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**Research Article**

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# Isolation and Performance Evaluation of Palm Wine Yeast

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**Abstract** The fermentation of carbohydrate sources into ethanol and carbon dioxide has become increasingly important to both industrialized and developing countries because of its numerous uses especially in the food industry. This research paper is objectively focused on the possibility of quantifying the carbon dioxide produced from the fermentative growth of yeast (Saccharomyces cerevisiae) and the probability of replacing the commercially imported baker's yeast with freshly isolated yeast strains. An average yeast colony count of $1.25x10^7$cfu/ml was formed after the inoculation of 0.1ml of freshly tapped palm wine from the oil palm specie (Elaeis Guineensis) under aseptic conditions. These colonies were tentatively identified as saccharomyces cerevisiae based on their colonial morphology, cellular characteristics and their abilities to ferment certain sugars. The gassing property of 3 pure yeast colonies were measured using the indirect method by inoculating them into a slant of potato dextrose agar in an Erlenmeyer flask and approximately 115ml of carbon dioxide was recorded in 2 days with rapid gas production obtained within the first 24 hours. Furthermore, the leavening property of freshly isolated yeast strains were been investigated by making a yeast solution with an average yeast colony of $5.00x10^7$ cfu/ml and it was discovered that able it proofed a dough weighing 300g with a proof time of 8hours.

**Keywords** Yeast, Carbon dioxide, Leaven, Fermentation

## Introduction

With the sudden inflation in the prices of commodities especially when they are manufactured or packaged internationally, the need for the development of less expensive locally made substitute has risen. Yeast is considered man's oldest and most industrious micro organism because of its numerous applications in the biotechnology industry. The fermentative abilities if yeast is been used in industries such as the pharmaceutical industry, the biochemical industry, the petroleum industry and especially the food industry where it is used by bakers to leaven dough, brewers in beer making and also in wine making. Since the evolution and advancement in the production of yeast, bakers have specialized in the use of dry yeast gotten from commercial yeast production processes to leaven bread and this brings up questions such as:

- Can this expensive commercial yeast be replaced by cheap propagated ones?
- How can the freshly isolated yeast be isolated and tested for their gassing properties been used to leaven dough in the food industry?
- Can storage conditions have adverse effects on the viability of the freshly isolated yeast cells?

Therefore the main objectives of this research paper are

- To determine the conditions under which yeast cells can be isolated and propagated from palm wine samples to achieve maximum fermentation abilities.
- To produce high carbon dioxide yielding yeast strains from palm wine to be compared to baker's yeast.
- To test the gassing property of yeast both *in vitro* (on Petri dishes) and *in vivo* (in dough).

- To compare the leavening property of baker's yeast and yeast isolated from palm wine to determine the similarities, differences, setbacks and areas for improvement as the case may be.

Palm wine is a whitish and effervescent liquid obtained from palm trees by the process of palm wine tapping. The unfermented sap is clean, sweet colourless syrup containing about 10-12% (w/v) sugar which is mainly sucrose [1]. Upon fermentation by the natural micro flora, the level of this sugar decreases as it converts to alcohol and other products whereas the sap becomes milky white due to the increased microbial suspension resulting from the prolific growth of fermenting organisms [2].

Fermentation sometimes refers specifically to the chemical conversion of sugars into ethanol producing alcoholic beverages such as wine, beer [3].

In order for an organism to make use of a potential source of food, it must be able to transport the food to its cells and it must have the proper enzymes capable of breaking the food's chemical bonds in a useful way [4]. Yeasts are capable of using some but not all sugars as a food source and it can metabolize sugars aerobically and anaerobically. In the course of this research paper, yeast will be isolated using the potato dextrose agar (PDA) as a medium since it contains all the essential requirements for the cultivation of the fungi.

Testing for the gassing property of the freshly isolated yeast *in vivo* will be carried out anaerobically in a slant of PDA by indirect method whereby the volume of gas produced during the fermentative growth of yeast displaces an equal volume of acid water from an air tight conical flask connected to the slant in the Erlenmeyer flask.

Finally, the freshly isolated yeast will be introduced into dough made of flour, sugar and butter and the proof pattern will be monitored and compared to dough leavened with the commercial baker's yeast. The net chemical equation for the production of ethanol from glucose is given as:

$C_6H_{12}O_6$ (glucose) → 2 $C_2H_5OH$ (ethanol) + 2 $CO_2$ (carbon dioxide)

**Materials and Methods**

In order to achieve the aim of this research paper, the experiments to be carried out are classified into three interrelated groups sequentially

1. Isolation of pure yeast strains from palm wine
2. Measurement of the carbon dioxide production from the fermentative growth of the freshly isolated yeast from 1 above
3. Investigating the leavening properties of the freshly isolated yeast in dough in comparison with commercially produced baker's yeast.

