Studies on the Microbial burden of Palm wine sold and consumed in Emohua local government area, Rivers State, Nigeria.

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ABSTRACT

The study was conducted to analyze and identify the microbes in *Palm wine* as a pre-requisite to attempts at increasing the shelf life of *Palm wine.*

The *Palm wine* was collected from three (3) different Locations in Emohua Local Government Area namely; Rumuji, Ovogo and Ibaa, which are the areas in which *Palm wine* is produced and consumed most. An aliquot (0.1ml) of diluted *Palmwine* from the 3 locations were inoculated on surface dried *Nutrient agar, Mcconkey agar* and *Sabouraud agar plates* incubated at room temperature and 37^0C under aerobic and anaerobic conditions yielding bacteria and fungi isolates. Physical and biochemical properties were used to identify the isolates. The organism isolated were *Saccharomyces cerevisae, Bacillus subtilis, Corynebacterium specie, Lactobacillus delhrueckii* and *Micrococcus varians*.

The organisms were present in all the *Palm* wine studied regardless of the sources and dates of collection. In Rumuji, a total of 143.32 x 10^{-6} colony forming units/ml (cfu/ml) were counted, Ovogo had 56.12 x 10^{-6} Cfu/ml and Ibaa 59.29 x 10^{-6} Cfu/ml were identified given an overall microbial burden of 258.73 x 10^{-6} Cfu/ml. There was no significant growth in bacterial count and decrease in yeast count with increasing distance from the location of the study. There was a steady decrease in the viable count of Microbes with age of the *Palm wine* ($P > 0.05$). The study hereby suggests the need to study the possibility of aseptic tapping and the blocking of the glycolytic pathway as a means of prolonging the Shelf life of *Palm wine.*

Keywords:

Microbes,
Palm wine,
Glycolytic,
Saccharonyces,
Probiotic, Pathogenic.

I. INTRODUCTION

Palm wine is an alcoholic beverage gotten from the sap of various species of Palm trees such as Palmyra, date palms and coconut palm (Rundel, 2002).

In some parts of Eastern Nigeria, and South-South Nigeria, especially Igbo and Ikwerre land, and Etche land, palm is called ***"Nkwu elu", Nmanya ocha (White drink), Maya Ngwo, Ucot Nsun, Palmy, Mmin Efik, emu, Oguro, tombo liquor, Nku ocha*** but in South Africa it called Ubusulu (Frank *et al., 2008)*. It is also called Nsamba in Democratic Republic of Congo and Nsafufuo in Ghana (Hogar, 2008). Palm wine is normally distilled to create a stronger drink such as local gin, kai-kai and country whiskey, ogogoro. In Ghana it is also called Akpeteshi or burukutu.

Palm sap begins to ferment shortly after collection, due to natural yeast in the pores of pot and air. Within 2 hours fermentation yields an aromatic wine of up to 4% alcohol content, mildly intoxicating and sweet. The wine may be allowed to ferment longer, upto 24 hours to yield a stronger, more sour and acidic taste which many people prefer. Longer fermentation produces vinegar of stronger wine (Frank *et al., 2008)*. *Palm wine* undergoes a process of distillation to produce local gin.

The difficulty of storing *Palm wine* to retain its normal characteristics is due to the fermentative ability of microbes present in the *Palm wine* and this has been a major problem in bottling of *Palm wine* in Nigeria.

Palm wine can be obtained from the young inflorescence either male or female ones. It is an alcoholic beverage that are made by fermenting the sugary sap from various *Palm plants.* It is generally collected by tapping either the top of the trunk by falling the palm trees or boring a hole into the trunk (Noll, 2008). It is cloudy whitish beverage with a sweet alcohol taste and very short life of only one day. The *Palm* is consumed in varieties of flavors varying from sweet unfermented to Sour, fermented and vinegary (Eric *et al.,* 1976).

Palm wine is an important source of nicotinic acid and vitamin c, protein, thiamine and riboflavin (Cunningham *et al.,* 1988). *Palm wine* is used as a symbol of unity by a popular club in Nigeria known as *Palm wine* drinkers club. *Palm wine* contains nutritious organic substance such as carbohydrate especially Sucrose. It also contains other substances such as Vitamins C, B, B1, B2, B12 and load of yeast recommended by natives as good for the eyes (Chouldhury *et al.,* 2013). Intoxication is as a result of alcohol fermentation carried out by Microbes in the Palm, utilizing the availably sugar as their source of substrate and producing alcohol as their metabolic waste.

A young man going for first introduction at his in-laws is required to come with *Palm wine*. There are specific gallons required depending on the custom of the various towns in some parts of Nigeria. In South East Asia the pen tailed tree shrew is consumed large amount of fermented palm nectar as part of their diet by some small pollinating mammals (Laws *et al.,* 2011).

