International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume: 09, Issue: 04 "July-August 2023"

MIXED FRUIT WINE PRODUCED FROM PINEAPPLE (*Ananas comosus*) AND WATERMELON (*Citrullus lanatus*) USING YEAST FROM RIPE SHADDOCK FRUITS

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DOI: https://doi.org/10.51193/IJAER.2023.9403

Received: 21 Jun. 2023 / Accepted: 04 Jul. 2023 / Published: 05 Aug. 2023

ABSTRACT

Mixed *"must"* from pineapple and watermelon fruits were used for table wine production using yeast *(Saccharomyces cerevisiae*) isolated from ripe shaddock fruits. Fresh watermelon and pineapple fruits were peeled, sliced, ground and sieved with muslin cloth. The juice obtained was mixed to obtain the "must" which was fermented. During the period of fermentation, aliquot of samples were analysed for pH, titratable acidity, specific gravity, alcoholic content, reducing sugar and total soluble solids using standard procedures. Vitamins and mineral contents of the wine were checked after clarification and aging and the wine produced was compared with a commercial wine sold in the market. Specific gravity of the wine was observed to reduce from 1.075 to 1.007 km/m^3, reducing sugar reduced from 0.713 to 0.047mg/ml while total soluble solids decreased from 7.0 to 1.6^o Brix. Titratable acidity showed an increase from 3.257 to 6.780 g/100ml, pH increased from 4.42 to 3.00 tending towards acidity and alcoholic content increased from 0.36 to 5.10% (v/v). The wine produced from the mixture of pineapple and watermelon "*must*" using yeast *(Saccharomyces cerevisiae*) isolated from ripe shaddock fruits also contained essential vitamins and mineral necessary for growth and replenishing the body. The research showed that a good table wine can be produced from the above ingredients, and this will help to reduce post-harvest loss and increase the economic utilization as well as consumption of the fruits. This channelled the fruits to an alternative use by converting them to table wine which will be easily available and affordable by an average Nigerian and if embarked upon will provide employment for youths and the unemployed.

ISSN: 2455-6939

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Keywords: Alcoholic Beverages, Fermentation, Shaddock fruits, Table wine, Wine Production.

1. INTRODUCTION

Fermentation is a form of anaerobic respiration during which there is incomplete breakdown of sugar extracts which leads to the fermentation of alcohol, carbon dioxide and the liberation of energy [1]. It is during such process that products such as wine, beer and other fermented products are produced.

Wine is an alcoholic beverage produced from fermentation of juices of different fruits by the action of microorganisms, especially yeast (*Saccharomyces cerevisiae*). It is among the most recognizable high value-added product from fruits. It is a complex mixture consisting of both organic and inorganic compounds [2; 1] including esters, alcohols, fixed acidity (malic, tartaric and citric acid), sugars, aldehydes, tannins, pectins, vitamins and minerals [3]. Wine making is one of the most commercially prosperous fermentation processes now. Although most commercially produced wines are usually made from fermented grapes; these are not known to grow naturally in Nigeria, this necessitates the need for alternative fruits native to the tropics [3, 5, 25, 27, & 28]. Fermentation process is not done by introducing any chemicals or sugar but by adding different species of yeast to the crushed grapes. Yeast has the capability of converting grape juice into an alcoholic beverage by breaking down the sugar content and the production of different types of wines. Tropical fruits like mango, pawpaw, banana, watermelon, pineapple, lemon, etc. had been used for wine production and the wine so produced bears the name of the fruit or fruit mixture from which it was made [4, 24, 25 & 27]. Pineapple and watermelon are among the tropical agricultural products largely grown in Nigeria. While they are abundant in Nigeria as a tropical country, several studies have been reported on their use in wine production but there is a paucity of reports of applications of yeast from shaddock fruits in wine production.

Pineapple (*Ananas comosus*) is rich in vitamins, minerals, enzymes and antioxidants. It helps to boost the immune system, build strong bones and aid indigestion [5]. Despite their sweetness pineapples are low in calories. Pineapple is grown in different parts of Nigeria either for export or for local markets. As a food crop, it has a lot of nutritional, medicinal and industrial importance. It is also fat-free, cholesterol-free and low in sodium.

