Original Research Article

APPLICATION OF ENRICHED YEAST SPECIES ISOLATED FROM PALMWINE IN BEER BREWING

ABSTRACT

Brewing is found to be one of the lucrative businesses in Nigeria. The industry is known to produce beer, wine or other alcoholic beverages. One important raw material for the industry is the brewer's yeast, commonly known as *Saccharomyces cerevisiae*. This raw material is always imported and its price affects the price of the products drastically. In this work, yeast strain was isolated from Palm Wine, identified as *Saccharomyces cerevisiae* and enriched to be used as an alternative in beer brewing. The isolate (SPW) was seen to poses many similar characteristics of the brewer's yeast (SCT) imported from foreign countries. The isolated and enriched yeast was employed in the laboratory for the production of beer. Results showed that both organisms failed to ferment melibiose and raffinose but fermented glucose, maltose, fructose, sucrose, and galactose. They survived at stress conditions for temperature and cell osmotic pressure in high concentration of ethanol and sugar. They showed good flocculation ability of 97%, and 82% respectively. Their percentage viability was 96.66% and 91.00% respectively with no production of hydrogen sulfide gas. The property of wort prepared for brewing was; pH of 5.82, specific gravity of 1.050 and iodine reaction showed yellow colour. After fermentation, the specific gravity was 1.001 and 1.010 for SPW and SCT respectively while alcohol content was 6.53% and 5.25% respectively. Wort was fermented at 28 ± 2^{o}C for six days and the product beer showed apparent extract of 3.4^{o}P and 4.8^{o}P, Real extract of 4.8^{o}P and 6.55^{o}P, real degree of fermentation of 58.44% and 61.22% for SPW and SCT respectively. Statistical analysis showed that yeast strain isolated from palm wine after enrichment had great potential when compared to brewer's yeast strain in beer production attributes ($p < 0{\cdot}05$).

Key words: Beer, Palm wine, Yeasts, *Saccharomyces cerevisiae*, fermentation, beverage.

INTRODUCTION

The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies (Ogbonna, 2013; Graeme and Graham, 2016). Fermentation is the process of converting carbohydrates to alcohol or organic acids using microorganisms such as yeasts or bacteria under anaerobic conditions. It is employed in many industries for transformation of raw materials into other products. For instance fermentation is employed in textile industries for the digestion of raw materials, in food industries for detoxification, softening etc. In brewing industries, it is employed in the breakdown of carbohydrate sources to produce beer, wine and other alcoholic drinks.

Beer is an alcoholic beverage made from cereal grains, usually barley as the first utilized grain, but now corn, sorghum, millet, rice, wheat, and oats are also used. During beer brewing, yeasts utilize sugars in the grain converting them to alcohol and carbon dioxide (Michael, 2004). It is one of the most widely consumed alcoholic beverages in the world. Beer is used in almost every ceremony or gathering such as parties, clubs, weddings and other entertainment activities. Nigeria has resorted to the use of available grains in veer production such as sorghum, millet, rice, etc. Almost all the raw materials used in beer brewing are obtained in this country except the major ingredient, the brewer's yeast. This yeast is costly and their price has contributed to the high cost of beer.

Yeasts are groups of unicellular microorganisms most of which belong to the fungi division of Ascomycota and Fungi imperfecti (Graeme and Graham, 2016). It is the microorganism that is responsible for fermentation of beer. The dominant types of yeast used to make beer are *Saccharomyces cerevisiae*, known as ale yeast, and *Saccharomyces pastorianus*, known as lager yeast (Walker, 2014). These yeast strains have certain characteristics that make them suitable for the beer production. The major differing attributes of the ale yeast and larger yeast is the flocculation reaction. Ale yeast was able to flocculate while larger yeast does not. Several strains of indigenous yeasts capable of producing beer have been isolated from different local sources such as fermented foods and fermented pineapple juices, but in most of the studies, the preferred candidate for industrial production of beer has been *Saccharomyces cerevisiae* (Okunowo *et al*., 2005; Agwuna *et al.,* 2019). This yeast has the ability to produce beer with no contamination by other products present in the substrate (Brooks, 2008).

