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Comparative Analysis of Red Wine Produced from *Hibiscus sabdariffa* l. and *Citrus sinensis* Juice Using *Saccharomyces cerevisiae* Isolated from Palm Wine and Different Brands of Commercial Wine

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Authors' contributions

This work was carried out in collaboration among all authors. Author FSI designed the study, performed the statistical analysis, Author AO wrote the protocol and managed the laboratory of analyses of the study. Authors FSI and NM managed the literature searches and wrote the first draft of the manuscript. Author FSI supervised the entire work while author AO used part of the work as her BSc Project in the Department of Microbiology Technology. School of Science Laboratory Technology, University of Port Harcourt, Nigeria. All authors read and approved the final manuscript.

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ABSTRACT

Roselle (*Hibiscus sabdariffa*) grows abundantly in some parts of Nigeria and the plant has high nutritional value to all ages. Over the years, Nigeria has depended on imported wines to satisfy the demands for wine consumption by her citizens and this has placed huge economic burden on her economy. Hence, the need to exploit the commercial availability of *Hibiscus sabdariffa* for wine production. This study was aimed at comparative evaluation of red wine produced from *Hibiscus sabdariffa* L. and *Citrus sinensis* juice using *Saccharomyces cerevisiae* isolated from palm wine

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and brands of commercial wine. The Roselle calyx extract and sweet orange juice in the ratio of 3:1 was inoculated with the palm wine yeast and allowed to ferment for 14 days at 28±2°C. Physicochemical and microbiological parameters of the fermenting 'must' were monitored at intervals using standard methods. Culturable microorganisms was not detected in the 'must' at Day 0, but only yeast cells which have a mean count of 2.77 x 10^4 Cfu/ml and 2.25 x 10^4 Cfu/ml were present in the fermenting 'must' at Day 7 and 14, respectively. There were significant differences ($p<0.05$) among the parameters monitored in this study except pH and specific gravity (SG) of the fermenting 'must'. The data obtained demonstrated that there was a decline in pH, reducing sugar, and SG of the fermenting 'must' which ranged from 3.8-3.5, 23-2.10 g/L and 1.08-1.00 ^{o}Brix % w/w, respectively. However, this study reported increase in alcohol content (0 - 10.87% v/v), titratable acidity (0.45 - 0.68 g/mL), and temperature (26.0 - 30.7°C) during the fermentation of the 'must'. The wine produced had a bright red colour, pH of 3.5, alcohol content of 10.87% v/v, SG of 1.00 ^{o}Brix % w/w, and titratable acidity (TA) of 0.68%. The outcome of this study indicated that the wine produced is slightly more preferable than some commercialized brands of wine based on sensory scores. Since, the alcoholic beverage developed during fermentation process is in generally of acceptable quality as a good table wine thus, it can be concluded that our wine is a good product recommendable.

Keywords: Red wine; roselle; sweet orange; palm wine; Saccharomyces cerevisiae; commercial wines.

1. INTRODUCTION

In the last few years, efforts towards commercial exploitation of indigenous fruits and vegetables in Nigeria is on the increase [1-3]. Tropical citrus fruits as well as a wide diversity of other tropical fruits such as banana, jackfruit, pineapple, etc are potential substrates for production of tropical fruit wines [4-7]. Currently, tropical and subtropical countries account for the largest quantity of fruits produced worldwide [8]. Traditionally, grape is a fruit used to produce wine [9]. It is among the beverages regarded as the oldest in the history of man [6,10,11]. Globally, the acceptability of wine has spanned for many centuries [12]. Although grape is a widely accepted fruit for wine production, it is usually not available and affordable in the tropics [13,14]. Interestingly, non-grape wines are receiving great attention in recent years because it is considered as a functional food [15].

Citrus sinensis is a fruit popularly known as sweet orange. The orange tree grows abundantly in tropical and subtropical countries. Annually, the total output of citrus fruit from estimated 3 million hectares of land in Nigeria is about 930 000 tons [16]. However, short shelf life of sweet orange and quick spoilage of the fruit which occur on transit is because the fruit is juicy and fragile. This is mainly responsible for the post-harvest wastages of sweet orange [8,17,18]. In Nigeria, it is estimated that fifty percent of citrus produced is not utilized due to wastages [4], sweet orange produced locally is mainly eaten fresh while a small portion of the harvested fruit is processed into canned orange juice. Utilizing citrus for wine making has economic potentials, yet it is underexploited [19]. However, [4] reported the production of orange wine using sweet orange fermented by four strains of yeast.

Roselle (*Hibiscus sabdariffa Linn*.) commonly called 'sorrel' or 'red sorrel' is a plant that grows in tropical and subtropical climates while in Nigeria it is abundant in north-east and middle belt regions [20-22]. It is suggested to be a native of West Africa or Asia [23,24]. In some African countries, the calyx of *H. sabdariffa* which is usually of three types (green, red, and dark red) is used in preparing drinks. Sorrel beverage is called zobo in Nigeria [24-26]. Zobo is a refreshing non-alcoholic drink which is not usually produced in a commercial quantity because of its short shelf life not exceeding 24 h from the time of preparation unless it is refrigerated [21,27]. This notwithstanding, sorrel is increasingly becoming more attractive in the food processing industries due to its potential health benefits [23,28,29]. Wines are alcoholic beverages resulting from the fermentation of grape juices by yeast. There are varieties of colours of wines ranging from white, red and rose wines. These can be grouped into different types such as dry and sweet, sparkling and still as well as brandy (those fortified with grape spirits) [7,30]. Red table wine of good quality was prepared by [1,31,32] using extract from *H. sabdariffa* and fermented by *S. cerevisiae* obtained from palm wine. Recent improvements in the preparation of zobo drink involves the addition of natural sweeteners such as pineapple

juice, orange juice or any other additive of choice to a drink which typically has a sharp sour taste [33]. Although [18,34-36], and few other researchers have produced table wines of good quality using either sweet orange juice or extract from *H. sabdariffa* (zobo drink) in combination with other fruit juices using palm wine yeast (*S. cerevisiae*). To the best of our knowledge, this study is the first to produce wine from sweet orange juice and extract from *H. sabdariffa* (zobo drink) using *S. cerevisiae* isolated from palm wine.