**Materials**

- Materials needed for the isolation of the pure yeast strains include; palm wine sample gotten from Alakahia Rivers State, the growth media- Potato Dextrose Agar (PDA), ethanol (minimum concentration of 80%), distilled water, beaker, conical flasks, weighing balance, Petri-dishes, Autoclave, lactic acid, pipette bulb, glass rod, incubator.
- Materials needed for the carbon dioxide measurement include; PDA, Erlenmeyer flask, conical flask (1000ml), rubber tubing, cork, inoculating loop, Bunsen burner, distilled water, hydrochloric acid, measuring cylinder.
- Materials required for the evaluation of the leavening properties of the freshly isolated yeast include; freshly isolated yeast with an average count of 5.0x107cfu/ml, dough weighing 300g, water.

**Isolation of Pure Yeast Strains from Palm Wine**

This involves isolation, culturing and propagation of yeast cells. The growth media (potato dextrose agar) is prepared under aseptic conditions by dissolving 39grams in 1000ml of distilled water to give a concentration of 0.039g/l of potato dextrose agar (PDA) solution. The prepared solution media and all other glass wares are been sterilized at $121^0$C and 103.4kPa for 15 minutes in an autoclave. The molten PDA solution is cooled to about $45^0$C after which a drop of 0.1%v/v lactic acid is introduced to inhibit the growth of bacteria.

The media is been dispensed into sterile Petri Dishes (also known as pouring of plates) labelled samples A,B,C and further cooled to room temperature. The residual media is been corked well and refrigerated for further use.

The palm wine to be inoculated is diluted serially to obtain a palm wine solution of 0.0001ml. This is done by introducing 9ml of sterile distilled water into 4 test tubes labelled A to D. 1ml of palm wine is introduced into test tube A to give a concentration of 0.1ml palm wine solution. 1ml of palm wine solution from test tube A is introduced into test tube B to give a concentration of 0.01ml palm wine solution. This is done till 0.0001ml palm wine solution is achieved in test tube D. The main aim of diluting the palm wine solution is to reduce the cluster of yeast cells so that they could easily be counted. Using a sterile glass rod, inoculate 0.1ml of the 0.0001ml palm wine solution on the surface of each sample in the Petri dishes, cover appropriately and incubate for 24 hours. After 24 hours, colonies of yeast strains become visible.

*Figure 1: 0.039g/l potato dextrose agar solution*

*Figure 2: Equipments to be sterilized in a pressure pot*

*Figure 3: Pouring of Plates*

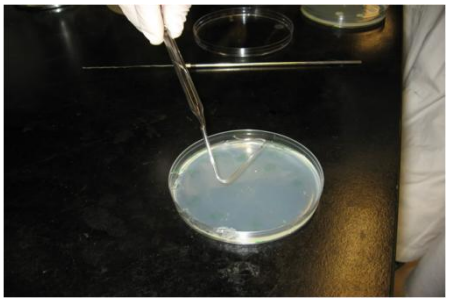

*Figure 4: Inoculation of Palm Wine Solution on Media Surface*

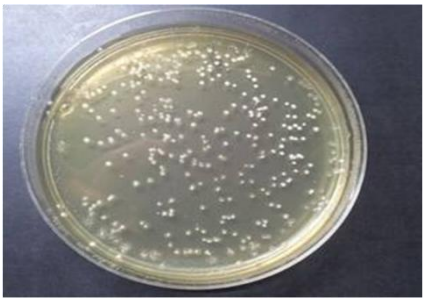

*Figure 5: Yeast Colonies on Media Surface after 24 hours*

**Measurement of the Carbon dioxide Production**

This is done after the isolation process. The residual media and all equipments to be used are sterilized. The media is dispensed into an Erlenmeyer flask and cooled to room temperature in a slanting position as labelled in figure 6.

