

Production of coconut sprout wine using *Saccharomyces cerevisiae* and its physico-chemical analysis

Abstract

Wine is a traditional fermented alcoholic beverage made using yeast. Coconut sprout is a big spongy cotton candy mass that is sweet and juicy, found inside coconut during germination produced as an endosperm to nourish the developing embryo. It is well known for its nutritional qualities and is of great medicinal value. The present study involves the production of coconut wine from coconut sprout using *Saccharomyces cerevisiae* under controlled conditions for a period of 20 days. Various physico-chemical analyses were performed at a 5 day interval to understand its benefits. The pH of wine during fermentation decreased to 2.5. The total yeast count shows a gradual increase from 0.202×10^7 cells/ml to 2.811×10^7 cells/ml and then decreased to 2.10×10^7 cells/ml. The total reducing sugar in wine was determined by DNS method which shows a gradual decrease from 8.510 mg/ml to 4.84 mg/ml. The total Polyphenolic content ranges from 24.648mg/ml on to 19.60 mg/ml during fermentation. The alcohol content of wine was estimated using iodoform test and was found to be 9.1% after 20 days. The electrical conductivity of wine was estimated as 5.24ms at 30°C. The antioxidant assay was carried out using DPPH method and found to be 58.024%. The organoleptic assay was carried out on the 20th day which gives a satisfactory result, with an overall acceptance of 7 out of 10. The Fourier Transform Infrared Spectroscopic analysis of wine detects the presence of ethyl alcohol in the fermented sample was well evidenced by the prominent C-O stretch observed at 1055 cm inverse and the deep and broad -OH stretch at about 3400cm inverse.

Keywords: alcohol, coconut sprout, polyphenols, *Saccharomyces cerevisiae*, wine

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Introduction

Wines are undistilled alcoholic beverages usually made from fruit juices which are nutritive, mild stimulants and are tastier than conventional fruit juice. These fruits undergo a period of fermentation where microbiological processes contribute significantly to the final quality of the product.¹ Wine usually has an alcohol content ranging between 5 to 13 percent. Wine has a flavour like fresh fruit which could be stored and transported under the existing conditions. Being an undistilled product, they contain most of the nutrients present in the original fruit juice.² A typical wine contains ethyl alcohol, sugar, acids, higher alcohols, tannins, aldehydes, esters, amino acids, minerals, vitamins, anthocyanins and minor constituents like flavoring compounds.³ The moderate consumption of wine has reduced the risks of dementia and cognitive decline.⁴ Wine has been a traditional drink used as a medicine for almost thousands of years and it is an important part of various religious myths.

Coconut sprouts are formed from the seeds during germination. A lot of benefits from coconut sprout have been identified so far such as high nutrient content, including proteins, low calories, higher content of fibers and enzymes which boost various metabolic processes as well as chemical reactions within the body aiding digestion and minimizing cardiac damage induced by isoproterenol.⁵ They are used to reduce blood pressure, treat anemia, lower cholesterol level and improve digestion. The inhibitory action of coconut sprout on ghrelin, the hunger hormone is also identified. Sprouts can clear conditions such as constipation and diarrhea and also helps to prevent

colorectal cancer. They are a great source of omega-3 fatty acids and are considered lower the risk of heart attack and strokes by reducing LDL cholesterol. The potassium in sprouts also helps to reduce blood pressure. By dilating the blood vessels it increases the circulation and oxygenation and hence reduce the risk of heart attack, strokes and also exhibit antidiabetic properties.^{6,7} Sprouts are rich source of antioxidants such as vitamin C and vitamin A and can boost our immune system. Coconut sprouts are enriched with various phytoconstituents which possess strong antibacterial, anti-inflammatory and antioxidant activities.⁸ GC-MS and docking studies on coconut sprout confirmed the presence of squalene, a triterpenoid having antiulcer affinity against *Helicobacter pylori*.⁹ Wine as an undistilled product can bring all the benefits of the drupe without losing its effect. Hence, the aim of the present study is to produce and evaluate wine from coconut sprout using *Saccharomyces cerevisiae* and spontaneous fermentation.

Materials and methods

Collection of coconut sprout

One kilo gram of coconut sprout was purchased from local market in Cherthala, Alappuzha district, Kerala state, India.

