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Analysis of the Physicochemical and Microbiological Quality of Unfermented Palm Sap Reserved for Infant Feeding

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Authors' contributions

This work was carried out in collaboration among all authors. Author VBRDR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ADNMC, CB and KKC managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study was conducted to evaluate the physicochemical and microbiological parameters that could contribute to the depreciation of the quality of unfermented palm sap used for infant feeding.
Study Design: A survey was conducted in 20 villages and camps to determine if the practice was still current. Also, unfermented sap was produced from 5 palms by resource persons to control the quality during the days of exploitation.
Place and Duration of Study: The survey was carried out from March 1st to 30th in villages and camps in Central and Central-Western Cote d'Ivoire. The production of the sap took place during one week in the village of Akpessekro.
Methodology: 30 people, without distinction randomly crossed were questioned on the food consumed at the age of 0 to 6 months. Also, in the unfermented sap produced acidity, sugar content, loads of GAM, thermotolerant coliforms, enterococci, yeasts and molds were determined and multiple correlations were established between all parameters.

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Results: In the villages and camps, palm sap is less and less used as infant food. During the first two days of palm farming, the acidity (6 < pH < 6.11) approximates that of breast milk or milk substitutes. However, after these two days, the acidity becomes high and the sugar content too low for infant feeding. In the unfermented sap, the loads of coliform fecal contamination germs are higher than the required standards. Also, the large load of lactic acid bacteria (≥1.5E+04 ufc/ml) and yeasts (1.4E+04 ufc/ml) present in the sap contribute to a depreciation of its quality over the days and when the sap is left to rest.
Conclusion: The quality of unfermented palm sap as infant food is not guaranteed. This is one of the reasons why this practice is in decline nowadays.

Keywords: Côte d'Ivoire; feeding; infant; microbiological parameters; unfermented palm sap.

1. INTRODUCTION

Special attention should be paid to the feeding of the newborn. Also, WHO and UNICEF recommendations advocate exclusive breast milk feeding for the infant for the first 6 months [1]. Breast milk should be the first natural food for infants [2]. Nevertheless, alternatives to breastfeeding have always been sought [3,4]. According to surveys worldwide, only 38% of infants 0-6 months are exclusively breastfed [3]. The substitute milk usually of animal origin undergoes transformation processes that tend to make it a rational and hygienic food for infant feeding [5]. However, before Pasteur's discoveries, the very high infant mortality was partly due to artificial feeding. The reasons were on the one hand poor hygiene of the bottles and on the other hand, poor preservation of milk [6,7]. Today, artificial feeding presents fewer risks if certain hygiene rules are followed.

This type of substitute food is not available in the rural landlocked areas of sub-Saharan Africa where access is difficult. In landlocked rural areas of Côte d'Ivoire, when a mother dies during or after childbirth or when a mother does not have sufficient milk secretion, unfermented palm sap is used to feed the newborn or infant. It is a non-alcoholic drink obtained from the unfermented sap of the oil palm (*Elaeis guineensis* L.). whose production process differs from that of fermented and alcoholic palm wine intended for the adult population [8]. Palm wine is produced from the incision of unhatched male inflorescences or the stem just below the apex, of felled palms. The palm sap is then collected in a container containing a palm wine-based ferment produced in a screen. This ferment has the role of accelerating the fermentation of the sap. As for the unfermented palm sap intended for the feeding of the newborn, the palm is prepared as previously mentioned, except that the sap is collected in a container that is carefully washed each time it is taken and closed to external contamination. In the villages and camps, especially in the forest area where livestock breeding is not very developed, this unfermented palm sap is produced exclusively for the consumption of the newborn. It thus contributes to saving the lives of newborns and infants who do not have access to breast milk or artificial milk substitutes. Thus, in Côte d'Ivoire, for the poorest rural populations of the Centre and Centre-West, unfermented palm sap plays the role of a substitute food for breast milk. This study was carried out with the objective of evaluating the physicochemical and microbiological parameters that could contribute to the depreciation of the quality of unfermented palm sap taken as a substitute food for the nutrition of newborns and infants.

2. MATERIALS AND METHODS

2.1 Evidence of use of Unfermented Palm Sap as a Substitute for Breast Milk

In order to determine the percentage of people in the population who were fed unfermented palm sap at the age of 0 to 6 months, a survey was conducted from March 1 to 30, 2021 in 20 villages and camps of 300 to 500 inhabitants in the Central and Central-Western zone of Côte d'Ivoire. 30 people, without distinction of sex or age, randomly crossed in the villages and representative of at least 5% of the population of each locality were questioned on the food consumed at the age of 0 to 6 months. Three possible answers were given: breastfeeding, artificial feeding or unfermented palm sap. Each person's answer was scored.

