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Investigation in the use of a Yeast Specie Isolated from a Fermented Beverage for Mixed Fruit Wine Production

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ABSTRACT

The juice from well-ripped pawpaw fruit (*Carica papaya*) and watermelon (*Citrullus lanatus*) were extracted, mixed and used to produce wine by fermentation using a yeast strain *Saccharomyces cerevisiae* isolated from a fermented local beverage drink "*burukuutu*". The "must" and wine were analyzed daily during fermentation to determine the pH, specific gravity, titratable acidity and reducing sugar content. It was fermented at 30±2°C for 12 days. The pH increased from 3·5 to 4·4, specific gravity decreased from 1·009 to 1·002%, reducing sugar content decreased from 0·11 to 0·00 g, while titratable acidity increased from 1·5 to 12·3% at the end of the fermentation. The alcoholic content of the wine increased to 3·96% after ageing while temperature was maintained at room temperature throughout the period of fermentation. The wine presented a brilliant yellow color with a slight sweet flavor. Sensory evaluation showed that the mixed juice of pawpaw and watermelon wine had slight potential when compared to other commercial samples ($p < 0·05$). These results showed that mixed juice of pawpaw and watermelon can be successfully used in the production of table wine. Using the yeast from a cheap local source makes the wine affordable and available. Also utilizing these fruits in wine production will help to reduce post harvest losses incurred on these fruits.

KEYWORDS: *Wine, pawpaw, watermelon, fermentation, "burukutu" yeast strain, mixed fruit, fruit juice*

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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, mixed acid (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). But recently most wines produced especially form tropical fruits had little alcoholic content (Okoro, 2007).

The nutritional role of wine is important since its average contribution to total energy intake is estimated to be 10 to 20% in adult males (Okaor *et al*., 2018). For the past few decades, grapes are the main fruits used for wine production. Despite that, several studies have investigated the suitability of other fruits as substrates for wine production (Joshi and Bhutani, 1991; Joshi et *al*., 1991; Ndip *et al*., 2001; Okunowo *et al*., 2005). Moreover, the non-availability of grapes, which is usually the fruit of choice for wine production in the tropics has necessitated the search for alternative fruit source in Nigeria and other tropical countries (Alobo and Offonry, 2009). In Nigeria, there is abundance of tropical fruit which includes passion fruit, watermelon, pineapple, pawpaw, orange etc., these fruits are highly perishable and susceptible to microbial spoilage as a result, they fail to reach the market due to the spoilage, mechanical damage and over ripeness (Ihekoroye and Ngoddy, 1985). Besides, these fruits are difficult to keep for

considerable length of time, hence the ripe fruits are utilized either as fresh or processed into juice and other specialty products (Oyeleke and Olaniyan, 2007). High rate wastage of these fruits especially at their peak of production during their season necessitates the need for alternative preservation of these fruits. The production of wines from common fruits could help reduce the level of post harvest losses and increase variety of wines (Okoro, 2007; Alobo and Offonry, 2009; Okafor *et al.,* 2018).

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.

Watermelon (*Citrullis lanatus*) is a subtly, crunchy, thirst quenching fruit that can be found in the market all year round. Though it has a season of abundance towards the end of rainy season, when they are sweet and of best quality. The flowering plant bears an accessory fruit of the type botanists call false berry. Water melon is an unusual fruit source of the carotenoid lycopene and a rich source of phenolic antioxidants. It contains cucurbitacin E, a triterpene anti-inflametory phytonutrient, and a large amount of amino acid, citrulline. Water melon has other health benefits and can be considered in the production of materials like wine to help preserve the fruits and add value to its consumption.

Generally, all wines are produced using wine yeast, *Saccharomyces cerevisiae* Var *ellipsoides* (Okoro, 2007, Amerine *et al*., 2012; Okafor *et al.,* 2018). This yeast is most times imported and is also expensive. Some authors had proved that the yeast can be obtained from palm wine which has the potentials of the imported ones and also has high alcoholic tolerance (Obisanya *et al*., 1987; Somari and Udo, 1993). Palm wine is not easily available now due to the high risk involved in climbing and tapping the wine. Therefore an alternative source of *Saccharomyces cerevisiae* was sourced from a local beverage drink, '*burukutu*'.

'Burukutu' is a local no-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. Sorghum, Guinea corn, is a local cereal grain extensively grown in Nigeria and other tropic regions of the world. It is called 'dawa' in Hause language and 'oka ajari' in Igbo. It is mainly employed in beer brewing and some other beverage drinks such as '*burukutu'* (Odibo *et al.,* 2002).

