Nutritional, biochemical and microbiological composition of *Borassus aethiopum* Mart. sap in Burkina Faso

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Abstract The palmyra palm *Borassus aethiopum* Mart. grow wild and gives natural stands in several localities of central-eastern and eastern regions of Burkina Faso. This work aimed to determine the nutritional, biochemical and microbiological composition of fresh palm sap from *B. aethiopum* Mart. during the first 4 days of tapping. The composition of palm sap was carried out by HPLC and standard methods. The sap collected during the first 4 days were sugary and contained less alcohol. The mean values of the pH, total and reducing sugars content were 4.84 ± 0.5 , 11.36 ± 3.97 and $2.93 \pm 1.22\%$ w/v respectively. Sucrose, glucose, fructose and Vitamin C values were 6.75% w/v, 4.99 g/L, 7.09 g/L, 8.93% w/v

respectively. Galactose and xylose were not detected. Soluble proteins, arabinose, phenols and ethanol were present in low concentration. Calcium, potassium, magnesium and ammonium were present in palm sap with highest potassium content (13.26 g/L). Lactate (2.41 ± 0.86 g/L), succinate (2.49 ± 1.46 g/L), acetate (0.01 ± 0.006 g/L), malate (0.17 ± 0.31 g/L), propionate (0.07 ± 0.04 g/L), citrate (0.19 ± 0.11 g/L), tartrate (0.08 ± 0.09 g/L) and pyruvate (0.05 ± 0.03 g/L) were detected in palm sap. The microbiological analysis of sap gave $1.23 \pm 1.01 \times 10^8$ cfu/mL for total aerobic flora, $7.27 \pm 1.19 \times 10^5$ cfu/mL for yeasts, $1.86 \pm 1.63 \times 10^7$ cfu/mL for lactic acid bacteria and $3.75 \pm 0.75 \times 10^5$ cfu/mL for acetic acid bacteria. The fresh sap from *B. aethiopum* presents good nutritional value and its consumption can help to improve dairy food intake of rural population. It can be used for the manufacture of various products like palm wine, syrups, sugars, functional foods, etc.

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Introduction

Palms are largely widespread in several tropical areas of Asia, America and Africa (Miège 1985). The most important product from palms trees is palm wine which is an alcoholic drink obtained by the natural fermentation of the sap from various types of palm species. This beverage is produced and consumed by population in tropical regions of the world (Santiago-Urbina and Ruiz-Terán 2014). In Africa, the sap is collected from various species of palm trees such as *Elaeis*

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guineensis, *Raphia hookeri*, *Phoenix dactylifera*, *Borassus aethiopum*, *Cocos nucifera*, *Nypa fruticans*, *Caryota urens* and *Borassus akeassii* (Mollet et al. 2000; Amoa-Awua et al. 2007; Ziadi et al. 2011; Ouoba et al. 2012; Santiago-Urbina and Ruíz-Terán 2014; Tapsoba et al. 2014; Karamoko et al. 2016). The palm sap is obtained through the process known as tapping, which involves a series of operations to stimulate the flow of sap 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) (Atputharajah et al. 1986; Dalibard 1999; Ouoba et al. 2012; Hebbar et al. 2015). According to Santiago-Urbina and Ruíz-Terán (2014), the methods of tapping palm trees depend on the locality but in general, two methods are practiced. In the first method, the sap is obtained from a lived standing tree and the second, the tree is fallen or cut down before tapping. The sap of the palm tree contains essentially sugar (10–12% of saccharose), soluble proteins, amino acids, amides, organic acids, phenolic compounds, flavonoids, minerals and vitamins (Bassir 1968; Karamoko et al. 2012; Tiépma et al. 2013; Santiago-Urbina and Ruíz-Terán 2014; Hebbar et al. 2015, 2018; Tapsoba et al. 2016; Zongo et al. 2017).

