

# Evaluation of the biochemical, physicochemical and microbiological quality of soft drinks sold in some elementary school of Williamsville (Abidjan, Côte d'Ivoire)

## ABSTRACT

The sale of soft drinks in and around primary schools is recurrent in Côte d'Ivoire. The objective of this work is to evaluate the rate of some biochemical and physicochemical parameters and the health risks related to the consumption of soft drinks. The biochemical and physicochemical parameters were determined according to the ~~standard methods using~~ SOXHLET technique, that of Kjeldhal and also that described by Dubois et al ~~in~~ (1956). Potentially ~~pathogeniques~~ pathogenic and pathogenic germs were enumerated and researched according to reference methods described by AFNOR. The moisture content ~~and sugar content~~ of the different beverages ranged from  $3.9 \pm 0.05$  to  $3.2 \pm 0.05$  ~~for moisture content, and the sugar content~~ from  $26.06 \pm 2.05$  to  $5.96 \pm 1.60$  ~~for sugar content~~. For the acidity, the higher value ~~was~~  $0.93 \pm 0.15$  (Bissap juice) and the lowest ~~was~~  $0.64 \pm 0.02$  (Tamarind juice). For proteins, the high value ~~was~~  $44.15 \pm 0.11$  (Milk Mint Juice) and the low value ~~was~~  $38.15 \pm 0.11$  (Ginger Juice). As for dry matter, the highest value ~~was~~ found in ginger juice ( $44.76 \pm 0.02$ ) and the lowest in bissap juice ( $40.96 \pm 0.01$ ). Soft drinks sold in Williamsville schools contain such potentially pathogenic species as *Staphylococcus aureus* and *Bacillus cereus* with a wide range of loadings in the drinks, ~~except for Escherichia coli was not identified~~. No *Salmonella* was observed in the different soft drinks analyzed. The presence of potentially pathogenic germs could pose a danger to school children.

**Keywords:** soft drinks, Primary Schools, juice, pathogenic germs, ~~power supply~~, children and adolescents

## 1. INTRODUCTION

Diets and lifestyles are changing rapidly, and urbanization has accelerated over the past decade. This evolution has caused changes in eating habits, a decrease in physical activity and a corresponding increase in chronic diseases and obesity risks related to poor nutrition [1]. While the developed countries are confronted with the problems of obesity and diseases of all sorts related to poor nutrition, the developing countries are now facing those of infectious and chronic degenerative diseases, increasingly associated with the nutritional transition. This coexistence of infectious and chronic diseases would be associated with the level of economic development of countries, low food quality and poor living conditions. Many developing countries around the world have undergone a nutritional transition ([2]; [3]) ~~urbanization and globalization~~ and ~~urbanization~~. This change has negative consequences even if access to food is increased and diversified, the problem of inappropriate food and chronic diseases is increasing. School age is an active growth phase of childhood [4], representing a dynamic period of physical growth as well as mental development. A healthy diet helps to improve or maintain good health and plays a crucial role in preventing the development of chronic diseases. Several individual and collective determinants [5] influence healthy eating in children and adolescents. Among these collective determinants, the school environment contributes to the establishment of eating habits because ~~they children~~ spend more time there. The latter is known to have a strong influence on their eating behavior and physical activity. In addition, elementary school are increasingly witnessing the emergence of food provided outside the family, especially fruit juices. This situation is particularly noticeable in and around schools. Good school feeding contributes to increased school enrollment, increased school attendance and improved nutritional status of the child population. However, this issue is now a major concern for both parents and school officials. The objective of this work is to evaluate the rate of some biochemical, physicochemical parameters and health risks related to its consumption of soft drinks.

## 2. MATERIAL AND METHODS

### 2.1. Material

#### 2.1.1. Description and selection of study sites

The schools were randomly selected from among the most **unhealthy** schools in the Williamsville suburbs. Thus, five elementary school were the subject of this study. They are (EEP Konaté, EEP LABAT A-B, EPP the Wonders, Saint School Group Kizito and School Group Jean Porquet).

