



ISOLATION AND SENSORY EVALUATION OF *SACCHAROMYCES CEREVISIAE* FROM PALM WINE (*ELAEIS GUINNEENSIS*) GOTTEN FROM DIFFERENT SITES IN ENUGU.

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ABSTRACT

Isolation and sensory evaluation of *Saccharomyces cerevisiae* from palm wine gotten from different sites in Enugu were analyzed on bread fermentation. The ability to get quality bread depends on its ingredients, skill and a very good fermentative agent. In this research, palm wine was gotten from four sites in Enugu, together with commercial yeast, which serves as the control yeast and they were cultured at 30°C to isolate *Saccharomyces cerevisiae* from each of the samples using the appropriate identification techniques like the colony morphology, microscopic observations, fermentative ability and temperature tolerance tests. The yeast isolates were then used to bake bread at 180°C for 8 minutes and the organoleptic or sensory properties of the bread like volume, crust, color, internal color, structure, texture, flavor, aroma, crumb clarity and elasticity were analyzed. The yeast isolate from palm wine sample 4 was used to bake bread 5 and it showed the highest mean when compared to volume (4.4), crust (4.0), color (4.3), internal color (4.4), structure (3.3), texture (3.6), flavor (3.7), aroma (4.1) and crumb clarity (3.4) more than the control which had volume (4.3), crust (3.5), color (3.7), internal color (4.0), structure (2.5), texture (3.1), flavor (3.5), aroma (4.0) and crumb clarity (3.4). Although some of the isolates did not have high leavening ability like the control, they influenced the aroma and flavor more than the control. Thus, these isolates may be combined to give varieties of sensory properties on bread and with the right industrial facilities would become an indigenous baker's yeast.

KEYWORDS: *Saccharomyces cerevisiae*, sensory properties, bread fermentation, dough, Agar.

INTRODUCTION

Bread in all its form is an important part of the human diet, but for many people, it is much more. Bread is a staple food from flour or meal mixed with other dry and liquid ingredients, usually combined with a leavening agent and kneaded, shaped into loaves and baked. The aroma and flavor of freshly baked bread remains as appealing today as in years gone by. Bread, along with cereals, rice and pasta, make up the foundation of a healthy diet. Derived from grains, these foods are rich sources of energy (carbohydrate), provide some protein, are economical and are naturally low in fat (Burrier and Legras, 2014). Not only is it an important source of carbohydrates, it's also portable and compact, which helps to explain why it has been an integral part of our diet for thousands of years (Loham, 2012). Bread is traditionally made from flour, water, salt and yeast. It has a honey comb structure and may be regarded as solid foam with a multitude of pockets of carbon dioxide distributed uniformly throughout its bulk (Lean, 2006).

The outer hard portion of bread is called the crust. The crumb's texture is greatly determined by the quality of the pores in the bread (Daobread, 2012). The bread ingredients are formulated to give bread its taste, structure, aroma, texture and nutrients. These ingredients include: flour, liquids, leavening agent, salt, sweeteners, fats or oil and additives (optional). (Burrier and Lucas, 2014; Ashton, 2009; Red Star Yeast Club, 2014; Young and Cauvain, 2007). In Nigeria, bread has become a special food to most families especially during breakfast. In 52 years of Nigeria's history, bread has transformed from being an elitist food to a staple food, mass produced and mass consumed (Laniyan, 2012). Palm wine is the fermented sap of various palm trees especially Palmyra, silver date palm and coconut palms. Palm wine is an alcoholic beverage produced from the sap of various palm tree species and usually consumed in parts of Africa, Asia and South America (Chandrasekhar *et al.*, 2012). In Africa, the sap is most often taken from wild date palms such as *Phoenix sylvestris* (the palmyra) and

