



## Production and Microbiological Assessment of Date Palm (*Phoenix dactylifera* L.) Fruit Wine

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### Authors' contributions

This work was carried out in collaboration between both authors. Author SA designed the study, managed the analyses of the study, performed the statistical analysis and wrote the protocol. Authors SNN wrote the first draft of the manuscript and managed literature searches. Both authors read and approved the final manuscript.

### Article Information

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### ABSTRACT

**Aim of the Study:** This study was undertaken to assess the possibility of using date palm fruit (*Phoenix dactylifera* L.) for wine production.

**Study Design:** Good quality, fresh and dried date fruit were gotten from Kankia village in Kastina State, Northern Nigeria, sliced to remove the seed and macerated using sterilised blender to give 8.5 kg of crushed date palm fruit used for the production.

**Place and Duration:** Date palm fruit (*Phoenix dactylifera* L.) wine production was studied from March to August, 2014 in Salem University Microbiology Laboratory, Lokoja, Nigeria.

**Methodology:** Date fruit wine was produced by fermenting the fruit must with *Saccharomyces cerevisiae* from Guangxi Danbaoli, China aerobically for 6 days with even stirring of the must twice daily and anaerobically for four weeks at  $28 \pm 2^\circ\text{C}$ . During both fermentation processes, the changes in pH, Titratable Acidity (TTA), sugar content, alcohol content, specific gravity, total yeast counts and total heterotrophic counts were monitored.

**Results:** During the aerobic fermentation, the pH dropped from 5.7 to 4.3, titratable acidity increased from 0.23 to 0.65, there was a notable decrease in the specific gravity from 1.070 sp.gr to 1.026, Alcohol content increased from 0 to 5.5%, Sugar content dropped from 17 to 0%, total yeast counts increased from  $4.69 \times 10^2$  to  $15.39 \times 10^2$  viable cells/ml while total heterotrophic

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bacterial count ranged from 2.0 to 9.0 cfu/ml. *Lactobacillus casei* and *Lactobacillus* spp. were encountered. During anaerobic fermentation pH ranged increased from 4.3 to 4.6, TTA decreased from 0.65 to 0.30. There was a notable decrease in the specific gravity from 1.070 sp.gr to 1.026 sp.gr while during anaerobic fermentation, there was a slight change in the specific gravity from 1.026 sp.gr to 1.025 sp.gr. Yeast population dropped from  $8.90 \times 10^2$  viable cells/ml to  $0.92 \times 10^2$  cells/ml at the end of week 2, sugar was not detected and the final percentage alcohol was 9.2%. Sensory evaluation shows 71.8% acceptance compared to imported white wine (78.3%). This study has shown the probability of using date palm fruit as raw material for fruit wine production.

**Keywords:** Date palm; wine; alcoholic fermentation; acceptability.

## 1. INTRODUCTION

Wine was derived from the Greek word 'oines' meaning wine while the science of wine is called 'oenology'. It starts with the harvesting of grapes, separation of the juice before fermentation and concludes with the variety of storage and ageing steps [1]. Apples, berries and blackcurrants are sometime also fermented for wine production. Grape berries have a natural chemical balance which allows a completely fermentation without the addition of sugar, acid, enzymes or other nutrients. It is a rich source of vitamins, many essential amino acids, minerals, fatty acid and others; however other fruits with same characteristics have been discovered and used effectively for wine production [2]. During fermentation, microscopic single-celled organism called 'yeast' such as *Saccharomyces cerevisiae* digest sugar found in fruit juice, producing alcohol and carbon dioxide gas in the process.

Wines are categorized using a number of different criteria, this include grape variety, region of origin, by colour, by name of the wine maker or viticulturalist, or by production technique. However, three basic groups of wines are most easily distinguishable for the consumer: table wines, sparkling wines and fortified wines. Wine has been produced and enjoyed by many people, from peasants to kings, for thousands of years [3]. The consumption of red wine is known to have a remarkable protective effect against oxidative stress in blood plasma [4]. Date palm (*Phoenix dactylifera* L.), production in Nigeria started at about 17<sup>th</sup> century ago but its cultivation and marketing has been subsistence level. It was reported that pilgrims brought Date palm in to Nigeria from North Africa during trans-Saharan trade [5]. Though Nigeria is not a major Dates producer in the world, the crop thrives in Northern parts of the country particularly regions above latitude 100 North of the equator [6]. It is propagated by seed, offshoot and tissue culture.

