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CAROB POD-A NOVEL SUBSTRATE FOR FRUCTOSE SYRUP AND ETHANOL PRODUCTION USING FRUCTOSE NEGATIVE MUTANTS BY SUBMERGED FERMENTATION

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ABSTRACT

Use of Carob pods for fructose syrup and ethanol production with two mutant strains *Zymomonas mobilis* and *Saccharomyces cerevisiae* were investigated. the carob pod was found to be a better substrate than sugarcane waste, pineapple waste, Banana waste and yielded maximum amount of fructose syrup and ethanol with the mutant strains of KLR5 and KLR3after 120 hrs of fermentation they yielded fructose 400g/lt and ethanol2.8% and *Saccharomyces cerevisiae* KLR3 yielded 380g/lt and ethanol2.4%. As such carob pod satisfies many parameters of suitability, hence it has been preferred & selected as the suitable substrate.

KEYWORDS: Carob pod, sugarcane Fructose syrup, Ethanol, *Zymomonas mobilis, Sacchromyces cerevisiae*, submerged fermentation.

INTRODUCTION

Fructose, also called Levluose or fruit sugar, is naturally occurring sweetest nutritionally sweetener,[1] it is widely used in the food industry. It is a caloric free compound, i.,e., it is scarcely hydrolysed by the digestive enzymes and not utilized as an energy source in the body.

Carob tree is an evergreen sclerophyllous plant, which naturally grows on barren, rocky and dry regions. it is accepted that c.siliqua is a native of Tunisia Algeria. Later the plant spread to middle east(Israel), Greece etc. Today the Carob tree also grows in the warmest regions of the Mediterranean and also in India. Carob fruit (Bean) is chocolate- brown in colour, lustrous and varies in weight(5-30g) and size. The bean size reaches in some cases 25cm in length and 4cm in width and bent like a horn (hence named as keratin =horn). Each pod may contain up to 15 seeds; carob seeds & pod are edible.

The chemical composition of the carob husk varies, depending on the carob variety& producing Country& District.(Soil, climatic & cultural conditions).

The sugar contents of the carob husk depend on the carob variety fillage of the trees and soil climate conditions. The water soluble sugars, mainly sucrose, fructose and Glucose are present in carob husk. Sucrose is the major constituent its level being16.2-47.3% on dry husk weight basis and constituents 50-80.7% of the total water soluble sugars. Thus treated carob pod is used in various food industries like confectioneries, beverages, bread &marcaroni making etc.The carob powder is used as a treatment of acute infantile diaahae due to the low cost of carob pod and high content of water-soluble sugars(up to 60%) it is used as feedstock for the production of industrial ethanol by fermentation, since the main fermentable sugar is sucrose.Thererfore several studies have been carried out to produce fructose syrup and ethanol at low cost.[11]

MATERIALS AND METHODS

In order to select a specific substrate for producing the fructose in a larger percentage various substrates like sugarcane waste, pineapple waste, banana waste as well as carob pods were finely chopped &maintained in a waring blender and fine pulps were obtained.

Analysis of chemical composition of carob pod

Collect the carob pods and they were deseeded & finely ground. Powdered carob pod as well as fruit pulp mixed with distilled water and adjust the sugar concentration. The supernatant were decanted & analysed for total carbohydrates, reducing sugars and Sucrose.[3]

Estimation of trace metal Elements

The collected supernatant were tested for the trace elements like Cu+ Fe+ Mn+& Zn+ by using an Atomic Absorption Spectrophotometer as per Arnold et al.,as shown in Table-1.

Isolation and screening of fructose negative mutants

Strains of *Zymomomnas mobilis* and *Saccharomyces cerevisiae* are used for screening fructose negative mutants by chemical mutation studies by using mutagenic agent Ethyl methane sulphonate (EMS) is treated in different concentration. These organisms are previously fructose utilizing organisms after chemical mutation they changed the character and those strains are fructose not utilizing and they utilizing only glucose, fructose as it remains.

