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Properties of palm wine yeasts and its performance in wine making

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Fresh palm wine samples were obtained from oil palm and raffia palm into sterile flasks. The samples were examined for yeasts properties and performance in wine making using grapes. The yeasts in the palm wine were characterized, identified, and screened for their sedimentation rate, ethanol tolerance, alcohol content, yeasts population, pH, total acidity (TA), total soluble solids, reducing sugar and total sugar at 6 h intervals. The yeasts from the samples were purified and used to inoculate grape must. Variation in temperature, alcohol contents, pH, specific gravity, total solids, titratable acidity and volatile acidity were determined. There were significant differences (P<0.05) between the yeast properties in oil palm and raffia palm. *Saccharomyces cerevisiae*, *Saccharomyces globosus* and *Saccharomyces carlsbergensis* were isolated from the palm wine samples. Ethanol tolerance was between 15 and 18% v/v in oil palm and 12 to 15% v/v in raffia palm after 24 h. Yeast population increased (10^8 to 10^9 cfu/ml) with increased level of ethanol and TA with corresponding reduction in pH level (6.7 to 4.6). Yeast performance in grape wine indicated significant differences (p<0.05) between oil palm and raffia palm samples. TA and ethanol production (9 to 11.4%) were lower as compared to that in the palm wine. The pH was fairly stable. This study indicates that palm wine yeasts have good properties for wine production from fruits.

Key words: Ethanol tolerance, grape wine, palm wine yeasts, *Saccharomyces,* yeasts properties.

INTRODUCTION

Palm wine is a nutritionally rich medium for the growth of many organisms among which is yeast species. Different species of yeast can be found in palm wine. Yeast population, among other organisms, have been found to vary in palm wine depending on the source. The yeasts quantitatively convert the sugars in the palm wine into alcohol. Hence, the physicochemical condition of palm wine is a function of the metabolic activities of the inherent yeasts in palm wine. The characteristics of palm wine is so unique that it has generated research interest (Nwachukwu et al., 2006, 2008; Naknean, 2010) to investigate the practical applications and industrial utilization. Bechem et al. (2007) determined the physiological characteristics of ten palm wine yeast isolates and some isolates showed tolerance to high sucrose and ethanol concentrations: a property that can be exploited. Similarly, Chilaka et al. (2010) evaluated the efficiency of yeast isolates from palm wine in diverse fruit wine production and concluded that acceptable wine could be produced from fruits with palm wine yeasts.

Saccharomyces species have been isolated from palm wine and used for bio ethanol products (Agu et al., 1993; Ezeogu and Okolo, 1994). Palm wine yeasts have been found to possess good sedimentation properties for high product recovery.

Palm wine is a cheap source of yeast that can augment

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Table 1. Palm wine sample sources and codes.

Sample	Wine type	Source
PW1	Palm Wine	Nsukka, Enugu State, Nigeria
PW2	Palm Wine	Enugu Ezike, Enugu State, Nigeria.
PW3	Palm Wine	Otukpa, Benue State, Nigeria.
RW1	Raffia Wine	Idah, Kogi State, Nigeria.
RW2	Raffia Wine	Ogbogbo, Kogi State, Nigeria.
RW3	Raffia Wine	Igabada, Kogi State, Nigeria.

for the more expensive commercial yeast. Utilizing palm wine yeasts for industrial processes requires a comprehensive knowledge of their technological properties. Available literatures in this regard are yet scanty. Desirable properties inadequate or lacking can be made up through genetic engineering. The production of palm wine yeasts can then be scaled up from which starters can be obtained for various industrial applications. This present study was aimed at examining the properties of palm wine yeasts for their suitability in wine making from grape fruits.

MATERIALS AND METHODS

Sample collection

Palm wine samples were collected fresh from the tappers early in the morning (Table 1). 50 ml from each sample was collected into flask containing 25 ml of 10% glycol solution. Samples were appropriately labelled with location, date and time of collection. The samples were immediately transported to the laboratory for analysis.

