PHYSICO-CHEMICAL PROPERTIES OF SOME PALM WINE SAMPLES OBTAIN FROM DIFFERENT LOCATIONS BENIN CITY

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CERTIFICATION

This is to certify that this project was carried out by OGHENEKEWE ISAAC (PSC1606960) of the Department of Chemistry, Faculty of Physical Science, University of Benin, Benin City.

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DEDICATION

This project is dedicated to the Almighty God for His infinite grace and favour upon me throughout the duration of this programme.

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None of my achievement would have been possible without ALMIGHTY GOD for His grace and protection during my four years sojourn in the university.

Special thanks go to my supervisor, Dr. E. N. Dibie, whose keen interest made this work a success.

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ABSTRACT

Palm wine is a beverage both alcoholic and non-alcoholic depending on whether fermentation has commerce. Some tropical plants including; date palm *(Phoenix dactylifera),* coconut palm (*cocos nucifera*), nipa palm (*nypa fruticans*), kithul palm and raffia palm (*Raphia*), and palm tree(*Elaeis guineensis*). The palm wine used was from freshly tapped palm tree (*Elaeis guineensis*), samples of which where obtained from Uhe and Ugbeghe both in Ikpoba-Okha Local Government Area in Edo. The samples were collected in clean sterile bottles and kept in a cooler containing ice block, and taken to the laboratory where selected physicochemical properties of the palm wine samples were examined. The examined physicochemical properties including; pH, specific gravity, alcohol levels, and mineral elements contents were investigated using standard methods. Qualitative analysis of alkaloids, saponnis, phenols**,** flavonoids, anthraquinone, terpenoids and Tanins in the palm wine studied was carried out using standard method. It was gathered from result that the pH values of the 4 samples ranges from 5.10 – 5.21, 4.18 – 4.04, 3.56 – 3.07, and 3.90 to 3.63 from 1 to 5 hrs interval. Specific gravity (NTU) were 1.20, 1.14, 1.23 and 1.96 Electrical conductivity (μScm^{-1}) were 0.36x104, 0.34x104, 0.31x104 and 0.33x104 in all samples. The alcohol content range were 3.7% - 3.2%., 4.3% - 3.9%, 4.9% - 4.5%, 5.3% - 4.9% and 5.9% - 5.3%. Findings also indicate the following respective values for the examined mineral elements; Ca [1.3-0.5], Zn [0.04-0.13], mg [1.58-1.51], Fe [0.37-0.41], Cu [0.15-0.08], K [1.46-1.42] ppm. It was also observed that the following phytochemicals were present in the studied palm wine alkaloids, saponnis, phenols**,** flavonoids, anthraquinone, terpenoids. However Tanins was not observed. From findings, it would appear that the consumption of palm wine will help meet the dietary needs in the examined nutritional induces.

CHAPTER ONE

1.1 INTRODUCTION

Human beings cannot survive without water for a long period of time, as normal functioning of the body requires a continual supply of fluid in various forms including beverages (Olufunke & Oluremi, 2015). Beverages are food items that are consumed in liquid state, but have lower food values relative to milk, and milk products. They are consumed either for their thirst quenching properties or for their stimulating effects. Two categories may readily be recognized: non-alcoholic and alcoholic beverage drinks (Omotoso *et al*., 2014).

1.1.1 Background of the problem

Africans have been making and imbibing alcoholic beverages from a wide array of fruits, grains, and other natural substances for as far back as the historical record goes, and continue to do so, ranging from palm-wine in coastal West and East Africa, banana beer in the Great Lakes region, to mead (tejj) in Ethiopia, and maize/sorghum beer across Southern Africa. In Nigeria, various types of alcoholic beverages are consumed ranging from beer to wine and spirit categories. Some of the alcoholic beverages traditionally produced include burukutu, pito, ogogoro, and palm wine (Dimelu, Agbo & Igbokwe, 2011). Palm wine is the collective name for a group of alcoholic beverages produced by the natural fermentation of the sap obtained from various tropical plants of the palmae family (Falegan and Akoja, 2014).

Indigenous people living in or close to swampy areas (such as the Ibos, Yoruba, Urhobos, Ijaws, and Itsekiris of Nigeria) prefer to tap their palm wine from raphia palm, while the Ibos prefer tapping their wine majorly from oil palm trees. The wine obtained from oil palm trees is called oil palm wine (OPW) while the palm wine obtained from raphia palm trees is called raphia palm wine (RPW) (Ikegwu, 2014). Fresh palm wine is sweet and contains little alcohol but, with fermentation, the alcohol content increases in time. Unbottled palm wine has lower alcohol content (around 3%) than bottled palm wine (around 4%). A litter of palm wine contains approximately 300 calories, 0.5-2.0 g of proteins, considerable amount of vitamins, a major component of which is vitamin A which helps to protect and improve consumer's eye sight (Amanchukwu *et al*., 2015). Raphia palm wine differs from oil palm wine in the following ways; it is sweeter and milkier, low alcohol level (intoxicating power), available all seasons and also relatively affordable (Aiyeloja *et al*., 2014).

It is commonly thought to be a very nourishing drink which greatly promotes lactation, the root and wine are used in local medicines as prevention and therapeutics for malaria fever, stomach pain and related diseases (Aiyeloja *et al*., 2014). Sweet taste is solely responsible for the acceptability of raphia palm wine among the female gender, hence it is preferred to other local wines (Aiyeloja *et* *al*., 2014). On the contrary, the ability to intoxicate due to the alcohol level accumulated during fermentation on exposure to air is one of the factors that attracts young people to oil palm wine (Aiyeloja *et al*., 2014). Oil palm wine is also erroneously consumed as energy drink particularly among the farmers (operating on local cutlass and hoe for land tilling) and artisans that engage in other energy sapping activities. Palm wine is enjoyed at birth celebrations, funeral ceremonies, and plays an integral role during traditional marriage ceremonies (Mbuagbaw and Noorduyn, 2012).

1.1.2 Statement of the Problem

The vast production and sale of the palm wine in different part of the country is not regulated and this makes the drink predisposed to food safety hazards which could be biological, chemical, physical, allergenic, nutritional or biotechnology-related. It has been reported that the drink can be contaminated at multiple steps in the production process and should be regulated for quality control to avoid health risks. In addition, plant material treated with herbicides and pesticides can be present in the palm wine processing environment, and it is not uncommon to see producers that use leaves to cover containers containing the drink. Leaves stem and bark can also get into the palm wine either when the incision is made to begin the tapping process or when the sap is flowing into a container. In some palm wine processing environments, insects could be a source of food safety hazards when they fly into bowls or containers containing the drink. A lot of characterization of palm wine has been carried out within and out the country, but it is difficult to identify research work done on the characterization of palm wine within this

1.1.3 Justification / Relevance of the Research Work

This study on the palm wine characterization will be relevant to the government, the general community and local consumers of palm wine, it will enable them to identify the constitute of the palm wine drink that they are consuming into their body.

1.1.4 Scope of the Work

The scope of the research was to determine the physiochemical properties of palm wine obtain from different two different communities in Ikpoba Local Government Are of Edo State, Nigeria.

1.1.5 Aim and Objectives of the Study

The aim of this research is to carryout characterization of locally tapped palm wine.

Objectives of the study

To achieve the aim of this study, the following objectives were set:

Obtain palm wine from different locations in Benin City.

Characterize the palm wine for the following physico-chemical properties:

pH Determination

Specific gravity

Heavy metal

Mineral content

Alcohol Content Determination

Phytochemical constituents including alkaloids, saponins, phenols, flavonoids, anthraquinone and saponins.

1.2 LITERATURE REVIEW

Palm wine is a whitish, effervescent, alcoholic beverage produced by the spontaneous yeast lactic acid fermentation of the sugary sap of palm trees. It can also be referred to as the collective name for a group of alcoholic beverages produced by the natural fermentation of the sap obtained from various tropical plants of the palmae family (Mavioga *et al*., 2009).

When palm wine is fresh it is sweet and refreshing because of the presence of sucrose but within 24 hours the concentration of the sucrose falls to less than 50% the initial amount. The sweetness is lost due to the spontaneous fermentation of its sugars which happens in a 24 hour period (Obire, 2005). Fermentation of the sap commences immediately by wild inoculum of yeast in the sap resulting into a milky-white product called palm wine that has increased microbial suspension caused by the prolific growth of the fermentation organism (Theophilus *et al*., 2015), thus the microorganisms in palm wine are alive when it is been consumed (Olawale *et al*., 2010).

Fresh palm wine is very sweet and refreshing because of the presence of sucrose, but within 24 hours the concentration of sucrose falls to less than 50% the initial amounts due to a rapid sugar fermentation by microorganisms. Fermentation virtually ends when the pH falls to 4.0; the whole process lasts about 48 hours resulting in an undesirable sour drink.

