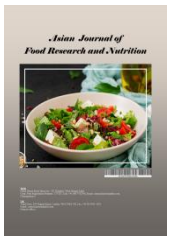

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Isolation and Characterization of Yeast Associated with Palm Wine Fermentation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Wine is a naturally occurring beverage that is produced via the action of yeast cells from fruit juices. The purpose of this study is to produce orange fruit wine and evaluate its quality utilising yeast that has been isolated from palm wine. Although the yeast were isolated from old palm wine and characterised using conventional methods, Saccharomyces cerevisae was verified as the main species present. Palm wine was characterised to discover its physicochemical features. Using recipes that included a blend of each fruit must with Saccharomyces cerevisae isolated from palm

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wine, orange fruit must was fermented for 14 days. Wine production was examined to assess its quality. For the yeast wine and commercial wine, respectively, the results indicate values of 3.67 and 3.38 for pH, 1.00 and 1.02 for specific gravity, 9.79 and 9.443 for percentage (%) alcohol (v/v), and 0.063 and 1.348 for percentage (%) titratable acidity. The results of the study shown that employing yeast (Saccharomyces cerevisae) isolated from palm wine, high-quality wine could be made from orange fruits for immediate consumption.

Keywords: Fermentation; isolation; palmwine; Upwine.

1. INTRODUCTION

The fermented sap of tropical plants of the palmae family is known as palm wine. It is both produced and consumed in enormous amounts in southeast Nigeria. It includes nutritionally significant elements such proteins, carbohydrates, vitamins, and amino acids [1]. These factors transform this wine into a true breeding ground for a variety of microorganisms, whose proliferation alters the physicochemical properties of the wine and fosters competition and successions of organisms.

Yeast is a single-celled, microscopic creature that belongs to the fungi family. Via the process of budding, in which a new cell starts as a tiny protrusion along the cell wall of a parent cell, individual yeast cells reproduce quickly. Huge numbers of yeast cells assemble in the presence of an ample food supply. Because of the brief two-hour budding period, the cells frequently resemble lengthy chains with freshly produced cells still linked to their parent cells [2].

Yeast are one of the few living things that can make energy without oxygen. Anaerobic refers to this absence of oxygen. Yeast breaks down starches and sugars into alcohol and carbon dioxide in these anaerobic conditions. Fermentation is the name given to this process. Enzymes, which function as catalysts in chemical processes and are akin to the digestive enzymes in the human body, are what cause yeast to ferment. The term "enzyme" really means "in yeast." The lengthy, chain-like molecules of starch are broken down into smaller sugar units by specific enzymes in yeast. Following that, different yeast enzymes change one type of sugar molecule into another [2].

The sugar molecule, which is made up of carbon, hydrogen, and oxygen atoms, is disassembled by further enzyme processes into ethyl alcohol and carbon dioxide. The chain of events gives yeast cells the energy they require for division and development (form of reproduction). In the natural world, yeast enzymes consume the sugar created by the breakdown of the intricate carbon compounds found in animal tissues and plant cell walls. Yeast serve as environmental natural decomposers in this way. Key Phrases Anaerobic: Residing or developing in an oxygen-deficient environment. Yeast is fed by the natural carbohydrates and sugars in the liquids. As a result of inadequate sugar breakdown when lacking oxygen during the fermentation process, yeast produces alcohol as a byproduct.

Indeed, several researchers have worked on projects to isolate and use palm wine yeasts in industrial operations. They include the generation of single cell proteins, portable ethanol production, and baking. *Saccharomyces cerevisiae* palm wine isolates were employed by Ogbonna [3] to make fake beer and fake palm wine, respectively. Characterizing these yeasts to produce fuel ethanol has not received much attention. Despite ongoing research into using bacteria to produce ethanol, yeast is still the preferred option for fermentation [4].

