Assessment of the Sanitary Quality of Fruit Juices Sold in Some Public Schools in the Agblangandan District in the Southern Part of Benin

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Received: July 14, 2022 Accepted: August 15, 2022 Online Published: September 7, 2022

doi:10.5539/ijb.v14n2p1 URL: https://doi.org/10.5539/ijb.v14n2p1

Abstract

Fruit juices are one of the favorite drinks for children these days. As a result, its production must be organized in such a way as to ensure quality and guarantee the safety of consumers in general and schoolchildren in particular. The objective of this study is to analyze the health quality of bissap, lemon and pineapple juices sold in some public schools in the district of Agblangandan. For this, we collected from the vendors, 12 pineapple juice, 14 bissap juice and 8 lemon juice during the morning recess. Then, the microbiological and physico-chemical analyzes were carried out on the juices. The microbiological analyzes made it possible to identify the presence of total mesophilic flora ($1.09.10^4$ CFU/ml), total Coliforms ($2.78.10^3$ CFU/ml), *Escherichia coli* ($4.44.10^2$ CFU/ml), Enterococci (86.1 CFU/ml) and Clostridium (13.2 CFU/ml) in bissap juices. These same germs have been identified in lemon and pineapple juices with varying loads. As for the physico-chemical parameters, the results revealed a high rate of turbidity, conductivity, temperature and pH of the juices. Factors such as failure to master hygiene rules when bagging juices, non-compliance with hygiene rules during handling and storage and then inappropriate hygiene practices are origin of the contamination of these juices. It is therefore urgent to prioritize the hygiene and health of school children through awareness-raising, education and regular and rigorous health control of the juices sold by sworn agents.

Keywords: Juice, sanitary quality, hygiene, school, Agblangandan, Benin

1. Introduction

Health and academic success are closely linked; schools cannot fulfill their primary educational mission if students are not in good health (De Ressources, 2010). School is one of the main social contexts in which lifestyles are developed, and it is also, for many other reasons, the ideal setting for encouraging healthy eating (Diarra, 2012). Schools can also be reservoirs of disease, especially in the case of a poorly organized informal food supply (Diarra, 2012). Poor hygiene and sanitation conditions in schools, resulting from lack of access to drinking water, inadequate sanitation and the prevalence of inappropriate hygiene behaviors and practices, affect not only the the child's state of health but also on their ability to learn (UNICEF, 2013). The food offer in Beninese schools in general and those of Agblangandan in particular is very wide ranging from hot dishes to different juices. However, according to Dansoko et *al*., 2016, fruit juices by their origins naturally contain many nutritional qualities (potassium, vitamins C and B9 and carotenoids, etc.) but also some microorganisms. In order to contribute to improving the health status of schoolchildren in Benin, the research entitled "Study of the health quality of fruit juices sold in certain public schools in the district of Agblangandan" was undertaken.

2. Materials and Methods

2.1 Setting

The collection of juices was done in the district of Agblangandan in seven (07) public primary schools (Davatin school complex, Agbalilamè school complex, Agbotta school complex, Sèkandji 1 school complex, Sèkandji 2 school complex, Sèkandji 3 school complex, Yémalin school complex) and microbiological analyzes in the Water Quality and Metrology Department of SONEB's water analysis laboratory.

