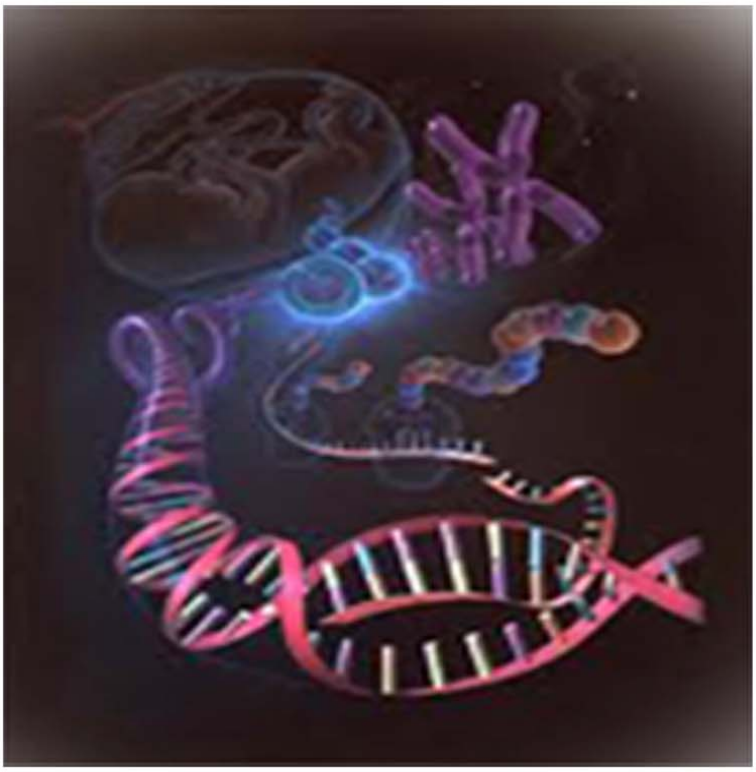
IJLBPR

ISSN 2250 - 3137
Vo1. 3, No. 4, October 2014

International Journal of
Life Sciences Biotechnology and Pharma Research



www.ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

Email : editorijlbpr@gmail.com or editor@ijlbpr.com

International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 3, No. 4, October 2014

Research Paper

ALCOHOL AND BIOMASS PRODUCTION FROM PINEAPPLE JUICE USING A COMBINATION OF PALM WINE YEAST AND BAKER'S YEAST

Mbajiuka Chinedu Stanley[1]*, Obeagu Emmanuel Ifeanyi[2], Agbo Ejiofor Christopher[3] and Enya Emmanuel[1]

*Corresponding Author: **Mbajiuka Chinedu Stanley** ✉ emmanuelobeagu@yahoo.com

Palm wine yeast and baker's yeast were used in combination to ferment the juice from pineapple fruits for biomass and alcohol production. The must was first upgraded to 22° brix content with sucrose. The fermentation was for 14 days at room temperature. The fermenting must and the wine produced were analyzed Physio-chemically. It showed that the combination when used as inoculums is very good. Soluble solids, sugars and pH decreased as the fermentation proceeds while the titrable acidity increased. Percentage alcohol yield was 8.4%. Information gotten from this research work shows that the combination has potentials for wine making. This can help wine makers develop fermentation inoculums from cheap sources.

Keywords: Palm Wine Yeast, Baker's Yeast, Pineapple, Biomass, Alcohol, Fermentation Biogas, Cassava, Plantain, Cowdung and digesters

INTRODUCTION

Fermentation was described by Prescott *et al.* (2008) as any process involving the mass culture of microorganisms either aerobically or anaerobically, and also the use of an organic substrate as the electron donor and the same partially degraded organic substrate as an electron acceptor.

According to Pelezar *et al*. (2000), Louis Pasteur found out that fermentation of fruits and a grain resulting in alcohol production was brought about by microorganisms.

Pineapple (*Ananas satirus*) is produced in large quantity in Nigeria. Madrid and Felice (2005) reported that about 4000 to 6000 tons of pineapple fruits are produced annually, most of them are wasted due to poor handling and poor storage methods. Fermenting pineapple juice goes a long way in reducing wastages and increasing local farmer's income.

