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MiniReview

Microbiology and biochemistry of traditional palm wine produced around the world

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Abstract

Palm wine is an alcoholic drink obtained by the natural fermentation of the sap of various types of palm trees; this beverage is produced and consumed in several tropical regions of the world. It plays an important role in traditional practices as an alcoholic beverage, so it is important to determine the physicochemical characteristics and microbiological aspects of its fermentation. During the tapping process of the palm wine production, lactic-alcoholic-acetic fermentation is conducted by the lactic acid bacteria (LAB), yeast and acetic acid bacteria (AAB), respectively. *Saccharomyces cerevisiae* is the main microorganism that has been identified as the responsible of the alcoholic fermentation and odorants production. On the other hand, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* have been reported as the predominant LAB. While, *Gluconobacter* and *Acetobacter* genera are the predominant AAB. The palm wine composition depends of the stage of tapping period in which it is consumed. Thus, ethanol concentration varies in the range of 1 to 6%, lactic acid concentration varies in the range of 0.1 to 0.5%, and acetic acid percentage varies between 0.02 and 0.4%. The principal components responsible for the odorants of the palm wine are higher alcohols, esters, acids, aldehydes and ketones.

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 (Okafor, 1978), such as those listed in the Table 1. 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 such as this shown in the Table 1. The sap of the palm trees, which is originally sweet (Atputharajah *et al.*, 1986; Amoa-Awua *et al.*, 2007; Naknean *et al*., 2010; Santiago-Urbina *et al.*, 2013) 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 (Amoa-Awua *et al*., 2007; Stringini *et al*., 2009; Ouoba *et al.*, 2012; Santiago-Urbina *et al.*, 2013). Palm sap fermentation has been reported to be an alcoholic, lactic and acetic fermentation (Okafor, 1978; Atputharajah *et al.*, 1986; Amoa-Awua *et al.*, 2007; Stringini *et al*., 2009; Ouoba *et al.*, 2012; Santiago-Urbina *et al*., 2013), therefore, yeasts, lactic acid bacteria and acetic acid bacteria are considered to play the most important role in the palm wine production.

The palm sap is obtained through the process known as tapping, which involves a series of operations to stimulate the flow of sap (Atputharajah *et al.*, 1986), 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) (Ouoba *et al*., 2012). 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 (Figure 1), this process implicates climbing very tall palm trees, and perforate the trunk in the top of the tree for Bandji production (Ouoba *et al.*, 2012), or cutting into the end of spadix from the tender inflorescence of the palm tree (inflorescence tapping) for Toddy production (Okafor, 1978; Atputharajah *et al*., 1986; Mbuagbaw and Noorduyn, 2012). In the second method the tree is felled or cut down before tapping (stem tapping), such as palm wine from Ghana and Taberna production (Figure 2). 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 (Balick, 1990; Amoa-Awua *et al*., 2007; Santiago-Urbina *et al.*, 2013). 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 (Amoa-Awua *et al*., 2007; Naknean *et al.*,

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Table 1. Palm wine names according to the country of origin

Palm tree	Traditional name	Country	References
Borassus akeassii	Bandji	Burkina Faso	Ouoba *et al*., 2012
Acrocomia aculeata	Taberna	Mexico	Santiago-Urbina *et al*., 2013
Cocos nucifera	Mnazi	Kenya	Kadere *et al*., 2008
Cocos nucifera	Toddy	Sri Lanka	Atputharajah *et al*., 1986
Cocos nucifera	Tuba	Philippines	Atputharajah *et al*., 1986
Cocos nucifera	Tuak	Indonesia	Atputharajah *et al*., 1986
Elaeis guineensis	Mimbo	Cameroon	Jepersen, 2003
Raphia hookeri	Emu	Nigeria	Jepersen, 2003
Elaeis guineensis	Palm wine	Ghana	Amoa-Awua *et al*., 2007
Elaeis guineensis	Palm wine	Cameroon	Stringini *et al*., 2009
Borassus flabellifer	Palmyrah toddy	Sri Lanka	Theivendirarajah and Chrystopher, 1987
Phoenix sylvestris	Toddy	India	Shamala and Sreekantiah, 1988
Borassus aethiopum	Palm wine	Republic of Guinea	Sambou *et al*., 2002
Nypa fruticans	Toddy	Malasya	Päivoké, 1985;Nur Aimi *et al*., 2013

