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SUITABILITY AND FERMENTATIVE PERFORMANCE OF INDIGENOUS PALM WINE YEAST (*SACCHAROMYCES CEREVISIAE*) USING APPLE

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ABSTRACT
Wine is a product of alcoholic fermentation by yeast on juice extract from ripe grapes or any fruit with suitable sugar content. The study was aimed at assessing the performance of the *Saccharomyces cerevisiae* isolated from palm wine in the production of wine. The yeast was isolated from palm wine and confirmed to be *Saccharomyces cerevisiae* by morphology, microscopy, and sugar fermentation tests. Fruit juice extracted from apple was inoculated with the S. cerevisiae isolated from palm wine and allowed to stand unperturbed for fourteen days. During the fermentation period, the fermenting 'must' was sampled at 2-day and 7-day intervals to monitor the physicochemical and microbiological conditions, respectively using standard methods. Five-point Hedonic scale was used to carry out a sensory evaluation of the apple wine. The yeast proved to be highly tolerant of alcohol and the pH of the apple wine produced at the end of fermentation was 3.7, which was slightly lower than that of the unfermented juice (3.8). While the temperature and alcohol content of the apple wine increased, the titrable acidity, reducing sugar and specific gravity decreased with the increasing length of fermentation. The final values with respect to reducing sugar, specific gravity, alcohol content, pH, and temperature of the wine were 24.45 g/l, 1.00, 12.23 % (v/v), 3.7, 30.3 ^{o}C, respectively. The result obtained from the sensory evaluation of the final product implies that the apple wine was generally acceptable. Hence, it is recommended that this indigenous yeast isolate (*S. cerevisiae*) should be utilized in subsequent brewing processes since it imparts a unique aroma and flavour to the wine.

1. INTRODUCTION

Wine is an undistilled alcoholic beverage prepared using only grape (Swami *et al*., 2014; Saranraji *et al.*, 2017). Utilization of various source materials such as pineapple, banana etc to produce 'fruit wines' also known as 'country wines' in some places (Jarvis, 1996) have broadened generally accepted definition of 'wine' by adding a prefix which recognizes fruit

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the wine was made from apart from grape (Velić *et al*., 2018; Emecheta *et al*., 2019). Basically all fruits can be used to produce an acceptable wine (Chakraborty *et al*., 2014). The use of apple fruit to produce 'apple wine' and 'cider' (Jarvis, 1996; Riekstina-Dolge *et al.*, 2012) instead of grape fruit is an age-old practice (Cousin *et al.*, 2017). The fermentation of apple juice to produce a pleasant alcoholic beverage is associated with people living in Eastern Mediterranean regions for over 2000 years (Alberti *et al.*, 2011). Apart from milk and water, wine is the only drink that has gained global acceptance for many centuries (Swami *et al*., 2014). Winemakers consider several factors during the production of wine of which sugar content of the juice and yeast strain deployed during the fermentation process are paramount (Okemini and Dilim, 2017). Generally, consumers' preference for a particular brand and/or type of wine is influenced by the aroma, colour, taste, quality, guaranteed origin, ecological production and other perceived sensory properties of the product (Saranraj *et al*. 2017). Traditionally, wine is consumed basically for pleasure until recently when certain health benefits was associated with it (Samson and Singh, 2017). Consumption of 1-2 glasses of wine per day considered to be moderate (Chakraborty *et al*., 2014) is associated with longevity, confers health benefits by preventing cardiovascular diseases and cancer. These benefits could be attributed to resveratrol present in wine. Reduced risk of stroke, increased cognitive performance and insulin sensitivity are other benefits that could be derived by drinking a moderate quantity of alcoholic wine. Meanwhile, the perception of Europeans towards wine is that the product is a food supplement since it contains carbohydrates, vitamins and minerals (Asuk *et al*., 2011; Wurz, 2019).

Winemaking is one of the prehistoric technologies practiced by man, currently among biotechnological processes with enormous commercial prospects is still attracting more contributions from researchers for improvements. Wine fermentation which involves biochemical processes and ecological considerations (Chilaka *et al*., 2010) could be categorized into pre-fermentation, fermentation, and post-fermentation stages (Saranraj *et al*. 2017). Fruit wines and grape wine production processes share similarities. It is important to note that extraction of sugars and other soluble compounds, lower sugar content and higher acid content in fruit juices compared with grape juices poses a challenge during fruit wine production (Velić *et al.*, 2018).

For ages, various ethanol-producing microorganisms have been identified and utilized through fermentation for the benefit of humans (Laplace *et al*., 1992 and 1993). Among such beneficial microorganisms, yeast belonging to *Saccharomyces cerevisiae* (Ngọc *et al*., 2013) popularly known as wine yeast have been used most commonly (Stanley *et al*., 2014; Parapouli *et al*., 2019). Palm wine is a popular refreshing alcoholic beverage obtained from the sap of oil palm (*Elaeis guiniensis*), Raphia palm (*Raphia hookeris* and *R. vinifera*) and other palm trees. A consortium of microorganisms ranging from lactic acid bacteria, acetic acid bacteria, Gram-negative bacteria and yeasts present in palm wine undergo microbial succession. Yeast genera present in palm wine are *Saccharomyces, Saccharomyeoides, Schizosaccharomyces, Endomycopsis, Kloekera, Pichia,* and *Candida* (Ogodo *et al.*, 2015). Exploiting the fermentative ability of indigenous palm wine yeast for the production of fruit wine could bring about greater economic gains than limiting its usefulness to palm wine which is a popular alcoholic drink among the people of Southern Nigeria (Ebana *et al*., 2019).

There is the presence of ethanol in drinks referred to as alcoholic beverages (Swami *et al*., 2014; Saranraji *et al.,* 2017). Bansal and Singh (2003) did a comparative study on ethanol production from molasses using *Saccharomyces cerevisiae* and *Zymomonas mobilis*. The yeast was found to be more ethanol tolerant and produced more ethanol than bacteria at sugar concentration above 15 % (v/v). The selection of the starter culture of yeast to be used in wine production is very important. According to Chilaka *et al*. (2010), different sources of yeast used in fermenting fruit juice contribute to variations in flavour and level of alcohol content in wine. Uma and Polasa (1990) isolated *S. cerevisiae* from palm wine which produced increased amounts of ethanol in yeast extract peptone dextrose medium. In a related study, Bertolini *et al.* (1991) isolated new strains of *S. cerevisiae* on basal medium containing 48 % sucrose from fermenting samples collected from Brazilian alcohol factories. Findings from their study showed that isolated strains fermented concentrated sugarcane syrups as well as high sucrose solution in synthetic medium with a conversion efficiency of 89-92 %.

