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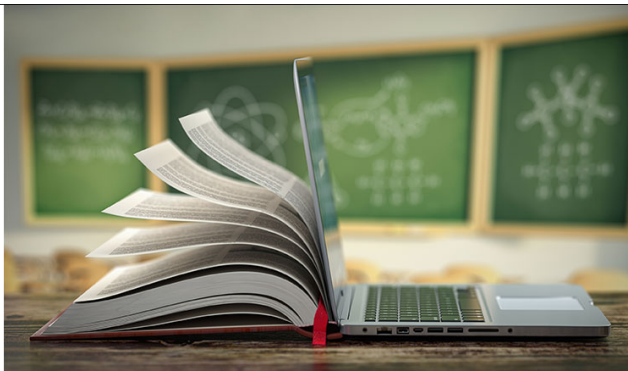
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# Production of fruit wine using a wild strain of *Saccharomyces cerevisiae* isolated from fresh palm wine for sustainable food security

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**Abstract.** The problems of post-harvest losses in the developing world have been of severe concerns especially when food security for the citizenry cannot be guaranteed. Thus, new means of the conversion of some of the periodic produce to novel stable products is an exceptional development. In this study, we carried out the production of wine from citrus fruits (*Citrus sinensis*) and Pineapple fruits (*Ananas Comosus*) using wild strains of yeast *Saccharomyces cerevisiae*. The *Saccharomyces cerevisiae* was isolated from fresh palm wine and identified using microscopic examination, morphological and biochemical characteristics. Pure yeast isolates were inoculated into sterile volumes of 200 ml and 500 ml of the orange and pineapple juice respectively and incubated for eight days. At regular intervals, we assessed some of the intrinsic properties of wine, such as pH, sulphur dioxide, titratable acidity (TA). From the results obtained, all the parameters measured were within the permissible limits for fruit wine. The mean pH values of orange and pineapple juice in 200ml and 500ml were between  $4.45 \pm 0.16$  and  $4.86 \pm 0.50$ ; while the mean Sulphur dioxide SO<sub>2</sub> (ppm) were  $18.36 \pm 0.20$  -  $39.49 \pm 3.68$  respectively. The titratable acidity (TA) obtained was between  $0.63 \pm 0.11$  -  $1.03 \pm 0.59$ , respectively. This study had shown that our strain of wild type of yeast could be used for producing homebrew fruit wine and may not present any hazard to the consumers.

**Keywords:** *Citrus sinensis*, *Ananas Comosus*, *Saccharomyces cerevisiae*, sulphur dioxide content, titratable acidity

## 1. INTRODUCTION

Wine is an alcoholic beverage obtained from fermented grape juice or a range of fruits [1]. Usually, the fruit type with the right amount of sugar and the strain of yeast used during the fermentation process will influence the name and the brand of the wine [2]. Wine typically contain about 79 – 89% water, 9 - 21 % alcohol, less than 1 % fruit acids, aromas and flavors in minimal quantities. The classification of wine is based on the fruit variety, the place of origin, colour, the name of the winemaker or viticulturist, or the production procedure [3]. Two main types of wine exist, namely, Natural wine with 9-14% alcohol and Fortified (Dessert and appetizer) wines with 15 - 21% alcohol [4]. The nutritional role/total energy contribution of wine is about 10 – 20 % in adult males [5]. Grapes are the most common fruit used in winemaking, however, many research studies have investigated other fruits as substrates for wine production [6].

In Nigeria for instance, the non-availability of grapes, has made it necessary to search for alternative fruit sources for wine production [7]. In most developing countries due to poor technical know-how and storage facilities, fruits spoil quickly however wine does not spoil readily. Thus, the production of wine from fruits is a form of extending the shelf life of the fruit [2]. A vast diversity of microbes is used in winemaking. These include various bacteria, yeasts, and molds. Prominent in this process are *Saccharomyces* species (predominantly *Saccharomyces cerevisiae*), which is primarily used in alcoholic fermentation, while the lactic acid bacteria (LAB), is used for malolactic conversion [8].



