Investigation of the Effects of Some Plant-Leaf Stem Preservatives on Palm Wine

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Abstract: A preliminary investigation was carried out on the effect of certain plant leaves; *Cola hispida, Mallotusoppositfoilus, Manihotesculenta* and *Newbouldia leavis* on palm wine during storage. The leaves are used by traditional palm wine tappers and retailers in the south eastern Nigeria for the preservation of palm wine. Palm wine from *Raphiaraphia* and *Elaensisguineensis* were used in this study. The effect of these leaves on ethanol levels, pH and total yeast count in these wines were monitored at 0,24,48 and 72 hours after taping. Results showed that the levels of ethanol and total yeast counts peaked at 24 hours for the treated samples while that of the control peaked at 48hours. The samples treated with *Manihotesculenta* leaves revealed much higher ethanol levels than the controls. The pH of the untreated control samples at 72 hours showed a higher acidity than that of the treated samples. ANOVA however showed no significant difference in the parameters monitored for treated and control samples during the period. P< 0.05.This study revealed that these leaves can be used to preserve the quality and extend the shelf life of palm wine from *Raphia raphia* and *Elaensis guineensis*

Keyword:

1. Introduction

Palm wine is a beverage produced by the fermentation of sugars present in sap of palm trees to ethanol and organic acids by yeasts and certain bacteria present (Nwachukwu[a] *et al*2006; Ogbulie *et al.* 2007; Chijioke and Ukaegbu –Obi 2014). These are wines obtained by methods previously described by Okafor (1977); Kovoor (1983); Essiamah (1992) and Gberikon *et al.*,(2016). These wines have been shown to contain nutritionally important compounds including amino acids, proteins, vitamins and sugars (Okafor 1987;Ogueri and Itumoh 2017). Although Ogueri and Itumoh (2017)revealed recently that palm wine have been shown to contain permissible level of chemical contaminants. The constituent nutrients however make palm wine a veritable medium for the growth of a consortium of microorganisms, whose activities rapidly change the physicochemical conditions of the wines, giving rise to competition and successions of microorganisms (Nwachukwu[b] *et al* 2006). These activities are responsible for the characteristic short shelf life and inability of the wine to keep for more than a day (Odunfa 1985), although differently aged palm wines have different flavours which individual consumers could classify as good or spoilt.

The organisms present in palm wine responsible for these conversions of sugars to pyruvate, acetaldehyde and then ethanol have been reported by Rassir (1962): Faparus; (1966); Okafor (1977) and Battok (1988).

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Nwachukwu[a] *et al.*,(2006) had further investigated physicochemical changes and successions of microorganisms form 0 hrs to 600hrs, for the raphia and oil palm wines.

In Africa the most popular palm wines are those obtained from Raphia species and *Elasensisguineensis* (Ibekwe at al 2006), where the wines are consumed by over 10 million people (ITD2003).It is expected to be served at every traditional event in south eastern Nigeria. Production and sale of this drink play a positive socioeconomic role in the lives of mostly, rural dwellers in southern Nigeria.

Different methods of palm wine preservation are practiced. The most common however is the use of plant leaves, barks, or roots. They are introduced whole or as grates into these wines. The aim generally is to increase the shelf life of the wines (high ethanol levels and acceptable organoleptic qualities) for as long as possible. Some of the plants in use in the South eastern Nigeria include *Cola hispida, Ficusspp, Mallotusoppositifolius, Manihotesculenta, Manniophytonflavum, Newbouidialeavis, Sacoglottisgabonensis, Milettiaspp, Costus afer, Vernoniaamygdalina*etc.

This practice underscores the need to investigate what effects these 'preservatives' may have on some characteristics of these wines. These could lead to development of standardized methods and large scale preservation of these wines avoiding the current spate of economic losses occasioned by the short shelf life of palm wines.

2.Materials and Methods

Collection of samples

Freshly taped palm wine from *Raphiaraphia (raphia palm tree)* and *Elaensisguineensis* (oil palm

tree) were obtained from traditional palm wine tappers at Mbaitoli and Ikeduru local government areas of Imo state Nigeria. The samples were collected and transported to the laboratory within one hour of taping in 2.5 litre sterile flasks for treatments and analyses. They were transported in coolers equipped with ice packs. This procedure kept the samples at about 2 - 4°C. (Ibekwe *et al.* 2006; Chijioke and Ukaegbu- Obi 2016).