In Igbo and Yoruba, a bride confirms a bridegroom as her husband by pretending to seek him out among the crowd and kneeling down to symbolically present a Calabash of *Palm wine* to him. It is also offered to the ancestor as a sign of worship in Africa.

Palm wine contains bacteria and yeast and other chemicals that are good for treating some common ailment like eye problem, malaria when soaked with herbs. Some eye Doctors and Optometrist say as yeast is good for the eye, *Palm wine* helps in enhancing sight but cautions that the high level of alcohol in *Palm wine* is injurious to organs of the body such as liver, kidney and eye (Ogbulie *et al.,* 2007).

Palm wine and its distillate are important solvent in herbal-medicinal administration, pregnant women consume it fresh for the sweetness and nutrition while nursing mothers drink it warm to enhance breast milk production.

Palm wine consist of many bacteria mainly lactic acid bacteria such as *Lactobacillus*, *streptococcus* etc. The pathogenic bacteria present in *Palm wine* that causes infection includes *Micrococcus varans*, *Serratia* etc.

The Probiotic bacteria that are beneficial to the host include *Lactobacillus*, *Leuconostic* etc. Fermentation of *Palm wine* start soon after the sap is collected and within an hour or two becomes reasonably high in alcohol (up to 4%) if allowed to continue to ferment for more than a day, it starts turning into vinegar (Awasum, 2007). Yeast degrades hydrocarbons such as kerosene and Oil spills as a Source of Carbon and energy source for growth and this suggest its potential application in oil cleanup as well as in single cell protein application using hydrocarbon feed stock (Ogbonna, 1984).

This study is aimed at identifying Microbes (Pathogenic and Probiotic) that are found in Palm so that people could be well guided.

II. MATERIALS AND METHODS

Study Area : The study was carried out in 3 rural communities in Emohua Local Government Area of Rivers State where *Palm wine* is produced, consumed and sold. The communities are Rumuji, Ovogo and Ibaa.

Collection of *Palm wine* samples and Transportation: Five different samples were collected from each of the different locations of Rumuji, Ovogo and Ibaa, from the *Palm wine* bars and transported in a sterile container with iced block to maintain its original status. It was then delivered to the laboratory and stored in the refrigerator before culturing.

Dilution and Plating of the Sample: Ten (10) fold dilution of the *Palm wine* were made using sterile normal saline (0.85%) as diluents. The dilutions were made in order 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}. From 10-4 dilution of each *Palm wine* an aliquot (0.1ml) was transferred into freshly prepared Nutrient agar, Mac Conkey and Sahauraud agar plates.

The plates were incubated aerobically and anaerobically at Room temperature for day one (1) and thereafter inoculated and incubated for day three (3) for 24 hours.

The plates were also incubated at 37^0c aerobically and anaerobically for day one (1) and day three (3).

Enumeration of Isolates: After incubation, the isolates were identified Morphologically, Gram's Method, Biochemically and viable cell count done in Colony forming unit/ml (CFU/ml).

III. RESULT

Table 1 shows the overall Microbial burden of *Palm wine* as 258.7 x 10^{-6} Cfu/ml with a mean value of 86.2. Rumuji had a rate of 143.32 x 10^{-6} cfu/ml, Ovogo 56.12 x 10^{-6} cfu/ml while Ibaa 59.29 x 10^{-6} cfu/ml.

Table 1: The microbial burden according to the villages under study

Microbial burden	Rumuji (10^{-6}cfu/ml)	Ovogo (10^{-6}cfu/ml)	Ibaa (10^{-6}cfu/ml)	Total no Microbe (10^{-6}cfu/ml)	Mean
Saccharomyces cerevisae	96.1	9.02	8.87	113.99	37.1
Bacillus subtilis	19.04	22.2	0.82	42.06	14.02
Corynebacterium sp	5.86	5.7	30.4	41.96	13.9
Lactobacillus delbruekii	11.1	7.1	6.5	24.7	8.2
Micrococcus varians	11.22	12.1	12.67	35.99	11.9
Total	143.32	56.12	59.29	258.7	86.2

Table 2 shows the microbial burden in *Palm wine* in Rumuji. Saccharonyces accounted for 96.1x10^{-6}cfu/ml, Bacillus sp 19.04x10^{-6}cfu/ml, Corynebacterium sp 5.86x10^{-6}cfu/ml, Lactobacillus 11.1x10^{-6}cfu/ml and Micrococcus 11.22x10^{-6}cfu/ml. All gave a total burden of 143.32x10^{-6}cfu/ml.

