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Fermentative production and optimization of wine from different vegetables

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Abstract
The study was focused on the fermentative production and optimization of wine from different vegetables. Wine can act as a nutrient supplement for seasonal fruits and vegetables throughout the year. Fermentation is carried out with *Saccharomyces cerevisiae* commonly known as baker's yeast. Daily monitoring was done to study the composition and characteristics of the wine. During the fermentation period, both the wines were analysed for pH, titrable acidity, specific gravity, alcohol and total sugar on a daily basis. The comparison of final analysis of homemade wine with commercial wine was done.Beetroot, Carrot, Ginger, Tomato and vegetable mix is a valuable vegetable and rich in nutrition, mainly it is rich in carbohydrate. The quality of wine depends up on the compositions of the juice and fermentation conditions. It also observed that total bacterial count, sugar and titrable acidity. Sensory evaluation revealed aroma, fine taste and the overall improved quality of the wine. The study concluded that the result to develop a small scale wine industry. All the vegetables are suitable for wine production. Among the vegetables tested, the beetroot is the best source for the production of wine.

Keywords: beetroot, carrot, ginger, tomato, wine, fermentation, alcohol and *Saccharomyces cerevisiae*

Introduction
Fermentation of grape juice into wine is a complex microbial reaction. Yeast are primarily responsible for the alcoholic fermentation of must, while many wines undergo another fermentation process mediated by lactic acid bacteria (Jolly *et al*., 2003) [6].

Wine making is one of the most ancient technologies and is now one of the most commercially prosperous biotechnological processes. Wine is an alcoholic drink made from fermented fruit juice. Generally, fruits contain quantities of sugar that can be used by yeast during the fermentation process. In addition to the inherent characteristics of fruit (pH values, sugar content and nitrogen contents), other factors must be taken into account during fruit wine production (Amerine *et al*., 1980) [1].

The term "wine" can also refer to starch- fermented or fortified beverages having higher alcohol content. During the past few decades, grapes have been the main fruit that were used for wine production. Despite that, several studies have investigated the suitability of other fruit as substrates for the purpose of wine production (Joshi *et al*., 2000) [7].

Saccharomyces cerevisiae the yeast used in alcoholic fermentation is primarily responsible for the formation of main metabolic products and also several other flavour compounds (Wensehke *et al.,* 1993).

Fermentation, the yeast species *Saccharomyces cerevisiae* convert carbohydrates to carbon dioxide and alcohols for thousands of the years the carbon dioxide has been used in baking and the alcoholic beverages. It is also a centrally important model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic microorganisms (Butzke *et al*., 2000) [2].

Hence the present study was carried out to collect the substrate *Zingiber officinale, Solanum lycopersicum, Beta valgaris, Daucu scaroto subsp* from local market at Pattukkottai, Tanjore District, Tamilnadu, to Prepare the must from collected vegetables. In oculate the *Saccharomyces cerevisiae* into the must. Then it allowedthe inoculated medium for fermentation process and analyse the physio-chemical properties of fermented wine such as pH, percentage of alcohol, total sugar and percentage of sugar. The wine production was optimizedwith different variables.

Materials and Methods
Collection of sample
The *Zingiber officinale, Solanum lycopersicum, Beta valgaris, Daucus caroto sub sp* were collected from local market at Pattukkottai, Tanjore district, Tamilnadu.

Saccharomyces cerevisiae was obtained from Microbiology Department of S.T.E.T. Women's College, Mannargudi, Thiruvarur district, Tamil Nadu.

Preparation of Vegetable Juice
All the vegetables were properly checked and washed. *Zingiber officinale* were crushed without removing the peels. *Solanum lycopersicum* were destemmed and crushed in the grinder. *Beta valgaris* and *Daucus caroto subsp* were peeled off and grinded.

Anaerobic Fermentation
Anaerobic fermentation glass beakers were used. The beakers were kept as air tight.

Process of Monitoring (daily)
Daily analysis of wine has been conducted. Various parameters such as pH, titratable acidity, specific gravity,

alcohol content, sugar concentration and biomass concentration etc., of each batch were determined day by day during the course of fermentation.

