

*Full Length Research Paper*

# Characterisation of palm wine yeast isolates for industrial utilisation

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Investigations were carried out on yeasts isolated from palm wines obtained from South Eastern Nigeria. The isolates were characterised for certain attributes necessary for ethanol production. Isolations were made from 600 hour-aged wines. The attributes investigated included ethanol tolerance and sedimentation rates. The effect of certain locally available supplements on ethanol tolerance was also investigated. Nine strains of *Saccharomyces cerevisiae*, two strains of *S. globosus*, and two strains of *Hanseniaspora uvarum* were isolated in this study. Results of the ethanol tolerance revealed a range of 10-20% (v/v), ethanol tolerance for the isolates. The sedimentation rates varied from 55.5 to 93.1%. Addition of local supplements enhanced ethanol tolerance of the isolates.

**Key words:** Palm wine yeast, industrial utilization, ethanol.

## INTRODUCTION

Palm wine is the fermented sap of the tropical plants of the *palmae* family. It is produced and consumed in very large quantities in the southeastern Nigeria. It contains nutritionally important components including amino acids, proteins, vitamins and sugars (Okafor, 1987). These make this wine a veritable medium for the growth of a consortium of microorganisms, whose growth in turn, change the physicochemical conditions of the wine, giving rise to competition and successions of organisms.

Many workers have indeed carried out studies aimed at isolating and exploiting palm wine yeasts for industrial processes. These include for baking, portable ethanol production and single cell protein production.

Ogbonna (1984) and Onyedinma (1983) used palm wine isolates of *Saccharomyces cerevisiae* to produce artificial palm wine and beer, respectively. Very few efforts have been made at characterizing these yeasts for

fuel ethanol production. Despite the continuing research efforts at utilising bacteria for ethanol production (Ingram and Burtke 1984), the yeast is still the primary choice for fermentation (Chandra and Panchal, 2003).

In selecting yeasts for the efficient production of fuel ethanol (as opposed to portable ethanol), workers have set out certain requirements for these yeasts. These include being ethanol tolerant, osmotolerant, acid tolerant, and possession of flocculating properties depending on process requirements (Stewart et al., 1984). This work aims at investigating palm wine for yeasts possessing certain attributes that could be necessary for efficient fuel ethanol production.

## MATERIALS AND METHODS

Fresh palm wine samples obtained from raffia palm (*Raphia raphia*) and oil palm (*Elaeagnis guineensis*) were collected in sterile 2.5 litre sample containers from palm wine tapers in some towns in Eastern Nigeria within 30-60 min of tapping. The sources and codes of the wines are as shown in Table 1. The samples were immediately transported to the laboratory for analysis in iced coolers. The wines were stored for 600 h in opaque conical flasks plugged with cotton

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**Table 1:** Sources and codes for palm wine samples.

Sample Code	Type of wine	Source
R <sub>1</sub>	Raffia Palm	Aronta Mbutu, Imo State
R <sub>2</sub>	Raffia Palm	Obowo, Imo State
P <sub>1</sub>	Oil Palm	Nsukka Urban, Enugu State
P <sub>2</sub>	Oil Palm	Ibagwa, Enugu State.

wool at 28-30°C to encourage fermentation.

### Isolation of ethanol tolerant yeasts

The 25-day-old wine samples were centrifuged in sterile centrifuge bottles for 5 min at low speed. One ml of the serially diluted sediment is inoculated by streaking on plates of GYA (These were supplemented with chloramphenicol (0.05 mg/l; Nwachukwu, 2001) and incubated at 28°C for 24 h (Okafor, 1972). The yeast colonies that developed are isolated and purified by further streaking on GYA. Standard methods and tables were employed in their identification, as described below.

