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Analysis of the Microbial Quality of Locally Consumed Palm Wine Sold in Elele Community of Rivers State Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author IMI designed the study and performed the statistical analysis. Author BCA wrote the protocol and first draft of the manuscript. Author CCU managed the literature searches. Author SON managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Palm wine is generally consumed due to its nutritive composition to the human body system particularly when fresh and unfermented state. A total of 20 Palm wine samples obtained from two different locations in Elele community of Rivers state, were analyzed for their microbiological qualities. A ten-fold serial dilution method was used. For Total Aerobic Plate Count (TAPC) nutrient agar was used, MacConkey for coliform count (CC), Eosin methylene blue for *Escherichia coli* count (EC), and Potato dextrose agar for the fungal count. Microbial counts in the palm wine sold in the drinking bar were higher than that of the palm wine tapper. TAPC, the sample from the drinking bar has a mean value (6.73+ 0.22 log10cfu/ml) which was higher than the value obtained from the palm wine tapper (6.70+0.15log10cfu/ml). The coliform count of palm wine from the drinking bar was (6.57+ 0.10log10cfu/ml) but not significantly different from those with minimum counts (6.56+ 0.9log10cfu/ml) obtained from the tapper. *Escherichia coli* of palm wine from drinking bar were (5.73+ 0.23 log10cfu/ml) which were higher than (5.71+ 0.18 log10cfu/ml). The Fungal counts of palm wine sampled from the drinking bar were higher but not significantly different from

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those obtained from the tapper. Bacteria isolated from the two respective palm wines sampled included *Staphylococcus* spp 50% and 30% respectively, *Klebsiella* spp 20% and 30% respectively, *Proteus* spp 40% and 10% and 30% respectively, *Aspergillus* spp 30% , 10% and *Saccharomyce cerevisae* 20% and 30% respectively. For the analysis of variance, bacteria and fungi count was not significant. Consumers of palm wine are advised to purchase the product from the tapper to reduce the chances of contamination.

Keywords: Palm wine; microbial; bacteria; fungi; tapper.

1. INTRODUCTION

Palm wine is described as an alcoholic beverage gotten from the sap of various species of palm tree such as the palmyra and coconut palm. Raphia palm wine popularly referred to as "Ogoro" is a traditional beverage of Yoruba in 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). It is fermented to ethanol and other minor constitutions by a complex mixture of wild yeast and bacteria [1]. Generally, palm wine is good for the body system, particularly when consumed fresh and unfermented. Palm wine is high in amino acids, potassium, magnesium, zinc, and iron. Palm wine constitutes the majority of carbohydrates, organic acid, protein, vitamin C, and ash. There are different species of palm trees among which are Elaeis guineensis, Raphia reghalis, R vinifera, and R. hookeri [2].

The fermentation of Raphia palm wine is considered inexpensive, fresh palm sap is usually converted to palm wine during storage. As a matter of fact, during the storage process when there is inadequate hygienic practice properly observed either by the tapper or bar seller it could subject such palm wine to bacteria and fungi contamination because the primary agent that is involved in contaminating the juice as it is tapped and thereby causing biochemical changes in the composition of the palm wine.

According to Faparusi (1994), Bassir and Okafor [3] the bacteria that are predominant in palm wine after fermentation are *Micrococcus* spp, *Leuconostoc* spp, *Lactobacillus* spp, and *Acetobacter* spp. while the predominant yeast usually identified are *Saccharomyces* and *Candida* spp. Palm wine is common in various parts of Asia and Africa, having a variety of flavors from the sweet unfermented to sour, fermented, and vinegary, having a very short shelf life of one. Palm tree belongs to the family of Palmaceae or Palmae. Palm wine is collected by tapping the top of the trunk after felling the palm tree; a hole is bored into the trunk. Palm wine is a cloudy whitish beverage with a sweet alcoholic taste; the wine is an excellent substrate for microbial growth. Fermentation starts soon after the sap is collected within an hour or two [4]. The sap should be collected from a growing palm, the sap is neutral in reaction and sweet, clear, colourless containing 10-12% of sugar mainly sucrose. The most abundant and consistently found organism is the yeast of the genera *Saccharomyces* spp. which converts palm sap to palm wine and the *Staphylococcus* spp.

