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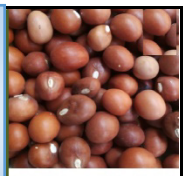

Isolation, Identification and Leavening Ability of Yeast from Local Fruits

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ABSTRACT

Yeast is the organism used in the leavening of dough in a bakery. The study was carried out to determine the leavening ability of yeast isolated from different fruits. Three different yeast species were isolated from different fruits and were identified using cultural and microscopic methods. The yeast species isolated were tested for their temperature tolerance, ethanol tolerance, carbohydrate fermentation and leavening ability. The isolates were able to tolerate different temperature ranges (25 °C, 30 °C and 37 °C), and different concentrations of alcohols (2%, 4%, 6%, and 8%). They were also able to grow and ferment glucose, sucrose, and fructose. The ability of the identified yeast species to increase the volume of the dough was examined by fermenting flour dough. The fermentation was conducted at 30 °C for 72 h. Yeast isolates from date, pineapple, mango and commercial beakers yeast were found to have a leavening ability of 66, 60, 40 and 58, respectively, and the control having 20 cm^3/g. This indicates that the fruits could be a potential source of indigenous yeast species which can serve as good leavening agents.

INTRODUCTION

Breaking down of sugars by yeast is the oldest application used in the leavening of flour dough. Babylonians (6000 BC) and Egyptians (5000 BC) have left written accounts of the production of bread by leavening flour dough, where all of them warranted the use of yeast [1]. Yeast is a unicellular eukaryotic fungus, very common in the environment and mostly saprophytic with about 1,500 species currently described. Yeast growth asexually by mitosis, and by an asymmetric division of the yeast cell called budding. It's often isolated from sugar-rich materials such as fruits [2–7].

Yeast is a biological leavening agent that is essential in raising flour dough. Biological leavening agents are organisms that can produce carbon dioxide from the breakdown of sugar [2]. Yeast plays a vital role in various fermentation processes including baking and brewing. In brewing, the alcohol produced by the fungus during fermentation is essential while carbon dioxide is of essential need for the rising of flour dough, maturation and development of fermentation flavour [7]. Leavening is the increase in the area of dough by aerating agents such as air, CO_2 and water vapour. The leavening of dough is a result of CO_2 produced by the fermenting organism which is usually *Saccharomyces* species. Yeast specifically *S. cerevisiae*, is used in baking as a leavening agent where it converts fermentable sugars present in the dough into carbon dioxide. This causes the dough to expand as the carbon dioxide forms bubbles [7].

MATERIALS AND METHODS

Sample Collection

Date fruit, Mango, and Pineapple were procured from Gombe's main market within Gombe Metropolis, Gombe State, Nigeria.

Isolation of yeast from Local fruits

Yeast cells were isolated by plating them onto a petri dish containing Yeast peptone agar. Plating was done by streaking a loopful of the organisms from enrichment cultures prepared by inoculating small amounts (about 1-2 g) of the fruit sample into tubes containing broth of the same medium with less agar and chloramphenicol [1]. The plates were incubated at 25 °C for 3 days.

Macroscopic identification of the Isolates
Pure cultures obtained were identified by visual examination of the growth, colour and texture of the isolates.

Microscopic identification
Colonies of yeasts were mixed in a droplet of distilled water on a glass slide and smeared until the smear dried off. The smear was then stained using lactophenol– cotton blue and observed under the microscope at 100× magnification.

Fermentation capacity test
The fermentation capacity test was conducted using 5 mL of yeast fermentation broth in a Durham tube with different carbon sources (glucose, sucrose, fructose) and incubated at 30 ˚C for 72 h. The changes in color from blue to yellow indicate the utilization of carbon sources by the yeasts [8].

Temperature tolerance test
Yeast isolates were plated in YPG medium and incubated at different temperatures of 25, 30, and 37 for 72 h.

Ethanol tolerance test
The ability of the yeast species to grow at different ethanol concentrations was determined by growing them on yeast peptone dextrose broth medium containing 4 different concentrations of ethanol, 2%, 4%, 6%, and 8% respectively and incubated at 30 ˚C for 72 h.

