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Effects of Selected Plant Preservatives on Microbial Load and Shelf Life of Palm Wine

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this study, ten different freshly tapped palm wine were collected at the point of tapping from traditional palm wine tappers and transported to the laboratory for analysis. Then 1.0 ml dilution was plated on nutrient agar for total heterotrophic bacterial count and on Saboraud dextrose agar for fungal count. It was incubated at 30^{o}C for 24 hours for bacteria and 48 hours for fungi.
The preservative potential of plants were determined by setting up sixteen sterile plastic bottles each containing 100ml of palmwine and four separate sterile plastic bottles containing 2.5, 5, 10 mg/ml of the dried blended plant preservative and the control. Aliquot (0.1ml) each for the test tubes containing mixture of palm wine and the preservative were plated out using spread plate technique

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on nutrient agar and Saboraud dextrose agar at constant intervals of 24, 48, 72 and 96 hours respectively. Incubation was done for 24 hours at 37^{o}C for bacterial and 48 hours at 30^{o}C for fungal growth. A ten member panel consisting of regular palm wine bar customers were drafted to evaluate the acceptability of the products based on the taste, colour and overall acceptability using a 9-point hedonic scale. There was a gradual reduction in the bacterial load of palmwine from 1.13 x10^{7} to 4.60 x10^{6} CFU/ml and 1.03 x10^{7} to 2.01 x10^{6}CFU/ml for ginger and nutmeg at 5g/ml after 72 hours. The fungal load of palmwine reduced from 1.30 x10^{7} to 1.80 x10^{6} CFU/ml and 1.03 x10^{7} to 1.80 x10^{6} CFU/ml for ginger and nutmeg at 5g/ml and 10g/ml after 72 hours. The scores of the colour, flavor and taste were high which shows their acceptability by consumers. There was a gradual reduction in the microbial load of palm wine treated with the plant preservatives thus suggesting their usefulness in extending the shelf life of palmwine which would make a significant contribution towards the search for low cost preservative for palmwine.

Keywords: Palmwine; Zingiber officinale; preservative; shelf life; microbial load.

1. INTRODUCTION

Palm wine is an alcoholic drink obtained by natural fermentation of the sap of various types of palm trees such as oil palm *(Elaeis guineensis),* raffia palm *(Raphia hookeri)* [1]. Palm wine is generally referred to as a group of alcoholic beverages obtained by fermentation of the saps of palm trees [2]. It is a refreshing beverage widely consumed in southern Nigeria and other parts of the world particularly Asia and Southern America [3]. Palm wine is a rich nutrient containing sugars, protein, amino acids, alcohols mineral and a lot of water-soluble vitamin[4]. It is a good source of vitamin B_1(thiamine) and C (ascorbic acid). The palm sap of the palm tree is a rich medium capable of supporting the growth of several types of microorganisms like high numbers of aerobic mesophilic bacteria, coliform bacteria, lactic acid bacteria, acetic acid bacteria and yeast [5]. This drink has a significant role in several nutritional, medical, religious and social uses such as traditional wedding ceremonies, religious ceremonies or festivals, prayers [6].The major hurdle to palm wine processing is usually lack of adequate and efficient storage and preservation. Plant derived preservatives have been documented to be useful in the preservation of beverages with little or no side effects. The sensory evaluation and quality of preserved palm wine have not been well documented. Ginger (*Zingiber officinale*), which is prominent as spices globally, especially in the South East Asian countries is a perennial plant that is used as well as a functional food due to its health promoting potentials [7]. Nutmeg has been used in cooking many years ago. It is used in soups, meats and vegetables. In some cases, they are usually blended with other spices like white pepper, cloves and ginger. Nutmeg contains many chemical compounds that are identified as anti-oxidant, health promoting properties and disease preventing. The aim of this study is to determine the effects of selected plant preservatives on the microbial load and shelf-lifeof palm wine.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Preservatives

Samples of Nutmeg (*Myristica fragrans*) and Ginger (*Zingiber officinale*) were commercially obtained from the market. The plant preservatives were transported to the laboratory and washed with sterile water and absolute ethanol. The ethanol was washed away with sterile distilled water and blended into fine powder.

2.2 Sample Collection

Freshly tapped palm wine from oil palm tree were collected at the point of tapping from traditional palm wine tappers from Agubia, Ikwo Local Government Area, Ebonyi State. The samples were transported to the laboratory for analysis using the ice cubed box.

2.3 Treatment of the Palm wine

The palm wine were disposed into 100ml capacity pre-sterilized bottles and treated by the addition of 2.5, 5.0 and 10g/ml of each powdered plant preservative (Ginger and Nutmeg) and the control (containing no treatment) were kept at room temperature. Analysis was carried out every 24 hours until 96 hours.

2.4 Isolation of the Microorganisms

One milliliters(1ml) of the palmwine was aseptically used for 10-fold serial dilution. Then 1.0 ml dilution was plated out using spread plate method on nutrient agar and Saboraud dextrose agar respectively [8,1].