2.2 Production of Unfermented Palm Sap

2.2.1 Preparation of palm trees for the production of unfermented sap

Five mature palm (*Elaeis guineensis* L.) trees in the savanna-forest transition zone of Yamoussoukro district (village of Akpessekro)

were used for the production of unfermented sap. The Yamoussoukro district is located in the center of Côte d'Ivoire between 6°40' and 7° North latitude and between 5°10' and 5°20' West longitude, its vegetation is a mosaic of Guinean savanna and semi-deciduous dense rainforest in which oil palms are frequently found [9]. The exploitation of the palm trees was carried out by resource persons from the villages who were used to producing unfermented sap for the feeding of newborns. The palm trees were completely uprooted and left for five days to concentrate the sap in the heart of the palm. The crown of leaves was then pruned to the terminal bud. An incision was made transversely in the terminal cabbage about 30 cm from its base revealing the section of the main stem evolving towards the trunk, a surface that will be notched at each sap collection. A hole was then made in the crucible not far from the part evolving towards the trunk. A bamboo pipe was inserted and the other end was plunged into a clean container to collect the flowing sap. The container is closed to the ambient air.

2.2.2 Collection of samples and their transport to the laboratory

Samples collection was done at about 6:00 a.m. every day for five days. The closed containers were stored in a cooler at 4°C and transported within 30 minutes to the laboratory for analysis. After each collection, the previous container was replaced with another clean one. This precaution was taken to avoid contamination of the sap by fermentative microorganisms probably present in the previous container.

2.3 Sample Analysis Technique

Each sample underwent two series of microbiological and physicochemical analyses. The first series of analysis was done as soon as the sample arrived at the laboratory at t0 (fresh sap). Then the second series, 2 hours after storage of the sample closed and left to rest under ambient conditions (28 and 30 °C) at t0 + 2 hours (rested sap). The interval of 2 hours corresponds to the time necessary between the first and the second intake of the unfermented sap by the infant during the day.

2.3.1 Analysis of unfermented palm sap physicochemical properties

2.3.1.1 Determination of pH

Samples pH was determined using a pH meter (pH meter P604 consort, bio block, France). The calibration of the apparatus was carried out thanks to two buffer solutions with pH 7 to 4 and this calibration is made systematically before the measurements of the pH. The measurement is made by dipping the electrode in 20 ml of fresh oil palm sap. The reading is repeated three times and the value retained is the average of the readings taken [10].

2.3.1.2 Determination of titratable acidity

The titratable acidity was obtained by the method described by [11]. Ten ml of palm sap was pipetted into a 50 ml beaker. The titration was carried out with 0.01 N NaOH and in the presence of 2-3 drops of 1% phenophthalein with stirring until pH = 7.00. Neutrality is marked by turning to a pale pink coloration of the sap at the pale white screen. The volume of 0.01 N NaOH used was noted and the value retained is the average of three trials. The titratable acidity (mg Eq Citric acid/100 ml) was determined according to the following formula:

$$Titratable\ acidity\ rate = \frac{\text{Volume NaOH} * \text{Normality NaOH} * 0.07 * 100}{\text{Volume of the test}}$$

0.07: Conversion factor of titratable acidity in citric acid equivalent.

2.3.1.3 Determination of the total sugar content of the palm sap

The total sugar contents were determined by the method proposed by Dubois et al. [12]. One (1) ml of phenol and 5 ml of sulphuric acid (as a dry spray) were added to 1 ml of palm sap collected for each test. The whole was heated in a boiling water bath for 5 minutes. After removal, the tubes were placed in the dark for 30 minutes and the optical density was read at a wavelength of 490 nm with a spectrophotometer (WANELENGHT). The total sugar content was determined by reference to a calibration curve previously established using a 1 mg/ml glucose solution.

2.3.1.4 Determination of reducing sugars

The content of reducing sugars was obtained according to the method described by Bernfeld et al. [13]. The determination of reducing sugars was carried out in two trials comprising 0.3 ml and 0.6 ml of palm wine respectively. The volumes of the tests were then made up to 2 ml with distilled water, 1 ml of DNS is added to each tube. The tubes were placed in a boiling water

bath for 5 minutes. After cooling the tubes, 10 ml of distilled water is added to the contents. The optical density of each tube is read at 546 nm with a spectrophotometer (GENESYS 5). The quantity of reducing sugars is determined by reference to a calibration curve previously established with a glucose + fructose solution at 1 mg/ml.

2.3.2 Analysis of microbiological contamination of unfermented palm sap

The spoilage germs are the microorganisms capable of contributing to the deterioration of the physical and chemical quality of the unfermented sap. In this study, the load of aerobic mesophilic germs, lactic acid bacteria and fungi (yeasts and molds) were determined.

The presence of faecal contamination germs in a food beyond the standards testifies to a lack of hygiene during the production, conservation or transport of the food. In this study, the load of total coliforms and enterococci were determined.

2.3.2.1 Preparation of the stock solution and the dilution series

The collected unfermented palm sap was used as a stock solution. From this stock solution, dilutions were made: 10^{-1}, 10^{-2}, 10^{-3}; 10^{-4}, 10^{-5}. To perform the 10^{-1} dilution, 1 ml of the stock solution (100) was transferred with a sterile propipette into a sterile test tube containing 9 ml of sterile distilled water under a laminar flow hood, in the presence of a flame. Thus, the dilution ranges up to 10^{-5} was performed.

