

NEWPORT INTERNATIONAL JOURNAL OF RESEARCH IN MEDICAL SCIENCES (NIJRMS) Volume 3 Issue 3 2023

Production and Evaluation of Fruit Wine from *Musa Sepientum* Puree using *Saccharomyces cerevisiae*

*Chinedu Christian Iheanacho¹, Alloysius Chibuike Ogodo¹, Ikenna Light Nkwocha², Rufus Emamoge Aso¹, Moses Adondua Abah³, Ale Ebenezer Morayo³, Enochone Roy Yohanna³, Mgbede Timothy³, Ugwuoke Kenneth Chinekwu³ and Briska Joycee¹

¹Department of Microbiology, Federal university Wukari, Taraba State,

²Department of Microbiology, University of Portharcourt, Choba, Rivers State

³Department of Biochemistry, Federal University Wukari, Taraba State

*Corresponding Author's Email: Chitray.1986@gmail.com

ABSTRACT

The feasibility of using an indigenous fruit, banana (*Musa sepientum* Linn), to produce wine was studied and compared to commercial standard wine through sensory evaluation. Selected ripe, but undamaged banana fruit purchased from Eke-Ukwu Owerri market was used to produce wine by fermentation with yeast (*Saccharomyces cerevisiae*). The banana was washed with sodium metabisulphite solution, peeled and blended. Primary and Secondary fermentation process lasted for 10 days, during which the specific gravity, total titratable acidity, pH and alcohol contents were measured daily, until constant values were obtained using standard techniques. The results show that the specific gravity of the fermenting must decreased from 1.099 to 0.980, pH dropped from 4.5 to 3.3, while total titratable acidity increased from 0.21% to 0.55%. The final alcoholic content was 10.2%. The banana wine was compared with a commercial wine for taste, colour, odour and overall acceptability on a 9-point hedonic scale. The wine was rated acceptable with the commercial wine > the banana wine in overall acceptability, though no significant difference ($P > 0.05$) was obtained with both wines in overall acceptability. The study has shown that acceptable wine can be obtained from banana puree using *Saccharomyces cerevisiae* and can serve as a way of reducing postharvest losses of banana due to inadequate storage facility.

Keywords: Wine, Banana, Fermentation, Alcohol, Acceptability

INTRODUCTION

Fruits are among the most important foods of mankind as they are not only nutritive but are also indispensable for the maintenance of health. Fruits both in fresh as well in processed form not only improve the quality of our diet but also provide essential ingredients like vitamins, minerals, carbohydrates etc. [1]. Various fruits have been used for the production of wine since the dawn of civilization. The fermentation with yeast is used for the production of wine that is considered as one of the oldest alcoholic beverages [2]. Usually, grapes were taken as substrate for wine making but in recent years, preferences have been given to other fruits such as apricot, banana, citrus fruits, and so on [3]. Moreover, there are reports indicate that home-made wines have been practiced with variety of fruits including apple, banana, cashew, watermelon, orange, plum, strawberry, guava,

Iheanacho *et al.*, 2023

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

cherries, pawpaw, cucumber etc. using *S. cerevisiae* which converts the sugar contents of the substrate to other products as alcohol, organic acids and esters. [4]; [5]; [6]; [7]. Wine is essentially the product of fermentation of hexose sugars of juice of grape and other fruit by the yeast, (*Saccharomyces cerevisiae*) to form alcohol and carbon dioxide [8]. Where wine is made from a fruit like Banana, paw-paw, Orange, Apple, Mango, Pineapple, etc, such wine carries the name of the fruit from which it is made, example; pineapple wine, paw-paw wine, banana wine, etc. [7]. Wine is a complex mixture consisting of both organic and inorganic compounds [9] The number of compounds found in wine has been estimated to be up to four hundred [10]. Banana (*Musa spp*) is a valued fruit across the world due to its flavor, high nutritional value, and availability throughout the year [11]. Banana is an important staple starchy food in Nigeria. Ripe bananas are consumed raw as a desert fruit. Banana serves as good nutritional sources of carbohydrates, minerals such as potassium and vitamins such as B₁, B₂, B₃, B₁₂, C and E [7]. Following the high nutritional content of banana, it is consumed in large quantity in a variety of ways in Africa. The banana fruit can be eaten raw or cooked, processed into flour or fermented for the production of beverages such as banana juice, beer, vinegar and wine [12]; [13]; [14]. The nutritional composition of banana can be preserved by processing them into wine to avoid wastage during their seasons. Therefore, the present study aims at producing acceptable wine from banana puree using *Saccharomyces cerevisiae* as starter culture.