The Date palm is dioecious perennial, the females of which normally begin to bear Date fruits after four years depending on the agronomic practices. It is a monocotyledonous plant with no tap root but fibrous root system. The trunk is vertical and columnar of the same girth all the way up. Dates production in Nigeria has two fruiting seasons (dry and wet seasons fruits), but only the dry season fruit is economically useful [7]. Large quantities of those fruits are disposed off yearly due to non-availability of, or poor, storage facilities. This results in loss of the vital nutrients (Vitamins) that are associated with them and loss of potential revenue source. If the fruits could be put to other use such as wine production, the nutrients that are so lost can be harnessed and made available all year round in addition to generating revenue.

This study is aimed at producing wine from Date palm fruit, evaluating its microbiological quality and acceptability as compared to a standard white wine (Carlo Rossi).

## 2. MATERIALS AND METHODS

The experiments were performed between February and July, 2014 in the Microbiology laboratory of Salem University, Lokoja, Kogi State, Nigeria.

### 2.1 Fermenting Organism

Pure culture of *Saccharomyces cerevisiae* was purchased and used to ferment the fruit must.

### 2.2 Preparation of Date fruit Wine

Good quality, fresh and dried date palm fruits were sliced using a sterile sharp knife to remove the seed and macerated using a clean sterilized grinding engine to produce a small granular texture of the date palm fruit so as to increase the surface area of the fruit for the activity of yeast for fermentation. After grinding, 6.5 kg of

crushed date palm fruit was gotten, Twenty seven (27) litres of water boiled at 100°C for 30 minutes and allowed to cool to about 45°C was mixed with the 6.5 kg of the crushed date palm fruit. After even mixing using a sterilized stirrer, the fruit pulp produced was evenly divided between two sterilized fermentors. Each fermentor held about 13.5 ltrs of the fruit must. The must was sterilized with Sodium metabisulphate solution to remove microbial contaminants by introducing the Standardized solution into the must 24 h before addition of pitching yeast [8].

### 2.3 Fermentation Process

Standardized amount of powder yeast purchased from Guangxi Danbaoli, China was added to the must in a fermenting Jar by sprinkling it over the surface of the juice then stirred; the inoculated must was covered with sterile muslin cloth and incubated at room temperature ( $28 \pm 2^\circ\text{C}$ ); it was aerated daily by stirring twice to encourage yeast multiplication [8]. Aerobic fermentation was terminated after 6 days and the must was sieved to remove the shaft and debris of the crushed fruits. During the Anaerobic phase of fermentation, the filtrate obtained after sieving the must was transferred into anaerobic fermentation jar and incubated at room temperature. An air trap was fixed to the fermenting jar. Sodium metabisulphate solution was added to the filtrate to supply sulfur dioxide gas. Fermentation was terminated after four weeks; the wine was then stored to allow the yeast to flocculate. The resulting wine was then aged for two months. The aged wine was then filtered using pressurized filtering kit, decanted into sterile bottles and corked.

### 2.4 Fermenting Organism Monitoring

The population of yeast in the fermenting must during aerobic and anaerobic phases was monitored by microscopic counting using Haemocytometer [9].

### 2.5 Enumeration of Total Heterotrophic Bacterial Count in the Wine

Populations of bacteria in the wine were assessed by standard pour plate method using nutrient agar. Ten fold serial dilution of the wine were made and 1 ml of desired dilution plated [2]. Isolated organisms were characterized and

identified using a series of biochemical test and identification keys [10].

### 2.6 Determination of Physicochemical Properties and Alcohol Content (pH, Titratable Acidity, Percentage Total Sugar and Specific Gravity)

The pH was measured with a pH meter model (Philips PW 9418). The titratable acidity was determined using wine maker's acid kit [11]. Total percentages of sugar were estimated using Berry method [8]. The Specific gravity was determined using a refractometer and the value taken from the calibration on it [8].