According to Ogodo *et al.* (2015), wine made from a grape usually involves *Saccharomyces cerevisiae* strains which are not genetically the same with twenty strains of *S. cerevisiae* mainly responsible for fermenting palm wine. However, the strains of *S. cerevisiae* isolated from palm wine have the capability to survive and continue fermentation process up to ethanol concentration of 18 % (v/v) making them ideal for producing wine (Nwachukwu *et al.,* 2006). Enidiok and Attah (2010) compared the quality of wine produced using *Syzygium malaccensis* (red) which is a locally produced apple in abundant supply in Nigeria and a similar product which involved the use of temperate apple fruits *Eugenia owariensis* (green) imported into the country in the form of fresh fruits and apple wine (finished product). Both wines had no significant difference in pH, specific gravity and alcohol contents (11 % on the average). Overall results from the study showed that wine obtained from *S. malaccensis* had slightly preferable qualities than the drink obtained from *E. owariensis.* Wine market in Nigerian wine market is dominated by imported white wines made from grape and *S. cerevisiae* var. ellipsoids which are expensive (Umeh *et al*., 2015). Therefore, the production of a similar product using locally sourced palm wine yeast and apple sold in open markets could offer consumers a cheaper product comparable in quality with exotic wines. Utilizing palm wine yeast for the industrial process requires acquisition of detailed knowledge about its technological and alcoholic fermentative performance (Mathew *et al.*,, 2017). Hence, this study was aimed at assessing the suitability and fermentative performance of palm wine yeast (*Saccharomyces* cerevisiae) using apple juice as a substrate for wine production.

2. MATERIALS AND METHODS

2.1 Collection of samples

Fresh palm wine was purchased directly from palm wine tappers in a sterilized container and promptly transported to the Food and Industrial Microbiology Laboratory, the University of Port Harcourt for analysis. Fresh apples and refined sugar used in the study were purchased from Choba Market in Obio/Akpor local government area.

2.2 Isolation of the yeast isolates

The method used by Ogodo *et al.* (2015) for isolation of yeast, *Saccharomyces cerevisiae* from palm wine with slight modification was adopted. Potato dextrose agar (PDA) medium was used for the isolation of the yeast. Exactly 4.8 g of PDA powder was weighed and suspended in 120 ml of distilled water in 250 ml Erlenmeyer flask, gently agitated until the powder was completely dissolved. The

medium was autoclaved at 121 ^{o}C and 15 psi for 15 min and allowed to cool to about 45 ^{o}C before adding 1 ml lactic acid to inhibit the growth of bacteria. Thereafter, the sterilized media was poured into Petri dishes. The fresh palm wine was serially diluted in peptone water (10^{-1}, 10^{-2} and 10^{-3}) and 0.1 ml of each dilution was dispensed in duplicates of the PDA plated and spread with the aid of sterile glass hockey stick. The plates were thereafter incubated at 37 ^{o}C for 48 h.

2.2.1 Subculture

Freshly prepared PDA culture plates were used to separately subculture four isolates from distinct colonies seen on the previous culture (from 10^{-3} culture plate). The subculturing was done using the streak method and the plates were labeled P1 to P4. The cultured plates were incubated for 48 h at room temperature (25-37 ^{o}C). Afterward, the isolates were stored on slants to avoid contamination or loss of the organism.

2.3 Microscopy of the isolates

2.3.1 Preparation of smears

Smears of the isolates were made on microscope slides by transferring a loopful of water to the surface of the slide. Using a well flamed loop, aliquot of the culture was collected from the plates and mixed in a whirl with water on the slide, then allowed to air dry. The smears were heat-fixed by passing the slide swiftly over the Bunsen flame 3 to 4 times.

i) Gram staining

The smears were stained using the Gram staining technique. The slides were first flooded with the primary stain, crystal violet for 60 s, after which it was rinsed off under running water and flooded with the mordant (Lugol's Iodine), for 60 s. and rinsed. Afterward, it was decolourized with ethanol, for 30 s., and rinsed, before the slides were flooded with the secondary stain, Safranin for 30 s. The slides were slanted to dry and viewed under the oil immersion objective lens of the microscope.

2.4 Carbohydrate utilization test on the isolates

1 % each of glucose, sucrose, fructose, galactose, maltose and ethanol was prepared. Peptone water was to all except the ethanol.

Procedure

Ten milliliter (10 ml) each of the prepared sugars were introduced into test tubes in four places. Durham tubes were introduced into the test tubes containing the sugars and autoclaved. Using a sterile wire loop, the colonies were introduced into the tubes and labeled accordingly. The set up was incubated for 48 h. Thereafter, the tubes were observed for acid and gas production

2.5 Preparation of apple juice

Mature, ripe and healthy apples were sorted, washed and de-seeded manually using a knife. The apples were then chopped into smaller quantities and blended. The slurry was filtered through a double folded cheesecloth to obtain the juice. 1.5 L of apple juice was produced after blending ten (10) large-sized apples. 0.1 % (v/v) potassium metabisulphite was added to the juice, alongside refined sugar (approximately 30 g/L). The apple juice was pasteurized at 60 ^{0}C for 3 min.

2.6 Fermentation of apple juice

The freshly prepared apple juice was poured into sterile conical flasks and was seeded with 3 % (v/v) of a 24 h culture of the isolated yeast. The flasks were covered with an airlock and the juice was allowed to ferment for a period of fourteen days. During this period, fermentation of the apple juice was monitored by carrying out microbiological and physicochemical (pH, temperature, reducing sugar, specific gravity, alcohol content and titratable acidity) analysis. On the stoppage of the fermentation,

the apple wine was racked, transferred into sterile bottles and stored.

Figure 1 shows a flow chart for apple wine production. The procedure described by Velić *et al.* (2018) with slight modification was adopted during the preparation of apple wine. Firstly, the preparation of apple juice followed by fermentation of apple juice.

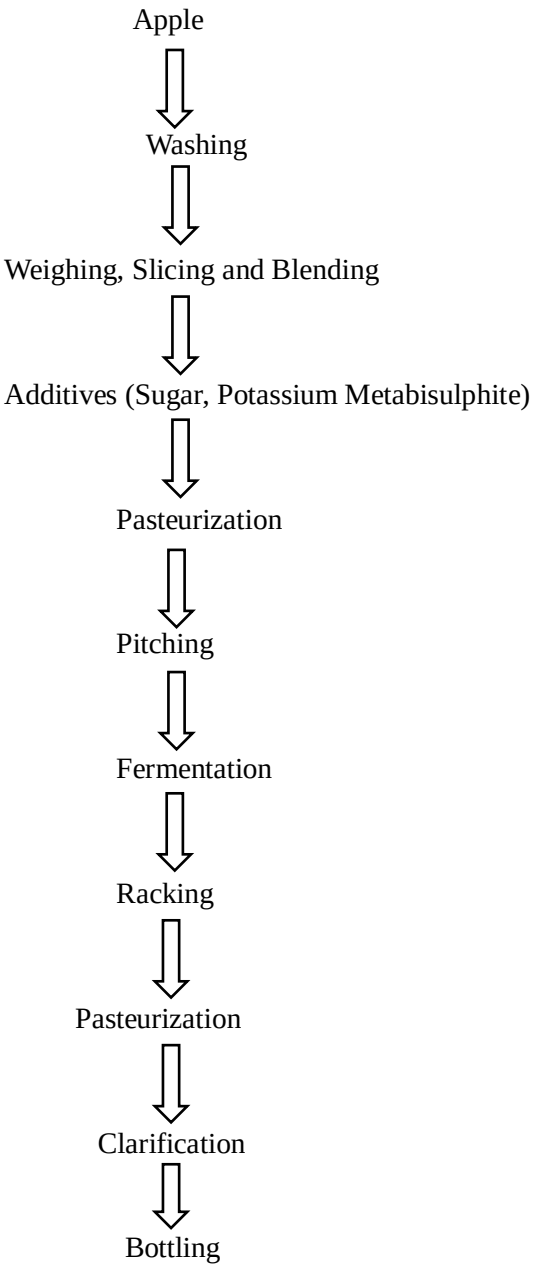


Figure 1: Flow chart for apple wine production

2.7 Microbiological analysis

Microbial analysis of the fermenting apple wine 'must' was carried out using MacConkey agar (MAC), Nutrient agar (NA), and Potato dextrose agar (PDA) and were prepared according to the manufacturers' instruction. During the fermentation process, at intervals, an aliquot portion of the fermenting 'must' was sampled and serially diluted. The dilutions were spread on prepared culture media of NA, PDA and MAC which were incubated for 24, 48 and 24 h respectively, then observed for colonies. The colonies in the culture plates were enumerated. The microbial population was

calculated, the result was expressed in CFU/ml.