2.3.2.2 Mesophilic aerobic germ counting (MAG)

Mesophilic aerobic germs enumeration was performed according to the AFNOR NF V08-051, 1999 standard [14]. The medium used for the enumeration is PCA (plate count agar). The plating was done by incorporation into the agar mass. One (1) ml of decimal dilutions 10^{-3} and 10^{-4} was first placed in an empty petri dish. Then 15 ml of the medium previously melted and maintained in supercooling at 47°C was poured into the dish containing the inoculum. The mixture was homogenized by slow shaking to prevent the medium in the dish from touching the lid. The mixture was allowed to cool and solidify on the laminar flow hood. A second 5 ml layer of the same medium was poured over the agar in the dish. Three Petri dishes were inoculated per dilution. The seeded plates were then incubated at 30°C for 72 hours. After this incubation period, plates with colony counts between 30 and 300 were selected for calculation of the AMG load of the unfermented palm sap.

2.3.2.3 Enumeration of lactic acid bacteria (LAB)

The enumeration of lactic acid bacteria was performed according to the ISO 17792:2006 [15]. The medium used for the enumeration of lactic acid bacteria is MRS agar (Man Rogosa Sharp). The plating was done by incorporation into the agar mass. One (1) ml of decimal dilutions 10^{-2} and 10^{-3} was first placed in an empty petri dish. Then 15 ml of the medium previously melted and maintained in supercooling at 47°C was poured into the dish containing the inoculum. The mixture was homogenized by slow shaking to prevent the medium in the dish from touching the lid. The mixture was allowed to cool and solidify on the laminar flow hood. A second 5 ml layer of the same medium was poured over the agar in the dish. Three Petri dishes were plated per dilution. The seeded plates were incubated for 72 hours at 30°C in a 5% CO2 atmosphere. After this incubation period, the plates with a colony count between 30 and 300 were retained for the calculation of the lactic acid bacterial load of the unfermented palm sap. A few well isolated characteristic colonies (15) randomly selected from the plates retained for enumeration were used to verify under the optical microscope after differential Gram staining that they were Gram-positive, non-spore forming bacilli [16].

2.3.2.4 Enumeration of yeasts and molds

Enumeration of yeasts and molds was carried out according to the NF 'ISO 6611, 1996 standard [17]. The medium used for the research and enumeration of yeasts and molds is Sabouraud agar with chloramphenicol. The inoculation was done by spreading 0.1 ml of 10^{-4} and 10^{-5} dilutions of palm sap on the surface of the agar previously poured and cooled in petri dishes. The incubation of the plates is done at 25°C for 72 hours. The colonies of molds have a fluffy appearance and those of yeast appear whitish smooth bulging. After this incubation period, the colonies present in the petri dishes respecting these characteristics were counted. The plates with a number of colonies between 30 and 300 were retained for the calculation of the yeast and mold load of the unfermented palm sap [17].

2.3.2.5 Enumeration of enterococci

The enumeration of enterococci was performed according to ISO 7899/1 [18]. The medium used was BEA (Bile, Esculin and Sodium Azide) agar. The inoculation was done by spreading 0.1 ml of 10^{-1} and 10^{-2} dilutions of palm sap on the surface of the agar previously poured and solidified in petri dish under a laminar flow hood around the flame of a Bunsen burner. Thus, three Petri dishes were inoculated per dilution. Incubation of the plates was done at 37°C for 24 hours. On BEA, enterococci form small translucent colonies surrounded by a black halo. After this incubation period, the colonies present in the petri dishes respecting these characteristics were counted. Petri dishes with a number of colonies between 15 and 150 were retained for the calculation of the enterococcal load of the unfermented palm sap. A few well-isolated typical colonies (15) randomly selected from the boxes retained for enumeration were used to verify under the light microscope after differential Gram staining that they were Gram-positive, non-spore forming cocci.

2.3.2.6 Enumeration of thermotolerant coliform populations

Neutral red crystal violet lactose agar (VRBL) was used for the enumeration of thermotolerant coliforms. To perform the enumeration, a quantity (1 ml) of the stock solution was placed in each of three sterile Petri dishes and 15 ml of the agar (VRBL) was added, previously melted, cooled and kept in supercooling at 47°C. Then the mixture was homogenized by gentle agitation. The Petri dishes were left to cool on the bench until the agar solidified. Incubation was performed at 44°C for 24 hours. The characteristic colonies which are usually red-violet, surrounded by a red halo were considered as positive. Counting was done by counting the colonies present in the Petri dishes. And the calculation of the number of colonies forming units was done with the Petri dishes presenting a number of colonies between 15 and 150. Some well isolated characteristic colonies (15) randomly selected on the dishes retained for the enumeration made it possible to check with the optical microscope after differential Gram staining that they are Gram negative bacilli, not spore forming [19].