Isolation and identification of yeasts

10 ml of wine was mixed thoroughly and centrifuged in sterile centrifuge tubes at 3000 rpm for 5 min. The sediment was re-suspended with sterile distilled water and centrifuged again. The sediment was inoculated by streaking on plates of glucose yeast agar (GYA) containing 0.05 mg/ml of chloramphenicol to inhibit bacterial growth. The plates were incubated at 28°C for 24 h (Okafor, 1972). Yeast colonies were further sub-cultured on GYA by streaking to obtain pure cultures. Standard morphological and physiological methods and identification keys described by Barneth et al. (1990), Deak and Beuchat (1987) and Phaff and Starmer (1987) were used.

Measurement of chemical properties

These properties were determined after every 6 h for a period of 24 h. The pH was measured using a pH meter (Satorious, USA). The total soluble solids were determined using hand refractometer and result expressed as degree Brix. Total acidity was by titration with NaOH and calculated in terms of lactic acid (Rangana, 1986). Total sugars and reducing sugars were determined by Lane and Eynon and volumetric method, by titrating with Fehlin reagents. Results were expressed as gram glucose per 100 g sample (Rangana, 1986).

Determination of ethanol tolerance

Ethanol tolerance of yeast isolates was determined based on visual assessment of turbidity and viability in tubes of basal medium (Bhajpai et al., 1988; Ohta et al., 1981). Sterile basal medium containing known percentages of ethanol was inoculated with actively growing yeast cells. They were incubated at 25°C for 48 h (Skinner et al., 1961; Nwachukwu et al., 2006). Evidence of turbidity/sedimentation indicated growth and tolerance. Percentage sedimentation was the ratio of the total drop in reading multiplied by 100 and the calorimeter reading at 0 h.

Plate count of yeast population

Serially diluted samples of palm wine were plated out on GYA containing chloramphenicol as previously described. Plates were incubated at 28°C for 48 h and counted using a colony counter.

Inoculation of grape must

Grape fruits were crushed, pasteurized at 80°C for 30 min, cooled and sulphited. Each of the isolated purified yeast [*S. cerevisiae, S. globosus* and *S. carlsbengensis*, and commercial yeast (control)], was used to inoculate sterile grape must in a fermenter with stirrers for agitation. The fermenting must was racked after 12 h, filtered and fermented further for 10 days. Samples were removed from the fermenter for analysis of alcohol content, specific gravity, volatile acidicty, titratable acidity and total solids using standard methods (Caputi and Wright, 1969; James, 1995 Bradly, 2003; George and Murphy, 2003). Temperature and pH were measured using a thermometer and pH meter, respectively.

Statistical analysis

All the data were analyzed using the SPSS statistical software. Data means were compared using one way analysis of variance (ANOVA). Means that differed significantly were separated using Duncan's multiple range test. Significant differences were accepted at $P<0.05$.

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 (Table 1). Both palms produce characteristically unique palm wine with different organoleptic properties.

Table 2. Characteristics and identification of yeasts from palm wine sample.

Sample	Type of yeast
PW1	*S. cerevisiae* *S. globosus*
PW2	*S. cerevisiae* *S. carlsbengensis* *S. globosus*
PW3	*S. cerevisiae*
RW1	*S. cerevisiae*
RW2	*S. cerevisiae*
RW3	*S. cerevisiae*

Table 3. Plate count of yeast (log_{10}cfu/ml) in palm wine sample.

Sample	Time (h)				
	0	6	12	18	24
PW1	3.7	5.6	7.8	8.7	8.8
PW2	4.8	5.2	6.9	8.2	8.5
PW3	4.7	5.0	6.2	8.3	8.9
RW1	3.6	4.5	6.7	7.9	8.2
RW2	3.8	4.6	5.7	7.5	7.9
RW3	3.9	4.2	5.9	6.8	7.6

Table 4. Time course of pH level of palm wine samples.

Sample code	Time (h)				
	0	6	12	18	24
PW1	5.83	5.72	4.23	4	3.65
PW2	5.17	5.13	4.25	3.92	3.5
PW3	5.52	5.31	4.92	4.26	3.82
RW1	6.92	6.73	5.28	4.93	4.25
RW2	6.24	6.05	5.52	4.95	4.3
RW3	6.36	6.02	5.64	4.2	3.97

Yeast cells were the most quantitatively abundant organisms in the palm wine samples. Various species of yeast were present in palm wine with *S. crevisiae* been the most predominant species (Table 2). Other yeast species present were *S. globosus* and *S. carlsbengensis*. *S. carlsbengensis* which is the brewery yeast was not commonly found in the palm wine samples.