Comment [AMN1]: Unhealthy schools seems to me a very harsh term, even if I understand its deep meaning

#### 2.1.2. Sampling

Altogether **50 samples** were collected from the different elementary school for this work. Ten samples were collected per school. The samples consisted of Tamarind juice, Milk Mint juice, Baobab juice, Bissap juice and Ginger juice.

Comment [AMN2]: Very small number of samples. The relevance and statistical significance of the results is questionable. 2 samples/juice / school?

### 2.2 Methods

#### 2.2.1. Determination of biochemical parameters

The fat of soft drinks **wais** extracted by the **A.O.A.C** method (**A.O.A.C**, [6]) using SOXHLET. Crude protein **wais** ~~determined~~ ~~deduced~~ from the determination of total nitrogen according to the Kjeldhal Method (B.I.P.E.A, [7]). The determination of total sugars was done according to the method described by Dubois *et al.* [8]. As for reducing sugars, they were determined according to the method described by Bernfeld [9].

#### 2.2.2. Determination of physico-chemical parameters

Moisture content, pH and ash content, ~~and~~ titratable acidity **and the Brix degree** were determined according to the method described by (A.O.A.C, [10]. **Brix degree was determined according to the method described by A.O.A.C, [10].**

#### 2.2.3. Microbiological analysis of samples

A 25 mL sample of soft drink **wais** collected under aseptic conditions and added to 225 mL of sterile buffered peptone water. The resulting solution **wais** stirred for 5 minutes and allowed to stand for thirty minutes. About 1 ml of the obtained suspension **wais** taken around the flame and transferred into a test tube containing 9 ml of sterile distilled water. Successive dilutions **wear** made until the desired ones **wear** obtained (NF EN ISO 6887-1, [11]).

- Listing of potentially pathogenic germs

A 0.1 mL of each decimal dilution concerned **wais** placed in a Petri dish containing 20 mL of ready agar. The 0.1 mL **wais** spread on the surface of the agar. Plates **wear** incubated at 45°C for 24 h for *E. coli* enumeration on *E. coli* Rapid 2 medium (NF/ISO 16649-2, [12]). **Coli Rapid 2 medium (NF/ISO 16649-2, [12])**, at 30°C for 24 to 48 h for the enumeration of *Bacillus* on Mossel medium (NF EN ISO 7932, [13]; NF EN ISO 21871, [14]) and at 37°C for 24 to 48 h for the enumeration of *Staphylococcus aureus* on Rapid Staph medium supplemented with egg yolk with potassium tellurite (NF EN ISO 6888/1, [15]).

- Search for *Salmonella*

For the **research of *S.salmonella***, the V08-52 standard provides 4 steps. These are pre-enrichment in EPT medium, enrichment in Vassiliadis Rappaport medium, isolation on Hektoen medium and identification from Leminor or API 20 E galleries.

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## 3. RESULTS AND DISCUSSION

### 3. 1. Results

#### 3.1.1. Monitoring of physicochemical parameters

The parameters analyzed **wear** all variable from one drink to another. Thus, the moisture and sugar content of different beverages also **varies** from one beverage to another. These ranged from 3.9±0.05 to 3.2±0.05 for moisture content, from 26.06±2.05 to 5.96±1.60 for sugar content. For acidity, the highest value **wais** 0.93± 0.15 (Bissap juice) and the lowest

wais 0.64± 0.02 (Tamarind juice). As for pH, the lowest value wais found in ginger juice (1.83±0.11) and the highest wais found in Bissap juice (3.93±0.43) (Table 1).

Table 1: Physicochemical parameters of soft drinks

	Non-alcoholic beverages				
Physicochemical parameters	Tamarind juice	Mint juice with milk	Baobab juice	Bissap juice	Ginger juice
Humidity level	3,4±0,03	3,2±0,05	3,7±0,03	3,9±0,05	3,2±0,03
Titrateable acidity (ml)	0,64± 0,02	0,73± 0,15	0,87± 0,14	0,93± 0,15	0,77± 0,14
pH	3,59±0,02	3,82±0,49	3,40±0,12	3,93±0,43	1,83±0,11
Ash content	3,20±2,4	2,19± 2,19	2,50±2,4	3,22± 2,19	3,2 ±0,1
Brix degree	5,96±1,60	9,00±2,00	10,90±0,36	26,06±2,05	25,26±0,64

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### 3.1.2. Monitoring of biochemical parameters

The biochemical composition of the different soft drink samples is presented in Table 2. The results show that the total sugar content, reducing sugar content, fat content and protein content of soft drinks are variable. In terms of protein, the highest value wais 44.15 ± 0.11 (Milk Mint Juice) and the lowest wais 38.15 ± 0.11 (Ginger Juice). As for dry matter, the highest value wais found in ginger juice (44.76 ±0.02) and the lowest in bissap juice (40.96 ±0.01).