Although, tropical fruits and several yeast strains have been screened for their suitability in wine production, most studies have either focused only on the suitability of the fruits or the yeast strains. This study is therefore aimed at investigating the suitability of two local fruits juice as substrates for wine production. The efficiency of indigenous yeast strains isolated from '*burukutu'* for alcoholic fermentation of the fruits was also investigated.

MATERIALS AND METHODS

Collection of samples

Sample of '*burukutu'* was purchased with a sterile bottle from sales point in Awka, Anambra State and taken to the laboratory immediately and refrigerated. Culture media and other chemicals and reagents (Oxoid) used were obtained from the Department of Applied Microbiology and Brewing laboratory and were of analytical grade. The flow chart below was used in the production of the wine. Two big freshly harvested fruits each of water melon (*Citrullus lanatus*) and pawpaw (*Carica papaya*) were purchase from local farmer and taken to the laboratory.

PREPARATION OF INOCULUM

The method of Fagbemi and Ijah (2005) was modified to isolate and identify the yeast strain from '*burukutu'*. Yeast strains were isolated by plating serially diluted samples of the '*burukutu'* on Sabouraud dextrose agar (SDA) 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.

PREPARATION OF "MUST"

The method of Okoro (2007) as used by Okafor *et al.,* (2018) was used in preparation of the 'must' during the study. Pulp of pawpaw and watermelon fruits were 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'). One liter each of 'must' was poured into a glass vessel and 100 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). The 'must' was enriched with 0.09g potassium dihydrogen phosphate to enhance rapid growth of the fermenting yeast.

WATERMELON AND PAWPAW "MUST" FERMENTATION

The method of Okoro (2007) as modified by Umeh and Achufusi (2014) was used for the wine fermentation. Two liters of standardized 'must' mixed in the ratio 1:1 was pitched with 160 ml of reconstituted yeast to give a pitching rate of 8% (v/v). Fermentation was carried out in a four liters glass jar and lasted for 12 days with the evolution of carbon oxide. This was carried out at room temperature 29±2ºC. During the period, pH, specific gravity, titratable acidity and reducing sugar were monitored every 48 h using appropriate methods (Amerine *et al*., 2012).

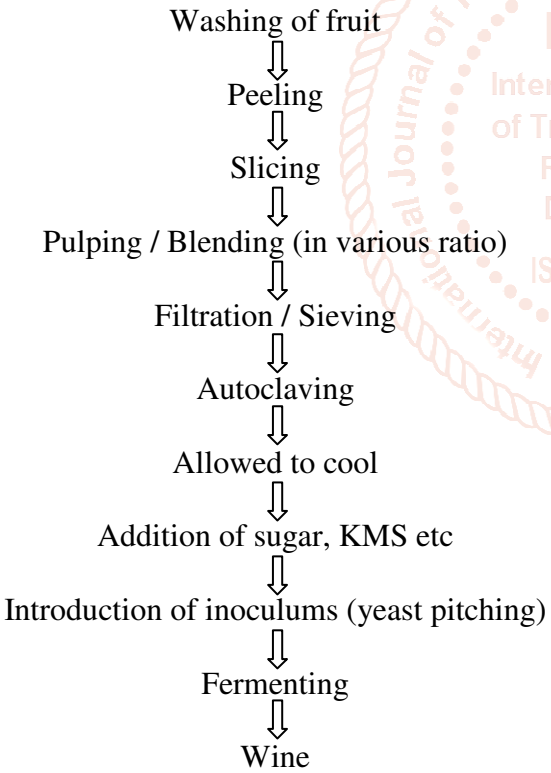

Figure1: Flow chart for the production of mixed watermelon and pawpaw wine.

Analysis of the wine chemistry

Specific Gravity

A specific gravity bottle was weighed empty to obtain W_1. The bottle was then filled with water and weighed W_2. The bottle was emptied, dried and filled with the sample solution and weighed W_3. The specific gravity was obtained as:

$$\frac{\text{Bottle Sample } (W_3) - \text{Bottle } (W_1)}{\text{Bottle Water } (W_2) - \text{Bottle } (W_1)}$$

Determination of Titratable Acidity

Exactly 2ml of the hydrolysate was titrated against 2% NaOH to its phenolphthalein end point, as described by (Mbajiuka *et al*., 2010). This was used to determine the volume of NaOH that will neutralize the HCl.