Total plate counts of yeast indicated high presence (3.7 to 4.8 log_{10} cfu/ml) of viable yeast which increased steadily with increase in storage time at ambient temperature (28 ± 2°C) to a maximum population range of 7.6 to 8.9 log_{10} cfu/ml after 24 h (Table 3). More yeast cells were observed in palm wine from oil palm (PW1-3) than that from raffia palm (RW1-3). The rate of multiplication of microbial cells in a medium is dependent on several factors which include physical factors such as pH, temperature, water activity and chemical factors which include nutrient, redox potential, antimicrobial agents, etc, as well as biotic factors such as antagonism. The combination of one or more of these factors resulted into series of successions and subsequently, predominance by yeast species in the palm wine samples.

The pH of fresh palm wine ranged from 5.17 to 6.92 which decreased progressively with increase in storage time to final pH of 3.50 to 3.97 after 24 h (Table 4). There were significant differences ($P<0.05$) in the pH level of palm wine samples from oil palm, which were higher than

Table 5. Time course of alcohol content (%) of palm wine.

Sample code	Time (h)				
	0	6	12	18	24
PW1	3.5	7.3	15.2	16.3	17.3
PW2	3.7	8.6	16.3	17.9	18.2
PW3	3	7.4	16	17.2	17.9
RW1	3.8	7.6	17.2	17.8	18.6
RW2	3.8	7.9	16.8	18.3	18.8
RW3	3.7	7.5	17.5	18.5	19.7

Table 6. Time course of total sedimentation rate (%)/ethanol tolerance (%v/v) of yeast in palm wine.

Sample code	Time (h)				
	0	6	12	18	24
PW1	80.3 (12.0)	82.4 (12.4)	87.6 (14.6)	89.7 (16.3)	90.6 (17.2)
PW2	75.8 (11.0)	78.6 (12.1)	82.7 (13.6)	84.2 (14.5)	89.3 (16.5)
PW3	76.5 (11.4)	79.1 (12.6)	84.2 (14.3)	86.5 (14.8)	88.9 (15.7)
RW1	62.5 (12.6)	68.9 (13.0)	72.5 (13.6)	75. 7 (14.0)	80.0 (14.7)
RW2	64.2 (11.7)	68.3 (12.4)	70.0 (13.0)	76.4 (14.1)	77.2 (14.8)
RW3	69.3 (12.0)	70.4 (12.5)	75.3 (13.2)	78.4 (13.9)	80.2 (15.2)

Table 7. Time course of total acidity (%) of palm wine samples.

Sample code	Time (h)				
	0	6	12	18	24
PW1	0.06	0.06	0.07	0.08	0.08
PW2	0.05	0.05	0.07	0.09	0.09
PW3	0.05	0.05	0.07	0.08	0.08
RW1	0.03	0.03	0.04	0.05	0.07
RW2	0.04	0.04	0.06	0.07	0.07
RW3	0.03	0.03	0.05	0.07	0.07

samples from raffia palm. Low pH favours wine production as it inhibits the growth of contaminating microorganisms, while favouring the growth of yeasts. This condition gives the fermenting yeast an edge over competing organisms. Table 5 confirms the alcohol production during fermentation by yeast. Alcohol levels which were initially low (3.0 to 3.8%) in the samples increased up to 15 to 17% after 12 h and up to 17 to 20% after 24 h. There were no significant differences ($P \geq 0.05$) in the alcohol content of the samples at any point in time during the fermentation in the palm wine.

Sedimentation rate and ethanol tolerance

The *Saccharomyces* species in the samples under test had high sedimentation rate (80 to 90.6%) and also high ethanol tolerance [14.7 to 17.2% (v/v)] and were not inhibited by that level of ethanol (Table 6). Both sedimentation rate and ethanol tolerance increased with storage. This indicates that the physiochemical condition and the genetic property had influence on each other. Sedimentation rate and ethanol tolerance are unique properties of the yeast that makes it exploitable for industrial applications. There were however, significant differences ($P<0.05$) between the sedimentation rate and ethanol tolerance of yeasts in different palm wine samples.

Chemical properties of palm wine yeast

Total acidity of the samples increased with storage (0.03 to 0.09%) (Table 7). Data indicated significant differences ($P<0.05$) in total acidity of samples. PW1-3 samples had higher acidity than the RW1-3 samples. On the other hand, progressive decrease was observed in total soluble solids (Table 8) as sugars were progressively been

Table 8. Time course of total soluble solid (°Brix) of palm wine samples.