Microorganism used

Yeast strain *Saccharomyces cerevisiae* from the culture collection of Department of Biotechnology and Research, K.V.M. College of Science and Technology, Kerala, India was used for fermentation process.

Preparation of starter culture

Saccharomyces cerevisiae obtained from culture collection were cultured in glucose yeast extract broth. It was then centrifuged at 6000 rpm for 10min and pellet was obtained. The cells were washed several times and resuspended in normal saline to obtain a concentration of 10^8 cells/ml and this was used as the pre-inoculum for wine making. The inoculum was prepared by transferring 10mL of pre-inoculum into 500ml conical flask containing 250ml mixture of coconut sprout juice. The mixture was then incubated overnight in shaking incubator at 60 rpm at 30°C.

Preparation of must

The coconut sprout was cut into small pieces and made into a paste by adding 500ml of distilled water. Then 250 ml was taken as control and another 250ml as sample for fermentation. Both the flasks were autoclaved at 121°C for 15 minutes at 15lbs. 1gm of yeast was weighed and dissolved in 10ml of water and mixed with wine except control and kept at room temperature.

The fermentation was initiated by the addition of 2 % starter culture, into the prepared must. The must was mixed well and kept for fermentation in a cool dry place. Fermentation was done for 20 days. Aliquots were taken from both the sample as well as control for various analytical tests at an interval of 5 days. This was achieved by filtering the samples using muslin cloth, sieve and syphon tubes sterilized by 70 % alcohol. The wine was syphoned into the sieve containing four layers of muslin cloth. The residues were removed and the filtrates were used for various analytical tests. After 20 days the fermentation was stopped by immersing the jar in 68-70°C water bath.

Test for pH

pH of the wine samples were checked using a digital pH meter (Eutech Cyber Scan pH 510) pre-calibrated with buffers of pH 4.0 and 7.0 as described by Ochai & Kolhatkar.¹⁰

Enumeration of yeast cells

5 ml of the wine sample was taken in a test tube and the absorbance was read using spectrophotometer at 600 nm.

Reducing sugar assay

2 ml of DNS reagent was added to 2 ml of wine sample taken in a test tube. Shaken well and heated the mixture at 100°C in a boiling water bath for 15 minutes. Allowed to cool at room temperature and read its absorbance at 540 nm. Standard curve was drawn using glucose as the standard and the amount of reducing sugar present in the sample was calculated.

Titrateable acidity

Titrateable acids represent the sum of all acids in the wine and titrateable acidity corresponds to the percentage of citric acid and the later was determined by the method of AOAC.¹¹

Estimation of alcohol

The estimation of alcohol was done using the Iodoform test.¹² 1ml of sample containing alcohol was taken in a test tube and 4 drops of 1N NaOH was added. Concentrated solution of iodine was added drop

by drop until the faint yellow colour persists. The tubes were allowed to stand for a minute and added excess amount of NaOH solution if excess colour developed. Shook the mixture well and allowed to stand for 2-3 minutes. The yellow precipitate settled at the bottom. Removed the precipitate at room temperature. Weighed and calculated the amount of alcohol present in the sample.

Determination of total phenolic content

The total phenolic content in wine was estimated by Folin-Ciocalteu's method.¹³ To 1.0 ml of the sample, 1.0 ml of Folin-Ciocalteu's reagent was added. After 3 min, 1.0 ml of saturated Na_2CO_3 solution was added and the final volume was made up to 10 ml with distilled water. The tubes were kept in dark for 30 min, after which its absorbance were read at 760 nm against a reagent blank. Standard curve was calculated using gallic acid standards and TPC were calculated.

Determination of antioxidant activity

The radical scavenging activity (RSA) of the wine was estimated using the DPPH assay.¹⁴ The method is based on the scavenging of DPPH (2, 2-diphenyl-1-picrylhydrazyl) by antioxidants, which upon a reduction reaction decolorizes the DPPH methanol solution. 5ml of wine sample was mixed with 5ml of 0.06 mill molar DPPH in methanol. The mixture was well mixed and allowed to stand in dark for 30 minutes. The absorbance was measured at 520nm. The capability to scavenge the DPPH radical was calculated using the equation

$$\text{Percentage RSA} = \frac{[1 - (\text{Absorbance of control} - \text{Absorbance of sample})]}{\text{Absorbance of control}} \times 100$$

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is a powerful tool for identifying the types of chemical bonds present in solids, liquids or gases. The tablets for FTIR spectroscopy (FTIR Shimadzu Prestige 21) were prepared in agate mortars, by mixing a drop of wine sample with potassium bromide (KBr) powder (1:100 p/p). The absorption spectra were measured between 400 and 4000 cm^{-1} .