Materials and Methods

Source of Materials

Ripe, undamaged banana fruits used for this were purchased from Eke-Ukwu Owerri market known as 'Ekeonuwa'. The banana fruits were kept in a sterile polyethene bags and transported to the laboratory for processing within an hour of purchase. The organism, *Saccharomyces cerevisiae* was a stock from the Microbiology laboratory, University of PortHarcourt. Other reagents are of analytical grade.

Preparation

The banana (2kg) was prepared aseptically by washing it with 1g of sodium metabisulphite dissolved in 1 liter of water to clear off the debris which might be attached to the fruit and at the same time, sterilize the fruit. Thereafter the fruit was peeled, blended and sprang with 4 liters of water to lower the viscosity of the puree. It was immediately poured into a sterilized fermenting bucket, followed by the addition of 1g of sodium metabisulphite to eliminate and suppress the growth of contaminating microorganisms and the microflora before the addition of the starter culture. The must was fortified with sucrose to raise the sugar content to about 22°brix.

Preparation of Starter Cultures

A selected ripe, but undamaged banana of about 5 inches in length was sterilized with a solution of sodium metabisulphite, aseptically peeled and blended with 20mls of sterile water. *Saccharomyces cerevisiae* was then diluted in 10-folds increment with distilled water making the resulting math relatively simple. The banana puree which is a propagative media was transferred into a sterile Petri dish and then over laid with the final dilution. The dish is then swirled to mix the wine yeast with the puree and the culture was left at 28°C for 24 hours to enable the yeast to grow well.

Primary Fermentation

The must was pitched with baker's yeast at 1% the volume of the must and primary fermentation proceeded for 4 days at room temperature varying between 25°C to 28°C. During fermentation, the must was stirred at least 3 times daily for aeration of the must and agitation of the fermenting yeast and aliquots taken daily to determine the specific gravity, pH, total titratable acidity and ethanol content of the fermenting must. At the end of primary fermentation, the wine was racked into a secondary fermentation container.

Secondary Fermentation

Secondary fermentation was carried out in an air-tight container with a small opening at the cork where a siphon tube was inserted above the wine level and the other end inserted into a sterile bottle filled with distilled water. This set-up will allow carbon dioxide escape from the secondary fermentor passing through the siphon into the water and does not allow the passage of oxygen into the fermentor [7]. During Secondary fermentation, the specific gravity, pH, total titratable acidity and ethanol content of the fermenting Must was also monitored daily until fermentation stopped after 6 days. At the end of fermentation, the fermented wine was decanted into a new sterile container for clarification.

Clarification

Clarification of the wine was achieved using bentonite. Exactly 120g of bentonite was dissolved in 100ml of water and then added to the wine at the rate of 1% the volume of must followed by a vigorous shaking. Clarification was allowed for a period of 21 days. After clarification, the wine was racked and allowed to age.

Chemical Analysis of the Wine

Specific gravity of the fermenting must was monitored daily using a brix hydrometer at 28°C [15]. About 100mls of the sample was transferred into a cylindrical jar. The brix hydrometer was inserted into it and allowed to float. This then measures the specific gravity in less than 5 minutes. The method used for the determination of total titratable acidity (TTA) was described by [15] and expressed as percentage tartaric acid. pH of the

Iheanacho *et al.*,2023

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

fermenting wine was monitored daily using analytical pH meter (Hanna instruments HI 9829 Multiparameter meter). The alcoholic content of the fermenting wine was determined using a vinometer (FIW simple model 0-25). About 100mls of the sample was poured into funnel-like equipment which was inverted immediately after pouring the sample and these measures the alcoholic content of the fermenting must in less than 2 minutes.

Sensory evaluation

The sensory evaluation of the wine was done following the method described by [7] with slight modification. The evaluation was done on a nine-point hedonic scale (where one represents very poor and nine represents excellent) to determine the taste, odour, colour, clarity and overall acceptability.

Statistical Analysis

The data were determined in triplicates and expressed as mean \pm standard deviation. The wine produced was compared to commercial wine using paired T-test with SPSS version 20.0. The significance was set at $P < 0.05$.

RESULTS

Figure 1 presents the specific gravity of the wines during the course of fermentation. The result shows that the specific gravity of the 'must' dropped from 1.099 on the one to 0.980 on day ten. The pH of the must dropped from the initial value of 4.5 to 3.3 (day 10) as presented on Figure 2. Figure 3 shows the total titratable acidity (TTA) of the must during fermentation. The TTA increased with increasing fermentation time and ranged from 0.21% (initial value) to 0.55% (day 10). The ethanol content of the must during fermentation showed a sharp increase from 0.0% to 10.2 %. (Figure 4). The sensory evaluation of the aged wine on a 9-point hedonic scale in comparison other commercial wines was presented on Table 1. The result shows that the present wine compared favourably with the commercial wine with no significant difference ($P > 0.05$) in taste, colour, odour and overall acceptability.