1.2.1 Tapping of palm wine

The palm sap is obtained through the process known as tapping, which involves a series of operations to stimulate the flow of sap, such as the perforation of the trunk, insertion of a tube in the hole and collection of the sap in a container (gourd, clay pot, plastic container, glass bottle or calabash) (Ouoba *et al*., 2012). There are diverse ways of tapping palm trees; they depend on the locality; but in general, two methods are practiced: in the first method the sap is obtained from a live standing tree, such as the Bandji and Toddy production, this process implicates climbing very tall palm trees, and perforate the trunk in the top of the tree for Bandji production (Mbuagbaw and Noorduyn, 2012), or cutting into the end of spadix from the tender inflorescence of the palm tree (inflorescence tapping) for Toddy production (Ouoba *et al.*, 2012; Mbuagbaw and Noorduyn, 2012).

In the second method the tree is felled or cut down before tapping (stem tapping), such as palm wine from Ghana and Taberna production. The cessation of the flow of palm sap varies according to the palm tree species and from tree to tree; for instance the shorter duration of tapping could be 2 weeks and the longest 8 weeks (Amoa-Awua *et al*., 2007; Santiago-Urbina *et al.*, 2013). Palm wine is collected twice a day, normally in the morning and the evening, it can be either immediately consumed or stored for later sale (Amoa-Awua *et al*., 2007; Naknean *et al.*, 2010; Karamoko *et al.*, 2012; Santiago-Urbina *et al*., 2013). Palm wine from Ghana is distilled for gin production called 'Akpeteshie'; similarly, Toddy is also distilled to produce the spirit known as Arrack (Amoa-Awua *et al*., 2007).

Tapping process from a live standing palm tree such as Bandji production from *Borassus akeassii*, has been reported that it is not significantly different of wine production from others types of palm trees where sap is collected from a live upright tree, as the palm wine from *Elaeis guineensis* produced in Ghana (Amoa-Awua *et al*., 2007).

1.2.3 Biochemical constituents of palm wine

The main characteristics of palm wine are whitish color, effervescent, sweet and acid taste. Palm wine is produced by natural lactic-alcoholic acetic fermentation of the sugary sap of palm tree (Amoa- Awua *et al*., 2007; Stringini *et al*., 2009;

Ouoba *et al*., 2012; Santiago-Urbina *et al*., 2013), it consist of an initial lactic acid fermentation, a middle alcoholic fermentation and a final acetic fermentation (Amoa-Awua *et al*., 2007). At each stage the microbial activity helping the activity of the microorganism in the next stage, i.e. members of the consortium communicative one another with trading metabolites. As a result each individual cell in the mixture responds to the presence of others in the consortium (Smid and Lacroix, 2013). An increase in the total acidity and decrease in the pH by the production of organic acids, probably enhance the growth and invertase activity of the yeasts (Naknean *et al*., 2010), and the ethanol produced by the yeasts serves as a substrate for the acetic acid production by the acetic acid bacteria (AmoaAwua *et al*., 2007).

The palm sap is transparent, with a sugar content in a range of 10-18% w/v approximately, which is mainly sucrose (Naknean *et al.*, 2010; Santiago-Urbina *et al.*, 2013), for example, Dibofori-Orji and Ali (2019) reported that the proportion of the sugars of the sap of *Phoenix dactylifera* consist of 95.27% of sucrose, 2.51% glucose and 1.61% fructose (dry matter basis). Palm sap has a pH near neutral, approximately 7 to 7.4; this value indicates the freshness of palm sap (Ezeagu *et al.*, 2003). During the tapping, palm sap changes the consistency and the color from transparent to whitish, due to the lactic acid bacteria produce a gum probably dextrans (Naknean *et al*., 2010). In addition a heavy suspension of yeast and bacteria also gives a milky-white appearance

(Lasekan *et al*., 2007). The composition of the palm wine depends of the state of the fermentation at which the wine is consumed (Dibofori-Orji and Ali, 2019).

1.2.4 Sugars identified and their concentrations in palm wine

In the first days of tapping the palm wine is very sugary and does not contain substantial concentration of alcohol. In Raphia palm wine, sucrose, maltose, glucose and fructose sugars were present in the first day of tapping (Ezeagu *et al*., 2003); while xylose and cellobiose were detected on the middle tapping period; and galacturonic acid, arabinose and rhamnose sugars appeared irregularly for a few days (Amoa-Awua *et al.*, 2007). The palm wine of *Borassus flabellifer* contains about to 9.29 to 17.44% of sucrose, glucose content between 0.50 and 1.85%, and fructose in a range of 0.50 and 1.81% (Naknean *et al*., 2010) in the first samples of tapping. On the other hand, in palm wine of *Elaeis guineensis* was found that the total sugars in the samples dropped from initial concentrations of about 14% to about 11% by the fourth day of tapping, and subsequently between 12% and 8%, this variation was observed during the 35 days of tapping period (Amoa-Awua *et al.*, 2007). This sugar concentration is maintained by the continual oozing of the sweet sap (Amoa-Awua *et al*., 2007). Meanwhile Karamoko *et al.* (2012) reported an initial concentration of total sugars of about 50% w/v, this sugar concentration decreased through the tapping process about 21% for the first week; then, 7.9%, 6.4% and 5.4% for the second, third and fourth week, respectively, in the palm wine of *Elaeis guineensis.*

The decrease in sugar content is a clear indication that a large portion of the sugars is fermented especially during the early stages of tapping. On the other hand, during the 15 days of tapping of *Acrocomia aculeata* was found an initial concentration of sucrose in the palm wine of 11.36%, this concentration dropped through-out the tapping process to 0.22%, as a result of the microbial metabolic activity (Santiago-Urbina *et al.*, 2013). In addition, this reduction in sugars can be also caused by the depletion of sugar reserve in the palm tree due to the fact that the trees are felled, and the leaves are cut off, hence the palm does not realize photosynthesis and does not produces sugar (Santiago-Urbina *et al.*, 2013). The variation in the sugar composition through the tapping process can be explained by different factors, such as different palm tree species, time of collection of the palm wine samples, different ways of make the tapping process.

1.2.5 pH and organic acids concentration in palm wine

Normally, natural palm sap shows approximately a neutral pH; however, in the first days of the tapping process, this value decreases between 5 and 4 and subsequently between 4 and 3 (Karamoko *et al.*, 2012). These changes on pH are due to the organic acids production as a result of the microbial metabolic activity. Lactic acid produced by the lactic acid bacteria has been reported as the main responsible for the acidic condition in palm wine (Ouoba *et al*., 2012; Santiago-Urbina *et al*., 2013). e.g. in the palm wine of *Elaeis guineensis* the percentage of lactic acid after the first few days of tapping was between 0.1 and 0.3% (Amoa-Awua *et al.*, 2007), similarly in the palm wine of *Acrocomia aculeata*, the lactic acid concentration varied in the range of 0.26 to 0.48%, through the tapping process (Santiago-Urbina *et al.*, 2013). This lactic acid concentration decreased the pH in the medium in an approximately 24 h and after that the pH is stabilized at 4 and 3 (Santiago-Urbina *et al*., 2013). The second organic acid produced in the palm wine is the acetic acid, with a concentration of about 0.02 to 0.4% (Ouoba *et al.*, 2012; Santiago-Urbina *et al*., 2013). According to Dibofori-Orji and Ali (2019), this acetic acid concentration in the palm wine are acceptable by the consumers but, when the concentration exceeds 0.6% the beverage becomes unacceptable. Thus, the acetic acid is considered as part of the aroma of palm wine (Amoa-Awua *et al*., 2007).

Moreover, in palm wine of *Elaeis guineensis* from Ivory Coast in addition to lactic (0.015-0.079%) and acetic (0.01-0.077%) acids, others organic acids have been reported such as oxalic (0.01-0.04%), citric (0.005-0.04%), tartaric (0.031-0.04%), malic (0.05-0.1%), ascorbic (0.005-0.024%) and fumaric (0.001-0.003%) acids (Karamoko *et al*., 2012). Lactic and acetic acids are produced throughout the tapping process by the lactic and acetic acid bacteria, respectively, however the tartaric and malic acids are considered as native to the exudates (Karamoko *et al.*, 2012).

pH of the analyzed palm wine samples ranged from 7.20 in RW2 to 7.40 in RW1. Normally, natural palm sap shows approximately a neutral pH; however these pH decreases with age due to the organic acids production as a result of metabolic activities (Ezeagu *et al*., 2003). The pH was between 7.2–7.4. Ezeagu and Fafunso (2003) observed that pH fell sharply after the first two days of storage and also that acidity was due to production of tartaric, acetic, and lactic acids. According to Tapsoba *et al*. (2014), the pH of different samples of palm wines collected from different producers (fresh sap) was 4.05 ± 0.61 to 4.90 ± 0.10. This result could be explained by the nature of the fermentation, the kind of palmyra and the sap extraction method.

