JORMAR 7(1&2) 2012, 14 - 25 ISSN: 1597-3204

Effects of Purified Yeasts From Natural Oil Palm and Raffia Palm Wine on Quality of Grape Fruit Wine

M. U. Ukwuru* and C. E. Nnadi

Department of Food Science and Technology, Federal Polytechnic,

P.M.B. 1037, Idah, Kogi State, Nigeria

ABSTRACT

Yeast *(Saccharomyces cerevisiae)* was purified from oil palm *(Elaeis guineensis)* and raffia palm *(Raphia vinifera)* wines and used to ferment grapefruit must (*Citrus paradise*). The Commercial Bakers' Yeast (CBY) was used as control. They were propagated and pitched at a rate of 4 % (v/v). The results of viability test showed that Oil Palm Wine Yeast (PWY) was 98 % viable with an initial cell count of 1.0×10^7,Raffia Palm Wine Yeast (RPWY) was 95 % viable with an initial cell count of 5.0×10^6 and commercial Bakers' yeast (CBY) was 96 % viable with an initial cell count of 7.0×10^6. The must had a pH of 3.59; titratable acidity of 0.69 % (as % tartaric acid) and soluble solids of 16 % and was fermented at 30±2 ^{0}C for 14 days followed by racking, fining and ageing (5±2 ^{0}C) for 15 days. The wines fermented (PWY, RPWY and CBY) had 10.0, 9.0 and 10.0 (%v/v) alcohol, residual soluble solids of 5.0 %, 6.1 % and 5.2 %; 3.71, 3.74 and 3.69 pH and 0.83 %, 0.79 % and 0.81 % tartaric acid, respectively. The results of sensory evaluation showed that except in flavour, there was no significant difference ($p>0.05$) in taste, colour and overall acceptability of the wine samples when compared with the control.

Keywords: Fruit wine, Oil Palm wine, Purification, Raffia palm, Yeasts.

**Correspondence Author: e-mail ukwuru@yahoo.com*

Introduction

Palm wine is a local alcoholic beverage obtained from different kinds of palm trees, such as the oil, raffia and date species. The most frequently tapped palms are raffia palm (*Raphia vinifera)* and the oil palm *(Elaeis guineesis) [*1]. The species *Elaeis guineesis* is the most exploited because of its oil and sap, which are widely consumed in southern part of Nigeria. Besides, its sap constitutes a good growth medium for numerous micro-organisms, especially yeast, lactic and acetic acid bacteria [1].

Yeasts are unicellular eukaryotic fungi which can be isolated from various sugar-rich sources including palm wine (2, 3). Nigerian palm wine yeasts have been used to develop baking yeast. The dough made with this yeast tasted better and had better quality when compared to dough made with a commercial strain of bakers' yeast (4). Generally, all wines are produced using wine yeast-*Saccharomyces cerevisiae* var, *ellipsoideus* [5]. However, Samori and Udo [6], have shown that oil palm yeast, *Saccharomyces cerevisiae,* has the potential of wine yeast and it is also alcohol tolerant. The oil palm *Saccharomyces* species have been used for bio-ethanol production at industrial level using different sources of fermentable sugars (7,8). It possesses better sedimentation properties for better product recovery [8]. Ogbonna [9] and Onyedinma [10] used palm wine isolates of *Saccharomyces cerevisiae* to produce artificial palm wine and beer. Very few efforts have been made at purifying these yeasts for wine production.

Despite the continuous research efforts at utilising bacteria for ethanol production [11], the yeast is still the primary choice for fermentation [12].

Micro-organisms necessary in food fermentation may be added as pure cultures or mixed cultures or in some instances, no cultures may be added, if the desired micro-organisms are known to be present in sufficient numbers in the original raw material (for example, in the production of fermented pickles sauerkraut, etc). On the other hand, controlled "starter" cultures, pure or mixed, usually are employed in the manufacture of certain dairy products, such as fermented milk, some brands of butter and most types of cheese and in the production of bread, malt beverages, wines, distilled liquors and vinegar (13).