2.8 Physicochemical analysis

2.8.1 Determination of pH and temperature

The method described by Stanley *et al.* (2014) with slight modification was adopted in determining pH and temperature of the fermenting must. The digital pH meter was standardized using a buffer solution, prepared using pH buffer powder of pH 4.00 at 25 ^{o}C, dissolved in 250 ml distilled water. The electrode of the pH meter was immersed in a glass beaker containing the wine sample, and thereafter the pH meter was adjusted to measure the temperature of the wine with the aid of a Celsius thermometer fixed together with the pH meter. Readings were obtained from the digital display of the pH meter.

2.8.2 Estimation of titratable acidity

This was determined by the methods described by Amerine and Ough (2014). Two hundred milliliter (200 ml) of distilled water was poured into a sterile 500 ml conical flask and boiled. 1 ml of 1 % aqueous alcoholic phenolphthalein indicator solution was dropped inside the conical flask with the content. This solution was titrated against 0.1M NaOH solution to give a faint pink colour. 5 ml of the "must" was pipette and introduced into the boiling neutralized solution followed by repeat titration until endpoint was achieved using 0.1M NaOH solution.

The titratable acidity was expressed as tartaric acid and was calculated thus:

$$\text{Tartaric acid g/100ml} = \frac{V \times M \times 75 \times 100}{v \times 100}$$

Where:

V= Volume of NaOH (final reading- initial reading).

M = Molarity of NaOH

v = Volume of "must"

Since malic acid constitute a larger portion of organic acid present in apple, therefore titratable acidity = tartaric acid value x 0.873

2.8.3 Determination of specific gravity

The method described by Okeke *et al.* (2015) was adopted to determine the specific gravity of the fermenting must. Fifty (50) ml specific gravity bottle was thoroughly cleaned with distilled water, dried in an oven at 50 ^{o}C and allowed to cool. The weight of the cooled dried bottle (W_1) was recorded. The dried bottle was filled with deionized water, the surface of the bottle was cleaned with cotton wool and weighed (W_2). The bottle was emptied, then rinsed twice using 10 ml of the "must". Thereafter, the bottle was filled to the brim with the 'must', then spilled 'must' outside the bottle was cleaned with cotton wool, and weighed (W_3). The specific gravity (S.G) was calculated as:

$$\text{S.G} = \frac{W3-W1}{W2-W1} = \frac{S}{W}$$

Where:

S = weight of 50 ml 'must' ($W_3 - W_1$)

W = weight of 50 ml water ($W_2 - W_1$)

2.8.4 Alcohol determination by specific gravity

The method assumes that the difference in Specific Gravity before and after fermentation is due solely

to the conversion of sugars before fermentation into alcohol after fermentation.

% (v/v) alcohol = $SG2 - SG1 / 0.0074$

Where:
SG_1 is the initial specific gravity measurement
SG_2 is the final specific gravity measurement.

2.8.5 Determination of reducing sugar

Reducing sugar otherwise called total soluble solids of the must were determined using the Rebelein process (Zoeckein *et al.*, 1990) and process of estimation of reducing sugars (Benedict, 1908). The reagent and procedure constituted as follows:

Rebelein Solution 1

One milliliter (1.0 ml) of concentrated H_2SO_4 was put in 600 ml of water. 41.92 g of copper sulphate ($CuSO_4$. $5H_2O$) was dissolved in some acid solution and quantitatively transferred to a 1.0 L volumetric flask making up to 1000 ml with distilled water.

Rebelein Solution 2

Two hundred and thirty grammes (230 g) of sodium potassium tartrate was put inside 600 ml of distilled water followed by the addition of 80 g of sodium hydroxide. **Caution:** Heat was generated and it was allowed to cool, then transferred to 1 L flask to make it up to 1000 ml.

Rebelein Solution 3

Hundred milliliter (100 ml) of 1 m sodium hydroxide was added to 600 ml of distilled water. 300 g of potassium iodide was dissolved in a portion of alkaline solution and was transferred to 1.0 L volumetric flask and diluted to volume.

Rebelein Solution 4

One hundred and seventy five milliliter (175 ml) of concentrated H_2SO_4 (sodium tetraoxosulphate VI) acid was added to 825 ml of cold distilled water (heat evolved), mixed very well and stored in a sealed glass container.

Rebelein Solution 5

Twenty grammes (20 g) of potassium iodide and 10 g of starch was put in a separate beaker followed by the addition of 10 ml of 1 M sodium hydroxide to 500 ml of distilled water and mixed very well. Sodium hydroxide solution was used to dissolve the potassium iodide and starch in solution and the volume of distilled water was titrated.

Rebelein 6 Solution

Sodium thiosulphate $Na_2S_2O_3.5H_2o$ was dissolved in water and transferred to 1.0 L volumetric flask. Thereafter, 500 ml of 1 M sodium hydroxide was added and then diluted with distilled water. 10 ml of Z_1 solution and 5 ml of Z_2 solution was pipette into an Erlenmeyer flask and boiling granular were added. 2 ml of wine sample was also pipetted into the flask and was heated until boiling which lasted for 30 sec. The flask was removed from the heat source and allowed to cool to room temperature. 10 ml of Z_3, Z_4, and Z_5 solution were added following the listed order. The burette was filled with Z_6 (standard Thiosulphate solution). The initial burette reading was recorded. The mixture in the Erlenmeyer flask was titrated by shaking the flask well to mix throughout the titration. The appearance of creamy colour marked the endpoint. The yellow-brown solution faded to become blue-grey which was a result of starch turning to cream. The final burette reading was recorded. Titration using 2 ml of distilled water was carried out instead of wine at the same time with the test

sample. The blank titre was treated identically following the above-mentioned procedure. The net titres for both the sample and distilled water blank were calculated. The blank titre was in the range of 29.3 ml and the sample titre was less. The reducing sugar was calculated using the formula below if no sample solution was formed.

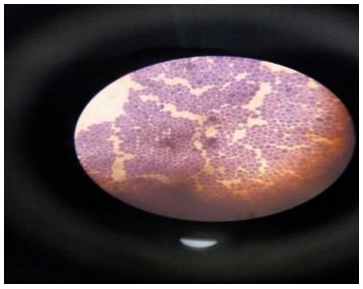
Reducing sugar = Dilution factor blank titre (ml) – sample titre (ml)

2.9 Sensory evaluation

Wine tasting involves sensory inspection and evaluation of wine. Wines contain many chemical compounds similar or identical to those in fruits, vegetables, and spices. The sweetness of wine is determined by the amount of residual sugar in the wine after fermentation, relative to acidity of the wine. Sensory parameters of the wine evaluated by a panel of seven judges using a five-point Hedonic scale ranging from strongly dislike (1) to strongly liked (5) were colour, flavour, taste, clarity, and overall acceptability.

