

Wine Production from Banana (*Musa sapientum*) Using Yeast (*Saccharomyces cerevisiae*) Isolated from Grape (*Vitis vinifera*).

Abstract

This research was carried out to produce wine from banana (*Musa sapientum*) pulp using yeast (*Saccharomyces cerevisiae*) isolated from grape (*Vitis vinifera*). The fermentation of the banana wine lasted for 21 days. Physico-chemical parameters were determined during fermentation using standard procedures. Liquor of the fermenting must was removed for every 48 hours from the fermentor for analysis of pH, titratable acidity, specific gravity and reducing sugar using standard procedures. The results from the experiment showed that specific gravity of the wine was observed to reduce drastically as the fermentation progressed. The pH of the banana wine during fermentation increased from 4.16-4.22 at day to the last day while the titratable acidity (% w/v tartaric acid) of the banana wine produced increased from 1.05-1.77. The alcohol content of the wine increased with time. The specific gravity values were observed to range from 1.266 to 1.184 kg/m³ which gradually decreased throughout the period of fermentation. At the end of the fermentation, alcohol content was observed to be 9%. The flavour and taste were appreciable, overall point obtained was 4.8. This study showed that the yeast *Saccharomyces cerevisiae* isolated from the grape have a good fermentative report with acceptable wine which suggested that it could be use banana wine production, its products or other industrial application.

Keywords: Banana, fermentation, grape, yeast, wine, must

Authors' Contributions: This work was carried out in collaboration among all authors. Although is AEJ is Dead. Author AEJ designed the whole study, and supervised it. OCA and ECC carried out the experiments and wrote the first draft of the manuscript. All the Authors managed the literature searches and analyses of the study. Author ECC performed the statistical analysis, wrote the protocol, managed editing and prepared the final manuscript. All authors read and approved the final manuscript.

1. Introduction

Wine is an alcoholic beverages produced by yeast fermentation of ripe grapes or any other fruit with a good proportion of sugar [1]. Wine is one of the most recognisable high value - added product from fruits. It can also be used as a substrate for the manufacture of vinegar, a by-product of wine manufacture. Fruit juices are fermented to produce wine, an alcoholic beverage. Grapes are usually preferred because of the natural chemical balance of the grape juice which aids their fermentation process without addition of sugars, acids, enzymes, or other nutrients.

However, fruits such as banana, cucumber, pineapple and other fruits are used in wine production [2].

Wine manufacture is challenging in which marketable product can be obtained, but the processes involved in its production are relatively straight forward [3]. Highly acceptable wines can be made from practically all fruits. Wine can be fermented with yeast that occurs naturally in grape which is the main organism responsible for alcoholic fermentation which belongs to the genus *Saccharomyces*. The yeast strains used also have a great influence on the overall quality of the wine [4] and Kukee [5]. Although many genera and species of yeast are found in must, *Saccharomyces cerevisiae* is the main yeast strain that is commonly reported to be alcoholic fermentation [1] and in other countries where grape is not produced, emphasis is usually placed on other fruits for wine making. There are some soft fruits from both temperate and tropical regions whose pigment stability and flavor profiles match those of any wine from grapes, but suffer from the lack of intensive research and development given to grape wine. Reports on tropical fruit wines have been mainly on exotic species such as banana, pineapple, citrus, mango, pawpaw, apple, strawberries etc.[5]. Wine represents a safe and healthy beverage; it also provides calories and vitamins. During period when life was often strenuous, it offered relaxation and relief from pains. Wine as a beverage assumes a vital place in human life, including in spiritual, economic, and societal contexts [6].

Bananas (*Musa sapientum*) is an important staple starchy food in Nigeria. It is a seasonal and highly perishable fruit, which can be available all year round. The large quantity of bananas and plantains provides the potential for industrial use [7]. In addition, this wine application to produce marketable, value-added products will improve banana farming economies and eliminate the large environmental problem presented by banana waste. Banana could then compete in the market, either as banana wine, juice or as mixtures with other juices because of its flavor and aroma to produce a palatable wine [8].

Bananas have a lot of nutritional benefits and a better choice for people suffering from potassium deficiency because of its impressive potassium content. Potassium is an important component of cell and body fluids that helps control heart beat and blood pressure, countering bad effects of sodium. Banana is considered as an important food to boost the health of malnourished children, it contains good amount of soluble dietary fiber that helps normal bowel movements; thereby reducing constipation problems. Medicinal uses of banana have positive contribution towards successful treatment of anemia, heartburn, temperature control, ulcer, overweight etc.