Watermelon is a sweet, popular fruit of summer, usually consumed fresh in slices, diced in mixed fruit salads, or as juice. It is an excellent source of immune-supportive vitamin C and free radical-scavenging vitamin A in several carotenoid forms especially in the form of beta-carotene [6; 7]. It is also grown in the northern part of Nigeria and available in all seasons.

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Fruits, vegetables and other agricultural products are important microhabitat for several yeast species. Shaddock fruit, generally known as local grape is one of such fruits. Shaddock plant is a wild plant in southeastern Nigeria harvested mainly for its medicinal use. Umeh and Okafor [8] isolated several yeasts including *Saccharomyces cerevisiae* from the ripe fruit of Shaddock plant. These agricultural fruits (pineapple, watermelon and shaddock) are mainly lost due to post harvest spoilage. They are not easily preserved because of their high-water content and therefore need for channeling them to other useful purposes such as juice and wine making.

The role of yeast in wine making is the most important element that distinguishes wine from juice. In the absence of oxygen, yeast converts sugar into alcohol and carbon dioxide through the process of fermentation. The yeast associated with wine making is *Saccharomyces cerevisiae* which has been favoured due to its predictable and vigorous fermentation capabilities, tolerance of relatively high levels of alcohol and sulphur dioxide as well as its ability to thrive in normal wine pH between 2.8 and 4.0. *Saccharomyces cerevisiae* was first identified in the late 19th century. Enologists called them *Saccharomyces ellipsoideus* due to the elliptical shape of the cells. Throughout the 20th century, more than 700 different strains of *Saccharomyces cerevisiae* were isolated and identified [4]. However, not all strains of *Saccharomyces* are suitable for winemaking and even among the strains that are, there is debate among winemakers and scientists about the actual magnitude of differences between the various strains and their potential impact on the wine [4].

Most wines sold in Nigeria are imported and are costly. Wine is supposed to be within the reach of broader group of people, so production of wine from abundant Nigerian fruits will help to bridge this gap. This will help in saving foreign exchange, further enlarge our economy by providing employment for Nigerians and better help to prevent waste of these fruits and also give the broader population an acceptable and affordable alternative wine.

The tropical fruits used in this work were purchased, washed, peeled and pulverized. Their juices were extracted to get the “must” which was fermented using the yeast isolated from ripe Shaddock fruits. During the period of fermentation and after aging the attributes of a table wine were tested on the product. It was confirmed that a good table wine was produced. Cost of the wine produced was low as the raw materials were locally sourced. This research achieves its aim which is to produce an acceptable table wine from a mixture of two local fruits using yeast isolates from a locally available shaddock fruit.

MATERIALS AND METHODS

Sample Collection

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Ten medium sized watermelon and pineapple fruits were purchased from a local market in Awka Anambra state, Nigeria. The samples were aseptically packed in different sterile cellophane bags and transported to the laboratory of the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University Awka. Eight big freshly ripe shaddock fruits were harvested from a farm at Umuezecoha village in Awka and also taken to the laboratory. The fruits were identified by a botanist from the Department of Botany of the same institution.

Yeast Isolation and Characterization

The shaddock fruits were washed, peeled, cut and squeezed using a juice extractor. The extracted juice was sieved into a beaker and stored in a refrigerator. The method of Barnett *et al*. [9] as used by Umeh and Okafor [8] was used in the isolation of the yeast, *Saccharomyces cerevisiae*.

Yeast identification

This was done based on the description of the gross morphological appearances of fungal colonies on the Sabouraud dextrose agar (SDA) culture medium and the colonies were gram stained for microscopic evaluation with references to the manual of fungal atlas. Also, some biochemical tests were carried out to ensure that the strain of yeast isolated is *Saccharomyces cerevisiae* which is suitable for wine production [9]. The identified yeasts were enriched, inoculated in bijou slants and stored in a refrigerator maintained at 10^{o}C.

Inoculums development

The watermelon and pineapple fruits were washed thoroughly with 0.1% sodium metabisulphite in water. The fruits were peeled and sliced, blended and filtered using muslin cloth to extract the must. Two hundred milliliters each of the filtered watermelon and pineapple juices (a 50:50 ratio) were introduced into a clean sterile 500ml conical flask and autoclaved at 100^{o}C for 15 minutes to sterilize the must and then allowed to cool. Yeast cells stored in bijou bottles were inoculated into the must and incubated at room temperature in a rotary shaker for 72hours.

