

WINE PRODUCTION FROM APPLE (*Malus pumila*) USING YEAST ISOLATED FROM PALMWINE

ABSTRACT

The study was aimed at the production of apple (*Malus pumila*) fruit wine with the use of yeast *Saccharomyces cerevisiae* isolated from palm wine. Both primary and secondary fermentation of the apple lasted 28 days. Aliquot samples were removed and used daily from the fermentation tank for analysis of alcohol content, specific gravity, *pH*, titratable acidity, and reducing sugar using standard procedures. During fermentation, *pH* of the fruit must range from 5.0 to 3.2. There was an increase in alcohol content, which was observed with time. Finally at the end of the 28th day's fermentation, the alcohol concentration in the fruit wine was observed to be 3.2%. Also titratable acidity concentration of the wine shows steady increase with time throughout the fermentation period. This study has revealed that much acceptable wine with quality could be produced from apple with *Saccharomyces cerevisiae* isolated from palm wine. Sensory evaluation results showed there were no significant differences ($p > 0.05$) in flavor, taste, clarity and overall acceptability between apple wine and a reference wine. The apple wine was generally accepted.

Keywords: apple, fermentation, *Saccharomyces cerevisiae*, wine, fruit.

Authors' Contributions: This work was carried out in collaboration among all authors. Author ECC designed the study, EAS performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ECC, EAS and ANV managed the literature searches and analyses of the study. Author ECC managed editing and prepared the final manuscript. All authors read and approved the final manuscript.

INTRODUCTION

Wine is an alcoholic beverage typically made of fermented fruit juice [1] Any fruit with good proportion of sugar may be used in producing wine and the resultant wine is normally named after the fruit [2].

In the European Union, wine is legally defined as the fermented juice of grapes [3] In the United Kingdom, wine is commonly called country wine. Wine can be made from virtually any plant matter which can be fermented [3]. It been noted that most fruits and berries have the potential to produce wine [4]. Few fruits other than grapes have the balanced quantities of sugar, acid, tannin, nutritive salts for yeast feeding and water to naturally produce a stable, drinkable wine. Recently, grapes are recommended as the main fruits that used for wine production. Despite that,

several studies have observed the suitability of other fruits as substrates for the purpose of wine production [5,6,7,8,4]. Grapes, which is usually the no 1 fruit of choice for wine production in the tropics has necessitated the search for alternative fruits source because of its non-availability both in Nigeria and other tropical countries [9].

In Nigeria, there are abundances of tropical fruit which includes; watermelon, pineapple, ugiri fruit etc. These fruits are susceptible to bacterial and fungal contamination, also highly perishable, as a result they fail to reach the market due to spoilage, not fresh and other mechanical damage [10]. Since, these fruit are difficult to keep for a very short period of time; hence the ripe fruits are utilized either as fresh or processed juice and other related products [11]. This wastage of these fruits especially at their peak of production during their season is very high, so it necessitates the need for alternative method to enhanced utilization of these fruit. The production of wines from available fruits could reduce the level of post harvest losses and also increase variety of wines [12,9,8].

Amerine and Kunkee, [13] observed that the type of wine to be produced dictates and strain of yeast to be involved and also the fruit to be used. Some of the preservatives used in wine making includes sulphur dioxide, potassium sorbate, sorbic acid and metabisulphites [14,2]. But high concentration of these preservatives in wine, causes off odors, can induce lots of systemic disorderliness such as breathing problems in asthmatic patients and gastrointestinal disturbances in allergic persons. The effects of bioaccumulation of these chemicals could further compound these situations [1].

Wine making involves the use of wine yeast to ferment the 'must' of fruits. This yeast which is the usually the main organism responsible for alcoholic fermentation belongs to the genus *Saccharomyces*. Although, many genera and species of yeast have been isolated from the musts, the main yeast strain that is commonly reported to be responsible for alcoholic fermentation is *Saccharomyces cerevisiae* [15,8].