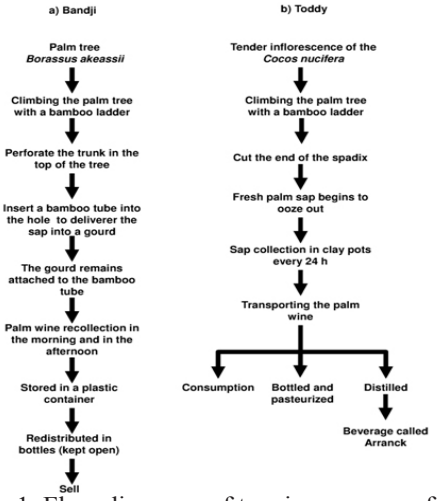

Figure 1. Flow diagrams of tapping process of live standing palm tree for Bandji (a) and Toddy (b) production

2010; Karamoko *et al.*, 2012; Santiago-Urbina *et al*., 2013). Palm wine from Ghana is distilled for gin production called Akpeteshie (Amoa-Awua *et al*., 2007); similarly, Toddy is also distilled to produce the spirit known as Arrack (Atputharajah *et al.*, 1986). 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 (Amoa-Awua *et al*., 2007).

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 (Olasupo and Obayori, 2003; Chandrasekhar *et al*., 2012). The aim of this review is to describe the biochemical and microbiological aspects of the traditional palm wine produced in some regions of Africa, Asia and America.

Biochemical constituents of palm wine

The main characteristics of palm wine are whitish color, effervescent, sweet and acid taste. Palm wine is produced by natural lactic-alcoholic-acetic fermentation of the sugary sap of palm tree (Okafor, 1978; Atputharajah *et al.*, 1986; Amoa-

Figure 2. Flow diagrams of tapping process of felled palm tree for the palm wine from Ghana (a) and Taberna (b) production

Awua *et al.*, 2007; Stringini *et al*., 2009; Ouoba *et al*., 2012; Santiago-Urbina *et al*., 2013), it consist of an initial lactic acid fermentation, a middle alcoholic fermentation and a final acetic fermentation (Atputharajah *et al*., 1986; Amoa-Awua *et al*., 2007). At each stage the microbial activity helping the activity of the microorganism in the next stage (Atputharajah *et al.*, 1986), i.e. members of the consortium communicative one another with trading metabolites. As a result each individual cell in the mixture responds to the presence of others in the consortium (Smid and Lacroix, 2013). An increase in the total acidity and decrease in the pH by the production of organic acids, probably enhance the growth and invertase activity of the yeasts (Atputharajah *et al.*, 1986; Naknean *et al*., 2010), and the ethanol produced by the yeasts serves as a substrate for the acetic acid production by the acetic acid bacteria (Atputharajah *et al*., 1986; Amoa-Awua *et al*., 2007).

The palm sap is transparent, with a sugar content in a range of 10-18% w/v approximately (Atputharajah *et al*., 1986; Eze and Uzoechi, 1988, Amoa-Awua *et al*., 2007; Naknean *et al.*, 2010; Santiago-Urbina *et al*., 2013), which is mainly sucrose (Eze and Uzoechi, 1988; Amoa-Awua *et al*., 2007; Obahiagbon and Oviasogie, 2007; Ben Thabet *et al.*, 2009; Naknean *et al.*, 2010; Santiago-Urbina *et al.*, 2013), for example, Ben Thabet *et al.* (2009) reported that the proportion of the sugars of the sap of *Phoenix dactylifera* consist of 95.27% of sucrose, 2.51% glucose and 1.61% fructose (dry matter basis). Palm sap has a pH near neutral, approximately 7 to 7.4; this value indicates the freshness of palm sap (Ezeagu *et al.*, 2003; Amoa-Awua *et al*., 2007; Karamoko *et al*., 2012; Santiago-Urbina *et al*., 2013). During the tapping, palm sap changes the consistency and the color from transparent to whitish (Naknean *et al.*, 2010), due

to the lactic acid bacteria produce a gum probably dextrans (Alcántara-Hernández *et al.*, 2010; Naknean *et al*., 2010). In addition a heavy suspension of yeast and bacteria also gives a milky-white appearance (Lasekan *et al*., 2007). The composition of the palm wine depends of the state of the fermentation at which the wine is consumed.

