



Evaluation of Physicochemical and Microbiological Standards for Quality Packaged Drinking Water in Plastic Bags Produced by Three Companies in Korhogo in Northern Ivory Coast

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Drinking water packaged in sachets is popular with the population of Korhogo in northern of Ivory Coast. The objective of this study is to evaluate the physicochemical and microbiological parameters of this water produced by three companies. Three packets of 40 sachets of water each were taken from each company and ten sachets of water were mixed to constitute the sample from each company. Physicochemical and microbiological analyzes were carried out according to ISO. Physicochemical parameters of water investigated were as follow: temperature ($25.30 \pm 0.40 - 25.53 \pm 0.25$), pH ($7.37 \pm 0.06 - 7.67 \pm 0.23$), bicarbonate ($41 \pm 0.50 - 48.53 \pm 0.85$ mg/L), chloride ($4.34 \pm 0.03 - 8 \pm 0.43$ mg/L), calcium ($5.60 \pm 0.40 - 8.23 \pm 0.15$ mg/L), magnesium ($4.08 \pm 0.51 - 6.62 \pm 0.05$ mg/L) and nitrates ($0.59 \pm 0.02 - 0.83 \pm 0.03$ mg/L). The results revealed that the physicochemical parameters of the water analyzed complied with WHO regulations. Concerning the microbiological parameters, results of the study are as follows: GAMs (22 °C) (6 – 113 CFU/mL), GAMs (37 °C) (6 – 89 CFU/mL), total coliforms (0 – 1100 CFU/100 mL), fecal coliforms (0 – 1100 CFU/mL), ASR (0 CFU/100 mL), *S. aureus* (190 – 310 CFU/100 mL) and streptococci fecal (0 – 3100 CFU/100 mL). These waters do not comply with the regulations and sample B is more loaded with microorganisms followed by sample C and finally A. However, no salmonella was identified in the samples analyzed. Consumption of this water exposes populations to health risks. It would be desirable to raise awareness among companies about hygiene conditions and employee training in order to reduce these risks.

Keywords: Bagged water; parameters; physicochemical; microbiological.

1. INTRODUCTION

Water is an essential element for human life and that of other living beings [1]. Water is importance of preponderant like other essential elements in human life, both for our diet and for our physiological needs. It performs several roles in the human body. Indeed, it allows the digestion of food and the transport of nutritional and hormonal substances [2].

Thus, water, the source of life, can become a danger for the environment and for users if it is not of acceptable quality [3] Poor water quality can be induced by anthropogenic activities or by natural phenomena [4].

Water quality is continuously deteriorating due to biological contamination by human waste, chemical pollutants from industries and agricultural inputs [5]. Drinking contaminated water containing pathogenic microorganisms is the cause of many diseases. WHO, through the International Drinking Water and Sanitation Decade, recommends good water quality for all by 2025 [6].

In Africa, lack of drinking water constitutes a major public health problem. Every year, 1.8 million people die from diseases caused by drinking poor quality water [7]. To circumvent the need for drinking water, water production units in plastic bags have multiplied in West Africa [8]. These sachet water production units are small

businesses that produce drinking water from river or borehole water. Thus, faced with this phenomenon, several studies were carried out by Ble et al. [9] to assess the quality and drinkability of these waters packaged in sachets. Water packaged in this material also presents bacteriological and physicochemical risks linked to its consumption.

In Ivory Coast, access to drinking water still remains a major issue for populations, unprotected wells and water sources increase the risk of water-borne diseases [10]. Despite the efforts made by state authorities, drinking water services record a national coverage rate of 48% in rural areas and 70% in urban areas. Efforts must be made to increase this rate [11]. Infections linked to the consumption of drinking water have been reported, and the problem of the health quality of drinking water sold in sachets on the markets has become one of the government's priorities [12].

In Korhogo in northern of Ivory Coast, production of drinking water plays an important role in the daily life of several people as a source of drinks, income and jobs. Drinking waters are most often produced at home or in inappropriate settings and these productions are semi-industrial or artisanal in nature. These waters are also exposed to multiple manipulations, hygiene of which is often questionable due to a lack of concept of quality control. To all this must be

added the use of production equipment often exposed to the ground and in permanent contact with flies and all types of ambient germs. Based on these findings, objective of this study is to evaluate the physicochemical and microbiological parameters of drinking water in sachets sold in the city of Korhogo with a view to making recommendations.

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Biological material

Biological material used in this study consists of water produced by three (03) sachet drinking water production units in the city of Korhogo named A, B and C.