Sample code	Time (h)				
	0	6	12	18	24
PW1	14.34	14.3	12.22	11.42	11.4
PW2	15.32	15.24	13.57	11.52	11.51
PW3	14.56	14.11	12.1	11.78	11.58
RW1	13.32	13.3	11.24	10.24	10.11
RW2	13.45	13.41	11.32	10.26	10.18
RW3	13.72	13.52	11.57	10.71	10.5

Table 9. Time course of reducing sugar (%) levels in palm wine samples.

Sample code	Time (h)				
	0	6	12	18	24
PW1	3.12	3.12	2.93	0.92	0.72
PW2	3.12	3.12	2.68	0.85	0.58
PW3	3.52	3.52	2.98	1.28	0.82
RW1	3.09	3.09	2.72	0.83	0.72
RW2	3.06	3.06	2.65	0.94	0.73
RW3	3.1	3.1	2.75	1.29	0.8

Table 10. Time course of total sugars (%) in palm wine samples.

Sample code	Time (h)				
	0	6	12	18	24
PW1	15.78	15.25	9.33	8.35	7.35
PW2	16.94	16.53	10.42	9.48	7.28
PW3	16.38	16.12	11.33	10.66	7.52
RW1	14.52	14.32	9.34	8.23	6.33
RW2	14.33	14.12	9.78	8.21	6.48
RW3	14.65	14.31	9.25	8.42	6.51

assimilated by the yeasts. The rate of assimilation of sugars varied among the test samples. Reducing sugar content also reduced progressively with storage (Table 9). There were no significant differences (P≥0.05) in the reducing sugar content among samples and all samples showed similar trend in reducing sugar content and utilization. The total sugar content showed a similar pattern of decrease after 24 h (14 to 6%) (Table 10). These decreases indicated high viability of palm wine yeasts and the potential to utilize them in industrial applications.

Properties of palm wine yeast in wine production

The purified yeasts from palm wine showed highly viable cells and good metabolic activity during grape must fermentation. Fermentation resulted in increase in temperature (28 to 32°C) and reduction in pH (4.3 to 3.1) (Table 11). The catabolic processes of sugars by yeast cells resulted in metabolic heat that ultimately increased the temperature, while at the same time reducing the pH. These metabolic activities resulted in the concomitant production of alcohol (Figure 1). The pattern of alcohol production by the *Saccharomyces* species were different but not significant (P≥0.05) even from the commercial wine yeast.

The specific gravity of the wines decreased gradually during fermentation in all the yeast species. Final specific gravity values of 0.09 kgm^{-3} in *S. cerevisive* and commercial yeast wine sample and 0.91 kgm^{-3} in *S. carlsbengensis* and *S. globosus* wine samples were observed at the end of fermentation (Figure 2). Differences in the specific gravity values among the different wines were not found to be significant (P≥0.05).

Total solid concentration in the wines decreased consistently during fermentation (21 to 5%) (Figure 3). All the yeast species had similar trend of total solid reduction

Table 11. Variation in temperature (°C) and pH during fermentation of wine using palm wine yeasts.

Time (day)	Palm wine yeast			
	S. cerevisiae	***S. globosus***	***S. carlsbengensis***	**Commercial yeast**
0	28 (4.3)	28 (4.3)	28 (4.3)	28 (4.3)
2	30 (3.5)	29 (4.1)	30 (4.0)	30 (4.0)
4	31 (3.4)	30 (3.6)	31 (3.8)	30 (3.7)
6	31 (3.1)	30 (3.4)	31 (3.5)	31 (3.4)
8	32 (3.1)	31 (3.3)	31 (3.5)	32 (3.2)
10	32 (3.1)	31 (3.3)	31 (3.3)	31 (3.2)

