

# Wine production using high nutritious value fruits as substrates

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## Abstract

**Aim:** The study was aimed to understand the correlation between the production of ethanol with carbohydrate utilization using various combinations of high nutritious substrates such as West Indian cherry, strawberry, grape, amla, and honey. **Materials and Methods:** High nutritious tropical fruits were selected as substrates and were taken in different combinations based on available sugar content. Individual combinations were allowed to ferment in a laboratory scale fermenter in presence of *Saccharomyces cerevisiae* (aerobic fermentation followed by anaerobic phase). On completion of fermentation, the wine samples were filtered, pasteurized and were analyzed for ethanol content using High-Performance Liquid Chromatography. Further, the antibacterial activity of the produced wine samples was assayed against human pathogen *Escherichia coli*. **Results and Discussion:** Among the tested combinations, Combination 1 (grape, West Indian cherry, and honey) was found to produce 16% of ethanol which is appropriate for wine production at industrial scale. The study depicts that production of ethanol is directly proportional to the rate of fermentation, similarly, the combination one was very effective in producing ethanol. In contrast, the other combinations were also found to produce significant concentrations of ethanol and showed antibacterial activity. **Conclusion:** We conclude that the combination of fruits like grape and West Indian cherry supplemented with honey can be employed in developing a high nutritious and healthy beverage.

**Key words:** Antibacterial activity, ethanol, High-Performance Liquid Chromatography (HPLC), tropical fruits, wine

## INTRODUCTION

Tropical fruits as a substrate for wine production have remained significant since ancient days (Muccillo *et al.*, 2013).<sup>[1]</sup> The beverages play a key role as a supplement in the human diet and also found to lower the mortality rate in people suffering from coronary heart diseases.<sup>[2]</sup> Grapes are one of the largely cultivated fruit crops from which huge proportions are used for wine production every year (International Organization of Vine and Wine, 2012, Toscano *et al.*, 2013).<sup>[3]</sup> For the production of wine, the European species of grapes *Vitis vinifera* has been widely used across the world.<sup>[4]</sup> They contain several compounds which harbor numerous traits such as antibacterial, and anticancerous activity, an especially phenolic compound like resveratrol in grapes has proven to show effective antimicrobial activity.<sup>[5]</sup>

Wine can be produced from a variety of substrates (fruits) such as strawberry, cherries, apple, and pomegranate and the produced wine is popularly known as fruit wine. Among all the fruit wines strawberry have a unique desirable flavor

which makes it one of the most popular summer fruit. Sugar and acids provide sweetness and tartness, whereas volatiles compounds give the fruity characteristic aroma (Sun *et al.*, 2014).<sup>[6]</sup> Strawberry wine is rich in anthocyanin, and the major anthocyanins includes cyanidin and pelargonidin glucosides.<sup>[7]</sup>

Amla (*Emblica officinalis*) is another euphorbiaceous plant which has vitamin C at higher concentrations. It is reported to have antioxidant activity, hypoglycemic, and hypolipidemic activity (Anila and Vijayalakshmi, 2000, Liu *et al.*, 2008).<sup>[8,9]</sup> Amla has a characteristic sour taste it provides the bitterness to the wine. Honey is often added to wine as a supplement, and it contains sugar such as fructose, sucrose and carbohydrates, and minerals. All these compounds suppress the growth of bacteria and provide stability to the honey. Honey has a

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healing property and can reduce inflammation it can stimulate angiogenesis and avoid various infections.<sup>[10]</sup> Natural unheated honey has a broad spectrum antibacterial activity.<sup>[11]</sup>

In this study, we have used various tropical fruits in various combinations for the production of nutrient-rich wine. The substrates used in this study are amla, grapes, West Indian cherries, strawberries, and honey. Further, the utilization of available sugar to the production of ethanol was correlated. The produced wine was also tested for their antibacterial activity against human pathogens like *Escherichia coli*.

