*Original Research Article*

Effects of Traditional Preservatives on the Acidity and Microbial Composition of Palm Wine in Tiko (South West Region) Cameroon

ABSTRACT

|  |
| --- |
| Palm wine is a natural alcoholic beverage tapped from palm tree (*Elaeis guinzensis*). It is consumed in most African countries; popularly known as matango and white stuff in Cameron. the main aim of this work was to analyze the effect of traditional preservatives on the acidity and microbial composition of palm Wine. The palm wine contains a heavy suspension of live yeast and bacteria. The microorganisms metabolize the sugar thus removing the pleasant sugary taste of the palm wine within 36-48 hours of production. This compels palm wine tappers to use some traditional preservatives like *Sacoglotis gabonensis* (mahoum), extract of *Elaeis Guinensis* to be able to prolong its storage time. The palm wine is served in different occasions like traditional weddings, njangi groups, funerals, baby-showers, etc. In order to be sure, it can be consumed after a day or two, the acidity level was determined using Phenolphthalein solution and Sodium hydroxide in three different samples, A, B and C (plain palm wine, palm wine with *Sacoglotis gabonensis* and palm wine with *Elaeis Guinensis* respectively. In day one the acidity level stood at a pH of 4.33 in all three samples. In day two and three, sample B had the highest level of acidity. For microbial growth, sample A was the highest with 4.8x107 UFC/ml; in day two the total microbial growth almost went to zero while in day three, it stood at 3.94x108 UFC/ml. For yeast/fungi, day one sample ‘A’ still had the highest with 2.9x106 UFC/ml, day two resurfaced very little while by day three Samples resurfaced. *Staphylococcus* *aureus* stood at 0 UFC/ml in all three samples in day one, day two, it surfaced in all three samples, though very high in sample B Compared to other samples; in day three, it stood very high in sample A; Salmonela, E. Coli and Coliform stood at 0 UFC/ml in all three sample for 3 days. The conclusion Drawn is that the use of these preservatives to prolong the storage of palm wine was far-fetched because microbes still manifested in the supposedly preserved palm wine |

*Keywords:* ***Effects, Preservative, Acidity, Microbial***

1. INTRODUCTION

Palm wine is an alcoholic beverage obtained from the fermented sap of tropical plants of the Palmae family, such as the oil palm (Elaeis guineensis), coconut palm (Phoenix dactylifera), date palm, nipa palm, kithul palm and raffia palm (Raphia hookeri) [1][2]. Palm wine, also known as palm toddy or simply toddy [3] is a refreshing beverage that is produced and consumed in large quantities in West Africa and Cameroon. It is also enjoyed by people in parts of Asia and South America and has continued to be the preferred sweetener in some Asian countries. Palm wine is known under various names, such as matango, white stuff or ‘mimbo’ in Cameroon, ‘nsafufuo’ in Ghana, ‘emu’ in Nigeria and ‘bandji’ in Côte d’Ivoire (Karamoko 2012). It is a very popular drink being consumed by over 10 million people in West Africa [4].

In Nigeria, palm wine is the most widely accepted and cherished natural traditional alcoholic beverage, especially in the Southern part of the country. Palm wine is of high economic and nutritional importance and plays a significant role in social and cultural practices in Africa. As a result of the nutritional value of the unfermented sap, it is often recommended to infants whose mother cannot produce the necessary milk for nursing. Ayernor and Mathews, (2015) [5] reported that as a good source of vitamins B1 (thiamine) and C (ascorbic acid), palm wine offers supplemental nutrition to a meal. The drink is a rich nutrient medium containing sugars, proteins, amino acids, alcohols and minerals. The unfermented palm sap is a sweet, transparent juice with a sugar content of 100 -144 g/kg, a pH of 7.0 - 7.4 and traces of ethanol [3] while the fermented sap, is whitish and has a pH of about 3.6 and alcohol content of 3.3 - 4.0%, depending on the stage of fermentation at which the wine is consumed [3]. Thus, palm wine is consumed for various reasons. It may be consumed for its thirst-quenching effect, sweetness or stimulating effect. Although it has a variety of flavours, ranging from sweet (unfermented) to sour (fermented) and vinegary [6] it is preferred by most people when sweet and fresh. Thus, one major problem still faced by the palm wine consuming public is preservation, which limits its distribution. It is therefore important that palm wine is preserved in its natural state. Most palm wine tappers in South East Nigeria get their palm wine from three types of palm trees namely, the palm oil tree *Elaeis guineensis* or *Raphia* palms, called *Raphia hookeri* and *Raphia vinifera* in Cameroon, it is obtained from Rhaphia tree, palm tree. which has been felt on the ground. Common in the South West Region; while in the North West Region still in Cameroon the tappers climb up the tree.

