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Full-Length Research Paper

Comparative Assessment of the Microbiology of Palm SAP from Life and Felled Oil Palm (*Elaeis guinensis*) Tree

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ABSTRACT: The sap of the oil palm tree (*Elaeis guinnesis*) serves as a rich substrate for various types of microorganism to grow. However, it is a source for producing the traditional wine, palm wine. This study was done with the aim to compare the microorganism found in palm wine samples obtained from living and felled oil palm tree at different time of fermentation. The palm sap samples were collected and allowed to ferment for three days (72 hours). Samples were collected twice a day for three days and analyzed for the presence of microorganism using standard microbiological procedure. The result reveals that the microbial load varies with fermentation time across each sample. With respect to the microbial population, aerobic mesophilic counts ranging between 7.08 x 10^7 and 6.20 x 10^8 cfu/ml and 4.01 x 10^7 and 1.60 x 10^9 cfu/ml respectively, lactic acid bacteria count ranging between 1.3 x 10^7 and 6.75 x 10^8 cfu/ml and 1.65 x 10^7 and 9.75 x 10^8 cfu/ml respectively. Yeast counts between 4.0 x 10^5 and 6.00 x 10^7 cfu/ml and 3.43 x 10^5 and 5.68 x 10^7 cfu/ml respectively. Across the sample from the two sources (living and felled oil palm tree) acetic acid bacteria counts between 5.6 x 10^7 and 6 x 10^7 cfu/ml and coliform bacteria between bacteria 5 x 10^3 and 7.50 x 10^3 cfu/ml. Further microbiological analysis revealed the various bacteria and yeast isolated from the palm wine samples. From the comparative analysis of the microorganism gotten from palm wine from life and felled oil palm tree at different time, the result revealed that at the beginning of fermentation on the first day, the isolates identified were mostly bacterial contaminants and the yeast *Saccharomyces cereveisae*. No lactic acid bacteria at the initial stage of tapping from either the palm wine from the living or felled oil palm tree. Lactic acid bacteria (*Lactobacillus spp*) was isolated from the 2nd day of fermentation from both living and felled palm sap and remained till the third day of fermentation in both palm wine samples. Acetic acid bacteria, *Acetobacter aceti* and *Gluconobacter spp* were present in both sample from the third day of fermentation due to the buildup in alcohol concentrations on the third day. *Zymomonas mobilis* was also present in the palm wine from living tree but absent in the felled tree palm wine. Generally, Lactic acid bacteria (LAB) and Acetic acid bacteria (AAB) were both present in the samples only after the onset of fermentation from the second and the third day of fermentation respectively. It is therefore recommended that the microbiology and the biochemistry of palm wine must be fully understood.

Keywords: Microbiological Analysis, oil palm tree, (*Elaeis guinnesis)* fermentation period

INTRODUCTION

Palm Wine**,** this alcoholic beverage is traditionally produced from the sugary sap of various palms (Tribe. Cocoinae. Family, Palrnae) throughout the tropics. In Nigeria it is obtained from the sap of the raphia palm (*Raphia hookeri and Raphia vinifera*) and the oil palm tree (*Elaeis guineensis)*. It is a sweet whitish liquid that gradually turns milky as a result of the growth of microorganisms which contaminate the sap as it oozes out of the tree and which causes the sap to undergo a spontaneous Fermentation. It is similar to the cereal-based alcoholic beverages because the microorganisms are alive when the wine is drunk (Nezuarni, 2014). The methods of procuring the unfermented sap from the palm tree vary with the tree and with the locality. They include Felling of the oil palm tree and collecting the sap from a cut on the stem or by cutting the terminal bud.

This type of palm wine is referred to as the “down wine” is not highly appreciated in Nigeria because of its high alcohol content and because it may result in the gradual elimination of the palm population.