Determination of the leavening ability of the yeast's species
The isolated yeasts were used to ferment the dough. Samples of dough were prepared in measuring graduated cylinder, each dough sample contained wheat flour, water and sugar. All the ingredients were properly mixed. The different yeast isolates were used to ferment the dough. Baker's yeast (*Saccharomyces cerevisiae*) was used separately as a positive control. Another set of dough formulations that did not contain any yeast samples was prepared as the negative control [9]. The dough samples were left at room temperature of 30 ºC for about 12 h. The height of the dough was measured from the graduated surface of the cylinder before and after fermentation and the net increased volume was observed as described by [4].

RESULTS

Isolation and identification of yeast from local fruits
Table 1 shows the result of isolation of yeast from different fruits. Four different species of yeast were isolated and identified from different local fruits.

Table 1. Cultural and morphological characteristics of yeast isolated from different fruits.

S/N	Sources	Cultural Characteristics	Morphological Characteristics	Inference
1	M	whitish	ovoid/circular	yeast
2	P	whitish and creamy	circular	yeast
3	D	creamy	circular	yeast

Key: M - Mango P - Pineapple D - Date

Temperature tolerance test
Table 2 shows the result of the temperature tolerance ability of the isolates. The isolates were able to survive at the different temperatures of (25 °C, 30 °C, and 37 °C).

Table 2. Temperature tolerance test.

	Temperature (°C)		
Yeast isolates	25	30	37
M	+	+	+
P	+	+	+
D	+	+	+

Key: Growth (+) No Growth (-)
M= Mango P= Pineapple D= Date

Fermentation capacity
Table 3 shows the result of the fermentation capacity of the different isolates. Yeast isolates were grown on different carbon sources (glucose, fructose and sucrose) and were able to ferment the different carbon sources.

Table 3. Fermentation capacity test of yeast isolates on different carbon sources.

	Isolates		
Carbon source	M	P	D
Glucose	+	+	+
Fructose	+	+	+
Sucrose	+	+	+

Key: Able to Ferment on Carbon Source (+)
Not Able to Ferment (-), M= Mango, P= Pineapple, D= Date

Ethanol tolerance test
Table 4 shows the result of the ethanol tolerance ability of yeast isolates. The yeast isolates were incubated at different ethanol concentrations (2%, 4%, 6% and 8%) and were found to grow at the different concentrations.

Table 4. Test for ethanol tolerance of yeast isolates at different concentrations of alcohol.

	Different concentration of alcohol (%)			
Yeast isolates	2	4	6	8
M	+	+	+	+
P	+	+	+	+
D	+	+	+	+

Key: Growth (+) No Growth (-)
M= Mango P= Pineapple D= Date

Leavening ability of the different Isolates
Fig. 1 shows the resulting ability of the different yeast isolates to leaven dough. The isolates were mixed with dough and put in separate graduated cylinders as described by Cauvian and Young [10]. Yeast strain from date and pineapple was found to have the highest leavening ability (**Fig. 2**).

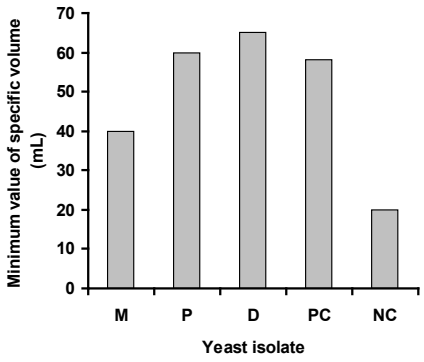

Fig. 1. Leavening capacity of the different yeast isolates (ml) .
Key: Mango (M), Pineapple (P), Date (D), Baker's Yeast Positive Control (PC), Negative Control (NC).`