In Cameroon, the flavour and aroma of palm wine obtained from *Elaeis* sp. is somewhat different from that of *Raphia* sp. To the best of the knowledge of the authors, the drink sourced from trees in Imo State has not been subjected to current analytical methods (HPLC, APCI-MS and GC-MS) to generate more information. However, volatiles from the southwest region and odorants of palm wine from *E. guineensis* sourced from other regions in Nigeria have been studied and it was found that no one compound is responsible for the characteristic palm wine odour. Odorants responsible for the intense aroma qualities perceived upon sample introduction into the mouth, while swallowing the drink and nasal aroma perception have been reported but quantification of the intensity of the main compounds responsible for the intense odour of fermenting palm wine in the atmosphere is yet to be explored by many workers. However, palm wine contains a heavy suspension of live yeast and bacteria [7]. These microorganisms metabolize the sugars thus removing the pleasant sugary taste of the fresh wine within 36 – 48 h of production, and produce various organic acids. Akinrotoye, reported that fresh palm wine is a sweet drink, which easily becomes sour when left for few hours before consumption [6]. This is due to excessive fermentation and acidity due to Malolactic/Acetic acid fermentation by the bacteria [8] resulting in the rapid deterioration of the organoleptic quality of the juice. If the palm wine is not consumed within a few days, it begins to develop a vinegary taste, making it unacceptable to consumers. In recognition of the significant role of palm wine in the lives of people, particularly in African society, studies over the years have focused on palm wine preservation. The use of chemical preservatives such as metabisulphite, benzoic acid, sodium benzoate diethylpyrocarbonate, and sorbic acid among others; pasteurization and refrigeration either singly or in combination with one another have been widely reported [9].

**1.1 PALM WINE**

Palm wine is a collective name for an assortment of alcoholic beverages prepared from fermented palm sap. [10]. defined palm wine as a generic name for a group of alcoholic beverages obtained by fermentation from the saps of palm trees. Palm wine was recently reported to be the phloem exudates from the palm tree [11]. It is probably the most popular naturally fermented alcoholic beverage in West Africa, and in some parts of Nigeria and Cameroon its production has developed into small-scale industries. During the peak production period of palm wine, most of this wine is wasted or distilled into a local gin (known as “Kaikai”, “Ogogoro” or “Akpeteshi”) due to a lack of a good and affordable preservation method. Palm sap is a sweet, clear colourless and nearly neutral juice containing 10-12% of sugar mainly sucrose [12].

It is collected by tapping the top of the trunk after felling the palm tree and boring a hole into the trunk. It is usually a whitish effervescent liquid. This property is derived from the metabolic activity of numerous microorganisms found in the wine. Okafor and Hai et al. [13][14] reported that fresh palm wine is a sweet drink which easily becomes sour when left for few hours before consumption. The sap contains approximately 0.23 protein, 0.02 fat. Half of the total sugars are fermented during the first twenty-four hours and the ethanol content of the fermented palm sap reaches a maximum of 5.0- 5.28 after forty-eight hours [15]. The sap is not heated and the wine is an excellent substrate for microbial growth. The palm sap fermentation involves alcoholic –lactic-acid fermentation, due to the presence mainly of yeast and lactic acid bacteria. It has been suggested that Saccharomyces Sapp is the most important for the formation of the characteristic aroma of the palm wine [16]. S Cerevisiae and Schizosacharomyces pombe have been reported to be the dominant yeast species [17]. Ethanol fermentation in palm wine is mediated by S. cerevisiae and S. carlsbergensis which are top and bottom fermenting yeasts respectively [18]. Olotu et al. [19]. reported that palm wine results from a yeast/lactic acid fermentation of the sugary sap of palms. Fermentation of the sugars and other nutrients present in the juice by endogenous microflora of the palm sap leads to alcohol and organic acid production. The alcohol content ranges from 0.5 to 7.0% (w/v) depending on the locality where the sap is obtained, and the extent of fermentation. The quality of the wine as indicated by the taste is highly variable and depends among other factors, on the variety of palm from which the sap is obtained [13].

Although some of these preservation techniques have made significant contributions towards extending the shelf life of palm wine, they are not without their attendant limitations. For instance, refrigeration which is effective in retarding microbial growth [20] is very expensive and not readily accessible to and affordable by over 98% of the low-income populace in rural communities where palm wine is produced. Thus, the search for an effective, suitable, safe and affordable technique(s) for palm wine preservation is still ongoing. The rapid deterioration in organoleptic quality of palm exudates by the natural flora of the fermenting sap constitutes the major problem facing palm wine production and distribution. This problem became evident ever since palm wine consumption became popular and has been a subject of intense study. Local tappers of palm wine often add the dry stem of a tree, Sacoglottis gabonensis, and extract of Elaeis guineensis in the container containing the palm wine immediately after collection to prolong the shelf life. This appreciably inhibits the growth of bacteria found in palm wine, such as Leuconostoc mesenteroides and Lactobacillus plantarum [13]. The palm wine becomes more alcoholic on standing. Other preservation methods were studied thereafter. Sorbic acid though found suitable, was disregarded on the ground that it is dangerous to man. Thus, research interest in the use of plant material such as S. gabonensis and others in palm wine preservation has been rekindled.