Table – 2 : Microbial burden in Palmwine sold in Rumuji.

Microbial burden	Room Temperature (10^{-6}cfu/ml)				37^{0}C (10^{-6}cfu/ml)				Total
	Day 1 aerobic	Day 3 aerobic	Day 1 anaerobic	Day 3 anaerobic	Day 1 aerobic	Day 3 aerobic	Day 1 anaerobic	Day 3 anaerobic	
Saccharomyces cerevisae	12.4	5.6	15.9	10.2	14.0	8.7	17.3	12.0	96.1
Bacillus Subtilis	1.6	0.04	5.5	0.2	3.6	0.2	7.7	0.2	19.04
Corynebacterum sp.	0.5	0.1	1.3	0.1	1.4	0.06	2.3	0.1	5.86
Lactobacillus sp.	1.4	0.1	3.0	0.3	2.2	0.2	3.6	0.3	11.1
Micrococcus varians	1.5	0.05	3.2	0.01	1.9	0.08	4.4	0.08	11.22
Total	**17.4**	**5.89**	**28.9**	**10.8**	**23.1**	**9.96**	**35.3**	**12.68**	**143.32**

Table 3 shows the overall microbial burden of Microbes in *Palm wine* sold in Ovogo as 56.12x10^{-6}cfu/ml with S. Cerevisiae accounting for 9.02x10^{-6}cfu/ml, Bacillus Sp as 22.2x10^{-6}cfu/ml, Corynebacterium sp. as 5.7x10^{-6}cfu/ml, Lactobacillus 7.1 x 10^{-6}cfu/ml and Micrococcus sp. as 12.1x10^{-6}cfu/ml.

Table – 3 : Microbial burden in Palmwine sold in Ovogo.

Microbial burden	Room Temperature (10^{-6}cfu/ml)				37^{0}C (10^{-6}cfu/ml)				Total
	Day 1 aerobic	Day 3 aerobic	Day 1 anaerobic	Day 3 anaerobic	Day 1 aerobic	Day 3 aerobic	Day 1 anaerobic	Day 3 anaerobic	
Saccharomyces cerevisae	1.1	0.01	1.7	0.01	2.6	0.1	3.4	0.1	9.02
Bacillus Subtilis	2.2	0.1	3.8	0.1	6.3	1.1	7.4	1.2	22.2
Corynebacterum sp.	0.7	0.2	0.8	0.3	1.4	0.3	1.7	0.3	5.7
Lactobacillus sp.	0.9	0.2	1.2	0.2	1.8	0.1	2.6	0.1	7.1
Micrococcus varians	2.7	0.2	1.8	0.2	3.1	0.2	3.7	0.2	12.1
Total	**7.6**	**0.71**	**9.3**	**0.81**	**15.2**	**1.8**	**18.8**	**1.9**	**56.12**

Table 4 shows the microbial burden of Microbe in *Palm wine* sold in Ibaa as 59.29x10^{-6}cfu/ml with Saccharomyces cerevisae accounting for 8.7x10^{-6}cfu/ml, Bacillus Sp as 0.87x10-6cfu/ml, Corynebacterium sp as 30.4x10^{-6}cfu/ml, Lactobacillus 6.5 x 10-6cfu/ml and Micrococcus sp. as 12.67x10^{-6}cfu/ml.

Table – 4 : Microbial burden in Palmwine sold in Ibaa.

Microbial burden	Room Temperature (10^{-6}cfu/ml)				37^{0}C (10^{-6}cfu/ml)				Total
	Day 1 aerobic	Day 3 aerobic	Day 1 anaerobic	Day 3 anaerobic	Day 1 aerobic	Day 3 aerobic	Day 1 anaerobic	Day 3 anaerobic	
Saccharomyces cerevisae	1.2	0.01	1.5	0.06	2.6	0.1	3.2	0.1	8.7
Bacillus subtilis	0.04	0.1	0.08	0.1	0.1	0.13	0.1	0.2	0.87
Corynebacterium sp.	2.2	1.8	3.7	2.0	6.3	4.1	6.6	4.2	30.4
Lactobacillus sp.	0.8	0.6	0.4	0.6	1.0	0.9	1.1	1.1	6.5
Micrococcus varians	3.7	0.07	1.9	0.1	3.2	0.1	3.4	0.2	12.67
Total	**7.9**	**2.58**	**7.58**	**2.86**	**13.2**	**5.33**	**15.4**	**5.8**	**59.29**

Figure 1 shows the viable count according to the days of incubation (day 1 and day 3). The day 1 in Rumuji shows viable microbial count of $104.7x10^{-6}$cfu/ml and 74.22 x 10^{-6} cfu/ml for day 3.

