African Journal of Biotechnology Vol. 6 (7), pp. 914-922, 2 April 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Comparative study on the microbiology and shelf life stability of palm wine from *Elaeis guineensis* and *Raphia hookeri* obtained from Okigwe, Nigeria

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Accepted 6 February, 2007

The microbiological and biochemical changes and shelf life stability of *Elaeis guineensis* and *Raphia hookeri* brands of palm wine were determined. *R. hookeri* brands were found to habour more heterotrophic and coliform population than the *E. guineensis*, whereas the later haboured more yeast species. Identification tests revealed the isolation of *Bacillus, Lactobacillus, Brevibacterium* and *Staphylococcus* from *E. guineensis* while *Escherichia coli and Micrococcus* species with the exception of *Brevibacterium* sp. was additionally isolated from *R. hookeri*. Furthermore heterotrophic count and pH were observed to decrease with increased fermentation days. The effect of the preservatives on the sensory properties of palm wine was dependent on the type of preservation used. The level of CO_2 as well as the effect of extracts from the plant preservatives on the isolates from the palm wine samples was also carried out. Percentage loss of CO_2 for each successive fermentation day was observed and there was significant difference in the effect of the plant preservatives used.

Key words: Microbiology, Biochemical changes, Shelf life, Palm wie, Nigeria.

INTRODUCTION

Palm wine is consumed throughout the tropics and appears as a whitish liquid produced by natural fermentation of the sap of *Elaeis guineensis* and *Raphia hookeri* (Uzogara et al., 1990; Uzochukuru et al., 1991). The unfermented sap is clean, sweet, colourless syrup containing about 10 - 12% sugar, which is mainly sucrose (Bassir, 1962; Okafor, 1975a). Upon fermentation by the natural microbial flora, the sugar level decreases rapidly as it is converted to alcohol and other products (Obire, 2005) whereas, the sap becomes milky-white due to the increased microbial suspension resulting from the prolific growth of the fermenting organism (Okafor, 1975a,b).

Palm wine is characterized by an effervescence of gas resulting from the fermentation of the sucrose content (Bassir, 1962), by the fermenting organisms. Previous studies on the microbiology of *E guineensis* and *R. hookeri* have incriminated several bacterial and yeast flora to be involved in the fermentation process (Faparunsi and Bassir, 1972a; Okafor, 1972ab; Okafor, 1975b; Eze and Ogan, 1987; Amanchukuru et al., 1989; Ejiofor, 1994; Orimaiye, 1997; Nester et al., 2004). These organisms have also been reported to originate from several sources, which include tapping equipment, con-tainers, the environment, etc (Faparunsi and Bassir, 1972a; Eapen, 1979).

Generally, both brands of palm wine have several nutritional, medical, religious and social uses which have been reported else where (Faparunsi, 1966; Odeyemi, 1977; Ikenebomeh and Omayuli, 1988; Uzogara et al., 1990; Iheonu, 2000), to have increasingly enhanced the demand for this natural product. Although attempts has been made towards the preservation and shelf-life extension of palm wine through bottling, use of chemical additives and addition of plant extracts have greatly affected the organoleptic quality of the product (Bassir, 1962; Okafor, 1975b; Odeyemi, 1977; Orimaiye, 1997; Iheonu, 2000; Nwokeke, 2001; Obire, 2005). Several factors however have been adduced for this variation and they include the indigenous microbial flora, the biochemical composition of the two brands of palm sap, the tap-

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ping and post tapping processes. This study is there-fore aimed at ascertaining the microbiological flora of the two brands of palm wine, the biochemical changes associated with the sap fermentation and the effect of the traditional plant preservatives on the shelf life of the products.

MATERIALS AND METHODS

Sample collection

Fresh palm wine samples from oil palm tree (*E. guineensis*) and Raphia palm (*R. hookeri*) was separately collected from traditional palm wine trappers from Okigwe, Imo State Nigeria. The freshly tapped samples were collected using 14 pre-sterilized labeled 100 ml capacity sample bottles with perforated screw caps. The perforated screw caps were 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 1 h of collection. This method of collection according to Bassir (1962) and Obire (2005), reduces fermentation rate considerably.

