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Fermentation of Grapes (*Vitis Vinifera*) and Orange (*Citrus Seninsis*) Wine by Using *Saccharomyces Cerevisiae* from Palm Wine

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ABSTRACT

Grapes (*Vitis vinifera*) and Orange (*Citrus sinensis*) are a fruit with good nutritional attributes but has a short shelf-life under the prevailing weather Conditions in tropical countries. Therefore, production of wine from this fruits can help increase wine Variety and reduce post-harvest losses. In this Study, We carried out the fermentation of wine from grapes (*Vitis vinifera*) and Orange (*Citrus sinensis*) using *Saccharomyces cerevisiae*. The yeast was isolated from fresh palm wine and identified using microscopic examination, morphological and characteristic. Brewer's yeast was found to be important features for wine production. Pure isolated yeast were inoculated into sterile volume of 200ml of the grape and orange juice respectively and incubated for eight days. The Wine was analysed during fermentation process For determining p^H, titrable acidity, temperature and alcohol content . The p^H values of grape and orange wine in 200ml between 6.02 – 3.43 and 5.79 -3.22. The titratable acidity obtained was between 3.270 - 6.023 and 3.025-5.665 respectively. The alcohol content of the wine increased with time. This study revealed that *Saccharomyces cerevisiae* from palm wine could be use liquor production or other industrial applications and may not present any hazards to the consumers. This will reduce the number of wastages and post yield losses that occurred in tropical fruits each year and also serve as a method of fruits preservation.

Key words: Grapes, Orange, Palm wine, *Saccharomyces cerevisiae*, Wine.

INTRODUCTION

Fruits are a important root of sugar, nutrients, micronutrients, and flavour. Also, they serve as a potential substrate for the fermentation processes that result in fruit wine. Tropic fruit is in abundance, especially citrus, which is highly perishable and difficult to store for an extended period of time, resulting in post-harvest losses of extra fruits and a loss of revenue for producers and resellers (**Baidya D., *et al.*, 2016)**. Alternative preservation food forms are required to increase the use of these fruits due to their high rate of wastage, particularly during their peak of production during their season. Newer food innovations with functional benefits for health, including fruit wines, have increased public interest in life's well-being. The Sweet drink from diverse palm trees, particularly those from the *Arecaceae,*wild date palm (*Borassus flabellifer*), and *Cocos nucifera*, is fermented to produce palm wine. A palm tree that is growing should have its sap harvested. Fresh palm juice is a sweet, clear, colourless juice that contains 10-12% sugar. The sap is a fantastic substrate for the development of microbe, and souring begins an hour or two after the sap is collected. If allowed to ferment for a longer period of time than a day, it starts to transform into vinegar and gets reasonably high in alcohol (up to 4%) as a result.

Grapes (*Vitis vinifera*) are more riched in antioxidants such as resveratrol, catechin, epicatechin, and proanthocyanins. The primary component of grape skin is resveratrol. Proanthocyanins are only found in the seeds. Resveratrol and proanthocyanin are the principal substances found in grapes and wines that are amountable for cardio protection, according to recent research findings. Because their fermentation process doesn't require any sugars, acids, enzymes, or other nutrients, grapes are often preferred for use in commercial wine production. The most popular fruit juice alcohol is probably grape wine. The most popular fruit to eat is citrus, which is also a strong source of Ascorbic acid and other supplements. Oranges are cultivated worldwide. In Asia, it is most abundantly grown in Japan, southern China and India. Only the orange is a more significant fruit crop than the mandarin. The current experiment has been carried out to investigate the viability of orange juice for the production of wine because it is typically consumed in raw form or in fruit salads in addition to juice(**Rajendra, 2009**). In the current investigation, attempts were also made to make wine from mixed cultures with the goals of examining the impact of mixed cultures on the preparation of wine from orange juice, contrasting the outcomes of the fermentation of orange juice in mixed cultures and monocultures, and enhancing the mixed culture fermentation conditions.(**Khandelwal *et al.*, 2006**).

MATERIALS AND METHODS

COLLECTION OF PALM WINE:

Fresh Palm wine was collected from Palm wine tapers within 30-60 minutes of tapping in 25ml screw capped bottles and transported to the Laboratory of Microbiology department, Kamban College of Arts and Science for Women, Tiruvannamalai for Analysis within Two hours of collection.

