Microbial Load of Ready to Drink Palm Wine Sold in Ekpoma, Edo State.

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Abstract - Palm wine is a popular traditional alcoholic beverage consumed by more than 10 million people in West Africa. It is a sweet, effervescent drink obtained from the sap of the oil palm, *Elaeis guineense* and raphia palm, *Raphia hookeri*. The aim of this study is to evaluate the microbial load of palm wine sold in Ekpoma, Edo State. Identification of yeasts, Lactic acid bacteria (LAB) and Acetic acid bacteria (AAB) was employed. The yeasts isolated were evaluated at different times of the day (Morning, Afternoon and Evening) by determining their pattern of fermentation and assimilation of several sugars and also the usage of various carbohydrates. The LAB and AAB were examined by Gram staining, catalase test, microscopic morphology, gas production, and growth in different selective culture medium. The yeasts identification in palm wine was 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. It was shown that the physical properties were significantly different among samples, except for the taste which all have sweet taste. The pH of the palm wine samples also ranged from 4.49 in sample PWB to 5.23 in sample PWD and temperature ranged from 31.8 in sample PWC to 34.1 in sample PWE. In the chemical analysis, the total alkalinity of the sample ranged from 0.03% in sample PWA, PWD and PWE to 0.06 in sample PWB. The total solids ranged from 10.670 Brix in sample PWA to 16.57 in sample PWC. Total reducing sugar shows that sample PWD has the lowest value of 0.88% and the value ranged from 10.81% in sample PWA to 18.94% in sample PWC while the protein ranged from 0.31mg/l in sample PWE to 0.34mg/l in sample PWB and PWC. According to the microbiological safety of the palm-wine observed in this research work, the palm-wine samples were not up to the standard required. Consumption of palm-wine in unhygienic environment is therefore not safe, as contaminants have been observed in the drinks and these contaminants are dangerous as they can cause diseases. When consumed e.g *Salmonellosis* / typhoid fever caused by *Salmonella* spp, *Listeriosis* caused by *Listeria spp*. It is therefore safer not to consume palm wine if it is observed by the consumer that the premises where the beverages were being prepared or sold is not hygenic and if the handlers are of poor personal hygiene.

Index Terms - Palm, Wine, Lactic, Acetic, Acid, Bacteria, Yeast.

1 Introduction

Palm wine is a popular traditional alcoholic beverage consumed by more than 10 million people in West Africa (FAO, 1998). It is highly valued among the lgbos in south eastern part of Nigeria as number one alcoholic drink in traditional ceremonies. It is a sweet, effervescent drink obtained from the sap of the oil palm, *Elaeis guineense* and raphia palm, *Raphia hookeri*. The drink is a rich nutrient medium containing sugars, protein, amino acids, alcohol and minerals (Ezeagu and Fafunso, 2003). It also contains a dense population of yeasts (Bassir and Maduagwu, 1978). Thus when it is allowed to stand, fermentation converts the sugars to ethanol and subsequently to acetic acid, leading to loss of sweetness, shortened shelf life and decreased acceptability (Odunfa, 1985). The major objective of bottling is to prolong the shelf life of palm wine by arresting yeast growth and taste deterioration. Essentially, the process involves filtration of fresh palm wine, dilution with water, bottling and pasteurization. Thus water is an important input in palm wine preservation although this is often denied or even masked by addition of artificial sweeteners. Indeed one of the most frequent complaints of palm wine consumers is the adulteration of the product by use of water and artificial sweeteners, which sometimes result in diarrhoea, abdominal pains and stomach problems (GRI, 2004). The objective of this study is to evaluate the microbial load of palm wine in in Ekpoma. Research Hypothesis: There are no microorganisms found in palm wine in Ekpoma, The microbial load of the organisms isolated from palm wine in Ekpoma is not high and The microbial isolates of palm wine in Ekpoma are not pathogenic.

2 MATERIALS AND METHODS

This study was carried out in in Ekpoma, the administrative headquarters of Esan West Local Government Area of Edo State, Nigeria. The area proper lies between latitudes $6^0$43′ and $6^0$45′ North of the Equator and longitudes $6^0$6′ and $6^0$8′ East of the Greenwich Meridian (Aziegbe, 2006). Ekpoma is made up of many quarters including Eguare, Iruekpen, Emaudo, Ujoelen, Ihumudumu, Illeh, Uke, Uhiele, Ujemen, Ukepnu, Idoa, Ukhun, Egoro, Emuhi, Igor and Idumebo (Aziegbe, 2006). The following quarters, Eguare, Ujoelen, Ihumudumu, Emuado, and Iruekpen are all considered in this study. Ekpoma has a population of 89,628 and 127,718 at the 1991 and 2006 population census respectively (NPCN, 2012), majority of which are civil servants, traders,

businessmen/women, transporters, farmers, teachers/lecturers and students by occupation. The samples were examined in the Research Diagnostic Laboratory, of the Department of Medical Laboratory Science, College of Medicine, Ambrose Alli University, Ekpoma.