Caryota urens, from oil palms such as *Elaeis guineensis*, or from raphia, kithul or nipa palms. The liquid collected, is a cloudy whitish beverage with a sweet alcoholic taste and very short shelf life of only one day. The wine is consumed in a variety of flavors varying from sweet unfermented to sour, fermented and vinegary (Chandrasekhar *et al.*, 2012). The microorganisms in the overnight palm wine have the effect on the palm wine and this effect is due to the increase in their population as well as their activities (Ogbuile *et al.*, 2007). Upon fermentation by the natural microbial flora, the sugar level decreases rapidly as it is converted to alcohol and other products (Obire, 2005). Alcohol has the ability to destroy microorganisms and prevent their multiplication, thus serving as a limiting factor for microbial growth (Bell *et al.*, 2001) (Mentz *et al.*, 2010). Palm wine contains good amount of microorganisms. The types and numbers of organisms encountered vary widely even from tree to tree (Theivendirarajah *et al.*, 1987). From the Microbial analysis of Palm wine, it includes both Yeast and Bacteria. Ezenroye and Okerentugba (2001), reported the genetically and physiologically different isolated yeasts from palm wine. Yeast populations have been reported in the palm wine in concentrations of about 10_4 to 10_7 CFU/mL. Palm wine yeast isolated from freshly tapped palm wine are mainly *Saccharomyces* and *Candida* from different palm trees. The *Saccharomyces spp* identified are as follows: *Schizo saccharomyces pombe*, *Saccharomyces cerevisiae*, *Debaryomyces hansenii* and *Zygosaccharomyces rouxii* (Chandrasekhar *et al.*, 2012). The main aim of this research work is to isolate *Saccharomyces cerevisiae* from palm wine in some towns in Enugu. To check the organoleptic analysis of these yeasts on dough and to cultivate the use of indigenous leavening agents and prevent the high cost of importing baker's yeast from other countries.

MATERIALS AND METHODS

The samples were collected from Mile 9, Obiagu; Eke-Out, Amaechi; Eke-Agbani and Ori-Emene markets all in Enugu State. The commercial yeast, used as control was gotten from Ogbete main market. The freshly tapped palm wine tapped from oil palm (nkwu enu) was purchased from a seller in the sites described above. The fresh palm wine samples were collected using sterile containers and were immediately transported to the laboratory in iced coolers. The samples were stored at 4°C until required for use. 260ml of For preparation of media Isolate: Potato Dextrose Agar was prepared according to manufacturer's guide.

The media was sterilized using the autoclave at 121°C for 15 minutes. The sterile media was poured into the Petri dishes to solidify. This media was used for isolating *Saccharomyces cerevisiae*. For serial dilutions: The test tubes were sterilized in hot oven at 160°C for 1 hour.

Ten sterilized test tubes were filled with 9ml of distilled water. 1ml of each sample (palm wines) was added into the test tube (10^{-1}) using a micropipette. 1ml from the

solution was transferred into a second test tube (10^{-2}) (Cheesbrough, 2005; Ezigbo *et al.*, 2014). 10g of the instant dry yeasts were dissolved in 200mls of water to get the stock. From the stock serial dilution was done just the way the palm wine samples were done for stock dilution of commercial yeast while for sample inoculation, From the dilution of 10^2 , 1ml was inoculated on the media by pour plating in triplicate (Harigan, 1998) at aseptic conditions. The plates were placed at 30°C \pm 2°C for 48 – 72 hours. From the dilution of 10^2 , 1ml was inoculated on the media by pour plating in triplicate (Harigan, 1998) at aseptic conditions. The plates were placed at 30°C \pm 2°C for 48 – 72 hours.

Temperature Tolerance Test

The media used for this biochemical tests is the yeast media called YP (10 g/L yeast extract, 10g/L peptone, 20g/L agar) supplemented with different 200g/L (glucose and sucrose) and 80ml/L ethanol. The YP medium supplemented with 20g/L glucose (YPG). Yeast isolates from the slant cultures were cultured on sterile PDA. A colony, each, was picked from the pure cultures grown on the PDA and were plated in YPG medium and incubated at temperatures 25, 30, 37 and 45°C for 72hrs (Maaruf, 2011).