ISOLATION OF YEAST FROM PALM WINE:

Sabouraud Dextrose Agar (SDA) or Potato Dextrose Agar (PDA) was prepared according to manufacturer's instruction and supplemented with 50mg of Chloramphenicol or Gentamicin to inhibit Bacterial growth. Serial dilution of the palm wine was carried out and inoculated using Pour Plate Techniques.

The identified yeast colonies were further purified by subculturing on Potato Dextrose Agar (PDA) and broth until pure culture was obtained. The pure culture was stored on PDA slants and broth culture, stored at 4℃ for further use. Microscopy examination of the isolate was carried out using Wet mount and Gram staining method.

CARBOHYDRATES UTILIZATION TESTS FOR YEAST:

One (1) % each of glucose, sucrose, lactose, galactose and maltose sugar were prepared using Yeast fermentation broth and dispensed 10ml volume into clean test tube. Clean Durham's tubes were introduced into the tubes, displaced all bubbles and then autoclave at 121℃ for 15 minutes and allow cooling.

The sterile broth was inoculated with 0.2 ml yeast culture broth and incubated at 37℃ for 24-72 hours and observed acid and gas production of carbohydrate fermentation. Change in colour (pink to yellow) that indicate acid production and trapping of gas trapped in the Durham's tube indicate the positive result; The presence of gas was taken as an evidence of a reasonably high rate of fermentation activity. The results were tabulated.

PROXIMATE ANALYSIS OF THE FRUITS:

DETERMINATION OF MOISTURE CONTENT:

Five (5) g of the sample was weighed into Petri plate and placed in air draught oven at 100℃ for 1 hour. The petriplate was then weighed after cooling. The process was repeated thrice until a constant weight was obtained. Loss in weight was calculated as the percentage moisture content and this was expressed by the following formula:

$$Percentage\ of\ moisture\ content = \frac{Loss\ in\ weight\ due\ to\ dryness}{Weight\ of\ sample\ taken} \times 100$$

$$= \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where; W_1 = weight of empty crucible,

W_2 = weight of crucible + sample before drying,

W_3 = weight of crucible + sample after attaining constant weight on drying.

DETERMINATION OF ASH CONTENT:

This was carried out as where porcelain crucible with lid was ignited in a hot Bunsen burner flame and transferred into desiccator to cool and the crucible was weighed. 5 g of the sample was accurately weighed into the crucible and gently placed in the Hot air oven set at 300℃ for 5 hours. The crucible was place in desiccator to cool. The ashed sample in the crucible was weighed after cooling without the lid and the process repeated thrice for the sample. The result was calculated using the following formula:

$$Percentage\ of\ Ash\ content = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where, W_1 = Weight of empty crucible,

W_2 = Weight of crucible + sample before ashing

W_3 = Weight of crucible + sample after ashing

DETERMINATION OF CRUDE FAT:

Two (2) g of the sample was transferred into a beaker and weighed as W, 10ml of water was added, and the solid was dispersed by agitating it. 10 ml of concentrated hydrochloric acid was added and immersed in a boiling water bath until the solid particle dissolved and the mixture become brown in colour. This was allowed to cool and 10 ml of alcohol added and agitated vigorously. A dried clean flask was weighed and recorded as W_1 and the ether layer was transferred into the flask and placed in a boiling water bath to evaporate the ether. The extraction was repeated by adding 50 ml diethyl ether in order to evaporate the ether living the fat behind. The fat and the flask was weighed and recorded as W_2, and then the fat content was calculated as follows:

$$Percentage\ of\ Crude\ fat = \frac{W_2 - W_1}{W} \times 100$$

Where, W = Weight of the sample

W_1 = Weight of dried flask

W_2 = Weight of dried flask fat residue.

METHODS OF FERMENTATION:

The fruit juices were placed in 500ml of conical flasks and sterilized by boiling at 100℃ for 30 minutes. The fruit juices were allowed to cool, then 10ml of the yeast were added to each sterilized juice and incubated at 27-30℃ for 8 days. The p^H, Acidity, Temperature, Alcohol content were measured every two days.