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*Saccharomyces cerevisiae* is a eukaryotic microorganism appearing microscopically as a globose cell with one to four ascospores, which are suave and ellipsoidal. The colonies appear translucent, smooth and raised [9]. *S. cerevisiae* can ferment glucose and sucrose. However, *S. cerevisiae* cannot utilize pentoses [10]. *Saccharomyces cerevisiae* on dry weight contains 3-5% phosphate, 2.5% potassium, 0.3 - 0.4% magnesium, 0.5% sulphur and trace amounts of calcium, chlorine, copper, iron, zinc, and manganese [11].

*Citrus sinensis* (orange fruit) is widely grown in tropical and subtropical climes. The orange fruit is a hybrid possibly between pomelo (*Citrus maxima*) and mandarin (*Citrus reticulata*) [12]. Orange fruit grows to 9–10 m in height, however could reach 15 m [12]. Orange fruit is hesperidium [13], with the leaves are arranged alternately and ovate with crenulate margins. It could be peeled or cut and eaten whole or processed to extract orange juice or for its fragrance from the peel. In 2008, 68.5 million tons of oranges were produced worldwide, mainly from Brazil, and the US states California and Florida [14].

Pineapple (*Ananas Comosus*) belong to the family Bromeliaceae with 2,000 species. It appears nearly white to yellow in colour [14]. It is an herbaceous perennial plant that grows to 1.0 - 1.5 m tall with about thirty trough-shaped and pointed leaves [14]. Pineapples contain the right sugar proportion, which makes it suitable for winemaking [15]. Pineapple is well-flavored and eaten fresh by consumers because it has very intense fruity fragrances and aromatic flavors [16]. The fruit contains vitamin C, iron, and other minerals. It could act as an effective laxative due to bromelain [14, 16], curing gastric irritability in fever and very helpful in jaundice. Pineapple is a good source of manganese, vitamin C and B1 needed for the body, bone and connective tissues [16]. This study aimed to produce fruit juice wine from the orange and pineapple juices with the view of contributing towards sustainable food production and addressing issues of food security.

## 2.0 MATERIALS AND METHODS

### 2.1 CHEMICALS/ REAGENTS

2.1.1. The analar grade chemicals and reagents used include Peptone water (Biolab), phenol red (Oxoid), Potato Dextrose agar LAB098 (Lab M), diluted sulphuric acid, 0.1M NaOH, grams iodine, crystal violet, safranin, and starch.

### 2.2 SAMPLE COLLECTION

Fresh orange and pineapple fruits were procured from Agbara market, Ogun State, Nigeria and moved to the laboratory for extraction of the juice.

#### PREPARATION OF FRUIT JUICES

The orange and pineapple fruits selected were only of good quality and cleaned with distilled water and 70% ethanol. The fruits were then macerated and filtered to get juice [1].

#### SUGAR FERMENTATION TEST MEDIUM

One (1) gram each of lactose, mannitol, glucose, sucrose, and maltose were weighed into separate conical flasks containing 10 ml of distilled water each. One drop of phenol red was added to each conical flask. The mixtures were dispensed into five different sterile test tubes and labeled. Five sterile Durham tubes were inserted into each test tube. The test tubes sterilized in an autoclave at 121°C for 15 minutes.

#### ISOLATION OF YEAST

Fresh palm wine was bought at Iju, Ota, Ogun State. The fresh palm wine was collected in a 2.5-liter sterile bottle and transported to the laboratory immediately.

A sterile pipette was used to withdraw some of the palm wine and few drops streaked over freshly prepared solid Potato dextrose agar (PDA) plates. The plates were covered and incubated at 27 °C for 72 hours. The Petri-dishes were examined for the growth of yeasts. A fleck of the growth was mounted on a glass slide and observed under the microscope for morphological characteristics.

### **IDENTIFICATION AND PURIFICATION OF ISOLATE**

The yeast identification was done using standard morphological and physiological tests, as described by [17]. These include cultural characteristics, ascospore formation, and vegetative reproduction. Fermentation test include the sugars maltose, glucose, sucrose, mannitol, and lactose. The identified yeast colonies were further purified by successive subculturing on potato dextrose agar until pure cultures were obtained. The pure culture was stored on PDA slants at a temperature of 8 °C.

### **SUGAR FERMENTATION TEST**

This was carried out as described by [18]. A speck of the isolate was inoculated into the sugars (lactose, maltose, mannitol, glucose, and fructose) and incubated at 37 °C for 24 hours. A yellow color indicates a positive test, while the red color denotes negative.