2.3.2.7 Calculation of the load of the microorganisms tested

For all the microorganisms tested, the colony-forming unit (CFU) load was calculated according to the following formula:

$$N\ (cfu/ml) = \frac{\sum C}{v(n1 + n2)d}$$

With: C: number of cfu (colony forming units) observed on all the selected and usable plates (plates from two successive dilutions); v: volume of the suspension spread on the surface of the media in ml; n1 : number of plates retained at the first dilution (the lowest); n2 : number of plates retained at the second (highest) dilution; d : lower dilution rate of the two successive dilutions retained [20].

Results are rounded to two significant digits after the decimal point and are expressed in cfu per ml.

2.3.3 Data analysis

In order to assess the evolution of physocochemical parameters and microbiological parametes in unfermented palm sap, data collected were subjected to analyses of variance (Generalized Linear Models) using Statistica 7.1. Means found to be significantly different at $p \leq 0.05$ were separated using Fisher's LSD (Least Significant Difference) test. Principal component analysis based on all parameters was performed to establish the interactions between physicochemical parameters and microbiological contamination of palm sap.

3. RESULTS AND DISCUSSION

3.1 Extent and Contemporaneity of the Practice

In all 20 villages, 600 people were interviewed and only 13 said they had been fed unfermented palm sap from 0 to 6 months of age (2.16%). These people were from 10 different villages. Also, this practice is less current because all of the people who have benefited from this food were born before the year 2000 (Table 1). The practice is therefore disappearing, even if it has not been totally abandoned. Modernization with the opening up of villages and camps would be one of the causes of the decline of this practice.

Table 1. Proportion of people who were fed palm sap at the age of 0 to 6 months in the villages and camps

Sub-prefectures	Camps, Villages	Food received at the age of 0 to 6 months			Years of birth
		Breastfeeding (%)	Formula feeding (%)	Palm sap (%)	
Zoukougleu	Belleville	93,34	3,33	3,33	1991
Bonon	Djahakro	93,33	-	6,67	1984/1980
Bouaflé	Benou	93,33	6,67	-	-
Soubré	Kobakro	100,00	-	-	-
Bonon	Yobouekro	86,67	10,00	3,33	1978
Bonon	Kouadiokro	96,67	3,33	-	1984
Yamoussoukro	Trakouadiokro	93,33	-	6,67	1970/1968
Brobo	Takassou	93,33	6,67	-	-
Brobo	Agbakro	96,67	-	3,33	1981/1975
Daloa	Affèliyaokro	93,33	3,33	3,33	1996/1994
Daloa	Zaibo	100,00	-	-	-
Gonaté	N'gbayakoffifro	96,67	3,33	-	-
Gonaté	Konankro	96,67	-	3,33	1999/1996
Daloa	Bolouguhé	96,67	-	3,33	1988
Daloa	Zebra	93,33	6,67	-	-
Bozi	Kouakougnanou	100,00	-	-	-
Daloa	Zintinkro	100,00	-	-	-
Daloa	Bowali	90,00	6,67	3,33	1993/1989
Bediala	Gnamanou	100,00	-	-	-
Yamoussoukro	Akpessekro	83,33	10	6,67	1976/1969

3.2 Evolution of Physicochemical Parameters of Palm Sap

The evolution of physicochemical parameters of freshly harvested unfermented palm sap (fresh sap) over successive days is shown in Table 2 and that of physicochemical parameters of sap rested two hours after harvesting (rested sap) is shown in Table 3. The pH of the fresh sap decreased from 6.11 to 4.95. The pH of the fresh sap decreased when the sap was rested for two hours. The pH of the rested sap decreased from 5.84 to 4.89. In contrast to the pH, the titratable acidity of both fresh and rested sap increased over the days. The titratable acidity of the fresh sap changed from 8.64 to 13.26 mg Eq Citric acid/100 ml while that of the rested sap increased from 9.45 to 13.90 mg Eq Citric acid/100 ml. When the sap was rested for two hours, the titratable acidity was higher than that of the fresh sap. The fresh sap is acidic. This acidity becomes more pronounced with each passing day. During the first two days, the acidity level could be suitable for feeding the newborn because the pH of breast milk oscillates between 6 and 7 [21]. However, after the first two days or when the sap is rested for two hours, the sap becomes more acidic and therefore less suitable for consumption by the newborn or infant. The gradual acidity of the sap could be caused by fermentation due to the activity of fermentative germs from the environment [22]. During these first two days of palm harvesting, these germs would be less present in the sap. Normally, palm sap has an approximately neutral pH [23,24]. However, in the following days, the sap residues that remain in the incision zone after harvesting would favor the proliferation of microorganisms, especially fermentative microorganisms. This inoculum, as well as the microorganisms carried by the different parts of the palm, would colonize the new sap in production that must pass through this incision zone. Thus, the sap would already be fermenting even before collection [8]. Also, when the sap is left to rest, these fermentative microorganisms would use the sugar it contains to carry out fermentation which further contributes to the acidification of the sap [25]. The increase in acidity indicates a production of organic acid at the expense of sugars in the palm sap as previously indicated by several works [25,26].