Local beverages such as fermenting palm wine, burukutu, pito, fura and nunu have been reported to be good sources of *Saccharomyces cerevisiae*. They contain nutritionally important components including amino acids, proteins, vitamins and sugars (Okafor, 2007). This makes the drinks veritable media for the growth of a consortium of microorganisms. These drinks are produced and consumed in very large quantities in Nigeria (Agwuna *et al.*, 2019). They are perishable and do not stay long on the shelf. Their storage difficulties brought about a large quantity to be wasted. Many researchers have indeed carried out studies aimed at isolating and exploiting yeasts from these local drinks for industrial purposes (Umeh *et al*., 2015; Umeh *et al.,* 2017), yet only little effort has been made at characterizing and enriching these yeasts to posses attributes necessary for beer production.

MATERIALS AND METHODS

Samples collection

Palm wine was purchased from a local market in Nibo, Awka South L.G.A. Anambra State using a sterile plastic bottle. Improved Sorghum variety (Sk5192) was obtained from Institute of Agricultural Research Zaria, Nigeria. Control *Saccharomyces cerevisiae* (Brewer's Yeast) was collected from Ama Brewing at 9th mile corner in Enugu State. All the samples were conveyed to laboratory of the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka.

SAMPLE PREPARATION

Preparation of inoculum

This was done using the method of Barnett *et al.,* (2000). Ten millilitres of the wine sample kept at room temperature were withdrawn daily for 7 days. Extra sucrose (1 g) was added to the remaining sample to increase the sugar concentration after each day's withdrawal.

Isolation and Identification of the yeast from palm wine

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 sample. Pour plate method was used to determine the colony count while streaking method was used to sub-culture, isolate and preserve pure colonies. Different isolated colonies were replicated on fresh plates of Yeast Peptone Dextrose (YPD) to get pure cultures of the isolates were characterized using colony shape and colour, colony surface 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.

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 glass slide and smeared until the smear dried off.

The smear was stained using diluted methylene blue dye, air dried and observed under light microscope at 100 x magnification.

Yeast characterization

Sugar fermentation test

The method of Atlas and Parks (1996) as used by Agwuna *et al.*, (2019) was used to test the abilities of the isolates to ferment different types of sugars.

Growth of the organisms to grow under Stress, high concentration of ethanol and at different temperatures

The ability of the organisms to grow under different stress conditions, high concentration of ethanol and at varying temperatures were conducted as described by Thais *et al*., (2006).

Flocculation test

The flocculation test (Helm's test) was performed according to the methods of D'Hautcourt and Smart (1999) and used by Agwuna *et al*., (2019)

Percentage flocculation was determined using the equation.

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

Yeast viability and consistency

Viability of the isolates was checked using the method of Singgih, (1998) as described by Umeh *et al.*, (2019).

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 done by Ono *et al.* (1991).

LABORATORY BEER PRODUCTION

Grain Sorting and Cleaning

The grains for malting were manually sorted to remove broken kernels, damaged kernels and other foreign materials.

Malt production

After washing the grain, brewing malt preparation procedures; steeping, germination and Kilning of the grains were done according to the recommended methods of analysis of the Institute of Brewing (1977).

Steeping

Four thousand grams (4000g) of the sorted grains were surface-sterilized by immersion for 40 minutes in sodium hypochlorite solution having 1% (v/v) available chlorine to reduce microbial contamination. The grains were subsequently drained and washed four times in tap water. Steeping was done in 3000ml of water. It was done in a total of nine hours comprising six hours wet and three hours dry. At the end of each 6 hours wet steeping, the grains were transferred to a sieve, previously sterilized with the hypochlorite solution for the 3-hour air-rest.

Germination

Germination of the steeped sorghum grains was carried out at 30^{o}C for a period of three days in a dark cupboard. The grains were sprayed with deionized water as required to prevent drying out and to ensure equal germination. Germinated samples were collected on daily (24-hour) basis for kilning and subsequent analysis.