[1] Department of Microbiology, Michael Okpara University of Agriculture, Umudike P M B 7267, Umuahia, Abia State, Nigeria.
[2] Diagnostic Laboratory Unit, University Health Services Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[3] Assistant Director, Department of Medical Laboratory Services, Federal Medical Centre, Umuahia, Abia State, Nigeria.

The nutritional values of pineapple fruits according to Ihekoronye and Ngoddy (1985) are 54 mg/100 mL of vitamin C, 12% (w/v) of total sugars, 0.3% of protein, 0.1% of fats, 12 mg of calcium, 0.3 mg of iron, 0.08% of thiamine and 0.1 mg of niacin.

Yeast species are very useful in many industrial processes such as the production of alcoholic beverages, biomass and various other metabolic processes (Frazier and Westhoff, 1978). In baking and alcohol production, palm wine yeast (*Saccharomyces cerevisiae*) is most commonly used to convert sugars into ethanol and as the yeast utilizes the sugar, carbon dioxide and alcohol are produced (Zhou, 1999).

Wine production is a product of old biotechnology (Alian and Musenge, 1976), although the modern methods of production are in carefully controlling the processes in bioreactors. The juice from the crushed fruit is usually fermented by pure cultures of certain strains of yeast (Maldonado *et al*., 1975). Processing steps includes crushing according to Querol *et al*. (2003). Must treatment; addition of sucrose and acidity adjustment, fermentation, clarification, aging, bottling and packaging. The aim of this research work is to study the fermentation of pineapple juice into alcohol and biomass using the combination of locally isolated yeast from palm wine and the baker's yeast, and investigate the effect of this combination.

MATERIALS AND METHODS

All the equipment and materials used in this work were available in the laboratories of microbiology and food science technology departments of Michael Okpara University of Agriculture, Umudike, Abia State except the pineapple fruits and the baker's yeast purchased from the open market of Umuahia town and the palm wine collected from a palm wine tapper in Umudike of Ikwuano Local Government Area of Abia State.

Must Preparation

Mature and ripe pineapples were washed, peeled and sliced manually with a knife, 100 g of pulp was blended with 300 mL of hot water (1:3) in a blender. The slurry was filtered with a muslin cloth. Sodium metabisulphite (0.1%) was added and the juice was pasteurized at 60^{o}C for 30 min. Six liters of the must was fortified with 1,200 g of granulated sugar. This increased the sugar content of the pineapple juice from 12.14^{o} to 22^{o} brix. This method was done according to Obisanya *et al*. (1987).

Preparation of the Inoculums

Yeast isolated from palm wine was cultured and pH was maintained at 3.5. The slurry of baker's yeast was prepared by stirring 1 g of the baker's yeast in 10 mL, of distilled water according to Anuna *et al*. (1990). Then a combination of palm wine yeast and baker's yeast culture was done. 2.5 mL each of palm wine yeast and that of the baker's yeast were mixed to give 5 mL of the yeast culture. This mixture was used to inoculate 45 mL of a sterile pineapple juice and incubated for 24 h at 30^{o}C. 5 mL of the above mixture was used to inoculate 500 mL of pineapple juice and incubated for 24 h at 30^{o}C. Then 200 mL of the final inoculums was used to ferment 2 L of the pineapple juice in the fermentation vat. The openings on the vat were closed with rubber stoppers fitted, with fermentation locks containing an aqueous solution of the sodium metabisulphite. This was done according to Anuna *et al*. (1990).

Primary Fermentation of the Must

This proceeded for 7 days at ambient temperature, during which the following vital fermentation parameters: specific gravity, total soluble solid, titrable acidity, ash content, sugar content and alcohol content were tested. The fermenting musts were agitated twice daily until the 7th day. At the end of the primary fermentation (7th day), the fermented must were decanted for secondary fermentation according to Lutchmedial *et al*. (2004).

