



Comparative assessment of three yeast samples for wine production from pineapple

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ABSTRACT Pineapple is an abundant fruit in Nigeria, which usually suffers post-harvest spoilage due to lack of preservation techniques. Its rich sugar content makes it a suitable substrate for wine production, which becomes a useful alternative to curbing its post-harvest spoilage. This research compared the production of wine from pineapple using *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, and *Saccharomyces ellipsoides*. Yeast viability was assessed on YPG medium. To each fermenting jar was poured sterilized 3000 ml must, comprising of a mixture of juice and 6 ml of each yeast inoculum. Sodium metabisulphite (0.4g/l) was also aseptically added to each of the fermenting jars as a preservative and the fermenting jars were sealed with corks. Fermentation was allowed for twenty-eight (28) days within which different physical analyses were carried out (pH, temperature, titratable acidity, specific gravity, reducing sugar, alcoholic content) for each day. Sensory evaluation of the finished products was conducted and the overall level of acceptance was determined. The viable counts of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, and *Saccharomyces ellipsoides* after growth on YPG medium were 3.3×10^6 , 3.5×10^6 , and 3.8×10^6 cfu/ml respectively. There was decreased pH, reducing sugar, and a specific gravity of the wine samples while, there was an increase in titratable acidity, alcohol content, and temperature. Significant differences ($p < 0.05$) existed in the overall acceptability of the wine samples with *S. bayanus* fermented wine having the least acceptance by taste panelists. Pineapple makes a good compatible raw material for wine production using varying yeast samples.

KEYWORDS: fermentation; saccharomyces; vinification.

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Avaliação comparativa de três amostras de levedura para produção de vinho de abacaxi

RESUMO O abacaxi é uma fruta abundante na Nigéria, que geralmente sofre deterioração pós-colheita por falta de técnicas de preservação. Seu rico teor de açúcar o torna um substrato adequado para a produção de vinho, o que se torna uma alternativa útil para conter sua deterioração pós-colheita. Esta pesquisa comparou a produção de vinho de abacaxi usando *Saccharomyces cerevisiae*, *Saccharomyces bayanus* e *Saccharomyces elipsoides*. A viabilidade da levedura foi avaliada em meio YPG. Em cada pote de fermentação foi depositado 3000 ml de mosto esterilizado, composto por uma mistura de sumo e 6 ml de cada inóculo de levedura. Metabissulfito de sódio (0,4g / l) também foi adicionado asépticamente a cada um dos jarros de fermentação como conservante e os jarros de fermentação foram selados com rolhas. A fermentação foi permitida por vinte e oito (28) dias, nos quais foram realizadas diferentes análises físicas (pH, temperatura, acidez titulável, peso específico, açúcar redutor, teor alcoólico) para cada dia. A avaliação sensorial dos produtos acabados foi conduzida e o nível geral de aceitação foi determinado. As contagens viáveis de *Saccharomyces cerevisiae*, *Saccharomyces bayanus* e *Saccharomyces elipsoides* após crescimento em meio YPG, foram de $3,3 \times 10^6$, $3,5 \times 10^6$ e $3,8 \times 10^6$ cfu/ml, respectivamente. Houve diminuição do pH, redução do açúcar e do peso específico das amostras de vinho, enquanto, aumento da acidez titulável, do teor de álcool e da temperatura. Houveram diferenças significativas ($p < 0,05$) na aceitabilidade geral das amostras de vinho com vinho fermentado de *S. bayanus*, tendo a menor aceitação pelos provadores. O abacaxi é uma boa matéria-prima compatível para a produção de vinho usando várias amostras de levedura.

PALAVRAS-CHAVE: fermentação; saccharomyces; vinificação.

Introduction

Wine production is traditionally performed using red grapes and apples as fruit sources both locally and internationally. Red grapes and apples are not successfully grown in Nigeria in reasonable commercial quantities; thus, local wine production will invariably largely depend on the importation of these fruits for the production of standard wine that will draw reasonable market acceptability (Archibong et al 2015). However, Nigeria has some fruits whose must serve as substrates for wine production, but are not being used. These local fruits are grown in commercial quantities in the country and most times are not properly preserved post-harvest; thus, a good number of the fruits are lost to post-harvest spoilage.

