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DISCOVERY

Comparative study of wines produced from Zobo/Pineapple peel using monocultures and mixed cultures of *Saccharomyces cerevisiae* and *Candida ethanolica*

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ABSTRACT

The high cost of imported wines and the limited supply of grape which is the fruit of choice in wine production have necessitated the search for alternative raw materials. This research evaluates the quality of wines produced from *Hibiscus Sabdariffa* (zobo) and

Ananascomosus (pineapple) peels, singly and combined, using monocultures and mixed cultures of *Saccharomyces cerevisiae* and *Candida ethanolica*. Musts were prepared with zobo and pineapple peels in the ratio 1:3 by dry weight and inoculated with starter cultures. Uninoculated zobo and a mixture of zobo and pineapple were used as controls. Microbiological and physico-chemical parameters were monitored during fermentation at 4 day interval for 12 days. The sensory attributes of the products were evaluated using 7-point hedonic rating. The ethanol concentration of the two most preferred samples and the controls were determined using gas chromatography - mass spectrometry (GC-MS). The total yeast count of the samples ranged from 7 to19 log cfu/ml. The pH ranged from 1.7 to 2.5 while temperature ranged from 26.5 to 27.8°C. The titratable acidity ranged from 4.3 to 9.0g tartaric acid/100ml. Specific gravity ranged from 0.91 to 1.03. The sensory evaluation showed that zobo/pineapple mixture fermented with *S. cerevisiae* was scored highest in terms of overall acceptability. The GC-MS analysis revealed that zobo/pineapple mixture fermented with the mixed cultures had the highest alcohol of 11.69% v/v. This study has shown that acceptable wine of good quality can be produced from hot water extracts of zobo and pineapple peels and *Saccharomyces cerevisiae* should be adopted as starter culture for a better product quality. The industrial potential of this product could also be further exploited.

Keywords: Monocultures, Mixed cultures, Ethanol concentration, Gas chromatography – Mass spectrometry

1. INTRODUCTION

Wine is a famous alcoholic beverage enjoyed by most adults. It is manufactured from fermentation of fruit juice using *Saccharomyces cerevisiae* (Awe *et al.,* 2013). The fruit juice may be fortified with sugar to improve the production of alcohol by yeasts (Duarte *et al.*, 2010; Idolo *et al.,* 2012). Grape has been the most utilized fruit for the production of wine but due to its unavailability, alternative raw materials have been harnessed (Alobo and Offonry, 2009; Chilaka *et al.*, 2010). Wine production is understood to depend on the effectiveness of yeast to convert sugar into alcohol and esters. Wine contributes a total energy intake of 10% to 20% in adult males (Chilaka *et al.*, 2010). Research has shown that moderate intake of alcohol is cardio-protective; however, excessive consumption causes alcohol-induced liver cirrhosis and depresses the central nervous system (Rall, 1990). It alters the intracellular NAD+/NADH ratio and this affects the equilibrium constant of a number of important metabolic reactions that utilize these two cofactors (Murray *et al.*, 1996). Alcoholism leads to fat accumulation in the liver, hyperlipidemia and ultimately cirrhosis (Ajani *et al.,* 2012).

Hibiscus sabdariffa is a plant which belongs to the order *Malvaceae* and has its source from East Africa (Ilondu and Iloh, 2007; Adesokan *et al.*, 2013). It is a yearly plant that grows to a height of 8 ft with smooth red stems (Da-costa-Rocha *et al.*, 2014). It is basically developed in the tropics; it grows majorly in the northern part of Nigeria because of the predominant ideal climatic condition. The plant is generally developed for its hard filaments and it is notable for its edibility and restorative properties. The calyx is the most utilized part of the plant. It is used to make a juice called "Karkade" in Sudan and "Zoborodo" in Nigeria respectively. The leaves and seeds are also made into servings of mixed greens, potherbs and curries (Da-costa-Rocha *et al.,* 2014; Okereke *et al.*, 2015). *Hibiscus sabdariffa* has been shown to have antibacterial (Liu *et al.,* 2005), antinociceptive (Ali *et al.*, 2011), hepato protective and anticancer properties (Mossalam *et al.*, 2011).

Pineapple (*Ananascomosus*) belongs to the family *Bromeliaceae*. It has therapeutic, financial, mechanical and sustenance values (Patrick, 2011). Pineapple peel is rich in hemicelluloses, cellulose and sugars. Another component of pineapple peels is bromelain which comprises of enzymes such as thiolendopeptidase, cellulase, escharase, phosphatase, peroxidase, and protease inhibitors (Bhattacharyya, 2008). Mensah and Twumasi (2016) demonstrated that pineapple waste can be used as substrate for the production of single cell protein. The proximate analysis of pineapple revealed that it has 81.2 – 86.2% water, 2 – 3% fiber, 13 – 19% aggregate solids (glucose, fructose, sucrose), 0.1 % lipid and nitrogenous mixes, 25 – 30% protein (Patrick, 2011). Ajani *et al.* (2012) reported that a three month, oral administration of pineapple drink to wister rats showed some hematological and biochemical advantages. It expanded the red platelet of the rats and filled in as a safe promoter by increasing the aggregate white platelet. This research is aimed at evaluating the quality of wines produced from *Hibiscus sabdariffa* (zobo) and *Ananascomosus* (pineapple) peels, singly and combined, using monocultures and mixed cultures of *Saccharomyces cerevisiae* and *Candida ethanolica*.

2. MATERIALS AND METHODS

Dry *H. sabdariffa* calyces (zobo) were purchased from Sabo Market, Kaduna while fresh pineapple peels and sugar (sucrose) were purchased from Choba Market, Port Harcourt, Nigeria. *Saccharomyces cerevisiae* and *Candida ethanolica* were isolated from palm wine.

