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Microbiological and Nutritional Status of Palm Wine from Umudike and Its Environs

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Abstract

The microbiological and nutritional status of twenty palm wine samples from Umudike environs was evaluated. The samples were serially diluted and inoculated by spread plate method and incubated at appropriate temperatures for the isolation of bacteria and yeasts. Five bacteria: *Staphylococcus aureus, Lactobacillus* spp., *Bacillus cereus*, *Streptococcus* spp and *Escherichia coli* and two yeasts namely *Saccharomyces cerevisiae* and *Candida* spp were isolated from the palm wine. The total viable bacterial count was highest for sample PLM10 (6.0 x 10^3cfu/ml) while lowest count was from sample PLM8 (2.4 x 10^4cfu/ml). The proximate composition of the palm wine isolates showed that the samples are low in carbohydrate (1.01±0.01 - 2.12±0.02), crude protein (0.51±0.03 - 0.90±0.01), lipid (0.01±0.00 - 0.07±0.04), ash (0.31±0.11 - 0.78±0.06) and crude fibre contents (0.21±0.04 - 0.37±0.01). The moisture of the palm wine was high for all the samples (96.35±0.01 - 98.74±0.01). However, the vitamins were high ranging from 21.48±0.02 - 22.76±0.08 for vitamin A and 6.60±0.80 - 7.42±0.02 for vitamin B. The mineral composition of the palm wine ranges from (3.24±0.28 - 3.85±0.02) for calcium; (30.02±0.00 - 31.35±0.01) for magnesium, (3.10±0.33 - 3.85±0.02) for iron; (0.25±0.04 - 0.31±0.03) for zinc and (0.28±0.08 - 0.48±0.08) for copper. The antimicrobial test shows that *Staphylococcus aureus* was inhibited by all the antibiotics tested while *E. coli, B. cereus*, *Streptococcus* spp and *Lactobacillus* spp showed lower degrees of susceptibility. Palm wine contains sufficient nutrients for the body but can be contaminated during handling.

Keywords

Microbiological Status, Nutritional Status, Palm Wine, Viable Counts

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1. Introduction

Palm wine is an alcoholic beverage produced by spontaneous fermentation of the sap of palm trees [1]. The sap is originally sweet and serves as a rich substrate for the growth of various types of microorganisms [2]. Fermentation of palm wine starts soon after the sap is collected and within an hour or two, it becomes reasonably high in alcoholic (up to 4%). If it's allowed to continue to ferment for more than a day, it starts turning into vinegar. It's reported that yeast and bacteria originate from the gourd, palm tree and tapping implements. However the high sugar content of the juice would seem to selectively favour the growth of yeasts which might originate from the air. This is supported by the fact that fermentation also takes place in plastic containers. Within 24hours the initial pH is reduced from 7.4-6.8 to 5.5 and the alcohol content ranges from 1.5-2.1%. The organic acids present are lactic acid, acetic acid and tartaric acid [4]. Although palm wine may be presented in a variety of flavours, ranging from sweet (unfermented) to sour (fermented) and vinegary, it is mostly enjoyed by people when sweet [5].

Generally, palm wine has several nutritional, medical, religious and social uses and these have increasingly

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enhanced the demand for this natural product [5]. Although attempts has been made towards the preservation and shelf-life extension of palm wine through bottling, use of chemical additives and addition of plant extracts have greatly affected the organoleptic quality of the product [3]. Several factors however have been adduced for this variation and they include the indigenous microbial flora, the biochemical composition of the two brands of palm sap, the tapping and post tapping processes.

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, coliforms bacteria, lactic acid bacteria, acetic acid bacteria and yeasts [4]. Palm wine contains about 10-12% sugar, mainly sucrose; about 0.36% protein; 10-19mg/100ml of vitamin C as well as about 160 µg/ml of vitamin B_{12} [6]. *Saccharomyces cerevisiae* is the dominant yeast species responsible for the fermentation of palm wine tapped from *Elaeis guineensis* in Ghana and Cameroon, as well as Bandji in Burkina Faso [7].

This research work aimed at isolation of microorganisms resident in and determination of the nutritional status of palm wine samples from Umudike town and its environs.