The main objective of this study is to produce wine from apple fruit using yeast isolated from palm wine, to utilize of agricultural product from perishing during fermentation study of apple wine. *pH*, temperature, titratable acidity and reducing sugar tests were assayed quantitatively and recorded.

MATERIALS AND METHODS

Sample collection

Twenty (20) apple fruit was obtained from local Eke Awka Market in Anambra State, (South east), Nigeria. Identified as *Malus pumila*, at Botany Department Nnamdi Azikiwe University, Awka. Other materials used were Sucrose, Ethanol, Sodium metabisulphite, Dinitrosalicylic acid (DNS) and distilled water.

Palm wine was purchased from Umuawulu, Awka in Anambra State and stored for fermentation for 24 hours to isolate the yeast. The yeast was isolated from palm wine, sub-cultured, characterized and kept for use.

Inoculum Development

Development was done to obtain large quantities of yeast cell for pitching. To build up the inoculums, 200ml of apple juice each were put into a 250ml conical flask separately and autoclaved at 121°C for 15 minutes. The mixture was allowed to cool then three loopful of stock culture from Sabouraud Dextrose Agar (SDA) plate was transferred into the 200ml standard 'must' in a conical flask and kept in the refrigerator [16].

Preparation of "must" juice:

The apple fruit (*Malus pumila*) were plucked and rinsed with distilled water; they were peeled for easy blending. The grated edible portions were blended in the electric blender with constant addition of water respectively. The overall water added during the blending was 2000ml distilled water to avoid friction in the blender. 2000ml of distilled water was added extract the "must" by filtering the juice with sterile muslin cloth. 4000ml of the whole "must" was poured into the fermenting jar for fermentation and then, 0.28g of sodium metabisulphite were added, transferred into the fermenting vessel of 5 liters volume, and corked then allowed to stay for 24hours [17].

Fermentation

The 200ml of developed inoculum was poured into the fermenter jar containing the "must" making it a total of 4.2 liters. 358.9g of sucrose was then added to the fermenter to fortify the

must, and then the mouth of the jar plugged tightly with cotton wool and kept on the bench for fermentation [16].

Determination of physico-chemical Test

The pH, titratable acidity, and alcohol percentage were determined. These were carried out in accordance with standard methods reported by (A.O.A.C., 1990). While reducing sugar (brix) was determined using Miller [18].

Organoleptic evaluation

This was carried out in accordance with the procedure reported by Maragatham and Panneerselvam [19] The sensory evaluation was done using 8 judge panels after aging for 28 days. Observations recorded for color, clarity, body and taste on a 5 point scale with 5 points for excellent quality and 1 point for bad quality.

Statistical analyses

These were carried out using comparative analysis at 95% confidence level.

RESULTS

Table 1a: PROXIMATE ANALYSIS OF APPLE JUICE BEFORE FORTIFICATION

Parameters	Values
<i>pH</i>	4.01
Reducing sugar(⁰ Brix)	2.803
Specific gravity	1.013

TABLE 1b: PROXIMATE ANALYSIS OF APPLE JUICE AFTER FORTIFICATION

PARAMETER	% QUANTITY
pH	5.6
Specific Gravity	0.081
Reducing sugar (⁰ Brix)	14.0

TABLE 2: PHYSICAL AND ORANOLEPTIC PROPERTIES OF APPLE JUICE

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

TABLE 3: WINE ANALYSIS OF APPLE JUICE

Days of fermentation	Specific gravity (kg/m³)	pH	Alcohol content (%)	Titrateable acidity	Reducing sugar (g)	Temp
1	1.0801	5.0	0	0.50	10.803	32
2	1.0564	4.9	0.2	0.55	6.738	31
3	1.0355	4.6	0.4	0.63	1.722	31
4	1.0215	3.9	0.8	0.65	0.671	31
5	1.0179	3.6	1.0	0.70	0.644	30
6	0.9140	3.5	1.4	0.76	0.563	30
7	0.8134	3.3	1.8	0.82	0.513	30
14	0.4105	3.3	2.4	0.88	0.474	29
21	0.4041	3.2	2.8	0.96	0.421	27
28	0.4002	3.2	3.2	1.00	0.384	27
Standard Wine	0.9722	3.5	6.0	0.63	0.546	32