2.1 Collection of Samples

Seven brands of Palm wine was purchased from retail outlets in Ekpoma, Edo State, Nigeria. Fresh, palm wine were bought from 5 different locations which include; Eromon in Ekpoma, Evboakhuala in Ekpoma, Ikhirolo in Ekpoma, Uhiele in Elpoma and Emaudo in Ekpoma. The palm wine were purchased in 250ml sterile bottles and transferred into the Research Diagnostic Laboratory, of the Department of Medical Laboratory Science, College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria for analysis of the microbial load.

2.2 Analysis of Samples for Microbial Load

Amoa-Awua *et al.* (2007) identification of yeasts, LAB and AAB was employed. 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, microscopic morphology, gas production, and growth in different selective culture medium (Ziadi *et al*., 2011). The yeasts identification in palm wine was 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).

2.3 Bacteria Isolation

Two (2) milliliters of Palm wine sample was aseptically dispensed into 18mls of sterile Nutrient molten agar, allowed to solidify and were incubated at 37 °C for 24 hours. Same procedure was carried out in palm wine for different times of the day (Morning, Afternoon and Evening). Distinct colonies were sub- cultured twice and pure cultures were stored in Nutrient agar slants and stored at -20 °C. Biochemical analysis, motility test and sugar test was done on the colonies isolated for identification to specie level.

2.4 Preparation of Media

Media to be used for the work would be Nutrient agar, blood agar and potato dextrose agar for the isolation of bacteria, mould and yeast respectively. The media were prepared using manufacturer's instruction and sterilized using autoclave at 121°C for fifteen minutes.

2.5 Characteristics of Samples

The consistency and color of the samples were observed and noted. The pH of each sample was determined using pH meter.

2.6 Isolation and Characterization of Microbial Load

This was carried out using the pour plate method. The samples were serially diluted up to 10-4 and 1ml of each dilution was introduced into dry agar medium. Nutrient agar and Blood agar were used for this purpose. A sterile glass spreader was used to spread the suspension onto the surface of the agar medium (Lateef *et al*., 2004). For isolation purpose, two media namely Nutrient agar (NA) and Blood agar (BA) were used for isolation purpose using pour plate method. The plates were incubated at 37°C for 18-48 hrs and the discrete colonies were selected and were re-inoculated into appropriate medium. All the isolates were kept at 4°C in the refrigerator for identification purpose.

2.7 Identification of Organisms

The bacterial isolates were then identified following standard microbiological procedure as described by Cheesbrough (2002). The colonial morphology of the isolates on different media was observed and noted. The procedures for identification of isolates were described below:

2.7.1 Gram Staining: Gram staining was done according to method as described in Cheesbrough (2002).

2.7.2 Biochemical tests: The Biochemical tests were performed according to the methods as described in Cheesbrough (2002).

3 RESULTS

Palm wine is produced by natural fermentations from the clear, sugary saps of various palm trees (Odunfa, 1987). After collection, the sap turns white from the growth of bacteria and yeast which are contaminants from the air, tapping utensils and normal flora of the tree. The wine usually turns sour within a short period due to the acid produced by the microorganisms (Odunfa, 1985). The pH of the palm wine samples also ranged from 4.49 in sample PWB to 5.23 in sample PWD and temperature ranged from 31.8 in sample PWC to 34.1 in sample PWE. In the chemical

analysis, the total alkalinity of the sample ranged from 0.03% in sample PWA, PWD and PWE to 0.06 in sample PWB. The total solids ranged from 10.670 Brix in sample PWA to 16.57 in sample PWC. Total reducing sugar shows that sample PWD has the lowest value of 0.88% and the value ranged from 10.81% in sample PWA to 18.94% in sample PWC while the protein ranged from 0.31mg/l in sample PWE to 0.34mg/l in sample PWB and PWC as was presented. It is generally known that the primary sources of invertase are from yeast such as *saccharomyces cerevisiae, saccharomyces carlsbergensis* and fungi such as *Aspergillus oryzae* and *Aspergillus niger*.

Table 1: shows the physical properties of the palm-wine sample. The palm wine bought have a pH range 4.40 – 5.23 with PWB having the lowest pH with pH 4.49 and PWD having the highest pH with pH 5.23. Temperature range was between 32.4 – 34.1 with PWA having the lowest temperature of 32.4 and PWE having the highest temperature of 34.1. The whole palm wine were having a sweet flavor.