2. Materials and Methods

2.1. Sample Collection

Twenty Palm wine samples from *E. guineensis* in Umudike were obtained during the taping of palm trees in Umudike, Ikwuano L. G. A between August and September, 2017. The samples were collected aseptically by 7am in sterile 200 ml bottles transported to the laboratory in a cooler containing freezing mixture of salt and ice-block for analysis within 1 h of collection. This method of collection according reduces fermentation rate considerably [3].

2.2. Microbiological Analysis

2.2.1. Isolation of Bacteria and Yeast

One mililitre of each palm wine sample was transferred aseptically into a test tube containing 9ml of sterile peptone water and 10 fold serial dilution was carried out. 0.1ml aliquot was added to sterile petri plates and spread evenly [8]. The inoculated plates for bacterial isolation were incubated for 24-48 hours at 35°C while the Sabourand Dextrose Agar (SDA) plates for yeast isolation were incubated at 22°C for 5 days [9]. Distinct bacterial colonies that grew on the plates for 24 hours were sub-cultured and later stored in Nutrient agar slants for future use. Yeast isolates that developed were sub-cultured on fresh SDA plates and then stored on SDA slants for future use.

2.2.2. Identification of Isolates

Identification of the organisms was done by the use of standard morphological characteristics and identification keys described by Barnett *et al.* (1990) [10]. The tests used in the identification of bacteria include morphology, gram reaction, spore production, biochemical test, and sugar fermentation. The tests used in the identification of yeasts includes, morphology, methylene blue staining and sugar fermentation.

2.3. Proximate Analyses of Palm Wine Samples

2.3.1. Determination of Moisture Content

Moisture content was determined using procedure described by A. O. A. C, (2000) [11]. 5 ml of each sample was weighed into an aluminum moisture can. The sample was then dried to constant weight at 105±2°C.

$$\text{Moisture content} = \frac{(\text{Weight of can} + \text{sample}) - (\text{weight of empty can}) \times 100}{\text{Weight of sample}}$$

2.3.2. Determination of Crude Protein

The Protein Content was determined using a Foss Tescator protein digestor and KJECTEC 2200 distillation apparatus (Kjeldahl method) according to the procedure of AOAC, (2000) [11]. 12 ml of concentrated sulphuric acid (H_2SO_4) and 2 tablets of catalyst were put into a Kjeldahl digestion flask containing 1 ml of the sample. The flask was placed in the digestor in a fume cupboard and switched on and digestion was done for 45 minutes to obtain a clear colorless solution. The digest was distilled with 4% boric acid, 20% Sodium hydroxide solutions were automatically metered into it in the KJECTEC 2200 distillation equipment until distillation was completed. The distillate was then titrated with 0.1M HCl until a violet color formation indicating the end point. A blank were run under the same condition as with the sample. Total nitrogen content was calculated using the formula:

$$\text{Crude protein} = \frac{(\text{Titre value (0f sample)} - \text{blank}) \times 0.01 \times 14.007 \times 6.25 \times 100}{1000 \times \text{Weight of sample}}$$

2.3.3. Determination of Crude Fat Content

Crude fat were extracted in a Soxhlet extractor with hexane and quantified gravimetrically. 1 ml of the sample was weighed into an extraction thimble and then stoppered with

grease-free cotton. Before extraction commenced, the round bottom cans was dried, cooled and weighed. The thimble was placed in extraction chamber and 80ml hexane was added to extract the fat. The extraction were be carried out at 135°C lasted for 1 hour 40minutes after which the fat collected in the bottom cans were cooled in a dessicators.

$$\text{Crude Fat} = \frac{\text{Weight of fat x 100}}{\text{Weight of sample}}$$

2.3.4. Determination of Ash Content

Two mililitres of the samples were weighed into well incinerated crucibles and then ashed in a muffle furnace at 600°C for 3 hours. The ash content was calculated as:

$$\text{Ash content} = \frac{\text{Weight of Ash x 100}}{\text{Weight of samples}}$$