RESULTS AND DISCUSSIONS

In this research, the choice of the fruit, apples (*Malus pumila*) was as a result of its high yield of sugar. The proximate composition of the fruit used was in agreement with reports by [20,21,16,8] as the case for fruits in general. In order to supplement the sugar content of the musts and granulated sugar which is a source of sucrose was added during the production of the wine. [9,8] have noted that the one of the major problem associated with the use of tropical fruits in wine production is their low sugar content.

This study revealed that much acceptable wine with quality could be produced from apple with *Saccharomyces cerevisiae* isolated from palm wine. Other similar researcher [16,17] Fermentation was achieved, although the must was inoculated with appreciable number of yeast cells. This was done to enhance fermentation rate and product formation.

The produced wine was characterized by measurements of parameters such as organoleptic attributes, physico-chemical properties, and physical screening. The physical and taste changes in the wines with in the period of fermentation observed are presented in Table 2. It was observed that there were appreciable changes in the apple wine during the fermentation period. Sensory evaluation results showed there were no significant differences ($p > 0.05$) in flavor, taste, clarity and overall acceptability between apple wine and a reference wine. The type and aroma of wine being produced is reported to depend on duration and physico-chemical characteristics of the musts and the strain of yeast used [8].

It was observed in Table 3, there is a low pH values, pH in the wines were within the acidic range. The pH of the apple juice, was 4.01, fermentation must 5.6 then after fortification, the pH decreased from 5.0 to 3.2. The changes in the pH of the wines could be due to production of acids with period of fermentation which can be attributed to the microbial succession. Amerine and Kunkee 2005 also suggest that production of carbondioxide during fermentation which dissolved in the wine during fermentation was the result of decrease in pH. This result agrees with the reports of other related researchers [1,2,22,16,17,23]. Reddy and Reddy [24] noted a similar observation in their study on mango fruit wine production and Noah *et al.* [26]; Idise [2] and Umeh *et al.* [23] also noted that optimum pH value for quality wine production is within the range of 4.0.

Reddy and Reddy [24], reported 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. Both Low pH and high acidity are known for creating an advantage in natural environments for fermenting yeasts [8]. Trend in titratable acidity in the result show a steady increase with time throughout the fermentation period. At the 28th day of apple fermentation, the acid concentration increased from the initial range of 0.5% (on the 1st day) to 1.00% (on the 28th day). The titratable acidity of the final wine was 1.00. This was in agreement with the report of [12,16,17,23], who found that there was an steady increase in acid concentration. that titratable acidity on fruit wine production is expected to be between the ranges of 0.5 to 1.0%.

On last day of the fermentation the 28th, the concentration of alcohol content of the wine was observed to range from 0% to 3.2%. The changes in specific gravity and % alcohol (v/v) of the wines with period of fermentation observed, support that it results is due to the occurrence of microbe with varying tolerance for metabolic end products. These results agree with reports of [14,25,1,16].

Remarkable amount of alcohol was produced from the apple fruit with the yeast strain used during fermentation and was consistent during the course of fermentation. The percentage alcohol produced from the apples fruits generally, towards the end of the fermentation by the yeast strain were above 2% which is equivalent and comparable to moderate grape wines produce [15,7]. In the present research, the amount of alcohol produced by the isolate from the palm wine did not show any difference from wines produced from yeast from other sources apart from palm wine. This work is contrary to the work of [23] who found that the alcohol content at the end of the fermentation during the production of wine from roselle (*Hibiscus Sabdariffa*) and Ugiri (*Irvingiagabonensis*) using *Saccharomyces cerevisiae* was 5.7%.