### **CHARACTERISATION OF YEAST**

#### ***Gram staining***

Gram stain was done as described by [12] and [19]. A drop of water was placed on a clean glass slide; a speck of yeast growth was taken from 24 hours growth culture to make a thin smear. The smear was allowed to air dry and the slide was flooded with crystal violet for 60 seconds. This was washed with distilled water thereafter flooded with Gram's Iodine for 60 seconds. The slide was washed with distilled water, and 70 % ethanol was added for decolourization and washed after 30 seconds. The smear was counter stained with safranin for 60 seconds, washed with water, and air-dried. The Ascospores of the yeast stained red while vegetative cells appeared purple when viewed under oil immersion objective lens.

#### ***Lactophenol stain***

This process was carried out as described by [18]. A speck of the yeast isolate from a 24-hour old culture was emulsified on a clean slide. The smear was covered with few drops of lactophenol blue, and a coverslip was placed and observed under the microscope.

### **STARTER CULTURE PREPARATION**

Four hundred (400) millilitre of distilled water supplemented with 80 g of sugar in conical flask was autoclaved. On cooling, pure cultures of *S. cerevisiae* was inoculated into the medium and incubated for 72hrs at ambient temperature [1].

### **FERMENTATION OF FRUIT JUICES AND DETERMINATION OF pH, SO<sub>2</sub> PRESENCE AND TITRATABLE ACIDITY**

#### ***Fermentation of fruit juices***

The fruit juices were placed in four 200 ml and 500 ml conical flasks and sterilized by boiling at 100 °C for 30 mins. The fruit juices were allowed to cool, then 10 ml of the yeast were added to each sterilized juice and incubated at 27-30 °C for eight (8) days. The pH, SO<sub>2</sub> presence, and titratable acidity were measured every two days.

#### ***pH***

The pH meter was used to record the pH changes over the course of the fermentation period. Before use, the pH meter was calibrated and the glass electrode immersed into the juice sample.

### SO<sub>2</sub> Presence

Fifty (50) millilitres of the wine to be tested was measured out. Two (2) millilitres of starch solution and Ten (10) millilitres of dilute sulphuric acid were added. The iodine solution which was measured in a clean syringe was added to the sample and swirled gently. There was a disappearance of the purple-black patch as the swirling was done. When the purple color persisted, the addition of iodine was stopped. How much of the iodine that was left in the syringe was noted and subtracted from the starting amount of 10 multiplied by 12.8. This calculation gave the number of ppm of free SO<sub>2</sub> in the wine.

### Determination of Titratable Acidity (TA)

This was done using the calibrated pH meter. About Hundred (100) millimetres of sterile distilled water of pH of  $8.2 \pm 0.2$  was poured into a clean 250 ml beaker. About 0.1 N NaOH and 5 ml of sample were added. The pH meter was immersed into the sample and stirred gently. About 0.1 N NaOH was slowly added to the sample and titrated to a pH of 8.2. The total titratable acidity was calculated using the formula

$$\text{TA (g/100 ml)} = \frac{[(V) (N) (75) (100)]}{(1000) (v)}$$

V = ml of sodium hydroxide solution used for titration

N = Normality of sodium hydroxide solution

v = sample volume (ml)

### 3.0 RESULTS

The pH values of 500 ml orange wine decreased from 4.60 at day 2 to 4.20; after that, it increased to 4.8 at day six then 4.4 on the final day of incubation. The pineapple wine showed a steady decline in pH from 5.2 - 4.0 during the period of incubation. Figure 1.0