The sugar content of both fresh and rested sap decreased sharply over successive days. It fell from 28.6 to 3.69 g glucose equivalent/L for fresh sap and from 14.4 to 2.06 g glucose equivalent/L for rested sap. Regardless of the day considered, the reducing sugar content of the fresh sap was higher when the sap was rested for two hours. Also, during the first two days (D1 and D2), the reducing sugar content of the fresh sap decreased by half two hours after resting. The total sugar content of the fresh sap decreased steadily over the days from 199.8 to 110.67 g glucose eq/L. On the other hand, when the sap was rested, the total sugar content of the successive days was almost the same, especially for days D1, D3, D4, D5. The total sugar content on day 2 (85.22.06 g glucose equivalent/L) was lower than on the other days. The reducing sugar contents of fresh palm sap from the first two days of palm exploitation (28 g glucose equivalent/L) are somewhat lower than the reducing sugar contents of animal milk ranging from (80 to 100 g glucose equivalent / L) [27]. From the fourth day of operation the reducing sugar contents of fresh sap (5.3 to 3.9 g glucose equivalent/L) becomes very lower than that of breast milk. The sugar content of unfermented palm sap would not be suitable for the feeding of newborns and infants. Contrary to the acidity which increases with each day, the contents of reducing sugars and total sugars decrease from one day to another. These results are in line with those obtained by other authors working on fermented palm sap commonly called palm wine [22]. It was also noted that the decrease in total sugar levels is less abrupt than that of reducing sugars, whose levels drop sharply from the first days of palm exploitation. The decrease in sugar levels over the days and when the sap is rested would indicate that some of the sugars would have been fermented during the exploitation of palm sap extracted from the palms [28]. In addition, the rapid decrease in reducing sugars could be explained by a transformation of these sugars into alcohol and then into organic acids [8]. Indeed, the fermentative microorganisms contained in the sap residues that remain in the incision zone would ensure an increasingly rapid metabolization of sugars in general but an even faster degradation of reducing sugars. This is because reducing sugars have a simpler chemical structure and are more easily metabolized by microorganisms than sugars with complex structures [29]. From the evolution of acidity and sugar content, it could be concluded that from the third day of exploitation of the palms, the sap collected even each time with new clean containers would be already in fermentation and, the chemical characteristics of this sap would be more similar to those of palm wine.

3.3 Evolution of the Microbial Load of Palm Sap

The evolution over successive days of the microbial load of freshly harvested unfermented palm sap (fresh sap) is shown in Table 4 and that of the microbial load of sap rested two hours after harvesting (rested sap) is shown in Table 5. Both the fresh and rested sap enterococci load increased over the days. The enterococci load of fresh sap changed from 5.28E+02 to 7.52E+03 cfu/ml, that of rested sap changed from

Table 2. Evolution of physicochemical parameters of freshly harvested unfermented palm sap according to successive days

Time (T0)	pH	Titratable acidity (mg Eq Citric acid/100 ml)	Reducing sugars (g glucose equivalent/L)	Total sugars (g glucose equivalent/L)
Day 1	6.11a ± 0.00	8.64e ± 0.1	28.6a ± 0.48	199.8a ± 5.63
Day 2	5.99b ± 0.00	10.84d ± 0.1	28.0a ± 0.90	150.4b ± 2.15
Day 3	5.52c ± 0.01	12.19c ± 0.05	8.4b ± 0.48	146.4b + 1.35
Day 4	5.40d ± 0.00	13.07b ± 0.6	5.3c ± 0.25	119.8c ± 1.16
Day 5	4.95e ± 0.03	13.26a ± 0.05	3.69d ± 0.01	110.67d ± 0.91
F (4 ; 20)	2465.3	1109.2	2303.6	599.21
P	0.00	0.00	0.00	0.00

Values in the same column with the same letter are not statistically different at the 0.05 threshold of the Fischer LSD test

Table 3. Evolution of physicochemical parameters of unfermented palm sap 2 hours after harvesting according to successive days

Time (T_{0+2h})	pH	Titratable acidity (mg Eq Citric acid/100 ml)	Reducing sugars (g glucose equivalent/L)	Total sugars (g glucose equivalent/L)
Day 1	$5.83^{a} \pm 0.01$	$9.95^{d} \pm 0.04$	$14.4^{a} \pm 0.80$	$101.6^{a} \pm 4.45$
Day 2	$5.59^{b} \pm 0.00$	$11.18^{c} \pm 0.20$	$12.2^{b} \pm 0.74$	$85.2^{b} \pm 1.72$
Day 3	$5.49^{c} \pm 0.00$	$12.87^{b} \pm 0.08$	$7.6^{c} \pm 0.50$	$101.2^{a} \pm 4.26$
Day 4	$5.32^{d} \pm 0.02$	$13.76^{a} \pm 0.07$	$4.38^{d} \pm 0.46$	$102.8^{a} \pm 1.32$
Day 5	$4.89^{e} \pm 0.00$	$13.90^{a} \pm 0.37$	$2.06^{e} \pm 0.23$	$98.8^{a} \pm 1.16$
F (4 ; 20)	2636.3	312.22	313.57	23.90
P	*0.00*	*0.00*	*0.00*	*0.00*

