Evaluation of Microbial Quality and Alcoholic Improvement of Natural and Fermented Raphia Palmwine ("Ogoro")

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Abstract: Microbial quality evaluation was carried out on 150 samples of raphia palmwine collected from five local government areas of Osun State. Three bacteria species; *Bacillus cereus, B.firmus and Enterococcus faecalis*, and yeasts; *Saccharomyces cerevisiae, S.chevalieris and Kloechera apiculata* were recovered from the palmwine samples. Although, the bacterial isolates from this study showed acquired resistance traits to a number of antibiotics, they were mostly susceptible to clinically relevant antibiotic (i.e ampicillin and vancomycin). Sterilized raphia palm sap fermented with only Saccharomyces yielded a significantly improved alcohol content compared to naturally fermented palmwine.The result of sensory evaluation showed that the laboratory fermented raphia palmwine was more preferred in overall acceptability to the naturally fermented one. The study showed that through sterilization and use of purified *Saccharomyces* in fermentation of palm sap a more quality and hygienic palm wine could be produced. [New York Science Journal. 2010;3(2):35-39]. (ISSN: 1554-0200).

Keywords: Palmwine, Microbial-quality, Alcohol, *Saccharomyces, Fermentation.*

Introduction:

Palmwine is an alcoholic beverage produced by natural fermentation of sap of various palms (*Elias guineensis* or *Raphia* spp.). Raphia palmwine popularly referred to as 'Ogoro' is a traditional beverage of Yoruba in the Western Nigeria and other palm growing countries.

The unfermented raphia palm sap contains 10 – 16.5% w/v sugar (mainly in the form of sucrose) is fermented to ethanol and other minor constituents by a complex mixture of wild yeasts and bacteria. The naturally fermented raphia palmwine contains about 5 to 6% v/v ethanol (Nwokeke, 2001). Generally, palmwine is good for the body. According to Bassir (1982), palmwine's major constituents are; carbohydrate, organic acid, protein, vitamin C and Ash. There are various species of palm trees among which are *Elias guineensis, Raphia regalis, R. sudanica R..vinifera and .hookeri* (Obire,2005).

The fermentation of raphia palmwine is considered an in-expensive and effective means of food production in Nigeria, fresh palm sap are usually converted to palmwine during storage. Bacteria and yeasts usually contaminate the juice as it is tapped and there are changes in biochemical composition of the palmwine.

According to Faparusi, Bassir and Okafor(1991 and 1987), the bacteria that are most predominant in palmwine after fermentation are *Micrococcus, Leuconostoc, Lactobacillus* and *Acetobacter;* while the predominant yeasts usually identified are *Saccharomyces* and *Candida* spp.

Raphia palmwine is made traditionally and usually diluted with untreated water available in most rural communities. Microorganisms in palmwine are alive when it is being consumed. Hence, it is essential to investigate their pathogenic potentials. Therefore, the survey of antibiotic resistant and possible pathogenic bacteria in palmwine sold in Osun State, Nigeria was carried out.

Materials and Methods

One hundred and fifty samples of raphia palmwine were collected at different stages of fermentation from three different outlets each, in 5 local government areas of Osun State, over a period of four Months. The samples were collected into 250ml sized sterile bottles. The bottles were tightly closed and transported to the laboratory in an ice-packed container. Analyses commenced within an hour after collection.

Isolation and Characterization of Yeast

Yeasts were isolated by direct plating of palmwine samples (after appropriate dilution) on (1) Medium containing (g/l of distilled water); glucose, 40; yeast extract, 5; peptone, 3; hydrolysed casein, 5; agar 25 (YPG agar medium) and (2) medium obtained by adding 3% (w/v) agar to the unfermented fresh Raphia palm-sap collected in sterilized polythene bags (Sap agar medium). Plates were incubated at room temperature (25 – 27^{0}C). Single colonies of the yeasts that developed after 48hrs were purified on TYG agar plates and stored under

refrigeration on slants of the same medium. The identification of yeasts was according to the general methods described by Lodder (1990).

Isolation of Bacteria

Bacteria were isolated by direct plating of raphia palmwine samples on Bile esculin agar, and *Bacillus cereus* medium (BCM). The plates were incubated at 37^0C for 24hours. Pure cultures of the isolates were kept on nutrient agar slants and placed inside refrigerator until used. The bacterial isolates were identified as described by Barrow and Feltham (1993).

Antibiotic Susceptibility Test

Susceptibility of the bacterial isolates to the antibiotics was determined using disc diffusion method (NCCLS, 2000). The antibiotic multidisc containing Ampicillin (16µg), Penicillins (16µg/ml), Tetracycline (16µg/ml), Chloramphenicol (30µg/ml), Ciprofloxacin (5µg/ml), Erythromycin (8µg/ml), Gentamycin (30µg/ml) and Vancomycin (32µg/ml) (Abtek Biological Lts, U.K.) were used.