Another method is to make an incision at the base of the male inflorescence after removing the bracts. It is left to dry for 2 days after which the hole is reopened and the sap is collected in a gourd. The hole is reopened twice daily for 2-3 weeks during which time the sap is tapped. This is the most acceptable method as it spares the life of the tree and produces a wine that commands a high price. Alternatively, the sap may be tapped through a hole under the terminal bud alter clearing the tree. The sap of the Raphia palm is obtained by cutting the terminal bud. The yield of sap varies with the season of tapping and with the type of palm. Up to 3 liters per day for 14 to 21 days of tapping the oil palm and 2 to 11 liters, per day from the bark of the male inflorescence, the tap holds and the huffy hairy out-growths that surround the tap holes, the tapping paraphernalia, the gourds used for collection and the air. As a result of this contamination, distribution of the products to distant places is difficult as it ferments rapidly, losing its sweet taste and becoming sour and milky-white within 24 h and becoming unacceptable to the consumer, (Olufunke and Oluremi, 2015). General both brands of palm wine are microbial communities that provide the wine several benefits (Nutritional, medical, religious and social) which have been reported, however, the aim of this research is to determine the microbiology (type and numbers of microorganisms) of palm sap collected from a life and filled oil palm tree, then make a comparative assessment.

MATERIALS AND METHODS

Sample Collection

Fresh Up and Down palm wine samples were collected separately twelve hourly from the palm tree by traditional palm wine trappers located within Auchi Etsako-West Local Government Area of Edo State, South-South, Nigeria, using to the modified method of Ogbulie *et al.,* 2007. These freshly tapped samples were collected using pre-sterilized, labeled 100 ml capacity universal sample bottles with perforated screw caps. The perforated screw caps will be plugged with sterile non-absorbent cotton wool. The samples were transported to the laboratory in a cooler equipped with packs of freezing mixture of salt and ice-block for analysis within 1h of collection.

Microbial enumeration and isolation

A one milliliter (1 ml) aliquot of each palm wine were taken aseptically from each sample and placed into separate sterile 250 ml conical flasks. A 10-fold serial dilution in 0.1% (w/v) bacteriological peptone was carried out and plated in triplicate into Nutrient Agar (NA), MacConkey Agar (MCA), Mannitol Salt Agar (MSA), De Mann, Rogosa and Sharpe Agar (MRS) and Potato Dextrose Agar (PDA) for the enumeration of total heterotrophic bacteria, coliform, *Staphylococcus species*, Lactic Acid Bacteria and Fungi respectively (Novella-RodrIguez *et al.,* 2004; Abdalla and Omer, 2017). This process was repeatedly done at 0, 12, 24, 48, 72 and 96 hrs intervals. The inoculated plates were incubated aseptically at 30°C for 24 h for bacteria and 24- 48 h for the yeast. Acceptable plates will be those that contained between 30-300 cfu /ml. In all samples of the Palm wine, bacterial and fungal colonies with varying morphologies were isolated, enumerated and stored at 4°C on Agar slants, for further use.

Characterization of palm wine isolates

The Isolates were grouped according to their colonial morphology and cell characteristics in pure culture using the medium on which they had grown as described by Njoku *et al.,* (1990). Identification of the various bacterial species will be confirmed, using conventional tests for biochemical and physiological characterization. These tests include: Gram staining, urease, catalase, oxidase, indole, coagulase and sugar fermentation. Further test to identify Lactic Acid Bacteria were bile hydrolysis, haemolysis, phosphate test and growth and the utilization of trehalose, lactose, ribose, sorbitol, raffinose and mannitol at 15^0C, 35^0C and 45^0C (Burdychova and Komprda, 2007). The probable identities of the isolates were determined as recommended by Holt (1984).

Characterization and identification of fungi isolates

The characterization and identification of the fungal isolates was carried out by using macroscopic and microscopic examinations depending on the colony color, shape, hyphae, conidia, conidiophores and arrangement of spores using the schemes of Salvamani and Nawawi, (2014) and Alsohaili, and Bani-Hasan (2018).

Macroscopic and microscopic characterization of isolated fungi

The morphology of the isolated fungi was identified based on their macroscopic colonial features (color, shape, size and hyphae) and microscopic features using a lactophenol cotton blue-stained slide mounted with a small portion of the mycelium. Four (4) drops of

Table 1: Viable cell count of microorganism isolated from palm wine samples gotten from a life oil palm tree (*E. guineensis*).