2.3.5. Determination of Crude Fiber

Two mililitres of the sample were transferred into 1 litre conical flask. 100 ml of sulphuric acid (12.5M) were heated to boiling and then introduced into the conical flask containing the sample. The contents then boiled for 30 minutes and he level of the acid was maintained by addition of distilled water. After 30 minutes, the contents were then filtered through a muslin cloth held in a funnel while the residue was rinsed thoroughly until its washing was no longer acidic to litmus. The residue was then transferred into a conical flask. 100 ml of sodium hydroxide (12.5M) was then allowed to boil and then introduced into the conical flask containing the sample. The contents were then boiled for 30 minutes and ensuring that the level of the acid were maintained by addition of distilled water. After 30 minutes, the contents were filtered through a muslin cloth held in a funnel and the residue was rinsed thoroughly until its washing was no longer alkaline. The residue was then introduced into an already dried crucible and ashed at 600°C ±200°C.

$$\text{Crude fiber} = \frac{\text{Final weight of crucible} - \text{initial weight of crucible x 100}}{\text{Weight of sample}}$$

2.3.6. Determination of Protein

Kjehdahl method will be used to determine crude protein content. This method involves stages of digestion, distillation and titration. 2 ml of the sample was transferred into a digestion flask. Half of selenium based catalyst tablet and a few anti-bumping agents (broken porcelain crucibles) were also added to the flask. 25 ml of concentrated sulphuric acid were added and the flask shaken to ensure that the sample was thoroughly wet. The flask were then placed on a digestion burner and heated slowly until boiling ceased and the resulting solution became clear. The flask and its content were cooled to room temperature. The digested solution was transferred into a 100 ml volumetric flask and distilled water was added to the mark. Distilled water was boiled in a steam generator of the distillation apparatus to flush out the apparatus before use, with the connections arranged to circulate through the condenser, for at least 10 minutes. 25 ml of 2% boric acid was pipetted into a 250 ml conical flask and 2 drops of mixed indicator added. The conical flask and its contents will be placed under the condenser in such a position that the tip of the condenser was completely immersed in solution formed in the conical flask. 10 ml of the digested solution sample were measured. The stopcock of the funnel on the steam jacket was opened and the 10 ml of the digested solution poured. Excess of 40% NaOH was added to the decomposition flask and the funnel stopcock closed. The stopcock on the steam trap outlet was shut to force steam through the decomposition chamber in order to drive the liberated ammonia into the collection flask. The distillate was titrated with 0.1 N HCl solution. The acid was added until the solution was colourless. Additional acid caused the solution to become pink. The same procedure was followed for the blank.

2.3.7. Determination of Mineral Elements

The mineral elements were manganese, calcium, iron, zinc and copper and were analyzed using the atomic absorption spectrophotometer (AAS) method outlined in AOAC (2000) [11].

Procedure:

Mineral analyses were done using the method of Benton and Vernon (1990) [12]. The palm wine was weighed into a porcelain crucible while an empty crucible was included as a blank. The crucibles were placed in a muffle furnace and heated to 600°C over a period of 2 hours and cooled in a desiccator. The ashed samples were transferred into 50 ml centrifuge tube and the crucibles were rinsed three times with 5 ml distilled water and 5 ml of *aqua regia*. 1200 ml of distilled water were poured into a 2 L volumetric flask. 400 ml concentrated hydrochloric acid and 133 ml of 70% nitric acid were carefully added and the volume will be made to 2 L to a total volume of 20 ml. The tubes were stoppered and vortexed to mix the contents thoroughly and centrifuged at 3000 rpm for 10 min. The supernatant were decanted into micro vials. The flame atomic absorption spectrophotometer were used in the determination the mineral contents.

2.4. Antibiotic Sensitivity Testing

The agar diffusion method standard disk diffusion method recommended by the National committee for clinical laboratory standards (2002) [13] were employed for antimicrobial susceptibility testing. Overnight cultures of each isolate were adjusted to 0.5, McFarlannd turbidity standard. A sterile cotton swab was dipped into the standardized suspension, drained and used for inoculating the culture plates (Sterilin, UK). The inoculated plates were air dried, and antibiotic discs (Oxoid, UK) were placed on the agar using flamed forceps and were gently pressed down to ensure contact. After proper diffusion of the antibiotics into the agar, the plates were incubated at 37°C for 18 – 24 hrs and the zones of inhibition were measured and compared with a zone-interpretation chart. The antimicrobial discs used include Rifampicin (30µg), ciprofloxacin (20µg), norfloxaxin (20µg), gentamicin (10µg), streptomycin (20µg) ampicillin (10µg). Erythromycin (10µg) levoflovacin (15µg), amoxicillin (25µg), ceftriaxone (30µg), ampiclox (30µg), and cefuroxime (30µg).