Preparation of Must

A modification of the method described by Ifie *et al.* (2012) was used to prepare the must for fermentation as presented in Figure 1. Must was prepared from *H. sabdariffa* calyces, solely and in combination with pineapple peels in the proportion of 1 part of *H. sabdariffa* to 3 parts of pineapple peels and 45 parts of water. Measure 75 g of *H. sabdariffa* and 225 g of pineapple peels were washed in tap water and boiled in 3375ml water for 5 min. The mixture was allowed to cool to room temperature after which it was filtered using a muslin cloth. The filtrate was fortified with 202.5 g of white granulated sugar and sterilized at 121 °C and 15 psi for 15 min.

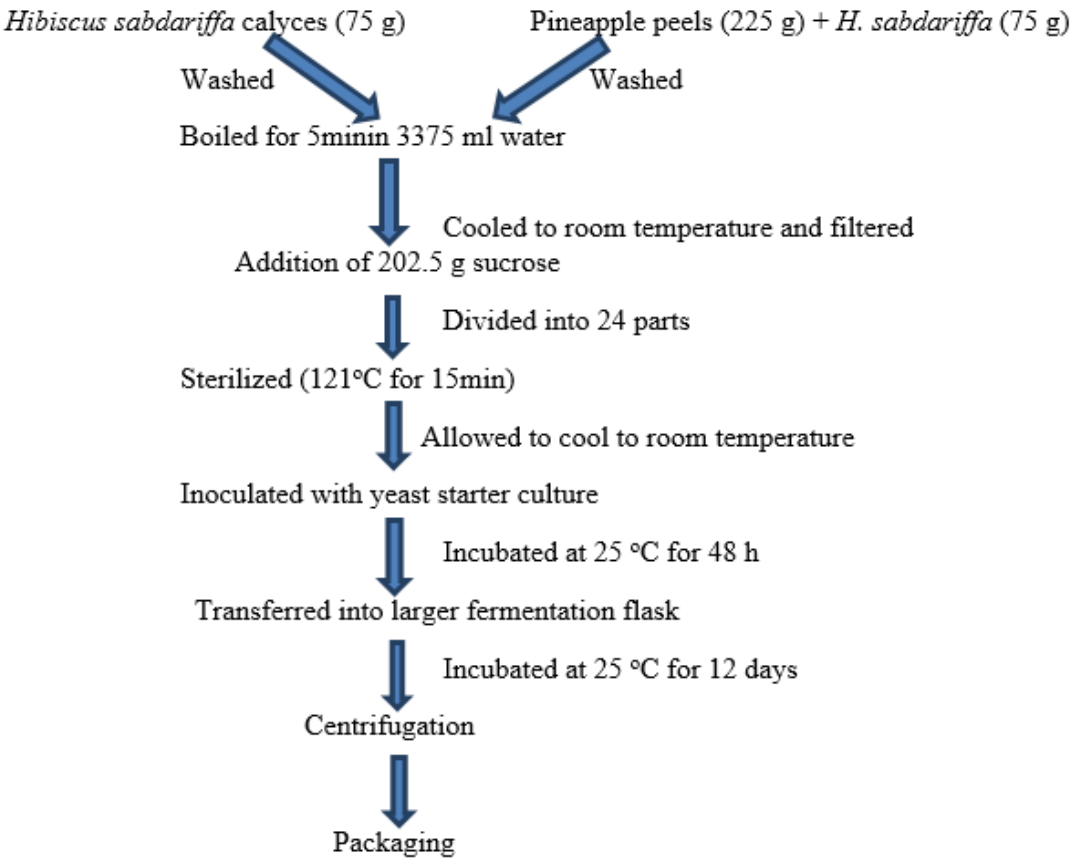

Figure 1 Production of zobo wine and zobo/pineapple wine

Inoculum Development

Exactly 200 ml of must were transferred to 500 ml conical flasks and inoculated with 1 X 10^8 cfu/ml *Saccharomyces cerevisiae* and *Candida ethanolica* as presented in Table 1. The samples were incubated at 25 °C for 48 h and shaken manually at 4 h intervals to aid aeration.

Table 1 Sample description

SAMPLE CODE	DESCRIPTION
Z	Zobo
ZP	Zobo + Pineapple
ZS	Zobo + *Saccharomyces cerevisiae*
ZC	Zobo + *Candida ethanolica*

ZSC	Zobo + *Saccharomyces cerevisiae*+ *Candida ethanolica*
ZPC	Zobo + pineapple+ *Candida ethanolica*
ZPS	Zobo + Pineapple + *Saccharomyces cerevisiae*
ZPSC	Zobo + Pineapple + *Saccharomyces cerevisiae*+ *Candida ethanolica*

Fermentation of Must

The method described by Alobo and Offonry (2009) was used for fermentation of must. Exactly 5ml of developed inoculum was aseptically transferred to 250ml must in 500ml conical flasks. Samples for mixed culture fermentation were inoculated with 2.5ml of each developed inoculum. The samples were incubated at 25 ^{o}C for 12 days with manual shaking at 4h intervals. Microbiological and physicochemical parameters were monitored at 4 day intervals.

Total yeast count

The total yeast count of the samples was monitored using the method described by Ebabhi *et al.,* (2013). Exactly 0.1ml of the sample was serially diluted to 10^{-10} in 9.9 ml physiological saline. The diluted sample was plated out in triplicates on glucose yeast agar containing 1% lactic acid. After incubation at 25^{o}C for 48h, colonies were counted and used to calculate total yeast count (TYC) as follows:

TYC= average number of colonies x dilution factor x correction volume

Physico-chemical Analysis

Determination of pH and Temperature

The samples were monitored for pH and Temperature change using the method described by AOAC (1984). Buffers (pH 4 and pH 7) were used to calibrate the pH meter (PHS – 25, UK) after which Samples were withdrawn at 4 day intervals for measurement of pH and temperature.