The four plant leaf preservativesinvestigated are the leave and stem parts of *Cola hispida* (*Ohailu*), *Newbouldialeavis* (*Ogirishi*) *Mallotusoppositifolius* (*Kamala*) and *Manihotesculenta* (*Cassava*). They were obtained from Orodo, Mbaitoli LGA. The leaves were transported wrapped in dry cellulose papers and used fresh (within 1 hour of plucking). The plant materials were authenticated at Department of crop science, Federal University of Technology, Owerri, Nigeria. On arrival at the laboratory the plant materials were picked and washed with sterilized water and absolute alcohol respectively. The ethanol was subsequently rinsed of with the sterilized distilled water and the plant materials dried before use.

The individual cleaned plant materials were introduced into the wines whole, at 1 gram to 300mls of palm wine in dark brown conical flasks with perforated stopper plugged with sterile cotton wool. The wines were held at 25 - 27°C. Another quantity of 300mls of the same untreated wine was kept under the same conditions on the bench as control. The samples were investigated for changes in ethanol level (% v/v), pH and total yeast counts. Readings were taken at 0hrs, 24hrs,48hrs and 72 hours after collection. There were four replicates for each treatment.

Determination of levels of ethanol:

The levels of ethanol in the treated and controlsamples of the palm wines was monitored by the boiling/iodometric method used by Gwarr (1987) and Amadi et al (2015). Into a 50ml flat bottomed flask was added 10ml of deionized water, 10ml of 10% solution of $K_2Cr_2O_2$ and 10ml of 1:1 dilution of 98.5% conc H_2SO_4. The boiling flask is mounted and covered with aluminum foil carrying delivery glass tubing leading to a receiving tube (B). A low flame was ignited under flask A, so that the liquid in it boils with little frothing. Distillation was continued until about half of the contents of flask A distils into tube B. The contents of tube B were subsequently transferred to another flask and rinsed with deionized water to make up to 60ml in a comical flask. A 5ml quantity of KI (10%) solution was added and allowed to stand for2mins. The flask was subsequently cooled to room temperature and2.0ml of 0.04% starch solution added and thoroughly mixed. The mixture was titrated against 1.0m solution of $Na_2SO_3.5H_20$ from a burette until a blue coloration appears. This represented the blank titre. A standard curve was obtained by replacing the l0ml deionized water with 3,6,10, and 15 ml of 99.6% ethanol. The levels of ethanol were plotted with the titre values to obtain the standard curve. The ethanol values for the treated and control samples were thus obtained at the previously indicated intervals by reading off the standard curve. The method described by AOAC (1980) was used to determine pH of the samples in this study at the previously indicated intervals.

Determination of total yeast count

One ml of each appropriately aged serially diluted sample was platted out on properly labeled Saboraud Dextrose Agar and Nutrient Agar (Oxoid) plates. The media were supplanted with chloramphenicol at 0.05mg/L of media to discourage the growth of bacteria. Cultures were incubated aerobically at 28°C for 48hours. The colonies were counted and re isolated in pure cultures. Acceptable plates were those that contained between 30 – 300 CFU/ml as in Njoku et al (1990), Nwachukwua *et al*(2006) and Chijioke and Ukaegbu- Obi (2014).These processes were repeated at the intervals earlier indicated.

The data obtained was subjected to Analyses of Variance (ANOVA)

3. Results

The results of the effects of the plant leaf preservatives on the ethanol concentrations attained in the palm wines are shown on figures 1 and 2. There was a clear deviation in the ethanol content of the treated samples from the control. While the control wines attained the highest ethanol levels for this study at 72hours, the treated samples all attained their highest levels of ethanol at 24hours. The oil palm wine treated with the *Manihotesculenta* showed the highest level of ethanol in this study 9.4% (v/v). This was closely followed by 8.3% (v/v) recorded for the raphia wine treated with same leaf. At this age the control oil wine attained only 5.0% (v/v), while its raphia counterpart attained 3.2% (v/v) ethanol. The lowest ethanol content (2.3% (v/v) at 24 hours was recorded for the raphia wine treated with *Mallotusoppositifolius.* At 72 hours, the control shared the highest levels of ethanol, 7.3 and 5.8% (v/v) respectively for the oil and raphia wines. Amongst the treated samples only the raphia wine treated with *Cola hispida* still had any trace of ethanol 1.2% (v/v) after 72 hours.