### Yeast Identification

Isolation and identification of yeasts was by the use of standard morphological and physiological tests and identification keys described by Barnett et al. (1990) and Kregger Van-Rij (1987). Incubation was at 28°C under aerobic and conditions. The morphological and cultural characteristics of the yeasts were studied after isolation on glucose yeast agar (GYA) and yeast malt agar (YMA) (Biolife). These tests included morphology, surface characteristics, presence of pseudohyphae, ascospore formation and vegetative reproduction. Fermentative tests included sugars such as glucose, galactose, sucrose maltose, cellobiose, trehalose, lactose, raffinose, soluble starch, D-xylose, L-arabinose, and D-ribose. Others tests include nitrate assimilation, growth in 10% NaCl + 50% glucose in yeast extract, growth at 37°C and growth in 50% w/w glucose yeast extract.

### Sedimentation rate determination

Cultures of the yeasts grown on malt yeast extract glucose peptone medium for 24 h were used for the experiment. The yeasts were harvested by high-speed centrifugation at 1600 rpm for 10 min and used to prepare a standard cell suspension of  $1.5 \times 10^8$  cells/ml in 0.9% NaCl (by aid of Neubauer counting chambers) solution. These were then used for measuring reduction/decrease in optical density reading over a period of 2 h at 650 nm using a corning colorimeter the sedimentation rate is expressed by the formula.

The sedimentation properties (Patil et al., 1981) of the ethanol tolerant yeasts were compared with a brewing strain from an International brewery in Jos Nigeria.

$$\% \text{ Sedimentation} = \frac{\text{Total drop in reading} \times 100\%}{\text{Colorimeter reading at 0 h}}$$

### Determination of ethanol tolerance

This was determined based on visual assessment of turbidity and viability in tubes of basal medium (Ohta et al., 1981) containing graded concentrations of 10-25% (v/v) ethanol inoculated with fresh yeast cells at  $1.5 \times 10^8$  cells/150 ml media. The cells were treated as in the sedimentation rate determination (Bhajpai et al., 1988). The tubes were incubated at 28°C and readings taken after 40 h (Sinner et al., 1961).

### Effect of soybean (*Glycine max*) and palm kernel from *Elaeagnis guineensis* on ethanol tolerance

The effect of soybean and palm kernel on ethanol tolerance was studied with yeast isolate designates R<sub>e</sub>, P<sub>a</sub>, P<sub>d</sub> and P<sub>f</sub>. The investigation was carried out using the method previously described by Ohta et al. (1981). The samples used as supplements were of food grade bought from the Relief Market Owerri, Nigeria. These were hand milled to very fine texture. These were then added to already prepared basal medium (as above) at the rate of 4 g/L of medium. Both are then sterilized at 115°C for 10 min. The sterilized media was subsequently filtered through pre-sterilized cotton wool to obtain a clear medium. This clear medium was then inoculated with actively growing cultures as in the test for ethanol tolerance above.

## RESULTS

### Isolation of ethanol tolerant yeasts

Ethanol tolerant yeasts were recovered from the wines after 600 h. These included six isolates of *S. cerevisiae* and one strain of *S. globosus* from the raffia palm wines. Three strains of *S. cerevisiae*, two isolates of *H. uvarum* and one isolate of *S. globosus* from oil palm wine (see Table 2). The frequencies of isolation were 62.9%, 15.3%, and 15.3% respectively for *S. cerevisiae*, *S. globosus* and *H. uvarum*.

### Sedimentation rate

The yeast isolate P<sub>d</sub> (*S. cerevisiae*) displayed the highest flocculation/sedimentation rate (93.1%). The lowest sedimentation rate observed was for the yeast designate R<sub>g</sub> (*S. globosus*; 54.5%). (Table 2). The sedimentation rate of the brewing strain of *Saccharomyces carlsbergensis* was 78.5% after 2 h. No significant difference was observed in sedimentation rates recorded for raffia wine and oil wine isolates using the ANOVA P = 0.05 (Nwachukwu and Egbulun, 2000).

