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RESEARCH ARTICLE

BIOTECHNOLOGY

COMPARATIVE EVALUATION OF WHITE WINE PRODUCTION FROM DIFFERENT CARBOHYDRATE RICH SUBSTRATES USING AIR-LIFT BIO-REACTOR

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ABSTRACT

In recent years, wine consumption rate has increased in India. Grapes are used as a primary source in wine production, all over the world. A number of studies have been conducted on apple, guava, banana, jamun, strawberry, apricot, kinnow and plum in order to find an alternate resource. The major objectives of this study are the production of wine using sweet potato and also to find an effective combinational resource. Initially, white wine was produced using sweet potato. Through a detailed analysis, it was confirmed that the rate of white wine production was higher in sweet potato when compare to grapes and figs. Finally, different combinational white wine productions were performed with the help of air-lift bio-reactors. Results suggest that a combination of sweet potato with grapes and sweet potato with beetroot and banana was effective. Anti-microbial activity was carried out in order to verify the fidelity of the produced wine.

KEYWORDS

white wine; sweet potato; Air-lift bio-reactor; combinational wine;

1. INTRODUCTION

Wine is the product of complex interactions between fungi, yeasts and bacteria that commence in the vineyard and continue throughout the fermentation process until packaging[1]. It is one of the most recognizable high value-added products from fruits. It can also be used as a substrate for the manufacture of vinegar, a by-product of wine manufacture. Wine manufacturing process is challenging, in the sense of obtaining a marketable product, but the processes involved in its production are relatively straightforward[2, 3].

Wine has been around for thousands of years. Historically, wine production and consumption have been at home in Europe. From ancient civilizations to modern times, wine has been produced and enjoyed by many, from peasants to kings. The Mesopotamians were the first known people to cultivate grapes[4]. In recent years, growing attention has been devoted to the conversion of biomass into ethanol, considered the cleanest liquid fuel as an alternative to fossil fuels[5].

Globally, a wide variety of carbohydrates rich sources are used in wine production. Among them, grapes are predominantly used in wine industries. Countries like Europe, America, Australia and Africa are highly dependent on their annual grape cultivation [6]. In recent years, a number of researches have been conducted, in order to find an alternate or new resource for wine production.

The rate of wine consumption in India has been increasing gradually in recent years. Grapes act as a primary source in wine production. Six grape varieties including Bangalore blue, Zinfandel, Cabernet, Chenin Blanc, Sauvignon Blanc and Shiraz are being used in India for wine making[7].

Recently, Kourkoutas et al experimented with the production of wine using apples. Cheirsilp et al tried to produce wine from banana. Presently wine is produced using carbohydrate rich fruits such as apple, guava, banana, jamun, strawberry, apricot, kinnow, plum [8]. Wine production using most of the above mentioned fruits are cost effective in India, due to the limited availability of raw materials.

The sweet potato (*Ipomoea batatas*) is a root vegetable with high starch content. It has a wide range of uses, including food, beverages, medicines, ceremonial and household objects, fishing bait, and animal feed. It is cultivated all over the world and especially in south India, it is very popular; the average temperature required for sweet potato cultivation is 24°C (75°F). Dr. Robert Cordell (2006), Emeritus Professor of cardiothoracic surgery at Wake Forest University School of Medicine, highly recommends sweet potatoes. Most remain unaware of the significant health benefits of this low-fat, high fiber vegetable that is such a rich source of vitamins A and E. Sweet potatoes, therefore, contain significant deterrents to heart disease and stroke, both of which tend to be higher in our part of the country. In addition, reports have suggested that it has high anti-oxidant property and anti-cancer effects.

The major objective of this study was to focus on white wine production from various carbohydrate rich substrates. Later, ethanol existence was confirmed through analytical works like spectrophotometric determination and gas chromatography. Finally, combinational wine productions using a low cost Air-lift bio-reactor [8,9,10] were performed.

2. MATERIALS AND METHODS

2.1 SCREENING OF SOURCE:

The process was initiated with the selection of carbohydrate rich vegetables and fruits, such as sweet potato (*Ipomoea batatas*), fig (*Ficus carica*), beetroot (*Beta vulgaris*) and banana (*Musa sapientum*).