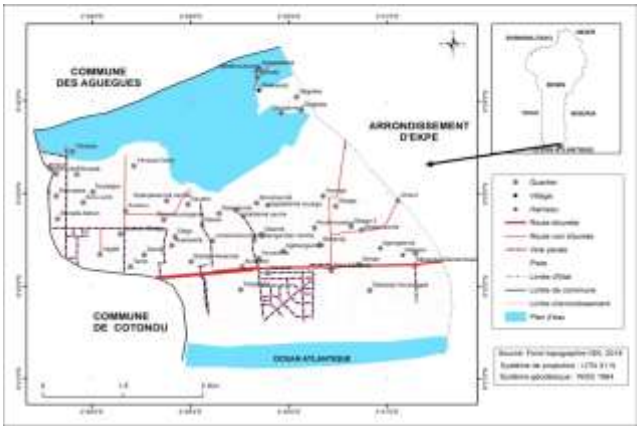

Figure 1. Borough location map: Elegbede, 2019

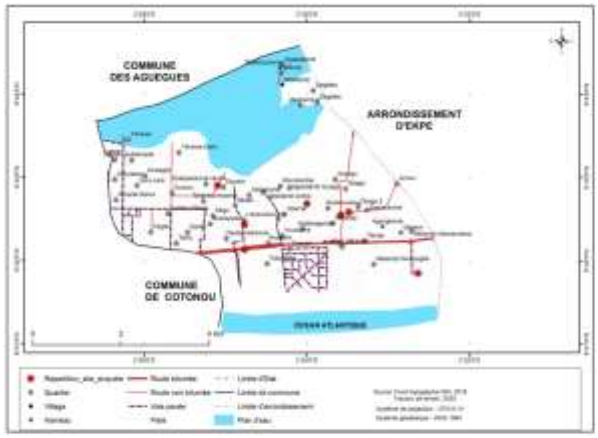

Figure 2. Map of the situation of the distribution of the schools surveyed: Elegbede, 2019

2.2 Sampling

The juices were collected in seven (07) public primary schools in the district of Agblangandan. A total of 34 juice samples were taken. In each school, three types of juice were sampled, namely pineapple juice, bissap juice and lemon juice.

On each site, a single passage is carried out and 100 ml of juice on sale in these public primary schools were sampled, collected in sterile bottles and stored at + 4 °C. The analyzes were carried out as soon as they arrived at the laboratory.

2.3 Enumeration of Contamination Bacteria

Two inoculation methods were applied for the microbiological analyses. The incorporation method was used for the enumeration of the Total Mesophilic Aerobic Flora and the membrane filtration method was used for the enumeration of total Coliforms, Staphylococci, enterococci, spores of sulphite-reducing anaerobes.

2.4 Enumeration of Total Mesophilic Aerobic Flora

The research methods used were chosen from the AFNOR reference techniques (1996 and 2004). The sample of the previously homogenized juice underwent a series of decimal dilutions (10^{-1} to 10^{-6}) in the buffered peptone water (IPA ®) medium according to the NF EN ISO 6887 Method. The count of the total mesophilic aerobic flora was carried out on standard agar for PCA counting (Plate Count Agar, IPA ®) by inoculation in depth and incubation of the cultures at 30 °C for 72 hours (standard NF-08-051).

2.5 Enumeration of Total Coliforms, and Escherichia Coli

After filtration of 100 ml of juice, the membrane was inoculated on the pre-poured TTC agar in the Petri dishes while avoiding the creation of air bubbles. The boxes were turned over and incubated at a temperature of 37 °C. for 48 hours ± 2 hours. The characteristic colonies were blue-greenish in color were counted. The search for oxidase and indole was done on these colonies to identify *E. coli* (Mackenzie test).

2.6 Enumeration of Enterococci

Enterococci were counted on Slanetz-Bartley agar. The Petri dishes containing the inoculated membranes after filtration were incubated at 37 °C. for 48 hours ± 2 hours. A volume of water of 100ml was filtered through the membrane. The characteristic colonies had a purple-red color. The confirmation test consists of transferring the membrane onto a Bile

Esculin Azide (BEA) agar plate followed by incubation for approximately 2 hours. The degradation of esculin made it possible to confirm the presence of enterococci by giving a black color to the colonies.

2.7 Sulfite-Reducing Anaerobic (asr) Spore Count

The counting of ASR spores was carried out by the membrane filtration technique following a volume of 20ml of water previously heated to 80 ℃. After filtration, the membrane was turned over and placed in the Petri dish. A volume of 20 ml of TSN agar was poured onto the membrane thus inverted in order to create the anaerobe. Incubation was done at 37 ℃ for 20H ±2H. The characteristic colonies had a black color.