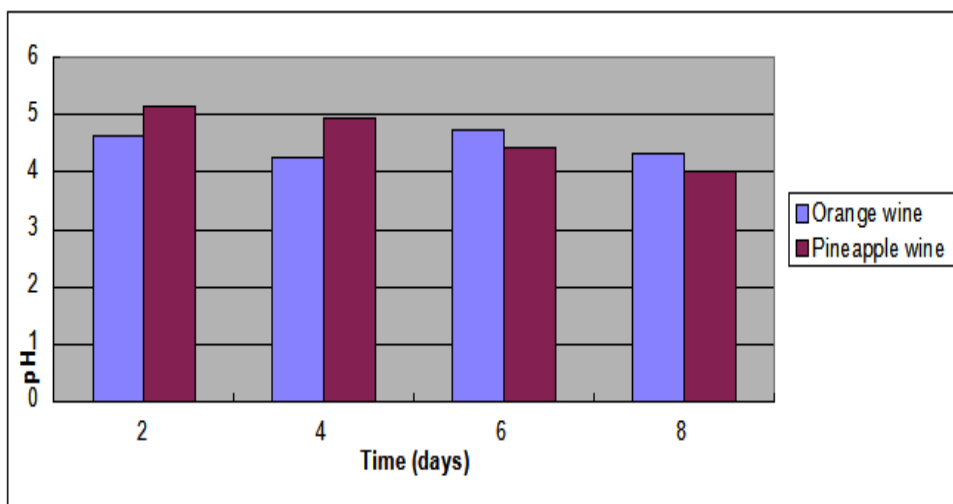


Figure: 1.0 Bar chart of pH changes in 500ml fruit wine.

For the 200 ml, of the wines, the pH of the pineapple wine was maintained at a similar pH of  $5.10 \pm 5.12$  between 2- 6 days. However, on day eight, there was a decline to a pH of 4.0. The pH of the orange had a slight reduction from 4.90 to about 4.40 within the period of incubation

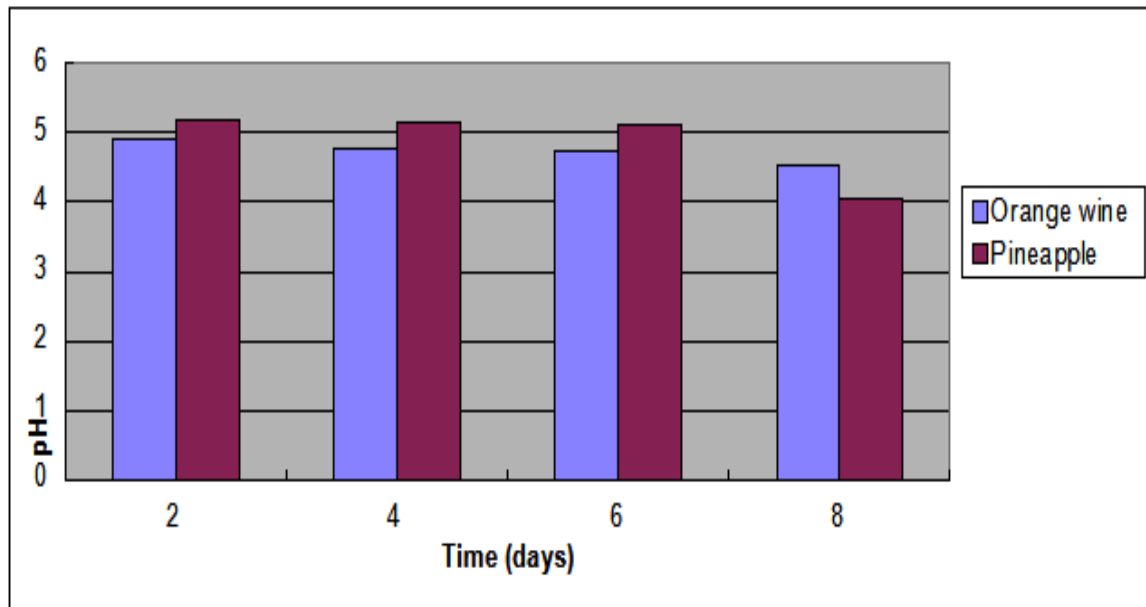


Figure 2: Bar chart of pH changes in 200ml fruit wine.

In the 200 ml wine, the concentration of  $\text{SO}_2$  in the pineapple wine ranged at 35 ppm for 2- 4 days, then on the 6<sup>th</sup> day, there was an increase to 45 ppm, which subsequently reduced to 40 ppm at the (8<sup>th</sup> day) final incubation period. The orange wine showed a steady decline in the concentration of  $\text{SO}_2$  from 35 - 10 ppm. (Figure 3). In the 500 ml juice, the  $\text{SO}_2$  of the pineapple wine reduced from 36 -24 ppm incubation period; consequently, the orange wine showed a decline in from 23 - 16 ppm.