DETERMINATION OF p^H VALVE:

The p^H was determined using a standard p^H meter. The p^H meter was standardized with buffer solution, prepared by dissolving the p^H buffer powder of p^H 4.0 at 25℃ in 250 ml distilled water. 10 ml of the juice was measured into a sterile beaker and electrode of the p^H meter was immersed into the beaker and readings were noted.

DETERMINATION OF ACIDITY:

This was determined by 1% of aqueous alcoholic phenolphthalein as indicator was added to 200ml of distilled water. It was titrated with 0.1M of NaOH. Titration was stopped when a faint but definite pink colour appeared and the titre value was noted that served as the initial titre. 5ml of the must was added to the neutralized solution. The same 0.1M NaOH was used to titrate it. The titration was stopped at the appearance of faint, but definite pink colour. The titre was taken. This served as the final titre.

DETERMINATION OF ALCOHOL CONTENT:

Take about 1ml or appropriate amount sample in a test tube. Add about 0.2 ml of 2% Potassium Dichromate solution followed by about 1ml of concentrated sulphuric acid. The yellow colour of dichromate changes to green indicates the presence of alcohol.

DETERMINATION OF TEMPERATURE:

A clean thermometer and measuring cylinder was used for temperature reading about 50ml of the sample was used. The periodic temperature change during fermentation were recorded.

MICROBIAL ANALYSIS OF THE FERMENTATED GRAPE AND ORANGE WINES:

Microbial quality of the grape and orange wines evaluated by Total coliform count, Total bacterial count, Total fungal count.

TOTAL COLIFORM COUNT:

The required amount of the MacConkey agar was prepared and sterilized at 121℃ for 20 minutes. The work benches were cleaned with 70% alcohol and the gas flame was also lighted. 1 ml of the fermented wine samples were introduced into 9ml of peptone water and homogenized. 1ml volume of the homogenised sample was inoculated into the petri plates and MacConkey agar was gently poured in to the petri plates and rocked and incubated at 37℃ for 24 hours. After incubation, colonies formed on the agar plates were counted.

TOTAL BACTERIAL COUNT:

One millilitre (1ml) of fermented wine were mixed with 9 ml of peptone water and homogenized by manual shaking. The liquid phase then forms the stock sample from which dilutions were made to obtain $10^{-1,}$ $10^{-2,}$ 10^{-3}upto 10^{-7} dilutions. After mixing each tube with the dilution, 0.1 ml of it transferred onto Nutrient agar then spread on the agar surface and immediately placed in an incubator. The plates were incubated at 37℃ over night. After incubation, colonies formed on the agar plates were counted.

TOTAL FUNGI COUNT:

The spread plate method was used. 1ml of the fermented wine sample was diluted in peptone water and homogenized. Potato dextrose agar supplemented with Chloramphenicol was used in the evaluation. The agar was prepared, poured in plates and allowed to solidify. 1ml of the test sample was transferred into the medium and spread over the surface of the agar plate with a sterile spreader until the liquid is completely absorbed into the medium and incubated aerobically in an upright position in the incubator at 25°C ±2°C for 5days. After incubation, colonies formed on the agar plates were counted.

RESULT AND DISCUSSION

The study aimed at evaluating the fermentative performance of *Saccharomyces cerevisiae* isolated from palm wine. The morphology of the yeast cells was observed on the culture plates. The colonies was observed to be flat with smooth edges, moist, creamy and glistening in appearance and were circular in shape. The identified yeast colonies were sub cultured on PDA and broth to obtain pure culture of yeast cells.

The *Saccharomyces cerevisiae* identified by Methylene blue staining techniques. The colourless viable yeast cells were observed. Dead yeast cells are stained in blue colour and budding was also observed. It was further characterized by gram's stain. The mature vegetative cells appeared purple brown . Budding cells was observed by wet mount technique . Macroscopic and Microscopic observation. The ability of yeast to ferment glucose, galactose, maltose, sucrose, and could not ferment lactose. So, confirmed for *Saccharomyces Cerevisiae*.

