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Estimation of sugar and bio ethanol from different decaying fruits extract

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ABSTRACT

Bio ethanol mainly produced from biological methods involving fermentation of cellulosic biomass in a broad spectrum. Since it is being widely recognized as an environmental friendly transportation fuel with powerful economic and strategic applications, here an attempt is made to explore the possibilities of bioethanol production from decaying fruits. Since they pose a major socio economic challenge and in turn by utilizing these kinds of wastes for fuel purpose reduce the burden on the authorities. Hence, bioethanol production from three different decaying citrus fruits like Citrus sinensis, Citrus limetta and Ananas comosus were studied. The sugar content before and after fermentation was analyzed by DNS method, the result showed that the sugar content was more before fermentation (Citrus limetta 21mg/mL, Ananas comosus 20mg/mL and Citrus sinensis 17 mg/mL,) when compared to after fermentation (Citrus limetta 16mg/mL, Citrus sinensis 12.5mg /mL and Ananas comosus11mg/mL). The production of ethanol was higher in Ananas comosus (13%) than the other two Citrus limetta (12%), Citrus sinensis (10%) which was calculated by distillation method.

Key words: Sugar, Bio Ethanol, Decaying Fruits.

INTRODUCTION

Rapid increase in population size and exponential growth in industrialization loads on fossil fuel resources and the resources are being depleted very fast. Some alternative fuels like Bio-fuel may reduce the load on conventional fossil fuel resources. In addition Brazil had a fleet of more than 10 million flexible-fuel vehicles regularly using neat ethanol fuel [1]. According to the International Energy Agency cellulosic ethanol could allow ethanol fuels to play a much bigger role in the future than previously thought [2]. Fuel ethanol production has been fascinated now, because many countries look for reducing oil imports, boosting rural economies and improving air quality so the world ethanol production has reached about 51,000 million liters [13]. Production of ethanol from sugary materials is easier than lignocellulosic materials, since it requires additional technical challenges such as pretreatment [3]. The production of ethanol from fermentative microorganism is the only possible way to meet the great demand for ethanol in the present situation of energy crisis [4]. The ripen fruit biomass as raw materials for fermentation, enzymatic hydrolysis using microbial enzymes could be a possible solution to reduce the energy and input costs in ethanol production [5]. Significant quantities of decaying fruits are discarded as wastes which cause real environmental problems [6], these can be used as potential feedstock for bioethanol production and this could also be an attractive alternate for disposal of the polluting residues [7]. However, little effort has been made in order to explore conversation of sugar present in different decaying fruits waste juice for potential application in bio-ethanol production by fermentation techniques.

India is the second largest producer of fruits in the world particularly pineapple and oranges which accounts for 30 million tones out of this seventy-two per cent of fruit waste due to lack of proper retailing and adequate storage

capacity. Overall fruit production has been consistently notching up impressive growth year after year despite because fruit is a key component of a healthy balanced diet. On a commercial note very little of fruit is exported in the absence of adequate processing and refrigeration/storage/cold chain facilities the situation is made worse by callous and antiquated post-harvesting practices, a staggering quantity of the produce rots in the fields or dangerously decays while in the process of transportation, storage and in the last leg of reaching the consumer. The value of this wastage is estimated at a whopping Rs 20,000 to Rs 50,000 crore annually. Worse, the pile up of the putrefying mass at assorted places becomes a breeding ground for disease and pests and poses a major challenge to the municipal authorities charged with keeping the civic areas clean. The developed world has better management of fruit waste. Now technology has made it possible to convert the decomposing fruit into an essential commodity a renewable resource bio-fuel.

Ethanol fuel has long been seen as a clean alternative fuel to petrol. However in the heated debate researchers have hit upon a novel idea i.e. using decaying fruit to make ethanol. These developments could not have come at a better time for India. Even as we struggle for infrastructure, post-harvesting, refrigeration and related solutions for farm produce the year-round supply of large quantities of rotting fruit currently a major disposal problem can well be turned on its head where decomposing and offending item can well be converted into ethanol. In this connection the purpose of this research was to investigate the feasibility of using decaying fruits for sugar and bio ethanol production in Bangalore city.

MATERIALS AND METHODS

Collection of the fruit wastes

Decaying citrus fruits like *Citrus sinensis* (Rutaceae), *Citrus limetta* (Rutaceae) and *Ananas comosus* (Bromeliaceae) were collected from Bangalore city market, brought in a sterilized plastic bag to the laboratory and stored in a refrigerator until further usage.