The palm wine results from an alcoholic, lactic and acetic fermentation as reported by most of the authors (Atputharajah et al. 1986; Amoa-Awua et al. 2007; Ouoba et al. 2012; Santiago-Urbina et al. 2013; Santiago-Urbina and Ruíz-Terán 2014). The palm sap of the palm tree is a rich medium capable of supporting the growth of several types of microorganisms like high numbers of aerobic mesophilic bacteria, coliforms bacteria, *Bacillus*, *Staphylococcus*, lactic acid bacteria, acetic acid bacteria and yeasts (Amoa-Awua et al. 2007; Karamoko et al. 2012; Santiago-Urbina et al. 2013; Santiago-Urbina and Ruíz-Terán 2014; Tapsoba et al. 2014; Zongo et al. 2017). However, yeasts, lactic acid bacteria and acetic acid bacteria are considered to play the most important role in the palm wine production. Depending on the country, the palm wine is known under various names including *toddy* in India, *emu* or *ogoro* in Nigeria, *lambanog* in Philippines, *taberna* in Mexico, *panam culloo* in China, *matango*, *fitchuk*, and *mbu* in Cameroon, *doka* in Ghana, *bandji* in Burkina Faso and Côte d'Ivoire (Noll 2008; Santiago-Urbina and Ruíz-Terán 2014). In West Africa, palm wine holds high sociocultural and traditional values. Palm wine is consumed by more than 10 million people in West Africa, and in lower frequencies in Asia, South America, the Middle East and North Africa (Mbuagbaw and Noor-duyn 2012). It is commonly thought to be a very nourishing drink which promotes lactation, treats conjunctivitis, improves eyesight, and also serves as a sedative (Obahigbon and Osagie 2007; Mbuagbaw and Noor-duyn 2012).

In Burkina Faso, previous studies have reported the existence of palmyra palm stands. These are western stands

consisting mainly of the *Borassus akeassii* species whose fruit are green at maturity and those of *Borassus aethiopum* in the east with yellow fruit (Bayton and Ouédraogo 2009). In the western part of the country, the main forms of exploitation of the green type palm (*Borassus akeassii*) are sap extraction for palm wine production (Yaméogo et al. 2008; Tapsoba et al. 2014). The sap is collected using a sustainable technique that keeps palm tree alive and always exploitable (Ouoba et al. 2012). However, the eastern palm is also important but is not highly valued. In this eastern part of the country, the palm (*Borassus aethiopum* Mart.) grows wild and gives stands in several localities. According to Guinko (1984) in the East, the palm still had no consideration in the exploitation of his products, unlike that of the West until the 1980s. The main forms of farming are the pulling out of seedlings, the collection of ripe fruit to produce seedlings called *koboula* for consumption or sale. The exploitation of the sap as a drink as well as the many possibilities of use in basketry offered by the palm leaves are unknown (Zongo et al. 2018). Studies have been performed on various aspects of socioeconomic importance of *B. aethiopum* Mart. palm but no studies have been carried out on the nutritional, biochemical and microbiological composition of its sap for beverage production. For a better valorization of this palm, the knowledge of the different characteristics of the sap is therefore necessary. This study aimed to determine the nutritional, biochemical and microbiological composition of fresh palm sap from *Borassus aethiopum* Mart. sap in Burkina Faso.

Materials and methods

Sampling of *B. aethiopum* palm sap

Samples were collected from seven different palmyra trees during 4 days of the tapping with local tapper in the province of Koulpélogo (central-eastern region of Burkina Faso, 11°47'21"North; 0°21'25"West) on February 2017. The sap is collected from a lived standing tree as described by Ouoba et al. (2012). The collection of the sap was normally done twice a day (morning and afternoon), while the sap collected in the morning (5:00 AM) was used in this study. A total of 28 samples (seven per day) were collected during the 4 days of tapping. Two aliquotes of samples (500 mL) were collected on each palm tree every day in sterile plastic containers and immediately immersed in an isothermal box (ice boxes), and transported to the laboratory. Once in the laboratory, samples aliquoted were maintained at 4 °C and – 20 °C before microbiological and physico-chemical analysis respectively.

Biochemical characterization of palm sap

Determination of pH and total titrable acidity

Total titrable acidity (TTA) and pH was determined as described by Amoa-Awua et al. (2007). Total titrable acidity was determined by titrating samples against 0.1 M NaOH using 1% phenolphthalein as indicator and expressed as percentage of lactic acid. The pH of each sample was measured directly with 15 mL of sample using a pH-meter (METTLER TOLEDO) calibrated with standard buffer solutions of pH 4 and pH 7 at 25 °C. All measurements including were repeated three times.

Determination of Vitamin C and sucrose concentration

Vitamin C content was determined by titration method using DIP (2,6-Dichlorophénolindophénol) as described by AOAC (1990) and Sucrose concentration was measured by refractometry method using a refractometer (METTLER TOLEDO, B/0311) (Obahiagbon and Osagie 2007). The results were expressed as percentage °Brix. All measurements were carried out in triplicate.