2.8 Physicochemical Analysis

The physico-chemical analyzes consisted of measuring the pH, conductivity, temperature and turbidity of the juices. The pH of the samples is measured with an electronic pH meter (HI 96107 instruments from Hanna), previously calibrated with buffer solutions of pH 4.1 and 7.1. The temperature of these samples was measured with a digital thermometer (VWR EU 620-2132 NA 98000-162) digital (-50 to 200 ℃)

The electrical conductivity is measured in situ using a HANNA HI 99300 conductivity meter. The probe of the conductivity meter previously calibrated in the laboratory is immersed in 100 ml of the sample of juice to be analyzed by making a slight rotation movement until the value stabilizes. Displayed on the screen.

The turbidity is measured in situ with a HANNA HI 93703C turbidimeter. After a blank test with distilled water, 10 ml of the sample to be analyzed is introduced into the tank of the device, carefully cleaned and this tank replaced in the device for reading. The turbidity corresponding to the sample is displayed on the screen after pressing the read key.

2.9 Statistical Analysis

The data relating to the microbiological and physicochemical parameters were encoded using the Excel 2013 spreadsheet. The Graph Pad Prism 8 software was used to determine the significant differences at the 5% threshold ($p < 0.05$) between the calculated means of the physicochemical tests and microbiological analyses. R software version 3.6.1 (R Development Core Team, 2017) was used to perform a PCA (Principal Component Analysis), in order to summarize the information contained in the data matrix and identify the best juice.

3. Results

3.1 Microbiological Quality of Juices

The total number of heterotrophic bacteria was counted in the different samples of bissap, pineapple and lemon juice in the 7 schools surveyed.

3.2 Bacterial Loads of Bissap Juice Samples

Table I presents the bacterial load of the bissap juice samples. The results showed that the bacterial load varied from 5.10^2 to $3.42.10^4$ (CFU/ml) for the Total Mesophilic Aerobic Flora. While for total coliforms, the bacterial load varied from 52.5 to $8.85.10^3$ (UFC/ml). Bissap number five juice was more contaminated with FAMT ($3.42.10^4$ UFC/ml), Total Coliforms ($8.85.10^3$ UFC/ml) and *Escherichia coli* ($1.05.10^3$ UFC/ml) while bissap juice No. 7(B7) was more contaminated with Enterococci ($2.47.10^2$ CFU/ml) and Clostridium (52 CFU/ml). The analysis of variance showed that there is no significant difference between the presences of bacteria in the bissap juices ($p > 0.05$). However, there is a highly significant difference between the bacterial load isolated from the bissap juice samples ($P<0.0096$). The results are summarized in Table I. It is found that all the bacterial loads of the bissap samples are higher than the WHO standard, so they are unfit for consumption.

Table 1. Bacterial loads of bissap juice samples

Bissap juice samples	***Bacterial load (CFU/ml)***				
	Total Mesophilic Aerobic Flora	***Total coliforms***	***Escherichia coli***	***Enterococci***	***Clostridium***
B1	$4.33.10^2$	$1.54.10^2$	37	5	0
B2	$1.75.10^2$	52.5	12	4.5	0
B3	5.10^2	$1.63.10^2$	43.5	12	0.5
B4	$4.5.10^2$	$1.13.10^2$	27	4	0
B5	$3.42.10^4$	$8.85.10^3$	$1.05.10^3$	$2.25.10^2$	12
B6	$2.21.10^4$	$5.51.10^3$	$9.46.10^2$	$1.05.10^2$	28
B7	$1.85.10^4$	$4.62.10^3$	$9.92.10^2$	$2.47.10^2$	52
Mean	$1.09.10^4$	$2.78.10^3$	$4.44.10^2$	86.1	13.2
WHO guidelines	**-**	**-**	**0**	**0**	**0**

3.3 Bacterial Loads of Pineapple Juice Samples

Table II below shows the bacterial load of pineapple juice samples from the 7 schools surveyed. The analysis of variance showed that there is no significant difference between the presences of bacteria in pineapple juice (p> 0.05). There is no significant difference between bacterial load isolated from pineapple juice samples (p>0.05). Pineapple juice No. 4 (A4) was more contaminated with FAMT ($24.4.10^3$ CFU/ml), total coliforms ($6.18.10^3$ CFU/ml), Escherichia coli ($1.54.10^3$ CFU/ml), Enterococci ($3.82.10^2$ CFU/ml) and Clostridium (43 CFU/ml) while Pineapple N°3(A3) juice was more contaminated by *Escherichia coli* (1.04.102 CFU/ml) and Enterococci (36 CFU/ml). The results are summarized in Table II. It is found that all the bacterial loads of the pineapple juice samples are higher than the WHO standard, so they are unfit for consumption.