3. Results

Table 1 shows the Morphological and biochemical identification of bacteria isolates from palm wine sample. Five microorganisms were isolated in this study and they include; *Staphylococcus aureus, Lactobacillus* spp., *Bacillus cereus*, *Streptococcus* spp and *Escherichia coli.*

Table 1. Morphological and biochemical identification of bacterial isolates from palm wine samples.

Morphology	Shape/arrangement	Gram reaction	Motility	Coagulase	Catalase	Oxidase	Indole	Citrate utilization
Milky, raised colonies on N. A	Cocci in clusters	+	-	+	+	-	-	-
Smooth pink colonies on MacConkey agar	Single flat rode	-	+	-	+	-	+	-
White smooth and small, with convex round colony on N. A	Spherical cocci in short chains	+	-	-	-	-	-	-
white large mucoid raised irregular on N. A	Large rods joined in chains	+	-	-	+	+	-	+
Small and greyish white colour on MRS	Short slender straight rod	-	-	-	-	-	-	-

Table 1. Continued.

Morphology	Shape/arrangement	Galactose	Sucrose	Fructose	Mannose	Xylose	Lactose	Maltose	
Milky, raised colonies on N. A	Cocci in clusters	**A**	A	A	A	-	A	A	*Staphylococcus aureus*
Smooth pink colonies on MacConkey agar	Single flat rode	AG	-	-	A	-	AG	A	*Escherichia coli*
White smooth and small, with convex round colony on N. A	Spherical cocci in short chains	-	-	A	A	A	-	-	*Streptococcus* spp.
white large mucoid raised irregular on N. A	Large rods joined in chains	-	A	-	AG	AG	+	+	*Bacillus cereus*
Small and greyish white colour on MRS	Short slender straight rod	**A**	A	A	A	-	+	+	*Lactobacillus* spp.

Table 2 shows the Morphological characteristics of yeast isolates from palm wine samples. *Saccharomyces cerevisiae* and *Candida* spp were isolated from the samples.

Table 2. Morphological characteristics of yeast isolates from palm wine samples.

MACROSCOPY	MICROSCOPY	YEAST ISOLATED
Round and creamy colonies on SDA.	Single oval cells were seen, some in pairs and elongate, Also budding was pronounced, spores seen	*Saccharomyces cerevisiae*
Whitish colonies, not well developed pseudomycellium	Single round cells seen, spores were absent	*Candida* spp.

Table 3 shows the pH and temperature of palm wine samples after collection. The sample PLM1 has the highest temperature (25°C) and pH (6.41).

Table 3. pH and temperature of palm wine samples.

S/N	Isolate	Temperature	pH
1	PLM1	25°C	6.41
2	PLM 2	22°C	6.13
3	PLM 3	22°C	5.81
4	PLM 4	21°C	5.51
5	PLM 5	22°C	5.02
6	PLM 6	21°C	4.00
7	PLM 7	25°C	6.21
8	PLM 8	22°C	6.13
9	PLM 9	22°C	5.10
10	PLM 10	21°C	5.51
11	PLM 11	22°C	6.31
12	PLM 12	21°C	6.13
13	PLM 13	25°C	5.11

S/N	Isolate	Temperature	pH
14	PLM 14	22°C	5.51
15	PLM 15	22°C	5.02
16	PLM 16	22°C	6.21
17	PLM 17	21°C	6.13
18	PLM 18	25°C	5.11
19	PLM 19	22°C	5.51
20	PLM 20	22°C	5.02
	MEAN	22°C	5.59

Table 4 shows the Microbial counts of palm wine samples (cfu/ml). The total viable count of the bacteria from palm wine was highest for PLM10 (6.0 x 10^3cfu/ml) while lowest count was for sample PLM8 (2.4 x 10^4cfu/ml). Highest coliform count was seen in Sample PLM5 (5.2 x 10^3cfu/ml) while the lowest value was observed in PLM4 (3.0 x 10^4cfu/ml). The total yeast count was highest in sample PLM 20 (2.6 x 10^3cfu/ml) while lowest count was recorded for sample PLM3 (9.0 x 10^3cfu/ml).

