

## HPLC determination of organic acids in palm saps throughout tapping process

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**ABSTRACT:** This study was carried out to determine organic acids in palm saps throughout tapping. Palm wine sample at different stages of processing namely tapping, were collected and analysed for physical, biochemical, nutritional and microbiological parameters (pH, total titratable acidity (TTA), fermenting microorganisms) extracted from two varieties of palm oil tree (*Dura* and *Tenera*). Microbiological and biochemical contents of palm wine were determined during the tapping of *Dura* and *Tenera* felled oil palm trees for 4 weeks. Some differences in chemical compositions between fresh palm wine samples of two palm trees varieties were observed. In addition the results of pH, total titratable acid and organic acids confirmed the importance of lactic acid bacteria in the tapping of palm wine. Organic acids detected in the freshly collected palm wine (first day) included oxalic ( $0.1 \pm 0.01 \text{ g L}^{-1}$ ), citric ( $0.05 \pm 0.02 \text{ g L}^{-1}$ ), tartaric ( $0.31 \pm 0.04 \text{ g L}^{-1}$ ), malic ( $0.5 \pm 0.02 \text{ g L}^{-1}$ ), ascorbic ( $0.05 \pm 0.002 \text{ g L}^{-1}$ ), fumaric ( $0.01 \pm 0.0005 \text{ g L}^{-1}$ ), lactic ( $0.15 \pm 0.02 \text{ g L}^{-1}$ ) and acetic ( $0.1 \pm 0.02 \text{ g L}^{-1}$ ) acids. Propionic acid was detected after 10 days of tapping. However, oxalic, propionic and acetic acids are not native to the exudates but are produced throughout the tapping process, though some may be present in the plant as anions. Throughout the tapping of palm wine, microorganisms population changed with undoubtedly influence on the palm wine quality.

**KEYWORDS:** organic acids, microorganisms, palm wine, palm oil trees.

### 1 INTRODUCTION

Palm wine is the collective name for a group of alcoholic beverages produced by the natural fermentation of the sap obtained from various tropical plants of the *Palmae* family [20]. Palm wine is an alcoholic beverage that is produced and consumed in different regions of the world, according to the country of origin; palm wine is called by different names. The sap of the palm trees, which is originally sweet [3] [1] [16] [24] serves as a rich substrate for the growth of various types of microorganisms. The sap undergoes spontaneous fermentation, which promotes the proliferation of yeasts and bacteria for the conversion of the sweet substrate into several metabolites mainly ethanol, lactic acid and acetic acid [1] [26] [22] [24]. Palm sap fermentation has been reported to be an alcoholic, lactic and acetic fermentation [22], therefore, yeasts, lactic acid bacteria and acetic acid bacteria are considered to play the most important role in the palm wine production. Natural palm sap is clean sweet colorless syrup containing about 10-20% of sugar that exudes from the trunk of the various species of palm tree during tapping operations. It is a cloudy whitish beverage with a sweet alcoholic taste and has a very short shelf life of only one day. The wine is an excellent substrate from a growing palm. It is obtained through the process known as tapping, which involves a series of operations to stimulate the flow of sap [3], such as the perforation of the trunk, insertion of a tube in the hole and collection of the sap in a container (gourd, clay pot, plastic container, glass bottle or calabash) [22]. There are diverse ways of tapping palm trees; they depend on the locality; but in general, two methods are practiced: in the first method the sap is obtained from a live standing tree, such as the Bandji and Toddy production, this process implicates climbing very tall palm trees, and perforate the trunk in the top of the tree for Bandji production [22], or cutting into the end of spadix

from the tender inflorescence of the palm tree (inflorescence tapping) for Toddy production [20] [3] [14]. In the second method the tree is felled or cut down before tapping (stem tapping), such as palm wine from Ghana and Taberna production. The cessation of the flow of palm sap varies according to the palm tree species and from tree to tree; for instance the shorter duration of tapping could be 2 weeks and the longest 8 weeks [4] [1] [24]. Palm wine is collected twice a day, normally in the morning and the evening, it can be either immediately consumed or stored for later sale [12] [24]. Palm wine from Ghana is distilled for gin production called Akpeteshie [1]; similarly, Toddy is also distilled to produce the spirit known as Arrack [3]. Tapping process from a live standing palm tree such as Bandji production from *Borassus akeassii*, has been reported that it is not significantly different of wine production from others types of palm trees where sap is collected from a live upright tree, as the palm wine from *Elaeis guineensis* produced in Ghana [1].

This drink has a significant role in several nutritional, medical, religious and social uses such as traditional wedding ceremonies, traditional religious ceremonies or festivals, prayers and it is good for malaria [21] [6]. The aim of this study was designed to investigate the microbiological and organic acids changes which occur in palm wine during the tapping of two varieties of felled oil palm trees.

## **2 MATERIALS AND METHODS**

### **2.1 MATERIALS AND SAMPLING**

Palm wine samples were obtained during the tapping of 20 palm trees (*E. guineensis*) of two varieties (*Dura* and *Tenera*) at the University Nangui Abrogoua (Côte d'Ivoire) over a period of 5 months. Collection of palm wine during tapping was normally done twice a day by the tapper, but in this study samples were collected at three day intervals each morning of sampling day at 7:00 AM in stomacher bags from the beginning until the end of tapping. These samples were immediately transported to the laboratory for analyses, carried out in replicates.

### **2.2 BIOCHEMICAL ANALYSIS**

#### **2.2.1 DETERMINATION OF PH AND TOTAL TITRATABLE ACIDITY**

The pH of palm wine samples was determined directly using a pH-meter (pH-meter P 107, CONSORT, Bioblock, France) after calibration with standard buffers. Total titratable acidity was determined by titrating samples against 0.1 M NaOH using 1% phenolphthalein as indicator as described by [1] and expressed as percentage of lactic acid.