Titrable Acidity

The titratable acidity was evaluated by the method described by Okeke *et al.*, (2015). Exactly 200ml of water was transferred into a 500ml conical flask and heated. The indicator (1ml, 1% phenolphthalein) was added to it and titrated against 0.1 M NaOH to give a light pink coloration. The solution was thereafter made acidic with 5 ml of sample followed by further titration with a solution 0.1 M NaOH to reach end point as indicated by pink coloration. The titratable acidity expressed as g/100 ml tartaric acid was mathematically calculated thus:

$$\text{g/100 ml tartaric acid} = \frac{M \times Vb \times 75 \times 100}{Vs \times 100}$$

Where:

M = Molarity of sodium hydroxide

Vs = Volume of the must

Vb = Volume of sodium hydroxide

Determination of Reducing Sugar

Reducing sugar was determined using the method described by Fakruddin *et al.*, (2015). Approximately five millimeter of the sample was centrifuged (SorvallGLC – 4, Germany) at 30,000xg for 5 min. 1 ml of the supernatant was mixed with 1 ml 3,5 – dinitrosalicyclic acid (DNS) in a test tube and heated in a water bath for 10 min. It was allowed to cool to ambient temperature. The volume of the solution was adjusted to 12ml with the addition of distilled water. A blank was prepared by mixing 1ml of distilled water with 1ml of DNS. The samples' optical density was read in a spectrophotometer (Spectrum lab 735, Germany) at 540 nm. The resultant reducing sugar concentration was estimated using a Glucose standard curve (Darman *et al.*, 2011).

Determination of Specific Gravity

The specific gravity of the samples was estimated using the method described by Ifie *et al.* (2012). A 25ml specific gravity bottle was washed with distilled water and oven-dried. The weight of the specific gravity bottle was recorded as R1. The bottle was then filled

to the brim with water and weighed. The weight was recorded as R2. The bottle was rinsed with the sample, there after filled to the brim with the sample and the external part of the bottle was dried with cotton wool. The sample was weighed and recorded as R3. The specific gravity of each sample was calculated thus:

$$\text{Specific gravity} = \frac{R3 - R1}{R2 - R1}$$

$$= \frac{\text{Weight of volume samples}}{\text{Weight of equal volume of water}}$$

Determination of Alcohol Concentration

The alcohol concentration of the more preferred samples, as determined by 7-point hedonic scale, was analyzed on the Head Space - Gas Chromatography–Mass Spectrometry (HS-GC-MS) manufactured by Agilent Chemstation, fitted with an oven. 10 ml of each wine sample was injected into vials, using the headspace-loop transfer approach at hotness level above 60°C. The samples were equilibrated and set for 20 min for it to contain the samples to be injected and pressurized at 15 psi for 9 s The MS transfer line was maintained at 280°C while the MS source and the quadruple were maintained at 230°C and 150°C, respectively. The MS electron multiplier voltage was set to a gain factor of 1. The test limit was set at 20 to 700 at a limit of 150 and a sample number of 4, at check-rate of 2.02 scans/s. The peaks were obtained by printing from the output on the screen.

Sensory evaluation of the wines

The quality attributes of the products were evaluated using the seven-point hedonic scale as described by Singh-Ackbarali and Maharaj (2014). The products were served to a 10-man panel that is familiar with the product. A commercial red wine was also presented to the panelists without revealing the identity of the products. The panelists evaluated the coded samples based on taste, aroma, sourness, color, and overall acceptability. The panelists were provided with water to rinse their mouths after each sample testing. The data recovered were analyzed using Statistical Package for Social Sciences, software version 20.0 at $p < 0.05$.

3. RESULT

Total Yeast Count

The total yeast count of the samples during fermentation is presented in Figure 2. The values ranged from 7 to 19 log cfu/ml.

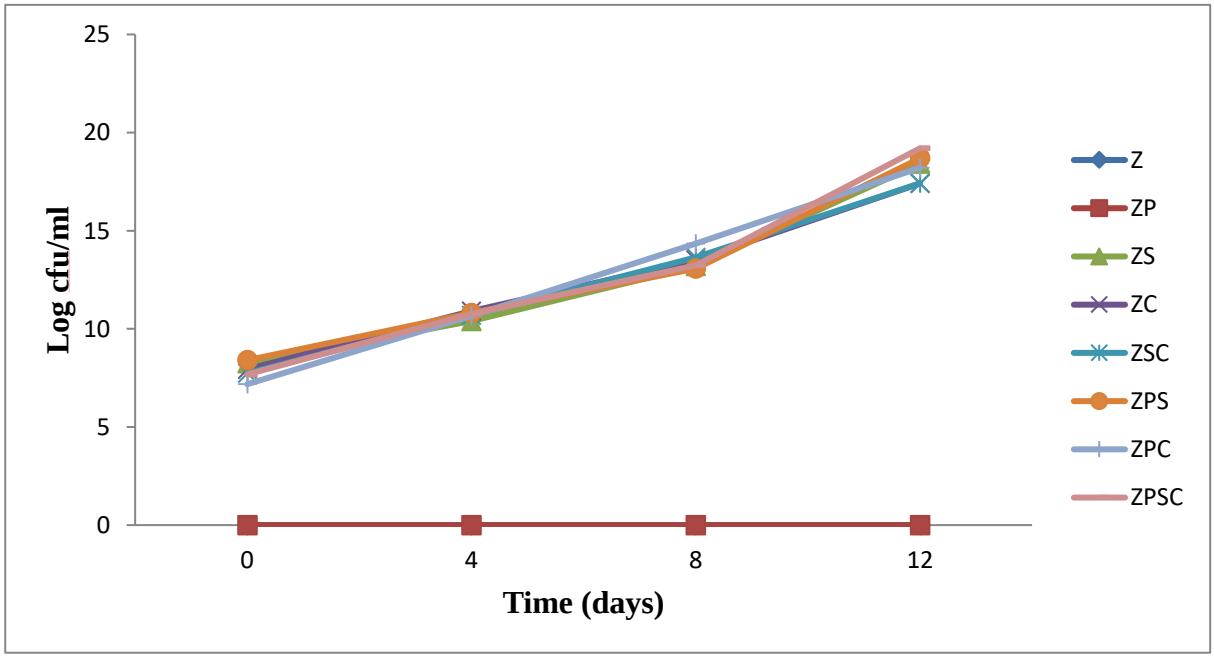

Figure 2 Total yeast count of samples during fermentation.