Time (hrs)	NA (cfu/ml)	Mrsa (cfu/ml)	PDA (cfu/ml)	MCA (cfu/ml)
0	$7.80x\ 10^{7}$	2.08×10^{7}	4.23×10^{5}	$.02 \times 10^{3}$
12	2.51×10^{10}	1.13×10^{7}	5.23×10^{6}	7.50×10^{3}
24	7.01×10^{10}	5.70×10^{7}	2.50×10^{7}	NG
48	6.20×10^{8}	1.31×10^{8}	5.68×10^{7}	NG
60	4.51×10^{8}	5.41×10^{8}	5.78×10^{7}	NG
72	4.15×10^{8}	6.75×10^{8}	6.00×10^{7}	NG

Table 2: Viable cell count of microorganism isolated from palm wine samples gotten from a felled oil palm tree *(E.guineensis).*

Time (hrs)	NA (cfu/ml)	Mrsa (cfu/ml)	PDA (cfu/ml)	MCA (cfu/ml)
0	4.01×10^{7}	1.65×10^{7}	3.43×10^{5}	NG
12	2.84×10^{9}	2.63×10^{7}	4.56×10^{6}	NG
24	6.23×10^{9}	1.98×10^{7}	1.65×10^{7}	NG
48	2.50×10^{8}	4.80×10^{8}	3.32×10^{7}	NG
60	3.05×10^{8}	6.51×10^{8}	4.51×10^{7}	NG
72	1.60×10^{9}	9.75×10^{8}	5.68×10^{7}	NG

Table 3: Cultural, Morphological and Biochemical Characteristics of Bacteria isolates gotten from Palm Wine from Life and Felled Oil Palm Tree at different times.

Parameter	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7
Cultural Characteristics	Small creamy colonies on nutrient agar	Large Creamy, circular, elevated on nutrient agar	Tiny creamy colonies on Nutrient agar, round and pinkish on MRSA and MCA respectively	Small creamy colonies on nutrient agar	Pale white, round, raised smooth colonies on nutrient agar	Cream smooth colonies on Nutrient agar	Brown pigmented colony which diffuses into the Nutrient agar
Morphological characteristics	Rod	Cocci	Rod	Cocci	Rod	Shorts rod	Rods
Ceel arrangement	Pairs	Clusters	Pairs	Singles	Chains	Pairs	Single
Gram reaction	-	+	+	+	-	-	+
Catalase	-	+	-	+	-	+	+
Coagulase	-	+	ND	-	ND	ND	ND
Oxidase	ND	ND	ND	ND	ND	ND	ND
Nitrate	ND	ND	ND	-	ND	ND	+
Urease	ND	ND	ND	ND	ND	ND	-
Motility	+	-	-	ND	+	+	+
Methyl Red	+	ND	ND	-	+	-	ND
Vogues Proskaur	-	ND	A	ND	+	+	ND
Glucose	A	AG		A	A	A	A
Lactose	A	AG	A	Aw	A	-	A
Sucrose	-	AG	A	-	A	-	A
Maltose	-	AG	A	Aw	A	A	-
Mannitol	-	AG	A	A	A	-	-
Probable bacteria	*Zymomonas mobills*	*Staphylococcus aureus*	*Lactobacillu spp*	*Micrococcus luteus*	*Acetobacier aceti*	*Gluconobacter Spp*	*Bacillus cereus*

lactophenol cotton blue was placed on a clean glass slide and small piece of mycelium free of medium was removed with sterile inoculating needle and transferred on to the stain on the slide. The mycelium was teased out

Table 4: Cultural and morphological characteristics of fungal isolates gotten from palm wine from life and felled oil palm tree at different times.

Character	1	2	3	4
Pigmentation	flat, smooth, moist, glistening or dull, and cream in color	cream colored to yellowish in colour	white to cream-colored, soft, dull, smooth or slightly wrinkled	Yellowish-tan, dull with fine wrinkles.
Growth rate	Slow to moderate	Rapid	Slow	Slow
Growth formation	Blastoconidia (cell buds) are observed, globose, and ellipsoid to elongate in shape	Blastoconidia (cell buds) are observed, globose, and cylindrical cells	Multilateral budding; ellipsoidal to cylindrical in chains. Pseudohypae seen arranged like short tree-like branches	cells are ovoid to elongate wich occur in pairs
Spore type	ascospores	NA	Blastospores	Ascospores
Probable Fungi	*Saccharomyces cerevisae*	*Candida fropicalis*	*Candida intermeridia*	*Pictia kluyveri*

Table 5: Occurrence of the isolates in palm wine from life and felled oil palm tree at different times.

