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Evaluation of Dough Sensory Properties Impacted by Yeasts Isolated from Cassava

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Abstract: This study is focused on isolating and identifying yeasts found in cassava as well as assessing the dough fermenting abilities of the isolates in term of leavening. A total of seven yeasts were isolated from the liquor of a four days fermented cassava. These are *Geotrichum lactis*, *Saccharomyces ellipsoideus*, *Candida tropicalis*, *C. robusta*, *C. intermidia*, *Debaryomyces hansenii* and *Zygosaccharomyces bailii*. They were used to ferment wheat flour doughs in order to test the fermentative ability of the isolates. The fermented doughs were baked and organoleptic analysis was carried out using some physical parameters namely: leavening, texture, aroma, taste and appearance. The analysis showed that *Saccharomyces ellipsoideus*, *Geotrichum lactis* and *Candida robusta* were best in leavening the flour doughs. Each of these isolates scored between 55 and 60% in all the attributes tested. In the sensory attributes applied, statistical analysis using ANOVA ($p<0.05$) and Duncan Multiple Range Test showed that about 71 and 80% of the tested isolates compared favourably with the commercial baker's yeasts STK Royal and Saf-instant used.

Key words: Cassava yeasts, fermentation, flour dough, sensory properties

INTRODUCTION

In Nigeria, over the last decade, efforts have been made by researchers to develop composite flours from crops such as cassava and a portion of wheat, which has yielded good results (Giami *et al*., 2004; Edema *et al*., 2005; Olaoye *et al*., 2006). Little attention has been paid to identifying and developing yeast strains that can compete with and even perform better than the commercially available baker's yeast which is a leavening agent.

Leavening agents, either chemical or biological, are important in raising flour dough. Chemically leavened flour products are however not as good as flour leavened biologically. Biological leavening agents are microorganisms that have the ability to produce carbon dioxide from the utilization of sugar (Rosada, 1998). Yeast plays an important role in various fermentation processes including baking and brewing. In brewing, the alcohol released by the fungus during fermentation is important while carbon dioxide is of utmost need for raising of flour dough, maturation and development of fermentation flavour (Norman and Joseph, 1996; Corriher, 2001).

Literatures have shown that bakers have used alternative dough leavening agents in the form of chemical leavening agents and palm wine from palm sap as substitutes for the commercial baker's yeast (Somiari and Udoh, 1993). Although these alternatives have been able to raise dough, but they have failed in the area of bringing out the desired taste, flavour and texture characterized by dough fermented with microbial agents, which consumers have always looked out for when choosing any fermented flour products. For example, bread produced using palm wine sap as substitute for yeast is usually characterized by a sharp sour taste, pronounced palm wine flavour and short shelf life (personal communication with Kole Akinkunmi, 2005, Kole bakery, Akure, Nigeria). This sour taste and short shelf life have been attributed to the large number of the lactic acid bacteria, which produce metabolites undesirable during the bread production.

Cassava (*Manihot esculenta*) is a common starchy substrate whose natural microflora has led to the production of different fermented staple foods such as Gari, Lafun, Fufu and Attieke. A lot of research works have been successfully carried out to develop the fermentation process of these foods through the development of cheap and adaptable technologies as well as to isolate and characterize the microflora involved in the fermentation of these foods. For example, Oyewole (2004) reportedly isolated six different strains of yeast, namely: *Candida crusei*, *C. tropicalis*, *Pichia soitoi*, *P. anomala*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* from fermented cassava water.

There are however scanty information on the dough leavening ability of yeasts isolated form cassava. The study reported here focused on isolating and identifying yeasts found in cassava as well as assessing the dough fermenting ability of these yeasts in term of leavening. Other organoleptic properties of the baked doughs made with the isolates were examined.

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MATERIALS AND METHODS

This study was carried out and completed at the end of 2007 in the Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

Microbial analysis: An aliquot (0.1 mL) of a serially diluted, four-day fermented cassava liquor was plated on Potato Dextrose Agar (PDA) medium by using pour plate method (Harrigan, 1998). The plates were placed at 30±2°C for 48-72 h to effect growth. Each type of the grown colonies was purified on fresh PDA by streaking. Each colony was stained with Lactophenol-in-cotton blue dye and observed under a light microscope using the oil immersion objective. Pure isolates were streaked on PDA slants and stored at 4°C until needed.