Throughout the period of fermentation, the specific gravity of the apple wine gradually decreases in values from day 1- 28 of fermentation, specific gravity values of the apple wine were observed to decrease from 1.0801 to 0.4002 kg/m³ as shown in table 3. This was due to the yeast activity in the degradation of sugars producing alcohol during the wine production [13] The specific gravity value of the wine was observed to diminish by significant value at $p \leq 0.006$.

The specific gravity on the last day was 0.4002; this might be to the fact that there were unfermentable sugars that could not be degraded by the *Saccharomyces cerevisiae*. This was similar to the findings of [26,27,17].

There were observation in the temperature values of the wines, these changes is in agreement with reports of previous researches [14,13,1,2,16].

In the case of reducing sugar of the wine, the values of the reducing sugar were observed to be decreasing. It was high on the first day (10.803g) but started to diminish from the second day till the 28th day. This was due to the result of fermentation by the fermenting yeast and the production of alcohol. The reducing sugar level showed that must contained a high amount of sugar . This corroborated the findings of [16,17,23]..

The research revealed the effectiveness of other yeast strains apart from the commercial yeast in wine produced from tropical fruits (apple), Also, an appreciable quality wine can also be produced using apple fruit.

CONCLUSION

This successful production of wine from apple (*Malus pumila*) using yeast *Saccharomyces cerevisiae* which was isolated from palm wine was found to have a good quality, analytically acceptable to potential consumers. This shows that another profitable utilization of apple fruits apart from consumption could be undertaken. Apple wine production in small-scale level should be encouraged instead of importing wines from other countries or relying on the traditional grape. This will be economical and also generate employment as well as to provide value addition to the agricultural produce.

This work has also given an insight towards role of local yeast strains and its efficiency during primary alcohol fermentation of apple fruits.

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REFERENCES

1. Okafor N. The technology of passion fruit and Pawpaw wines. American Journal of Enology and Viticulture. 2007;17:27.
2. Idise OE. Studies of wine produced from pineapple (*Ananas comosus*). International Journal for Biotechnology and Molecular Biology Research. 2012;3(1):1-7.
<http://www.academicjournals.org/IJBMBR> DOI: 10.5897/IJBMBR11.034 ISSN 2141-2154 ©2012 Academic Journals
3. Harding GA Wine Miscellany" Clarkson Potter Publishing, New York. 2005; 5-9. ISBN 0-307-34635-8
4. Okafor UC, Edeh, JI, Umeh, SO. Table Wine Production From Mixed Fruits of Sourp (Annona Muricata) And Pineapple (Ananas Comosus) Using Yeast From Palm Wine. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 2018;(12):52-56.
5. Joshi VK, Bhutani VP. The influence of enzymatic clarification in fermentation behavior and qualities of apple wine. Science des Aliments. 1991;11:491-498.
6. Ndip RN, Akoachere JF, Dopgima LL. Ndip LM. Characterization of yeast strains for wine production: effect of fermentation variables on quality of wine produced Journal of Applied Biochemistry and Biochemistry and Biotechnology. 2001; 95(3):209-220.
7. Okunowo WO, Okotore RO, Osuntoki AA. The alcoholic fermentative efficiency of indigenous yeast strains of different origin on orange juice. African Journal of Biotechnology. 2005;4: 1290- 1296.
8. Chilaka, CA, Uchechukwu N, Obidiegwu JE, Akpor OB. Evaluation of the efficiency of yeast isolates from palm wine in diverse fruit wine production. African Journal of Food Science 2010; 4(12): 764 - 774, <http://www.academicjournals.org/ajfs> ISSN 1996-0794 ©2010 Academic Journals
9. Aloba, AP, Offonry SU. Characteristics of coloured wine produced from roselle (*Hibiscus sabdariffa*) calyx extract. Journal of Institute of Brewing. 2009; 115(2): 91-94.
10. Ihekoronye AI, Ngoddy PO. Integrated Food Science and Technology for The Tropics. Oxford Macmillian Education, Ltd., London; 1985:65-78
11. Oyeleke FI, Olaniyan AM. Extraction of juice from some tropical fruits using a small scale multi-fruit juice extractor. America Crop Science Processes, 2007; 8:1803- 1808.

