International Journal of Biotechnology and Food Science
Vol. 3(2), pp. 17-22, March 2015
ISSN: 2384-7344
Research Paper

Pawpaw (*Carica papaya*) wine: With low sugar produced using *Saccharomyces cerevisiae* isolated from a local drink '*burukutu*'

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Accepted 15th February 2015

Abstract. The juice from well-ripped pawpaw fruit *(Carica papaya)* was used to produce wine by fermentation using a yeast strain *Saccharomyces cerevisiae* isolated from a fermented local beverage drink '*Burukutu'*. The 'must' and wine were analyzed daily during fermentation and after ageing respectively: determining pH, specific gravity, titratable acidity and reducing sugar content. Initially, the pH was 6.8, and after fermentation it decreased to 5.5, specific gravity decreased from 20.6 to 8.0%, reducing sugar concentration decreased from 16.7 to 1.10 g, while titratable acidity increased from 0.33 to 1.25% at the end of secondary fermentation. The alcoholic content of the wine was 10.12% after fermentation and increased to 10.14% after aging while temperature during fermentation rose from 28.3 to 29.4°C. The wine presented a brilliant yellow color with a slight sweet flavor. Sensory evaluation showed that pawpaw wine had slight potential when compared to other commercial samples ($p < 0{\cdot}05$). These results showed that pawpaw can be successfully used in the production of low sugar table wine. It is therefore recommended that wine producers should use pawpaw in wine production. This will reduce the amount of wastages that occurred in Pawpaw fruits every year and also serve as a method of pawpaw fruit preservation.

Keywords: Table wine, must, pawpaw, '*burukutu'*, *Saccharomyces cerevisiae*, low sugar drink.

INTRODUCTION

Wine is a complex mixture, consisting of both organic and inorganic compounds (Odibo et al., 2002; Amerine et al., 2012), including esters, high alcohols, fixed acidity (malic, tartaric and citric acid), sugars, aldehydes, tannins, pectins, vitamins and minerals. It can be defined as an alcoholic beverage made from grape juice or other fruits through fermentation of 'must' by wine yeasts (Archer and Castor, 2006). Most wines have a total acidity content ranging from 0.3 to 0.55% (as tartaric acid and acetic acid). The European Economic Community recommends that the alcoholic content for table wines should range from 8.5 to 19.5% (Austin, 2008; Amerine and Ough, 1980).

Wine can be classified as table wine, sparkling wine, fruit wine, fortified wine, dry wine or sweet wine. They may also be classified on the basis of the countries of origin or fruit type from which they were obtained. For example red table wines are made from black grapes while white wines are made from black or white grapes. It is now known that it can be produced from other fruits such as oranges, bananas, mangos, pineapples, lemons, etc. and the wine so produced bears the name of the fruit used in its production (Robinson, 2006; Amerine et al., 2012).

Most wines consumed in Nigeria are completely fermented, aged, bottled and imported ones. The temperature restriction of most grapes to temperate regions predisposes this trend (Okoro, 2007). Imported products are costly now due to high duties paid on them. This had made imported wines too expensive to local consumers and for these reasons, there arose the need for more wine from other plants species.

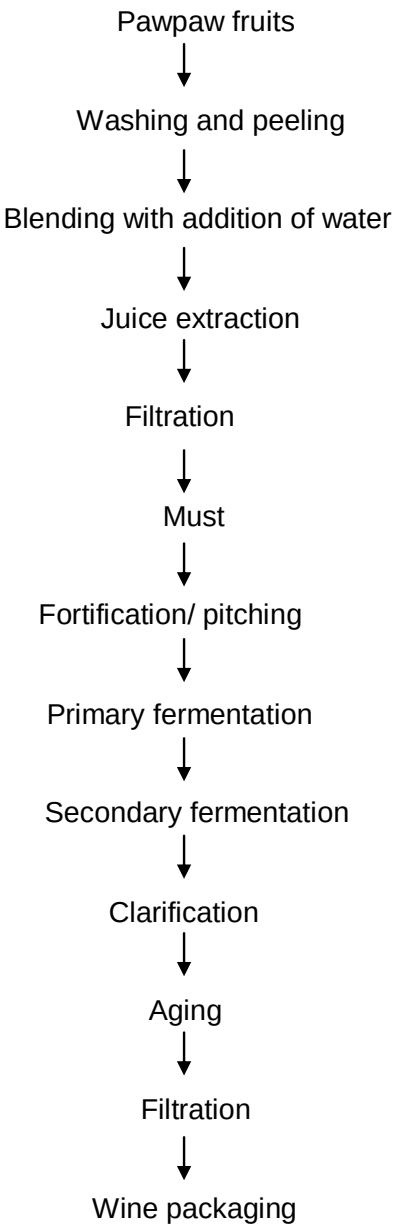

Figure 1. The flow chart for pawpaw wine production.