Therefore, there is a need to channel these fruits away from spoilage to other value chain processes such as wine production. Some researchers (Idise 2012; Archibong et al 2015; Dellacasa et al 2016; Qi et al 2017; Chaudhary et al 2019) have pointed out the potential of harnessing pineapple as an indigenous Nigerian fruit for wine production due to its richness in sugars that can support yeast proliferation and consequent fermentation. A successful standard wine production using indigenous fruits will turn out to be a source of foreign exchange, grow the local currency, and contribute immensely to the gross domestic product (GDP) margin of the country. The quality of wine produced greatly depends on the yeast strains (Idise 2012). Published research articles (Archibong et al 2015; Qi et al 2017; Chaudhary et al 2019) on the use of pineapple for wine production have employed *Saccharomyces cerevisiae* for wine production. However, this research seeks to analyze a comparative wine production using other different yeast samples (*Saccharomyces bayanus* and *Saccharomyces ellipsoides*) to find out if industrialists stand a chance of producing quality wine with pineapple using some known brewing-grade yeasts aside from widely known *Saccharomyces cerevisiae*; as this will help in the expansion of winery as an industry in Nigeria.

Material and Methods

Sample collection

Pure culture of wine yeasts (*Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces ellipsoides*) were sourced from an indigenous wine production company in Imo State, Nigeria. Pineapple fruits were purchased from Eke-Awka Market, Anambra State, Nigeria.

Viability test of the yeast samples

The viability test of the yeast samples was determined with the viable count method according to Mirek and Tecza (2014). A one ml aliquot of the slurry containing each yeast samples was transferred into a test tube containing 5 ml phosphate buffer solution to form a stock solution, then 1ml from each tube was serially diluted using a tenfold serial dilution, inoculated onto YPG medium, and incubated at 28°C for 2 days after which colonies were counted.

Preparation of the must

Pineapple fruits (*Ananas comosus*) were washed, peeled, and sliced. The sliced fruits were then poured into a sterile juice extractor (in bits), connected to a power source, and switched on to blend and extract the juice. A 1000 ml aliquot of the fruit juice was collected in turns into a sterile 1000 ml fermenting flask, properly labeled, and was then pasteurized at 60°C for twenty minutes in a water bath (Jana 2011).

Fermentation

Twenty-one (21) different fermenting jars (3800 ml capacity) were clearly labeled with the name of the fruit and the corresponding yeast samples. To each fermenting jar was poured sterilized 3000 ml must comprising of a mixture of juice and 6 ml of each yeast inoculum. Sodium metabisulphite (0.4g/l) which serves as a preservative was also aseptically added to each of the fermenting jars and the fermenting jars were sealed with corks. Fermentation was allowed for twenty-eight (28) days within which different physical analyses were carried out (pH, temperature, titratable acidity, specific gravity, reducing sugar, alcoholic content) for each day (Ogodo 2015).

Determination of pH

This was determined by using the pH meter as described by Idise (2012).

Determination of titratable acidity

This was determined by the volumetric method, according to Analysis of Association of Analytical Chemist AOAC, (2005).

Determination of reducing sugar

This was done by the method of Miller (1959).

Determination of specific gravity

This was determined by the gravimetric method as recorded in AOAC (1980) using a relative density bottle

Determination of alcohol content

This was determined by the methods of the AOAC (1980).

Determination of temperature

This was done as described by Idise (2012).

Sensory and organoleptic evaluation

A nine-point scale hedonic test was conducted on the finished products by a panel of 25 trained and experienced wine tasters. The purpose was for both characterizations of the wine flavor profile and comparison with an existing brand. The test wine and control wine samples were anonymously presented to the panel along with a questionnaire to document their observations David et al (2013). They characterized the wine produced based on some attributes which were taste, aroma, color and texture.

Statistical Analyses

One-way Anova was employed to analyze the significant differences amongst the test samples at 95% confidence interval, using SPSS software version 22.

Results

Viability test of yeast samples

The result of the viability test of the yeast samples is as shown in Table 1. The viable counts of *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces ellipsoides* after growth on YPG medium were 3.3×10^6 , 3.5×10^6 and 3.8×10^6 cfu/ml respectively.

Table 1 Viability tests of pure cultures of yeast samples.

