ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue III March 2025

Characteristics of *Saccharomyces Cerevisiae* Isolated from an Indigenous Fermented Beverage (Burukutu) as Potential Brewer's Yeast for Beer Production

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DOI: https://doi.org/10.51584/IJRIAS.2025.10030048

Received: 21 February 2025; Accepted: 26 February 2025; Published: 13 April 2025

ABSTRACT

Beer is one of the regular drinks produced and consumed in many countries including Nigeria. Its cost has become so high making it unaffordable to low income earners. One major reason for the high cost of beer is the cost of imported brewer's yeast. The potential application of *Saccharomyces cerevisiae* isolated from burukutu (a Nigerian local fermented beverage drink) was investigated. The Sorghum variety used in this study was developed from Sk5192 variety obtained from the Institute of Agricultural Research Zaria, Nigeria. Burukutu sample was purchased from community market in Enugu State, Nigeria. Standard Microbiological techniques were employed in the isolation, characterization and identification of the yeasts. Beer production attributes such as stress exclusion, flocculation, hydrogen sulphide production, yeast viability and consistency tests were checked. The yeast isolated from burukutu (SBB) exhibited identical morphological characteristics as the control brewer's yeast (SCT), showed multiple budding capacities and fermented all tested sugars except melibiose and raffinose. The flocculation capacity and viability rate of SBB was recorded as 82% and 83% respectively against the control yeast (SCT) which was 82% and 91%. None of the isolates produced hydrogen sulphide when tested. Malt production involving steeping, germination, kilning, preparation of wort for alcohol production, inoculum development and pitching were the processes used in the laboratory beer production. Properties of the beers produced with SBB and SCT showed that the specific gravity of the fermented wort for SBB and SCT were the same with no significant difference (1.010 and 1.010). The apparent and real extract of the isolated yeast (SBB) were 6.70% and 8.50% respectively and the control yeast (SCT) recorded 4.80% and 6.55% which were greater than the standard minimum values (2.50%, 4.40%) for extra strong beer. The pH values of beer produced with SBB and SCT fell within the standard value of 36-48 for normal beer. Thus, Burukutu can be a good source of *Saccharomyces cerevisiae* which can be enriched and used for industrial and home brewing.

Key words: *Saccharomyces cerevisiae*, Burukutu, Beer, Fermentation, Brewer's yeast.

INTRODUCTION

Beer is one of the most widely consumed alcoholic beverages in the world (Ogu *et al.,* 2022). It is made from Barley, but Indigenous African beers are fermented drinks made with Sorghum, Maize or Millet and its production depends solely on imported Brewer's yeast.

Brewer's yeasts are starter *Saccharomyces* spp produced in foreign countries like Europe and France. They are preserved in many ways like lyophilization (Freeze drying) and sold to developing countries such as Nigeria for use in beer brewing. Most times, these yeasts lose their characteristics in the preservation process and yield abnormal products when used. They are sometimes contaminated with harmful bacteria and if not detected cause beer spoilage. Importation of brewer's yeast contributes to the high cost of beer. There is need to reduce the cost of beer production which will as well reduce the cost of beer sold in the markets for consumption. This will make beer cheap and affordable to low income earners.

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'Burukutu' is an indigenous alcoholic drink made from sorghum by the Savannah region of Nigeria (Arnold, 2005). It is produced using traditional fermentation techniques, where indigenous organisms ferment the grains. Consumption of burukutu has spread to other parts of Nigeria due to intermingling of tribes. They contain nutritionally important components including amino acids, proteins, vitamins and sugars (Okafor, 2007) which make the drink a veritable media for the growth of a consortium of microorganisms.

In Nigeria, large quantities of Burukutu are produced daily but are difficult to preserve for considerable length of time. The high wastage rate of these drinks necessitates the need for alternative use and the economic situation of the nation necessitates the adoption of simple, inexpensive brewing techniques that can result in quality improvement and be carried out at household and community levels for the production of beer that is safe and affordable. Isolating yeast strains from local materials is expected to reduce the cost of importation of brewer's yeast as well as reduce the cost of beer.

