FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 4 No. 2, June, 2020, pp 758 – 766
DOI: https://doi.org/10.33003/fjs-2020-0402-327

BREAD PRODUCED FROM COMPOSITE FLOURS USING *Saccharomyces cerevisiae* ISOLATED FROM LOCAL FERMENTED BEVERAGE (BURKUTU)

***[1]Ayoade, B. R., [2]Mohammed, S. S. D and [3]Auta, R.**

[1]Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna State, Nigeria
[2]Department of Biology, Microbiology and Biotechnology, Faculty of Natural and Applied Sciences, Nile University of Nigeria, Jabi, FCT, Abuja, Nigeria
[3]Department of Biochemistry, Faculty of Science, Kaduna State University, Kaduna State, Nigeria

*Corresponding Author's Email: ayoadebiodun6@gmail.com

ABSTRACT
Composite flour/bread technology is an important area to be properly harnessed in food industries to help reduce the cost of wheat importation in Nigeria. In this study, bread produced from composite flours using *Saccharomyces cerevisiae* isolated from local fermented beverage (Burkutu) was investigated. Sugar fermentation, carbohydrate utilization and ethanol tolerance tests were carried out in identifying and characterizing the yeast isolates. Composite breads were produced from Wheat Flour (WF) (100%) and its blends with Rice Flour (RF) (40%), Plantain Flour (PF) (40%) and Cassava Flour (CF) (40%) using the straight dough method. The yeast survived in 25% glucose and 15% ethanol, suggesting its usefulness in industrial application. Loaf weights and loaf volumes of the composite bread samples ranged from 318.4 - 355.7 g, and 420.6 - 457.3 mL respectively, with 100% Wheat Bread ranking highest while Composite rice bread the least, using a significant difference of $P<0.05$. The total yeast count recorded after 4 days showed 0.25 x 10^{-3} CFU/mL (100% W bread), 0.40 x 10^{-3} CFU/mL (W/R (bread), 0.21 x 10^{-3}CFU/mL (W/P bread) and 0.32 x 10^{-3}CFU/mL for W/C bread. Sensory assessment revealed that there was no significant difference ($P>0.05$) in the bread samples analysed. Shelf life assessment of the bread samples at room temperature (26±1°C) within the first three days revealed no microbial growth/cell count. Bread produced from the incorporation of cassava with wheat flour was found to be acceptable, with insignificant variation when compared to bread made with 100% wheat flour.

Keywords: Burkutu, *Saccharomyces cerevisiae*, yeast isolates, composite flour, bread sample.

INTRODUCTION

Bread is the most common among all baked products of wheat. It is consumed and enjoyed by both children and adults from different socio economic status in Nigeria, therefore leading to its high daily demand (Inyang and Asuquo, 2016; Nwanekezi, 2013). It contains a rich source of energy, protein, vitamins especially the B vitamins, minerals and dietary fibre, making it highly nutritive. Recent developments for over 20 years has focused on healthy eating, enhancing the utilization of indigenous produce such as whole wheat, local cereals and legumes in baking industries (Therdthai and Zhou, 2014). In very few developing countries, wheat which is distinctive among other cereals usually employed to make bread and other aerated baked products is produced in low yields (Ayele *et al*., 2017). Exceptions include temperate areas induced by high latitude or high altitude or both (examples are Mexico, Northern India, Eastern Africa). Nigeria, due to her unfavourable climatic condition is unable to grow wheat in large quantity (Nwanekezi, 2013).

Wheat is imported from nations that grow it in abundance, as a result of favourable temperature for its cultivation in these regions. Nigeria imported 4.1 million metric tons of wheat in 2011, according to the U.S Department of Agriculture. Scarce foreign currency is used to pay for these imports and this, no doubts is depleting income and reserves from Nigeria's foreign currency. In an effort to correctly reduce or stop wheat importation, the Nigerian government and the Food and Agriculture Organization (FAO) encouraged the use of composite flours and blends of wheat less flours or meals to produce aerated products like bread, cookies, cake, doughnuts, etc. Luckily, there are enormous fertile arable lands in Nigeria where food produce such as cassava, maize, rice, millet and sorghum can be cultivated in large quantities (Adesina, 2011).

Composite flours are blends of various cereals, legumes and tuber flours, rich in starch, protein and other nutrients integrated with or without wheat flour. The replacement or incorporation of wheat flour with indigenous raw materials is ever increasing as a result of the high demand for baked products and pastries (Noor-Aziah and Komathi, 2009). Hence, many developing countries have promoted the introduction of the strategy to harness the possibility of replacing wheat flours with indigenous flours (Abdelghafor *et al*., 2011; Jensen *et al*., 2015).

Isolation and exploitation of suitable microbes (from indigenous sources) of economic importance employed in diverse manufacturing processes, is an important facet of biotechnology. Burkutu is an indigenous alcoholic beverage produced from the grains of guinea corn (*Sorghum vulgare*-red corn and *Sorghum bicolor*) and millet (*Pennisetum glaucum* or *P. americanum*) which is consumed locally in Nigeria and some African countries (Alo *et al*., 2012; Chikodili *et al*., 2015). *Saccharomyces cerevisiae*, a unicellular yeast is usually isolated from indigenous alcoholic drinks (Palm wine and Burkutu) and sugary foods. It originates from specific selection of short generation time (fast growing) naturally occurring yeast strains, with excellent yielding capability for storage in terms of stability making it unique from other yeasts (Olowonibi, 2017).

