Microbiological Quality of Palm wine (*Elaeis guineensis*) sold within Ikwo, Ebonyi State, South East, Nigeria.

Abstract

Palm wine is of high nutritional and economic importance and plays a significant role in cultural and social practices in Africa. Since the nutritional content is high, there is proliferation of microorganism which results in spontaneous fermentation. The microbiological quality of different palm wine samples was investigated and it was found to harbor microorganisms such as *Lactobacillus* sp, *Micrococcus* sp, *Staphylococcus* sp, *Streptococcus* sp and *Bacillus* sp. This had a count of $0.8 \times 10^3 - 1.8 \times 10^3$ CFU/ml and $1.2 \times 10^3 - 3.8 \times 10^3$ CFU/ml for different samples. The isolation of these microorganisms from these product is of public health concern because this product is on high demand in this area and beyond. With the result of this study, public awareness has to be created on the risk factors involved in the consumption of palm wine and the need to imbibe proper sanitary hygienic practice in tapping, processing, diluting and distribution of the product.

Keywords: Palm wine, contamination, fungal count, fermentation, hygienic practice.

Introduction

Palm wine is referred to as a collective group of beverages that are obtained after the fermentation of sap of palm trees (Agu *et al*., 1999). Over many years, the utilization of fermentation techniques has been a prominent as well as inexpensive preservation method especially during food shortages. Different kinds of locally fermented beverages exist worldwide in developing countries (Obafemi *et al*., 2022). Microbial growth converts major and minor components by enzymatic actions which produced fermented beverages (Marco *et al*., 2021). In tropical regions of Asia, Africa and Southern America where palm trees grow, fermented drink from palm trees is of much significance to people (Karamoko *et al*., 2012). In Nigeria, most palm wine is tapped from three types of palm trees: *Elaeis guineensis* (palm oil tree), *Raphia vinifera* and *Raphia hookeri* (raphia palms) (Nwaiwu *et al*., 2016). Palm wine is collected twice a day, in the morning and evening where is can be consumed immediately or stored for sale the same day or next day (Santiago-urbina and Ruiz-terla, 2014). The knowledge associated with the applied method will influence the quality attributes of the collected palm wine. Palm wine beverage has a high content of amino acid, zinc, iron and potassium (Eze *et al*., 2019). It is equally a good source of vitamin B1, B2, B3 and B6 and associated with increased sperm and breast milk production (Mbuagbaw and Noorduyn, 2012). The nutritional profile of palm wine makes it a good medium for microbial growth. Due to rapid fermentation by microbes, hydrolysis of sucrose to fructose and glucose occurs within 24h. They are then converted to lactic acid, acetic acid and ethanol. This action reduces the pH of palm sap to 5 making it unacceptable to consumers as a drink.The aim of this study is to evaluate the microbial quality of some locally tapped palm wine within Ikwo Local Government Area, Ebonyi State.

Materials and Methods

Sample collection

Five (5) were samples of palmwine(*E. guineensis*) were collected from different local palm wine tappers in Ikwo, Ebonyi State, Nigeria using pre-sterilized 100ml capacity sterile

sample bottles with perforated screw caps. The samples were immediately transported to the Laboratory in mixture of salt and ice block.

Isolation and microbial Succession in palm wine

One milliliter (1ml) aliquots of each palm wine was collected and used for 10-fold serial dilution with 0.1% (w/v) bacteriological peptone. 1ml dilutions each of the samples were plated out in duplicates using spread plate method (Cheesbrough, 2006) on nutrient agar for total heterotrophic bacterial count, MacConkey agar for total coliform count and Sabouraud dextrose agar containing 0.05mg/ml streptomycin for yeast count. The inoculated petri dishes were incubated at 30^{o}C for 24 h for bacteria and 48 h for yeast. Discrete colonies were subcultured and stored in agar slants.

Characterization of Isolates

The isolates were grouped according to their colony morphology and microbial cell characteristics which were further subjected to biochemical tests (Collins and Lyne, 1984). The suspected identities of the isolates were described by (Cheesbrough, 2006).