2.5 Characterization of the Isolates

The isolates were characterized by their colony morphology, cell characteristics, Gram staining and biochemical tests [9].

2.6 Estimation of the Microbial Load

The 1.0 ml dilutions of the sample (palm wine) were plated out using spread plate method on nutrient agar for total heterotrophic bacterial count on Saboraud dextrose agar for fungal count. It was incubated at 30^{o}C for 24 hours for bacteria and 48 hours for fungi [9].

2.7 Determination of the Preservative Potential

This was determined by setting up sixteen sterile plastic bottles each containing 100ml of palmwine. It was carried out in sets of four (4) separate sterile plastic bottles containing 2.5, 5, 10 mg/ml of the dried blended plant preservative and the control [10]. Aliquot (0.1ml) each for the test tubes containing mixture of palm wine and the preservative were plated out using spread plate technique on nutrient agar and Saboraud dextrose agar at constant intervals of 24, 48, 72 and 96 hours respectively. Incubation was done for 24 hours at 37^{o}C for bacterial and 48 hours at 30^{o}C for fungal growth [11].

2.8 Sensory Evaluation

The palmwine was evaluated after preservation studies for the organoleptic properties. A ten member panel consisting of regular palm wine bar customers were drafted to evaluate the acceptability of the products based on the taste, colour and overall acceptability using a 9-point hedonic scale [12]. The descriptive terms and their rating were such that below 5 points indicated poor or dislike extremely, 5-6 indicated fair or dislike moderately, 7-8 points stood for good or like moderately, whereas 9-10 points indicated very good or like extremely.

3. RESULTS AND DISCUSSION

Six different bacterial isolates were obtained from the palmwine and they are *Lactobacillus*sp, *E. coli*, *Streptococcus*sp, *Staphylococcus*sp, *Micrococcus*sp and *Bacillus*sp while *Candida*sp and *Saccharomyces*sp were the fungi isolated. This corroborates the work of Nwachukwu *et al*., 2016; Uzoh et al.[13] who isolated same organisms from palmwine. The isolation of *Staphylococcus*sp, *E. coli* and *Micrococcus*sp from palmwine is of serious public health concern as it is attributed to being responsible for unstable bowel movement upon the consumers of the palmwine as attested to by the drinkers interviewed during this study while others attribute it to a sign that their stomach likes the palmwine. This possible microbial contamination probably could be from the handlers and unhygienic practices during the storage of the palmwine or due to the exposure of the freshly tapped palmwine which poses a health challenge like gastrointestinal disorders associated with drinking of palmwine. It was observed that there was gradual decrease in bacterial load. No growth was observed for 10g/ml of ginger at 72 hours and at 96 hours for both ginger and nutmeg. There was absence of growth for 5g/ml of nutmeg at 96 hours (Table 3). There was an observed decrease in the fungal count as the days progressed. There was no observable growth for 10g/ml of ginger from 72 hours to 96 hours (Table 4). Comparing the effect of the plant preservatives on the bacteria and fungi, it was observed that there were much higher counts of bacteria than fungi at the early stage. The effect was more on the bacteria than the fungi. Although it was noted that 10g/ml of ginger produced the same results at 72 hours (ie no growth) for both bacteria and fungi, that of nutmeg at 96 hours on bacteria produced no growth for both 5g/ml and 10g/ml of nutmeg. This result showed that the nutmeg prolonged the shelf life of the palmwine more than the ginger. The loss of viability by the isolates were related to the preservative effect of the plant preservatives (ginger and nutmeg). The preservative effect of the nutmeg on bacterial isolates was more pronounced on the 4th day. The survival pattern of the isolates from the palmwine from 0 hr to 96 hrs showed that the *Lactobacillus*sp, *Saccharomyces*sp and *Bacillus*sp survived till the last day (96 hours) while *Staphylococcus*sp, *Micrococcus*sp, *Candida*sp and *E. coli* were not isolated on subsequent days of isolation. The scores of the taste, flavor, colour and overall acceptability for the different treated palmwine with different traditional plant based preservatives revealed that the palmwine treated with ginger (*Zingiber officinale*) maintained acceptable foaming and was stable up to 96 hours after collection and the palmwine maintained most of its organoleptic qualities. Since there is paucity of information in this line of research, this study will provide a baseline for the advancement of research on palmwine and most effective preservation methods.

Table 1. Morphological characteristics, gram reaction and biochemical characteristics of bacterial isolates

Isolates	Edge	Shape	Colour	Cellshape	Gram	Cat	Ind	Urease	Cit	Mot	Lac	Glu	Probable Isolates
A	Entire	Circular	Colourless	rod	_	+	_	_	_	+	+	+	*Escherichia coli*
B	Entire	Irregular	White	Rod	+	+	_	+	+	+	+	+	*Bacillus sp.*
C	Entire	Circular	Yellowish	Cocci	+	+	_	_	+	_	+	+	*Streptococcus sp.*
D	Lobate	Circular	Creamy	Cocci	+	+	_	+	+	_	+	+	*Staphylococcus sp.*
E	Entire	Circular	White	Rod	+	_	_	-	+	+	+	+	*Lactobacillus sp.*
F	Undulate	Round	Yellowish	Cocci	+	+	_	+	_	_	_	_	*Micrococcus sp.*

Key:+ Positive, - = Negative, Ind = Indole, Cat = Catalase, Cit = Citrate, Mot = Motility, Lac = Lactose, Glu = Glucose

Five bacteria Escherichia coli, Bacillus, sp., Streptococcussp., Staphylococcus sp. Micrococcus sp. and Lactobacillus sp. were isolated.