12. Okoro CT. Production of red wine from roselle (*Hibiscus sabdariffa* and Pawpaw (*Carica papaya*) using palm-wine yeast (*Saccharomyces cerevisiae*). Niger Food Journal. 2007; **25**(2): 158-164.
13. Amerine MA, Kunkee RE. Microbiology of Wine making. Ann.Rev.Microbiology. Academic Press; London. 2005;223-258
14. Idise OE, Izuagbe YS. Microbial and chemical changes in bottled palm wine during storage. Niger. J. Microbiol., 1988; 8(1): 175-184.
15. Querol A, Fernandez-Espinar TM, Olmo ML, Barrio E. Adaptive evolution of wine yeast. International Journal of Food Microbiology, 2003;86:3-10.
16. Archibong EJ, Ezemba CC, Chukwujama IC, Archibong EJ. Production of wine from mixed fruits: Pineapple (*Ananascomosus*) and orange (*Citrus sinensis*) using yeast isolated from palmwine. World Journal of Pharmacy and Pharmaceutical sciences. 2015; 4(08):126-136.
17. Ezemba CC., Archibong E J. Comparative Studies of Wine Produced from Coconut (*Cocos Nucifera*) and Mango Fruit (*Mangiferaindica*) using Yeast Isolated from Palm Wine International Journal of Research in Pharmacy and Biosciences. 2017; 4(8):44-49 ISSN 2394-5885 (Print) & ISSN 2394-5893 (Online)
18. Miller GL. Use of dinitrosalylic acid reagent for detection of reducing sugars. Analytical chemistry. 1959;31:427-431
19. Maragatham C, Panneerselvam A. Isolation, identification and characterization of wine yeast from rotten papaya fruits for wine production. Adv. Appl. Sci. Res. 2011; 2(2): 93-98.
20. Pearson D. The Chemical Analysis of Foods. Churchill LivingStone; Edinburgh. 1976;353-355.
21. Okaka JC. Tropical Plant Perishable Processing, Storage and Handling, Silicon Valley, New Heaven, Enugu. 1997

22. Agbor AA, Ben UM, Ubana EM, Olayinka JC, Okon AE. Production, characterization and safety of wine obtained from a blend oftomato, almond, orange, lemon and African star apple extract. Scholars Research Library. Annals of Biological Research. 2011;2(5):492-503.
23. Umeh SO, Okafor U C, Awah N S, Obasi C J, Asogwa B. Preliminary Investigation into the Use of Rosselle (*Hibiscus sabdariffa*) and Ugiri (*Irvingia gabonesis*) Fruits in Wine Production International Journal of Life Science and Engineering. 2018; 3(3):64-68 <http://www.aiscience.org/journal/ijlse> ISSN: 2381-697X (Print); ISSN: 2381-6988 (Online)
24. Reddy LVA, Reddy OVS Production and characterization of wine from mango fruit (*Mangifera indica* L). World J. Microbiol. Biotechnol. 2005; 21: 1345-135025.
25. Robinson J. The Oxford companion to wine (3rd Ed), Oxford University Press; USA. 2006; 84.
26. Noah AA, Alechenu JO, Abiaziem CV, Oduwobi OO. (Comparative Studies of Wine Produced From Pawpaw Juice and Coconut Milk at Different Proportions. Current Research on Nutrition Food Science. 2013; 1(2).
27. Idise OE, Ofiyai O. Studies on Wine Production from Pawpaw (*Carica papaya*). Journal of Brewing Distilling. 2011; 2 (4)56-62. ISSN 2141-21972 @Academic journals.