A pure yeast culture can be obtained from various sugar-rich substrates by isolation and propagation on a special medium. Attempts so far made to produce yeast in commercial quantities locally have been limited by technical difficulties.

Oil palm and raffia palm wine are cheap and important alcoholic beverages in Nigeria consumed by more than 10 million people. In Nigeria, dried yeast is imported from Europe and America at the expense of her overstretched exchange earnings. Consequently, the cost of wine yeast is usually very high, amounting to about 25 % of total raw material cost in wine making. The prospects of using natural yeasts inherent in grapes in the wine production are limited by the fact that grapes are not grown in the country. Due to high cost incurred in importation of dried yeast from Europe and America, various approaches have been employed in cultivation of yeast from various sugar-rich substrates for diverse utilization. The aim of this work was to develop a yeast starter culture from oil palm wine and raffia palm wine to be used as alternative and cheaper starter culture for wine production.

Materials and Methods

Sources of Raw Materials

Oil palm wine was obtained from Nnadi Ugwu farm, Ezimo, Udenu L.G.A, Enugu State, Nigeria. Raffia palm wine was obtained from Odolu, Kogi State, Nigeria. Grapefruits and commercial Bakers' yeast were bought from Ega Main market, Idah, Kogi State.

Must Preparation

The ripe grapefruits were sorted, washed, peeled manually and cut into halves with sharp, clean knife. Each half was then extracted manually by pressing out the juice into a bowl. The juice containing seeds, rags and pectic substances was then filtered through a cheese cloth (0.002 mm) and standardized by adding granulated sucrose (England, UK) to raise its specific gravity from 9 to 16% (W/W). The must was then pasteurized at 68°C for 15 min, cooled to 23±2°C, sulphited (150ppm) and stored in a clean bottle until used for analysis and wine production (Fig 1)