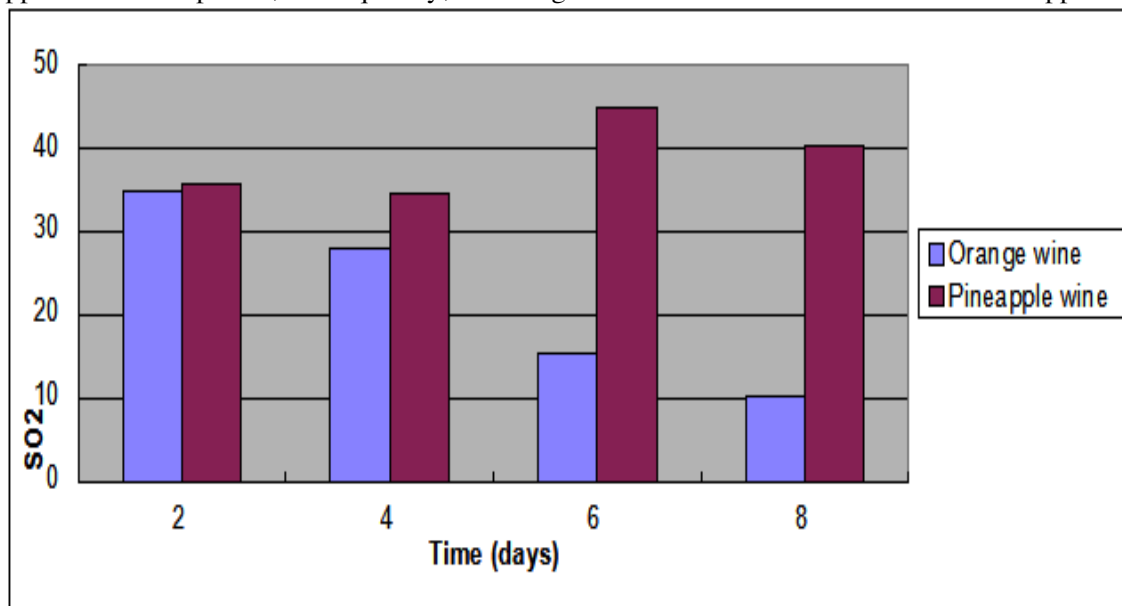


Figure 3: Bar chart of  $\text{SO}_2$  changes in 200ml fruit wine.

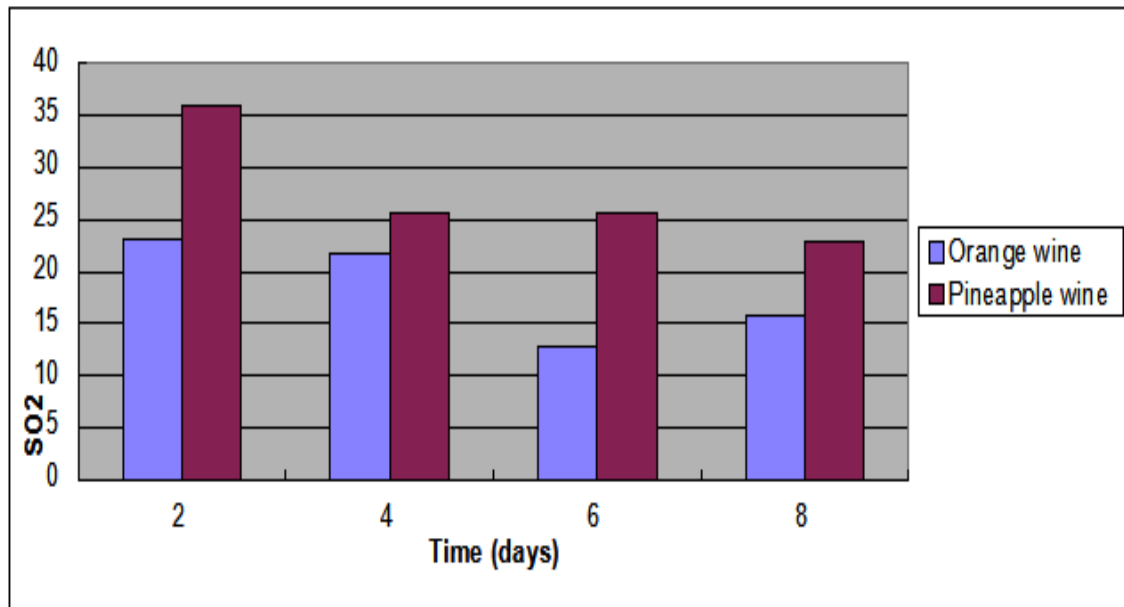


Figure 4: Bar chart of SO<sub>2</sub> changes in 500ml fruit wine.