#### **2.2.2 ORGANIC ACIDS DETERMINATION**

##### **2.2.2.1 SAMPLES PREPARATION**

Samples were centrifuged at 4000 rpm for 20 min and supernatants were filtered through a 0.20 mm Millipore membrane filter (Sartorius AG, Goettingen, Germany) and then stored at 20 °C until analysis.

##### **2.2.2.2 ORGANIC ACIDS CONTENT**

Organic acid were determined by High-Performance Liquid Chromatography (HPLC) as previously described by [17]. Analyses were carried out with an ion-exclusion ORH-801 column (300-6.5 mm) (Interchrom, France) preceded by a Universal Guard Cartridge- Holder column. The High-Performance Liquid Chromatograph system (LC-6A, Shimadzu Corporation, Japan) was equipped of a Shimadzu LC-6A pump. Column effluents were monitored by a UV detector (SPD-6A, Shimadzu Corporation, Japan) set at 210 nm. The mobile phase (0.004 N H<sub>2</sub>SO<sub>4</sub>) used at a low rate of 0.8 ml/min was filtered through a 0.45 mm Millipore membrane filter (Sartorius AG, Goettingen, Germany). A 20 ml injection volume was used for HPLC samples and the analyses were done in duplicate. The organic acids standards were dissolved in distilled water at concentrations ranging to 0.05-0.4 g L<sup>-1</sup>, filtered and injected as the samples. Organic acids were identified and quantified by comparison of their retention times and peak areas with those of standards.

### **2.3 ENUMERATION OF MICROORGANISMS**

The palm wine samples were shaken by hand in the stomacher bag and tenfold serial dilutions were prepared and spread-plated for determination of micro-organism counts. After dilutions, enumeration of total aerobic mesophile was carried out using plates of Plate Count Agar (PCA, Difco 0479-17-3; Difco Laboratories, Detroit, MI, USA) which were incubated at 30°C

for 2 days. Lactic acid bacteria (gram positive catalase negative rods, cocci and coccoids) were enumerated by pour plate on DeMan, Rogosa and Sharpe Agar (MRS, Merck 10660; Merck KGaA, Darmstadt, Germany) containing 10 mg mL<sup>-1</sup> cycloheximide (ICN 100183 Biomedical Inc., Aurora, OH, USA) to suppress yeast growth after incubation at 30°C for 3 days in an anaerobic jar with anaerocult A (Merck). Yeasts and moulds were enumerated on plates of Sabouraud Chloramphenicol agar (BIO-RAD, France) which were incubated at 30°C for 3-5 days. Enumeration of total and faecal coliforms was carried out using plates of Violet Red Bile Lactose agar (VRBL, Merck 10660, Merck, Darmstadt, Germany) which were incubated for 24 h at 30°C for total coliforms and 44°C for faecal coliforms. Enterococci species were enumerated on plates of Bile Esculin Azide agar (AES Laboratoire, COMBOURG France) after incubation at 37°C for 2 days.

#### 2.4 STATISTICAL ANALYSIS

The data obtained were subjected to analysis of variance (Statistica, 99 Edition, Alabama, USA) and mean differences determined by Duncan's multiple range tests. Significance of variations in the analyzed data was tested at 95% confidence limit.

### 3 RESULTS AND DISCUSSION

Organic acids in fresh palm wines collected at every three sampling day intervals during tapping of two varieties of palm tree (*Dura* and *Tenera*) is showed in Table 1. The initial pH were respectively  $5.2 \pm 0.18$  and  $4.64 \pm 0.2$  in *Dura* and *Tenera* variety. Normally, natural palm sap shows approximately a neutral pH; however, in the first days of the tapping process, this value decreases between 5 and 4 and subsequently between 4 and 3 [8] [7] [11] [24]. These changes on pH are due to the organic acids production as a result of the microbial metabolic activity. Lactic acid produced by the lactic acid bacteria has been reported as the main responsible for the acidic condition in palm wine [1] [26] [22] [24]. Throughout the tapping, a subsequent drop of pH associated with an increase of the titratable acidity were observed. During the collecting process, it is highly susceptible to spontaneous yeast-lactic fermentation of the sugary sap. This process is reported to be rapid under sunlight. Sources of fermenting microorganisms are tapping implements (knife and bamboo tube) and air. The weak rate of sugars observed in *Tenera* variety would be had to either to the important chemical changes in the sap herself or either to high loads of microorganisms in a container which converted the fermentable sugars into lactic and acetic acids. Ours results were similar with results obtained by [29] [5] and [1]. The process of tapping of palm wine from felled trees can be considered as semi-continuous fermentation. Periodically (everyday, twice a day), samples were collected and added to the previous samples in a container placed near the tree. In the traditional setting, palm wine tapping instruments, especially receiving vessels are used repeatedly without cleaning the inner surfaces to remove the microbial deposits. So, rapid deterioration of sugary taste is observed shortly after the fresh exudate is obtained. This is the result of fermentation of endogenous sugars by the natural flora of the fermenting sap which is whitish in appearance and also exhibit vigorous effervescence. The results are similar to those obtained by [1]. The pH and total acidity of all palm sap samples were significantly different among the samples ( $P < 0.05$ ) (Fig 1). The pH values of all palm sap samples varied from 5.23 to 3.43 while total acidity found in a range from 0.17% to 0.88%, as calculated based on lactic acid equivalence, since lactic acid is the main organic acid presented in palm sap [23] [12]. Microorganisms, mainly lactic acid bacteria produced organic acids (mainly lactic acid), which then increase in total acidity and decrease in pH value. Hence, a high percentage of total acidity and low pH indicates the initial fermentation step of palm sap, for example, during collecting time. In addition the results of pH, total titratable acid and organic acids confirmed the importance of lactic acid bacteria in the tapping of palm wine. In fact, lactic acid bacteria were considered to be responsible for the rapid acidification of the product as the acetic acid bacteria were not isolated in the palm wine samples on the first days of tapping (Fig 2). The results are similar to those obtained by [1].