In order to lengthen the shelf life of palm wine, a number of preservation measures have been adopted. These include the use of dry stem of bark of trees such as *Saccoglottis gabonensis*, *Vermonia amydalina*, *Euphobiasp*., *Nauclea sp*. and *Rubiacae sp*. [21]. Sulphite and Benzoate pasteurization, have all been used for the preservation of palm wine. All these attempts resulted in a change of taste or not being completely able to curb the actions of the fermenting microbes [22].

2. materialS and methods

Tiko is a small town found in the South West Region of Cameroon. It has an estimated population of 78.885 inhabitants. For the climatic condition, Tiko in the wet season is warm, the Dry season is hot and mostly cloudy and is oppressive year-round. The temperature over the years varies from 74oF to 90oF and it's fairly below 72oF or 74oF. Fresh Palm wine (3 litres), Elaeis guinensis (ripe fruits extract) Sacoglottis gabonensis (dry stem) Distilled water, Sodium hydroxide, Phenolphthalein, Buffer solution, Plastic bottles (3) Autoclave Beaker Test Tubes Drying Oven Electric plate heater Stirrer A scale PH Meter Volumetric flask Petri dish Funnel Bowls Gloves

**2.1 SAMPLES COLLECTION**

The Samples were collected as follows:

* Fresh palm wine was bought from a taper, and a litre each was put in the three different plastic bottles that were sterilized to destroy microbial growth, labelled A, B and C representing the three samples and where immediately carried to the lab.
* The stem of dry *Sacoglottis gabonensis* was also bought from a palm wine tapper. Fresh palm nut fruit was bought from the market.

**2.2 METHODOLOGY**

The samples were carried to the laboratory together with the preservatives. On day one, only sample A (control sample) was used and the other two samples B and C with the preservatives, were immersed for day 2 and 3. Sample A which was the control, the pH and acidity were analysed. To obtain the pH, the pH scale was placed in a buffer solution to have a standard value of 7. For the acidity, distilled water, sodium hydroxide solution and phenolphthalein solution small quantity of palm wine was used. While for the microbial growth the different algars were used at varying quantities to target different microorganisms after 24 hours. The same procedure was carried out on three different days.

***2.2.1 To determine the presence of natural preservatives of palm wine***

Here two ways were used. That is an interview and questionnaire with the focus groups who were all palm wine tappers with a lot of experience in tapping from 5 years and above. They were posed questions on the different preservatives and how they are used while with the questionnaire they were guided on how to answer the questions.

***2.2.2 To determine the effect of traditional preservatives on the acidity of palm wine***

To obtain the acidity of the palm wine with the preservatives and the control with no preservative, the researcher in the laboratory used drops of phenothalein solutions and NaOH (sodium hydroxide solution which together with a dilution of small quantities of the samples gave the acidity level.

***2.2.3 To analyse the effect of the natural preservatives on the microbial load of palm wine***

In the laboratory different agar media were used and different quantities were prepared. Portions of diluted samples were spread in petri dishes and dried in the oven. After 24 hours, they were removed, and the number of samples was counted to obtain the total microbial growth.

***2.2.4 Determination of Acidity***

pH is an indicator of the acidity of a product and can also provide information on its microbiological stability (at pH 4, the growth of most bacteria is inhibited). The measurement of the pH in each sample of palm wine was carried out by potentiometry according to the reference protocol NE V05- 108 using a pH meter brand. For its realization, the pH meter is initially calibrated by introducing the probe for 1 mm in 50 mL of distilled water, then in a buffer solution of pH 7. Subsequently, the probe is rinsed in distilled water and introduced into 50 mL of the sample. The pH value is read directly on the display panel of the device.

***2.2.5 Determination of Titratable***

Acidity is associated with a lot of organic acids from the fermentation of palm wine, mainly acetic, citric and lactic acid. It was determined by titrimetry according to the reference protocol NP V04 — 206 carried out in three stages. Concentration 0.1 M, Volume = 100 ml in a beaker, 0.4 g of anhydrous NaOH was weighed and gradually dissolved in distilled water. The whole was transferred to a 100 ml volumetric flask and filled with water up to the gauge mark. In a beaker containing a volume of 22.5 ml of distilled water, 2.5 ml of each wine was introduced (this is a 10th dilution). The experiment was repeated 3 times in 3 different beakers to perform the three tests. To each solution, 2 to 3 drops of phenolphthalein were introduced, and then the whole was homogenized and titrated. The acidity contained in each sample of diluted palm wine was gradually titrated with the 0.1 M NaOH solution until the solution was changed to Rose.

