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EFFECT OF ADDITIVES ON ALCOHOL PRODUCTION AND KINETIC STUDIES OF *S.CEREVISIAE* FOR SUGAR CANE WINE PRODUCTION.

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ABSTRACT:

In the present study, growth kinetics of *S.cerevisiae* (brewer's yeast) was studied for sugar cane wine production. The specific growth rate and generation time for fermentation on sugar cane juice was calculated. The growth and alcohol production by *S.cerevisiae* was investigated in presence of two additives, biotin and guava leaves. The specific growth rate and generation time for sugar cane wine production by *S.cerevisiae* were found to be 0.772 h^{-1} and 0.89 h^{-1}. Optimum concentration of biotin and guava leaves for alcohol production was 50µg/ml and 600µg/ml respectively. Under optimized condition, the alcohol produced was unaffected with biotin while it increases in presence of guava leaves. The rate of fermentation was decreased and volatile acidity was affected in presence of additives.

Keywords: *Saccharomyces cerevisiae, biotin, guava leaves, generation time, growth rate, volatile acidity.*

1.INTRODUCTION:

Sugarcane is grown in over 110 countries and India stands second in the sugar cane production in the world. Sugar cane is used as the source of table sugar, rum, fuel ethanol and directly as fresh sugar cane juice. The high sugar content of sugar cane makes it an ideal source for the production of alcoholic beverages [1].

Saccharomyces cerevisiae (brewer's yeast) has been used in classical food fermentation applications such as production of beer, bread, yeast extract/vitamins, wine, saké, and distilled spirits. [2] Alcoholic fermentation is the main activity of yeasts, while *Saccharomyces cerevisiae* is the major species used in winemaking [3]. It utilizes sucrose, glucose, fructose, maltose and maltotriose as carbon sources [4] to produce alcohol under anaerobic conditions. Thus the sugarcane juice is an excellent medium for fermentation in order to elaborate alcoholic beverages as it is a rich source of sucrose, glucose and fructose. Fresh sugarcane juice has been used as a thirst quenching drink in some places such as South East Asia and also in Mexico and some parts of South America [5]. However, there are no reports about the kinetics of the fermentation of sugarcane juice in order to elaborate a wine.

Biotin is an essential vitamin in the carboxylation and decarboxylation reactions in yeast cells. If only insufficient quantities of biotin are available, cell growth may be limited and cell membrane is damaged [19]. The enzymic transfer of CO, within living systems has been shown to be mediated by biotin in a number of reactions, namely, the synthesis of fatty acids, the carboxylation of pyruvic acid to oxaloacetic acid, the

decarboxylation of oxaloacetic and succinic acids and the deamination of certain amino acids . The organisms incapable of synthesizing an adequate amount of their own biotin, require biotin,for rapid growth as in the case of *Saccharomyces cerevisiae* [3] Higher biotin levels are not usually associated with an increase in the fermentation tempo, but when nitrogen shortages are experienced, higher levels of biotin can accelerate the course of fermentation. Biotin also plays an important role in the production of higher alcohols and esters [19]. Supplementing the vitamins in the medium at increased concentration with exponential feeding increases the production of ethanol [6].

In traditional Philippines Basi(sugar cane wine) production, guava leaves are used to enhance the flavor. Guava leaves can also be used in various food and beverages as flavor enhancing agents [7]. Along with its flavor enhancing effect of, there strong antimicrobial action on Gram-positive and Gram-negative organisms has been reported [8]. The basi with addition of guava leaves received significantly higher scores in all sensory attributes such as clarity, color, flavor and general acceptability than basi produced by traditional method [9].

2. MATERIALS AND METHODS:

2.1. Substrate:

The sugar cane species under study is Co86032. The fresh sugar cane juice was used in the study and it is obtained from the sugar cane crusher. The juice obtained was then subjected to analyze total sugars, total solid content and pH.

2.2. Preparation of inoculum:

The cake yeast strains *Saccharomyces cerevisiae* (Brewer's yeast) was used in the study. Inoculum was prepared by adding a 5gm of yeast to 100 ml of sterile MPYD liquid media taken in 250 ml flask under sterile conditions. The media was incubated at 26^0C for 24 hrs. The cultures were maintained on MPYD (Malt extract 0.3%, peptone 0.5%, yeast extract 0.3%, dextrose 2% and agar 1.5%) slants at 4^0C [10].

The number of viable cells was measured using haemocytometer and tryphan blue (0.4%) dye.

2.3 Effect of additives:

In order to study effect of additives on rate of alcohol production and also the hedonic taste, two additives biotin and guava leaves were used. To obtain the optimum concentration of biotin and guava leaves added, fermentation of the cane juice with different concentration of additives was carried out for five days. Optimum biotin concentration was determined by using various concentrations (0.002, 0.004, 0.006, 0.008, 0.01mg/ml) and for guava leaves the range used was between 0.2-1.0 mg/ml.