Preparation of Fruit Juice Extract

The collected decaying fruits were surface sterilized by washing in 5% Potassium Permanganate and rinsed well in distilled water. About 10g of decaying fruit sample was taken in a pestle and mortar, grinded well with the addition of about 20.0 mL of Distilled water. The liquid extract was obtained by filtering the grinded content using a cheese cloth. The liquid extract was suitable diluted to 1:100 with distilled water (v/v).

Estimation of Sugar content

Estimation was done both before and after fermentation to determine the reducing concentration of sugar in different extract, DNS Method was followed. Standard graphs have been plotted by using Glucose solution (200µg/mL of working standard). To the above samples 2 ml of DNS reagent was added into a lightly capped test tube. (To avoid the loss of liquid due to evaporation, cover the test tube with a piece of paraffin film if a plain test tube is used). The mixture was heated at 90ºC for 15 minutes to develop the red brown color. Thereafter 16 ml of distilled water was added to stabilize the color. After cooling it to room temperature in a cold water bath, absorbance was measured with a spectrophotometer at 540nm. Hence, the above procedure was repeated for 1.0mL of extract and 1.0mL of water has been taken for unknown estimation.

Fermentation of fruits extracts

First the fruits extracts were kept for aerobic fermentation with yeast (*Saccharomyces cereviseae*), 5% of the starter culture were inoculated into the sterilized extract after 24 hours. The fermentation was allowed to take place for 5 days at 30±2°C. The fermenter was designed such that a muslin cloth was used to cover the fermenter to allow the supply of oxygen [8], later it was subjected to anaerobic phase of fermentation and the fermenting vessel was made air tight by covering it with a lid and sealing the edge with paper tape. The fermentation was allowed to last for 9 days and terminated on the 9^{th} day. Then it was racked, settled yeast and other debris was clarified using Gelatin a fining agent [8].

Determination of Alcohol percentage in fermented extracts

Simple distillation method followed, add 150 mL of room temperature fermented extracts to a 250 mL graduated cylinder, transfer contents of the graduated cylinder to the 250 mL boiling flask, rinse down walls of the graduated cylinder with approximately 50 mL of distilled water and add this rinsed water to the boiling flask (This will capture virtually all of the alcohol still remaining in the graduated cylinder. The extra volume of water will also prevent the

flask from boiling dry), Add a few boiling stones to the boiling flask to prevent violent bumping while boiling, turn-on the cold water intake to keep the condenser cool, place the 250 mL graduated cylinder under the condenser's output to collect the distillate, light the Bunsen burner, set to a low flame and bring the sample to a slow boil, keep boiling the fermented juices until approximately 140 mL of clear distillate has collected in the graduated cylinder (This takes approximately 30 min depending upon the boiling rate). Make sure that do not allow more than 150 mL of distillate to collect. Remove the graduated cylinder from the apparatus and allow its contents to cool to room temperature (A cold water bath can be used to accelerate the cooling process), Once cool, carefully add distilled water to the distillate until the volume reaches 150 mL (The same volume as the original sample), Use short-range hydrometer to measure the specific gravity (SG) of the room temperature distillate, next the specific gravity measure to %Alcohol by volume. Alternatively use a special alcohol hydrometer that's calibrated to read %Alcohol directly (thereby eliminating the need to use the lookup table). Finally using a SG hydrometer use the following equation to convert SG to %Alcohol [9].

%Alcohol (v/v) = 8610.6 – (16584 x SG) + (7973.3 x SG2)

Where SG = specific gravity of the distillate returned to the original 150 mL volume.

The experimental design was completely randomized, with three replicates all data were expressed as mean values ± SE. The comparison between the mean values were tested using Duncan's new multiple range test and the ANOVA was also performed to find out the LSD ($p < 0.05$).

RESULTS AND DISCUSSION

Estimation of Sugar

The standard graph for the estimation of reducing sugar have been plotted by using glucose as working standard solution (200ug/mL) and the values have shown in table 1 and figure 1 and 2. The sugar content of decaying fruit extracts have been calculated by comparing their optical density values at A_{540} with the standard graph. The individual values were taken in triplicates and the mean values have been entered. Among the three extracts used for the analysis of reducing sugar content (glucose level), the result shows that the glucose level was high in *Citrus limetta* (210 µg/mL) when compared to *Ananas comosus* (200 µg/mL) and *Citrus sinensis* (170 µg/mL)before fermentation. Since the extracted juices were high in sugar content, they had been diluted to 1:100 with distilled water.