The equipment required for the fermentation process was autoclaved at 15-lb/sq inch pressure for 15 min. The fruits and vegetables were washed in clean water, then smashed and made into juice. In the case of beetroot and sweet potato, instead of smashing directly, they were cut into pieces, boiled with distilled water and smashed.

200ml of three juices namely, grape, sweet potato and fig were collected in different flasks. The micro-organism used for inoculation was *Saccharomyces cerevisiae* (baker's yeast). The inoculation medium was kept in a sterilized dark place for 28 days at 20-25°C for the fermentation process. During fermentation, factors such as colour, odour, pH, and air bubbles were monitored at frequent intervals.

2.2 MAINTENANCE OF CULTURE:

The yeast strain, *Saccharomyces cerevisiae* (Baker's yeast), was maintained in potato dextrose agar medium (PDA - medium was prepared with peeled potato- 200 g/L, dextrose- 20 g/L, pH- 6.5□0.2 and Agar- 20 g/L).

2.4 SCREENING OF COMBINATIONAL WINE:

Combination wine was prepared by mixing fruits and vegetables in different ratios. The following combinations were tried:

1. Sweet Potato + Grapes + *Saccharomyces cerevesiae*.
2. Sweet Potato + Fig + *Saccharomyces cerevesiae*.
3. Sweet Potato + Fig + Grapes + *Saccharomyces cerevesiae.*
4. Sweet Potato + Beetroot + Banana + *Saccharomyces cerevesiae.*

2.5 *BIO-REACTOR DESIGN:*

The type of bio-reactor used in this study was the Air-Lift bio-reactordesigned with a minimum of 1 litre capacity using the following parts:

- ✓ An inoculum inlet.
- ✓ An air inlet with sparger.
- ✓ An air outlet.
- ✓ A rubber corkfor all inlets and outlet.
- ✓ A cylindrical barrier of glass.
- ✓ An outer cylindrical flask.
- ✓ An outlet for product collection.
- ✓ A motor for air inlet process.
- ✓ Connecting tubes.
- ✓ A clip

Complete aeration leads to a good mixture of solution and it eventually results in excellent fermentation.

2.6 ANTIMICROBIAL ACTIVITY:

In the nutrient agar medium, *Salmonella typhi* was inoculated using streaking method. Later four inoculation wells were bored, the first being kept as control with distilled water, the second with the non-inoculated medium (juice) and the other two wells filled with the wine samples. Then the plate was kept for incubation and observed after two days.

2.7 ANALYTICAL METHODS:

2.7.1 Titration techniques for alcohol estimation:

The fermented wine was filtered using a tea strainer. The filtrate was centrifuged at 10,000 rpm for 10 minutes. The clear supernatant was collected and refrigerated. The clear supernatant (5ml) was taken in a beaker to which 5ml of distilled water and a few drops of 1% phenolphthalein indicator were added. The mixture was filtrated against 0.1N NAOH taken in the burette and the appearance of pale pink color was noted as the end point.

- ✓ Burette solution – sodium hydroxide
- ✓ Flask solution – 5ml of distilled water + 5ml of clear filtered wine solution.
- ✓ Indicator – phenolphthalein.

Estimation of Tartaric acid:

$$\text{Percentage of tartaric acid} = \frac{\text{Amount of Alkali X Normality of Alkali X 7.5}}{\text{Amount of Sample}}$$

2.7.2 Spectrophotometric determination of ethanol: *(Caputi et al 1968)*

One millilitre of the fermented wash was taken in a 500ml pyrex distillation flask containing 30 ml distilled water. The distillate was collected in a 50 ml flask containing 25 ml of potassium dichromate solution (33.768 g of K2Cr2O7 dissolved in 400 ml of distilled water with 325 ml of sulphuric acid and volume raised to 1 litre). About 20 ml of the distillate was collected for each sample and the flasks were kept in a water bath maintained at 62.5ºC for 20 minutes. They were then cooled to room temperature and the volume raised to 50 ml. 5 ml of this was diluted with 5ml of distilled water for measuring the optical density at 600nm using a spectrophotometer.

A standard curve was prepared under a similar set of conditions using standard solution of ethanol containing 2 to 10% (v/v) ethanol in distilled water. Ethanol content of each sample was estimated and graph was plotted.

2.7.3 Estimation of reducing sugars:

The DNS method of Miller (1959) was used to estimate reducing sugars.