According to Kanter et al. [37], yeast and its metabolic products are essentially responsible for the development of preferred sensory properties in wines. This has endeared more people to prefer alcoholic wines to other products. High fermentative ability is responsible for wide acceptability of *S. cerevisiae* used mainly for the production of wine and other alcoholic beverages [35]. The role of *S. cerevisiae* in the wine industry is extensive [38]. Palm wine is usually the source of *S. cerevisiae* (palm wine yeast) for the fermentation industries [8,18]. According to Nwachukwu et al. [39], palm wine yeasts isolated from palm wine sourced from south-eastern Nigeria have the potentials to be used for industrial ethanol fermentation applicable in wine making. As far back as 1983, Nigeria recorded a major breakthrough when cocoa wine was patented. Since then till date, many researchers have developed varieties of wine such as kola wine, coconut wine, cashew-apple wine, pineapple wine [18]. It is estimated that Nigeria imported champagne (sparkling wine) estimated at N9 billion in 2016. Consequently, high duty was imposed which resulted in a decline of imported wine by 24% and shifted people's interest to locally prepared drinks [21]. Based on this context, this study aimed to prepare fruit wine from Roselle (zobo drink) and sweet orange juice made from abundant local raw materials using yeast (*S. cerevisiae*) obtained from fresh palm wine and compare it with the various commercial wine brands in the Nigerian markets.

2. MATERIALS AND METHODS

2.1 Sample Collection

Roselle Calyx (*Hibiscus sabdariffa*) and sweet orange (*Citrus sinenesis*) were purchased in Choba market, Port Harcourt, Rivers state using sterile polyethene bags. Freshly tapped palm wine was purchased from Alakahia market along East/West Road, Rivers state using a sterile plastic container with a cork. All the samples were quickly transported to Food and Industrial Microbiology Laboratory, the University of Port Harcourt, Port Harcourt, Nigeria for analyses. Also taken to the Laboratory for analyses is a sealed bottle of Baron de Vall and Nigerian concord wine purchased from a wine shop in Alakahia.

2.2 Preparation of Roselle Calyces Extract

Healthy, ripped and dried *Hibiscus sabdariffa* flowers were sorted. Exactly 12.7 g of the flower was weighed, washed using potable water, sieved and boiled in 1000 ml of hot distilled water using a pressure pot at 80°C for 30 min. The boiled flower was filtered using muslin cloth and the residual calyces was re-extracted once more using 100 ml of hot distilled water. The filtrate and the rinse water were blended which yielded a 10% (w/v) Roselle calyces hot water extract.

2.3 Sweet Orange Fruit Extract and 'Must' Preparation

About 250 g of sweet orange fruit was washed, rinsed with potable water and table salt. The rinsed sweet orange was peeled with a knife sterilized with 70% ethanol. A sterilized juice extractor was used to extract orange juice from the pulp. The juice was strained through a muslin cloth. Roselle calyces extract (2100 ml) was mixed with the orange juice extract (700 ml) in the ratio 3:1. A pinch of sucrose was added to the mixture to increase sugar level of the 'must', then it was pasteurized at 68°C for 15 min and allowed to cool to room temperature (28±2°C).

2.4 Serial Dilution

Peptone water contain 10 g of peptone, 5 g of sodium chloride (NaCl) in 1 000 ml of distilled water. Nine millilitre (9 ml) of peptone water was dispensed in test tubes labeled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, autoclaved at 121°C for 15 min at 15 psi and allowed to cool. One millilitre (1 ml) of 24 h old palm wine was added to already prepared 9 ml peptone water in a 10-1 labeled test tube and gently mixed. From the 10-1 dilution, 1 ml solution was aseptically transferred to 10-2 labelled test tube using a sterile pipette. The same procedure was repeated until the 10-5 labelled test tube was reached, using sterile pipette for each transfer.

2.5 Isolation of Yeast from Palm Wine

Yeast was isolated from 24 h old palm wine using a modified method described by Olorunfemi and Adetuyi [40]. Aliquot (0.1 ml) of each dilution of palm wine was pipetted and spread on potato dextrose agar (PDA) in duplicates. The inoculated PDA plates were incubated for 72 h at room temperature (28±2°C) and observed afterwards for possible growth. Distinct colonies obtained from the PDA culture plates were picked and subcultured on freshly prepared plates by streaking method, incubated for 48 h at room temperature and observed. Repeated subculturing was carried out until pure isolates were obtained.

2.6 Identification of the Yeast Isolates

The characteristics of the yeast isolates were determined macroscopically and microscopically.

2.6.1 Macroscopy

It involves observing and taking note of the surface, shape, elevation, colour, and morphology of the yeast isolates.

2.6.2 Microscopy

The yeast isolates were prepared using the wet mount techniques and Gram staining techniques. Clean glass slides were sterilized and lactophenol blue was placed on the clean glass slides. A sterilized inoculating needle was used to cut a portion of the agar containing the yeast colonies and placed on the slide, then covered with a cover slip. The preparation was viewed under x40 objective lens of microscope.

2.6.3 Gram staining techniques

Gram staining technique was used to stain the yeast isolates. A smear was made on the slide for each of the yeast isolates, allowed to air dry and heat fixed. Crystal violet was flooded on the slide and stained for one minute, then gently rinsed with water. The slides were stained a second time with Gram's iodine and allowed to stay for one minute, then gently rinsed with water. The slides were decolourized with 95% ethanol for 30 s, then rinsed with water. Safranin was used to counterstain the already stained isolate for 30 s, then rinsed with water and drained. The slides were air-dried and viewed under x100 oil immersion lens of the microscope.

