also be explained by the germination, after the juice cooling, of thermoresistant spores of certain microorganisms. This aerobic mesophilic flora would be acidophilic (optimum pH for growth between 0.1 and 5.4) [16]; given the low pH of the bissap juice samples analysed.

In addition, the average yeast and mould loads varied between $1.21\times10^2 \pm 0.22$ CFU/mL and $2.91\times10^2 \pm 0.91$ CFU/mL. Statistical analyses show a significant difference ($p < 0.05$) between the samples from Groupe Scolaire Résidentiel 3 and Groupes Scolaires Haoussabougou, Jean Delafosse, Nanguin, Soba, Collège Moderne and Lycée Houphouet-Boigny (Figure 4).

Table 1. Physicochemical Characteristics of the Bissap Juice Sold in the Various Schools in Korhogo

Schools	pH	Titratable acidity (% citric acid)	Brix degree
Collège Moderne	2.66 ± 0.17^{a}	0.17 ± 0.04^{a}	16.11 ± 4.14^{bcd}
Lycée Moderne	2.68 ± 0.18^{a}	0.16 ± 0.06^{a}	14.25 ± 4.56^{abc}
Lycée Houphouet-Boigny	2.79 ± 0.58^{a}	0.17 ± 0.06^{a}	16.51 ± 3.86^{bcd}
Groupe Scolaire Haoussabougou	2.76 ± 0.04^{a}	0.19 ± 0.01^{ab}	18.95 ± 2.47^{cd}
Groupe Scolaire Jean Delafosse	2.79 ± 0.01^{a}	0.17 ± 0.03^{ab}	10.6 ± 1.27^{d}
Groupe Scolaire Notre Dame de l'Assomption	2.62 ± 0.06^{a}	0.26 ± 0.01^{b}	10.6 ± 0.42^{ab}
Groupe Scolaire Nanguin	2.52 ± 0.09^{a}	0.18 ± 0.06^{ab}	14.55 ± 4.11^{abcd}
Groupe Scolaire Résidentiel 3	2.56 ± 0.06^{a}	0.24 ± 0.03^{ab}	10.15 ± 0.35^{a}
Groupe Scolaire Soba	2.59 ± 0.1^{a}	0.16 ± 0.06^{ab}	10.4 ± 1^{a}
Mean ± standard deviation	2.68 ± 0.31	0.17 ± 0.06	15.09 ± 4.32

Averages followed by the same lowercase letters (a, b, c, d) are not statistically different at the 5% level

Table 2. Average Loads of Microorganisms in the Bissap Juice Samples Sold in the Various Schools of Korhogo

Schools	Total coliforms (UFC/mL)	*E. coli* (UFC/mL)	*S. aureus* (UFC/mL)
Collège Moderne	< 1	< 1	< 1
Lycée Moderne	< 1	< 1	< 1
Lycée Houphouet-Boigny	< 1	< 1	< 1
Groupe Scolaire Haoussabougou	< 1	< 1	< 1
Groupe Scolaire Jean Delafosse	< 1	< 1	< 1
Groupe Scolaire Notre Dame de l'Assomption	< 1	< 1	< 1
Groupe Scolaire Nanguin	< 1	< 1	< 1
Groupe Scolaire Résidentiel 3	< 1	< 1	< 1
Groupe Scolaire Soba	< 1	< 1	< 1