Table 1 : Organic acids obtained in palm in every day during tapping of two varieties of palm tree

Varieties	Days of sapping										
	1	4	7	10	13	16	19	22	25	28	
Ac. Ox	Dura	0,09 ± 0,01	0,1176 ± 0,02	0,129 ± 0,02	0,156 ± 0,01	0,196 ± 0,01	0,109 ± 0,02	0,087 ± 0,01	0,052 ± 0,01	0,049 ± 0,01	0,026 ± 0,01
	Tenera	0 ± 0	0,3173 ± 0,02	0,3462 ± 0,01	0,253 ± 0,02	0,1929 ± 0,02	0,1259 ± 0,02	0,1022 ± 0,01	0,0519 ± 0,01	0,0797 ± 0,01	0,0659 ± 0,01
Ac. C	Dura	0,0467 ± 0,002	0,0929 ± 0,014	0,201 ± 0,033	0,413 ± 0,011	0,453 ± 0,0135	0,484 ± 0,024	0,397 ± 0,04	0,374 ± 0,05	0,358 ± 0,02	0,324 ± 0,04
	Tenera	0,0302 ± 0,021	0,0712 ± 0,013	0,0879 ± 0,026	0,121 ± 0,04	0,181 ± 0,03	0,183 ± 0,04	0,272 ± 0,027	0,411 ± 0,013	0,597 ± 0,03	0,662 ± 0,019
Ac. T	Dura	0,312 ± 0,04	0,342 ± 0,03	0,442 ± 0,01	0,513 ± 0,03	0,785 ± 0,06	1,018 ± 0,04	0,864 ± 0,02	0,7797 ± 0,03	0,78 ± 0,04	0,705 ± 0,02
	Tenera	0,22 ± 0,02	0,259 ± 0,03	0,282 ± 0,02	0,161 ± 0,01	0,137 ± 0,02	0,136 ± 0,02	0,136 ± 0,03	0,113 ± 0,01	0 ± 0	0 ± 0
Ac. M	Dura	0,469 ± 0,02	0,598 ± 0,05	0,803 ± 0,07	0,952 ± 0,06	0,725 ± 0,09	0,698 ± 0,05	0,632 ± 0,04	0,469 ± 0,08	0,366 ± 0,09	0,336 ± 0,08
	Tenera	0,68 ± 0,05	0,855 ± 0,08	1,255 ± 0,07	0,895 ± 0,06	0,801 ± 0,06	0,75 ± 0,05	0,646 ± 0,03	0,567 ± 0,03	0,503 ± 0,02	0,351 ± 0,03
Ac. Asc	Dura	0,025 ± 0,002	0,03 ± 0,002	0,03 ± 0,014	0,04 ± 0,003	0,05 ± 0,004	0,072 ± 0,021	0,09 ± 0,008	0,13 ± 0,002	0,14 ± 0,001	0,17 ± 0,003
	Tenera	0,685 ± 0,02	0,57 ± 0,03	0,38 ± 0,02	0,23 ± 0,06	0,16 ± 0,04	0,13 ± 0,02	0,12 ± 0,02	0,131 ± 0,03	0,09 ± 0,02	0,13 ± 0,04
Ac. L	Dura	0,149 ± 0,02	0,197 ± 0,06	0,359 ± 0,11	0,396 ± 0,12	0,638 ± 0,13	0,928 ± 0,13	0,74 ± 0,11	0,54 ± 0,12	0,473 ± 0,1	0,402 ± 0,11
	Tenera	0,197 ± 0,02	0,274 ± 0,24	0,621 ± 0,03	1,687 ± 0,06	0,885 ± 0,09	0,597 ± 0,03	0,429 ± 0,03	0,38 ± 0,05	0,3 ± 0,04	0,17 ± 0,05
Ac. A	Dura	0,101 ± 0,06	0,299 ± 0,06	0,326 ± 0,09	0,406 ± 0,02	0,521 ± 0,02	0,654 ± 0,05	0,698 ± 0,08	0,735 ± 0,08	0,752 ± 0,03	0,767 ± 0,05
	Tenera	0 ± 0	0,15 ± 0,05	0,227 ± 0,05	0,455 ± 0,08	0,713 ± 0,06	0,824 ± 0,05	0,87 ± 0,02	0,921 ± 0,02	1,091 ± 0,02	1,24 ± 0,06
Ac. F	Dura	0,012 ± 0,0005	0,011 ± 0,0005	0,0009 ± 0,0001	0,007 ± 0,0001	0,007 ± 0,0005	0,005 ± 0,0006	0,004 ± 0,0005	0,004 ± 0,0002	0,004 ± 0,0005	0,003 ± 0,0004
	Tenera	0,014 ± 0,0002	0,013 ± 0,0006	0,009 ± 0,0005	0,009 ± 0,0005	0,009 ± 0,0008	0,007 ± 0,0005	0,006 ± 0,0001	0,005 ± 0,0004	0,005 ± 0,0001	0,004 ± 0,0005
Ac. P	Dura	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0,006 ± 0,0004	0,008 ± 0,005	0,009 ± 0,003	0,022 ± 0,006	0,06 ± 0,005	0,072 ± 0,004
	Tenera	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