Electrical conductivity

Electrical conductivities of the wine samples were determined using electrical conductivity meter (Systronics 308).

Organoleptic assay

After the 20th day of fermentation, the wine samples collected from the fermentation flask were filtered and subjected to sensory evaluation. The characters such as taste, odour, flavor, clarity and overall acceptance of wine were evaluated out by organoleptic parameters tested among five individuals (5 point hedonic scale).¹⁵

Results and discussion

Coconut sprout is a rich source of essential nutrients like amino acids, minerals, proteins, vitamins and large amount of phytoconstituents which are mainly required for maintaining human health. The properties of coconut sprout after fermentation are given in Table 1. The pH of the must was in the acidic range throughout the period of fermentation. The initial pH of the wine was 4.0 and on the

20th day the value was 2.5, showing a drastic change during the growth of yeast on the culture. On the first day, yeast cell count was found to be 0.202×10^7 cells/ml and decreases on the 20th day (Table 1). Yeast grows on the media by utilizing the fermentable sugar and results in the production of alcohol and carbon dioxide. The initial amount of

reducing sugar in the medium was 10.931 mg/ml on the 1st day, 8.510 mg/ml on the 5th day, 7.987 mg/ml on the 10th day, 6.404 mg/ml on the 15th day and 4.84 mg/ml on the 20th day. Yeast converts the sugars present in the culture and produces alcohol and carbon dioxide which results in the depletion of sugar in the must.

Table 1 Results of physicochemical analyses

Physicochemical parameters	Days				
	0	5	10	15	20
pH	4	3.6	3.2	2.8	2.5
Yeast cell count (cells/ml)	Nil	0.202×10^7	1.938×10^7	2.811×10^7	2.10×10^7
Alcohol (%)	Nil	2.01	4.9	8	9.1
Reducing sugar (mg/ml)	10.931	8.51	7.987	6.404	4.84
Total phenolic content (mg/ml)	25.8	24.648	22.921	21.878	19.6
Titratable acidity (g/l)	0.071	0.121	0.282	0.374	0.412

The acidity of the wine shows an increase from the first day to the last day. The wine had an initial acidity of 0.071 g/l, 0.121 g/l on the 5th day, 0.282 g/l on the 10th day, 0.374 g/l on the 15th day and 0.412 g/l on the 20th day. The acidity of the wine increases as the fermentation progresses. Titratable acidity can also result in the change in sensory perception of wine. The alcohol content in the wine was estimated using iodoform test, which was 2.01% on the 5th day, 4.9% on the 10th day, 8% on the 15th day and 9.1% on the 20th day. The amount of alcohol in wine increases in proportion to yeast cells. The antioxidant activity of the wine was estimated using DPPH assay and it was found to be 58.024%. Antioxidants are molecules which can prevent effects of oxidation in tissues and can protect cell from damaging free radicals.¹⁶ Antioxidant capacity of wine on human low-density lipoprotein (LDL) and also in antiplatelet properties are related to the content of polyphenols, which improve aortic biomechanical properties.¹⁷

The total phenolic content of the wine was estimated using Folin-Ciocalteu's method. The initial value was 25.8 mg/ml on the 5th day, 24.648 mg/ml on the 10th day, 21.878 mg/ml on the 15th day and 19.6 mg/ml on the 20th day. Phenolic compounds may interact with volatiles and contribute to the release of specific aromas in wine.¹⁸ Content of polyphenols, composition of phenolic complex and antioxidative or antiradical capacity of wines could be affected by many extrinsic and intrinsic factors, such as variety, fruit growing area and climatic conditions, quality of fruit, and, not least, technological procedures during wine making.¹⁹ The results showed a decrease in phenolic contents of wine from the first day to the last day. It is believed that phenolic contents in wine provide antioxidant activity and also affects the taste, color and mouth feel. The electrical conductivity of the wine was found to be 5.24 ms. As the solution becomes more concentrated, the proximity among ions depressed their activity and consequently their ability to transmit current, although the amount of dissolved solids is the same.

