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Full Length Research Paper

Assessing the Efficiency of Yeast Isolates from Palm Wine in the Production of Various Fruit Wines

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This study was aimed at investigating the suitability of three local fruits as substrates for wine production and the efficiency of four indigenous yeasts strains isolated from palm wine in comparison with the commercial *Saccharomyces cerevisiae* for alcoholic fermentation of fruits. A total of five yeast strains (*S. cerevisiae, Schizosaccharomyces octoporus, Pichia* spp and *Saccharomyces paradoxus*) isolated from palm wine and commercial *S. cerevisiae* and three fruits (passion fruit, water melon and pineapple) were used for this investigation. Primary and secondary fermentation of the fruit must lasted for 12 and 8 days, respectively. During fermentation, aliquot samples were removed daily from the fermentation tank for analysis of alcohol content, specific gravity, total solids, titratable acidity, volatile acidity and fixed acidity, using standard procedures. Proximate analysis of the test fruits revealed them to be poor sources of protein but with high moisture content that ranged from 72 to 84%. Temperature and pH of the fruit must during the period of fermentation ranged from 28 to 32°C and from 3.0 to 4.8, respectively. During the fermentation period, consistent increases in alcohol content were observed with time. At the end of 20 days fermentation, the concentration of alcohol in the fruit wines was observed to range from 10.14 to 12.80%. Also, titratable, volatile and fixed acid concentrations were observed to show steady increase with time throughout the period of fermentation. The study has revealed that acceptable wine could be produced from these fruits with the test yeast strains.

Key words: Fruit wines, yeast, fermentation, alcohol, acidity.

INTRODUCTION

The ability to produce palatable effervescent beverage by alcoholic fermentation of natural fruit juices is a demonstration of inherent ingenuity of man. 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 (Macrae et al., 1993).

During the past few decades, grapes are the main fruits that were used for wine production. Despite that, several studies have investigated the suitability of other fruits as substrates for the purpose of 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, plum, orange etc., these fruits are highly perishable, and susceptible to bacterial and fungal contamination as a result they fail to reach the market due to 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 specialty products (Oyeleke and Olaniyan, 2007).

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High rate wastage of these fruits especially at their peak of production during their season necessitates the need for alternative preservation food forms towards an enhanced utilization 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).

Although, many genera and species of yeasts are found in musts, *Saccharomyces cerevisiae* is the main yeast strain that is commonly reported to be responsible for alcoholic fermentation (Pretorius, 2000; Querol et al., 2003). However, many studies have investigated the use of other yeast strains (Ciani and Maccarelli, 1998; Okunowo et al., 2005) and also combination of yeast strains (Clemente-Jimenez et al., 2005) in fermentation especially in wine production. Reports have shown that the fermentation of fruit juices using yeast from different sources creates variety in flavour and varying levels of alcoholic contents in wines. Clemente-Jimenez et al. (2005) in their work reported *Pichia* fermentans as good starter strains for most fermentation as *Pichia* fermentans in mixture with *S. cerevisiae* improve the aroma as well as the characteristics features of the wine.

The fermentation of wine is known to be a complex process with various ecological and biochemical processes involving yeast strain (Fleet, 2003). Palm wine is a naturally sweet fermented beverage obtained from the sap of *Elaeis* spp. and the sap of *Raphia* spp, which contains a heavy suspension of live yeasts and bacteria (Okafor, 1975). It is mostly common in Africa, especially Nigeria. Most studies on palm wine have reported its potentials as source of yeast isolate for the fermentation industries. Okafor (1972) in his study isolated seventeen yeast strains, four belonging to the species of *Candida*, twelve to the genus of *Saccharomyces* and one to *Endomycopsis* spp. In grapes, yeast strains, such as *Hanseniaspora uvarum, Kloeckera apiculata, Candida, Pichia, Rhodotorula and Kluyveromyces* are known to be predominant (Duart et al., 2010).

The fermentation for the elaboration of beverage is known to depend on the performance of yeast to convert the sugars into alcohol and esters. Besides, the different species of yeast that develop during fermentation determine the characteristic of the 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 (Fleet, 2003; Duart et al., 2010). Although, tropical fruits and several yeast strains have been screened for their suitability in wine production, most studies have either focussed only on the suitability of the fruits or the yeast strains. This study is therefore aimed at investigating the suitability of three local fruits as substrates for wine production. The efficiency of four indigenous yeasts

strains isolated from palm wine in comparison with the commercial *S. cerevisiae* for alcoholic fermentation of the fruits was also investigated.

MATERIALS AND METHODS

A total of five yeast strains and three fruits (passion fruit, water melon and pineapple) were used for this investigation. The fruits were purchased from local markets in Umudike, Abia State, Nigeria. Of the five yeast strains, four were isolated from palm wine, using standard microbiological techniques (Bessey, 1974). The isolated strains were identified as *S. cerevisiae, Schizosaccharomyces octoporus, Pichia* spp and *Saccharomyces paradoxus*. A fifth strain (*S. cerevisiae*), which served as a reference strain was purchased from a retail shop in Umuahia, Abia State, Nigeria. In this study, this strain is referred to as commercial *S. cerevisiae.*

Prior use, the fruits were physically examined for presence of defects. Fresh, unspoiled fruits were chosen for the production of wines. Proximate composition of the fruits was determined using standard procedures. The protein and fat contents were determined using the Kjeldahl and continuous solvent extraction methods, respectively. The concentration of crude fibre, total ash, carbohydrate and moisture contents were estimated using the Wende, incineration gravimetric, nitrogen free extractive and gravimetric methods, respectively (Pearson, 1976; James, 1995).