The alcohol content of the must was determined using Triple Scale Hydrometer for beer and wine (Model HY110).

### 2.7 Sensory Evaluation

This was determined by evaluation point system [12] and compared with standard white wine (Carlo Rossi).

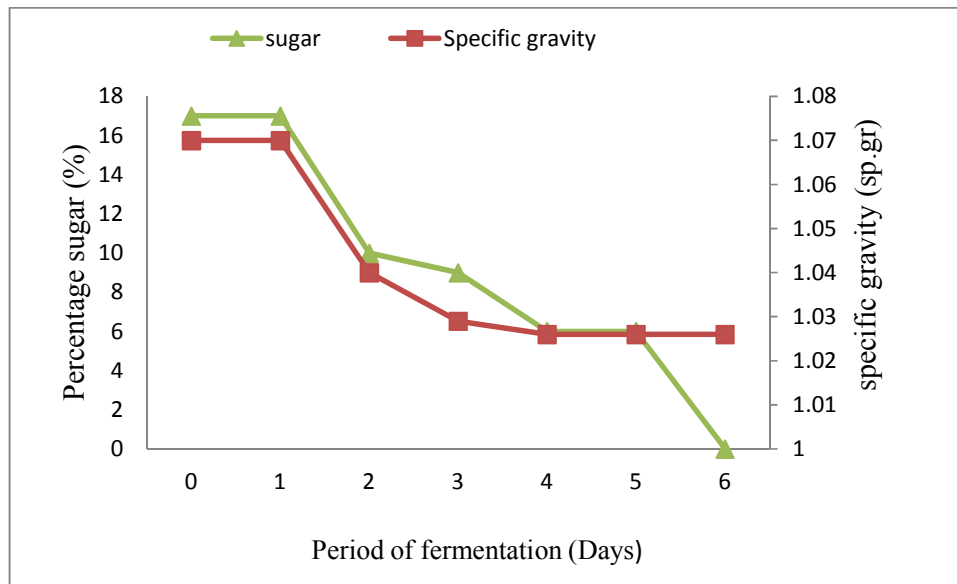
### 2.8 Statistical Analysis

The data were analyzed using one way ANOVA followed by Duncan multivariable post-hoc test for comparison between control and wine produced. P values less than 0.05 were considered statistically significant.