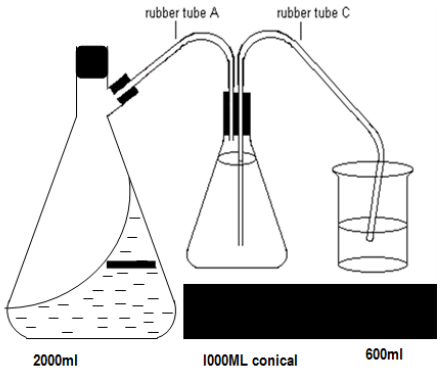


*Figure 6: Equipment Set-up for Measurement of $CO_2$ production*

Three yeast colonies from sample A is inoculated onto the surface of the media in the Erlenmeyer flask using an inoculating loop. The Erlenmeyer flask is corked airtight with a rubber tubing connected to the cork. The rubber tubing leads to an airtight conical flask filled with water to be displaced by the produced gas. A rubber tubing C is connected from the conical flask into a beaker or a measuring cylinder. The gas production is measured by indirect method. The equipments are arranged as below:

The rate of carbon dioxide production is determined by measuring the amount of water displaced every hour to give a rate curve.

**Performance Evaluation of Freshly Isolated Yeast**

This involves investigating the leavening properties of the freshly isolated yeast. Yeast broth is made from Sample B which gave an average yeast colony count of $1.25x10^7$cfu/ml by making a solution of the yeast solution in distilled water and this is added to bread dough weighing 800 g to act as the leavening agent. The dough is kept at a temperature slightly above room temperature to enhance the fermentation process. The characteristics of the leavening process are recorded.

**Results and Discussion**

The yeast cells that were isolated from palm wine were identified based on three properties; their appearance/characteristics, cellular morphology and their ability to ferment certain sugars. The isolated yeast cell growth on the PDA media revealed an average colony count of $1.25x10^7$cfu/ml (colony forming unit per ml). Most of the colonies were cream with raised appearance as in figure 5. As said earlier, yeast grows fermentatively utilizing substrate from the growth media. During the fermentative growth of yeast, the substrate is metabolized to produce ethanol and carbon dioxide. The rate of production of carbon dioxide was measured from the time of the yeast inoculation for about 24 hours and the data obtained is given as follows:

**Table 1**: Data of volume of water displaced (ml) with time (hour)

| S/N | Time (hour) | Volume of water displaced (ml) | S/N | Time (hour) | Volume of water displaced (ml) |
|---|---|---|---|---|---|
| **1** | 0.0 | 9.00 | **15** | 10.68 | 57.00 |
| **2** | 0.50 | 9.00 | **16** | 11.18 | 60.50 |
| **3** | 1.00 | 10.00 | **17** | 12.18 | 64.00 |
| **4** | 1.50 | 10.50 | **18** | 13.18 | 67.00 |
| **5** | 2.00 | 12.00 | **19** | 14.18 | 70.00 |
| **6** | 2.50 | 13.00 | **20** | 15.18 | 74.00 |
| **7** | 3.00 | 15.00 | **21** | 17.18 | 77.00 |
| **8** | 4.50 | 26.00 | **22** | 18.18 | 79.00 |
| **9** | 5.00 | 28.00 | **23** | 19.18 | 81.00 |
| **10** | 5.50 | 31.00 | **24** | 20.18 | 83.00 |
| **11** | 6.50 | 37.00 | **25** | 21.18 | 84.00 |
| **12** | 7.08 | 40.00 | **26** | 22.18 | 86.00 |
| **13** | 7.68 | 43.00 | **27** | 24.18 | 90.00 |
| **14** | 8.18 | 47.50 | **28** | 25.00 | 92.00 |

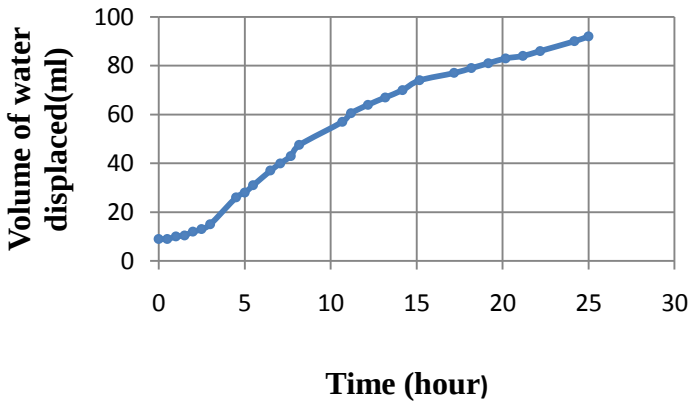


*Figure 7: Graph of volume of water displaced (ml) against time (hour)*

The fermentative growth of yeast lasted for a few days. To get a clearer view of the growth curve, a graph of volume of water displaced is plotted against time in days:

**Table 2**: Data of volume of water displaced (ml) with time (day)

| S/N | Time (day) | Volume of water displaced (ml) |
|---|---|---|
| **1** | 0 | 9.00 |
| **2** | 0.021 | 9.00 |
| **3** | 0.042 | 10.00 |
| **4** | 0.500 | 60.50 |
| **5** | 1.000 | 90.00 |
| **6** | 1.500 | 106.00 |
| **7** | 2.000 | 115.00 |
| **8** | 2.167 | 115.00 |
| **9** | 3.750 | 113.00 |

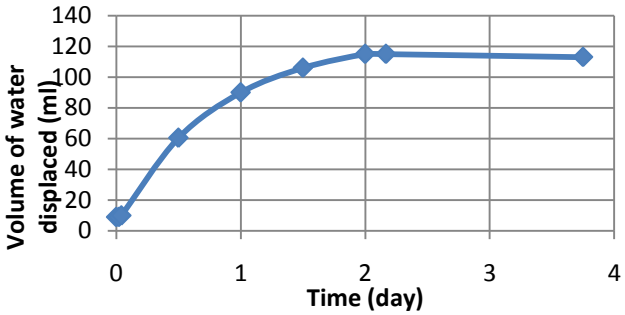


*Figure 8: Graph of volume of water displaced (ml) per unit time (day)*

The curve obtained from the carbon dioxide measurement is a typical example of the growth curve which shows the different phases of growth in a microorganism. It was observed that after the inoculation of the microorganism, the lag phase was experienced first. During this phase, no carbon dioxide was produced this is due to some number of factors such as adaptation time of the organism, previous conditions of the organism etc. the next phase is the exponential or logarithmic phase during which the yeast strains are rapidly growing and dividing. This is followed by the stationary phase caused by depletion in the nutrients in the media due to large population of the fungus. This finally leads to the death phase during which the yeast cells lose their ability to reproduce and therefore individual cells begin to die.

This therefore implies that yeast isolated from palm wine can metabolize substrates to give ethanol and carbon dioxide. Therefore the carbon dioxide producing property of yeast should be utilized in industries and areas where carbon dioxide gas is needed. Such areas include enhanced oil recovery in the petroleum industry, urea fertilizer production, beverage carbonation, winemaking etc.

**Leavening Properties of the Freshly Isolated Yeast**

The fresh yeast isolates produced a good quantity of carbon dioxide. This confirms the fact that it possesses good leavening properties. Approximately $5.0x10^7$ cfu of yeast were used to proof dough weighing 300g and it was observed that the dough actually rose with a proof time of 8 hours under a temperature of 28 $^oC$. The dough is shown below:

*Figure 7: Dough before leavening*

*Figure 8: Dough after leavening*

**Table 3**: Comparison between Baker's yeast and Freshly Isolated Yeast

| | |
|---|---|
| Leavens dough in a very aggressive, fast and random way. | Leavens dough gradually and in a well defined manner |
| Leavens dough in a short time (about 1 to 3 hours) depending on the quantity of yeast and the size of the dough | Takes a very long time to leaven dough (about 6 to 12 hours) depending on the quantity of yeast and the size of the dough |
| No palm wine smell present though flavour enhancers could be added to enhance the aroma of the bread | Bread proofed with fresh isolated yeast from palm wine has a characteristic palm wine smell and therefore will need addition of flavour enhancers |
| Easily quantified | Difficult to quantify |

**Conclusions**

The following conclusions were deducted from this research:

1. Yeast can be isolated from palm wine and these isolated yeast strains possess good gassing property that can be utilized to leaven bread in the food industry and also in order areas where carbon dioxide gas is needed.
2. Lag phase for freshly isolated yeast is higher in vivo (dough) than in vitro (PDA media in Petri dishes).
3. Bread proofed with freshly isolated yeast from palm wine has a characteristic palm wine smell.

**Recommendations**

More research should be carried out in some areas to be able to achieve the replacement of baker's yeast with freshly isolated yeast.

1. More research should be done on removing the palm wine smell from the bread besides addition of flavour enhancers.
2. More research should be done on establishing the particular quantity of yeast needed to leaven a particular quantity of dough at a particular time.
3. People should be enlightened on new ways to carry out things and how to oppose the resistance to change.

**References**

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