Fermentation of sugars and the production of alcohol (mainly ethanol) and carbon dioxide in the absence of oxygen is a major attribute of the yeast, making it useful in industries due to its fermentative and oxidative ability, globally (Farid *et al*., 2019). *S. cerevisiae* is usually inoculated into bread dough at about 2% of the entire ingredients in the baking industry. The main function of the yeast is to transform the available carbohydrate (mainly glucose, fructose, sucrose, and maltose) into CO_2 gas. With the lowering of dough pH, the production of reducing compounds like glutathione which affects dough rheology and strength, and volatile components; *S. cerevisiae* enhances dough and flavor development (Stewart, 2014).

MATERIALS AND METHODS

Samples Collection and Processing

Fresh burkutu (local beverage) was obtained from within Kaduna metropolis and was deposited into sterile air-tight container which was allowed to ferment for 24 hours. Local varieties of unprocessed wheat and rice grains were obtained from Anchau market in Kubau local Government Area, Kaduna State. Bunch of unripe plantain, tubers of sweet cassava, sugar, salt, fat, and milk were purchased from Central market, Kaduna. All samples were transported to Microbiology Laboratory of Kaduna State University for analyses. Cereal and tuber samples were sorted, washed, dried, ground into powder and sieved in 2mm sieve aperture size to obtain uniform particles, then packaged and stored in air tight plastic bags until required (Papageorgiou and Skendi, 2018; Ojure and Quadri, 2012).

Isolation and Identification of Yeast from Burkutu

Potato Dextrose Agar (PDA) was used to culture the burkutu sample. The medium was prepared according to manufacturer's instructions and supplemented with 40 mg/L chloramphenicol for selective enumeration of yeast. The isolates obtained were sub-cultured on fresh medium of PDA to obtain pure cultures (Olowonibi, 2017).

Carbohydrate Utilization and Ethanol Tolerance

Potato Dextrose Agar (PDA) was used for sugar tolerance test. The medium was autoclaved in bijou bottles at 121^{o}C for fifteen (15) minutes. Various concentration of sucrose ranging from 5 - 25% were added to different bottles of the same medium to constitute varying percentages of the sugar. The medium were poured into various Petri dishes, allowed to solidify and then inoculated with the yeast isolates. All cultures were incubated at 27^{o}C for 48 hours (Khaing *et al.,* 2016). For ethanol tolerance, yeast isolates were grown in Yeast Extract–Peptone Glycerol (YPG) broth containing different concentrations of ethanol ranging from 5 - 25% (v/v) respectively and were incubated at 30^{o}C for 72 hours (Karki *et al*., 2017).

Molecular Identification of Yeast Isolates

DNA Extraction

In order to amplify the target genes, standard fungal primers, Internal Transcribe Spacer (ITS 4 and 5): 5'TCCTCCGTCTATTGATATGC3' and 5'GGAAGTAAAAGTCGTTAACAAGG3', were used. Polymerase Chain Reaction (PCR) was carried out to amplify the target gene. DNA denaturation (94^{0}C for 30 seconds) annealing (54^{o}C for 30 seconds), extension (72^{o}C for 45 seconds), and elongation (72^{o}C for 7 minutes) were carried out utilising 36 cycles. The PCR was performed in a 50µL reaction containing 25µL master mix, 5µL forward primer, 5µL reverse primer, 5µL template and 13.4 µL nuclease free water to make up 50µL (Looke *et al*., 2011).

Gel Electrophoresis of the DNA

The different gDNA fragments of base pairs were amplified and the PCR products was visualized employing a safe view stain on 1.5% -agarose gel electrophoresis, then the gel images were digitally captured. The DNA ladder used was Hyper ladder from Bioline (Looke *et al*., 2011).

Sequencing of the DNA

Standard ABI sequencing reactions were performed on clean (dNTP) and primer free DNA template using the Big Dye kit (P.E- BiosystemsInc). Unincorporated dyes were removed by precipitation with 95% ethanol in the presence of 3M Sodium aceteate pH 5.2. Sequencing gels were performed by MU seq (Massey University DNA Analysis Service). The Amplified PCR Products was sequenced thus; each single strand of DNA fragment isolated was combined in a tube with the adequate primers, DNA polymerase and DNA nucleotides, [dideoxyribonucleoside triphosphate (ddNTP)]. The mixtures was heated to denature the template strands, then cooled so that the primer can bind to the single stranded template (Janitz, 2011).

Basic Local Alignment Search Tool (BLAST)

After construction of the retrieved sequence, the whole sequence was used for searching a well suited nucleotide-nucleotide sequence from the database of National Centre for Biotechnology Information (Muhopadhyay *et al*., 2017).

Inoculum Development

Yeast culture was picked from PDA plate with a sterile stick and placed in sterile distilled water of 5 mL to make a turbid suspension in a test tube. The contents was filtered with a Whatman filter paper, then the density of the suspension was adjusted with spectrophotometer at a wavelength of 520 nm (Santos *et al*., 2006).