Results

Table 1: Total fungi count and Total bacterial count (cfu/ml) in palm wine samples

Sample	Total fungi count	Total bacteria count
A	3.2×10^3	1.0×10^3
B	3.8×10^3	1.8×10^3
C	2.1×10^3	0.9×10^3
D	1.6×10^3	1.1×10^3
E	1.2×10^3	0.8×10^3

Key: CFU/ml= colony forming unit per ml.

The table shows the total fungal and bacterial count in palm wine samples sold in Ikwo, Ebonyi State. Sample B had the highest microbial contamination of 3.8×10^3 and 1.8×10^3 CFU/ml while sample E had the least contamination with the value of 1.2×10^3 and 0.8×10^3CFU/ml respectively.

Table 2: Morphology and biochemical characteristics of the bacterial isolates from palm wine

Colony morphology	Gram's reaction	Coagulase test	Oxi	Cit	Ind	Glu	Suc	Lact	Identified Organism
Smooth colonies with metallic sheen on EMB	+ve rod	–	–	–	–	A	A	A	*Lactobacillus* sp
Round yellow colonies on NA	Cocci	–		–	–	–	A	–	*Micrococcus* sp
Smooth round colonies	+ve cocci in cluster	+	–	–	–	AG	A	A	*Staphylococcus* sp
Round colourless colonies	+cocci in chains	–	–	–	–	AG	–	A	*Streptococcus* sp
Irregular colonies	+ve rod	–	–	+	+	AG	A	G	*Bacillus* sp

Key: G = Gas, A = Acid, + = positive, – = negative, EMB – Eosine Methylene Blue, NA = Nutrient agar, cit = citrate test, Ind = Indole test, oxi = oxidase test, Glu = Glucose test, suc = Sucrose test , Lact = lactose test

This table showed the biochemical characteristics of the isolates (bacteria) from palm wine. A total number of five isolates were identified. This includes *Lactobacillus* sp, *Micrococcus* sp, *Staphyloccus* sp, *Streptococcus* sp, *Bacillus* sp.

Table 3: The Culture and morphology of fungi isolated from palm wine

Isolates	**Appearance of Cultural**	**Morphological**	
X	Moist smooth and creamy colour on SDA	Formation of pseudohyphae on incubation with human serum after 3 hours and they are irregular in shape.	*Candida tropicalis*
Y	Flat, smooth, glistening and creamy in colour	Multilateral budding, pseudohyphae morphology	*Saccharomyces cerevisiae*

From the table above, two fungal isolates were isolated and identified from the palm wine samples.

DISCUSSION

The sap of palm wine trees is usually sweet and serves as a rich substrate for the growth of different types of microorganisms immediately after it is tapped. The sap undergoes rapid fermentation as a result of the proliferation of bacteria and yeasts which rapidly converts the sugar into different metabolites like lactic acid, ethanol and acetic acid (Santiago-urbina *et al*., 2013). From the microbiological quality of the palm wine investigated, the result showed that the samples harboured, different strains of microorganisms which consists of bacteria and fungi. The highest and lowest fungal count were for isolate B and isolate E with values of 3.8×10^3CFU/ml and 1.2×10^3 CFU/ml. Similarly, the result of the bacterial count showed that isolate B had the highest count (1.8×10^3 CFU/ml) while isolate E had the lowest count (0.8×10^3 CFU/ml). These result corroborates the work of (Obi *et al*., 2015; Karamoko *et al*., 2021) reported the presence of yeasts and bacteria in palm wine samples. The presence of different organisms that are not involved in the fermentation of the palm wine is an indication of the poor hygienic practices of the palm wine tappers, the materials and methods they used to perform this task. The level of awareness of the palm wine tappers or their knowledge on hygiene and hygienic practices influences the microbial content of the palm wine. However, the methods employed in tapping, collection and storage of palm wine determines the microbial content of the sweet cell sap of palm wine trees. The presence of these organisms is of public health concern since the palm wine is consumed in most parts of the state and outside the state due to its health, nutritional and social importance. Another area of public health concern is the questionable stream water used by the tappers to dilute the palm wine. There has been misconceptions among palm wine tappers and those who regularly patronize them that the degree of housefly or other flies that petch around where palm wine are stored makes the palm wine sweet instead of adopting sanitary measures to prevent the flies. However, most palm wine tappers believe that palm wine has traditionally inherent cleansing ability which makes it unnecessary to rinse the cups or containers used to dispense wines after use by an individual. There is need to sensitize the public on the consumption of these wines which will promote the quality of these products and avoid the health risk posed by the consumption of palm wine contaminated by microorganisms.