Determination of total and reducing sugars

The total sugar (TS) content of the samples was measured by spectrophotometric assay using phenol sulphuric acid method as described by Fox and Robyt (1991). The reducing sugars (RS) concentration was determined by a colorimetric method with the 3,5-Dinitrosalicylic acid (DNS) reagent according to Miller (1958). All measurements were repeated three times.

Soluble proteins and total phenols determination

Total phenolic compounds (TP) was measured by spectrophotometry using the Folin–Ciocalteu reagent (Sigma Aldrich) according the procedure described by Singleton et al. (1999). The results were expressed as g/L of gallic acid. The soluble proteins content in each sample was carried out using the standard method of Bradford (1976) with bovine serum albumin (BSA) (Sigma Aldrich) as reference. All measurements including standards were carried out in triplicate.

Analysis of sugars and minerals contents by HPIC

Sugars (glucose, fructose, arabinose, xylose, galactose) and minerals (calcium, magnesium, potassium, ammonium) contents were determined by high-performance ionic chromatography (HPIC). Analyses and quantification of different sugars were performed by injecting 20 µL of filtered samples

diluted 1/300 in HPIC devise (Dionex ICS-3000) equipped with column CarboPac PA1 (4 × 250 mm) preceded by a guard column CarboPac PA1 (4 × 50 mm) and coupled to an Amperometer detector (ED 40). The running was carried out in isocratic mode with NaOH 200 mM as mobile phase at a flow rate of 1.0 mL/min at 30 °C. Sugars were identified and quantified by comparison of their retention times and peak areas with those of standards (external standard method). Minerals content was analysed using 10 µL of filtered samples of palm sap. The HPIC system (Dionex ICS-3000) was equipped with column Ion Pac CS16 (3 mm × 250 mm) preceded by a guard column Ion Pac CG16 (3 mm × 50 mm) provided with a suppressor (CERS—40 mA) upstream of the conductimetric detector. The mobile phase was Methane Sulfonic Acid 30 mM at a flow rate of 0.36 mL/min at 40 °C. The external calibration was done with standards (Fig. 1) in order to quantify the different ions in palm sap samples (Fig. 2). All the analyses were carried out in duplicate.

Analysis of organic acids, alcohol and glycerol by HPLC

Organic acids (pyruvate, succinate, lactate, acetate, propionate, citrate, tartrate, malate), alcohols (ethanol, isopropanol) and glycerol were quantified by high-performance liquid chromatography (HPLC). The samples of palm sap were first diluted 1/3, filtered with 0.20 µm pore size filter (Startorius CA, UK) and 10 µL of filtered samples were injected into HPLC system (Dionex Ultimate 3000) equipped with an ion-exchange column (Aminex HPX-87H, 300 × 7.8 mm, BIORAD, CA, USA) protected with a guard column (Aminex, 30 × 4.6 mm, BIORAD) and coupled to a RI detector and an UV detector ($\lambda = 210$ nm). The column was eluted in isocratic mode with a degassed mobile phase containing 5 mM H₂SO₄ at 50 °C at a flow rate of 0.5 mL/min. The organic acids standards, alcohol and glycerol were dissolved in ultra-pure water (MilliQ) and filtered in a 0.20 µm pore size filter at concentrations ranging to 0.5–10 g/L, and injected as the samples. Organic acids were detected in UV (210 nm), alcohol and glycerol by refractometry. Individual components were identified by comparing their retention times with certified standards, and concentrations were determined according to an external calibration methodology (Fig. 3). All the samples from each palm tree were analysed in duplicate.

Enumeration of micro-organisms in palm sap

Tenfold serial dilution of each palm sap sample was carried out using sterile peptone water (Biomerieux, France) and 0.1 mL of appropriate dilutions were poured into plates in

Fig. 1 Chromatograms of HPIC analysis of standard for minerals contents

Fig. 2 Chromatograms of HPIC analysis of fresh palm sap sample for minerals contents

duplicate on appropriate selective media then enumerated. The total aerobic mesophilic flora was carried out using Plate Count Agar (PCA) (Biokar, France) incubated aerobically at 30 °C for 48–72 h. Lactic acid bacteria (LAB)

were enumerated on De Man, Rogosa and Sharpe Agar (MRS) (Sigma Aldrich, USA) containing Nystatin (100 mg/L) which were aseptically added to suppress yeast growth and plates incubated in anaerobic condition at

Fig. 3 Chromatograms of HPLC analyses of standard for organic acids, ethanol and glycerol