Hence, the actual values of sugar concentrations in *Citrus limetta*, *Ananas comosus* and *Citrus sinensis* were 21mg/mL, 20mg/mL and 17mg/mL respectively (Table 1 and figure1).

Table 1: Estimation of sugar by DNS method

Vol. of Std. glucose solution (ml)	Vol. of distilled water in ml	Amount of glucose in µg/mL	Vol. of DNS reagent in ml		Vol. of distilled water in ml	Absorbance at 540nm
0.0	2.0	0.0	2ml	Keep the tubes in boiling water bath for 15min	16.0ml	0.00
0.2	1.8	40				0.11
0.4	1.6	80				0.22
0.6	1.4	120				0.32
0.8	1.2	160				0.41
1.0	1.0	200				0.51

Table 2: Estimation of sugar in decaying fruit extracts before fermentation

Vol. of sample (ml)	Vol. of distilled water in ml	Vol. of DNS reagent in ml		Dist. H_2O ml	Absorbance at 540nm
Citrus sinensis 1.0	1.0	2ml	Keep the tubes in boiling water bath for 15min	16.0	0.45
Citrus limetta 1.0	1.0				0.50
Ananas comosus 1.0	1.0				0.49

Values are the means ±SE of three replicates each. Data were subjected to analysis of variance and compared for significance according to DMRT (P=0.05).

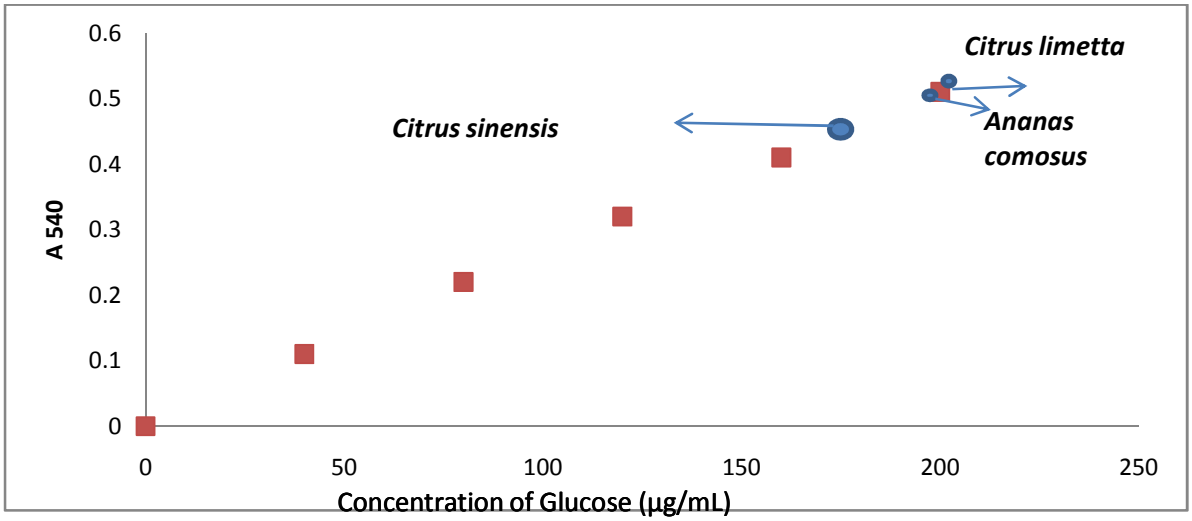

Figure 1: Estimation of sugar in decaying fruit extracts before fermentation

After 9 days of fermentation sugar concentrations of *Citrus limetta* 16mg/mL, *Citrus sinensis* 12.5mg/mL and *Ananas comosus* 11mg/mL respectively (Table 3 and figure 2).

Table 3: Estimation of sugar in decaying fruit extracts after fermentation

Vol. of sample (ml)	Vol. of distilled water in ml	Vol. of DNS reagent in ml	Keep the tubes in boiling water bath for 15min	Dist. H_2O ml	Absorbance at 540nm
Citrus sinensis 1.0	1.0	2ml		16.0	0.30
Citrus limetta 1.0	1.0				0.41
Ananas comosus 1.0	1.0				0.28

Values are the means ± SE of three replicates each. Data were subjected to analysis of variance and compared for significance according to DMRT (P=0.05).