TABLE 1: THE PHYSICAL PROPERTIES OF THE PALM-WINE SAMPLED IN THE STUDY

SAMPLES	pH	TEMPERATURE	TASTE
PWA	5.10	32.4	Sweet
PWB	4.49	33.1	Sweet
PWC	4.60	33.8	Sweet
PWD	5.23	32.9	Sweet
PWE	4.87	34.1	Sweet

Key:
PWA- Isolates from Palmwine sample collected at Eromon - Ekpoma
PWB- Isolates from Palmwine sample collected at Evboakhuala - Ekpoma
PWC- Isolates from Palmwine sample collected at Ikhirolo - Ekpoma
PWD- Isolates from Palmwine sample collected at Uhiele - Ekpoma
PWE- Isolates from Palmwine sample collected at Emuado – Ekpoma

Table 2: shows the chemical quality of palm wine samples showing the total alkalinity, total solids, reducing sugar, total sugar and protein content. PWA, PWD and PWE had the lowest total alkalinity of 0.03% and PWB has the highest total alkalinity of 0.06%. PWA has the lowest content of total solid of 10.67% and PWC has the highest total solids of 16.57. PWD has the lowest content of reducing sugar of 0.88% and PWB has the highest content of reducing sugars of 1.85%. PWA has the highest content of total sugar of 10.81% and PWC has the highest content total sugar of 18.94%. PWE has the highest content of protein of 0.31mg/l while PWB and PWC has the same highest value of 0.34mg/l.

TABLE 2: CHEMICAL QUALITY OF PALM WINE SAMPLED IN THE STUDY

Parameter	PWA	PWB	PWC	PWD	PWE	Mean± SD	P value
Total Alkalinity (%)	0.03	0.06	0.05	0.03	0.03	0.04±0.01	0.05
Total Solids % (BRIX)	10.67	15.93	16.57	12.07	12.40	13.5±2.58	0.05
Reducing Sugar (%)	0.99	1.85	1.74	0.88	1.15	1.40±0.44	0.05
Total Sugar (%)	10.81	14.32	18.94	11.72	12.60	13.7±3.21	0.05
Protein (mg/l)	0.33	0.34	0.34	0.32	0.31	0.33±0.01	0.05

Key:
PWA- Isolates from Palmwine sample collected at Eromon - Ekpoma
PWB- Isolates from Palmwine sample collected at Evboakhuala - Ekpoma
PWC- Isolates from Palmwine sample collected at Ikhirolo - Ekpoma
PWD- Isolates from Palmwine sample collected at Uhiele - Ekpoma
PWE- Isolates from Palmwine sample collected at Emuado – Ekpoma

Table 3: shows the total plate count and the total yeast count in the palm wine samples. The total plate count (TPC) (105cfu/ml) in this study was between the range of 0.9 - 2.3 (105cfu/ml) with PWC having the lowest TPC of 0.9 (105cfu/ml) and PWA having the highest TPC of 2.3(105cfu/ml) with a total mean of 1.7 (105cfu/ml). The total yeast count (TYC) (104cfu/ml) in this study was in the range of 1 - 6 (104cfu/ml) with PWB having the lowest TYC

of 1 (104cfu/ml) and PWD having the highest of 6 (104cfu/ml), the mean in this study is 3.2.

TABLE 3: TOTAL PLATE COUNT AND THE TOTAL YEAST COUNT IN THE PALM WINE SAMPLED IN THE STUDY

Parameters	PWA	PWB	PWC	PWD	PWE	Mean± SD	p-value
Total Plate Count (TPC) 105cfu/ml	2.3	1.4	0.9	2.1	1.8	1.7±0.56	0.05
Total Yeast Count 104cfu/ml	2	1	4	6	3	3.2±1.92	0.05

Key:
PWA- Isolates from Palmwine sample collected at Eromon - Ekpoma
PWB- Isolates from Palmwine sample collected at Evboakhuala - Ekpoma
PWC- Isolates from Palmwine sample collected at Ikhirolo - Ekpoma
PWD- Isolates from Palmwine sample collected at Uhiele - Ekpoma
PWE- Isolates from Palmwine sample collected at Emuado – Ekpoma

Table 4: shows the frequency and the percentage distribution of bacterial and the yeast isolated from the palm wine. The bacteria organisms isolated in the course of this study were *Leuconostoc spp.* (3) (17.6%), *Lactobacillus spp.* (6) (35.3%), *Acetobacter spp.* (4) (23.5%), *Corynebacterium spp* (3) (17.6%) and *Listeria spp* (1) (5.9%) and the yeast isolated are *Saccharomyces cerevisiae* (6) (50%) and *Saccharomyces carlbsbergensis* (6) (50%).