Preparation of Composite Flour Blends

Wheat and composite flour preparation were carried out according to the methods adopted by Papageorgiou and Skendi (2018); and Ojure and Quadri (2012). The formulation of its mixes as described by Cauvain (2015) is shown in Table 1.

Table 1. Formulation of Wheat Flour and Composite Flour Mixes

Sample	Wheat Flour (g)	Composite Flour (g)	Fat (g)	Salt (g)	Sugar (g)	Yeast (mL)	H_2O (mL)
(WF)	100	-	20	2.5	12.5	2.5	50
(RCF)	60	40	20	2.5	12.5	2.5	50
(PCF)	60	40	20	2.5	12.5	2.5	50
(CCF)	60	40	20	2.5	12.5	2.5	50

Key:
WF - (Wheat Flour): 100% Wheat Flour
RCF - (Rice Composite Flour): 60% Wheat plus 40% Rice Flour
PCF - (Plantain Composite Flour): 60% Wheat plus 40% Plantain Flour
CCF - (Cassava Composite Flour): 60% Wheat plus 40% Cassava Flour

Bread Production

Bread Dough Formation and Specific Volume Determination

The bread dough was produced based on the Straight dough. This method involves the addition of all ingredients; Wheat/ Composite flour (100g), sugar (12.5g), salt (2.5g), milk (12.5g), yeast (0.5 Mc Farland standard), fat (20g) and water (50mL) at the mixing stage, then kneading to form the dough (Cauvain, 2012). Specific volume was measured by dividing the volume by the weight; Specific volume =v/wt (cm^3/g) as described by Maneju *et al*. (2011).

Production of Bread Using Composite Flour Blends

The bread samples were produced in batches by mixing and kneading manually each of the above flour blends with the principal bread ingredients in a stainless steel bowl. After thorough kneading in each case, the dough was allowed to ferment and develop for an hour at 35^{o}C before being knocked back and then moulded into cylindrical shape. After moulding in each case, the dough was placed in a well-oiled baking pan where it was proofed for 30 minutes at room temperature before it was baked in an oven pre-heated and set at 210^{o}C. After baking, the dough was brought out in each case from the oven and immediately de-panned by knocking out. The knocked out bread was allowed to cool, weighed and measured before being packed in polyethylene bag and stored at room temperature (Ayo *et al*., 2014).

Determination of the Proximate Composition of Produced Bread

The Association of Official Analytical Chemist (A.O.A.C, 2010) procedure was used to determine the proximate compositions of the produced bread. These analyses include: percentages of moisture content, ash content, crude protein, fat content, total carbohydrate and fibre respectively.

Microbial Analysis of Bread Produced

The American Public Health Association (A.P.H.A, 2015) method for the microbiological examination of foods was used. Potato dextrose agar (PDA) was used for enumeration of yeasts, Nutrient Agar for bacteria and MacConkey Agar for Total Coliform Count of bacteria (TCC).

Sensory Evaluation of Bread Produced from Composite Flour

The Organoleptic evaluation of the bread was carried out by a panel of 10 judges, using a 9-point hedonic scale, with 9 representing "liked extremely" and 1 "dislike extremely". Parameters such as crumb colour, crust colour, texture, flavor, taste, and general acceptability of the bread were evaluated (Iwe, 2010).

Statistical Analysis of Data

Data generated from the Sensory evaluation were subjected to one-way analysis of variance (ANOVA) and Duncan multiple range test was employed to compare the mean values at $p< 0.05$ level of significance (Chim *et al*., 2015).

Shelf Life Extension of the Produced Bread

This was carried out to determine the period the bread would remain free from microorganisms (especially moulds) at good eating quality for the consumers as well retaining most of the Organoleptic properties of the bread (Jideani and Vogt, 2015).

RESULTS AND DISCUSSION

Isolation of *Saccharomyces cerevisiae* from Local Fermented Beverage (Burkutu)

Molecular characterization of the yeast isolated from Burkutu confirmed the isolate to be *Saccharomyces cerevisiae* strain TSA66 with an amplicon size of about 1500bp. This is comparable with the result obtained by Chinedu *et al*. (2010) who reported *S. cerevisiae* as one of the organisms associated with the fermentation of sorghum in Burkutu production. Similarly, Atter *et al*. (2014) and Anaukwu *et al*. (2015). recorded the isolation of pure cultures of *S. cerevisiae* and *Aspergillus aculeatinus* from Burkutu, and found variants of *S. cerevisiae* to be the most dominant yeast isolated from the fermented beverage. The isolation of yeast strains from Burkutu could be attributed to high level of fermentation of the cereals utilised and the ability of the yeast to ferment the substrate to produce carbondioxide as gas (Chikodili *et al*., 2015; Alo *et al*., 2012).