Palm wine contamination is linked to certain factors such as the collection of palm sap with an unwanted utensil, repeatedly used without proper sterilization of the instrument, and unhygienic storage conditions which in turn facilitate the multiplication of microbial growth within the palm wine hence exposing the potential consumers. The environmental conditions of a palm wine bar and storage facilities are not adequate particularly in this study area because they expose the palm wine to the attack of a housefly which is regarded as one of the disease vectors with these storage bottles that often time are not properly sterilized. The numbers of genera of microorganisms found in this kind of sap are high, unlike those collected with a sterile instrument. Attempts have been made towards the preservation and shelf life extension of palm wine through bottling, use of chemicals additives, and addition of plant extracts which greatly affect the organoleptic quality of the product [5] Most times, palm wine is being diluted by the taper or the bar seller with water which may harbour some water pathogens as a result of inadequate treatment or purification of these water which eventually be utilized in the process of palm wine dilution thereby endangering the health safety of the consumers thus, leading to different ill- health and disease outbreak in such area if proper management is not put to check. Therefore, it is imperative, to investigate the microbial quality of locally consumed palm wine sold in Elele community since it is generally consumed by the

people and used during major traditional events activities such as traditional marriage rights coupled with other events that require palm wine.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted within Elele community in Ikwerre local government area of Rivers State, Nigeria. The study was undertaken between July 10th – September 15, th 2021.

2.2 Collection of Samples

Twenty (20) samples of palm wine (from oil palm tree, *Elaeis guineensis*) were collected from two different locations in Elele which include (Drinking bar and palm wine tapper). A sterile container was used to put the sample after which the samples were taken to the laboratory in a covered container filled with ice block and freezing mixture of staff microbial analysis within sample [6].

2.3 Preparation of Dilution and Samples for Bacterial Enumeration Analysis

About 9mls normal saline was dispenses into each of the ten (10) capped tube using sterile 10mls pipette and they are sterilized for 15minutes at 121^{o}C in autoclave. Thereafter, 1ml of each sterilised diluents suspension was introduced into the test tubes using the tenfold serial dilution technique and labelled 10^{-2}. It was thoroughly mixed by shaking the tube with its cap properly closed, after which 1ml was removed from the test tube labelled 10^{-2} and dispensed in the one labelled 10^{-3} this dilution continues until the last labelled 10^{-10}, were all mixed. 1ml was withdrawn and discarded.

2.4 Enumeration of Microorganism

After tenfold serial has been done, 0. 1ml aliquots from 10^{-1} – 10^{-10} dilutions were aseptically transferred on a Petri dish and 18-20ml of nutrient agar was poured on the Petri dish and swirled clockwise, anticlockwise for distribution and it's been left to solidify. The MacConkey agar plate is been used for coliform count, Nutrient agar for total aerobic plate count (TAPC), Eosin Methylene Blue (EMB) for *Escherichia coli* count, Potato Dextrose agar for fungal count and also for fungal isolation. The agar plates were inverted, but the fungal plates were not and incubated at 37^{o}C for 24hrs while the potato dextrose agar plate incubated at 25^{o}C for 72-120hrs. Then the colonies were counted after incubation time for plates which yield counts between 30-300 colonies [7].

2.5 Purification and Preservation of Colonies

A sterile wire loop was used to pick discrete from the nutrient agar based on shape, opacity, elevation and edge and subsequently sub-cultured into nutrient agar plate to obtain pure culture. These plates were incubated at 37^{o}C for 24hrs after which pure colonies were obtained and then sub cultured into nutrient agar slant. After 24hrs incubation the nutrient agar slants were preserved in the refrigerator at 4^{o}C.

2.6 Macroscopic Identification

Isolates were observed and recoded based on their colonial morphology (colour, shape, edge, elevation and its consistency) on the agar plate according to Bergey's Manual of Determinative Bacteriology (Holt, 1994).

2.7 Gram Staining

A sterile wire loop was used to transfer small portion of the isolate under aseptic condition onto a clean dry, grease free slide containing a drop of sterile normal saline. The inoculums were well emulsified with the loop a thin film of smear was obtained. The smears were allowed to air-dry and then heat-fixed by passing them thrice over the blue flame of the Bunsen burner. Each smear was then stained for 60secs using crystal violent stain. Then the stain was washed off gently in a running tap water and then flooded with lugols iodine solution (mordant) and was allowed to stay for 60secs and the excess stain was washed off in running tap water. Acetone was used to flood the stain to decolorize the film for 25secs and immediately washed with running tap water and then counter stained with safranin red for 120secs. Finally, the slides were washed, blotted dry and observed under oil immersion objective of the microscope. The colour for gram negative organism is pink while colour for gram positive organism is purple [8].