1.2.6 Ethanol concentrations in palm wine

The concentration of ethanol in palm wine during the taping depends of several factors such as the presence of microorganisms responsible for the alcoholic fermentation, composition of sap, species of palm tree, environmental conditions e.g. temperature and velocity of the wind (Santiago-Urbina *et al*., 2013), type of tapping, flow rate of the sap, and the time in which the palm wine samples are taken, and time between collection and analysis of the samples. Thus, in palm wine of *Elaeis guineensis*, the ethanol concentration fluctuates, the palm wine collected between the day contains less alcohol (1.4% and 2.82%) than the palm wine which has been accumulated overnight (3.24% and 4.75% and even over 6% in few cases) (Amoa-Awua *et al*., 2007), it is probably due to the microbiota that has colonized the walls of the receptacle is removed during tapping in the morning, when the tapper cuts a thin slice off the walls of the receptacle or canoe, which reduces the microbial load (Amoa-Awua *et al*., 2007; Santiago-Urbina *et al.*, 2013) therefore reducing the ethanol production. On the other hand, the palm wine which is stored has higher levels of ethanol than palm wine recollected directly from the tree. e.g. Amoa-Awua *et al.* (2007) reported alcohol content about 8.16% in 24 h. Moreover, Dibofori-Orji and Ali (2019) reported a percentage of ethanol about 10.80% in 60 h of storage (*in vitro* fermentation); this concentration is higher than 4.78% ethanol content reported in an *in vivo* fermentation (Santiago-Urbina *et al.*, 2013). The variation aforementioned in the ethanol content is due to that in an *in vivo* fermentation there is a constant dilution of the metabolites produced as the flow of sap is accumulated in the receptacle or canoe, this process is considered as fed-batch fermentation (Santiago-Urbina *et al*., 2013).

The ethanol production is attributed to several microorganisms, such as *Saccharomyces cerevisiae* and *Saccharomyces chevalieri* (Ouoba *et al*., 2012). In addition, *Zymomonas mobilis* also have been reported as responsible in the ethanol production (Amoa-Awua *et al*., 2007). Alcohol contents falls between

2.10−2.30 (%v/v), in the two wine samples studied, there were slight difference in their alcoholic content with 0.10 mean deviation. It should be understood that the alcoholic content increases with the age of the palm wines (Santiago-Urbina *et al*., 2013).

Fresh palm sap usually contains no alcohol but levels could rise to 4.5–5.2gm/100 ml after 72 hours and may fall slightly after the fifth day of storage due probably to oxidation of alcohol by invading microorganisms (Ezeagu and Fafunso, 2003).

The values of alcohol content are lower than those obtained by Amoa-Awua *et al*. (2007) and Tapsoba *et al*. (2014). This result could be explained by several factors such as the nature of fermentation, the type and extraction period of sap, biodiversity of microflora, the collection time and the time between the collection of sap and analyzes However, the alcohol content of fresh sap are close to those obtained by Ouoba *et al*. (2012) that were 0.30 to 2.73% (v/v) and Amoa-Awua *et al*. (2007) between 1.4 and 2.82% during the first days of sap collection.

1.2.7 Odorants in palm wine

During the fermentation of palm wine, various organic acids and alcohols are produced due to microbial metabolic activity, and do not correspond to metabolites of the palm tree because the major components are not present in the fresh palm sap; all the metabolites play an important role in palm wine characteristic aroma. For instance, in the palm wine of *Elaeis guineensis* has been identified 73 compounds. There are 23 esters, 11 carbonyls, 14 alcohols and phenols, 10 acids, 5 sulphur compounds, 3 terpenes, 2 hydrocarbons, 2 acetals, 2 nitrogen compounds and 1 lactone. The higher alcohols and esters (more than 70% of total volatiles), as well as acids, aldehydes and ketones are the major groups of compounds found, and they are considered the main volatile components responsible for the palm wine aroma (Ukwuru and Awah, 2013). Similarly, Lasekan *et al.* (2007) reported that the volatile profile is largely dominated by alcoholic substances such as ethanol, 2-3-methylbutanol and 2-phenylethanol, as well as acetic acid. In addition, methyl butanoate, acetoin, diethyl succinate, ethyl lactate have also been reported, and several acids such as isobutanoic acid, 2-methyl butanoic acid, 3-methylpentanoic acid, phenylacetic acid and pentanoic acid. The most potent odorants in palm wine are earthy-smelling: 3-isobutyl- 2metoxipyrazine, buttery-smelling acetoin, fruity ethylhexanoate, 3-methylbutylacetate and popcornsmelling 2-acetyl-1-pyrroline. Furthermore, Dibofori-Orji and Ali (2019) identified the volatile compounds responsible for the aroma in fermented nipa sap (*Nypa fruticans*), which consists of alcohols such as ethanol, 1-propanol, 2-methylpropanol, 2-methylbutanol; acetoin, acetic acid, diacetyl, and esters such as ethyl acetate and ethyl lactate.

1.2.8 Specific gravity of palm wine

Specific gravity, also called relative density, ratio of the density of a substance to that of a standard substance. Specific gravity is the ratio of the density of a substance to the density of a reference substance; equivalently, it is the ratio of the mass of a substance to the mass of a reference substance for the same given volume. Apparent specific gravity is the ratio of the weight of a volume of the substance to the weight of an equal volume of the reference substance. The reference substance is nearly always water at its densest (4°C) for liquids; for gases it is air at room temperature (21°C). Nonetheless, the temperature and pressure must be specified for both the sample and the reference. Pressure is nearly always 1 atm (101.325 kPa). Temperatures for both sample and reference vary from industry to industry. In British beer brewing, the practice for specific gravity as specified above is to multiply it by 1000 (Vengaiah *et al.,* 2017).

Also the Specific gravity of the wine samples were observed to be the same (0.99), Turbidity ranged from (8.00−8.50NTU) with 0.25 mean deviation. This is a parameter that indicates the content or amount of suspended organic particles present in the palm wines (Singaravadivel *et al*., 2012).

1.2.9 Turbidity of palm wine

Turbidity is the amount of cloudiness in the water. This can vary from a river full of mud and silt where it would be impossible to see through the water (high turbidity), to a spring water which appears to be completely clear (low turbidity).Turbidity can be caused by : silt, sand and mud ; bacteria and other germs ; chemical precipitates (Ogueri and Itumoh, 2017).

Turbidity can be measured using either an electronic turbidity meter or a turbidity tube. Both methods have advantages and disadvantages, as shown below. Turbidity is usually measured in nephelometric turbidity units (NTU) or Jackson turbidity units (JTLJ), depending on the method used for measurement. The two units are roughly equal. However, according to Singaravadivel *et al.* (2012), the turbidity values of the samples were within the acceptable limit of fresh palm wines (7.5NTU).

1.2.10 Minerals and trace elements present in the palm wine

Macro and micro mineral elements content have been reported in the palm wine. significantly, Magnesium and Phosphorus were the most abundant minerals, in concentrations of 32.0 and 59.75 mg/L, respectively, in palm wine from *Elaeis guineensis* (Ezeagu *et al.*, 2003). Cadmium, Plumb and Cobalt were detected in low levels ≤ 0.1 ppm. Copper, Manganese, Zinc, and Calcium have also been reported in concentrations of 3.78, 2.63, 1.26, 1.95 and 0.48 mg/L, respectively

(Ezeagu *et al*., 2003). While, in palm wine from *Phoenix dactylifera* in addition to P and Mg (41.49 and 330 mg/100 g of dry matter basis, respectively) the Potassium is also reported (522.92 mg/100 g of dry matter basis), and it is the most abundant element. Others mineral elements, in decreasing order are Ca, Na, Fe, Cu, and Zn (Thabet *et al.*, 2009).

Ezeagu and Fafunso (2003) stated that the total nitrogen and free amino acids varied between 85.12–119.7 and 50.5–66.5 mg/100 ml, respectively. Mean protein content was 39.03 mg/100 ml. The proteins may have been produced by the plant and are being translocated in the palm sap. Occurrence of high protein levels, coupled with the oil and high sugar contents, could be of nutritional significance. The amino acids are probably of plant origin too, but microorganisms also may contribute to amino acid production. Mean lipid content was 62.65 mg/ml. *E. guineensis* is a well-known economic source of edible red palm oil, which is very rich in palmitic acid, a precursor of vitamin A. Ash content ranged from 0.12−0.21(g/100ml) of palm wine samples. This indicates the amount of inorganic residues in the palm wine samples, which invariably provides a measure of the total amount of minerals in the palm wines. However, the results were in close agreement with those of the works of Singaravadivel *et al*. (2012), (0.12g/100ml−0.38g/100ml) and Chandrasekhar *et al*. (2012), (0.11−0.41g/100ml).