Microbial isolation and succession in palm wine

A 1 ml aliquot of each palm wine was taken aseptically at 0, 24, 48, 72, 96 and 240 h of fermentation. These samples were serially diluted 10-fold in 0.1% (w/v) bacteriological peptone. A 1.0 ml dilutions were plated out in duplicated using spread plate methods (Cheesbrough, 1994), on tryptone soy agar (Oxoid) for total heterotrophic bacterial count, MacConkey agar (Oxoid) for the total coliform count and Sabouraud dextrose agar (Oxoid) containing 0.05 mg/ml chloramphenicol for yeast count as described by Cruickshank et al. (1982), Ojomo et al. (1984) and Okpokwasili and Ogbulie (1999). The inoculated plates were incubated aseptically at 30°C for 24 h for bacteria and 24 – 48 h for the yeast. Acceptable plated were those that contained between 30 - 300 cfu /ml. They were stored on agar slants at 40°C for characterization.

Chacterization of isolates

The Isolates were grouped accorded to their colonial morphology and cell characteristics. The colonies were counted and re-isolated in pure culture using the medium on which they had grown as described by Njoku et al. (1990). Isolates were thereafter subjected to biochemical tests as described by Collins and Lyne (1984) and Ogbulie et al. (1994). The probable identities of the isolates were determined as recommended by Holt (1984).

Preservation treatment of raphia and oil palm wine

Six-60 ml sample of each raphia and oil palm wine were treated with a total of 5 traditional plant preservatives namely *Saccoglottis gabonensis, Vernonia amygdalina, Euphobia* sp.*, Nauclea* sp. and *Rubiacae* sp., which were processed by drying under sunlight and ground to powdery form. The treatment was carried out by adding 10 mg of each powdered traditional preservative to the sterile sample bottles. Thereafter, 60 ml of fresh palm wine sap were added, gently shaken to mix and allowed to stand in a laboratory glass cabin sterilized using 2.5% acid alcohol as in Njoku et al. (1990).

Chemical analysis

The method described by AOAC (1980) was adopted to determine the rate of CO_2 evolution and pH of the sample.

Sensory evaluation

The two brands of palm wine were evaluated after preservation studies for the organoleptic properties. A ten member panel consisting of regular palm wine bar customers was drafted to evaluate the acceptability of the product based on the taste, color and over all acceptability using the 9-point hedonic scale as described by Njoku et al. (1991). The descriptive terms and their rating were such that below 5 points indicates poor or dislike extremely; 5 - 6 indicates fair or disliked moderately; 7 - 8 points stands for good or like moderately whereas 9-10 points indicates very good or like extremely.

Evaluation of the activity of the plant extracts on the palm wine isolates

Extracts were obtained from the plant preservatives using water and ethanol as solvents. This was achieved using the methods described by Obi and Onuoha (2000). For water extraction, 20 g of the grounded plant samples was dissolved in water in a sterile conical flask. The mixture was heated to boil and transferred to a water bath at 100°C for 15 min. The solution was thereafter, allowed to settle for about 2 h and later filtered with sterile Whatman filter paper.

For ethanol extraction, 20 g of each of the ground plant preservatives used was dissolved in 250 ml of 99% ethanol in a sterile conical flask and stirred with a glass rod every 20 min for 6 h. This was stoppered and allowed to stand for 24 h, after which it was filtered with Whatman filter paper.

All the solutions of the plant extracts were evaporated to remove the solvents used for extraction and to ascertain the yield in gramme of each extraction method per weight of the powdered sample extracted (Ntiejumokwu and Onwukaeme, 1991). This was however done using a rotary evaporator. Furthermore, crude extract for evaluation was obtained using the method of Akujiobi et al. (2004), where 50 mg/ml of each extract from ethanol and water was prepared in 30% dimethylsulphoxide and sterile saline solution, respectively.

The agar diffusion method as described by Cheesbrough (1994) was adopted for the evaluation. However, the organisms isolated from the test samples were first prepared by inoculating a loop full of each isolate into Nutrient broth and Potato Dextrose broth for bacterial and yeast isolates respectively, in different Mac Carthney bottles. After incubation at 37°C for 24 h (for bacterial culture) and 30°C for 24 h (for yeast culture), 1.0 ml of the Nutrient broth and Potato Dextrose broth containing the organisms was inoculated on Nutrient agar and Saboraud Dextrose agar plates respectively using pour plate method as described by Cheesbrough, (1994).