Kilning

Germination was arrested by kilning (drying) the germinated grains in electric oven at 50^{o}C for 48 hours. The rootlets and shoots were separated from the kilned malt immediately after kilning. The malt obtained was subsequently milled to powdery form using a grinder. This was used for beer production.

Inoculums Development and Pitching

The method of Olu *et al*. (2011) was used for this process. This was done by pouring 5 ml of sterile wort into a test-tube. A loop full of yeast from YPD agar was inoculated in the sterile wort in a test tube and shaken vigorously. The test tube was allowed to stand for 24h and the wort transferred into a flask containing 20 ml sterile wort. This was allowed to stand for another 24 h and also transferred into flask containing 50 ml sterile wort and subsequently to 100ml and 200 ml sterile wort and each was allowed to stand for 24 h. At the end 200 ml of fermenting wort was obtained and this was used as the inoculants for the fermentation process. Fermentation lasted for five days and the chemistry of the beer produced was determined.

METHODS USED FOR WORT AND PRODUCED BEER ANALYSIS

Determination of Alcohol content

This was done according to the recommended methods of analysis of the Institute of Brewing (1977) and as done by Okafor (2007).

Determination of specific gravity

This was carried out according to the official methods of the Association of Official Analytical Chemists (2000). The specific gravity (S.G) was calculated as:

S.G = S

$$\frac{S}{W}$$

Where S = W3 – W1= (weight of sample + bottle) – (weight of empty bottle)

W = W2 – W1= (weight of water + bottle) – (weight of empty bottle)

pH determination

The pH was determined using a Jenway 3015 pH meter. Ten millilitre of fermenting wort was taken and the electrode of the pH meter was inserted into the wort sample. The reading on the screen of the pH meter was observed and recorded daily as described by Umeh *et al*. (2015).

Determination of Alcohol content of fermenting wort

This was determined according to the official methods of the Association of Official Analytical Chemists (2000). It was done by subtracting the original gravity from the final gravity and then multiplying by 131.25. (Original gravity – Final gravity) × 131.25 = ABV% (ABV means alcohol content by volume).

Determination of Real and Apparent extract of fermented wort

This was determined by using official methods for the determination of alcohol by Official Analytical Chemists (AOAC, 2000).

Determination of real degree of fermentation of fermented wort

This was done according to Official Analytical Chemists (AOAC, 2000)

Statistical Analysis.

The result were analyzed statistically using ANOVA (SPSS version 20) to see the relationship in the beer production attributes of the yeast isolated from local drinks and the conventional brewer's yeast and are significant at $p<0.05$.

RESULTS AND DISCUSSION

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The yeast isolated from the Palm wine, SPW, was identified and characterized as *Saccharomyces cerevisiae.* It showed the same morphological identity with commercial yeast (SCT) being spherical and creamy, flat, smooth with single budding cells (Table 1). This is in agreement with the work of Berhanu *et al*. (2017) who worked on isolation and characterization of *S. cerevisiae* from 'Tella'. The isolate showed good fermentative capability as compared to the commercial *S. cerevisiae*. They fermented all sugars tested except melibiose and raffinose (Table 1) as also found by Berhanu *et al*., (2017) from their isolate from 'Tella'.

The isolation of these yeast strains from a local beverage and their identification as *Saccharomyces cerevisiae* have confirmed earlier reports that palm wine is a good source of *Saccharomyces cerevisiae* to be used in brewing and baking industries (Jideani and

Osume, 2001; Umeh *et al*., 2015). The finding is also in agreement with the report that palm wine is the major source of *Saccharomyces cerevisiae* (Okoli and Ezenweke, 1989).