**2.3 Determination of the Microbiological quality of palm wine**

The principle of the method of certain microorganisms such as bacteria and fungi (viable and cultivable) present in a product can be isolated, by forming after multiplication, a cluster of characteristic cells on a culture medium. Each cell cluster called a colony or colony-forming unit (CFU) comes from a single cell, which makes it possible to detect the presence or to determine the number of microorganisms present in the product. In addition, it is possible to carry out an identification of the isolated microorganisms using selective culture and identification media. The analysis was carried out according to standard methods and the Colony Forming Unit (CFU)/mL values have been determined. The following work steps were undertaken: Sabouraud Dextrose Agar (SDA), Muller Hinton Agar (MIJA), Mannitol Salt Agar (MSA), and Mac Conkey agar were prepared according to each manufacturer’s directions. The appropriate masses of powder for each agar were weighed and solubilized in the corresponding volumes of distilled water then heated. Sterilization was carried out at 121°C for 15 min for the first three culture media. Before inoculation, l ml of each wine sample was diluted in sterile distilled water contained in tubes, to 100th for inoculation on Mac Conkey Agar and Mannitol Agar and to 10000 for inoculation on Muller Hinton Agar and Sabouraud Dextrose Agar. The solution obtained was homogenized and was used for subsequent inoculations to search for the different microorganisms.

***2.3.1 Search for microbiological parameters.***

The fungal flora represented by yeasts and molds was sought in the sample by the standard method NF V 08-059. A volume of 50 mL of the sample diluted to 10,000th was inoculated onto Sabouraud Dextrose Agar supplemented with chioramphenicol and incubated for 1 day at 3 5°C. At the end of this time, the number of colonies on the surface of the agar was determined and reported for a volume of 1 ml of sample. Average Colony Forming Unit per ml (CFU/ml) values were determined for 3 trials.

***2.3.2 Total aerobic mesophilic flora (FMAT)***

The FMAT represents all the microorganisms present in a product and capable of multiplying in the presence of air at temperatures between 20 and 40°C. These microorganisms represent the spoilage flora and provide information on the state of degradation of the product. The FMAT was determined according to the standard method NF EN ISO 4833. To do this, a volume of 50 mL of sample of each wine diluted to 10,000th times was inoculated onto Muller Hinton Agar and incubated for 48 hours. At the end of this time, the number of colonies present on the surface of the agar was determined and reported for a volume of 1 mL of sample. The average CFU/ml values were determined for 3 trials

***2.3.3 Total coliforms***

Total coliforms, also called enterobacteriaceae, were determined by the standard method NP V 08-050. For its production, a volume of 50 ml of the sample diluted to 100th was inoculated on Mac Conkey Agar and incubated at 35°C for 72 hours. After incubation, the number of colonies present on the surface of the agar was determined for 1 mL of the sample. Average CFU/ml values were determined for 3 trials.

***2.3.4 Escherichia coli***

The search for E. coli was carried out concomitantly with that for total coliforms on Mac Coonkey agar. Inoculation and culture conditions were carried out as described above for total coliforms. After incubation, the number of colonies referring to E. coli according to the indications of the manufacturer of the culture medium was evaluated per mL of sample. Average CFU/ml values were determined for 3 trials.

**2.3.5 Staphylococcus aureus**

The search for Staphylococcus aureus (Pathogens) or Staphylococcus aureus was carried out on Mannitol Salt Agar according to the standard method NF EN ISO 68882. For its production, a volume of 50 ml of each wine sample diluted to 100th was inoculated onto Mannitol Salt Agar and incubated at 35°C for 48 hours. After incubation, the number of golden yellow colonies was determined per ml of sample.

***2.3.6 Salmonella and Shigella***

The search for Salmonella and Shigella was done concomitantly with that for total coliforms on Mac Coonkey agar. Inoculation and culture conditions were carried out as described above for total coliforms. After incubation, the number of colonies referring to Salmonella and Shigella according to the manufacturer’s instructions for the culture medium (white colonies) was evaluated per ml of sample. Average CFU/ml values were determined for 3 trials.

3. results and discussion

## **3.1 Determination of Traditional Preservatives Used in Palm Wine Preservation.**

These traditional preservatives were determined through interviews and questionnaires. The results obtained from the people interviewed indicated the different preservatives and the quantities used in a litre of palm wine. The discussion was obtained and presented on a pie chart as shown below.