1. ***Substrate solution:***

Standard solution of 1000 µ g/ml concentration was prepared by dissolving 100 mg of glucose in 100ml distilled water.

2. 3, 5 dinitrosalicylic acid (DNS) solution:

The reagent was prepared by dissolving 10.0g of 3, 5-DNS, 2.0g of phenol and 0.5 g of sodium sulphite in 500 ml of 2% NaOH solution and then diluting it to 1 litre with distilled water. The reagent was filled and stored in a dark coloured bottle.

3. Potassium sodium tartarate (Rochelle salt):

40 g of potassium sodium tartarate was dissolved in distilled water and the volume made upto 100ml.

One ml of the appropriately diluted sample solution (500-1000 µg ml-1) was taken in a test tube to which 3ml of DNS reagent was added. The tubes were boiled in a boiling water bath for 15 minutes. One ml of Rochelle salt was added to these test tubes and they were cooled to room temperature and used for measuring optical density at 575 nm.

A standard curve of glucose was prepared by using 100-1000g concentration prepared in distilled water.

2.7.4 Gas chromatography:

Ethanol in the fermentation broth was estimated by GC method. The name of the instrument used for GC was GC 1000. A computer related Nucon series gas chromatograph equipped with flame ionization detector (FID) was employed for the separation and quantification of ethanol. A column was fitted into the instrument to provide column injection. The detector and injector temperature was maintained at 260°C and 280°C respectively. The gas chromatograph was connected to an integrator and computer system to determine the area of ethanol and the internal standard peak. For analysis of ethanol, the following program has been standardized:

Nature of the column: Liquid phase (100% Polyethylene Glycol)
Dimensions : Zebron ZB WAX 30ml X 0.25mm ID X 0.25m df
Carrier Gas : Nitrogen.
Flow rate : 0.3ml/min.

Standard solutions of ethanol were prepared. The standards contained 2, 3, 4, 5, 6, 7 and 8% ethanol. The standard curve was prepared with

a retention time of 2.5 minutes. 10 µL of the sample was taken and filtered using micron filter paper to remove fine residues. From that, 0.5 µL was syringed out and injected. The area under the peak was determined for samples by comparing it with the standard curve and the percentage of ethanol was measured.
Finally, the percentage of produced wine was calculated using the following formula:

$$\text{Quantification} = \frac{\text{Sample area X standard weight X sample dilution X purity of the standard}}{\text{Standard area X sample weight X standard dilution}}$$

3. RESULTS

3.1 SCREENING OF SOURCES:

Different substrates were inoculated with baker's yeast. The inoculated media was kept under incubation for fermentation and as a result, the change in pH, odour, colour and presence of air bubbles which showed the evolution of carbon-di-oxide were observed. In this, sweet potato was selected as the best substrate which got a good sugar content and easy fermentation and also early air bubble occurrence (Fig 1) .The pH, odour and colour change in grape wine was more significant and was taken as control to compare the fermentation efficiency of the other substrates during screening. Wine from figs had odour and colour change, but lacked suitable pH and fermentation odour. The observation was done, once in 5 days, and the results tabulated (Table 1 & 2).

Figure 1
Screening of sources

A - Inoculated Grape juice.
B - Inoculated Sweet potato juice.
C - Inoculated Fig juice

Table 1
Screening of sources

Parameter	Sources			
	Grapes	**Sweet potato**	**Fig**	**Beet -Root**
pH	3.9	4.02	3.75	4.9
Colour	Blood Red	Pale Yellow	Baby Pink	Dark red
Amount of micro organism inoculated	1.25ml	1.25ml	1.25ml	1.25ml
Air bubbles observed on	3rd Day	4th Day	5th Day	6th Day
odour	Fermentation smell	Starch smell	Fruit smell	Starch smell

Table 2
Screening of sources:

Parameter	Sources			
	Grapes	Sweet potato	Fig	Beet -Root
pH	3.9	4.02	3.75	4.9
Colour	Blood Red	Pale Yellow	Baby Pink	Dark red
Amount of micro organism inoculated	1.25ml	1.25ml	1.25ml	1.25ml
Air bubbles observed on	3rd Day	4th Day	5th Day	6th Day
odour	Fermentation smell	Starch smell	Fruit smell	Starch smell

3.1 SCREENING OF COMBINATIONAL WINE:

The screening of different combinational wines was done since the screening of individual sources like sweet potato, fig and beetroot confirmed the occurrence of fermentation. As a result of screening (Table 3 & 4), the best combinations were found to be sweet potato with grapes and sweet potato with beetroot and banana because of their good pH, colour and odour (Fig 2). The two combinations of sweet potato with fig and sweet potato with fig and grape were not that efficient when compared to the previous two.