Table 4. Microbial counts of palm wine samples (CFU/ml).

S/N	SAMPLES	TVC (cfu/ml)	TCC (cfu/ml)	TYC (cfu/ml)
1	PLM 1	$4.5x10^3$	$3.4x10^3$	$1.0x10^3$
2	PLM 2	$4.7x10^4$	$3.2x10^4$	$1.1x10^4$
3	PLM 3	$3.5x10^3$	$4.1x10^3$	$0.90x10^4$
4	PLM 4	$5.1x10^3$	$3.0x10^4$	$1.5x10^3$
5	PLM 5	$3.7x10^4$	$5.2x10^3$	$2.1x10^3$
6	PLM 6	$4.2x10^3$	$4.9x10^3$	$1.9x10^3$
7	PLM 7	$3.6x10^3$	$4.3x10^3$	$1.8x10^4$
8	PLM 8	$2.4x10^4$	$3.3x10^4$	$1.3x10^3$
9	PLM 9	$5.2x10^4$	$4.6x10^3$	$1.9x10^4$
10	PLM 10	$6.0x10^3$	$5.1x10^3$	$2.1x10^3$
11	PLM 11	$5.0 x10^3$	$3.7x10^4$	$1.9x10^3$
12	PLM 12	$4.8 x10^3$	$4.2x10^3$	$1.8x10^4$
13	PLM 13	$4.0 x10^3$	$5.1x10^3$	$2.1x10^3$
14	PLM 14	$5.0 x10^4$	$3.7x10^4$	$1.9x10^3$
15	PLM 15	$4.2x10^3$	$4.8 x10^3$	$2.1x10^3$
16	PLM 16	$4.7x10^4$	$4.0 x10^3$	$1.7x10^3$
17	PLM 17	$5.2 x10^4$	$5.0 x10^4$	$2.1x10^3$
18	PLM 18	$4.7 x10^3$	$4.2x10^3$	$2.1x10^3$
19	PLM 19	$4.5 x10^4$	$4.8 x10^3$	$1.4x10^3$
20	PLM 20	$4.8 x10^3$	$4.8 x10^3$	$2.6x10^3$
	MEAN	$4.4x10^4$	$4.2x10^4$	$1.7x10^3$

Table 5 shows the proximate composition of palm wine. The samples are low in carbohydrate (1.01±0.01 - 2.12±0.02), crude protein (0.51±0.03 - 0.90±0.01), lipid (0.01±0.00 - 0.07±0.04), ash (0.31±0.11 - 0.78±0.06) and crude fibre contents (0.21±0.04 - 0.37±0.01) while the samples are high in moisture.

Table 5. Proximate composition of palm wine.