The selected fruits were washed with distilled water and allowed to drain dry before weighing and manually removal of peels. All fruit pulps were cut and pomace removed before crushing and homogenising the edible portions in a blender. The respective homogenised masses were then transferred to clean two-fold muslin cloth in large plastic containers to obtain juices. The resulting juice was pasteurised at 80°C for 30 min before cooling to room temperature, followed by the addition of ammonium sulphate, citric acid and sucrose to serve as additives to the must. To ascertain sterility, aliquot samples of the pasteurized must were plated on potato dextrose agar plates and nutrient agar plates, for fungi and bacteria detection, respectively. Only must corresponding to plates were no growth was observed were ascertained as sterile and only reported in study.

For primary fermentation, the respective yeast cultures were inoculated into the must in a tank made of plastic and fitted with stirrers for agitation and thermometer for temperature measurement. Primary fermentation lasted for 12 days after which the fermenting must was racked by scooping the must together with the solids using sterile plastic mugs. After filtration, the racked wine was transferred back to the fermentation tank for secondary fermentation, which lasted for 8 days.

During the two fermentation periods, aliquot samples were removed daily from the fermentation tank for analysis of alcohol content, specific gravity, total solids, titratable acidity, volatile acidity and fixed acidity, using standard procedures (Caputi and Wright, 1969; James, 1995; Bradly, 2003; George and Murphy, 2003). Temperature and pH were estimated using a thermometer and electric pH meter, respectively.

Statistical analyses were carried out using the SPSS statistical software. Comparison of means was done using the One-Way Analysis of Variance (ANOVA). All statistical analyses were carried at 95% confidence interval.

RESULTS

As shown in the Table 1, the test fruits were poor sources of protein (0.24 to 2.57%) but with high moisture content that ranged from 72 to 84%. The highest protein and moisture contents were observed in passion fruit and water melon, respectively.

Temperature and pH of the fruit wines during the period of fermentation were not observed to follow any particular trend. This was irrespective of the test yeast strains. In all

Table 1. Proximate composition of the test fruits.

% composition	Passion fruit	Water melon fruit	Pineapple fruit
Protein	2.57	0.47	0.24
Fat	2.27	0.24	0.17
Crude fibre	4.25	0.27	0.51
Ash	1.25	0.55	3.83
Moisture content	72.11	92.81	84.39
Carbohydrate	17.55	5.65	10.87

Table 2. Temperature (°C) and pH variation in the fruit wines during fermentation with the test yeast strains.

Time (d)	A	B	C	D	E
Passion fruit wine					
0	29 (4.4)	29 (4.4)	29 (4.4)	29 (4.4)	29 (4.4)
1	30 (3.9)	29 (4.0)	30 (4.1)	30 (4.1)	30 (4.0)
4	30 (3.6)	30 (3.7)	30 (3.7)	31 (3.9)	30 (3.8)
8	31 (3.4)	31 (3.4)	31 (3.5)	31 (3.6)	31 (4.6)
12	31 (3.1)	31 (3.3)	31 (4.5)	32 (3.5)	31 (3.5)
16	29 (3.3)	30 (3.2)	29 (3.3)	29 (3.3)	29 (3.3)
20	28 (3.3	29 (3.2)	28 (3.3)	29 (3.3)	29 (3.3)
Water melon fruit wine					
0	29 (4.8)	29 (4.8)	29 (4.8)	29 (4.8)	29 (4.8)
1	30 (4.5)	30 (4.7)	30 (4.6)	29 (4.5)	30 (4.6)
4	32 (4.1)	31 (4.4)	31 (4.4)	30 (4.0)	31 (4.0)
8	32 (3.8)	32 (4.1)	31 (4.0)	31 (3.6)	32 (3.8)
12	30 (3.6)	31 (3.8)	30 (3.8)	31 (3.6)	30 (3.5)
16	29 (3.5)	29 (3.4)	28 (3.6)	29 (3.4)	29 (3.4)
20	29 (4.5)	28 (3.4)	28 (3.5)	28 (3.4)	28 (3.4)
Pineapple fruit wine					
0	29 (4.7)	29 (4.7)	29 (4.7)	29 (4.7)	29 (4.7)
1	30 (4.1)	29 (4.2)	29 (4.4)	30 (4.2)	29 (4.5)
4	30 (3.9)	30 (3.8)	30 (3.9)	31 (3.7)	30 (4.1)
8	31 (3.1)	31 (3.4)	31 (3.5)	32 (3.4)	31 (3.8)
12	30 (3.2)	31 (3.2)	32 (3.5)	30 (3.2)	31 (3.4)
16	29 (3.0)	30 (3.0)	30 (3.2)	29 (3.0)	30 (3.2)
20	28 (3.0)	29 (3.0)	29 (3.2)	28 (3.0)	29 (3.2)

Values in parenthesis represent pH. All values are averages of duplicate samples. A, B, C, D and E represent commercial *S. cerevisiae*, *Saccharomyces cerevisiae* isolated from palm wine, *Schizosaccharomyces octoporus*, *Pichia* spp and *Saccharomyces paradoxus*, respectively.

the fruit wines, temperature of the fruit wines were observed to range from 28 to 32°C. Also, throughout the period of fermentation, pH in the fruit wines were within the acidic range. This was also irrespective of the test yeast strain used for fermentation. pH ranged from 3.1 to 4.6, 3.4 to 4.8 and 3.0 to 4.7 in the passion, water melon and pineapple fruit wines, respectively (Table 2).