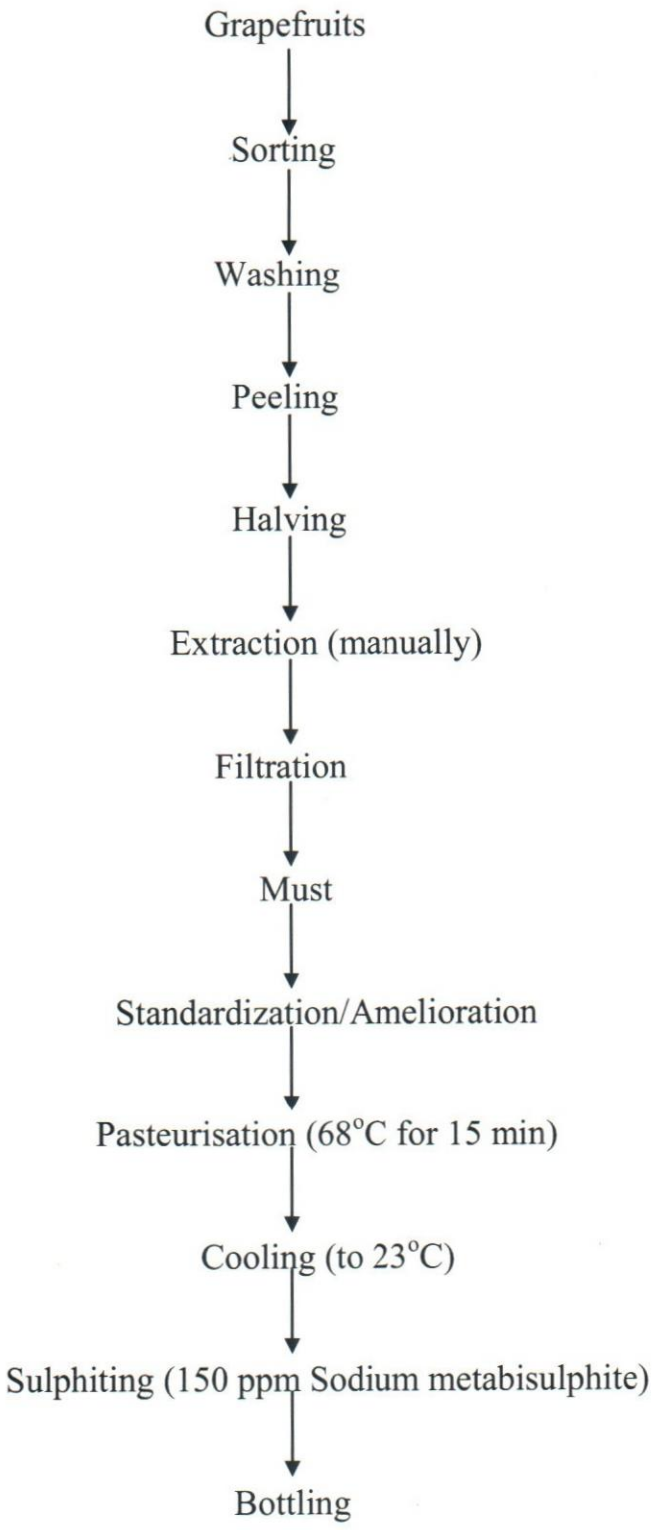

Fig 1: Flow chart for Must Preparation

Analysis of Must

The *must* was analyzed for its total titratable acidity (as tartaric acid), pH, soluble solids and specific gravity. The procedures are given in 3.50 (Chemical Analysis of Wine).

Standardization/Amelioration of Must

The degree Brix of the must was not high enough for favourable fermentation, so it was increased by the addition of granulated sugar made into solution. About 250g of fine granulated sucrose was properly dissolved in distilled water (500 ml). This increased the Brix° of the Must from 9 % to 16 %. This addition of sugar solution increased the volume of the Must to 6.5L.

Isolation, Identification, Purification and Propagation of Palm wine and Raffia Palm Wine Yeasts.

Isolation of Palm wine and Raffia Palm Wine Yeasts.

Yeast was isolated from 24 h old palm wine and raffia palm wine using the modified method of Olorunfemi and Atueyi [14]. One ml of each – palm wine and raffia palm wine was diluted with 10 ml of sterile water and agitated for even distribution. One ml of the solutions (10^{-6}) was cultured on acidified (pH 4.0) Malt Extract Agar (MEA) and incubated at 37±1°C for 24 h. The colonies were counted using Quebec colony counter.

Identification of Oil palm and Raffia Palm wine Yeast

This was carried out by observing the colonies for some characteristics like colour, size and shape using a compound microscope (Nikko Model). The spherical colonies of *Saccharomyces cerevisiae* of oil palm and raffia palm wine yeasts were identified. The identified yeast colonies were further sub-cultured using acidified (pH 4.0) MEA to obtain pure strain of *Saccharomyces cerevisiae.* This was confirmed by carrying out sugar fermentation test using Maltose (M), Sucrose (S) and Glucose (G). The medium was prepared with the composition of 1.0 % peptone, 0.1 % sodium chloride and 1.0 % each of the fermentable sugar.

Purification of Oil palm and Raffia Palm Wine *Saccharomyces cerevisiae*

The yeasts were purified by washing the colonies with normal saline into sterile centrifuge tubes. It was then centrifuged for 10 min at 6000 rpm. The supernatant was decanted and sterile water added and re-centrifuged to obtain purified cells. Purified yeast was then grown on acidified MEA slant and stored at 3±1°C as the stock culture.

Storage Stability Test

The storage stability of the stock culture was studied everyday by growing it on acidified MEA. The stock culture was stored for 3 days at 37±2 °C without being affected by bacteriophage.

The stock culture was sub-cultured every 3 days to maintain the purity of the culture.

Propagation of the Yeasts

The propagation involved transferring stock cultures from four acidified MEA slants into 200 ml standard Must in an Elenmeyer flask mounted on an orbital shaker (ProlaboOscil 12, France) for 24 h and subsequently topped with 200 ml portions of the standard must after 48 h and 72 h respectively.

Preparation of Commercial Yeast Culture

About 10 g of dry Commercial bakers' yeast (*Saccharonyces cerevisiae*) was dissolved in 300 ml of standardized Must pre-heated at 37°C and the mixture was held in a culture propagation bottle plugged with cotton wool slightly and made to stand at room temperature (30±2 °C) for 48 h.

