Original Research Article

ISOLATION AND CHARACTERIZATION OF YEAST ASSOCIATED WITH PALM WINE FERMENTATION.

ABSTRACT

Wine is a natural fermented product from the juices of fruits, by the action of yeast cells. The aim of this study is the production and quality assessment of wine from orange fruits using yeast isolated from palm wine. Palm wine were characterize to ascertain its physicochemical properties while the Yeast were isolated from aged palm wine and characterize using standard method from which Saccharomyces cerevisae was confirmed as the major species present. Fermentation orange fruits must for 14days was carried out using recipes that contained a mixture of each fruit must with Saccharomyces cerevisae isolated from palm wine. Wine produced was analysed to determined there quality. The result shows values of 3.67 and 3.38 for pH, 1.00 and 1.02 for specific gravity, 9.79and 9.443 for percentage (%) alcohol (v/v), and 0.063 and 1.348 for percentage (%) titratable acidity respectively for the yeast wine and commercial wine respectively. The study have shown that quality wine could be produced from orange fruits for immediate consumption using yeast (Saccharomyces cerevisae) isolated from palm wine.

Keywords: Fermentation, Isolation, Palmwine, Upwine

INTRODUCTION

Palm wine is the fermented sap of the tropical plants of the *palmae* family. It is produced and consumed in very large quantities in the southeastern Nigeria. It contains nutritionally important components including amino acids, proteins, vitamins and sugars (Okafor, 2017). These make this wine a veritable medium for the growth of a consortium of microorganisms, whose growth in turn, change the physicochemical conditions of the wine, giving rise to competition and successions of organisms.

Yeast is microscopic, single-celled organisms that are classified in the family Fungi. Individual yeast cells multiply rapidly by the process of budding, in which a new cell begins as a small bulge along the cell wall of a parent cell. In the presence of an abundant food source, huge populations of yeast cells gather. The cells often appear as long chains with newly formed cells still attached to their parent cells, due to the short budding time of two hours (Ingram and Burttke 2014).

Yeast are among the few living organisms that do not need oxygen in order to produce energy. This oxygen-independent state is called anaerobic. During such anaerobic conditions, yeast convert carbohydrates—starches and sugars—to alcohol and carbon dioxide gas. This process is known as fermentation. The fermentation process of yeast is caused by enzymes, catalysts in chemical reactions similar to the digestive enzymes in the human body. In fact, the word enzyme means "in yeast." Certain enzymes in yeast act on starch to break down the long chainlike molecules into smaller units of sugar. Then other yeast enzymes convert one kind of sugar molecule to another(Ingram and Burttke 2014).

Still other enzyme reactions break apart the sugar molecule (composed of carbon, hydrogen, and oxygen atoms) into ethyl alcohol and carbon dioxide. The series of reactions provides the yeast cells with the energy necessary for their growth and division (form of reproduction). In nature, yeast enzymes break down the complex carbon compounds of plant cell walls and animal tissues, feeding on the sugar produced in the process. In this way, yeast function as natural decomposers in the environment. Words to Know Anaerobic: Living or growing in an atmosphere lacking oxygen. The natural starches and sugars in the liquids provide food for the yeast. Deprived of oxygen during the fermentation process, yeast produce alcohol as a by-product of incomplete sugar breakdown.

Many workers have indeed carried out studies aimed at isolating and exploiting palm wine yeasts for industrial processes. These include for baking, portable ethanol production and single cell protein production. Ogbonna (2014) used palm wine isolates of *Saccharomyces cerevisiae*to produce artificial palm wine and beer, respectively. Very few efforts have been made at characterizing these yeasts for fuel ethanol production. Despite the continuing research efforts at utilising bacteria for ethanol production, the yeast is still the primary choice for fermentation (Anyaegbu et al., 2021).

Since the beginning of the 1980s, the use of *Saccharomyces cerevisiae*yeast starters has been extensively applied in the industrial and homemade beverage production processes. Currently, most ofthe wine production processes rely on *S. cerevisiae* strains that allow rapid and reliable fermentations, reduce the risk of sluggish or stuck fermentations and prevent microbial contaminations. Yeast startercultures that are specifically selected for the winemaking process onthe basis of scientifically verified characteristics typically complement and optimise the raw material quality and individual characteristicsof the wine, creating a more desirable product. Generally, wines produced with selected yeasts have a higher quality than wines produced by spontaneous fermentation (Ejimofor and Oledibe, 2021).

Wine is a product of alcoholic fermentation by yeast of the juice of ripe grapes or any fruit with a good proportion of sugar (Okafor, 2017). Wine is one of the most recognizable high value added products from fruits. It can also be used as a substrate for the manufacture of vinegar, a by-product of wine manufacture. Wine manufacture is challenging in which marketable product can be obtained, but the processes involved in its production are relatively straight forward (Amerine *et al.*2020). Highly acceptable wines can be made from practically all fruits. Wine can be fermented with yeast that occurs naturally in grape and in other countries where grape is not produced, emphasis is usually placed on other fruits for wine making.