Yeast Samples	Viable Counts (x10 ⁶ cfu/ml)
<i>Saccharomyces cerevisiae</i>	3.3
<i>Saccharomyces bayanus</i>	3.5
<i>Saccharomyces ellipsoides</i>	3.8

Determination of pH

There was a reduction in the pH of the different fermented pineapple wines. The pH of pineapple wine produced from *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces ellipsoides* reduced from 5.20 to 5.00, 5.20 to 5.04 and 5.20 to 5.00 respectively across the 28 days of fermentation (Figure 1).

Determination of titratable acidity

The comparative analysis results of the titratable acidity of pineapple wine were shown in Figure 2. There was an increase in the titratable acidity of the pineapple wines. The titratable acidity of the pineapple wine from *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces ellipsoides* increased from 0.41 to 1.10, 0.45 to 0.61, and 0.45 to 1.18 respectively across the 28 days of fermentation.

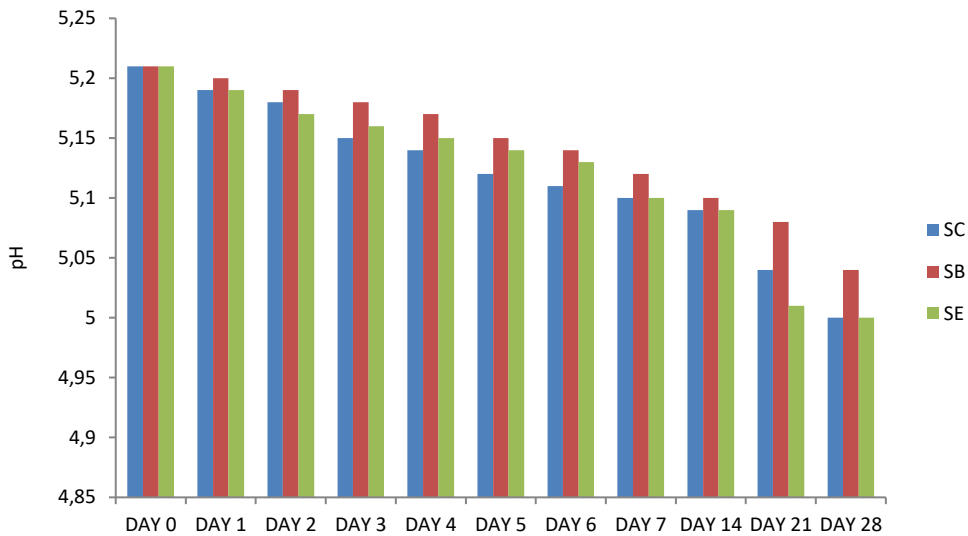


Figure 1 Comparative analyses of the pH of pineapple wine. SC: *Saccharomyces cerevisiae*. SB: *Saccharomyces bayanus*. SE: *Saccharomyces ellipsoides*.

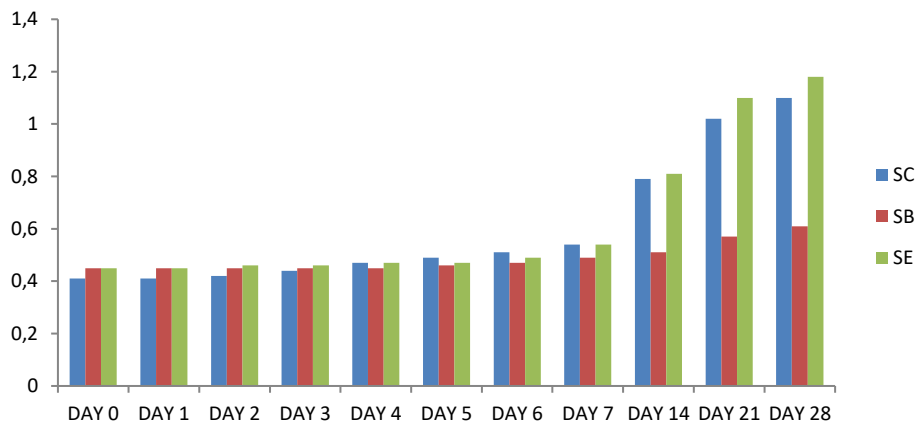


Figure 2 Comparative analyses of titratable acidity of pineapple wine. SC: *Saccharomyces cerevisiae*. SB: *Saccharomyces bayanus*. SE: *Saccharomyces ellipsoides*.

