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Fermentation of wine from different fruit juices and Its Quality analysis

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Abstract

Vitamins, minerals, and fiber-rich fruits are good sources for the production of wine. Grapes are the most commonly utilized resource for making wine across the globe. The objective of this study was to determine total acidity, volatile acidity, and alcohol content using the acid dichromate method. For the winemaking process, S. *ellipsoideus* was used. Around nine different fruits including some locally available underutilized fruits have been utilized. During fermentation, aliquot samples were taken for analysis of pH, aroma, acidity, alcohol estimation, etc. using standard procedure. During the period of fermentation, the temperature and pH of the fruits must be 28° -32° C and 3-4 respectively. Throughout the fermenting process, there was a constant increase in the amount of alcohol with time, but the multiplication of acetic acid-producing bacteria in a few samples altered the taste and quality of the wine. The color change in the chromic acid solution showed contamination in certain wine samples. After 21 days of fermentation, the alcohol percentage in the fruit wines ranged from 9-12 percent whereas it was below 5 percent in contaminated samples. The greatest concentration of alcohol was discovered in the bilimbi sample, the cashew apple samples (11-11.8%) while the least with green grapes, apple, and carambola which was having a higher rate of acidity and were sour.

Wine is considered the most ancient fermented, traditional, and effective alcoholic beverage on the planet and the process of fermentation is aged food preservation practice[20,9]. Fermented beverages and foods contain a wide range of environmental microbes, including molds with mycelia, yeasts, and bacteria, primarily LAB (lactic acid bacteria), micrococci, bacilli and they induce slow decomposition of organic substances. During the process of fermentation, microorganisms turn the chemical constituents of raw materials into nutrition-rich products; which improves flavor and texture; thereby preserve perishable foods; add vital amino acids, health-promoting bioactive compounds,

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vitamins, and minerals to the product; destroy unwanted substances and antinutritional factors; offer antioxidant capabilities, they also enhance probiotic properties[4].

In the Neolithic period, between 8500 and 4000 BCE, the process of making and storing wine was invented. Today, wine is consumed all over the world[8]. According to "The World Atlas of Wine" there are two worlds: traditional wine-producing countries around the Mediterranean area as the "old world" and countries producing wine after the European colonial expansion as the "new world"[14]. For the past few decades, grapes have been the primary raw material utilized in wine production. However, many research groups have looked into the suitability of fruits other than grapes for winemaking, such as apricots, apples, and palm sap[24]. Underutilized fruit trees were expected to have an essential role in reducing hunger and poverty in developing and underdeveloped countries. Because of ignorance, knowledge, availability, and difficulty in harvesting and preserving these fruit trees, they may be ignored[23].

There are different types of food fermentation which include alcoholic beverages fermented by yeasts, vinegar fermentation, pickling fish fermentation with lactobacilli, and so on[2].

Fermentation extends the shelf life of food while requiring less energy than refrigeration or other forms of food preservation. As a result, it is an excellent technique for usage in underdeveloped countries and rural places where sophisticated equipment is scarce[5].

Fresh and processed fruits are a great source of different vital nutrients such as minerals, carbohydrates, vitamins, etc. As India is one of the world's leading fruit producers, the post-harvest loss of fresh fruits is a major problem. The fermentation technique can be used to solve this issue and it is suitable for developing new products with altered sensory and physicochemical properties[22].

Sample collection/preparation :

Winemaking starts with the selection of substrate/fruits. For wine preparation, we chose 2 kg each of orange (*Citrus sinensis*), red grapes, green grapes (*Vitis vinifera*), pineapple(*Ananas comosus*), apple (*Malus*), and indigenous fruits such as carambola (*Averrhoa carambola*), rose apple (*Syzygium jambos*), cashew fruit (*Anacardium occidentale*), and bilimbi (*Averrhoa bilimbi*). These samples were collected from nearby fruit stalls and local markets respectively. These samples were transported safely and aseptically in sterile sample bags to the Microbiology laboratory, Sree Ayyappa College, and were stored at refrigerator temperature before the process of fermentation.