Hours	Living oil palm tree	Felled oil Palm Tree
0	*Saccharomyces cerevisae, Staphylococcus aureus, Bacillus cereus, Micrococcus lutes, Zymomonas mobilis*	*Saccharomyces cerevisae, Bacillus cereus, Staphylococcus aureus*
12	*Saccharomyces cerevisae, Candida intermedia, Bacillus cereus, Lactobacillus spp, Zymomonas mobilis*	*Saccharomyces cerevisae, Candida tropicalis, Bacillus cereus, Lactobacillus spp, Micrococcus letus*
24	*Saccharomyces cerevisae, Candida tropicalis, Lactobacillus spp, Micrococcus letus Zymomonas mobilis*	*Saccharomyces cerevisae, Candida tropicalis, Lactobacillus spp, Pitcia kluyveri, Candida tropicalis.*
48	*Saccharomyces cerevisae, Acetobacter aceti, Glucobacter spp, Lactobacillus spp Zymomonas mobilis*	*Saccharomyces cerevisae, Bacillus cereus, Acetobacter aceti., Micrococcus letus, Glucobacter spp*
60	*Pitchia kluyveri, Saccharomyces cerevisea, Zymomonas mobilis, Acetobacter aceti Glucobacier spp*	*Pitchia lduyveri, Saccharomyces cerevisea, Candida tropicalis, Lactobacillus spp, Acetobacter aceti*
72	*Pitchia kluyveri, Saccharomyces cerevisea, Zymomonas mobilis, Acetobacier aceli, Glucobacter spp*	*Pitchia kluyveri, Saccharomyces cerevisea, Candida tropicalis, Acetobacier aceti, Glucobacter spp*

with the needles and covered with cover slip carefully avoiding air bubbles (Salvamani and Nawawi, 2014; Alsohaili and Bani-Hasan, 2018).

RESULTS AND DISCUSSION

The tables below will show microbial load, characterization of bacterial and fungal isolate identified. The result reveals that the microbial load varies with fermentation time across each sample (Tables 1-5). With respect to the microbial population, aerobic mesophilic counts ranging between 7.08 x 10^7 and 6.20 x 10^8 cfu/ml and 4.01 x 10^7 and 1.60 x 10^9 cfu/ml respectively, lactic acid bacteria counts ranging between 1.3 x 10^7 and 6.75 x 10^8 cfu/ml and 1.65 x 10^7 and 9.75 x 10^8 cfu/ml respectively. Yeast counts between 4.0 x 10^5 and 6.00 x 10^7 cfu/ml and 3.43 x 10^5 and 5.68 x 10^7 cfu/ml respectively. Across the sample from the two sources (living and felled oil palm tree) acetic acid bacteria counts between 5.6 x 10^7 and 6 x 10^7 cfu/ml and coliform bacteria between bacteria 5 x 10^3 and 7.50 x 10^3 cfu/ml. Further microbiological analysis revealed the various bacteria and yeast isolated from the palm wine samples. The various bacterial isolates encountered include *Staphylococcus aureus, Bacillus cereus*, *Micrococcus lutues*, *Acetobacter aceti, Glucobacter spp, Lactobacillus spp and Zymomonas mobilis*. The yeast isolated in this study include *Pitchia kluyveri*, *Saccharomyces cerevisae, Candida tropicalis*, and *Candida intermedia.*

Staphylococcus aureus was found in both samples at the initial tapping but disappeared as fermentation time

increased to 12 hours. The presence of *Staphylococcus aureus* in samples of fresh palm sap reveals poor hygienic conditions during the extraction or conditioning of the sap by producers. According to Olawale *et al*. (2010), these germs could come from the water used in the extraction, or to wash the container used for the collection of the sap in order to dilute sap to increase their income. They might also come from a manual contamination of the collector or the environment (Tapsoba *et al*., 2014).