As shown in Figure 1, a steady increase in alcohol content was observed in the fruit wines throughout the period of fermentation with the test yeast strains. This increase was irrespective of the test yeast strain used and fruit. At the end of the 20 days fermentation, the concentration of alcohol in the fruit wines were observed to range from 10.46 to 12.42%, from 10.14 to 10.44%and from 11.60 to 12.80%, for passion, water melon and pineapple fruit wines, respectively. In the passion fruit wine, the highest and lowest alcohol levels were observed in the presence of the commercial *S. cerevisiae* and *S. cerevisiae* isolated from palm wine. In the case of water melon and pineapple fruit wines, alcohol contents

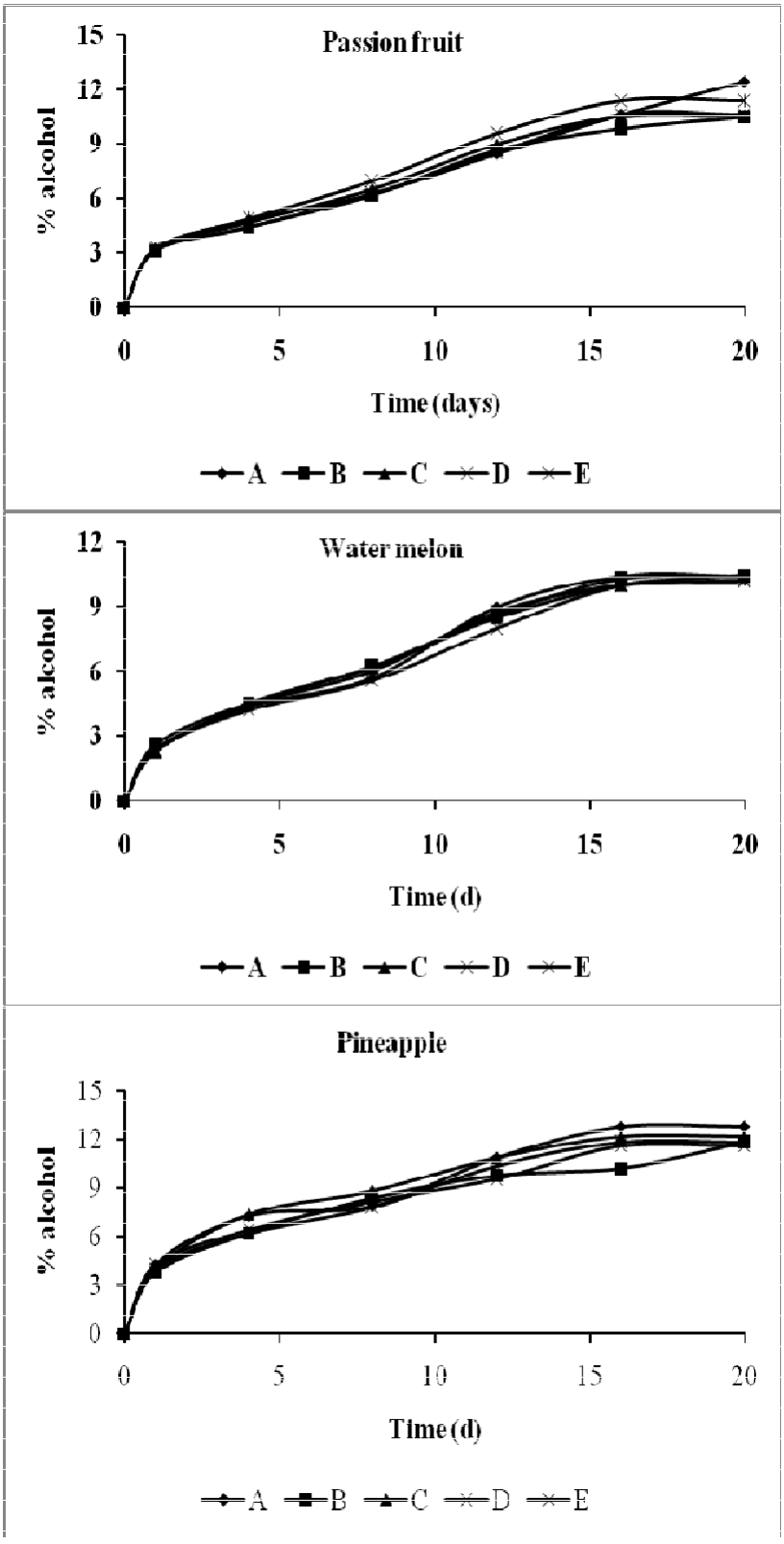

Figure 1. Variation in alcohol content of the test fruit wines when fermented with the yeast strains. A, B, C, D and E represent commercial *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* isolated from palm wine, *Schizosaccharomyces octoporus*, *Pichia* spp and *Saccharomyces paradoxus*, respectively. All values are averages of duplicate analysis.