2.10 Statistical analysis

The data obtained from the study were subjected to a completely randomized analysis of variance (ANOVA). SPSS version 20.0 was used to separate the mean as well as do a comparison. If $P < 0.05$, then there is a significant difference. The result obtained were expressed as mean ± standard deviation away from the mean.



3. RESULTS AND DISCUSSIONS

Table 1 shows the reference standard for carbohydrate utilization test used for proper identification of *Saccharomyces cerevisiae*. Shown in Table 2 is the result of the carbohydrate utilization status of the isolates palm wine. The yeast isolates had white and creamy texture (colony characteristics), ovoid shape (viewed under a microscope), presence of ascospore, and budding pattern (multipolar). Lodder (1970), and Boekhout and Kurtzman (1996) proved that the four isolates (P1, P2, P3 and P4) belong to saccharomyces type unicellular ascomycete. Further testing of the isolates for possessing the ability to ferment carbohydrates revealed that Isolate P4 was *Saccharomyces cerevisiae.* After making a smear and Gram staining, isolate P4 appeared as Gram-positive oval-shaped cells, under the microscope. Plate 1 shows the photograph of *Saccharomyces cerevisiae* viewed under the microscope.

Plate 1: Isolate P4 (*Saccharomyces cerevisiae*) viewed under the microscope

Table 1: Reference standard for carbohydrate and ethanol utilization test

Specie	Glucose	Maltose	Galactose	Sucrose	Lactose	Fructose	Ethanol
S. cerevisiae	+	+	V	+	-	-	-
S. veirellipso	+	+	V	+	-	+	-
S. fragilis	+	-	+	+	+	+	-
S. lactis	+	-	+	+	+	+	+
S. rouxi	+	+	-	-	-	+	-
S. mellis	+	-	-	-	-	-	-
S. carlsbergensis	+	+	+	+	-	+	-

*V – variable (could be (+) positive (+) or negative (-)

Table 2: Carbohydrate and ethanol utilization capability of the isolates

Isolates	Glucose	Fructose	Sucrose	Lactose	Maltose	Galactose	Ethanol
P1	+	+	+	+	+	+	-
P2	+	+	+	+	+	+	+
P3	+	+	+	-	+	+	+
P4	+	-	+	-	+	+	-

Table 3 shows the microbial load of the fermenting 'must' monitored at 7 Days interval. At Day 0, culturable microorganisms were not detected in the pasteurized apple juice prior to inoculating wine yeast into the juice. Between Day 7 and 14, our results show that there was increase in the mean count of fungal colonies in the fermenting apple 'must' from 3.95 x 10^5 Cfu/ml to 5.15 x 10^5 Cfu/ml. In a related study, Zainab et al. (2018) reported a rapid increase in population of the yeast cells (*S. cerevisiae* isolated from palm wine) involved in the fermentation of watermelon wine within 54 h from the time the process commenced. The increase in the population of the yeast is an indication that an adequate amount of sugar in the fermenting must be available for the organism to utilize. This could be attributed to granulated sugar obtained from cane sugar which was used as an additive to serve as a source of sucrose during the fermentation process involved in the production of apple wine (Okeke *et al.*, 2015). According to Awe and Nnadoze (2015), a viable organism in the fermenting date palm must decrease significantly from 9.0 Cfu/ml to 1.0 Cfu/ml during aerobic fermentation of the must whereas the microbial population during anaerobic fermentation of the must show a constant value (2.0 Cfu/ml). The yeast count of the fruit 'must' increased from 4.69 x 10^2 cells/ml to 15.39 x 10^2 cells/ml during aerobic fermentation of the must but it reduced from 15.39 x10^2 cells/ml to 0.0 x 10^2 cells/ml during anaerobic fermentation of the must. It is remarkable that the population of bacteria in the fermenting apple must at Day 7 drastically reduced to the extent that no culturable bacteria colonies were reported on Day 14. This trend could be a result of increasingly unfavourable growth conditions for bacteria present in the fermenting must in terms of alcohol content, pH and competition with yeasts for available nutrients. According to Mathew et al. (2017), the increase in concentration of alcohol content of the fermenting must could This probably was not the case in this study as the population of the yeast cells increased during the fermentation period. rupture the cell membranes of the wine yeast resulting in the decline in population of the yeast cells. Absence of culturable bacteria in the apple wine is in agreement with the study carried out by Zainab *et al.* (2018) which reported absence of bacteria and coliforms in the watermelon wine made from watermelon juice which was fermented by *S. cerevisiae* isolated from palm wine, and remained in that condition for 72 h after it was produced. They attributed the absence of contaminant in the finished product to pasteurization and hygienic conditions maintained during the production process. This corroborates the findings from this study. The microbiological quality of wine should not be neglected by winemakers because the use of contaminated water in preparing wine, winemaking in a dusty environment infested with houseflies and fruit flies are possible sources of microbial contamination of the product. Also, dressing with ice could impact the product. Critical findings by *Oladipo et al.* (2014) showed that *Bacillus cereus, B. pumilus, Aeromonas hydrophila, Flavobacterium aquatile, Enterobacter aerogenes, Pseudomonas putida* and *P. fluorescens* were present in samples of local fruit wines purchased from Ogbomoso, Oyo State, Nigeria. Shown in Plate 2 is a sample of the apple wine prepared using apple juice fermented by palm wine yeast (*S. cerevisiae*).

Plate 2: Apple wine

Table 3: Microbial population of fermenting 'must' monitored at intervals

Day	Dilution	Growth Medium	Mean Count	Microbial population (Cfu/ml)
0	10^{0}	NA	-	-
	10^{0}	MAC	-	-
	10^{0}	PDA	-	-
7	10^{-2}	MAC	-	-
	10^{-2}	NA	41	4.1×10^{3}
	10^{-4}	PDA	39	3.9×10^{5}
	10^{-4}	NA	5	5.0×10^{4}
	10^{-4}	MAC	-	-
	10^{-4}	PDA	40	4.0×10^{5}
14	10^{-1}	MAC	-	-
	10^{-3}	NA	-	-
	10^{-3}	PDA	234	2.3×10^{5}
	10^{-3}	MAC	-	-
	10^{-4}	NA	-	-
	10^{-4}	PDA	80	8.0×10^{5}

Key: MAC-MacConkey, NA – Nutrient agar, PDA – Potato dextrose agar

The physicochemical parameters monitored during the period of fermentation of apple must is presented in Table 4. Among all the parameters (pH, specific gravity, titratable acidity, alcohol content, temperature and reducing sugar) monitored at 2-Day intervals for 14 days, statistical analysis of the results obtained for each parameter revealed that only specific gravity and pH of the fermenting 'must' do not show significant difference throughout the 14 -Day fermentation period.

In terms of pH of the fermenting must, our study shows that the values slightly decreased from 3.8-3.7 within the fermentation period. Since the pH of the fermenting must between Day 4-6, 8-10, and 12-14 was 3.5, 3.6 and 3.7, respectively, the overall result could be described as being relatively stable.