Values in the same column with the same letter are not statistically different at the 0.05 threshold of the Fischer LSD test

3.48E+03 to 1.46E+04 cfu/ml. The enterococci load of the rested sap was higher than when the sap was fresh. On the other hand, the load of thermotolerant coliforms in fresh sap was higher than in rested sap. Also, the load of thermotolerant coliforms in fresh sap as well as in rested sap regressed over the days. This load decreased from 1.58E+03 to 3.21 E+02 cfu/ml and that of the rested sap decreased from 2.82E+02 to 8 cfu/ml.

This study revealed a significant diversity of microorganisms in unfermented palm sap. These include yeasts, molds, lactic acid bacteria, thermotolerant coliforms, and enterococci. These results are corroborated by those obtained by other authors working on fermented palm sap (palm wine) [22]. This set of different microorganisms living in the same medium as complex as palm sap, would promote a competition between them for available nutrients. And these competitions between microorganisms in this complex medium would determine the load of each group of microorganisms. Thus, the coliform loads are the lowest compared to the other groups of microorganisms. However, this average remains higher than the coliform loads of raw milk as defined by the French standard (AFNOR standards). This standard provides for less than 103 CFU/ml of coliforms in raw milk [30]. In the rested sap, the increase in acidity causes the fall of thermotolerant coliform loads in the sap. Indeed, the coliforms are affected by the acidity of the environment linked to the production of organic acids by the alteration germs present in the sap during fermentation. Similar results were found by other authors working on food supplements in Ghana [31]. The presence of thermotolerant coliforms in palm sap appears to be transient and the coliforms found in fresh sap would be the result of contamination at harvest. These coliforms could come from various origins, including poor hygiene control around the exploitation of the palms but also poor handling during harvesting [32].

Also, the loads of enterococci are higher than those provided for by the standards, in particular French standard [30] which provides for an absence of fecal streptococci in 100 µl of raw milk. The loads obtained with freshly collected sap range from 5.28E+02 to 7.52E+03 cfu/ml. Also, when the sap is left to rest these loads increase further. A high population of enterococci was identified in the different unfermented palm sap collected over the days. The presence of enterococci in palm sap is thought to be due to various causes, including human contamination during sap harvesting, or animal contamination by insects attracted by the sugars in palm sap [22,33], or by microorganisms present in the palm tree environment. On the other hand, the persistence of enterococci in palm sap that has become acidic could be explained by the capacity of enterococci to resist extreme environmental conditions. Most enterococci are adapted to environments and ecological conditions different from their natural habitat [34]. These environmental microorganisms therefore readily contaminate environments when hygienic conditions are not met [35]. Thus, their presence in palm sap reflects a lack of hygiene.

The spoilage germs are present in the palm sap with higher loads than the spoilage germs. Their loads increased over successive days and when the sap was left to rest for two hours. The lactic acid bacteria load of fresh sap changed from 1.5E+04 to 6.3E+04 cfu/ml and that of rested sap increased from 5.5E+04 to 2.2E+06 cfu/ml. Yeast and mold load of fresh sap increased from 1.4E+04 to 8.25E+05 cfu/ml and that of rested

Table 4. Evolution of the fecal contamination and spoilage germ load of fresh unfermented palm sap according to successive days

Time (T_0)	Fecal contamination germs		Alteration germs		
	Enterococci (cfu/ml)	Thermotolerant coliforms(cfu/ml)	lactic acid bacteria(cfu/ml)	Yeasts, moulds(cfu/ml)	MAG(cfu/ml)
Day 1	$528^{d} \pm 21$	$1580^{a} \pm 172$	$14994^{b} \pm 373$	$14093^{d} \pm 967$	$19187^{d} \pm 829$
Day 2	$1341^{c} \pm 136$	$1310^{b} \pm 20$	$17856^{b} \pm 561$	$30600^{d} \pm 3929$	$50687^{d} \pm 1526$
Day 3	$3470^{b} \pm 157$	$1086^{c} \pm 81$	$56531^{a} \pm 4124$	$210125^{c} \pm 1777$	$365625^{c} \pm 2072$
Day 4	$7297^{a} \pm 249$	$706^{d} \pm 67$	$63750^{a} \pm 4292$	$397750^{b} \pm 1483$	$526562^{b} \pm 4609$
Day 5	$7516^{a} \pm 358$	$321^{e} \pm 66$	$63125^{a} \pm 4146$	$827000^{a} \pm 1990$	$1027343^{a} \pm 5927$
F (4 ; 20)	913.3	127.6	79.89	601.78	1397.2
P	0.00	0.00	0.00	0.00	0.00

