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Comparative Evaluation of the Sensory Properties of Doughs Fermented with Yeasts Isolated from Orange

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Abstract: Five different yeasts were isolated from the juice of orange. The yeasts were identified as *Brettanomyces bruxellensis, Hanseniaspora uvarum, Saccharomyces rosei, Pichia fermentans* and *Hypopichia burtoni.* Yeast population (1.41×10^9 cfu mL^{-1}) of each of the isolates was used to ferment wheat flour dough in order to determine their individual fermentative abilities. Sensory evaluation of the baked fermented doughs using parameters namely: leavening, texture, aroma, taste and appearance revealed that the yeasts, *Saccharomyces rosei and Pichia fermentans* produced loaves having sensory properties ($p \leq 0.05$) comparable with two baker's yeasts (Fermipan and Sat-instant) commonly used in many of the bakeries in Ondo State, Nigeria.

Key words: Orange yeasts, fermentation of dough and organoleptic properties

INTRODUCTION

Yeasts have been isolated from various fermenting sources for dough leavening. Such include yeasts isolated from Nigerian palm wine (Somiari and Udoh, 1993; Adenaike *et al*., 2006; Boboye *et al*., 2008) and pineapple (Olorunfemi and Adetuyi, 2005). Orange (*Citrus cinensis*) juice is an acidic beverage (pH 3 to 4) with high sugar content (~15^0 Brix). Under this condition, acidolactic bacteria, yeasts and molds comprise the typical microbiota present in the orange juice (Arias *et al*., 2002). Yeast species associated with orange juice have been investigated. According to Hatcher *et al*. (2000) typical yeast species associated with citrus juices are *Candida parapsilosis*, *Candida stellata*, *Saccharomyces cerevisiae*, *Torulospora delbruecki* and *Zygosaccharomyces rouxii.* Despite the rich yeast flora of orange juice, there are little or no information on the dough leavening ability of yeasts isolated from orange juice. This study was directed to assessing the fermentative abilities of yeasts isolated from orange juice as well as their effects on the sensory characteristics of bread.

MATERIALS AND METHODS

Isolation of yeasts from orange juice: Fully ripe orange fruits purchased from Akure metropolis, Nigeria in year 2007, were washed and rinsed many times in sterile distilled water. The fruits were cut, squeezed and the juice was collected into a sterile air-tight container. An aliquot (0.1 mL) of the serially diluted juice was plated on acidified Potato Dextrose Agar (PDA) by using pour plate method (Harrigan, 1998). The plates were placed at 30±2°C for 48-72 h. After good growth of the colonies, each type of colony was purified on fresh PDA by streaking. Each colony was confirmed to be yeasts by examining them under a light microscope using the oil immersion objective after staining with lactophenol-in-cotton blue dye. Pure isolates were plated on PDA slants and stored at 4°C until needed.

Characterization and identification of the yeast isolates: The yeast isolates were characterized by conventional methods based on their cultural characteristics and their morphological properties as described by Barnett *et al*. (2000). Further identification of the isolates was done in accordance to the proposed scheme of Deak and Beuchat (1993). The cultural properties of the yeast isolates were determined by observing their distinct colonies on PDA. Colony shapes, pigment, elevation, edge and surface appearance were the cultural characteristics considered. Biochemical characterization of the yeasts was done by testing for ability of the isolates 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).

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Cultivation of yeast isolates for dough fermentation: The yeasts isolated were cultured separately at 25±2°C in pasteurized basal medium containing 20% (w/v) glucose and lactic acid at concentration of 0.2% (v/v) contained in 1 L conical flasks equipped with air locks. The set up was agitated continuously for 72 h in rotary shaker regulated at 150 rpm. After good growth was observed, the biomass concentrate for each yeast specie was obtained by centrifuging the culture in a MSE centrifuge machine at $12,168\times10^3$ g for 10 min. The yeast concentrates were washed sufficiently with sterile distilled water after which they were resuspended in 10 mL of sterile distilled water. Biomass concentration of each yeast and commercial yeasts (Fermipan and Saf-instant) used as positive control was calculated from the absorbance measured at 630 nm. Dilutions were made as necessary to obtain an optical density between 0.3 and 0.9.

Determination of fermentative ability of the yeasts: Samples of doughs were prepared as described by Cauvian and Young (1998). Each dough sample contained wheat, flour, salt, water, sugar and fat. Each yeast isolate (1.41×10^9 cfu mL^{-1}) was used to ferment the dough. Baker's yeasts (Fermipan and Saf-instant) were used separately as positive control to ferment the dough. Another set of dough formulation that did not contain any yeast sample was also prepared as the negative control. The dough samples were left to ferment at room temperature of 37±2°C for about 1 h. The doughs were baked in an oven for 1 h at 200°C.

Comparative analysis of the baked dough samples: The baked dough samples were subjected to sensory analyses by using panel of enlightened 26 judges to evaluate the following physical parameters: leavening, taste, texture, appearance and aroma. 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 Multiple Range Test. Significance of variations in the analyzed data was tested at 95% confidence limit.