Key: Z = Zobo; ZP = Zobo + Pineapple; ZS = Zobo + *Saccharomyces cerevisiae;* ZC = Zobo + *Candida ethanolica;* ZSC = Zobo + *Saccharomyces cerevisiae*+ *Candida ethanolica;* ZPC = Zobo + pineapple+ *Candida ethanolica;* ZPS =Zobo + Pineapple + *Saccharomyces cerevisiae;* ZPSC = Zobo + Pineapple + *Saccharomyces cerevisiae*+ *Candida ethanolica*

Change in pH

The change in pH of the samples during fermentation is presented in Figure 3. The pH ranged from 1.7 to 2.5.

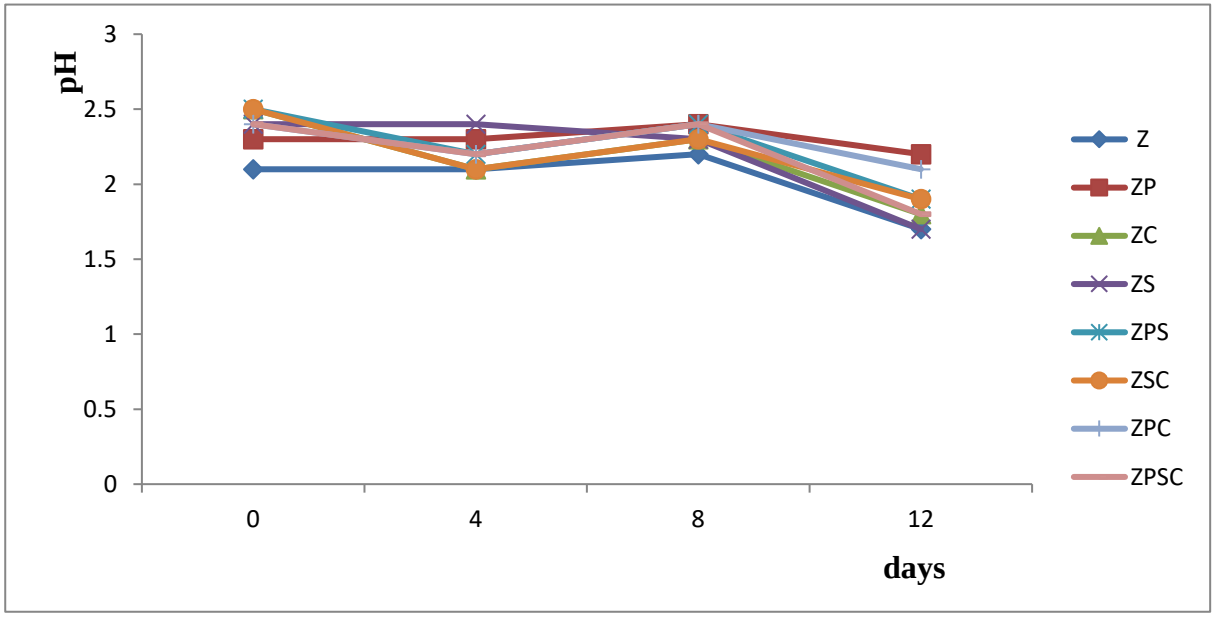

Figure 3 Changes in pH of the samples during fermentation

Key: Z = Zobo; ZP = Zobo + Pineapple; ZS = Zobo + *Saccharomyces cerevisiae;* ZC = Zobo + *Candida ethanolica;* ZSC = Zobo + *Saccharomyces cerevisiae*+ *Candida ethanolica;* ZPC = Zobo + pineapple+ *Candida ethanolica;* ZPS =Zobo + Pineapple + *Saccharomyces cerevisiae;* ZPSC = Zobo + Pineapple + *Saccharomyces cerevisiae*+ *Candida ethanolica*

Temperature

The change in Temperature of the samples during fermentation is presented in Figure 4. The temperature ranged from 26.5 to 27.8°C.