The yeasts's isolates were characterized based on their cultural characteristics (colony shapes, pigment, elevation, edge and surface appearance). Morphological and biochemical characterization of the isolated yeasts was done by testing for ability of the yeasts to ferment and assimilate sugars, utilize nitrate, form pellicle, spore, pseudomycelium and mycelium according to the methods of Olutiola *et al.* (1991), Harrigan (1998), Barnett *et al.* (2000) and Fawole and Oso (2001).

Cultivation and determination of fermentative ability of the yeast isolates: The yeasts isolated were cultured individually at 25±2°C in sterilized peptone broth medium containing 2% (w/v) glucose and lactic acid at concentration of 0.2% (v/v) in 1.0 L conical flask equipped with air lock. The set up was agitated continuously on rotary shaker regulated at 150 rpm for 72 h when good growth was observed. The cells were cropped by centrifuging in MSE centrifuge machine at $12{,}168\times10^{3}$ g for 10 min. The yeast concentrate was washed with sterile distilled water after which it was resuspended in 10 mL sterile distilled water.

Dough was prepared as described by Cauvian and Young (1998). Each dough contained wheat, flour, salt, water, sugar and fat. Each yeast isolate (1.41×10^{9} cfu mL^{-1}) was added to these ingredients. Baker's yeasts (STK Royal, China and Saf-instant, France) were used separately as positive control to ferment the doughs. Another set of dough that lacked yeast was prepared as the negative control. The dough samples were left to ferment at room temperature (dry season) 37±2°C for about 1 h and baked in an oven at 200°C for 1.0 h.

Organoleptic analysis of the baked dough samples: The baked dough samples were subjected to sensory analysis by using trained panel of 26 judges to evaluate the leavening, taste, texture, appearance and aroma properties of the baked samples. Five points grade was used in the analysis starting with Excellent = 5, Very Good = 4, Good = 3, Satisfactory = 2 and Poor = 1.

Analysis of data: All data obtained from sensory evaluation were subjected to statistical analysis by using analysis of variance and Duncan's multiple range test. Significance of variations in the analyzed data was tested at 95% confidence limit.

RESULTS

Seven types of yeast representing five genera isolated from fermented cassava liquor were *Geotrichum lactis*, *Saccharomyces ellipsoideus*, *Candida tropicalis*, *C. robusta*, *C. intermidia*, *Debaryomyces hansenii* and *Zygosaccharomyces bailii* (Table 1). Results of the sensory evaluation are shown in Fig. 1-5. The Fig. 1-5 showed that the dough samples compared well with those produced with commercial yeasts namely: STK Royal and Saf-instant in all attributes (leavening, taste, aroma, texture and appearance) considered at $p<0.05$.

Table 1: Morphological and biochemical characteristics of the yeasts isolated from cassava

	Morphology							Biochemical properties: Fermentation/assimilation											
		Ascospore																	
S. No.	Cell shape	Present/ Absent	Shape	Spore	Ps	My	Pe	Gl	Ga	Su	Fr	La	Ma	Ra	Ce	Xy	Ar	Mn	Yeast identity
A	Cylindrical	ND	ND	+	-	+	+	FA	-A	-A	-A	-A	-A	-A	-A	FA	-A	FA	*Goetrichum lactis*
B	Oval	+	Oval	+	+	-	-	FA	FA	FA	-A	- -	FA	-A	-A	-A	-A	FA	*Saccharomyces ellipsoideus*
C	Spherical	ND	ND	+	+	+	+	FA	-A	FA	FA	-A	-A	- -	- -	- -	-A	FA	*Candida tropicalis*
D	Oval	ND	ND	+	-	+	+	FA	FA	FA	FA	-A	FA	-A	-A	-A	-A	-A	*Candida robusta*
E	Oval	+	Oval	+	+	-	-	FA	FA	- -	FA	-A	-A	FA	- -	- -	-A	FA	*Debaryomyces hansenii*
F	Cylindrical	ND	ND	+	-	-	+	FA	FA	FA	FA	-A	-A	- -	- -	- -	-A	FA	*Candida intermidia*
G	Spherical	+	Spherical	+	+	-	-	FA	-A	FA	FA	-A	- -	-A	-A	-A	-A	FA	*Zygosaccharomyces bailii*