RESULTS AND DISCUSSION

A total of five yeast strains representing five genera were isolated from the orange juice (Table 1). The research of Arias *et al*. (2002), who isolated a total of 99 strains of yeasts from a variety of orange juice sources using five different identification methods confirms that orange juice is a suitable substrate where yeasts can be sourced for. The variation in species of yeasts that were present in the orange juice used in this study is related to the fact that the yeasts were isolated under natural and uncontrolled environmental conditions.

Figure 1 shows the leavening ability of the five different yeasts on doughs. Mean leavening activity ratings of the isolates range between 2.15 (*Brettanomyces bruxellensis*) and 3.00 (*Saccharomyces rosei*) while the commercial yeasts had leavening activities of 2.5 and 2.8, respectively. These correspond to good leavening abilities on the sensory scale used at significant level of ($p\leq0.05$). Dough baked without yeast (negative control)

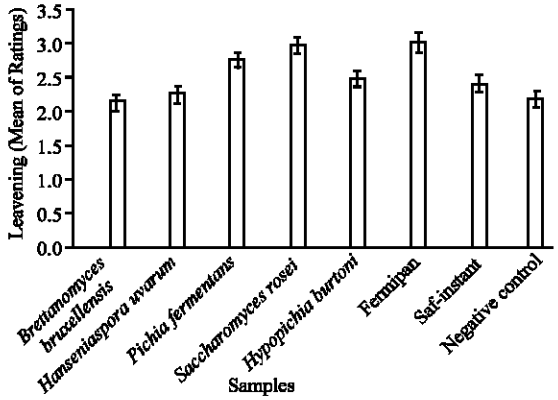

Fig. 1: Dough leavening activities of the yeasts isolated from orange

Table 1: Morphological and biochemical characteristics of the yeasts isolated from the fermented orange juice

		Ascospore		Morphology				Biochemical properties: Fermentation/Assimilation											
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	+	Spherical	+	+	+	+	FA	FA	FA	FA	FA	-A	--	FA	-A	-A	FA	*Brettanomyces bruxellensis*
B	Oval	ND	ND	+	-	-	-	FA	FA	-A	-A	-A	-A	-A	-A	--	-A	FA	*Hanseniaspora uvarum*
C	Oval	+	Sherical	+	+	-	+	FA	-A	FA	FA	-A	-A	-A	-A	-A	-A	FA	*Pichia fermentans*
D	Cylindrical	ND	ND	+	+	-	-	FA	-A	--	FA	-A	FA	FA	FA	--	-A	FA	*Saccharomyces rosei*
E	Cylindrical	ND	ND	+	-	-	+	FA	FA	-A	FA	-A	-A	--	--	-A	-A	FA	*Hypopichia burtoni*

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

had leavening activity of 2.20. The high leavening performance of *Saccharomyces rosei* indicates that the organism was the best biological wheat dough leavener obtained in this study. Next to it in leavening performance is *Pichia fermentans* that compared favourably with the commercial yeasts tested. The metabolism of simple sugars derived from flour and sucrose added as ingredients by the yeasts can be said to be responsible for the evolution of carbon dioxide into the fermenting doughs, thereby leading to the expansion of the doughs. This confirms the statement made by Rosada (1998) that the leavening performance of yeasts can be said to be dependent on their sugar fermentative abilities. The yeasts, *Brettanomyces bruxellensis* and *Hanseniaspora uvarum* having a leavening rating of 2.15 and 2.27 respectively, performed least in the leavening of the flour implying that they are poor dough leaveners. According to Beuchat (1988), strains of *Brettanomyces* exhibit characteristic negative Pasteur effect i.e., oxygen stimulate fermentation of glucose. Yeasts belonging to this genus are normally short-lived due to the production of copious acetic acid. This property may have accounted for the poor leavening ability of this yeast isolate.

The mean texture ratings of the doughs range between 1.96 (*Brettanomyces bruxellensis*) and 2.72 (*Saccharomyces rosei*) (Fig. 2). The yeast *Hanseniaspora uvarum, Hypopichia* and *Pichia fermentans* produced doughs with textures rated between 2.46 and 2.27. This range corresponds to good texture ratings in the sensory scale used. Furthermore, it was observed from the result that the texture ratings are at very close range to their leavening activity ratings. This suggests that besides causing dough to rise, the yeasts activities also have effect on the texture of doughs they fermented. This confirms the statement made by Corriher (2001) that expansion of dough due to the carbon dioxide produced by yeast leads to a characteristic porosity and texture of baked doughs.