Table 2. Macroscopic and microscopic characteristics of fungal isolates

Isolates	Morphology	Microscopic features	Glucose	Lactose	Sucrose	Isolates
G	Round and creamy colonies	Single oval cells	+	_	+	*Saccharomyces spp*
H	Whitish colonies not well developed	Single and round cells were seen	+	_	+	*Candida spp.*

Key: + = Positive, - = Negative

The probable organisms are Saccharomyces spp and Candida spp.

Table 3. Effect of plant preservatives on the bacterial count of palm wine sample

	Duration (hours)							
Concentration (g/ml)	**24**		**48**		**72**		**96**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Control	2.93×10^7	2.93×10^7	2.12×10^7	2.12×10^7	1.13×10^7	1.13×10^7	1.03×10^7	1.03×10^7
2.5	2.54×10^7	1.50×10^7	1.54×10^7	1.36×10^7	1.30×10^7	1.20×10^7	3.20×10^6	1.14×10^7
5	9.60×10^6	1.30×10^7	4.95×10^6	1.01×10^7	4.60×10^6	1.01×10^7	2.01×10^6	-
10	5.61×10^7	1.12×10^7	4.56×10^6	4.78×10^6	-	1.10×10^6	-	-

Key: A = Ginger, B = Nutmeg, - = Not growth

A gradual decrease in bacterial load was observed with respect to time and concentration. No growth was observed in both treatment after 72 hours at both 5.0 and 10.0g/ml.

Table 4. Effect of plant preservatives on the fungal count of palm wine sample

	Duration (hours)							
Concentration (g/ml)	**24**		**48**		**72**		**96**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Control	7.8×10^6	7.8×10^6	1.07×10^7	1.07×10^7	1.30×10^7	1.30×10^7	2.58×10^7	2.58×10^7
2.5	1.8×10^7	5.1×10^6	1.22×10^7	3.0×10^6	1.07×10^7	1.20×10^6	7.9×10^6	1.01×10^6
5	3.0×10^6	5.0×10^6	2.8×10^6	4.8×10^6	1.8×10^6	2.8×10^6	1.3×10^6	1.0×10^6
10	2.0×10^6	2.8×10^7	1.3×10^6	1.1×10^6	-	1.6×10^6	-	2.3×10^6

Key: A = Ginger, B = Nutmeg, - = Not growth

No growth was observed after 48 hours with the ginger treatment at 10g/ml.

Table 5. Survival pattern of microbial isolates

	Hours of Isolation				
Organisms	**0**	**24**	**48**	**72**	**96**
Micrococcus sp	+	+	+	-	-
E. coli	+	+	+	+	-
Bacillus sp.	+	+	+	+	+
Lactobacillus sp	+	+	+	+	+
Streptococcus sp	+	+	-	-	-
Saccharomyces sp	+	+	+	+	+
Candida sp	+	+	+	-	-
Staphylococcus sp	+	+	-	-	-

Key: – = Absent, + = Present

This showed the survival pattern of microbial isolates from palm wine sample from 0 to 96 hours of isolation. Bacillus spp., Lactobacillus sp., and Saccharomyces sp. survived till the last day of isolation while E. coli, Streptococcus sp., Staphylococcus sp. Micrococcus sp and Candida sp. were eliminated.

Table 6. Sensory evaluation of palmwine

Plant preservative	Conc. (g/ml)	Colour	Flavor	Taste
	Control	7.8	6.5	6.3
Ginger	10	6.5	6.1	4.8
	5	7.5	6.8	6.5
	2.5	8.2	7.7	6.4
Nutmeg	10	4.8	4.0	2.3
	5	5.6	3.0	2.0
	2.5	6.0	2.5	2.5

Key: Conc = Concentration
Score = Interpretation
< 5 = Poor/Extremely disliked
7-8 = Good/Moderately liked
5-6 = Fair/Moderately disliked
9-10 = Very good/Extremely liked

From the scores in the table, ginger gave a higher score for colour, flavor and taste and the palmwine preserved with ginger had a better organoleptic quality.

4. CONCLUSION

This study showed that plant preservatives have potential of extending the shelf life of palmwine hence can be developed extensively towards providing a low cost and acceptable source of alcohol that can be acceptable even 96 hours after it was tapped. This could further be applied in combination with other preservation methods to further prolong the shelf life and this will contribute rapidly to expanding the alcoholic beverage market.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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