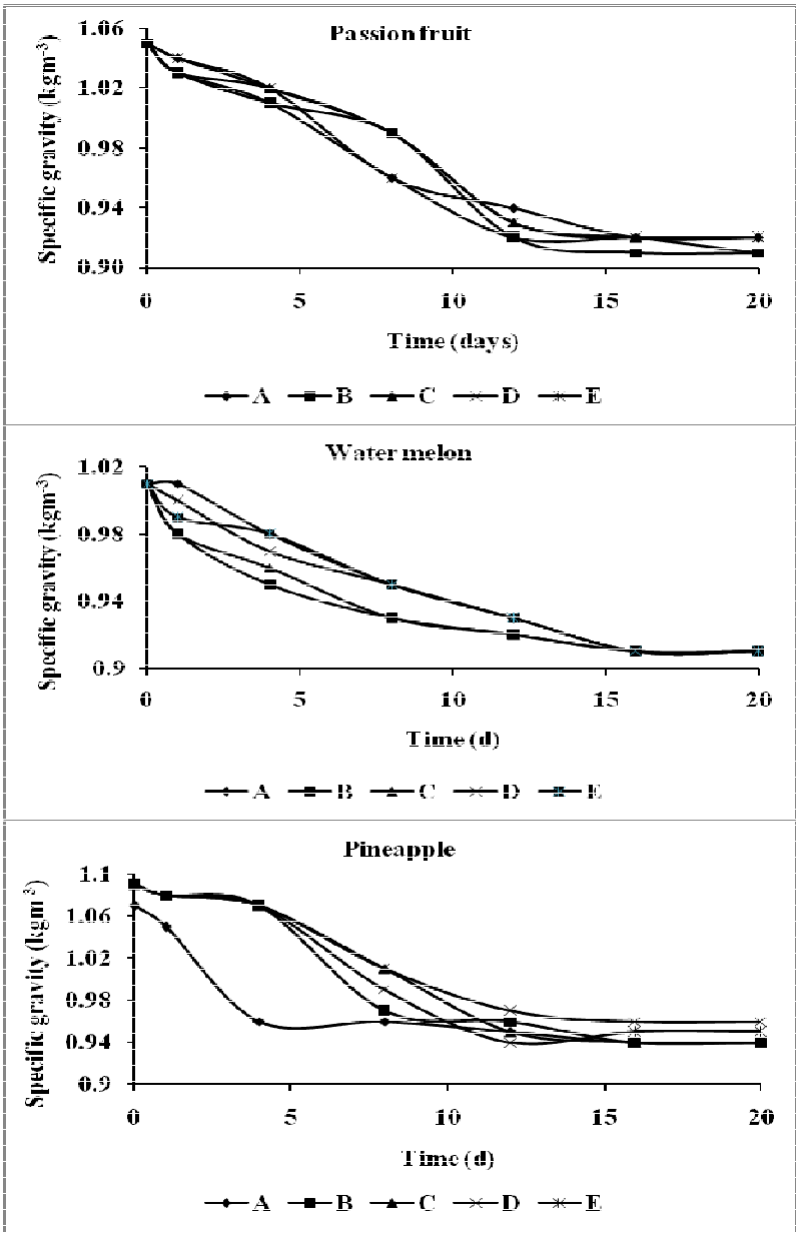

Figure 2. Variation in specific gravity of the test fruit wines when fermented with the yeast strains. A, B, C, D and E represent commercial *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* isolated from palm wine, *Schizosaccharomyces octoporus*, *Pichia* spp and *Saccharomyces paradoxus*, respectively. All values are averages of duplicate analysis.

were observed to be highest and lowest in the presence of *S. octoporus* and *Pichia* spp (for water melon) and in the presence of the commercial *S. cerevisiae* and *Pichia* spp (for pineapple), respectively (Figure 1). Although the alcohol contents in the fruit wines were observed to be different in presence of the different yeast strains, these differences were not observed to be significant ($p \leq 0.05$). This trend was irrespective of the fruit wines.

In the case of specific gravity of the fruit wines, gradual decreases in values were observed throughout the period of fermentation. These decreases were observed to be irrespective of the test fruit wines and yeast strain used. After 20 days fermentation, specific gravity values were observed to range from 0.91 to 0.92 kgm^{-3} (passion fruit wine), from 0.90 to 0.910.91 to 0.92 kgm^{-3} (water melon fruit wine) and from 0.94 to 0.96 kgm^{-3} (pineapple fruit wine). In all the test fruit wines, the lowest specific gravity values were observed in the presence of *Saccharomyces cerevisiae* isolated from palm wine (Figure 2). As was observed in the case of alcohol content, although the