Banana juice can also be applied to wine production; however, banana juice is turbid, gray in color, very viscous, tends to settle during storage and, therefore must be clarified prior to commercialization [8]. The turbidity and viscosity of banana wine are caused mainly by the polysaccharides in banana juice such as pectin and starch and therefore make the clarification

process harder. Application of pectinase and α -amylases that affect the quality of wine is important for improving the process of banana wine production. In the present work, banana fruit pulp was used for wine production using *S. cerevisiae*, and the physicochemical as well as sensory evaluation of banana wine was also evaluated.

2. Materials and Methods

2.1. Sample collection

Over-ripe banana and grape fruits used for this experiment were bought from Eke Market, Awka, in Anambra State and were identified at Botany Department, Nnamdi Azikiwe University, Awka.

2.2. Isolation of yeast from grape

The yeast was isolated from grape. The fruits were washed thoroughly with 0.1% sodium metabisulphite in water. The fruits were cut, manually deseeded, blended and filtered to obtain the juice. One hundred (100ml) of the grape must was kept in a sterile conical flask. The solution was cultured on Sabouraud Dextrose Agar (SDA) plate and incubated at 37°C for 72hrs. The identified yeast colonies were sub-cultured on SDA to obtain pure cultures of yeast cells [9]

2.3. Inoculum Development

The ripe bananas, about 2kg of were thoroughly disinfected with cotton wool soaked in ethanol before peeling, then were washed thoroughly with 0.1% sodium metabisulphite in water. The banana slices were blended with water in a Super Mark blender and pulp obtained was used for further experiment. The slurry was autoclaved, cooled later it was poured into the fermenting vessel. About 250 mL of the juice was introduced into a clean sterile 500 ml conical flask, Ammonium sulphate and Potassium di-hydrogen phosphate (0.12%) were added as yeast nutrient. 0.14% Sodium metabisulphite was also added to inhibit the growth of microorganism. and sterilized by autoclaving. Upon cooling, three {3} loopful of the yeast (*Saccharomyces cerevisiae*) stock culture isolated from the grape that was preserved in SDA slant was used to inoculated into the juice and incubated in a rotary shaker {attemperated Gallenkamp} for 48 hours.[9] All procedures were done in triplicates and under aseptic condition method described by Archibong.

2.4. Preparation of wine fermentation

The Aliquots of must was analysed for reducing sugar, titratable acidity (tartaric acid %w/v), specific gravity and alcoholic content before fermentation and at intervals. Aliquots were drawn from the fermentation flask and control flasks by siphoning with sterilized tubes through layers of muslin cloth. The 250 mL of developed inoculum was poured into the 3 Liter fermenting jar

containing the earlier autoclaved juice making it a total of 2400 mL. 200g of sucrose (for fortification) was then added to the fermentation medium. Then the mouth of the jar was plugged tightly with cotton wool and kept on the bench at room $\pm 28^{\circ}\text{C}$ temperature in a cool dark environment for 21 days for fermentation to commence. After the fermentation, settled yeast and other debris were clarified using Gelatin as fining agent prior to the packaging [9] After 21 days, the wine flask was immersed in $70\text{--}80^{\circ}\text{C}$ water bath to stop the fermentation. The wine was filtered as before and the sensory evaluation was carried out.

2.5 Determination of pH value

The pH was determined using a standard pH meter (Model: pH S-25). The pH meter was standardized with buffer solution, prepared by dissolving the pH buffer powder of pH 4.0 at 25°C in 250 ml distilled water. 10 mL of the juice was measured into a sterile beaker and electrode of the pH meter was immersed into the beaker and readings were noted.

2.6 Determination of Reducing Sugar

The quantitative estimation of reducing sugar of the juice was determined using the method described by Miller [10]. 1 mL of 3,5-Dinitrosalicylic acid (DNS) is added to 1 mL of supernatant of wort (sample) in a test tube and the mixture heated in boiling water for 10 minutes. The test tube is cooled rapidly in tap water and the volume adjusted to 12 mL with distilled water. A blank containing 1 mL of distilled water and 1 mL of DNS is also prepared. The Optical Density (OD) of sample is read against the blank in a Spectrophotometer at 540 nm absorbance. The concentration of reducing sugar is estimated from a glucose standard curve.