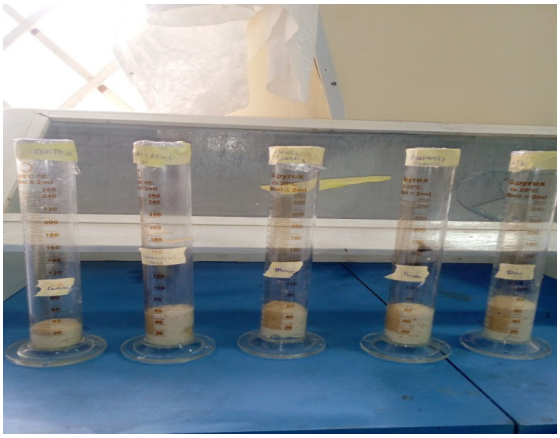

Fig. 2. Volume of fermented dough by Isolated fruit Yeast Mango (M), Pineapple (P), Date (D), Positive Control (PC, Negative Control (NP).

DISCUSSION

Three different species of yeast were isolated and identified using cultural and morphological characteristics. Yeast was isolated from fruits because they are known to be sugar lovers. This result is in agreement with the work of [7] who determine the ability of yeast isolates from Indigenous Sources for Dough Leavening. Ethanol, temperature and fermentative capacity tests were conducted on the isolates. The identified yeast were incubated at different temperatures (25 °C, 30 °C, and 37 °C) and were able to survive the different temperatures.

The result of this finding is in line with the work of [11], who reported that yeast species isolated from different local fruits could withstand a temperature up to 37 °C. The ability of yeast to withstand high temperatures suggests that the yeast isolates can resist high heat associated with fermentation processes and therefore can be used to accomplish fermentation at a wide range of temperatures. They may also be used in bread making to speed up the baking process and increase carbon dioxide production and the production of flavor and aroma.

The yeast isolates were tested for their ability to ferment glucose, sucrose, and fructose and to produce carbon dioxide (**Table 3**). The result indicated that all the yeast isolates were able to ferment all the sugars provided and release carbon dioxide gas as observed in the tube as seen from the color change of carbon sources from green to yellow. The breakdown of sugars releases carbon dioxide that leavens the dough according to [12]. This indicates that it has enzymes capable of breaking down most of the sugars. These indicate that the isolated strains may initiate the fermentation process immediately after inoculation into the dough and can produce more carbon dioxide, causing the dough to rise.

The isolates were also tested for their ability to tolerate different concentrations of alcohol. All the identified strains from local fruits grown well in a medium containing ethanol concentrations of 2%, 4%, 6% and 8%. These showed that the isolates can tolerate ethanol toxicity during fermentation. The results agreed with the findings of [13], who reported that the yeast species can thrive in a medium containing low ethanol concentration while the high concentration of ethanol is reported to be toxic to the yeast by inhibiting the cell's growth due to the destruction of the cell membrane.

The leavening ability of the yeast isolates from local fruits is shown in **Figs. 1** and **2**. All the yeast isolates showed good fermentation or leavening ability giving specific volumes of 40 cm^3/g, 60 cm^3/g, and 66 cm^3/g, respectively with the commercial yeast and control having 58 cm^3/g and 20 cm^3/g respectively. The results showed that all the yeast isolates have the ability to leaven dough comparable to or even better than the commercial yeast. This is supported by the fact that yeasts are capable in fermenting sugars especially glucose, sucrose, and fructose (**Table 3**). The breakdown of sugars will release carbon dioxide that leavens the dough causing it to expand or rise according to [10,12,14]. Yeast isolates from date and pineapple showed the highest leavening ability (**Figs. 1** and **2**) and would be considered the most active yeasts to ferment dough compared to other strains including commercial yeast strain.

CONCLUSION

The result of this study indicates that yeast isolated from local fruits such as mango, pineapple and date were able to tolerate different temperature ranges, and ethanol concentrations and ferment glucose, sucrose, and fructose. The yeast strains have the ability to leaven the dough by releasing carbon dioxide which causes the dough to rise. The yeast isolates from date and pineapple ($66cm^3/g$ and $60cm^3/g$) have the highest leavening ability compared to commercial yeast ($58cm^{3/}/g$). This indicates that the fruits could be a potential source of yeast species for baking purposes. Future recommendations include that the yeast isolates should be identified to the species level and the yeast isolates should be further investigated to determine the possibility of using them for industrial purposes.

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