Doughs fermented with the isolated yeasts showed varying acceptance levels of aroma as revealed in (Fig. 3). The aroma ratings range between 2.38 (*Brettanomyces bruxellensis*) and 2.81 (*Hypopichia burtoni*). *Hypopichia burtoni* produced dough with the best aroma. This was followed by *Saccharomyces rosei* (2.76) and *Pichia fermentans* (2.69). *Saccharomyces rosei* and *Pichia fermentans* produced doughs that were comparable in aroma ($p \leq 0.05$) with one of the commercial yeast (Saf-instant yeast) (3.0). When compared with the aroma rating of dough that lacked yeast (2.20), it could be deduced that all the yeasts were able to produce compounds which have imparted appealing flavours to the baked fermented doughs. Figure 4 shows the organoleptic level of the taste of doughs produced using the isolated yeasts and commercial yeast as well as dough that lacked yeast. *Saccharomyces rosei* and *P. fermentans* with taste ratings of 3.12 and 3.04 produced doughs

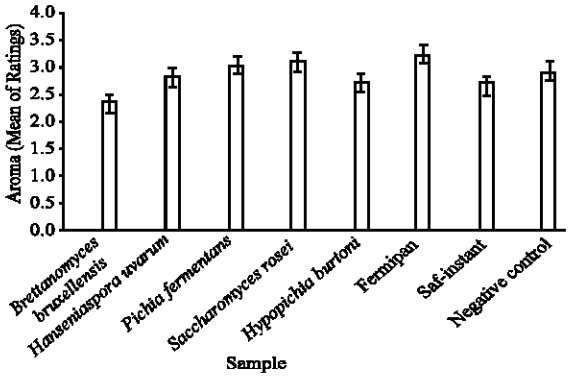

Fig. 3: Aroma of baked doughs prepared with the various yeasts isolated from orange

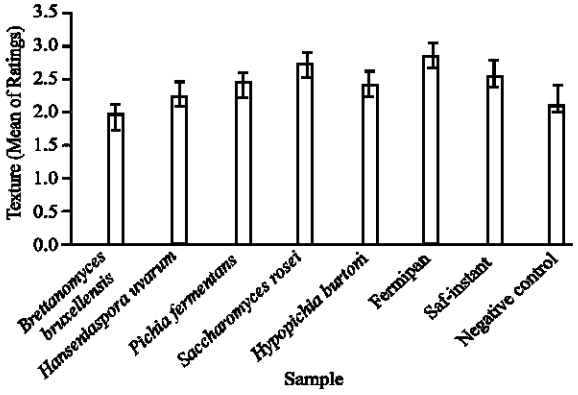

Fig. 2: Texture of baked doughs prepared with the various yeasts isolated from orange

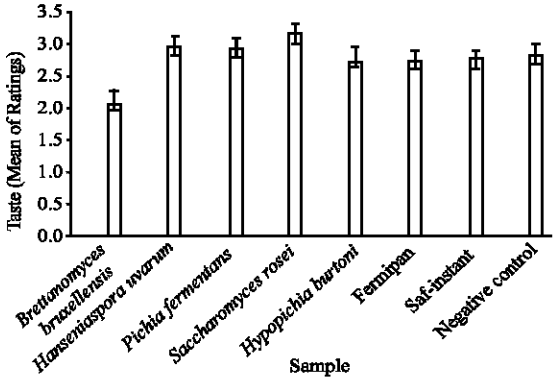

Fig. 4: Taste of baked doughs prepared with the various yeasts isolated from orange

with tastes rated better than the taste of doughs fermented with the two commercial yeasts. These ratings fell within the very good range of the sensory scale used. The taste ratings of baked dough fermented with *Brettanomyces bruxellensis* was significantly lower ($p \leq 0.5$) when compared with the taste ratings of doughs fermented with the remaining yeasts. This implies that all the yeasts except *Brettanomyces bruxellensis* improved the taste of the doughs they fermented making them to be more palatable after baking. Furthermore, with the exception of dough fermented with *B. bruxellensis*, the doughs fermented with the isolated and the commercial yeasts had appearance ratings that were comparable with the appearance of the negative control dough that lacked yeast. This could imply that the appearance of fermented doughs after baking was not greatly influenced by the activities of the isolated yeasts. Appearance of baked doughs prepared with the various yeasts isolated from oranges is shown in Fig. 5.

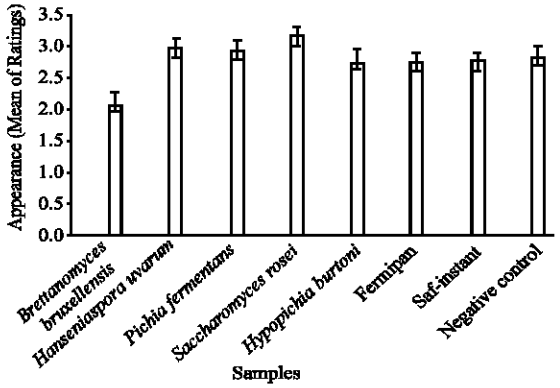

Fig. 5: Appearance of baked doughs prepared with the various yeasts isolated from orange

The result obtained in this work have shown that the juice of orange which is one of the most economically important fruit that is cultivated extensively in Akure, Nigeria, is an excellent source of yeasts having dough fermenting abilities. It is therefore recommended that research works aimed at isolating and developing yeast strains with biotechnological importance from indigenous fruits should be intensified.

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