2.7 Determination of Specific Gravity

Fifty{50}mL pycnometer bottle was thoroughly cleaned with distilled water, dried in an oven for 50°C and allowed to cool. The weight of the cooled dried bottle (W_1) was recorded. The dried pycnometer bottle was filled with deionized water and surface of the bottle was cleaned with a cotton wool and weighed (W_2). The pycnometer bottle was emptied and cleaned twice with 10 mL of the juice thereafter the bottle was filled to the brim with the “must” and the bottle cleaned with cotton wool and weighed (W_3). The specific gravity (Kg/m^3) was calculated.

$$S.G = \frac{W_3 - W_1}{W_2 - W_1} = \frac{S}{W}$$

Where

S= weight of volume of must ($W_3 - W_1$)

W= weight of volume of water ($W_2 - W_1$)

2.8 Estimation of total titrable acidity {Tartaric Acid (g/100ml)}

This was determined by the methods as described by Amerine and Ough [11]. 1% of aqueous alcoholic phenolphthalein as indicator was added to 200ml of distilled water. It was titrated with 0.1M of NaOH. Titration was stopped when a faint but definite pink colour appeared and the titre value was noted that served as the initial titre. 5mL of the must was added to the neutralized solution. The same 0.1M NaOH was used to titrate it. The titration was stopped at the appearance of faint, but definite pink colour. The titre was taken. This served as the final titre. The titratable acidity was calculated with reference to tartaric acid.

2.9 Determination of alcohol content

The distillation method according to the Association of Official Analytical Chemists Methods of Analysis[12]. Fractional distillation of the wine was carried out and 100 mL of the wine was measured into a round- bottom flask with side arms. The flask was connected to the fractioning column and an anti-bumping chip was added to the wine. This ensured adequate dispersal of heat in the wine. It was then heated and the distillate was collected. The distillate was poured into a clean dry 100mL volumetric flask and made up to 100 mL with distilled water. It was left to stand overnight on the bench. The specific gravity of the distillate was determined and the result was taken to determine the alcoholic strength.

2.10 Determination of Temperature

A clean thermometer and measuring cylinder was used for temperature reading. about 50ml of the sample was used.

2.11. Organoleptic Evaluation

The organoleptic traits such as color, clarity flavor, taste, odor and the overall acceptance of the sample were evaluated on the 20th day by Maragatham and Panneerselvam [13]. A group of panelist of 10 judges was selected and these characteristics was based on a five points scale (Excellent quality=Five points while Poor Quality=One point).

3. Results

The study aimed at evaluating the fermentative performance of *S. cerevisiae* isolated from grape on banana wine production. The result of the banana wine produced by the fermenting with yeast strains isolated from grape (*S. cerevisiae*) shows high rate of alcoholic production at the end of day 21. This result is similar to the observation reported by [9, 14] who recorded that pure

culture of *S. cerevisiae* produces more ethanol and give a faster fermentation. This result is in consistent with the work of [15] for sugar fermentation using *S. cerevisiae* isolated from fermented grape pomace. Anuna *et al.*[16] noted, that almost all the fruits that contain sugar (fermentable sugar) can be used for the production of wine. The major problem with the use of tropical fruits in wine production is their low content sugar [17].

Saccharomyces cerevisiae isolated from grape fruit has been reported to reduce specific quality of fruit wines during fermentation. The resultant increase in the alcohol concentration from 0 to 8.4 (%v/v) for the beetroot must under anaerobic fermentation, shows the efficiency of the *S. cerevisiae* (brewer yeast) in utilizing the sugar as source of carbon and energy.

Throughout the period of fermentation, pH of the must was within the acidic range. The pH of the fermenting wine as shown in Table 1 and Figure 1 indicate a gradual decrease in the pH as the fermentation time increases and increased at the end of fermentation. At day 0, the pH is 4.16. While at day 21 the pH is 4.22. The result of titrable acidity carried out on the sample as shown in table indicates that the titrable acidity ranges from 1.05 at 0 day to 1.77 at day 21 in the fermenter. Therefore, the titrable acidity increases as fermentation time increases.

In this research, it is observed that the fermentation period agrees with the result of [18] who observed that *Saccharomyces cerevisiae* could thrive under low pH. The result from this research shows that pH values of banana wine decreased progressively as the fermentation period increases till the end of the fermentation. This shows that the wine became more acidic with the period of fermentation. The drop in pH also records the utilization of the sugar present in the must for growth. This observation is similar to that reported by [9,14,19].