2.1.2 Chemical and culture medium

Chemicals used consist of ethylene diamine tetraacetic acid, Erichrome Black T, silver nitrate and sodium hydroxide (NaOH). These reagents are analytical grades.

Culture media used consist of Plate Count Agar, Bair-Parker, Lactose bile medium with crystal violet and neutral red, Salmonella-Shigella Agar, Bile Esculin Azide and Tryptone sulphite neomycin.

2.2 Methods

2.2.1 Sample preparation

9 packets containing 40 sachets of water each were taken from the production units at a rate of 3 packets per unit. Various packages were sent to the laboratory. 10 sachets of water per packet of each unit were taken randomly then the sachets were rinsed thoroughly under a jet of water and immersed in 10% bleach water for 30 min. After 30 min, the sachets were rinsed with plenty of water. Bags were opened with a platinum handle heated with a Bunsen burner. Contents were mixed to constitute the sample of each production unit. The sample was split into 2 batches. The first batch was used for microbiological analyzes and the second for physicochemical analyses.

2.2.2 Determination of physicochemical parameters

2.2.2.1 Temperature and pH

Temperature and pH were determined according to the ISO [13] method. Temperature was determined by dipping the probe of a

thermometer into 50 mL of sample contained in a beaker. After stabilization the value is read. As for pH, it consisted of immersing the previously calibrated electrode in 50 mL of sample. After stabilization the value is read.

2.2.2.2 Calcium and magnesium

Calcium and magnesium were measured according to the ISO [14] method. Calcium was evaluated by successively adding to 5 mL of sample to be analyzed 45 mL of distilled water, 3 mL of 2 M NaOH, and 0.2 g of carboxylic calcon and 1 g of NaCl. Mixture obtained is titrated with a 0.01 M EDTA solution until it turns purple. Regarding magnesium, 45 mL of distilled water, 4 mL of pH 10 buffer and 4 drops of NET are added to 5 mL of sample. Solution obtained is titrated with EDTA at 0.01 M until it turns blue.

2.2.2.3 Chloride

Chloride dosage was carried out according to standard [15]. 100 ml of sample was placed in a capsule placed on a white background to which 1 ml of potassium chromate indicator was added. Mixture was titrated with a 0.5 M silver nitrate solution until a reddish color was obtained.

2.2.2.4 Bicarbonate

ISO [16] standard was used to determine alkalinity. To 20 ml of samples were added 3 drops of phenolphthalein and the mixture was titrated until the solution was colorless. To the colorless solution, 4 drops of helianthin are added and titrated again with 0.2 N sulfuric acid until brick red coloring appears.

2.2.2.5 Nitrates

Nitrates are measured according to standard [17] To 10 ml of sample were added 3 drops of 30% NaOH and 1 ml of sodium salicylate. The mixture is heated in an oven between 75 and 88°C until total evaporation. After 10 min of cooling to room temperature, 15 ml of distilled water and 15 ml of double sodium and potassium tartrate are added and the reading is taken at 420 nm against the blank. The nitrite content was determined using a 0.1 mg/mL sodium nitrate solution.

2.2.3 Determination of microbiological parameters

2.2.3.1 Research for Aerobic Mesophilic Germs (AMG)

AMG were evaluated using the ISO [18] method. 15 mL of PCA (Plate Count Agar) medium are

poured into a petri dish containing 1 mL of sample. After slow shaking of the petri dish stuck to the bench and solidification, the dishes are incubated at 22 °C for 72 hours for GAMS (Aerobic Mesophilic Germs) developing at water temperature and at 37 °C for 24 hours for those developing at body temperature. After incubation the plates containing 30 to 300 colonies were retained for enumeration using the following formula:

$$N = \sum C/V (n1 + 0.1n2)*d$$

N: number of colony forming units (CFU) per ml of product;

n1: number of boxes retained at the first dilution;

V: volume of inoculum applied to each plate, in milliliters

$\sum C$: sum of the colonies counted on all the plates retained as countable from two successive dilutions

n2: number of boxes retained at the second dilution

d: dilution rate corresponding to the first dilution retained

2.2.3.2 Research for total Coliforms and fecal Coliforms

Determination of total coliforms. and fecal coliforms were carried out according to standard [19]. 100 ml of sample are filtered through a membrane with a porosity of 0.45 µm. Membrane is then placed on the Crystal Violet Neutral Red Bile and Lactose (VRBL) or (CVRBL) agar medium. Boxes are incubated at 37°C for 48 hours for total Coliforms. and at 44°C for 24 hours for fecal Coliforms.