The Physical Properties and Appearance of the Flours and Bread Produced

The physical properties and appearance of the wheat and composite flour indicated that WF (Wheat Flour), RCF (Rice Composite Flour), and CCF (Cassava Composite Flour) were slightly coarse; while PCF (Plantain Composite Flour) was smooth in texture. Table 2 shows the observed physical properties of the produced bread samples. The crumb colour of the bread produced from WF and RCF appeared creamy while that of PCF was brownish in colour. Weight loaves of the composite bread samples ranged from 320.1 - 350.3 g, loaf volume ranged from 421.3 - 451.2 mL and loaf specific volume from 1.29 - 1.35 mL g^{-1} respectively, using significant difference of ($P < 0.05$). This result inferred that the higher the loaf volume and loaf weight, the lower the specific volumes of the loaves (Erikson *et al*., 2014; Mongi *et al*., 2011). This could be attributed to the utilisation of the substrate by the yeast and the release of carbondioxide by the action of the enzyme amylase with respect to wheat protein (glutein). This is in accordance with Abdelghafor *et al*. (2011) and Julianti *et al*. (2015) who reported that higher loaf weight of composite bread samples was as a result of less retention of

carbondioxide gas in the blended dough thus, producing dense bread texture.

Proximate Composition Assessment of the Produced Bread

There was significant difference ($p<0.05$) in the moisture, ash, protein, and carbohydrate content, but no significant difference ($p>0.05$) in lipid content of bread made from WF, RCF, PCF and CCF as shown in Table 3. In all the bread samples [Wheat Bread (WB}, Rice Composite Bread (RCB), Plantain Composite Bread (PCB), and Cassava Composite Bread (CCB)], the following were obtained for their proximate assessment. Percentage moisture: 2.53, 2.66, 2.52 and 2.75%; percentage ash: 1.88, 1.79, 1.56 and 1.74%.; percentage protein: 10.76, 11.34, 9.21 and 8.31%; percentage lipid 22.0, 21.14, 20.40 and 21.93%; and percentage carbohydrate: 62.84, 63.08, 66.32 and 65.27% respectively. The proximate composition revealed that bread samples produced from CCF had the highest moisture content of 2.75% while that of PCF had the least moisture content of 2.52%. This could be attributed to the fact that cassava flour has higher tendency to retain more water than the other flours due to the high moisture content of its root of values between 65 and 70% (Omosuli *et al*., 2017). This is in accordance with Olaoye *et al*. (2006) who reported that moisture content of composite breads increased with non wheat flour substitution, as a result of higher levels of water holding capacity of non wheat flours as compared to wheat flour. High moisture content indicates high levels of water activity within the dough (a_w) which gives the high spoilage tendency (Smith *et al*., 2010).

The ash content percentages ranged from 1.56 to 1.83% with WB having the highest percentage due to the nutritional content of wheat flour (Ayele *et al*., 2017; Olaoye *et al*., 2011). Higher percentage of crude fat recorded in WF could be attributed to fat content present in the germ of wheat. With respect to protein content, higher percentages recorded in WB and RCB could occur as a result of high composition of protein in wheat grain and unprocessed (local rice variety) utilised in composite flour blends. Crude fibre results revealed that there was no record of undigestible fibres in all the composite bread samples. This could be attributed to the removal of bran from wheat grain and particle size reduction as a result of the Endicott test sieves (600 to 53 μm) employed in sieving the flours after processing. This result differs from the findings of Olaoye *et al*. (2006) who recorded significant percentages in crude fibre on cocoyam composite bread. Higher percentages of carbohydrate recorded in PCB and CCB could be due to their higher compositions of starch as root and tuber crops, making them important as energy or calorie giving foods (Boboye and Owoyemi, 2009). This result is contrary to that of Mongi *et al*. (2011) who reported significant increase in carbohydrate, crude fibre and ash content but decrease in moisture and protein content of cocoyam composite bread.

Microbiological Assessment of the Produced Bread

The result of the microbial analysis is shown in Table 4. This revealed that there was no bacterial and coliform growth on the bread samples. Total viable yeast count shows 0.25×10^{-3} CFU/mL for WB, 0.40×10^{-3} CFU/mL for RCB; 0.21×10^{-3}CFU/mL for PCB and 0.32×10^{-3}CFU/mL for CCB respectively after 5 days of observation and counting. The absence of viable microbial growth of cells within 5 days of counting and examination is similar to the result obtained by Oloyede, (2013) who also recorded no microbial growth and count on bread samples produced from wheat-fermented unripe plantain flours after 4 days of microbiological examination. Absence of microbial cells until day 5 could be attributed to good sanitation practises employed during production of the bread. However, the presence of microbial growth (yeast count) after day 5 could be attributed to the primary source of the yeast (Burkutu) since it is an indigenous micro flora for the isolation of different strains of yeast (Olowonibi, 2017).

Table 2. Physical Properties and Appearance of Produced Bread Samples

Sample	Crumb colour	Crust colour	Loaf volume (mL)	Weight (g)	Width (cm)	Loaf specific volume (mL g^{-1})
A	Creamy	Brownish	451.2^a	350.3^a	7.0^a	1.29^c
B	Creamy	Light Brown	421.4^c	320.1^c	5.0^d	1.32^b
C	Brownish	Dark Brown	421.3^c	321.5^c	6.0^c	1.31^b
D	Pale yellow	Light Brown	445.7^b	330.5^b	6.3^b	1.35^a

Values with similar superscripts in a column do not differ significantly ($P > 0.05$)

Keys:
A - WB plus Burkutu yeast;
B - RCB plus Burkutu yeast;
C - PCB plus Burkutu yeast;
D - CCB plus Burkutu yeast.