Macro and micro mineral element contents are Magnesium and P (32.0 and 59.75mg/L, respectively) were the most abundant minerals. Cadmium, Pb, and Co occurred in very low levels (<0.1 ppm), though levels may vary widely with location. Some elements such as Pb, Cd, and Zn could act as cumulative poisons if long-term, low-level exposure and possible build-up to threshold levels occur (Onianwa *et al*., 2001). However such a situation usually is averted by the homeostatic mechanism of the body, except in disease conditions or where the exposure is too high for the body to handle. Palmwine usually is taken occasionally, although an average consumer may take up to 2.5 L at a time. Based on a 2.5 L daily consumption of palmwine, the potential intake of heavy metals was compared to the normal acceptable daily intake (ADI). Only Co and Cu (1.2–2.5 and 6.25–12.5 mg/2.5L, respectively) intake exceeded recommended normal ADI levels, even though these levels were lower than the toxic levels. Such occasional consumption of palmwine therefore is not considered hazardous.

Other constituents variously reported in palmwine include nitrates (18.16–91.26 mg/L), nitrite (0.00–2.81mg/L) dimethylamine (4.06–28.36 mg/L), (Ezeagu, 1995) and tyramine (11.27 mg/100 ml). Fresh palmwine is not ulcerrogenic as might be thought despite its content of 5% ethanol (Ezeagu and Fafunso, 2003). Some samples of fresh sap has a vitamin C content close to that obtained in Nigeria by Dioha *et al*. (2009) on the fresh sap of the raffia palm which averaged 8.8% and 9.01% (w/v). Freshly collected sap is an important source of sugars and vitamin C.

1.2.10 Health Concerns about palm wine

Obesity and Diabetes

Due to its sugary nature, palm wine may not be suitable for regular consumption by people that are obese or those living with diabetes. The sugar content is highest when the drink is freshly harvested but decreases as fermentation progresses (Ubi *et al*., 2017). It would be helpful if commercial producers of the drink can indicate the quantity and specific type of sugar in the processed product's label to enable consumers to make informed decisions on the quantity to consume in order not to exacerbate any underlying adverse condition (Ochuko *et al*., 2019).

1.1.11 Extraneous contaminants

The biggest health concern in palm wine consumption comes from intentional and unintentional contamination of the drink with extraneous materials, which are harmful to the human body. In communities with an abundance of palm trees, there is a steady supply of palm wine and little adulteration occurs. However, in places where the drink is scarce, it is common knowledge that some palm wine sellers try to boost yield by adding water to the drink and then supplement with artificial sweeteners to maintain the sugary taste. Unintentional contamination that occurs during processing has been summarized in a review (Ogueri and Itumoh, 2017), and it was emphasized that chemical contaminants associated with palm wine are potential food safety hazards. It was recommended that a detailed hazard analysis that covers palm wine processing and the supply chain would be beneficial to public health. More regulatory oversight was also suggested (Stephen, 2013).

Regulation for private consumption may be difficult to enforce hence it would be beneficial to enforce food safety standards on entrepreneurs that sell to the public. Presently the drink is sold in restaurants, roadside kiosks, and shacks. In many communities, the product is sold under a tree with a few benches and tables. Regulatory enforcement should cover all categories of sellers, no matter how small to help safeguard public health (Deborah *et al*., 2014).

1.2.12 Heavy metal contaminant in palm wine

Palm wine is essentially stored in plastic kegs, glass jars and calabash. One of the most frequent complaints of palm wine consumers is adulteration of the product by using water and artificial sweetness, which sometimes result in diarrhea, abdominal pains, and other stomach problems. The presence of metals in foods and beverages, except for accidental or criminal action reflects the environment. Water is the main component of beverages; as a consequence, the concentrations of metals in beverages may be related to the purity of the water of used in the production processes (Ukhun *et al*. 2005).

The content of metals in palm wine can be attributed to the natural sources, the atmospheric deposition of airborne particulate matter on palm trees and transfer of metals from the soil through roots to the palm trees and finally to the palm wine. Metals of primary natural origin comes from soil on which the oil palm tree is grown and reach the sap (wine) through roots and stems (Malu, 2014).

The concentration of primary metals is characteristic and comprises of the largest part of the total content in wine, it is connected with the maturity of oil palm tree, their variety, the type of soil in the plantation and the climatic conditions during growth. The contribution of metals of secondary origin is associated with external impurities that reach sap (wine) during growth of oil palm trees. During the growth of oil palm trees in a plantation, contaminations can be classified as geogenic (originating in the soil) from protection and growing practices, or from environmental pollution (Ismail, 2011). Differences in Cu, K, Ca, content can be due to fertilizers used for cultivation. Application of fertilizers, rodenticides, fungicides, and pesticides containing Cd, Cu, Mn, Pd and Zn compounds during growing season of plantation leads to increase in the amount of these metals in wine. Wine from plantations that are found close to the road traffic or located in industrial areas might contains higher levels of Cd and Pb because vehicles exhaust fumes or other emissions to air, water and soil.

Determination of other elements such as Pb, As, and Cd is of considerable importance due to their potential toxic effect. (Ape *et al*., 2015).

Ape *et al*. (2015) investigate the degree of contamination of toxic elements in industrial areas comparatively to non industrial areas with special interest to lead (Pb) and cadmium (Cd) which are hazardous to human health. The concentration of some heavy metals (K, Mg, Ca, Na, Fe, Zn, Mn, Cu, Cr, Co, Cd, Ni, and Pb) in palm wines obtained from two non- industrial sites (NIDS) and two industrial sites (IDS) in Enugu state Nigeria was determined using Atomic Absorption Spectrophotometer (AAS). The result showed the concentration of the metals from NIDS and IDS as follows: NIDS 1 and 2; Cr (0.01ppm, 0.03ppm), Cu (0.13ppm, 0.15ppm), Cd (ND), Fe (1.99ppm, 2.95ppm), Mn (0.21ppm, 0.17ppm), Ni (0.02ppm, ND), Pb (ND), Zn (0.89ppm, 0.24ppm), K (1.25ppm, 1.45ppm), Co (0.02ppm, 0.03ppm) Mg (0.97ppm, 0.79ppm), Ca (0.42ppm, 0.35ppm), Na (1.02ppm, 0.87ppm), while that of IDS 1 and 2; Cr (0.24ppm, 0.26ppm), Cu (0.45ppm, 0.36ppm) Cd (ND) Fe (2.98ppm, 2.75ppm), Mn (0.5ppm, 0.6ppm), Ni (0.02ppm, 0.01ppm), Pb (0.002ppm, ND), Zn (0.3ppm, 0.41ppm), K (2.05ppm, 1.99ppm), Co (0.04ppm, 0.02ppm), Mg (1.05ppm, 0.96ppm) Ca (0.93ppm) Na (1.62ppm, 1.53ppm). Comparison of the concentration of these heavy metals in the palm wine with those of the international/national standards of heavy metals for food, vegetables, cereals and drinking water shows that all metal were within allowable limit. Though Zn showed a little variation as it was higher in concentration in the NIDS 2 as compare to the IDS 1&2 even in NIDS 1, this variation however did not affect the standards for consumption since it is still within the permissible limit. This shows that the palm wine obtained from both non-industrial sites and industrial sites from oil palm trees is suitable for consumption.

1.2.13 Insect contamination and link with Nipah virus

After harvesting of palm wine, it is not uncommon to see dead flies or bees floating in the receptacle that was hung on the palm tree to collect the palm sap. There are concerns that pathogens on the dead insects, which may be harmful to humans may get into the palm wine. Other animals may also try to gain access to the palm wine during tapping. Most prominent is the case of Nipah viral disease, first reported in Malaysia in 1998 (Stephen, 2013). It is a zoonotic infection caused by Nipah virus, a paramyxovirus biosafety level-4 pathogen, belonging to the genus Henipavirus of the family Paramyxoviridae. Transmission to humans from the vector *Pteropus* spp, a fruit bat which harbours the virus has been demonstrated and fatalities in some Asian countries have been reported. During tapping of palm wine, it was found that the bat invades the palm wine receptacle on the tree to drink and pass excreta into the drink. Preventive control is the use of mesh and appropriate coverage to ensure insects and bats do not have access to the palm wine before, during or after tapping (Vikrant *et al*., 2018).

1.2.14 Preservation of palm wine

The greatest obstacle to the distribution of palm wine is the fact that once fermentation starts, it usually continues. The microorganisms utilize the sugars producing organic acids and alcohol and after a certain period, spoilage microorganisms render the wine undrinkable. It is therefore essential that proper hygienic collection procedures be employed to prevent contaminating bacteria from competing with the yeasts, producing acids instead of alcohol. The main control points are the extraction of a high yield of palm sap without excessive contamination by spoilage microorganisms and proper storage to allow natural fermentation to take place (Aniekpeno and Ojimeluke , 2013).