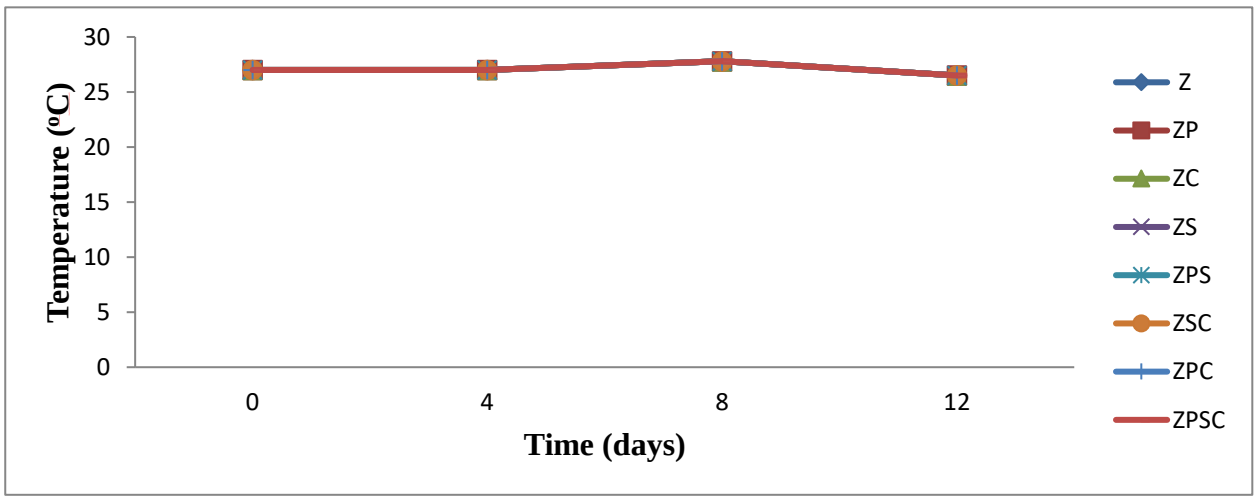

Figure 4 Changes in Temperature of samples during fermentation

Key: Z = Zobo; ZP = Zobo + Pineapple; ZS = Zobo + *Saccharomyces cerevisiae;* ZC = Zobo + *Candida ethanolica;* ZSC = Zobo + *Saccharomyces cerevisiae*+ *Candida ethanolica;* ZPC = Zobo + pineapple+ *Candida ethanolica;* ZPS =Zobo + Pineapple + *Saccharomyces cerevisiae;* ZPSC = Zobo + Pineapple + *Saccharomyces cerevisiae*+ *Candida ethanolica*

Titratable Acidity

The change in titratable acidity of the samples during fermentation is presented in Figure 5. The titratable acidity ranged from 4.3 to 9.0g tartaric acid/100ml.

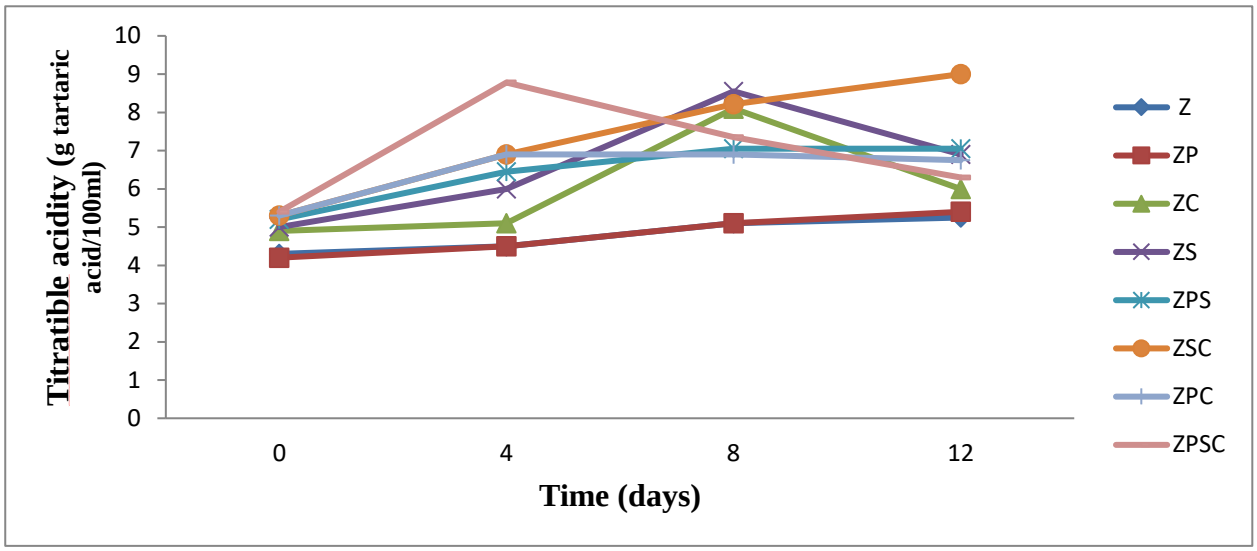

Figure 5 Changes in titratable acidity of samples during fermentation

Key: Z =Zobo; ZP = Zobo + Pineapple; ZS = Zobo + *Saccharomyces cerevisiae;* ZC = Zobo + *Candida ethanolica;* ZSC = Zobo + *Saccharomyces cerevisiae*+ *Candida ethanolica;* ZPC = Zobo + pineapple+ *Candida ethanolica;* ZPS =Zobo + Pineapple + *Saccharomyces cerevisiae;* ZPSC = Zobo + Pineapple + *Saccharomyces cerevisiae*+ *Candida ethanolica*

Specific Gravity

The change in specific gravity of samples during fermentation is presented in Figure 6. The specific gravity ranged from 0.91 to 1.03.