Must fermentation

Three hundred millilitres each of the filtered and sterilized juice of both fruits were introduced into a clean sterile 9 litre fermenter and a mixture containing 0.4g (per 400ml) sodium meta-bisulphite, 100g (per 400ml) of granulated sugar for fortification, 29.4g 0f 0.84% (per 400ml) Ammonium sulphate, 4.2g of 1.012% (per 400ml) potassium dihydrogen was added to enhance yeast growth. The juice was inoculated with yeast obtained from inoculums development and the set up allowed to ferment [10].

Wine Production Process:

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Primary fermentation

Primary fermentation of the "must" lasted for 4 days with the evolution of carbon (iv) oxide and liberation of alcohol. This occurred at room temperature (30^{o}C) after which the "must" was racked for secondary fermentation.

Secondary fermentation

One hundred grams of sugar dissolved in 1 litre of boiling water was cooled and added in the "must" for secondary fermentation to start. Secondary fermentation lasted for another 4 days. After this the wine was siphoned and the sediment discarded. The wine was allowed to stay for another 24 days completing 28 days for clarification and aging. During these processes aliquots of the sample were taken aseptically at 2 days intervals for analysis of parameters such as pH, reducing sugar, titratable acidity, specific gravity, total soluble solids and alcoholic content. Vitamins and mineral contents of the wine were also checked after clarification and aging.

Determination of pH

Ten (10) ml each of the "must" and wine were put into a clean sterile beaker, and the pH determined using a digital pH meter (Model No: pH S-25). All the samples for each analysis was performed in triplicate.

Determination of Reducing Sugar

The quantity estimation of reducing sugar of the "must" and wine were determined using the method described by AOAC [11]. One milliliter of the sample was pipette into a sterile test tube on a rack. Also1ml starch solution in 0.2M phosphate buffer of pH 6.9 was added in the tube. The mixture was incubated in a water bat set at 30^{o}C for 30 minutes. Reducing sugar was checked by adding 1ml of DNS reagent and boiling for 5minutes.The mixture was cooled under running tap water after which 12 ml of distilled water added. It was mixed and allowed to stand and stabilize for 5minutes at room temperature (28±2^{o}C). The absorbance of the solution was determined using a Jenway 6405 UV/Visual spectrophotometer set at 540nm absorbance after zeroing the spectrophotometer with a reagent blank. The concentration of reducing sugar in the sample was estimated from the standard glucose curve.

Determination of Specific Gravity

Fifty millilitre specific gravity bottle was thoroughly cleaned with distilled water, dried in an oven at 50^{0}C, allowed to cool and weighed (W_1). The dried bottle was filled with deionized water, its surface cleaned with cotton wool and weighed (W_2).

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The bottle was emptied and cleaned twice with 10ml of the sample. Thereafter the bottle was filled to the brim with the sample and its surface cleaned with cotton wool and weighed (W_3). The specific gravity (S.G) was calculated.

$$S.G = \frac{W_3 - W_1}{W_2 - W_1} = \frac{S}{W}$$

Where S=weight of volume of sample (W_3-W_1)

W=weight of volume of water (W_3-W_2)

Estimation of Titratable Acidity

This was determined by the method describe by Amerine and Ough [12]. 200ml of distilled water was introduced into a sterile 500ml conical flask and boiled. 1ml of 1% aqueous alcoholic phenolphthalein indicator solution was added. This was titrated with 0.1M NaOH solution to give a faint pin colour. 5ml of the sample was taken using a pipette and introduced into the boiling neutralized solution and titrated again to the end point using the same 0.1m NaOH solution. The titratable acidity was expressed as tartaric acid and was calculated with the formula;

$$Tataric\ Acid g/100ml = V\ x\ M\ x\ 75\ x100/100\ x\ V$$

Where V = Volume of NaOH (final reading – initial reading)

M = Molarity of NaOH

V = Volume of the Must used.

Determination of Alcoholic Content

Alcoholic content of the sample was determined using the method of AOAC [11].