Table 1: Interview Results of Palm Wine Tappers Using the Various Preservatives

|  |  |  |
| --- | --- | --- |
| Preservative | Tappers Involved | Percentage |
| Veromia amydalina | 1 | 10% |
| Elaies guinensis | 2 | 20% |
| Sacroglotis gabonensis | 7 | 70% |
| Total | 10 | 100% |

Figure 1: Chart showing the number of palm wine tappers interviewed per traditional preservative used

From a total of ten persons interviewed, 1 person (10%) of the correspondents used *Veronia amydalina* as a traditional preservative for palm wine; 2 persons (20%) of correspondents used *Elaesis guinensis* for this same purpose and 7 persons (70%) of the correspondents used *S. gabonensis* as a traditional preservative for palm wine. Thus, gobonensis is the most used traditional preservative for palm wine in the Tiko community whereas amydalina is the least used traditional preservative for palm wine in this same locality.

## **3.2 To Determine the Effect of Traditional Preservatives on The Acidity of Palm Wine**

Table 2 shows a standard deviation of 0.2 for acidity tests and measurements carried out on day one, the day the wine was fetched from the palm tree. This low standard deviation indicates that the acidity of the fresh palm wine is about 4.03. There was no addition of substances in the fresh palm wine on day one but acidity was measured in all the bottles to be certain that the bottles were all clean. On the following day, the pH of the wine was 3.84 and the standard deviation was as low as 0.03. Adding *sacoglotis gabonesis* to the palm wine on one hand and nut extract on the other hand, and making it sleep for a day stabilizes its pH to about 3.95 and 3.74 respectively for a standard deviation of 0.1. After keeping a harvested palm wine to sleep for two days, the measurements of day three indicated that, the acidity rate of the sample with *Sacoglotis gabonesis* remains lower with a pH of 3.66 compared to that with palm nut extract with a pH of about 3.57 and 3.42 respectively.

Table 2: Acidity for day 1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample | **Description** | 1st Trial | 2nd Trial | 3rd Trial | Mean | SD |
| A | Palm wine | 4.04 | 4.04 | 4.0 | 4.03 | 0.02 |
| B | Palm wine with Sacoglotis gabonesis | 4.04 | 4.04 | 4.0 | 4.03 | 0.02 |
| C | Palm wine with palm nut extract | 4.04 | 4.04 | 4.0 | 4.03 | 0.02 |

**Table 3: Acidity for day 2**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample | **Description** | 1st Trial | 2nd Trial | 3rd Trial | Mean | SD |
| A | Palm wine | 3.87 | 3.84 | 3.82 | `3.8 | 0.03 |
| B | Palm wine with sacoglotis gabonesis | 3.95 | 3.95 | 3.94 | 3.9 | 0.01 |
| C | Palm wine with palm nut extract | 3.74 | 3.74 | 3.75 | 3.74 | 0.01 |

Table 4: Acidity for day 3

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample | Description | 1st Trial | 2nd Trial | 3rd Trial | Mean | SD |
| A | Palm wine | 3.52 | 3.74 | 3.46 | 3.57 | 0.15 |
| B | Palm wine with sacoglotis gabonesis | 3.66 | 3.66 | 3.66 | 3.66 | 0.00 |
| C | Palm wine with palm nut extract | 3.46 | 3.40 | 3.39 | 3.41 | 0.04 |

**Figure 2: Acidity Evaluation for Samples A, B and C for three days**

The observation of the evolution of acidity rate of the sample palm wine for the three days is summarized in Figure 2. Results indicate that palm wine freshly harvested has an acceptable acidity for consumption. In most societies today, many people keep palm wine to stay for days and some people even like to consume but the one that has stayed for a long time. This practice is justified by the fact that harvesters and sellers have some practices they do in the name of conserving the wine safely. The two common methods are the addition of *Sacoglotis gabonesis* or palm nut extract in the fresh palm wine.

Results show that pH for fresh palm wine with or without conservation substances is the same and acceptable compared to that of some commercialized drinks. A day-old palm wine has a pH reduced. After two days, the pH of all samples kept decreasing. Contrary to what people believe, the results of this research indicated that traditional methods used to conserve palm wine do not succeed in stabilizing the pH of the palm wine. Results still show that *Sacoglotis gabonesis is* capable of reducing the acidity of the wine compared to wine kept for days without any substance applied but cannot maintain it at an acceptable rate for days. The results show that, palm nut extract used for the conservation of palm wine is just a fallacy. Figure 1 shows that the pH of wine with palm nut extract used as the preservative substance is like a pH booster. It decreases pH of the wine even more than when the wine is kept without any substance. This makes this practice more dangerous for those who consume palm wine conserved for days using this method. As reported by Ziadi and colleagues, [23], palm wine has acid and the acidity level is due to excessive fermentation of the palm wine as days go by.