Secondary Fermentation

Sugar syrup of 50 g of sucrose in 150 mL of water was added to the fermenting musts and kept for secondary fermentation in anaerobic fermentation cats at ambient temperature for 5 days after which the vital parameters were determined and recorded. The fermented wine were racked off the sedimented yeasts and clarified with 1.2 g of bentonite crystals.

Bottling and Aging

After clarification, the wine were bottled and pasteurized at temperature of 70°C for 30 min after which they were kept for aging at a temperature of about 4°C.

PHYSIO-CHEMICAL ANALYSIS OF PARAMETERS

Determination of pH and Temperature

The pH of the substrate was determined using a digital pH meter. The pH meter was standardized using phosphate buffer. The substrate was measured by dipping the electrode into the sample and the value read. The temperatures of the samples were measured directly using a Celsius thermometer attached to the pH meter. The electrode of the temperature was dipped and the value read off the temperature meter according to James (1995).

Determination of Specific Gravity

This determines the amount of fermentable sugar (solutes) in wine. It was determined by the gravimetric method of James (1995).

Determination of Titrable, Fixed and Volatile Acidity

This was done using the alkaline titrimetric methods (Okunowo *et al*., 2005).

Determination of Ash Content

Dry empty crucibles were weighed and 5 g of the sample added. The crucibles and their contents were weighed and ashed for 1 – 2 h using an electric stove. They were allowed to cool in desiccators and then weighed. The percentage ash content was calculated according to James (1995).

Determination of Biomass Concentration

The biomass concentration during fermentation was determined by measuring the intensity of the light in the musts through electric detectors of the spectrophotometer at wavelength of 620 nm.

Determination of Sugar and Alcohol Content

The alcohol content was done using distillation method as described by James (1995), while sugar content was determined with the aid of a Refractometer. A drop of the sample was placed on the lens of the Refractometer and directed to a source of light and viewed through the eyepiece. The soluble sugar content was read off from the calibrated inner lens.

RESULTS

The physiological characteristics summarized in

Table 1 shows that the palm wine yeast grew as a creamy white on the yeast extract agar with ovoid colonies with smooth edges. These facts confirmed that the palm wine yeast used belonged mainly to the genus *Saccharomyces*. This was also confirmed when the baker's yeast used were cultured. This conforms with the description of *Saccharomyces* by Chawla (2005).

The sugar concentration of the pineapple juice used before fortification was 12.14° brix. This was increased to 22° brix and after fermentation the sugar concentration fell to 5.6° brix. The specific gravity of the must before fortification was 0.300 and it rose to 1.060° brix after fortification, but it decreased to 1.025° after fermentation. The pH of the must before fortification was 4.6 and it became 4.9 after fortification with sucrose and decreased as the days of fermentation progressed as shown in Figure 1.

The titrable acidity of the must was 0.40% before fortification. It increased for the first 4 days of fermentation to 1.65% as shown in Figure 2. The alcohol production profile is shown in Figure 3. The fermentation of sugars by this combination

Table 1: Identification of Palm Wine Yeast

Physiological characteristics	Saccharomyces cerevisae (control)	Palm wine yeast
Glucose	++	++
Galactose	++	++
Gluconate	-	-
Sucrose	++	++
Raffinose	+(+)	+(+)

Note: ++ = fermentation positive; - = fermentation negative; +(+) = poorly fermented