Saccharomyces cerevisiae yeast starters have been widely used in both commercial and homebrewing beverage manufacturing procedures since the 1980s. S. cerevisiae strains are currently used in the majority of wine production methods because they enable dependable and quick fermentations, lower the danger of slow or blocked fermentations, and guard against microbial contamination. In general, yeast starter cultures that are particularly chosen for the winemaking process on the basis of traits that have been scientifically validated complement and optimise the quality of the wine's distinctive qualities. In general, wines made using certain yeasts are of a higher calibre than those made by spontaneous fermentation [5].

The most well-known high-value fruit products are wine and fruit juice. The production of vinegar, a byproduct of making wine, may also employ it as a substrate. Although the methods

required in producing wine are rather simple, it can be difficult to create a commercial product (Amerine et al. 2020). Almost any fruit may be transformed into a wine that is excellent. Yeast that naturally exists in grapes may be used to ferment wine, albeit in nations where grapes aren't grown, other fruits are typically preferred for wine production. Wine is a product of alcoholic fermentation by yeast of ripe grapes or any fruit with a good proportion of sugar [1].

1.1 Statement of Problem

Researchers Anyaegbu et al. [4], Ejimofor et al. [5], Ejimofor and Oledibe [4], and many others have noted the presence of numerous microorganisms, particularly the bacteria and yeasts responsible for the fermentation of palm wine. The sugars in palm sap are converted during fermentation into alcohol and organic acids, which makes the sap less sweet. The sorts of bacteria that are present seem to be influenced by the sap's composition and fermentation stage [1]. Although yeasts frequently produce alcohol, bacteria seldom do so (Ingraham and Ingraham, 2014). For the majority of alcoholic drinks, yeast is employed. Pulque, however, is an exception. The alcoholic beverage pulque is made from the agave plant's juice and *Zymomonas mobilis* [6]. Due to the ability of likely Zymomonas species and other microorganisms present in the wine to ferment, it is difficult to store palm wine and maintain its normal characteristics. This has been a significant issue in the bottling of palm wine in Nigeria and subsequently its distribution for consumption. So, the existence of yeast species in palm wine may be advantageous to man in addition to their capacity for fermentation.

So, the goal of the current study is to isolate and identify the yeast in palm wine and to ascertain how the bacteria contributes to the fermentation of carbohydrates during wine production.

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 sites within Awka.

- Determining and separating the microbes in palm wine.
- Using the yeast isolate to make wine
- Making a comparison between commercial yeast and isolated yeast.
- Gathering data on the results, comparing it to the literature that is accessible, and providing the required suggestions.

1.2 Significance of Study

The microorganisms found in palm wine have significant economic value and have helped both people and enterprises. The findings of this research will be significant in the following ways:

- The research will provide additional information about the nutritional value of palm wine and the need to boost consumption. The sales revenue for producers and marketers of palm wine will rise as a result.
- This effort will demonstrate the value of yeast in the creation of wine.
- The outcome of this investigation would be beneficial to the beverage industries since it will suggest several locations where the detected microorganisms may be used as a catalyst in the industries. The research will also present new techniques for fermentations that use less dangerous microbes. This action will gain more for such industries.
- Researchers that wish to pursue the isolation of the numerous bacteria discovered via this effort will have a solid basis thanks to this work. These bacteria can be utilised for biotechnology in the alcoholic beverage industry. Lastly, the government and our community will gain from this effort in the form of new businesses being started by individuals who have learned about the fantastic nutritional value of this palm wine.

2. MATERIALS AND METHODS

2.1 Sample Collection

From tappers in Awka, five samples from each of the two sources of palm wine (Raffia palm and palm tree) were chosen at random. The palm wine was gathered by the tappers utilising natural wood during tapping process using bamboo tube. Following that, the palm wine was collected in sterile bottles and transported (30 minutes) to the laboratory while being preserved at 40°C in the icebox.

2.2 Preparation of Samples

Within a day, the physical and chemical characteristics of each sample of palm wine were established. The samples were sterilised using Watman filter paper and aseptically filtered before being stored at 40°C for analysis.

2.3 Physicochemical Properties

Visual inspection of the samples of palm wine was required for this.