Statement of problem

The presence of various microorganisms especially the bacteria and yeasts responsible for the fermentation of palm-wine has been reported by Anyaegbu et al. (2019), Ejimofor et al., (2021) and

Ejimofor and Oledibe (2021) and many other researchers. During fermentation, the sugars in the palm-sap are metabolized to alcohol and organic acids with the result that the sap loses its sweetness. The types of bacteria present appear to depend on the stage of fermentation and the composition of the sap (Okafor, 2017). Although alcohol production is common among yeasts, it is rare among bacteria (Ingraham and Ingraham, 2014). Yeasts are used to make most alcohol beverages. But *pulque* is an exception. Pulque is an alcoholic beverage from the juice of the agave plant fermented by *Zymomonasmobilis* (Uzochukwu *et al.,* 2019).

The difficulty of storing palm-wine to retain its normal characteristics due to the fermentative ability of probably *Zymomonas* species and other microorganisms present in the wine has been a major problem in the bottling of palm-wine in Nigeria and consequently its distribution for consumption. In addition to their fermentative ability, the presence of yeast species in palm-wine may thus be beneficial to man.

The present study therefore is aimed at the isolation and identification of *yeast in palmwine*and to determine the contribution of the bacterium to the fermentation of sugars in wine production.

Objectives of the study

The major aim of this project work is to identify and isolate yeast commonly found in palm wine and evaluates their role in production of wine.

The specific objectives are

- Collection of palm wine samples from different locations within Awka.
- Identification and isolation of the microorganisms in palm wine.
- Use of the yeast isolate for wine production
- Comparing the yeast isolated and the commercial yeast.
- Collection of result data, correlating it with available literatures and making necessary recommendations.

Significance of study

Palm wine have great economic important and the microorganisms it contains have been very useful to man and industries. The result from this work will have many significance in the following areas:

- The study will give more insight into the nutritional composition of palm wine and the need for increase in its consumptions. This will increase the sales revenue of palm wine tappers and sellers.
- This work will show how valuable yeast can be in wine production.
- Beverage industries will benefit from the result of this work as it will recommend various areas at which the microorganisms identified could be employed as a catalyst in the industries. The work will also provides novel methods of fermentations with less harmful microorganisms. This move will boast more revenue for such industries.
- This work will give a valuable foundation to researchers who will further into isolation of the various microbes identified from the work. This microbes can be usefully employed into biotechnology in alcohol industries.
- Finally the government and our society will benefit from this work in terms of creation of new business by people having been enlighten on the great nutritional content of this palm wine.

Materials and methods

Sample collection

Five samples each from two sources of palm wine (Raffia palm and palm tree) were randomly collected from tappers in Awka. The palm wine was harvested by the tappers using natural wood during tapping process using bamboo tube. After that sterile bottles were use to collect the palm wine and was kept in an icebox (40C) during transportation (30mins) to the laboratory.

Preparation of samples

The physical and chemical property of each of the palm wine sample was determined within a day. The samples were aseptically filtered (with sterile Watman filter paper) and kept at 40C until analyses were carried out.

Physicochemical properties

This involved visual examination of the palm wine samples.

- Color measurements of samples were carried out using a hunter lab clorflex colorimeter.
- The turbidity of the palm wine was estimated by measuring the transmittance at 650nm using a spectrophotometer as described by Taipaiboon (13).
- The taste and odour of the palm wine was also determined.
- The pH value was measured at ambient temperature with pH meter which was calibrated with pH 4.0 and 7.0.
- The total acidity was determined by titration with NaOH and phenolphytalin was used as an indicator which was calculated in term of lactic acid.
- The total soluble solids of palm wine sugar syrup were determined as degree Brix using hand refractometer.
- Total sugar and reducing sugar were quantified by titration with Fehling reagents. The results were expressed as gram of glucose per 100 gram of sample.

- Conductivity measurements were carried out with an Orion 4 Stars conductometer. The procedure consisted in calibrating the instrument with 1413 µS and 12.9 mS/cm standards, and, subsequently, the sensor was immersed in tequila and the conductivity was measured in triplicate. The electrode was rinsed with abundant water before and after each immersion. All experiments were performed at room temperature.

- Density and ultrasound velocity measurements were carried out with an Anton Paar DSA5000 densimeter and sound velocity analyzer equipped with a new-generation stainless-steel cell. Temperature control was maintained with a Peltier element with a resolution of 0.001°C, giving rise to uncertainties in density of ca. g/cm3. Errors in ultrasound velocity measurements arise mainly from temperature variations, and in this study the resolution was 10−2 m/s. The densimeter was cleaned following the routine, consisting of injecting Alconox at a concentration of 40% for several times. Later, ultrapure water was injected in order to calibrate at 20°C until density reached a value of 0.998203 g cm−3. Once this measurement was achieved, density and viscosity of tequila samples were measured. The determination of the density and sound velocity in the tequilas was carried out at 25°C in triplicate. Both density and sound velocity measurements were performed at the same time.

- Measurements of the refractive index in the tequila were carried out with a refractometer of the company Abbe, model 2WA. The analysis consisted in initially cleaning the prism with ethylic alcohol, followed by calibration with a drop of pure ethylic alcohol. The visual field of the equipment was adjusted to illuminate half of the field while the other half remained dark. Once the equipment was working properly, a measurement of 1.36 was obtained. Later, a drop of tequila was put on the sample holder in order to obtain its refractive index. All measurements were performed in triplicate at 25°C.