Table 1: Morphological characteristics and sugar fermentation abilities of the isolates

	Morphology identification				Sugar fermentation						
Yeast Isolate	**Colony shape and colour**	**Colony surface and appearance**	**Vegetative morphology cell shape arrangement**	**Budding**	**Glucose**	**Maltose**	**Fructose**	**Sucrose**	**Melibiose**	**Galactose**	**Raffinose**
SPW	Creamy and spherical	Smooth and flat	Spherical cell	Single	+	+	+	+	_	+	_
SCT	Creamy and spherical	Smooth and flat	Spherical elongated cell	Single	+	+	+	+	_	+	_

Key: Assimilate carbon source and simultaneously release of carbon dioxide (+), did not assimilate carbon source and not release carbon dioxide (-).

Table 2 showed the Stress tests for temperature and cell osmotic pressure in high concentration of ethanol and sugar. Both isolates were able to tolerate high temperature range of up to 30^{o}C and high concentrations of ethanol.

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

Yeast Strain	**Growth in different media**				
	YPG	Temperature 30%	Ethanol (8%v/v)	YPG (Glucose 20% w/v)	YPS (Sucrose 20% w/v + ethanol 8%v/v
SPW	+++	+++	+++	+++	+++
SCT	+++	+++	+++	+++	+++

Key: Intensive growth (+++) YPS- yeast peptone sucrose medium

YPG- yeast peptone glucose medium

Table 3: Ethanol and temperature tolerance ability.

Yeast strain	Ethanol tolerance			Temperature tolerance (^{o}C)			
	10%	**13%**	**15%**	**15**	**20**	**25**	**30**
SPW	+++	+++	+++	+++	+++	+++	+++
SCT	+++	+++	+	+++	+++	+++	+++

Key: Intensive growth (+++), moderate growth (++), low growth (+).

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

Yeast strain	Flocculation (%)	Viability (%)	Hydrogen sulfide
SPW	$97.00^{a} \pm 0.54$	$96.66^{a} \pm 0.58$	-
SCT	$82.00^{b} \pm 0.10$	$91.00^{b} \pm 0.10$	-

Key: no response (-)

Means in the same column with same superscript are not significantly different (p>0.05)

Table 5: Specific Gravity and Alcohol content of fermented wort.

Isolates	**Specific Gravity**		**Alcohol% (v/v)**
	Initial (SG)	Final (SG)	
SPW	1.050	$1.001^{a} \pm 0.00$	$6.530^{a} \pm 0.08$
SCT	1.050	$1.010^{a} \pm 0.02$	$5.250^{b} \pm 0.04$

Key: Means in the same column with same superscript are not significantly different (p>0.05)

Table 6: Apparent extract, Real extract and Real degree of fermentation

Isolates	Apparent extract (°P) (%)	Real extract (°P) (%)	Real degree of fermentation Mean (%)
SPW	$3.40^{c} \pm 0.1$	$4.80^{c} \pm 0.3$	$58.44^{b} \pm 0.2$
SCT	$4.80^{b} \pm 0.1$	$6.55^{b} \pm 0.4$	$61.22^{a} \pm 0.2$

Key: Means in the same column with same superscript are not significantly different (p>0.05)