Sugars identified and their concentrations in palm wine

In the first days of tapping the palm wine is very sugary and does not contain substantial concentration of alcohol (Ezeagu *et al*., 2003; Amoa-Awua *et al.*, 2007; Karamoko *et al.*, 2012; Santiago-Urbina *et al*., 2013). In Raphia palm wine, sucrose, maltose, glucose and fructose sugars were present in the first day of tapping (Faparusi and Bassir, 1972; Faparusi, 1981); while xylose and cellobiose were detected on the middle tapping period; and galacturonic acid, arabinose and rhamnose sugars appeared irregularly for a few days (Faparusi, 1981). The palm wine of *Borassus flabellifer* contains about to 9.29 to 17.44% of sucrose, glucose content between 0.50 and 1.85%, and fructose in a range of 0.50 and 1.81% (Naknean *et al*., 2010) in the first samples of tapping. On the other hand, in palm wine of *Elaeis guineensis* was found that the total sugars in the samples dropped from initial concentrations of about 14% to about 11% by the fourth day of tapping, and subsequently between 12% and 8%, this variation was observed during the 35 days of tapping period (Amoa-Awua *et al.*, 2007). This sugar concentration is maintained by the continual oozing of the sweet sap (Amoa-Awua *et al*., 2007). Meanwhile Karamoko *et al.* (2012) reported an initial concentration of total sugars of about 50% w/v, this sugar concentration decreased through the tapping process about 21% for the first week; then, 7.9%, 6.4% and 5.4% for the second, third and fourth week, respectively, in the palm wine of *Elaeis guineensis*. The decrease in sugar content is a clear indication that a large portion of the sugars is fermented especially during the early stages of tapping. On the other hand, during the 15 days of tapping of *Acrocomia aculeata* was found an initial concentration of sucrose in the palm wine of 11.36%, this concentration dropped through-out the tapping process to 0.22%, as a result of the microbial metabolic activity (Santiago-Urbina *et al.*, 2013). In addition, this reduction in sugars can be also caused by the depletion of sugar reserve in the palm tree due to the fact that the trees are felled, and the leaves are cut off, hence the palm does not realize photosynthesis and does not produces sugar (Santiago-Urbina *et al*., 2013). 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, different ways of make the tapping process.

pH and organic acids concentration in palm wine

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 (Eze and Uzoechi, 1988; Ezeagu *et al.*, 2003; Amoa-Awua *et al*., 2007; Karamoko *et al*., 2012; Santiago-Urbina *et al*., 2013). 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 (Atputharajah *et al.*, 1986; Amoa-Awua *et al*., 2007; Stringini *et al.*, 2009; Ouoba *et al*., 2012; Santiago-Urbina *et al*., 2013). 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% (Amoa-Awua *et al.*, 2007), 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 (Santiago-Urbina *et al.*, 2013). 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 (Eze and Uzoechi, 1988; Amoa-Awua *et al.*, 2007; Santiago-Urbina *et al*., 2013). The second organic acid produced in the palm wine is the acetic acid, with a concentration of about 0.02 to 0.4% (Amoa-Awua *et al.*, 2007; Ouoba *et al.*, 2012; Santiago-Urbina *et al*., 2013). According to Faparusi (1973), this acetic acid concentration in the palm wine are acceptable by the consumers but, when the concentration exceeds 0.6% the beverage becomes unacceptable. Thus, the acetic acid is considered as part of the aroma of palm wine (Amoa-Awua *et a*l., 2007). Moreover, in palm wine of *Elaeis guineensis* from Ivory Coast in addition to lactic (0.015-0.079%) and acetic (0.01-0.077%) acids, others organic acids have been reported 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 (Karamoko *et al*., 2012). 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).

