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Research Article

Study on the Wine Production Attributes of *Saccharomyces cerevisiae* Isolated from Sucrose Enriched Palm Wine and Non-Sucrose Enriched Palm Wine

Agwuna, Linus Chukwuma, Umeh, Sophina Ogonna and Egbuim, Timothy Chukwudiegwu

Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, P.M.B 5025, Awka, Anambra State, Nigeria.
***Corresponding author:** linuschukwuma10@gmail.com

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ABSTRACT

Saccharomyces cerevisiae is a common yeast of economic importance in food and brewing industries. They are obtained commercially from foreign suppliers and are costly; their high cost renders the cost of the brewed products too high for consumers. In this study, wine production attributes of *Saccharomyces cerevisiae* isolated from palm wine enriched with sucrose and fresh palm wine not enriched with sucrose, were checked. The isolates (SCE for palm wine enriched with sucrose and SCN for palm wine not enriched with sucrose) were characterized based on morphology and sugar fermentation tests. The attributes important for wine production investigated include; ability to ferment simple sugars, resistance to different stress conditions, ethanol tolerance, growth at elevated temperatures, flocculation ability, viability and low or no hydrogen sulfide production. Results showed that none of the isolates fermented melibiose and raffinose but they all fermented glucose, maltose, fructose, sucrose and galactose. They survived at different stress conditions of high temperature and cell osmotic pressure in high concentrations of ethanol and sugar, but SCE showed more intense growth. The isolates tolerated 15% (v/v) ethanol with different growth rates; growth was more intensive for SCE and low for SCN. They grew well at the temperature range of 20ºC to 37ºC, but at 45ºC, SCE showed low growth while SCN had no growth. They showed good flocculation ability of 97% and 82% for SCE and SCN, respectively. The results of the viability test showed percentage viability of 96.66% and 83.00% for SCE and SCN, respectively. There was no production of hydrogen sulfide gas for all the isolates. Statistical analysis showed that yeast strains isolated from palm wine enriched with sucrose had greater potential when compared to yeast strain isolated from palm wine not enriched with sucrose in wine production attributes (P<0·05).

Key words: Palm wine, *Saccharomyces cerevisiae*, Wine, Wine production

INTRODUCTION

Wines are un-distilled alcoholic beverages usually made from grapes or other fruits such as peaches, plums or apricots, banana, elderberry or black current etc. which are nutritive, more tasty and mild stimulants (Swami *et al.*, 2014). Wine is a product of alcoholic fermentation by yeast of the juice of ripe grapes or any fruit with a good proportion of sugar (Okafor, 2007). Wine is one of the most recognizable high value-added products from fruits. It can also be used as a substrate for the manufacture of vinegar, a by-product of wine manufacture. Highly acceptable wines can be made from practically all fruits and can be fermented with yeast (Boodle, 2010). Wine has been part of human culture for over 6,000 years, serving dietary and socio-religious functions; its production takes place on every continent and has been enjoyed by many people from peasants to kings and its chemical composition is profoundly influenced by enological techniques **(**Nikhil *et al.*, 2009). Wine is produced by fermentation of the juice of ripe grapes using yeasts (*Saccharomyces cerevisiae)*; they digest sugars found in fruit juice, producing alcohol and carbon dioxide gas in the process.

Palm wine is the collective name for a group of alcoholic beverages produced by the natural fermentation of the sap obtained from various tropical plants of the *Palmae* family (Okafor, 1978). It is produced and consumed in very large quantities in the South-Eastern Nigeria and other parts of the world particularly Asia and Southern America (Nwachukwu *et al.*, 2016). According to Ikegwu (2014), Palm wine is extracted in South-Eastern Nigeria mostly from oil palm (*Elaeis guineensis*) and

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raphia palm (*Raphia hookeri* and *Raphia vinifera*). Although palm wine may be presented in a variety of flavours, ranging from sweet (unfermented) to sour (fermented) and vinegary, it is mostly enjoyed by people when sweet (Elijah *et al.*, 2010). Most studies on palm wine have reported its potentials as a source of yeast for the fermentation industries. Yeasts play a prominent role in wine fermentations, which can strongly affect the quality and flavour of the final product (Querol and Fleet, 2006). Commercial wine yeast strains are primarily selected for their ability to ferment grape must to dryness, whereby residual sugars are reduced to less than 4 g/L (Pretorius, 2000). For complete fermentation, the yeast strain is required to adapt and respond to a multitude of environmental stresses, either simultaneously or successively (Fairbairn, 2012).