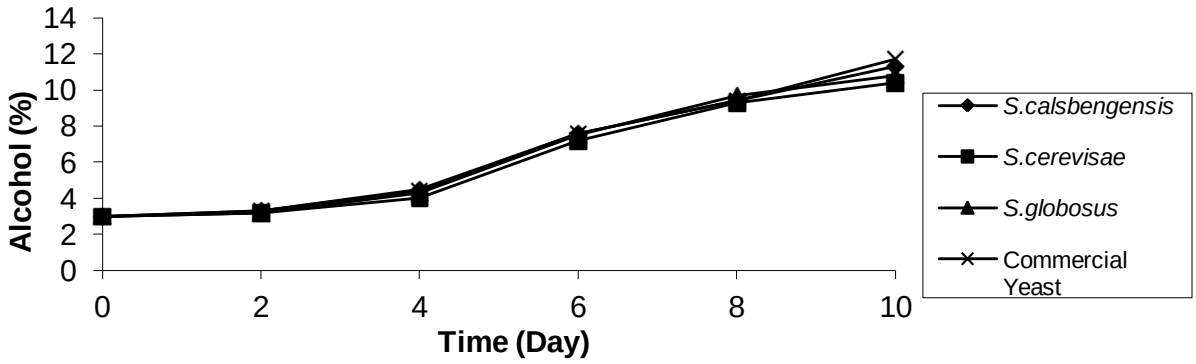

Figure 1. Variations in alcohol content of grapewine fermented with palmwine yeasts.

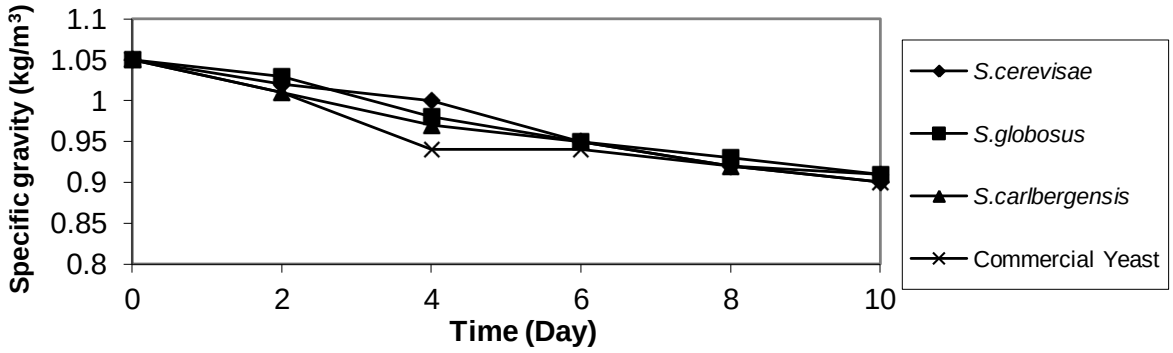

Figure 2. Variations in specific gravity of grape wine fermented with palm wine yeasts.

during fermentation. Total solid values among samples were not significant (P≥0.05). The grape fruits had enough sugar which is a prerequisite for alcohol fermentation and high alcohol production. Titratable acidity increased during fermentation from 0.44 to 0.82% (Figure 4). The differences in the final acidity after fermentation were not significant in the test samples and the control. These acidity values of the fermented wine agreed with those published by Snell and Eltre (1974). Similarly, volatile acidity of the samples followed a similar pattern of increase. The final volatile acidity ranged from 0.60% in *S. globosus* wine to 0.64 in *S. carlsbengensis* fermented wine (Figure 5). The wine fermented by commercial yeasts had volatile acidity of 0.65%. These differences in values were also not significant (P≥0.05).

In the present study, the performances of the yeast cells were compared to that of the commercial yeast. This was evident in high alcohol content and the other various properties measured. Their alcohol levels were high enough for ester formation which contributed to the flavour of wines. The quality of the finished wine may be influenced by the other by-products of fermentation other than ethanol depending on their composition (Plutowska and Wardencki, 2008; Duarte et al., 2010).

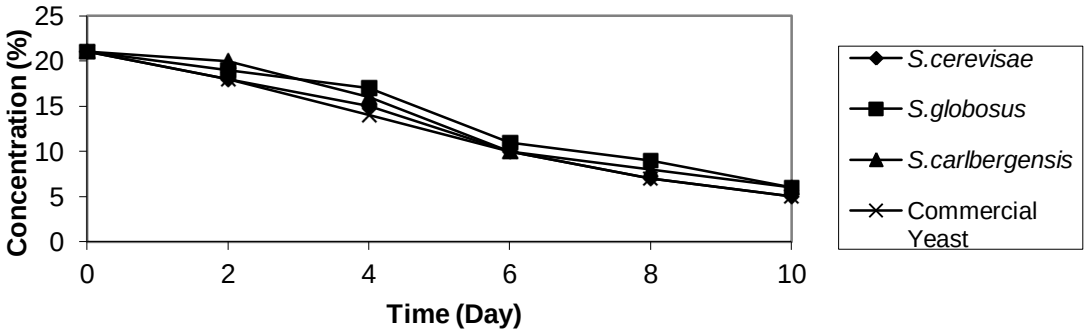

Figure 3. Variations in total solid concentration of grape wine fermented with palm wine yeasts.