Table 2. Bacterial loads of pineapple juice samples

Pineapple Juice	***Bacterial load (CFU/ml)***				
	Total Mesophilic Aerobic Flora	***Total coliforms***	***Escherichia coli***	***Enterococci***	***Clostridium***
A1	$2.30.10^3$	$1.22.10^3$	61	19	0
A3	$2.88.10^3$	$7.69.10^2$	$1.04.10^2$	36	1.5
A4	$24.4.10^3$	$6.18.10^3$	$1.54.10^3$	$3.82.10^2$	43
A5	$0.950.10^3$	$1.13.10^2$	47	8	1
A6	$1.40.10^3$	$1.50.10^2$	56	14	4
A7	$1.10.10^3$	$2.72.10^2$	69	18	1
Moyenne	$5.50.10^3$	$1.45.10^3$	$3.12.10^2$	79.5	8.42
WHO guidelines	**-**	**-**	**0**	**0**	**0**

3.4 Bacterial Loads of Lemon Juice Samples

Table III shows the bacterial loads of the different samples of lemon juice. This table shows that regardless of the type of bacteria involved, the bacterial loads varied from one sample to another. The analysis of variance showed that there is no significant difference between the presences of bacteria in lemon juice (p> 0.05). There is no significant difference between bacterial loads isolated from lemon juice samples (p>0.05). Lemon juice No. 3 (C3) was more contaminated with FAMT ($6.80.10^3$ CFU/ml), total coliforms ($1.69.10^3$ CFU/ml), *Escherichia coli* ($2.53.10^2$ CFU/ml), Enterococci ($1.17.10^2$ CFU/ml) and Clostridium (8.5 CFU/ml). The results are summarized in Table III. It is found that all the bacterial loads of the lemon juice samples are higher than the WHO standard, so they are unfit for consumption.

Table 3. Bacterial loads of lemon juice samples

Lemon juice	***Bacterial load (CFU/ml)***				
	Total Mesophilic Aerobic Flora	***Total coliforms***	***Escherichia coli***	***Enterococci***	***Clostridium***
C1	$1.15.10^3$	$2.86.10^2$	82.5	23.5	0.5
C2	$1.15.10^3$	$2.86.10^2$	82.5	23.5	0.5
C3	$6.80.10^3$	$1.69.10^3$	$2.53.10^2$	$1.17.10^2$	8.5
C4	1.10^2	28	9	1	0
Moyenne	$2.30.10^3$	$5.73.10^2$	$1.07.10^2$	41.3	2.38
WHO guidelines	**-**	**-**	**0**	**0**	**0**

3.5 Physicochemical Quality of Juices

The physico-chemical parameters of the juices from the 7 surveyed schools revealed that the pH values varied between 2.1 and 3.8 (table IV). The lemon juice samples were more acidic than the other juices tested. There is a highly significant difference (P <0.0001) between the pH of the different juices analyzed. Concerning the temperature (°C), the values varied between 23.3 and 24.2 °C. There is also a significant difference between the temperatures (P<0.0001) of the different juices analyzed. Regarding the Conductivity (µS/cm), the bissap juices have a higher conductivity than the other juices analyzed with a lower turbidity (Table IV). Pineapple juices have higher turbidity than other juices. There is a highly significant difference between the conductivity of the juices tested (P<0.0001), there is the same for the turbidity of the juices tested (P<0.0001).