Ethanol concentrations in palm wine

The concentration of ethanol in palm wine

during the tapping depends of several factors such as the presence of microorganisms responsible for the alcoholic fermentation, composition of sap, species of palm tree, environmental conditions e.g. temperature and velocity of the wind (Santiago-Urbina *et al*., 2013), type of tapping, flow rate of the sap, and the time in which the palm wine samples are taken, and time between collection and analysis of the samples. Thus, in palm wine of *Elaeis guineensis*, the ethanol concentration fluctuates, the palm wine collected between the day contains less alcohol (1.4% and 2.82%) than the palm wine which has been accumulated overnight (3.24% and 4.75% and even over 6% in few cases) (Amoa-Awua *et al*., 2007), it is probably due to the microbiota that has colonized the walls of the receptacle is removed during tapping in the morning, when the tapper cuts a thin slice off the walls of the receptacle or canoe, which reduces the microbial load (Amoa-Awua *et al*., 2007; Santiago-Urbina *et al.*, 2013) therefore reducing the ethanol production. On the other hand, the palm wine which is stored has higher levels of ethanol than palm wine recollected directly from the tree. e.g. Amoa-Awua *et al.* (2007) reported alcohol content about 8.16% in 24 h. Moreover, in Taberna, Alcántara Hernández *et al.* (2010) reported a percentage of ethanol about 10.80% in 60 h of storage (*in vitro* fermentation); this concentration is higher than 4.78% ethanol content reported in an *in vivo* fermentation of Taberna (Santiago-Urbina *et al.*, 2013). The variation aforementioned in the ethanol content is due to that in an *in vivo* fermentation there is a constant dilution of the metabolites produced as the flow of sap is accumulated in the receptacle or canoe, this process is considered as fed-batch fermentation (Santiago-Urbina *et al*., 2013).

The ethanol production is attributed to several microorganisms, such as *Saccharomyces cerevisiae* (Amoa-Awua *et al*., 2007; Stringini *et al.*, 2009; Ouoba *et al*., 2012) and *Saccharomyces chevalieri* (Atputharajah *et al.*, 1986). In addition, *Zymomonas mobilis* also have been reported as responsible in the ethanol production (Obire, 2005; Alcántara-Hernández *et al.*, 2010).

Minerals and trace elements present in the palm wine

Macro and micro mineral elements content have been reported in the palm wine. Where, Magnesium and Phosphorus were the most abundant minerals, in concentrations of 32.0 and 59.75 mg/L, respectively, in palm wine from *Elaeis guineensis* (Ezeagu *et al*., 2003). Cadmium, Plumb and Cobalt were detected in low levels ≤ 0.1 ppm. Copper, Manganese, Zinc, and Calcium have also been reported in concentrations of 3.78, 2.63, 1.26, 1.95 and 0.48 mg/L, respectively (Ezeagu *et al*., 2003). While, in palm wine from *Phoenix dactylifera* in addition to P and Mg (41.49 and 330 mg/100 g of dry matter basis, respectively) the Potassium is also reported (522.92 mg/100 g of dry matter basis), and it is the most abundant element. Others mineral elements, in decreasing order are Ca, Na, Fe, Cu, and Zn (Ben Thabet *et al.*, 2009).

Odorants of palm wine

During the fermentation of palm wine, various organic acids and alcohols are produced thanks to microbial metabolic activity, and do not correspond to metabolites of the palm tree because the major components are not present in the fresh palm sap; all the metabolites play an important role in palm wine characteristic aroma. For instance, in the palm wine of *Elaeis guineensis* has been identified 73 compounds. There are 23 esters, 11 carbonyls, 14 alcohols and phenols, 10 acids, 5 sulphur compounds, 3 terpenes, 2 hydrocarbons, 2 acetals, 2 nitrogen compounds and 1 lactone. The higher alcohols and esters (more than 70% of total volatiles), as well as acids, aldehydes and ketones are the major groups of compounds found, and they are considered the main volatile components responsible for the palm wine aroma (Uzochukwu *et al*., 1997). Similarly, Lasekan *et al.* (2007) reported that the volatile profile is largely dominated by alcoholic substances such as ethanol, 2-3-methylbutanol and 2-phenylethanol, as well as acetic acid. In addition, methyl butanoate, acetoin, diethyl succinate, ethyl lactate have also been reported, and several acids such as isobutanoic acid, 2-methyl butanoic acid, 3-methylpentanoic acid, phenylacetic acid and pentanoic acid. The most potent odorants in palm wine are earthy-smelling: 3-isobutyl-2metoxipyrazine, buttery-smelling acetoin, fruity ethylhexanoate, 3-methylbutylacetate and popcorn-smelling 2-acetyl-1-pyrroline. Furthermore, Nur Aimi *et al.* (2013) identified the volatile compounds responsible for the aroma in fermented nipa sap (*Nypa fruticans*), which consists of alcohols such as ethanol, 1-propanol, 2-methylpropanol, 2-methylbutanol; acetoin, acetic acid, diacetyl, and esters such as ethyl acetate and ethyl lactate.