Fermentation Capacity Test

The media used for this test was carried out using 2ml Yeast Fermentation Broth (4.5g of powdered yeast extract, 7.5g of peptone in 1 litre demineralised water, 50mg/75ml of bromthymol blue in distilled water). 2ml of the YFB media was poured inside tubes containing Durham tubes in addition to different carbon sources 1.0mL sterile carbohydrate solution (glucose, sucrose, fructose and maltose) and incubated at 30°C for 72hours. Yeast isolates from the slant cultures were cultured on sterile PDA. A colony, each, was picked from the pure cultures grown on the PDA and were inoculated in the yeast fermentation broth. The changes from green to yellow indicate that yeast using the carbon sources. A positive and a negative control were also set up for each isolate (Maaruf, 2011).

Cultivation Of Yeast Isolates For Dough Fermentation

Yeast isolates from the slant cultures were cultured on sterile PDA. The isolates were cultured separately at 30°C + or – 2°C in peptone broth medium containing 25% (w/v) glucose in 100ml of conical flask equipped with air locks. The set-up was agitated continuously for 72hours in rotary shaker regulated at 150 rpm. After good growth was observed, the biomass concentrate for each isolate was obtained by centrifuging it in an MSE centrifuge machine at 10,000 for 15 minutes. The resulting cells were then washed with cold sterile distilled water after which it was resuspended in 10ml sterile distilled water.

Dough Fermentation

All the yeast isolates and the control yeast were used to ferment dough by baking bread in order to test for their fermentative ability. A negative control (dough without yeast) was also used. Samples of the dough were prepared according to the basic method described by Young and Cauvian, (2007). The samples were left in a warm place about 30°C for about 1¹/₄ and baked in an oven at 180°C for 8 minutes.

Analysis Of Baked Dough Sample

The baked dough was subjected to various dough sensory properties like volume, crust, color, internal color, structure, texture, flavor, aroma, crumb clarity and elasticity by 10 enlightened judges. Five points grade was used in the analysis starting with Excellent =5, Very Good = 4, Good = 3, Satisfactory = 2 and Poor = 1.

RESULTS

TABLE 1: Isolates recovered from the palm wine samples.

ISOLATES	COLOR	TEXTURE	SHAPE	ELEVATION	SUSPECTED ORGANISMS
SAMPLE 1					
Isolate 1	Creamy	Smooth	circular	Raised	<i>S. cerevisiae</i> Suspected
Isolate 2	White	Smooth	punctiform	Raised	<i>S. cerevisiae</i> Suspected
Isolate 3	White	Rough	Circular	Flat	<i>S. cerevisiae</i> Suspected
Isolate 4	Yellow	Smooth	Circular	Raised	<i>S. cerevisiae</i> Not Suspected
SAMPLE 2					
Isolate 1	White	Smooth	punctiform	Raised	<i>S. cerevisiae</i> Suspected
Isolate 2	Creamy	Smooth	Circular	Raised	<i>S. cerevisiae</i> Suspected
Isolate 3	white	fluffy	Irregular	Raised	<i>S. cerevisiae</i> Not suspected
Isolate 4	Yellow	Smooth	Circular	Raised	<i>S. cerevisiae</i> Not suspected
SAMPLE 3					
Isolate 1	White	Smooth	Circular	Raised	<i>S. cerevisiae</i> Suspected
Isolate 2	Creamy	Smooth	Circular	Raised	<i>S. cerevisiae</i> Suspected
Isolate 3	Yellow	Smooth	Circular	Raised	<i>S. cerevisiae</i> Not Suspected
SAMPLE 4					
Isolate 1	White	Rough	Irregular	Raised	<i>S. cerevisiae</i> Suspected
isolate 2	Creamy	Smooth	Circular	Raised	<i>S. cerevisiae</i> Suspected
Isolate 3	Dark creamy	Rough	Irregular	Flat	<i>S. cerevisiae</i> Suspected
Control sample	Creamy	Smooth	Circular	Raised	<i>S. cerevisiae</i> Suspected

Tables 2: Microscopic observations of the colonies.

COLONIES	MICROSCOPIC OBSERVATION (PRESENCE OF BUDS)
SAMPLE 1	
Isolate 1	+
Isolate 2	-
Isolate 3	-
SAMPLE 2	
Isolate 1	-
Isolate 2	+
SAMPLE 3	
Isolate 1	+
Isolate 2	-
SAMPLE 4	
Isolate 1	-
Isolate 2	+
Isolate 3	-
Control sample	+

KEYPOINTS

+ = Presence of buds

- = Absence of buds.