Starter culture preparation :

S. ellipsoideus from the laboratory culture collection were cultured and validated in potato dextrose broth (PDB) in a 1 L conical flask at a suitable temperature for about 24 hours accompanied by suitable biochemical assays. The verified isolates were primed in respective fruit juice for 4 hours, standardized (cell concentration of 10^6 CFU/mL), and stored at 4ºC before inoculation[3]. During the primary fermentation, the sugar components present in the fruit degrade to form alcohol and carbon dioxide.

The reaction takes place as follows:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2\ CO_2$$

Preparation of the substrate :

a) Selection process :

The quality of the fruit determines the quality of wine therefore mature and undamaged fruits were selected for the fermentation process.

b) Sterilization :

All the selected fruits are then washed with distilled water and then blanched for 30 seconds to avoid any chance of contamination. Pat dried the fruits with a sterile towel and spread them on a baking sheet to get completely dried, also maintained the sterile condition during the process.

Wine preparation :

Fruit samples were collected

⇓ **Selected fresh samples**

Sterilized and blanched

⇓

500 ml of pasteurized fruit juice (must) taken in a 1 liter sterilized flask

⇓ **Add 20 g of sucrose (2 days of an interval) and 50 ml of starter culture**

For anaerobic conditions, used sterile cotton to close the flask

⇓ **Filtered and incubated for 21 days at 25ºC**

Samples were taken after 7 days of interval for analysis

Total acidity and volatile acidity :

Previous studies show that a wine's total acidity is determined by the presence of non-volatile or fixed acids like malic and tartaric, as well as acids formed from the steam volatilization method. Carbon dioxide in the form of carbonic acid, sulfur dioxide as sulfurous acid, and lactic, formic, butyric, and propionic acids can all add considerably to the volatile acid content. The optimal overall acidity of finished wine, defined as tartaric acid, is between 0.6 and 0.8 percent. Because some acidity is lost throughout the process, fresh juice should be 0.1 percent to 0.3 percent higher[21,26].

The volatile acidity is a measurement

of the low molecular weight (or steam distillable) fatty acid in wine, which gives a scent of vinegar to the product. More than 93 percent of the distillable acids in wine are acetic acid and generally, winemakers are more concerned. Acetic acid bacteria use ethanol for the production of acetic acid by oxidizing the alcohol. Volatile acidity is referred to as a sign of wine deterioration[7,26].

To determine the total acidity, pour 500ml of the pasteurized fruit juice (must) into a 1 L sterilized flask and 20 g of sucrose was added into each must.50 ml of *S.ellipsoidens* fruit juice broth culture (10% starter culture) were added into the flask and closed the lid with a sterilized stopper containing cotton plugged air vent for producing anaerobic condition to the yeast. Incubated the inoculated fermented wine flask at 25ºC for 21 days .20g of sucrose was added after 2 days of incubation and filtered. Samples from fermenting wine were taken after 7 days of an interval to test the aroma. To determine the total acidity and volatile acidity 10 ml of wine were taken from each fruit sample to 100 ml conical flask and added 10 ml of sterile water into it.

The titration method was carried out by adding 5 drops of phenolphthalein indicator to the solution and titrating against 0.1 N NaOH and calculating the acidity and volatility[6].

Using the following equations, Total acidity and volatile acidity can be determined

$$\text{Total Acidity} = \frac{(\text{Volume of alkali used (ml)} \times \text{Normality of alkali} \times 7.5)}{(\text{Weight of the sample in g})}$$

$$\text{Volatile Acidity} = \frac{(\text{Volume of alkali used (ml)} \times \text{Normality of alkali} \times 6.0)}{(\text{Weight of the sample in g})}$$

Ethanol estimation by the chromic acid method :

As in the fermentation of wine, beer, and fuel ethanol, the overall concentration of alcohol and reducing sugar are the two important factors.[11,25] Chemical methods for determining ethanol rely on colorimetric changes caused by chemicals like potassium dichromate reacting with ethanol.[19] The majority of chemical oxidation techniques are based on the full oxidation of ethanol by dichromate in the presence of sulfuric acid, resulting in the production of acetic acid. Because potassium dichromate is commonly available in high purity and the solution is indefinitely stable in air, this reaction is highly recommended.[13] The chromic acid technique was used to determine the amount of alcohol in the wine samples in two distinct methods.