## MATERIALS AND METHODS

### Collection of Substrates

The freshly harvested fruits such as strawberries (*Fragaria ananassa*), amla (*E. officinalis*), and grapes (*V. vinifera*) were procured from nearby fruit market. The unprocessed honey and West Indian cherries (*Malpighia emarginata*) were acquired from the field station of Tamil Nadu Agricultural University, Thadiyankudisai, Dindigul, Tamil Nadu, India. The substrates except honey were surface sterilized using 70% ethanol followed by repeated washing with sterile deionized water and were allowed to air dry.

### Culture Conditions

Commercially available lyophilized *Saccharomyces cerevisiae* (baker's yeast) was procured. 1 g of lyophilized powder was added in 100 ml of autoclaved distilled water containing 2% of glucose. The mixture was incubated at 35°C in a temperature controlled orbital shaker operated at 120 rpm for 4 h to activate the yeast cells. Thus, obtained metabolically active cells were used as an inoculum.

### Combination of Substrates

The substrates were initially sorted and taken in various combinations based on their nutrition and sugar levels. The Combination 1 included grapes, West Indian cherries, and honey (2:1:1), Combination 2 included West Indian cherries, amla, and honey (2:1:1), Combination 3 included West Indian cherries, grapes, amla, and honey (1:1:1:1), Combination 4 included strawberries, amla, and honey (2:1:1), Combination 5 and 6 containing grapes alone served as positive and negative control.

### Detection of Sugar Concentration

The substrates were taken in various combinations and manually crushed and pressed to extract the sugar and other organic acids which were followed by homogenization. The available concentration of the sugar was determined using Brix meter. The sugar concentrations were recorded before and after the fermentation to monitor the utilization rate.

### Fermentation Process

The batch fermentation process was opted for the production of wine, where 400 g of the homogenized substrate was taken in a sterile Erlenmeyer flask which acted as a fermenter to carry out the fermentation process. 2% of the metabolically active yeast culture was added to all the combinations except Combination 6 to study the effect of indigenous bacteria on fermentation.

### Aerobic Fermentation

On addition of the inoculum, the mouth of the conical flask was sealed with cotton plug allowing the free passage of the air. The conical flasks were allowed to incubate at 25°C in a temperature controlled incubator in a static condition for 3 days to carry out the aerobic phase of fermentation.

### Anaerobic Fermentation

After the aerobic fermentation, the cotton plugs of the conical flasks were replaced with center holed rubber stoppers to restrict the entry of air thus creating the anaerobic condition. One end of a hollow tube was fixed into the rubber stopper and another end into a beaker containing sterile distilled water to facilitate the escape of carbon dioxide produced as a by-product during the fermentation process. The anaerobic fermentation was carried out for 12 days at 25°C in a temperature controlled orbital shaker operated at static condition. All the fermentation processes were conducted in triplicates, and the mean value was taken for statistical analysis.

### Clarification Process

On completion of the fermentation process, the slurry was filtered using sterile Whatman filter paper aseptically. The filtrate was collected in screw capped tubes and stored at 4°C until further processing.

### Pasteurization

To inactivate and arrest the further fermentation by yeast or other lactic acid producing bacteria, the samples were pasteurized with slight modification as given by Marangon *et al.*, 2012.<sup>[12]</sup> The screw-capped tubes containing wine samples were exposed to 72°C in hot water bath for 15 s. The process was repeated thrice to achieve the maximum result.