Viability Test of the Yeast Cultures

The viability of the three yeast (*S. cerevisiae)* cultures was carried out using the Institute of Brewing (IOB) method of 1989 (15), used in brewery industries. This was done by smearing a drop of 48 h yeast culture on a clean slide. The slide was allowed to dry and a drop of 1 % methylene blue indicator was smeared on top of the slide. After drying, the slide was viewed with a compound microscope (Nikkon Model) with an objective lens of x 100. The live or viable yeast cells remain colourless while the dead cells retained the colour of the methylene blue. The percentage of the viable cells was calculated as:

$$\frac{\text{Number of colourless cells}}{\text{Total number of yeast cells}} \times \frac{100}{1}$$

Wine Production/Fermentation

Three (3) litres of the standardized Must were pitched with 400 ml of the propagated oil palm and raffia palm wine yeasts to give a pitching rate of 4 % (V/V) and fermented in a 5 L glass jar at 30±2°C for 14 days. The wines were racked, fined with 1 % gelatin (England, UK) and aged at 5°C for 15 days. The aged wines were filtered through a cheese cloth sieve (0.002 mm), pasteurized (80°C, 3 min) and bottled. For the control sample, the same fermentation procedure was repeated except that the starter culture was propagated commercial strain of *Saccharomyces cerevisiae* (Bakers' yeast).

Chemical Analysis of Wine

The Brix°, pH, titratable acidity, specific gravity and alcohol content of the fermenting Must were determined every 48 h for the 14 days of fermentation and subsequently determined after 15 days of ageing. The soluble solids of fermenting Must were determined using hand refractometer. (The result was expressed in % soluble solids). An electronic pH meter (equip – Tronics Digital meter model EQ-610) was used to determine the pH. Titer acidity was carried out as outlined by AOAC [16]. Alcohol content was determined according to the method of Pearson [17]. Specific gravity was determined according to IOB [18]. Sensory evaluation was done for taste, flavour, colour and general acceptability using 10 panelists randomly selected. Data were analysed using analysis of variance. Means that differed

significantly were separated by Duncan's multiple range tests.