ANOVA showed no significant difference between the treated and control samples at $P<0.05$

The pH of the wines also deviated from that of the controls with time. These are shown on figures 3 and 4. The trend however was towards lower pH values with time. The decline however was more pronounced with the control than with the treated samples. The lowest value recorded for the treated sample was pH5 recorded for the raphia wine treated with *New bouldialeavis* at 72hours. The pH of the control samples at 72 hours was 4.2 for the oil and raphia wines. The difference in pH with time between the treated and

untreated samples were not significantly different (P<0.05)

The yeast count of the treated and control samples with respect to age are shown on tables 1 and 2. There were variations between the treated and control samples with age. However, these values were not significantly different at P<0.05. The counts were equal at zero hours but ageing of the wines witnessed stronger deviations from the control. The treated samples attained their peak yeast counts at 24 hours whereas the control samples attained peak counts at 48hours. Subsequently, the counts declined.

4. Discussion of results

The results of ethanol levels attained with time shows that the leaves had an effect on the wines empirically. The treatments enabled the wines attain their highest ethanol levels within 24 hours of taping, as against the controls that attained their highest levels at 72 hours. The oil wines treated with the *Manihotesculenta* produced high levels of ethanol at 24hrs.A value higher, even than the controls at 72 hours. The results of the control agree with the results of Ibekwe *et al.* (2006) and Nwachukwu *et al.* (2006).

Ebuehi (2005) found cassava leaves to be rich in protein, fat, ascorbic acidand ash. His work also revealed presence of alkaloids, tannins, *anthroquinones, saponins,* reducing sugars etc. *Mallotusoppositifolius* has revealed the presence of alkaloids, flavoniods, phenols (Burkill 1994): *Newbouldia* leaves; contain terpenoids and derivatives, naphtaquinones, isocourmarins, lignins, and steroids (Bondyopadhyay et al 1999), while *Cola hispida* has also revealed caffeine, the obromine, kolatin and glucose content (Randy and Thydall 1997). The effects that these leaves have shown on the wines could be as a result of biologically active compounds from the leaves being extracted by the water and ethanol mixture in to the wines.

Some of these constituent compounds have biological and chemical actions ranging from inhibition of microorganisms, increasing amount of sugars available for fermentation, to enhancing the ethanol tolerance of the fermenting microorganisms in the wines. These could be related to the highest yeast counts for the treatments being obtained at 24 hours (rapid attainment of high cell mass compared to the control).

The pH values of the treated samples remained within the values of 6.8 and 5, 6.7 and 5.1 for *raphia* and oil wines respectively while the pH for the control declined to 4.2 for the same period. This suggests presence of a buffering component in the leaves or a product of their interaction with the wines.

This study revealed a decline in yeast viability after 24 and 48 hours for both treated and control samples respectively. This supports the findings of Chijioke and Ukaegbu-Obi (2014). This decline may have been a result of increase in ethanol concentration, lowering of pH, and accumulation of other bio active waste products produced by the resident microflora in the wine.

There was however no significant difference between the treatments and the control using 2 way analysis of variance (Nwachukwu and Egbulonu 2000) at P = 0.05. *Obire, (2005) indicated that preservation of palm wine* could be achieved by deactivation of the microorganisms at 15 min after tapping. A combination of this method and subsequent application of benign plant preservatives may enhance the shelf life of the wines

The results of this study have implications for the continued use of these plants as agents for increasing the shelf life of these wines

5. Conclusion

This study has shown that some local plants used in preservation of palm wine in South Eastern Nigeria have empirical positive effects on the shelf life of palm wine. The use of these benign plants in combination with other preservation methods may prove beneficial.