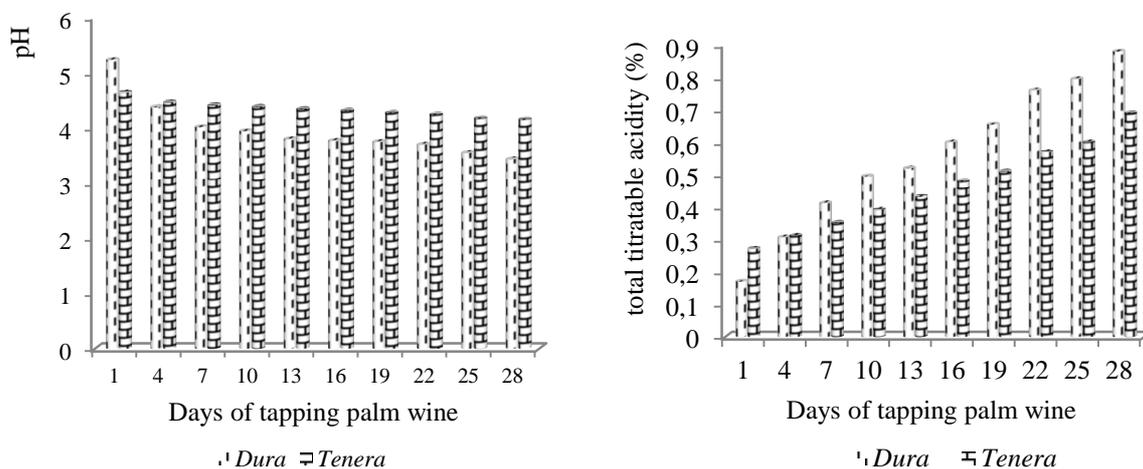
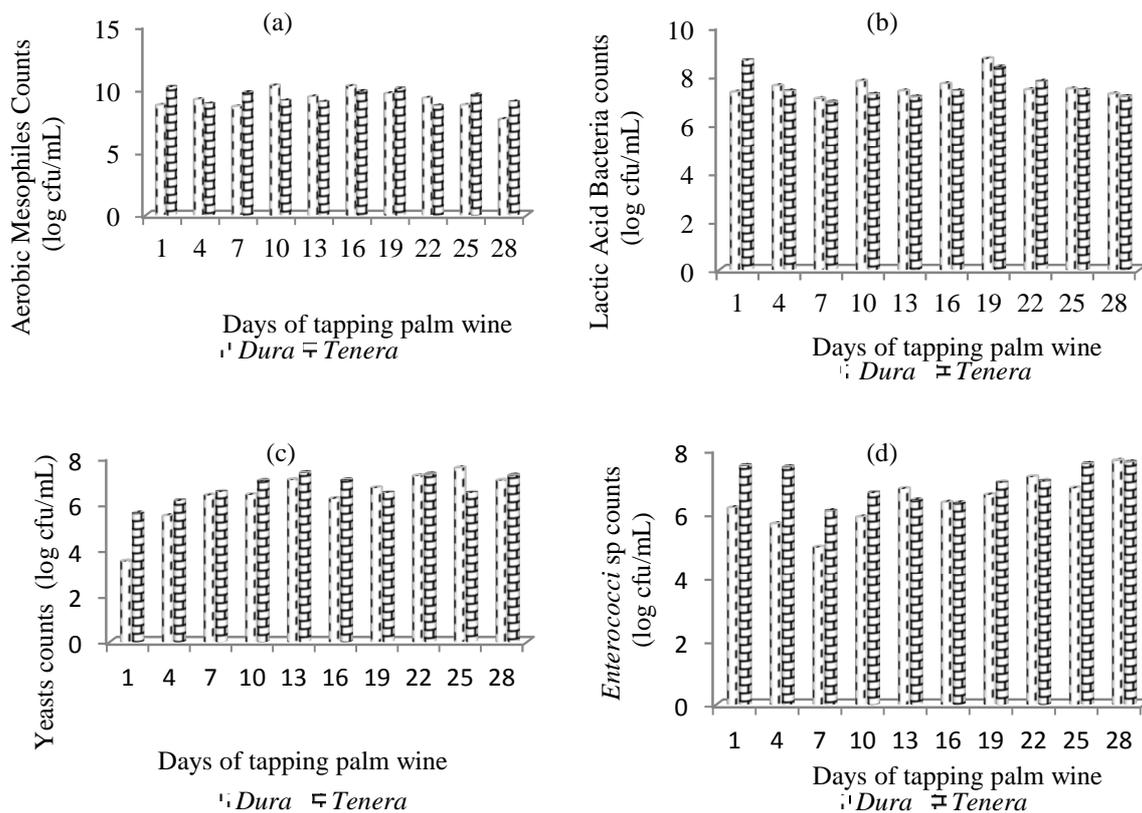


Fig. 1 : Changes in pH and total titratable acidity content during the tapping of different palm wine samples.



**Fig. 2: Mean values of the population of various microorganisms in different samples of palm wine during the tapping (a) Aerobic mesophiles, (b) lactic acid bacteria, (c) yeasts, (d) Enterococcus sp.**

The organic acids of fresh palm wines collected during tapping are showed in Fig 3. Organic acids detected in the freshly collected palm wine (first day) included oxalic ( $0.1 \pm 0.01 \text{ g L}^{-1}$ ), citric ( $0.05 \pm 0.02 \text{ g L}^{-1}$ ), tartaric ( $0.31 \pm 0.04 \text{ g L}^{-1}$ ), malic ( $0.5 \pm 0.02 \text{ g L}^{-1}$ ), ascorbic ( $0.05 \pm 0.002 \text{ g L}^{-1}$ ), fumaric ( $0.01 \pm 0.0005 \text{ g L}^{-1}$ ), lactic ( $0.15 \pm 0.02 \text{ g L}^{-1}$ ) and acetic ( $0.1 \pm 0.02 \text{ g L}^{-1}$ ) acids. Propionic acid was detected after 10 days of tapping. Throughout the tapping, a subsequent drop of fumaric acid (Fig 3 h) content associated with an increase of acetic, propionic and ascorbic acid rate were observed. Ascorbic acid (Fig 3 g) content in *Tenera* oil palm trees was higher than this of *Dura*. The high concentration of ascorbic acid detected in *Tenera* freshly palm wine could be explained by the fact that some microorganisms during their growth, produced organic acids such as ascorbic acid. Moreover, the increase of other organic acids throughout the tapping can be explained by the fact that they are present naturally in palm wine and can be produced in sufficient quantity in this process. The fact that palm wine contained ascorbic acid could be of great interest and might have positive effects on maternal iron deficiency [31]. The other organic acids increased within 16 days and then decreased throughout the tapping. The increase of the other organic acids throughout the tapping could suggest that these acids are native to the exudates and are also produced abundantly during the process. The results were similar to those obtained by [2] [25] [11].