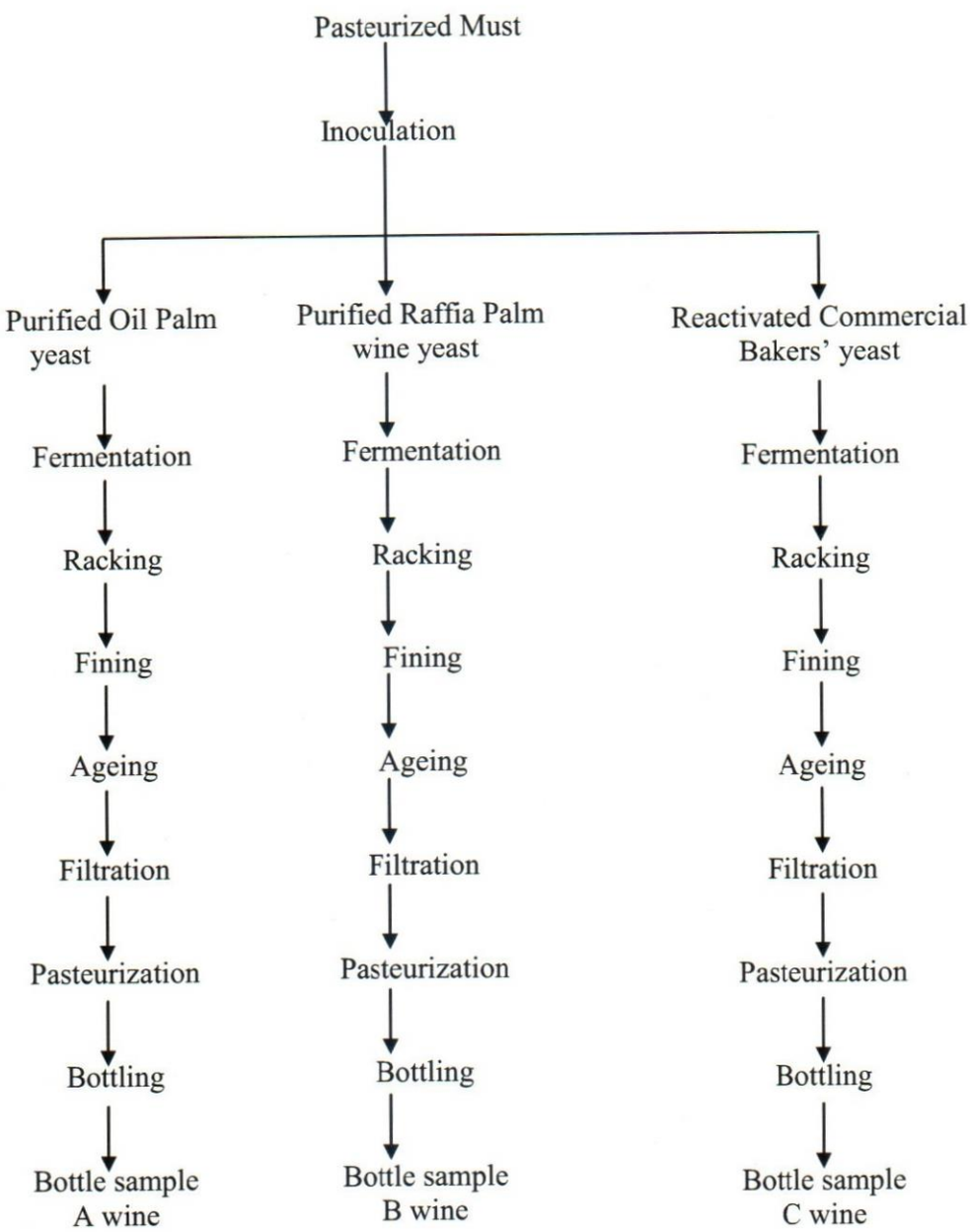

Fig. 2: Flow chart for wine production

Results and Discussion

Viability of the three Yeasts Culture

All the yeasts cultures under investigation had high levels of viability (>95%) (Table I). The oil palm yeast (PWY) culture had the highest percentage viability (98 %) with an initial viable cell count of 1.0 x 10^7 cfu/ml followed by the commercial bakers' yeast (CBY) culture with 96 % viability with an initial viable cell count of 7.0 x 10^6 cfu/ml and the raffia palm wine yeast (RPWY) with 95 % viability and initial viable cell count of 5.0 x 10^6 cell cfu/ml. This showed that the three yeast cultures were very active, hence, their ability to ferment the Must when it was innoculated with the yeast culture.

Must Analysis

The Must had chemical characteristic that compared well with grapefruits Must (5), although its specific gravity was augmented with granulated sucrose. The amelioration led to an increase in degree Brix from 9 % to 16 % to provide enough room for yeast fermentation and pH from 3.32 to 3.59. These conditions which came about as a result of sugar addition favoured fermentation process as reported by Amerine *et. al.* [5]; that sugar could be added to Must that has low sugar content in order to favour alcoholic production of about 13 % by volume. The attained Must pH (3.59) fell within the pH level of 3.5 – 4.0 that was reported to be optimal for palm wine yeast activity [6]. The high tartaric acid level may be due to the initial low pH of the grapefruit juice (Table II).

Chemical Changes of the Fermenting Must

Chemical Changes of oil Palm Wine Yeast (PWY) Fermenting Must

The observed reduction in soluble solid/Brix° from 16% to 5.0% and resultant increase in the alcohol concentration from 0 to 10.0% (v/v) with fermentation showed the efficiency of the *Saccharomyces cerevisiae* isolated and purified from oil palm wine. Its attenuation between 10 and 10 (%v/v) alcohol implies that the yeast was alcohol tolerant (19). The observed low variation in pH and titratable acidity underscored the ability of pure strain of *S. cerevisiae* from oil palm wine to yield alcohol at acceptable levels in fruit wine production without objectionable high acidity (Table III).