2.6.4 Sugar fermentation test

The isolated yeasts were subjected to sugar fermentation test which involves the use of 1% each of glucose, sucrose, fructose, galactose, lactose, maltose and ethanol prepared using 0.1% peptone water. The peptone water was added to the sugars leaving the ethanol. Ten millilitres (10 ml) each of the prepared sugars was introduced into test tubes. Durham tubes were introduced into the test tubes and autoclaved. Sterile wire loop was used to pick colonies of each yeast isolate and aseptically inoculated into the test tubes, then incubated for 48 h at 37°C.

2.7 Preservation of Identified Yeast Isolates

The identified yeast isolates were subcultured on potato dextrose agar (PDA) and stored on PDA slants at 37°C.

2.8 Preparation of Broth for Fermentation

Potato dextrose agar (PDA) was prepared according to manufacturer's specification. The yeast slant inoculated with *S. cerevisiae* was selected among the yeast isolates stored on slants and inoculated onto PDA broth, incubated at 37°C for 48 h before it was seeded into the 'must'.

2.9 Fermentation Process

The method described by Kiin-Kabari and Igbo [41] was adopted with some modifications was adopted. The pasteurized 'must' was seeded with 10 ml yeast (*S. cerevisiae*) in a fermentation broth. An aqueous solution of potassium metabisulphite (0.095 g/L) was added to the 'must' and the fermentation bottles were covered with cotton wool and allowed to ferment at room temperature (primary fermentation). Aeration of the fermenting 'must' was performed daily by shaking the fermentation bottles to encourage yeast cell multiplication. This aerobic fermentation was carried out for 7 days. At the end of the primary fermentation, the fermenting 'must' was racked and transferred to the sterile fermentation bottles. Thereafter, the fermentation bottles were closed with a rubber stopper fitted with fermentation air locks and allowed to ferment at room temperature (28±2°C) for another 7 days (secondary fermentation). Samples of the fermenting 'must' were withdrawn from the bottle at different time intervals (Day 0,

7 and 14) for microbiological analysis. Physicochemical parameters of the fermenting 'must' were monitored at intervals throughout the fermentation period. After the 14th day of fermentation, the wine was fined with 1% bentonite (England, UK) and pasteurized at 68°C for 15 min. Thereafter, filtration and packaging of the wine was done.

2.10 Microbiological Analysis

At Day 0, samples of the fermenting 'must' were aseptically withdrawn from the fermenting flasks and serially diluted using 0.1% peptone water. Exactly 1 ml of dilution 10^{-2} and 10^{-3} were spread plated on freshly prepared nutrient agar (NA) and potato dextrose agar (PDA) plates. The NA culture plates were incubated at 30°C for 24 h for bacterial growth while the PDA culture plates were incubated at 28 ^{c}o. for 72 h for growth of molds and yeasts. The bacterial and fungal colonies observed on the culture plates after the incubation periods were enumerated. Further identification of the isolates was carried out.

2.10.1 Total coliform count

The method described by Kiin-Kabari et al. [41] was adopted. Exactly 1 ml of the fermenting 'must' was aseptically withdrawn from the fermenting flaks and diluted in 9 ml distilled water. Subsequently, serial dilution up to 10 -3 dilution was carried out. One millilitre (1 ml) of the diluted sample was cultured on MacConkey agar and incubated at 37°C for 48 h. The colonies observed on the culture plates after incubation were enumerated.

2.11 Physicochemical Analysis

2.11.1 Reducing sugar

Reducing sugar of the fermenting 'must' was determined using the Rebelein process [42] and process of estimating reducing sugars [43] as earlier by in Ire et al. [12].

2.11.2 Titratable acidity

Titratable acidity (TA) of the fermenting 'must' was determined by the method of [44]. Two hundred millilitre (200 mL) of distilled water was measured into a sterile 500 ml flask and boiled. One millilitre (1 mL) of 1% aqueous phenolphthalein indicator was poured into the solution with the addition of 5 mL of the fermenting 'must' and titrated with 0.1M NaOH to end point (faint pink colour). The TA was calculated using the formula below:

$$\text{Titratable acidity (TA)} = \frac{\mathbf{V \times M \times 0.75}}{\mathbf{v}}$$

Where:
M = Number of moles of 'must'
V = Volume of titre
V = Volume of 'must'
0.75 = Constant

2.11.3 Determination of temperature and pH

The method described by Ire et al. [12] was adopted in determining the pH and temperature of the fermenting 'must' during the fermentation period with the aid of pH and temperature scale (Hanna instrument). A buffer solution of pH 4.00 was used to standardize the digital pH meter followed by dipping the electrode inside the must. Adjustment of the pH meter was made to determine temperature of the 'must' using a thermometer fixed by the manufacturer. Both readings were taken after 1 min.

2.11.4 Specific gravity

Specific gravity of the fermenting 'must' during the fermentation period was determined using the procedure described by Ire et al. [12]. A specific gravity bottle (50 ml) was thoroughly cleaned using distilled water, dried in an oven at 50°C and allowed to cool. The weight of dried empty specific gravity bottle (Mo) was taken. Thereafter, the dried specific gravity bottle was filled with deionized water, spilled liquid on the bottle was thoroughly cleaned using a cotton wool and content of the bottle was weighed (M2). The distilled water inside the specific gravity bottle was discarded, replaced with the must and reweighed (M1).

$$\text{Specific gravity} = \frac{M_1 - Mo}{M_2 - Mo}$$

Where:
Weight of specific gravity bottle = M_o
Weight of bottle + water = M_2
Weight of bottle + 'must' = M_1

2.11.5 Determination of alcohol

Alcohol content of the fermenting 'must' during the fermentation period was determined using specific gravity method as described by Egan et al. [45].