Determination of reducing sugar

There was a decrease in reducing sugar of the pineapple wines. The reducing sugar of the wine produced from *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces ellipsoides* decreased from 4.50 mg/ml to 2.10 mg/ml, 4.50 mg/ml to 2.10 mg/ml and 4.50 mg/ml to 2.30 mg/ml respectively across the 28 days of fermentation (Figure 3).

Determination of specific gravity

There was a reduction in the specific gravity of the pineapple wine. The specific gravity of the pineapple wine from *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces ellipsoides* reduced from 1.005 to 0.9906, 1.005 to 0.9904, and 1.005 to 0.9907 respectively across the 28 days of fermentation (Figure 4).

Determination of alcohol content

The results of the comparative analyses of the alcohol content of pineapple wine were shown in Figure 5. The alcohol content of the pineapple wine produced from *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces ellipsoides* increased from 0.13% to 6.60%, 0.20% to 6.75% and 0.26% to 6.53% respectively across the 28 days of fermentation.

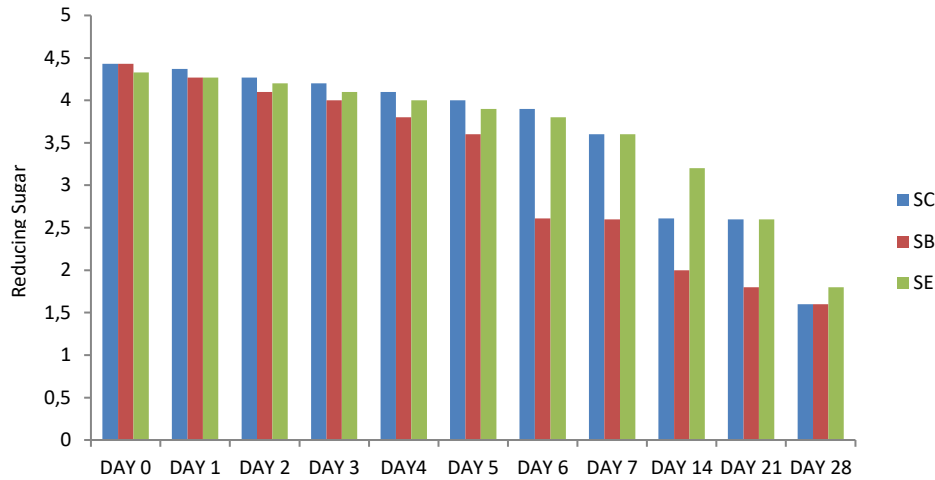


Figure 3 Comparative analyses of reducing sugar of pineapple wine. SC: *Saccharomyces cerevisiae*. SB: *Saccharomyces bayanus*. SE: *Saccharomyces ellipsoides*.

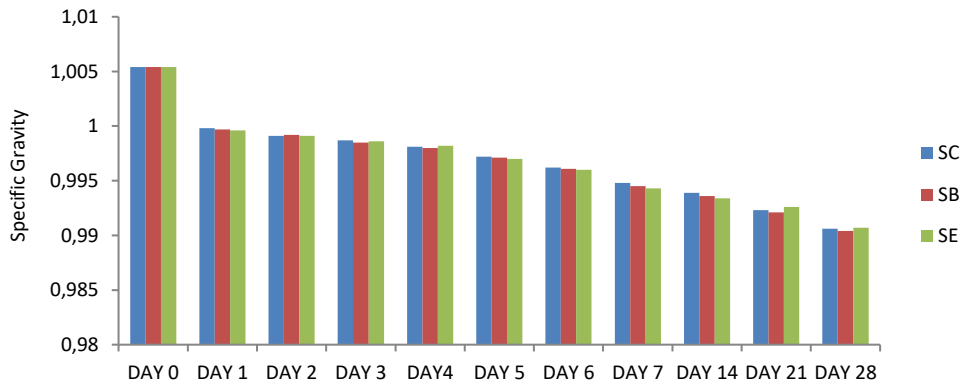


Figure 4 Comparative analyses of specific gravity of pineapple wine. SC: *Saccharomyces cerevisiae*. SB: *Saccharomyces bayanus*. SE: *Saccharomyces ellipsoides*.