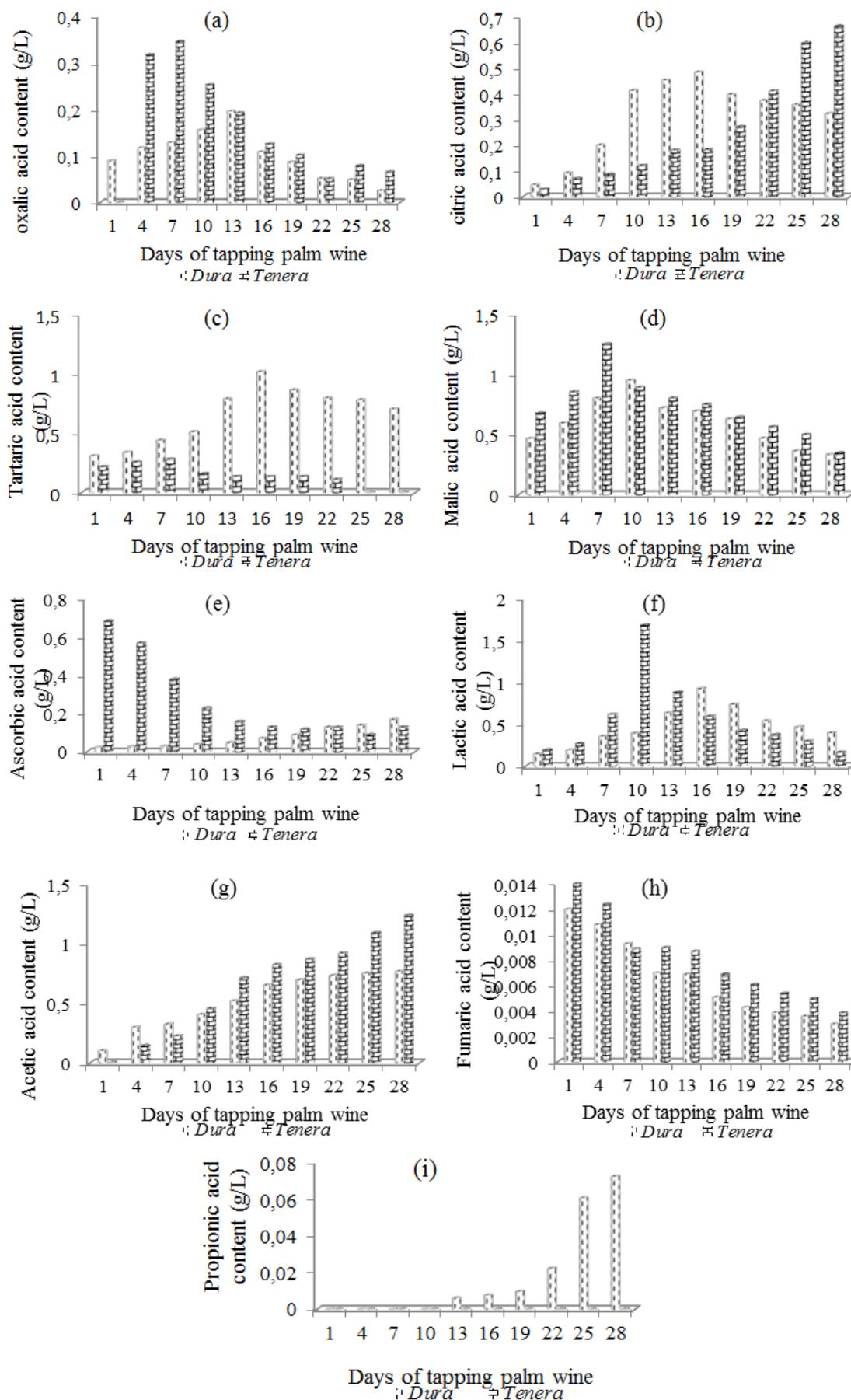


Fig. 3 : Changes in organic acids content during the tapping of different palm wine samples.(a) : oxalic, (b) : citric, (c) : tartaric, (d) : malic, (e) : ascorbic, (f) : lactic, (g) : acetic, (h) : fumaric, (i) : propionic acids.

Lactic acid produced by the lactic acid bacteria has been reported as the main responsible for the acidic condition in palm wine [1] [26] [24] e.g. in the palm wine of *Elaeis guineensis* the percentage of lactic acid after the first few days of tapping was between 0.1 and 0.3% [1], similarly in the palm wine of *Acrocomia aculeata*, the lactic acid concentration varied in the range of 0.26 to 0.48%, through the tapping process [24]. This lactic acid concentration decreased the pH in the medium in an approximately 24 h and after that the pH is stabilized at 4 and 3 [8] [1] [24]. The second organic acid produced in the palm wine is the acetic acid, with a concentration of about 0.02 to 0.4% [1] [22]. According to [9], this acetic acid concentration in the palm wine are acceptable by the consumers but, when the concentration exceeds 0.6% the beverage becomes unacceptable [1].