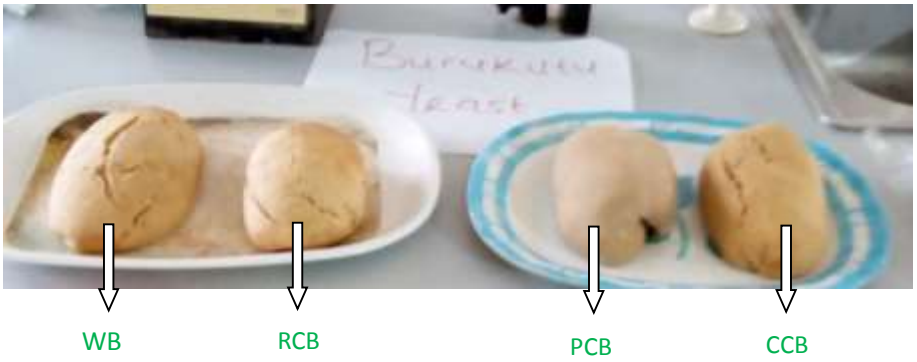

Figure 1. The Finished Product of Produced Bread using *S. cerevisiae* isolated from Burkutu

Key: WB – Wheat Bread; RCB – Rice Composite Bread; PCB – Plantain Composite Bread; CCB – Cassava Composite Bread.

Table 3. Proximate Composition of the Produced Bread

Parameters	**Bread Samples**			
(%)	**WB**	**RCB**	**PCB**	**CCB**
Moisture	2.53±0.01^{b}	2.66±0.01^{a}	2.52±0.02^{b}	2.75±0.07^{a}
Ash	1.83±0.03^{a}	1.79±0.11^{a}	1.56±0.02^{b}	1.74±0.02^{a}
Protein	10.76±0.03^{b}	11.34±0.05^{a}	9.21±0.02^{c}	8.31±0.00^{d}
Lipid	22.04±0.02^{a}	21.14±0.52^{ab}	20.40±0.57^{b}	21.93±0.62^{a}
Fibre	0.00	0.00	0.00	0.00
Carbohydrate	62.84±0.35^{b}	63.08±0.56^{a}	66.32±0.60^{a}	65.27±0.71^{a}

Values are Mean ± SD, value with different superscript across the rows are statistical significant at $p<0.05$ using Duncan multiple range test

Keys:
WB - Wheat Bread plus Burkutu yeast
RCB - Rice Composite Bread plus Burkutu yeast
PCB - Plantain Composite Bread plus Burkutu yeast
CCB - Cassava Composite Bread plus Burkutu yeast

Table 4. Microbiological Profile of the Produced Bread

Bread Samples	TYC (**x 10^{-3}**)	TCC	TVC
WB	0.25	NIL	NIL
RCB	0.40	NIL	NIL
PCB	0.21	NIL	NIL
CCB	0 32	NIL	NIL

KEY: TYC- Total Yeast Count **, TCC-** Total Coliform Count**, TVC-** Total Viable Count**, NIL** – Absence of Micro organism.
WB - Wheat Bread plus Burkutu yeast;
RCB - Rice Composite Bread plus Burkutu yeast;
PCB - Plantain Composite Bread plus Burkutu yeast;
CCB - Cassava Composite Bread plus Burkutu yeast

Sensory Assessment of the Produced Bread

The sensory perception of the produced bread samples from the different flours are presented in Figure. 2. This shows the different views of the ten (10) panellists on a nine (9) point hedonic scale ranging from dislike extremely to like extremely of the produced bread. Composite breads produced from Plantain Composite Flour and Rice Composite Flour had lower mean scores in terms of crust colour and flavour, while that of RCF had the highest mean score in terms of texture. Review of all the organoleptic properties revealed that WB averagely had the highest mean score of 7.03 while PCB had the least mean score of 6.57 There was no significant difference at (P>0.05) between the perception of different qualities (crumb colour, crust colour, flavour, texture, sweetness and general acceptability) of the produced bread samples. CCB was found to be comparable to WB with respect almost all the organoleptic properties. Similar result was obtained by Aboaba and Obakpolor, (2010) and Eddy *et al*. (2007) who reported no significant difference in sensory attributes of CCB when compared to 100% Wheat Bread (WB). Bread produced from the incorporation of cassava flour (about 30%) with wheat flour have been found to be acceptable, with insignificant variation when compared to bread made with 100% wheat flour (Jensen *et al*., 2015) The preference of WB with respect to crumb colour, sweetness and general acceptability could be attributed to the familiarisation of panellists with the conventional wheat bread (Olaoye, 2006). Also, composite breads produced from other flours aside wheat flours has the tendency to have hard crust and crumb structure of cake unlike the conventional bread, thus agreeing with the result of Mongi *et al*., (2011).

Shelf life Assessment of the Produced Bread

The shelf life assessment of the bread samples after seven (7) days of storage at the experimental temperature (26±1^{o}C) is shown in Table 5. The data generated showed that there was no contamination after production of the bread. There was no evidence of spoilage organisms within the first three days of storage in WB (Wheat Bread), RCB (Rice Composite Bread) and PCB (Plantain Composite Bread) and CCB (Cassava Composite Bread). However spoilage organisms were observed to occur in the bread samples within the last three days of storage. The presence of microbial growth recorded in CCB could be attributed to high moisture content of cassava (Omosuli *et al*., 2017). Microorganisms identified to have infected the bread samples includes *Aspergillus sp*, *Rhizopus*, and *S. cerevisiae*, this is similar with the result obtained by Dzomeku *et al*. (2012) who reported *Aspergillus flavus, A.niger, Rhizopus*, *Peniccilium* and *S. cerevisiae* as the spoilage microorganisms associated with the spoilage of bread at room temperature.