- Viscosity measurements of different tequilas were performed with an ARES rheometer TA-22 G2 using a Couette geometry double-wall type. The inner and outer diameters of the hollow cylinder are 29.51 and 32 mm, respectively, while the inner and outer diameters of the cup are 27.94 and 34 mm, respectively. All measurements were performed at 25°C. An amount of 8 mL of tequila was placed in the sample holder and maintained at a shear velocity of 10 s^{-1}.

Isolation of yeasts from palm wine

The 25-day-old wine samples were centrifuged in sterile centrifuge bottles for 5 min at low speed. One ml of the serially diluted sediment is inoculated by streaking on plates of Glucose Yeast Agar (Anyaegbu et al., 2019) and incubated at 28°C for 24 h. The yeast colonies that developed are isolated and purified by further streaking on GYA.. Yeast isolates were tentatively identified by determining their pattern of fermentation and morphological characteristics according to Kunkee and Amerine, (2010).

Yeast Identification

Isolation and identification of yeasts was by the use of standard morphological and physiological tests and identification keys described by Ejimofor et al., 2021.

Incubation was at 28°C under aerobic and conditions. The morphological and cultural characteristics of the years were studied after isolation on glucose yeast agar (GYA) and yeast malt agar (YMA) (Biolife). These tests included morphology, surface characteristics, presence of pseudohyphae, ascospore formation and vegetative reproduction. Fermentative tests included sugars such as glucose, galactose, sucrose maltose, cellobiose, trehalose, lactose, raffinose, soluble starch, D-xylose, L-arabinose, and Dribose. Others tests include nitrate assimilation, growth in 10% NaCl + 50% glucose in yeast extract, growth at 37°C and growth in 50% w/w glucose yeast extract.

Evaluation of yeast strains Isolated with commercially sold yeast

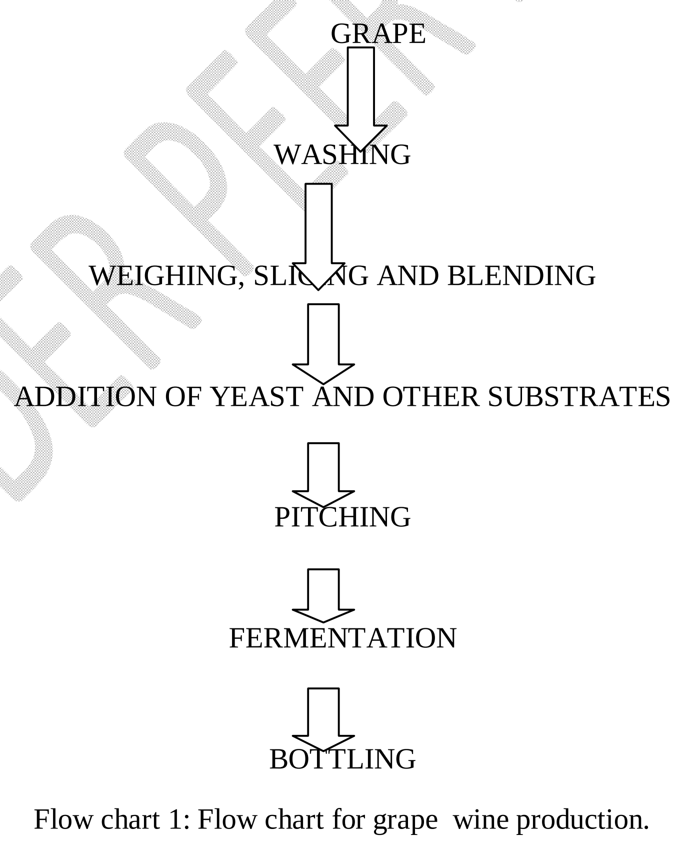
The yeast isolated was used in the production of wine and compared with commercially sold yeast.

Sample preparation

Fresh fruits of orange purchased were sorted, washed thoroughly with clean water to remove any adhering substances, peeled and its seeds removed. The flesh (3.3 kg) was sliced into small pieces using sharp stainless steel knife and blended until it becomes pure juice. A mesh cloth was used to remove solid materials from the juice. The juice extracted were then filled into sterilized glass bottles and then pasteurized using a heating mantle at 70°C for 30 min. 10g of citric acid was added and 10g of aspertine was also added.

GRAPE Wine Fermentation

FLOW CHART FOR ORANGE WINE PRODUCTION



Flow chart 1: Flow chart for grape wine production.

Procedure for wine production

- Sugar, lemon juice (to enhance distinct aroma/flavor), and distilled sterile water was added to the banana must.
- 5g of dried yeast cells was inoculated into the orange must contain in a fermenter, a process known as pitching.
- The experiment was observed for seven days at 32°C.

Analytical Assay

Sampling was carried out every 48 hrs for yeast count, total suspended solids, total dissolved solids, titrable acidity, pH determination, specific gravity and alcohol content.

RESULTS

The physicochemical properties of the palm wine and up wine used in the production of wine are presented in table 1.