2.8 Biochemical Test

Biochemical tests were used in the differentiation of isolated organisms preserved on nutrient agar slant. The slants were brought to room

temperature and sub-cultured unto fresh nutrient agar to obtain fresh cultures that were used for biochemical test. The bacterial isolates were characterized using biochemical techniques such as Catalase test, Coagulase, citrate utilization test, indole test, oxidase test, methyl red, voges proskaeuer, Sugar fermentation test, urease, motility, Triple sugar iron agar (TSIA) respectively [9].

2.9 Wet Preparation of Fungi

A needle was used to smear colonies on a clean grease free glass slide, this was then stained lightly using lacto phenol cotton blue and covered with a cover slip and examined under the light microscope using x 40 objective.

3. RESULTS AND DISCUSSION

Microbial counts of microorganisms from the palm wine gotten from drinking bar and palm wine tapper in Elele community as showed in Table 1: The microbial load of these organisms gotten from the palm wine sold in the drinking bar was higher than the one obtained from the palm wine tapper. For Total Aerobic Plate Count (TAPC), mean count of bacteria from drinking bar is (6.73+ 0.22log10 cfu/ml); which is higher but not significantly different from those obtained from the tapper which is (6.70+ 0.15 log10 cfu/ml). The coliform count of palm wine from drinking bar is (6.57+0.10 log10 cfu/ml) which was high but not significantly different from those obtained from the tapper which is (6.56+0.09 log10 cfu/ml) From MacConkey agar, the *Escherichia coli* of palm wine from drinking bar is (5.73+0.23 log10cfu/ml) was higher but not significantly different from that gotten from the tapper (5.71+0.18log10 cfu/ml) but not significantly different from those isolated from palm wine tapper (6.48+0.09 log10 cfu/ml).

The percentage distribution of microorganisms isolated from the palm wine drinking bar and palm wine obtained from tapper as showed in Table 2 are as follows: *Staphylococcus aureus* with 5(50%), *Proteus* spp with 4(40%) and *Klebsiella* spp with 2(20%) which was from the drinking bar was high and occurrence was significantly different from other isolated microorganism from the palm wine tapper. *Escherichia coli* with 5(50%) and *Micrococcus* spp with 3(40%) gotten from palm wine from the drinking bar was significantly higher than that gotten from the palm wine obtained from the tapper. The fungi *Aspergillus* spp 3(30%) which was isolated from palm wine obtained from the drinking bar was higher than the fungi isolated from the palm wine obtained from the tapper 1(10%) but was significantly different while *Saccharomyces cerevisae* 3(30%) isolated from the palm wine obtained from the tapper was higher than that isolated from the palm wine obtained from the drinking bar 2(20%) but not significantly different.

Table 3: Shows the statistical analysis, indicating the Analysis of various (ANOVA). Were the value of the Total aerobic plate count (TAPC) and Fungi count (FC) was significant at $P<0.05$. While the values for the Coliform count (CC) and *Escherichia coli* count (EC) was not significant at $P<0.05$.

3.1 Discussion

Palm wine is generally consumed due to its nutritive composition to the human body system particularly when fresh and unfermented state. The study investigated and analyzed the microbial quality of locally consumed palm wine gotten from the tapper and drinking bar seller in two different popular locations at Elele Ikwerre, Rivers state. A total of 40 bacteria and fungi organisms consist of five (5) different bacterial organisms and two (2) fungi organisms were isolated respectively. The result obtained in (Table 2) signifies that bacteria like *Staphylococcus* spp, *Klebsiella* spp, *Proteus* spp, *Escherichia coli*, *Micrococcus* spp, and also, fungi like *Saccharomyces cerevisiae* and *Aspergillus* spp were adequately isolated from locally consumed palm wine from drinking palm wine bar and palm wine tapper all at Elele in Ikwerre, Local Government, Rivers State. This result is in harmony with the result obtained by Bassire.

