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Production of Bioethanol From *Muntingia calabura* – An Under Exploited Fruit

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Abstract

The generation of bio-fuels from underexplored fruits forms an attractive solution towards energy generation. The utility of less utilized fruits as a possible source of ethanol production in a process without aeration was investigated by using fruits of Muntingiacalabura. The fruit juice was subjected to fermentation by Saccharomyces cerevisiae. The amount of ethanol produced after fermentation was analyzed by gas chromatography and the ethanol production is almost equal to any commercial fruit ethanol production. The results indicate the promising future for generation of ethanol from underexploited fruits on a large scale.

Keywords: bioethanol production, *Muntingia calabura*

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INTRODUCTION

Bioethanol as an alternative source of energy has received special attention worldwide due to depletion of fossil fuels. In India, sugar cane molasses is the main raw material for ethanol production. But the short supply and increased cost is the main hindrance for its use. The cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and is therefore expensive.[1,2] Fruit waste can also be used for ethanol production, but the demerit is its collection and the waste also contains unwanted microorganisms. Under such circumstances a novel approach is essential to use renewable substrates such as underutilized or underexplored fruits.[3-5] Among underexploited fruits, Singapore cherry (*Muntingiacalabura*) was chosen to examine its potential for production of ethanol. It is one of the less-known and less popular fruits. It belongs to the family Eleaocarpaceae. The *M. calabura*is a fast growing fruit tree, draught tolerant plant, capable of thriving in poor soils and it is also acidic and alkaline soils. It is known to be high fruit yielding, nearly all through the year.[6,7] It is popular in several South American countries. In developing countries like India it is being introduced as a shade provider or as avenue tree and not so much as a valuable fruit-yielder.[8,9] The fruits are rich in carbohydrates (14.28%).

MATERIALS AND METHODS

Collection of sample- Well-ripened fruits of Japanese cherry (*Muntingiacalabura*) were handpicked from the trees, washed with tap water and stored in refrigerator until taken up for further processing.

Determination of reducing sugar- 500gms of fruits was mashed using blender and suspended in 1liter of water. The total reducing sugar content of the sample was determined by DinitroSalicyclic Acid (DNSA) assay method described by Miller (1959).[10]

Microorganism and culture media- Pure cultures of *Saccharomyces cerevisiae* was obtained from MTCC and culturedon Saboraud dextrose agar (SDA) media at 30˚C. The cultures were stored at 4˚C and subcultured every 30 days.

Inoculum preparation: The inoculum was prepared by inoculating the slant culture in 10ml test tube incubated overnight then 3% inoculums transferred to 100ml of sterile Saboraud dextrose broth medium taken in 250ml flask and cultured on a rotary shaker (90rpm) for 48 hours.

Fermentation-The slurry was supplemented with 250gms of cane sugar, 10gms of ammonium chloride and 0.58 gms of potassium metabisulfite and pasteurized at 85^0C-90^0C for 5 minutes.After adding the developed inoculums (10%) to the bulk of the fruit mash, fermentation was allowed for 15 days. Small aliquots were drawn once in 5 days for analysis. At the end of 15 days the fermented material was filtered by suction through a pad of washed filter paper pulp. This yielded a clear pale brown colored fluid. The ethanol produced was determined by Gas Chromatography.

Physico-chemical analysis: The TSS content was determined using Erma hand refractometer.
The pH was determined using and ELICO model digital pH meter. The acidity and total sugar were determined as per the standard protocols.

Physico-chemical analysis was carried out once in five days during fermentation. The observations were also recorded. The parameters of observations were acidity, alcohol, microbial count and TSS.

RESULTS AND DISCUSSION

Total soluble solids(TSS)- The final TSSafter fifteen days of fermentation in *Muntingiacalabura* was varied between 15 °Bx(DegreesBrix) to 10 °Bx. The TSS of the fruit juice on the initial day of fermentation was 15 °Bx. It kept on decreasing during fermentation. As the alcohol content increases, the content of TSS decreases. The results are shown in the table and in the graph-1.

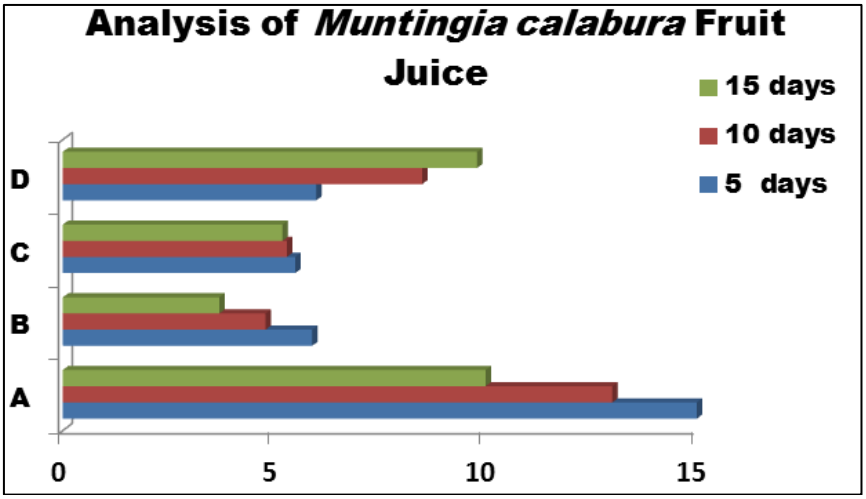
pH- The pH of must on initial day of fermentation was determined(5.9). Then the decline of pH was rapid up to 15 days. The results are shown in the table and in the graph-1. The pH of the fruit juice varied between 5.9 to 3.7. Subsequently this value is decreased in the fifteenth day of fermentation indicating an increase in acidity.