A hunter lab clorflex colorimeter was used to measure the samples' colour.

- According to Taipaiboon's instructions, the transmittance at 650 nm was measured using a spectrophotometer to assess the turbidity of the palm wine (13).
- The palm wine's flavour and aroma were also assessed.
- A pH metre calibrated with pH 4.0 and 7.0 was used to measure the pH value at room temperature.
- Using phenolphytalin as an indicator and calculating the total acidity in terms of lactic acid, the total acidity was evaluated by titration with NaOH.
- Using a hand refractometer, the total soluble solids in the palm wine sugar syrup were calculated as a degree Brix.
- By titrating with Fehling reagents, total sugar and reducing sugar concentrations were determined. Grams of glucose per 100 grammes of sample were used to express the results.
- An Orion 4 Stars conductometer was used to assess conductivity. The process involved calibrating the device with standards of 1413 S and 12.9 mS/cm, then submerging the sensor in tequila and measuring the conductivity in triplicate. Each time the electrode was submerged, it was thoroughly cleaned with water. At room temperature, all tests were carried out.
- An Anton Paar DSA5000 densimeter and sound velocity analyzer with a new-generation stainless-steel cell was used to measure density and ultrasonic velocity.
- Using a Peltier element that had a precision of 0.001°C for temperature control, errors in density of around g/cm3 resulted. The resolution in this investigation was 102 m/s, and temperature fluctuations are the primary source of errors in ultrasonic velocity measurements. After the prescribed procedure, which involved repeatedly injecting Alconox at a 40% concentration, the densimeter was cleaned. Following that, ultrapure water was pumped to calibrate at 20°C until density was measured at 0.998203 g cm3. When this measurement was made, the samples of tequila's density and viscosity were determined. At 25°C, duplicate measurements of the tequilas' density and sound velocity were made. The measurements of density and sound velocity were done simultaneously.
- A refractometer made by the manufacturer Abbe, model 2WA, was used to measure the refractive index of the tequila. Ethylic alcohol was used to first clean the prism before being calibrated with a drop of pure ethylic alcohol. The equipment's viewing field was modified to light up half of the area while leaving the other half in the dark. When everything was in working order, a measurement of 1.36 was made. Tequila was afterwards dropped into the sample holder to measure its refractive index. At 25°C, each measurement was made three times.
- An ARES rheometer TA-22 G2 was used to evaluate the viscosity of several tequilas utilising double-wall Couette geometry. The cup's inner and outer diameters are 27.94 and 34 mm, respectively, while the hollow cylinder's inner and outer diameters are 29.51 and 32 mm, respectively. At 25 °C, all measurements were made. The sample container was filled with 8 mL of tequila, which was kept moving at a shear rate of $10s^{-1}$.

2.4 Isolation of Yeasts from Palm Wine

The 25-day-old wine samples were centrifuged for five minutes at a low speed in sterile centrifuge bottles. By streaking on Glucose Yeast Agar plates, one ml of the serially diluted sediment is inoculated and incubated at 28°C for 24 hours [4]. By additional streaking on GYA, the yeast colonies that formed are separated and purified. According to Kunkee and Amerine [7], physical traits and patterns of fermentation were used to identify yeast isolates.

2.5 Yeast Identification

Ejimofor et al. [8] identification keys and common morphological and physiological tests were used for the isolation and identification of yeasts. Incubation took place at 28°C under aerobic circumstances. After isolation on glucose yeast agar (GYA) and yeast malt agar (YMA), the morphological and cultural traits of the years were examined (Biolife). These examinations covered morphology, surface traits, the development of ascospores, the presence of pseudohyphae, and vegetative reproduction. Sugars such glucose, galactose, sucrose, maltose, cellobiose, trehalose, lactose, raffinose, soluble starch, D-xylose, L-arabinose, and Dribose were tested for their ability to ferment. Another experiments include nitrate absorption, growth in 10% NaCl + 50% glucose in yeast extract, growth at 37°C and growth in 50% w/w glucose yeast extract.