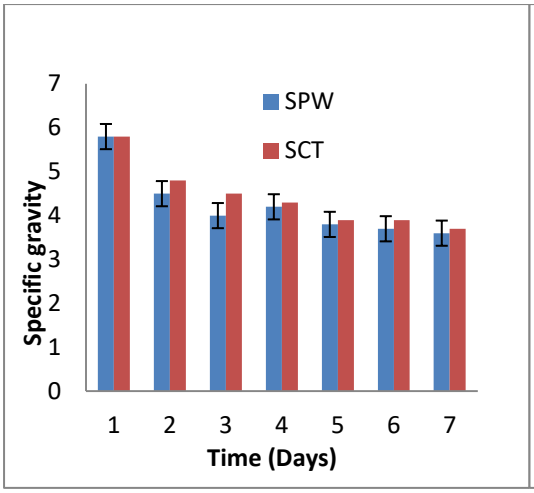

Figure 1: Comparison of changes in Specific gravity during fermentation

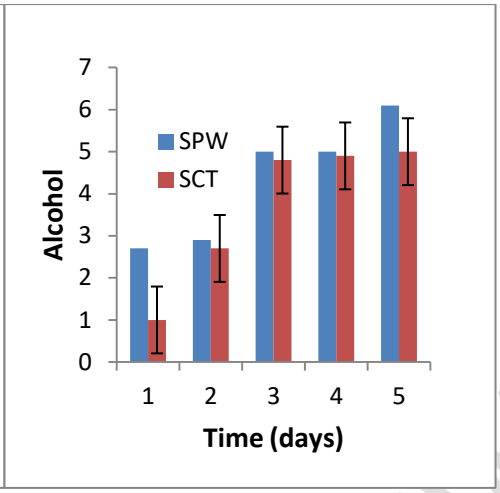

Figure 2: Comparison of changes in alcohol content during fermentation.

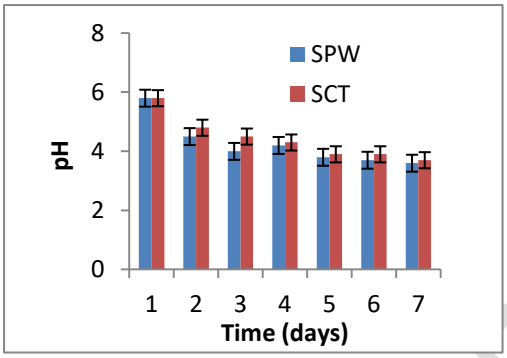

Figure 3: Comparison of change in pH during fermentation

During beer brewing, yeasts usually does not find an environment of optimal conditions, because they are continuously exposed to several stress conditions, especially osmotic and ethanol stress due to the constant production of ethanol in the broth (Querol *et al*., 2003). In this study, the isolates were cultured for 15 days to observe for cell viability due to each stress condition and they showed high viability. The findings were in agreement with Pataro *et al*. (2000) who reported that most *S. cerevisiae* strains isolated from traditional fermentation processes can physiologically adapt to extreme conditions. In this case, the strains were able to grow on yeast peptone medium (YP) containing 20% (w/v) glucose and 8% (v/v) ethanol incubated at 30°C. Glucose is the most acceptable carbon source for *S. cerevisiae* growth but is able to repress genes that code for metabolic enzymes such as invertase (Gancedo, 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 beer 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 SPW was greater than SCT yeast strains. It is a well documented fact that different *S. cerevisiae* strains have different capacity for resisting concentration of alcohol. *S. cerevisiae* isolates in this study were in line with the report of Chi and Ameborg (2000) in respect to capacity of alcoholic resistance. They reported high ethanol tolerance for *Saccharomyces cerevisiae* to be between 10 – 15%v/v. One of the best qualities of yeast isolates for beer production is the capability of tolerating high alcohol concentration. It is well known that the cell volume of yeast is significantly altered with increasing ethanol concentration. This is because the ethanol stress can reduce cell volume (Chi and Ameborg, 2000).

High flocculation capability of beer 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 behavior of yeast isolates is significant to get appropriate yeast isolates for beer production. In brewing, flocculation occurs towards the end of primary fermentation (Berhanu *et al*., 2017). In the present findings, flocculation capacity of SPW (97%) was higher than that of SCT (82%) and the result differs statistically ($p<0.05$). The flocculation is an important characteristic that allows an 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; Agwuna *et al.,* 2019).The high viable count of the isolated yeast strains in this study and other good qualities have shown that the yeast cells were viable and can actually carry out good fermentation in the process of beer brewing. The beer produced in the laboratory using the isolated and enriched yeast from palm wine showed some better qualities than the one produced with the conventional commercial brewer's yeast as shown in Tables 5 and 6; and Figures 1 to 3 above.

Conclusion

Enriched yeast from palm wine can serve as an alternative for imported brewer's yeast can be preserved and sold as a type of brewer's yeast to beer brewers and wine makers.

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APPENDICES

Appendix I: Colonies of the isolates and fermentation setup

SPW **SCT**

SPW **SCT**