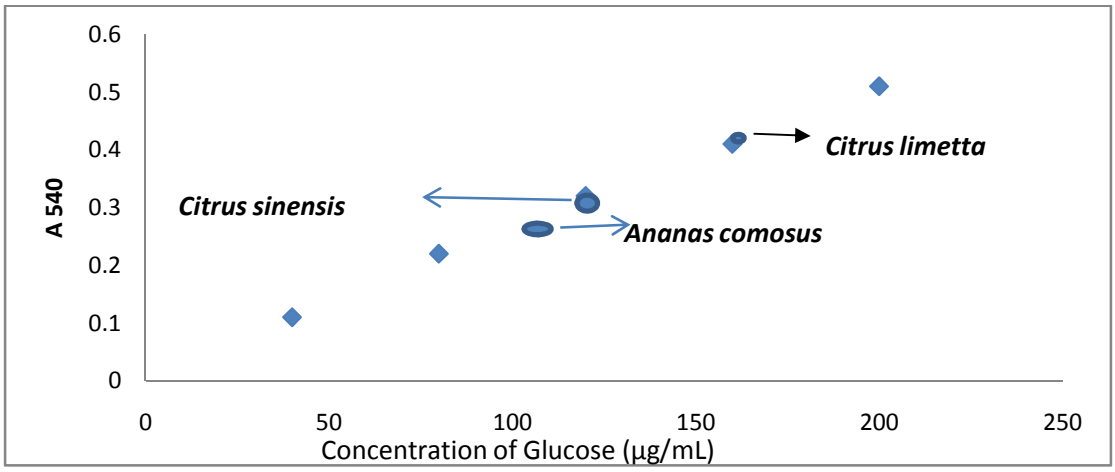

Figure 2: Estimation of sugar in decaying fruit extracts after fermentation

Determination of Alcohol percentage in fermented extracts

After 9 day of fermentation total volume of 200ml ethanol was obtained from a total volume of 1000ml of substrate after distillation. In this present study efforts were made to identify which decaying fruit is potential raw material for bioethanol production and the results showed that *Ananas comosus* gives highest percentage of alcohol (13%) compare to *Citrus limetta* (12 %) and *Citrus sinensis* (10 %). Using higher grade distillation assembly a more

concentrated product can be recovered by re distillation which can be used as a bio fuel. The substrates used are very cheap raw material and the process was found to be very easy and less cost effective (Table 4).

Table 4: Alcohol percentage in fermented extracts

Decaying Fruits	Percentage of alcohol
Citrus sinensis	10
Citrus limetta	12
Ananas comosus	13

Values are the means ± SE of three replicates each. Data were subjected to analysis of variance and compared for significance according to DMRT (P=0.05).

The present investigation on sugar and ethanol estimation from decaying fruits extracts shows that, they will play an important role in providing a cheap source for ethanol production these observations concur with [10] who has shown that Pineapple (*Anamas Checa* L) Papayas (*Carica papaya* L) are potential sources for alcohol production the data from the present study reveals the same that maximum production of ethanol is in *Ananas comosus* using yeast than the other two fruits. In our studies extraction and fermentation have evinced maximum ethanol production which is reflected the studies of [10].

Ethanol production from spoiled starch rich vegetables by sequential batch fermentation was studied by [11] where as studies in decaying fruits like *Citrus limetta*, *Ananas comosus* and *Citrus sinensis* has not been done so far, so in the present investigation in production of with standard physiochemical parameters and saccharification process are studied

This preliminary study showed that ethanol production from fruit wastes residue is possible and the inhibitors may reduce the extent of fermentation (Lignin prevents the degradation of cellulose mainly by acting as a physical barrier between the cellulolytic enzymes and their substrate [12] where the present study shows a significant removal of lignin from the fruit waste residues which resulted in higher production of ethanol. Further research is needed to carry out to determine the optimum conditions for maximum production of ethanol from fruit waste residues.

CONCLUSION

The production of ethanol from these decaying fruits can be further improved by using suitable technologies i.e., using genetically engineered strains that are capable of converting multiple sugars in to ethanol. The major bottlenecks are feedstock collection, storage, transportation and pretreatment, hence optimizing these unit operations through using un-marketed decaying fruit wastages can be proved beneficial.

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