Some of these organisms isolated from palm wine are due to the factors such as unhygienic or unsterilized equipment used during tapping, poorly storage facilities, inadequate filtration process, and unpurified water for dilution process that facilitate, enhance and proliferate microbial growth within the palm wine products thereby endangering the safety of the potential consumers. The microbial load in palm wine decrease as fermentation continues, this is due to an increase in acidity and ethanol concentration. Therefore, the microbial load in palm wine can be attributed to the concentration of ethanol present, which gradually decreases as fermentation drops. Similarly, some of these organisms especially *Saccharomyces cerevisiae*

are regarded as industrial yeast capable of fermenting pentose and hexose sugar into ethanol, immune with the efficacy to withstand increased in ethanol concentration as reported by Bassire *et al*. [5].

Some of the organisms isolated from this study are referred to as pathogenic microbes that have the capacity in causing ill-health conditions among humans when they are in contact. Also, some of them are seen as microbes capable of causing waterborne diseases, as result of this fact, palm wine processing would not be successful without the addition of water which exposes the palm wine to harbouring these pathogens especially when such water is not adequately treated or purified.

Percentage occurrence of microorganism's results in this study in (Table 2) revealed that *Staphylococcus* spp and *Escherichia coli* were the most occurring organisms from both samples that are palm wine from the drinking bar and palm wine from the tapper itself. The total number of *Staphylococcus* spp from the drinking bar is 5(50%) while from the palm wine tapper is 3(30%), therefore, the total number of *Staphylococcus* spp in both samples were eight (8). Meanwhile, *Escherichia coli* from the drinking bar is 5(50%) while from the palm wine tapper is 3(30%) which signify eight (8) Escherichia coli for both samples which are in agreement with a similar work carried out by Batra and Miiner [10] indicating neglects in maintaining adequate hygienic practice. The increase of *Escherichia coli* particularly in palm wine drinking bars could as a result of untreated or unpurified water used for the palm wine dilution process which leads to contamination and proliferation of microbe's water pathogens. Furthermore, it could also be as a result of storage facilities being exposed to disease vectors such as housefly which visit faecal materials thereafter having contact with these storage facilities (containers) thereby transmitting or injecting the feacal deposit into them hence causing palm wine contamination.

More also, the high percentage of *Staphylococcus* spp could be attributed to the fact that they are normal flora of the nostrils and skin. There could be a tendency of the drinking bar seller sneezing and having contact with moist skin without proper hygiene practice being observed can autoinnoculate these pathogens into the prepared palm wine on the process of service making it unsafe for human consumption. But in the aspect of palm wine gotten from the tapper, there is a reduction in microbial population indicating observation of personal hygiene practice by the tapper though there still some fraction of microbial growth in that palm wine from the tapper which could be a result of inadequate sterilization of tapping equipment but it was greatly better than that of palm wine gotten from drinking bar.

Even though *Klebsiella* spp, *Proteus* spp, *Micrococcus* spp were the lowest organisms in this study with 2(20%) for palm wine gotten from the drinking bar and 3(30%) from the tapper respectively, they can cause disease conditions in humans such as diarrheal, urinary and septic infections, bacteremia, endocarditis, pneumonia. Likewise, fungi organisms that were isolated in this study were *Saccharomyces cerevisiae* and *Aspergillus* spp. *Saccharomyces cerevisiae* occurs with a percentage frequency of 2(20%) in palm wine gotten from drinking bar and 3(30%) from palm wine tapper thus with a total of five (5) *Saccharomyces cerevisiae*. Meanwhile, *Aspergillus* spp recorded 3(30%) in palm wine gotten from drinking bar and 1(10%) from palm wine tapper, therefore, mounted as four (4) *Aspergillus* spp from both samples which agreed with the finding of Eme [11].

Saccharomyces cerevisiae was higher in palm wine gotten from the tapper than that of drinking bar reason been that the one gotten from the palm wine tapper has not been diluted with any substance in other hands they are referred to as fresh palm wine whereas, the one gotten from drinking bar has been diluted with water or other substances that could reduce the palm wine normal flora which is the *Saccharomyces cerevisiae* which serves as palm wine fermenting indicator fungi or yeast. The process of diluting this palm wine may eventually reduce this palm wine fermenting indicator yeast thereby leading to the degradation of biochemical and nutritional qualities of the palm wine from the original composition.

More so, *Aspergillus* spp found in the palm wine in both samples signified unfitness of such palm wine for human consumption because the presence of this fungi organism clearly shows the toxicity of this palm wine when consumed. Hence, the fungi organism *Aspergillus* spp are regarded as pathogenic fungi which can cause several unhealthy conditions to a human when being ingested through drinking of this contaminated palm wine into the body system resulting to Aspergillosis, difficulties in breathing Bassire and Obire [6].

