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ASSESSMENT OF THE PHYSICO-CHEMICAL PROPERTIES OF OIL PALM WINE (*Elaeis guineensis*) AND RAPHIA PALM WINE (*Raphia vinifera*)

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Abstract
This study was conducted to evaluate the physicochemical properties of oil palm wine (*Elaeis guineensis*) and Raphia palm wine (*Raphia vinifera*). Samples of fresh palm wine were collected from Ikwuano and Isiala Ngwa local government councils both in Abia state. They were stored in a cooling jar and were analyzed in their fresh state. Test for concentrations of pH, Ethanol concentration, Acetic Acid concentration, Lactic Acid concentration was initiated immediately and was repeated after 24 and 48 hours respectively. Results obtained were then subjected to two way completely randomized analysis of variance tests. From the study, it was observed that the results gotten vary from pH, Sugar, Lactic acid, Ethanol and Acetic of the oil palm wine and the raphia palm wine. Acetic acid concentration of *Ngwo* (Raffia) juice was 0.01 for 0 hour, 0.01 for 24 hours and 0.05 for 48 hours while that of *Nkwu* (Oil palm) juice was 0.01 for 0 hour, 0.04 for 24 hours and 0.04 for 48 hours. Effect of difference in plant, time and interaction was not significant ($P \geq 0.05$). From the result, there was a positive strong correlation between pH and Sugar, negative strong correlation between pH and Ethanol, weak correlation between pH and Acetic acid and negative strong correlation between pH and Lactic acid.

Keywords: Acetic acid, lactic acid, palm wine, pH, Sugar and Ethanol

Introduction

Palm wine is a whitish, sparkling, alcoholic drink produced by the impulsive yeast-lactic acid fermentation of the sugary juice of palm trees (Mavioga *et al*., 2009). The juice of the tropical plants of the Arecaceae family, when fermented, produces palm wine. The southeastern part of Nigerian produces and consumes it in large quantities. It is rich in nutritionally essential components which include vitamins, amino acids, sugars, and proteins (Okafor, 1987). These make this wine an authentic medium for the growth of a group of microbes, whose growth, causes a change in the physicochemical conditions of the wine, resulting in rivalry and successions of organisms. Palm wine is an alcoholic drink produced by natural fermentation of the juice of different palms, which include *Elias guineensis, Raphiaregalis, R. sudanica, R.vinifera*and *R. hookeri* (Obire, 2005). Raphia palm wine commonly referred to as 'Ogoro' is a traditional drink of the Yorubas in the Western part of Nigeria and other palm-growing countries. Palm wine is a whitish liquid, consumed throughout the tropics is consumed throughout the tropics (Uzochukuru *et al*., 1991). The presence of various microorganisms especially the bacteria and yeasts which are responsible for the fermentation of palm-wine has been reported by Bassir (1962), Faparusi (1966) and Okafor (1977). Palm wine is produced and consumed in very large quantities in the southeastern part of Nigeria. This makes the wine a veritable medium for the growth of a consortium of microorganisms, whose growth in turn, changes the physicochemical conditions of the wine, giving rise to competition and successions of organisms. During fermentation, the sugars in the palm-sap are metabolized to alcohol and organic acids which results to the sap losing its sweetness (Okafor, 1975). The type or nature of yeasts with leavening activity from palm wine samples from Abia State (Nigerian eastern region) may have peculiar characteristic information on physiochemical properties of palm-wine from Ikwuano/Ngwa and environs considering that this is not available; this information is required and may provide reason for yeast isolation and domesticating such yeasts for industrial use. This current study aimed at assessing the change in physiochemical of oil palm wine (*Elaeis guineensis*) and raphia palm wine (*Raphia vinifera*) properties over time, the degree of relationship between the physiochemical properties and difference between the physiochemical properties of oil palm wine (*Elaeis guineensis*) and raphia palm wine (*Raphia vinifera*).

Materials and Methods

Palm wine

Samples of freshly taped Palm wine were purchased from different locations in Ikwuano and Ngwa area in Abia State, South East Nigeria. Samples were set up on the day of purchase for physico-chemical analysis.

