IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 3 Ver. I (March. 2018), PP 52-56
www.iosrjournals.org

Table Wine Production From Mixed Fruits Of Soursop (Annona Muricata)And Pineapple(AnanasComosus)Using Yeast From Palm Wine.

Okafor Ugochukwu C.*, Edeh Jude I., Umeh Sophina O.
Dept. of Applied Microbiology & Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria
Corresponding author:Okafor Ugochukwu C

Abstract:*This Study Was Aimed At Investigating The Suitability Of Two Fruits (Pineapple And Soursop) As Substrates For Wine Production And The Efficiency Of Yeast Isolated From Palm Wine For Alcoholic Fermentation Of Fruits. During Fermentation Aliquot Samples Were Removed Daily From The Fermentation Tank For Analysis Of Ph, Temperature, Alcohol Content And Reducing Sugars, Using Standard Procedures. Ph Of The Fruit "Must" During The Period Of Fermentation Ranged From 4.91 To 4.25. During The Fermentation Period, Consistent Increase In Alcohol Content Was Observed With Time. At The End Of 7days Fermentation, The Concentration Of Alcohol In The Fruit Wine Was Observed To Be 4.6%.The Reducing Sugar Content Of The Wine Was Observed To Be 0.2632. During The Course Of The Fermentation, The Titrable Acidity Of The Wine Was Observed To Show A Steady Trend With Time. Based On The Level Of The Nutritional Composition Of Pineapple And Soursop Juice, Their Relative Affordability, Their Ability To Support Yeast Growth, The High Alcoholic Content And Palatability Of The Wine Produced, This Study Showed That Acceptable Wine Can Be Produced From Mixed Fruits Of Pineapple (Ananas Comosus) And Soursop (Annona Muricata).*
Keywords:*Wine, Yeast, Fruits, Fermentation, Alcoholic Beverages.*

Date of Submission:03-02-2018 Date of acceptance: 19-03-2018

I. Introduction

Wine is any alcoholic beverage produced from juices of variety of fruits by fermentative action of microorganisms either spontaneously or seeding with a particular strain mainly of yeast species to adopt a particular quality of wine[1]. Wine is one of the most recognizable high value added products from fruits. Most commercially produced wines are usually made from fermented grapes; this fermentation process is not done by introducing any chemicals or sugar but by adding different species of yeast to the crushed grapes.

According to Garrison [1], the process of fermenting is basically feeding sugars and nutrients in solution to yeast, which return the favour by producing carbon dioxide gas and alcohol. This process goes on until either all the sugar is gone or the yeast can no longer tolerate the alcoholic percentage of the beverage. Different yeasts produce different results, and have different tolerance levels. Fermentation is a process of deriving energy from the oxidation of organic compounds, such as carbohydrates, and using an endogenous electron acceptor, which is usually an organic compound [2], as opposed to respiration where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. The risk of stuck fermentation and the development of several wine faults can also occur during this stage which can last from 5 to 14 days for primary fermentation and potentially another 5 to 10 days for a secondary fermentation. Fermentation may be done in stainless steel tanks, which is common with many white wines like Riesling, in an open wooden vat, inside a wine barrel and inside the wine bottle itself as in the production of many sparkling wines. [3] [4]

Fermentation is a cheap and energy efficient means of preserving perishable raw materials such as pineapple juice [5]. Harvested fruits may undergo rapid deterioration if proper processing and storage facilities are not provided, especially in the humid tropics where the prevailing environmental conditions accelerate the process of decomposition [6].Although there are several options for preserving fresh fruits, which may include drying, freezing, canning and pickling, many of these are inappropriate for the produce and for use on small-scale in developing countries. For instance the canning of fruits at the small-scale has serious food safety implications and contamination especially botulism [7]. Freezing of fruits and vegetables is not economically viable at the small-scale.

Fermentation requires very little sophisticated equipment, either to carry out the fermentation or for subsequent storage of the fermented product. It is a technique that has been employed for generations to preserve fruits in the form of drinks and other food for consumption at a later date and to improve food security. Basically most fruits can be fermented; if not all provided they are well prepared [1].