Table 4. Physicochemical parameters of the different juices analyzed

Juice samples	*pH*	*Temperature (°C)*	*Conductivity (µS/cm)*	*Turbidity (NTU)*
A1	3.8	23.7	1559	1076.7
B 1	2.7	24.1	2336.3	331.3
C 1	2.8	23.7	1473.7	614.7
B 2	3.05	23.9	3030	321.5
C 2	2.3	23.5	1540	733
A 3	3.65	24.2	1667.5	1293.5
B 3	3	23.75	2262.5	345.5
C 3	2.1	24.35	1207.5	689
A 4	3.4	23.8	1445	1512
B 4	2.8	24.2	2170	312
C 4	2.8	23.7	1155	603
A 5	3.8	24.1	1412	1303
B 5	2.8	23.7	3012	412
A 6	3.2	23.5	1645	1212
B 6	3.1	23.6	3175	288
A 7	3.6	23.3	1507	1200
B 7	2.7	23.6	2111	277
WHO guidelines	-	-	-	5

3.6 Principal Component Analysis

A principal component analysis was carried out with, on the one hand, the matrix relating to pineapple, lemon and bissap juices from the different schools, taking into account the physico-chemical parameters as variables. The following table presents the eigenvalues and the percentages of inertia associated with each axis. With regard to this table, we note that the first two axes concentrate more than 75% of the information relating to the data matrix, which is sufficient to guarantee a good interpretation.

Table 5. Matrix of eigenvalues

	Axis 1	**Axis 2**	**Axis 3**	**Axis 4**	**Axis 5**
Variance	4.69	2.129	1.079	0.651	0.301
Variance in %	52.116	23.66	11.987	7.231	3.349
Cumulative variance	52.116	75.776	87.763	94.993	98.343

3.7 Sign of the Correlations of the Variables on the Axes

We note that the microbiological characteristics are associated with axis 1 and that the physico-chemical characteristics are associated with axis 2. Axis 1 is therefore the axis of microbiological characteristics and axis 2 represents the physico-chemical characteristics.

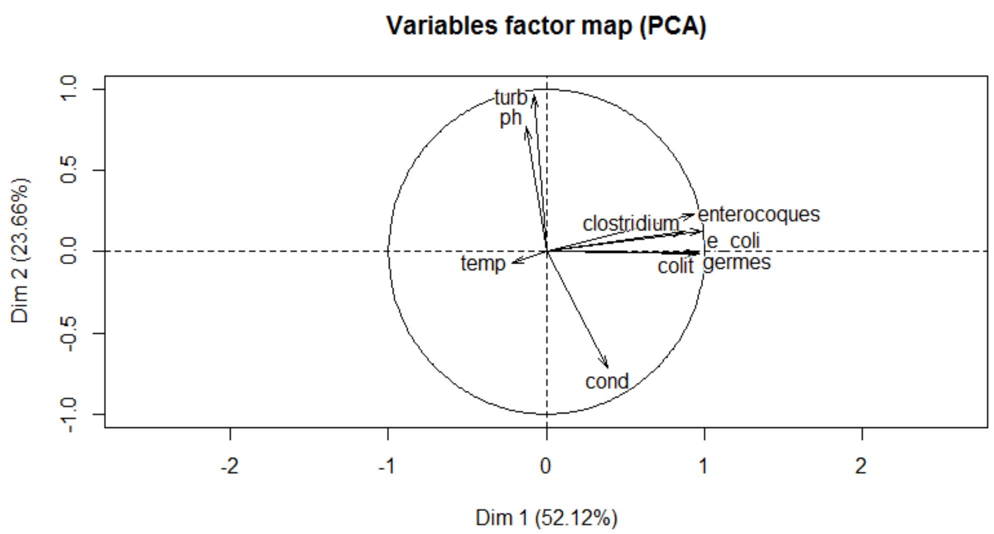

Figure 3. Representation of chemical characteristics in the system of axes 1 and 2

On axis 1, the correlation coefficients of the first three components indicate that an increase in Total Mesophilic Aerobic Flora (germ) is associated with an increase in *E.coli*, Enterococci, Clostridium and total Coliforms. On axis 2, an increase in turbidity is associated with a high pH, but on the other hand these two characteristics are associated with a decrease in conductivity.