Represented by:

% Alcohol content by volume = % ABV

$$\% \text{ ABV} = \frac{\text{Original specific gravity} - \text{Final specific gravity}}{7.36} \times 1000$$

2.11.6 Sensory evaluation

The procedure described by Omoya and Akharaiyi, [46] was adopted. The red wine produced was subjected to sensory test using 10-man panelist familiar with the taste of wine drawn from undergraduates and staff of the Department of Microbiology Technology, University of Port Harcourt, aged between 20-45 years. The colour, taste, flavour, appearance and general acceptability of the wine produced were evaluated by the Sensory panelist. In an open space and broad daylight, each of the assessors was served chilled (20±2°C) coded sample of the wine produced in a transparent tumbler requesting them to evaluate the samples using a 9-point Hedonic scale ranging from 9-like extremely to 1- dislike extremely. One bottle of imported red wine and a similar product made in Nigeria were also evaluated as a comparative reference sample. A cup of potable water was served each panelist to rinse their mouth before tasting the first sample, and subsequent testing of each sample.

2.12 Data Analysis

In the course of this study, data generated were subjected to statistical analysis making use of One-Way Analysis of Variance (ANOVA). Least significant differences (LSD) were used to evaluate the differences between means. The statistical analysis was performed at 95% confidence level using IBM SPSS software version 22.

Table 1. Morphology of the yeast isolates

Isolate identity	Surface	Shape	Elevation	Colour	Cell morphology
A 10^{-1}	Smooth	Circular	Convex	Cream	Cocci
B 10^{-1}	Smooth	Circular	Convex	Cream	Cocci
A 10^{-2}	Smooth	Circular	Convex	Off white	Cocci
B 10^{-2}	Smooth	Circular	Convex	Cream	Cocci
A 10^{-3}	Smooth	Circular	Convex	Cream	Cocci
B 10^{-3}	Smooth	Circular	Convex	Cream	Cocci
A 10^{-4}	Smooth	Circular	Convex	Off white	Cocci
B 10^{-4}	Smooth	Circular	Convex	Off white	Cocci
A 10^{-5}	Smooth	Circular	Convex	Cream	Cocci
B 10^{-5}	Smooth	Circular	Convex	Cream	Cocci

Table 2. Macroscopic and microscopic identification of the yeast isolates

Isolate identity	Macroscopic description	Microscopic and morphology	Tentative genera
A 10^{-1}	Off white to cream, dry smooth colonies, heavy growth around the plate.	Oval to convex, budding yeast cells, purple coloured colonies, cocci shape.	*Saccharomyces*
B 10^{-2}	Cream, smooth colonies, heavy growth on all the plates, dry surfaces.	Spherical cells packed in clusters, convex elevation.	*Saccharomyces*
C 10^{-3}	Creamy colonies, dry surface, discrete growth.	Oval shape, convex in elevation, budding yeast, cocci shape.	*Saccharomyces*
D 10^{-4}	Off white, discrete, dry surface.	Convex elevation, cocci shape, purple colonies.	*Saccharomyces*
E 10^{-5}	Cream, smooth, discrete and scanty growth.	Oval shape, convex elevation, purple colonies.	*Saccharomyces*

Table 3. Sugar fermentation and ethanol tolerance test on the yeast isolates

Isolate identity	Glucose	Fructose	Sucrose	Lactose	Maltose	Galactose	Ethanol	Probable organism
1	+	-	+	-	+	+	-	*Saccharomyces cerevisiae*
2	+	-	+	-	+	+	-	*Saccharomyces cerevisiae*
3	+	-	+	-	+	+	-	*Saccharomyces cerevisiae*
4	+	+	+	+	-	+	+	*Kluyveromyces lactis (formerly Saccharomyces lactis)*
5	+	-	+	-	+	+	-	*Saccharomyces cerevisiae*
6	+	+	-	-	+	-	-	*Saccharomyces rouxii*
7	+	-	+	-	+	+	-	*Saccharomyces cerevisiae*
8	+	+	+	-	+	+	-	*Saccharomyces carlsbergensis*
9	+	+	+	+	-	+	-	*Kluyveromyces lactis (formerly Saccharomyces lactis)*
10	+	-	+	-	+	+	-	*Saccharomyces cerevisiae*

3. RESULTS

The Table 1 shows the results of the morphology of the yeast isolates from palm wine. Table 2 shows the macroscopic and microscopic identification of the yeast isolates from the PDA cultured plates. Microscopic view of the yeast indicated that the isolate belongs to the genus *Saccharomyces*. The result of sugar fermentation tests carried out on the yeast isolates to identify their species is presented in Table 3. The percentage frequency of occurrence of the yeast isolates identified which belongs to four species of *Saccharomyces* were *S. cerevisiae* (60%), *S. lactic* (20%), *S. rouxii* (10%) and *S. carlsbergensis* (10%).

Microbiological analysis of the 'must' during the fermentation is presented in Table 4. The result obtained showed that no culturable bacteria or fungi was detected in the fermenting 'must' at Day 0. However, yeast cells were detected in the fermenting 'must' at Day 7 and the mean yeast cell population was 2.77 x 10^4 cfu/ml. At Day 14, the mean yeast cell population of the fermenting 'must' decreased to 2.25 x 10^4 cfu/ml. Coliforms and bacteria were not detected in the fermenting 'must' at Day 7 and 14, respectively.

The physicochemical parameters of the fermenting 'must' monitored for 14 days is depicted in Table 5. Our result demonstrated that there was an increase in temperature, titratable acidity and alcohol content of the 'must' during the fermentation period which were within the range of 26.0-30.7°C, 0.45-0.68 g/mL and 0.00-10.87%, respectively. It was remarkable that alcohol was not detected in the fermenting 'must' at Day 0. Within the fermentation period, increase in temperature, titratable acidity and alcohol content of the 'must' were significantly different ($p<0.05$) with the exception of the values obtained between Day 13-14. On the contrary, the pH, reducing sugar and specific gravity of the 'must' decreased during the fermenting period and the values were within the range of 3.8-3.5, 23.00-2.10 g/L and 1.08-1.00 ^{o}Brix %, respectively. Notably, the decrease in pH and specific gravity of the 'must' during the fermentation period were not significantly different ($p>0.05$). However, the decrease in reducing sugar content of the 'must' within the fermentation period were significantly different ($p<0.05$) with the exception of the values obtained between Day 13-14.