Figure 1: pH Variation with Time

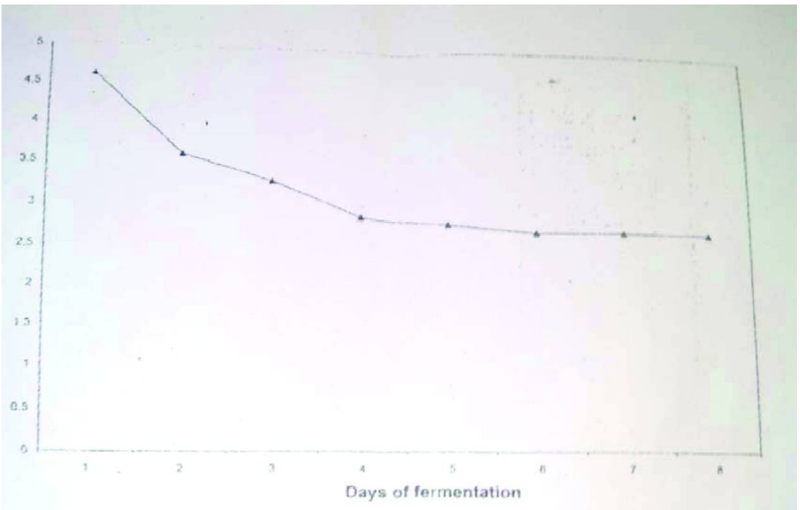


Figure 2: Fermentation Profile for Total Titratable Acidity Variation with Time

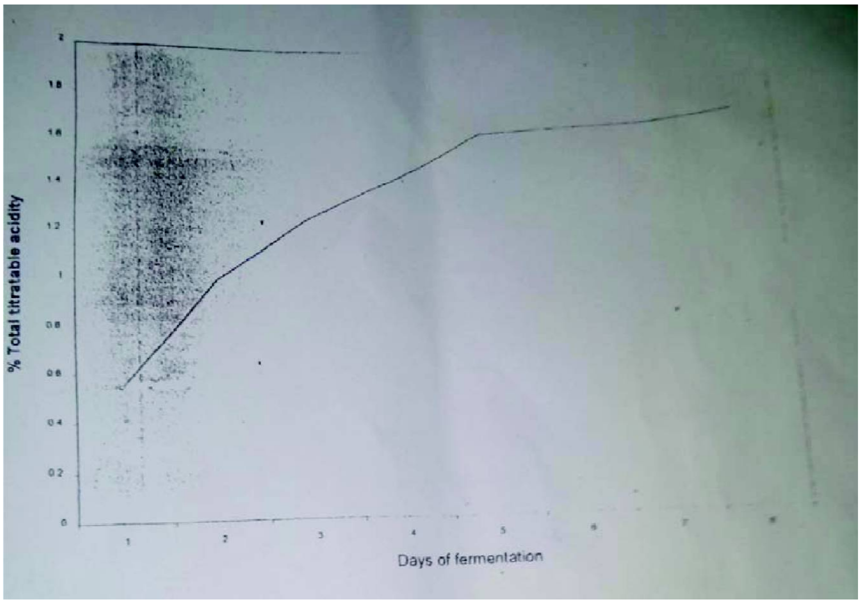
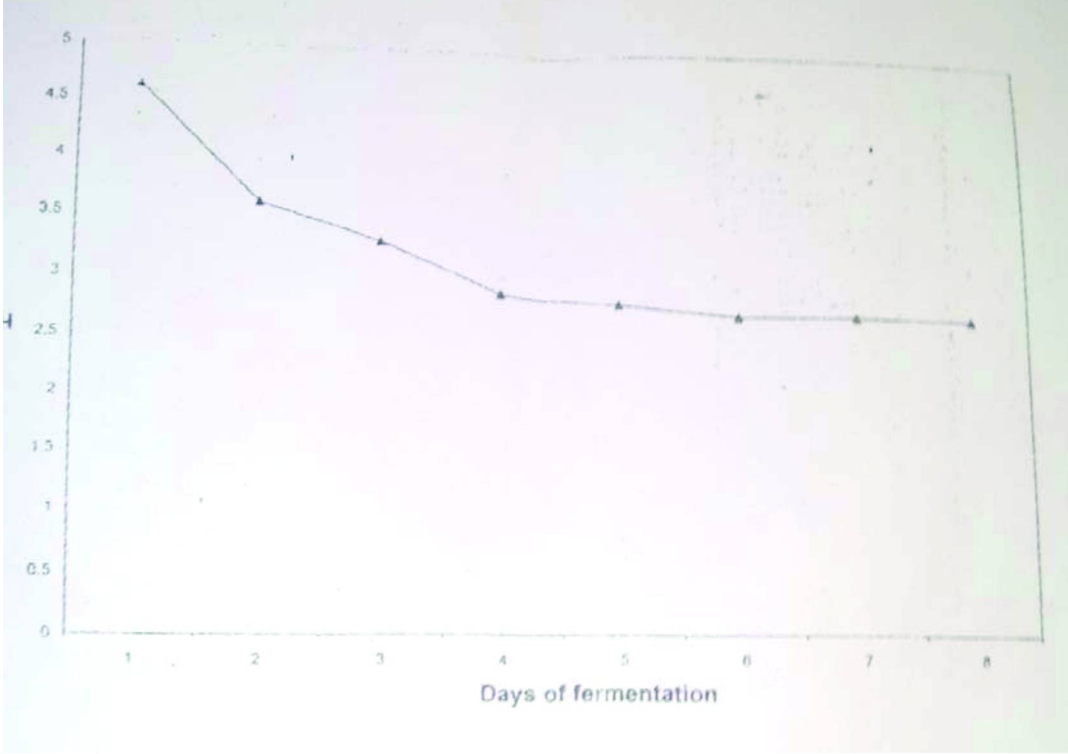