Determination of the wine vitamins and minerals

The Atomic Absorption Spectrometric method of the American Public Health Association as used by Umeh *et al.* [13] was used to determine the mineral elements of the product wine. And the method of Plummer [14] also used by Umeh *et al*. [13] was used to determine the vitamin contents of the wine.

Sensory and organoleptic evaluation

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The product wine was contrasted with a store-bought, commercial mixed fruit wine. To assess the sensory and organoleptic qualities of the product wine based on taste/aroma, flavour, colour, and general acceptability, a 10-man panel of judges who are familiar with table wine's qualities were selected. They were given questionnaires to complete in which they were asked to rank the product on a 5-point Hedonic scale (excellent = 5, very good = 4, good = 3, bad = 2, and very bad = 1). The Kruskal-Wallis test was used to statistically analyze the results.

The flow chart below was used as a guide for the table wine production.

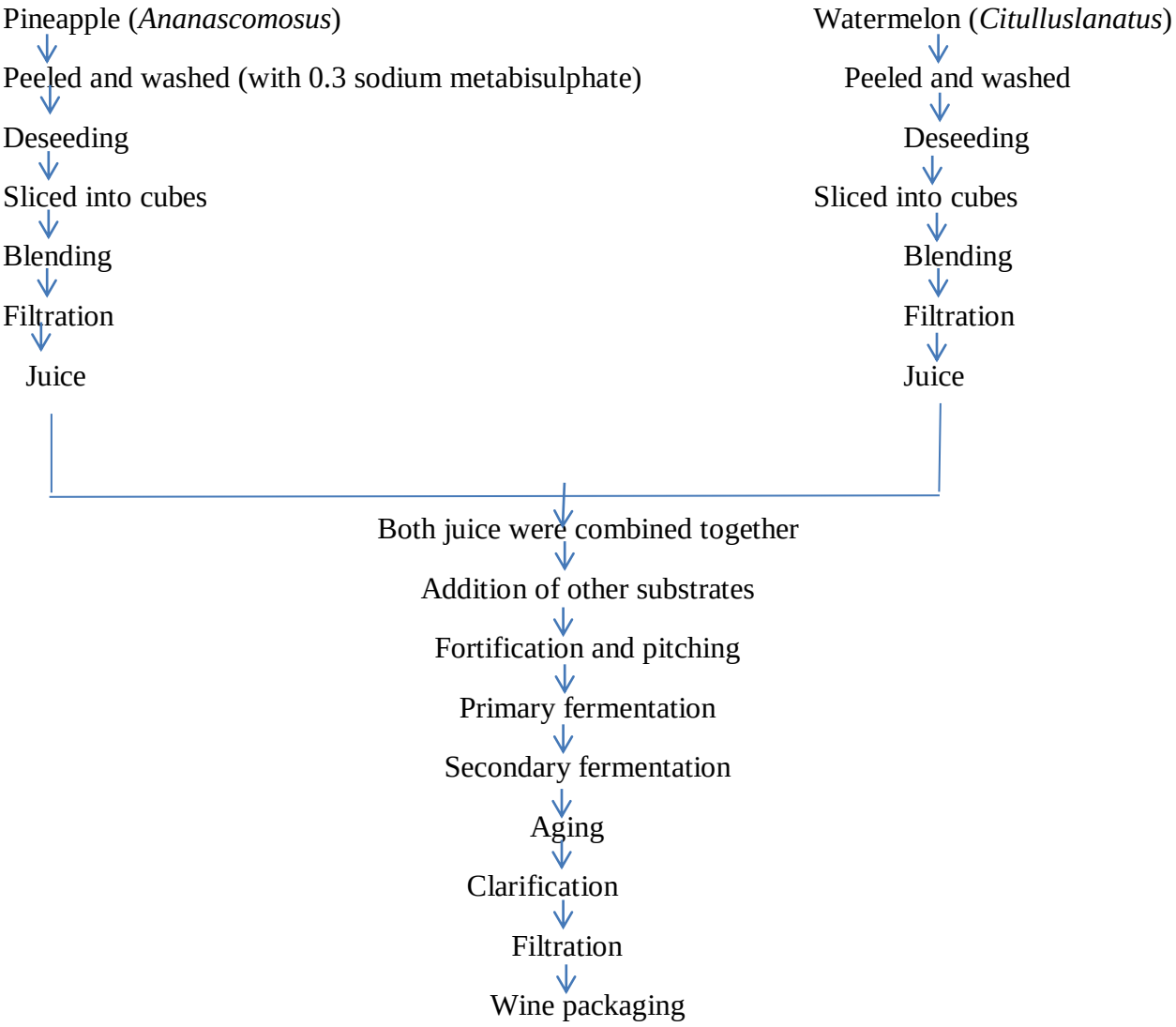

Figure 1: Flow Chart of Wine Production Using Watermelon and Pineapple.