Table 1: Physicochemical properties of palm wine and up wine

Parameter	**PALM WINE**	**UP WINE**
Colour	Milky	Cloudy
Alcohol Content (g/100ml)	4.0	4.3
Density	1.02	1.03
pH	7.20	6.0
Glucose (g/100ml)	0.60	0.75
Fructose (g/100ml)	0.80	1.05
Sucrose (g/100ml)	2.50	2.90
maltose (g/100ml)	0.09	1.80
Total Sugar (mg/100ml)	3.99	6.50

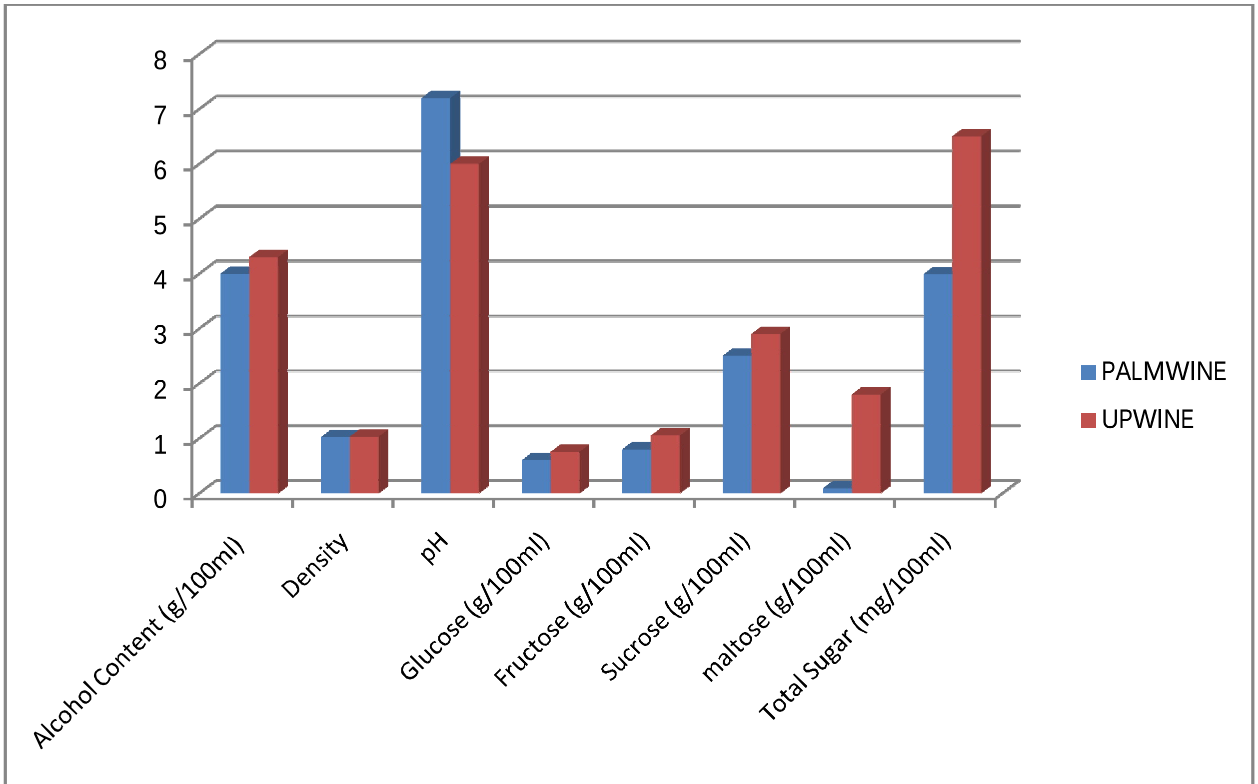


Fig 1:Physicochemical properties of palm wine and up wine

MICROBIAL COUNT

The Total Plate Count of Yeast (X 10^3cfu/ml) carried out at different times viz; 0, 6, 12, 18 and 24 hours respectively are presented in table 2.

Table 2: Total Plate Count of Yeast (X 10^3cfu/ml) carried out at different times

Time (Hrs)	0	6.0	12.0	18.0	24.0
Palm wine	2.0 X 10^3	4.5 X 10^3	6.2 X 10^3	7.8 X 10^3	8.2 X 10^3
UP wine	5.4 X 10^3	5.6 X 10^3	6.4 X 10^3	6.9 X 10^3	7.3 X 10^3