Pawpaw, *Carica papaya*, is a fast growing but short-lived herbaceous plant that grows widely in the tropics. It bears clusters of fruits round its stem very close to the leaves when mature. It can bear fruit throughout the year and the fruits mature and ripe within three months. The fruits are yellow or red when ripe and spoil quickly if not utilized after about five days. Though the fruits can be eaten raw, there is virtually no method of preservation of ripe pawpaw fruit pulp. Hence, to overcome this hurdle there is great need that pawpaw fruit pulp be used in the production of several value added products like wine, jam, etc to avoid fruit wastage after harvest.

Generally, all wines are produced using wine yeast, *Saccharomyces cerevisiae* var *ellipsoids* (Okoro, 2007; Amerine et al., 2012) which is most times imported and is found expensive. Some authors had proved that the yeast can be obtained from palm wine which has potentials of imported ones and also has alcoholic tolerance (Obisanya et al., 1987; Somari et al., 1993). Palm wine is not easily available due to high risk involved in climbing and tapping the wine. Therefore an alternative source of *S. cerevisiae* was sought from a fermented local beverage drink, '*burukutu'*.

'*Burukutu*' is a local non-alcoholic beverage drink produced from sorghum (*Sorghum vulgare*). It was mostly produced by the Hausa tribe of Nigeria before now but recently, almost everybody in Nigeria including foreigners can produce and consume it. It is very cheap to get and always available. The aim of this study therefore is to use local cheap raw materials to produce wine and also find an alternative way of preserving pawpaw.

MATERIALS AND METHODS

Raw material procurement

Ten ripe pawpaw fruits were bought from a native market and transported to the laboratory in a clean sterile polythene bag. Sample of '*burukutu'* was purchased with sterile bottles and taken to the laboratory immediately and kept for refrigeration. Culture media (Agar) and other chemical additives used were obtained from the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka. Figure 1 is a Schematic diagram used for the production of the wine.

Experimental procedure

Isolation and identification of yeast from burukutu

The method of Fagbemi and Ijah (2005) was used to isolate and identify the yeast strain from '*burukutu'*. Yeast strains were isolated by plating serially diluted samples of the '*burukutu'* on Potato dextrose agar (PDA) plates. The plates were incubated at room temperature (28 ± 2°C) for 48 h. Different isolated colonies were replicated on fresh plates to get pure cultures of the isolate. The isolated yeast cells were characterized using colonial morphology, cellular characteristics, ascospore formation, vegetative reproduction and sugar utilization. The organisms were further identified by comparing them with known taxa using the method of Barnett et al. (2000). The choice isolate was stored in a slant culture and preserved in a refrigerator maintained at 4°C.

Processing of must

The method of Okoro (2007) was used in preparation of

'must' during the study. Pulp of pawpaw fruits was collected in a clean sterile basin after washing, peeling and removing the seeds and pulverized using a sterile Monilex electric blender with the addition of water. The slurry was further diluted in ratio of 1:1 (water and pulp) and sieved with a muslin cloth of pore size 0.8 mm to obtain the filtrate, 'must'. About 3.5 L of 'must' was poured into a glass vessel and 1.3 g of sodium bisulphate was introduced into it and allowed to stand for 24 h. They were sterilized according to the methods of Amerine and Kunkee (2002) as used by Robinson (2006). About 4.8 g of sucrose was dissolved in 300 ml of the must. The sugar solution was made by heating the mixture over a Bunsen flame and stirred until complete dissolution. It was allowed to cool at room temperature and poured into whole 'must' in the glass fermenter. The 'must' was enriched with 29.4 g (0·84%) of ammonium sulphate and 4.2 g (0.12%) of potassium dihydrogen phosphate to enhance rapid growth of fermenting yeast.