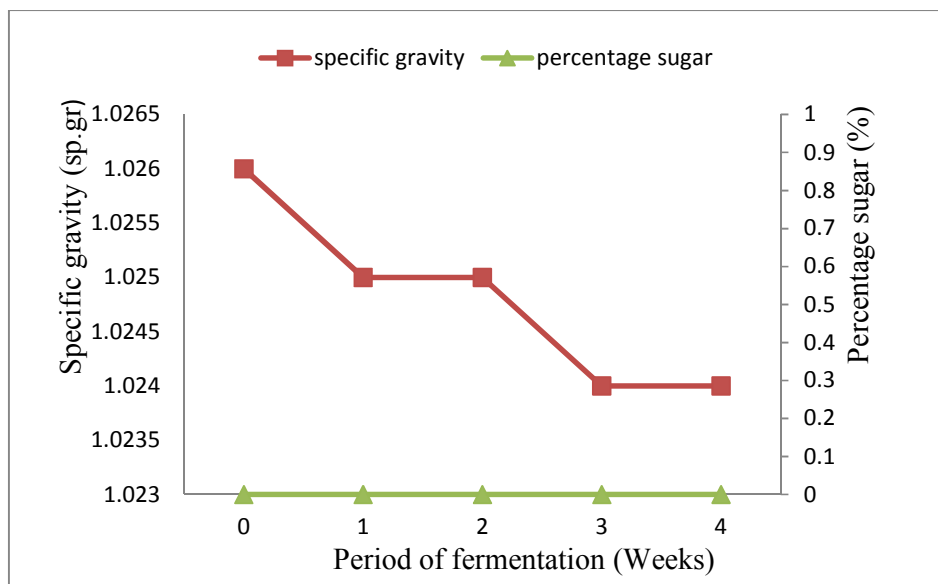
## 3. RESULTS

The variations in the specific gravity and percentage sugar of the fermenting date fruit must during aerobic fermentation are shown in Fig. 1. There was a notable decrease in the specific gravity from 1.070 sp.gr to 1.026 sp.gr and also a decrease in the percentage sugar content of the fermenting must from 17.0% to 0.0% while during anaerobic fermentation, there was a slight change in the specific gravity from 1.026 sp.gr to 1.025 sp.gr and the percentage sugar remained at 0% as shown in Fig. 2.

A change in the pH and Titratable acidity of the fermenting date fruit must during aerobic fermentation is shown in Fig. 3. Generally there was a drop in pH from 5.7 to 4.3 and an increase in the titratable acidity from 0.23 to 0.65. The pH increased from 4.3 to 4.6 and the titratable acidity decreased from 0.65 to 0.30 during anaerobic fermentation as shown in Fig. 4. The Relationship between number of days and total



**Fig. 1. Relationship between specific gravity and percentage sugar during aerobic fermentation of must**

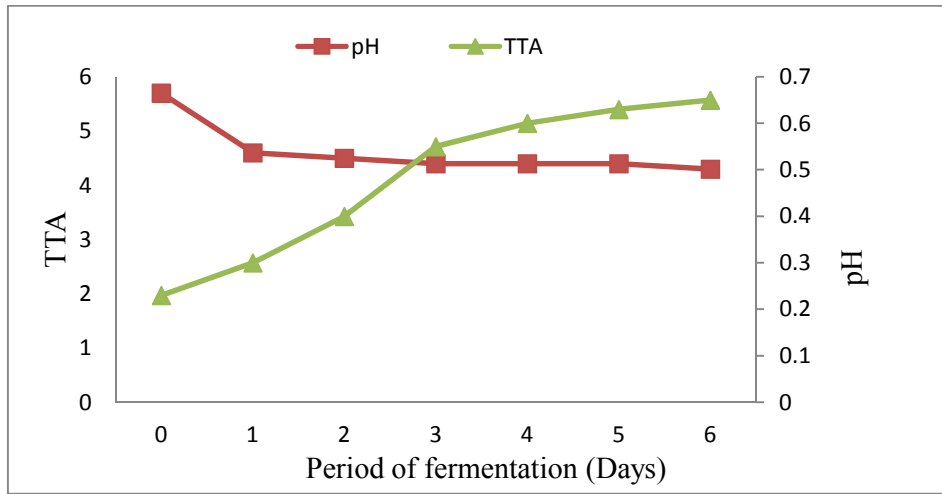


**Fig. 2. Relationship between specific gravity and percentage sugar during anaerobic fermentation of must**

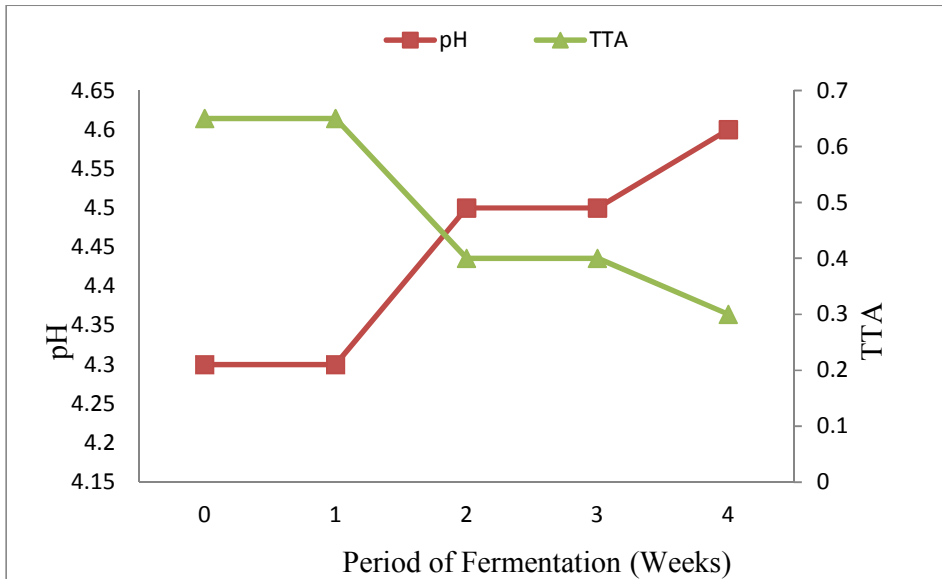
viable count during aerobic fermentation is shown in Fig. 5. There is a notable decrease in the number of viable organism from 9.0 cfu/ml to 1.0 cfu/ml during aerobic fermentation while a constant value of 2.0cfu/ml was recorded during anaerobic stage Fig.6. Two bacterial species are isolated from the wine during aerobic