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RESULTS

The yeast isolated from the ripe shaddock fruits and characterized revealed one of the isolates as *Saccharomyces cerevisiae.* The chosen yeast strain had the characteristics presented in Table 1. Result of analysis of the fruit "must" before fermentation is presented in Table 2. The Results of the wine analysis during fermentation of the "must" after pitching with the isolated yeast are as shown in Table 3 and Figure 2.ThepH of the fermenting "must" showed a gradual decrease as fermentation time increases and in the range of 4.42 on day 2 to3.00 on day 28. Titratable acidity increased from 3.257 to 6.780g /100mlon the 28th day while specific gravity decreases from 1.075 to 1.017. Reducing sugar decreased from 0.713 to 0.047mg/ml and alcoholic content of the fermented wine increased from 0.36to 5.10% (v/v) on the day 28. Results of the vitamins and mineral contents of the wine as well as the sensory and organoleptic qualities were as presented in Tables 4 and 5 respectively.

Table 1: Morphological and biochemical characteristics of the isolated yeast

Isolates	Colony Morphology (Macroscopic and Microscopic)	Sugar Fermentation Test						Probable organism
		Fru	Gala	Mal	Glu	Suc	Lac	
Plate 1	Creamy-white, oval shape with raised elevation, Gram-positive cells occurring in pairs with slight buds.	+	+	+	+	-	-	*Saccharomyces cerevisiae*
Plate 2	Creamy-white, oval shape with raised elevation, Gram positive cells in clusters pairs.	+	+	+	+	-	-	*Saccharomyces cerevisiae*

Key: Fru-Fructose, Gala - Galactose, Mal - Maltose, Glu- Glucose, Suc -Sucrose, Lac - lactose.

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Table 2: Analysis of the fruit "musts" before fermentation

Parameters	Pineapple Juice	Watermelon Juice	Must
pH	3.73	3.51	3.97
Specific gravity (kg/m^3)	1.034	1.017	1.024
Total soluble solids (^{0}Brix)	8.42	6.30	10.00
Alcoholic content (%v/v)	0.00	0.00	0.00

Table 3: Characteristics of the product wine during fermentation and aging

Days	Specific gravity (kg/m^3)	Reducing sugar (mg/ml)	Titratable acidity (g/100ml)	pH	Alcoholic content (%v/v)	Total soluble solids (^{0}Brix)
2	1.175	0.713	3.257	4.42	0.36	7.0
4	1.067	0.641	3.743	4.24	0.39	7.8
6	1.063	0.577	3.937	4.30	0.97	4.4
8	1.035	0.444	4.381	4.15	1.76	4.0
10	1.028	0.404	4.846	4.12	2.13	3.8
12	1.026	0.284	5.067	4.08	2.36	3.6
14	1.025	0.257	5.256	3.32	2.94	3.0
19	1.024	0.226	5.461	3.27	3.32	2.6
22	1.024	0.108	5.689	3.14	3.78	2.4
25	1.022	0.089	6.194	3.02	4.36	2.2
28	1.007	0.047	6.780	3.00	5.10	1.6