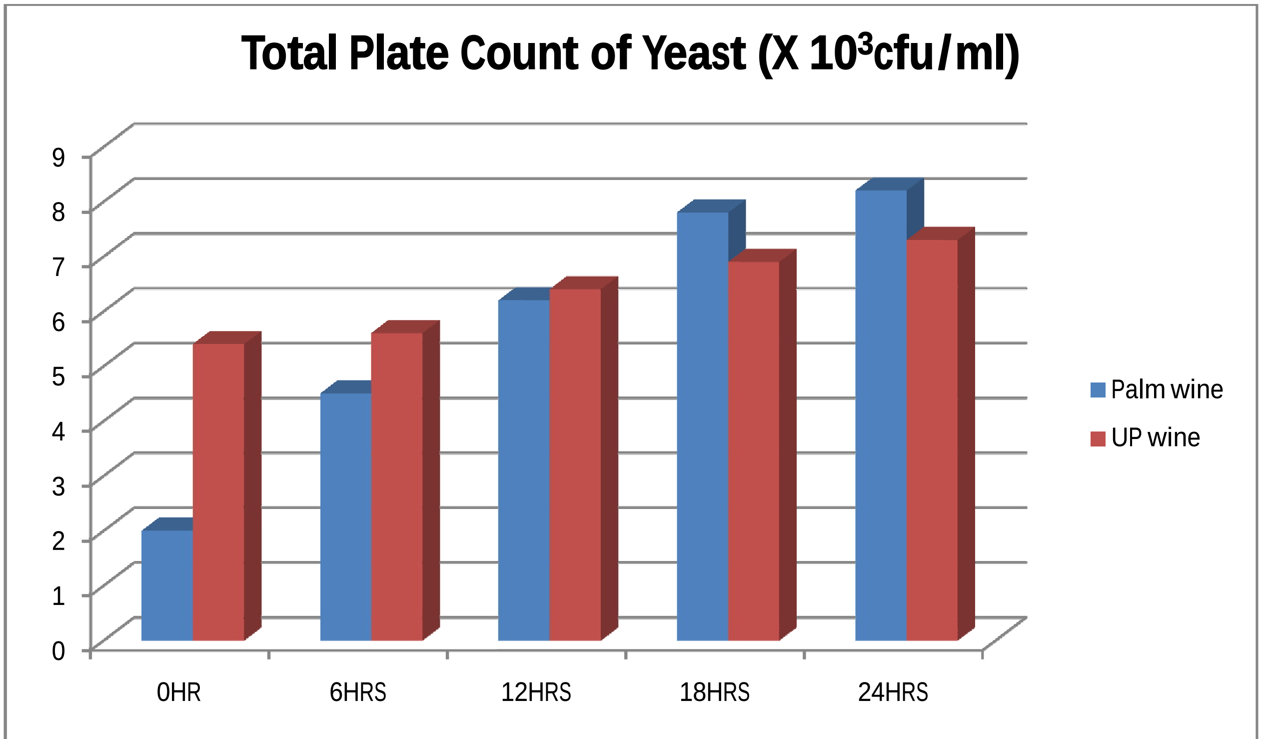


Fig 2: Total Plate Count of Yeast (X 10^3cfu/ml) carried out at different times

List 1 : Morphological Characteristics of yeast cells

Isolates	Surface	Margin	Colony Size (mm)	Shape	Vegetative Reproduction	Probable Isolates
A	Smooth	Entire	0.5 cream	Spherical	Budding	*S. cerevisiae*
B	Smooth	Entire	0.5 cream	Spherical	Budding	*S. cerevisiae*
C	Smooth	Entire	0.5 cream	Spherical	Budding	*S. cerevisiae*
D	Smooth	Entire	0.5 cream	Spherical	Budding	*S. cerevisiae*
E	Smooth	Entire	0.5 cream	Spherical	Budding	*S. globosus*

F	Smooth	Entire	0.3 cream	Elipsoidal	Budding	*S. cerevisiae*

List 2: Carbohydrates Fermentation By Yeast Isolates

Carbon Source	**A**	**B**	**C**	**D**	**E**	**f**
Glucose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Lactose	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+
Xylose	-	-	-	-	-	-
Raffinose	+	+	+	+	+	+

List 3 : Yeast isolates, sources, Name, sedimentation rate and ethanol tolerance

ISOLATE	**SOURCE**	**NAME**	**SEDIMENTATION RATE**	**ETHANOL TOLERANCE**
A	PALM WINE	*S. cerevisiae*	57.5	12.0
B	PALM WINE	*S. cerevisiae*	56.5	10.0
C	PALM WINE	*S. cerevisiae*	83.6	12.0
D	PALM WINE	*S. cerevisiae*	82.0	17.0
E	UP WINE	*S. cerevisiae*	90.0	16.0
F	UP WINE	*S. globosus*	64.5	15.0

Physiochemical properties of wine

The wine produced after 14days fermentation with yeast isolated from palmwine and commercial yeast were compared in other to evaluate its quality. The result shows values of 3.67 and 3.38 for pH, 1.00 and

1.02 for specific gravity, 9.64 and 9.44 for percentage (%) alcohol (v/v), and 0.63 and 1.34 for percentage (%) titratable acidity respectively for the yeast wine and commercialwine. The physiochemical properties of the yeast wine and commercialwinene are presented in Table 3.

Table 3: Physiochemical properties of yeast wine and commercial wine

Parameters	**Yeast wine**	**Commercial wine**
pH	3.67	3.38
Specific gravity	1.00	1.02
Titratable acidity	0.63	1.34
Residual ºBx	0.54	0.54
Alcoholic content percentage (%) (v/v)	9.46	9.44