Sample Code	Carbohydrate	Crude protein	Lipids	Ash	Moisture	Crude fibre
PLM 1	1.12±0.01^a	0.61±0.04^a	0.07±0.0^a	0.70±0.14^a	98.74±0.01^a	0.29±0.03^a
PLM 2	1.15±0.01^a	0.51±0.03^d	0.06±0.0^a	0.31±0.11^f	97.74±0.02^a	0.26±0.01^a
PLM 3	1.19±0.02^b	0.73±0.03^b	0.03±0.03^b	0.77±0.03^b	96.45±0.01^a	0.21±0.04^b
PLM 4	1.01±0.01^d	0.77±0.00^c	0.01±0.00^c	0.66±0.01^c	98.74±0.02^b	0.23±0.02^d
PLM 5	1.13±0.01^a	0.81±0.00^c	0.07±0.04^a	0.53±0.03^e	97.64±0.02^a	0.37±0.01^c
PLM 6	1.20±0.03^b	0.85±0.00^c	0.06±0.01^a	0.61±0.00^d	96.55±0.01^a	0.31±0.03^a
PLM 7	2.10±0.14^c	0.56±0.06^d	0.03±0.03^b	0.67±0.01^c	98.64±0.01^b	0.35±0.01^c
PLM 8	2.12±0.02^c	0.56±0.06^d	0.01±0.00^c	0.56±0.01^e	97.64±0.02^a	0.26±0.06^a
PLM 9	1.17±0.01^a	0.61±0.02^a	0.07±0.04^a	0.57±0.03^e	96.35±0.01^a	0.26±0.05^a
PLM 10	1.19±0.04^b	0.90±0.01^e	0.06±0.01^a	0.78±0.06^b	98.64±0.02^b	0.21±0.02^b
PLM 11	1.19±0.02^b	0.73±0.03^b	0.03±0.03^b	0.77±0.03^b	96.45±0.01^a	0.21±0.04^b
PLM 12	2.10±0.14^c	0.56±0.06^d	0.03±0.03^b	0.67±0.01^c	98.64±0.01^b	0.35±0.01^c
PLM 13	2.12±0.02^c	0.56±0.06^d	0.01±0.00^c	0.56±0.01^e	97.64±0.02^a	0.26±0.06^a
PLM 14	1.19±0.02^b	0.73±0.03^b	0.03±0.03^b	0.77±0.03^b	96.45±0.01^a	0.21±0.04^b
PLM 15	1.13±0.01^a	0.81±0.00^c	0.07±0.04^a	0.53±0.03^e	97.64±0.02^a	0.37±0.01^c
PLM 16	1.20±0.03^d	0.85±0.00^c	0.06±0.01^a	0.61±0.00^d	96.55±0.01^a	0.31±0.03^a
PLM 17	1.13±0.01^a	0.81±0.00^c	0.07±0.04^a	0.53±0.03^e	97.64±0.02^a	0.37±0.01^c
PLM 18	1.20±0.03^d	0.85±0.00^c	0.06±0.01^a	0.61±0.00^d	96.55±0.01^a	0.31±0.03^a
PLM 19	2.10±0.14^c	0.56±0.06^d	0.03±0.03^b	0.67±0.01^c	98.64±0.01^b	0.35±0.01^c
PLM 20	1.19±0.02^b	0.73±0.03^b	0.03±0.03^b	0.77±0.03^b	96.45±0.01^a	0.21±0.04^b
MEAN	1.13±0.03	0.70±0.02	0.04±0.02	0.63±0.03	97.48±0.01	0.28±0.03

Values are mean ±SD; values with different superscript along the column are significantly different (P≤0.05).

Table 6 shows the vitamin composition of palm wine. The values are in the range: 21.48±0.02 - 22.76±0.08 for vitamin A and 6.60±0.80 - 7.42±0.02 for vitamin B.

Table 6. Vitamin composition of palm wine.

Sample codes	Vitamin A	Vitamin B
PLM 1	21.76±0.03^a	7.42±0.02^a
PLM 2	21.86±0.01^c	6.00±0.20^c
PLM 3	21.48±0.02^d	6.60±1.20^b
PLM 4	21.86±0.04^c	6.90±0.83^b

Sample codes	Vitamin A	Vitamin B
PLM 5	21.76±0.03 [a]	6.80±0.80 [b]
PLM 6	22.75±0.01 [a]	6.90±0.83 [b]
PLM 7	21.48±0.02[d]	7.20±0.52 [a]
PLM 8	21.68±0.02 [b]	6.90±0.83 [b]
PLM 9	22.76±0.08 [a]	7.40±0.04 [a]
PLM 10	21.86±0.01[c]	6.60±0.80 [b]
PLM 11	21.76±0.03 [a]	7.42±0.02 [a]
PLM 12	21.76±0.03 [a]	6.80±0.80 [b]
PLM 13	22.75±0.01 [a]	6.90±0.83[b]
PLM 14	21.48±0.02 [d]	7.20±0.52 [a]
PLM 15	21.76±0.03[a]	7.42±0.02 [a]
PLM 16	21.67±0.02 [b]	6.00±0.20[c]
PLM 17	21.86±0.04[c]	6.90±0.83 [b]
PLM 18	22.76±0.08 [a]	7.40±0.04 [a]
PLM 19	21.67±0.02[b]	6.60±0.80[b]
PLM 20	21.76±0.03 [a]	7.42±0.02 [a]
MEAN	21.94±0.02	6.9±0.50

Values are mean ±SD; values with different superscript along the column are significantly different (P≤0.05).