The high yield of alcohol is attributed to the ability of palm wine yeasts to break down the fermentable sugars in the Must. This characteristic of palm wine yeasts has been effectively employed in the production of wine from mango Must by Somari *et al.* [6], production of red wine from Roselle (*Hibiscus sabdariffa)* and Pawpaw (*Carica papaya)* by Okoro [20] and in leavening of dough by Somari and Udo [6], Ejiofor *et. al.* [4] and Olorunfemi and Adetunyi (14).

Chemical Change during Must Fermentation using Yeast from raffia palm wine

The observed reduction in soluble solids from 16% to 6.1% and resultant increase in the alcohol concentration from 0 to 9.0 (%v/v) with fermentation showed the ability and efficiency of the *Saccharomyces cerevisiae* purified from raffia palm wine. Its attenuation between 7.5 and 9.0 (%v/v) alcohols implies that the yeast was alcohol tolerant [19]. However, its attenuation rate was lower than that of oil palm wine yeasts (Table IV). Again, the observed low variation in pH and titratable acidity underscores the ability of pure strain of *S. cerevisiae* from raffia palm wine to yield alcohol at acceptable levels in fruit wine production without objectionable high acidity. The high yield of alcohol was attributed to the ability of RPWY to breakdown the fermentable sugars in the Must [5].

Chemical Changes during the Fermentation of Grape fruits Must by Commercial Bakers' Yeast (CBY)

The observed decrease in Brix° from 16% to 5.2% and resultant increase in the alcohol concentration from 0 and 10.0 (v/v) with fermentation showed the ability and efficiency of the Commercial Bakers' yeast (*Saccharomyces cerevisiae*). Its attenuation at between 9.0 and 10.0 (% v/v) alcohol implies that the yeast is alcohol tolerant [19]. However, its attenuation rate was higher than raffia palm wine yeast (Table V).The observed low variation in pH and titratable acidity underscore the ability of Commercial Bakers' yeast (*S. cerevisae)* to yield alcohol at acceptable levels in fruit wine production without objectionable high acidity. The high (10 %v/v) alcohol yield was attributed to the ability of CBY to break down the fermentable sugars in the must [5].

Chemical Changes after Ageing

The wines produced using Oil Palm Yeast (PWY), Raffia Palm Wine Yeast (RPWY) and Commercial Bakers' Yeast (CBY) had 10.0%, 9.0 % and 10.0% alcohol (v/v); residual soluble solid of 5.0%, 6.1% and 5.2%; 3.71, 3.74 and 3.69 pH and 0.83%, 0.79% and 0.81% tartaric acid respectively after ageing for 15 days. The attained alcohol content of the three wine samples fell within the range acceptable for table wine [5], though the wine had a high residual tartaric acid. The pH was also within the range acceptable for table wine [5] and was low to inhibit the microbial growth. The residual soluble solids contributed to the flavour of the wine (Table VI).

Sensory Evaluation

The result of sensory evaluation showed that there was no significant difference among the taste, colour and overall acceptability but the samples differed in terms of flavour at 5% probability level ($p<0.05$) (Table VII). However, the wine fermented with raffia palm wine yeasts was comparable to the control sample in terms of

flavour. The wine samples had bright light orange colour. The two developed wines according to the assessors had tonic-like flavour with marked absence of oil palm and raffia palm wine taste and flavour unlike wines produced using other fruits and fermented with oil palm yeast that had mild palm wine taste [21]. The marked absence of oil palm, raffia palm wine taste and flavour may be due to the purified starter cultures of *S. cerevisiae* from oil palm and raffia palm wine used (Table VII).

Conclusion

An acceptable tropically-sourced wine was produced from grapefruit (*Citrus paradise)* using purified oil palm and raffia palm wine yeasts (*Saccharomyces cerevisiae)*as starter culture. The result of this research showed that the oil palm and raffia palm wine yeasts have the potential of wine yeasts. They were alcohol and acid tolerant. Purified oil palm yeast had a higher percentage viability and attenuation rate compared to the raffia palm wine yeasts and both produced good quality wine with marked absence of oil palm and raffia palm wine taste and flavour.