Values in the same column with the same letter are not statistically different at the 0.05 threshold of the Fischer LSD test

Table 5. Evolution of the load of fecal contamination germs and spoilage germs in rested palm sap according to the successive days

Time (T_{0+2h})	Fecal contamination germs		Alteration germs		
	Enterococci(cfu/ml)	Thermotolerant coliforms(cfu/ml)	Lactic acid bacteria(cfu/ml)	Yeasts moulds(cfu/ml)	MAG(cfu/ml)
Day 1	$3484^{c} \pm 103$	$282^{a} \pm 24$	$55687^{c} \pm 4812$	$416200^{d} \pm 2540$	$546250^{d} \pm 3442$
Day 2	$3629^{c} \pm 529$	$117^{b} \pm 2$	$75062^{c} \pm 4262$	$568100^{d} \pm 3613$	$751780^{d} \pm 5760$
Day 3	$16812^{a} \pm 414$	$59^{c} \pm 1$	$416250^{b} \pm 2487$	$1885000^{c} \pm 54151$	$2667447^{c} \pm 2174$
Day 4	$13179^{b} \pm 409$	$23^{d} \pm 1$	$2156640^{a} \pm 8324$	$2732500^{b} \pm 5894$	$3796875^{b} \pm 1565$
Day 5	$14593^{b} \pm 546$	$8^{d} \pm 2$	$2190234^{a} \pm 9587$	$3560000^{a} \pm 5979$	$4226562^{a} \pm 2591$
F (4 ; 20)	155.98	391.19	886.03	180.35	471.91
P	0.00	0.00	0.00	0.00	0.00

Values in the same column with the same letter are not statistically different at the 0.05 threshold of the Fischer LSD test

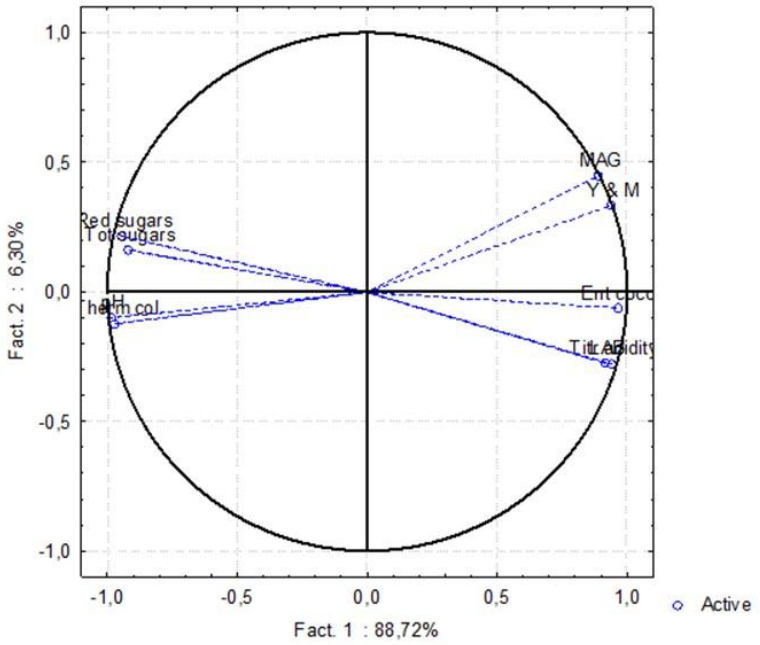

Fig. 1. Correlations between the physicochemical quality of fresh unfermented sap and microbial contamination

Red sugars: reducing sugars; Tot sugars: total sugars; Therm col: thermotolerant coliforms; MAG: Mesophilic aerobic germs; Y&M: yeast and mould; Ent cocci: Enterococci; LAB: Lactic acidic bacteria; Titr acidity: titratable acidity

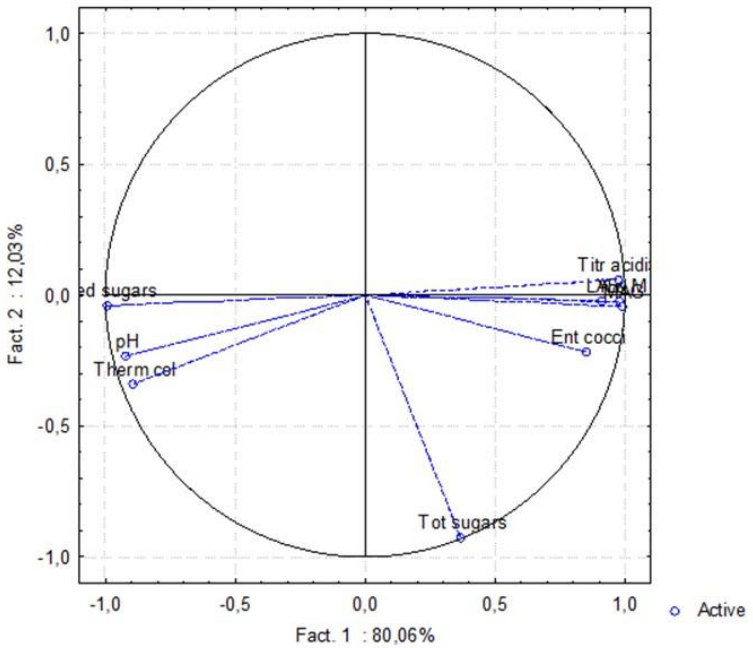

Fig. 2. Correlations between the physicochemical quality of sap rested for two hours and microbial contamination