### Detection of Ethanol Using High-Performance Liquid Chromatography (HPLC)

The samples were subjected to HPLC analysis using C-18 reverse phase column for the detection of ethanol, where commercially available 99.9% ethanol served as a standard

reference.<sup>[13]</sup> The conditions used were: Flow rate: 1.2 ml/min; detector: Ultraviolet 220 nm; mobile phase: acetonitrile and water (4:1 ratio) and sample injection volume: 20 µl. Further, the quantitative analysis of the ethanol was performed by peak height and peak area measurements. The formula for calculating the concentration is as follows:

$$\% = \frac{\text{Area under peak}}{\text{Sum of the total area of the peaks}} \times 100$$

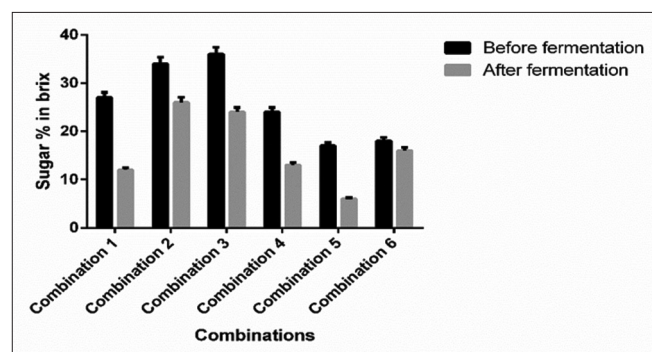
## Antibacterial Activity

The effective samples were analyzed for their antibacterial activity against known human pathogen *E. coli* using well diffusion method, where streptomycin and distilled water served as positive and negative control, respectively.<sup>[14]</sup>

## RESULTS

### Utilization of Available Sugar

Figure 1 depicts that the supplemented yeast culture was metabolically active and have utilized the available sugar in all the tested combinations. However, the major variance in the sugar level was observed in combination 1 where 15 brix sugar was fermented within 12 days of the fermentation period. Although other combinations such as 2, 3, 4, and 5 showed a considerable amount of utilization. In contrast, the



**Figure 1:** The utilization of available sugar before and after fermentation

Combination 6 without yeast showed a negligible utilization of sugar (2 brix), which adds to the importance of the yeast cells in the fermentation process. Preliminary observations like aroma, color indicated the presence of ethanol and different flavoring compounds.

### Detection of Ethanol Using HPLC

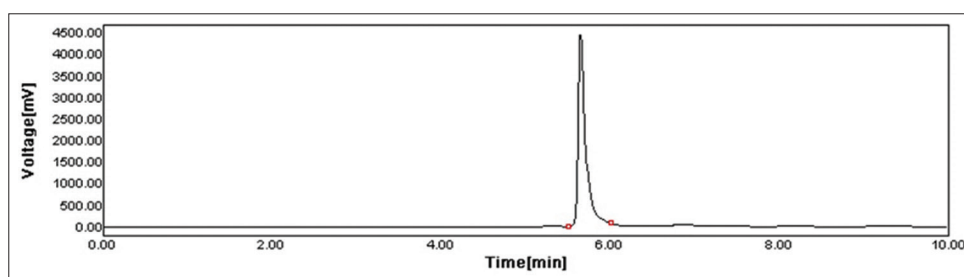
The ethanol standard on injection gave a single sharp peak at a retention time of 6 min as shown in Figure 2. The fermented samples from all the fermenters (Combination 1, 2, 3, 4, and 5) on injecting into HPLC operating in similar conditions showed a sharp peak at retention time 6 min as shown in Figure 3a-e were identical to the standard peak. Thus, confirming the production of ethanol in all the combinations. Further, the quantification of ethanol revealed that Combination 1 was an effective combination with 16% ethanol production, whereas Combinations 3 and 4 were found to produce 13% of ethanol. The Combination 5 (only grapes) showed 14% of ethanol production as shown in Figure 4.