Ezenwa *et al* 2020 reported that beetroot must pH reading 4.7 to 3.9 decreased gradually during fermentation. Similar observation was reported by The initial pH (4.16) falls within the pH range of 4.0 that was reported to be optimal for yeast activity [21]. These results were in agreement with that of Ifie *et al.* [22] who reported the decrease in pH, and increase in alcohol content during the fermentation of roselle wine. Studies have shown that during fermentation of fruits, low pH is inhibitory to the growth of spoilage organisms but creates conducive environment for the growth of desirable organisms. Also, low pH and high acidity are known to give fermenting yeast competitive advantage in natural environment [11]. The pH of the wine samples fall within the range 4 to 4.22. so is acidic; which confers stability on the wine sample [24]. Reddy and Reddy [25] noted that the pH of wine determines the microbial and physicochemical stability of the wines. Low pH and high acidity are known to give fermenting yeasts competitive advantage in natural environments [11].

The result shows an increase in the alcohol content of the fermenting sample as shown in the Table 1. The alcohol content ranges from 0.0 to 9.0% v/v at day 21 for the banana must, shows a very good performance of the yeast in degrading sugar as their carbon and energy sources.

Similar increase was reported by [20] who noted that beetroot must increased after 20 days of fermentation from 0 - 8.4%. The conversion of reducing sugar in to ethanol and carbon dioxide is due to the activity of microbes. The increase in the alcohol content can be attributed to yeast metabolism, continuous utilization of the sugar content ethanol is then produced. The increase in the alcohol content of the fermenting must, from Day 1 until all the available sugar in the fermenting must has been utilized is in support with the work of [9,14,20, 24]. This agrees with the findings of [26] who reported that wines that has 7 -14% of alcohol are considered as table wine. They also reported that alcohol content in wine is influenced by wine preparation method and type of yeast used. Alcohol contributes to taste, mouth-feel and sweetness to the wine, but at a very high level of alcohol content the taste will be suppressed, This causes both burning sensation in the nostril and a sense of bitterness [20]. Reports have shown that alcoholic fermentation leads to a series of by-products in addition to ethanol. Some of the by-products include carbonyl compounds, alcohols, esters, acids and acetals, all of them influencing the quality of the finished product [20].

The specific gravity values were observed to range from 1.266 to 1.184 kg/m³ which steady decrease throughout the period of fermentation. These decreases were observed to be irrespective of the yeast strain and the fruits used in the wine production. The steady decrease in the specific gravity of the samples was due to the activities of the yeast which fed on the sugar to produce alcohol and carbon dioxide. This result is in consistent with the work of [20], specific gravity of the beet root wine samples ranged 4.55 to 4.71% and 1.009 to 1.014 respectively. This was supported by Nidli [27]. This values for specific gravity fall within EC Wine Regulations reported by [28].

Result of this study also revealed consistent increase in titratable acidity of the wine throughout the period of fermentation. Total acidity (% w/v tartaric acid for both juices for the wine sample). The titratable acidity is recorded as 1.05% at Day 0 which increased to 1.77% at day 21. This result is found to conform to that of [29] who observed gradual increase in the titratable acidity and the alcohol content in the fermentation of plantain. A similar phenomenon was reported by [20] who reported that total titratable acidity increased from 0.15 to 0.34 for the beetroot must which might be as a result of increase in the production of organic acids. Reports have been noted that during the fermentation of must, organic acids such as acetic acid and pyruvic acids were produced [9]. A similar work of [30], revealed that there is a corresponding reduction in pH as the acidity increased in sour-sop juice. Studies have shown that acidity plays a vital role in determining wine quality by aiding the fermentation process, enhancing the overall characteristics and balance of the wine [31].

As stated by [20] there exists a correlation between pH and acidity of the sample, higher the acidity, lower the pH of wine. This is because as the organic acids in the wine increased the pH lowers and total titratable acidity also increases. This agrees with work of [32]. The pH of wine, is a direct reflection of the total titratable acidity, if low, the wine will have good shelf stability.

This was supported by Awe *et al* [31] who stated that lack of acidity might result to the production of a poor fermentation process.

It is observed that the temperature fluctuate within the period of fermentation Table 2. This corroborates with the work of [34], who observed the temperature of 28⁰C at Day 7 during the production wine from mixed fruits of Pineapple and Soursop.

The sensory evaluation was evaluated in Table 3, after the wine aging. The parameters that were determined were color, odor, taste, and flavor The overall point obtained was 4.8 out of 5. The wine produced was completely watery at the end of fermentation. This result is similar to that recorded by [9,11,14]. As fermentation rate proceeded, gas was formed which rose through the liquid forming forth on the surface. The rising gas carries the cells through the fermenting must cause it to be cloudy and as a result, a strong odor of alcoholic fermentation developed. The aroma obtained was as a result of fermentation; so no additional flavor enhancers were added. The short shelf-life of beverages are however a major problem faced by their producers and consumers in Africa [35].