Yeast has the capability of converting grapes into an alcoholic compound and removing the sugar content in it for the production of different types of wines. Sometimes wines are produced from different types of fruits like; Paw-Paw, mango, Pineapple, Banana, Lemon, Watermelon etc., here the wine so produced bears the name of the fruit or fruit mixture used in its production [3].

Wine can be made from virtually many plant matters that can be fermented [8].Most fruits and berries have the potential to produce wine. Wine making involves the use of yeast to ferment the 'must' of a chosen fruit or fruits for a number of days, depending on the objective of the winemaker. The yeast which is the main organism responsible for alcoholic fermentation usually belongs to the genus *Saccharomyces*.

The aim of this study is to produce wine from mixed fruits of Pineapple (*AnanasComosus*) and Soursop (*Annonamuricata* L.).

II. Materials And Methods

Sample Collection

The pineapple and Soursop used in this fermentation studies were sourced from a local market, Ogbete-Enugu, while the palm wine used for yeast isolation was obtained from Umuawulu, Awka metropolis both in South East, Nigeria. The *Ananas comosus* and *AnnonamuricataL.* were identified at Botany Department, Nnamdi Azikiwe University, Awka.

Isolation of yeast from Palm wine

The yeast was isolated from 24 hours fermented palm wine [28]. One hundred (100ml) of the palm wine was kept in a sterile conical flask. The sediment of the solution was cultured on Sabouraud Dextrose Agar (SDA) plate and incubated at $37^{o}C$ for 72hrs. The identified yeast colonies were subcultured on SDA to obtain pure cultures of yeast cells [9].

Preparation of "mixed must"

The 'pineapple and soursop' fruit were weighed, washed, peeled, sliced, rewashed, with the removal of the seeds for soursop and then reweighed. The fruit were then blended with a sterile blender into puree, and then filtered. The overall water added during the blending was 600mls distilled water to avoid friction in the blender. 300mls of distilled water was added to extract the "must" by filtering the juice with muslin cloth. 2150mls of the whole "must" was poured into the fermenting jar prefilled with CO_2 for fermentation and then, 0.28g of sodium metabisulphite was added.

Inoculum development

Inoculum development was done to obtain large quantities of yeast cell for pitching to build up the inoculums. 100ml of each must (pineapple and soursop) was mixed in a 500ml conical flask. Ammoniumn sulphate and Potassium di-hydrogen phosphate (0.12%) were added as yeast nutrient. The mixture was autoclaved al $121^{o}C$ for 15 minutes. Three loopfuls of the stock culture from an SDA slant was transferred into the 200ml standard "must" in a conical flask and shaken in an attemperated Gallenkamp rotary shaker for 48hours [10].

Must Fermentation

The 150mls of developed inoculum was poured into the fermenter jar containing the "must" making it a total of 2300ml. 179.5g of sucrose was then added to the mixed fruit fermenter and then the mouth of the jar stuffed tightly with cotton wool and kept on the bench for 7days. After the fermentation, settled yeast and other debris were clarified using Gelatin as fining agent prior to the packaging [9].

III. Sample Analyses

- **Determination of Reducing Sugar**

The'must' was placed in a 50ml burette. 25ml of Benedict's quantitative reagent was pipetted into a 250ml conical flask and boiled gently. During boiling, sample from burette was added slowly until traces of blue color of Benedict's solution disappeared and red precipitate formed. The reducing sugar was monitored for 7days of fermentation [28].

The weight in gram of reducing per 100ml

$$\text{Reducing Sugar} = \frac{K \times 100 \times N}{V}$$

K = Reducing Sugar constant for glucose = 0.050

N = Number of time the sample 'must' was diluted

V = Volume of sugar solution required for titration (titre) i.e
Final Volume – Initial Volume

- **Determination of Titrable Acidity**

200ml of distilled H_2O was poured into a 500ml conical flask and boiled 1ml of 1% aqeous alcoholic phenolphthalein indicator was added. This was titrated with 0.1m NaOH solution to faint but definite pink color. 5ml of 'must' was pipetted and dropped into the boiling neutralized solution again titrated to the end point using the same 0.1m NaOH solution. The titrable acidity was expressed as tartaric acid and calculated according to the method of [19].

$$\text{Tartaric Acid (g/100ml)} = \frac{V_1 \times M \times 75 \times 100}{1000 \times V_2}$$

V_1 = Volume of NaOH solution (Final Reading – Initial Reading)
M = Molarity of NaOH solution
V_2 = Volume of 'must'

- **Determination of Specific Gravity**

25ml measuring cylinder was used to measure 25ml of the sample. A clean brix hydrometer was dipped gently into the sample and the specific gravity read and recorded at room temperature.

