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EFFECT OF PALM-WINE ON COLIFORM BACTERIA

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ABSTRACT

The inhibiting property of palm wine on coliform bacteria was investigated. The inhibiting property of palm wine on coliform generally was confirmed. It was established that this property was neither due to the alcohol content nor to any biological agent. Faecal coliform was inhibited. Three samples of palm wine type were used. It was found that all the three samples of palm wine possess this inhibitory property.

Keywords: *Palm wine, faecal coliform, inhibition alcohol*

Introduction:

Palm wine has been defined as an alcoholic beverage produced from the sap of various palms and which contain heavy suspension of live yeasts and bacteria. These organisms give the drink milky - white appearance (Okafor, 1974). Faparusi (1973) defined palm wine as a fermented palm juice which is consumed mainly by the peasants in the Southern Region of Nigeria. This local traditional beverage is derived from two principal sources; the oil palm and the raphia, in Nigeria (Bassir, 1968).

Consumption of palm wine is not limited to Nigeria alone but also to other parts of the world such as Brazil, Nicaragua, Philippines, Middle East, Venezuela including Bay of Bengol (Okafor, 1972). These countries including some Africa countries derive their palm wine from various species of palm such as cocoa nucifera, date palm, *Corypha umbraculifera* L., *Raphia hookeri*, *Nypa fruitcans*, *wurmb* and *Movenia montana*. However, only some of these palms are really productive in relationship to palm wine. The species most associated with good yield of palm wine include *Eleais guinensis*, *Raphia vimfera* and *Raphis hookeri*.

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The commonest varieties is *Eleais guinensis* which is associated with production of palm wine in Nigeria are Dura, Macrocarya, Pisifera and Tenera (Bassir, 1968). Thus the quality of wine as indicated by the taste is highly variable depends among other factors on the genus of palm from which the sap is obtained (Okafor, 1972a).

The fresh palm juice which has not been fermented is whitish in appearance due to the presence of a suspension of yeast. The yeast cells of various types including *Saccharomycess cereviciae* are said to be very rich in high grade of protein, amino acids and vitamins B complex. Yeast which are among micro-organisms in palm juice anaerobically ferment sugar into alcohol (Okafor, 1974a).

Samarajeewa, U., Adams, M. R. & Robinson, J. M. (2008) reported that wine undergoes spontaneous two stages of fermentation. The first is the lactic acid fermentation and subsequently fermented by yeast to produce ethanol. Basir (1962b) also reported that fermentation of sugar by the yeast yield alcohol, which rises to its peak within 3 days with evolution of carbondioxide. The preliminary identification results of palm wine gives an idea of presence of Saccharomyces in frostily collected palm wine sample. The important organisms in palm wine responsible for alcoholic fermentation and for characteristic odorant production is *S. cerevisiae* (Olabisi, 2017).

Palm wine is usually sweetish and has a variable alcohol content of 0.5 – 7.1% by volume (Faparusi & Basir, 1971); (Van Pce and Swings, 1971) depending on a number of factors including the source of sap and the length of fermentation. At consumption, the most favour palm wine has a pH of 3-5 (Okafor, 1978).

Laboratory analysis has shown that palm wine no matter the origin contains varied chemical substance and micro-organisms. The chemical substance includes various classes of sugar, protein, lipid, vitamin, acid and alcohol.

Gradual changes occur in an unfermented palm wine as it is left to ferment. Between 24hrs of tapping, rapid biochemical changes take place (Faparusi 1968), Okafor (1975) stated that the original colourless of palm wine changes to milky-white condition as a result of microbial growth.

Changes in the composition of fermenting wine as a result of metabolic activities of micro-organism have also affected the taste. The wine usually turn sours within a short period due to the acid produced by the micro-organism Odunfa (1985).

Micro-organism mainly lactic acid bacteria have produced organic acid (Lactic Acid) which then increases in total acidity and decrease in pH value. Normally, natural palm wine showed neutral pH approximately 7 as reported by Adams and Moss (2010) and Lasekan, O., Ruettner, A. and Christbaure, M. (2011).

The extensive study on the change in palm wine have enable researchers to produce various products such as vinegar and locally made traditional gin called "Ogogoro". The product of distillation of palm wine is called "Kain-Kain" (Ogogoro) in Nigeria (Akinrele, 1968). Composition of fermenting palm wine changes as a result of metabolic activities of micro-organism which had given the wine another taste. After 48hours of fermenting, palm wine has sharp and bitter taste (Bassir, 1962). At this stage the taste is acidic and it contains various acids including acetic acid, malic acid, pyruvic acid, lactic acid and tartaric acid.