The proximate composition of grape and orange fruit . The proximate composition of fruits revealed that moist content values 84.24 for orange fruit and 79.12 for grape fruit. Where as, the ash content value is 3.0 for orange fruit and 2.18 for grape fruit. The crude fat values 0.285 for orange fruit and 0.521 for grape fruit. The proximate composition revealed high percentage moisture content in both the fruits. And this according to (**Okaka 2010**) accounts for their high perishable nature and their short shelf life under normal storage condition. The proximate composition of fruits in this investigation is in agreement with the general case for fruits as reported by (**Pearson 2007**).

The present study revealed low p^H values 3.43 for grape juice and 3.22 for orange juice, in the fruit wines at end of the fermentation period. Throughout the period of fermentation, p^H of the wine was within the acidic range. It indicates a gradual decrease in the p^H as the fermentation time increase, throughout the period of fermentation **(Chilaka *et al.*, 2010)**. The studies have shown that during fermentation of fruits, low p^H is inhibitory to the growth of spoilage organisms, but conducive and competitive advantage environment for the growth of desirable organisms. **(Reddy and Reddy 2009)**

The Measure of the amount of acidity in wine is known as the " Titratable acidity or Total acidity" which refers to the test that yields the total of all acids present which the strength of the acidity is measured according 1- 0 p^H. Acidity was estimated by the titration method by the use of Sodium hydroxide as titrant against the diluted fermented wine. The acidity values of grape and orange wine. The end of the fermentation period acidity value is 6.023 for grape wine and 6.030 for orange wine. As started by **Ezenwa *et al.*, 2020** these exists a correlation between p^H and acidity of the wine, higher the acidity, lower the p^H of wine. In this condition, the wine will have good shelf stability **(Ough C.S Amerine, *et al.*, 1988)**. This was supported by **Awe *et al*** who started that lack of acidity might result to the production of a poor fermentation process.

The present study revealed that yellow colour of potassium dichromate changes to green colour indicates the presence of alcohol in the fermented grapes and orange wine. The conversion of reducing sugar into ethanol and carbon dioxide is due to the activity of microbes. The increase in the alcohol content can be attributed to yeast metabolism, continuous utilization of the sugar content ethanol is then produced. They also reported that alcohol content in wine influenced by wine preparation method and type of yeast used. Alcohol contributes to taste, mouth-feel and sweetness to the wine, but at a very high level of alcohol content the taste will be suppressed.(**Ezenwa *et al.*, 2020**).

The Temperature measures with a Thermometer. The Temperature of the fruit wines throughout the period of fermentation ranged from 19 - 31℃. **(Reddy and Reddy 2009)**. The Grape wine temperature value was first day at 26℃, 20^{th} day at 29℃. The Orange Wine temperature value was first day at 28℃ and 20^{th} day at 28℃. The rises in Temperature recorded may be due the catabolic processes of sugars by yeast cells resulting in metabolic heat that ultimately increases the temperature **(Ukwuru and Awah 2013).**

The microbial analysis of the orange and grape wine revealed that there was no microbial contamination of the wine. This implies that wine was produced under aseptic condition and it is safe for human consumption. The Heat treatment was sufficient to destroy microbial load in the wine.(**Carter *et al.*, 2007**) reported that many products that could safely be maintained sterile by a heating process alone could be doubly preserved by the addition of potassium metabisulphates. The sulphites inhibit Coliform, Bacteria and Fungi (**Doughari and Elmahmood 2007**).

In the world of medicine and health, wine has long history of use. Wine has been part of social, religious and cultural events for centuries. Wine has a long record of use as an early form of medicine, being suggested variously as a secure substitute for drinking water, an antiseptic for treating wounds and a digestive aid, as well as a cure for a wide range of illness from lethargy and diarrhoea to easing the pain of childbirth.