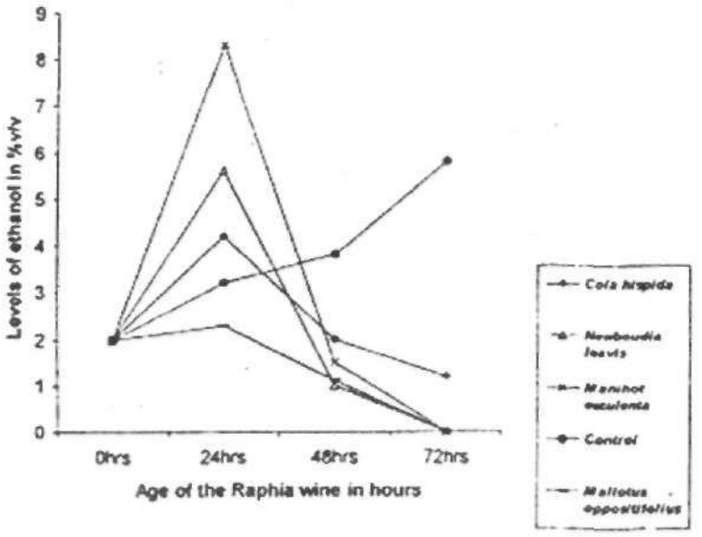

Fig. 1: Changes in the ethanol levels of the treated and control raphia palm wine samples with age

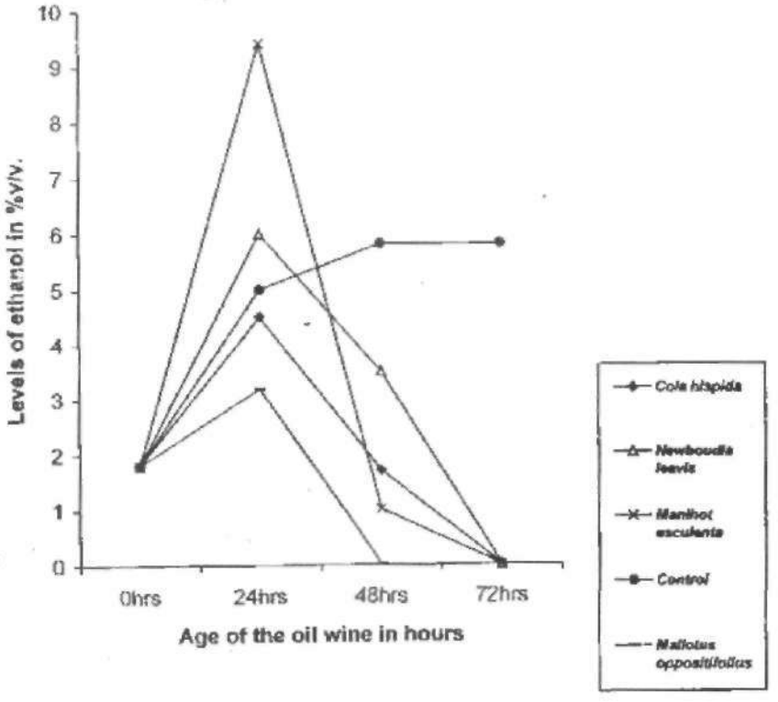

Fig 2: Changes in the ethanol levels of the treated and control oil palm wine samples with age