Reconstitution of the yeast

The method of Amerine et al. (2012) was used. Sabouraud dextrose agar plate was prepared according to manufacturer's instructions with the addition of an antibiotic, chloromphenicol, to eliminate bacterial growth and allowed to set. The stock yeast (*S. cerevisiae*) was reactivated by inoculating with sterile wire loop into the solidified medium using streaking and incubated for 48 hat 37°C. The yeast biomass was produced by inoculating colonies from the pure culture into a solid media by streaking to cover the face of culture plate. This was incubated at room temperature for 96 h. 400 ml of sterile distilled water was used to wash out yeast from plates and inoculated into 'must'. The fermenter was plugged with sterile cotton wool and the set up was allowed for fermentation at room temperature.

Primary fermentation

The method of Okoro (2007) was used for primary as well as secondary fermentations. In primary fermentation, 5 L of standardized must was pitched with 400 ml of reconstituted yeast to give a pitching rate of 8% (v/v). Primary fermentation of the 'must' was carried out in a ten liters glass jar which lasted for 168 h with the evolution of carbon oxide. This was carried out at room temperature after which the 'must' was racked for secondary fermentation. During primary fermentation, pH, specific gravity and reducing sugar were monitored every 24 h using appropriate methods (Amerine et al., 2012).

Secondary fermentation

After primary fermentation, the wine was siphoned; 800 g of sucrose was dissolved and added to remaining 'must' for secondary fermentation. This lasted for another 168 h after which the wine was siphoned. Bentonite solution was prepared and used for clarification and the wine was aged at 5 ± 2°C for 30 days. The aged wine was filtered through a cheese cloth of 0.002 mm pore size and bottled.

Analysis of the wine chemistry

Chemical parameters such as alcoholic content, pH, titratable acidity, specific gravity and reducing sugar were determined (AOAC, 2000).

Analysis for vitamins and mineral elements

Atomic Absorption Spectrophotometer (AAS) method of APHA 1995 (American Public Health Association) as used by Umeh et al. (2013) was used to determine the mineral elements. The sample was thoroughly mixed and 100ml of it was transferred into a 250 ml glass beaker. Five milliliters of conc. nitric acid was added and heated to boil till the volume reduced to about 15 to 20 ml. Concentrated nitric acid was added in increments of 5 ml till all the residue dissolved completely. The mixture was cooled and made up to 100 ml using metal free distilled water. The sample was aspirated into the oxidizing air-acetylene flame of the AAS. When the aqueous sample is aspirated, the sensitivity was read from the detector.

The method of Plummer (1979) as used by Umeh et al. (2013) was used to check the vitamin contents of the wine.

Sensory evaluation

Ten man panelists who were conversant with the test of table wine were chosen to evaluate the sensory features (color, taste, flavor and general acceptability). Questionnaires were given to them to rate the product as excellent - 5, very good - 4, good - 3, bad - 2 and very bad - 1. The scores were analyzed statistically using the Kruskal – Wallis test.

RESULTS

Pawpaw wine fermentation lasted for 336 h, 168 h for primary fermentation and 168 h for secondary fermentation. Aging period lasted 30 days at a temperature of 5 ± 2°C. The results obtained from the analysis were as shown in the following tables. The pH decreased from 6.8 to 5.5, titratable acidity increased from 0.33 to 1.25%, specific gravity and reducing sugar decreased from 20.6 to 8.0% and 16.7 to 1.10%

Table 1. Changes in daily *pH*, titratable acidity, specific gravity, alcoholic content and reducing sugar of the 'must' during fermentation and after ageing of the wine.

Days	pH	Titratable acidity (%w/w)	Specific gravity (%w/w)	Alcohol (%v/v)	Reducing sugar (%)
0	6.80	0.33	20.60	0.58	16·70
1	6.78	0.35	20.50	0.97	16·60
2	6.72	0.40	18.47	1.46	14·50
3	6.71	0.48	15.42	1.98	14·80
4	6.69	0.56	14.40	2.56	13·80
5	6.69	0.66	13.37	2.99	8.70
6	6.66	0.75	12.33	3.05	6.10
7	6.66	0.86	11.30	4.44	5.63
8	6.64	0.95	10.28	4.98	16.14
9	6.63	0.98	9.84	5.51	15.66
10	6.61	1.00	9.77	6.75	14.47
11	5.99	1.04	8.84	7.66	12.55
12	5.97	1.07	8.43	8.46	8.68
13	5.65	1.08	8.20	9.77	5.48
14	5.50	1.25	8.00	10.12	1.10
Post aging	5.44	1.24	7.89	10.14	1.10

Table 2. Comparison of the characteristics of wine with an imported wine.