Method 1:

By oxidizing ethanol to ethanoic acid with an excess of acidified potassium dichromate, the ethanol content of a solution of ethanol in water can be determined.[12] By adding potassium iodide solution and freeing the resultant iodine with a standard solution of sodium thiosulfate, the quantity of untreated dichromate may be measured.

$$2Cr2O_7^{2-} + 3C_2H_5OH + 16H+ \longrightarrow 4Cr^3 + + 3CH_3COOH + 11H_2O$$

$$2Cr_2O_4^{2-} + 14H + + 6I \longrightarrow 2Cr^3 + + 3I_2 + 7H_2O$$

$$2S_2O_3^{2-} + I2 \longrightarrow S_4O_6 + 2I$$

The wine may contain other oxidizable substances but they are less volatile than ethanol.

Preparation of potassium dichromate solution :

The preparation of the solution is initiated by adding 70 ml of Conc. $H_2 SO_4$ to 125 ml of water in a 250 ml conical flask (carefully with constant stirring) and cooled by placing it below the tap water. Then add 0.75 g of potassium dichromate and makeup to 250 ml using sterile water.

Preparation of Sodium thiosulfate solution:

7.44 g of $Na_2SO_3.5H_2O$ is dissolved in 1L of distilled water.

Starch indicator solution :

1 g soluble starch, dissolved in 100 mL freshly heated water, stirred until thoroughly dissolved.

Preparation of Potassium Iodide solution:

In 25 mL of distilled water, 5g potassium iodide is added.

Procedure :

Diluted the wine samples to 1:50 (20 ml in 1000ml) and then transferred a volume of 10 mL acid dichromate solution in a conical flask of 250 mL capacity and closed with a suitable rubber stopper.1 mL of diluted sample was pipetted to a glass vial or 5 ml beaker. As shown in **Figure 1**, suspend the vial over the dichromate solution and secure it with a rubber stopper. The flask was stored at 25-30ºC overnight. On the next day, the flask was allowed to come to room temperature and then the stopper was loosened carefully to remove the sample holder. After rinsing the flask's walls, 100 mL sterile water and 1 mL potassium iodide solution were added and well mixed.[12]

The titrimetry procedure is used; it is one of the ancient analytical techniques, and it is often used in practice.[18] For each sample, three blank titrations were made by adding 10 mL of acid dichromate solution to a conical flask.100 mL of water and 1 mL of potassium iodide solution were added to it and mixed well. Every flask was titrated using sodium thiosulfate, and when the brown iodide color faded to yellow, 1 mL starch solution was added, and the titration continued until the blue hue disappeared. Repeated the process until concordant values are obtained and then did the same procedure for the wine samples.

The color of the wine samples turned from respective wine color to **orange to blue-green** as the dichromate (VI) ion, $Cr_2O_7^{2-}$, is reduced to the chromium (III) ion, Cr^{3+}.

Method 2 :

Reduced chromic acid determines the color of dichromate, which is often yellowish. The absorption spectra of dichromate and chromite ions are quite similar. To compute the individual concentration of dichromate and chromic ions in a mixture, Beer's law is not followed; instead, the spectra of the solution of interest must be studied at several wavelengths. Given the material balance that the total number of chromium atoms must be conserved, a conserved appropriate concentration of H_2SO_4 in the surrounding solution will lead to ethanol oxidation to acetic acid rather than aldehyde.

Preparation of the 34% chromic acid reagent :

Add 3.25 ml of concentrated H_2SO_4 to 3 ml of $K_2Cr_2O_7$ along the side of the flask; make up the volume by adding 675 ml of distilled water.

Alcohol standard :

Alcohol standard of 0.1-2% is used.

Procedure :

To 100ml of each concentration, standard, and wine samples 5 mL of the chromic acid solution is added. Mix thoroughly and keep each tube in a boiling water bath for 15 minutes. The tubes were cooled and measured the OD value was at 600nm. A standard graph was prepared using the OD value obtained for alcohol standards.

pH analysis :

pH analysis has been conducted at different intervals like 7, 14, and 21 days of fermentation. The pH of the solution at various fermentation stages changed depending on the day of production and the substrate is chosen[16]. Results are shown in **Table 1**. It usually increased and decreased towards the final stage of fermentation while in some samples pH value was high at the initial stages, then dropped and showed an increase in the value.