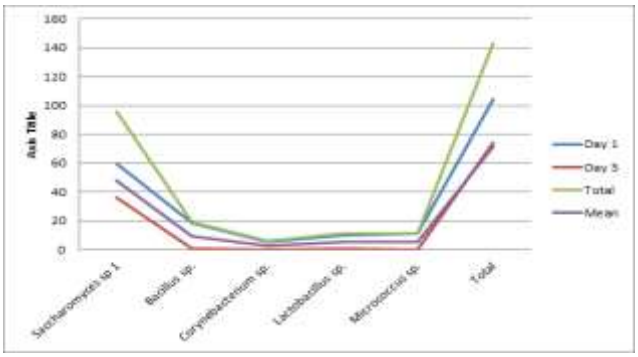

Figure 1: Number of Viable count according to days in Rumuji

Figure 2 shows the viable count in Ovogo as day 1, 50.9x10-6 cfu/ml and day 3 5.22x10-6cfu/ml.

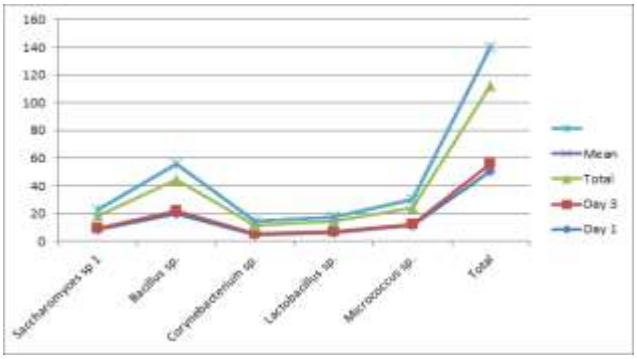

Figure 2: Number of Viable count according to days in Ovogo

Figure 3 for Ibaa shows viable count of 42.62 x 10-6cfu/ml for day 1 and 16.67x10-6cfu/ml for day 3.

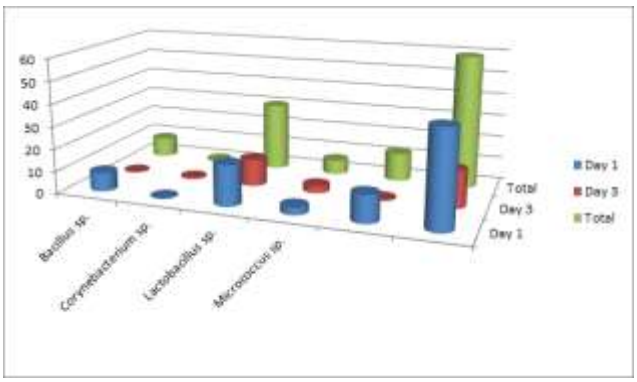

Figure 3: Number of Viable count according to days in Ibaa

IV. DISCUSSION

The organisms isolated from *Palm wine* were *Saccharomyces cerevisae, Bacillus subtilis, Corynebacterium sp., Lactobacillus sp*. and *Micrococcus varian*, most of whom are human pathogens while others are beneficial to man. There was a decrease in microbial count as the *Palm wine* becomes older. This could be as result of fermentation carried out by yeast and bacteria present in the *Palm wine*. Yeast is known to produce alcohol and carbon dioxide as products of fermentation of sugar, change in pH as a result of acid production. These conditions are inimical to bacteria growth and survival. The presence of most of Pathogenic Microbes may expose individual to diarrhea.

The variation in the microbial count in *Palm wine* obtained from the same area may have been due to the fact that the *Palm wine* was not collected from the same tapper. Microorganism could also be from utensils, containers used by the tapper. The count could also be qualitatively variable because of the personal habit of the tapper. It is also usual habit in Emohua that *Palm wine* be diluted after tapping before getting to the point of consumption and this may lead to increase the volume. This dilution should ultimately cause reduction in the microbial count per mil. The decrease in bacterial count could be that the water used for the dilution may contain some of these micro-organisms or as a result of the transfer of the *Palm wine* from container to container each harbouring its own microbial population. This could also cause increase in the bacterial load.

V. CONCLUSION

The microbial burden analysis was done to isolate and identify the microbe in *Palm wine* and also to find a lasting solution to the change in taste of a day old *Palm wine* from surgery taste to the sour taste of older *Palm wine*. Another way is by blocking the metabolic Pathway of yeast and other sugar fermenting microbes. It is necessary to advise tapper to adopt aseptic precaution while tapping in order not to endanger the lives of their customers as a result of inherent risk associated with consumption of *Palm wine* as a result of Pathogenic Microbes.

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