- **pH Determination**

A 10ml of the 'must' was measured into a sample bottle and placed on the curvette of the digital pH meter. The reading was taken when the pointer became steady.

- **Determination of Temperature**

The temperature reading was taken using a clean thermometer.

IV. Results And Discussion

Results of the analysis conducted during the fermentation of "must" after pitching with the culture yeast showed that the alcoholic content of the wine increased as the fermentation progressed. The pH level of the wine decreased from 4.91 at 0-23 hours to 4.85 after 24 hours. It continued decreasing as fermentation continued. The reducing sugar in Gram was 2.5500 at the start of the fermentation but decreased to 0.8333 by the second day. It kept decreasing as the fermentation progressed.The temperature of the wine was rather steady, maintaining a value of 28 ± 1 from the start to the finish of the fermentation. Titrable acidity decreased over time as fermentation progressed.

This study revealed that there was a continuous drop in *pH* values in the fruit wine as fermentation progressed. Studies have shown that during fermentation of fruits, low *pH* is inhibitory to the growth of spoilage organisms but create conducive environment for the growth of desirable organisms. Also, low *pH* and high acidity are known to give fermentation yeast comparative advantage in natural environments. A similar observation has been reported by Reddy and Reddy[11], in their study on mango fruit, optimum *pH* and temperature values for quality wine production was 5.0 and 30^oC, respectively. [12] noted that maximum pH for mixed fruit wine is 3.5.

During fermentation, the results obtained are shown in table 1. The changes in specific gravity and reducing sugar (^{0}Brix) of each sample wine decreased from the initial day to the end of the fermentation. This could be due to microbial succession, available nutrients, sugar and alcohol resulting in the production of acid. This result agree with the reports of [13]**;** [3]; [14]; [15]; [16]; [17].

In the case of specific gravity of the fruit wines, gradual decreases in values were observed throughout the period of fermentation. The observed reduction in specific gravity from 1.0299 SG and the resultant increase in alcohol concentration in with fermentation show the efficiency of the yeast cells isolated from palm wine. [18]

It was observed that the alcoholic content decreased from the initial day to the end of the fermentation. This is due to production of ethanol during fermentation process through metabolic activities. These results agree with the reports of Amerine and Kunkee[19]; [20]; [15]; [21]; [22]; [12]; [17]

Sucrose was a part of the additives, in order to supplement the sugar content of the "must" the level of sugar in the reactor decreased with the progress of the fermentation process. Reports have also shown that the major problem associated with the use of tropical fruits in wine production is their low sugar content [23]. Remarkable amount of alcohol was produced from the fruit wines during fermentation with the palm wine yeast, which is comparable with moderate grape wines, [24] [25] [18].Acidity plays a vital role in determining wine quality by aiding the fermentation process and enhancing the overall characteristics and balance of the wine. Lack of acidity will mean a poor fermentation [26] The changes in the % titratable acidity of mixed wine within the period of fermentation shows the occurrence of Malo-lactic fermentation [22] These results agree with the report of Child[27]; [15]; [22]; [12]; [28].

Competing Interests
The authors declare that no conflict of interests exists regarding the publication of this article.

Acknowledgements

The authors appreciate Okafor Ugochukwu C., Edeh Jude I., whose efforts led to the realization of this research. We also appreciate the laboratory technologists of the Department of Applied Microbiology and Brewing Science, Nnamdi Azikiwe University, Awka.

Author's Contributions
Okafor, U.C designed the whole study, supervised the intellectual content and revised it and Edeh J.I. carried out the experiment. The other author assisted in the research methodology. All the authors approved the final manuscript.