Table 6. Classification of the physico-chemical characteristics taken into account according to the components

Signs	**Axis** 1	**Axis** 2	**Axis** 3
Negative		Conductivit é	
Positive	*E.coli*, Coliforms Totals, Flora Aerobic Total, enterococci, Mesophile Clostridium	Turbidity, pH	Temperature

3.8 Projection of the Juices on the Axes

This figure 4 shows that the juices A4, B7, B5 and incidentally B6 are located on the positive side of axis 1 and therefore have high values in Total Mesophilic Aerobic Flora, enterococci, Clostridium, total Coliforms and *E. coli*. The pineapple juice from school 4, and the bissap juice from schools 7, 6 and 5 cannot therefore be considered to be good quality juices if we refer to WHO standards (2000). Juices on the positive side of axis 2 (A5, A7, A3, A1) have higher pH and turbidity than other juices, but have lower conductivity. Juices located on the negative side of the axis (B2, B1) have higher conductivity but have lower turbidity and pH values.

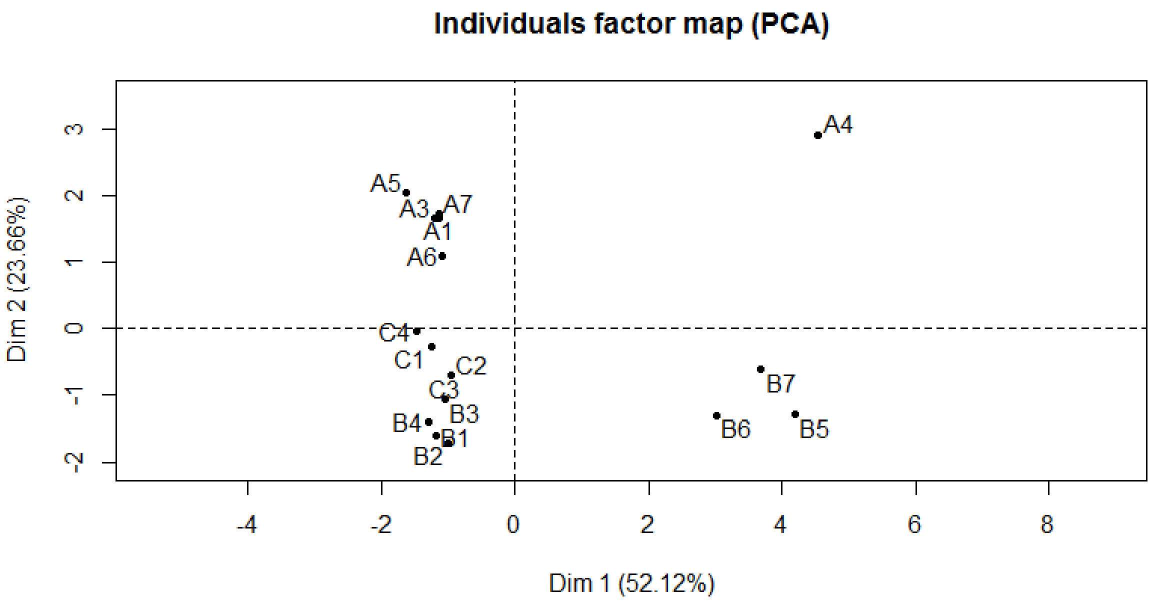

Figure 4. Representation of bissap (B), lemon (C) and pineapple (A) juices on the different axes

4. Discussion

Despite the benefits offered by fruit juices, concerns about their safety and quality have been raised, particularly due to their manufacturing and sales process.

The physico-chemical characteristics (temperature, pH, conductivity, turbidity) were determined. In our study, the results obtained show that the temperature does not vary greatly from one juice to another, with a minimum of 23.3 ℃ (pineapple juice from school 7) and a maximum 24.35 ℃ (lemon juice from school 3). Our results have higher values than those of Belghiti et al. (2013), who found a minimum of 20 ℃ and a maximum of 21.04 ℃. The pH values are 3.2 to 3.8 for pineapple juice; from 2.7 to 3.05 for bissap juices and from 2.1 to 2.8 for lemon juices; these values are similar to those of Ocaña de Jesús et al. (2021), which had a minimum of 3.4 and a maximum of 3.7. This parameter is reported as one of the necessary characteristics for colonization by certain microbial groups such as fungi and yeasts, which are more acid tolerant, while certain bacteria can also contaminate the product due to their acid tolerance. Acid and sugar concentration typical of the product (Aneja et *al*., 2014; Kaczmarek et *al*., 2019).