Furthermore, using a sterile cork borer, wells were created in the plates. Thereafter, the wells were filled with equal volumes of the plant extracts. The plates were kept at room temperature for prediffussion for 1 h, after which the plates were incubated at the 37°C for 24 h and 30°C for 48 – 72 h for bacterial and yeast, respectively. At the end of incubation, zones of inhibition were measured and results recorded in millimeter.

Data analysis

The analysis of variance (ANOVA) was used to statistically analyze the data obtained while the Fishers teats significant difference (LSD) was used to separate the means of sensory results obtained with significant F-values as described by Spiegel and Stephens (1999).

RESULTS

Microbiological assay revealed that more total heterotrop-

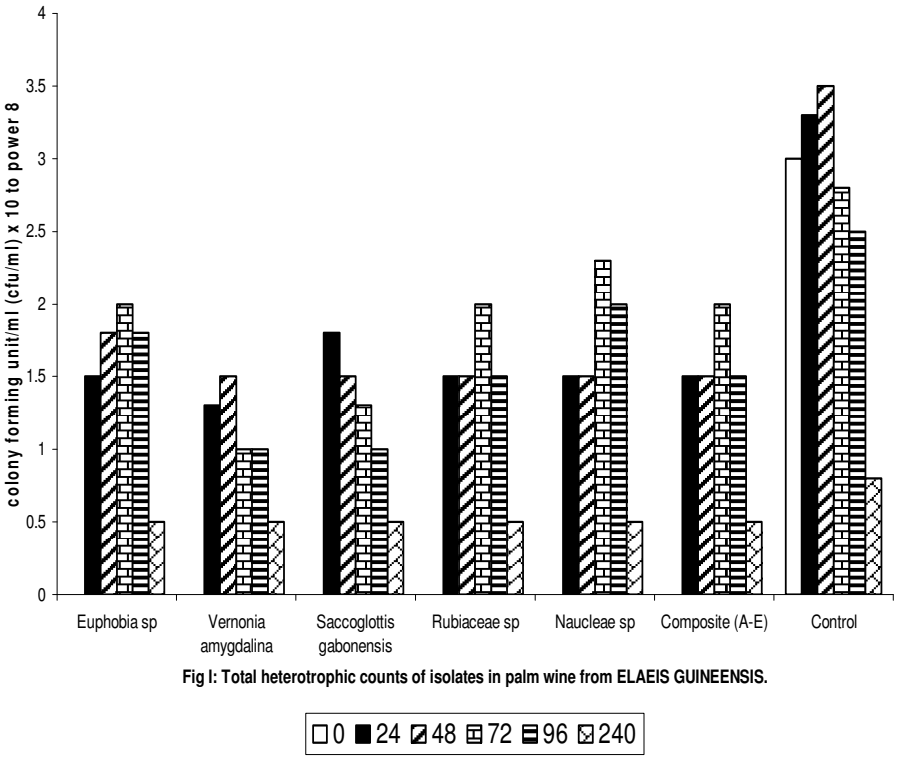

Figure 1. Total heterotrophic counts of isolates in palm wine from *E. guineensis*.