1.2.14.1 Refrigeration

With increasing industrialization and the availability of modern preservation methods, efforts were directed towards the use of chemical preservatives and other preservation methods known to be effective in food preservation. The earliest attempt made in this regard was to preserve the wine by refrigeration and pasteurization. Refrigeration was not very effective as microorganisms continued to grow even at refrigeration temperature (Uraih and Izuagbe, 1990). The method only retards microbial growth while the wine gets sour over time.

Besides, this method is very expensive and not readily affordable by over 98% of the low income populace in tropical countries (Agu *et al*., 1999).

1.2.14.2 Pasteurization

Okechukwu *et al.* (1984) used heat pasteurization at 68°C for 30 min to preserve palm wine without adverse effect on its flavour. However this method did not gain universal acceptance since a dark sediment formed at the bottom of the container. This problem was overcome by the addition of 70 mg/l of sodium metabisulphite before pasteurization.

1.2.14.3 Sulphite

Levi and Oruche (1957) tried unsuccessfully to preserve palm wine with sulphite. Faparusi (1969) reported the failure with sulphite as being due to unfavourable pH and the very high numbers of microorganisms. Sulphur dioxide (SO_2).

1.2.14.4 Fermenticide Powder

Busch's fermenticide powder containing benzoic acid, sodium benzoate and potassium or sodium bisulphate were also tried for palm wine preservation (Uriah and Izuagbe, 1990). The results obtained were unsatisfactory.

1.2.14.5 Diethylpyrocarbonate, sodium metabisulphite and sorbic acid

Diethylpyrocarbonate, sodium metabisulphite and sorbic acid were tested both individually and in combination with heat treatment. It was found that sorbic acid was the most effective. Metabisulphite has been reported to preserve the taste and flavour of palm wine (Aniekpeno and Ojimeluke, 2013).

1.2.14.6 Pasteurization in combination with sorbic acid

Pasteurization in combination with sorbic acid treatment was then recommended for preserving the wine since diethylpyrocarbonate was not suitable because the effective dose was above the safe level of 0.35 mg/kg of body weight (Mossel, 1971).

1.2.14.7 Centrifugation and pasteurization

A subsequent attempt was the use of centrifugation in addition to pasteurization at lower temperature (60°C), lower sorbate and metabisulphite concentrations (0.05%, w/v), to achieve virtually sterile palm wine (Uriah and Izuagbe, 1990). This method could keep the wine in a fresh and acceptable form for periods of between 6 and 9 months without formation of a deposit, but produced a clear liquid lacking the whitish colour of palm wine, thus unacceptable to the consumer (Okafor, 1974). The last attempt showed that pasteurization at 70°C for 40 min without chemical treatment preserved palm wine for longer than 9 months.

Such beverage possessed the three characteristic (fresh sugary taste, whitish colouration and vigorous effervescence) required of a good palm wine (Uraih and Izuagbe, 1990). This method was recommended for the palm wine bottling industry in Nigeria.

1.2.14.8 Bottling of Palm Wine

Currently, palm wine is bottled on a commercial scale in Nigeria at the Nigerian Institute of Industrial Research, Oshodi (FIIRO), Lagos and at the Nigerian Institute for Oil Palm Research (NIFOR) Benin. At NIFOR, the palm wine is first pooled, filtered through mesh to remove leaves, treated with 3.75 mg/l potassium metabishulphite, bottled and pasteurized at 65°C for 35 min (Uriah and Izuagbe, 1990). This wine has been found to have a shelf life of more than 6 months, although a dark sediment usually formed at the bottom after this period.

1.2.14.9 Preservation with Plant Materials

There is a drift towards the use of plant materials possessing antimicrobial properties in food preservation. Studies on the use of plant materials (functional foods) have thus increased with the view of incorporating them into food systems. Traditional attempts to preserve palm wine imply the introduction of natural preservatives mostly barks and leaves from edible or medicinal plants like *Vernonia amygdalina, Cymbopogon citratus,* etc. So, palm wine is supplied and drank with those preservatives inside. Due to their chemical composition, these preservatives offer chemical compounds (essential oils, alkaloids, phenolic compounds, etc.) preventing the microbial activity and therefore increasing the shelf life of palm wine (Udochukwu *et al.,* 2015).

1.2.14.10 Sacoglottis gabonensis

The preservative effect of *S. gabonensis* in palm wine is widely reported (Ojimelukwe, 2000). Maduka *et al*. (1999) has reported that is a palm wine additive in Southern Nigeria especially among the rural communities of Ngwa and Umuahia in Abia, Akwa Ibom, Cross River, Rivers, Bayelsa, Imo, and Delta States of Nigeria. The tree bark is known as “Nche” in Igbo, “Edat” or “Mkpaeto” in Akwa Ibom. The tree bark is beaten into mesh and placed inside the gourd before being hung for collection of the palm sap, or the pulverized dust is added to fresh palm wine. It has been reported that the tree bark extract delays souring of palm wine, lowers its titratable acidity (Ojimelukwe, 2000), and the palm wine becomes more alcoholic on standing (Morah, 1995). Okafor (1975a) suggested that the tree bark extract probably inhibited acid producing organisms. Ogan (pers; comm.) however suggested a probable depression of acidity by direct chemical action and not by the inhibition of bacteria growth. However, it has been shown that the tree bark extract of *S. gabonensis* inhibited the growth of certain bacteria isolated from palm wine such as *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. The inhibitory principle was found to be a phenol. Aniekpeno and Ojimeluke (2013) also reported that it inhibits the growth of *Sarcina lutea*. While investigating the scientific merits of the use of *S. gabonensis* in preserving palm wine, Ojimelukwe (2000) observed that the optimum concentration of this plant product is 0.625% (w/v). At this concentration the wine was still generally acceptable on the 4th day of storage when sensory evaluation was carried out. Any increase in the concentration of the additive beyond that reported, is considered a waste. Neither the tree bark of *S. gabonensis* nor a crystalline, C-glucoside, bergenin isolated from it, has been shown to possess any significant mammalian toxicity (Aniekpeno and Ojimeluke, 2013).

1.2.15 Nutritional importance of palm wine

Palm wine is a rich nutrient medium containing sugars, proteins, amino acids, alcohols and minerals. It contains a lot of water soluble vitamins (Ukhun *et al*., 2005). Ibegbulem *et al*. (2013) reported that it is a goodsource of vitamins B1 (thiamine) and C (ascorbic acid). According to the authors, palm wine yeast, *Saccharomyces cerevisiae*, is able to concentrate large quantities of thiamine, nicotinic acid and biotin and thus form enriched products. West African palm wine is particularly rich in vitamins B12, which is very important for people with low meat intake and those who subsist primarily on vegetarian diets. Since vitamin B- deficiency is widespread amongst pregnant women and teenagers in Nigeria, palm wine is considered an important additive to their diet (Ezeagu and Fafunso, 2003).

Palm sap contains many simple sugars such as sucrose, glucose, fructose and maltose (Ezeagu *et al*., 2003). Onianwa *et al*. (2001) reported the presence of raffinose in addition to amino acids and organic acids such as lactic acid, acetic and tartaric acids. Another report (Thabet *et al.*, 2009) indicated that oil palm sap contains 4.29% (w/v) sucrose and 3.31% (w/v) glucose while Singaravadivel *et al*. (2012) reported 3.92% (w/v) glucose and 9-11% (w/v) sucrose in raphia palm sap. Subsequently, Dioha *et al*. (2009) reported a total sugar content of about 10-12% in pure oil palm sap and an appreciable amount (0.35% , w/v) of raffinose. The authors also noted that sugar composition varies according to tapping conditions and is also influenced by the fact that as soon as the sap drips from the tree, it undergoes uncontrolled inoculation by the natural atmospheric flora of yeasts and bacteria.

Report shows that a litre of good portable palm wine provide approximately 300 kcal of energy from sugars and alcohol, 0.5-20 g protein and considerable vitamins for the consumer (Ezeagu *et al*., 2003). In addition, a maximum vitamin C content of 85 mg/l was obtained within the first 24 h of fermentation. Vitamin B2 (Riboflavin) increased from approximately 35 to 50 µg/l within the same period. Agu *et al*. (1999) reported that the protein content of palm wine ranges from 7.2 – 8.6% while the ash content ranges from 4.4 - 5.0%. The mineral content of the palm wine was 2.0 -2.5 mg/l iron, 0.18 - 0.19 g/l sodium, 0.10 - 0.13 g/l potassium, 0.12 – 0.16 g/l calcium and 6.2- 7.1 mg/l phosphorus.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

Beaker

pH meter

Hydrometer

I liter measuring cylinder

Alcohol meter

Test tube

2.2 REAGENTS USED

pH buffers

Hydrochloric acid

Wagner Reagent

Hager Reagents

Dragendorff Reagents

Mayer Reagent

Distilled water

Ferric chloride

Sodium hydroxide solution

Lead acetate solution

Chloroform

2.3 Sample Collection

Palm wine samples from palm tree will be freshly collected from the tappers at the point of tapping, from Uhe and Ugbeghe Ikpoba-Okha Local Government

Area of Edo. Two samples were collected from different palm trees from each communities making a total of four samples collected and they were. The samples were kept in clean sterile bottles properly labeled (AB, AC, AD and AE).