The shelf life of palm wine is about 48hours after which it is not fit for consumption. Many researchers have tried to find way of preserving palm wine so that its shelf life will be extended without loss of its natural qualities (Okafor, 1974). Various preservatives have been tried. Preservatives such as sodium metabisulphites, sorbic acid, benzoic acid and bark of *Sacoglottis gabonensis* extracted with water were used to extend the shelf life of palm wine.

Series of ways of preserving palm wine was unsuccessful but FIIRO has developed a method of preservation so as to extend the shelf life of palm wine to over 24months. This is bottling of pasteurized palm wine without lost of its natural characteristics. This goal was achieved by keeping bottle palm in a water bathe at a carefully controlled temperature which will deactivate the metabolism of the yeast.

Palm wine is a social drink which is consumed predominantly by villagers and the low income earners in the urban areas. Palm wine has become more important drinks in occasions such as wedding, burial and even for

birthday celebration. The importance of palm wine is well pronounced in Nigeria especially the Yoruba speaking tribe. In Yoruba tribe, palm wine is used culturally as a special drink during the celebration of "Ogun Festival". Furthermore, palm wine has been embraced by the institution of higher learning. It social acceptance has leads to formation of club such as palm wine drinkers club and Kegit Club by the students. In order to boost the production of palm wine and encourage people that are taking it, the Federal Government has replace the use of champaign in official parties with FIIRO palm wine (Onyekwe and Kokosho, 1981).

The importance of palm wine are many, apart from nutritive ingredient obtained from it, it has been known to act as an extractant of materials from the leaves, the root and certain stem of plant which are employed in the treatment of malaria. For example, farmer believe that the mixture of extract of the leaves of pawpaw plant (*Carica papaya*) are mixed with palm wine could be used to cure malaria. Okafor (1978) have worked on the bacterial floral of palm wine. He indicated that Gram positive bacteria are the predominantly and frequently occurring species. Only four Gram negative bacterial geneva had been isolated from palm wine. These are Acetobacter, Aerobacter, Serratia and Zymomonas.

Okafor, (1978) had acknowledged the dilution of palm wine, by middle men with water of dubious sanitary quality. The dilution is twofold; one with the tapper and the other with bar owner. The dilution is usually done in the rural area where there is no potable pipe-borne water. The dilution is done with river or well water that are bacteriologically polluted. He also acknowledged the inability of two gram negative enteric bacteria pathogen-Salmonella and Shigella to survive in palm wine. The report of Akinyanju and Oloruntoba (1986) also confirmed the Okafor observation and extend it in general to the coliform.

The coliform bacteria are among those that inhabit the intestines of humans and animals. They are gram negative short bacilli measuring some 2-5 micrometer x 0.4micrometers. They ferment lactose with acid and gas production in 48hrs at 37^0c. Member of this group include genera *Citrobacter*, *Escherichia*, *Enterobacter*, *Hafnia* and *Klebsiella*. The coliform can be differentiated into two groups, the non-faecal coliform and faecal

coliform on the basis of their ability to grow at elevated temperature. Both group grows at 37^0C but only the faecal coliform are able to grow at 44.5^0C. The two groups can also be differentiated by four biochemical tests which are indole, methyl red, voges Proskauer, Citrate (IMVIC) series. In bacteriology coliform presence is regarded as "presumptive" indicators of pollution and should be absent from disinfected water sample.

The enteric disease caused by coliform bacteria are transmitted almost exclusively by faecal contamination of water and food materials. Transmission through contaminated water samples is by far the most serious sources of infection and responsible for the massive epidemic of the more serious enteric disease (particularly typhoid fever and cholera) that periodically scourge all countries until the beginning of the present century. Today, these diseases are almost unknown in most parts of Western World. This is achieved through effective sanitary controls. *E. coli* inhabit the intestinal tract, and as such serve as principal indices of contamination of faecal origin. Though the *E. coli* itself is not an agents of disease.

Akinyanju (1985) reported that water from the shallow well are not all that safe for domestic use. He stated further that shallow wells were polluted and regarded as light sewage. He attributed the source of pollution to include human and animal wastes which littered the surrounding of the wells.

Okafor (1978) attributed the inability of the two Gram negative enteric pathogen – Salmonella and Shigella to survive due to increae in acidity and lowering of pH and the rising ethanol content. Akinyanju and Oloruntoba (1986) has got a contrary view. They were able to show that palm wine with neutral pH was capable of inhibiting coliform growth while the alcohol content in their sample did not rise up to 2.0%. Furthermore, they show that heated palm wine under steam lost its inhibiting properties but acidity and alcohol content were not altered. Thus the aim of the work is to ascertain the inhibitory properties of palm wine on coliform bacteria.