2.6 Evaluation of Yeast Strains Isolated with Commercially Sold Yeast

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

2.7 Sample Preparation

Purchased fresh oranges were separated, properly cleaned with clean water to eliminate any clinging materials, peeled, and had their seeds removed. With a sharp stainless steel knife, the flesh's (3.3 kg) little pieces were divided into pure juice. The fluid was filtered through a mesh cloth to eliminate any remaining solids. After being extracted, the juice was put into clean glass bottles and pasteurised for 30 minutes at 70°C using a heating mantle. In addition to 10g of aspertine, 10g of citric acid was added.

2.8 Grape Wine Fermentation

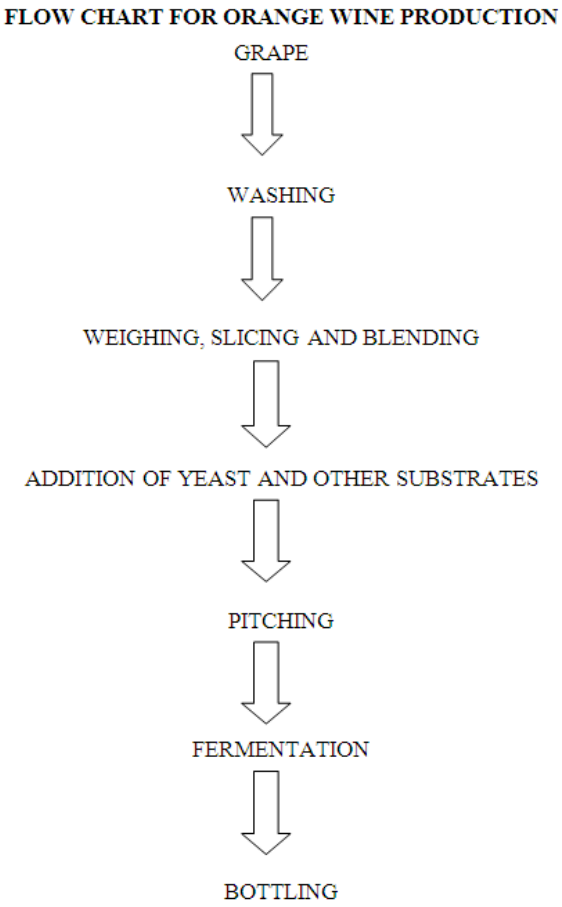

Fig. 1. Flow chart for grape wine production

2.9 Statistical Test

Yeast count, total suspended particles, total dissolved solids, titrable acidity, pH estimation, specific gravity, and alcohol concentration were all sampled every 48 hours.