30 °C for 72 h. Yeasts and moulds were enumerated on Sabouraud Chloramphenicol agar (Biokar, France) incubated at 30 °C for 72 h. Acetic acid bacteria (AAB) were enumerated by spread plating on YPM (yeast extract, peptone and mannitol) agar at 30 °C 72 h aerobically according to modified method described by Ouoba et al. (2012). To the YPM media, 20 mL/L of absolute ethanol was added, as well as Penicillin (12.5 mg/L) and Nystatin (100 mg/L) to inhibit growth of LAB and yeasts respectively. Enumeration of total and faecal coliforms was carried out using VRBL Agar (Violet Red Bile Lactose Agar) (Biokar, France) and plates were incubated aerobically for 24–48 h at 37 °C for total coliforms and 44 °C for faecal coliforms. Chapman's Agar medium (Sigma Aldrich, USA) were used for *Staphylococcus aureus* count at 37 °C for 48 h. *Bacillus* sp. were counted on Trypcase Soy Agar (TSA) (Biomerieux, France) using the thermal shock method. The sap samples were first heated in a Water bath at 90 °C for 10 min, and cooled using the classical technique. Plates were incubated aerobically at 37 °C for 48 h. All experiments were performed in duplicate. Counts were expressed as colony forming units per mL (cfu/mL).

Statistic analysis

The data obtained were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics 20 software program. The Tukey's multiple comparison test was used to determine significant differences at $p < 0.05$.

Results and discussion

Physico-chemical characteristics of fresh palm sap

The biochemical composition of *B. aethiopum* fresh palm sap collected during the first 4 days of tapping is shown in Tables 1, 2 and 3. The sap collected in this period was very sugary, tangy taste and contains less alcohol as reported previously by Karamoko et al. (2012) and Santiago-Urbina et al. (2013). The pH, total titrable acidity (TTA) and total sugars contents were respectively ranged from 4.70 ± 0.34 to 5.06 ± 0.83 , $0.32 \pm 0.06\%$ to $0.54 \pm 0.11\%$ w/v, 9.65 to 12.64% w/v (Table 1). The result showed a non-significant difference in the mean values of pH and TTA ($p > 0.05$) but the difference was significantly in total sugars values ($p < 0.05$). The Vitamin C, reducing sugars and sucrose concentration varied from 7.59 to 10.32% w/v; 2.51 to 3.27% w/v; 5.05 to 10.02% w/v ($p < 0.05$) (Table 1). Similar results were previously reported by Zongo et al. (2017) on fresh sap samples from *Borassus akeassii* with pH ranged from 4.05 ± 0.15 to 4.86 ± 0.04 ; total acidity from 0.2 ± 0.01 to $0.48 \pm 0.03\%$, total sugar content was between 8.00 ± 0.00 and $12.5 \pm 0.10\%$ and vitamin C content between 1.57 ± 0.22 and $11.01 \pm 0.22\%$. According to Kalaiyarasi et al. (2013) the pH of the fresh sap collected from the inflorescence of *Borassus flabellifer* (Palmyra palm) was 4.5 and for *Cocos nucifera* was 4.7 (similar to values obtained in this study). The average content of Vitamin C ($8.93 \pm 3.48\%$)

Table 1 Nutritional and biochemical composition of fresh sap from *B. aethiopum*

Parameters	Days of sap collection			
	1	2	3	4
pH	4.78 ± 0.29 ^a	4.81 ± 0.37 ^a	4.70 ± 0.34 ^a	5.06 ± 0.83 ^a
TTA (%w/v)	0.48 ± 0.11 ^a	0.54 ± 0.11 ^a	0.34 ± 0.07 ^c	0.32 ± 0.06 ^c
Total sugars (% w/v)	9.65 ± 2.97 ^a	11.09 ± 4.29 ^b	12.07 ± 3.23 ^c	12.62 ± 5.24 ^d
R sugars (%w/v)	2.51 ± 0.68 ^a	3.27 ± 1.00 ^b	3.11 ± 1.23 ^b	2.86 ± 1.83 ^a
Vitamin C (% w/v)	9.74 ± 4.38 ^a	8.11 ± 4.44 ^b	10.32 ± 1.45 ^c	7.56 ± 2.75 ^d
Sucrose (°Brix)	5.30 ± 1.66 ^a	5.05 ± 0.76 ^a	6.62 ± 1.74 ^b	10.02 ± 2.06 ^{cd}
Soluble proteins (g/L)	0.89 ± 0.01 ^a	0.90 ± 0.01 ^a	0.91 ± 0.01 ^a	0.90 ± 0.02 ^a
Total phenols (g/L)	0.08 ± 0.04 ^b	0.09 ± 0.06 ^b	0.08 ± 0.04 ^b	0.12 ± 0.09 ^b
Calcium (g/L)	0.79 ± 0.16 ^d	0.72 ± 0.20 ^d	0.60 ± 0.25 ^d	0.60 ± 0.39 ^d
Magnesium (g/L)	0.40 ± 0.22 ^c	0.32 ± 0.12 ^c	0.25 ± 0.13 ^c	0.26 ± 0.17 ^c
Potassium (g/L)	14.58 ± 4.88 ^a	13.56 ± 3.76 ^b	12.87 ± 5.28 ^c	12.02 ± 9.92 ^d
Ammonium (g/L)	0.83 ± 0.24 ^b	0.78 ± 0.25 ^b	1.16 ± 0.97 ^b	0.94 ± 0.94 ^b