## **3.3 To Analyse the Effect of Traditional Preservatives on the Microbial Composition of Palm Wine**

Results presented in Table 5 revealed that, fresh palm wine harvested is practically safe from microorganisms. A test carried out on the same day the palm wine was fetched indicated that it had only yeast present. The concentration of the yeast was close to 2.1 x107 UFC/ml. Nevertheless, the growth of yeast in the palm wine still left it safe for consumption. Tankoano [24], reported that, 70% of the total yeast population in palm wine is *Saccharomyces Cerevisiae*.

**Table 5: Microbial results for day 1** **for A, B and C (UFC/ml)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter in UFC/ml | A | B | C | Mean | SD |
| TMG | 9.6 x 107 | 1.14 x 107 | 1.41 x 107 | 4.1 x 107 | 4.8 x 102 |
| Coliform | 0 | 0 | 0 | 0 | 0 |
| Fungi/yeast | 1.8 x 107 | 2.22 x 107 | 2.36 x 107 | 2.1 x 107 | 2.9 x 102 |
| E.Coli | 0 | 0 | 0 | 0 | 0 |
| Staphylococcus aureus | 0 | 0 | 0 | 0 |  |
| Sallmonella shigella | 0 | 0 | 0 | 0 | 0 |

*A =Wine simple, B = Wine + S. gabonensis, C = Wine + P*

**Table 6: Microbial results for day 2** **for A, B and C (UFC/ml)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter  UFC/ml | | TMG | Coliform | Fungi  /Yeast | E. coli | S. aureus | Salmonella  shigella |
| A | Box 1 | 1. 6 x 104 | 0 | 0 | 0 | 8.0 x 10 | 0 |
| Box 2 | 5.6 x 104 | 0 | 0 | 0 | 6.0 x 102 | 0 |
| Mean | | 3.6 x 104 | 0 | 0 | 0 | 7.0 x 102 | 0 |
| B | Box 1 | 3.62 x 105 | 0 | 0 | 0 | 5.2 x 103 | 0 |
| Box 2 | 9.4 x 104 | 0 | 0 | 0 | 2.0 x 102 | 0 |
| Mean | | 2.28 x 104 | 0 | 2.0 x 103 | 0 | 2.61 x 104 | 0 |
| C | Box 1 | 4.0 x 107 | 0 | 0 | 0 | 2.0 x 102 | 0 |
| Box 2 | 2.2 x 104 | 0 | 6.0 x 103 | 0 | 1.0 x 102 | 0 |
| Mean | | 19.19 x 103 | 0 | 3.0 x 103 | 0 | 1.5 x 102 | 0 |
| General Mean | | 6.76 x 104 | 0 | 1.33 x 103 | 0 | 8.98 x 103 | 0 |
| SD | | 1.63 x 102 | 0 | 500 | 0 | 200 | 0 |

*A =Wine simple, B = Wine + S. gabonensis, C = Wine + P*

Table 6 reveals that after 24 hours of incubation, fungi/yeast disappeared from the wine. Staphylococcus aureus was reported at rate of 7 x 102 UFC/ml. The Sacoglotis gabonesis added to the palm wine for preservation decreased the presence of yeast in the wine but did not affect staphylococcus aureus present in the wine. Adding palm nut extract in the palm and keeping for a duration of 24 hours does not protect the wine from taking microbes. Even though many microorganisms had increased in the palm wine, their growth rate was slightly decreased compared to day one. The difference in the growth is estimated at 3.42 x 107 UFC/ml.

Table 7: Microbial result for day 3 for A, B and C (UFC/ml)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter in CFU/ml | | TMG | Coliform | Fungi/yeast | E. coli | S. aureus | Salmonella  shigella |
| Wine simple | Box 1 | 7.2 x107 | 0 | 3.5x106 | 0 | 1.2 x 104 | 0 |
| Box 2 | 6.8 x 106 | 0 | 4.2 x 106 | 0 | 1.1 x 105 | 0 |
| Mean | | 3.94 x 108 | 0 | 3.85 x 106 | 0 | 6.1 x 104 | 0 |
| Wine + S. gabonensis | Box 1 | 3.7 x 106 | 0 | 6.7 x 106 | 0 | 1.4 x 10 | 0 |
| Box 2 | 4.8 x 106 | 0 | 8.5 x 106 | 0 | 1.6 x 10 | 0 |
| Mean | | 4.25 x 106 | 0 | 7.6 x 106 | 0 | 1.5 x 103 | 0 |
| Wine + P | Box 1 | 8.9 x 106 | 0 | 4.6 x 106 | 0 | 2.4 x 103 | 0 |
| Box 2 | 8.9 x 106 | 0 | 2.3 x 106 | 0 | 2.2 x 103 | 0 |
| Mean | | 8.9 x 106 | 0 | 3.45 x 106 | 0 | 2.3 x 103 | 0 |
| General Mean | | 1.75 x 107 | 0 | 4.97 x 106 | 0 | 3.96 x 104 | 0 |
| SD | | 2.44 x 102 | 0 | 2.06 x 102 | 0 | - | 0 |