Bacillus cereus and *Micrococcus luetus* was present in both samples even after 12 hours of fermentation. Their presence *Bacillus cereus* is as a result of contamination from air and poor sanitation in and around the environment (Badau *et al*., 2018). The dusty and windy conditions of the environment may be a contributor to the presence *of Bacillus cereus* as the study was done during the dry season. *Micrococcus luteus* and *Bacillus cereus* have been reported to be mostly found as food contaminants and their presence in large amount could result to food poisoning (Obi *et al*., 2015). The fruit fly (Drosophila melanogaster) is an important factor in the contamination of the product abinitio. The insect habours yeasts on its body and transfers the same from ripped fruits to the palm wine which attracts its attention via the natural aroma of the fresh product (Obi *et al*., 2015).

The presence of *Lactobacillus spp* in the palm wine samples from both sources is no surprise as 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; Oluoba *et al*., 2012). They appeared in the palm wine samples 12 hours after initial tapping of the palm wines.

The predominant LAB reported in palm wine fermentation are *Lactobacilius plantarum* and *Leuconostoc mesenteroides* (Amoa-Awua *et al.,* 2007). 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-Hemández *et al*., 2010; Naknean *et al*., 2010; Santiago-Urbina *et al*., 2013). This may be the reason there is a decrease in the number to the complete absence of coliform in the samples analyzed. This report is in line with that of Santiago-Urbina *et al.* (2013) who reported that the total coliforms population in Taberna decreased during the tapping period with the increased of lactic acid production.

Zvmomonas mobilis was also isolated but only from the palm wine from the life tree. This bacterium alongside *Saccharomyces cereviseae* 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 3.53) and tolerate high ethanol concentration (10.33% vfv) (AlcántaraHernández *et al*., 2010), similar results are reported in palm wine obtained by “inflorescence tapping” from *Elaeis guineensis* in Nigeria (Obire, 2005).

Additionally, the acetic acid bacteria (AAB) of the genera *Acetobacter aceti* and *Gtuconobacter spp* were identified in all palm wine samples only after 2 days of fermentation. They have also been identified in palm wine by Amoa-Awua *et al.,* (2007) and Kadere *et al*., (2008). AAB such as *Acetobacter pasteurianus, Acetobacter indonesiensis*, and other species have been identified (AlcántaraHemández *et al*., 2010). 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 (Oluoba *et al*., 2012).

The yeasts identified. *Saccharomyces cerevisiae* is the dominant yeast species responsible for the fermentation of palm wine tapped from both Living and Felled *Elaeis guineensis* in this study. This is in correlation with the findings of AmoaAwua *et al*., (2007), Stringini *et al*., (2009) and Oluoba *et al*., (2012) who identified *Saccharomyces cerevisiae* as the dominant fermentative yeast in palm wine from Ghana, Cameroon and Burkina Faso respectively. 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). Also as reported by Nur Aimi *et al*. (2013) the higher alcohols in fermented nipa (Nypa fru ticans) sap is by cause of the metabolism of *S. cerevisiae* through two metabolic pathways; amino acids such as isoleucine and leucine, and glycolysis. Other identified yeasts during the tapping process probably play a determinant role in the fermentation. For Example, in wine fermentation is reported that the yeasts such as *Pitcia kluyveri*, Candida tropicalis, and *Candida intermedia* have the capacity to influence, in a positive way, the aromatic profile of wines (Moreira *et al*., 2011).

Conclusion

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. From the comparative analysis of the microorganisms gotten from palm wine from living and felled oil palm tree at different time, the result revealed

that at the beginning of fermentation on the first day, the isolates identified were mostly bacterial contaminants and the yeast *Saccharomyces cereveisae*, no Lactic acid bacteria at the initial stage of tapping from either the palm wing from the living or felled oil palm tree. Lactic acid bacteria (*Lictobadilus spp*) was isolated from the 2nd day of fermentation from both life and felled palm sap and remained till the third day of fermentation in both palm wine samples. Acetic acid bacteria, *Acetobacter aceti* and *Gluconobacter spp* were present in both samples from the third day of fermentation due to the buildup in alcohol concentrations on the third day. *Zymomonas mobilis* was also present in the palm wine from living tree but absent in the felled tree palm wine. Generally, Lactic acid bacteria (LAB) and Acetic acid bacteria (AAB) were both present in the samples only after the onset of fermentation from the second and the third day of fermentation respectively.

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