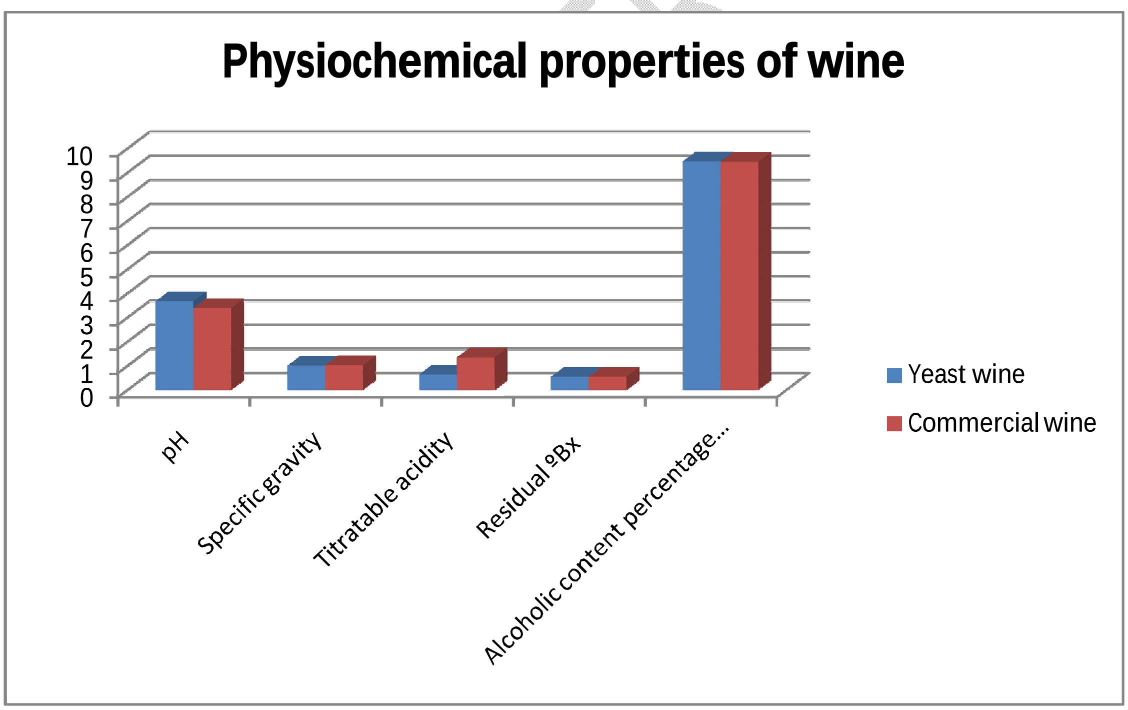


Fig 3: **Physiochemical properties of yeast wine and commercial wine**

Sensory properties

The sensory evaluation result of yeast wine and commercial wine are presented in in Table 4. The result revealed that wine fermented with yeast differed significantly in terms of color, odour, taste, and overall acceptability when compared with the control sample.

Table 4. Summary of the mean sensory score for the yeast wine and commercial wine

Parameter	**Yeast wine**	**commercial wine**
COLOUR	6.80± 0.11	7.50± 0.25
ODOUR	7.50± 1.03	7.40± 0.20
TASTE	7.30± 1.20	6.70± 0.11
OVERALL ACCEPTABILITY	7.20± 0.33	7.90± 0.20

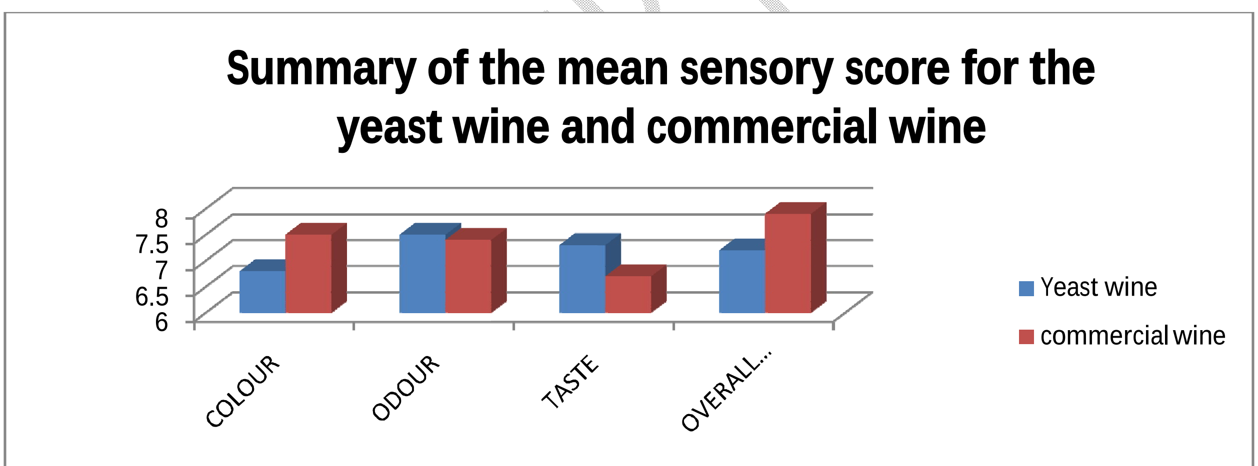


FIG 4: Summary of the mean sensory for the yeast wine and commercial wine

DISCUSSION

We studied the production of wine through fermentation using yeast isolated from palm wine. All the processes were carried out with crushed fruit. The fresh pulp was also pressed in some of the experiments with orange. The initial sugar concentration of the orange fruit mash was 110.1 g/L. The yeast inoculated alcoholic fermentation was faster than the spontaneous one because the lag phase was shorter and the fermentation rate similar. Although yeast inoculation was not really needed to produce these fruit wines, specific yeast for inoculation will be highly recommended in the industrialization of both the wine and the vinegar process. The industrialization requires shorter production periods and a repetitive product, which could be obtained by the practice of inoculating selected strains (Ejimofor and Oledibe, 2021).