Alcoholic content determination

The methods used to determine the alcoholic improvements of the sampled palmwine were according the methods described by Theivendirarajah and Chrystopher (1987).

(i).Naturally fermented palmwine

Representative samples of raphia palm-sap were obtained from different localities in Osun State and put inside clean calabash which was normally used for the collection of raphia palmwine. After 1st, 5^{th} and 7^{th} day of fermentation, the alcohol contents of the samples were measured with an ebulliometer.

(ii).Laboratory fermented palmwine.

A 24hour culture of *S.cerevisiae* was prepared in 500ml of YPG broth and the yeast cells separated by centrifugation and washed with sterile distilled water. The yeast residue was transferred into clean plastic pots. Secondly, unfermented raphia palm-sap was obtained by using a collection pot lined with slaked lime. The lime was removed by sedimentation and later by precipitation as calcium phosphate by adding superphosphate. Precipitation was enhanced by heating to about 40 – 50% and also by centrifugation. The decalcified sweet sap having pH of 6.2 was sterilized by autoclaving at 121^0C for 15min. (Theivendirarajah andChrytopher, 1987). The medium was then inoculated with *S.cerevisiae* 10^7 cell/ml. Alcohol content of the medium was measured after 1^{st}, 5^{th} and 7^{th} day.

Sensory Evaluation of Palmwine Samples

The Natural and Laboratory fermented palmwine were subjected to sensory evaluation using a 5 point hedonic scale (Ihenkoronye and Ngoddy, 1985). Questionnaire was prepared to assess the flavour, taste, colour, foaming and overall acceptability of the palmwine. A ten member panel was selected to express their likes for each of attribute. The samples were presented to each of the assessors in two appropriately labeled glass cups.

Statistical Analysis

Analysis of variance (ANOVA) was used to compare the mean percentage data for alcohol content of the Natural and Laboratory fermented palmwine and the data obtained from the sensory evaluation. Significant differences between sample means were determined using Turkey's test (Ihekoronye and Ngoddy, 1985).

Results and Discussion

From various samples of palmwine examined, three groups of yeast strains were isolated. The yeasts were identified as, *S.cerevisiae*, *S.chevalieri*, and *Kloeckera apiculata*. Also, bacteria isolates fall into three groups; *Bacillus cereus*, B. *firmus* and *Enterococcus faecalis*.

From the totality of microbial isolates, yeasts show the highest numerical predominance, while the least was *E.faecalis* (Fig.1).The dominance of yeast may be due to the fact that the fermentation is not controlled, and the yeast inocullum comes mainly from unsterilized containers, previously used for the collection of sap. The genus *Saccharomyces* was the organism of importance in palmwine as revealed in this study, by its numerical predominance. Due to its superior fermentative ability, Saccharomyces may have adapted to growth in the special condition of the palm-sap (Faparusi and Bassir, 1991). The occurrence of *Bacillus* spp and *E. faecalis* are evidence of poor hygiene conditions of some of the palmwine sampled. These organisms may be contaminants from untreated water normally used in diluting palmwine in the villages, in order to increase the volume for high profitability.

Bacillus cereus has been reported to be mostly found as food contaminants and that its present in large amount could result in food poisoning (Whong *et al*., 2006). Adding to the problem is emergence of *E. faecalis* strain resistant to some test antibiotics (CHL, ERT & CIP), which is an indication of faecal contamination. It is difficult to assess the impact of antibiotic resistant *Enterococci* from foods on potential human pathogenicity. It is clear that in hospital environment, antibiotics may influence selection of pathogenic *Enterococci*, which

may lead to infections or super infections (Murray, 1990). Most problematic are strains that have acquired multiple antibiotic resistance, especially resistance to vancomycin and to the synergistic action of β lactams and aminoglycosides (Murray, 1990) which leaves few therapeutic options. Although, the *Enterococci* in this study showed acquired resistance traits to a number of antibiotics, they did generally not show resistance to the clinically relevant antibiotics, ampicillin or vancomycin and a low incidence of resistance towards gentamycin was observed. These results indicated that these food bacterial strains were mostly still susceptible to clinically relevant antibiotics. This study may be significant in the evaluation of the effectiveness of monitoring the spread of antibiotic resistant pathogenic bacteria in locally fermented alcoholic drinks which is highly important for community health.

The result of sensory analysis (table. 3) showed that laboratory fermented palmwine was more preferred to natural palmwine in almost all sensory attributes evaluated except in flavour. Palmwine flavour depends on the culture organisms, their metabolism during fermentation (Venkataramu *et. al.*, 1987). The low score obtained for laboratory fermented palmwine may be due to the absence of bacterial species which may have their contribution to the flavour of natural palmwine.

The pH value of naturally fermented palmwine was lower to the laboratory fermented palmwine. Acidity of palmwine imparts a sour taste to it as fermentation progresses, this is due to the presence of acid produced bacteria (i.e. *Bacillus cereus* and *B. firmus*) and hence, they make the taste unsuitable for human consumption as affirmed by Theivendirarajah and Chrystopher, 1987). The differences obtained from sensory evaluation of palmwine samples may be attributed to the removal of contaminated microorganisms through sterilization and the use of purified *Saccharomyces cerevisiae*. This may form a new technique for the production of a high quality and hygienic brand of palmwine.