fermentation and are identified to be *Lactobacillus casei* and *Lactobacillus* sp.

Yeast count and percentage alcohol produced during aerobic and anaerobic fermentations are shown in Figs. 7 and 8 respectively. The yeast counts increased from  $4.69 \times 10^2$  cells/ml to  $15.39 \times 10^2$  cells/ml and the percentage alcohol



**Fig. 3. Changes in pH and Titratable acidity (TTA) during aerobic fermentation of must**

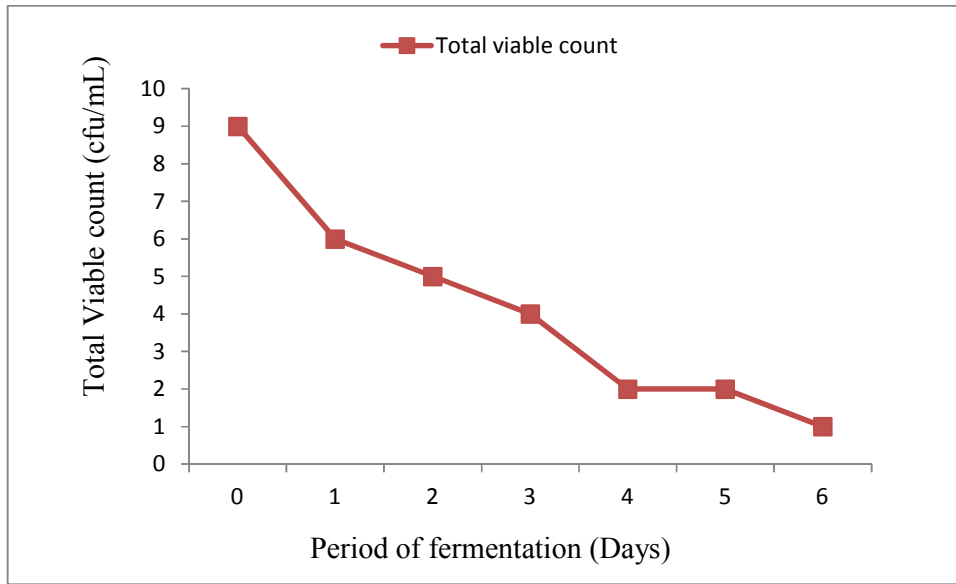


**Fig. 4. Relationship between pH and Titratable acidity (TTA) during anaerobic fermentation of must**

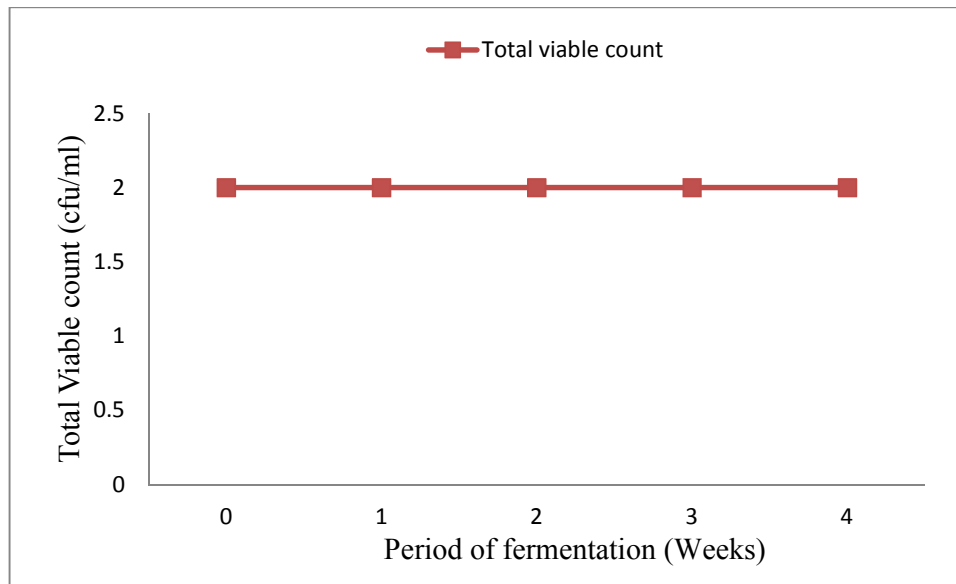
increased from 0.0% to 5.5% during aerobic fermentation while during anaerobic fermentation there was a drop in the yeast count from  $15.39 \times 10^2$  cells/ml to  $0.0 \times 10^2$  cells/ml and the alcohol content increased from 5.5% to 9.2%.

The percentage acceptability level of the wine produced as compared to white wine is shown in Fig. 9 with date fruit wine having 71.8% acceptability and Carlo rossi wine having 78.3% acceptability as assessed by human volunteers.

Table 1 shows the proximate composition of fruit sample (Date palm) analyzed; the parameters used include vitamin C (88.57  $\mu\text{g/g}$ ), Protein (2.13%), Glucose (11.08%), Ash (9.66%), Total dissolved solid (2.47g/l), Total solid(28.67g/l), Fat(0.88%), and Moisture (28.67g/l) while proximate analysis of the date palm fruit wine in comparison with a white wine is shown in Table 2. Vitamin C, protein and glucose content in the date palm fruit are higher than white wine.



**Fig. 5. Relationship between number of days and total viable count during aerobic fermentation of must**



**Fig. 6. Changes in bacterial total viable count during anaerobic fermentation of must**

#### 4. DISCUSSION

The general and consistent decline in the percentage sugar of the fermenting must during aerobic fermentation and rapid increase in the number of yeast cell can be attributed to the effective utilization of the available sugar component and the daily aeration of the fermenting must leading to their cell propagation and rapid multiplication [2]. However, it was