Physico-Chemical Analysis

Titratable acidity determination

10 ml aliquot of the sample solution was taken and titrated with 0.1 N NaOH using phenolphthalein solution as indicator. Titratable acidity was calculated as percent citric acid (AOAC, 2005).

TTA (%) = V × N × 0.0064/ Volume of sample analyzed

Where V = is the titre value obtained

N = is the normality of the titrant

Total sugars determination (brix^0)

The concentration of soluble sugars was determined using a handheld Bellingham and Stanley refractometer (Bellingham and Stanley limited, 61 Markfield Road, London, England) at 20 °C (AOAC, 2005). Few drops of each sample were placed on the surface of the lens and the lens covered. The sugar content was read from the digital scale directly.

pH determination

The pH of the wine was determined following the method described by AOAC (2005). The pH reading was done by direct measurement using an electronic pH meter. The pH meter was standardized with a buffer solution of pH value of four. The electrodes were dipped into the alcohol and the reading were allowed to stabilize and then recorded.

Acetic acid determination

50 ml aliquot of the wine was taken and titrated with 0.1 N NaOH using phenolphthalein solution as indicator. Titratable acidity was calculated as percent acetic acid (AOAC, 2005).

Acetic acid (%) = V × N × 6.004/ Volume of sample analyzed

Where V is the titre value obtained

N is the normality of the titrant

6.004 is the equivalent weight of acetic acid

Lactic acid determination

Ten ml aliquot of the wine was taken and titrated with 0.1 N NaOH using phenolphthalein solution as indicator. Titratable acidity was calculated as percent acetic acid (AOAC, 2005).

Acetic acid (%) = V × N 0.98/ Volume of sample analyzed

Where V is the titre value obtained

N is the normality of the titrant

0.98 is the equivalent weight of lactic acid × normality of the titrant × 100

Determination of ethanol content

A 50.0 ml sample of wine was added to a distillation flask and made just alkaline with 0.1 N NaOH. The sample was distilled until the temperature of the distillate reached 100°C or until half the original volume had been distilled. The distillate was diluted with distilled water to exactly 50.0ml in a pre-weighed pycnometer bottle. The pycnometer bottle and liquid were then weighed and the density of the solution calculated. A series of five standard solutions of ethanol in distilled water was prepared covering the range 5-25 per cent (V/V) ethanol. The densities of these solutions were determined by weighing accurately, known volumes and a calibration curve of density versus ethanol concentration plotted was then used to determine the concentration of ethanol in the wine.

Statistical analysis and data presentation

Data collected was subjected to analysis of variance test to compare the effect of difference in time on the physiochemical properties. Correlation analysis was used to check the relationship between physiochemical properties. Difference in the physiochemical properties of palm wine was done using student t-test. All analysis was done using Genstat 12 statistical package. Data analysed was presented in tables.

Results and Discussion

Results

Table 1 shows the analysis of variance of the acetic acid concentration of palm wine. From the results, mean Acetic acid concentration of *Ngwo* juice was 0.01 for 0 hour, 0.01 for 24hours and 0.05 for 48 hours while that of *Nkwu* juice was 0.01 for 0 hour, 0.04 for 24 hours and 0.04 for 48 hours. Effect of difference in plant, time and interaction was not significant (P ≥ 0.05).

Table 1: Analysis of variance of the acetic acid concentration (%) of palm wine

	0 hour	24hours	48hours	Mean total	SEM
Ngwo	0.01	0.01	0.05	0.02	0.01
Nkwu	0.01	0.04	0.04	0.03	0.01
Mean total	0.1	0.52	0.06	0.20	

Nkwu (Elaeis guineensis) = Oil palm wine
Ngwo (Raffia hookeri) = Raffia palm wine
LSD 0.05 (plant) = 0.88NS
LSD 0.05 (Time) =0.989NS
LSD 0.05 (Interaction) =1.399NS