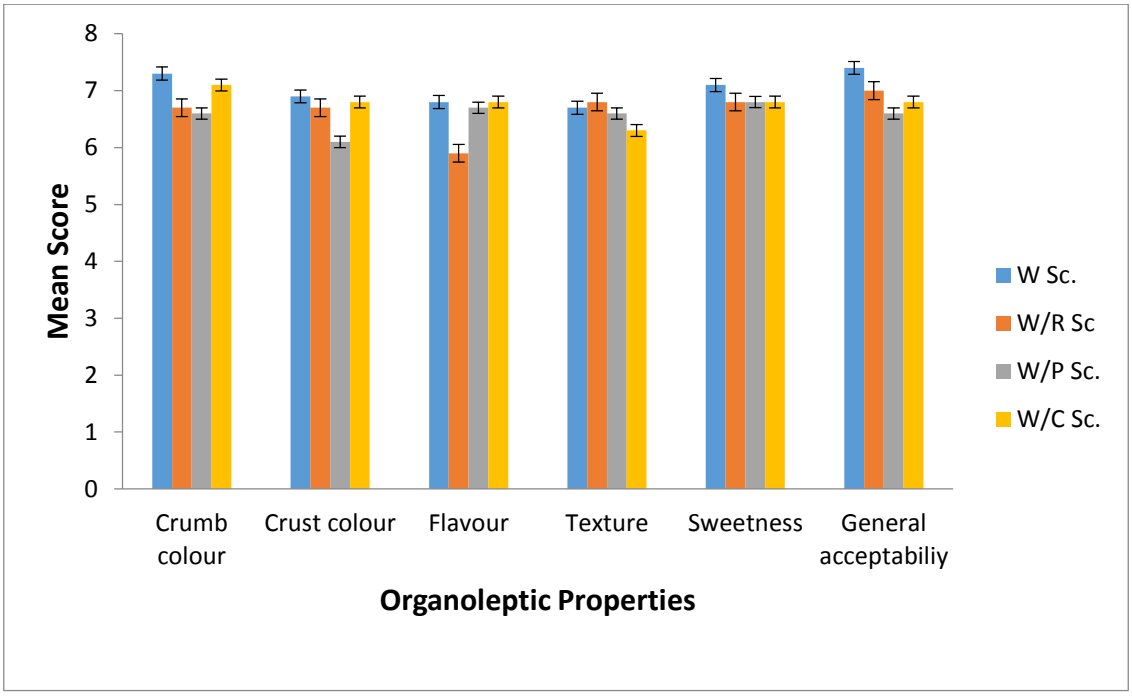

Figure 2. Organoleptic Properties of Different Produced Bread Samples

Table 5. Assessment of Shelf Life of the Produced Bread Samples at Room Temperature (26±1°C)

Shelf life (Days)	WB		RCB		PCB		CCB	
	NA	**PDA**	**NA**	**PDA**	**NA**	**PDA**	**NA**	**PDA**
1	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
2	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
3	NIL	NIL	NIL	NIL	NIL	NIL	NIL	*S. cerevisiae*
4	NIL	*S. cerevisiae*	NIL	*S. cerevisiae*	NIL	*S. cerevisiae*	NIL	*S. cerevisiae*
5	NIL	*S. cerevisiae, Rhizopus*	NIL	*S. cerevisiae*	NIL	*S. cerevisiae*	NIL	*S. cerevisiae*
6	NIL	*S. cerevisiae, Apergillus sp.*	NIL	*S. cerevisiae*	NIL	*S. cerevisiae*	NIL	*S. cerevisiae*
7	NIL	*S. cerevisiae, Rhizopus, Aspergiluus sp.*	NIL	*S. cerevisiae*	NIL	*S. cerevisiae*	NIL	*S. cerevisiae*

KEY: NA - Nutrient Agar; PDA - Potato Dextrose Agar; and NIL- No growth.

CONCLUSION

The utilization of *S. cerevisiae* strain TSA66 isolated from local fermented beverage (Burkutu) in composite bread production was successful and yielded good result in terms of loaf weight and loaf volume. Weight loaves of the composite bread samples ranged from 320.1 - 350.3 g, loaf volume ranged from 421.3 - 451.2 mL and loaf specific volume from 1.29 - 1.35 mL g^{-1}, with significant difference of ($P < 0.05$). Bread produced from the incorporation of cassava flour with wheat flour was found to be acceptable, with insignificant variation when compared to the bread made with 100% wheat flour. There was no microbial growth of cells in the bread samples within 3 days of counting and examination. Viable microbial cell growth and count recorded after day 3 revealed the presence of *Aspegillus sp, Rhizopus, S. cerevisiae,* which were the indigenous yeast isolates from the isolation source (Burkutu). The exploitation of indigenous cultures from cheap and readily available substrates is an important facet to be properly harnessed in Biotechnology. Other native flours like cassava having suitable potentials to partly replace wheat flour could be blended with wheat in bakery products. This would help in reducing the high cost of wheat importation and also improve the livelihoods of citizens engaged in agriculture, thereby promoting the cultivation of local raw materials. Finally, bread from composite flour is a welcomed initiative for countries where high cost of wheat importation poses a great challenge.