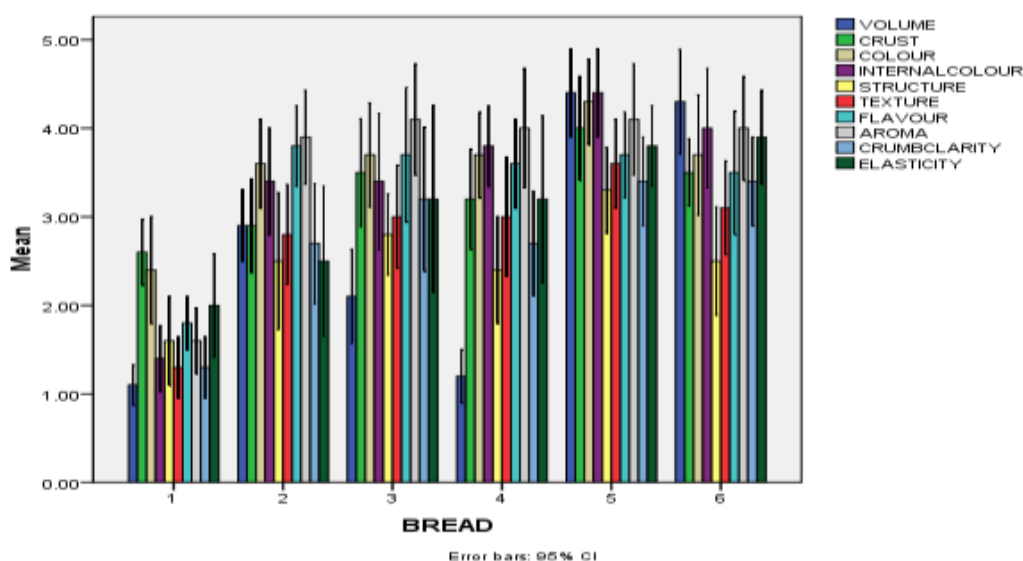


Figure 1: A Graph Of The Mean Against The Different Breads Baked With The Isolate.

DISCUSSION

Fifteen isolates including the control was gotten after the isolation but only 12 isolates were suspected to be *Saccharomyces cerevisiae*. The result of the morphology character is presented on Table 1 above. In this study, fresh palm wine samples were gotten from four different sites in Enugu namely from Mile 9 market, Obiagu; Eke- Agbani market, Agbani; Ori- Emene market, Emene and Eke-Out market, Amaechi. The commercial yeast used as the control was bought from Ogbete Main market. The palm wines were gotten in sterile containers and placed in coolers with ice packs to prevent further fermentation as this could alter the microbial quality of the palm wine because if their growth is not inhibited, the proliferation of yeast continues (fermentation) which makes the palm wine acidic with the release of CO₂ and alcohol. At this stage, the yeasts keep multiplying, but a stage is reached where the alcohol produced starts inhibiting the yeast community and this is achieved at a very high concentration of alcohol and the sugar in the palm wine serving as a carbon source to the yeasts are used up (Chandrasekhar *et al.*, 2012; Obire, 2005; Amoa-Awua *et al.*, 2007; Ogbuile *et al.*, 2007; Bell *et al.*, 2001). The fresh palm wines in the coolers were immediately transported to the lab where they were inoculated in a media prepared the previous day that has shown no signs of contamination. They were inoculated in triplicates to get the various types of microorganisms (colonies) found in palm wine which would be subjected to identification tests to get the required microorganism. Since fungi grow by either branching and elongating or budding which takes longer time, it took three days to observe the growth of fungi on the PDA media which was manifested by formation of 14 colonies (see Table 2).