noted that the yeast cell multiplied progressively till day 3 of the aerobic fermentation before a notable decline by day 4. Decline in the number of yeast cells in the fermenting must may be due to the notable decline in the sugar content as a result of rapid and effective utilisation of the sugar available in the must by the yeast cells leading to the fermentation of the must while increase in the alcohol content recorded will also affect the rate of yeast growth, this can be

corroborate by Awe submission [2]. No trace of viable yeast cells was noticeable in the fermenting must by week three of anaerobic fermentation. The yeast utilizes intermediate products like acetaldehydes as hydrogen acceptors and alcohol production [13].

**Table 1. Proximate composition of fruit sample (date palm fruit)**

Parameters	Proximate composition (%)
Vitamin C (µg/g)	88.57
Total dissolved solid (g/l)	2.47
Total solid (g/l)	28.67
Protein (%)	2.13
Glucose (%)	11.08
Ash (%)	9.66
Fat (%)	0.88
Moisture (g/l)	28.67

The drop in pH and corresponding increase in titratable acidity of must during the aerobic and anaerobic fermentation stages are attributable to yeast metabolism. These also show acidification of the medium during the fermentation stages, which is very important in wine production. The decrease in the pH of the fermenting must makes the must acidic and thereby leading to the increase in the Titratable acidity of the must. Acidity plays a vital role in determining wine quality by aiding the fermentation process and enhancing the overall characteristics and balance of the wine. Lack of acidity will result to the production of a poor fermentation process [8].