REFERENCES

Abdelghafor, O. R. F., Mustafa, A. I., Ibrahim, A. M. H., and Krishnan, P. G. (2011) "Quality of Bread from Composite Flour of Sorghum and Hard White Winter Wheat," *Advance Journal of Food Science and Technology* ***3****(1) 9-15.*

Aboaba, O.O. and Obakpolor, E.A. (2010). The Leavening Ability of Baker's Yeast on Dough Prepared with Composite Flour (Wheat/ Cassava). *African Journal of Food Science* ***4****: 325-329*

Adesina, A. (2011). Weekly Federal Executive Committee press briefing www. Africa.com ((Accessed 02/12/11).

Alo, M. N. Eze U. A. and Eda N. E. (2012). Microbiological Qualities of Burkutu Produced from a Mixture of Sorghum and Millet. *American Journal of Food and Nutrition* ***2****(4): 96-102*

A.P.H.A. (2015). Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association Washington, DC, USA.

Anaukwu, C.G., Nwagu, F.C., Okafor, O.I., Ezemba, C.C., Orji, K.C., Agu, K.C., and Archibong, E.T. (2015). Burukutu (Brief Overview, Health Importance, Isolation). *European Journal of Experimental Biology* ***5****(8) :18-22*

A.O.A.C (2010). *Official Methods of Analysis. Association of Official Analytical Chemists* (18th edition) Washington D.C, U.S.A.

Atter, A., Obiri-Danso, K., and Amao-Awua, W.K. (2014). Microbiological and Chemical Processes Associated with the Production of Burukutu a Traditional Beer in Ghana. *International Food Research Journal* ***21****(5):1769-1776*

Ayele, H.H., Buttosa, G., Abera, T. and Astatkie, T. (2017). Nutritional and Sensory Quality of Wheat Bread Supplemented with Cassava and Soybean Flours. *Cogent Food and Agriculture* ***3****(1): 1-14*

Ayo J.A., Ayo, V.A., Popoola, C., Omosebi, M., and Joseph, L. (2014). Production and Evaluation of Malted Soybean-acha composite Flour Bread and Biscuit. *Africa Journal Food Science and Technology.* ***5****(1):21-28.*

Boboye, B., and Dayo-Owoyemi, I. (2009). Evaluation of Dough Sensory Properties Impacted by Yeasts Isolated from Cassava. *Journal of Applied Sciences* ***9****(4):741-776*

Cauvain, S. (2012). Breadmaking *:* An Overview 9-31 BakeTran , U.K.

Cauvain, S. (2015). Bread; The Product. In Technology of Breadmaking. Springer International Publishing pp 1-22

Chikodili, G. A., Franklin, C. N., Onyedika I. O.,Chinyere C. E., Chinedu, C. O., Kingsley, C. A. and Etim J. A. (2015). Microbiological Analysis of Burkutu Beverage Produced in Southern Part of Nigeria. *European Journal of Experimental Biology* ***5****(8):18-22*

Chim, C., Erlinda, I.D., Elegado, F.B., Hurtada, A.W., Charkrya, N., and Raymundo, G.L. (2015). Traditional Dried Starter Culyure (Medombae). For Rice Liquor Production in Cambodia. *International Food Research Journal* ***22****(4) 1642-1650*.

Chinedu, S. M. Yusuf, O. S and Ezeja M.I. (2010). Fermentation of Sorghum Using Yeast *Saccharomyces cerevisiae* as a Starter Culture for Burkutu Production. *Continental Journal Biological Sciences* ***3***: *63 - 74*

Dzomeku, M.B., Makimilua, T.B., Akweiba, B., Adalo., J.K., and Appiah, K.B. (2012). Assessing the Proximate of Bread on the Market in Ghana. *Annals Food Science and Technology.* ***13****(1): 29-36*

Eddy, N.O., Udofia, P.G. and Eyo, D. (2007). Sensory Evaluation of Wheat/ Cassava Composite Bread and Effect of Label Information on Acceptance and Preference. *African Journal of Biotechnology* ***6****: 2415 – 2418*

Erikson, E., Koch, K., Tortoe, C., Akonor, P.T. and Oduro-Yeboah. (2014). Evaluation of the Physical and Sensory Characteristics of Bread Produced from Three Varieties of Cassava and Wheat Composite Flours. *Food and Public Health* ***4****(5): 214-222*

Farid, F., Sideeq, O., Khan, F., and Niaz, K. (2019).*Saccharomyces cerevisiae*; Non vitamin and Non mineral Nuritional Supplements.Chp 5: pp 1-8

Inyang, U.E. and Asuquo, I.E. (2016). Physico-Chemical and Sensory Qualities of Functional Bread Produced from Wholemeal Wheat and Unripe Plantain Composite Flours. *MOJ Food Processing and Technology* ***2****(2): 48-53*

Iwe, M.O. (2010). Handbook of Sensory Methods and Analysis. PROJOINT Communicatios Services Ltd. Enugu. Pp 70-72

Janitz, M. (2011).Next Generation Genome Sequencing.Towards Personalized Medicine .Pp 113-114.