Microbial communities in palm wine

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, lactic acid bacteria, acetic acid bacteria and yeasts (Amoa-Awua *et al.*, 2007; Karamoko *et al.*, 2012; Santiago-Urbina

Table 2. Microorganisms identified in several types of palm wine

Microorganisms	Bandji[1]	Taberna[2]	Palm wine from Cameroon[3]	Palm wine from Ghana[4]	Palm wine from Nigeria[5]	Toddy[6]	Toddy[7]
Saccharomyces cerevisiae	x		x	x	x		x
Saccharomyces ludwigii			x			x	
Saccharomyces bayanus					x		
Saccharomyces uvarum					x		
Saccharomyces bailii						x	
Saccharomyces chevalieri						x	
Candida tropicalis	x				x	x	
Candida pararugosa	x						
Candida quercitrusa	x						
Candida parapsilopsis			x			x	
Candida fermentati			x				
Candida krusei				x			
Candida utilis					x		
Candida guilliermondii						x	
Candida valida						x	
Pichia etchellsii						x	
Pichia farinosa						x	
Pichia membranaefaciens						x	
Pichia ohmeri						x	
Pichia guilliermondii						x	
Pichia fermentans			x				
Zygosaccharomyces bailii			x				
Schizosaccharomyces pombe	x				x	x	x
Issatchenkia orientalis	x						
Arthroascus fermentans	x						
Trichosporon asahii	x						
Hanseniaspora uvarum	x		x				
Kodamaea ohmeri	x						
Trichosporom asteroides	x						
Trigonopsis variabilis	x						
Galactomyces geotrichum	x						
Kloeckera apiculata				x			
Kloeckera javanica						x	
Rhodotorula glutinis						x	
Kluyveromyces lactis					x		
Lactobacillus plantarum	x			x			
Lactobacillus fermentum	x						
Lactobacillus paracasei	x						
Lactobacillus nageii	x	x					
Lactobacillus sucicola		x					
Lactobacillus sp		x				x	
Leuconostoc mesenteroides	x			x			
Leuconostoc dextranicum							x
Leuconostoc sp						x	
Fructobacillus durionis	x	x					
Fructobacillus fructosus		x					
Streptococcmitis	x						
Acetobacter indonesiensis	x						
Acetobacter tropicalis	x						
Acetobacter estunensis	x						
Acetobacter ghanensis	x						
Acetobacter aceti	x					x	x
Acetobacter lovaniensis	x						
Acetobacter orientalis	x						
Acetobacter pasteurianus	x	x					
Acetobacter cerevisiae	x						
Acetobacter rancens							x
Acetobacter suboxydans							x
Acetobacter sp				x			
Gluconobacter oxydans	x						
Gluconobacter saccharivorans	x						
Gluconobacter sp				x			
Zymomonas mobilis		x					

1: Ouoba *et al*., 2012; 2: Alcántara-Hernández *et al*., 2010; 3: Stringini *et al*., 2009; 4: Amoa-Awua *et al*., 2007; 5: Ezeronye and Ekerentugba, 2001; 6: Atputharajah *et al*., 1986; 7: Shamala and Sreekantiah, 1988. x: Identified microorganisms

et al., 2013). Yeast populations have been reported in the palm wine in concentrations of about 10^4 to 10^7 cfu/mL, while, LAB ranging between 10^7 and 10^9 CFU/mL, AAB from concentrations of 10^5 to about 10^8 cfu/mL, total aerobic mesophiles ranging between 10^6 and 10^9 cfu/mL, and total coliforms have been reported in a range of 10^3 to 10^7 cfu/mL (Atputharajah *et al*., 1986; Amoa-Awua *et al*., 2007; Stringini *et al*., 2009; Karamoko *et al*., 2012; Ouoba *et al*., 2012; Santiago-Urbina *et al*., 2013).