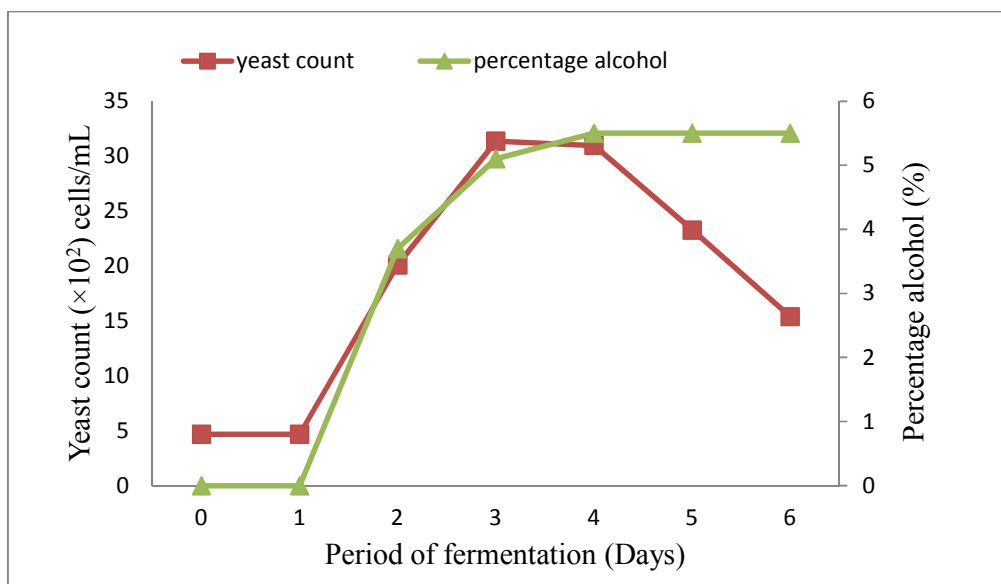
Acidity for wines lies between pH 3 and 7 (dry wine), pH 3.5 to 4.5(sweet wine) while higher acidities are sometime encountered in wines that have undergone fortification [14].

**Table 2. Proximate composition of wine samples**

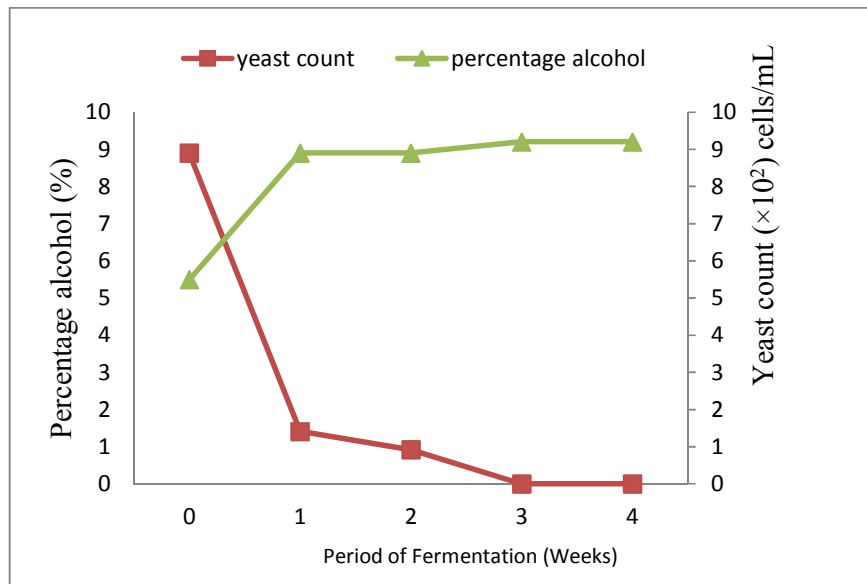
Parameter	White wine (Carlo Rossi)	Date fruit wine
Vitamin C (µg/g)	12.98	24.61
Total dissolved solid (g/l)	2.47	2.66
Total solid (g/l)	2.61	2.82
Protein (%)	0.56	1.40
Glucose (%)	4.10	6.03
Ash (%)	ND	ND
Fat (%)	ND	ND