*A =Wine simple, B = Wine + S. gabonensis, C = Wine + P*

It is illustrated in figure 3 that the total growth of microbes in the palm wine decreases as days went ahead irrespective of the preservative method used. However, conserving palm wine using Sacoglotis gabonesis drastically slows the development of microbes in the wine. Palm nut extract preservative method also makes microbes in the wine to grow slowly.

**Figure 3: Evolution of fungi/yeast in palm wine**

Figure 4 presents the evolution of fungi/yeast during the three days of the tests. From the results, fungi/yeast are abundant in palm wine when harvested from the palm tree. After a day of adding preservatives, the microbes are reduced. It resurfaces and starts growing after 2 days.

**Figure 4: Evolution of staphylococcus aureus in palm wine**

Salmonella and Shigella which were absent in the freshly harvested palm wine from its tree, appeared in the drink the next day. Farmers using *Sacoglotis gabonesis* to conserve the drink indirectly cultivate *staphylococcus aureus* as shown in results in figure 4.



**Figure 5: Spread samples of algae ready for the drier Source: Field Work**



**Figure 6: Identified Bacteria colonies after 24 hours of heating. Source: Field Work 2022**

# 4 CONCLUSIONS

The pH of palm wine decreases as time goes on, irrespective of the traditional method used in conserving, making the wine to contain more acid. Moreover, the idea of using traditional methods in conserving palm wine to keep its qualities stable has been proven contrary by results which showed that, regardless of the traditional method applied to the wine, microorganisms still find their way to develop themselves in the wine as days go by. It has also been shown that palm wine does not contain microorganisms like Coliforms, Salmonella + Shigella and Escherichia coli.

Palm wine is a natural alcoholic beverage that is drunk in its natural state immediately after it is tapped. In Tiko, South West Region, where the research was carried out, it is consumed by both the young and the elderly. Since it is tapped on a large scale, some of it is being transported to big cities like Douala where it is sold. Palm wine is consumed for different reasons by different people – as a thirst quencher for its nutritional content; in baby showers; during traditional wedding ceremonies; local Njangi Group meetings; as a stimulant; consumed for pleasure and others use it to prepare traditional concoctions. The majority of men like to consume palm wine when it has become acidic while women prefer to consume it at its early and fresh state, when it is still sweet. The storage of this liquor became a problem to the palm wine tappers because it yields a smaller income, making it difficult for them to buy the necessary equipment like refrigerators to store the palm wine, which will help reduce the acidity level and even inhibit the growth of microorganisms in the liquor. This difficulty made the palm wine tappers practice the use of traditional preservatives like the dry stem of *Sacoglotis gabonensis*; extract of *Eleais guinensis* (ripe palm nut fruits), which they believe will greatly influence the acidity level and microbial growth. The use of these traditional preservatives by palm wine tappers has no specific dosage. The quantities used are determined by each individual palm wine tapper.

During the research and analysis, the researcher came to discover that the use of traditional preservatives is just a fallacy since they do not attain their intended objectives. This is explained more by the fact that on day 2 of the analysis, the sample containing the extract of Eleais guinensis had the highest level of acidity, followed by sample A and lastly sample B. Day 3 still showed an increase in acidity in all three samples, respectively. For the microbial growth, coliform, E. coli and Salmonella sligella registered zero in all three days, while yeast/fungi, *Staphylococcus aureaus* featured in all three days of sample analysis for the three samples.

# 5 REFERENCES

[1] Olotu E.J., Oluyele O., Ojo O. R., Osinowo I. (2020), Therapeutic Potential of Palm Wine on Selected Diarrhoeagenic Bacteria. International Journal of Clinical Chemistry and Laboratory Medicine (IJCCLM), 6(1):17-24.

[2] Karamoko, D., Djeni, N.T., N’Guessan, K.F., Bouatenin, K., M.J. P. (2012). The biochemical and microbiological quality of palm wine samples produced at different periods during tapping and changes that occur during their storage food control, 2b, 504-511

[3] Lasekan O., Abbas K. A. Flavour chemistry of palm toddy and palm juice: a reviewTrends in Food Science & Technology Volume 21, Issue 10, October 2010, Pages 494-501

[4] FDA, Food and Drug Administration. Data on benzene in soft drinks and other beverages. Available online: http://www.fda.gov/Food/ (assessed on 12 December 2015).