However, oxalic, propionic and acetic acids are not native to the exudates but are produced throughout the tapping process, though some may be present in the plant as anions neutralized by cations such as potassium [30]. Their content increased to reach  $0.07 \pm 0.1 \text{ g L}^{-1}$  of propionic acid in the sap collected in *Dura* palm wine,  $0.19 \pm 0.02 \text{ g L}^{-1}$  of oxalic and  $1.24 \pm 0.06 \text{ g L}^{-1}$  of acetic acid in *Tenera* palm wine throughout the tapping. Moreover, the finding of a considerable amount of acetic acid in palm wine is not an indication of spoilage by acetic acid bacteria as is the case in conventional wines and as previously assumed for palm wine. Also, as acetic acid was present in all the mature palm wine samples, it was considered as part of the aroma of palm wine. [32] has shown that when fructose is available in wines and lactic acid bacteria are able to grow, they can produce equimolar amounts of acetic acids from fructose and this could constitute a serious source of acetic acid in the wine. The fructose produced early in palm wine fermentation as a by-product of dextran synthesis by lactic acid bacteria were likely to have been used in this way by the same bacteria to produce lactic and acetic acids. This indicated the existence of acetic acid bacteria such as *Acetobacter* sp. In addition, acetic acid was also produced by lactic acid bacteria through heterofermentation [28] [16]. The important increase in organic acid contents could undoubtedly have inhibitory effects on microbial growth, particularly those which do not support high acid conditions. Indeed, an initial increase followed by a decrease in coliforms loads and the disappearance of sulphite-reducing bacteria after only 4 days of tapping were observed (Fig. 4). This is in accordance with the death kinetics reported in similar natural fermented plant materials [18] [13]. According to [12], only  $1 \text{ g L}^{-1}$  of undissociated citric acid can inhibit a significant concentration of enterobacteria. Moreover ascorbic acid, like many other edible organic acids, has been reported to inhibit the growth of many foodborne pathogenic and spoilage bacteria [10].

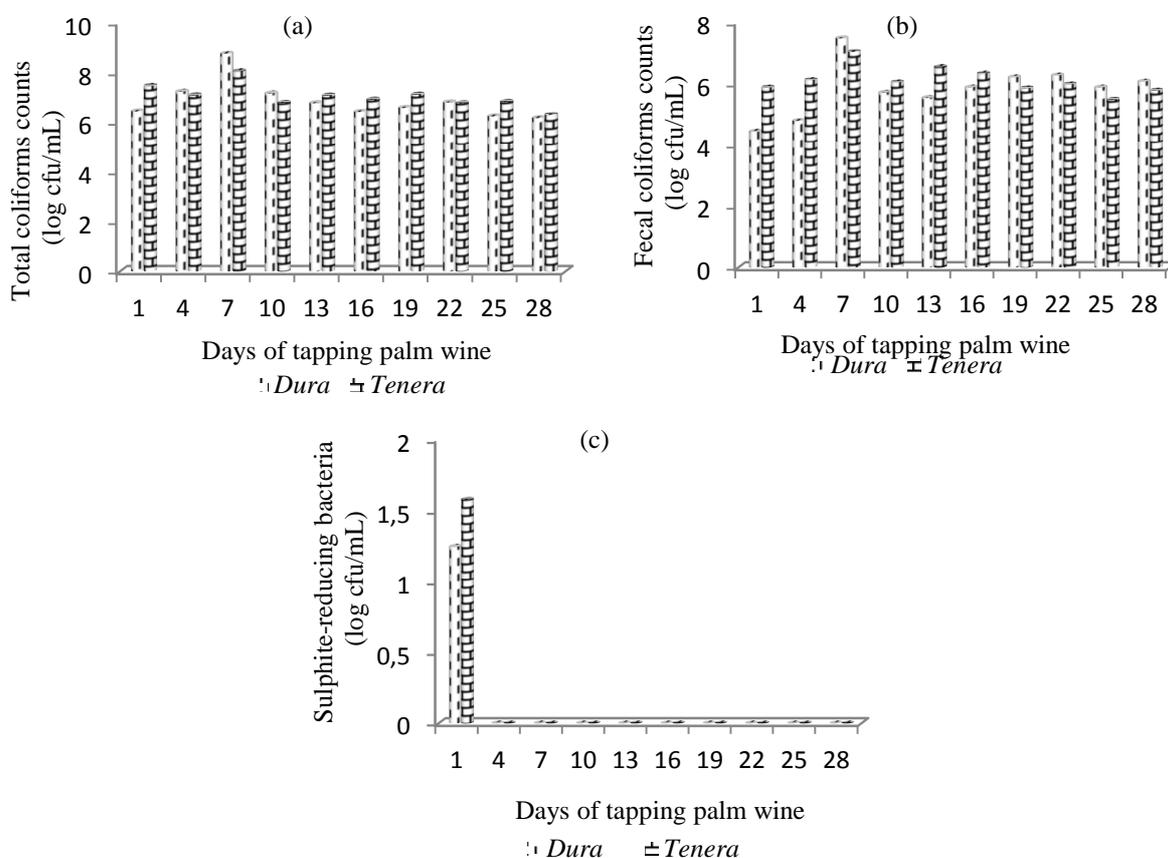


Fig. 4: Mean values of the population of various micro-organisms in different samples of palm wine