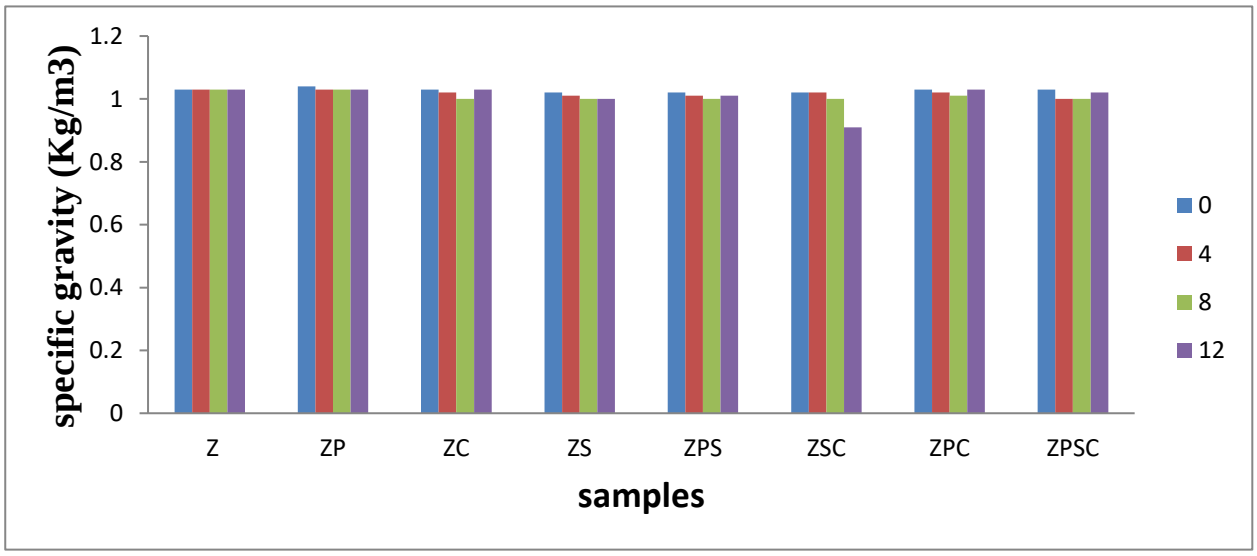

Figure 6 Changes in specific gravity of samples during fermentation

Key: Z = Zobo; ZP = Zobo + Pineapple; ZS = Zobo + *Saccharomyces cerevisiae;* ZC = Zobo + *Candida ethanolica;* ZSC = Zobo + *Saccharomyces cerevisiae* + *Candida ethanolica;* ZPC = Zobo + pineapple+ *Candida ethanolica;* ZPS =Zobo + Pineapple + *Saccharomyces cerevisiae;* ZPSC = Zobo + Pineapple + *Saccharomyces cerevisiae* + *Candida ethanolica*

Reducing Sugar

The change in reducing sugar of the samples during fermentation is presented in Figure 7. The reducing sugar ranged from 0.011 to 0.1 g.

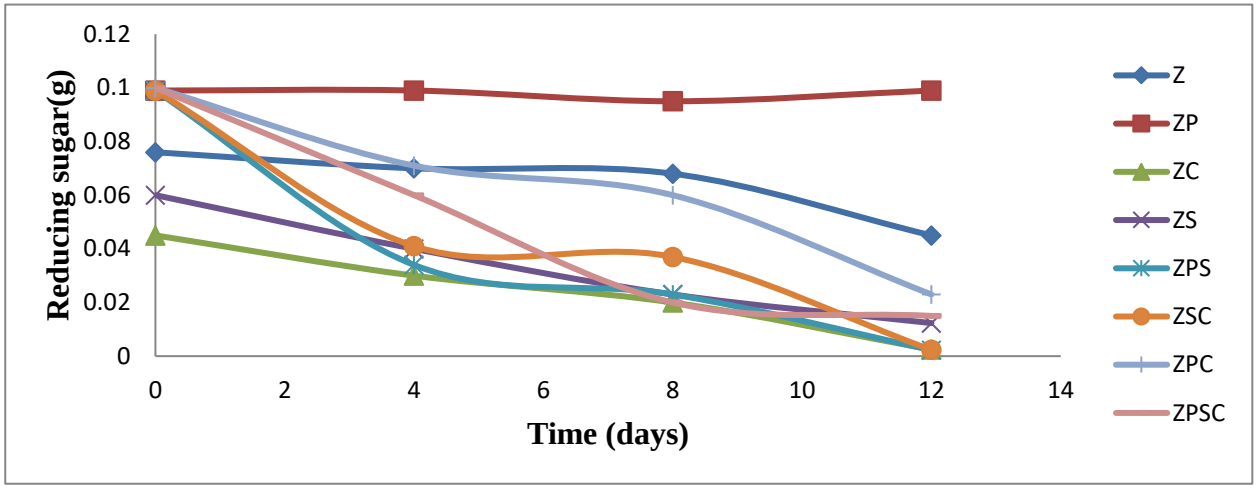

Figure 7 Change in reducing sugar of samples during fermentation

Key: Z = Zobo; ZP = Zobo + Pineapple; ZS = Zobo + *Saccharomyces cerevisiae;* ZC = Zobo + *Candida ethanolica;* ZSC = Zobo + *Saccharomyces cerevisiae* + *Candida ethanolica;* ZPC = Zobo + pineapple+ *Candida ethanolica;* ZPS =Zobo + Pineapple + *Saccharomyces cerevisiae;* ZPSC = Zobo + Pineapple + *Saccharomyces cerevisiae* + *Candida ethanolica*

Sensory Evaluation

The sensory scores of the samples by the 7-point hedonic scale is presented in Figure 8. Zobo/pineapple mixture fermented with *S. cerevisiae* and *C. ethanolica* (ZPSC) was scored highest (taste = 6.00±1.054; sourness = 6.60±0.516; aroma = 6.10±0.738; colour = 6.60 ± 0.516 and overall acceptability = 6.60±0.516).