Table 2: Biochemical parameters of soft drinks

	Non-alcoholic beverages				
Biochemical parameters	Tamarind juice	Mint juice with milk	Baobab juice	Bissap juice	Ginger juice
Protein	41,07 ± 0,47	44,15 ± 0,11	34,15 ± 0,11	42,15 ± 0,11	38,15 ± 0,11
Total sugars	5,77± 0,14	6,83± 0,15	0,77± 0,14	0,83± 0,15	0,77± 0,14
Reducing sugars	5,06±0,10	4,32 ± 0,08	4,06±0,10	5,32 ± 0,08	5,06±0,10
Fats	42,76 ±0,02	39,96 ±0,01	43,76 ±0,02	40,96 ±0,01	44,76 ±0,02
Dry matter	90,45±4,76	92,32±4,37	94,85±4,76	95,32±4,37	94,65±4,76

### 3.1.3. Monitoring of potentially pathogenic germs

The soft drinks sold at the various Williamsville schools contained potentially pathogenic bacterial species such as *Staphylococcus aureus* and *Bacillus* with widely varying loads in the drinks. with the exception of *Escherichia coli* was not present in any of the soft drinks tested (Table 3).

Table 3: Microbial load of potentially pathogenic germs in soft drinks

	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
Tamarind juice	3,1.10 <sup>3</sup> ± 144,6	5,9.10 <sup>3</sup> ± 964,4	0 ± 0
Mint juice with milk	8,6.10 <sup>3</sup> ± 122,3	4,5.10 <sup>3</sup> ± 482,4	0 ± 0
Baobab juice	5.10 <sup>3</sup> ± 192,7	3,5.10 <sup>3</sup> ± 421,2	0 ± 0
Bissap juice	2,5.10 <sup>3</sup> ± 330,4	1,2.10 <sup>3</sup> ± 628,2	0 ± 0

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Ginger juice	1,6.10 <sup>3</sup> ± 122,4	1,2.10 <sup>3</sup> ± 204,2	0 ± 0
<b>Microbiological criteria</b>	<b>10<sup>3</sup> CFU/g</b>	<b>10<sup>3</sup> CFU/g</b>	<b>10 CFU/g</b>

### 3.1.4. Monitoring of strict pathogens: *Salmonella*

Non-alcoholic beverages sold at the various Williamsville elementary schools did not contain pathogens, specifically the genus *Salmonella* as shown in Table 4 below.

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Table 4: Search for *Salmonella* genus in non-alcoholic beverages analyzed

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Sample sites	Denominations	Presence of <i>Salmonella</i>
EEP Konaté	Ginger Juice	-
	Tamarind Juice	-
	Baobab Juice	-
	Mint Milk Juice	-
	Bissap Juice	-
EEP LABAT A-B	Ginger Juice	-
	Tamarind Juice	-
	Baobab Juice	-
	Mint Milk Juice	-
	Bissap Juice	-
EPP the Wonders	Ginger Juice	-
	Tamarind Juice	-
	Baobab Juice	-
	Mint Milk Juice	-
	Bissap Juice	-
Saint Kizito School Group	Ginger Juice	-
	Tamarind Juice	-
	Baobab Juice	-
	Mint Milk Juice	-
	Bissap Juice	-
Jean Porquet School Group	Ginger Juice	-
	Tamarind Juice	-
	Baobab Juice	-
	Mint Milk Juice	-
	Bissap Juice	-

### 3.2. Discussion

Comment [AMN3]: Maybe an analysis of these parameters per school might be interesting