The titratable acidity values from the 500 mL of the different wines, showed that the pineapple wine showed an increase from 0.4g/ml -1.7g/mL on day 6, after that there was a reduction to 1.3g/mL on day 8. The orange wine increased 0.6 - 0.8 g/mL within day 2-6, on day 8, the titratable acidity reduced to 0.4 g/mL (Figure 5)

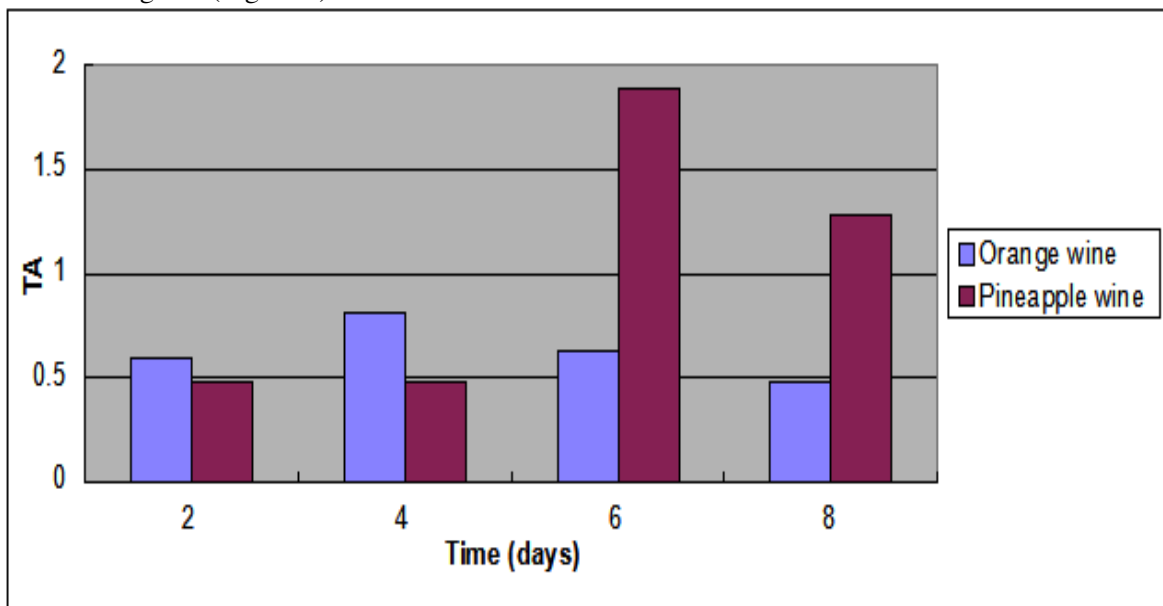


Figure 5: Titratable acidity (TA) changes in 500ml fruit wine.

In the 200 mL wine, the total acidity or Titratable acidity (TA) of orange wine increased from 0.45 - 0.89 g/mL and the pineapple showed a decline from 0.47 g/mL to 0.40 g/mL which increased to 0.46 g/mL (Figure 6)