Values are means of seven measurements for each sample taken at different day ± standard deviation. Samples were collected from seven different palmyra tree. Means values followed by the same letter in the same line are not significantly different ($p > 0.05$), according to Tukey's multiple comparison test. TTA: Total Titrable Acidity; R Sugars: Reducing Sugars

Table 2 Organic acids, alcohol and sugars content of fresh sap from *B. aethiopum*

Parameters (g/L)	Days of palm sap collection			
	1	2	3	4
Lactate	2.44 ± 0.66 ^a	2.39 ± 0.69 ^a	1.92 ± 0.83 ^c	2.91 ± 1.08 ^d
Acetate	0.01 ± 0.00 ^{bd}	0.01 ± 0.00 ^{bd}	0.01 ± 0.01 ^{bd}	0.02 ± 0.01 ^{bd}
Pyruvate	0.05 ± 0.02 ^c	0.04 ± 0.02 ^c	0.04 ± 0.03 ^c	0.06 ± 0.05 ^c
Propionate	0.04 ± 0.04 ^b	0.05 ± 0.04 ^b	0.05 ± 0.04 ^b	0.11 ± 0.05 ^c
Succinate	2.06 ± 0.81 ^c	2.33 ± 1.03 ^c	2.28 ± 1.17 ^c	3.28 ± 2.35 ^b
Citrate	0.05 ± 0.02 ^a	0.16 ± 0.08 ^b	0.17 ± 0.08 ^b	0.22 ± 0.16 ^c
Tartrate	0.06 ± 0.05 ^d	0.07 ± 0.06 ^d	0.09 ± 0.07 ^d	0.11 ± 0.16 ^d
Malate	0.11 ± 0.28 ^b	0.11 ± 0.28 ^b	0.28 ± 0.37 ^b	0.19 ± 0.34 ^b
Ethanol	0.08 ± 0.02 ^a	0.09 ± 0.03 ^a	0.09 ± 0.02 ^a	0.24 ± 0.25 ^a
Isopropanol	nd	nd	nd	nd
Glycerol	nd	nd	nd	0.02 ± 0.04 ^d
Arabinose	0.005 ± 0.01 ^b	0.007 ± 0.01 ^b	0.005 ± 0.001 ^b	0.004 ± 0.001 ^b
Glucose	4.92 ± 3.41 ^a	3.23 ± 1.70 ^b	4.78 ± 2.91 ^a	7.03 ± 4.41 ^d
Fructose	6.72 ± 3.58 ^c	6.89 ± 3.54 ^c	6.90 ± 4.59 ^c	7.86 ± 5.90 ^c
Xylose	nd	nd	nd	nd
Galactose	nd	nd	nd	nd

Values are means of seven measurements for each sample taken at different day ± standard deviation. Samples were collected from seven different palmyra tree. Means values followed by the same letter in the same line are not significantly different ($p > 0.05$), according to Tukey's multiple comparison test. nd: not detected

correlated with the values obtained in Nigeria by Dioha et al. (2009) on the fresh sap of the *Raphia* palm which averaged 8.8 and 9.01% (w/v). The high percentage of total acidity and low pH indicates the initial fermentation step of palm sap during collection (Karamoko et al. 2016). The process of tapping of *B. aethiopum* palm tree can be considered as fed-batch fermentation since every 12 h and

fresh sap is the sap accumulated from 6:00 PM to 6:00 AM as reported in our former studies (Zongo et al. 2017) and study performed by Santiago-Urbina et al. (2013). The results show that sucrose is the main sugar in palm sap from *B. aethiopum*. The similar values of total sugars, reducing sugars was previously reported in sap from other palm trees species. Indeed, Naknean et al. (2010) reported