2.4 Methods

2.4.1 pH determination

This was carried out according to the method described by AOAC (1996). In particular, 20ml of each sample was measured using 50ml capacity measuring cylinder and transferred to a 100ml beaker. After calibration of the pH meter with pH buffers of 4.0 and 7.0, the pH electrode was dipped into the sample in a beaker and the value was recorded. The measurement was made at ambient temperature. Also the pH meter was recalibrated for its sensitivity after each measurement.

2.4.2 Specific gravity

The specific gravity was measured using a floating glass hydrometer that was sanitized and calibrated. The palm wine sample was placed in the hydrometer test jar and the instrument was spin to remove any bubble that might cling to it. With the sample at eye level, the reading was taken at where the liquid crosses the marks.

2.4.3 Alcohol content determination

Alcohol meter was dipped into the palm wine sample in a 250ml beaker and the lever of the pump was pressed to suck up alcohol into the portable meter. The reading was made using the display screen.

2.4.4 Conductivity

The conductivity meter was first standardized with the KCl solution. Afterwards, the electrode was rinsed with distilled water and cleaned with tissue paper. Thereafter, some of the palm wine was collected in a 10ml glass beaker and the electrode immersed in it. The samples were stirred with the electrode gently to prevent air bubbles from adhering to the electrode surface and the reading was taken at a stable value. The electrode was removed, dipped into distilled water and cleaned with tissue before immersing it into the palm wine samples again.

2.4.5 Phytochemical Analysis of Palm wine Samples

2.4.5.1 Test for alkaloids

The method of Odebiyi and Ramstard (1978) was used.

One (1) ml of hydrochloric acid was added to 3 ml of palm wine in a test tube. The mixture was heated for 20 mins, cooled and filtered.

i. Two (2) drops of Wagner Reagent was added to 1 ml of palm wine and observed for brown precipitate.

ii. Two (2) drops of Hager Reagents were added to 1 ml of the palm wine and observed for a yellow precipitate.

iii. Two (2) drops of Dragendorff Reagents was added to 2 ml of palm wine and observed for reddish brown precipitate.

iv. Two (2) drops Mayer Reagent were added to 2 ml of palm wine and observed for a milky Precipitate.

2.4.5.2 Test for Saponins

Three (3) ml of distilled water was added to 2 ml of palm wine in a test tube and was shaken vigorously for 2 minutes and observed for persistent foaming (Waterman, 1993).

2.4.5.3 Test for phenols

Ferric chloride test

To 2 ml of palm wine was added 3 ml of distilled water followed by 2 drops of 5% ferric chloride solution. Observe for the formation of intense coloration.

Alkaline reagent test

To 2 ml of the palm wine was added few drops of 20% sodium hydroxide solution followed by a few drops of dilute hydrochloric acid solution. Observe for the formation of intense yellow precipitate dissolve on addition of dilute acid.

Lead acetate test

To 2 ml of palm wine was added few drops of lead acetate solution. Observe for the formation of milky precipitate.

2.4.5.4 Test for flavonoids

To 4ml of palm wine, 2 ml of 10% NaOH was added and observed for yellow colouration.

2.4.5.5 Test for triterpenoids

Ten milligrams (10 mg) of the palm wine was dissolved in 1ml of chloroform, 1 ml of acetic anhydride were added followed by addition of 2 ml of Concentrated H_2SO_4. Formation of reddish-violet colour indicates the presence of triterpenoids.

2.4.5.6 Test for tannins

To 2 ml of the palm wine, 10 ml of distilled water was added and allowed to stand for 5 min.

i. To about 2 drops of the palm wine, ferric chloride ($FeCl_3$) solution was added; formation of a bluish precipitate is required for hydrolysable tannin.
ii. To about 5 drops of the palm wine, 2ml dilute HCl was added and allowed to stand for 5 minutes. Red precipitate was observed indicating positive.

2.4.5. 7 Test for anthraquinone

An aliquot (0.1 ml) of palm wine was measured into conical flask and 10 ml of 5% ferric chloride and 5 ml of hydrochloric acid was added. Absorbance concentration of the solution was measure at 515 nm using anthraquinone glycoside as reference standard (Harborne, 1998).

2.4.6 Determination of mineral elements contents

Mineral elements contents were analyzed in accordance with the atomic absorption spectrophotometric methods described by AOAC (1995). Remarkably, flame photometer was used to analyze for Sodium and Potassium. On the other hand, Calcium, Iron, Magnesium, Zinc and Copper were analyzed using Atomic Absorption Spectrophotometer.

2.4.7 Determination of heavy metals contents in the sample

20 ml aliquot of each of the bulk samples was measured using measuring cylinder (50 ml) and carefully transferred to a 250ml digestion flask. 10 ml of concentrated (stock) acid mixture (HNO_3/$HClO_4$) freshly prepare in the ratio of 3:1 was added to the flask. The flask was shaken slightly to mix the mixture, the flask with the acid-sample mixture was then placed on a heating mantle, the temperature was first set at 60°c and slightly increased to 180°C; the heating continued until the solution became clear and colourless. It was allowed to cool after switching off the heating mantle. The cooled digest was carefully filtered

and the filtrate was made up to 50ml volume with distilled water. Each of the digest was properly labeled and kept in a refrigerator until the time of analyses by atomic absorption spectrophotometer (AAS). Bulk scientific model 210 VEP (East Norwalk, USA) atomic absorption spectrophotometer was used to analyze the analyte metals in accordance with standard method of operation (ASTM, 1996).

CHAPTER THREE

3.1 RESULTS AND DISCUSSION

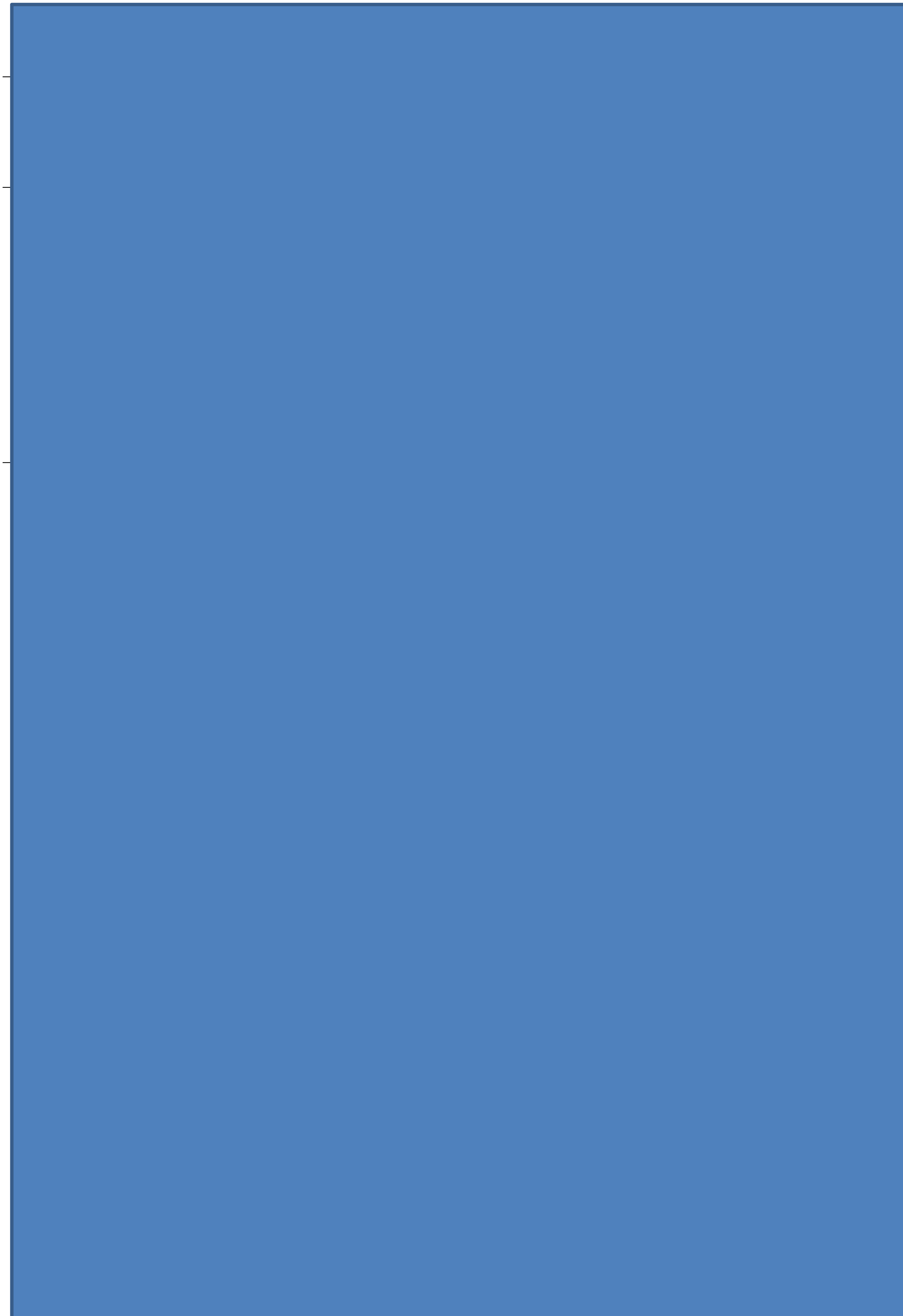
Palm wine is an abundant product in Nigeria. Some areas have palm wine from oil palm while others have it from raffia palm. Both palms produce characteristically unique palm wine with different organoleptic properties.