Table 1: Representation of Temperature results from the start of the fermentation to finish

Days of Fermentation	Temperature
0	29
1	28
2	28
3	28
4	27
5	28
6	27
7	28
Standard wine	29

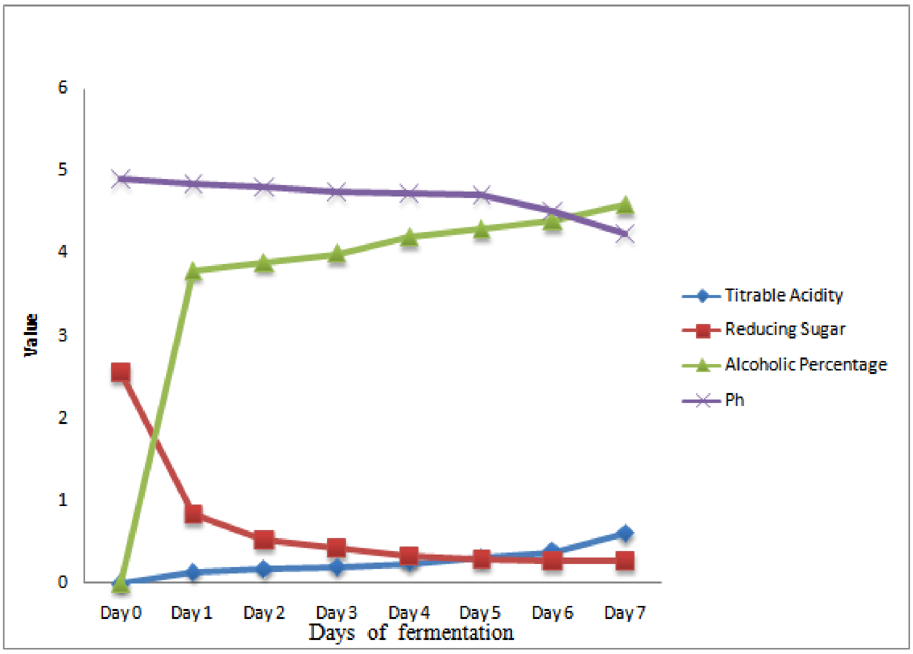

Fig 1: Graphical representation of Physicochemical parameters from the fermentation

V. Conclusion

Fruits both in fresh as well as in processed form not only improve the quality of our diet but also provide essential ingredients like vitamins, minerals, carbohydrates etc. An acceptable tropically sourced wine can be produced from the combined use of ripe pineapple and soursop fruit juice and pure culture of yeast cells obtained from palm wine. This study also gave an insight into the efficiency and role of local yeast (palm wine yeast) during alcohol fermentation of fruits. For this successful fermentation, wine making should be encouraged commercially in Nigerian using our locally available fruits. This will earn income and revenue for both our citizens and government, reduce dependency on foreign and imported wines and grow our economy.