The sap of the palm tree has been shown to be a rich medium capable of supporting the growth of various types of microorganisms, as high numbers of aerobic mesophiles, lactic acid bacteria, yeasts, *Enterococci*, coliforms and sulphite reducing bacteria were found in palm wine during tapping. In discussing the population of the various microorganisms in palm wine, the following factor appears important. That to prevent insect and larvae infestation and also facilitate the oozing of the sap, the tapper cuts thin slices off the walls of the receptacle daily to expose a fresh layer. This physically removed the microbiota that had colonized the walls of the receptacle, thus reducing the microbial load in the chamber. In spite of all measures, the microorganism colonizes the palm wine. This could be explained by the results presented in Fig. 2 and 4, which showed that these micro-organisms in fresh palm wine might have originated from those that colonize those parts of the palm stalk of the male inflorescence, the leaf petiole, the felt (afabric-like outgrowth of the frond petiole used to cover the tapping hole), the cross strips and xylem stream which are covered with fluffy hairy outgrowths. Our results are similar to those obtained by [9]. Some of them were also brought by the tapper, tapping materials and insects attracted by the sap sugar (Fig. 2 and 4). Our results showed high loads of microorganisms already the first day in palm wines of both varieties (*Dura* and *Tenera*). Microbiological examination of *Tenera* palm wine samples recorded aerobic mesophilic counts of  $10.2 \pm 0.1 \log \text{ cfu mL}^{-1}$ . Yeast counts was  $5.6 \pm 0.3 \log \text{ cfu mL}^{-1}$ , lactic acid bacteria counts was  $8.6 \pm 0.2 \log \text{ cfu mL}^{-1}$ , *Enterococci* species counts  $7.5 \pm 0.2 \log \text{ cfu mL}^{-1}$ . Total coliforms and fecal coliforms counts from palm wines were  $7.5 \pm 0.2 \log \text{ cfu mL}^{-1}$  and  $5.9 \pm 0.2 \log \text{ cfu mL}^{-1}$  respectively. Throughout tapping of *Dura* palm wine, a drastic increase was observed in yeast loads from average values of  $3.5 \pm 0.3$  to  $7.5 \pm 0.2 \log \text{ cfu mL}^{-1}$  after 25 day (Fig. 2c). It is possible that differences in the chemical composition of palm wine tapped from *Dura* and *Tenera* oil palm trees favour the evolution of yeast biota. During the first week of competition between microorganisms in successive fermentations was unfavorable of *Enterococci*, coliforms (total and fecal) and sulphite reducing bacteria. The second week was characterized by a decrease in the profit of coliforms and *Enterococci* training decrease in pH and an increase of ethanol in palm wines. The decrease of pH and increase of titratable acid allow acidification palm wine provided by the population of lactic acid bacteria founded in the palm wine samples, which was very little during the semi-continuous fermentations. Indeed, microorganisms such as lactic acid bacteria produce organic acids including lactic allowing increased titratable acidity and therefore lower pH.

Therefore, high titratable acidity and the low pH of palm wine obtained indicate that initial fermentation of the sap was done before collection. As the results of pH, titratable acidity and lactic acid confirm the presence of lactic acid bacteria in palm wine. Similar observations were reported by [1], who confirm that the lactic acid bacteria were considered to be responsible for the rapid acidification of the product as the acetic acid bacteria were not isolated in the palm wine samples on the first day of tapping. The increase in acidity indicates production of acid in palm wine as had previously indicated work done by [21] and [19]. A substantial part of coliforms and sulphite reducing bacteria were present in the different saps with the highest loads in those collected in *tenera* palm wine when compared with corresponding values for *dura* palm wine (Fig. 4). The presence of coliforms in the palm wines could be explained itself by a human or animal fecal contamination on the place of tapping and also by the presence of bugs attracted by sugars contained in palm wines. Our results agree with those of [27]. In addition the occurrence of coliforms and sulphite reducing bacteria are evidence of poor hygiene conditions of some of the palm wine sampled. These microorganisms may be contaminants from untreated container normally used in tapping palm wine by the tappers [15].

#### 4 CONCLUSION

Palm wine is the collective name for a group of alcoholic beverages produced by the natural fermentation of the sap obtained from various tropical plants of the *Palmae* family (Okafor, 1978). Palm wine is an alcoholic beverage that is produced and consumed in different regions of the world, according to the country of origin; palm wine is called by different names. The chemical composition of the palm sap is very similar among different species of palm trees. Palm wines collected during tapping contains many organic acids such as oxalic, citric, tartaric, malic, ascorbic, fumaric, lactic, acetic and propionic but propionic acid was detected after 10 days of tapping. The composition of palm wine depends of several factors such as the acidity and microorganisms. The important increase in organic acid contents could undoubtedly have inhibitory effects on microbial growth, particularly those which do not support high acid conditions. Therefore, further studies on their technological properties could help to elucidate the involvement of these micro-organisms in the food safety and the improvement of the consumer digestion; as palm wine is consumed with its wild micro-organisms generally without any toxicity has been observed.