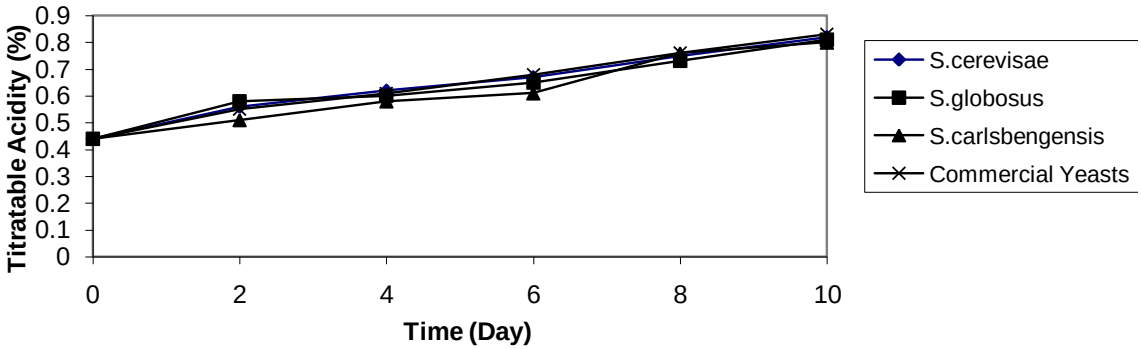

Figure 4. Variations in titratable acidity of grape wine fermented with palm wine yeasts.

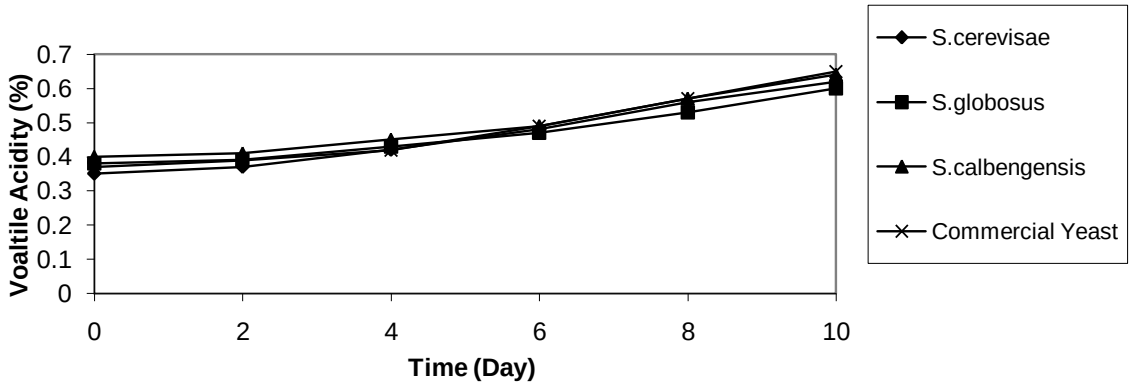

Figure 5. Variations in volatile acidity of grape wine fermented with palm wine yeasts.

Conclusion

This work evaluated the properties of palm wine yeasts obtained from oil palm and raffia palm for application in wine production from grape fruits. The palm wine yeasts isolated were *S. cerevisiae*, *S. globosus* and *S. carlsbengensis*. The yeast cells had high viability, sedimentation rate and ethanol tolerance. The yeast species produced high levels of alcohol in the palm wine. These desirable properties were expressed when the yeast species were used to ferment grape must. A good wine was produced from each of the yeast species isolated from palm wine. The wine samples were characteristically similar to that fermented with commercial wine yeast.

This study therefore indicates that palm wine yeasts can be purified and used to make fruit wines in place of the expensive commercial wine yeasts. Process optimisation and scale up will be required for a better application of this study.

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