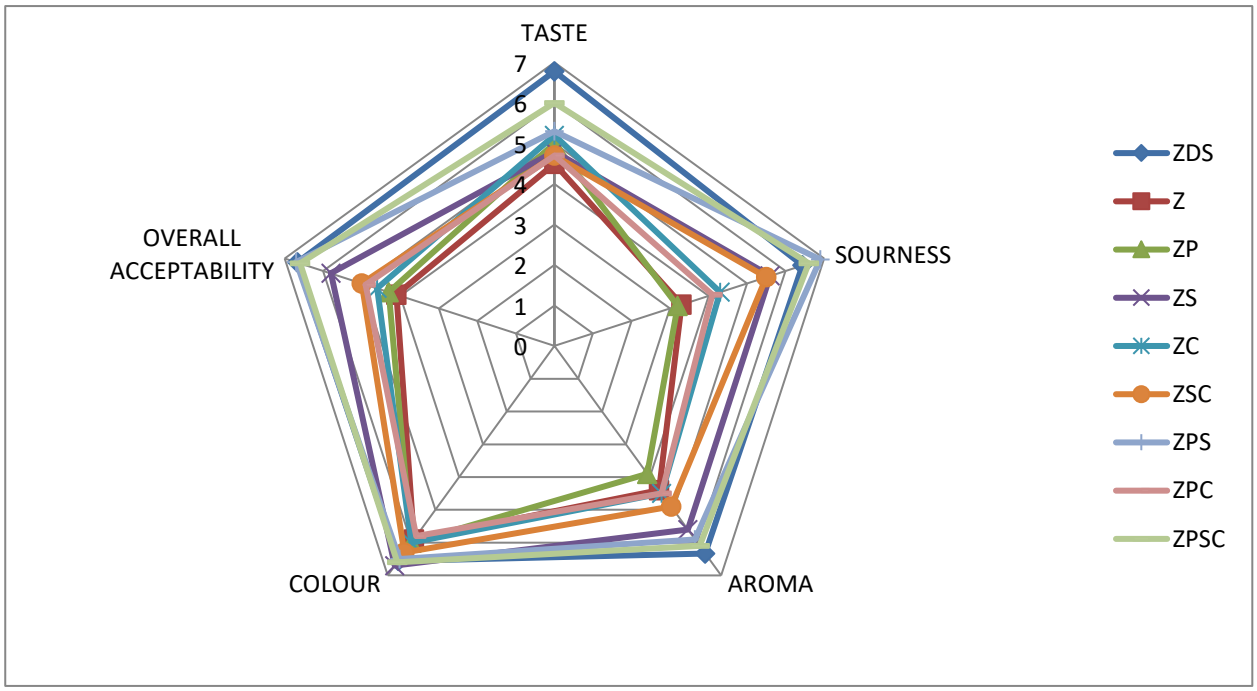

Figure 8 Sensory scores of samples by 7-point Hedonic scale

Key: ZDS = Commercial red wine; Z = Zobo; ZP = Zobo + Pineapple; ZS = Zobo + *Saccharomyces cerevisiae;* ZC = Zobo + *Candida ethanolica;* ZSC = Zobo + *Saccharomyces cerevisiae*+ *Candida ethanolica;* ZPC = Zobo + pineapple+ *Candida*

ethanolica; ZPS =Zobo + Pineapple + *Saccharomyces cerevisiae;* ZPSC = Zobo + Pineapple + *Saccharomyces cerevisiae*+ *Candida ethanolica*

Ethanol Concentration

The ethanol concentration of the controls and the more preferred samples is presented in Figure 9. Samples ZPSC and ZPS had 11.69 % and 9.19 % alcohol respectively.

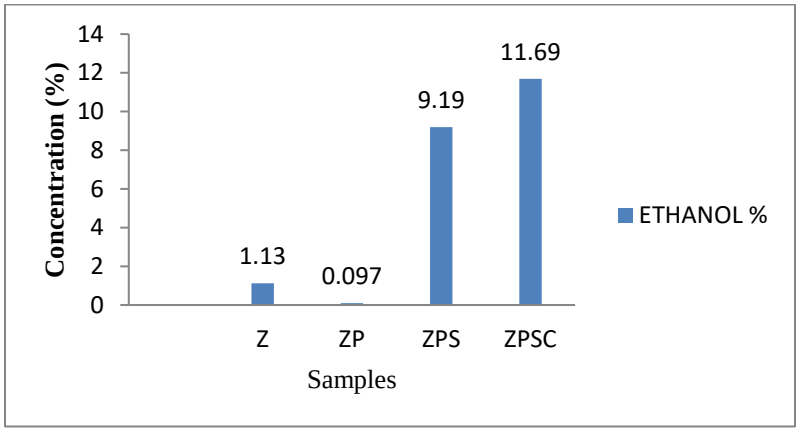

Figure 9 Ethanol concentrations of the controls and the more preferred samples

Key: Z = Zobo; ZP = Zobo + Pineapple; ZPS =Zobo + Pineapple + *Saccharomyces cerevisiae;* ZPSC = Zobo + Pineapple + *Saccharomyces cerevisiae*+ *Candida ethanolica*

Other Components of the Zobo/Pineapple Wine

The other components of the Zobo/Pineapple Wine as determined using GC-MS are presented in Figure 10. The samples were determined to contain formic acid, acetic acid, glycerol, furfural, hydroxymethyl furfural, xylose, glucose and arabinose.