Ps: Pseudomycelium, My: Mycelium, Pe: Pellicle, Gl: Glucose, Ga: Galactose, Su: Sucrose, Fr: Fructose, La: Lactose, Ma: Maltose, Ra: Raffinose, Ce: Cellobiose, Xy: Xylose, Ar: Arabinose, Mn: Mannose, +: Positive or Present, -: Negative or Absent, FA: Fermentation and Assimilation, -A: Assimilation only, ND: Not Determined

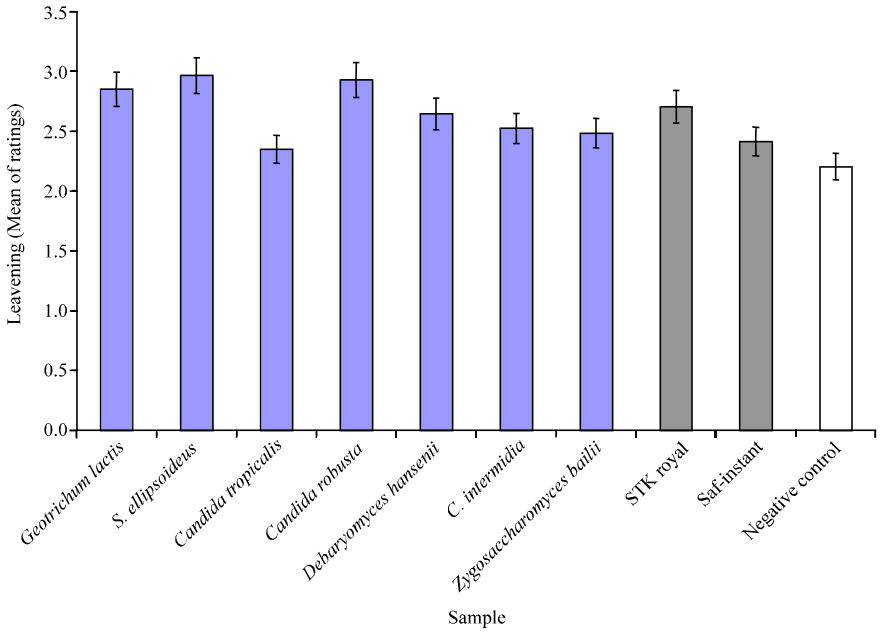

Fig. 1: Leavening activity of the various yeast isolates

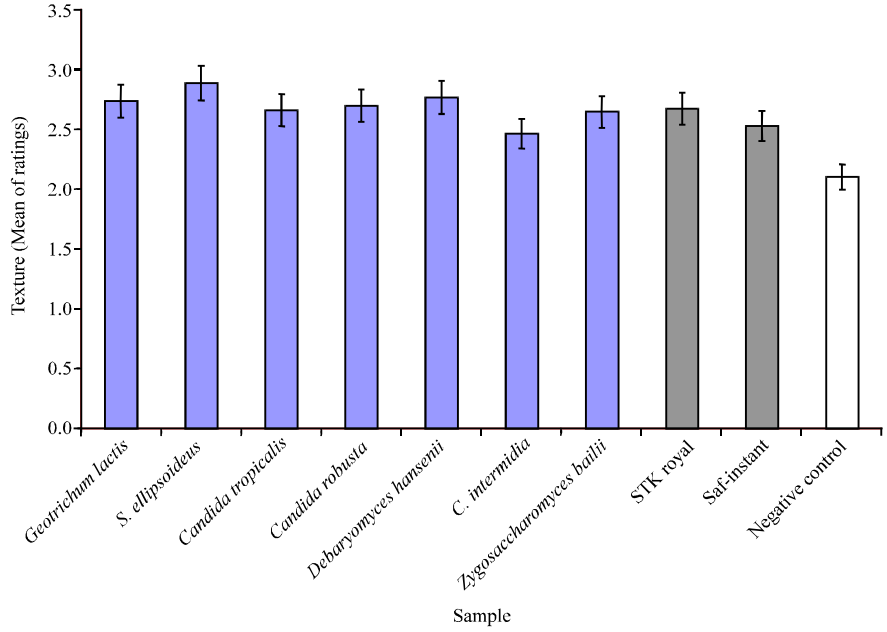

Fig. 2: Texture of baked doughs made with the various yeast isolates

Mean ratings of the leavening activity of the isolates range between 2.35 (*Candida tropicalis*) and 3.0 (*Saccharomyces ellipsoideus*) (Fig. 1). The *S. ellipsoideus*, *C. robusta* and *G. lactis* showed fermentative ability with leavening rating between 2.85 and 3.0. They performed better than the two commercial baker's yeasts. The remaining yeast isolates had mean leavening activity rated below 2.5 with *C. tropicalis* having the least score of 2.35. The commercial yeast STK Royal and Saf-instant had leavening activities of 2.7 and 2.4, respectively, while the dough baked without yeast (negative control) had lowest leavening activity rating of 2.28.

The mean texture rating of the yeast isolates range between 2.46 (*C. intermidia*) and 2.89 (*S. ellipsoideus*) with the latter having the highest mean texture rating followed by *Debaryomyces hansenii* (2.76), *Geotrichum lactis* (2.73), *Candida robusta* (2.70), *C. tropicalis* (2.65)