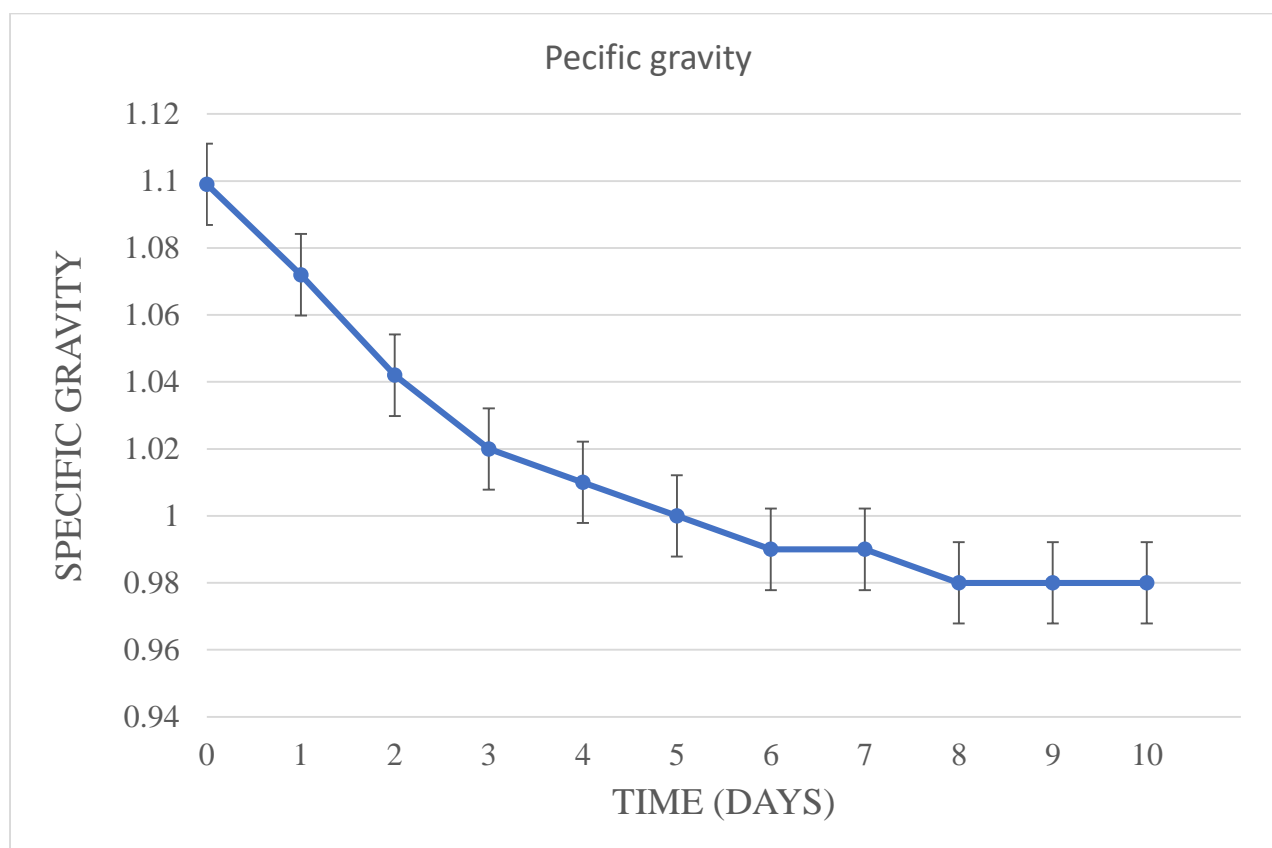


Fig. 1 Specific gravity of the must during fermentation. The values are mean of triplicate determination. The error bars represent standard error.