Table 1. Microbial counts of microorganisms from the palm wine gotten from drinking bar and palm wine tapper in Elele community

Locations	TAPC	CC	EC	FC
Drinking bar	6.73±0.22log10cfu/ml	6.57±0.10log10cfu/ml	5.73±0.23log10cfu/ml	6.50±0.11log10cfu/ml
Palm wine tapper	6.70 ± 0.15log10cfu/ml	6.56 ± 0.09log10cfu/ml	5.71 ±0.18log10cfu/ml	6.48±0.09log10cfu/ml

Keys: TAPC= Total Aerobic Plate Count, CC= Coliform Count EC= Escherichia coli Count FC= Fungi Count

Table 2. Percentage occurrence of microorganisms isolated from palm wine

Isolates	A	B	TNOI	% Occurrence
Staphylococcus spp	5(50)	3(30)	8	20%
Klesbsiella spp	2(20)	3(30)	5	12.5%
Proteus spp	4(40)	1(10)	5	12.5%
Escherichia coli	5(50)	3(30)	8	20%
Micrococcus spp	3(30)	2(20)	5	12.5%
Saccharomyces cerevisiae	2(20)	3(30)	5	10%
Aspergillus spp	3(30)	1(10)	4	10%
Total	24	16	40	100%

KEYS: A= Drinking bar, B= Palm wine tapper, TNOI= Total Number of Isolates

Table 3. Statistical analysis of the microbial variance in relation to palm wine consumption

		Sum of squares	Df	Mean square	F	Sig
	Between Groups	3797.733	2	56.067	397	.329
CC	Within group	3781.600	12	511.800		
	Total	16.33	14			
	Between Groups	341.733	2	3.267	.014	.004
TAPC	Within groups	339.600	12	229.933		
	Total	2.133	14			
	Between groups	2765.733	2	1.067	.038	.241
EC	Within groups	2.759.200	12	28.300		
	Total	6.533	14			
	Between groups	6253.733	2	8.067	.026	.003
FC	Within groups	6141.600	12	315.133		
	Total	112.133	14			

KEYS: TAPC (Total aerobic plate count), FC (Fungi Count), CC (Coliform Count), EC (Escherichia coli count)

In this study, the results in (Table 1) revealed the different microbial counts obtained in two samples that are, the palm wine gotten from the drinking bar and the palm wine gotten from the palm wine tapper. The microbial counts from the palm wine gotten from the drinking bar range from 5.73+0.23log10cfu/ml- 6.73+log10cfu/ml while palm wine gotten from palm wine tapper ranges from 6.48+ 0.09log10cfu/ml-6.70+0.15log10cfu/ml which is in agreement with the findings of Batra and Miiner (2014).

This result proves that there were high microbial load counts in the palm wine gotten from the drinking bar than that of palm wine tapper possibly as a result of unsterilized storage equipment, lack aseptic filtration process, untreated/unpurified water used for dilution process, and general personal hygiene practice that can stir –up microbial load and proliferation on the palm wine gotten from drinking bar thereby making it unfit for human consumption. Similarly, the decrease in microbial load on the palm wine gotten from the palm wine tapper could be linked to a very large extent to proper sterilization of equipment used in the tapping process and observation of personal hygiene by the personnel.

Therefore, it pertains to know that the personnel's who produce and manage these local wine producers are a major contributing factor concerning low and high microbial load count, presence of other microbes aside from the normal palm wine flora with their different levels of food safety precautional enlightenment on the palm wine products.

4. CONCLUSION

Palm wine obtained from the drinking bar tends to have a high microbial load compared to that gotten or obtained from the palm wine tapper, as a result of the storage equipment used, filters, and also water added to dilute the palm wine. Therefore, the study of microbial quality of palm wine is very paramount as it aids in the enlightenment of microbial content/load in palm wine to the producer, sellers, and potential consumers to improve the sanitary condition of the palm wine.

5. RECOMMENDATIONS

1. The National Agency for Food and Drug Administration (NAFDAC) and other health bodies should take appropriate measures to ensure proper inspection of areas where palm wine is sold to reduce or eliminate chances of contamination.
2. Consumers should try their best to acquire palm wine from tappers because of its freshness and palatability compared to palm wine gotten from retailers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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