pH of palm wine

Table 3.1 shows pH values of four palm wine samples within different time intervals in the first 1 hour, the pH was within 5.10 – 5.21, while in 2 hours, the pH was 4.18 – 4.04, while 3 hours the pH value was 3.56 – 3.07, while 4 hours the pH value was within 3.90 to 3.63. The pH of the palm wine sap was observed to decrease from pH of 5 to pH of 3 across the four samples as a result of the continue fermentation of the occurring in the palm wine. This finding is similar with Tapsoba *et al*. (2014) who studied the impact of technological diagram on biochemical and microbiological quality of *Borassus akeassii* wine produced traditionally in Burkina Faso. They discovered that the pH of different samples of palm wines collected from different producers (fresh sap) was 4.05 ± 0.61 to 4.90 ± 0.10. These values are similar to our values. This result could be explained by the nature of the fermentation. Normally, natural palm sap shows approximately a neutral pH; however, in the first days of the tapping process, this value decreases between 5 and 4 and subsequently between 4 and 3 (Karamoko *et al*., 2012; Santiago-Urbina *et al*., 2013). Ezeagu *et al.* (2003)

stated that these changes on pH are due to the organic acids production as a result of the microbial metabolic activity.

Amoa-Awua *et al*. (2007) and Tapsoba *et al*. (2014) said that natural palm sap shows approximately a neutral pH; however these pH decreases with age due to the organic acids production as a result of metabolic activities. This could be explained by several factors such as the nature of fermentation, the type and extraction period of sap, biodiversity of microflora, the collection time and the time between the collections of sap and analyzes (Ouoba *et al*., 2012).



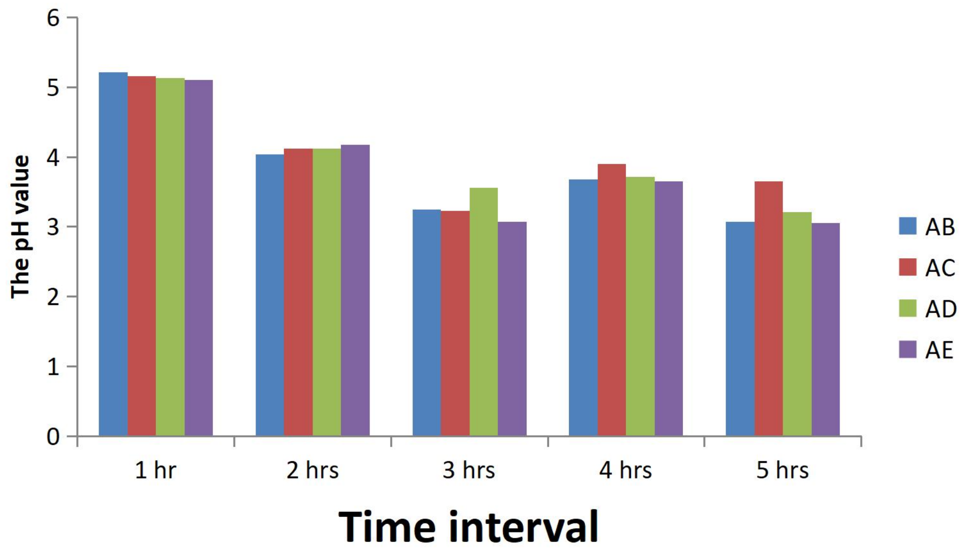


Figure 3.1: The pH value of palm wine samples tested at time intervals.

Mineral elements contents of palm wine

In Table 3.2, the mineral content of palm wine samples were analyzed, the following mineral was tested for which are potassium, copper, Iron, Magnesium, Zinc and Calcium.

Potassium content was 1.46 – 1.22. Magnesium content was 1.58 – 1.46, calcium content was 1.67 – 0.5, Zinc content was 1.35 – 0.04. Iron content was 1.02 – 0.29 and Copper content was 0.27 - 0.08.

In the findings of Ezeagu *et al*. (2003) reported that macro and micro mineral elements content have been reported in the palm wine. Where, Magnesium and Phosphorus were the most abundant minerals, in concentrations of 32.0 and 59.75 mg/L, respectively, in palm wine from *Elaeis guineensis*. Copper, Manganese, Zinc, and Calcium have also been reported in concentrations of 3.78, 2.63, 1.26, 1.95 and 0.48 mg/L, respectively (Ezeagu *et al*., 2003).

While, in palm wine from *Phoenix dactylifera* in addition to P and Mg (41.49 and 330 mg/100 g of dry matter basis, respectively) the Potassium is also reported (522.92 mg/100 g of dry matter basis), and it is the most abundant element. Others mineral elements, in decreasing order are Ca, Na, Fe, Cu, and Zn (Thabet *et al*., 2009).

Table 3.2: Mineral element content of palm wine samples (ppm)

	Palm wine Samples codes			
	AB	**AC**	**AD**	**AE**
Potassium	1.46	1.22	1.27	1.42
Copper	0.15	0.16	0.27	0.08
Iron	0.37	0.29	1.02	0.41
Magnesium	1.58	1.46	1.49	1.51
Zinc	0.04	0.14	1.35	0.13
Calcium	1.3	0.8	1.67	0.5

KEY:

AB: Palm Wine Sample 1　**AC: Palm Wine Sample 2**

AD: Palm Wine Sample 3　**AE: Palm Wine Sample 4**

Phytochemical Analysis of Palm wine Samples

Phytochemical analysis of palm wine samples showed the presence of saponin, phenols, flavonoids, triterpenoids and anthraquinone but tannins was absent in all the test samples. This finding is similar with Okwu and Nnamdi (2008) who investigated the phytochemical contents and medicinal values of *Dacroydes edulis* and *Raphia hookeri* exudates (palm wine), phytochemical screening of the sap showed that they contain bioactive compounds comprising saponins (2.08-3.98mg 100g-1), alkaloids (0.28-0.49 mg 100g-1), flavonoids (0.26-0.39 mg 100g-1), and phenolic compounds (0.01- 0.05 mg 100g-1).

Table 3.3: Phytochemical Analysis of Palm wine Samples

	Palm wine Samples codes			
	AB	**AC**	**AD**	**AE**
Alkaloid	+	+	+	+
Saponins	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Triterpenoids	+	+	+	+
Tannins	-	-	-	-
Anthraquinone	+	+	+	+

KEY:

+ = present;

- = absent

AB: Palm Wine Sample 1 AC: Palm Wine Sample 2

AD: Palm Wine Sample 3 AE: Palm Wine Sample 4

Specific gravity and Turbidity of palm wine

Specific gravity of palm wine samples across the four samples was within 1.96 – 1.14 and electrical conductivity was 0.36×10^4 – 0.3×10^4**.** Also Singaravadivel *et al*. (2012) reported the Specific gravity of the wine samples were observed to be the same (0.99), Turbidity ranged from (8.00−8.50NTU) with 0.25 mean deviation. This is a parameter that indicates the content or amount of suspended organic particles present in the palm wines

Also the Specific gravity of the wine samples were observed to be the same (0.99), This is a parameter that indicates the content or amount of suspended organic particles present in the palm wines (Ogueri and Itumoh, 2017).

Table3.4: Specific gravity, Alcohol content and electrical conductivity of palm wine samples

	Palm wine Sample codes			
	AB	**AC**	**AD**	**AE**
Specific gravity (NTU)	1.20	1.14	1.23	1.96
Electrical conductivity (μScm^{-1})	$0.36x10^4$	$0.34x10^4$	$0.31x10^4$	$0.33x10^4$

KEY:

AB: Palm Wine Sample 1 AC: Palm Wine Sample 2

AD: Palm Wine Sample 3 AE: Palm Wine Sample 4

Specific gravity of palm wine samples across the four samples was within 1.96 – 1.14 and electrical conductivity was $0.36x10^4$ μScm^{-1} – $0.3x10^4$. μScm^{-1}

Alcohol content

The alcohol content of fresh palm wine sap of four different samples shown in Table 3.5 indicate across the four samples. At the 1 hour the alcohol content of the palm wine samples was 3.7% - 3.2%. The 2 hours ranges from 4.3% - 3.9%. the 3 hours ranges from 4.9% - 4.5%. The 4 hour range from 5.3% to 4.9% and the 5 hour range from 5.9% to 5.3%. This indicate that as the storage time continues to increase there was an increase in the fermentation time of the four palm wine sample saps, which may be due to the presence of microorganisms.