CHART 1: DETERMINATION OF P^H OF GRAPE WINE AND ORANGE WINE

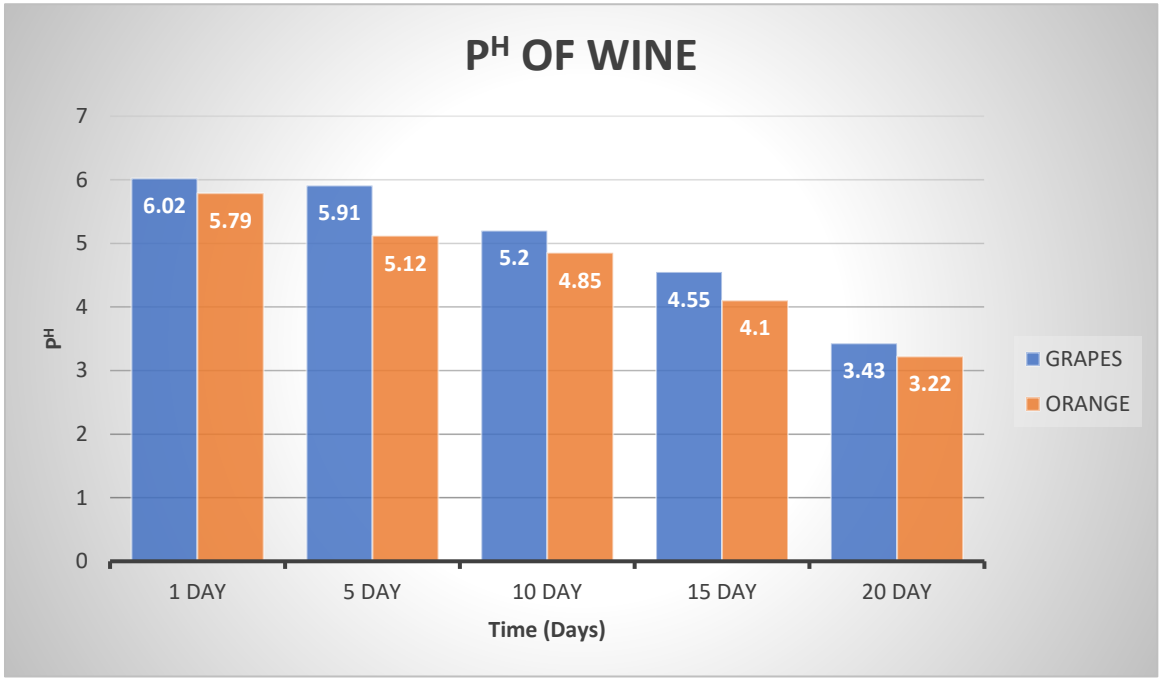


CHART 2 : DETERMINATION OF ACIDITY OF GRAPE WINE AND ORANGE WINE

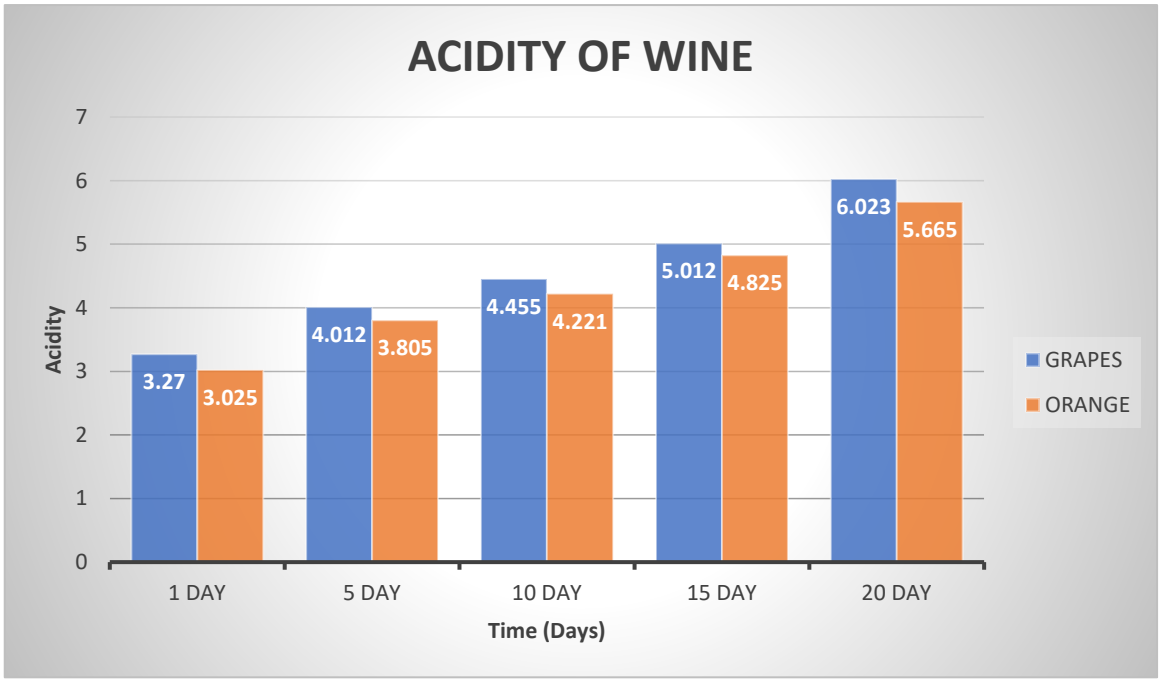
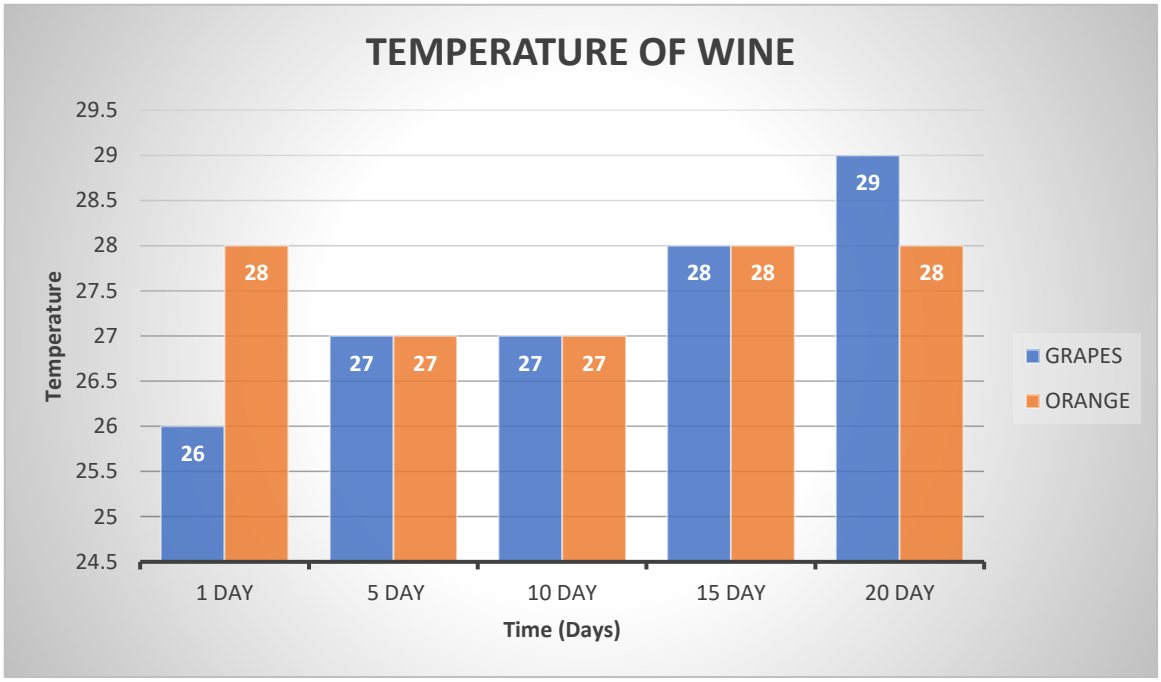


CHART 3: DETERMINATION OF TEMPERATURE OF GRAPE WINE AND ORANGE WINE



CONCLUSION

The Result of this work has shown that wine making from grape and orange juice by using *Saccharomyces cerevisiae*. For microbial investigation used some microscopic techniques for identification of yeast. Biochemical analysis includes test for p^H , Temperature, Total acidity, Ethanol content etc. It was concluded that the p^H of sample was decreased where as the titrable acidity of sample was increased during fermentation process. Temperature control during fermentation is crucial to the preservation of delicate fruit flavours in the resulting wine. Wine was prepared after 21 days of fermentation. It is possible to produce wines from locally available fruit with good microbiology standard and high acceptability.

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