11 colonies including the control sample were suspected have as same colony morphology as *Saccharomyces cerevisiae* which are: white or cream colored colony that

is circular in shape, with a raised elevation that has both rough and smooth edges. The colonies were compared to the control which was cream in color, circular in shape, raised in elevation and smooth edges. The nine colonies, including the control, were subjected to microscopic observation using the microscope after being stained with lacto-phenol in cotton blue using. Lacto phenol acts as a cleaning agent: as phenol is fungicidal in nature so kills the organism. Lactic acid acts as a preservative and it preserves the structure of fungus, whereas, cotton blue stains the cytoplasm or chitin in the fungal cell walls in light blue color so here we can observe the fungal structure colorless with light blue background. Only five colonies (see Table 3), including the control yeast, were found to be budding yeasts. The cells were oval in shape. Various stages of budding were seen when they were viewed with microscope at $\times 10$ and $\times 40$ respectively. The buds appeared smaller at $\times 10$ but a little bigger at $\times 40$ objectives. Some of the buds were still small while others are bigger than others because they are almost forming out from the original cell. The isolates observed to be *Sacchromyces cerevisiae* were then stored in PDA slants and allowed to grow for three days before the slants were transferred into the refrigerator to prevent further growth. Other biochemical tests were carried out to check the fermentative and temperature capability of the isolates. This was done to understand the yeast behavior. Temperature can influence the growth of the yeast and the test to check the temperature at which the various yeast would grow is important. Table 4 illustrates the various temperatures, the isolates can withstand. At the temperature of 30°C and 25°C, the yeast isolates had intense growth but at 37°C, the yeast isolates 1 and 3, did not show intense growth but moderate growth. Those that were able to grow at high temperature showed that they may be used in bread making to speed up the proofing process, increased carbon dioxide production and formation of flavor and aroma (Ma'aruf *et al.*, 2011). The media used for this test

was the Yeast Peptone Glycerol which provided the environment that is undergoing fermentation and compares the yeast behavior with the various temperatures.

The yeast fermentative capacity test was used to check the fermentative ability of the test isolate. Yeasts bring about fermentation whenever it comes to a favorable environment i.e. with a sugar source. Sugar's main function is to provide food for the yeast. The various sugars used for the tests are the sugars found in dough and so the yeasts' ability to ferment them will show that they possess the various enzymes which ferment these sugars. The flour contains starch with the enzyme, Amylase, which was produced during the milling process. Once water comes in contact with the flour, the action of the enzyme is activated which turns the starch into maltose. The enzymes possessed by the yeasts then come to play, first starting off with maltase which converts maltose to simple sugar like the glucose which is then acted upon by zymase to give carbon dioxide and alcohol. But if sucrose (table sugar) was added in the dough, the yeast then provides the enzyme, invertase, which breaks it down to glucose and fructose, then subsequently to carbon dioxide and alcohol. Therefore, yeasts usually used for baking should possess the enzymes: maltase, zymase and invertase to be able to break down the various sugars and give the end products which are carbon dioxide and alcohol. With these in mind, it goes to show that the fermentative capacity test was used to show if the different yeast possess these enzymes that can break these sugars. Table 5 shows the results of the fermentative capacity test, with the yeast isolates fermenting the sugars with acid and gas production shown by the acid production which produced a colour change and the spaces on the Durham tubes. The media used for the fermentative test was the Yeast Fermentation Broth, with bromthymol blue, as the indicator. The capacities to ferment the various sugars were manifested by the color change from green to yellow and gas production shown by Durham tubes. The color change was due to the acids produced during the fermentation together with the other by-products. The fermentative capacity test agrees with Cofalec, (2014); Ma'aruf, (2011) and Chukwuka, (2013). The time interval at which these isolates fermented the sugars are also of great importance as it could determine the rate of fermentation of the various isolates. Most of the isolates were able to ferment glucose, sucrose and fructose within few hours that they were inoculated. Most of them took fermenting maltose like up to 1 day. Although isolate 4 was able to ferment within few hours followed by the control yeast and the others followed sequentially except for isolate 3 that took a whole day to ferment the sugar. The fermentation intensity depends on the form of the yeast and the availability of fermentable sugars in the flour, including maltose produced by starch hydrolysis (Hutkins, 2006). It is possible to say that this form of yeast has low fermentation intensity, as it could ferment the simpler sugars but took a longer time to ferment the