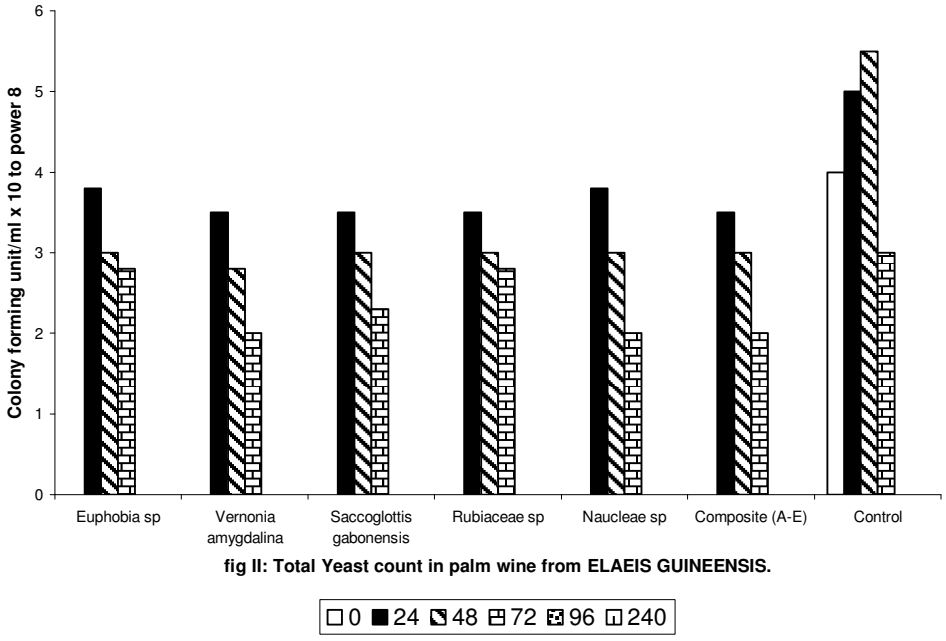

Figure 2. Total yeast count in palm from *E. guineensis*.

hic bacteria and coliform counts were obtained from the *R. hookeri* samples than the *E. guineeneses* while the later haboured more yeast counts than the former (Figures 1 to 6). The mean occurrence of the bacterial genera and yeast revealed a sharp increase from 0 – 72 h for the total heterotrophic bacteria, while coliform and

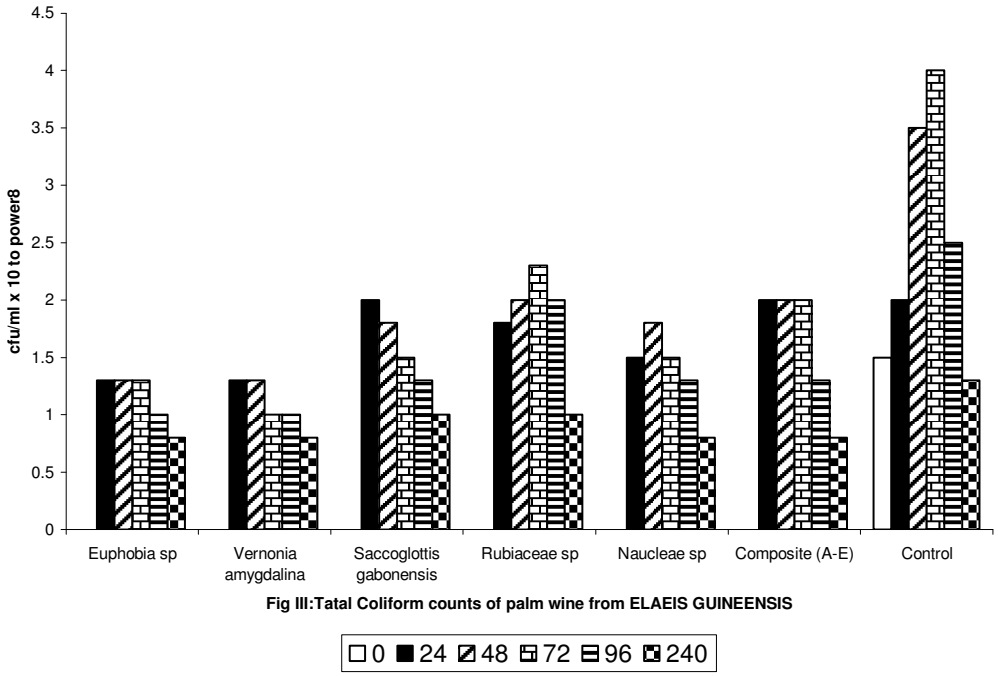

Figure 3. Total coliform counts of palm wine from *E. guineensis*.