3. 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

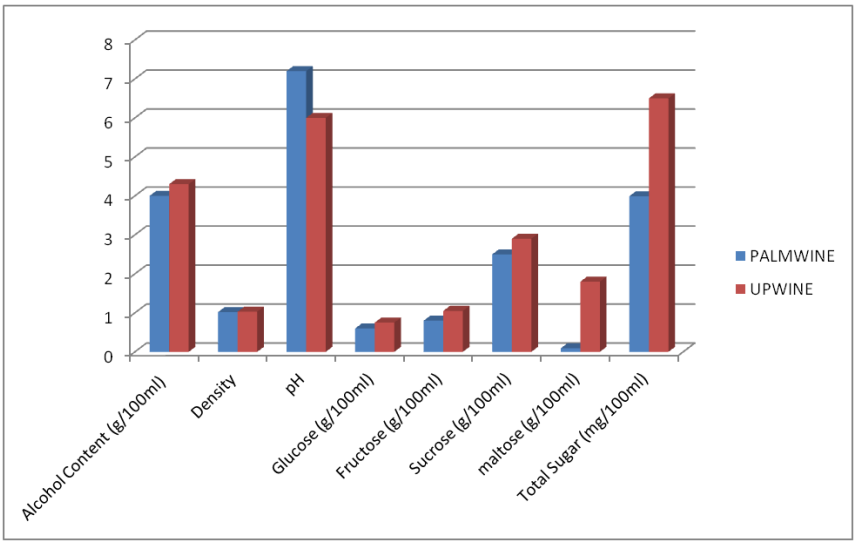

Fig. 2. Physicochemical properties of palm wine and up wine

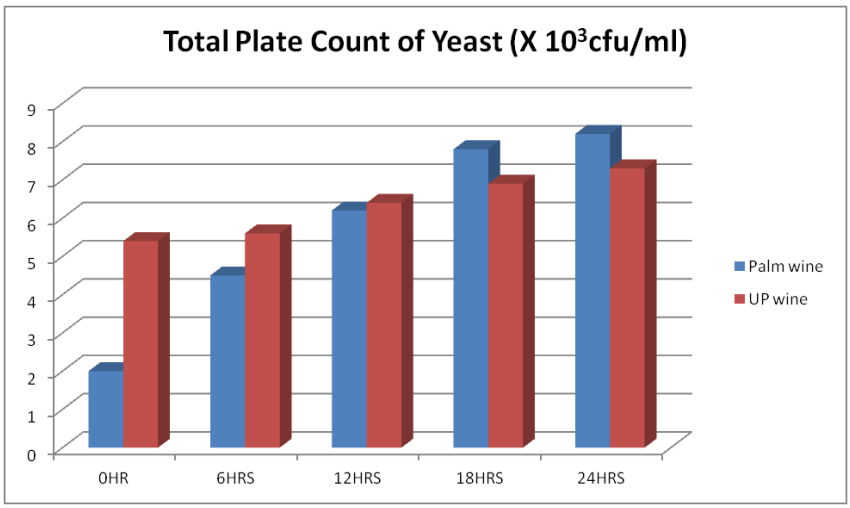

Fig. 3. Total Plate Count of Yeast (X 10^3cfu/ml) carried out at different times

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

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*

Carbohydrates Fermentation by Yeast Isolates

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

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

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

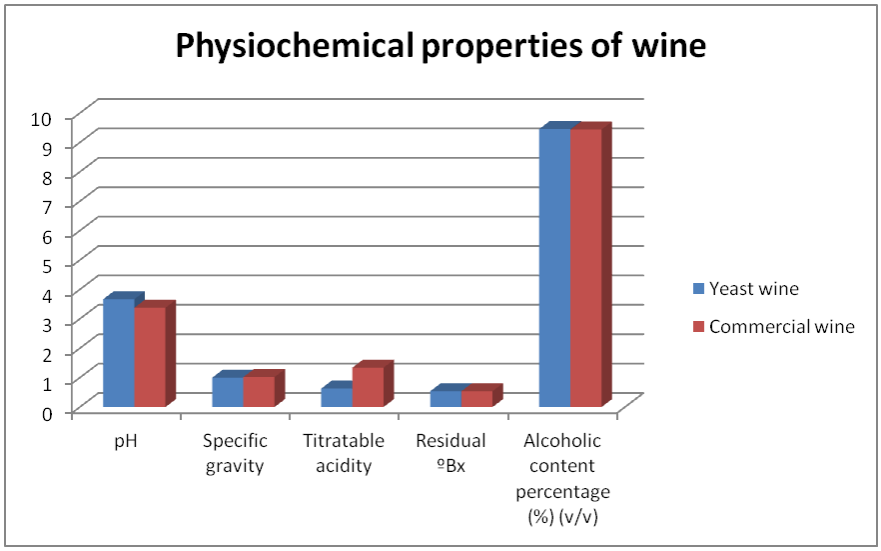

Fig. 4. Physiochemical properties of yeast wine and commercial wine

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

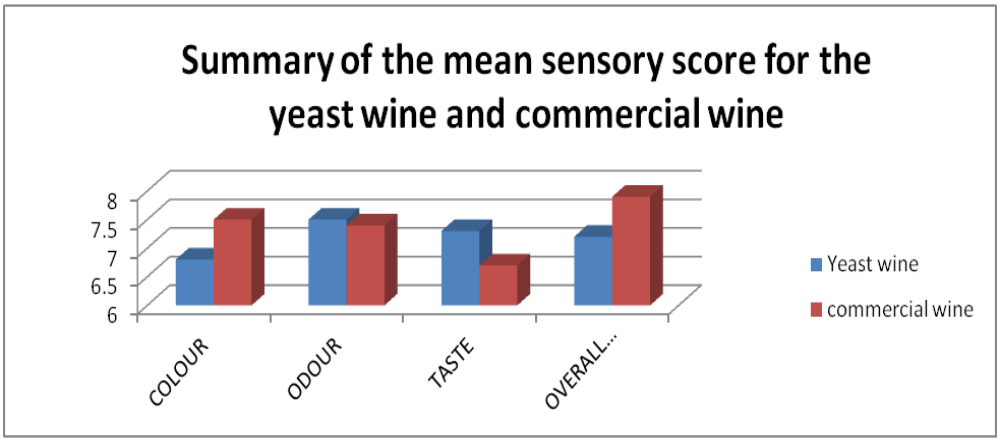

Fig. 5. Summary of the mean sensory for the yeast wine and commercial wine

3.1 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.

3.2 Physiochemical Properties of Wine

The wine produced after 14 days 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.

3.3 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.

4. DISCUSSION

Using yeast that was isolated from palm wine, we investigated the fermentation process used to make wine. Fruit that had been crushed was used in every step of the procedure. Some of the orange experimentation also used the fresh pulp that had been squeezed. The orange fruit mash had an initial sugar content of 110.1 g/L. While the lag phase was shorter and the fermentation rate was comparable, the yeast-inoculated alcoholic fermentation occurred more quickly than the spontaneous one. Although particular yeast for inoculation will be highly recommended in the industrialization of both the wine and vinegar processes, yeast inoculation was not actually necessary to generate these fruit wines. Shorter production cycles and a repeatable product are required by industrialization, and these requirements might be met by the process of inoculating certain strains [8].