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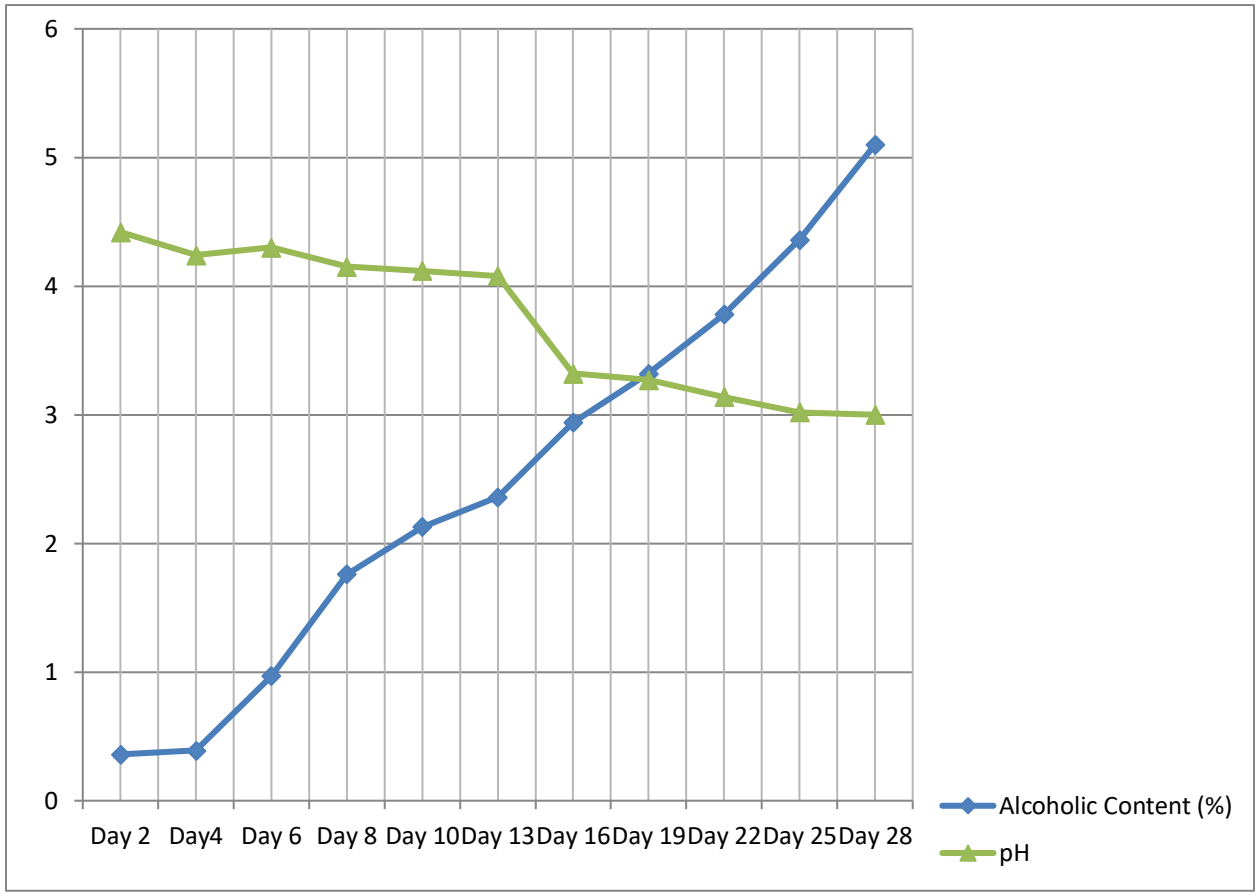

Fig 2: Increase in alcoholic content and decrease in pH as fermentation progresses.

Table 4: Vitamins and mineral present in the product wine

Parameters	Inference
Vitamin A	+
Vitamin B	-
Vitamin C	+
Thiamine	+
Riboflavin	+
Beta-carotene	+
Niacin	+
Potassium	+
Calcium	+
Iron	+

Key: + = present; - = not present

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Table 5: The sensory and organoleptic qualities of the product and commercial wine

Parameters	Product wine	Commercial wine
Colour	4.8	5.0
Flavour	4.5	4.5
Taste/aroma	4.4	4.3
General acceptability	4.9	4.9

Rating inference: 5.0 – excellent, 4.0 – very good, 3.0 – good, 2.0 – bad and 1.0 – very bad

DISCUSSION

The morphological and biochemical properties of the isolate utilized for the fermentation is shown in Table 1.Table 2 displays the findings of the analysis performed while the must was fermenting.

A vivid yellow coloured wine was produced, following fermentation with yeast obtained from mature shaddock fruits.