Key: ND= Not Detected

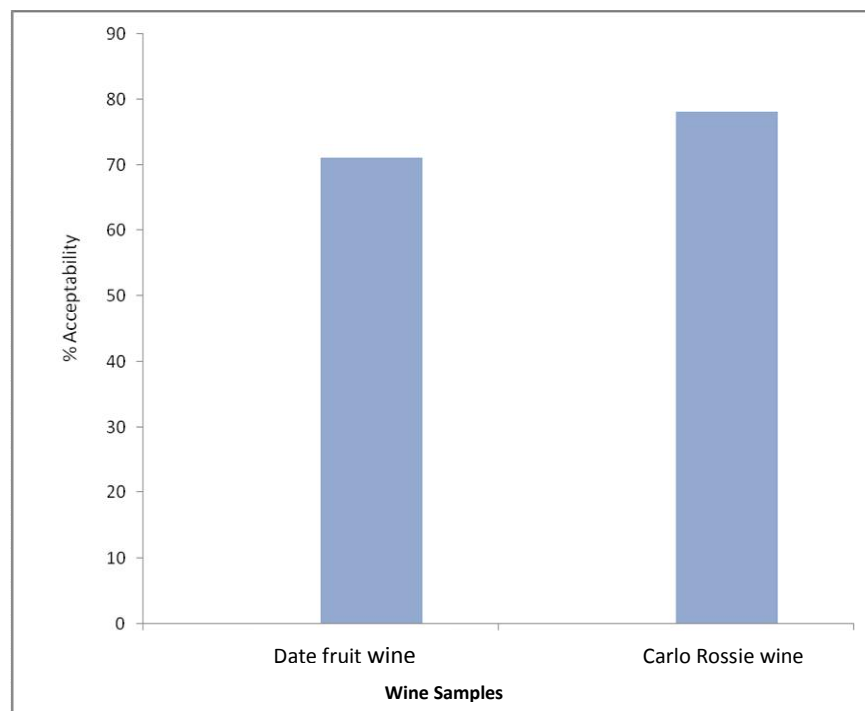
Generally with progression in days there was a notable decline in the number of total viable cells during aerobic and anaerobic fermentation, this can be said to be due to the decline in the available nutrients in the fermenting medium as fermentation occurs, also (reduction in the pH level) the increase in the alcohol content of the fermenting medium made it un conducive for growth of those organism because the pH level of the fermenting must does not support the growth of the organisms present in it, thereby helping in eradication of wild yeast and the presence of any other unwanted organism [2].



**Fig. 7. Relationship between percentage alcohol and total yeast count during aerobic fermentation of must**



**Fig. 8. Relationship between percentage alcohol and yeast count during anaerobic fermentation of must**



**Fig. 9. Percentage acceptability of wine produced compared to standard white wine**

The drop in the specific gravity of the fermenting must during aerobic and anaerobic fermentation falls within the range for wine.

The alcohol content of the fermenting must increased progressively during both aerobic and

anaerobic fermentation. The increase in the alcohol content can be attributed to yeast metabolism by continuous utilization of the sugar content, ethanol is produced and thus there is an increase in the alcohol content of the fermenting must, this continued until all the available sugar



in the fermenting must has been utilized. The final alcohol content of the wine (9.2%) ranks it among good table wines. A good table wine must have alcohol content between 8 and 14% [3].

The Sensory evaluation of the date fruit wine produced in regards to flavor, colour, appearance, clarity, aroma, taste, aftertaste and overall impression by twenty member panel rated the wine acceptable with 71.8% acceptability as compared to Carlo Rossi (control) 78.4%.

The proximate analysis of the wine produced as compared to Carlo Rossi white wine shows that the wine produced compares favourably in all parameters of the comparative components with vitamin C, glucose and protein having higher content than the control.

## 5. CONCLUSION

It can be concluded from this study that a locally available fruit in Nigeria like Date palm is suitable for fruit wine production with high acceptability and good microbiological standard

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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