Many researchers have indeed carried out studies that aimed at isolating and exploiting yeasts from local drinks for industrial processes (Umeh *et al.,* 2017; Umeh *et al.,* 2015). The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies. Yeast plays a vital role in the production of all alcoholic beverages and the selection of suitable yeast strains is essential not only to maximize alcohol yield, but also to maintain beverage sensory quality (Graeme and Graham, 2016).

Thus, the adoption of indigenous and inexpensive processing techniques that can be carried out at household and community levels for the production of products (e.g. Beers) that are safe and affordable becomes paramount. The aim of this study is to investigate the potential application of *Saccharomyces cerevisiae* isolated from Burukutu in beer production.

MATERIALS AND METHODS

Collection of Samples

The sorghum variety used in this study is an improved variety (Zm-Dandam) developed from Sk5192 variety (obtained from Institute of Agricultural Research Zaria, Nigeria). The control *Saccharomyces cerevisiae* (Brewer's yeast) was collected from Ama Brewing at 9th mile Corner in Enugu State, Nigeria. Samples of "Burukutu" was purchased with sterile bottles at 82 Divison in Enugu State Nigeria. The containers for sample collection were sterilized by immersion in 1% (v/v) sodium hypochlorite solution having available chlorine for 40 minutes and rinsed severally with sterile water. The burukutu samples were conveyed to the Department of Applied Microbiology and Brewing Laboratory of NAU Awka in an ice-packed container.

Methods

Isolation Characterization and Identification of Yeasts from Burukutu

The method of Fagbemi and Ijah (2005) as reported by Umeh *et al.* (2015) was used to isolate, characterize and identify the yeast strain from burukutu. Each sample was streaked on Sabouraud dextrose agar (SDA) medium containing 0.05mg/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.

Microscopic observation

This was carried out as done by Thais *et al.* (2006) and modified by Ogu *et al.,* (2022). A single colony of yeast was suspended in a drop of sterile distilled water placed on glass slide and smeared until the smear dry

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

Test for Beer production attributes

Sugar fermentative test

Yeast fermentation test was conducted as described by Atlas and Parks (1996) as used by Ogu *et al.*, 2022.

Stress exclusion tests

The ability of the organisms to grow under different stress conditions; high concentration of ethanol and at varying temperatures were conducted as described by Thias *et al.*, 2006 and modified by Ogu *et al.*, (2022).

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.

The percentage (%) flocculation was then determined using the equation.

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

Where,

A is the absorbance of tube A

B is the absorbance of tube B

Yeast Viability and Consistency

The viability of the isolates was checked using the mehod of Singgih, 1998 and used by Ogu *et al.*, 2022.

Hydrogen sulfide production test

The ability of the yeasts 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 Ogu *et al.*, 2022.

Laboratory Scale 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 grains, brewing malt production procedures such as steeping, germination and kilning of grains were done according to the recommended methods of analysis of the Institute of Brewing IOB, 1977

Steeping

This was done according to the recommended methods of analysis of the Institute of Brewing (1977). 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

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subsequently drained and washed four times sterile water. Steeping was done in 5 litres of water. It was done in a total of sixty 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/malting

This was done according to the recommended methods of analysis of the Institute of Brewing (1977). Germination of the steeped sorghum grains was carried out at about 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

This was done according to the recommended methods of analysis of the Institute of Brewing (1977). Germination was stopped 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.