Table 3 Microbiological composition of fresh sap from *B. aethiopum*

Parameters (cfu/mL)	Days of palm sap collection			
	1	2	3	4
Total aerobic flora	$1.36 \pm 1.18 \times 10^{8a}$	$1.05 \pm 0.85 \times 10^{8b}$	$1.47 \pm 1.25 \times 10^{8a}$	$1.05 \pm 0.83 \times 10^{8b}$
Yeasts	$1.35 \pm 1.68 \times 10^{6a}$	$1.00 \pm 1.47 \times 10^{6a}$	$1.90 \pm 3.10 \times 10^{5b}$	$3.60 \pm 4.83 \times 10^{5c}$
L AB	$2.46 \pm 1.17 \times 10^{7c}$	$2.10 \pm 1.34 \times 10^{7c}$	$2.69 \pm 2.01 \times 10^{7c}$	$1.79 \pm 3.94 \times 10^{6b}$
AAB	$6.85 \pm 3.21 \times 10^{5a}$	$1.55 \pm 1.23 \times 10^{5b}$	$2.07 \pm 1.84 \times 10^{5c}$	$1.61 \pm 1.51 \times 10^{4b}$
Faecal coliforms	nd	nd	nd	nd
Total coliforms	$7.04 \pm 9.65 \times 10^{2a}$	nd	nd	nd
<i>S. aureus</i>	$2.99 \pm 7.90 \times 10^{2a}$	nd	nd	nd
<i>Bacillus</i> sp.	nd	$3.73 \pm 6.04 \times 10^{3b}$	$8.01 \pm 8.97 \times 10^{2c}$	nd

Values are means of seven measurements for each sample taken at different day $\pm$ standard deviation. Samples were collected from seven different palmyra tree. Means values followed by the same letter in the same line are not significantly different ($p > 0.05$), according to Tukey's multiple comparison test. nd: not detected

LAB lactic acid bacteria, AAB acetic acid bacteria, *S. aureus* *Staphylococcus aureus*, cfu colony forming units

sucrose contains about 9.29–17.44% in the sap samples from *Borassus flabellifer* during the first days of tapping. Santiago-Urbina et al. (2013) found an initial concentration of sucrose in the palm wine between 5.72 and 11.36% at the first 4 days during the 15 days of tapping of *Acrocomia aculeata*. Meanwhile Karamoko et al. (2012, 2016) reported an initial concentration of total sugars of about 50% w/v the first day, this sugar concentration decreased through the tapping process about 21% for the first week in the palm wine of *Elaeis guineensis*. The variation in the sugar composition through the tapping process can be explained by different factors, such as, different palm tree species, time of collection of the palm wine samples and different ways of make the tapping process (Santiago-Urbina and Ruíz-Terán 2014).

Soluble proteins (0.90 ± 0.01 g/L) and total phenols (0.09 ± 0.06 g/L) was found in low concentration in palmyra fresh sap (Tables 1 and 4) and no difference were observed between means values of sample collected at different days ($p > 0.05$). Minerals such as Calcium, Magnesium, Potassium and Ammonium were found in *B. aethiopum* palm sap at average concentrations of 0.68 ± 0.26 , 0.31 ± 0.17 , 13.26 ± 6.12 and 0.93 ± 0.67 g/L respectively (Table 4). There was significant difference in Potassium content but no difference in other minerals content (Table 1). The protein content was consistent because it is not degraded or hydrolysed by microbial activity to its constituent (Obahiagbon and Osagie 2007). The low content in proteins and total phenols was observed by some author (Obahiagbon and Osagie 2007; Ogbonna et al. 2013; Hebbbar et al. 2018). However, Tiépma et al. (2013) reported higher protein average of fresh sap from *Raphia* (6.69 ± 1.41 g/L). According to the same author this could be due to the influence of climatic and soil factors that influence the nutritive composition of palm sap.

Magnesium and Phosphorus were the most abundant minerals, in concentrations of 32.0 and 59.75 mg/L, respectively, in palm sap from *Elaeis guineensis* (Ezeagu and Fafunso 2003). While, in palm sap from *Phoenix dactylifera* in addition to Phosphorus and Magnesium (41.49 and 330 mg/100 g of dry matter basis, respectively), the Potassium is also reported as the most abundant element (522.92 mg/100 g of dry matter basis).