Table 2: shows the analysis of variance of the Ethanol concentration of palm wine. From the results, mean Ethanol concentration of *Ngwo* juice were 0.00 for 0 hour, 1.06 for 24hours and 4.45 for 48 hours while that of *Nkwu* juice was 0.00 for 0 hour, 2.25 for 24 hours and 4.20 for 48 hours. Effect of difference in plant and interaction was not significant. ($P \geq 0.05$) but was significant in time ($p \leq 0.05$)

Table 2: Analysis of variance of the Ethanol concentration of palm wine

	0 hour	24hours	48hours	Mean total	SEM
Ngwo	0.00	1.06	4.45	1.84	1.34
Nkwu	0.00	2.25	4.20	2.15	1.21
Mean total	0.00	1.66	4.33	1.99	

Nkwu (Elaeis guineensis) = Oil palm wine
Ngwo (Raffia hookeri) = Raffia palm wine
LSD $_{0.05}$ (plant) = 0.925^{NS}
LSD $_{0.05}$ (Time) =1.133***
LSD $_{0.05}$ (Interaction) =1.602^{NS}

Table 3 shows the analysis of variance of the Lactic acid concentration of palm wine. From the results, mean Lactic acid concentration of *Ngwo* juice where 0.04 for 0 hour, 0.09 for 24 hours and 0.17 for 48 hours while that of *Nkwu* juice was 0.06 for 0 hour, 0.11 for 24 hours and 0.21 for 48 hours. Effect of difference in wine and time was significant ($p \leq 0.05$), but the interaction was not significant ($p \geq 0.05$).

Table 3: Analysis of variance of the Lactic acid concentration of palm wine

	0 hour	24hours	48hours	Mean total	SEM
Ngwo	0.04	0.09	0.17	0.10	1.34
Nkwu	0.06	0.11	0.21	0.13	0.04
Mean total	0.05	0.10	0.19	0.12	

Nkwu (Elaeis guineensis) = Oil palm wine
Ngwo (Raffia hookeri) = Raffia palm wine
LSD $_{0.05}$ (wine) = 0.01631***
LSD $_{0.05}$ (Time) =0.01998***
LSD $_{0.05}$ (Interaction) =0.02825^{NS}

Table 4: shows the analysis of variance of the sugar concentration of palm wine. From the results, mean sugar concentration of *Ngwo* juice were 15.85 for 0 hour, 13.05 for 24hours and 7.35 for 48 hours. *Nkwu* juice was 14.33 for 0 hour, 11.00 for 24 hours and 5.50 for 48 hours. Effect of difference in plant and time was significant ($p \leq 0.05$), but the interaction was not significant ($p \geq 0.05$).

Table 4: Analysis of variance of the sugar concentration of palm wine

	0 hour	24hours	48hours	Total mean	SEM
Ngwo	15.85	13.05	7.35	12.08	2.50
Nkwu	14.33	11.00	5.50	10.28	2.58
Total mean	15.09	12.03	6.43	11.18	

Nkwu (Elaeis guineensis) = Oil palm wine
Ngwo (Raffia hookeri) = Raffia palm wine
LSD 0.05 (plant) = 0.1240 ***
LSD 0.05 (Time) =0.1519***
LSD 0.05 (Interaction) =0.2148NS

Table 5 shows the analysis of variance of the pH concentration of palm wine. From the results, mean pH concentration of *Ngwo* juice were 4.25 for 0 hour, 3.94 for 24hours and 3.39 for 48 hours while that of *Nkwu* juice was 4.06 for 0 hour, 3.67 for 24 hours and 3.20 for 48 hours. Low pH favours wine production as it inhibits the growth of contaminating microorganisms, while favouring the growth of yeasts. Effect of difference in plant and time was significant ($p \leq 0.05$), but the interaction effect was not significant ($p \geq 0.05$).