Figure 3: Fermentation Profile for Alcohol Productivity by the Various Yeasts with Time



of yeasts as shown in Figure 4 gave a gradual decrease from 22° brix down to 5.6° brix which was the final level at the end of the fermentation period.

The Physio-chemical composition of the must before fermentation is shown in Table 2 while the temperature of the fermentation profile is shown in Table 3. The Physio-chemical composition of the alcoholic beverage produced after the fermentation is shown in Table 4 whole Table 5 shows the values obtained from the Spectrophotometric readings of the musts 31.

Table 2: Physiochemical Composition of the Pineapple Juice before Fermentation

Parameters	Results
pH	4.6
Specific gravity	0.30
Soluble solids (°brix)	12.14
Proteins (%)	1.43
Total sugars (°brix)	22
Titratable acidity (% citric)	0.56
Volatile acidity (% acetic)	0.08
Fixed acidity (% citric)	0.90
Ash content (%)	0.43

Note: * Means values of three determinations.

Table 3: Temperature Changes of the Fermenting Must with Time

Days	Temperature (°C)
0	30
1	31
2	31
3	30
4	31
5	31
6	30
7	30

DISCUSSION

In this research work, the amount of sulphite added to the must was according to the work of Dubois *et al*. (1956). The excess will delay fermentation and increase the fixed sulphur dioxide content which will affect the finished wine. The addition of sucrose to the must was to raise the sugar concentration in the must to a level that

Table 4: Physiochemical Composition of the Alcoholic Beverage produced after the Fermentation Process

Parameters	Results
Alcohol (% volume)	8.4
Specific gravity	1.015
Total titratable acidity (% citric)	1.65
pH	2.7
Volatile acidity (% acetic)	0.48
Total soluble solids (°brix)	1.00

Note: * Means values of three determinations.

Table 5: Biomass Concentration of the Must Fermented with time Spectrophotometric readings at 620nm

Days	Biomass Concentration
0	0.74
1	0.80
2	0.90
3	1.00
4	1.15
5	1.10
6	1.11
7	0.87

will help obtain a desired alcoholic content of the produced wine. According to Prescott *et al*. (2008), adequate amount of yeast must be inoculated so as to get a high value product.

The observed decrease in specific gravity and total dissolved solids is in accordance with the work of Singh (1960). This indicates the usage of sugar by the inoculums. The drop in the pH is desirable as it helped to inhibit the growth of undesirable microbes and the rising titrable activity could be due to the conversion of organic acids to lactic acid and CO_2 as stated by Dennis (2003) in his work. The decrease in acidity after 7 days of fermentation is possibly because of conversion of some of the acids to alcohol (Kunkee and Amerine, 1970). It could also be due to precipitation of the acids in the form of salts or due to formation of basic compounds in the must.

The finished wine had the alcohol content of 8.4% which was in agreement with the work of Obisanya *et al*. (1987). Biomass concentration and ethanol tolerance is considered important attributes of yeasts considered for wine, biomass and alcohol productions.