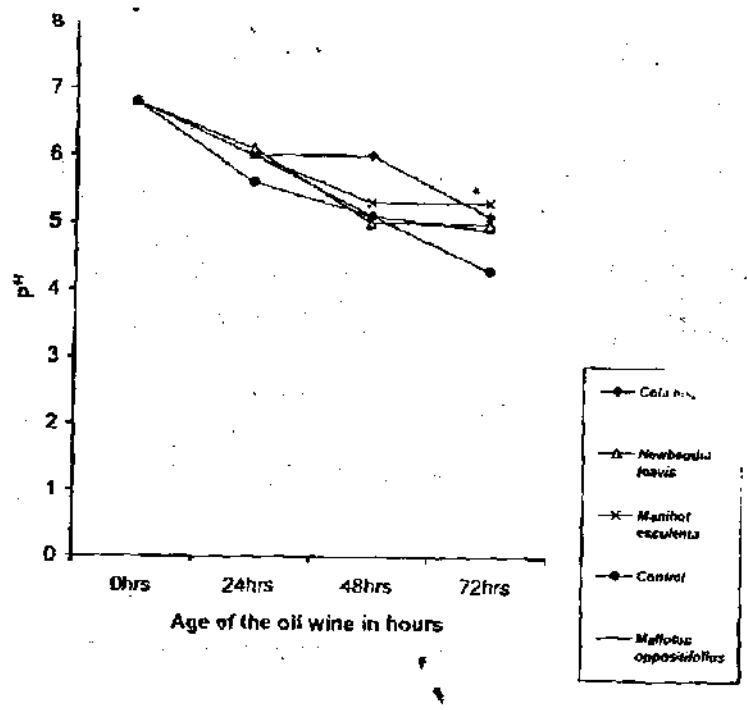

Fig 3: Changes in the pH of the treated and control raphia palm wine samples with age

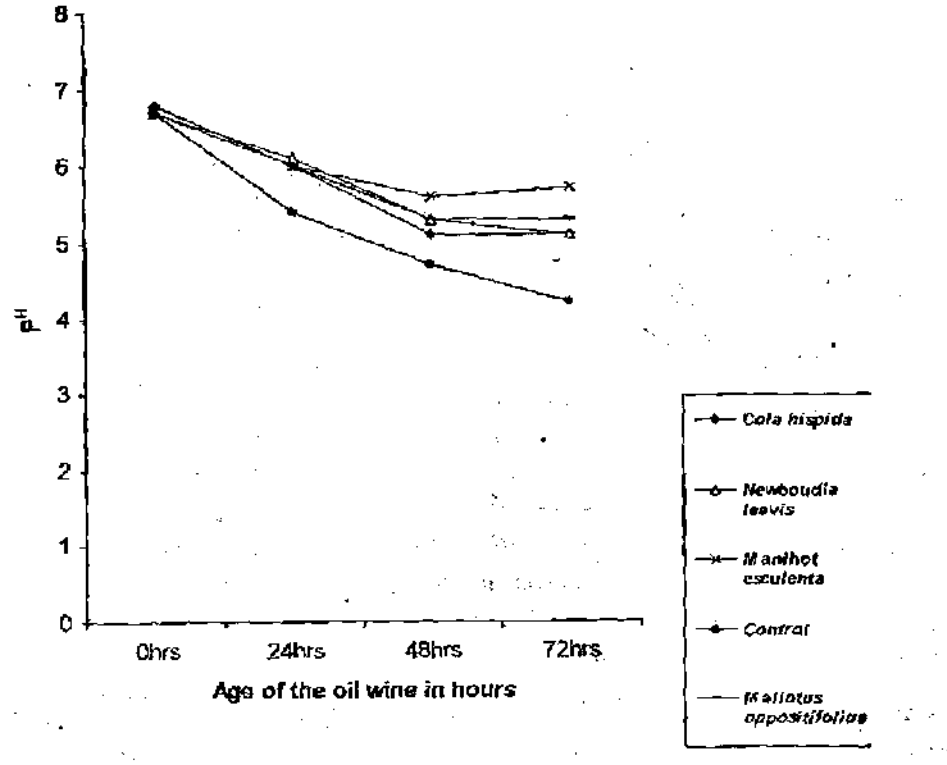

Fig 4: Changes in the pH of the treated and control oil palm wine samples with age

TABLE 1:Mean Yeast counts of the treated and untreated raphia palm wine with respect to age at 28 °C in Log_{10} CFU ML^{-1}

Age Hrs	Cola hispida	Newbouldia laevis	Mallotus oppositifolius	Manihot esculenta	Control
0	6.9	6.9	6.9	6.9	6.9
24	8.4	8.6	8.2	9.2	8.3
48	8.5	8.1	8.3	9.0	8.5
72	4.4	4.1	3.8	4.0	5.3

TABLE 2: Mean,Yeast counts of the treated and untreated oil palm wine with respect to age at 28 °C in Log_{10} CFU ML^{-1}

Age Hrs	Cola hispida	Newbouldia laevis	Mallotus oppositifolius	Manihot esculenta	Control
0	8.7	8.7	8.7	8.7	8.7
24	8.3	8.9	7.0	9.3	8.3
48	8.0	8.7	5.6	9.5	8.9
72	4.1	4.2	3.9	3.3	8.4

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