It is known that coconut meat contains complex ligno-cellulosic matrix which contains fats, proteins and moisture along with traces of vitamins and minerals. During germination or sprouting of the seeds this complex structure breaks down into simpler components, especially the cellulose breaks down into simple sugars. From the

FTIR spectrum of the unfermented endosperm (after sprouting) it is clear that cellulosic structure degraded. This is evidenced by the absence of absorption peaks at 3434, 2924 and 2854 cm⁻¹ inverse, the first one corresponding to -OH stretching in hemicellulose and the second and third values corresponding to -CH stretching in methyl and methylene of cellulose. Also lignin if present in the endosperm will show a strong and wide absorption peaks between 3500-3100 cm⁻¹ inverse, which is less prominent in the FTIR spectrum (Figure 1) of the unfermented sample. This suggests that the partial hydrogen bonding in the lignocellulosic material has been lost during germination, because of the structural degradation.

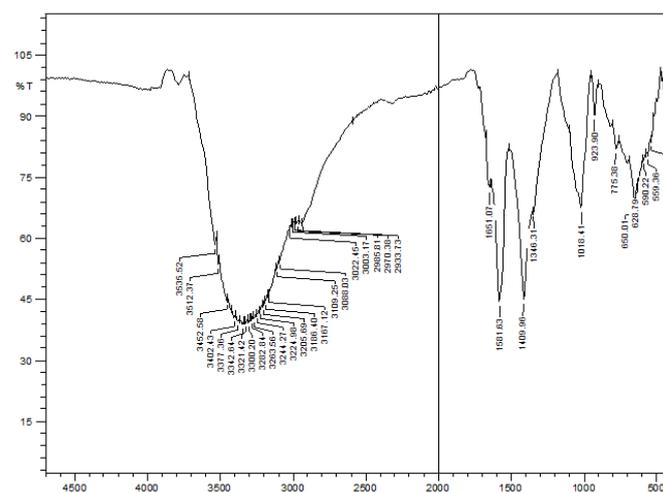


Figure 1 FTIR spectrum of unfermented coconut sprout.

The absence of absorption peaks at 1396 cm⁻¹ inverse, and 1248 cm⁻¹ inverse, the former corresponding to aromatic hydroxyl groups and the latter to the C-O stretching of ether linkages indicate that the lignin content in the endosperm reduced to a greater extent during germination. Higher concentrations of simple water soluble sugars such as glucose and fructose are expected during the degradation of cellulose and hemicellulose structures while sprouting of the coconut seed.²⁰ Some of the marker bands of glucose and fructose which

corresponds to the spectral range between 1400 and 900 cm inverse are present in the spectrum of unfermented sample. The absorption bands in the region 1153- 900 cm inverse are assigned to C-O and C-C stretching modes while those in the 1199-1400 cm inverse region are due to O-C-H, C-C-H and C-O-H bending vibrational modes of carbohydrates. However, there can be a conclusion drawn from the spectrum of the unfermented endosperm that it is devoid of the disaccharide, which is evidenced by the absence of the marker band of sucrose at 993 cm inverse.

The carbonyl (C-O) stretching of unconjugated ester which mainly exists in the side chains of lignin structural units, producing an absorption band at 1746 cm inverse is also absent, indicating the breakdown of lignin during sprouting /germination of the coconut seed. Another important aspect found from the spectrum of the unfermented endosperm is the absence of the absorption peaks at 1638 cm inverse which corresponds to the C-C stretching of aromatic groups (which are part of lignin structure). Also it is to be noted that the absorption band near 1379 cm inverse associated with C-H bending vibration in cellulose and hemicellulose are also absent. Moreover, the prominent band at 1365 cm inverse, corresponding to the methoxyl stretching, expected in a ligno-cellulosic material is also absent, showing the degradation of lignin structure during germination. An absorption peak at 1158 cm inverse, which represents an ester bond (C-O-C) stretching found usually in cellulose and hemicellulose is also absent in the spectrum of the unfermented sample. An overall conclusion from the FTIR analysis of the germinated coconut endosperm hints