Parameter	Product wine (after ageing)	Imported brand
pH	5.44	3.54
Specific gravity (%)	7.89	6.65
Titratable acidity (%)	1.22	0.99
Alcohol content (%)	10.14	7.88
Colour (visual)	Bright yellow	Deep yellow

respectively (Table 1). The alcoholic content of the produced was 10.12% at the temperature of 29.4°C and increased to 10.14% after ageing. Table 2 showed the comparison of the chemical characteristics of the product wine and purchased imported wine. The wine contained vitamins A, C, thiamine, riboflavin and niacin with mineral elements as potassium, calcium and iron (Table 3). Table 4 presents the sensory attributes of the wine and that of an imported brand, which showed the product is acceptable by the panelists who also indicated interest to buy the product if sent in the market.

DISSCUSION

A very good table wine was produced from ripe pawpaw fruit pulp using yeast (*S. cerevisiae*) isolated from a local fermented beverage drink *burukutu*. The product had chemical properties comparable with those of other fruit wines (Okoro, 2007; Amerine et al., 2012) as observed in Table 1.

The reduction in pH may be as a result of the production of carbon oxide which dissolved in the 'must' to form a weak acid. The fall could also be as a result of the production of acetic acid by acetic acid bacteria. The pH of the wine is close to the findings of Somari et al. (1993) and Okoro (2007) who also produced fruit wine with pH range of 3.76 to 4.0.

At the end of fermentation process alcoholic content of the wine stood at 10.12% and increased to 12.14% after ageing. The high yeast inocula used for pitching may be what resulted in rapid accumulation of alcohol (10.12%) at the fermentation temperature of 29.4°C and this agreed with the findings of Amarine et al. (1980) and Amerine et al. (2012). The value also falls within the European Economic Community recommended alcoholic content for table wine of 8.5 to 19.5% (Amerine and Ough, 1980; Amerine and Kunkee, 2005). This therefore classifies the wine as a good table wine, confirming that a good table wine can be obtained from pawpaw fruit pulp.

The result of the analysis at various stages of production shows that the percentage reducing sugar content falls from 16.7 to 1.10%. The general reduction in specific gravity, and reducing sugar were due to the constant utilization of sugar by yeast for their metabolic activities. This was in conformation with the results obtained by Cavalieri et al. (2003) and McGovern (2003). *S. cerevisiae* isolated from *burukutu* had high alcoholic

Table 3. Some vitamins and mineral elements contained in the wine.

Parameters	Inference
Vitamin A	+
Vitamin B	-
Vitamin C	+
Thiamine	+
Riboflavine	+
Niacin	+
Potassium	+
Calcium	+
Iron	+

Key: + Present**; -** Not present

Table 4. The organoleptic attributes of the product and an imported wine.

Parameters	Product wine	Imported wine
Colour	4.4	4.3
Taste	4.6	4.8
Flavour	4.5	4.5
General acceptability	4.9	4.9

Retting inference: 5 – excellent, 4 – very good, 3 – good and 2 – bad and 1- very bad

tolerance (Obisanya et al., 1987; Somari et al., 1993; Zoecklein et al., 1990; Fagbemi and Ijah, 2005). This indicates the utilization and depletion of sugar during the fermentation process until there was little or no sugar left in the 'must'.

Titratable acidity was decreasing daily during the fermentation process as seen in Table 1. The final titratable acidity of 1.25% was similar to that obtained by Akingbala and Oguntimehin (1992), Amerine and Kunkee, (2005) and Okoro (2007).

There is great need for the development of industries that will make use of local and cheap raw materials to produce wine to take care of the increasing rate of wine consumption in the country. Also, to reduce wastage of pawpaw fruit recorded after every harvesting season as a result of high yield of the pawpaw tree, this research was carried out to open a way towards the development of an efficient method of wine production, using pawpaw (*C. papaya*) fruits as the sole source of pulp materials. The pawpaw wine produced from this work when compared with the already existing wines in the market competed favorably in terms of flavor, color, taste and general acceptability.

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