### Antibacterial Assay

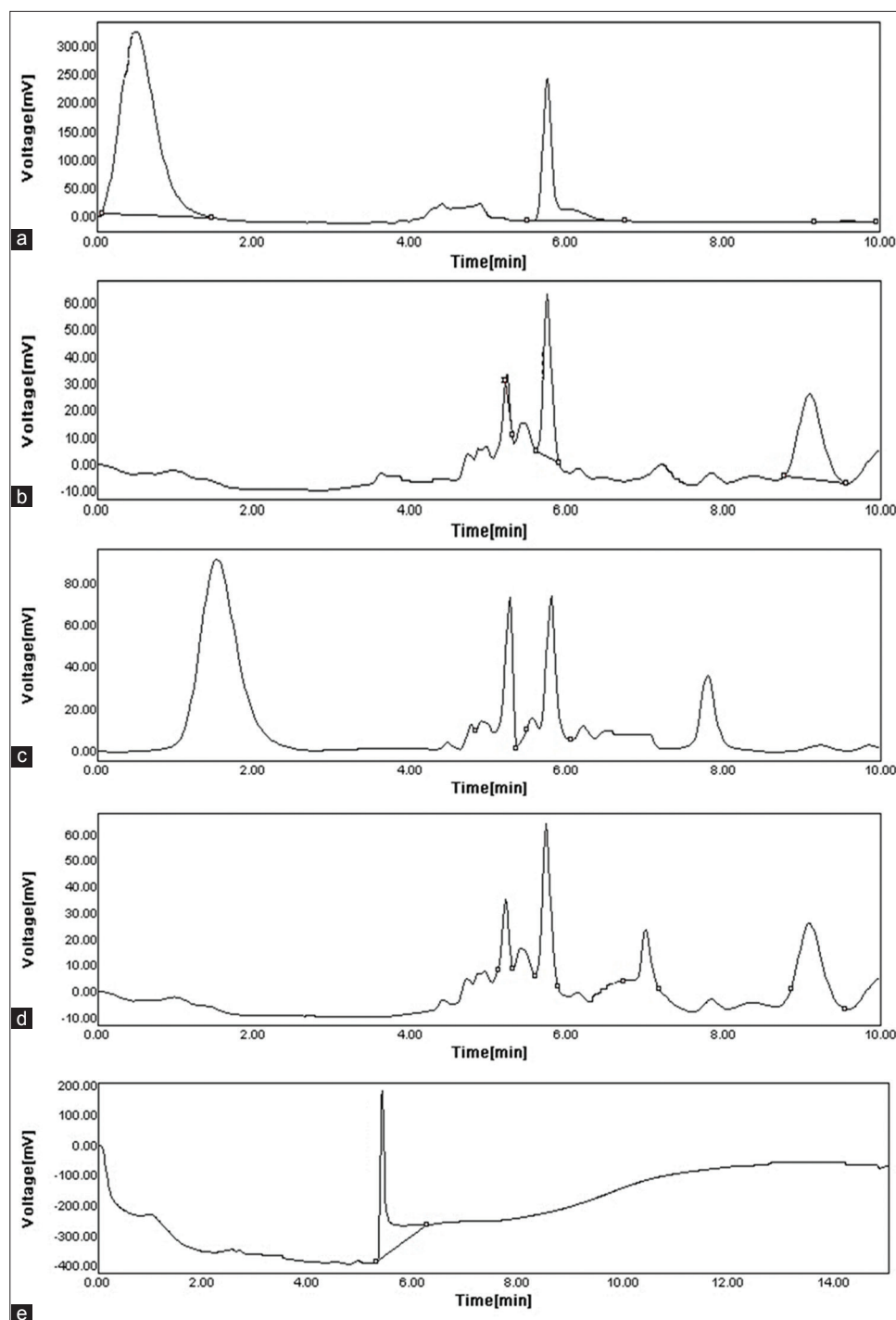
Among all the tested samples, the Combination 1 was found to have high antibacterial activity showing a 0.4 mm zone of inhibition as shown in Table 1. The positive control streptomycin (antibiotic) showed a zone of inhibition around 1.1 cm whereas the negative control failed to restrict the growth of *E. coli*.