The ultimate product yield (wine) is satisfactory because it was consistently well above 60%. We carried out the entire procedure in the lab, with such constraints as the press's force and the small-scale recovery of fruit pulp. Higher yields will result by scaling up to larger amounts and using industrial machinery, similar to what is seen in wine. In both instances, the finished product had pleasing colour and organoleptic qualities.

According to Table 5, the yeast wine had a lower acidity than commercial wine. This rise in acidity is likely caused by a few organic acids that are present in yeast wine and are mostly used as preservatives. According to Ough [9], main acids produced during fermentation include lactic, malic, succinic, and acetic acids. At a final acidity of 0.63%, spoiling organisms can be prevented. As reported by Pozo-Bayón et al. [10], table wines have titratable acidity in the range of 0.6-0.9%. These acids must be present in wine for it to function properly; otherwise, the beverage would taste unpleasant and deteriorate with a bad colour and flavor [11].

In line with expectations, the pH dropped from 3.67 in sycamore wine to 3.38 in yeast wine. This ought to follow from its inverse relationship to acidity. Yet it's crucial to note that there is no direct correlation between pH and total titratable acidity due to the fermenting liquor's variable buffer capacity [12]. As a result, the fact that yeast created few acids throughout fermentation accounts for the low change in fixed acidity [3]. For the microbiological stability of sycamore wine aroma and flavour developments, this PH value of 3.67 is crucial.

While fermentation continues, the alcohol content gradually rises. From the 14th day, which was the final day of fermentation, it thereafter became steady. The majority of the fermentable carbohydrates have been transformed to alcohol, which is the cause. Also, the toxicity of the created additional alcohol rendered the yeast dormant for further synthesis [13]. Its alcohol concentration was 9.67%, whereas that of commercial wine was 9.44%, mostly as a result of the further conversion of residual sugars to alcohol. Our findings are consistent with table wines' typical alcohol concentration, which runs from 6 to 10% [14].