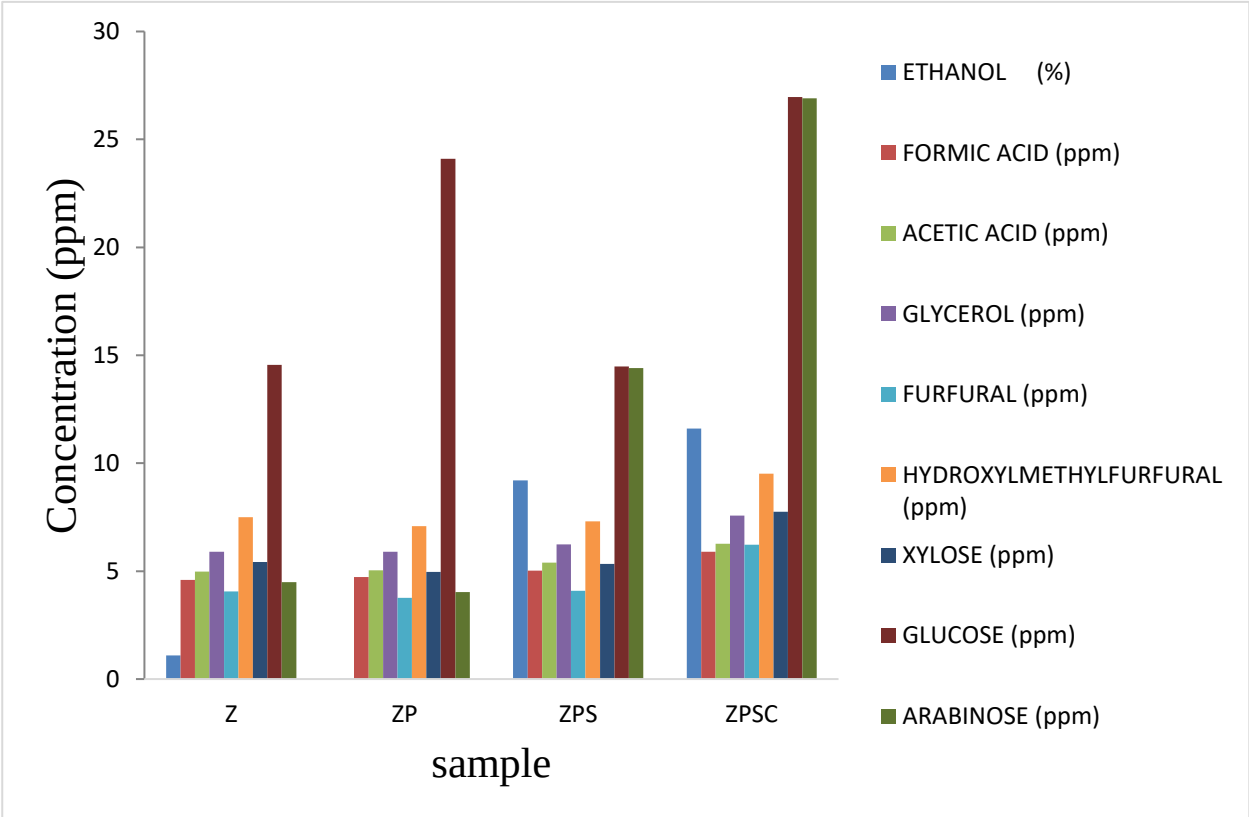

Figure 10 Other components of the Zobo/Pineapple Wine

4. DISCUSSION

In Nigeria, zobo drink is used as refreshment in social gatherings and also serves as an appetizer due to its fruity taste (Obi, 2015).The fermentation of zobo and pineapple extracts is based on the efficiency of yeast to convert sugar into alcohol and esters (Okoro, 2007).The total yeast count of the samples increased during fermentation. This is in agreement with the findings of Mishra *et al.* (2010) who reported an increase in microbial population during fermentation of tropical fruits. The increase in total yeast count of the samples inferred that the samples provided a suitable nutrient environment which the starter cultures utilized for metabolic activities and growth.

Physico-chemical analysis of the samples during fermentation showed that pH and specific gravity reduced while the titratable acidity increased with increase in fermentation time. This collaborates with the findings of Ifie *et al.* (2012) and Okeke *et al.* (2015) who reported a similar trend in vegetable wine and mixed fruit wine respectively. The decrease in pH and specific gravity was attributed to the utilization of sugars by the fermenting yeasts to produce acid and alcohol. In this study, the final products had specific gravity ranging from 0.91 to 1.03. Samples fermented with *Saccharomyces cerevisiae* had lower specific gravity compared to those fermented with *Candida ethanolica*. This implies that *Saccharomyces cerevisiae* utilized more sugar and consequently produced more ethanol than *Candida ethanolica*.

Titratable acidity and pH both measure acidity but in an unrelated manner. While pH measures the amount of acid in the wine, titratable acidity measures how tart the acid is, irrespective of the amount present. The best quality wine should have pH value of about 3.6 (Jacobson, 2006) and titratable acidity between 0.5% and 1.0% (Chilaka *et al.,* 2010). The samples had very low pH values ranging from 1.7 to 2.5. This therefore suggests that the products should not be consumed on empty stomach in order to avoid acidic reflux (Adesokan *et al.*, 2013).The titratable acidity ranged from 0.69 % to 0.9% tartaric acid. These are within the limits of a good quality wine.

There was a slight drop in temperature of the sample from 27.8^{o}C to 26.5^{o}C at the end of the fermentation period. The control samples had the same temperature as the test samples. It can therefore be inferred that the starter cultures had no effect on the temperature of the substrate. The level of reducing sugar in the samples decreased with increase in fermentation time, while the ethanol concentration increased. This is in line with the findings of Okeke *et al.* (2015) who reported a similar trend during wine production from pineapple and watermelon. The inverse relationship between reducing sugar and ethanol concentration denotes the utilization of reducing sugars for the production of ethanol and also explains the reason for the decrease in specific gravity with increase in fermentation time.

A very important aspect of new product development is consumer acceptability of the product. In this study, wines produced using various formulations were compared and sensory evaluation of the products revealed that products fermented with *S. cerevisiae* monoculture had better overall acceptability compared to monoculture samples fermented with *C. ethanolica* and mixed culture of *S. cerevisiae* and *C. ethanolica*. Samples fermented with monoculture of *C. ethanolica* were the least preferred and they differed significantly at ($p < 0.05$) from commercial red wine. Samples fermented with either mixed culture or monoculture of *S. cerevisiae* compared favorably with commercial red wine. This is in agreement with the findings from physicochemical analyses that *S. cerevisiae* utilized more fermentable carbohydrates than *C. ethanolica* thereby producing more ethanol. Consequently, more volatile organic compounds that improve the sensory attributes of the wine were produced by *S. cerevisiae*. It was also observed that fortification with pineapple peel extract improved the sensory scores of the wine. This implies that pineapple peels provided more fermentable carbohydrates that enhanced the performance of the starter cultures used in wine production. These results are similar to the findings of Mishra *et al.* (2010) who showed that fermentation with *Saccharomyces cerevisiae* monoculture produced wine of better sensory attributes than those with mixed culture of *Saccharomyces cerevisiae* and other non-*Saccharomyces* yeasts. Analysis of the most preferred samples with gas chromatography – mass spectrophotometry showed that ZPS (zobo + pineapple + *S. cerevisiae*) had alcohol concentration of 9.2% and sample ZPSC (zobo + pineapple + *S. cerevisiae*+ *C. ethanolica*) had 11.6% alcohol while the controls (Z and ZP) had 1.13 % and 0.097 % alcohol respectively. This confirms the utilization of fermentable carbohydrates for the production of ethanol.

5. CONCLUSION

Wine is a widely consumed beverage. Therefore, the use of alternative raw materials for the production of wine will improve a country's economy. This study has shown that wine of acceptable quality can be produced from hot water extracts of zobo and pineapple peels, and *Saccharomyces cerevisiae* should be adopted as starter culture for a better product quality. The industrial potential of this product could also be further exploited.

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