References

[1] Garrison, E. C., (1993). Making Simple Fermented Beverages [online]. Available from: http://www.homebrew.net/ferment/
[2] Klein, D.W., Lansing, M. and Harley, J., (2005). Microbiology. New York: McGraw Hill [online]. Available from: www.bdu.ac.in/syllabi/affcol/pg/mb8.pdf
[3] Robinson, J., (2006). *The Oxford Companion to Wine*. 3rd Ed. Oxford University Press. pp. 268-780.
[4] Kunze, W., (2004). Technology Brewing and Malting. VLB Berlin, Germany [online]. Available from: http://en.wipidia.org/wiki/special.
[5] FAO, (2002). Tropical foods commodity notes, Statistical Database [online]. Available from: http://www.fao.org/statisticedatabas.
[6] FAO, (2010). Fermented Fruits and Vegetables [online]. Available from: http://www.fao.org/docrop/x056oe14.htm.
[7] FAO, (1998). Fermented Fruits and Vegetable: A Global Perspective. *FAO Agricultural Services Bulletin No. 134* [online]. Available from: http://www.fao.org/agap/frg/afris/espanol.
[8] Harding, G., (2005*). "Wine Miscellany"*. Clarkson Potter Publishing New York. 5-9
[9] Archibong, E.J., Ezemba, C. C., Chukwujama, I. C. and Archibong, U. E., 2015. Production of wine from mixed fruits: pineapple (Ananas comosus) and orange (Citrus sinensis) using yeast isolated from palm wine. *world journal of pharmacy and pharmaceutical sciences*, **4**:126-136.
[10] Okeke, B.C., Agu, K.C., Uba, P.O., Awah, N.S., Anaukwu C.G., Archibong, E.J., Uwanta, L.I., Ezeneche, J.N. Ezenwa, C.U. Orji, M.U., (2015). Wine Production from Mixed Fruits (Pineapple and Watermelon) Using High Alcohol Tolerant Yeast Isolated from Palm Wine. Universal Journal of Microbiology Research **3**(4): 41-45.
[11] Reddy, L.V.A. and Reddy, O.V.S., (2005). Production and characterization of wine from mango fruit. *World journal of microbiology and biotechnology*, **21**: 1345-1350.
[12] Agbor, A.A., Ben, U.M., Ubana, E.M., Olayinka, J.C. and Okon, A.E., (2011). Production, characterization and safety of wine obtained from a blend oftomato, almond, orange, lemon and African star apple extract. Scholars Research Library, *Annalsof Biological Research*, **2**(5): 492-503.
[13] Anononymos, (2008). Making Wine at Home Using Wild Yeast. Available at www.diversity. makingwinefromwildyeast/html.
[14] Okafor, N., (2007). Modern Industrial Microbiology and Biotechnology. Science Publishers, Enfield, 263-308.
[15] Idise, O. E. and Ofiyai, O., (2011). Studies on Wine Production from Pawpaw (*Carica papaya*). *Journal of Brewing Distilling*. Vol. **2**(4) Pp. 56 – 62. ISSN2141 – 21972 @Academic journals.
[16] Noah, A.A., Alechenu, J.O., Abiaziem, C.V., Oduwobi, O.O., (2013). Comparative Studies of Wine Produced From Pawpaw Juice and Coconut Milk at Different Proportions. Current Research on Nutrition Food Science, **1**(2).
[17] 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*, **4**:1290-1296.
[18] Amerine, M.A. and Kunkee, R.E. (2005). Microbiology of Wine making. *Annals Review Microbiology*. Academic Press, London. Pp 223-258.
[19] Harrias, H.C., (2005). The Evolution, Dissemination and Classification of *Cocos nucifera*. *Botany Review*, **44**: 265-320.
[20] Anononymos, (2010). Yeast. Encyclopædia Britannica. *Ultimate Reference Suite*. Chicago: Encyclopædia Britannica
[21] Idise, O. E., (2011). Studies On Wine Production From Coconut (*Cocos nucifera*). *Journal of Brewing and Distilling*. Vol. **2**(5), Pp. 69 – 74 Online http:www.academic journal.org/jbd.ISSN2141-2197 @Academic journals.
[22] Alobo, A.P. and Offonry, S.U., (2009). Characteristics of coloured wine produced from Roselle I (*Hibiscus sabdariffa*) calyx extract. *Journal of Institute of Brewing*, **115**(2):91-94.
[23] Ayogu, T.E., (1999). Evaluation of the performance of yeast isolate from Nigerian palm wine in wine production from pineapple fruits. *Bioresource Technology*, **69**: 189-190.
[24] Querol, A., Fernandez-Ezpinar, T.M., Olmmo, M.L. and Barrio, E., (2013). Adaptive evolution of wine yeast. *International Journal of Food Microbiology*, **86**:3-10.
[25] Berry, C. J. J., (2000). First steps in wine making, published by G.W. Kent,inc. 3667 morgan road, Ann Arbor M I 48108, pp:235
[26] Child, R., (2002). Coconuts (2nd ed.). Longman Group, London. pp. 335. Commonwealth Scientific and Industrial Research Organization. The health benefits of citrus fruits. Horticulture Organization, Sydney.
[27] Umeh, S.O., Agwuna, L.C., Okafor, U.C.(2017). Yeasts from Local Sources: An Alternative to the Conventional Brewer's Yeast. World Wide Journal of Multidisciplinary Research and Development, 3(10): 191-195.

Okafor Ugochukwu C "Table Wine Production From Mixed Fruits Of Soursop (Annona Muricata)andPineapple(AnanasComosus) Using Yeast From Palm Wine."IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 12.3 (2018): 52-56.