## REFERENCES

- [1] Amoah-Awua, W.K., Sampson, E. & Tano-Debrah, K., 2007. Growth of yeasts, lactic and acetic bacteria in palm wine during tapping and fermentation from felled oil palm (*Elaeis guineensis*) in Ghana. *Journal of Applied Microbiology*. 102, 599-606.
- [2] Akalin, A.S., Gönç, S. & Akbast, Y., 2002. Variation in organic acids content during ripening of pickled white cheese. *Journal of Dairy Science*. 85, 1670-1676.
- [3] Atputharajah, J. D., Widanapathirana, S. and Samarajeewa, U., 1986. Microbiology and biochemistry of natural fermentation of coconut palm sap. *Food Microbiology* (3) 4: 273-280.
- [4] Balick, M.J. 1990. Production of coyol wine from *Acrocomia mexicana* (arecaceae) in Honduras. *Economic botany* 44 (1): 84-93.
- [5] Borse, B.B., Rao, J.L., Ramalakshmi, K. & Raghavan, B., 2007. Chemical composition of volatiles from coconut sap (neera) and effect of processing. *Food Chemistry*. 101, 877-880.
- [6] Chandrasekhar, H., Sreevani, S., Seshapani, P. and Pramodhakumari, J. 2012. A review on palm wine. *International Journal of Research in Biological Science* 2 (1): 33-38.
- [7] Ezeagu, I.E. & Fafunso, M.A., 2003. Biochemical constituents of palm wine. *Ecology and Food Nutrition*. 42, 213-222.
- [8] Eze, M. O. and Uzoechi, O. 1988. Sugars of the unfermented sap and the wine from the oil palm, *Elaeis guineensis*, tree. *Plant Foods for Human Nutrition* 38 (2): 121-126.
- [9] Faparusi, S.I., 1973. Origin of initial microflora of palm wine from oil palm trees (*Elaeis guineensis*). *Journal of Applied Bacteriology*. 36, 559-565.
- [10] Guenier, A.S., Caillet, S., Ouattara, B., Laleye, L. & Lacroix, M., 2010. Antibacterial capacity of a reconstituted fortified beverage used as a nutritional supplement. *Food Research International*. 43, 2321-2328.
- [11] Karadeniz F., 2004. Main organic acid distribution of authentic citrus juices in Turkey. *Turkey Journal of Agriculture and Forest*. 28, 267-271.
- [12] Karamoko D., Djeni N.T., N'guessan K.F., Bouatenin K. M.J-P. & Dje K.M., 2012. The biochemical and microbiological quality of palm wine samples produced at different periods during tapping and changes which occurred during their storage. *Food Control*. 26, 504-511.
- [13] Masha, G. G. K., Ipsen, M. A., Petersen, M. A., Jakobsen, M., 1998. Microbiological, rheological and aromatic characteristics of fermented 'uji' (an East African sour porridge). *World Journal of Microbiology and Biotechnology*. 14, 451-456.
- [14] Mbuagbaw, L. and Noorduyn, S. G., 2012. The palm wine trade: occupational and health hazards. *International Journal of Occupational and Environmental Medicine* 3 (4): 157-164.
- [15] Murray B. E., 1990. The life and Time of the *Enterococcus*. *Clinical Microbiology Reviews*. 3, 46-47.
- [16] Naknean, P., Meenune, M. and Roudaut, G., 2010. Characterization of palm sap arvested in Songkhla province, Southern Thailand. *International Food Research Journal*. 17, 977-986.
- [17] N'guessan K. F., 2009. *Analyse de quelques paramètres de la fermentation alcoolique conduisant au tchapalo (bière traditionnelle à base de sorgho) et des potentialités à la formulation d'une culture starter à partir de levures isolées*. Thèse de doctorat unique : Université Abobo-Adjamé 1-190.
- [18] Nout, M.J.R., Rombouts, F.M. & Havelaar, A., 1989. Effect of natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. *International Journal of Food Microbiology*. 8, 351-361.
- [19] Obire O., 2005. Activity of yeast species in palm sap obtained from three areas in Edo state, Nigeria. *Journal of Applied Science Environmental Management*. 9, 25-30.
- [20] Okafor, N., 1978. Microbiology and biochemistry of oil palm wine. *Advanced in Applied Microbiology*. 24, 237-256.
- [21] Olasupo, N.A. & Obayori, O.S., 2003. Utilization of palm wine (*Elaeis guineensis*) for the improved production of Nigerian indigenous alcoholic drink Ogogoro. *Journal of Food Processing and Preservation*. 27, 365-372.
- [22] Ouoba, L., Kando, C., Parkouda, C., Sawadogo-Lingani, H., Diawara, B. and Sutherland, J. P. 2012. The microbiology of Bandji, palm wine of *Borassus akeassii* from Burkina Faso: identification and genotypic diversity of yeasts, lactic acid and acetic acid bacteria. *Journal of Applied Microbiology* 113 (6): 1428-144.
- [23] Phaichamnan, M., Posri, W. and Meenune, M., 2010. Quality profile of palm sugar concentrate produced in Songkhla province, Thailand. *International Food Research Journal*. 17, 425-432.
- [24] Santiago-Urbina, J. A., Verdugo-Valdez, A. G. and Ruiz- Terán, F. 2013. Physicochemical and microbiological changes during tapping of palm sap to produce an alcoholic beverage called "Taberna", which is produced in the south east of Mexico. *Food Control* 33 (1): 58-62.
- [25] Soyer, Y., Koca, N. & Karadeniz, F., 2003. Organic acid profile of Turkish white grapes and grape juices. *Journal of Food Composition and Analysis*. 16, 629-636
- [26] Stringini, M., Comitini, F., Taccari, M. and Ciani, M., 2009. Yeast diversity during tapping and fermentation of palm

- wine from Cameroon. *Food Microbiology* 26 (4): 415-420.
- [27] Tchiendji, C. 1985. *Le vin de palme d'Elaeis guineensis*. Thèse Ecole Supérieure Agronomique et des industries alimentaires, Université de Nancy (France) 1-218.
- [28] Tesfaye, W., Morales, M. L., Garcia-Parrila, M. C. and Troncoso, A. M., 2002. Wine vinegar: technology, authenticity and quality evaluation. *Trends in Food Science and Technology*. 13, 12-21.
- [29] Uzochukwu S.V.A., Balogh E., Tucknot O.G., Lewis M.J. & Ngoddy P.O., 1994. Volatiles Constituents of Palm wine and Palm Sap *Journal of the science of food and agriculture*. 94, 405-411.
- [30] Uzochukwu, S.V. A., Balogh, E., Tucknot, O.G., Lewis, M.J., & Ngoddy, P.O., 1997. Volatiles of palm wine using solvent extracts. *Journal of Food Quality*. 20, 483-494.
- [31] Wijaya-Erhardt M., Muslimatun S. & Erhardt J.G., 2011. Fermented soyabean and vitamin C-rich fruit: a possibility to circumvent the further decrease of iron status among iron-deficient pregnant women in Indonesia. *Public Health Nutrition*. 14, 2185-2196
- [32] Whiting, G.C., 1973. Acetification in ciders and perries. *Journal Instrumental Brewing*. 79, 218-226.