Preparation of wort for alcohol production test

This was done according to the recommended methods of analysis of the Institute of Brewing (1977) and as done by Okafor (2007). Three thousand grams of malted sorghum was weighed and mashed with water inside a basin and dispensed in conical flasks. This mash was placed inside a water bath and the temperature rose between 50 and 55°C for about 20 min, to allow for optimum activity of the enzymes present in the malt. The temperature was later raised to 72°C for 45 min. to terminate enzymatic activities. The mash was then allowed to rest for 45 min to allow the mash to undergo complete saccharification (Okafor, 2007). Saccharification, which is the complete hydrolysis of starch to simple sugar is shown by a brown or colourless reaction with 2% iodine solutions (Okafor, 2007). After saccharification, the mash was filtered and aqueous solution collected that is, wort. After boiling, the wort was allowed to cool and the following parameters were determined; pH, Color, specific gravity, reducing sugar, and temperature. The cooled wort was transferred into fermenting bottles and allowed to stand for inoculation after development of inoculums. .

Inoculums Development

The method of Olu *et al*. (2011) modified by Ogu *et al.*, 2022 was used for this process. This was done by pouring 5 ml of sterile wort into a test-tube. Five loopfulls of the yeasts from YPD agar was used to inoculate the sterile wort and shaken vigorously. The test tube was allowed to stand for 48h. The inoculated wort was transferred into a 20 ml flask containing sterile wort and this was also allowed to stand for another 48 h. This was also transferred into another flask containing 50 ml of sterile wort and subsequently to 100 and 200 ml sterile wort and each was allowed to stand for 48 h. At the end 200 ml of fermenting wort was obtained and this was used as the inoculants for the fermentation process.

Pitching

The cooled wort in the fermenting jars were inoculated with the developed inoculums and mixed thoroughly and allowed to stand and ferment. Fermentation lasted for six days and the chemistry of the beer produced was determined.

Methods for wort and produced beer analysis

Determination of Alcohol Content

This was done according to the Institute of Brewing Recommended Methods of Analysis IOB, 1977.

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Determination of specific gravity

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

$$S.G = \frac{S}{W}$$

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 milliliter 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 = 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 was 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

The yeast isolates from Burukutu, SBB was identified and characterized as Saccharomyces cerevisiae and it showed similar morphological characteristics (spherical, creamy, flat, smooth) with the Commercial brewer's Yeast (SCT) except the multiple budding seen in SBB. The isolates fermented all the sugars tested except melibiose and raffinose as shown in Table 1. The chosen isolate and the commercial brewer's yeasts were able to withstand different stress conditions and grow profusely in the presence of different stress as shown in Table 2.

The isolates were able to grow at high temperature ranges as well as ethanol concentrations as seen in Table 3. The isolates showed acceptable flocculation ability, viability count and were unable to produce hydrogen sulfide as recorded in Table 4. The specific gravities of the worts produced by the isolates were 1.010±0.01 and 1.010±0.02 respectively which showed no significant difference as shown in Table 5. The same concentrations of ethanol were found in both worts as seen in Table 5.

Apparent extract, Real extract and Real degree of fermentation results were as presented in Table 6.

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Table 1: Morphological characteristics and Sugar fermentation ability

Yeast Isolate	Morphology identification				Sugar Fermentation						
	Colony shape and colour	Colony surface and appearance	Vegetative morphology cell shape arrangement	Budding	Glucose	Maltose	Fructose	Sucrose	Melibiose	Galactose	Raffinose
SCT	Creamy and spherical	Smooth and flat	Spherical Elongated cell	Single	+	+	+	+	-	+	-
SBB	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
SBB	+++	+++	+++	+++	+++
SCT	+++	+++	+++	+++	+++

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

Table 3: Ethanol and temperature tolerance ability

Yeast strain	Ethanol tolerance			Temperature tolerance (^oC)			
	10%	**13%**	**15%**	**15**	**20**	**25**	**30**
SBB	+++	+++	++	+++	+++	+++	+++
SCT	+++	+++	+	+++	+++	+++	+++

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

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

Yeast strain	Flocculation (%)	Viability (%)	Hydrogen sulfide
SBB	$82.00^b \pm 0.10$	$83.00^c \pm 0.10$	-
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)	
SBB	1.050	$1.010^a \pm 0.01$	$5.250^b \pm 0.01$
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 ($p>0.05$)

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

Isolates	Apparent extract (°P) (%)	Real extract (°P) (%)	Real degree of fermentation Mean (%)
SBB	$6.70^{a} \pm 0.1$	$8.50^{a} \pm 0.1$	$56.45^{c} \pm 0.1$
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 ($p>0.05$)

Specific gravities of the worts during fermentation were determined daily and results showed that the specific gravities were highest on day 3 as shown in Fig 1. While the alcoholic contents and pH were presented in Fig 2 and 3.