Table 7 shows the Mineral composition of palm wine. The values are in the range: 3.24±0.28 - 3.85±0.02 for calcium; 30.02±0.00 - 31.35±0.01 for magnesium; 3.10±0.33 - 3.85±0.02 for iron; 0.25±0.04 - 0.31±0.03 for zinc and 0.28±0.08 - 0.48±0.08 for copper.

Table 7. Mineral composition of palm wine.

Sample codes	Calcium	Magnesium	Iron	Zinc	Copper
PLM 1	3.38±0.02[a]	31.35±0.02[a]	3.38±0.02[a]	0.36±0.05[a]	0.48±0.08[a]
PLM 2	2.87±0.01[b]	31.22±2.06 [c]	2.87±0.01[b]	0.25±0.01 [b]	0.41±0.03 [b]
PLM 3	3.85±0.02[b]	30.02±0.00 [b]	3.85±0.02[b]	0.27±0.08 [b]	0.47±0.08 [a]
PLM 4	3.22±2.06[c]	30.02±0.00 [b]	3.22±2.06 [c]	0.31±0.03[c]	0.47±0.08 [a]
PLM 5	3.24±0.28 [c]	31.00±0.03 [b]	3.24±0.28 [c]	0.27±0.08 [b]	0.45±0.04 [a]
PLM 6	3.10±0.33 [d]	31.35±0.01[a]	3.10±0.33[d]	0.25±0.04 [b]	0.46±0.05 [a]
PLM 7	3.38±0.02 [a]	31.35±0.02 [a]	3.38±0.02 [a]	0.36±0.05 [a]	0.48±0.08 [a]
PLM 8	2.87±0.01 [b]	31.22±2.06 [c]	2.87±0.01 [b]	0.25±0.01 [b]	0.41±0.03[b]
PLM 9	3.85±0.02 [b]	30.02±0.00[b]	3.85±0.02[b]	0.27±0.08 [b]	0.47±0.08 [a]
PLM 10	3.33±0.02 [a]	31.0±0.003[b]	3.17±0.01 [c]	0.31±0.03 [c]	0.45±0.04 [a]
PLM 11	3.38±0.02 [a]	32.35±0.01[d]	3.38±0.02 [a]	0.36±0.05 [a]	0.48±0.08 [a]
PLM 12	3.37±0.03 [a]	31.35±0.02[a]	3.37±0.02 [a]	0.36±0.05 [a]	0.48±0.08 [a]
PLM 13	2.87±0.01[b]	31.22±2.06 [c]	2.87±0.01 [b]	0.25±0.01 [b]	0.41±0.03 [b]
PLM 14	3.85±0.02[b]	31.02±0.00 [b]	3.85±0.02 [b]	0.27±0.08 [b]	0.47±0.08 [a]
PLM 15	3.38±0.02 [a]	31.35±0.02 [a]	3.38±0.02 [a]	0.36±0.05 [a]	0.48±0.08 [a]
PLM 16	2.87±0.01 [b]	31.22±2.06[c]	2.87±0.01 [b]	0.25±0.01 [b]	0.41±0.03[b]
PLM 17	3.24±0.28[c]	31.02±0.02 [b]	3.24±0.28[c]	0.27±0.08 [b]	0.45±0.04 [a]
PLM 18	3.10±0.33[d]	31.35±0.01 [a]	3.10±0.33 [d]	0.25±0.04[b]	0.46±0.05 [a]
PLM 19	3.38±0.02 [a]	31.35±0.02 [a]	3.38±0.02 [a]	0.36±0.05 [a]	0.47±0.06 [a]
PLM 20	3.37±0.01 [a]	31.33±0.01 [a]	3.38±0.02 [a]	0.36±0.05 [a]	0.48±0.08 [a]
MEAN	3.29±0.17	29.60±0.42	3.29. ±0.04	0.29±0.04	0.45±0.06

Values are mean ±SD; values with different superscript along the columns are significantly different (P≤0.05).

Table 8 shows the antimicrobial susceptibility result of the bacterial isolates. *S. aureus* was inhibited most by all the antibiotics while *E. coli, B. cereus*, *Streptococcus* spp and *Lactobacillus* spp showed lower degrees of susceptibility.