In Table 3 a general decrease in specific gravity, reducing sugar and total soluble solids as fermentation progressed was observed. This is in conformity with the findings reported by several researchers [16; 17; 3]. The specific gravity decreased from 1.175 to 1.007km/m^3 on the 2^{nd} and 28^{th} day respectively. In addition, the reducing sugar also decreased from 0.713 to 0.047 mg/ml on the 2^{nd} and 28^{th} day respectively while total soluble solids fall from 7.0 to 1.6 (^{o}Brix) during the same period. The decrease in total soluble solids with succeeding days could be attributed to the utilization of polysaccharides by yeast in the must. The *Saccharomyces cerevisiae* isolated from the ripe shaddock fruits had high alcoholic tolerance. It tolerated the high accumulation of alcohol in the fermenting wine and utilized the sugars present until very little sugar content remained in the wine. The alcohol tolerance as a suitable attribute for wine yeasts was also reported by some authors [18; 19; 20].

The *Saccharomyces cerevisiae* isolated had all the attributes of the wine yeast as postulated by Amerine *et al.* [15]. Studies have shown that the major problem associated with the use of tropical fruits in wine production is their low sugar content [5, 24, 25, 27]. The main justification for the additions of sugar to the "must" before fermentation was to boost its sugar level, thereby overcoming the low sugar content in tropical fruits. According to Umeh *et al.* [3], the isolated yeast fermented the sugar in the "must" from watermelon and pineapple as well as the granulated sugar used for fortification.

It was observed that the pH of the fermenting "must" rose and teetered toward acidity. Prior to fermentation, the "must" had a pH of 3.97 (Table 1). The pH dropped gradually from 4.42 to

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4.08 midway through the fermentation. The final pH was 3.00 after 28days fermentation, following clarity and aging. This lies within the acceptable acidic pH range [15] and could be attributed to the carbon dioxide produced during fermentation and dissolved in the wine as fermentation went on [10; 19]. This increase in acidity could also be the result of acetic acid bacteria, which are mostly prevalent during the fermentation of food goods, producing acids like acetic acid. Somari and Udoh [18] and Okoro [21] reported pH values of 3.7 and 4.0, respectively. The pH of the wine produced falls within the high acid range puts it clearly in the white wine classification both in colour and other important characteristics [15, 16, 17].

The titratable acidity and alcoholic content of the fermenting "must" were increasing during the period of fermentation. They increased from 3.257 to 6.780g/100ml and 0.36 to 5.10% (v/v) respectively from the 2nd and 28th day respectively. Acidity plays a vital role in determining wine quality, aiding fermentation process and enhancing the overall characteristics of the wine. Lack of acidity in the production process will mean a poor fermentation [`22]. The changes in value of the titratable acidity of the mixed wine during the fermentation period showed the occurrence of malo-lactic fermentation. This agrees with the finding of other researchers [23; 24; 25]. The wine product had a lower alcohol concentration than what the European Economic Community advised. Table wine should have an alcohol concentration of between 8.5 to 19.5%, according to their recommendations [26]. Additionally, the alcohol content obtained falls within the acceptable range for white wine with ranges from 5-14%. Still, the wine produced is suitable for the tropics, where the climate is particularly hot, due to its lower alcohol content. Due to their cold climate, Europeans who live in a temperate location may require wine with a high alcohol concentration. This characteristic designated the wine as being excellent for table use in Nigeria and other tropical countries.

CONCLUSION

The fruits used in this work (pineapple and watermelon) have been used singly in producing table wine [27, 28]. They showed good qualities singly, but the mixed wine produced from them had the most efficient characteristics, especially in its low alcoholic content. The wine also had the combined nutritional qualities of the fruits from which it is produced (Table 4) and compared favourably with purchased table wine in all the attributes tested (Table 5). The successful production of wine from the mixture of watermelon and pineapple fruit "must" using *Saccharomyces cerevisiae* isolated from ripe shaddock fruits was found to have a good quality, acceptable and healthy to potential consumers. The wine as beverage contained unique aroma, differentiating it from other juice product.

This research was successful in producing an acceptable table wine from a mixture of two local fruits using yeast isolates from locally available shaddock fruit. Production of wine using locally

available agricultural products should be encouraged in the country instead relaying on the imported wine or relying on grapes which are none-native to Nigeria. This will help to reduce post-harvest loss of the fruits, boost the economy of Nigeria, reduce cost of importation of wine and subsequently create jobs for the youths and unemployed.

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