Table 6 shows the comparison between the physicochemical parameters of the fermented 'must', wine (product) and the imported wine. Our result indicated that there is a significant difference ($p<0.05$) between alcohol content of the 'must', wine (product) and the imported wine. The level of alcohol development increased with

Table 4. Microbiological analysis of the 'must' monitored during fermentation

Days	Dilutions	Growth medium	Microbial count	No of colonies (cfu/ml)
0	10^{0}	NA	-	-
	10^{-1}	NA	-	-
	10^{-1}	PDA	-	-
	10^{-2}	PDA	-	-
	10^{-2}	MAC	-	-
	10^{-3}	MAC	-	-
7	10^{-1}	NA	-	-
	10^{-3}	NA	-	-
	10^{-1}	PDA		
	10^{-2}	PDA	274	2.74×10^{4}
	10^{-2}	MAC	-	-
	10^{-3}	MAC	-	-
	10^{-3}	PDA	28	2.8×10^{4}
14	10^{-2}	NA	-	-
	10^{-3}	NA	-	-
	10^{-2}	PDA	220	2.2×10^{4}
	10^{-3}	PDA	23	2.3×10^{4}
	10^{-2}	MAC	-	-
	10^{-3}	MAC	-	-

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

Table 5. Physicochemical parameters of 'must' during fermentation

Day	Temp (^{o}C)	pH	TA (g/mL)	RS (g/L)	Alcohol (% $^{v}/_{v}$)	SG (^{o}Brix % $^{w}/_{w}$)
0	26.0±0.28^{a}	3.8±0.14^{a}	0.45±0.04^{a}	23.00±0.71^{h}	0.00±0.00^{a}	1.08±0.09^{a}
2	27.0±0.57^{ab}	3.7±0.42^{a}	0.47±0.09^{ab}	21.15±0.95^{g}	1.36±0.11^{b}	1.07±0.03^{a}
3	27.5±0.71^{bc}	3.7±0.28^{a}	0.48±0.03^{ab}	19.40±0.64^{f}	4.08±0.28^{c}	1.05±0.04^{a}
5	27.9±0.71^{bcd}	3.6±0.28^{a}	0.52±0.04^{abc}	15.50±0.83^{e}	5.43±0.64^{c}	1.04±0.03^{a}
7	28.0±0.42^{bcd}	3.5±0.57^{a}	0.55±0.07^{abcd}	11.20±0.34^{d}	5.43±0.39^{c}	1.04±0.01^{a}
9	28.5±0.14^{cd}	3.6±0.28^{a}	0.60±0.04^{bcd}	8.70±0.64^{c}	8.15±0.35^{d}	1.02±0.03^{a}
11	29.0±0.42^{d}	3.6±0.42^{a}	0.65±0.06^{cd}	5.40±0.57^{b}	9.51±0.72^{e}	1.01±0.01^{a}
13	30.5±0.42^{e}	3.5±0.28^{a}	0.67±0.04^{d}	3.20±0.71^{a}	10.87±0.83^{f}	1.00±0.00^{a}
14	30.7±0.21^{e}	3.5±0.42^{a}	0.68±0.06^{d}	2.10±0.17^{a}	10.87±0.98^{f}	1.00±0.00^{a}

Values show means of duplicate analysis ±SD. Values with different superscript down the column are significantly different (P = 0.05). Key: TA-Titratable acidity; RS-Reducing sugar; SG-Specific gravity, Temp-Temperature

Table 6. Comparison between the physicochemical parameters of the 'must', wine produced and imported wine

Parameters	'Must'	Product (wine)	Imported wine
pH	3.8±0.127^{a}	3.5±0.099^{a}	3.54±0.092^{a}
AC (% $^{v}/_{v}$)	0.00±0.00^{a}	10.87±0.106^{c}	8.75±0.071^{b}
SG (^{o} Brix % $^{w}/_{w}$)	1.08±0.057^{a}	1.00±0.00^{a}	6.57±0.071^{b}
TA (% Tartaric acid)	0.45±0.042^{a}	0.68±0.057^{b}	0.51±0.042^{a}
Colour (visual)	Dull red	Bright red	Rose to red hue

Values show means of duplicate analysis ±SD. Values with different superscript across the row are significantly different (P = 0.05). Key: TA-Titratable acidity; RS-Reducing sugar; SG-Specific gravity, AC-Alcohol content

Table 7. Description of sample code for sensory evaluation

Sample Code	Brand	Colour	Country of origin
RORW	Developed product	Bright light red	Nigeria
BDV	Baron de Vall	Bright light rose	Spain
CRW	Concord red wine	Dull deep red	Nigeria

Table 8. Comparison between sensory attributes of the wine produced, imported and made in Nigeria wine

Attributes	RORW	BDV	CRW
Colour	6.40±0.52^{b}	5.60±0.52^{a}	5.60±0.52^{a}
Taste	7.00±0.67^{c}	6.00±0.67^{b}	5.40±0.52^{a}
Flavour	6.70±0.82^{b}	6.00±0.94^{ab}	5.70±0.67^{a}
General acceptability	7.40±0.84^{a}	6.10±0.99^{b}	5.30±0.67^{c}

Values show means of sensory scores by 10-man panelist ±SD. Values with different superscript across the row are significantly different (P = 0.05). Key: RORW-Wine produced; BDV- Imported baron de Vall wine; CRW-Nigerian concord wine. 9-point Hedonic scale: 9-like extremely, 8-like very much; 7-Like moderately; 6-Like slightly; 5-Neither like nor dislike; 4-Dislike slightly; 3-Dislike moderately; 2-Dislike very much; 1-Dislike extremely

increase in fermentation time with the highest alcohol content recorded on day 13 and day 14. The alcohol content of the wine produced (10.87%) is higher than the value for imported wine (8.75%) whereas alcohol was not detected in the 'must'. There is no significant difference ($p>0.05$) between pH of the 'must', wine (product) and imported wine which ranged from 3.54 - 3.8. There is no significant difference ($p>0.05$) between specific gravity of the 'must' (1.08 ^{o}Brix %) and the wine produced (1.00 ^{o}Brix %) but the values were significantly different ($p<0.05$) from SG of 6.57 ^{o}Brix % for the imported wine. The titratable acidity of the 'must' (0.45 g/mL) and imported wine (0.51 g/mL) were not significantly different ($p>0.05$) but these values were significantly different ($p<0.05$) from TA of the wine produced.