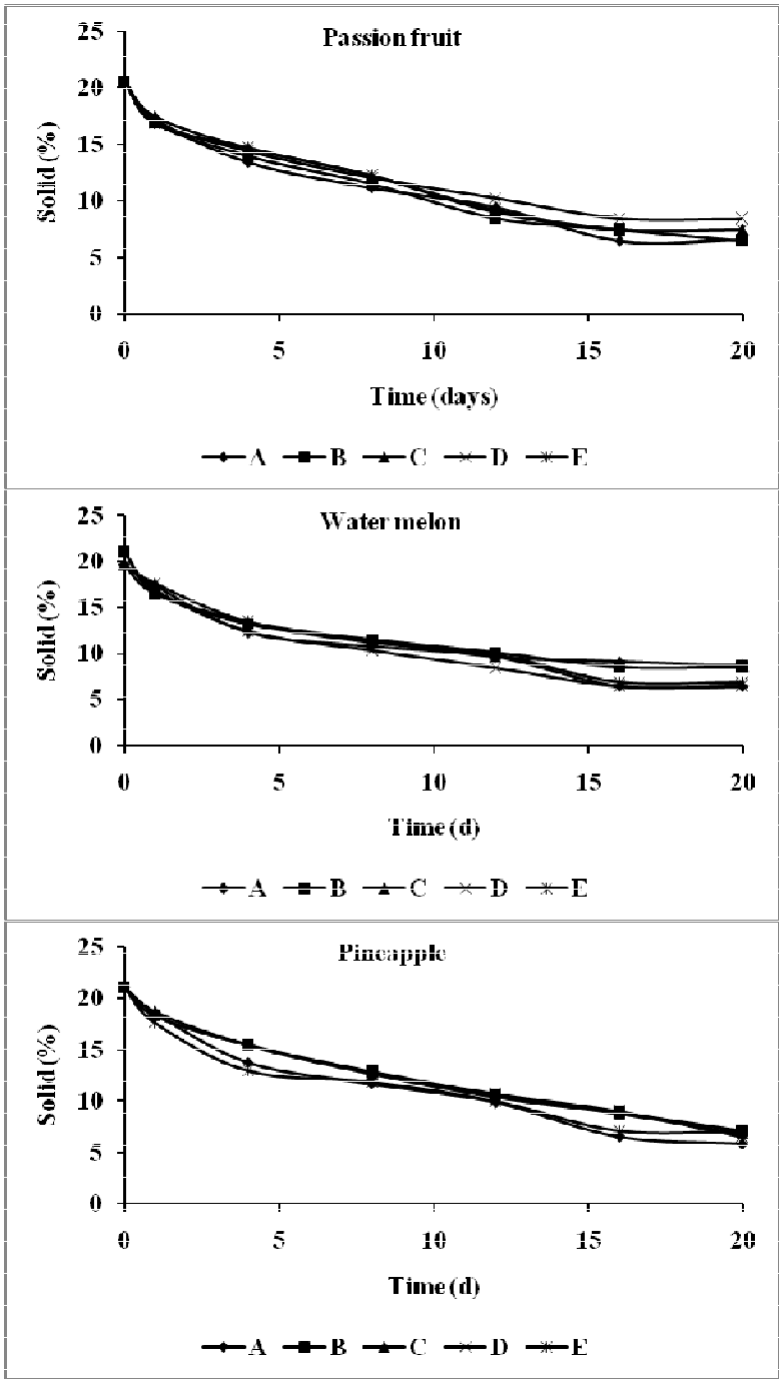

Figure 3. Variation in total solid concentration of the test fruit wines when fermented with the yeast strains. A, B, C, D and E represent commercial *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* isolated from palm wine, *Schizosaccharomyces octoporus*, *Pichia* spp and *Saccharomyces paradoxus*, respectively.

specific gravity values of the different fruit wines were observed to differ, these differences were not observed to be significant among the isolates ($p \leq 0.05$).

With respect to total solid concentration in the fruit wines, values were observed to decrease consistently throughout the period of fermentation. This trend was similar in the presence of the test yeast strains and irrespective of the fruit wines (Figure 3). From initial ranges of 20.63 to 20.64%, 19.62 to 21.08% and 21.06 to 21.08%, total solid concentrations were observed to