Table 5: Analysis of variance of the pH concentration of palm wine

	0 hour	24hours	48hours	Total mean	SEM
Ngwo	4.25	3.94	3.39	3.86	0.25
Nkwu	4.06	3.67	3.20	3.64	0.25
Total mean	4.16	3.81	3.29	3.75	

Nkwu (Elaeis guineensis) = Oil palm wine
Ngwo (Raffia hookeri) = Raffia palm wine
LSD $_{0.05}$ (plant) = 0.03079***
LSD $_{0.05}$ (Time) =0.03771***
LSD $_{0.05}$ (Interaction)=0.05333^{NS}

Table 6: shows the correlation analysis from physicochemical properties of Raphia palm wine. From the result, there was a positive strong correlation between pH and Sugar, negative strong correlation between pH and Ethanol, weak correlation between pH and Acetic acid and negative strong correlation between pH and Lactic acid. Sugar had negative strong correlation with Ethanol, weak correlation between Sugar and Acetic acid and negative strong correlation between Sugar and Lactic acid. Ethanol had negative correlation with Acetic acid and negative strong correlation with Lactic acid. Acetic acid had negative strong correlation with Lactic acid.

Table 6: Correlation analysis of physicochemical properties of Raphia palm wine

	pH	Sugar	Ethanol	Acetic Acid	Lactic Acid
pH	1	0.998**	-0.940**	0.079	-0.993**
Sugar		1	-0.940**	0.093	-0.993**
Ethanol			1	-0.383	0.896*
Acetic Acid				1	0.023
Lactic Acid					1

Nkwu (Elaeis guineensis) = Oil palm wine
Ngwo (Raffia hookeri) = Raffia palm wine
*****. Correlation is significant at the 0.01 level (2-tailed).***
****. Correlation is significant at the 0.05 level (2-tailed).***

Table 7: shows the correlation analysis from physicochemical properties Oil palm wine. From the result, there was a positive strong correlation with between pH and sugar, negative strong correlation with Ethanol, negative strong correlation with Acetic and negative strong correlation with Lactic. Sugar had negative strong correlation with Ethanol, negative strong correlation with Acetic acid and negative strong correlation between Sugar and Lactic acid. Ethanol had a negative strong correlation with Acetic acid and a negative strong correlation with Acetic acid. Acetic acid had a positive strong correlation with Lactic acid.

Table 7: Correlation analysis of physicochemical properties of Oil palm wine

	pH	Sugar	Ethanol	Acetic Acid	Lactic Acid
pH	1	0.996**	-0.994**	-0.956**	-0.971**
Sugar		1	-0.982**	-0.955**	-0.984**
Ethanol			1	0.965**	0.962**
Acetic Acid				1	0.974**
Lactic Acid					1

Nkwu (Elaeis guineensis) = Oil palm wine
Ngwo (Raffia hookeri) = Raffia palm wine
*****. Correlation is significant at the 0.01 level (2-tailed).***
****. Correlation is significant at the 0.05 level (2-tailed)***

Table 8 shows the t-test comparison between the physicochemical parameters of raffia palm wine (*Ngwo*) and oil palm wine (*Nkwu*). From the results, pH mean concentration was 3.64 for *Nkwu* and 3.86 for *Ngwo*. They did not show any significant difference (P≥0.05). Sugar mean concentration was 10.28 mg/l for *Nkwu* and 12.08 mg/l for *Ngwo*. They did not show any significant difference (P ≥ 0.05). Ethanol mean concentration was 2.15 mg/l for *Nkwu* and 1.84 mg/l for *Ngwo*. They did not show any significant difference (P≥0.05). Acetic acid mean concentration was 0.04 mg/l for *Nkwu* and 0.36 mg/l for *Ngwo*. They did not show any significant difference (P ≥ 0.05). Lactic acid mean concentration was 0.13 mg/l for *Nkwu* and 0.10 mg/l for *Ngwo*. They did not show any significant difference (P≥0.05).