[5] Ayernor, G.K.S. and Mathews, J.S. (1971) The Sap of the Palm Elaeis guineensis Jacq as Raw Material for Alcoholic Fermentation in Ghana. Tropical Science, 13, 71-83.

[6] Akinrotoye, Kehinde Peter. (2014), Effect of fermented palm wine on some diarrhoeagenic bacteria. Elite Research Journal of Biotechnology and Microbiology, 2(1): 4 – 14.

[7] Ogueri N. M. I, (2017) Molecular phytogenic of yeast from palm wine and enological potentials of the drink. Annual research and review in biology 2017, 20, 1-12, 10.9734/ARRB/2017/37748.

[8] Adedayo M. R., Ajiboye A. E. (2011), Antimicrobial property of palm wine. International Research Journal of Microbiology (IRJM), 2(8):265-269.

[9] Etejere H. K., Robinson, R., Olayinka E., Aderemi, R. (2017). Phytochemical analysis of aqueous extract and proximate composition of Chromolaena odorata. 232. 173-182.

[10] Ojo O.C., Agboola S. A. (2019), Antibacterial Effects of Palm Wine (Elaeis guineensis) on Salmonella typhi Isolated from Different Sources. International Journal of Pathogen Research, 2(4): 1-12.

[11] Ojimelukwe P. C., Okechi A. and Ojinnaka M. C. (2011). Physicochemical characteristics of fermenting castor seeds containing lime and NaCl as additives African Journal of Food Science Vol. 5(14), pp. 754-760, 30 November, 2011

[12] Bassir,O. (1998), Observation of the Fermentation of Palm wine. West African Journal Biology, Applied Chemistry.10: (2) 42 – 45.

[13] Okafor N. microbiology of Nigerian palm-wine with particular reference to nutrients. Journal of Applied Bacteriology 38(2): 81-5, 2017

[14] Hai A., Rambabu K., Al Dhaheri A S., Kurup S.S., Banat F. Tapping into Palm Sap: Insights into extraction practices, quality profiles. fermentation chemistry, and preservation techniques. Heliyon. 2024 Aug 2;10(15): e35611.

[15] Detto K.., Theodore N. D., Aboya Moroh J. C. (2016), Biochemical and Microbial Properties of Palm Wine: Effect of Tapping Length and Varietal Differences. Food and Nutrition Sciences, 7: 763-771.DOI: [10.4236/fns.2016.79077](https://doi.org/)

[16] Meera C., Satish Deokar, Nitin Deshmukh, et al. (2017), Study of Antibacterial Activity of Palm Wine against Bacterial Pathogens Isolated from the Clinical Samples. Int. J. Pharm. Sci. Rev. Res., 45(2):138-141.

[17] Ouoba, L., Kando, C., Parkouda, C., Sawadogo-Lingani, H., Diawara, B., 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, 1428–1441.

[18] Okerentugba P., and Ezeronye O.U. (2003) Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. Journal of Biotechnology},2003},2},288-292

[19] Olotu E.J., O. Oluyele, O. R. Ojo, I. Osinowo. (2020), Therapeutic Potential of Palm Wine on Selected Diarrhoeagenic Bacteria. International Journal of Clinical Chemistry and Laboratory Medicine (IJCCLM), 6(1):17-24.DOI: [10.20431/2455-7153.0601004](https://doi.org/)

[20] Gacheru P.K., Abong G.O., Okoth M.W, Lamuka P.O, Shibairo S.A and Katama C.K.M. African Crop Science Journal, Vol. 24, Issue Supplement s1, pp. 137 - 143

[21] Uwaezuoke J. C. and N. Ogbulie, “Antibiotic sensitivity pattern of urinary tract pathogens in Port-Harcourt, Nigeria,” Journal of Applied Sciences and Environmental Management, vol. 10, no. 3, pp. 103-107, 2006.

[22] Suzanne M K., Grischa B., Ariane C., Abdon A., Susanne F. (2020) Use and Cultural Significance of Raphia Palms. Economic Botany, 2020, 74 (2), pp.207-225.

[23] Ziadi, M., Gaabeb, N., Mrabet, A. and Ferchichi, A. (2014) Variation in physicochemical and microbiological characteristics of date palm sap (Phoenix dactylifera) during the tapping period in oasian International Food Research Journal 21(2): 561-567 (2014)

[24] Tankoano. A. G. F, and Savadogo A. (2016) African cassava Traditional Fermented Food: The Microorganism’s Contribution to their Nutritional and Safety Values-A Review International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 5 Number 10 (2016) pp. 664-687