Figure 2
Screening of combinational wine

A – Sweet Potato + Grape + Saccharomyces cerevesiae.
B - Sweet Potato + Fig + Saccharomyces cerevesiae.
C - Sweet Potato + Fig + Grapes + Saccharomyces cerevesiae.
D - Sweet Potato + Beetroot +Banana+ Saccharomyces cerevesiae.

Table 3
Screening of combinational wine:

Parameters	Different Combinations			
	Flask 1	Flask 2	Flask 3	Flask 4
	Sweet potato + Grapes	Sweet potato + Fig	Sweet potato + Grapes + Fig	Sweet potato + Beetroot+Banana
pH	3.5	4.5	3.6	3.4
Colour	Chocolate Brown	Yellowish	Light Brown	Dark Red
Odour	Fermentation Smell	Fermentation Starting Smell	Fermentation Starting Smell	Fermentation Smell
Air Bubbles	Presence	Presence	Presence	Presence

Table 4
Screening of combinational wine

Days	pH For Different Combinations			
	Sweet potato + Grapes	Sweet potato + Fig	Sweet potato + Grapes + Fig	Sweet potato + Beetroot+Banana
10	5.2	4.0	5.9	3.9
15	4.3	3.7	4.7	3.75
20	4.0	3.3	3.6	3.6
25	3.5	3.0	3.2	3.52

3.2 TITRATION TECHNIQUE

As the result of titration different combinations of wine gave various ranges of tartaric acid percentage (Fig 3).Tartaric acid is a major by-product formed during fermentation by yeast in alcohol formation. It gives a characteristic flavor to the wine and also helps in its preservation. The lesser amount of acid produced is an indicator of proper and complete fermentation and good quality wine with better ethanol production. So the combinational wines of sweet potato with grapes and sweet potato with beetroot and banana were selected as the best when compared to the rest. These were taken and fermented using the designed bio-reactor (Fig 4).The best was chosen at the end of the trial process and used for bulk production.

Figure 3
Fermentation Efficiency in Combinational wine

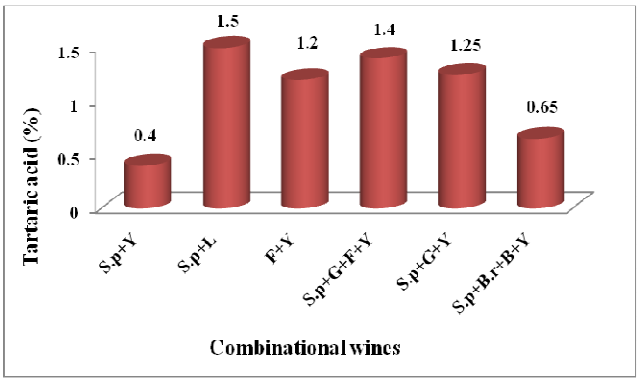
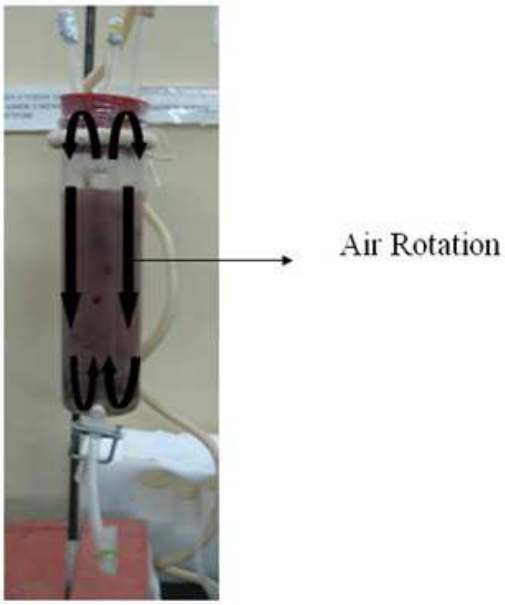