Among several yeasts, *Saccharomyces cerevisiae and S. bayanus var. uvarum* are the most important species present during the fermentation process (Pretorius, 2000; Querol and Fleet, 2006). According to Arroyo-López *et al*. (2009), recently, interspecific hybrid strains between Saccharomyces species have been described as involved in wine fermentations. González *et al*. (2006) described wine yeast hybrids between the species *S. cerevisiae* × *Saccharomyces kudriavzevii* and *S. cerevisiae* × *S. bayanus*. Several Saccharomyces hybrids are used as commercial wine yeast (González *et al*., 2006; Bradbury *et al*., 2006), for instance, the hybrid *S. cerevisiae* × *S. kudriavzevii* Lalvin W27 (Arroyo-López *et al.,* 2009). Hybrid strains appear well adapted to the stress conditions (low pH, high sugar concentration and ethanol content) occurring during wine fermentations (Belloch *et al*., 2008), and their enological characterization confirmed their interesting properties according to the new trends in winemaking (González *et al*., 2006).

According to Arroyo-López *et al.* (2009), different factors can affect the course of fermentation, influencing the ecology and adaptation of the microbiota present. The temperature is a variable that directly affects the growth rate of the microorganisms (Charoenchai *et al*., 1998), and the final composition of wine (Torija *et al*., 2003). Another significant variable is the concentration of fermentable sugars in musts, ranging between 125 and 250 g/L (Fleet and Heard, 1993). It is likely that the initial concentrations of glucose and fructose (main grape sugars) will selectively influence the species and strains of yeast present during fermentation (Arroyo-López *et al.,* 2009). Must pH, ranging from 2.75 to 4.25, is also considered an important factor for the survival and growth of yeasts (Fleet and Heard, 1993). Due to climatic change, glucose and fructose are increasing their concentrations in grapes meanwhile the acidity decreases, affecting the global wine quality (Jones *et al*., 2008). This fact according to Arroyo-López *et al.* (2009) originates musts with a higher initial amount of fermentable sugars and higher pH. Therefore, these factors must be studied critically with more details, especially the interactions between them and their influence on fermentation microorganisms. Several studies have modelled the wine fermentation process (Malherbe *et al*., 2004; Colombié *et al*., 2005; Coleman *et al*., 2007), but it is more important to select and improve the features of yeast to enable it to adapt to those changing variables during wine production.

Although wine industries are available in Nigeria, they are expensive to establish especially using imported *Saccharomyces cerevisiae* and materials; this makes the product very expensive for most families. In Nigeria, large quantities of palm wine are produced daily and are difficult to preserve for a considerable period of time. There is, therefore, the need for alternative usage and applications. The economic situation in our country demands the adoption of local materials for the production of products; this will help improve the economy of the nation.

MATERIALS AND METHODS

Samples

Palm wine was obtained from palm wine tapper in Oji River Local Government Area, Enugu State about 10 minutes after tapping, and was taken to the laboratory in a sterile bottle. It was conveyed to the laboratory in a basin containing ice.

Enrichment of palm wine with sucrose

The method described by Agu *et al*. (1993) was used for enrichment of the palm wine with sucrose. The palm wine was held at room temperature, and then samples (100 ml) were withdrawn daily for 7 days. After each day's withdrawal, extra sucrose was added to the bulk sample to increase the concentration to 3% (w/v).

Inoculum preparation

The method of Fagbemi and Ijah (2005) as reported by Umeh *et al.* (2015) was used to isolate and identify the yeast strain from the enriched palm wine and un-enriched palm wine. Each sample was streaked on Sabouraud dextrose agar (SDA) medium containing 0.05 mg/ml chloramphenicol (to inhibit bacterial growth) and incubated for 48 h at room temperature. Different isolated colonies were replicated on fresh plates of Yeast Peptone Dextrose (YPD) to get pure cultures of the isolates. The isolated yeast cells were characterized using colony shape and colour, colony surface and appearance, vegetative morphology, types of budding and sugar utilization. The choice isolate was stored in a slant culture, and preserved in a refrigerator maintained at 4°C.