Microbial count- Microbial count (yeast cell count) during fermentation was determined by haemocytometer. Then the decline of microbial count was rapid up to 15 days. The microbial population showed logarithmic increase in number initially and subsequently there was decline in its populations. This could be due to the fact that higher concentration of ethanol inhibited the growth and multiplication of yeast during fermentation. The results are shown in the table and in the graph-2.

Acidity: The volatile acidity constituted majority of the total acidity was determined. Then thedecline of acidity was rapid up to 15 days. The results are recorded in the Table and shown in the graph-1. In all the treatment the trend of volatile acidity was similar to that of Total acidity. The Nonvolatile acidity showed an initial increase followed by decrease in all fermentation broth. The decreased in the acidity during fermentation in the juice could be due to its utilization by the yeast for production of carbon dioxide and water.

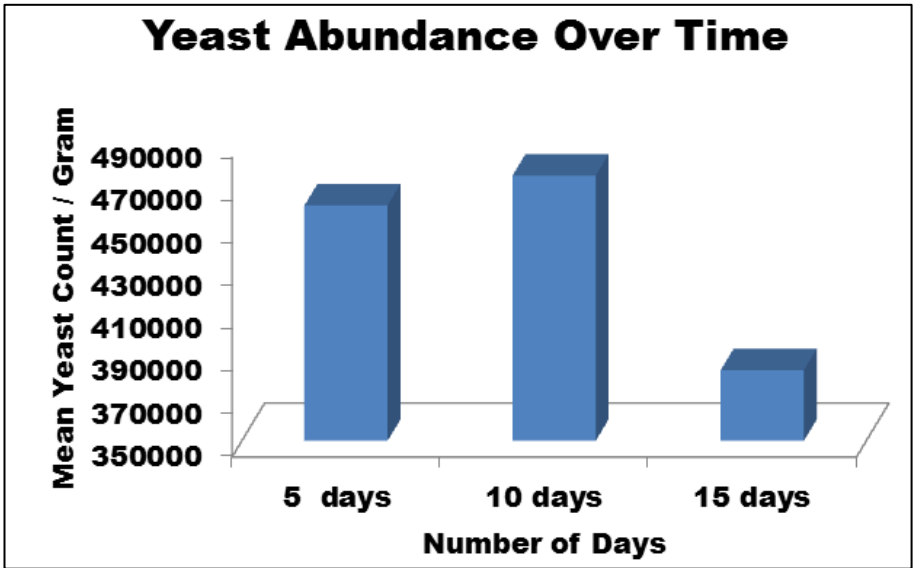
Alcohol content: The alcohol content of fermented broth was determined. It was shown in Table and in the graph-1. The alcohol content in fruit juice showed an increasing trend during fermentation.

Sl. no.	Analysis of *Muntingia calabura* fruit juice	No. of days		
		5	10	15
1	Analysis of Total Soluble Solids(TSS) during fermentation in (°Bx)	15	13	10
2	Analysis of pH during fermentation	5.9	4.8	3.7
3	Analysis of microbial count	460×10^3	474×10^3	383×10^3
4	Analysis of acidity during fermentation	5.5	5.3	5.2
5	Alcohol Production (%)	6	8.5	9.8



Graph 1. Analysis of Muntingiacalabura Fruit Juice

A. Analysis of Total Soluble Solids(TSS) during fermentation (Degrees Brix)
B. Analysis of pH during fermentation
C. Analysis of acidity during fermentation
D. Alcohol Production (%)



Graph 2. Yeast abundance over time

From above table results, it was shown that, the alcohol content after fifteen days was 9.8%. In this fruit's fermented broth significant differences in the content of TSS, acidity, pH and alcohol content was noticed. This also depends upon content of alcohol in the wine. As the alcohol content increases in the wine, the content of the TSS, microbial count and pH decreases.

SUMMARY

The present report has indicated the potential of *M. calabura*fruit for economic exploitation and the ethanol production is almost on par with commercial fruits like papaya, grapes, orange, banana etc. As *M. calabura* is a less edible fruit, almost all the fruits are wasted. Now a days it is spreading fast in all the regions, its number is increasing exponentially because of entomophily and fast growth of the plant. It is also becoming popular in India. Instead of wasting the fruits we can use them for cost effective production of ethanol throughout the year at the industrial scale.

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