The yeast wine's specific gravity was 1.00 as opposed to commercial wine's 1.02, but the difference was due to the conversion of sugar to alcohols, which has a lower specific gravity than sugar. This outcome supports the findings of Akubor et al. [15], who created wine using sycamore and bush mango juice, respectively.

The asessors gave the wine good marks for its sensory quality features and confirmed this by saying they would buy the wine if it were put up for sale. The assessors stated that this wine had a fruity-like flavour with a noticeable orange taste. According to Olorunfemi et al. [16]'s research, yeast, ambient conditions, and physiochemical processes all have a role in the kind and scent of wine that was created.

The resulting orange wine is clear and brown in hue. This was brought on by the orange pulp must's brown tint and prolonged age time. Moreover, it was seen that particle sedimentation happened quite quickly. This may have happened because they included denser, insoluble particles, which sink to the bottom. The papain protease enzyme's activity guaranteed that the proteins, peptides, and polypeptides present in the wine were broken down. After the wine was allowed to stand for a while, the

protein-tannin complex, which also included the peptide and polypeptide components, settled out. This improved wine's ability to clarify as it aged. The wine's peptide and polypeptide components formed a complex with the tannis (protein tannin complex), which settled out throughout one month of maturing, resulting in the mean value of colour acceptability derived from the sensory assessment being 6.80 and 7.50 for the standard [15].

For the odour of orange wine, sensory assessment mean scores were 7.4 for commercial wine and 7.5 for yeast wine. This demonstrated that there is no discernible difference between the yeast wine and the control wine in terms of aroma. There is no discernible difference between the commercial wine and yeast wine in terms of taste, according to Table 1, which is consistent with Mounigan et al. [17] observations in sensory acceptance, quantitative descriptive, and physicochemical examination of wines. For the standard, this was done with 7.2 and 7.9. This demonstrated that there are no appreciable differences.

5. CONCLUSION

This investigation proved that many Saccharomyces species were isolated from old palm wine and upwine. The ability of the isolate to develop on 10% sodium chloride + 50% glucose medium revealed that the isolate is *Saccharomyces cerevisae*. The isolated species displayed significant similarities. *Saccharomyces cerevisae* may be distinguished from all other species by the growth test. The potential exists that yeast isolated from palm wine might be used to make orange wine. As compared to a typical egg wine sample, the wines generated did not significantly differ in terms of pH, specific gravity, percentage (%) alcohol (v/v), or percentage (%) titratable acidity. The outcome of this research has demonstrated that the process of manufacturing orange wine, which began with the harvesting of healthy sycamore and garden egg fruit, washing, crushing, adding sulphites, fermenting, racking, clarifying, packing and pasteurization and finally aging of the wine can be achieved successfully. The wine made held up well against a table wine purchased from the market, which prompted measures to fully halt the importation of fruit wines. The capability of its production on an industrial scale is confirmed by the successful manufacture of orange wine from palm wine yeast. It will go a long way towards resolving the issue of all fruits being wasted in the nation, particularly seasonal fruits, and by doing so waste management will be improved. So, it is feasible to produce high-quality, delectable, and acceptable wine from sycamore pulp.

6. RECOMMENDATIONS

The effectiveness of locally isolated yeast (*Saccharomyces cerevisiae*) from palm wine for the creation of fruit wine serve as the foundation for this study. The findings of the fermentation indicate that palm wine must might be used to make respectable wine. During the alcoholic fermentation as suitable and acceptable substrates for wine production, the study also shed light on the effectiveness and function of local yeast strains. This study has shown that it is feasible to create wines with high acceptance and appropriate microbiological standards from fruit that is readily available locally.

In this investigation, yeast from palm wine was isolated and identified. It was demonstrated that the enrichment culture approach is effective at encouraging yeast growth. It was advised that these fruits be fully ripe in order to be used for the enrichment procedure. The isolates generated the maximum acetic acid content and could grow at ethanol concentrations of 4–10%, indicating their viability for making vinegar. To determine the wines' shelf life, however, more investigation is required.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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