Confirmation of fructose negative nature by TLC method

To confirm whether the mutants were unable to utilize fructose, the confirmation of presence of fructose in the culture filtrates was done by employing Ascending Thin Layer Chromoto graphy (TLC) run with standard controls as per Lobanok et al.,[5]

Fermentation studies

Collected the fermented sample and further tested for the production of fructose syrup and ethanol production by using the mutants strains of *Zymomonas mobilis and Saccharomyces cerevisiae* for submerged fermentation process. The inocula 18-24 hrs suspension is used to yield 1x105 cfu/ml in the experiment (6).fermentation is carried out at 37°C for 5-7 days with frequent gentle agitation.

Required quantity of samples were withdrawn from each flask at regular intervals of every 24hrs under aseptic conditions and analysed for the fructose[3] Sucrose and Ethanol.[4]

Estimation of Fructose

2ml of the fermented sample was added to 2ml of 0.1%resorcinol followed by 6ml of 0.075% ferric chloride and the contents were mixed thoroughly. The samples were kept in a water bath at 80-90oC for 6 min, cooled and read optical density at 480nm in a Spectrophotometer as per Snell and Snell.[3]

Estimation of Ethanol

The fermented sample 1.0ml was diluted with 25ml of distilled water then distilled in a distillation unit and 15ml of distilled sample were collected in 25ml K2Cr2O7. The colour developed sample was read at 600nm in the Spectrophotometer as per Gunashekaran.[4]

Table -1

Chemical Composition	Carob pod(%)
Sucrose	47
Reducing Sugar	37.5
Fructose	8.0
Protein	2.5
Trace Metals(ppm/100g)	
Fe2+	26.39
Cu2+	3.56
Mn2+	8.14
Zn2+	5.43