According to Won *et al.* (2015), the fermentation process which has a pH lower than 4.0 is regarded as homofermentative devoid of contamination. Based on this assertion, the apple wine fermented using indigenous palm wine yeast (*S. cerevisiae*) is safe for human consumption. According to Chilaka *et al.* (2010), during the fermentation process, low pH inhibits the growth of spoilage organisms but makes the environment favourable for the growth of desirable microorganisms. Therefore, the final product which is apple wine could be considered safe for consumption due to the absence of undesirable bacteria. Low pH and high acidity are known to give fermenting yeasts a competitive advantage in natural environments (Reddy and Reddy, 2005; Okeke *et al*., 2015). If the pH of wine is below 3.50, it is considered as having a high amount of acids. Otherwise, the pH of wine could reach up to 4.00 (Radovanović *et al*., 2007). In terms of acidity of wines, a glass of dry wine and sweet wine has a pH ranging from 3-7 and 3.5-4.5, respectively. After fortification of wine has taken place, higher acidities of the product occur. Considering the pH of apple wine prepared from apple fruits using palm wine yeast (*Saccharomyces cerevisiae*), the product evaluated in this study is a sweet wine (Awe and Nnadoze, 2015). According to Umeh *et al.* (2015), the reduction in pH of 'musts' undergoing fermentation could be attributed to the production of carbon oxide which forms a weak acid after being dissolved in the musts. In addition, the production of acetic acid by acetic acid bacteria could also have contributed to the decrease in pH of the fermenting musts. During a study which involved the production of apple wine using mash subjected to various pre-treatments (adding lactic acid and pectinase, chaptalization), Won *et al.* (2015) reported that pH of the fermenting apple wine mash decreased with increase in fermentation period which ranges from 3.07-3.90 while the control was within the range 3.80-3.97. In a related study, Chilaka *et al.* (2010) reported a similar trend which involved a reduction in pH during fermentation of passion, watermelon and pineapple fruit must by yeast isolated from palm wine which ranges were 3.1-4.6, 3.4-4.8 and 3.0-4.7, respectively.

Our results show that the alcohol content of fermenting apple wine 'must' increased from 0.00 – 12.23 % during the fermentation period which lasted for fourteen days. However, between Day 2-4 and 10-12 of the fermentation period, its alcohol content maintained the same value. According to Won *et al*. (2015), during nine days' fermentation period, there was an increase in the alcohol content of apple wine mash subjected to different pretreatments which were fermented by a commercialized yeast (20g; Fermivin, DSM, Heerlen, Denmark) generally used for wine fermentation. The values were ranging from 0 – 11.97 % (v/v) while the control was 1.37-6.47 % (v/v). Chilaka *et al.* (2010) also reported an increase in an alcohol content of passion, watermelon and pineapple wines produced using yeast isolated from palm wine within twenty (20) days fermentation of the fruit musts. The alcohol content of passion, watermelon and pineapple fruit wines at the end of the fermentation process were within the range 10.46-12.42 %, 10.14-10.44 % and 11.60-12.80 %, respectively. Both reports coming from Won *et al*. (2015) and Chilaka *et al*. (2010) is in agreement with the results presented in this study. Generally, *Sacchromyces* species are the most ethanol tolerant eukaryotic organisms which can tolerate 20 % ethanol and above. In a previous study, Casey and Ingledew, (1986) reported that yeast strain TGY2 could tolerate up to 16 % (v/v) ethanol. According to Teramoto *et al.* (2005), *S. cerevisiae* demonstrated the ability to tolerate 16.5 % (v/v) ethanol. In terms of alcohol content for table wine, the European Economic Community (EEC) recommends that it should be within the range of 8.5-19.5% (Umeh *et al*., 2015). Interestingly, the apple wine produced using palm wine yeast met the alcohol content requirement of EEC which qualifies the product as a good table wine.

Based on the quantity of reducing sugar in the fermenting apple wine must be monitored within the

fermentation period, our results show that steady decreases occurred which range from 24.45-52.3 g/l. This could be attributed to metabolic activities of *Saccharomyces cerevisiae* which breakdown available sugar present in apple wine must as fermentation period increases. In a related study, Umeh *et al.* (2015) reported that during fermentation of pawpaw wine must using *Saccharomyces cerevisiae* isolated from 'burukutu' which is a local drink, the reducing sugar content steadily decreased from 16.70 – 1.10 % between Day 0-14. Although reducing sugar content of the pawpaw wine was lower compared with apple wine produced using palm wine yeast (*Saccharomyces cerevisiae*), both results showed the same trend.

Results obtained from this study show that titratable acidity of the fermenting apple wine mash was on the decrease during the period of fermentation which ranges from 0.33-0.69 g/ml except between Day 0-2 when a sharp increase from 0.39-0.69 g/ml occurred. Between Day 8-12, the titratable acidity of the fermenting must remain unchanged, but reduced to 0.33 g/ml on Day 14. A slightly higher titratable acidity (0.53-1.10 g 100 g^{-1}) compared with the result obtained from this study was reported by Riekstina-Dolge *et al*. (2012) which involved using five apple varieties and commercial yeast *Saccharomyces bayanis* EC-1118 to produce apple wine which is also known as cider. The varieties of apple and yeast strain used to produce the apple wine might have influenced higher titratable acidity of the product compared with the samples analyzed in this study. Meanwhile, Awe and Nnadoze (2015) reported that titratable acidity during anaerobic fermentation of date fruit must which involve *Saccharomyces cerevisiae* increased from 0.23 to 0.65. According to Okeke *et al.* (2015) and Snell and Ettre (1974), titratable acidity of final wine is expected to range from 0.5 – 1.0 %. Rajković *et al*. (2007) stated that titratable acidity of finished wines is a significant parameter to give attention to because it influences sensory evaluation of the product. Stipulated regulations for wines require that the titratable acidity of the product evaluated should be within the range of 4.0-8.0 g/dm^3 expressed in tartaric acid. The origin of wine becomes suspicious of whether some illegal acts were involved during the production of the wine when the titratable acidity is less than 4 g/dm^3.

Starting from the beginning of the fermentation period (Day 0), the specific gravity of the fermenting apple 'must' decreased from 1.09 – 1.00. However, between Day 2-4 and 10-12, the specific gravity of the fermenting 'must' remained unchanged. According to Umeh *et al*. (2015), the decrease in specific gravity during fermentation of the musts could be attributed to metabolic activities resulting from the steady utilization of sugar present in the must by the yeast. Gradual decreases in specific gravity of passion fruit wine, watermelon fruit wine and pineapple fruit wine during fermentation of the musts reported by Chilaka *et al.* (2010) though in agreement with the trend observed in this study had lower values which ranges were from 0.91-0.92, 0.90-0.91, 0.94-0.96 kgm^{-3}, respectively. The metabolic activities of the fermenting yeast present in the must which utilized sugar present in the substrate and produced alcohol and carbon dioxide could be responsible for the decrease in specific gravity of the fermenting must be reported in this study.