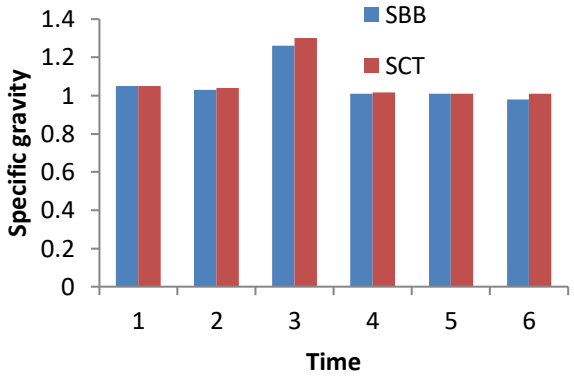

Fig 1: Comparison of Changes in specific gravity during fermentation

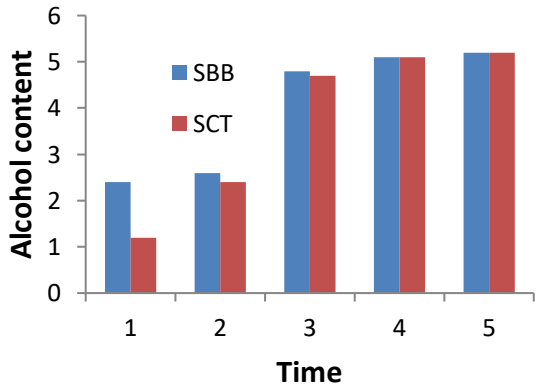

Fig 2: Comparison of changes in Alcohol content

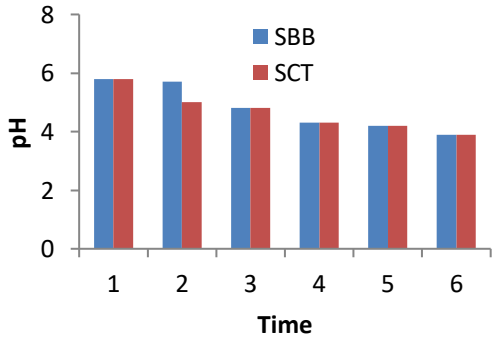

Fig 3: Changes in pH during fermentation

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DISCUSSION

Yeasts were isolated from an indigenous drink (Burukutu) and identified as Saccharomyces *cerevisiae* (SBB). Characterization of the choice yeast and comparison with the control brewer's yeast (SCT) showed that the isolates were found to be morphologically identical. Based on colony shape and colour, the Control yeasts (SCT) were spherical and creamy while SBB strains were also spherical but white to creamy in colour. Apparently, they were all flat, smooth with SCT growing in single budding yeast cell but SBB in multi 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 other drinks.

One of the most important technologically valuable characteristics of yeast is its ability to ferment simple sugars. According to the present finding, the various yeast isolates behaved in similar ways in their fermentative capability of simple sugar fermentation. The isolated yeast (SBB) showed good fermentative capability as compared to the commercial *S. cerevisiae* strains (SCT) which was used as controls for this study. They fermented all sugars tested on except melibiose and raffinose (Table 1). This also complied 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 report. Moreover, the finding of the present study provides a promising source of good beer yeast. This was also used in identifying the yeast as *Saccharomyces cerevisiae*.