2.2.3.3 Research for fecal Streptococci

ISO [18] method was used for the enumeration of fecal Streptococci. 100 ml of sample are filtered through a membrane with a porosity of 0.45 µm. Membrane is placed on selective Bile-esculin-azide agar containing sodium azide intended to inhibit the growth of Gram-negative bacteria. Plates are incubated at 44°C for 24 to 48 hours. After incubation, the red, brown or pink colonies were counted.

2.2.3.4 Research for *Staphylococcus aureus*

Enumeration of *S. aureus* was carried out according to the ISO [20] method. 100 ml of

sample are filtered through a membrane with a porosity of 0.45 µm. Membrane is placed on Baird Parker agar. Colony counting was carried out after 24 hours of incubation at 35°C.

2.2.3.5 Research for Anaerobic Sulphite-Reducing germs (ASR)

Anaerobic Sulphite-Reducing Germs were counted according to the ISO [21] method. 100 ml of sample are filtered through a membrane with a porosity of 0.45 µm then the filtrate is heated to 75°C for 15 minutes and finally cooled in an iced water bath. Cooled filtrate is filtered through a membrane with a porosity of 0.2 µm and the membrane is placed on the TSN agar face down, avoiding any incorporation of air. Colonies are counted after incubation at 37°C for 48 hours.

2.2.3.6 Research for Salmonella

Salmonella enumeration was carried out according to the ISO [22] method. It was carried out in 4 steps: pre-enrichment, enrichment, isolation and identification. Pre-enrichment consisted of mixing 1 mL of sample in 9 mL of buffered peptone water. Mixture is incubated at 37°C for 20 hours. As for enrichment, 0.1 mL of the pre-enriched medium is incorporated into 10 mL of Vassiliadis Rappaport broth and the whole is incubated at 42 °C for 18 to 24 hours. Isolation was done by streaking SS (Salmonelle Shigella) agar using Vassiliadis' Rappaport broth. Plates were incubated at 37°C for 18 to 24 hours. Colonies were subjected to biochemical tests before any confirmation of salmonella

2.3 Statistical Analysis

STATISTICA 17.1 software was used to process the physicochemical parameter data obtained. Means plus or minus standard deviation of the different repetitions were obtained using one-way ANOVA. ANOVA was followed by Tukey's HSD test to classify the means. Statistical significance threshold considered was 5% ($p < 0.05$).

3. RESULTS AND DISCUSSION

Table 1 presents the physicochemical parameters of the different sachet water samples analyzed. Temperature of the different water samples are statically identical with an average of 25.40 °C. These values are in agreement with those of Kanouté [23] who recorded values between 25.10 and 26.70 °C in drinking water in

Mopti and Sévaré in Mali. These temperature values comply with the standard [1]. Analysis results show statically identical pH of the different samples. These values vary from 7.37 to 7.67. Our values are different from those of Kordowou et al. [24] in water samples in sachets (4.95 - 7.25) sold in Togo. But similar to those of Dieng et al. [25] in sachet waters (7.12 – 7.36) sold in Dakar in Senegal. Despite these slightly basic pH values, they are between 6.5 and 8.5 as recommended by WHO [1].

Bicarbonate contents of the 3 samples are statically different. These values oscillate between 41 and 48.53 mg/L with the highest value for C and the lowest for A. As for B, it records an intermediate value (45.57 mg/L). Our values are approximately equal to those of Kouame et al. [26] in the drilling waters of Daloa in Ivory Coast with an average of 41.10 mg/L. These values are well below the maximum standard (250 mg/L) recommended by WHO [1]. These low values would be due to the treatments that this water would have undergone before conditioning, which would indicate a low alkalinity of this water. This low alkalinity could be advantageous for the consumer because it would facilitate digestion while maintaining the natural acidity of the stomach. Chloride content of sample A differs statically from those of samples B and C. These contents are between 4.34 and 8 ±0.43 mg/L. these values are included in those of Kordowou et al. [24,27] in drinking water sold respectively in Togo (2 to 20 mg/L) and Burkina Faso (0.44 to 166 mg/L).

These values are lower than the maximum standard (≤ 200 mg/L) recommended by the WHO. Low chloride content of our samples may not affect the taste of the water analyzed. Regarding calcium and magnesium, the contents are statically different. These contents vary respectively from 5.60±0.40 to 8.23±0.15 mg/L and from 4.08±0.51 to 6.62±0.05 mg/L for

calcium and magnesium. These values are higher than those recorded by Kordowou et al. [24] in sachet drinking water sold in Togo (Calcium: 0.4 to 6.40 mg/L; Magnesium: 0.24 to 3.40 mg/L). These low calcium and magnesium contents could be due to the use of softeners which considerably eliminate calcium and magnesium [24].