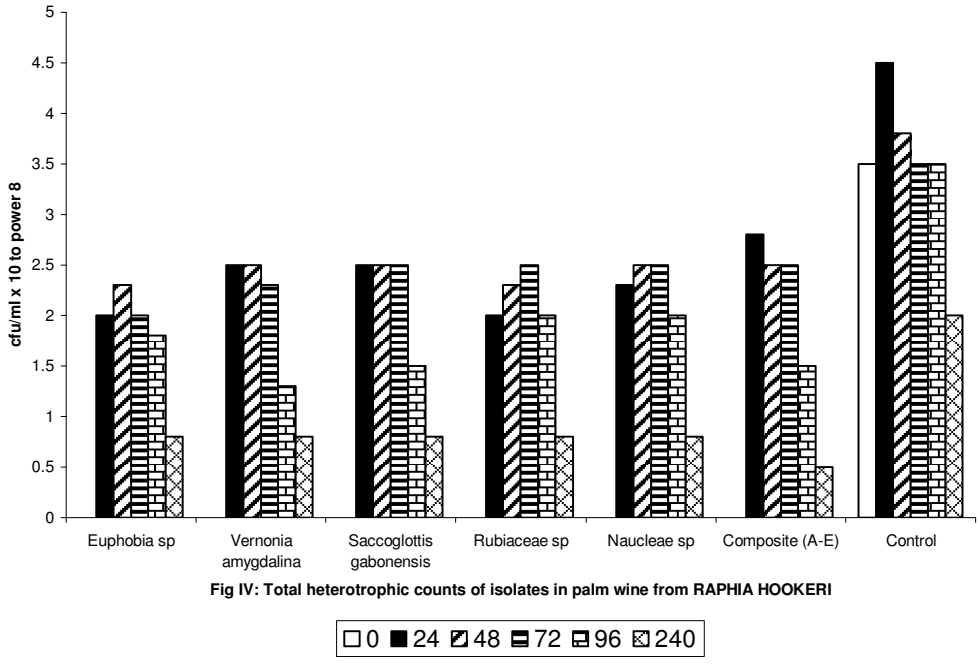

Figure 4. Total heterotrophic counts in palm wine from *R. hookeri.*

yeast population showed a progressive increase from 0 h of fermentation to the 48 h. Thereafter, a sharp progressive decrease was observed from 72 h. This trend followed the same order till the signs of spoilage of the palm wine sample were observed. The survival pattern of the isolates was monitored from 0 - 240 h and obvious disappearance of some organism was recorded as shown in Table 1. Identification test revealed the isolation of more bacteria genera in the Raphia palm wine than in the oil palm wine. While *Lactobacillus, Brevibacterium,*

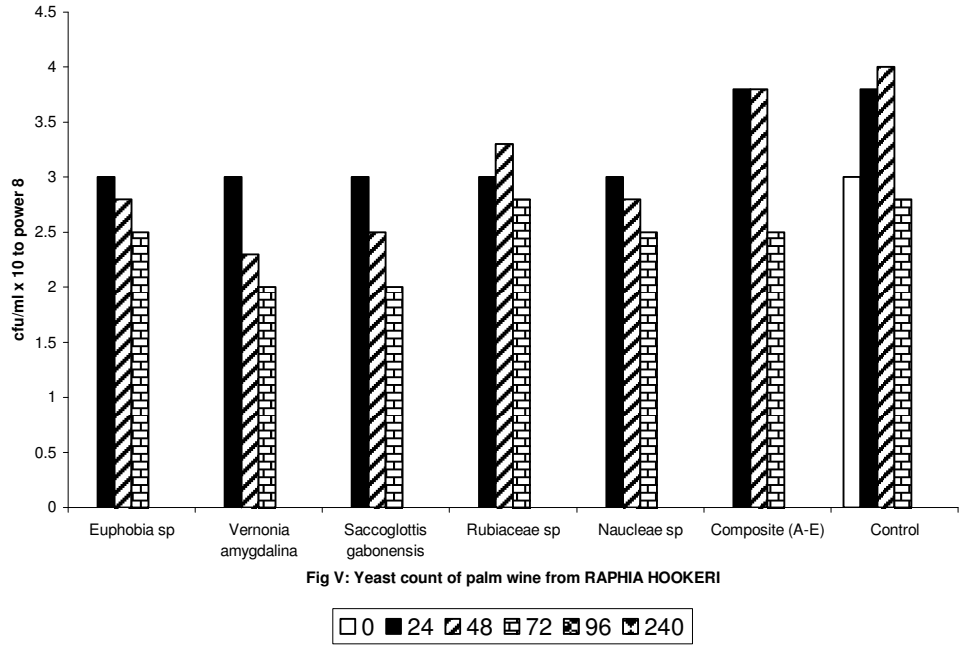

Figure 5. Yeast count of palm wine from *R. hookeri*.