Materials and Methods

Palm Wine Sample: The diluted bar palm wine was obtained from the palm wine seller in Yoruba road Ilorin and we reached an agreement that no additive should be added and that she should supply us with fresh palm wine that she normally sold to her customers. We requested that she should supply a day old palm wine which have to be the same sample we got from fresh sample. Fresh palm wine was collected around 9-10am the following day. These samples were collected into sterile glass containers. The bottled palm wine was purchase from beer parlors in Ilorin.

Well Water Sample: Well water from a well which is about 3m deep and ascertained to have high coliform counts was used. The well is situated along Asa river side along Taiwo Road in Ilorin. The well water was collected employing a sterilized bottle weighted with a heavy metal. Well water obtained was used within 3-4 hours of collection.

Pure Culture of E. Coli: Pure Culture of E. Coli: which was faecal in origin was obtained from Baptist hospital Ogbomoso. This was transferred to the nutrient agar slants for preservation. This was used for subsequent experiment by taking inoculum of the bacteria using it to seed plate of nutrient agar and then incubated at 37^0c for 24hrs.

General Procedure: All the glass wares and the media used for the analysis were thoroughly washed with detergent and rinsed well with tap water. They were then autoclaved at $1kg/cm^3$ pressure at 121^0C for 15 minutes. The nutrient media and broth used were prepared according to directive from DIFN Manual or manufacturer directives.

Effect of Pure Ethanol on E. Coli

Five millitres of 24hrs old culture of *E. coli* suspension was added to 495ml of sterile distilled water. From this, 98ml of *E. coli* Solution (ESC) was added to 2ml of potable alcohol (PA). This thus represent 2% solution of ethanol 96ml of (ESC) plus 4ml (PA); 94ml of ESC plus 6ml of PA and 92ml plus 8ml of PA were prepared to represent 4, 6 and 8% solution of ethanol respectively. The control was also set up by using 2, 4, 6 8ml of sterile distilled water instead of potable alcohol. These solution were allowed a minimum contact time of 90 minutes.

To determine the effect of alcohol on *E. coli* cells were enumerated by the five-tube most Probable Number Procedure. Aliquots (10ml, 1ml and 0.1ml) of the *E. coli* cell solution prepare were seeded into quantuplicate tubes of 10ml double strength and two sets of 10ml of single strength Mac Conkey Broth. The tubes were incubated at 37^0C for 48hrs. Gas positive and negative tubes yielded the Most Probable Number (MPN) of *E. coli* cells.

Effect of Ethanol on Well Water Borne Coliform

Ninety eight millilitres of well water (ww) was added to 2ml of pure ethanol (PE) to represent 2% solution of ethanol. 96ml of (ww) plus 4ml of (PE): 94ml of ww plus 6ml of PE: 92ml of ww plus 8ml of PE were also prepared to represent 4, 6 and 8% ethanol solution respectively. The duplicate of each dilution were prepared but in this case sterile distilled water was used instead of ethanol as control. The dilutions were left for 90mins contact time before they were seeded into the Mac Conkey Broth medium to obtain the MPN of coliform cell.

Assay of inhibitory properties of palm wine on well water borne coliform

Fifty millitres of bar palm wine (PW) was mixed with fifty milliliter of (ww) that contained high coliform and faecal coliform count. This mixture was left for 90mins the recommended minimum contact time (Akinyanju and Oloruntoba, 1986).

The coliform content of palm wine/ well water (PW/WW) mixture and well water/sterile distilled water (WW/SDW) mixture were estimated using the five tube MPN procedure. In addition to this, bottled palm wine and a day old palm wine were subjected to the same procedure. Gas positive and negative tubes yielded were recorded.

Assay of inhibitory properties of dilute palm wine on well water borne faecal coliform:

One hundred milliliters of diluted palm wine was added to one hundred millilitres of well water (PW/WW). Also one hundred milliliter of palm wine was added to sterile distilled water (PW/SDW) and finally 100ml of well water was added to 100ml of sterile distilled water (WW/SDW). These mixture were left to observe the contact time of 90mins.

The coliform content of palm wine/well water (PW/WW); palm wine/sterile distilled water (PW/SDW) and well water/sterile distilled water (WW/SDW) mixture were estimated using the five tube MPN procedure. These tubes were incubated in the water bathe maintained at 44^0C for 48hrs. Gas positive tubes were recorded and the negative tubes yielded the MPN of the mixture at elevated temperature.