Dioha *et al*. (2009) noted that the fresh sap of the raffia palm wine the alcohol content averaged 8.8% and 9.01% (w/v). Santiago-Urbina *et al*. (2013) stated that the alcohol contents of palm wine which was which is produced in the south east of Mexico falls between 2.10−2.30 (%v/v), in the two wine samples studied, there were slight difference in their alcoholic content. They noted that the alcoholic content increases with the age of the palm wines. However, the alcohol content of fresh sap are close to those obtained by Ouoba *et al*. (2012) that were 0.30 to 2.73% (v/v) and Amoa-Awua *et al*. (2007) between 1.4 and 2.82% during the first days of sap collection.

Table 3.5: The percentage (%) of alcohol content of palm wine samples tested at time intervals.

	Palm wine Samples codes			
Time interval	**AB**	**AC**	**AD**	**AE**
1 hr	3.2	3.7	3.5	3.7
2 hrs	3.9	4.2	4.0	4.3
3 hrs	4.5	4.9	4.7	4.9
4 hrs	4.9	5.2	5.1	5.3
5 hrs	5.3	5.7	5.8	5.9

KEY:

AB: Palm Wine Sample 1 **AC: Palm Wine Sample 2**

AD: Palm Wine Sample 3 **AE: Palm Wine Sample 4**

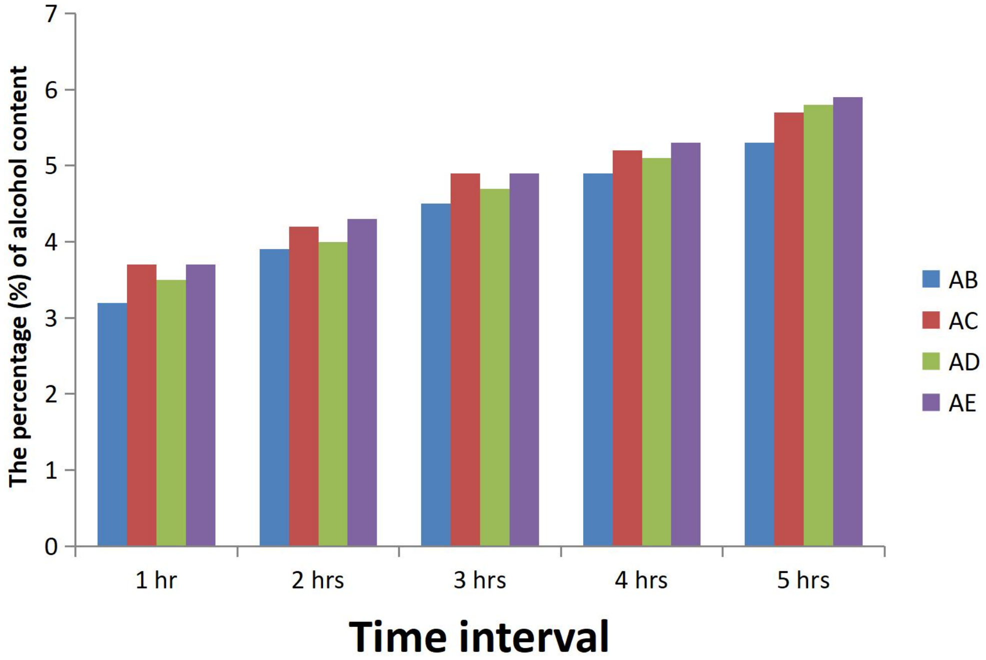


Figure 3.2: The percentage (%) of alcohol content of palm wine samples tested at time intervals.

Heavy metals concentrations of palm wine samples

The heavy metal concentration of palm wine samples was determined, Mn ranges from 0.13 – 0.10, Co was 0.03 – 0.02, Cr was 0.03 – 0.01. Ni was only present in sample AC and Ni was absent in all the test samples.

Ape *et al*. (2015) investigate the degree of contamination of toxic elements in industrial areas comparatively to non-industrial areas with special interest to lead (Pb) and cadmium (Cd) which are hazardous to human health. The concentration of some heavy metals (Mn, Cu, Cr, Co, Cd, Ni, and Pb) in palm wines obtained from two non- industrial sites (NIDS) and two industrial sites (IDS) in Enugu state Nigeria was determined using Atomic Absorption Spectrophotometer (AAS). The result showed the concentration of the metals from NIDS and IDS as follows: NIDS 1 and 2; Cr (0.01ppm, 0.03ppm), Cd (ND), Mn (0.21ppm, 0.17ppm), Ni (0.02ppm, ND), Pb (ND), Co (0.02ppm, 0.03ppm) while that of IDS 1 and 2; Cr (0.24ppm, 0.26ppm), Cd (ND), Mn (0.5ppm, 0.6ppm), Ni (0.02ppm, 0.01ppm), Pb (0.002ppm, ND), Co (0.04ppm, 0.02ppm). Comparison of the concentration of these heavy metals in the palm wine with those of the international/national standards of heavy metals for food, vegetables, cereals and drinking water shows that all metal were within allowable limit. This shows that the palm wine obtained from the four different locations from oil palm trees is suitable for consumption.

Malu (2014) stated that metals of primary natural origin comes from soil on which the oil palm tree is grown and reach the sap (wine) through roots and stems. Water is the main component of beverages; as a consequence, the concentrations of metals in beverages may be related to the purity of the water of used in the production processes (Ukhun *et al.* 2005). The contribution of metals of secondary origin is associated with external impurities that reach sap (wine) during growth of oil palm trees. During the growth of oil palm trees in a plantation, contaminations can be classified as geogenic (originating in the soil) from protection and growing practices, or from environmental pollution (Ismail, 2011).

Table 3.6: Heavy metal concentration in the palm wines (ppm)

Time interval	AB	AC	AD	AE
Mn	0.13	0.15	0.11	0.10
Co	0.02	0.02	0.02	0.03
Pb	-	-	-	-
Ni	-	0.02	-	-
Cr	0.03	0.01	0.02	0.01

KEY:

AB: Palm Wine Sample 1 **AC: Palm Wine Sample 2**

AD: Palm Wine Sample 3 **AE: Palm Wine Sample 4**

3.2 Conclusion

Findings has revealed in this work indicates that palm wine is rich in the examined phytochemicals and mineral elements. Therefore, the consumption of palm wine will help in meeting the nutritional requirements of the examined phytochemicals and mineral elements of consumers. It is also nutritionally desirable that the toxicologically relevant lead was not detected in the studied palm wine which suggests that consumption of palm wine will not lead to cases of lead (Pb) poisoning.

3.3 Findings

The following are findings from the study:

i. The pH of palm wine to decrease continuously at room temperature which indicate that when no preservative medium is added to it.
ii. The mineral content of palm wine samples were analyzed, the following mineral was tested for which are potassium, copper, Iron, Magnesium, Zinc and Calcium.
iii. Phytochemical analysis of palm wine samples showed the presence of saponin, phenols, flavonoids, triterpenoids and anthraquinone but tannins was absent in all the test samples.
iv. Specific gravity of palm wine samples across the four samples was within 1.96 – 1.14 and electrical conductivity was $0.36x10^4$ – $0.3x10^4$.

v. The alcohol contents of the palm wine sap indicated that as time increases there was an increase in the alcohol content in all the samples which is due to continuous microbial fermentation.

vi. Heavy metals such Mn, Co, Pb, Ni and Cr was determine and they were within the permissible limits and their sources of contamination are use of fertilizer, herbicide, pesticides and runoff water.

3.4 Contribution to knowledge

The study has contributed to knowledge in the following ways:

- The present study has provided practical result on the phytochemical analysis of palm wine samples showed the presence of saponin, phenols, flavonoids, triterpenoids and anthraquinone but tannins was absent in all the test samples.
- The pH of the palm wine sap was observed to decrease from pH of 5 to pH of 3 across the four samples as a result of the continue fermentation of the occurring in the palm wine.
- The heavy metal concentration of palm wine samples was determined, Mn, Co, Cr and Ni but Ni was only present in sample AC.
- The research have provided information on the mineral contents of palm wine, the following mineral was detected which are Potassium, Copper, Iron, Magnesium, Zinc and Calcium

3.5 Suggestion for further studies

- Due to the continuous fermentation of palm wine sap which lead to the increase of acidity content different perseverative methods should be investigated in order to increase the shelf life of palm wine.
- Palm wine are mainly stored in gallons and served with glass cups at point of sales which may lead to a source of contamination, commercial bottling and canning of this products that is widely consumed all over the country should be carried.

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