### Ethanol tolerance of the yeasts isolates at 600 h

The ethanol tolerance of the isolated yeasts after 600 h revealed a wide range of tolerance levels, between 10 and 20% (v/v) ethanol for the isolates. The yeast designate R<sub>e</sub> (*S. cerevisiae*) tolerated the highest-level

**Table 2.** Characteristics of ethanol tolerant palm wine yeasts isolated at 600 h.

Isolate	Source	Name	Sedimentation rate %	Ethanol tolerance (% v/v ethanol)
R <sub>a</sub>	Raffia palm	<i>S. cerevisiae</i>	81.7	15
R <sub>b</sub>	''	''	76.7	12.0
R <sub>c</sub>	''	''	83.6	12.0
R <sub>d</sub>	''	''	59.6	11.0
R <sub>e</sub>	''	''	64.2	20.0
R <sub>f</sub>	''	''	58.0	12.0
R <sub>g</sub>	''	<i>S. globosus</i>	55.5	13.0
P <sub>a</sub>	Oil Palm	<i>S. cerevisiae</i>	86.9	19.0
P <sub>b</sub>	''	''	84.6	17.0
P <sub>c</sub>	''	<i>H. uvarum</i>	61.1	12.0
P <sub>d</sub>	''	<i>S. cerevisiae</i>	93.1	18.0
P <sub>e</sub>	''	<i>H. uvarum</i>	61.3	12.0
P <sub>f</sub>	''	<i>S. globosus</i>	64.8	10.0
	Brewery	<i>S. carlsbengensis</i>	78.5	-

**Table 3.** The effect of certain supplements on ethanol tolerance.

Yeast	Ethanol tolerance (% v/v ethanol)		
	Soy bean	Palm kernel	Soy bean + Palm kernel
R <sub>e</sub>	22	22	22
P <sub>g</sub>	21	21	21
P <sub>d</sub>	21	21	21
P <sub>f</sub>	14	15	15

ethanol. It showed growth in 20% (v/v) ethanol. The least tolerant isolate was P<sub>f</sub>, tolerating only 10% v/v ethanol (Table 2).

### Effect of supplements on ethanol tolerance

The effect of soybean and palm kernel on ethanol tolerance was that of enhancement of the tolerance level of all the tested isolates. The isolate designate R<sub>e</sub> appreciated from a tolerance level of 20 to 22% (v/v) ethanol while the yeast designate P<sub>f</sub> improved from 10 to 15% (v/v) ethanol. A combination of the two supplements did not lead to further enhancement of tolerance (Table, 3).

## DISCUSSION

The organisms isolated after 25 days clearly tolerated the physicochemical conditions of the wines from which they were isolated. They are thus better adapted to those conditions than the cocktail of the other organisms that had been previously reported at the onset of palm wine

fermentation. The increased rate of isolation of saccharomyces yeasts at 25 days further proves them the most ethanol tolerant organisms available (Nwogu, 1983). The level of ethanol tolerance between 15 and 20% compared favourably with levels already described for brewing, sake and distillers yeasts (Casey and Ingledew, 1986).

The sedimentation and flocculation rate of yeasts is of great importance in choosing yeasts for industrial fermentations (Patil et al., 1989). In this study, five yeast isolates flocculated better than the *S. carlsbengensis* obtained from a brewery. The implication is the possibility of isolating from palm wine, novel yeasts, which, with or without further work may be used for various industrial processes which otherwise uses imported yeasts. The supplements evaluated enhanced ethanol tolerance of the tested yeast strains significantly. It may be due to their content of certain saturated and unsaturated, oils, vitamins and other essential nutrients, important in stress tolerance, which helped the yeasts survive such extreme condition. Fatty acids however has been proven to improve ethanol tolerance and consequently product yield (Thomas et al., 1987)

This study raises very strong hope for the industrial ethanol fermentation industry of reduced production costs using locally available substances. Further work is at conclusion stage on the suitability of these isolated strains (including their genetic modifications) and optimization of processes for their use in fermenting some locally available organic waste substrates to ethanol.

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