CONCLUSION

The result gotten from this research work shows that a combination of palm wine yeasts and baker's yeast can ferment pineapple juice to produce alcohol and biomass at acceptable quantities. Therefore, this analysis could be an incentive for an eventual commercial production of alcohol and biomass from pineapple juice.

REFERENCES

1. Alian A and Musenge H M (1976), "Utilization of pineapple waste for Wine making", *Zambian Journal of Food Science and Technology*, Vol. 2, No. 1, pp. 10-17.
2. Anuna M I, Sokari T G and Akpapuunam M A (1990), "Effect of Source of Yeast on Alcohol Content and Quality of Pineapple (*Ananas comosus*) Wine", *Nigerian Journal of Food Science,* Vol. 2, pp. 80-83.
3. Chawla H S (2005), *Introduction of Plant Biotechnology* (3rd edition), Oxford and IBH Publishing Com. New Delhi, p. 537.
4. Dennis A E (2003), *Food and Beverage Services* (5th Edition) British Library Cataloguing in Publication Data, p. 390.
5. Dubois M, Giles K A, Hamilton J K, Rebers P A and Smith F (1956), "Colorimetric method for determination of sugars and related substances", *Analytical Chemistry*; No. 28, pp. 350-356.
6. Frazier H and Westhoff J C (1978), *Food Microbiology*, McGraw Hill Publishers, New Delhi, pp. 331-334.
7. Ihekoronye A I and Ngoddy P O (1985), "Integrated Food Science Technology for the Tropics", Macmillan Press, London, pp. 178-180.
8. James C S (1995), "Experimental methods on Analytical Chemistry of food", Chapman and Hull, New York, pp. 114-116.
9. Kunkee R E and Amerine M A. (1970), "Yeasts in Wine Making", *Nigerian Journal of Nutritional Sciences,* Vol. 3, pp. 27-28.
10. Lutchmedial M, Ramlal R, Badrie N and Chan-Yen I (2004), "Nutritional and sensory quality of stirred soursop (Annona muricata L) youghurt*", International Journal of Food Science and Nutrition*; Vol. 55, pp. 407-414.
11. Madrid R E and Felice C J (2005), "Microbial

Biomass Estimation", *Journal of Micro-biological Methods*; Vol. 351, pp. 37-42.

12. Maldonado O, Roll C, Cabrera S S and Schneider de Cabrera S (1975), "Wine and Vinegar production from tropical fruits", *Journal of food science*, No. 40, pp. 262-264.

13. Obisanya M O, Aina J O and Oguntimein G B. (1987), "Production of Wine from Mango (*Mangifera indica*) using *Saccharomyces* and *Schizosac charomyces* isolated from palm wine", *Journal of Applied Biotechnology,* Vol. 63, pp. 191-193(22).

14. Okunowo W O, Okotore R O and Osuntoki A A (2005), "The alcoholic fermentative efficiency of indigenous yeast strains of different origin on orange juice", *African Journal of Biotechnology,* Vol. 4, pp. 1290-1296.

15. Pelzar M J, Chain E C and Noel R K (2000), *Microbiology* (5th Edition). McGraw Hill Publishers, New Delhi, pp. 183-186.

16. Prescott M L, Harley J P and Klein D A (2008), *Microbiology* (7th Edition). McGraw Hill Publishers, New York, pp. 207-210.

17. Querol A, Fernández E T M, Olmo M L and Barrio E (2003), "Adaptive evolution of wine yeast", *International Journal of Food Microbiology*; No. 86, pp. 3-10.

18. Singh L B (1960), "The Mango. Botany, Cultivation and Utilization", In: *World Food Crops*, Academic Press, London, pp. 105-116.

19. Zhou O (1999), "High efficient production of alcohol using molasses as raw materials", Academic Press, Tsinghua University, China, p. 58.

International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
E-mail: editorijlbpr@gmail.com or editor@ijlbpr.com
Website: www.ijlbpr.com

ISSN 2250-3137