During the fermentation process, the sensitivity of yeasts to growth rate, alcohol concentration, rate of fermentation, length of lag phase, viability, enzyme and membrane function could be affected by temperature. At higher temperature, Samson and Singh (2017) reported that alcohol production becomes higher. This corroborates the findings from this study. In the course of fermenting apple wine must, the temperature of the product increased from 26.4 ^oC at the beginning (Day 0) to 30.3 ^oC at Day 14 when the process was stopped. This result to some extent is in agreement with temperature ranging from 28-32 ^oC reported by Chilaka *et al*. (2010) from a study that involved fermentation of

passion fruit wine watermelon fruit wine and pineapple fruit wine using yeasts isolated from palm wine. A combination of environmental factors, fermentation conditions, yeast strain, and source substrate used for wine production could influence variations in the temperature of the fermenting 'must'. In a related study which involved the production of water melon (*Citrullus lanatus*) wine using *S. cerevisiae* isolated from palm wine, Zainab *et al.* (2018) attributed the increase in temperature to metabolic heat generated by *S. cerevisiae* during catabolic breakdown of sugar present in the fermenting must while the fluctuation in temperature suggests that biochemical changes occurred while the fermenting organism was metabolizing the substrate. Mathew *et al.* (2017) stated that the production of heat during fermentation is an exothermic process which increases the temperature of the fermenting must inside a vessel. According to Ezeama and Ebia (2015), quality wine should have an optimum temperature of 30 ^{o}C. Therefore, our product meets this requirement which makes it a wine of good quality. However, the optimum temperature suitable for the growth of most wine yeast is 25 ^{o}C. Hence, excessively low or high temperature will affect the process of fermentation.

Table 4: Physicochemical parameters of the fermenting 'must' monitored at intervals

Day	TA (g/ml)	RS (g/l)	SG	AC (% v/v)	pH	Temp (^{o}C)
0	0.39±0.015^{b}	52.3±1.80^{f}	1.09±0.036^{a}	0.00±0.000^{a}	3.8±0.173^{a}	26.4±0.346^{a}
2	0.69±0.030^{d}	51.0±2.00^{de}	1.08±0.035^{a}	1.37±0.101^{b}	3.7±0.231^{a}	28.0±0.093^{c}
4	0.52±0.026^{c}	50.5±1.35^{de}	1.08±0.026^{a}	1.37±0.075^{b}	3.5±0.361^{a}	27.2±0.361^{b}
6	0.40±0.025^{b}	49.0±1.00^{d}	1.05±0.046^{a}	5.43±0.518^{c}	3.5±0.306^{a}	27.2±0.341^{b}
8	0.39±0.021^{b}	45.9±1.73^{c}	1.03±0.031^{a}	8.15±0.601^{d}	3.6±0.289^{a}	27.6±0.721^{bc}
10	0.39±0.026^{b}	43.2±1.31^{b}	1.01±0.150^{a}	10.87±0.300^{e}	3.6±0.200^{a}	27.1±0.380^{ab}
12	0.39±0.020^{b}	25.7±0.90^{a}	1.01±0.190^{a}	10.87±0.573^{e}	3.7±0.265^{a}	27.9±0.130^{bc}
14	0.33±0.020^{a}	24.45±0.87^{a}	1.00±0.100^{a}	12.23±0.332^{f}	3.7±0.208^{a}	30.3±0.557^{d}

Values show means of triplicate analysis ± SD. Different letters (a-f) used as superscript along the column indicate significant difference at $P<0.05$ by Fisher's least significant difference test.
Key: TA –Titratable acidity, RS – Reducing sugar, SG – Specific gravity, AC – Alcohol content

Presented in Table 5 is the average sensory score the panelist assigned to apple juice and apple wine considering various quality parameters. The overall result shows that apple wine aged for 24 hours was slightly preferable than freshly prepared apple juice on Day 0. The overall acceptability indicated that apple wine aged for 24 h was strongly liked (5) than apple juice of day 0 which was liked (4). This result could be attributed to the fermentative role played by palm wine yeast during winemaking. High alcohols are known to be important precursors for the formation of esters which are associated with pleasant aromas (Clemente-Jimenez *et al*., 2005; Okeke *et al*., 2015). Reports have shown that alcoholic fermentation leads to a series of byproducts in addition to ethanol. Some of the byproducts of alcoholic fermentation which influence the quality of the finished product including sensory properties are carbonyl compounds, alcohols, esters, acids and acetals. According to Plutowska and Wardencki (2008) and Duarte *et al*. (2010), the composition and concentration of the byproducts can vary widely (mg/L to hundreds of mg/L). In addition to contributing to the sensory characteristics of wine, its acid content also plays a significant role in preserving the product (Radovanović *et al*., 2007).

Table 5: Sensory evaluation scores assigned to apple juice and apple wine

Quality	Apple juice (Day 0)	Apple wine aged for 24 h
Colour	3.29±0.488	4.00±0.000
Taste	4.14±0.690	5.00±0.000
Aroma	5.00±0.000	4.00±0.000
Texture	3.14±0.900	3.29±1.000
Overall acceptability	4.14±0.690	5.00±0.000

5 - point Hedonic scale: 5-Strongly liked, 4-Liked, 3-Moderately liked, 2-Disliked, 1-Strongly disliked

Fig. 2 illustrates the fact that specific gravity (density) of a must is largely dependent on the sugar content of the must. During alcohol fermentation, yeast converts sugars into carbon dioxide and alcohol. The decrease in sugar content and the presence of ethanol (which is appreciably less dense than water) is capable of reducing the density of the must. According to Idoko *et al.* (2020), specific gravity shows an inverse relationship with alcoholic content. In other words, during fermentation, increase in alcohol content, acids and other ethyl compounds will result in decrease in specific gravity of the must. In order to supplement the sugar content of the must for sustainable growth of the yeast, refined sugar was part of the additives used in the course of preparing apple wine. Several studies have identified low sugar content as a limitation towards utilization of tropical fruits in wine production (Alobo and Offonry, 2009). Considering the relationship between the alcohol and reducing sugar content of fermenting apple wine must, the result obtained from this study shows that alcohol content gradually increased but the reducing sugar content was on the decrease with an increase in fermentation time. As fermentation progressed, Figure 2 suggests that there is a level of relationship between the reduction in quantity of reducing sugar and specific gravity of the fermenting must.

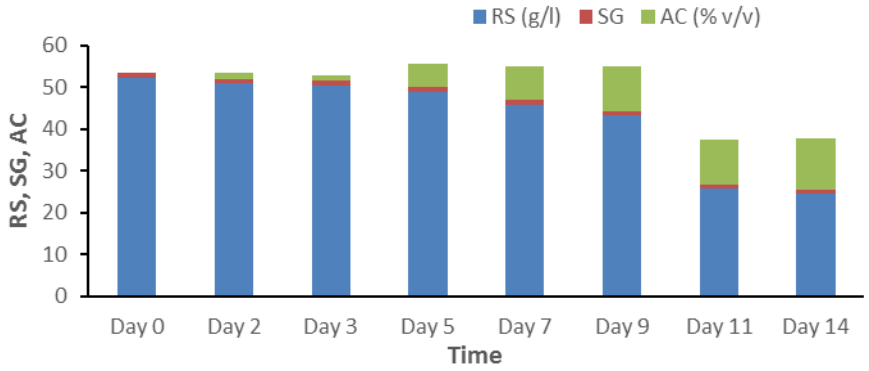


Figure 2: Comparison between reducing sugar, specific gravity and alcohol content of fermenting 'must'.
Key: RS – Reducing sugar, SG – Specific gravity, AC – Alcohol content

The inherent relationship between the titratable acidity of the fermenting musts and its pH value is illustrated in Fig. 3. pH is a logarithmic measure of the concentration of free hydrogen ions in a chemical or biological system whereas titratable acidity is a measure of the (related) amount of acid 'anions' in a juice. As a general rule, pH value increases whereas the titratable acidity reduces,

vice-versa.