Table 8: T-test comparison of the physicochemical properties of palm wine from raffia palm wine (*Ngwo*) and oil palm wine (*Nkwu*)

	Wine	Mean	Std.Deviation	Sig.(2tailed)
pH	*Nkwu*	3.64	0.39	0.361^{NS}
	Ngwo	3.86	0.39	
Sugar	*Nkwu*	10.28	3.99	0.444^{NS}
	Ngwo	12.08	3.87	
Ethanol	*Nkwu*	2.15	1.88	0.797^{NS}
	Ngwo	1.84	2.20	
Acetic Acid	*Nkwu*	0.04	0.03	0.361^{NS}
	Ngwo	0.36	0.81	
Lactic Acid	*Nkwu*		0.07	0.489^{NS}
		0.13		
	Ngwo	0.10	0.06	

Nkwu (*Elaeis guineensis*) = Oil palm wine
Ngwo (*Raffia hookeri*) = Raffia palm wine
NS= not significant

Discussion

Acetic acid

The acetic acid is considered as part of the aroma of palm wine (Amoa-Awua *et al*., 2007). Acetic acid concentration increased after 24 hours in *Ngwo* (Raffia palm wine) and increase after 0 hour in *Nkwu* (oil palm wine). Also, the acetic concentration was observed to be higher in *Nkwu* than it is in *Ngwo*. This might be connected to differences in the physiological and genetic makeup of the different palm trees. This finding was in agreement with the findings of Amoa-Awua *et al*. (2007) who studied growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm (*Elaeis guineensis*) in Ghana. Acetic acid concentration had positive strong correlation with Lactic acid which is an indication that a change in lactic acid levels affects the Acetic acid concentration of the sap. Also the Acetic acid concentration of Raphia palm wine sap had positive strong correlation with Lactic acid, which is an indication that changes in lactic acid levels affect the Acetic acid concentration of the sap.

Ethanol

Ethanol is a natural byproduct of plant fermentation and also can be produced through the hydration of ethylene. It is a common ingredient in many cosmetics and beauty products. It acts as an astringent to help clean skin; in lotion as a preservative and to help ensure that lotion ingredients do not separate (Maria Verhagen, 1993). From the study, it was observed that ethanol concentrations in palm wine increased after 0 hour and also after 24 hours, the increase in ethanol was higher in *Nkwu* than in *Ngwo* in 24 hours and higher in *Ngwo* than *Nkwu* in 48 hours. The study revealed that Ethanol of Raphia palm wine sap had positive strong correlation with Lactic acid, which indicates that as the Ethanol concentration of the sap increases, Lactic acid increases. The study revealed that Ethanol of oil palm wine sap had positive strong correlation with Acetic acid and Lactic acid which indicate that as the Ethanol concentration of the sap increases, Acetic acid and Lactic acid increases. Initial concentration of ethanol at the date of collection is a clear indication that ethanol increase after some time is directly connected to the presence of yeast. Hence, the significance difference ($p \leq 0.05$) on ethanol levels over time. Furthermore several other factors such as the presence of other microorganisms, composition of sap, species of palm tree, and environmental conditions e.g. temperature, may cause changes in the ethanol levels. Palm wine tappers collect their wine in the morning and evening, but wine collected in the morning would have accumulated throughout the night. According to Amoa-Awua *et al*. (2007) Ethanol content was found to increase with time of ageing to a peak of 5.1 % (v/v) at 72 hours, after which it diminish gradually to a level of 3.0% v/v after seven (7) days of ageing. Thus, 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). The finding of this present study was in agreement with the findings of Amoa-Awua *et al*., 2007; Akande *et al.,* (2013) who concluded that changes and rate of ethanol production in palm wine is directly connected to the yeast availability, nature and palm wine type. On the other hand, this was further explained by the report of Amoa-Awua *et al.,* (2007) who stated that palm wine which is stored has higher levels of ethanol than palm wine collected directly from the tree. In addition it may also be connected to the fact that other organisms aids in ethanol production other than *Saccharomyces cerevisiae.*

Lactic acid

Lactic acid is an organic acid. It is white in solid state, miscible with water and a colorless solution in liquid state. It is used as a synthetic intermediate in much organic synthesis (The Royal Society of Chemistry, 2014). From the study, it was observed that Lactic acid concentrations in palm wine increased after 0 hour and 24 hours, this maybe as a result of fermentative process of palm wine after some times. Also, the ratio of increase is higher in *Nkwu* than in *Ngwo*. There was a significant difference on the effect of difference in palm wine type and time ($P \leq 0.05$). This may be as a result of

fermentative process of the palm wine after some time. This finding was in agreement with the findings of Amoa-Awua *et al.* (2007).