The yield in terms of final product (wine) is acceptable as it was always well over 60%. We performed the whole process at the laboratory level, with such limiting factors as the strength of the press and the recovery of fruit pulp on a small scale. Scaling up to higher volumes and with industrial equipment will produce higher yields, similar to those observed in wine. The final product obtained in both cases showed good colour and good organoleptic characteristics.

From table 5 the acidity of the yeast wine was lower than the commercial wine. This increase in acidity is probably as a result of certain organic acids present in yeast wine mostly use as preservative. Ough (2010) reported that during fermentation, major acids formed include lactic, marlic, succinic and acetic acids. The final acidity of 0.63% is favourable for a check against spoilage organisms. Since table wines have titratable acidity in the range of 0.6-0.9% (Pozo-Bayón *et al.;* 2012). Presence of these acids in wine is necessary because without them the wine would taste unpalatably flat and spoil with a poor colour and flavour. (Reddy *et al,;* 2015)

The pH was on the decrease as expected from 3.67 in sycamore wine to 3.38 in yeast wine. This should be a result of its inverse proportionally with acidity. But it is important to point out that is no direct relationship between pH and total titratable acidity because of varying buffer capacity of the fermenting liquor (Lea *et al.;* 2013). So, little variation in fixed acidity is explained by the fact that yeast produced little acids during fermentation, (Ogbonna, 2014). This PH value of 3.67 is very important for microbial stabilization of sycamore wine aroma and taste developments.

There was gradual increase in alcohol content as fermentation continues. It later became constant from the 14th day which was the last day of fermentation. The reason is that most of the fermentable sugars have been converted to alcohol. Also toxicity of increased alcohol content produced made the yeast inactive for more production (Gambelliand Santaroni, 2014). Its alcohol content was 9.67% but commercial wine has alcohol content was 9.44% mostly due to further conversion of residual sugars to alcohol. Our result is in agreement with normal alcoholic content of table wines which ranges from 6-10% (Ferreira *et al.;* 2015).

Specific gravity of the yeast wine was 1.00 as compared to commercial Wine of 1.02 but the value was lower due to conversion of sugar to alcohols since alcohols have less Specific gravity than sugar. This result corroborates with Akubor *et al.;*(2013) observations when he produced wine using bush mango juice and sycamore respectively.

The sensory quality attributes of the wine highly rated; this is corroborated by the asessors who indicated their willingness to buy the wine if it was offered for sale. This wine was reported by the assessors to have a fruity-like flavour with marked presence of orange taste. The work of Olorunfemi, *et al*., (2019) confirmed that the type and aroma produced during wine making depends on yeast, environmental factors and physiochemical characteristics of the "must".

The color of the orange wine produced is brown and clear. This was because of the color of must of orange pulp which is brown and longer period of aging. It was also observed that sedimentation of particles was very fast. This could have been as a result of the occurrence of denser insoluble particles in them which settles to the bottom. The action of the protease enzyme such as papain ensured a breakdown of the proteins, peptides and polypeptides present in the wine. The peptide and polypeptide compounds also formed a complex with the tannin (protein –tannin complex) which settled out when the wine was left to stand for some time. This enhanced clarification of wine during aging. The mean value of color acceptability obtained from the sensory evaluation was 6.80 and 7.50 for the standard, Reason for this is the fact that peptide and polypeptide compounds present in the wine have formed a complex with the tannnis (protein tannin complex) which settled out during one month of aging (Akubor *et al*., 2013).

Sensory evaluation mean values obtained for odor of orange wine were 7.5 and 7.4 for standard wine. This showed that there is no significant different in odor between the orange wine and the control wine. Table 1 shows the mean values obtained in sensory evaluation of orange wine for taste are 7.3 and 6.7, for standard (control) wine, there is no significant difference in taste between the standard wine and it corroborates with Mounigan *et al.;* (2016) observations in sensory acceptance, quantitative descriptive and physicochemical analysis of wines. This was done with 7.2 and 7.9 for standard. This indicated that there is no significant different in acceptability.

CONCLUSION

This study has establisheddifferent species of Saccharomyces were isolated from aged palm wine and upwine. The isolated species had many similarities; The Ability of the isolate to grow in 10% Sodium chloride + 50% glucose medium confirmed that the isolate is *saccharomyces cerevisae*. The growth test distinguishes *saccharomyces cerevisae* from all other species.There is the possibility of orange wine production using yeast isolated from palm wine. The wines produced showed no appreciable differences in the tested parameters – pH, specific gravity, percentage (%) alcohol (v/v) and percentage (%) titratable acidity – when compared with Garden egg wine sample. The result of this work has shown that orange wine making which started with the collection of wholesome sycamore and garden egg fruit, cleaning, crushing, sulphite addition, fermentation, racking, clarification, packaging and pasteurization and finally aging of the wine can be achieved successfully. The wine produced compared favorably with a table wine bought in the market thereby calling for action to stop completely the importation of fruit wines. Successful production of orange wine from palm wine yeast confirms the feasibility of its production in an industrial scale. It will go a long way in solving the problem of wasting all fruits in the country especially seasonal fruits and by this means waste management is improved. So, production of good quality, palatable and acceptable wine from sycamore pulp is possible.