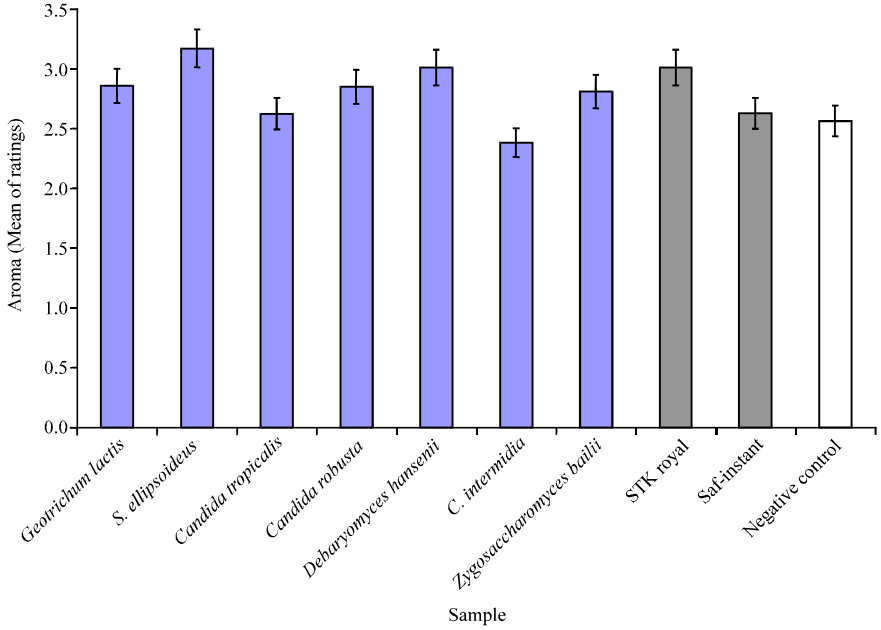

Fig. 3: Aroma of baked doughs made with the various yeast isolates

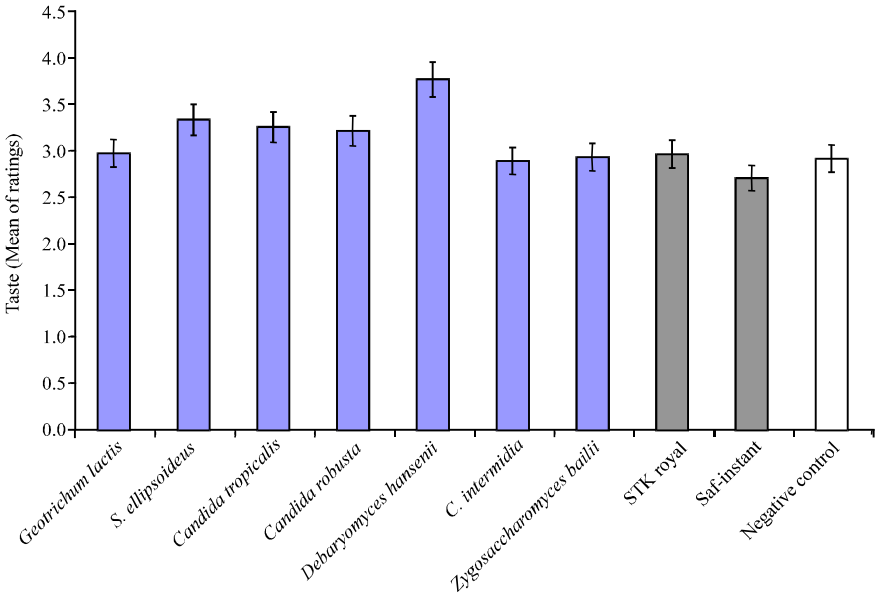

Fig. 4: Taste of baked doughs made with the various yeast isolates

and *Zygosaccharomyces bailii* (2.64) (Fig. 2). These yeasts produced doughs with texture better than and similar to those made with the commercial yeasts.

Doughs fermented with the yeasts showed varying acceptance levels of aroma as shown in Fig. 3. *Saccharomyces ellipsoideus* produced dough with the best aroma (3.16). This was followed by *Debaryomyces hansenii* (3.0) and *Geotrichum lactis*, *Candida robusta* and *Zygosaccharomyces bailii* with aroma ratings of 2.85, 2.84 and 2.80, respectively. *Candida intermidia* had the lowest score for aroma (2.4). All the yeasts except *Candida intermidia* produced baked doughs similar in aroma to that of STK Royal and Saf-Instant.

Figure 4 shows the organoleptic levels of the taste of the doughs produced using the isolated fungi and commercial yeasts as well as baked doughs

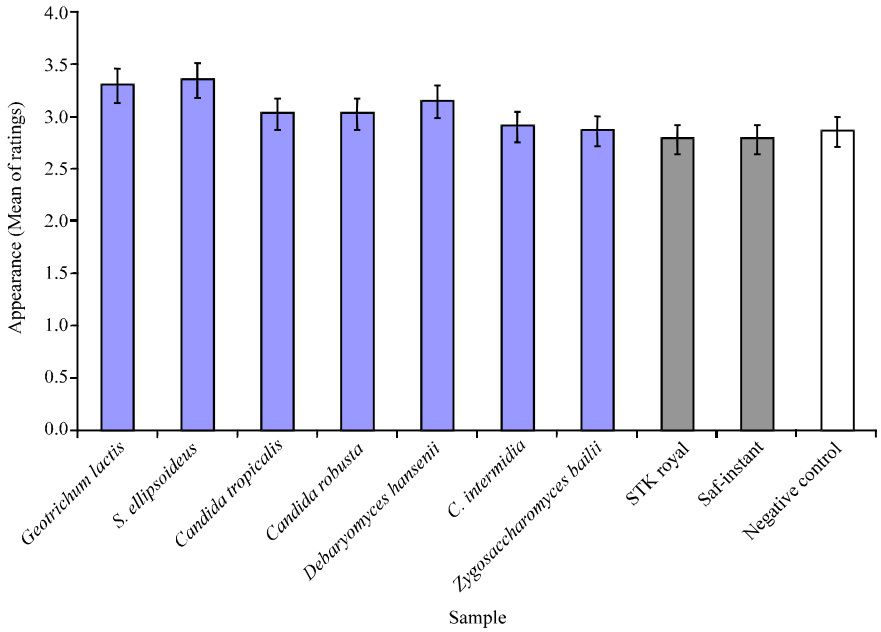

Fig. 5: Appearance of baked doughs made with the various yeast isolates

produced without adding yeast. The yeast isolates produced doughs with taste rating ranging from 2.88 (*Candida intermidia*) to 3.75 (*Debaryomyces hansenii*) corresponding to good and very good grades. Baked doughs fermented with the *C. intermidia* and *Z. bailii* as well as the commercial baker's yeasts have taste that were not significantly different ($p<0.05$) from the negative control.