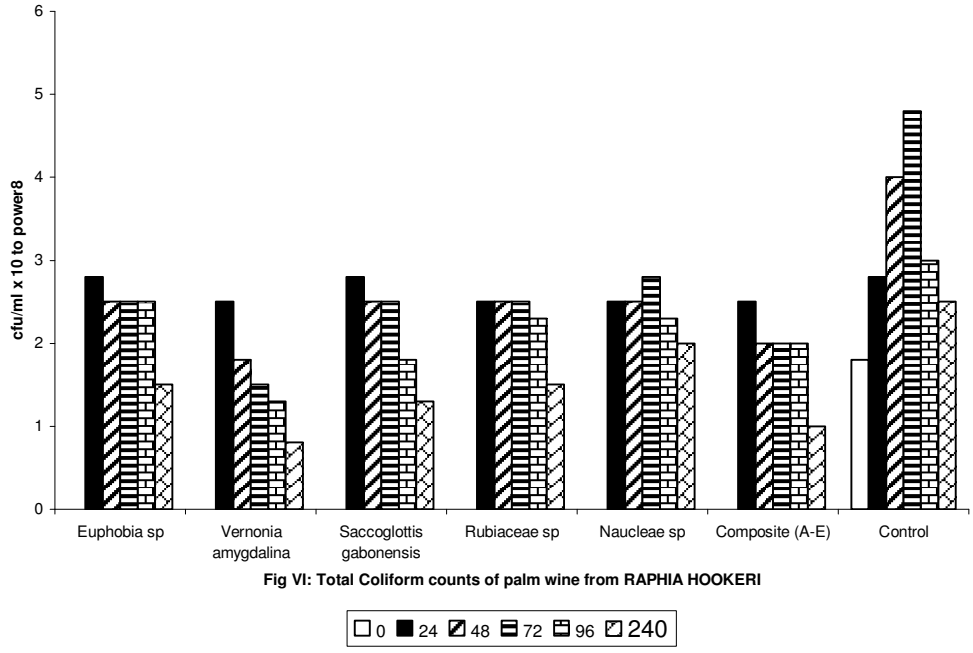

Figure 6. Total coliform counts of palm wine from *R. hookeri.*

Bacillus and *Staphylococcus* sp were isolated from *E. guineensis*, *E. coli* and *Micrococcus* sp in addition to the other isolates observed from *E. guineensis* though with the exception of *Brevibacteria* obtained from *R. hookeri.* The total heterotrophic counts and the pH level (Table 2) were observed to decrease as the fermentation time progresses. Statistical analysis at 95% confidence level showed that there are significant differences in the two samples between the percentage loss of CO_2 for each successive fermentation day as shown in Tables 3 and 4. Sensory evaluation of the properties of the preserved and unpreserved palm wine was determined. The level of in-

Table 1. Survival pattern of micro-organisms in palm wine.

Organisms	Hour of Isolation					
	0	24	48	72	96	240
Saccharomyces sp.	x	x	x	x	o	o
Lactobacillus sp.	x	x	x	x	o	o
Bacillus sp.	x	x	x	x	x	x
Staphylococcus sp.	x	x	o	o	o	o
Escherishia coli	x	x	x	x	o	o
Micrococcus sp.	x	x	o	o	o	o
Brevibacterium sp.	x	x	x	o	o	o

x = Presence.
o = Absence.

Table 2. Effect of plant preservative on the pH of palm wine.

Time (h)	A	B	C	D	E	F	G
0	6.30	6.30	6.30	6.30	6.30	6.30	6.30
24	6.10	5.68	5.93	6.00	6.12	5.72	5.20
48	4.62	4.57	4.55	4.62	4.56	4.50	4.10
72	3.83	4.25	4.50	4.12	4.25	4.35	3.40
96	3.32	3.25	3.30	3.46	3.30	3.35	3.20
240	2.20	2.45	2.40	2.35	2.34	2.35	2.10

Samples A-F are palm wine samples treated with different plant preservatives. A – Euphobia sp; B – Vernonia amygdalina; C – Saccoglottis gabonensis; D – Rubiaceae sp; E – Naucleae sp; F – composite (A-E); G – Control (no preservative).

Table 3. Percentage CO_2 evolved from palm wine obtained from *R. hookeri* and preserved with five different preservatives.