Results

Table 1

Effect of Ethanol on E. Coli

% of Alcohol used	Coliform count/100ml (ET/ECS)	Coliform count/100ml (SW/ECS) Control
2.0	2,400	2,400
4.0	2,400	2,400
6.0	2,400	2,400
8.0	2,400	2,400

Table I shows the effect of ethanol on the *E. coli* cell

It was observed that the coliform count of the well water was not reduced by the addition of ethanol. This result indicated that 8% ethanol solution was not capable of inhibiting coliform growth. Both experimental tubes and control have the same number of coliform count after incubation at 37^0C for 48hrs.

Table II

Effect of ethanol on well water borne coliform

% of Alcohol used	Coliform count/100ml (ET/WW)	Coliform count/100ml (SDW/WW)
2.0	2,400	2,400
4.0	2,400	2,400
6.0	2,400	2,400
8.0	2,400	2,400

Table II shows the effect of ethanol on well water borne coliform. It was observed that up to 8% solution of ethanol could not cause the inhibition

of coliform in well water. The control and the experimental reading have the same coliform counts after the incubation at 37^0C for 48hrs.

Table III

Assay of inhibitory properties of palm wine on well water borne coliform.

Type of Palm Wine Sample	Coliform count/100ml (PW/WW)	Coliform count/100ml (SDW/WW)
Bottled palm wine	7.0	2,400
A day old palm wine	6.0	2,400
Fresh palm wine	9.0	2,400

Table III shows the inhibitory properties of palm wine on water borne coliform. It was observed that all the palm wine types were effective in inhibiting coliform bacteria. There was a drastic reduction in the coliform count of the well water. The control experiment shows a high coliform count of 2,400 whereas the bottled palm wine, a day old palm wine and fresh palm wine shows 7, 6 and 9 coliform counts respectively.

Table IV

Assay of inhibitory properties of dilute palm wine on well water borne faecal coliform

Type of Palm Wine	Coliform count /100ml (SDW/PW)	Coliform count /100ml (PW/WW)	Coliform count /100ml (SWD/WW)
Diluted bar palm wine	7.0	17.0	2,400

Table IV shows that coliform count of well water with the addition of palm wine was drastically reduced. The bar palm wine itself was shown to contain low coliform count. It was also observed that well water has a high coliform counts which are mostly faecal in origin.

Discussion

Generally the initial findings were as follows:

Okafor (1978) was able to ascertain that the Gram negative bacteria could not survive in palm wine. This view was supported by Akinyanju and Oloruntoba (1986) which was the reason Salmonella and Shigella bacteria

were not found in palm wine despite dilution of palm wine with water of dubious sanitary quality by the middle men. The middle men do this to increase their profit.

Okafor also believed that the inhibition of these Gram negative bacteria was largely due to the increase in volume of alcohol produced by action of yeast in palm wine with increase acidity (i.e reduction in pH). The finding of Akinyanju and Oloruntoba was contrary. They were able to show that neutral palm wine with low volume of alcohol could perfectly inhibit coliform bacteria.

Basir (1972) reported that the fermentation of sugar in the palm wine by the yeast yield alcohol which rises to its peak within 3 days with evolution of carbondioxide which was also agreed upon by other researchers till today. With this, other researcher Van Pee and Swings (1971) reported that the content of alcohol in palm wine usually vary within 0.5-7.1% by volume. It could be observed however that the alcohol content of palm wine ranging from 0.5-7.1% by volume with reduction in pH which was attributed to the inhibition of Gram negative bacteria in palm wine as believed by Okafor (1978).

The finding in this work revealed that even alcohol of 8.0% by volume could not inhibit the coliform bacteria. Hence, rises in the alcohol volume was not responsible for coliform inhibition. This is evident in the result shown in table I and II respectively. Pure faecal coliform obtained from Baptist Medical Centre Ogbomoso and the well water (WW coliform) were not affected by the volume of alcohol (i.e 8.0% vol).

The three types of palm wine, bottled palm wine: fresh palm wine; and a day old palm wine were able to inhibit coliform bacteria. This shows the efficacy of palm wine of any type to act as inhibitor to coliform bacteria. This was evident in table III. Since it was believed that palm wine is further diluted by the middle men with water of dubious sanitary quality, the diluted palm wine was also used. It was evident in the result shown in table IV that diluted palm wine also has the ability to inhibit coliform bacteria.

In conclusion, it is likely that certain inhibitor is present in palm wine which inhibits coliform bacteria. Probably that is largely due to the reason that despite the fact of diluting palm wine with polluted water, there is no

major food poisoning that has ever been attributed to palm wine. Contact time with palm wine with polluted water by the tappers and sellers is far exceeding minimum contact time established by Akinyanju and Oloruntoba (1986).

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