Parameters monitored during fermentation period

- Variation in pH (Robinson, 2003) [11].
- Sugar concentration (Miller, 1959) [9].
- Alcohol percentage (Berry, 2000) [2].
- Titratable acidity (Rodriguez *et al*., 1971) [12].

Total Soluble Solid (TSS) adjustment (Srivastava and Kumar, 2009) [13].

The Total Soluble Solid (TSS) of both juices was adjusted by adding cane sugar in powder form.

Optimization of fermentation processes (Singh and Puyo, 2014) [14].

- Incubation period
- Size of inoculum
- pH of the juice
- Adjustment of brix
- Incubation temperature
- Alcohol adapted and Non- adapted culture
- Age of culture

Final analysis of wine

Tannin content was estimated by Folins – Denis method in mg/100ml. Phenol content was determined by Folins Lowry method in mg/100ml. Free and total SO_2 was done by Ripper method in g/L. Total suspended solids was calculated in Degree Brix. Final analysis of all parameters such as pH, alcohol content specific gravity, sugar content, titratable acidity, and biomass were conducted using the methods described in daily analysis.

Sensory Evaluation (Fessler, 1988) [3].

The sensory parameters of wine like taste, colour, aroma, appearance and flavour were evaluated by using an evaluation system. Hence the improvement is required with respect to the taste as well as overall aromatic quality of the beverages.

Bacterial count (Dhiva, 2017)

Preparation of sample for bacterial count in the case of a homogeneous sample involves proper dilutions of facilitates easy identification and counting. To 20ml of distilled water in a 25ml graduated cylinder added 5ml of sample by displacement and shacked thoroughly. Placed the haemocytometer slide under microscope and using 400 to 500 magnification counts in four small size squares from each corner of ruled chamber and central Medium Square.

Isolation and Identification of Microorganism from the Vegetable Wine Serial Dilution (Lozano, 2009) [8].

Microbiological analysis of the vegetable wine was also carried out to analyze the microbial population. Serial dilution was performed by using the collected vegetable wine to isolate the bacteria. 1ml of vegetable wine was diluted in the tube containing 9 ml of sterile distilled water and mixed thoroughly to make 1:10 dilution (10^{-2}). 1ml of diluted sample was transferred to the next test tube and serially diluted in to the series of test tubes having 9ml of sterile pipettes up to 10^{-7} dilution. The isolated bacterial organisms were identified by Gram's staining, motility and biochemical characteristics (Han's Christain Gram, 1884) [5].

Calculation of microbial load (Mohammad Abul Mansur *et al.,* 2013)

The microbial load of five different vegetable wines was calculated by using the following formula.

$$\text{Colony Forming Unit (CFU/g)} = \frac{\text{No. of colony} \times 10n \times 10 \times \text{Vol.of Soon}}{\text{Weight of Sample}}$$

Comparison of commercial wine (Giri Nandagopal and Praveen, 2013) [4].

Parameters were estimated such as pH, alcohol content, specific gravity, sugar content and titrable acidity. The parameter of the homemade wine was compared with that of the commercially available wine. The pH of the homemade wine was measured by taking 1ml of fresh sample and read with the pH meter.

Statistical Analysis (Mohony, 1985) [10].

All the experiments were carried out in the mean values and standard deviation was calculated. The data was presented as mean ± standard deviation for each sample.

Results

In this study, the home made wine was produced from *Zingiber officinale, Solanum lycopersicum, Beta valgaris, Daucus caroto subsp* using *Saccharomyces cerevisiae* and it was compared to the commercial wine, then the microbial and chemical characteristics of the wine was also analysed.

Production of Wine

The wine produced after the completion of the fermentation of the reconstituted vegetable juice at combination of amylase and yeast in the selected ranges. The fermentation temperature of 25^0c and pH of 4.5 was found to be favourable for the fermentation of vegetable juice, on 14^{th} day when the wine was ready, it had a very good taste and aroma with good body. The wine also had on acceptable good after taste and colour.

pH of wine

Variation in pH in the fermentation medium during the course of process was analysed. pH showed a decrease trend then attains minimal increased showed table-1.