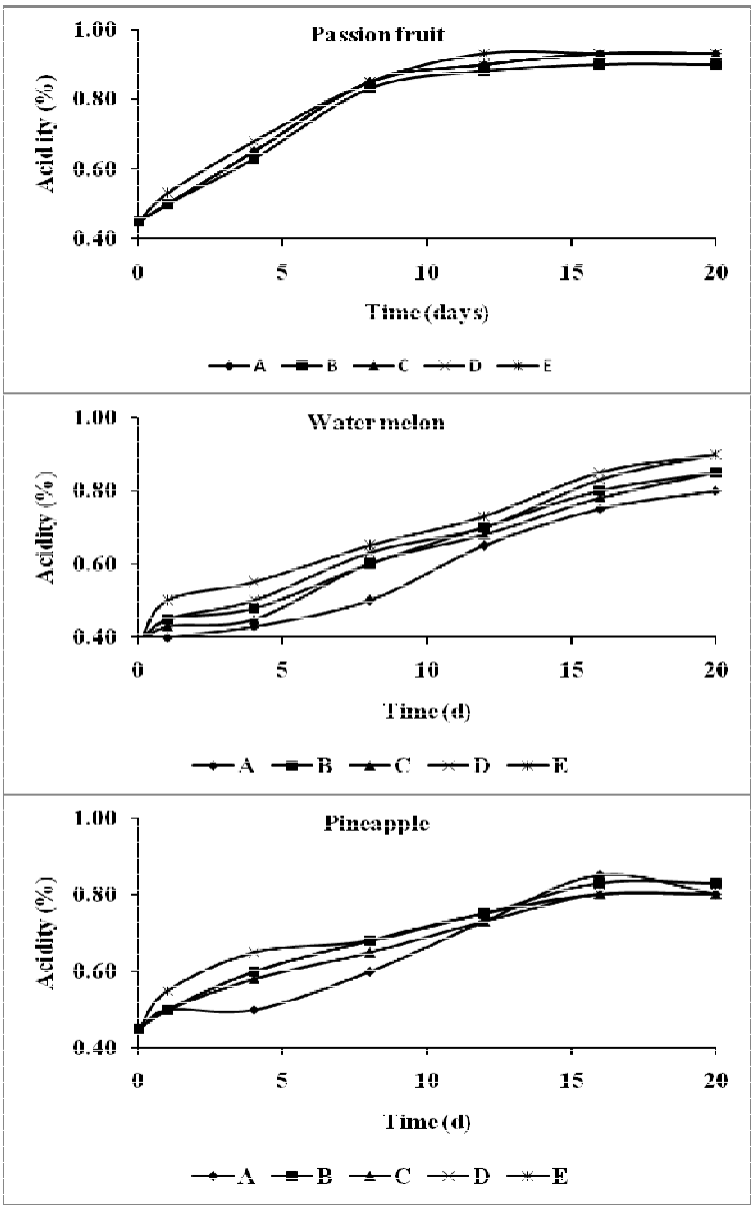

Figure 4. Variation in titratable acidity of the test fruit wines when fermented with the yeast strains. A, B, C, D and E represent commercial *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* isolated from palm wine, *Schizosaccharomyces octoporus*, *Pichia* spp and *Saccharomyces paradoxus*, respectively.

decrease to final ranges of 6.42 to 8.40%, 6.42 to 8.48% and 5.82 to 7.12%, for passion, water melon and pineapple fruit wines, respectively (Figure 3). The highest and lowest total solid concentrations were observed in the presence of *Pichia* spp and *S. cerevisiae* isolated from palm wine (passion fruit wine), *Saccharomyces paradoxus* and *Pichia* spp (water melon fruit wine) and the commercial *S. cerevisiae* and *S. cerevisiae* isolated from palm wine (pineapple fruit wine). As observed previously for the earlier parameters, the differences in solid concentrations were not observed to differ significantly among the test yeast strains. This observation was irrespective of the test fruit wines.

Figures 4 and 5 show the trend in titratable and volatile acid concentrations in the fruit wines during fermentation with the test yeast strains. As shown in the figures, titratable and volatile acid concentrations were observed to show steady increases with time throughout the period of fermentation. These increases were irrespective of the test fruit wines and yeast strains used. At the end of 20 days fermentation, acid concentrations in the passion fruit wine were observed to increase from initial concentration