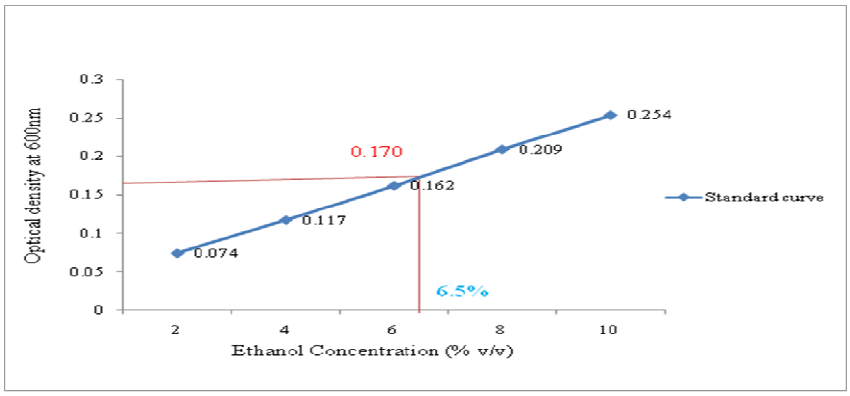
Figure 4
Airlift Bio-Reactor



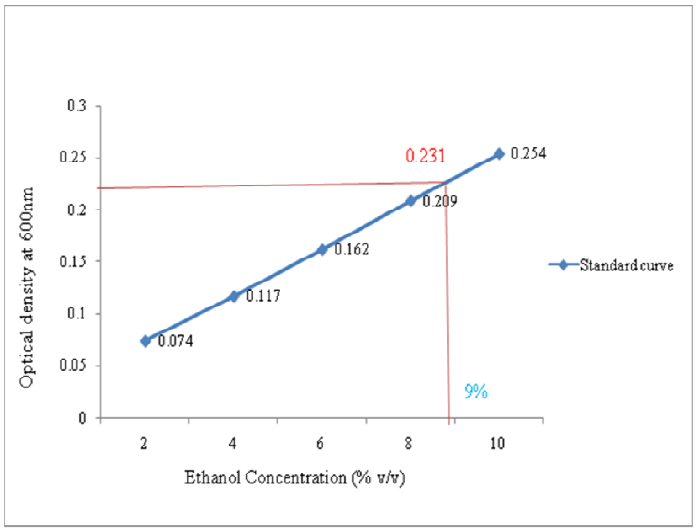
3.3 SPECTROPHOTOMETRIC DETERMINATION OF ETHANOL:

After the spectrophotometeric determination for ethanol in the samples, the values were plotted in a graph against the standard curve of ethanol concentration. The O.D at 600nm for sweet potato with grapes was 0.170 and for sweet potato with beetroot and banana, it was 0.231. Thus by plotting the values, the percentage of ethanol was found to be 6.5% and 9% respectively for sweet potato with grapes and sweet potato with beetroot and banana (Fig 5)

Figure 5
Spectrophotometric determination of ethanol
Ethanol concentration of Sweet potato+Grapes



Ethanol concentration of Sweet potato+Beetroot +Banana

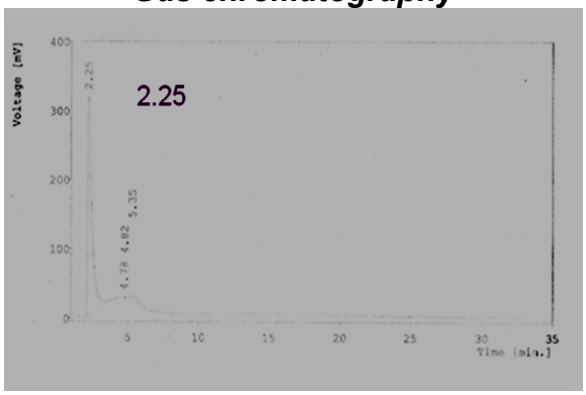


3.4 GAS CHROMOTOGRAPHY:

As the result of gas chromatography, the standard ethanol retention time was observed and noted. This was compared to the retention time of the samples and the ethanol content quantified. The retention time of sweet potato with grape sample was 2.25 and the retention time of sweet potato with beetroot and banana sample was 2.49. Then following the quantification formula, the ethanol percentage of the two samples were 6.3 and 9.15 respectively for sweet potato with grapes and sweet potato with beetroot and banana (Fig 6 & 7).The ethanol content determines the efficiency of any wine. The wines produced here contain a reasonable amount of ethanol. Sweet potato with beetroot and banana sample contained pure ethanol, but the sweet potato with grape showed some small deviation of curves indicating impurity

.