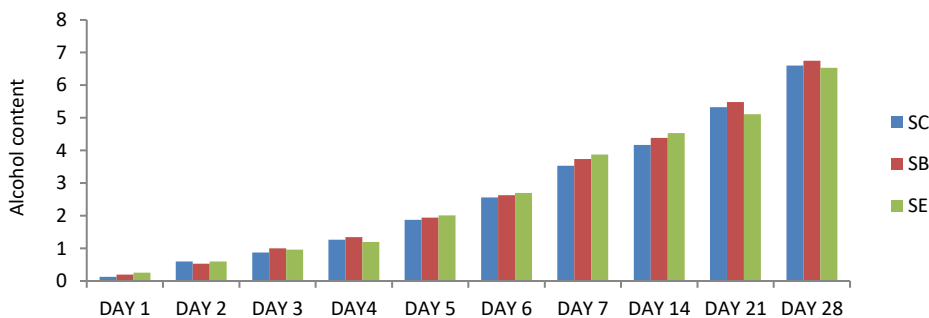


Figure 5 Comparative analyses of alcohol content of pineapple wine. SC: *Saccharomyces cerevisiae*. SB: *Saccharomyces bayanus*. SE: *Saccharomyces ellipsoides*.

Determination of Temperature

The results of the comparative analyses of the temperature of pineapple wine were given in Figure 6. The temperature of pineapple wine produced from *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, and *Saccharomyces*

ellipsoides increased from 24.0°C to 24.2°C, 24.0°C to 24.2°C, and 24.0°C to 24.1°C respectively across the 28 days of fermentation.

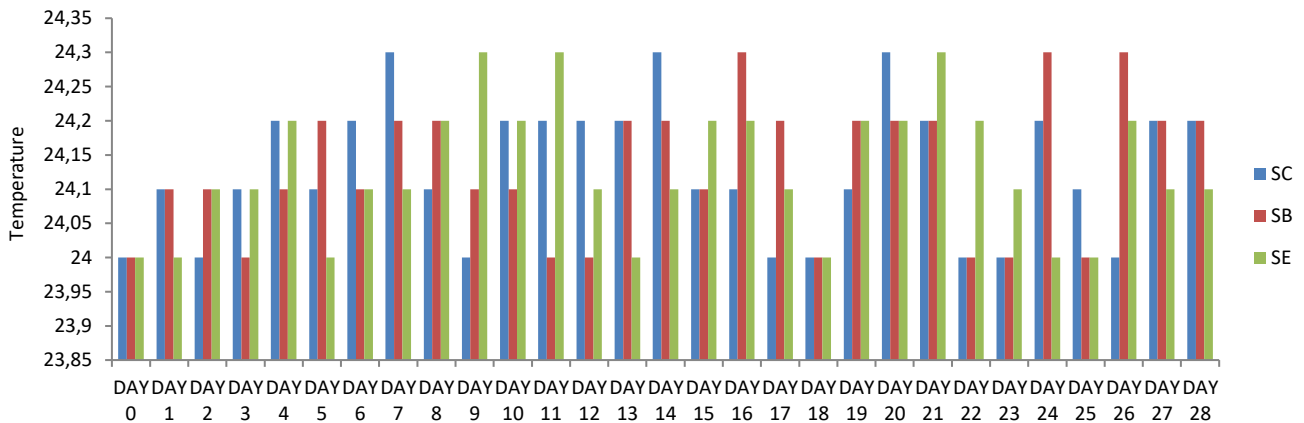


Figure 6 Comparative analyses of alcohol content of pineapple wine. SC: *Saccharomyces cerevisiae*. SB: *Saccharomyces bayanus*. SE: *Saccharomyces ellipsoides*.

Sensory and Organoleptic Evaluation

Taste panelists agreed on the wine taste from the test samples as all sweet. The aroma was graded as fruity smell, the color as golden yellow color and they all had smooth texture (table 2). Overall acceptability of the wine samples in comparison with standard table wine showed significant difference ($p < 0.05$) in *Saccharomyces bayanus* wine, while no significant differences existed amongst other test wine groups as shown in table 3.