Table-1. Analysis of pH at a different interval

Fruit sample	Day 7	Day 14	Day 21
Standard	3.8	3.5	3.1
Grapes(red)	3.8	3.6	4
Grapes(green)	3.5	3.15	2.5
Pineapple	3.7	3.4	3.2
Apple	4	3.7	2.9
Carambola	3.4	3.2	2.8
Rose apple	4.2	3.9	3.4
Cashew apple	3.6	3.8	3
Bilimbi	3.3	3.6	3.2
Orange	4.1	3.5	3.9

In the case of green grapes, apples, and carambola initial pH was in the range of 3.4-4 and then it dropped at the final stage. Among these 9 fruits, pineapple wine was having appropriate alcohol and acid content which impart good taste as well as better texture as in **figure 2**.

Test for Acidity in wine :

During the fermentation of fruit juice in the presence of yeast and bacteria, important organic acids such as succinic, tartaric, pyruvic, lactic, and acetic acid are formed[10]. A similar study[15] found that when the acidity of sour-sop juice rose, the pH decreased correspondingly. Acidity has been found to have an important part in establishing wine quality by assisting the fermentation process and increasing the wine's overall qualities and balance[1].

As was reported in previous studies by Kuriakose et.al, during the fermentation phases, different fruits showed a various range of acid content. The acidity of 9 wine samples ranged from 0.3-to 0.8%. In the present study, total acidity was found high on bilimbi (0.72)

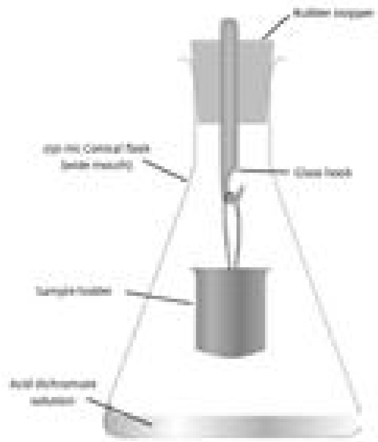

Figure 1. Diagrammatic representation of dichromate method 1

Figure 2: pineapple wine

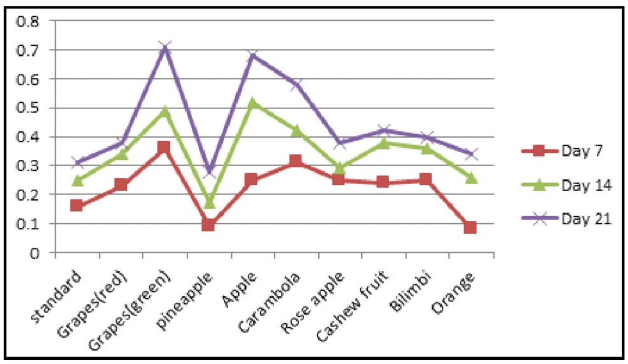

Figure 3. Volatile Acidity (VA) obtained from different wine samples during three stages of the fermentation process

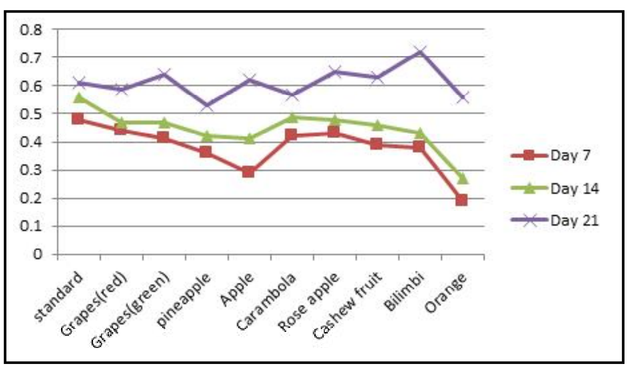

Figure 4. Total acidity (TA) obtained from ten wine samples during different fermentation stages

(a) (b)

Figure 5: Analysis of clear and cloudy wine as an indication of spoilage
(a) wine from bilimbi sample (b) spoiled wine from apple