Red sugars: reducing sugars; Tot sugars: total sugars; Therm col: thermotolerant coliforms; MAG: Mesophilic aerobic germs; Y&M: yeast and mould; Ent cocci: Enterococci; LAB: Lactic acidic bacteria; Titr acidity: titratable acidity

sap changed from 4.16E+05 to 3.5E+06 cfu/ml. The GAM load of fresh sap increased from 1.9E+04 to 1.02E+06 cfu/ml and that of rested sap increased from 5.46E+05 to 4.22E+06 cfu/ml.

The loads of AMGs are very high and exceed the standards in force for raw milk and infant food [22]. In fact, the GAMs represent all the bacteria, yeasts and molds that are capable of growing in the presence of air (aerobic) at temperatures between 25 and 30°C (mesophilic). These include enterococci, coliforms, other bacteria [22] but also yeasts and molds. The AMGs that colonize the unfermented palm sap would come from the environment of the palms, from the different parts of the oil palm which are among others the male inflorescences, the leaf petiole, the used felt, the transverse bands and the xylem which are covered with growing downy hairs, but also from the harvesters [36]. The presence of fermentative germs such as yeasts and lactic acid bacteria in fresh palm sap indicates that fermentation of the sap would begin before collection. These microorganisms would use the sugars present in the unfermented sap to transform them into organic acids such as lactic acid or acetic acid [37]. The activity of these microorganisms is thought to be responsible for the increase in acidity and decrease in sugar content over the days that the felled palms are harvested and when the sap is left to rest [38]. The average lactic acid bacteria load is high from the first days and increases over the following days. Similarly, the even higher yeast load increases over the days of palm harvesting.

3.4 Impact of Microbial Contamination on the Organic Quality of Unfermented Sap

A principal component analysis was performed to establish the interactions between physicochemical parameters and microbiological contamination of palm sap. At the level of fresh sap (Fig. 1), on axis 1 with a contribution of 88.72%, the well-represented variables (reducing sugars, total sugars, thermotolerant coliforms and pH) have negative coordinates while the variables (GAM, yeasts and molds, lactic acid bacteria, enterococci and titratable acidity) have positive coordinates and are also well-represented on this axis. The increase in spoilage germ loads is related to the decrease in reducing sugars and total sugars, but is also related to the increase in sap acidity, which is reflected in a decrease in pH and an increase in titratable acidity. Regarding the germs of fecal contamination, the increase in acidity, the decrease in reducing sugars and total sugars is associated with a decrease in the loads of thermotolerant coliforms. However, enterococci loads are positively correlated to the increase in acidity, the decrease in reducing sugars and total sugars.

At the rested sap level (Fig. 2), on axis 1 with a contribution of 80.06%, the variables (reducing sugars, thermotolerant coliforms and pH) have negative coordinates and are well represented on this axis while the variables (GAM, yeasts and molds, lactic acid bacteria, enterococci and titratable acidity) well represented have positive coordinates. The variable (total sugars) is not well represented on axis 1. The increase in spoilage germ loads is associated with the decrease in reducing sugars, but related to the increase in sap acidity reflected by a decrease in pH and an increase in titratable acidity. As regards the germs of fecal contamination, the increase of acidity, the fall of the content of reducing sugars is associated with the fall of the loads of thermotolerant coliforms which tend towards the disappearance. However, the enterococci load evolves in the same direction as the increase in acidity, the decrease in the content of reducing sugars and total sugars. The different chemical parameters observed in the palm sap could favor the evolution of the yeast load. In addition, the sharp increase in yeast load could be related to the sugar-rich chemical composition of the palm sap [39]. Degradation of sap quality by fecal contamination and fermentative spoilage microorganisms would be another cause for the decline in the practice of using unfermented palm sap as infant food.

4. CONCLUSION

The chemical and microbiological quality of unfermented palm sap for infant feeding was analyzed in this study. The physicochemical quality of the sap is strongly depreciated by the microbiological contamination. Thus, after more than two days of exploitation of fresh sap or in sap rested for two hours, the acidity becomes too high for infant feeding. Also, the loads of microorganisms of fecal origin (thermotolerant coliforms and enterococci) are beyond the standards required for infant feeding. Also, the large number of fermentative germs (lactic acid bacteria and yeasts) present in the unfermented sap before its collection contribute to the degradation of organic compounds (reducing

sugars and total sugars) and their transformation into organic acids over the days of exploitation of the palm trees. This sap could therefore be harmful to the health of infants, given the acidity and loads of microorganisms it contains.

CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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