The conductivity values recorded in the juices studied vary from 1155 µs/cm to 3175 µs/cm, the minimum is recorded at the level of lemon juice from school 4 and the maximum at the level of bissap juice from school 6 This increase is the sign of an input of dissolved, mineral or polluting substances. The juices studied have a turbidity that varies from 277 NTU (bissap juice from school 7) to 1512 NTU (pineapple juice from school 4). These values denote the very cloudy aspect of the juices sold in these schools. The PCA of these physico-chemical parameters revealed that an increase in turbidity is associated with a high pH, on the other hand these two parameters are associated with a decrease in conductivity. These results are comparable of Soma Massieke et al (2017) and Baba-Moussa et al (2006).

Concerning the microbiological quality of the juices, the results obtained indicate a significant difference between the bacterial load of the juices analyzed. The Total Mesophilic Aerobic Flora is significantly present in the juice samples analyzed. This value indicates a fairly high level of contamination. This may be due to the juice production or sales environment. With the composition of the juices, it would constitute a good broth for the development of the Total Mesophilic Aerobic Flora. These results are comparable with those of Ndiaye et al. (2015) and Bayoi et al. (2014) who concluded that bissap juices are mostly of non-compliant microbiological quality due to their high microbial load.

The results of the analysis of pineapple juice sold in schools reveal that learners run a risk by consuming this type of juice because the proportions of germs found are likely to cause poisoning or diarrheal diseases. This result is in contradiction with that of Tchango (1996) who revealed that pineapple juices are of good microbiological quality. The analysis of the lemon juice samples shows that the results are unsatisfactory. This contamination is due to a strong presence of common germs which can be explained by poor conservation of lemon before its transformation into juice.

The presence of *Escherichia coli* strains and total coliforms in the juice samples collected indicate recent faecal contamination and the potential presence of ecologically similar pathogens. In addition, coliforms of the genus Escherichia contaminate the juice directly, or multiply following poor cleaning of juice manufacturing or selling utensils (Hamiroune et *al*., 2014). This indicator is a measure that calls for the adoption of good hygiene practices by women during the juice manufacturing process, for the prevention of the occurrence and spread of faecal contamination during the sale of juice. Our results are similar to those found by Ahoyo et al. (2010) who revealed that the microbiological quality of food and beverages sold on the Abomey-Calavi campus analyzed are unsatisfactory with a contamination rate of 74% by bacteria indicative of general spoilage. These results are comparable of Soma Massieke et al (2017) and Baba-Moussa et al (2006).

They are also comparable to those made by Barro et al. (2001) in Burkina Faso who found high numbers of microbes in all foods and beverages compared to French regulatory figures. Factors that were found to be significant causes of bacterial contamination in juices included juice type, juice pH, sales sites, and storage containers (Simforian et *al*., 2015). Poonam (2013) reported that high pH combined with high ambient temperatures (>28 C) appeared to promote bacterial growth and reduce the shelf life of juices. Low pH of juices could inhibit microbial contamination of juices significantly. This is highlighted in the report by Zahra (2010) who studied the antagonistic activity of lemon and lime juices against certain bacteria and concluded that the low pH of both juices had inhibitory effects on the bacteria. This result is in disagreement with our study, where lemon juice C3 with a pH of 2.1 which has a higher bacterial load (6.80.103 CFU/ml) than other juices of the same school (A3: pH 3 .65 and bacterial load 2.88.103 CFU/ml and B3: pH 3.0 and bacterial load 5.102 CFU/ml). This could be because some bacteria can also continue to grow at low pH due to their acid tolerance.

5. Conclusion

Fruit juices are fat-free beverages that are nutrient-dense and rich in natural vitamins, minerals and poly-nutrients that provide health and therapy benefits. The presence of different bacteria in fruit juices is of great concern. This work has shown that fruit juices sold in primary schools contain bacteria at high levels. But the isolation of coliforms and *E. coli* in some of the samples in this study calls for serious concern and a rapid response from relevant stakeholders. It is therefore preferable to monitor the proper management of raw materials, production and sales to prevent or minimize microbial contamination of fruit juices.

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