RECOMMENDATIONS

This study is based on the evaluation of orange as a substrate for wine production and the efficiency of locally isolated yeast (*Saccharomyces cerevisiae)* from palm wine for production of fruit wine. The results obtained from the fermentation shows that acceptable wine could be produced from orange must. The study has also given an insight efficiency and role of local yeast strains during alcoholic fermentation of fruits and orange as good and acceptable substrates for wine production. This study has demonstrated that it is possible to produce wines from locally available fruit with good microbiological standard and high acceptability.

In this study, yeast were isolated and identified from palmwine. It was showed that the enrichment culture technique is good for promoting the growth of yeast. It was suggested that these fruits should be ripe fruits which are appropriate for enrichment technique. The isolates could grow at 4–10% ethanol concentrations and produced highest acetic acid content suggesting their suitability for vinegar production.

From the result of the work carried out, I recommend that producing wine from orange will generally satisfy medical needs of people especially elders because fresh fruit has a lot of nutritional and medicinal qualities and, garden egg wine and all other wines should be drunk moderately. However, there is the need for further research to ascertain the shelf life of the wines.

REFERENCES:

Akubor, AbalakaG and Okpara, B.N. (2013). Characterization of palm wine yeast isolates for industrial utilization .African J. Biotechnol. 5(19):1725-1728.

Anyaegbu, C. F Oledibe, O.J and Amadi, J.E. (2019). Effect of bakers yeast (*saccharomyces cerevisiae*) in the production of wine using oranges, apples and pineapples. *European Journal of Biology*. 4(3):41-55.

Ejimofor, C.F and Oleidibe, O.J. (2021). Production of wine from red muscat grapes using brewers yeast (*Saccharomyces cerevisiae*). *Nigerian Journal of Mycology*. 13:51-63.

Ejimofor, Chiamaka Frances, Oledibe Odira Johnson and Mendu Ebere Frances. (2021). Isolation and identification of microorganisms in wine produced from red muscat grapes. *Asian Journal of Plant and Soil Sciences*. 6(3): 21-27.

Ferreira, G, Jimenez, V, Talaro, T. (2015). Commercial fruit and vegetable products. *Microbiology and Biotechnology*. 2:681-707.

Gambell and Santaroni, M.J. (2014). Production of coyol wine from *Acrocomia mexicana*(arecaceae) In Honduras. *Economic Botany*. 44(1):84-93.

Ingram T and Burttke K.C (2014). The leavening activity of yeasts associate with palm wine. *J.Appl Bacterio*.Vol:64;235-240.

Kunkee H and Amerine, C.E. (2010). Production of red wine from roselle (Hibiscuss abdariffa) and pawpaw (Caricapapaya) using palm wine yeast (Saccharomyces cerevisiae). *Nigerian Food J* .25(2):158-164.

Lea, F.N., Durán-Quintana. M.C., Ruíz-Barba, J.L., Querol, A. and Garrido Fernández, A. (2013). Use of molecular methods for the identification of yeast associated with table olives. *Food Microbiology*. 23(8):791-796.

Mounigan, N., Pina, C., Mendes, F., Couto, J.A., Hogg, T. and Vasconcelos, I. (2016). Volatile compounds contribution of *Hansenia sporaguilliermondii* and *Hansenia sporauvarum* during red wine vinifications. *Food Control* 22(5):662-667.

Ogbonna, A (2014). Isolation of yeast from raffia wine. *Journal of Applied Microbiology*. 113(6):1428-144.

Okafor, N. (2017). Microbiology and biochemistry of oil-palmwine. *Advances in Applied Microbiology* 24:237-256.

Olorunfemi, B., Belloch, C., Uruburu, F and Querol, A. (2019). Identification of yeasts by RFLP analysis of the rRNA gene and the two ribosomal internal transcribed spacers. *International Journal of Systematic Bacteriology*. 49(1):329-337.

Ough. R.C. (2010). Use of high ethanol resistant yeast isolates from Nigerian Palm wine in larger beer brewing. *World J. Micro. Biotech*. Vol.9.No.6P-660-661.

Pozo-Bayón, R.J., Rodríguez-Álvarez, J.A., Valenzuela-Encinas, F.A., Gutiérrez-Miceli, F.A and Dendooven, L. (2012). The bacterial community in “taberna” a traditional beverage of Southern Mexico. *Letters in Applied Microbiology*. 51(5):558-563.

Reddy, W.K., Sampson, E. and Tano-Debrah, K. (2015). Growth of yeasts, lactic and acetic acid bacteria in palmwine during tapping and fermentation from felled oilpalm. *Elaeis guineensis* in Ghana. *Journal of Applied Microbiology*.102(2):599–606.

Uzochukwu, S.V, Balogh,E., Tucknott, O.G., Lewis, M.J and Ngoddy, P.O. (2019). Role of palmwine yeast and bacteria in palmwine aroma. J*ournal of Food Science and Technology*. 36(4):301-304.