The yeast isolates were coded as follows: •SCE (*Saccharomyces cerevisiae* from Enriched palm wine); **•SCN** (*Saccharomyces cerevisiae* from palm wine not enriched)

Microscopic observation

This was carried out as done by Thais *et al*. (2006). A single colony of yeast was suspended in a drop of sterile distilled water placed on a glass slide and smeared until the smear dry off. The smear was then stained using diluted methylene blue dye, air dried and observed under a light microscope at X100 magnification.

Yeast characterization: Sugar fermentative test

In this test, Yeast fermentation broth (YFB) was prepared and the test was conducted as described by Atlas and Parks (1996). They were grown on Yeast fermentation broth (YFB) which contains peptone 7.5 g/L, yeast extract 4.5 g/L, 1 ml of 1.6% (w/v) bromothymol blue as an

indicator and separately 6% (w/v) glucose, sucrose, fructose, maltose, raffinose, but 12% (w/v) melibiose. The yeast cells were grown at 25ºC for 3 days. Durham tubes were placed into the media to trap the carbon dioxide released.

Stress exclusion test

Stress exclusion test was conducted as described by Thais *et al*. (2006). The stress exclusion test was done for 15 days' by incubation onto different media. The ability to grow under different stress conditions was conducted by inoculating the yeast isolates onto Yeast Peptone Glucose (YPG) (10 g/L yeast extract, 10 g/L peptone, 20 g/L glucose and 20 g/L agar) medium and incubated at 25ºC for 3 days. A single colony was then transferred and continuously grown on YPG medium and incubated at 30ºC for another 3 days, before further subculture of the isolated yeast colony on YPG medium containing 8% (v/v) ethanol and incubated at 30ºC for 3 days. A single isolated colony on YPG with 8% ethanol was further subcultured on YPG supplemented with 20% (w/v) glucose and incubated under the same conditions as above. Finally, the yeast cells were transferred onto YP (10 g/L yeast extract, 10 g/L peptone) medium supplemented with 20% (w/v) sucrose and 8% (v/v) ethanol and incubated under the same conditions as above.

Ethanol tolerance test

The ability of the isolated yeast strains to grow in higher ethanol concentrated media was tested by growing them in YPG broth containing 3 different concentrations of ethanol, 10%, 13% and 15% (v/v), respectively and incubated at 30ºC for 72 hours (Thais *et al*., 2006).

Temperature tolerance test

The ability of the yeast isolates to grow at higher temperatures was verified by plating the yeast isolates onto YPG medium and incubated at 4 different temperatures i.e. 25, 30, 37 and 45ºC for 72 hours (Thais *et al*., 2006).

Flocculation test

The flocculation test (Helm's test) was performed according to the methods of D'Hautcourt and Smart (1999). A suspension of yeast cells was centrifuged at 3000 rpm for 5 minutes. The pellets were washed 3 times in distilled H_2O. The cells were resuspended in distilled H_2O and diluted to achieve a cell concentration of $1x10^8$ cells/ml. 1 ml of cell suspension was aspirated into six eppendorf tubes, three of the tubes were labelled A and three labelled B.

Tubes A – The eppendorf tubes were centrifuged at 3,000 rpm for 4 minute and the supernatant removed. 1 ml of 0.05M EDTA was added and resuspended by vortexing for 15 seconds. The tubes were inverted five times and left to sediment for 20 minutes. The top 100 µl of suspension was carefully removed using a pipette and put directly into a cuvette. 900 µl of distilled H_2O was added and the OD_{600} was measured. The spectrophotometer was zeroed with distilled H_2O.