RESULTS AND DISCUSSION

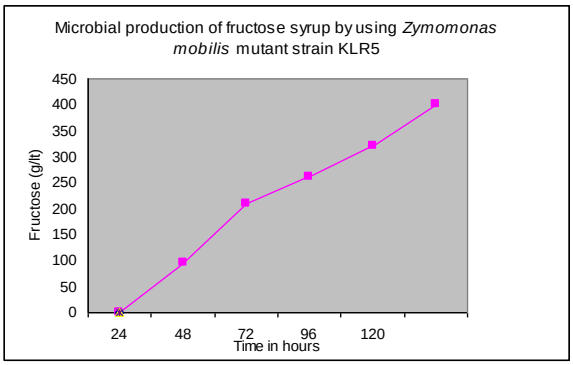

Figure-1

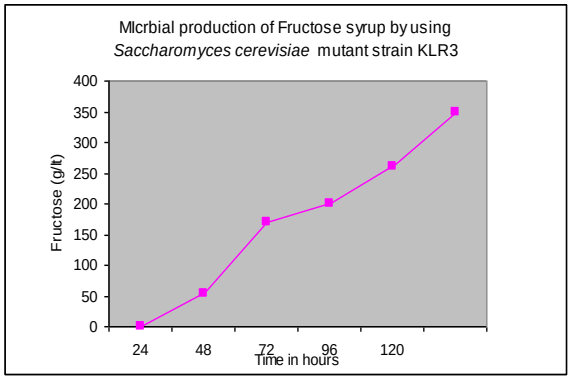

Figure-3

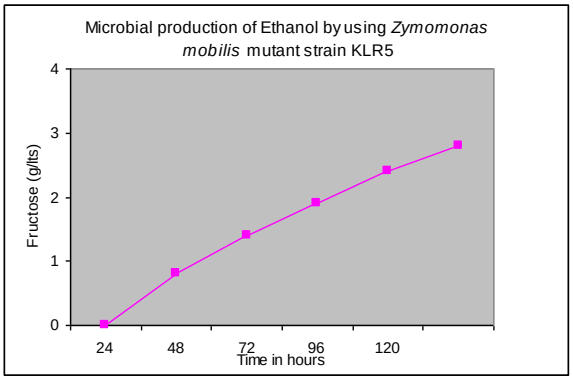

Figure -2

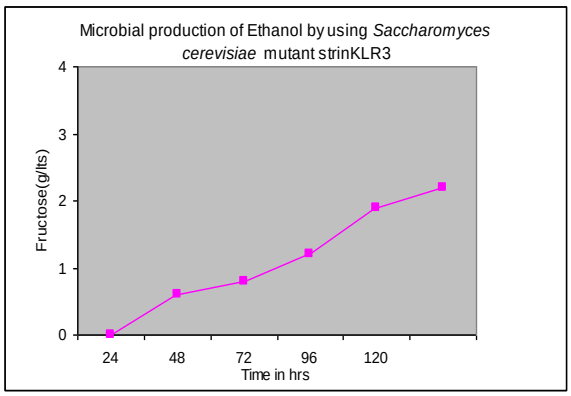

Figure-4

Totally five mutant strains of *Zymomonas mobilis* with confirmed Fructose negative capability over two generations were obtained through chemical mutagenesis and are KLR1,KLR2,KLR3,KLR4 and KLR5 as well as mutant strains of *Saccharomyces cerevisiae* KLR1,KLR2&KLR3 were obtained. Among all five mutants of Zymomonas mobilis KLR5 is the potent organism and in case of Saccharomyces cerevisiae mutant KLR3 is high yielding further these two strains are used fermentation process.

Fructose production gradually increases in the medium reaching maximum level at 120hr period further fructose accumulation was almost stagnant. Maximum fructose accumulation 400g/lt was KLR5 *Zymomonas mobilis and Saccharomyces cerevisae* KLR3 is 340g/lt and sucrose content in the fermentation medium decreases as the process continued, being positively propositional to the fructose production some quantity of sucrose still remain in the medium at the end of experimental period, i.e., nearly1/3rd of the initial sucrose concentration. The production of ethanol also KLR5 and KLR3 yields maximum2.8% and 2.4% produced.

*Zymomonas mobilis*KLR5 and *Saccharomyces cerevisiae*KLR3 mutants have been recognized as the most potents for fructose and ethanol producers and have been used for different substrates that are rich in sugars. Since the aim of the present study is to produce both fructose and ethanol simultaneously from the same substrate and several attempts have been made to alter their normal metabolic pathway producing fructose and ethanol simultaneously from the same substrate rich in sucrose, completely restricting their capacity to utilize fructose. Few researchers have made similar attempts successfully by inducing mutations in Saccharomyces cerevisiae[6,7,8,9] and Zymomonas mobilis[10,11] these workers could successfully induce mutations in these organisms so that they could produce both fructose and ethanol.

Among the two mutants *Zymomonas mobilisKLR5* (Fig1,2)yielded more 400g/lt fructose and ethanol 2.8% from the carob pod substrate fermentation, while *Saccharomyces cerevisiae*KLR3(Fig 3,4) yielded slightly less fructose and ethanol 380g/lt and 2.4% ethanol in the carob pod sustrate.During the present study in such a way that the mutants lose their capacity to convert fructose, still capable of using glucose alone and convert it into ethanol.

As such carob pod satisfies many parameters of suitability, hence it has been preferred & selected as the suitable substrate for production of fructose and ethanol through submerged fermentation.

CONCLUSION

The main aim of the present study is the production of fructose and ethanol by using carob pod as substrate by submerged fermentation process. In the study the three agro based wastes (sugarcane, banana, pineapple) and the carob pod have been analysed to select a suitable substrate for fructose and ethanol.

Apart from the biochemical composition, carob pod also satisfies the condition suggested by Jackson (1987).It also excess proportion of fermentable sugars (47%) is sufficient nutrient for the growth of the fermenting organism and also not contains any toxic components.

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