

# Bioethanol Production by Sub Merged Fermentation from Carob Pod Extract by using *Saccharomyces Sps*

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## Abstract

The exploration of rich in sugar residues of carob pod (*Ceratonia siliqua*) for bioethanol production has been investigated. The main types of feedstocks for the production of bioethanol are raw materials contain different form of fermentable sugar. The aim of this study is to evaluate the bioethanol production in submerged fermentation from carob pods. The extraction of sugars from the carob pod was conducted, achieving very good yields in a short period of time. *Saccharomyces sps* were isolated from different fruit samples and identified. Among the isolated *Saccharomyces sps* 01 showed the 20% , *Saccharomyces sps* 02 produced 11%, and *Saccharomyces sps* 03 showed minimum of 9% of ethanol production. The sugar syrup was allowed to fermentation process with different pH and temperature. At pH 5 showed maximum production compared to other pH condition. The maximum production of ethanol was seen at 30°C compare with different temperatures. The outcome of this experiment revealed that carob pod which is neglected and considered as agro sated can be used for large scale production of bioethanol by using specific yeast strains.

**Keywords** - Carob pod, Bioethanol and Yeast

## I. INTRODUCTION

The carob pod is the fruit of the carob tree (*Ceratonia siliqua*), which is one of the Raw materials for production of ethanol by microbial fermentations. Carobs is found not only in wild form but also in cultivated forms [1][2]. Carobs have been cultivated for 4.000 years. Carob trees have many distinct advantages over traditional crops such as high carbohydrate yield, good growth in poor soil under favorable dry farming conditions. Due to high carbohydrate content, it is possible to use carob as an interesting source for bioethanol production. Carob pod has usually been neglected for a long time alternative utilization especially about biotechnological processes and fermentation. In recent years, carob has attracted considerable attention because of high carbohydrate and mineral content.[3][4] The demand of alternative energy in developed societies to join the development process

of other countries is accelerating a decrease in fossil energy stocks and an increase in environmental degradation. Bioethanol production from agro wastes requires several methods, including the pretreatment of biomass with subsequent enzymatic hydrolysis followed by fermentation.[5] The ethanol production from carob pod extract by using *Saccharomyces cerevisiae*. During the fermentation process, sugars are converted into ethanol and carbon dioxide with the help of yeast.[4] Microorganisms such as yeasts play an essential role in bioethanol production by fermenting a wide range of sugars to ethanol. They are used in industrial scale due to valuable properties in ethanol yield, ethanol tolerance, ethanol productivity, growth in simple and inexpensive media and undiluted fermentation broth with resistance to inhibitors and retard contaminants from growth condition[6]

The aim of the present study is to evaluate the suitability and utility of carob pods as best substrate for the production of ethanol (Alcohol) employing locally isolated yeast strain. The carob pod contains about 50-87% of sugars (sucrose) and proteins about 2.5% which can be easily converted into ethanol (Alcohol). Although the utility of other agricultural wastes or agro based materials have been studied, literature on the suitability of carob pods is scanty. Further, the use of carob pods for the production of ethanol (Alcohol) is nowhere reported. These carob pods are easily available and the costs of production are moderate when compared to other conventional substrates used for ethanol (Alcohol) production. Therefore, in the present study efforts have been made to use carob pods for the production of ethanol (Alcohol) by employing yeast strain under submerged fermentation (SmF).

## II. MATERIALS AND METHODS

### A. Isolation of yeast strains

Different fruit samples like grapes, chikoo and sugarcane were collected from different parts of Bengaluru. The collected samples were immediately brought to laboratory and serially diluted, plated on yeast isolation agar medium (10 g/L yeast extract, 10 g/L peptone, 20 g/L agar) and were incubated at 30°C for 48 hours. The colonies appeared were further identified on the basis of colony characters. The

selected colonies were identified based on their microscopic features by Lugol's iodine. The colonies showing standard characters were picked up and subjected to further studies.

### B. Characterization of yeast isolates

Characterization of yeast is done by morphological and biochemical characteristics. Morphological characteristics show flat, smooth, moist, glistening or dull, and cream in color. Biochemical characteristics show assimilation of nitrogen with carbohydrates. Further tests show fermentation tests with different sugars.

### C. Sugar fermentation test

Carbohydrate fermentation broth with bromocresol purple as an indicator was prepared with different sugars like glucose, maltose, mannitol and sucrose. The test isolates were inoculated and incubated at 30°C for 24 hrs and acid production in medium was recorded by change in color from purple to yellow and gas production by observing the gas in Durham's tube.

### D. Collection of carob pods

Carob pods were obtained from in and around Bengaluru, brought to laboratory, shade dried and stored in room temperature. The pods were chopped into small pieces.

### E. Extraction and Estimation Sugars

Sugar extraction from carob pods were carried out with water at different ratio such as 10 g of ground carob pod was mixed with appropriate amount of water and boiled at 80°C for 1hr. Then the mixture was filtered and the extract was analyzed for its content of total sugars. Sugar level was checked using a Brix meter.[6]

### F. Production of alcohol

The filtrate was inoculated with three different yeast strains at 3%. The filtrate was mixed well. The mixture was kept in aerobic condition for two days. From the third day fermented flask is maintained in anaerobic condition and incubated for five days at 30°C temperature

### G. Estimation of alcohol

After fermentation the yeast cells were separated by filtration. The liquid part was distilled by Claisen condenser (distillation) apparatus. The fractions were boiled up to 70°C and collected fractions were obtained. The analyzed fraction was estimated for ethanol percentage (v/v) by specific gravity method at 20°C.[1]

### H. Fermentation kinetics

The sugar syrup was obtained by boiling method and varied with different parameters such as pH and temperature.

### I. Effect of pH on production of alcohol

Sugar syrup was prepared and was set with different pH (5,6,7,8) using NaOH and HCl and subjected to fermentation followed by distillation process. We also checked the productivity of alcohol from specific gravity method.

### J. Effect of Temperature on production of alcohol

Sugar syrup was prepared and set at different temperature such as 25, 30, 35, 40° C and kept in incubator and allowed for fermentation to occur. After fermentation we subjected it to distillation process and checked the productivity of alcohol using specific gravity method.

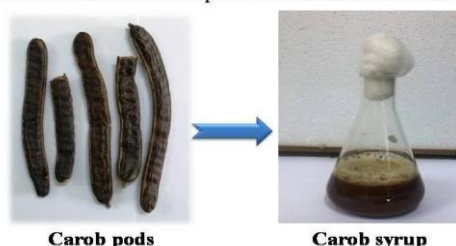
## III. RESULTS AND DISCUSSION

The concerns of rapid growth of human population, the ever-depleting natural resources, environmental pollution and industrialization lead to the critical need for alternative sources for fuel and energy production.[7] The global production of large-scale bioethanol is increasing as it can be used as an octane booster or fuel additive, even as a neat fuel source. Alcoholic fermentation by *S. cerevisiae*, a robust yeast strain, is significantly affected by the presence of pretreatment process products The paper indicate that the degree of inhibition of toxic compounds is primarily dependent on the nature, classification and concentration of the inhibitor compound as well as the growth phase of the microorganism. [8][9]

Three species of yeast were isolated from the fruit samples like grapes, chikoo and sugarcane on Yeast isolation agar. Based on the colony characters and the microscopic observations, the isolates were identified and labeled as *Saccharomyces sp* 01., 02 and 03. Carbohydrate utilization test for the yeast isolates showed positive result for glucose, sucrose & maltose and but *Saccharomyces sp.* 03 showed negative to Mannitol fermentation.

The carob pod (Fig. 1) which was processed for the extraction of fermentable sugars, showed 14% of sugar content which was observed using the Brix meter. The sugar solution extract was used for the fermentation using the three isolates to produce alcohol. The substrate carob pod satisfies many parameters of suitability, hence it has been preferred & selected as the suitable for production of ethanol through submerged fermentation.[9]

Figure 1 Collection of carob pods and extraction of carob syrup

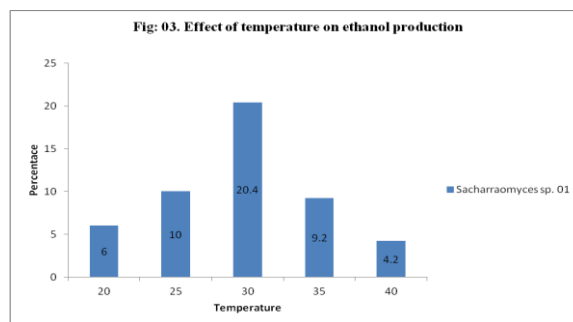
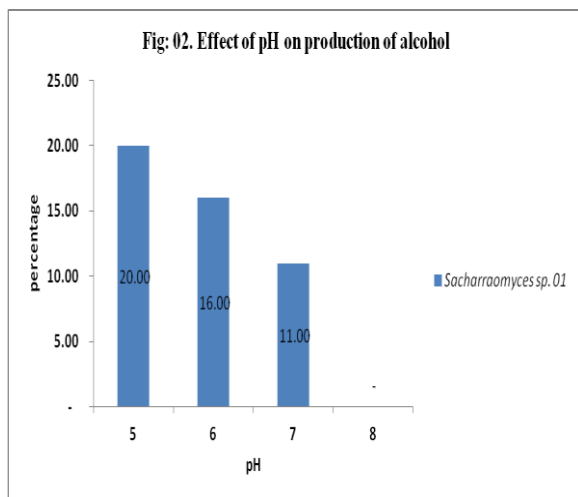


The fermented broth was subjected to distillation and then estimated by the specific gravity method and the percentage yield was finally compared from the association of analytical communities (AOAC) chart (Table 2). Among three isolates *Saccharomyces* sp. 01 produced maximum ethanol production of 20%, where as *Saccharomyces* sp. 02 produced 11 % and minimum ethanol (9%) was produced by *Saccharomyces* sp. 03.

**Table 2: Ethanol Production by Yeast isolates**

MICRO ORGANISMS	ALCOHOL PRODUCED Specific gravity	ACCORDING TO AOAC CHART
<i>Saccharomyces</i> sp. 01	0.9732	20.40%
<i>Saccharomyces</i> sp. 02	0.9842	11.44%
<i>Saccharomyces</i> sp.03	0.9972	9.12%

The sugar syrup was allowed to fermentation process with different pH (Fig: 02) and temperature (Fig: 03) parameters and distilled using distillation apparatus and checked the productivity using specific gravity method. Fig; 02 shows pH 5 maximum production of 20 % alcohol compared to pH 6 (16%) pH 7 (11%) and pH 8 no alcohol production. The sugar syrup was further allowed to fermentation process with different temperature, where maximum production of 20% ethanol was seen at 30° C, compared to other temperature (Fig:03). The results obtained was similar to those of results reported by Singh and Bishnoi (2013)[10] and Turhan et al. (2010)[11] that optimum pH value for ethanol fermentation by *S.cerevisiae* was 5.5 and temperature 30° C.



The outcome of the project reveals that Carob pod which is neglected and considered has an agro waste can be used for large scale production of ethanol. Further a promising strain *Sacharraomyces* sp. 01 can also be exploited to covert material into useful product in large scale production of bioethanol due to its maximum production.

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