Time (h)	A	B	C	D	E	F	G
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	1.82	3.19	3.59	2.00	2.50	3.00	2.90
48	0.44	1.41	1.72	0.40	0.44	1.00	1.82
72	0.12	0.60	1.44	0.20	0.22	0.44	0.19
96	0.09	0.12	0.72	0.11	0.10	0.10	0.09
240	0.03	0.05	0.06	0.03	0.04	0.04	0.00

Samples A-F is palm wine samples treated with different plant Preservatives A – Euphobia sp; B – Vernonia amygdalina; C – Saccoglottis gabonensis; D – Rubiaceae sp; E – Naucleae sp; F – Composite (A-E); G – Control (no preservative)

hibition exhibited by the extracts from the plant presservatives on the isolates as shown in Table 5 revealed that all the plant water extract did not inhibit the growth whereas the plant ethanol extracts showed obvious inhibition except *Euphobia* sp. and *Nauclea* sp., which had no inhibitory effect on *Bacillus* sp. Statistical analysis at 6 and 21 degree of freedom for 95% confidence level revealed that the effect of the preservation to be significant (Tables 6 and 7). Though the rate of significant was observed to be dependent on a particular preservative used.

Table 4. Percentage CO_2 evolved from palm wine obtained from *E. guineensis* and preserved with five different preservatives.

Time (h)	A	B	C	D	E	F	G
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	1.00	2.12	2.90	1.50	1.89	2.00	1.90
48	0.22	0.79	1.60	0.42	0.52	1.60	0.82
72	0.20	0.40	0.64	0.20	0.30	0.64	0.42
96	0.07	0.19	0.37	0.09	0.10	0.22	0.16
240	0.02	0.03	0.06	0.02	0.02	0.03	0.15

Samples A-F is palm wine samples treated with different plant Preservatives A – Euphobia sp; B – Vernonia amygdalina; C – Saccoglottis gabonensis; D – Rubiaceae sp; E – Naucleae sp; F – Composite (A-E); G – Control (no preservative)

DISCUSSION

The total heterotrophic bacterial counts were generally low in the palm wine samples with counts higher at 0 h than at subsequent periods as fermentation progresses. The higher bacterial count obtained at 0 h corroborates the report of Okafor (1972 a, b) and Ikenebomeh and Omayuli (1988). Thus, the bacterial and yeast count decreased with time suggesting a progressive loss of viability. This viability loss was more pronounced from the 4th day till the end of fermentation period. Seven microbial isolates were obtained; this consists of six probable bacterial genera which included *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Lactobacillus* sp., *Brevibacterium, Escherichia coli* and a yeast isolate identified to belong to the genera *Saccharomyces*. The occurrence of these microbial isolates in the palm wine samples, however, supports the reports made by Faparunsi and Bassir (1971), Okafor (1975a, b) and Ikenebomeh and Omayuli (1988), and lends more weight to the present finding.

Furthermore, the isolation of *Lactobacillus* sp. and *Saccharomyces* sp. corroborates the earlier report of Bassir (1962), Faparunsi and Bassir (1971, 1972 a, b). Thus, the isolation of *E. coli* from the raphia palm sample and *Micrococcus, Staphylococcus* from the two palm wine pose obvious public health questions. Hence, the unstable bowel movement associated with the consumption of raphia palm wine, as reported by some palm wine drinkers interviewed during the study could be associated with the pathogenic species of microbial contaminants such as *E. coli*. The parity in the microbiological quality of the 2 brands of palm wine may not be surprising since palm wine from *E. guineensis* is rarely diluted with water in this area of study as stated by the palm wine tapper, whereas the raphia palm is generally diluted with stream-

Table 5. Degree of inhibition (in zone diameters (mm)) of the isolates by the plant preservatives.

Preservatives	Extract concentration (50mg/ml)	Staphylococcus sp.	*Bacillus* sp.	*Micrococcus* sp.	*Lactobacillus* sp.	Escherichiae coli	*Brevibacterium* sp.	*Saccaromyces cerevisae*
Euphobia sp.	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	3.50	0.00	1.00	1.90	1.50	1.60	2.50
Vernonia amygdalina	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	8.00	12.00	10.00	10.00	11.00	8.50	10.00
Saccoglotti gabonensis	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	6.50	4.00	8.00	8.00	4.50	7.50	6.00
Rubiaceae sp.	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	8.00	8.90	10.00	10.00	8.00	9.00	8.00
Naucleae sp.	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	3.00	0.00	2.00	2.00	1.20	1.50	1.00

1 = Extracts obtained using hot water extraction method.
2 = Extracts obtained using ethanol extraction method.