Tube B - The eppendorf tubes were centrifuged at 3,000 rpm for 4 minute and the supernatant removed. 1 ml of washing solution Helms A ($CaSO_4$ 0.51 g/l) was added to the cell pellet and resuspended by vortexing for 15 seconds. The eppendorf tubes were centrifuged again and the supernatant discarded. The cell pellets were resuspended in 1 ml of suspension solution Helms B ($CaSO_4$ 0.51 g/l: sodium acetate 6.8 g/l: glacial acetic acid 4.05 g/l: ethanol 4% in one litre of deionised water). Where appropriate, mannose, maltose or glucose was added to achieve sugar inhibition profiling (D'Hautcourt and Smart, 1999). The tubes were inverted five times and left to sediment for 20 minutes. The top 100 µl of suspension was carefully removed using a pipette and put directly into a cuvette. 900 µl of distilled H_2O was added and the OD_{600} was measured. The spectrophotometer was zeroed with distilled H_2O.

In order to calculate the % flocculation, the mean OD_{600} of the A tubes was determined. The % flocculation was then determined using the following equation.

$$\frac{(A - B)}{A} \times 100 = \% \text{ Flocculation}$$

Yeast viability and consistency

Five milliliters of fermenting wort was placed in a test tube, two drops of methylene blue was added to the sample, the sample was vigorously shaken, and two drops of the stained sample was placed on the haemocytometer, mounted on the microscope and observed. The dead cells which absorbed the stain and retained the blue stain of the methylene blue were expressed as the percentage of the living cells which absorbed the blue colour of the methylene blue and digested it to become colourless (Singgih, 1998).

Hydrogen sulfide production test

The ability of the yeast to produce hydrogen sulphide (H_2S) was examined by growing the yeast isolates on lead acetate medium (40 g/L glucose, 5 g/L yeast extract, 3 g/L peptone, 0.2 g/L ammonium sulfate, 1 g/L lead acetate and 20 g/L agar) and incubated at 30ºC for 10 days as described by Ono *et al.* (1991).

RESULTS AND DISCUSSION

The two yeast strains were separately isolated from palm wine enriched with sucrose and the one not enriched. They were coded SCE and SCN, i.e. yeast strains from palm wine enriched with sucrose and yeast strains from palm wine not enriched with sucrose, respectively. The result of their morphology and biochemical properties showed that they are *Saccharomyces cerevisiae*. Based on colony shape and colour, SCE was spherical and creamy while SCN was also spherical but white to creamy in colour, apparently, they were all flat and smooth. SCE was a single budding yeast while SCN was multi budding. This is in agreement with the work of Berhanu *et al*. (2017) who worked on isolation and characterization of *S. cerevisiae* from "Tella", they reported *S. cerevisiae* to be spherical and creamy. A similar finding was also observed by Kevin (2005) who reported that typical *S. cerevisiae* colonies were creamy and spherical in shape.

One of the valuable characteristics of yeast is its ability to ferment simple sugars. According to the findings of this study, the various yeast isolates behaved in similar ways as far as their fermentative capability of simple sugars is

concerned. The isolated yeast (SCE and SCN) showed good fermentative capability. They fermented all sugars tested on except melibiose and raffinose (Table 1). This also complies with the report of Berhanu *et al*. (2017) who worked on isolation and characterization of *S. cerevisiae* from "Tella", their report of sugar fermentation by *Saccharomyces cerevisiae* is the same as recorded in this study. Moreover, the finding of the present study provides a promising source of good wine yeast. The findings is also in agreement with the report that 89-92% of the total *Saccharomyces* spp found in palm wine is *Saccharomyces cerevisiae* and that palm wine is the major source of *Saccharomyces cerevisiae* (Okoli and Ezenweke, 1989).

During fermentation for wine production, the yeast usually does not find an environment of optimal conditions, it's being continuously exposed to several stress conditions, especially osmotic and ethanol stress (Querol *et al*., 2003). The isolated yeast cells were grown continuously for 15 days to observe for cell viability due to each stress condition. The strains were able to grow on medium (YP) containing 20% (w/v) glucose and 8% (v/v) ethanol after incubation at 30°C. Glucose is the carbon source of first choice for *S. cerevisiae* but is also able to repress genes that code for metabolic enzymes as invertase (Gancedo, 1998). The findings were in agreement with Pataro *et al*. (2000) who reported that most of *S. cerevisiae* strains isolated from traditional fermentation processes were physiologically adapted to extreme conditions. In this case, the resistance to glucose repression could be interesting for wine production as well as a high invertase activity (Pataro *et al*., 1998).