Determination of Reducing Sugar

The presence of reducing sugar (glucose) in the hydrolysate was determined by method of Fehling's solution described by (Onwuka, 2002)

pH determination

The pH was determined using a Jenway 3015 pH meter. Ten milliliter of fermenting wort was taken and the electrode of the pH meter was inserted into the wort sample. The reading on the screen of the pH meter was observed and recorded daily.

Alcoholic content Analysis

The table of Association of Official Analytical Chemist (AOAC, 2000) of Brewing method of specific gravity against percentage of alcohol at a specific temperature was used to determine the alcoholic content 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

During the course of this study, the analytical assays carried out comprise pH determination, titratable acidity, specific gravity, reducing sugar, alcoholic content and sensory evaluation of the product wine. The 'must' showed a gradual increase in pH as the fermentation time increased from 3.5 on 2nd day to 4.4 on 12th day. The titratable acidity ranged from 1.5to 2.3(%w/w) showing increase as fermentation time increased. Alcoholic content of the sample showed an increase to 2.96% at the end of fermentation. There was a gradual decrease in specific gravity from 1.009 to 1.002%. Reducing sugar concentration decreased from 0.11 to 0.00 g, while the temperature was maintained at room temperature throughout the period of fermentation (Table 1). The sensory evaluation attributes of the wine produced is as shown in table 2.

Table 1 Changes in pH, specific gravity, alcoholic content, titratable acidity and reducing sugar of the 'must' during the period

Days	pH	Titratable acidity (%w/w)	Reducing sugar (%)	Specific gravity (%w/w)	Temperature (C)	Alcohol content%
0	3.50	1.50	0.11	1.009	31	
2	3.80	1.80	0.11	0.999	30	
4	4.02	2.25	0.12	1.002	31	
6	4.23	1.80	0.09	0.988	29	
8	4.25	1.80	0.09	0.996	29	
10	4.40	2.25	0.03	0.999	28	
12	4.40	2.30	0.00	1.002	28	3.96

Table 2: Sensory Evaluation of the fermenting must

Sensory attributes	period of fermentation	
	0day	18day
Colour	3	4
Taste	4	5
Aroma	5	4
Texture	3	3
Overall acceptability	4	5

Sensory scale used = 5 - point Hedonic scale
5 - Strongly liked
4 - Liked
3 - Moderately liked
2 - Disliked
1 - Strongly disliked

DISCUSSION

Table wine was produced from mixed ripe pawpaw and watermelon fruit pulp using yeast (*S. cerevisiae*) isolated from a local beverage drink "palm wine". Currently, most of the perishable fruits are lost during their journey through the agric-food chain, due to spillage, physiological decay, water loss, mechanical damage during harvesting, packaging and transporting, or due to transportation (Okafor *et al.,* 2010). The present investigation has revealed the usefulness of pawpaw and watermelon in wine making, hence a way of reducing wastages. The present study revealed low pH values in the fruit wines throughout the fermentation period. Also revealed are consistent increases in acidity (titratable, volatile and fixed) of the fruit wines throughout the period of fermentation. Studies have shown 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. Also, low pH and high acidity are known to give fermenting yeasts competitive advantage in natural environments (Reddy and Reddy, 2005). Reports have shown that the major problem associated with the use of tropical fruits in wine production is their low sugar content (Alobo and Offonry, 2009). No remarkable amount of alcohol was produced from the fruit wines during fermentation with the test yeast strains. The percentage alcohol produced from the fruits at the end of fermentation by the test yeast strain was above 3%, which is not comparable with moderate grape wines (Ayogu, 1999; Querol *et al*., 2003; Okunowo *et al*., 2005). This may be because supplement in the form of sugar was not added. In this study, pH and temperature of the fruit wines throughout the period of fermentation ranged from 3.5 to 4.4 and from 28 to 31°C, respectively. A similar observation has been reported by Reddy and Reddy (2005). In their study on mango fruit, optimum pH and temperature values for quality wine production was 5.0 and 30°C, respectively. The type and aroma produced during

wine making is reported to depend on yeast, environmental factors and physicochemical characteristics of the musts. The result of the analysis at various stages of production showed that the percentage reducing sugar content falls gradually during the fermentation period. 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). 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 since mixed pawpaw and watermelon wine produced from this work has shown that it can compete favorably in terms of flavor, color, taste and general acceptability with already existing market wine.

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