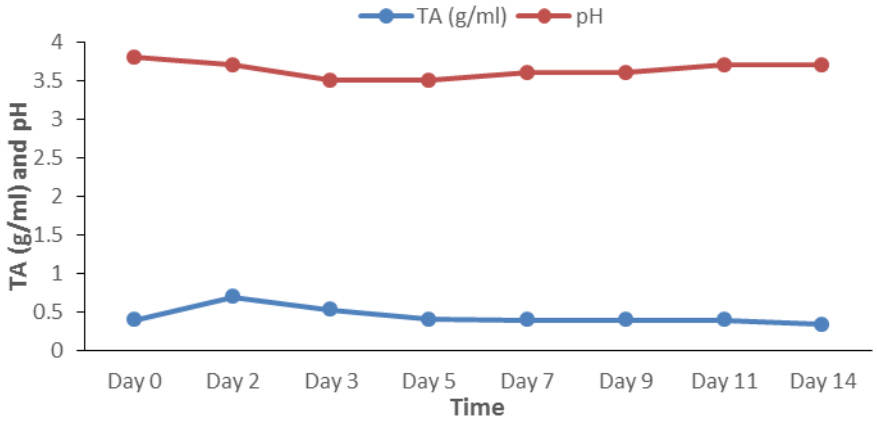


Figure 3: Comparison between titratable acidity (TA) and pH of fermenting must

4. CONCLUSIONS

Apple wine was produced using apples gotten from the market and indigenous palm wine yeast (*Saccharomyces cerevisiae*). Physicochemical analysis of the apple 'must' fermented by palm wine yeast (*S. cerevisiae*) shows that titratable acidity, reducing sugar and the specific gravity were within the ranges of 0.39-0.33 g/ml, 52.3-24.45 g/l and 1.09-1.00, respectively were decreasing continuously as fermentation progressed with few exceptions. Similarly, pH of the fermenting apple 'must' ranging from 3.8-3.7 steadily decreased while maintaining some level of stability but slightly increased a few days before the stoppage of the fermentation process. Alcohol content of the fermenting must range from 0-12.23 % (v/v) of which in the course of fermentation time, the values steadily increased except at short intervals when it was stable. That notwithstanding, the mean count of the palm wine yeast fermenting the 'must' increased from 3.95 - 5.15 x 10^5 Cfu/ml with an increase in fermentation time and the alcohol content is an indication that the fermenting palm wine yeast is highly alcohol-tolerant. A decrease in the mean bacterial count (2.7 x 10^4 Cfu/ml) of the fermenting must until no culturable bacteria were detected in the apple wine is an indication that the alcoholic drink is safe for consumption. The pH (3.7) of the apple wine also gives a level of assurance that the product is safe. The temperature of the fermenting must range from 26.4-30.3 ^oC of which as the fermentation progressed, the values gradually increased and maintained stability over a period. The sensory evaluation shows that apple wine was slightly preferable than apple juice based on overall acceptability, colour and taste. Therefore, the indigenous yeast strain *S. cerevisiae* isolated from palm wine which was used to ferment apple juice which turned into apple wine is a product of acceptable quality. Based on European Economic Community recommendation for wines, the product is a good table wine considering its alcohol content (12.23 % v/v).

Conflict of interests

The authors declare no conflict of interest.

REFERENCES

Alberti, A., Vieira, R. G., Drilleau, J. F., Wosiacki, G. and Nogueira, A. (2011). Apple wine processing with different nitrogen contents. *Brazilian Archives of Biology and Technology* 54(3):551-558.

Alobo, A. P. and Offonry, S. U. (2009). Characteristics of coloured wine produced from roselle

(Hibiscus sabdariffa) calyx extract. *Journal of the Institute of Brewing* 115(2): 91-94.

Amerine, M. A., Berg, H. W., Kunkee, R. E., Ough, C. S., Singleton, V. L. and Webb, A. D. (1980). The Technology of Winemaking, (4th Ed). AVI, Westport Connecticut. pp. 185-703

Asuk, A. A., Umoh, I. B., Eteng, M. U., Jayeola, C. O. and Akpanyung, E. O. (2011). Production, characterization and safety of wine obtained from a blend of tomato, almond, orange, lemon and African star apple extract. *Annals of Biological Research* 2(5):492-5003.

Awe, S. and Nnadoze, S. N. (2015). Production and microbiological assessment of date palm (*Phoenix dactylifera*) fruit wine. *British Microbiology Research Journal* 8(3):480-488.

Benedict, S. R. (1908). A regent for the detection of reducing sugars. *Journal of Biological Chemistry* 5:485-487.

Bertolini, M. C., Ernandes, J. R. and Laluce, C. (1991). New yeast strains for alcoholic fermentation at higher sugar concentration. *Biotechnology Letters* 13:197-202.

Boekhout, T. and Kurtzman, C. P. (1996). Principles and methods used in yeast classification, and an overview of currently accepted yeast genera, In: Non-conventional Yeasts in Biotechnology. A Handbook (Wolf, K.). Springer-Velag, Berlin, Heidelberg. pp. 1-99.

Casey, G. P. and Ingledew, W. M. M. (2008). Ethanol tolerance in yeasts. *CRC Critical Reviews in Microbiology* 13(3):219-280.

Chakraborty, K., Saha, J., Raychaudhuri, U. and Chkraborty, R. (2014). Tropical fruit wines: A mini review. *Natural Products* 10(7):219-228.

Chilaka, C. A., Uchechukwu, N., Obidiegwu, J. E. and Akpor, O. B. (2010). Evaluation of the efficiency of yeast isolates from palm wine in diverse fruit wine production. *African Journal of Food Science* 4(12):764-774.

Clemente-Jiménez, J. M., Mingorance-Cazorla, L., Martínez-Rodríguez, S., Heras-Vázquez, F. J. L. and Rodríguez-Vico, F. (2005). Influence of sequential yeast mixtures wine fermentation. *International Journal of Food Microbiology* 98:301-308.

Cousin, F. J., Guellec, R. L., Schlusselhuber, M., Dalmasso, M., Laplace J. and Cretenet, M. (2017). Microorganisms in fermented apple beverages: current knowledge and future directions. *Microorganisms* 5(39):1-22.

Duarte. W. F., Dias, D. R., Oliveira, M. J., Teixeira, J. A., Silva, J. B. D. and Schwan, R. F. (2010). Characterization of different fruit wines made from cacao, cupuassu, gabiroba, jaboticaba and umbu. LWT-*Food Science and Technology* 43:1564-1572.

Ebana, R. U. B., Edet, U. O., Anosike, K. I. Etok, C. A. & Kanu, T. O. (2019). Nutritional analysis and wine production potentials of *Telfairia occidentalis* (fluted pumpkin) leaves and *Cucumis sativus* L. (cucumber) using baker's and palm wine yeast strains, *World News of Natural Sciences*, 22, 12-30.

Emecheta, R. O., Maduka, N., Ahaotu, I. and Odu, N. N. (2019). Assessment of date wine clarified using commercial pectinase and *Aspergillus tamarii* strains immobilized on biomatrix. *Science Park Journal of Applied Biotechnology Research* 3(1):019-031.

Enidiok, S. E. and Attah, L. E. (2010). Chemical composition in relation to the quality of wines produced from Nigerian *Syzygium malaccensis* and *Eugenia owariensis* apples. *African Journal of Food Agriculture Nutrition and Development* 10(2):2124-2138.

Ezeama, C. F. and Ebia, O. O. (2015). Yeast performance, chemical and sensory properties of wine produced from Chinese star apple (*Averrhoa carambola*). *American Journal of Food and Nutrition* 5(1):9-15.