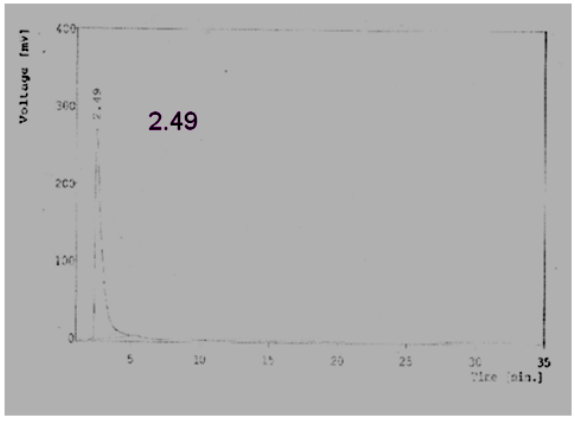
Figure 6
Gas chromatography



Result table – Calculation Method

Peak no:	Retention Ti	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	2.247	6345.7897	314.6311	0.3000	99.9553	99.4470
2	4.780	0.2109	0.3850	0.0200	0.0033	0.1217
3	4.820	0.4661	0.5543	0.0200	0.0073	0.2561
4	5.353	2.1589	0.8102	0.0533	0.0341	0.2561
-	Total	6348.6255	316.3808			

Figure 7
Gas chromatography



Result table – Calculation Method

Peak no:	Retention Time:	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	2.487	9202.1492	272.7982	0.4400	100.0000	100.0000
-	Total	9202.1492	272.7982			

3.5 ESTIMATION OF REDUCING SUGARS:

The percentage of reducing sugar present in the samples was estimated by Miller's method. During the procedure, the colour change in the samples was observed. The O.D of those samples and sweet potato with beetroot and banana was 0.398 and 1.009 respectively (Fig 8&9). and it was concluded that sweet potato with grapes contained 0.8% and sweet potato with beetroot and banana contained about 2% reducing sugar.

Figure 8

Estimation of reducing sugar

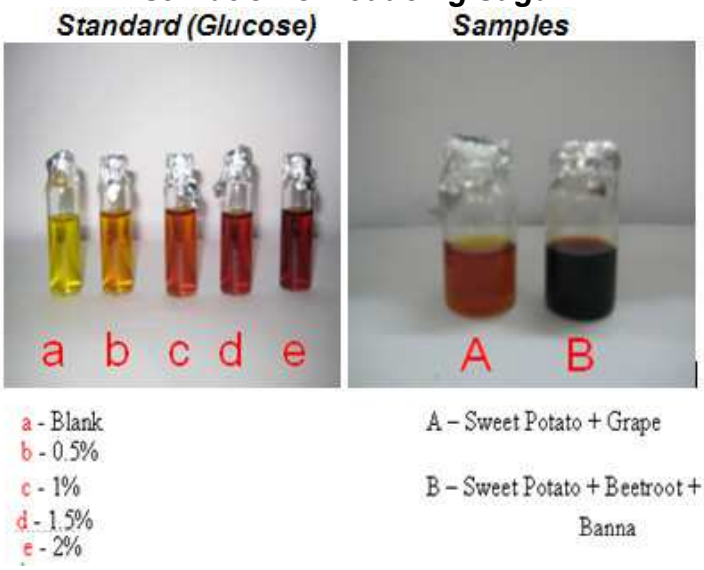
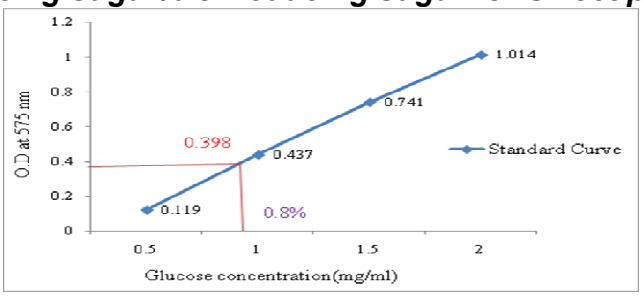
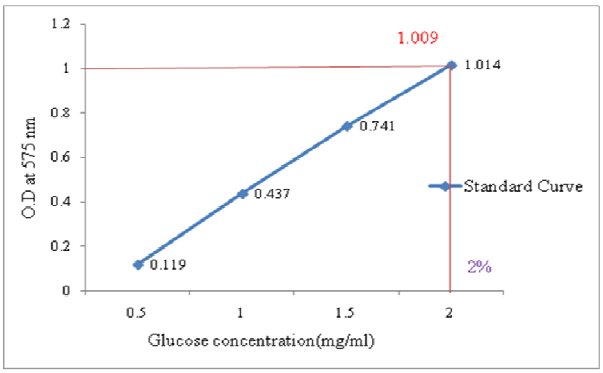