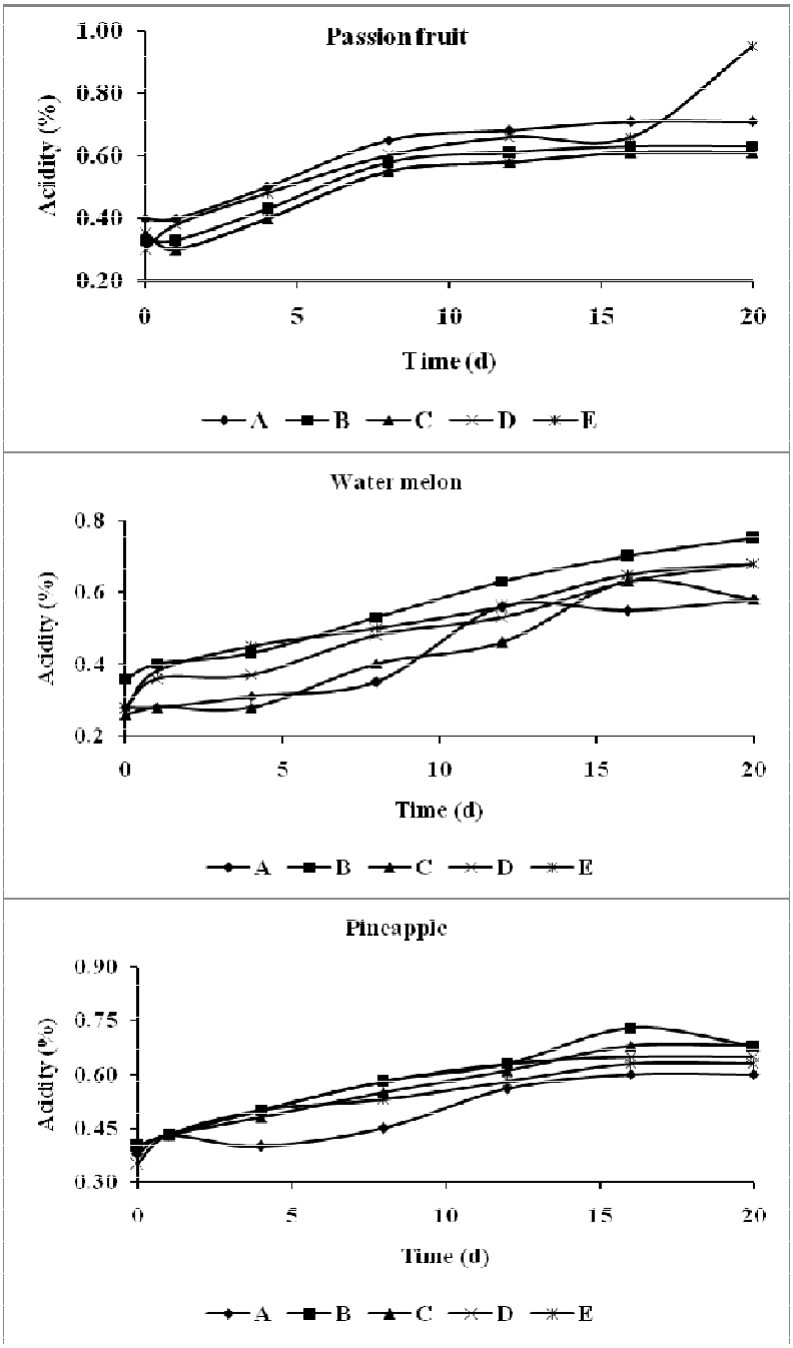

Figure 5. Variation in volatile acidity of the test fruit wines when fermented with the yeast strains. A, B, C, D and E represent commercial *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* isolated from palm wine, *Schizosaccharomyces octoporus*, *Pichia* spp and *Saccharomyces paradoxus*, respectively

ranges of 0.40 to 0.45% and 0.30 to 0.40% to final ranges of 0.90 to 0.93% and 0.61 to 0.95% for titratable and volatile acidity, respectively. Similarly, titratable acid concentrations were observed to increase from initial concentrations of 0.38 and 0.45% to final concentration ranges of 0.85 to 0.90% and 0.80 to 0.83%, for water melon and pineapple fruit wines, respectively (Figure 4). Also, volatile acid concentrations were observed to increase from initial concentration ranges of 0.26 to 0.36% and 0.35 to 0.40% to final ranges of 0.58 to 0.75% and 0.60 to 0.68%, for water melon and pineapple fruit wines, respectively (Figure 5).

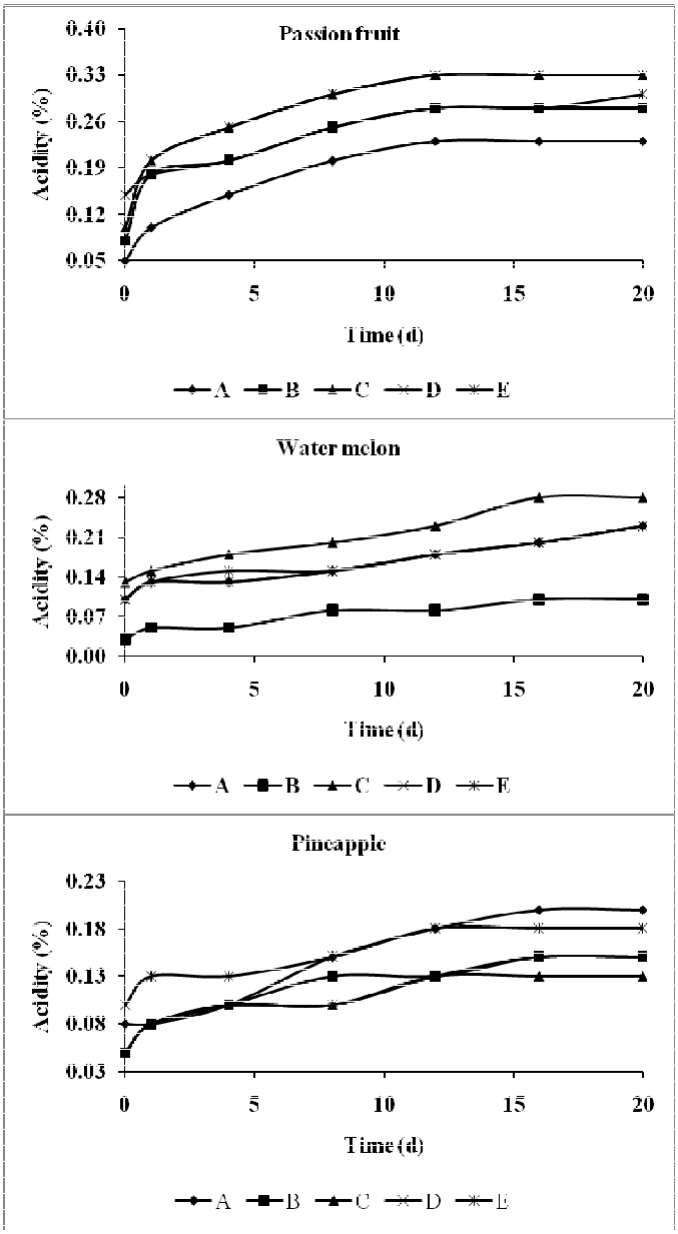

Figure 6. Variation in fixed acidity of the test fruit wines when fermented with the yeast strains. A, B, C, D and E represent commercial *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* isolated from palm wine, *Schizosaccharomyces octoporus*, *Pichia* spp and *Saccharomyces paradoxus*, respectively.