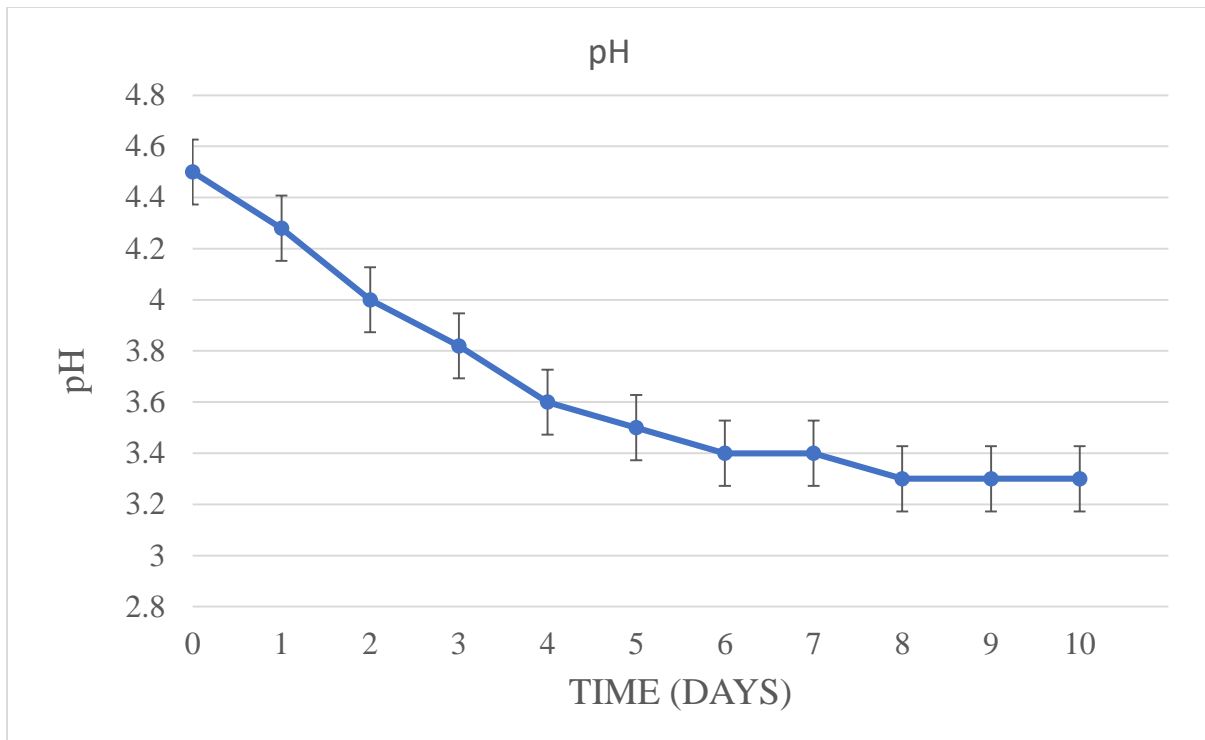


Fig. 2 pH of the must during fermentation. The values are mean of triplicate determination. The error bars represent standard error.