Sugar (Brix)

Sugar is a generic name for sweet-tasting, soluble carbohydrate, many which are used in food. Sugar is found in the tissues of most plants. Honey and fruit are abundant natural sources of unbounded simple sugar (World Health Organization, 2015). The present study confirmed that sugar availability is very important for fermentation performance. When fermentation was performed rapid and complete sugar utilization was observed. There was also a significant effect ($P \leq 0.05$) in the palm wine type and time on the sugar levels. 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. The decrease rate of sugars observed in both varieties is as a result of important chemical changes in the sap or either to high loads of microorganisms (Yeast cells) in a container which converted the fermentable sugars into alcohol. The findings in this study indicate that, for high sugar fermentation, increase fermentation performance occurs which lead to the production of sugar. Also the sugar content dropped indicates that some sugar was fermented during the taping of palm wine. In addition, the rapid decrease in content of sugars could be explained by a change in alcohol and other products. The variation in the sugar composition through the tapping process can be explained by different factors, such as difference palm tree species, time of collection of the palm wine samples, different ways of make the tapping process. This finding is in agreement with the findings of Karamoko *et al*. (2012) that 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; 7.9 %, 6.4 % and 5.4 % for the second, third and fourth week, respectively, in the palm wine of *Elaeis guineensis*.

pH

pH measures Hydrogen ion potential which is used to determine the acidity and alkalinity of a substance**.** From the study, it was observed that pH concentration in palm wine decreased after 24 hours and 48 hours, there was also a significant difference in the plant and time ($p \leq 0.05$). It was observed that pH of the palm wine decreased slowly from 4.25 to 3.39 in *Nkwu* and 4.06 to 3.20 in *Ngwo* within 48 hours study period, which suggest that the fermented palm wine was acidic. The current study revealed that pH of oil palm wine sap had a positive strong correlation with Sugar, Which is an indication that changes in sugar levels affects the hydrogen ion concentration of the sap. The current study revealed that pH of Raphia palm wine sap had a positive strong correlation with Sugar and Acetic acid which is an indication that changes in sugar levels affects the hydrogen ion concentration of the sap. The presence of other organism such as Lactic acid bacteria in the sap which produces lactic acid may lead to potential increase in the acidic levels of the sap over time. Normally, natural palm sap showed neutral pH approximately 7 as reported by Jitbunjerdkul (1989) and Lasekan (2007). Hence, a high acid concentration after 24 hours indicates the fermentation steps of palm sap, after collection led to increase in acid levels over time. The changes on pH are due to the organic acids production as a result of the microbial metabolic activity in the palm wine after some time. This finding was in agreement with the findings of Ukpaka and Farrow (2015) that investigated the yield of ethanol using palm wine in CSTR connected in series.

T-test comparison of the physicochemical properties of palm wine from raffia palm wine (Ngwo) and oil palm wine (Nkwu)

The result of the T-test comparison of physiochemical parameters of *Nkwu* and *Ngwo* did not differ significantly ($P \leq 0.05$). This is an indication that the yeast and the chemical constituent of wine irrespective of the plant source do not cause significant change. Hence, the yeast type and its performance in either of the wine(s) were at same rate and pattern.

Conclusion

The physicochemical properties of the wines of two palm species: *E. guineensis* and *R. vinifera,* has been studied. The results have shown the presence and levels of ethanol, lactic acid, acetic acid, sugar and the pH of the solutions. It was also discovered that the available properties depend on each other for a normal physiological function of the plants as well as normal nutritional function to man. Certain microorganisms are responsible for the chemical changes that occur in the sap according to the study. It was also seen from the study that time is an important factor for the changes.

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