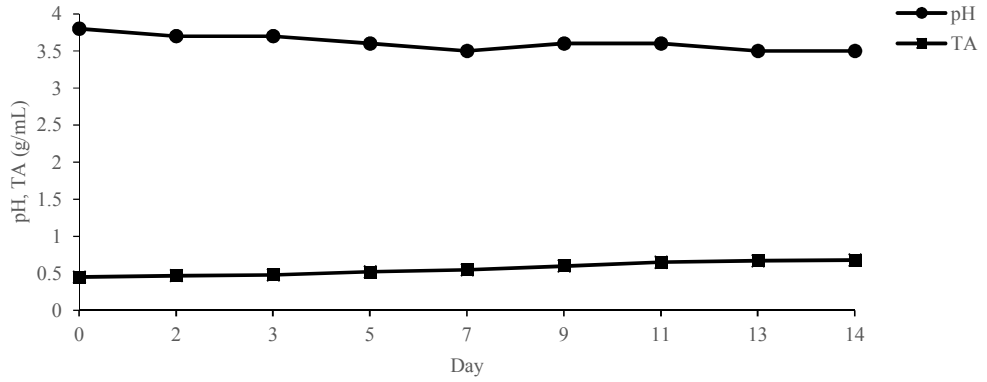

Fig. 1. Comparison between the titratable acidity and pH of the fermenting 'must'

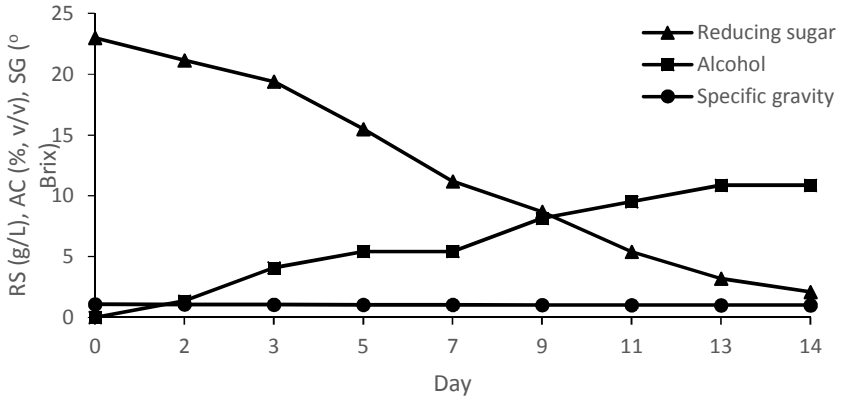

Fig. 2. Comparison between reducing sugar, alcohol content and specific gravity of the fermenting 'must'

Shown in Table 7 is the description of three brands of wine subjected to sensory evaluation including wine produced from orange juice and extract of *Hibiscus sabdariffa* using *S. cerevisiae* isolated from palm wine. Two of the products were of Nigerian origin and the other was a product of Spain. Presented in Table 8 is a comparison between sensory attributes of the wine produced, imported (Baron de Vall) and made in Nigeria wine (Concord). The sensory results obtained, showed that all the sensory attributes of the wine produced (RORW) were significantly different *(P = 0.05)* from that of Baron de Vall (BDV) and Concord red wine (CRW) except flavour which share a relationship with Baron de Vall.

Fig. 1 shows the relationship between titratable acidity (TA) and pH of the fermenting 'must'. The graph demonstrated that both physicochemical parameters of the fermenting 'must' were directly dependent on each other such that as pH decreases, the TA increases and vice versa. Fig. 2 shows the relationship between reducing sugar content, alcohol content and specific gravity of the fermenting 'must'. The result indicated that the reducing sugar content of the fermenting 'must' decreased as alcohol content of the 'must' increased. Meanwhile, the specific gravity of the 'must' remained relatively stable within the fermentation period.

4. DISCUSSION

The frequency of occurrence of yeasts isolated from fresh palm wine were *Saccharomyces cerevisiae* (60%), *S. lactic* (20%), *S. rouxii* (10 %) and *S. carlsbergensis* (10%). *S. cerevisiae* was selected for wine production using orange juice and extract from *Hibiscus sabdariffa* (Roselle drink). According to Nwaiwu et al. [47], the frequency of occurrence of yeast isolates from palm wine sourced from different locations in south east Nigeria were *Saccharomyces cerevisiae* (70.40%), *Pichia kudriavzevii* (15.14%), *Candida ethanolica* (9.76%) and

C. tropicalis (4.70%). Other yeasts isolated from palm wine obtained from the same region were *Saccharomyces globosus* and *S. carlsbergensis* [48]. According to Onwumah et al. [49], *S. cerevisiae* is recognized as the most popular yeast present in palm wines available in Nigerian market which is in agreement with the findings from this study.