The ethanol stress is probably one of the most interesting conditions to be analyzed due to high amount of this substance produced during the wine fermentation process (Chi and Ameborg, 2000). Ethanol is the main extracellular metabolite of *S. cerevisiae* in anaerobic fermentation. It exerts a very notable influence on growth velocity and fermentation rate of yeasts. In this study, ethanol tolerance (15%) of SCE was greater than SCN yeast strains. It is a well-documented fact in literature that different *S. cerevisiae* isolates have different capacities for resisting concentrations of alcohol. *S. cerevisiae* isolates of this study were in line with the report of Chi and Ameborg (2000) in respect to capacity of alcohol resistance. The higher ethanol tolerance of SCE is however due to enrichment with sucrose.

High flocculation capability of wine yeast strains is another parameter for selection of yeast for commercial purpose. Flocculation occurs because of interactions between surface proteins on one cell and carbohydrate receptors on another cell (D'Hautcourt and Smart, 1999). Determination of the flocculation behaviour of yeast isolates is significant to get appropriate yeast isolates for wine production. In the present findings, flocculation capacity of SCE (97%) was higher than SCN (82%) and the result differs statistically ($p<0.05$). Flocculation is an important characteristic that allows easy separation of the final product at the end of the fermentation without additional filtration/centrifugation steps and also allows the utilization of immobilized yeasts on fermentation processes (Berhanu *et al*., 2017).

Table 1: Morphology and sugar fermentation of the isolates

Yeast	Morphology Identification				Sugar Fermentation						
Isolate	Colony shape and colour	Colony surface and appearance	Vegetative morphology; cell shape arrangement	Budding	Glucose	Maltose	Fructose	Sucrose	Melibiose	Galactose	Raffinose
SCE	Creamy and spherical	Smooth and flat	Spherical cell	Single	+	+	+	+	-	+	-
SCN	White to creamy spherical	Smooth and flat	Spherical elongated cell, Oval cells	Multi polar	+	+	+	+	-	+	-

Table 2: Stress exclusion tests for temperature and cell osmotic pressure in high concentration of ethanol and sugar.

Yeast strain	Growth into different media				
	YPG	Temperature 30%	Ethanol (8%v/v)	YPG (Glucose 20% w/v)	YPS (Sucrose 20% w/v + ethanol 8%v/v
SCE	+++	+++	+++	+++	+++
SCN	+++	+++	+++	+++	++

Key: Intensive growth (+++), moderate growth (++). YPG- yeast peptone glucose medium; YPS- yeast peptone sucrose medium

Table 3: Ethanol and temperature tolerance ability.

Yeast strain	Ethanol tolerance			Temperature tolerance (°C)				
	10%	13%	15%	20	25	30	37	45
SCE	+++	+++	+++	+++	+++	+++	+++	+
SCN	+++	++	+	+++	+++	+++	+++	-

Key: Intensive growth (+++), moderate growth (++), low growth (+), no growth (-)

Table 4: Flocculation test, viability count and hydrogen sulfide test

Yeast strain	Flocculation (%)	Viability (%)	Hydrogen sulphide
SCE	$97.00^{a} \pm 0.54$	$96.66^{a} \pm 0.58$	-
SCN	$82.00^{b} \pm 0.10$	$83.00^{b} \pm 0.10$	-

Response (+), no response (-).

The viability test showed that all the isolates had good viability capacity. SCE had 96.66% while SCN had 83%; though SCE was higher, the result differs statistically ($p<0.05$). The high viable count of the yeast isolates from this study proves that the yeast cells were viable and can carry out good fermentation. Yeasts with high production of hydrogen sulfide are undesirable for wine production because it confers flavour and taste that compromise the quality of the wine obtained (Ribeiro and Horii, 1999). Thus, the yeasts were tested for hydrogen sulfide production; none of the isolates produced hydrogen sulfide.

Conclusions

The present study has provided information on the possibility of improving *S. cerevisiae* through enrichment using sucrose for industrial applications. Although this investigation cannot be considered to be exhaustive, the results obtained showed that yeast from palm wine enriched with sucrose can compete favourably when tested for attributes necessary for wine production.

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