The yeasts, LAB and AAB which have been identified in the different palm wine are presented in the Table 2. *Saccharomyces cerevisiae* is the dominant yeast species responsible for the fermentation of palm wine tapped from *Elaeis guineensis* in Ghana and Cameroon, as well as Bandji in Burkina Faso (Amoa-Awua *et al*., 2007; Stringini *et al*., 2009; Ouoba *et al*., 2012). On the other hand, *Saccharomyces chevalieri* has been reported the yeast specie predominant in the Toddy from Sri Lanka (Atputharajah *et al*., 1986). *S. cerevisiae* predominance in the palm wine production is attributed by the selective medium regarding pH, ethanol content, and anaerobic conditions, which favors the fermenting yeasts (Stringini *et al*., 2009). The major total volatiles and alcohols are produced by *S. cerevisiae* and *S. chevalieri* (Uzochukwu *et al*., 1999). e.g. the higher alcohols in fermented nipa (*Nypa fruticans*) sap is by cause of the metabolism of *S. cerevisiae* through two metabolic pathways; amino acids such as isoleucine and leucine, and glycolysis (Nur Aimi *et al*., 2013). Moreover, *Zymomonas mobilis* is also considered as the microorganism responsible for the palm wine fermentation and has been reported in Taberna, considering that this microorganism has ability to grow in acidic condition (pH about to 3.53) and tolerate high ethanol concentration (10.33% v/v) (Alcántara-Hernández *et al*., 2010), similar results are reported in palm wine obtained by "inflorescence tapping" from *Elaeis guineensis* in Nigeria (Obire, 2005).

Other identified yeasts during the tapping process probably play a determinant role in the fermentation. e.g. in wine fermentation is reported that the apiculate yeasts such as *Hanseniaspora guilliermondii* and *Hanseniaspora uvarum* have the capacity to influence, in a positive way, the aromatic profile of wines. *H. guilliermondii* has been reported to produce high levels of 2-phenylethyl acetate and 1-propanol (Moreira *et al*., 2011).

Thus also, the predominant LAB reported in palm wine fermentation are *Lactobacillus plantarum* and *Leuconostoc mesenteroides* (Amoa-Awua *et al*., 2007). These microorganisms are responsible for the sour taste of palm wine and are responsible for the pH decrease during the tapping through the organic acids production (Amoa-Awua *et al*., 2007; Ouoba *et al*., 2012). These bacteria also control the growth of undesirable microorganism such as enterobacteria by acid and H_2O_2 production (Amoa-Awua et al., 2007; Alcántara-Hernández *et al*., 2010; Naknean *et al*., 2010; Santiago-Urbina *et al*., 2013). e.g. Santiago-Urbina *et al.* (2013) reported that the total coliforms population in Taberna decreased during the tapping period with the increased of lactic acid production. Similar results are reported in Bandji where the predominant genus is *Lactobacillus* representing 86.67% of the LAB total isolates followed by the genera *Leuconostoc* (10%). Hence, *Lactobacillus plantarum* is the dominant species represented

46.67% of the total isolates (Ouoba *et al*., 2012). In addition, other species are described and are listed in the Table 2. Furthermore, in Taberna has been reported that *Lactobacillus nagelii* and *Lactobacillus sucicola* are present in the fermentation process (Alcántara-Hernández *et al.*, 2010). Additionally, the acetic acid bacteria of the genera *Acetobacter* and *Gluconobacter* have been identified in palm wine (Amoa-Awua *et al.*, 2007; Kadere *et al*., 2008), such as *Acetobacter pasteurianus* in Taberna (Alcántara-Hernández *et al.*, 2010), *Acetobacter indonesiensis* in Bandji, and other species listed in the Table 2. The role of AAB during the palm wine fermentation is related with the acetic acid production, which comprises part of the aroma volatiles. However, AAB can be considered as spoilage microorganisms, when the palm wine becomes unacceptable to consumers. In addition, like LAB, AAB also can contribute to the acidification and inhibition of undesirable microorganism (Ouoba *et al*., 2012).

Methods employed for identification of microorganisms in palm wine

Many microorganisms can be present during the palm wine production; however, they cannot be detecting because a good identification technique is not apply, and there are only a limited number of advanced identification studies. The microbial population identification in palm wine has been performed by the application of traditional methods and some molecular techniques.