The results of sugars, organic acids and alcohol content of palm sap collected during 4 days are presented in Table 2. Different sugars including arabinose, glucose fructose were detected in palm sap at concentration varied from 0.004 to 0.007 g/L, 3.23 to 7.03 g/L and 6.72 to 7.86 g/L respectively (Table 2). According to Naknean et al. (2010) glucose content of *B. flabellifer* palm sap was between 0.50 and 1.85%, and fructose in a range of 0.50 and 1.81% in the first days of tapping. Xylose and galactose were not detected in *B. aethiopum* palm sap. In *Raphia* palm sap, sucrose, maltose, glucose and fructose were present in the first day of tapping; while xylose and cellobiose were detected on the middle tapping period; and galacturonic acid, arabinose and rhamnose appeared irregularly for a few days (Santiago-Urbina and Ruíz-Terán 2014). The ethanol content was low in palm sap and ranged from 0.08 to 0.24 g/L (0.01–0.03% v/v) ($p > 0.05$). Isopropanol was not detected in *B. aethiopum* fresh palm sap and glycerol was present only in sap samples collected on the fourth day at low concentration (0.02 g/L). Presence of glucose and fructose in palm fresh sap is due to result of the microbial metabolic activity of yeasts (inversion of sucrose). Similar concentration of ethanol was reported previously by Karamoko et al. (2012; 2016) and Santiago-Urbina et al. (2013) during the first 4 days of tapping (15–28 days). The low concentration or absence of glycerol in palm sap can be explained by insignificant level of initial fermentation during the tapping. Eight organic acids were

Table 4 Means values of biochemical and microbiological content of fresh sap from *B. aethiopum*

Biochemical parameters	Mean values	Microbiological count (cfu/mL)	Means values
pH	4.84 ± 0.50	Total aerobic flora	1.23 ± 1.01 × 10 ⁸
TTA (%w/v)	0.42 ± 0.13	Yeasts	7.27 ± 1.19 × 10 ⁵
Total sugars (% w/v)	11.36 ± 3.97	LAB	1.86 ± 1.63 × 10 ⁷
Reducing sugars (%w/v)	2.93 ± 1.22	AAB	3.75 ± 0.75 × 10 ⁵
Vitamin C (% w/v)	8.93 ± 3.48	Faecal coliforms	nd
Sucrose (% °Brix)	6.75 ± 2.53	Total coliforms	2.53 ± 6.65 × 10 ²
Soluble proteins (g/L)	0.90 ± 0.01	<i>S. aureus</i>	1.71 ± 6.31 × 10 ²
Total phenols (g/L)	0.09 ± 0.06	<i>Bacillus</i> sp.	1.20 ± 3.26 × 10 ³
Calcium (g/L)	0.68 ± 0.26		
Magnesium (g/L)	0.31 ± 0.17		
Potassium (g/L)	13.26 ± 6.12		
Ammonium (g/L)	0.93 ± 0.67		
Lactate (g/L)	2.41 ± 0.86		
Acetate (g/L)	0.01 ± 0.006		
Pyruvate (g/L)	0.05 ± 0.03		
Propionate (g/L)	0.07 ± 0.04		
Succinate (g/L)	2.49 ± 1.46		
Citrate (g/L)	0.19 ± 0.11		
Tartrate (g/L)	0.08 ± 0.09		
Malate (g/L)	0.17 ± 0.31		
Ethanol (g/L)	0.13 ± 0.12		
Isopropanol (g/L)	nd		
Glycerol (g/L)	0.04 ± 0.02		
Arabinose (g/L)	0.005 ± 0.004		
Glucose (g/L)	4.99 ± 3.36		
Fructose (g/L)	7.09 ± 4.27		
Xylose (g/L)	nd		
Galactose (g/L)	nd		

Values are means of different sample taken during 4 day ± standard deviation. Samples were collected from seven different palmyra tree every day