The physicochemical analysis carried out on the acidity of soft drinks showed very variable and very acidic results. However, the acidity of ginger juice was 1.83±0.11, higher than the other drinks. This acidity is thought to be due to the release of numerous organic acids such as succinic, oxalic, tartaric, and malic acids contained in the rhizome into water (Shimoda *et al.* [16]; Colleen *et al.* [17]). Nevertheless, the pH was satisfactory according to CODEX-STAN 243-2003, which recommends that the pH of fruit juices and soft drinks should be below 4.5. Also, the average Brix level of ginger and bissap juice was very high. The Brix degree gives information on the sugar content in the dry matter. These high values of Brix degree could be explained by the fact that during the manufacture of the drink, the producers would add the quantities of sugar at their convenience. For many producers, the sweeter the drink, the better it is appreciated by consumers. But over-consumption of these highly sweetened juices could have adverse health effects and lead to cardiovascular disease, tooth decay, obesity (Yang *et al.* [18]), and especially diabetes (Basu *et al.* [19]). Microorganisms could therefore grow easily in the said drink despite the relatively low pH. Indeed, at pH close to 4, germs are not eliminated but their growth is slowed down.

The analysis revealed a high water content in soft drinks. This water content leads to the hydrolysis of sugars and consequently to an increase in the content of reducing sugars and total sugars during sale. This increase in sugar content would make the substrate available for the development of microorganisms. This development of microorganisms is perfectly in agreement with the work of Omoruyi *et al.* [20] which showed that, the more a food contains sugars the more quickly it will degrade.

Regarding proteins, a low temperature favors the increase of their levels in soft drinks. This assertion contradicts those of Bimbinet *et al.*, [21] who demonstrated that proteins risk denaturation each time the temperature of the wet product approaches or exceeds the maximum threshold of 80°C. The total ash content of soft drinks was observed to vary between 2.19± 2.19 and 3.22± 2.19. This total ash content could probably be due to the stability of the total ash during storage when the soft drinks are sold which gives them this stability character (Bimbinet *et al.* [21]).

The different analyses performed on the soft drink samples revealed the absence of *Salmonella* in the samples. This is due to the fact that the water used to make the beverages *was* water that *was* not contaminated with *Salmonella*. These results are consistent with those obtained by (Oulaï *et al.* [22]) who also showed the absence of these germs in some foods sold in the streets.

For the potentially pathogenic species, the different non-alcoholic beverages analyzed *we* are contaminated by *Staphylococcus aureus* and *Bacillus cereus*, ~~but not by and an absence of *E. coli*~~. These results are in conformity with the norm (10<sup>2</sup> CFU/g). Our results are similar to those of (Berghofer *et al.* [23]; Le Loir & Gautier, [24]) who have shown in recent studies that the presence of *Staphylococcus aureus* could also be due to human contamination of foodstuffs, given that this bacterium is commensal to the skin and mucous membranes of humans. This would indicate non-compliance with good hygienic practices and inefficiency of the product in these different markets (Berghofer *et al.* [23]).

The results of the microbiological analysis of *Escherichia coli* revealed an absence of these germs in all the non-alcoholic beverages, these results respect the microbiological criteria (10 UFCCFU/g). The absence of *Escherichia coli* in all samples attests ~~to~~ the absence of contamination of fecal origin. The results of this study are consistent with those performed by Degnon *et al.* [25].

#### 4. CONCLUSION

This study was conducted to assess the health risk associated with the consumption of soft drinks sold in some elementary school in *W*illiamsville, a suburb of Abidjan. The lack of good hygiene practices in the places of sale and manufacture of the juice of these drinks would predispose to all forms of contamination. Poor preparation, use of water for drinks, sieves, use of hands for mixing are all subject to microbial contamination. Microbiological analyses showed that soft drinks sold in elementary schools were highly contaminated with potentially pathogenic germs. It should be noted that the presence of pathogenic species such as coagulase-positive *Staphylococcus aureus* and *Bacillus cereus* in soft drinks sold in elementary school would represent a danger to school children.

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Comment [AMN5]: An additional phrase pointing out to biochemical & physicochemical findings in the juices should be added, for a better illustration of the title of the study.

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