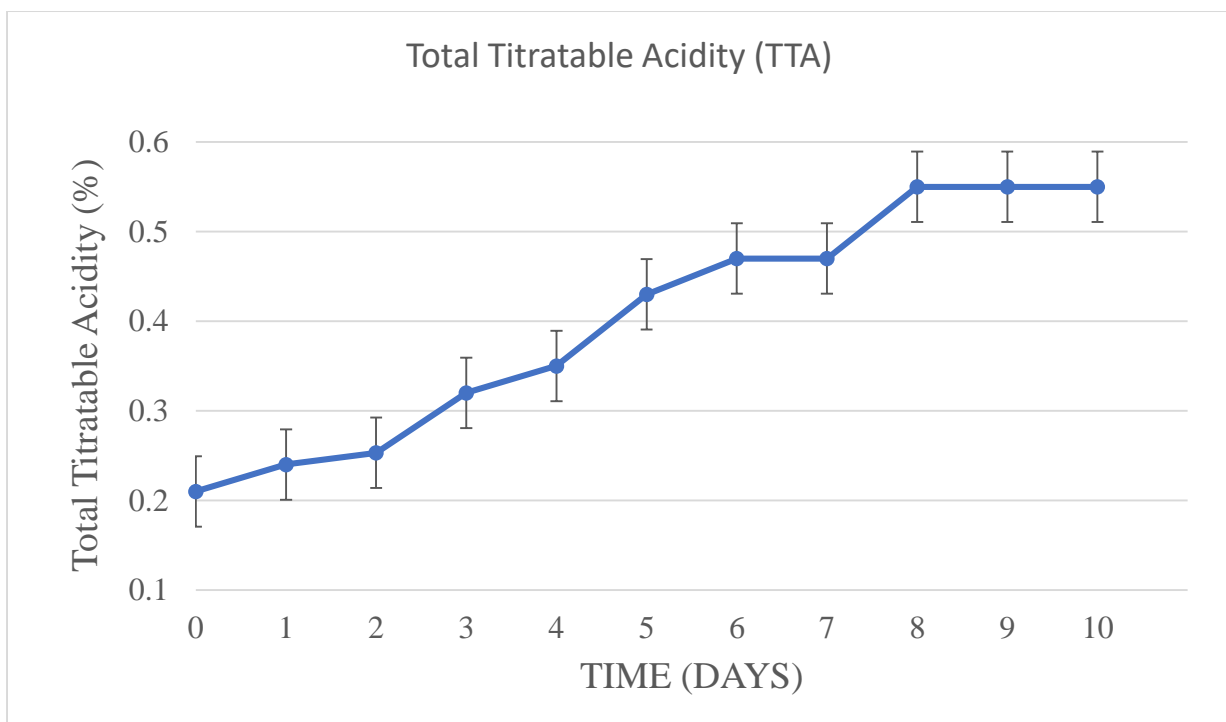


Fig. 3 Total Titratable acidity (TTA) the must during fermentation. The values are mean of triplicate determination. The error bars represent standard error.

Iheanacho *et al.*,2023

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

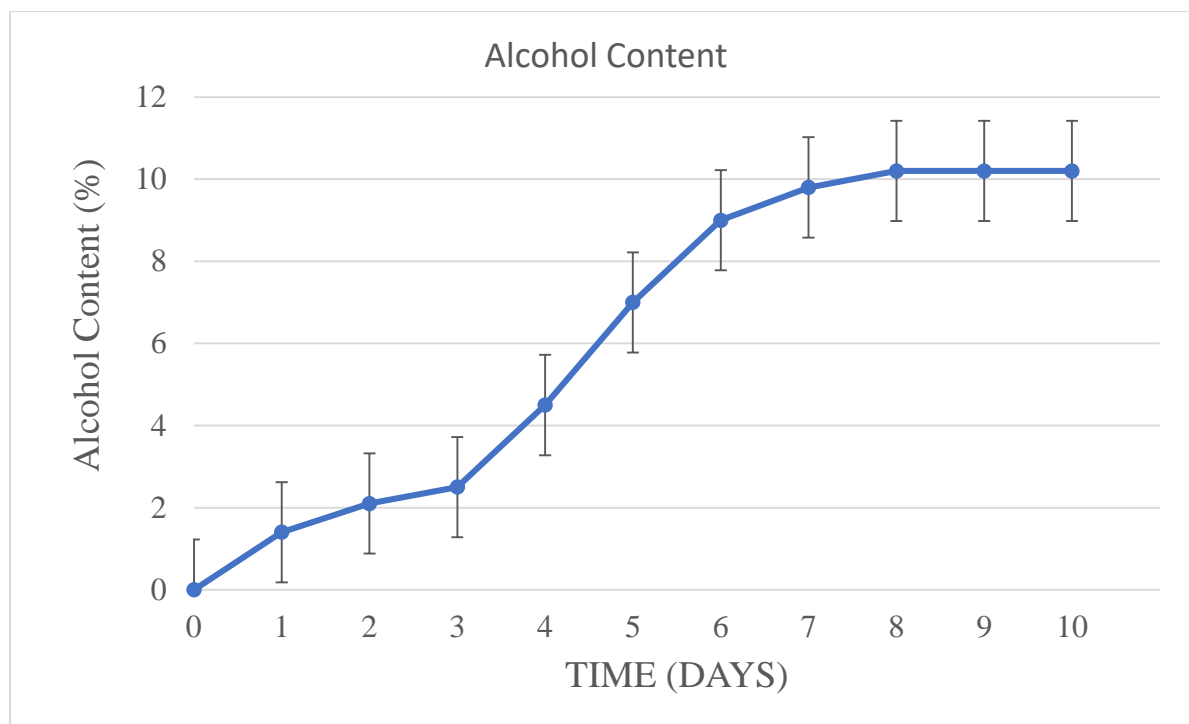


Fig. 4 Alcohol content of the must during fermentation. The values are mean of triplicate determination. The error bars represent standard error.