The lowest titratable acid concentrations in the fruit wines were observed in the presence of *S. cerevisiae* isolated from palm wine, commercial *S. cerevisiae* for passion and water melon fruit wines, respectively. Also, volatile acid concentrations were observed to be lowest in the presence of *S. octoporus* and *Pichia* spp for the passion fruit wine; *S. octoporus* and commercial *S. cerevisiae* for the water melon fruit wine and commercial *S. cerevisiae* for the pineapple fruit wine (Figure 5). Despite the observed differences in the titratable and volatile acid concentrations of the fruit wines in the presence of the test yeast strains, these differences were not observed to be significant ($p \leq 0.05$).

In terms of fixed acidity contents of the test fruit wines during fermentation, values were also observed to increase consistently with time throughout the period of fermentation (Figure 6). From initial ranges of 0.05 to 0.15%, 0.10 to 0.13% and 0.05 to 0.10%, fixed acidity values in the fruit wines were observed to increase to final ranges of 0.23 to 0.33%, 0.10 to 0.23% and 0.13 to

0.20% for passion fruit, water melon and pineapple fruit wines, respectively (Figure 6).

The highest and lowest fixed acid concentrations were observed in the presence of the commercial *S. cerevisiae* and *Pichia* spp (passion fruit wine), *S. octoporus* and *S. cerevisiae* isolated from palm wine (water melon fruit wines) and in the presence of the commercial *S. cerevisiae* and *S. octoporus* (pineapple fruit wines).

In the passion fruit wine, the concentration of fixed acidity in the presence of the commercial *S. cerevisiae* was observed to be significantly higher than in the presence of *S. octoporus* and *Pichia* spp ($p \leq 0.05$). In the water melon fruit wine, fixed acidity in the presence of *S. cerevisiae* isolated from palm wine was observed to be significantly lower than those in presence of the other yeast strains ($p \leq 0.05$). Also, fixed acidity in the presence of *S. octoporus* was observed to be significantly lower than that in the presence of *S. octoporus* and *Pichia* spp ($p \leq 0.05$).

DISCUSSION

In the present investigation, the choice of fruits (passion fruit, water melon and pineapple fruit) was deliberate. The proximate composition of the test fruits was in agreement with the general case for fruits as reported by Pearson (1976). The low protein and mineral contents of the fruits as reported in this study is a probable indication that the fear of over accumulation due to consumption of the fruits do not arise (Okegbile and Taiwo, 1990). From the results, the mean moisture content of the fruits ranged from 72-84 %, and accounts for their high perishable nature and their short shelf life under normal storage conditions (Okaka, 1997). The fruits contained reasonable amount of carbohydrate, which gives an account of their high caloric value.

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). The titratable acidity of final wine is expected to be between 0.5 to 1.0% (Snell and Ettre, 1974). In this study, the results of titratable acidity in the test fruit wines fell within this limit.

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 content (Alobo and Offonry, 2009). Remarkable amount of alcohol was produced from the fruit wines during fermentation with the test yeast strains. This trend was consistent in all the test yeast strains. In general, the percentage alcohol produced from the respective fruits at the end of fermentation by the test yeast strains was above 11%, which is comparable with moderate grape wines (Ayogu, 1999; Querol et al., 2003; Okunowo et al., 2005).

The performance and potential of the test yeasts strain as substitute for the commercial bakers' yeast was measured by the amount of alcohol produced. High alcohols are known to be important precursors for the formation of esters, which are associated with pleasant aromas (Clemente-Jimenez et al., 2005). In the present study, the amount of alcohol produced by the respective isolates was not observed to differ significantly. Reports have shown that alcoholic fermentation leads to a series of by-products in addition to ethanol. Some of the by-products include carbonyl compounds, alcohols, esters, acids and acetals, all of them influencing the quality of the finished product. The composition and concentration levels of the by-products can vary widely (ng/L to hundreds of mg/L) (Plutowska and Wardencki, 2008; Duarte et al., 2010).

In this study, pH and temperature of the fruit wines throughout the period of fermentation ranged from 3.0 to 4.8 and from 28 to 32°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 physico-chemical characteristics of the musts. The present study revealed the effectiveness of other yeast strains, apart from the commercial bakers' yeast in wine production from the test tropical fruits. Several studies have indicated the effectiveness of non-Saccharomyces yeasts in must fermentation. Although this was not observed in this study, non-Saccharomyces are reported to lack the ability to complete fermentation due to their low ethanol tolerance, but are effective in improving wine quality (Toro and Vaquez, 2002; Clemente-Jimenze, 2004).

Conclusion

This study which was based on the evaluation of three indigenous fruits as substrates for wine production and the efficiency of locally isolated yeast strains from palm wine for fruit wine production have revealed the following:

- The three test fruits (passion fruit, water melon and pineapple) are good substrates for wine production.
- The amount of alcohol produced by the test yield strains during fermentation of the fruit juices did not differ significantly, even in comparison with commercial *S. cerevisiae*.

 The acid produced during fermentation of the fruit must by the test yeast strains fell within acceptable limits and did not differ significantly from each other.

Although this investigation cannot be considered to be exhaustive, the results obtained show that acceptable wine could be produced from these fruits with the different yeast strains. The study has also given an insight into the efficiency and role of local yeast strains during alcoholic fermentation of fruits.

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