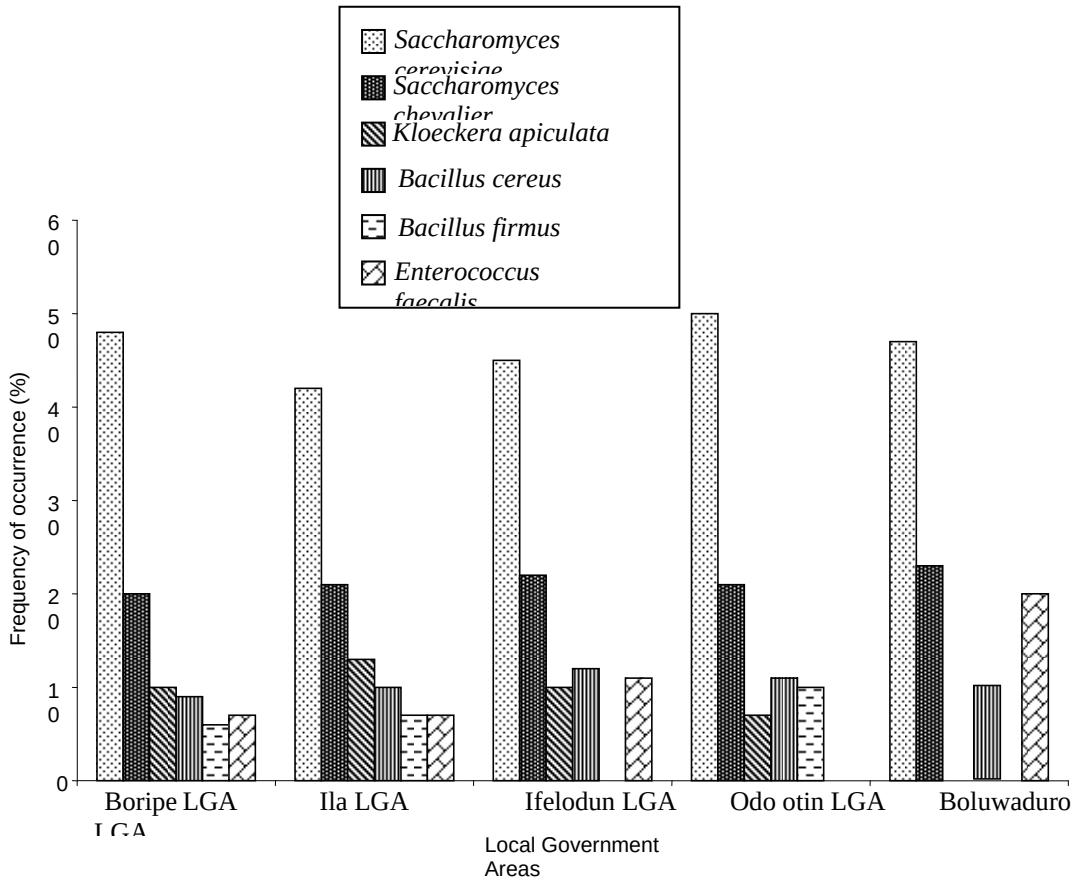

Fig. 1: Distribution of Microflora associated with raphia palmwine in five local government areas of Osun State.

Table 1: Antibiotic resistance of the bacterial isolates from raphia palmwine in Osun State.

Antibiotics	Percentage Resistance (%)		
	Bacillus cereus n=15 **(%)**	***B. firmus*** n =12 **(%)**	***E. faecalis*** n=10 **(%)**
Ampicillin	0	0	10
Penicillin	10	0	20
Tetracycline	20	10	40
Chloramphenicol	38	26	50
Erythromycin	25	22	40
Ciprofloxacin	26	30	40
Gentamycin	5	2	20
Vancomycin	0	0	0

Table2: Improvements inalcoholproduction of laboratory fermented, over naturally fermented raphia palmwine.

Day	Palmwine sample	Mean alcohol ($\%^{v}/_{v}$)	
1	Natural palmwine	4.2	a
	Laboratory palmwine	5.3	b
5	Natural palmwine	4.8	a
	Laboratory palmwine	5.8	b
7	Natural palmwine	5.6	a
	Laboratory palmwine	7.2	b

The values denoted by the letters a and b are statistically significantly different

Table 3: Mean scores of sensory evaluation of naturally and laboratory fermented palmwine

Sensory attribute	Natural palmwine	Laboratory palmwine
Flavours	3.5a	3.3a
Taste	3.1a	3.6b
Colour	2.5a	2.8b
Foaming	3.7a	2.5b
Overall acceptability	3.7a	3.9a

Mean on the same row having different letters are significantly different ($p<0.05$).

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