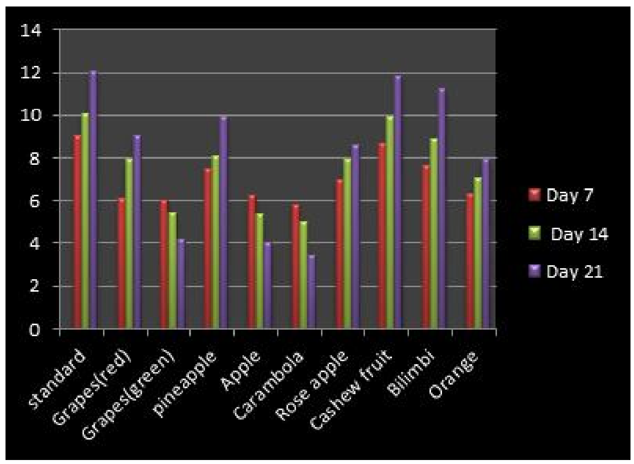

Figure 6. Analysis of alcohol content using the dichromate method

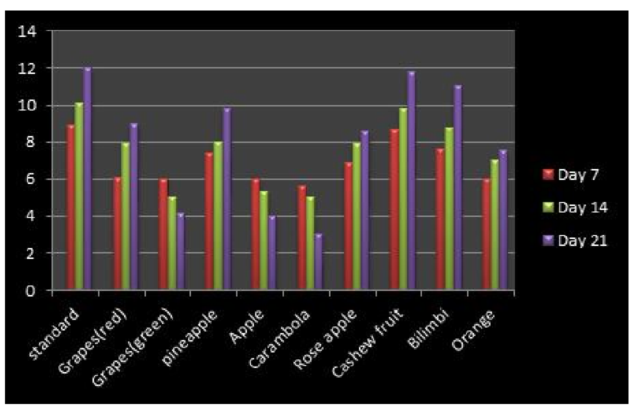

Figure 7. Analysis of alcohol content using the dichromate method

and least on pineapple (0.53) on 21 days of fermentation shown in figure 3, whereas green grape, carambola, and apple samples showed high volatile acidity when compared to the standard after 21 days of fermentation which is evident from figure 4. Volatile acidity is considered a sign of spoilage by microbial contamination. Generally, clear wine is obtained after the filtration process, but the green grape, carambola, and apple wine were cloudy due to the microbial growth and it tasted much more tart and sour than the other wines figure 5.

Alcohol estimation using the dichromate method :

The potassium dichromate method is used to determine the amount of ethanol in the fermented product. Acidity and the amount of ethanol in a wine are the two parameters that give a strong influence on the overall quality and flavor. Samples having high acid content generally exhibit low alcohol content. Alcohol adds to the wine's flavor, mouthfeel, and sweetness, but at high levels of alcohol, the taste is repressed, resulting in a burning sensation in the nostrils as well as a bitter taste[17]. The wine produced from green grape, carambola, and apple had low ethanol content, as they are having a high acidity which ranged between 0.6-0.7 percent.

Two methods have been used to determine the ethanol content in 9 different fruits, method one is the hanging method where the sample is suspended/hanged in the flask, and the other one is by measuring the OD. By using the hanging method color change during titration could be used to determine the quality of the wine. Yellow acid dichromate solution and iodine fade from brown to pale yellow after titration. Due to the production of a starch-iodine combination, which shows the presence of alcohol, the solution takes on a blue-black hue (which fades later on titration). The results showed that the sample with high acidity tends to have low alcohol content. This was backed up by Awe *et al.*,[1], who claimed that a lack of acidity might lead to a poor fermentation process.

Figures 6 and 7 depict that maximum alcohol content was obtained in cashew apple wine with 11.8% and low ethanol content was observed in the green grape at 4.2%, carambola at 3.4%, and apple at 4%.

While in other samples the alcohol concentration of fruit wine increased as it matured. During primary fermentation, the alcohol production was at its peak; however, during secondary fermentation, the rate of alcohol development was slow.

The study demonstrates the various parameters such as color, odor, and flavor influencing the quality of wine samples. The result of this research revealed that the bilimbi sample and the cashew apple sample were having high alcohol content whereas green grapes, apple, and carambola having high acid content. The amount of acid and alcohol determines the shelf life of finished products. There exists a correlation between acid and alcohol content, higher the acidity lowers the pH of the wine sample. However, more research is needed to determine how long the wines may be stored.

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