Figure 9

Estimation of reducing sugar% of reducing sugar for Sweet potato+Grapes



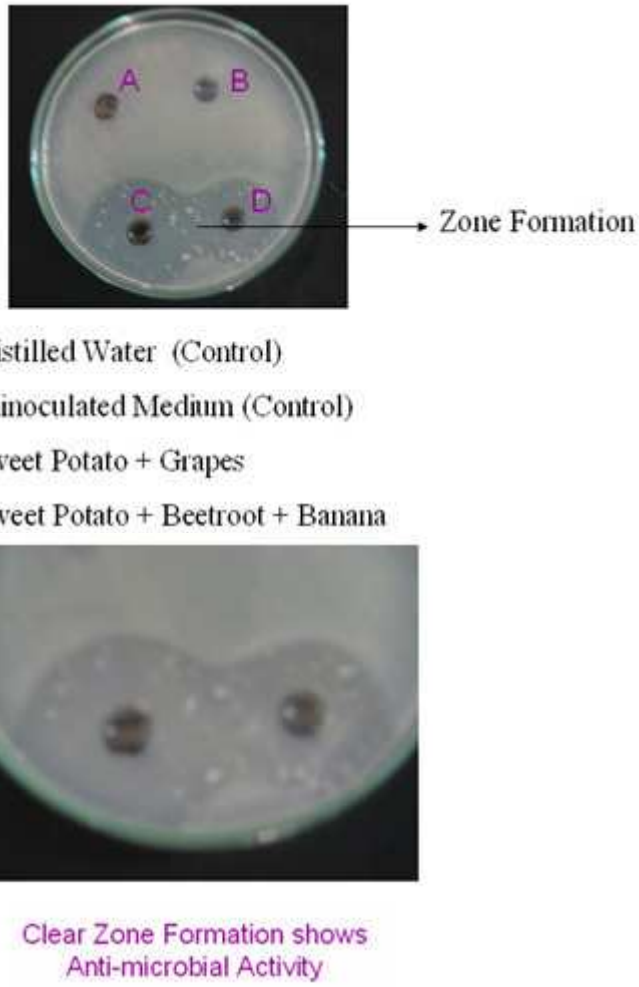
% of reducing sugar for Sweet potato+Beetroot+Banana



3.6 ANTI-MICROBIAL ACTIVITY

The anti-microbial test gave a positive result with clear zone formation (Fig 10) .The presence of ethanol in the wine inhibits microbial growth. Both the wine samples satisfied the anti-microbial test by forming an inhibition zone. Thus the wines have an anti-microbial ability.

Figure 10
Antimicrobial activity of wine



4. DISCUSSION

Recently, L. V. A. Reddy *et al.*, 2005 performed wine production with the most popular and the choicest fruit of India. They reported an ethanol concentration of 7–8.5% w/v in mango, that was slightly higher than that of grape wines and other volatile compounds were present in comparable amounts.

In this study, sweet potato combination gave an ethanol concentration of 7-9.5% w/v. Thus, sweet potato with beetroot combination worked better than mango. As wine is a costly alcohol beverage, the sweet potato combinations could act as a novel substrate and use of the air-lift bio-reactor would be a new methodology in fermentation that can be recommended to industries, in order to change the trend in wine production and upgrade it to a cost-effective product.

5. CONCLUSION

Wine is much more expensive than all other alcoholic beverages. Preparation of wine from a cheaper source could change its value. The market price of sweet potatoes is much lower, when compared to that of grapes and apple. Hence, wine produced from sweet potato can be cost effective. The use of sweet potato' with fruits is a novel substrate and Air-lift bio-reactor is a new method in fermentation, which would help industries to achieve cost-effective wine production.

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