Table 1: Analysis of the fermenting wine from banana.

Number of Days	pH	Reducing sugar (mg/ml)	Titrateable acidity (% w/v)	Specific Gravity (Kg/m ³)	Alcohol content (%v/v)
0	4.16	0.20	1.05	1.266	0.0
2	3.91	0.18	1.09	1.224	0.3
4	3.72	0.09	1.12	1.218	2.4
6	3.53	0.05	1.23	1.149	5.8
8	3.92	0.02	1.32	1.112	7.5
14	4.00	0.02	1.44	1.112	7.6
21	4.22	0.01	1.77	1.184	9.0

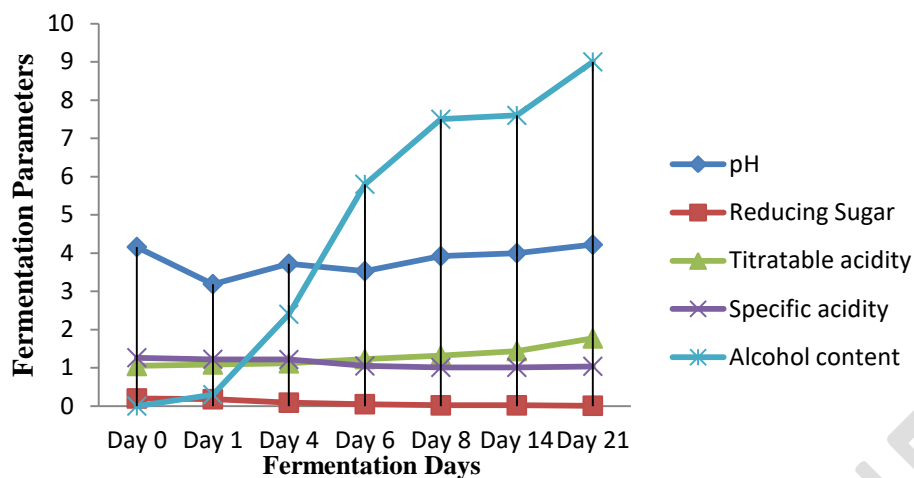


Figure 1: Graphical representation of banana wine analysis

Table 2: Temperature results for banana wine production.

Days of Fermentation	Temperature (°C)
0	27
1	28
2	28
3	28
4	27
5	28
6	27
7	28
14	27
21	27
Standard wine	29

TABLE 3: physical and organoleptic properties of banana wine

Days	SWEETNESS	COLOUR
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1	+	CREAMY
2	+	CREAMY
3	+	CREAMY
4	-	DIRTY CREAMY
5	-	DIRTY CREAMY
6	-	DIRTY CREAMY
7	-	DIRTY CREAMY
14	-	PALE CREAMY
21	-	PALE CREAMY
28	-	PALE CREAMY

5.0 Conclusion

Banana wine is very nutritious and easy to produce and could then compete in the market with other wines because of its flavour, taste, aroma and the successful production of using indigenous fruits as substrates for wine production and the efficiency of locally isolated yeast *Saccharomyces cerevisiae* strains from grape (*Vitis vinifera*) for fruit wine production. The pH of the fermenting must was shown to increase at the last day of fermentation ranging from 4.16 to 4.22. The alcohol content also gradually increased ranging from 0 to 9% showing the strength of the fermenting must. Banana wine has a lot of nutritional benefits, vitamins including B5, B6, C, A are all present in banana wine and this makes it one of the high ranking beverage over other alcoholic ones. Vitamin A helps in restrain of eye sight. Banana wine is infused with high taste and health. Commercially produced banana wine is a clear, slightly sparkling alcoholic beverage with a long shelf life than banana juice, which is spoiled easily and therefore not stored for long periods. In the present study all the parameters analyzed, is expected to improve the quality of wine during large scale production. The overall point obtained for the sensory analysis was 4.8 out of 5. However, there is the need for further research to ascertain the shelf life for the wines.

ACKNOWLEDGEMENT : The authors appreciate **Late Associate Professor. Archibong, Etim, Joseph** for his supervision and mentorship during the research period. We also appreciate Chychy Gilgal Laboratories and Consultancy Services Anambra State, Nigeria, for providing necessary assistance for the realization of this research work.

COMPETING INTERESTS: Authors have declared that no competing interests exist.

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