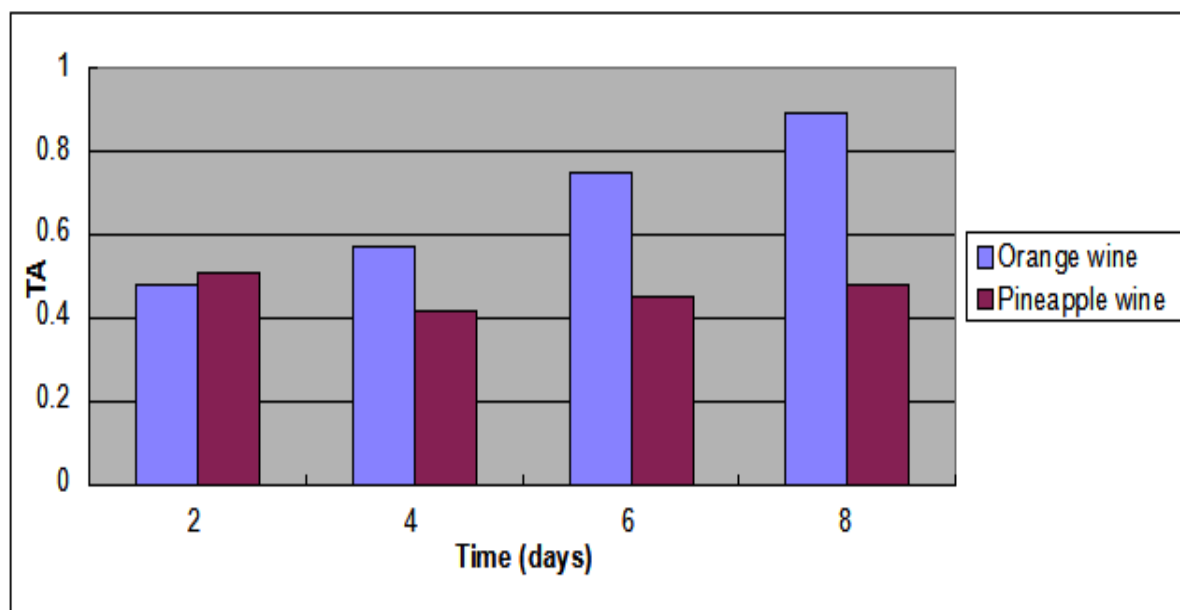


Figure 6: Titratable acidity (TA) changes in 200ml fruit wine.

**Table 1 Morphological and Biochemical reactions of the isolated yeasts**

+ positive, - negative

1. Morphological characteristics	Observed reactions
Elevation	Raised
Consistency	Moist
Colour	Creamy
Shape	Oval
Size	Moderate
Budding	+
<b>Sugar fermentation tests</b>	
Lactose	+
Maltose	+
Sucrose	+
Manitol	-

## DISCUSSION

*Saccharomyces* species isolated from fresh palm wine was characterized and identified according to [20]. It was further characterized by grams stain, which validated the organism isolated (See Table 1). The observed changes in the pH, orange, and pineapple wine in Figures 1-2 could be due to the production of acids during the fermentation process by the yeast. The data obtained agree with the reports of [2, 21]. Results showed that orange wine had higher total acid, especially at 200 mL, when compared to the 500 mL volume (See Figures 5 and 6). These behavior may be due to the physiological responses of our yeasts, possibly because they are top fermenters. The high titratable acid content reveals the maturity of the wine. In the 200 ml wine, the concentration of  $SO_2$  in the



pineapple wine ranged at 35- 45 ppm within 6 days, after that it reduced to 40 ppm at the (8<sup>th</sup> day) final incubation period.

The orange wine showed a steady decline in the concentration of SO<sub>2</sub> from 35 - 10 ppm. In the 500 ml juice, the SO<sub>2</sub> of the pineapple wine reduced from 36 -24 ppm within the study period, consequently the orange wine showed a decline in SO<sub>2</sub> from 23 - 16 ppm. Sulphur dioxide adds antimicrobial action and antioxidant effects on the wine. However, it causes browning or off-flavors in the wine. It was evident that the volume of media influences the behavior of the yeast cells. From this study, orange juice presents as the right choice for wine production, especially when the parameters that affect its production is taken into account. It was reported by [22] that the yeast showed outstanding stability without a decrease in activity at 37°C for wine production within eight days. Thus the yeast *Saccharomyces cerevisiae* plays a critical role in converting the nutrients such as starch and sugar in the juice into sulphur dioxide and organic acids that contribute to the shelf life of wine [23]. Overall, the capacity of our yeast to produce the essentials in wine shows that can efficiently serve as a starter culture for wine production. Wines are categorized based on the fruit variety, the place of origin, colour, the name of the winemaker or viticulturist, or the production procedure [3]. Thus depending on the desire of the viticulturist, different brands of wine could be produced even as accounted for in this study. This study has shown the possibility of reducing spoilage and wastage of fruits by fermentation preservation techniques. Consumption of fruit wines is encouraged as no preservatives or chemicals are added. In conclusion, this study has shown how volume dynamics could play a significant role in the production and maturity of the wine.

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