complexed ones or could not access the maltose immediately as the other ones did. Although Harding and Nicholson reported that some baker's yeast loses its maltase activity almost completely after standing a few days at room temperature. The isolates were then used to bake bread to check their sensory properties on dough. From table 6, comparing the volume of the different breads, it was obvious that bread 5 had the highest mean. This shows that the yeast isolate increased the volume of the bread even more than the commercial yeast which is due to its ability to produce carbon dioxide and this agrees with Cofalec (2014) and Francisca *et al.*, (1999) who stated that yeast produces carbon dioxide that results in dough leavening and contributes to the flavor and crumb structure of bread. Bread 2 and bread 3 were significantly different from the negative control (Bread 1-bread produced without yeast) and positive control (Bread 6-bread produced with commercial yeast). This means that they were not able to yield good results like the positive control but not as bad as the negative control. This agrees to Chukwuka, (2013), who stated that different strains of *Saccharomyces cerevisiae* produce different proportions of carbon dioxide and alcohol. Therefore it is possible to say that those isolates did not produce enough CO₂ for the quantity of flour used. Bread 4 was very similar to bread 1, due to the fact that it produced little or no CO₂. The crust, color and internal color of the breads were checked to get the overall acceptability of the bread since it could dictate the yeast's interaction with the ingredients of the bread. The bread ingredients are formulated to give bread its taste, structure, aroma, texture and nutrients. These ingredients include: flour, liquids, leavening agent, salt, sweeteners, fats or oil and additives (optional). (Burrier, 2014; Ashton, 2009; RSYC, 2014; Young, 2007). Bread 5 gave the best results regarding the three parameters listed. Bread 3 was as good as bread 6 based on the three parameters. The structures of the different breads were checked and bread 5 was significantly different from bread 1 and bread 6, as it gave a good bread structure because it produced enough CO₂ which helped in the development of the gluten network. As a result, the dough gets fatter and bigger, and rises, of course. Thus when the dough is baked, you have a 'bold' loaf, light and airy; when you cut it you can see all the tiny holes formed by the gas, so that it looks like a sponge. The structure of bread depends on dough ingredients, yeast activity, fermentation temperature and gas bubble formation (Lassoued *et al.*, 2007). Bread 3 gave a better structure than bread 6. The breads were also checked on texture and they were all significant from bread 1 meaning they improved the texture of the bread. The holes produced due to the leavening of the yeast helps to give the yeast the light and spongy texture. Bread 5 had the highest mean for texture.

The flavor and aroma of the breads were analyzed and it showed that all the breads excluding bread 1, affected the flavor and aroma of the bread. This agrees with Chukwuka, (2013) that During dough fermentation, yeast

produces secondary by products like the ketones, higher alcohols, organic acids, aldehydes and esters. Most of the alcohols are cooked off during baking. Then the others react with one another and other elements contained in the fermenting dough to form new and more complexed flavored compound (BRIT, 2014). Bread 5 and bread 3 had more impact on the flavor and aroma of the bread while the others had more impact than bread 6. Crumb clarity and the elasticity of the breads were checked and all the breads except bread 1 showed a positive impact on dough based on these two parameters. These parameters occur as a result of the bread's ability to leaven the dough. These qualities come from the ability of the yeast to produce CO₂ and the strength of the dough to hold the gases produced and ability to be stable enough to hold its shape and cell structure.

It is therefore obvious that yeast isolate 5 has the characteristics of a good baker's yeast and can be produced in large quantities in fermentors for industrial production. This yeast isolate was gotten from Eke-Otu-Amechi. The other yeast isolates may not be as good as isolate 4 in leavening but they were able to produce nice flavor and aroma in the breads. It was observed, therefore, from the results that the palm wine isolates vary in their sensory properties. This could be attributed to the fact that different strains of *Saccharomyces cerevisiae* are found in palm wine samples from different sites and they vary from tree to tree. Therefore, these yeasts can be isolated and packaged appropriately as our indigenous baker's yeast. It is therefore recommended that yeasts isolated from different palm wines in Enugu be combined to produce our indigenous baker's yeast, as it has been shown to affect the sensory properties of the bread they were used to produce. Appropriate screening of the strains of *Saccharomyces cerevisiae* isolated from palm wine could be done to identify the particular strains of yeast that affects a specific sensory property.

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