Idoko, B. O., Ukayima, M. T., Gberikon, G. M. and Olaleye, O. O. (2020). Physico-chemical and sensory properties of wine produced from blends of roselle calyces extract and pineapple juice. *Asian Journal of Biotechnology and Bioresource Technology* 6(1):28-38.

Jarvis, B. (1996). Cider, perry, fruit wines and other alcoholic fruit beverages In: Fruit Processing. Arthey, D. and Ashurst, P. R. editors. Blackie Academic and Professional pp. 98.

Laplace, J. M., Delgenes, J. P., Molleta, R. and Navarro, J. M. (1992). Fermentation of lignocellulosic sugars to ethanol-selection of mutants of *Pichia stipitis* affected for D-glucose utilization. *Enzyme and Microbiology Technology* 14:644-648.

Laplace, J. M., Delgenes, J. P., Molleta, R. and Navarro, J. M. (1993). Ethanol production from glucose and xylose by separated and co-culture processes using high cell density systems. *Process Biochemistry* 28:519-525.

Lodder, J. (ed) (1971). *The yeasts: A taxonomic study.* Northern Holland and Publishing, Amsterdam.

Ngọc, N. M., Minh, N. P. and Dao, D. T. A. Isolation and characterization of yeast strains for palm (*Borassus flabelliffer*) wine fermentation. *International Journal of Engineering Research and Technology (IJERT)* 2(11):3602-3615.

Mathew, B., Datsugwai, M. S. S., David, E. S. & Harriet, U. (2017). Production of wine from fermentation of grape (*Vitis vinifera*) and sweet orange (*Citrus seninsis*) juice using *Saccharomyces cerevisiae* isolated from palm wine, *International Journal of Current Microbiology and Applied Sciences*, 6(1), 868-881.

Nwachukwu, I. N., Ibekwe, V. I., Nwabueze, R. N. and Anyanwu, B. N. (2006). Characterization of palm wine yeast isolates for industrial utilisation. *African Journal of Biotechnology* 5(19):1725-1728.

Ogodo, A. C., Ugbogu, O. C., Ugbogu, A. E. and Ezeonu, C. S. (2015). Production of mixed fruit (pawpaw, banana and watermelon) wine using *Saccharomyces cerevisiae* isolated from palm wine. *SpringerPlus* 4(683):1-11.

Okeke, B. C., Agu, K. C., Uba, P. O., Awah, N. S., Anaukwu, C. G., Archibong, E. J., Uwanta, I. I., Ezeneche, J. N., Ezenwa, C. U. and Orji, M. U. (2015). Wine production from mixed fruits (pineapple and watermelon) using high alcohol tolerant yeast isolated from palm wine. *Universal Journal of Microbiology Research* 3(4):41-45.

Okemini, O. F. and Dilim, I. C. (2017). Physico-chemical properties and sensory evaluation of wine produced from tiger nut (*Cyperus esculentus*). *International Journal of ChemTech Research* 10(12):155-164.

Oladipo, I. C., Akinsola, O. E. & Ojerinde, B. O. (2014). Microbiological analysis of some locally

produced fruit wine in Nigeria, *Global Journal of Biology, Agriculture and Health Sciences*, 3(2), 75-80.

Parapouli, M., Vasileiadis, A., Afendra, A. and Hatziloukas, E. (2020). *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiology* 6(1):1-31.

Plutowska, B. and Wardencki, W. (2008). Application of gas chromatography-olfactometry (GCeO) in analysis and quality assessment of alcoholic beverages-a review. *Food Chemistry* 107(1):449-463.

Rajković, M. B., Novaković, I. D. and Petrović, A. (2007). Determination of titratable acidity in white wine. *Journal of Agricultural Sciences* 52(2):169-184.

Reddy, L. V. A. and Reddy, O.V. S. (2005). Production and characterization of wine from mango fruit (*Mangifera indica* L.). *World Journal of Microbiology and Biotechnology* 21:1345-1350.

Riekstina-Dolge, R., Kruma, Z. and Karklina, D. (2012). Sensory properties and chemical composition of cider depending on apple variety. *Research for Rural Development* 18:102-108.

Samson, A. K. S.m Singh, G. (2017). Optimum parameters for wine production from pomegranate fruit juice, *International Journal of Pharmaceutical Sciences and Research*, 8(11), 4826-4831.

Saranraj, P., Sivasakthivelan, P., Naveen, M. (2017). Fermentation of fruit wine and its quality analysis: A review. *Australian Journal of Science and Technology* 1(2):85-97.

Snell, F. D. and Ettre, I. S. L. (1974). *Wine and Must:* In Encyclopedia of Industrial Chemical Analysis, vol. 14. Interscience Publishers, John Wiley and Sons Inc, New York.

Snopek, L., Mlcek, J., Sochorova, L., Baron, M., Hlavacova, I., Jurikova, T., Sochor, J. (2018). Contribution of red wine consumption to human health protection, *Molecules*, 23(1684), 1-16

Stanley, M. C., Ifeanyi, O. E., Christopher, A. E. and Emmanuel, E. (2014). Alcohol and biomass production from pineapple juice using a combination of palm wine yeast and baker's yeast. *International Journal of Life Sciences Biotechnology and Pharma Research* 3(4):214-221.

Swami, S. B., Thakor, N. J. and Divate, A. D. (2014). Fruit wine production: a review. *Journal of Food Research and Technology* 2(3):93-100.

Teramoto, Y., Sato, R., Ueda, S. (2005). Characteristics of fermentation yeast isolated from traditional Ethiopian honey wine, ogol. *African Journal of Biotechnology* 4(2):160-163.

Uma, V. and Polasa, H. (1990). *S. cerevisiae* of palm wine enhanced ethanol production by using mutagens. *Journal of Industrial Microbiology and Biotechnology* 5:1-4.

Umeh, S. O., Udemezue, O., Okeke, B. C. and Agu, G. C. (2015). Pawpaw (*Carica papaya*) wine: with low sugar produced using *Saccharomyces cerevisiae* isolated from a local dink 'burukutu'. *International Journal of Biotechnology and Food Science* 3(2):17-22.

Velić, D., Velić, N., Klarić, D. A., Klarić, I., Tominac, V. P., Košmerl, T. and Vidrih, R. (2018). The production of fruit wines-a review. *Croatian Journal of Food Science and Technology* 10(2):1-12.

Won, S. Y., Seo, J. S., Kwak, H. S., Lee, Y., Kim, M., Shim, H. S., Jeong, Y. (2015). Quality

characteristics and quantification of acetaldehyde and methanol in apple wine fermentation by various pre-treatments of mash. *Preventive Nutrition and Food Science* 20(4):292-297.

Wurz, D. A. (2019). Wine and health: a review of its benefits to human health. *Bio Web of Conferences* 12(04001):1-3.

Zainab, A., Amos, Y., Datsugwai, M. S. S., Mathew, B. (2018). Quality assessment of water melon (*Citrullus lanatus*) wine produced using *Saccharomyces cerevisiae* isolated from palm wine, *Journals of Biomaterials*, 2(2), 65-73.

Zoecklein, B. W., Fugelsang, K. C., Gump, B. H. and Nury, F. S. (1990). *Phenolic Compounds and Wine Color. In: Production Wine Analysis*. Springer, Boston, MA. pp. 129-168.