A red wine made from sweet orange juice and extract from *Hibiscus sabdariffa* (Roselle drink) using *Saccharomyces cerevisiae* isolated from palm wine was successfully developed in this study. Since the wine produced is a fruit based fermented and undistilled product, it is expected that most of the nutrients originally found in the Roselle drink and sweet orange juice are also present in the wine produced [50,51]. During the wine making process, our result showed that no culturable microorganism was present in the fermenting 'must' at Day 0 whereas at Day 7 and 14, only yeast cells were detected which decreased from mean yeast count of 2.77×10^4 Cfu/ml – 2.25×10^4 Cfu/ml. Absence of culturable microorganism in the 'must' could be attributed to pasteurization of the 'must'. In a related study which involved production of watermelon wine, Zainab et al. [52] reported that bacteria and coliforms were not detected in the fermenting 'must' except yeast cells which increased from 0-5.0×10^7 Cfu/ml within the fermentation period. In this study, undetected bacteria in the fermenting 'must' comprising of sweet orange juice and extract of *H. sabdariffa* throughout the fermentation period is an indication that the finished product is safe for human consumption. Ifie et al. [1] reported that 2 Log_{10}Cfu/ml was the mean count of bacteria in the fermenting 'must' during Roselle wine making (wine made from extract of *H. sabdariffa*) which they generally considered to be of no significance. Also, reported in the study was 2 Log Cfu/ml spore count in the fermenting 'must'. Meanwhile, during the production of Roselle wine, [32] reported an increase in viable count in the fermenting 'must' from 1.64×10^6 Cfu/ml at Day 0 to 8.80×10^8 Cfu/ml at Day 11. A recent study carried out by Baba et al. [53] reported that juice from sweet orange (*Citrus sinensis*) demonstrated a remarkable inhibition against clinical bacteria species isolated from wounds namely *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* which they attributed to the presence of secondary metabolites. The result from that study was collaborated by the findings reported by [54] which involved testing the inhibitory properties of sweet orange juice against bacteria wound isolates, namely *Klebsiella pneumonia, Proteus mirabilis, Acinetobacter baumannii,* and *S. aureus*. Both reports suggested that the sweet orange juice used in this study to prepare red wine influenced the absence of nonculturable bacteria in the fermenting 'must'. The low pH of the fermenting 'must' also may have contributed to the non-detection of bacteria due to the inhibition of the growth of some pathogenic microorganisms. According to Saranraj et al. [7], at pH below 3.5, only few microorganisms involved in fermentation process survived whereas most microbes were eliminated. Critically, the increasing concentration of alcohol in the fermenting 'must' most likely created an unconducive environment for microorganisms to thrive with the exception of yeast cells which decreased in population between Day 7 and 14 [12]. Bacterial growth was inhibited by alcohol by plasmolyzing the cell wall of the bacteria [41]. According to Mathew et al. [36], the possible reasons for the decrease in population of yeast cells as reported in their study include high cell density, depletion of nutrients, suspected presence of toxic metabolic byproducts as well as rupturing of cell membrane of the yeast cells by increasing concentration of alcohol.

A combination of pH and titratable acidity is a critical parameter known to influence the colour, flavour and aroma of fermented products. The levels of both parameters for a fermented product could be used as an index of its shelf life [55]. According to Okoro [34], titratable acidity (TA) as tartaric acid of fermenting 'must' comprising of Roselle and pawpaw for wine production increased from 0.56-0.70% (w/w) within 14 Day of the fermentation period. The TA of the final product was 0.71% (w/w). In a related study that involved the production of vegetable wine from *H. sabdariffa,* Ifie et al. [1] reported that the TA of the fermenting 'must' steadily increased from 0.52 g/L at Day 0 to 0.73 g/L at Day 12. A study carried out by Archibong et al. [18] which involved wine making from pineapple and orange juice using palm wine yeast reported that TA of the fermenting 'must' increased from 0.768-0.973%. Meanwhile, the results reported in this study showed that TA of the fermenting 'must' steadily increased from 0.45-0.68 g/mL within the 14 days fermentation period. According to Kiin-Kabari and Igbo [41], wine is supposed to have a titratable acidity within the range 0.5-1.0% and the wine produced in this study met this requirement.

During fermentation of 'must' which comprised of sweet orange and pineapple juice, Archibong et al. [18] reported that the pH decreased from 3.5-3.0 between Day 0-14. Although the pH of the fermenting 'must' was lower than the values reported in this study which ranged from 3.8-3.5, both results followed the same trend. According to Ifie et al. [1], pH of fermenting 'must' during production of Roselle wine (made from extract of *H. sabdariffa*) decreased from 3.78-3.09. The trend in pH is also in agreement with our results, although, some of the values were lower than what is reported in this study. According to Okoro [34], pH of the fermenting 'must' during wine production involving Roselle drink and pawpaw (*Carica papaya*) using *S. cerevisiae* isolated from palm wine decreased from 3.76-3.61 within 14 days fermentation period which further reduced to 3.57 after 30 days aging of the wine. This result supports the findings reported in this study. Based on our results, the finished product is referred to as a sweet wine because its pH falls within the range of 3.5-4.5 [56]. Lowering of pH of the fermenting 'must' encountered in this study could be explained theoretically by stating that sugars present in the 'must' were converted to alcohols, followed by conversion of the alcohols to aldehydes, then aldehydes to ketones and finally, the ketones were converted to acids [32]. It is also suggested that increased alcohol production by yeast is favoured by acidic pH of the fermenting 'must'. In wine making, acidity plays an important role by influencing the quality of the wine. This happens by regulating fermentation, improving balance as well as the overall characteristic traits possessed by the wine. However, in the absence of acidity, fermentation process will be affected and poor quality wine will be produced [57].

In evaluating the quality of fruit wine, its alcohol content is a critical parameter to put into consideration [55]. It also influences the level of acceptability of the alcoholic beverage among consumers [3]. During the fermentation of 'must' (sweet orange fruit juice using *Saccharomyces cerevisiae* for wine production, [6] reported that the alcohol content was 10.47% (v/v) at Day 14. This result is in agreement with the alcohol content of 10.87% (v/v) at Day 14 of the fermenting 'must' reported in this study. In a related study, [34] also reported that the alcohol content of red wine made from Roselle drink and pawpaw was 10.50% (v/v) after aging for 30 days. According to [1], alcohol content of fermenting 'must' during vegetable wine making from *Hibiscus sabdariffa L*. steadily increased from 0.0-9.6% vol. within 12 days fermentation period. The alcohol content (10.87% v/v) of the wine produced from sweet orange juice and extract from *H. sabdariffa* using *S. cerevisiae* isolated from palm wine met the criterion recommended by European Economic Community for alcohol content of wine which should be within the range of 8.5-19.5% [58]. The red wine produced is regarded as a good table wine because its alcohol content falls within the range of 7-14% [52]. Alcohol not detected in the 'must' at Day 0 is consistent with several reports on wine making from different researchers [12,18,32,34]. Kanter et al. [37] had also reported that fermentation of wine by hybrid yeast, *S. cerevisiae* × *S. paradoxus* (SC × SP was completed on day 14.