Conclusion

Palm wine, as a whitish liquid produced by the natural fermentation of the sap of palm tree (*Elaeis guineensis*) has been consumed widely among which is pregnant mothers to enhance breast milk production. It is also consumed during ceremonies and other social activities. If not properly handled, they will constitute a major public health problem. However, there is need to monitor and promote the quality of these products because of their health and nutritional benefits.

REFERENCES

Agu, R. C.; Okenchi, M. U.; Ude, C. M.; Onyia, A. I.; Onwumelu, A. H. and Ajiwe, V. I. E. (1999). Fermentation kinetic studies of Nigerian palm wines; *Elaeis guinensis* and *Raphia hookeri* for preservation by bottling. *J. Food Sci. Technol* 36(3): 205-209.

Cheesbrough M.(2006).District Laboratory Practice in Tropical Countries. 2nd Edition Cambridge University Press, Cambridge Edition pp 72-75.

Karamoko, D., Djeni, N. T., N'Guessan, K. F., Bouatenin, K. M. J.-P., and Dje, K. M. (2012). The biochemical and microbiological quality of palm wine samples produced at different periods during tapping and changes which occured during their storage. *Food control* 26: 504–511.

Marco, M. L., Sanders, M. E., Gänzle, M., Arrieta, M. C., Cotter, P. D., de Vuyst, L., Hill C, Holzapfel W, Lebeer S, Merenstein D, Reid G, Wolfe B. E and Hutkins R.(2021). The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on fermented foods. *Nat. Rev. Gastroenterology Hepatology* 18 (3): 196–208.

Nwaiwu, O., Ibekwe, V. I., Amadi, E. S., Udebuani, A. C., Nwanebu, F. C., Oguoma, O. I., and Nnokwe, J. C. (2016). Evaluation of fermentation products of palm wine yeasts and role of Sacoglottis gabonensis supplement on products abundance. *Beverages*, **2**(2), 9

Obafemi, Y. D., Oranusi, S. U., Ajanaku, K. O., Akinduti, P. A., Leech, J., and Cotter, P. D. (2022). African fermented foods: overview, emerging benefits, and novel approaches to microbiome profiling. *NPJ Sci. Food* 6 (1): 15.

Obi, C. N.; Ogbulie, J. N. and Nkwo, A. M. (2015). Assessment of microbial growth and survival in fresh rafia palmwine from Umuariaga community, Ikwuano L. G. A. Abia State, Nigeria. *Int.J.Curr.Microbiol.App.Sci* 4(1): 484-494

Ouoba, L., Kando, C., Parkouda, C., Sawadogo-Lingani, H., Diawara, B. and Sutherland, J. P. (2012). The microbiology of Bandji, palm wine of Borassus akeassii from Burkina Faso: identification and genotypic diversity of yeasts, lactic acid and acetic acid bacteria. *J. Appl Microbiol* 113 (6): 1428-144.

Santiago-Urbina, J. A., and Ruíz-Terán, F. (2014). Microbiology and biochemistry of traditional palm wine produced around the world. *Int. Food Res. J*. 21 (4): 1261–1269.

Santiago-Urbina, J. A., Verdugo-Valdez, A. G. and Ruíz-Terán, F. (2013). Physicochemical and microbiological changes during tapping of palm sap to produce an alcoholic beverage called “Taberna”, which is produced in the south east of Mexico. *Food Control* 33 (1): 58-62.