Table 1: Analysis of pH and Titratable Acidity of wine

S. No	Wine sample	Ph	Titratable acidity (g/l tartaric acid)
1	Carrot	$3.78^a \pm 0.02^b$	$2.60^a \pm 0.40^b$
2	Ginger	4.76 ± 0.07	3.20±0.42
3	Tomato	5.40 ± 0.19	3.30±0.40
4	Beetroot	5.20 ± 0.23	1.3 ± 0.22
5	Vegetable mix	6.1 ± 0.12	2.1 ± 0.14

Mean = a, Standard deviation = b

Values are expressed as mean ± standard deviation.

Titratable acidity

Titratable acidity of carrot, ginger, tomato, beetroot and

vegetable mix was determined. The titratable acidity of wine shows a fluctuating trend as the number of days increase. The titratable acidity ranges from 3.5 g/l to 7 g/l tartaric acid. Titratable acidity of carrot ranges 7.85 g/l. Similarly ginger 7.4 g/l, tomato 5.8 g/l, beetroot 4.3 g/l, and vegetable mix was 3.45 g/l.

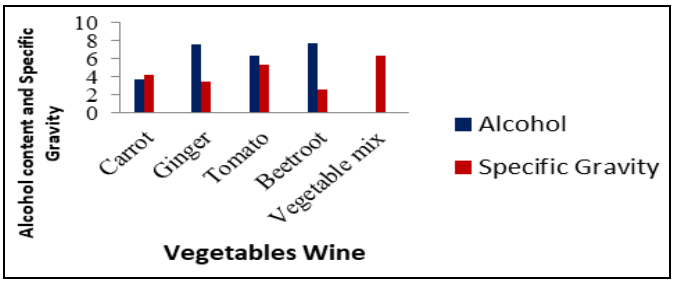

Fig 1: Analysis of Alcohol Content and Specific Gravity in Wine

Alcohol percentage

In this study of alcohol content in volume percentage of carrot, ginger, tomato, beetroot and vegetable mix was analysed. It can be concluded that the alcohol volume percentage increased as the number of day's increases. The percentage of alcohol between 0 to 8 during the fermentation period of each batch. The initial level of alcohol was 0 % for all the wine samples – carrot, ginger, tomato, beetroot and vegetable mix. Final alcohol content for carrot was 12.57% on 14th day, ginger was 12.00% on 8th day, tomato was 10.52% on 10th day, beetroot was 10.02 on 14th day, and vegetables mix was 15.7% on 15th day.

Optimization of fermentation processes

Incubation Period

The longer time of incubation result of ethanol content was increased. The highest level of ethanol was produced on 12th day with ethanol content of 18.71%. The maximum ethanol concentration achieved during fermentation at the 18th day and began to decline at 32 hours. This is because on the 24th hours yeast entered the phase exponentially, while at the 32 hours yeast cell begin to enter stationary phase. And also the ethanol content of wine is influenced by method of wine preparation.

Size of Inoculum

Different levels of inoculum (5, 7, 10, 12 & 15%) were used to inoculate the fermentation mixture. The maximum ethanol production was observed at 7% inoculum size. Further increase in inoculum size did not favour ethanol production. It was concluded that 7% inoculum selected for the further study.

Incubation temperature

A different level of temperature was studied to optimize wine preparation. They are 20, 25 and 30ºC. As the temperature increases initial fermentation rate are increased due to the enzyme activity of the metabolic pathway. And also higher temperature had negative effect on stability of enzymes or any other biomolecules and decreases the enzyme activity. The higher yield of alcohol is showed the 30ºC in fermentation period when compared to other temperature.

Age of culture

The 24 hours old yeast culture show the maximum (15.38%) alcohol production when compare to 48 hours old culture. This may be due to decline in the growth rate of cells after 24 hours and also the transition of population into the stationary phases. So, for further study 24 hours old yeast culture was used.

Final Analysis of Wine

Alcohol percentage, tannin content, phenol content, pH, specific gravity, titratable acidity and total suspended solid were estimated. Final analysis of wine was conducted after the fermentation period (i.e. after 15 days).