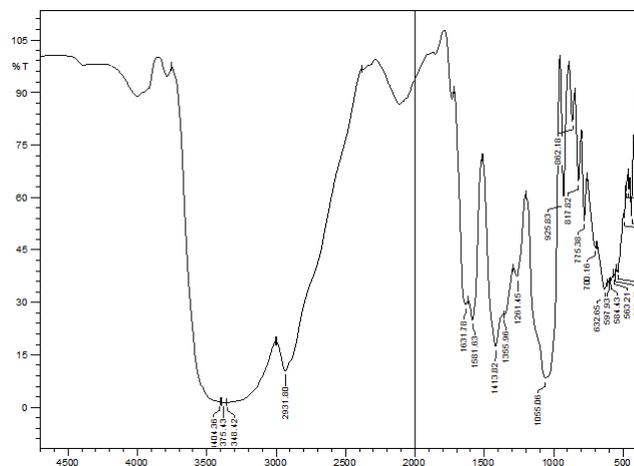


Figure 2 FTIR spectrum of wine sample.

Conclusion

The present study which was based on the evaluation of coconut sprout as substrate for wine production has revealed that the coconut sprout was a good substrate for wine production. The biochemical and sensory attributes of the wine was acceptable by the consumers. Coconut sprout wine combines the effect of both sprout and wine together to bring out an effective result during consumption. The wine showed good antioxidant activity. Antioxidants have potential efforts in preventing several diseases such as, neurodegenerative diseases, anemia etc. and also reduce the process of ageing. A desirable ethanol

at the presence of highly degraded ligno-cellulosic matrix combined with simples sugars such as glucose and fructose, but not sucrose in it.

The FTIR spectrum of the fermented sample contains a strong absorption band at 1056 cm inverse due to the formation of ethyl alcohol, which corresponds to the C-O stretching vibrations along with O-H stretching vibrations around 3404 cm inverse and C-H stretching vibrations at about 2931 cm inverse. Other absorption peaks related methyl and or methylene stretching vibrations at 1413, 1355 and 1261 cm inverse are also prominent. However the absorption bands in the region between 1400 and 900 cm inverse due to C-C stretching modes of carbohydrates and those in the region 1400-1199 cm inverse, which are the specific bending vibrational modes of O-C-H, C-C-H and C-O-H (again characteristics of carbohydrates) are missing. Nevertheless broader peaks between 3340 cm inverse and 3400 cm inverse (Figure 2) are prominent in the spectrum of the fermented sample, which could be due to the absorptions from -OH groups from alcohols as well as water molecules, both showing intermolecular hydrogen bonding (Figure 3).

The wine was tasted by 5 individuals with a mean age of 23.5 and their opinions were recorded as points out of 5. The results showed an average of 4.18 for color, 4.4 for taste, 4.16 for flavor, 4.32 for clarity and 4.32 for overall acceptability. Wine drinkers have long acknowledged the link between taste and terroir, the often unmistakable connection between the flavor of a wine and the particular patch of ground in which the corresponding fruits were grown.²¹

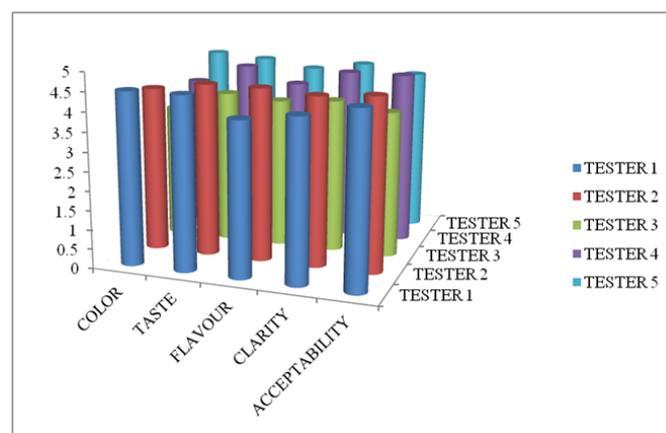


Figure 3 Sensory evaluation of wine.

content and significant quantities of phenolic content showed that the wine is a new potential candidate of functional beverage class. The organoleptic assay shows that color, flavor, taste and clarity were acceptable and the wine can be assumed good for consumption. Thus the value-added coconut sprout wine can be a prospective contender of expanding class of health beneficial beverages.

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Conflicts of interest

The authors declare none.

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