TABLE 4: FREQUENCY AND THE PERCENTAGE DISTRIBUTION OF BACTERIAL AND THE YEAST ISOLATED FROM THE PALM WINE

Microorganisms	Frequency	Percentage Distribution (%)
BACTERIA		
Leuconostoc spp.	3	17.6
Lactobacillus spp.	6	35.3
Acetobacter spp.	4	23.5
Corynebacterium spp.	3	17.6
Listeria spp.	1	5.9
YEAST		
Saccharomyces cerevisiae	6	50
Saccharomyces carlbsbergensis	6	50

4 DISCUSSION

Growth of micro-organisms in palm wine during fermentation. The sap of the palm tree has been shown to be a rich medium capable of supporting the growth of various types of micro-oganisms, as high numbers of aerobic mesophiles, lactic acid bacteria, yeasts and acetic acid bacteria were found in palm wine during the tapping of felled palm trees for up to 5 weeks. In discussing the population of the various micro-organisms in palm wine, the following factor appears important. Despite this, the microbial population found in the palm wine samples appeared to be fairly stable.

Palm wine tappers collect their wine in the morning and evening, but wine collected in the morning would have accumulated throughout the night. Such samples were found to contain much higher concentrations of alcohol ranging from 3% to 5% and this is in agreement with the alcohol concentrations reported by Herzog *et al.,* (1995).

Microbial species responsible for the fermentation of palm wine. *Saccharomyces cerevisiae* has been confirmed in the present work as the dominant yeast species responsible for the fermentation of palm wine tapped from the felled palm trees. In mature palm wine samples, only *S. cerevisiae* were isolated and this species appear to completely dominate the fermentation of palm wine in the felled palm trees. This is in agreement with other studies carried out in Ghana (Okraku-Offei 1968; Owusu 1982; Brown 1990, 1994).

In Nigeria, Owuana and Saunders (1990) isolated both S. cerevisiae and K. apiculata from the palm wine, whilst Ezeronye and Okerentugba (2000) reported the presence of

S. cerevisiae. Enwefa *et al*., (1992), however, reported the presence of several genera of yeasts including *Saccharomyces, Candida, Endomycopsis, Hansenula, Kleoclera, Pichia, Saccharomycoides and Schizosacchromyces* in palm wine.

From the table 1, it was shown that the physical properties were significantly different among samples, except for the taste which all have sweet taste. The pH of the palm wine samples also ranged from 4.49 in sample PWB to 5.23 in sample PWD and temperature ranged from 31.8 in sample PWC to 34.1 in sample PWE. In the chemical analysis, the total alkalinity of the sample ranged from 0.03% in sample PWA, PWD and PWE to 0.06 in sample PWB. The total solids ranged from 10.670 Brix in sample PWA to 16.57 in sample PWC. Total reducing sugar shows that sample PWD has the lowest value of 0.88% and the value ranged from 10.81% in sample PWA to 18.94% in sample PWC while the protein ranged from 0.31mg/l in sample PWE to 0.34mg/l in sample PWB and PWC as was presented in table 2. It is generally known that the primary sources of invertase are from yeast such as *saccharomyces cerevisiae, saccharomyces carlsbergensis* and fungi such as *Aspergillus oryzae* and *Aspergillus niger*.

Moreover, an increase in total acidity and decrease in pH are also responsible for the inversion reaction. The inversion reaction occurs when the glucosidic linkage of disaccharide is hydrolyzed, releasing the monosaccharide units. In the chemical quality parameters analyzed such as pH, total acidity, total soluble solids, reducing sugar, it was determined that the pH and the total acidity of all palm wine samples were significantly different among the samples, since lactic acid is the main organic acid present in palm wine samples which leads to the differences.

In the total plate count, observed in palm-wine sample analyzed, the total bacteria count ranged from 0.9 X 105cfu/ml in sample PWC to 2.3 X105cfu/ml in sample PWA while the mean values is 1.8X105cfu/ml. For the total yeast count, the value ranged from 1.0 in PWB to 6.0 in PWD, with the mean value of 3.2.

5 CONCLUSION

According to the microbiological safety of the palm-wine observed in this research work, the palm-wine samples were not up to the standard required. Consumption of palm-wine in an unhygienic environment is therefore not safe, as contaminants have been observed in the drinks and these contaminants are dangerous as they can cause diseases. When consumed e.g *Salmonellosis* / typhoid fever caused by *Salmonella* spp, *Listeriosis* caused by *Listeria spp*. It is therefore safer not to consume palm wine if it is observed by the consumer that the premises where the palm wine is being sold is not hygienic and if the handlers have poor personal hygiene.

6 CONTRIBUTION TO KNOWLEDGE

Results from this study will help to access the microbial load of ready to drink palm wine sold in Ekpoma and environ. This will help to create awareness on the microbial load for the public due the hygienic conditions of the sale points and to recommend the best management and preventive measures to reduce the microbial load in the palm wine via personal and environmental hygiene, better public awareness and public policies. It will also help the government to sensitize the general public on hygienic status of business owners in the study area. This research work is expected to add to the already existing or available literatures that will be assessable to individuals and organizations when the final work is published.

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