Tannin Content

Tannin content for carrot, ginger, and tomato was 2.706mg/ml, 1.14mg/ml and 0.19 mg/ml. Similarly for beetroot and vegetable mix were 0.74mg/ml, and 3.71mg/ml respectively.

Phenol Content

Phenol content of carrot is 0.39mg/ml, followed by ginger-0.42mg/ml, tomato-0.88mg/ml, beetroot-1.36mg/ml and for mix vegetable was 2.56 mg/ml respectively.

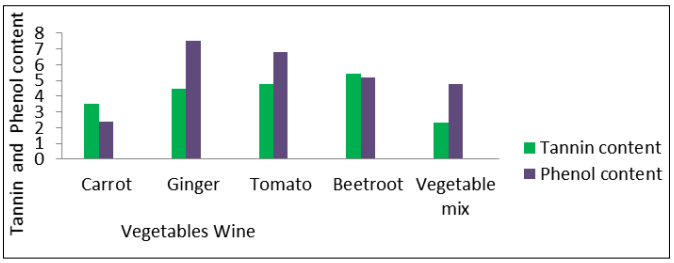

Fig 2: Analysis of Tannin and Phenol content in wine

Isolation and identification of bacteria from wine

From carrot, ginger, tomato, beetroot and mix vegetables wine, the bacteria were isolated and identifed by agar plate method. The bacteria were identified using Gram's staining. The isolated bacteria namely, *Staphylococcus aureus*, *Pseudomonas fluorescencs*, *E. coli*, *Bacillus subtilis*, *Streptococcus pyogens* and *Lactobacillus plantarum. Staphlococcus aureus* was identified in carrot wine, *Pseudomonas fluorescen* was identified in ginger wine, *E.coli* was identified in tomato wine, *Bacillus subtilis* was identified in beetroot wine, *Stapthylococcu spyogens* was identified in vegetable mix wine.

Total bacterial colonies of vegetable wine

In carrot 32 colonies were counted. Similarly ginger 12, tomato 16, beetroot 12 and mix vegetables 24 were counted using colony counter. Totally 96 colonies were counted. The highest numbers of colonies were counted in carrot, followed by mix vegetables, tomato, ginger and beetroot.

Sensory parameters

The wine was analysed according to taste, colour, aroma, appearance and odour. These sensory parameters of both the wines were analysed separately. A bottle of red wine was

purchased from a supermarket in Pattukkottai. This was used as reference sample of a preference test between commercial wine and the wine prepared. A panel of twenty judges was drawn from the STET Women's College, Mannargudi, Thiruvarur (Dt), India for the sensory evaluation. The panellists were familiar with all the quality attributes of a good wine.

Comparison

The comparison of final analysis of homemade wine with commercial wine was conducted and can be concluded that the pH (except ginger), specific gravity and alcohol content of commercial wine is higher than homemade wine.

Yeast count of homemade wine was higher than the commercial wine. Itwas increased from 0 to 4.5×10^6cells when compared with commercial wine.

Discussion

This study were reported that 10g of dry yeast was mixed with 250 ml of *Zingiber officinale, Solanum lycopersicum, Beta valgaris, Daucus caroto subsp* which had been preheated to 37˚C. All the parameters such as sugar and pH were checked before the inoculum. *Saccharomyces cerevisiae* was added in the juice and kept for incubation at 25˚C for 15 days. Our study was agreed to Kaiser Younis *et al.,* (2014), *Saccharomyces cerevisiae* inoculum were prepared by inoculating loopful culture into 10 ml test tubes containing medium broth under sterile conditions. The tubes were kept at 30^0C temperature for 24 hours and a full test tube along with media is poured into one litter juice of guava and beer juices and incubated at 30°C for 24 hours under aerobic conditions.

Summary and Conclusion

Homemade wines have relatively low alcohol content than the commercially available wine and there is no usage of either any preservative or any additives, so homemade wines are not harmful for health and are acceptable for daily usage. The results of process monitoring and final analysis will help a small scale wine industry or can refer the results to develop a small scale wine industry. The study concluded that the result to develop a small scale wine industry. All the vegetables are suitable for wine production, finally compared to all the vegetable, the beetroot is the best vegetable for the production of wine.

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