The result obtained from this study revealed that the specific gravity of the fermenting 'must' decreased from 1.086-1.000 ^{o}Brix % (w/w). In a related study, Okoro [34] reported a similar trend in the fermenting 'must' in the course of producing red wine from Roselle and pawpaw using palm wine yeast which ranged from 22.1-7.2 ^{o}Brix % (w/w) whereas the specific gravity of the final product was 5.1 ^{o}Brix % (w/w) after ageing for 30 days. [36] also reported a decrease in specific gravity from 1.086 to 1.000 in the fermenting 'must' which involved using sweet orange juice for the production of orange wine. This result is in agreement with the findings reported in this study. The efficiency of the yeast cells involved in the fermentation process is indicated by reduction in specific gravity and increase in alcohol content [18]. The decreases in specific gravity could be attributed to microorganisms being able to utilize the nutrients primarily sugars available in the substrate to carry out metabolic activities and in the process releases CO_2 and heat [50]. According to Ifie et al. [1], the reduction in soluble solids in the fermenting 'must' resulted in alcohol being produced which was the case in this study.

The internal temperature of the fermenting 'must' is a parameter winemakers should take into consideration since fermentation is a biochemical process that generates a lot of heat. Generally, yeast remain active between a wide temperature range of 0-50°C, and 20-30°C being the optimum temperature range. According to Saranraj et al. [7], temperature not exceeding 29.4°C for red wines and 15.3°C for white wines is recommended because higher temperatures will cause the yeast cells to stop growing. On the

contrary, slightly lower temperatures are preferable because production of esters, other aromatic compounds as well as alcohol increases. This condition makes the wine less susceptible to bacterial infection and easier to clear. Results from this study showed that the temperature of the fermenting 'must' increased from 26-30.7°C within the fermentation period which is in agreement with the recommended temperature range for red wine production. Zainab et al. [52] reported that temperature of the fermenting 'must' used for the production of watermelon wine increased from 25-29°C which later declined from 29 to 28°C towards the end of the fermentation period.

The results obtained from this study showed that reducing sugar of the fermenting 'must' decreased from 23 g/L to a lower concentration (2.10 g/L). According to Kiin-Kabari and Igbo [41], the decrease in sugar content as fermentation progressed could be attributed to microbial succession, available nutrients, sugar and alcohol which lead to acid production. Opara and Rexford [32] reported that reducing sugar of the fermenting 'must' during production of wine from Roselle drink (zobo) reduced from an initial concentration of 0.43 mg/ml to 0.176 mg/ml on the eleventh day of fermentation. In the course of producing wine from pineapple and orange juice using palm wine yeast, Archibong et al. [18] also reported that the reducing sugar content of the fermenting 'must' decreased from 12.928-0.082 g within 21 days fermentation period. These reports corroborate with the findings from this study.

Sensory analysis of the wine produced indicated that the mean sensory score for colour is interpreted as 'like slightly'. The taste and overall acceptability of the red wine produced is interpreted as 'like moderately' were higher than the values assigned to the same sensory parameters for both imported wine and the wine produced in Nigeria. Although, the interpretation of sensory score for flavour of the red wine produced (Like slightly) is the same with Baron de vall (imported wine), the mean sensory score was slightly higher. The overall sensory analysis of wine produced in comparison with Baron de vall and Concorde wine indicated that the wine made from sweet orange juice and extract from *H. sabdariffa* using palm wine yeast (*S. cerevisiae*) is preferable than the two brands of wines evaluated in this study. A blend of two source materials (sweet orange juice and extract from *H. sabdariffa*) for wine production possibly influenced higher sensory scores for the alcoholic beverage compared with Concorde wine and Baron de vall wine. This report is in agreement with a similar study carried out by Omoya and Akharaiyi [46]. The preference of the wine produced over the two brands of wine already commercialized could also be attributed to many volatile and non-volatile compounds released into the product which gives it a typical taste and odour. According to [50], the concentration of ethanol in the alcoholic beverage will determine the extent the olfactory system will perceive the volatile compounds released.

The relationship between titratable acidity and pH demonstrated in this study is such that as pH of the fermenting must decreases, the titratable acidity increases. This occurrence could be attributed to accumulation of organic acids during the fermentation process. Different researchers have also reported a similar trend in studies involving fruit wine making [41,59]. Considering the relationship between reducing sugar, alcohol content and specific gravity, our study shows that as the reducing sugar decreases due to increased utilization of sugar by the yeast cells, the concentration of alcohol released increases which result in decreases in the specific gravity.

5. CONCLUSION

Saccharomyces cerevisiae was the dominant yeast isolated from palm wine and was selected for the production of wine using a blend of sweet orange juice and extract from *Hibiscus sabdariffa* (Roselle drink). The pH, alcohol content, specific gravity, titratable acidity and colour of the wine produced was 3.5, 10.87% v/v, 1.0 ^{o}Brix % (w/w), 0.68% and bright red, respectively. Meanwhile, the pH, alcohol content, specific gravity, titratable acidity, and temperature of the fermenting 'must' were within the range of 3.8-3.5, 0-10.87% v/v, 1.08 -1.00 ^{o} Brix % w/w, 0.45-0.68 g/mL, and 26.0-30%, respectively. At Day 0, no culturable microorganism was detected in the fermenting 'must', but at Day 7 and 14, there was presence of yeast cells with a mean count of 2.77 x 10^4 Cfu/ml and 2.25 x 10^4 Cfu/ml, respectively. The wine produced had a higher sensory score for color, taste, flavour and overall acceptability compared with a local and imported brand of wine. Therefore, this study has demonstrated that a blend of sweet orange juice and Roselle drink fermented by indigenous palm wine yeast (*S. cerevisiae*) is a generally acceptable red wine of good quality which contains 10.87% v/v alcohol. It is interesting to report that the quality

of the red wine produced is comparable with the imported and local brand of wine already commercialized.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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