These levels comply with WHO standards which recommend maximum values of 100 mg/L and 50 mg/L respectively for calcium and magnesium. Concerning nitrates, the contents are statically different. They vary from 0.59±0.02 to 0.83±0.03 mg/L. these values are largely low compared to those of Moussa [27 and 24] who recorded values oscillating between 2.20 and 37.8 mg/L and 45 and 159.5 mg/L respectively in drinking water in sachet sold in Burkina Faso and Togo. These levels are lower than the standard accepted by the WHO which is estimated at 50 mg/L.

Table 2 presents the results of the microbiological parameters of the different sachet drinking water samples analyzed. AMG counts vary from 6 to 113 CFU/mL and 6 to 89 CFU/mL at 22°C and 37°C, respectively. Samples from company A (6 CFU/mL) are less contaminated compared to those from companies B (49 CFU/mL) and C (89 CFU/mL). These values are much lower than those of Kordowou [24] and [25] respectively in sachet drinking water sold in Togo (3300 CFU/mL <) and in Senegal (200 CFU/mL <) . Values recorded are lower than the standard which is estimated at 100 CFU/mL according to the WHO. This low load of AMG could be due to the storage duration which would influence the proliferation of microorganisms and could not affect the organoleptic characteristics. Regarding total Coliforms, fecal Coliforms, ASR and fecal Streptococci, they were not counted in sample A while in sample C only total C. (410 CFU/100 mL) were counted.

Table 1. Physicochemical parameters of drinking water in sachets sold in Korhogo

	A	B	C
Temperature (°C)	25.53±0.25a	25.37±0.06a	25.30±0.40a
pH	7.37±0.06a	7.60±0.00a	7.67±0.23a
Bicarbonate (mg/L)	41.00±0.50c	45.57±1.06b	48.53±0.85a
Chloride (mg/L)	8.00±0.43a	4.34±0.03b	4.47±0.13b
Calcium (mg/L)	8.23±0.15a	5.60±0.40b	7.63±0.45a
Magnésium (mg/L)	6.62±0.05a	4.08±0.51b	4.67±0.12b
Nitrates (mg/L)	0.76±0.01b	0.59±0.02c	0.83±0.03a

Data are represented as Means values of tests ± SD (n = 3). Means in the line with no common letter differ significantly (p<0.05)

Table 2. Microbiological parameters of drinking water in sachets sold in Korhogo

	A	B	C
GAMs (22°C) CFU/mL	6	113	13
GAMs (37°C) CFU/mL	6	49	89
Total Coliforms CFU/100 mL	0	1100	410
Fecal Coliforms CFU/100 mL	0	1100	0
ASR CFU/100 mL	0	0	0
<i>S. aureus</i> CFU/100 mL	210	310	190
Streptococci. fecal CFU/100 mL	0	3100	0
Salmonella	Absence	Absence	Absence

By contrast in sample B, with the exception of ASR, the enumeration revealed values of 1100 CFU/100 MI for total Coliforms and fecal Coliforms and 3100 CFU/100 MI for fecal Streptococci. Presence of these bacteria in drinking water was revealed by the work of Ble [9] and [27] in drilling water. Massive presence of fecal Coliforms, total coliforms and fecal Streptococci in sample B indicates fecal pollution. This could be explained by the non-compliance with good hygiene practices by staff before, during and after packaging and the lack of UV lamp on the packaging rolls during bagging [24]. Results of analyzes revealed the presence of *S. aureus* in the different samples. This presence is greater in sample B (310 CFU/100 MI) followed by A (210 CFU/100 MI) and finally by C (190 CFU/100 MI). Presence of *S. aureus* indicates manual handling without any protection. The presence of coliforms, streptococci and staphylococci in these drinking waters could cause health damage leading to diarrhea, nausea, weakening of the immune system and could lead to serious cases such as pulmonary, skin and nervous infections [28,29]. One solution would be to boil this water before use. Regarding Salmonella, the results show their absence in all samples. Therefore, the consumption of this water could not transmit salmonellosis.

4. CONCLUSION

This study revealed that the drinking water in sachets produced by three companies based in Korhogo in northern of Ivory Coast is of acceptable physicochemical quality but of unacceptable microbiological quality in accordance with WHO recommendations. Presence of microorganisms at an abnormal level indicates a significant risk to the health of the consumer. As a result, public authorities must require health certificates from these companies, maintenance of premises and training of staff on hygiene rules and good practices.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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