Table 6. Analysis of Variance (ANOVA) on data from the sensory evaluation of the properties of preserved palm wine (*Raphia hookeri*).

Sources of Variance	Degree of freedom	Sum of square	Mean square	F cal
Treatment	6	33.25	5.54	8.16
Error	21	14.23	0.68	
Total	27	47.48		

Tabulated value of F (Ftab) at 6 and 21 degree of freedom (DF) for 95% confidence level (i.e. Ftab (6,21.0.95)) is 2.57, while the calculated value of F (Fcal) is 8.16 which, is much higher than the value for Ftab. It shows therefore that the effect of preservatives on the palm wine samples (*R. hookeri*) is not equal and is dependent on the type of preservatives used.

Table 7. Analysis of Variance (ANOVA) on data from the sensory evaluation of the properties of preserved palm wine (*E. guineensis*).

Sources of Variance	Degree of freedom	Sum of square	Mean square	F cal
Treatment	6	37.95	6.33	10.38
Error	21	12.80	0.61	
Total	27	50.75		

Tabulated value of F (Ftab) at 6 and 21 degree of freedom (DF) for 95% confidence level (i.e. Ftab (6,21.0.95)) is 2.57, while the calculated value of F (Fcal) is 10.38 which, is also higher than the value for Ftab. It also shows that the effect of preservatives on the palm wine samples (*E. guineensis*) is not equal and is dependent on the type of preservatives used.

water of questionable microbiological quality. Studies on the association of these organisms with water bodies in this area have been reported elsewhere (Blum et al., 1987; Nwanebu, 2001; Eme, 2003).

The gradual but progressive decrease in the level of the individual isolates as fermentation progresses could be associated with the progressive decrease in the level of the fermentation sugar as fermentation progresses as well as the obvious changes in the physicochemical quality that characterize the quality of palm wine sap. Amongst this physico-chemical quality that may affect the microbial succession in palm wine as fermentation progresses includes temperature, pH, oxygen tension, acidity, high-sugar concentration, alcoholic content and water activity (Rogomier et al., 1980; Eze and Ogan, 1987; Ikenebomeh and Omayli, 1988; Mmegwa et al., 1988).

The scores for taste and overall acceptability for the different treatment of oil palm and raphia palm wine with different traditional plant preservatives revealed that the palm wine treated with *Saccoglottis gabonensis* maintained acceptable foaming of the palm wine samples. It was as well found to be stable up to 96 h of collection. Nonetheless, the analysis of variance (ANOVA) on data obtained from different treatments of palm wine were found to be significantly different at 95% confidence limit from the controlled palm wine without any preservative.

Palm wine treated with plant preservatives were able to maintain some of its characteristic organoleptic qualities. Based on the microbial counts obtained and zones of inhibition by the extracts observed, loss of viability by the isolates could be associated with the preservative effect of the plant preservatives. Though the loss was more pronounced on the 4th day, the shelf life stability of the palm wine samples using the preservatives were maintained for four days after tapping. Similar reports of this finding has been made (Iheonu, 2000), and *Bacillus* sp. has been discovered to be the persisting organism in palm wine even after 96th hour of collection. However, Obire (2005) reported that preservation of palm wine could be achieved through inactivation of microorganisms at about 15 h after tapping when the density of these organisms is at its peak. Combination of such method with subsequent treatment using plant preservatives could however, extend the shelf life of palm wine more.

This study therefore showed that the plant preservatives have the potentials of extending the shelf life of two types of palm wine. Its development as a possible means of extending the shelf-life of palm wine in the rapidly expanding alcoholic beverage would make a significant contribution towards providing a low cost acceptable source of alcohol which hitherto can only be acceptable within 96 h after tapping.

ACKNOWLEDGEMENT

The authors are grateful to Mr. B. White the palm wine taper for providing fresh palm wine samples and the different traditional plant preservatives.

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