The mean ratings of appearance of the doughs fermented with the isolated yeasts range between 2.84 (*Z. bailii*) and 3.3 (*S. ellipsodeus*) (Fig. 5). They all rated better at ($p<0.05$) than the commercial yeasts in forming doughs with good appearance.

DISCUSSION

The complexity and variation in the species of yeast that were present in the fermenting-cassava water used in this study are similar to the findings of Oyewole (2004) who reported that six different strains of yeasts were found present in cassava fermenting-water among which are *Candida krusei*, *C. tropicalis*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. This observation is related to the fact that these yeasts were isolated under natural uncontrolled fermentation and that the yeasts were indigenous microflora of the cassava. Cassava root contains a heavy concentration of 18 to 20% protein and 80 to 90% carbohydrates. This relatively high concentration of carbohydrates provides a suitable nutritional medium, which supports the growth and therefore presence of yeasts in fermenting cassava.

The mean leavening activity rating profile of the yeast isolates showed there were some differences in the leavening performances of these yeasts. The considerable increase in the size of the dough samples leavened with the yeast isolates when compared with the unleavened samples (Negative control) is supported by the ability of most of the isolates to ferment sugars such as glucose, fructose and sucrose; the breakdown of which brings about the release of carbon dioxide that caused each dough to rise. The main simple sugars: glucose and fructose represent about 5% of wheat flour and these sugars are transformed into alcohol and carbon dioxide by Zymase, an enzyme naturally present in yeast (Rosada, 1998). The metabolism of simple sugars derived from the flour and the sucrose added as an ingredient is responsible for the evolution of CO_2 into the fermenting doughs, thereby leading to the expansion of the doughs.

The higher leavening abilities of *Saccharomyces ellipsoideus*, *Candida robusta* and *Geotrichum lactis* than other isolates indicate that these yeasts are the best biological wheat dough leaveners obtained in this work since they were better dough leaveners comparing favourably with the commercial baker's yeast. The lowest leavening performance of *Candida tropicalis* when compared to those of other yeasts suggests that it is weak in its ability to ferment dough. The low texture score recorded for *C. tropicalis* further confirms the effect of yeasts on fermented baked doughs.

The doughs fermented with *S. ellipsoideus*, *Debaryomyces hansenii* and *Geotrichum lactis* had leavening activity ratings that are close to each other; this

is supported by texture ratings obtained for the doughs made with these yeasts. The expansion of dough due to the carbon dioxide produced by yeasts leads to a characteristic porosity and texture of fermented baked dough (Corriher, 2001). Except for *Candida robusta*, the *Candida* species isolated in this work can be said to be weak dough fermenters.

Saccharomyces ellipsoideus and *D. hansenii* and other yeasts that produce doughs with very good and good aroma ratings indicate that the yeasts were able to produce compounds such as organic acids, alcohols, aldehydes and carbonyls which have imparted appealing flavour to the doughs. The taste ratings of baked doughs fermented with the isolated yeasts are comparable to each other and to those of the commercial baker's yeasts means that the taste of the fermented doughs after baking is more dependent on the ingredient formulation than on yeast activity.

Furthermore, the very good appearance ratings of about 71% of the yeast isolates imply that the appearance of baked fermented dough is influenced substantially by the previous activities of the yeasts in the doughs.

CONCLUSION

Generally, results of the sensory evaluation presented in the figures showed that the dough samples compared well with those produced with commercial yeasts namely: STK Royal and Saf-instant in all attributes (leavening, taste, aroma, texture and appearance) considered at $p<0.05$. It has been revealed in this work that cassava is an excellent habitat where yeasts with potentials for industrial uses can be isolated, particularly yeasts with dough fermenting ability. In all the attributes considered, the yeast, *Saccharomyces ellipsoideus* had the overall best performance. This could be linked with the superior fermentative ability possessed by the *Saccharomyces* yeasts (Walker, 1998). Following this yeast in performance are *Geotrichum lactis*, *Candida robusta*, *Debaryomyces hansenii* and *Candida intermidia* respectively. They had sensory qualities that were similar to those made with commercial baker's yeast, therefore, they show prospect of being developed into commercial yeasts.

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