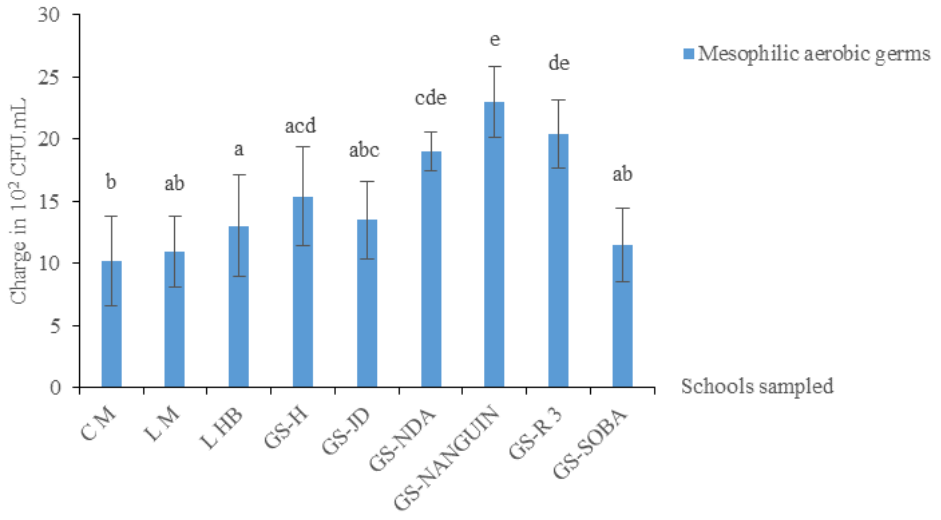

Figure 3. Average Loads of Mesophilic Aerobic Flora in Bissap Juice According to the Different Schools in Korhogo. CM=Collège Moderne; LM=Lycée Moderne; LHB=Lycée Houphouet-Boigny; GS = Groupe Scolaire; GS-Haoussabougou; GS-Jean Delafosse; GS-Notre Dame de l'Assomption; GS-Résidentiel 3. Means followed by the same lowercase letters (a, b, c, d, e) are not statistically different at the 5% level

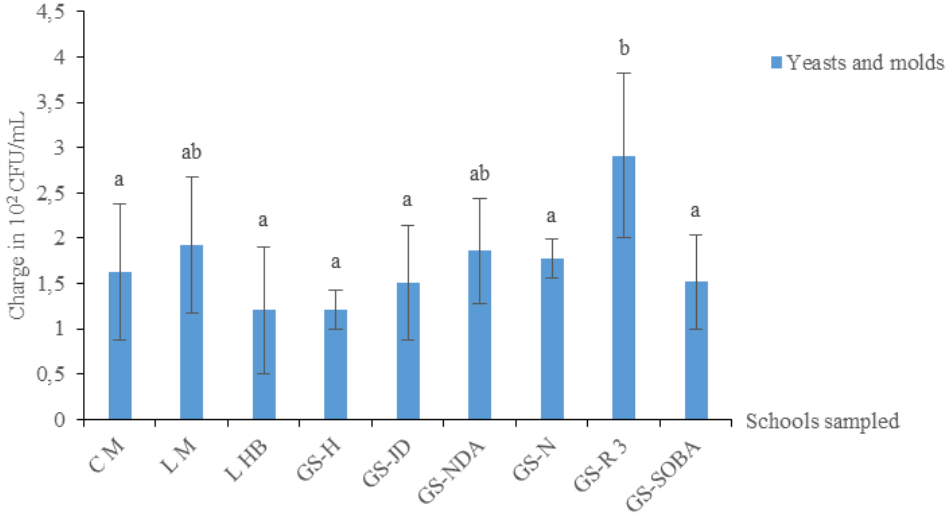

Figure 4. Average Load of Fungal Flora in Bissap Juice Acording to the Various Schools in Korhogo. CM=Collège Moderne; LM=Lycée Moderne; LHB=Lycée Houphouet Boigny; GS=Groupe Scolaire; GS Haoussabougou, GS Jean Delafosse, GS Notre Dame de l'Assomption; GS Nanguin; GS Résidentiel 3. Mean Values Followed by the Same Lowercase letters (a,b) are not statistically different at the 5% level

This study reveals that these average fungal flora values are higher than those prescribed by the Tanzanian standard (TZS 585: 2011) which recommends a value of 10 CFU/mL. Also, these values are lower than those obtained by [17] in Cameroon in the Foléré (*Hibiscus sabdariffa*) juice. The fungal flora in bissap juice is thought to come from the calyxes of *H. sabdariffa*. Indeed, these calyxes are harvested from the plants (which are often infected by fungi [18]), then dried and sold in the markets. The storage conditions (temperature, humidity) could favour the development of fungal flora [18-20]. The environmental framework of production (open air cooling), the water and the utensils used could also be the source of contamination of bissap juice by yeasts and moulds. Moreover, the yeasts and moulds proliferation could be favoured by the acidic pH of the bissap juice, as most of these microorganisms are acidophilic [21,22]. The sugar added to the juice, in the manufacturing process, constitutes a substrate which would help the proliferation of fungal flora [19,21].

4. Conclusions

This study shows that the bissap juice sold in schools in the city of Korhogo is very acidic. This low pH prevents the proliferation of pathogenic microorganisms and protects the health of consumers. However, this acidity promotes the growth of yeasts and moulds which constitute a danger to the consumer health. The use of potable water and the observance of good hygiene and production practices would help to reduce the fungal load of bissap juice. Isolation and identification of yeasts and moulds, as well as their various possible pathogenic metabolites, should be carried out with a view to the risk of illness to the consumer.

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