Table I:Viability Test of Yeast Culture

Yeast Culture	Initial viable cell count (cfu/ml)	No of colourless cells	No of stained cells	Total No of cells	% viability
Palm wine Yeast	1.0×10^7	49	1	50	98
Raffia palm Wine yeast	5.0×10^6	38	2	40	95
Commercial Bakers' yeast	7.0×10^6	48	2	50	96

Table II: Physicochemical analysis of the Must

Parameter	Composition
pH	3.59
Soluble solids (%w/w)	16
Titratable acidity (as % tartaric acid)	0.69
Specific gravity	1.0179
Alcohol (%v/v)	0
Colour (visual)	Light yellow

Table III: Chemical changes during the fermentation of grapefruits Must by palm wine yeasts (PWY)

Day	pH	°Brix/Soluble solids	Specific Gravity	Alcohol Concentration (%v/v)	Titratable Acidity
0	3.59	16.0	1.01790	0.0	0.69
2	3.60	13.9	0.99712	1.5	0.70
4	3.62	12.6	0.99342	4.5	0.71
6	3.63	10.2	0.99280	5.0	0.74
8	3.65	8.8	0.98725	7.5	0.78
10	3.71	7.8	0.98746	9.0	0.79
12	3.72	7.5	0.98746	9.0	0.81
14	3.72	7.2	0.98684	10.0	0.82

Table IV: Chemical changes during the fermentation of grapefruits Must by raffia palm wine yeasts (RPWY)

Day	pH	°Brix/Soluble solids (%/w/w)	Specific Gravity	Alcohol Concentration (%v/v)	Titratable Acidity
0	3.59	16	1.01790	0	0.69
2	3.62	14.5	0.99778	0.5	0.70
4	3.64	13.8	0.99630	2.5	0.72
6	3.64	11.7	0.99486	3.5	0.73
8	3.69	10.0	0.98280	5.0	0.75
10	3.71	9.2	0.98198	6.0	0.75
12	3.72	8.6	0.98198	6.0	0.75
14	3.72	8.0	0.98725	7.5	0.78

Table V: Chemical changes during the fermentation of grapefruits Must by Commercial Bakers' Yeasts (CBY)

Day	pH	^{0}Brix/Soluble solids	Specific Gravity	Alcohol Concentration (%v/v)	Titratable Acidity
0	3.59	16.0	1.01790	0.0	0.69
2	3.59	14.1	0.99704	2.0	0.71
4	3.61	13.0	0.99630	2.5	0.73
6	3.63	10.9	0.99342	4.5	0.74
8	3.64	9.0	0.99198	6.0	0.78
10	3.65	8.7	0.98725	7.5	0.78
12	3.67	8.0	0.98746	9.0	0.79
14	3.68	7.5	0.98746	9.0	0.80

Table VI: Chemical changes after ageing

Parameter	A (PWY)	B (RPWY)	C (CBY)
pH	3.71	3.74	3.69
Soluble solids (%)	5.0	6.1	5.2
Specific gravity	0.98684	0.98746	0.98684
Alcohol Concentration (%v/v)	10.0	9.0	10.0
Titratable Acidity (% tartaric acid)	0.83	0.79	0.81

A (PWY) – Wine Developed Using Palm Wine Yeast, B (RPWY) – Wine Developed Using Raffia Palm Wine Yeast and C (CBY) – Wine Produced Using Commercial Baker's Yeast.

Table VII: Sensory Means Score of Wines Fermented with Palm Wine and Raffia Palm Wine Yeasts.

	Wine Sample		
Attribute	**A (DWY)**	**B (RPWY)**	**C (CBY)**
Taste	4.8 ± 1.76^a	45.1 ± 1.25^a	5.8 ± 1.57^a
Flavor	5.9 ± 1.01^b	4.9 ± 1.20^c	5.0 ± 1.70^a
Colour	4.9 ± 1.27^d	4.8 ± 1.08^d	5.4 ± 1.11^d
Overall Acceptability	5.3 ± 1.76^e	5.1 ± 1.03^e	5.3 ± 1.48^e

Values are mean of three determinations. Means with the same superscripts along the rows are not significantly different ($p>0.05$). A (PWY) – Wine developed using palm wine yeast, B (RDWY) – Wine developed using raffia palm wine yeast and C (CBY) – Wine developed using Commercial Bakers' yeast.