TTA total titrable acidity, nd not detected, LAB lactic acid bacteria, AAB acetic acid bacteria, *S. aureus* *Staphylococcus aureus*, cfu colony forming units

detected in palm sap including lactate (1.92–2.91 g/L), acetate (0.01–0.02 g/L), pyruvate (0.04–0.06 g/L), propionate (0.04–0.11 g/L), succinate (2.06–3.28 g/L), citrate (0.05–0.22 g/L), tartrate (0.06–0.11 g/L) and malate (0.11–0.28 g/L). Some of the organic acid produced is a result of the microbial metabolic activity. Lactic and acetic acids are produced throughout the tapping process by the lactic and acetic acid bacteria respectively, however the tartaric and malic acids are considered as native to the exudates (Karamoko et al. 2012). In *Elaeis guineensis* palm sap the percentage of lactic acid after the first few days of tapping was between 0.1 and 0.3% as reported by Amoa-Awua et al. (2007). The lactic acid concentration of *B. aethiopum* sap varied in the range of 0.26–0.48%, through the tapping process similarly in the palm wine of *Acrocomia aculeate* and fresh sap from *B. akeassii* (Santiago-Urbina et al. 2013; Zongo et al. 2017). Study performed by Karamoko et al. (2012) in palm

wine of *Elaeis guineensis* from Ivory Coast has been reported in addition to lactic (0.015–0.079%) and acetic (0.01–0.077%) acids, other organic acids such as oxalic (0.01–0.04%), citric (0.005–0.04%), tartaric (0.031–0.04%), malic (0.05–0.1%), ascorbic (0.005–0.024%) and fumaric (0.001–0.003%) acids. The high concentration of succinate and lactate in our samples can explain the low of pH of fresh palm sap collected during first days of tapping.

Microbiological characteristics of *B. aethiopum* palm sap

The microbiological analyses including count of total aerobic flora, yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB), faecal and total coliforms, *S. aureus* and *Bacillus* sp. of palm sap samples collected is presented in Table 3. Our results showed high loads of total aerobic

flora count varied from 1.05 to 1.47×10^8 cfu/mL with average value of $1.23 \pm 1.01 \times 10^8$ cfu/mL (Table 4). Yeasts, LAB and AAB populations were between 1.90×10^5 and 1.35×10^6 cfu/mL, 1.79×10^6 and 2.69×10^7 cfu/mL, 1.61×10^4 and 6.85×10^5 cfu/mL. Faecal coliforms were not detected in palm sap samples but total coliforms (7.4×10^2 cfu/mL) was found only in sap samples collected on the first day. *Staphylococcus aureus* was also detected in this sample at low number of 2.99×10^2 cfu/mL. *Bacillus* sp. was present only in sap tapped the second days (3.73×10^3 cfu/mL) and third days (8.01×10^2 cfu/mL). The results show the presence of high number of total aerobic population, LAB, yeasts and AAB. Coliforms, *S. aureus* and *Bacillus* were found in few samples of fresh palm sap. The sap of the palm tree has been shown to be a rich medium capable of supporting the growth of various types of micro-organisms, as high numbers of aerobic mesophiles, lactic acid bacteria, yeasts, *Enterococci*, coliforms, *Bacillus*, *Staphylococcus* and sulphite reducing bacteria found in palm wine during tapping (Karamoko et al. 2012, 2016; Tapsoba et al. 2014; Zongo et al. 2017). Similar results have been reported by Karamoko et al. (2016) who showed high loads of microorganisms already the first day in palm wines of both varieties (*Dura* and *Tenera*). These micro-organisms in fresh palm wine might have originated from those that colonized 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. Some of them were also brought by the tapper, tapping materials, insects attracted by the sap sugar and environment (Faparusi 1973; Karamoko et al. 2016). In a former work, Zongo et al. (2017) reported on fresh sap from *B. akeassii* total mesophilic flora count from 1.6×10^7 to 5.6×10^8 cfu/mL, yeasts from 1.2×10^6 to 2.5×10^8 cfu/mL, LAB was between 5.7×10^5 and 1.8×10^7 cfu/mL and AAB between 1.3×10^5 and 3.2×10^6 cfu/mL. Total and faecal coliforms, *Staphylococcus aureus* and *Bacillus* sp. were previously found in palm sap from *B. akeassii* (Tapsoba et al. 2014; Zongo et al. 2017). *Bacillus* spore are present in the environment and occasionally contaminate the sap during tapping.

Conclusion

The nutritional, biochemical and microbiological characteristics of fresh sap from palmyra *Borassus aethiopum* collected during the fourth days of tapping has been reported in this study. The palm sap from *B. aethiopum* of Burkina was sugary, contained sugars mainly sucrose, minerals with high Potassium content, organic acids,

Vitamin C, low proteins and ethanol. Yeasts, LAB and AAB were the main microorganisms. Another study could help to elucidate the microbiological and biochemical changes which occur in palm sap during the normal tapping period (3 or 4 weeks).

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