Jensen, S., Skibsted, L. H., Kidmose, U. and Thybo, A. K. (2015). Addition of Cassava Flours in Bread-making: Sensory and Textural Evaluation. *LWT - Food Science and Technology,* ***60***: *292–299.*

Jideani, V.A., and Vogt, K. (2015). Antimicrobial Packaging for Extending the Shelf Life of Bread. A Review. *Critical Reviews in Food Science and Nutrition* ***56****(8): 1313-1324*.

Julianti, E., Tharise, N., and Nurininah, M. (2014). Evaluation of Physico-Chemical and Functional Properties of Composite Flour of Cassava, Rice, Potato, Soybean and Xanthan Gum as Alternative of Wheat Flour. *International Food Research Journal* ***2****(4): 1641-1649*

Karki, T.B., Timilsina, P.M., Yadav, A., Pandey, G.R., Joshi, Y. And Bhujel, S. (2017).Selection and Characterization of Potential Baker's Yeast from Indigenous Resources of Nepal. *Biotechnology Research International* ***17****:1-10*

Khaing, T.W., Weine, W. And Mya, M.O. (2016). Isolation and Screening of Ethanol Tolerance Yeast for Bio-ethanol Production in Ethopia. *Global Journal of Life Sciences and Biological Research* ***2****(2): 1-7*

Looke, M., Kristjuhan, K., andKristjuhan, A. (2011). Extraction of Genomic DNA from Yeast for PCR-Based Applications. *BioTechniques* ***50*****:** *325-328*

Maneju, H., Udobi, C.E., and Ndife, J. (2011). Effects of Added Brewers Dry Grain on the Physicochemical, Microbial and Sensory Quality of Wheat Bread. *American Journal Food Nutrition* ***1****(1): 39-43*

Mongi, R.J., Ndabikunze, B.K., Chove, B.E., Mamiro, P., Ruhembe, C.C and Ntwenya, J.A. (2011). Proximate Composition, Bread Characteristics and Sensory Evaluation of Cocoyam- Wheat Composite Breads. *African Journal of Food, Agriculture, Nutrition and Development* ***11****(7): 5586-5599*

Muhopaddhyay, C.S., Choudhary, R.K., andIquebal, M.A. (2017). Basic Local Alignment Search Tool for Nucleotide (BLASTn). Chp 12.

Noor-Aziah, A. A. and Komathi, C. A. (2009). Acceptability Attributes of Crackers Made From Different Types of Composite Flours. *International Food Research Journal* ***16****: 479-482.*

Nwanekezi, E.C. (2013). Composite flours for baked products and possible challenges *–A Review Nigerian food journal* ***31****(2): 8-17*

Ojure, M.A. and Quadri, J.A. (2012). Quality Evaluation of Noodles Produced From Unripe Plantain Flour. *International Journal of Research and Reviews in Applied Science* ***13****: 740-752*

Olaoye, O.A. and Ade-Omowaye, B.I.O. (2011). Composite flours and bread: Potential of Local crops Developing Countries. Flours and Breads and their fortification in Health and Diseases Prevention. Chp 17: pp 183-192

Olaoye, O.A., Onilude, A.A., and Idowu, O.A. (2006). Quality Characteristics of Bread Produced from Composite Flours of Wheat, Plantain and Soybeans. *African Journal of Biotechnology* ***5****(11): 1102-1106*

Olowonibi, O.O (2017). Isolation and Characterisation of Palm wine Strains of *Saccharomyces* cerevisiae Potentially Useful as Bakery Yeast. *European Exp. Biology* ***7****:11-15*

Oloyede, O.O., Ocheme, O.B. and Nurudeen, L.M. (2013). Physical, Sensory and Microbiological Properties of Wheat-Fermented Unripe Plantain Flour. *Nigerian Food Journal* ***31****(2) :123-129*

Omosuli, S.V., Ikujenlola, A.V and Abisuwa,A.T. (2017). Quality Assessment of Stored Fresh Cassava Roots and Fufu Flour Produced from Stored Roots. *Journal of Food Science and Nutrition* ***3****(1): 9-13*

Papageorgiou, M, and Skendi, A. (2018). Introduction to Cereal Processing and By-Products. Sustainable Recovery

and Reutilization of Cereal Processing By-Products. pp 1-25

Santos, A.D., Barros, S.E.M. and Hamdan, S.J. (2006). Establishing a Method of Inoculum Preparation for Susceptibility Testing of *Trichophyton rubirum* and *Trichophyton mentagrophtes*. *Journal of Clinical Microbiology* ***44****(1): 98-101*

Smith, J.P., Daifas, D.P., El-khoury, W., Koukoutsis, J. And El-khoury, A. (2010). Shelf life and Safety Concerns of Bakery Products: A Review. *Critical Review in Food Science and Nutrition* ***44****(1): 19-55*

Stewart, G.G. (2014). Saccharomyces *cerevisiae*. Encyclopedia of Food Microbiology. pp 309-314.

Therdthai, N. and Zhou, W. (2014). Manufacture. *Bakery Products Science and Technology* ***27****: 473-488*