Table 8. Antimicrobial susceptibility of bacterial isolates.

Isolates	Cotrimazole	Cefixime	Erythromycin	Gentamycin	Augmentin	Streptomycin	Tetracycline	Chloramphenicol
Staphylococcus aureus	19 (S)	20 (S)	15 (I)	20 (S)	27 (S)	15 (I)	18 (S)	18 (S)
Escherichia coli	13 (R)	20 (S)	20 (S)	5 (I)	27 (S)	15 (I)	19 (S)	13 (R)
Bacillus spp	0 (R)	14 (I)	20 (S)	12 (R)	0 (R)	20 (S)	16 (I)	20 (S)
Streptococcus spp	0 (R)	0 (R)	20 (S)	14 (I)	0 (R)	0 (R)	20 (S)	20 (S)
Lactobacillus spp	12 (R)	18 (S)	19 (S)	5 (I)	21 (S)	14 (I)	18 (S)	11 (R)

Key: R= Resistance; S = Sensitive; I = intermediate

4. Discussions

The microbiological and nutritional status of palm wine from Umudike environments was evaluated. 20 samples of palm wine were used in this study.

Five microorganisms were isolated in this study and they include: Staphylococcus aureus, Lactobacillus spp., Bacillus cereus, Streptococcus spp and Escherichia coli. Lactobacillus, Leuconostoc, Bacillus, Streptococcus, Saccharomyces are probable probiotic organisms present in palm wine and will help to produce vitamins, digestive enzymes and stimulate the immune system [14]. Studies on the association of these organisms with water bodies in this area have been reported elsewhere [15].

The occurrence of these microbial isolates in the palm wine samples in this study supports the reports [16-18]. Furthermore, the isolation of *Lactobacillus* spp. and *Saccharomyces cerevisiae* corroborates the earlier report [19-21]. Thus, the isolation of *E. coli* from the palm sample and *Staphylococcus* from the two palm wine poses obvious public health questions. Hence, the unstable bowel movement associated with the consumption of raphia palm wine, as reported by some palm wine drinkers interviewed during the study could be associated with the pathogenic species of microbial contaminants such as *E. coli*.

Two yeasts were isolated in this study and they include; *Saccharomyces cerevisiae* and *Candida* spp. The presence of similar organisms in palm wine sample from Ikwuano local government area of Abia State has been reported [4, 22]. However, the methods of palm wine tapping and collection of palm sap influence the microbial content of the sap [2].

The high count obtained in viable coliform and yeast counts shows that the palm wine sample harboured several strains of microorganism which may be as a result of the poor hygienic condition involved in the tapping, collection and distribution of the wine. This agrees with earlier reports [18, 23- 24].

Sample PLM1 had the highest temperature (25°C) and pH (6.41). Other samples studied had other degrees of temperature ranging from 21°C -22°C.

The proximate composition of the palmwine isolates showed that the sample are low in carbohydrate (1.01±0.01 - 2.12±0.02), crude protein (0.51±0.03 - 0.90±0.01), lipid (0.01±0.00 - 0.07±0.04), ash (0.31±0.11 - 0.78±0.06) and crude fibre contents (0.21±0.04 - 0.37±0.01). The moisture of the palm wine was high for all the samples ranging from 96.35±0.01 - 98.74±0.01. The nutrient composition of the fresh sap has been reported by several workers [25]. The fresh palm sap is composed of sugars, proteins, titratable organic acids, alcohol, vitamins (ascorbic acid, thiamine, riboflavin etc) mineral elements and water.

Staphylococcus aureus was inhibited by all the antibiotics used in this study while *Escherichia coli, Bacillus cereus*, *Streptococcus* spp and *Lactobacillus* spp showed lower degrees of susceptibility.

5. Conclusion

The presence of these organisms in the wine is an indication of the poor hygienic state of the tappers, materials used and the method of tapping involved. This occurrence is of public health importance as the wine is consumed due to its nutritional significance. There is therefore the need for public awareness in the consumption of these wines to help promote the quality of these products as well avoiding the health risk that may be associated with the consumption of contaminated palm wine.

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