Table 1 Comparative sensory evaluation of the wine with commercial wines

PARAMETERS	BANANA WINE	COMMERCIAL WINE (Jacob's sanctuary communion wine)	P-VALUE
TASTE	6.0 ± 1.2	7.3 ± 1.6	>0.05
COLOUR	6.9 ± 2.1	7.5 ± 1.7	>0.05
ODOUR	6.5 ± 0.8	7.5 ± 1.3	>0.05
OVERALL ACCEPTABILITY	6.0 ± 1.0	7.6 ± 0.6	>0.05

DISCUSSION

Wine is a safe and nutritious beverage which provides calories of energy and vitamins as well as offer drink for relaxation [15]. In Nigeria, substantial quantities of various fruits are grown, of which many do not reach the market due to spoilage from mechanical damage and over-ripeness [16]. However, processing these fruits into wine has helped a lot in preserving them as finished products. In the present study, acceptable fruit wine was produced from indigenous banana. Previous reports from other locations have shown that wine of acceptable quality can be produced from banana fruits. For instance, [17] and [18] have respectively reported the production of acceptable wines from banana fruits. Similarly, the production of acceptable wines from the mixture of banana, watermelon and pawpaw have been reported by [7]. This shows that the nutritive values of bananas can be preserved by converting them into wine.

In the present study, there was gradual fall in specific gravity during fermentation of the must to wine. This observation is consistent with the report of [19] who reported decrease in specific gravity of passion fruit, watermelon and pineapple during fermentation into wine using palm wine yeasts and commercial *Saccharomyces cerevisiae*. This is indicative of total absorption of oxygen in the must by yeast for cell growth, sugar uptake and formation of alcohol until there was no fermentable sugar in the must [20]. The sugar uptake by the fermenting yeast results to decrease in the fermentable sugar with resultant increase in alcohol content of the wine. The decrease in fermentable sugar during fermentation of fruits to wine has been reported by other researchers such

Iheanacho *et al.*,2023

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

as [10], [21], [22] and [7]. The decrease in the fermentable sugar could be due to the fact that fermenting yeasts utilize them as sources of energy [23] [21].

In the present study, there was a gradual steady fall in the pH (towards acidity) of the fermenting must during the primary fermentation. This observation agreed with the reports of previous researchers on watermelon, passion and pineapple [19], mango juice fermented with *Saccharomyces cerevisiae* and *Schizosaccharomyces* species [24], mango wine [22] as well as on mixed fruit wines of pawpaw, banana and watermelon wines fermented with *S. cerevisiae* isolated from palm wine [7]. Similarly, decrease in pH of musts during fermentation of other tropical fruits into wine, such as sapota fruits [25]; [26], banana fruit [17] and tundu fruits [27]. The low values in pH observed in the present study could be as a result of organic acid production by the fermenting organisms and its accumulation in the must. This is also a sign that the wine would have a good keeping quality since low pH can inhibit the growth of spoilage microorganisms.

There was a gradual steady increase in the total acidity of the wine during primary fermentation. The acidity ranged from 0.21% to 0.55%. This trend of increase in the total acidity during fermentation of fruits into wine have been reported by [7] during mixed fruit wine fermentation, [28] on orange juice and [22] during mango wine production. The acidity reported in the present study is lower than the reports of [22], [25] and [29] on mango fruit, sapota fruit and sweet potato wines respectively but higher than 0.15g/100ml reported on bael wine [26]. According to [19], total acidity of wines should be found between the range of 0.5% to 1.0% and the present study acidity fall within this range. The gradual increase in acidity and consequent decrease in pH could be attributed to the fact that the fermenting organism produced organic acid, phenolic compounds and esters which contributed to increase in acidity and lowering of pH [30]; [35]; [36].

The alcohol content of the wine obtained in the present study was 10.2%. This compared favourably with the reports of [22] and [19] who reported 10.5% and 10.46% respectively on mango wines fermented with commercial yeasts. During alcoholic fermentation, products such as ethanol, acids, esters, acetyls and carbonyl compounds which contributes to the flavour and overall characteristic quality of the final wine [31]; [32]; [33]. Moreover, maximum alcohol production can be enhanced by taking into consideration, initial cell and sugar concentration on the viability of baker's yeast [34]; [35]; [36].

Sensory evaluation of the aged wine was done and compared to a commercial standard wine purchased from a supermarket. The evaluated rated the wine produced in this study acceptable. The comparison of the commercial wine and the produced wine for tastes, colour, odour and overall acceptability shows that there was no significant difference ($P > 0.05$) between the two wines. The favorable comparison of the two wine samples with respect to overall acceptability may be attributed to the ageing period. The banana wine was aged for a long time when this evaluation was performed, and since wine colour, taste and odour is relative to age, the prolonged ageing period of the banana wine enabled it to compare favorably with the commercial wine with respect to overall acceptability.

CONCLUSION

The present study had shown that acceptable fruit wine which compares favourably with commercial standard wine could be produced from banana puree using *Saccharomyces cerevisiae*. This is a breakthrough in converting the excess banana fruits which are wasted due to lack of storage facilities into wine in Nigeria in addition to boosting the economy and creating more jobs thereby reducing unemployment.