REFERENCES

1. Odunfa, S.A. (1985). African Fermented Foods: In *"Microbiology of Fermented Foods"*, Elsevier Applied Science Publishers, USA.

2. Okafor, N. (1972). Palm-wine yeast from parts of Nigeria. *Journal of Science. Food and Agriculture*. 23: 1399 – 1407.

3. Okagbu, R.N. (1988). A note on the activity of yeast isolated from Nigerian Palm wine. *J. Appl. Bacteriol*. 64: 234 - 240.

4. Ejiofor, A.O., Okafor, N. and Ugwueze, M. (1994). Development of baking yeast from Nigerian Palm-wine yeast. *World Journal of Microbiology and Biotechnology* 10:199 - 202.

5. Amerine, M.A., Berg., H.W., Kunkee, R.E., Ough, C.S., Singleton, V.L. and Webb, A.C. (1980). *The Technology of Wine Making*. 4th ed. Avi Publishing Co., Inc. Westport, C.T., U.S.A. pp. 359 - 380.

6. Somari, R.I. and Udo, A.E. (1993). Evaluation of the performance of Yeast isolated from sap of *Elaeis guineensis* in dough leavening. *Nigerian Food Journal*. 11:34 - 44.

7. Agu, R.C., Anyanwu, T.U. and Onwumelu, A.H. (1993). Use of high ethanol-resistant yeast isolates from Nigerian palm wine in Lager beer brewing. *World Journal Microbiology and Biotechnology*. 9: 660 -661.

8. Ezeogu L.I. and Okolo, B.N (1994). Effect of Molasses Concentration and Medium supplementation of the adaptability and viability of high level ethanol – tolerant palm-wine *Saccharomyces* isolate; *Biotechnology Letters* 16 (1). 95 - 100.

9. Ogbonna, A. (1984). Isolation of Alcohol Production. *M. Sc. Thesis*, University of Nigeria, Nsukka, Nigeria.

10. Onyedinma, J. (1983). Production of Palm wine Analogue from Synthetic Medium. Thesis, University of Nigeria, Nsukka, Nigeria.

11. Ingram L. and Buttle, C. (1984). Effect of ethanol on Micro-organisms. Advances in Microbial Physiology. 22: 233 - 330.

12. Chandra, J. and Panchal, F. (2003). Yeast strain selection, *Bioprocess Technology Series*, Marcel Dekka Pub. New York.

13. William, C.F. and Dennis, C.W. (2005). *Food Microbiology* 4th ed., Teta McGraw-Hill Publishing Co., Ltd, New Delhi, India. pp 321 - 328.

14. Olorunfemi, O.B. and Adetuyi, F.C. (2005). Isolation of Baking Yeast from naturally fermented Pineapple. *Journal of Food, Agriculture. & Environment*. 3: (1): 115 - 117.

15. Crompton, J.M. (1977). *Practical Brewer*. Master Brewers Association of the American, Madison, pp. 53.

16. AOAC (1984). "Official Methods of Analysis". Association of Official Analytical Chemists, (AOAC) Inc.,14th ed., Washington, DC. USA

17. Pearson, D. (1976)."The Chemical Analysis of Foods".Edinburgh, London, New York. Pp. 112 – 125.

18. IOB (1989). "Recommended Methods of Analysis". Institute of Brewing, London.

19. Zoecklein, B.W, Fugelsang, K.C, Cump, B.H and Nurry, F.S (1990). "Production wine analysis" 1st ed. Avi Publication Co; Inc;, Westport, Connecticut. pp. 71 – 243.

20. Okoro, C.E. (2007). Production of red wine from Roselle (*Hibiscus sabdariffa)* and pawpaw (*Carica papaya)* using palm-wine yeast (*Saccharomyces cerevisiae). Nigerian Food Journal*, 25: (2): 158 - 164.

21. Obayanju, V.S. and Ademokeya, A.A. (1991). Utilisation of Pawpaw juice for wine making. *Nigeria Journal of Technical Education*, 8: 125 - 128.