Amoa-Awua *et al.* (2007) made a tentative identification of yeasts, LAB and AAB. They employed culture dependent methods, where the yeasts isolated were evaluated by determining their pattern of fermentation and assimilation of several sugars and also the usage of various carbohydrates in ID 32 C galleries. The LAB and AAB were examined by Gram staining, catalase test, gas production, and growth in different selective culture medium. LAB were also identified by determining their pattern of carbohydrate fermentation in API 50 CHL galleries. Similarly, Kadere *et al*. (2008) identified the AAB present in Mnazi, they used Gram staining, catalase test, biochemical and physiological test. On the other hand, LAB was phenotypically characterized by Gram staining, catalase test, microscopic morphology, and carbohydrate fermentation pattern using API 50CH (Ziadi *et al*., 2011). Thus, the yeasts identification in palm wine has been also carried out using standard morphological and physiological tests. These tests include morphology, surface characteristic, and presence of pseudohyphae, ascospore formation and vegetative reproduction; as well as, fermentative test of several sugars (Nwachukwu *et al.*, 2006). These identification methods are commonly called traditional methods, and their disadvantage is that only easily culturable microorganism can be detected, and members of microbial communities that need elective enrichment are not identified (Stringini *et al*., 2009). Moreover, this phenotypic identification is time consuming and there is possible inaccuracy in the results (Martín-Platero *et al.*, 2009). However, others techniques applied to isolated microorganisms have been employed in the studies of the palm wine microbiology, those are based on polymerase chain reaction (PCR) amplification and analysis of restriction of the complex Internal Transcribed Spacer (ITS) regions (non-coding and variable) and the 5.8S rRNA gene (coding and conserved) useful in measuring close fungus phylogenetic relationships (Kurtzman, 1992; Esteve-Zarzoso *et al.*, 1999; Arroyo López *et al.*, 2006). e.g. Stringini *et al.* (2009) identified yeasts population present during the tapping of palm wine from Cameroon using the RFLP (restriction fragment length polymorphism) analysis of the 5.8S rRNA gene and the two internal transcribed spacers (5.8S-ITS). The DNA is extracted of the isolated yeasts, then, through the PCR the 5.8S-ITS region is amplified, using the primers ITS1: 5'-TCCGTAGGTGAACCTGCGG-3'; and ITS4: 5′-TCCTCCGCTTATTGATATGC-3'. After that, the PCR products are digested using restriction endonucleases such as *Cfo*l, *Hae*lll and Hinfl. For the microorganism identification, the restriction patterns are compared with previously published studies, such as in Esteve-zarsoso *et al.*, 1999; Sabate *et al*., 2002; de Llanos Frutos *et al*., 2004; Arrollo López *et al*., 2006). Ouoba *et al.* (2012) made identification and genotypic diversity of the yeasts, LAB and AAB isolated from Bandji. The isolated microorganism were grouped by amplification of the 5.8S-ITS region and 16S-23S rDNA ITS region for yeast and bacteria, respectively. The yeasts, first grouped by phenotypic characteristic were analyzed using ITS-PCR, 11 groups representing different species were founded. For yeasts identification, sequencing of the D1/D2-region of the 26S rRNA was performed, using the NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') primers for the amplification. Correspondingly, the bacteria (LAB and AAB) were grouped phenotypically and then, clustered by mean of ITS-PCR. For bacteria identification, sequencing of a 940 bp portion of conserved region of the 16S rRNA gene was amplified using the pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pE (CCGTCAATTCCTTTGAGTTT-3') primers. In addition, the *gyrB* gen that encodes the subunit

B protein of DNA gyrase, an enzyme important in DNA replication was sequencing. In the other hand yeasts and bacteria were further differentiated by repetitive sequence-based PCR (rep-PCR) using the primer GTG5 (5'-GTGGTGGTGGTGGTG-3'). Rep-PCR allowed a clear differentiation of some yeast species, which using ITS-PCR, it was not possible to differentiate. Some LAB were not clearly differentiate by 16S RNA gene sequencing but, *gyrB* sequencing allowed a clearly distinction (Ouoba *et al*., 2012). Although these molecular PCR methods have improved the identification of the culturable microorganisms, there are still problems associated with selective cultivation and isolation of microorganisms from natural samples. Considering the lack of knowledge of the real conditions under which most microorganisms are growing in the natural habitat and the difficult to develop media for cultivation that accurate resembles these conditions, the culture-independent techniques have been used (Ercolini, 2004). Denaturing gradient gel electrophoresis (DGGE) is perhaps the most commonly used among the culture-independent fingerprinting techniques (Ercolini, 2004). The PCR-DGGE is usually employed to assess the structure of microbial communities in environmental samples without cultivation, and to determine the community dynamics in response to environmental variations (Ercolini, 2004). DGGE is an electrophoretic method capable of detecting differences between DNA fragments of the same size but with different sequences (Ercolini, 2004). e.g. Stringini *et al.* (2009) in addition to PCR-RFLP analysis of isolated yeasts, they also evaluated the yeasts population in palm wine by means of PCR-DGGE analysis, they used a polyphasic approach, i.e. both culture-dependent and culture-independent strategies. Total DNA was extracted from each samples of palm wine and approximately 250 nucleotides of the 5'-end region of the 26S rRNA gene were amplified by PCR using the primers NL1 with GC clamp, 5'CGCCC GCCGCGCGCGGCGGGCGGGGCGGGGGCCA TATCAATAAGCGGAGGAAAAG-3', and reverse primer LS2, 5´-ATTCCCAAACAACTCGACTC-3', the addition of a GC clamp to one of the primers insures that the fragments of DNA will remain partially double-strand and that the region screened in is the lowest melting domain (Myers *et al*., 1985; Sheffield *et al*., 1989). The PCR products were analyzed in 8% polyacrylamide gels containing a 20-50% urea-formamide gradient. After the DGGE analysis, selected bands were excised and sequenced. Thereafter, molecular identification and phylogenetic analysis were performed. This culture-independent method (DGGE) achieves the detection of additional species, which were not detected using culture based methods (Stringini *et al*., 2009). On the other hand, another culture-independent method used in the identification of microorganism in palm wine is the clone library of the 16S rDNA, this technique was employed by Alcántara-Hernández *et al.* (2010) for identification of bacterial community in taberna. The library was constructed from metagenomic DNA extracted from samples collected during the in vitro fermentation of Taberna. Approximately 1500 bp were amplified from 16S rDNA via PCR using the 46F (5'-GCCTAACACATGCAAGTC-3') and 1540R (5'-AAGGAGGTGATCCAGCCGCA-3') bacterial specific primers. PCR products were cloned directly into a vector using the TOPO TA cloning kit, and restriction analysis with *Eco*Rl was performed to detect the insertion. Then the 16S rDNA was sequenced and molecular identification and phylogenetic analysis were carried out (Alcántara-Hernández *et al*., 2010).

Conclusions and perspectives

The chemical composition of the palm sap is very similar among different species of palm trees. Sucrose is the main sugar presents in the sap and is the substrate in the natural fermentation conducted by lactic acid bacteria, yeasts and acetic acid bacteria. The palm wine involves three types of fermentations: lactic, alcoholic and acetic, making this traditional beverage an interesting environment where microorganism or genes with potential biotechnological applications can be isolated. Palm wine contains ethanol, lactic acid, acetic acid, as well as higher alcohols, esters, aldehydes and ketones. The composition of palm wine depends of several factors such as the source of the sap and the length of the fermentation. Microorganism identification in this beverage has been performed using traditional techniques of identification which involves isolation of the microorganism, and some molecular techniques that do not need microorganism culture such as PCR-DGGE. Therefore the structure of the microbial community probably is not completely identified. The microbiology and the biochemistry of palm wine must be fully understood. e.g. determine changes in the structure and the metabolic activity of the microbial community. Microbial community metabolism can be evaluated by monitoring the relative expression of messenger RNA (mRNAs). Ratio of microorganism in the fermentation during tapping of palm tree can be performed by quantitative monitoring of microorganisms using real-time PCR technology. Other important point is, knowing the

origin of the microorganism and microbial vectors. On the other hand, many authors have reported that yeasts are the responsible for the ethanol production mainly *S. cerevisiae*, however, others authors have attributed this to *Zymomonas mobilis*, therefore is necessary that this bacteria is studied.

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