REFERENCES

1. Swami, S.B., Thakor, N.J., and Divate, A.D. (2014). Fruit Wine and Health Benefits. *Journal of Food Research and Technology*, **2**(3): 93 – 100.
2. Gavimath, C.C., Kalsekar, D.P., Raorane, C.J., Kulkarni, S.m., Gavade, B.G., Ravishankar, B.E., and Hooli, V.R. (2012). Comparative Analysis of Wine from different Fruits. *International Journal of Advanced Biotechnology and Research*, **3**(4): 810 – 813.
3. Satav, P.D., and Pethe, A.S. (2017). Production and Optimization of Wine from Banana Fruits. *International Journal of Pharma and Bio Sciences*, **8**(2): 790 – 794.
4. Fleet, G.H. (2003). Yeast interaction and Wine flavor. *International Journal of Food Microbiology*, **86**: 11 – 22.
5. Isitua, C.C. and Ibeh, I.N. (2010). Novel method of wine production from Banana (*Musa acurninata*) and pineapple (*Ananas cosmosus*) waste. *African Journal of Biotechnology*, **9**: 7521 – 7524.
6. Duarte, W.F., Dias, D.R., Oliverira, M.J., Teixeira, J.A., Schwan, R.J. (2010). Characterization of different fruit wines made from cocoa, cupuassu, gairoba, jaboticaba and umbu. *Food Science Technology*, **30**: 1 – 9.

Iheanacho et al.,2023

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

7. Ogodo, A.C., Ugbogu, O.C., and Ezeonu, C.S. (2015). Production of mixed fruit (pawpaw, banana and watermelon) wine using *Saccharomyces cerevisiae* isolated from palm wine. *SpringerPlus*, 4: 683
8. Frazier, W.C. and Westhoff, D.C. (1991). *Food Microbiology*. 3rd Edition, Mc Graw Hill company Ltd, New Delhi; Pp: 350 – 355.
9. Amerine, M.A. and Ough, C.S. (1980). "Methods for Analysis of Musts and Wines," New York.
10. Kunkee, R.E. (1974). "Malo-lactic fermentation and winemaking". In, The chemistry of winemaking, Adv. Chem. Ser. 137, A.D. Webb, Ed, pp. 151-170.
11. Syriac, G.M., Mishra, S., Prasad, V.M., Saravanan, S. and Topno, S.E. (2017). Response of different sources of sugar on production and quality analysis of banana (*Musa paradisical*) peel wine CV. Grand Naine. *Journal of Pharmacognosy and Phytochemistry*, 6(4): 591 – 595.
12. Pillay, M. and Tripathi, L. (2007). Banana. In: Kole C(ed) Genome mapping and molecular breeding in plants, fruits and nuts, vol 4. *Springer, Varlay Berlin*, 281 – 301.
13. Nelson, S.C., Ploetz, R.C., and Kepler, A.K. (2006). *Musa species* (banana and plantains). Permanent agricultural resource, Holoa, Hawaii. <http://www.agro-forestry.net/tti/musa-banana-plantain/>. Accessed 12/06/2012
14. Pillay, M.A., Tenkouano, A, Ude, G, and Irtiz, R. (2004). Molecular characterization of genomes in *Musa* and its application. In: Jain SM, Swannem R(eds) *Banana improvement: cellular molecular biology and induced mutations*. Science Publishers Inc., Enfield, 124 – 185.
15. Amerine, M.A.; Berg, H.W., Kunkee, R.E., Ough, R.E., Singleton, V.C. and Webb, A.D. (1980). *Technology of wine making*. 4th Edition. Avi. Pub. Co. Westport Connecticut, 50 – 75.
16. Keay, R.W.J., Onochie, C.F.A. and Stanfield, D.P. (1964). *Nigerian trees. Vol.II*. Department of forest Research Ibadan.
17. Obaedo, M.E. and Ikenebomeh, M.J. (2009). Microbiology and production of banana (*Musa sapientum*) wine. *Nigerian Journal of Microbiology*, 23(1):1886–1891
18. Akubor, P.J., Obio, S.O., Nwadamere, K.A. and Obiomah, E. (2003). Production and quality evaluation of banana wine. *Plant Food for Human Nutrition*, 58:1–6
19. Chilaka, C. A., Uchekukwu, N., Obidiegwu, J. E. and Akpor, O. B. (2010). Evaluation of the efficiency of yeast isolates from palm wine in diverse fruit wine roduction. *African Journal of Food Science*, 4(12): 764 - 774
20. Amerine, M.A.; Berg, H.W.; and Kunkee, R.E. (1999). *Technology of wine making*. 4th Edition. Avi, Westport, 523 – 544.
21. Kamassah AKQ, Saalia FK, Osei-Fosu P, Mensah-Brown H, Sinayobye E, et al. (2013) Fermentation Capacity of Yeasts Using Mango (*Mangifera indica* Linn.) as Substrate. *Food Sci Qual Manag* 22: 69-78.
22. Ogodo, A. C., Ugbogu, O. C., Agwaranze, D. I. and Ezeonu, N. G. (2018). Production and Evaluation of Fruit Wine from *Mangifera indica* (cv.Peter). *Applied Microbiology*, 4 (1): 144. doi:10.4172/2471-9315.1000144.
23. Ribéreau-Gayon P, Dubourdieu D, Donèche B, Lonvaud A (2006). *Handbook of Enology: The Microbiology of Wine and Vinifications*. (2nd edn), John Wiley & Sons, Ontario.
24. Obisanya, M.O., Aina, J.O. and Oguntimein, G.B. (2002). Production of wine from mango (*Mangifera indica*) using *Saccharomyces* and *Schizosaccharomyces* species isolated from palm wine. *Journal of Applied Microbiology*, 63: 191-196.
25. Panda, S.K., Sahu, U.C., Behera, S.K. and Ray, R.C. (2014a) Fermentation of sapota (*Achras sapota* linn.) fruits to functional wine. *Nutrafoods*. doi:10.1007/s13749-014-0034-1
26. Panda, S.K., Sahu, U.C., Behera, S.K. and Ray RC (2014b). Bio-processing of bael [*Aegle marmelos* L.] fruits into wine with antioxidants. *Food Biosci* 5:34–41.
27. Sahu, U.C., Panda, S.K., Mohapatra, U.B. and Ray, R.C. (2012). Preparation and evaluation of wine from tendu (*Diospyros melanoxylon* L) fruits with antioxidants. *International Journal of Food Fermentation Technology* 2(2):167–178
28. Okunowo, W.O., Okotore, R.O. and Osuntoki, A. A. (2005). The Alcoholic fermentation efficiency of indigenous yeast strains of different origin on orange juice. *Afri J Biotechnol* 4: 1290-1296.
29. Ray, R.C., Panda, S.K., Swain, M.R. and Sivakumar, S.P. (2011). Proximate composition and sensory evaluation of anthocyanin-rich purple sweet potato (*Ipomoea batatas* L.) wine. *International Journal of Food Science and Technology*. doi:10.1111/j.1365-2621.2011.02861.x
30. Onwuka, U.N. and Awam, F. N. (2001). The potential for baker's yeast (*Saccharomyces scerevisiae*) in the production of fruit wine from banana, Cooking banana and plantain. *Food Sci Technol* 1: 127-132.
31. Amerine, M.A. and Ough, C.S. (1974). Wine and Must Analysis. *Journal of Chromatographic Science*, 12 : (5)19A.