The isolation of these yeast strains from local beverages and their identification as *Saccharomyces cerevisiae* have confirmed earlier reports that burukutu is a good source of *Saccharomyces cerevisiae* to be used in brewing and baking industries (Jideani and Osume, 2001; Umeh *et al*., 2015).

During fermentation for beer production, the yeast usually does not find an environment of optimal conditions, being continuously exposed to several stress conditions, especially osmotic and ethanol stress (Querol *et al*., 2003). The yeast isolated in this study were able to posses good beer producing attributes such as stress tolerance to different stress conditions as shown in Tables 2 and 3. The findings of this research 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 strains were able to grow on medium (YP) containing 20% (w/v) glucose and 8% (v/v) ethanol after incubation at 30°C (Table 2). The resistance to glucose repression could be interesting for beer production as well as a high invertase activity (Pataro *et al*., 1998). When subjected to high glucose concentration all the colonies followed the same pattern and still maintained their viability (Table 5).

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. Different *S. cerevisiae* isolates have different capacity for resisting concentration of alcohol. *S. cerevisiae* isolates of this study were in line with the report of Chi and Ameborg, (2000) in respect to capacity of alcoholic resistance (Table 3).

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, the flocculation capacity of SBB and SCT gave the same result (82%) (Table 4).

Investigation in viability test showed that the isolate and commercial strain had good viability capacity, SCT had 91% and SBB had 83% (Table 4). The high viable count of the isolated yeast strains in this study has shown that the yeast cells were viable and can actually carry out good fermentation.

Yeasts with high production of hydrogen sulfide are undesirable for beer production because it confers flavor and taste that compromise the quality of the beer obtained (Ribeiro and Horii 1999). Thus, none of the isolates produced hydrogen sulphide when tested (Table 4).

The specific gravity of the fermented wort using SBB and SCT were 1.010 for both yeasts (Fig 1) and there was no significant difference ($p<0.05$).

The alcohol content of fermented wort by isolates of SBB (5.25%) was comparable with that of SCT as shown

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in Fig 2. Therefore, yeast isolates of this finding produced reasonably high concentration of alcohol and at the same time had good alcohol tolerance capacity to solve influence on growth velocity and fermentation rate of yeasts.

All the yeast isolates investigated in this study was in line with desired extract for apparent and real extract of given beer as also reported by Berhanu *et al*. (2017). This value is within the standard value of extra strong beer (12.51-14.50^{o}P) (ES833, 2012). Therefore, the isolate (SBB) can be used for production of industrial extra strong beer.

Real degree of fermentation refers to the percentage of reductions in wort's specific gravity caused by the transformation of the sugars into alcohol and CO_2 and yeast biomass. There real degree of fermentation capacity of the yeast isolates tested in this investigation were a little bit less than that of the commercial yeast (SCT). The minimum standard of apparent and real extracts of extra strong beer is 2.50% and 4.42%, respectively (ES 842, 2012). The apparent and real extract of the isolated yeast (SBB) were 6.70%, 8.50% respectively. The control (SCT) apparent and real extract values were 4.80% and 6.55% respectively. Therefore, the apparent and real extract values of yeast isolates in this study were greater than the standard minimum values for extra strong beer. The isolated yeast organisms in the present study may be used for production of commercial extra strong beer and there was significant difference in the results ($p<0.05$).

The pH values of beers produced with different isolates of the study were within the standard value (3.6-4.8) of (ES830, 2012) and Indian standard beer specification value (3.8– 4.5) (BIS, 2001). The pH in such a range (Fig 3) can reduce the risk of contamination and secondary fermentation.

CONCLUSION

This investigation cannot be considered to be exhaustive, but, it has given an insight into the efficiency and availability of yeast in local drinks and its potential application in beer production. It is therefore recommended that Production of beer using our local materials should be modified in such a way that it can be industrialized so that this yeast will be available in the market to encourage home brewing and also be sold as brewer's yeast to beer producing industries.

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