**Table 1:** The zone of inhibition of wine samples from various combinations (in mm)

Sample	Zone of inhibition (in mm)
Positive control	1.1 cm
Sterile distilled water	0.0 mm
Combination 1	0.4 mm
Combination 2	0.1 mm
Combination 3	0.2 mm
Combination 4	0.1 mm
Combination 5	0.3 mm



**Figure 2:** Chromatogram showing the standard peak of ethanol standard



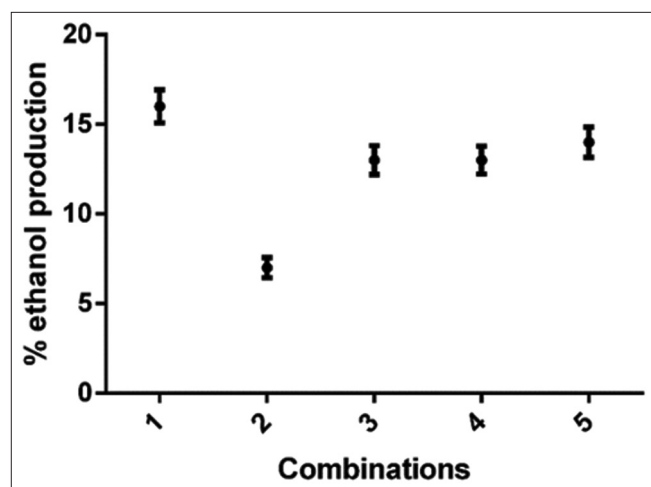
**Figure 3:** (a) Chromatogram showing the peaks of Combination 1 (grapes, west Indian cherries, and honey), (b) chromatogram showing the peaks of Combination 2 (west Indian cherries, amla, and honey), (c) chromatogram showing the peaks of Combination 3 (west Indian cherries, grapes, amla, and honey), (d) chromatogram showing the peaks of Combination 4 (strawberries, amla, and honey), (e) chromatogram showing the peaks of Combination 5 (grapes alone)

## DISCUSSION

Usage of beverages across the world is increasing significantly in which wine is one of the oldest and largely consumed beverage. A variety of substrates is being employed for the production of wine to meet this requirement. Several substrates were previously reported,

however, this study is the first of its kind where combinations of one or more substrates were used for the production of wine. A report by Sun *et al.*, 2014<sup>[6]</sup> showed that sugar and acids aids in sweetness of wine, similarly the wine produced from the combination of grapes, West Indian cherries and honey showed increased sweetness, tartness, and aroma. Further, the choice of different combinations of the fruits





**Figure 4:** The quantitative analysis of ethanol production (%)

was deliberately selected based on their nutritional and bioactive properties.

The usage of amla as substrate was reported by Anila and Vijayalakshmi,<sup>[8]</sup> 2000 to have high antioxidant, hypoglycemic, and hypolipidemic activity. A similar study by Oliveira *et al.*, 2013<sup>[5]</sup> unveiled the application of grapes which contains phenolic compound like resveratrol capable of showing antibacterial and anticancerous activity, whereas strawberries and honey were selected for their sweet texture and aroma.<sup>[7,11]</sup> The combination one (grape, West Indian cherries, and honey) was found to show enhanced antibacterial activity over the other combinations can be related to the presence of phenolic compounds such as resveratrol, gallic acid, (+)-catechin, epicatechin, dimeric procyanidin, proanthocyanidins commonly found in grapes, and West Indian cherries.<sup>[2]</sup>

The study also revealed the simultaneous production of ethanol during the fermentation process, among all the tested combinations, the combination one was found to utilize high concentrations of available sugar and produce 16% of ethanol. The observed pattern can be related to the observations by Chilaka *et al.*, 2011.<sup>[15]</sup> The study depicts that production of ethanol is directly proportional to the rate of fermentation, similarly, the combination one was very effective in producing ethanol. In contrast, the other combinations were also found to produce significant concentrations of ethanol and showed antibacterial activity.

Thus from this study, it is evident that the combination one consisting of grape, West Indian cherries, and honey could be effectively used for the production of wine with high nutritive value to meet this requirement.

## CONCLUSION

From this study, we conclude that the combination of fruits like grape and West Indian cherries supplemented with honey

can be employed in developing a nutritious beverage like wine which can replace the existing expensive substrates.

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