32. Clement-Jimenez, J. M., Mingorance-cazorla, L., Martinez-Rodoriguez, S., Las Heras, V. F. J. and Rodriguez-vico, F. (2005). Influence of sequential yeast mixtures on wine fermentation. *Int J Food Microbiol* 98: 301-308.
33. Reddy, L.V. and Reddy OVS (2009) Production, optimization and characterization of wine from mango (*Mangifera indica* L.). *Natural Product Radiance*, 8(4):426-435
34. Ayrapaa, J. (1987). Higher Alcohols in Fermentation. *J. Inst. Brew.*, 17: 20 – 73.
35. Ugwu Okechukwu P.C. and Amasiorah V.I. (2020). Review on Health Implications, Benefits and Biochemistry of Alcohol Intoxication. *INOSR Experimental Sciences*,6(1): 62-74.
36. Ebugosi RS, IN Achara and OPC Ugwu (2023). Evaluation of the effects of Maternal alcohol consumption on some selected biochemical parameters. *IAA Journal of Biological Sciences*,10(1): 87-95.

Chinedu Christian Iheanacho, Alloysius Chibuikwe Ogo, Ikenna Light Nkwocha, Rufus Emamoge Aso, Moses Adondua Abah, Ale Ebenezer Morayo, Emochone Roy Yohanna, Mgbede Timothy, Ugwuoke Kenneth Chinekwu and Briska Joyce (2023). Production and Evaluation of Fruit Wine from Musa Sepientum Puree using *Saccharomyces cerevisiae*. NEWPORT INTERNATIONAL JOURNAL OF RESEARCH IN MEDICAL SCIENCES (NIJRMS) 3 (3): 18-25.

Iheanacho *et al.*,2023

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited