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Production of red wine from *Ananas comosus, Musa acuminata* and *Mangifera indica* using *Sacchromyces cerevisiae* isolated from plam wine

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Abstract
Mixed fruit wine (pineapple, banana, mango) was using *Saccharomyces cerevisiae* was isolated from wine. Exactly 609 and 406g each of the fruit in two mixed and three mixed fruit fermentation respectively were crushed using laboratory blender, mixed with distilled water (1:1 w/v) and heated for 30 minutes with subsequently addition of sugar (0.656 kg). The fruit must were subjected to primary (aerobic) and secondary (anaerobic) fermentation for 4 and 20 days respectively. During fermentation aliquots were removed from the fermentation tank for analysis. During primary fermentation, consistent increases in alcohol contents (ranging from 0.0 to 15.0%) and total acidities (ranging from 0.20 to 0.80%) were observed with gradual decrease in specific gravities (ranging from 1.060 to 0.9800) and pH (ranging from 0.80 to 2.90). Temperature ranged from 27ºC to 29ºC. The alcoholic content of the final wines were17.50±0.02% (pineapple and banana) 16.00±0.02% (pineapple and mango) 18.50±0.02% (banana and mango wine). The alcoholic content of the wines did not different significantly (p> 0.05). The pH of all the wines were acidic and ranged from 2.5±0.01 to 3.8±0.01 (p>0.05). The acid concentration (residual and volatile acidity) were within the acceptable limit and ranged from 0.35±0.02 to 0.88±0.01% (p>0.05). Sensory evaluation (p>0.05) rated the wines acceptable as pineapple and banana wine, pineapple and mango wine, pineapple and banana and mango wine, banana and mango wine. This study has shown that acceptable mixed fruit wine could be produced from the fruit with *Saccharomyces cerevisiae* from wine.

Keywords: wine, pineapple, banana, mango, yeast, fermentation

Introduction

Wine is an alcoholic drink typically made from fermented grapes. Yeast consumes the sugar in the grapes and converts it to ethanol, carbon dioxide, and heat. Different varieties of grapes and strains of yeasts produce different styles of wine. These variations result from the complex interactions between the biochemical development of the grape, the reactions involved in fermentation, the grape's growing environment (Terroir), and the production process. Many countries enact legal appellations intended to define styles and qualities of wine. These typically restrict the geographical origin and permitted varieties of grapes, as well as other aspects of wine production. Wines not made from grapes involve fermentation of additional crops including, rice, wine and other fruit wines such as plum, cherry, pomegranate, currant and elderberry.

Wine, an alcoholic beverage is prepared by different fruit juices with appropriate processing and additions (Amerine and Singleton, 2000) [2]. The conventional process of wine making involves the fermentation of grape juice. However, there are numerous reports available on wine preparation from other fruits such as apple, plum, apricot, pomegranate, strawberry, kinnow, guava, jamun, sapota, litchi, etc (Awe, 2004; Ayogu, 2003; Ay00la 2002; Bamett, 2000) [10, 11, 12, 13]

Wine is an alcoholic beverage typically made of fermented fruit juice (Okafor, 2007) [3]. Any fruit with good proportion of sugar may be used in producing wine and the resultant wine is normally named afterthe fruit. The type of wine to be produced dictates the fruit and strain of yeast to be involved (Amerine and Kunkee, 2005) [1]. Preservatives used in wine making include sulphur- dioxide, potassium sorbate, sorbic acid and metabisulphides (Chilaka, 2010) [15]. High concentration of these preservatives in wine, aside causing 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 (Bechem, 2000) [14]. Fermentation is a process of extracting energy from the oxidation of organic compounds such as carbohydrates using an endogenous electron acceptor, usually pyruvate, an organic compound. Before fermentation takes place, one glucose molecule is broken down into two pyruvate molecules during glycolysis. Fermentation is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of Adenosine tri-phosphate (ATP) by glycolysis. During alcoholic fermentation, usually carried out by yeasts, pyruvate is then converted into ethanol and carbon dioxide thus:

$$C6H12O6 \rightarrow 2C2H5OH + 2CO2.$$

During this process, the carboxylic carbon atom is released in the form of carbon-dioxide with the remaining components becoming acetaldehyde. The acetaldehyde in the absence of oxygen will then be further reduced by alcohol dehydrogenase to form ethanol along with carbon-dioxide (Reddy, 2005) [9]. This research was aimed at producing wine from pineapple and banana and mango for immediate consumption.

Materials and Methods

Collection of fruit

Saccharomyces cerevisiae was cultured in sterilized glucose yeast extract broth (glucose 1%, yeast extract 0.3%, malt extract 0.3%, peptone 0.5% and pH 4.5) for 24 hours at 30℃ on a rotary shaker at 60 rpm. The cells were then separated by centrifugation at 6000 rpm at 4℃ for 10 min. The cells were washed twice and resuspended in normal saline to obtain a concentration of 108 cells/ml and this was used as the pre-inoculum. The inoculum was prepared by transferring 10 ml of pre-inoculum in 250 ml Erlenmeyer flask containing 100 ml mixture of *Musa acuminata* and *Ananas comosus* and *Mangifera indica* fruit juice. The mixture was then incubated overnight at 30℃ in shaking incubator at 60rpm.

Isolation of *S. cerevisiae* from palm wine (Amova-Awua, 2003)

Culturing of the fresh wine was done on PotatoDextrose Agar (PDA) and incubated at room temperature for 24 h. Isolates were obtained and subcultured on fresh medium to obtain pure cultures. The yeast cultures were transferred to modified Malt Extract Agar (MEA) containing yeast extract and 2 % glucose and then incubated for 24 h. organisms were identified *S. cerevisiae* based on their cultural characteristics, microscopy and their pattern of fermentation and assimilation of glucose, sucrose, raffinose, galactose, maltose, dextrose, trehalose and meliobiose as described by Amoa-Awua *et al*. (2003) [7]. The different isolates of *S. cerevisiae* were further screened for their ability to tolerate different concentrations of sugar and alcohol by inoculating on MEA supplemented with 10-60, and 5-30 %, sucrose and ethanol respectively. The isolate with the highest sugar and alcohol tolerance was selected and used as the starter culture. The identified organism was maintained on MEA slant.

Multiplication of starter culture

The isolated organism was multiplied prior to fermentation by culturing them on Malt Extract Broth (MEB) and incubating for 48-72 h at 27.0 °C ± 0.02. The broth cultures of the organism were centrifuged at 500 rpm for 5 min. The sediments were collected and used for must fermentation.

Preparation of must for mixed fruit fermentation

The must was prepared for two mixed fruit and three mixed fruit fermentation respectively. The fruits were washed thoroughly with distilled water and then peeled. Exactly 609 and 406 g each of the fruit samples, banana, pineapple and mango were weighed for two-mixed fruit and three-mixed fruit fermentations respectively. This was then chopped into smaller pieces using a clean knife before transferring them quantitatively into laboratory blender for crushing. The crushed sample was transferred into a clean new transparent bucket and mixed with distilled water (1:1 w/v). Exactly 0.656 kg of sugar was added to the must followed by vigorous stirring. Exactly 4 g of sodium metabisulphate (Na2S2O5) was dissolved in 400 ml of water and poured in 100 ml aliquots to each of the mixtures and stirred properly. Sodium metabisulphate serve as a sterilizer and prevents fermentation before the addition of the yeast starter. The sugar concentrations were measured and the musts were mixed in the combination of 'pineapple and mango' (30.4 °Brix), 'pineapple and banana' (29.3 °Brix), 'pineapple, banana and mango' (32.1 °Brix) and then 'banana and mango' (31.2 °Brix).

Fermentation (Joshi, 2012)

The primary fermentation was initiated by the addition of the starter culture. The must was stirred every 12 h with subsequent reading of the specific gravity, pH, temperature and alcohol content for 4 days. After 4 days, the wine was racked into the secondary fermenter. The secondary fermentation was done in an air tight container in which a tube was passed into a clean bottle containing clean water. The essence was to monitor the course of fermentation. This was allowed until completion of fermentation was evidenced by lack of the appearance of bubbles in the container within 3 weeks. Secondary fermentation was done for 20 days. When fermentation stopped, the wine was promptly racked off the lees ensuring mini- mum exposure to oxygen. After secondary fermentation, the wines were clarified. The clarification/fining were done using bentonite Isclarifying agent. Exactly 500 g of bentonite was dissolved in two litres of boiling water and stirred properly to a gel. This was allowed to stand for 24 h. Then 150 g of the gel-like bentonite was transferred into each of the wine followed by stirring to dissolve properly.

A small quantity of the mixture was collected in a clean bottle which was covered tightly and was used to monitor the process of clarification. This was done for a period of 3 months. Filtration was done after the wines had completed clarification using muslin cloth, sieve and syphon tubes sterilized by 70 % alcohol. The wines was syphoned into the sieve containing four layers of muslin cloth. The residues were removed and the filtrates were allowed to mature for a period of 5 months before other chemical analysis was carried out.

Isolation of microorganisms from the fermentation broth

Microbial analysis of the fermentation broth was performed as described by using Nutrient Agar (NA), MacConkey Agar (MA) and Potato Dextrose Agar (PDA). The nutrient agar used was treated with fulcin (50 mg/20 ml of NA) to suppress fungal growth while the PDA was treated with chloramphenicol. The cultured plates were incubated at room temperature and pure cultures were obtained by streaking and identified based on colonial characteristics, microscopy, biochemical reactions and carbohydrate utilization.

The fungi were identified only on the basis of their cultural characteristics and microscopy.

Chemical analysis of the wines (McClements, 2003)

The volatile acidity was determined using the method described by McClements (2003) [6], total acidity of the wines was determined by titration and concentration of the acid was calculated. The residual acidity of the wines was also determined as described the alcohol content was determined using the density method. The specific gravities of the wines were determined using the hydrometer method and the results were determined from the reading on the stem.

The total solid and total sugar content of the wines were determined the pH and temperature were determined using a digital pH metre and an analytical thermometer respectively.

Sensory evaluation (Nissen, 2000)
The wines produced were compared for colour, flavour, taste, clarity, and overall acceptability by seven point hedonic scale where seven denote.

Statistical analysis (Winner, 2004)
The completely randomized analysis of variance (ANOVA) was used as described by (Winner 2004) [8] to analyze the data obtained. Mean separation and comparison was done using SPSS version 16.0. Significance was accepted at $P < 0.04$ and results were expressed as mean ± standard deviation from the mean.

Results
There were fluctuations in the temperature of the mixed fruit wines throughout the period of fermentation. These variations were observed for all the wines. In all the mixed fruit wines, the temperatures were observed to range from 27.0 °C ± 0.02 to 29.0 °C ± 0.02. The pH in the mixed fruit wines was acidic throughout the period of fermentation. This was also irrespective of the fruit wine. The pH ranged from 4.0± 0.01 to 4.8 ± 0.01 in pineapple and mango wine, 2.9± 0.01 to 3.8 ± 0.01 in pineapple and banana wine, 3.4 ± 0.01 to 4.0 ± 0.01 in pineapple, banana and mango wine and 3.6 ± 0.01 to 4.3 ± 0.01 in banana and mango wine.
A steady increase in alcohol content was observed in the mixed fruit wines throughout the period of primary fermentation. This increase was observed in all the mixed fruit wines irrespective of the fruits used. The concentration of alcohol in the mixed fruit wines at the end of primary fermentation were observed to range from 0 to 15, 0 to 14, 0 to 15.5 and 0 to 15 % in pineapple and mango wine, pineapple and banana wine, pineapple, banana and mango wine and banana and mango wine respectively. The highest alcohol content was observed in the wine produced by the mixture of pineapple, banana and mango (15.5 %), while the least alcohol content was observed in pineapple and banana wine (14 %). The specific gravities of the mixed fruit wines gradually decreased throughout the period of primary fermentation. After primary fermentation, specific gravity values were observed to range from 0.9800 to1.0600, from 0.9820 to 1.0300, from 0.9800 to 1.030 and from 0.9810 to 1.0900 in pineapple and mango wine, pineapple and banana wine, the three mixed fruit wines and banana and mango wine respectively. showed the trend in total acid concentrations in the mixed fruit wines during the primary fermentation period with the test yeast. Total acidity was observed to show steady increase with time throughout the period of primary fermentation. These increases were irrespective of the test fruit wine. At the end of primary fermentation, acid concentration in the pineapple and mango wine was observed to increase from initial concentration of 0.20 ± 0.01 to final concentration of 0.32 ± 0.02 %. Similarly, total acidity was observed to increase from initial concentration of 0.40 ± 0.02 to a final concentration of 0.80 ± 0.02 %, 0.41 ± 0.01 to 0.71 ± 0.01 % and 0.29 ± 0.02 to 0.62 ± % for pineapple and banana wine, the three mixed fruit wine and banana and mango wine respectively.
After secondary fermentation, the temperature of the wines were observed to range from 27 ± 0.07 °C for pawpaw and banana wine to 28 ± 0.07 °C for pineapple, banana and mango wine and banana and mango wine. The pH of the wines maintained an acidic range of 2.7 ± 0.1 for pineapple and banana wine to 3.9 ± 0.1 for pineapple and mango wine. There were little increases in the alcoholic content of the mixed fruit wines after secondary fermentation. The alcohol content to pineapple and watermelon wine increased from 15±0.02% in primary fermentation to 16.5 ± 0.02 % after secondary fermentation, 14 ± 0.02 to 15.20 ± 0.02 %, 15.5 ± 0.02 to 18.50 ± 0.02 % and15 ± 0.02 to 18 ± 0.02 % for pineapple and banana wine, pineapple, banana and mango wine and banana and mango wine respectively. The highest alcohol content was observed in banana and mango wine (19.00 ± 0.02 %) while pineapple and banana wine recorded the lowest alcohol content (15.20 ± 0.02 %). In the case of specific gravities, little decreases were also observed in all the wines after secondary fermentation with banana pineapple wine having the lowest value (0.9870 ± 0.00) and, pineapple and banana wine having the highest value (0.9800 ± 0.00) while the acid concentrations ranged from 0.34 ± 0.02 (pineapple and mango wine) to 0.86 ± % (pineapple and banana wine)(Table 1).
The general chemical parameters of the mixed fruit wines after maturation compared favourably. The result indicated that the final alcohol concentration of pineapple and m a n g o wine, pineapple and banana wine, pineapple, banana and mango wine, banana and mango wine, were 16.50 ± 0.02, 15.00 ± 0.02, 16.00 ± 0.02 and 18.50 ± 0.02 % respectively (Table 2). These variations do not show any significant difference ($p > 0.04$).
Sensory evaluation ($p > 0.04$) rated the acceptability of the wines as pineapple and banana wine > pineapple an watermelon > pineapple, mango and banana > banana and mango wine (Table 3).

Discussion
The fermentation of wine is known to be complex with various ecological and biochemical processes in volving yeast strains. The fermentation for the elaboration of beverage is known to depend on the performance of the yeast to convert the sugars into alcohol and esters. Besides, the different species of yeast that develop during fermentation determine the characteristics flavour and aroma of the final product. Also, because different fruits have different composition, there is the need for yeast strains to adapt to different environments, such as sugar composition and concentration of acetic acid.
The mixed fruit wines (pineapple and mango wine, pineapple and banana wine, pineapple, banana and mango wine and banana and banana wine) produced in the present investigation revealed low pH values (in the range of 2.5–3.8) throughout the fermentation periods and in the final product. Similar observations have been reported for other tropical fruit wines such as wine (sweet potato wine, sapota fruit wine and banana wine. Studies have shown that during fermentation of fruit, low pH is inhibitory to spoilage organisms but increases conductive environment for the growth of desirable organisms. Also, low pH is known to give fermenting yeasts a competitive advantage in natural environment. The decrease in pH could be due to accumulation of organic acids during fermentation and this reduces the influence of bacteria that can lead to spoilage. Therefore the wines have a good keeping quality.
Fluctuations in temperature of the must were observed during the period of fermentation. This could be as a result of biochemical changes occurring during the metabolism of the substrates by the fermenting organism. Temperature of the final mixed fruit wines ranged from 27.00 ± 0.07 to 28 ±

0.07. The present study also revealed a consistent increase in the total acidity of the mixed fruit wines throughout the period of fermentation. The total acidity of final wine is expected to be between 0.5 and 1.0 %. In this study, the result of the total acidity in the mixed fruit wines fell within this limit ranging from 0.35 ± 0.02 to 0.88 ±0.01 %. However, the acidity is lower than the reports for sweet potato wine (1.34 g/100 ml) and sapota fruit wine (1.29 g/100 ml) but is consistent with the reported 0.15 ± 0.07 g/100 ml In order to supplement the sugar content of the musts, sucrose was part of the additives. Reports have shown that the major problem associated with the use of tropical fruits in wine production is their low sugar contents. In the present study, the fermentation was nearly complete with total sugar content of 0.76 ± 0.02, 0.94 ± 0.02, 0.64 ± 0.02, and 0.54 ± 0.02 % in 'pineapple and mango wine', pineapple and banana wine', 'pineapple, banana and mango wine' and banana and banana wine respectively. This observation did not correspond with the reports of, Ray *et al*. (2011)who reported higher values for sapota fruit wine (3.28 g/100 ml), purple sweet potato wine (1.35 g/100 ml), tendu wine (3.78 g/100 ml) and bael wine (2.05 ± 0.12 g/100 ml) respectively. The result revealed that the total sugar contents of the wines in the present study are less than 1 %. This is an indication that the wines will have a good keeping quality since the fear of further fermentation during storage which could lead to spoilage will not arise. This result also showed that the wines could be classified as dry table wines because of low total sugar content of less than 1 %. The variations in the total sugar content of the wines were not observed to differ significantly ($p > 0.05$).

In the present investigation, the test fermentation yeast (*S. cerevisiae*) was the only organism isolated from pineapple and mango wine as well as pineapple and banana wine while neither pineapple, banana and mango wine not banana and mango wine showed the presence of any microorganism. This is an indication of good quality. This observation may be attributed to low pH values, high acidity and high alcohol contents of the wines which are known to inhibit the growth of pathogens and gives fermenting yeast a competitive advantage in natural environment as reported by Reddy and Reddy (2005) [9]. The absence of the growth of the yeast in pineapple, banana and mango wine and banana and mango wine could be due to the high alcoholic content which exceeded the ethanolic tolerance level of the yeast used for fermentation.

Sensory evaluation rated the wines acceptability as pineapple and banana wine > pineapple and mango > pineapple, mango and banana > banana and mango wine. These attributes compared favourably with the reports for other tropical wines (Ray *et al*., 2011) [5]. Also, the sensory evaluation of the wines in the present study do not differ significantly ($P > 0.05$).

Table 1: Temperature, pH, specific gravity, Alcohol content and total acidity of the wines after secondary fermentation

Wines	Temperature	pH	Specific gravity	Alcohol (%)	Total acidity
A	28.00±0.6	3.80±0.1	0.9890±0.00	14.40±0.3	0.33±0.01
B	20.00±0.6	2.0±0.1	0.9700±0.00	15.20±0.3	0.86±0.02
C	22.00±0.6	3.70±0.1	0.9800±0.00	18.50±0.3	0.73±0.01
D	27.00±0.6	3.20±0.1	0.9770±0.00	19.00±0.3	0.65±0.01

Values were expressed as mean ± standard deviation
A. Pineapple and watermelon wine,
B. Pineapple and banana wine,
C. Pineapple, banana and watermelon wine,
D. Banana and watermelon

Table 2: Chemical parameters of the final wines

Chemical parameters	wines				P value
	A	B	C	D	
Alcohol content (%)	16.50±0.02	15.00±0.02	16.00±0.02	18.50±0.02	>0.04
Total acidity (%)	1.35±0.02	0.99±0.002	0.88±0.02	0.66±0.01	>0.04
Residual acidity (%)	0.13±0.02	0.40±0.02	0.38±0.02	0.23±0.02	>0.04
Volatile acidity (%)	0.024±0.00	0.78±0.02	0.58±0.02	0.45±0.02	>0.04
Specific gravity (kg/l)	0.9785±0.00	0.8800±0.00	0.9880±0.00	0.8740±0.00	>0.04
Density (kg/l)	0.9950±0.00	0.9850±0.00	0.9800±0.00	0.9980±0.00	>0.04
Total solids (%)	0.18±0.02	0.45±0.02	0.47±0.02	0.39±0.02	>0.04
Total sugars (%)	0.78±0.02	0.84±0.02	0.65±0.02	0.64±0.02	>0.04

Values were expressed as mean ± standard deviation;
Significant different are taken at $P < 0.05$
A. Pineapple and watermelon wine,
B. Pineapple and banana wine,
C. Pineapple, banana and watermelon wine,
D. Banana and watermelon wine

Table 3: Sensory evaluvation of wines

Parameters	wines				P value
	A	B	C	D	
Taste	5.4	5.8	5.1	4.7	>0.04
Clarity	5.7	5.7	5.5	4.6	>0.04
Colour	5.5	6.0	5.7	4.9	>0.04
Flavor	5.7	5.6	5.3	4.8	>0.04
Over all acceptability	5.6	5.7	5.6	5.1	>0.04

Conclusion

The present study which was based on the evaluation of three indigenous fruits as substrates for wine production and the efficiency of isolated *S. cerevisiae* from palm wine for mixed fruit wine production has revealed that the three test fruits (Pineapple, banana and watermelon) are good substrates for wine production. The biochemical and sensory attributes of the wines were acceptable by the consumers. The study has also given an insight into the efficacy and role of *S. cerevisiae* from palm wine during alcoholic fermentation of fruits. Pineapple, banana and watermelon have short shelf life under the prevailing temperature and humidity condition in Nigeria. Therefore, this study provides an avenue to preserve their nutrients,

minerals, vitamins, aroma and taste to the consumers by fermenting them into wines.

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