Table 2 Sensory attributes of wine samples.

Sensory Parameters	<i>S. cerevisiae</i> wine	<i>S. bayanus</i> wine	<i>S. ellipsoides</i> wine	Table wine
Taste	Sweet	Sweet	Sweet	Sweet
Aroma	Fruity smell	Fruity smell	Fruity smell	Fruity smell
Colour	Golden-yellow	Golden-yellow	Golden-yellow	Pink
Texture	Smooth	Smooth	Smooth	Smooth

Table 3 Mean overall acceptability of the test wine samples.

Wine Samples	Mean \pm SD
<i>S. cerevisiae</i> wine	8.04 \pm 0.16
<i>S. bayanus</i> wine	6.88 \pm 0.28*
<i>S. ellipsoides</i> wine	8.44 \pm 0.17
Table wine	8.72 \pm 0.12

*stands for the significantly different acceptance level of wine sample

Discussion

The viable counts of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, and *Saccharomyces ellipsoides* as shown in Table 2, shows that the yeast samples were active and fit for use for the winemaking process. A comparative analysis of the changes in pH of the product wine showed a reduction in the pH within the period of fermentation from 5.20 to 5.00 this finding did not agree with the work of Idise (2012) who produced pineapple wine with *Saccharomyces cerevisiae* in which the pH was as low as 3.0-3.5. His reported low pH value could be as a result of the increased fermentation rate of the pineapple must because it was augmented with 6 L sugar solution; while our research method used just pure pineapple must with no sucrose augmentation hence, the pH range of 5.00-5.20. The reduction in pH could be attributed to the metabolic activities of the yeasts involved in the production of carbon-dioxide which is dissolved in the must to form carbonic acids thus, lowering the pH. The increase in titratable acidity is due to the production of carbon-dioxide which is dissolved in the must forming carbonic acids which add to the acids naturally found in the fruits. An excessive increase in the titratable acidity could lead to the production of wines with a vinegar-like taste and the eventual death of the yeast isolates. Titratable acidity reported by Idise (2012) falls in the same range as that obtained from our fermentation using the three yeast samples. The general reduction in reducing sugar and a

specific gravity of the wine is due to constant utilization of the sugar in the must by the yeasts isolates and subsequent conversion to alcohol.

A comparative analysis of the alcohol content of the product wines showed a steady increase in all the produced wines. This could be as a result of the production of ethanol through the conversion of sugars in the must due to the yeast's metabolism. Idise (2012) and Qi et al (2017) reported an increased percentage of alcohol concentrations from their pineapple wine production experiments. However, Qi et al (2017) reported 10.2% alcohol which was higher than our alcoholic content of 6.53-6.75%. Likewise, Idise (2012) reported a different alcohol concentration of 1.35% which is notably lower than the alcohol concentration of our wine. These noted differences in alcohol concentrations could be attributed to yeast strains and also to the duration of fermentation. Idise (2012) fermented for 6 days, Qi et al (2017) fermented for 7 days while our fermentation lasted for 28 days. There was no significant difference ($p>0.05$) in the alcohol content amongst our different product wine. There was no significant ($p>0.05$) difference in the temperature amongst the compared wine samples. The temperature ranges of 24.0°C to 24.2°C, have been posited by Thias et al (2006), to support good growth of yeast samples during winemaking. This is because the excessive increase in temperature of the wine could lead to the sudden death of the yeast isolates thereby halting the fermentation process. The taste panelist's recommendations show a notable level of acceptance to the sensory attributes of the test wine samples ranging from their flavor to appearance, and their overall level of likeness and acceptability is suggestive of the fact that pineapple serves as a good locally sourced raw material for indigenous wine production. Idise (2012) reported good acceptability of pineapple wine based on its flavor and aroma, Archibong et al (2015) and Dellacassa et al (2016) reported its acceptance based on flavor while Qi et al (2017) reported pineapple wine acceptance based on it being a low alcoholic wine.

Conclusion

There is a great need for the development of industries that will make use of local and cheap raw materials like pineapple to produce wine to take care of the increasing demand of wine consumption and cut off post-harvest preservation losses of fruits in the country. Pineapple makes a good compatible raw material for wine production using varying yeast samples.

Conflict of Interest

The authors declare no conflict of interests

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