2.4. Growth kinetics:

The rate of alcohol production was studied by fermenting the sugar cane juice for 10 days. The filtered and pasteurized juice adjusted to pH 5, inoculated with inoculum and incubated at 30^OC. The juice was analyzed daily for alcohol production, sugar utilization and number of viable cells. The wine sample was analyzed pH, total acidity, volatile acidity and fixed acidity. Same procedure is repeated to study the effect of additives on alcohol production.

2.5. Analysis of sugar cane juice and wine:

Analysis of sugar cane juice and wine was done for total soluble solids, pH, reducing sugar and specific gravity. The measurement of total soluble solids is done with the refractometer. pH of the juice and wine was measured with pH meter. The amount of total sugar was calculated using phenol-sulphuric acid method [11, 12]. For wine analysis, total acidity, volatile acidity and fixed acidity was determined by titrating with 0.1 N NaOH [2]. Presence of ethanol and methanol was detected by gas chromatography. The wine samples were analysed by Agilent 6890 N instrument with HP-5 (fused silica capillary)

column. Carrier gas used was H_2 gas. Oven temperature set was 35°C for 50 minutes with flow rate 20°C /min. It was ramped to 300°C and the injection temperature was 28°C. The eluted compounds were detected by Flame ionisation detector (FID). The amount of ethanol produced was measured by specific gravity method, potassium dichromate method and ebullometer.

3. RESULT AND DISCUSSION:

Effect of different concentration of biotin on the rate of fermentation of sugar cane juice was shown in figure 1. The maximum alcohol production was observed at concentration 0.005mg/ml. Similarly the effect of different concentration of guava leaves on the rate of fermentation of sugar cane juice was shown in figure 2. The concentration of guava leaves where maximum alcohol was produced was found to be 0.06mg/ml.

During first seven days of fermentation, there is significant utilisation of sugar. After seventh day, no change was detected in sugar concentration(figure 3). The results are similar to previous studies where fermentation of sugar cane juice and grape juice was performed and it was reported that there is decrease in total soluble solids during first seven days of fermentation [13, 14]. In the present study the specific growth rate, generation time and product yield calculated are as 0.7(h-), 0.89(h) and 0.18(g/g). The specific growth rate of wine yeast is found to be in the range 0.4-0.66 when grown at 34°C and pH 4.7. [15].

The production of alcohol and sugar utilization during fermentation was compared with addition and without addition of additives. It was observed that the amount of alcohol produced was same in presence and absence of biotin. Previously it was studied that the yeast cells will not show any requirement of biotin for growth and ethanol production [16]. Also it was observed that yeast cells grown in presence of biotin lowered the ethanol yield [17].Absence of biotin makes growth slow and sluggish fermentation rates [18, 6]. As shown in figure 4, the amount of alcohol produced was increased in presence of guava leaves. The guava leaves improved the test and aroma of the wine. The results are similar to previous studies which showed improved hedonic taste of Philippine sugar cane wine, Basi [9].

The total sugar present after fermentation was found to be decreased in presence of both biotin and guava leaves as compared to the wine produced in absence of additives (figure 6).

4. CONCLUSION:

S. cerevisiae can be used effectively for production of sugar cane wine. Biotin has no effect on sensory characteristics and alcohol production. While guava leaves can be used as additive for wine production as it increases the alcohol production and improves sensory characteristics.

5. ACKNOWLADGMENT

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Table 1: growth kinetics parameters for *S.cerevisiae*

Parameters	*S.cerevisiae*
Specific growth rate (h-1)	0.772
Generation time (h)	0.89
$Y_{p/s}$(g/g)	0.1872

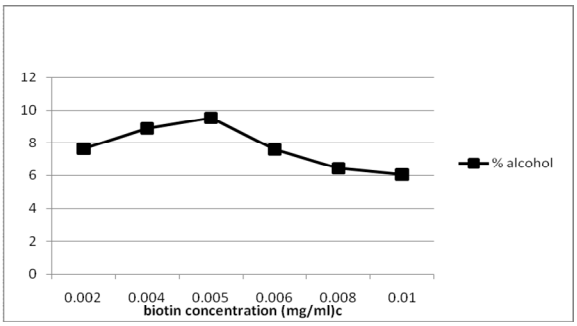

Figure 1: effect of biotin concentration on production of alcohol

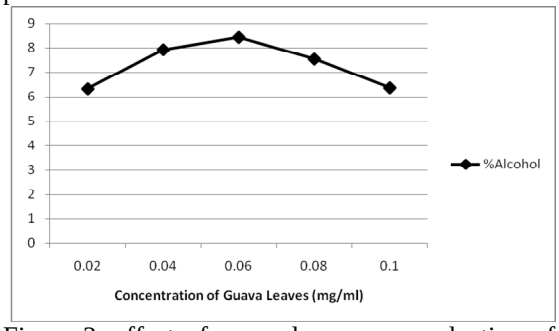

Figure 2: effect of guava leaves on production of alcohol

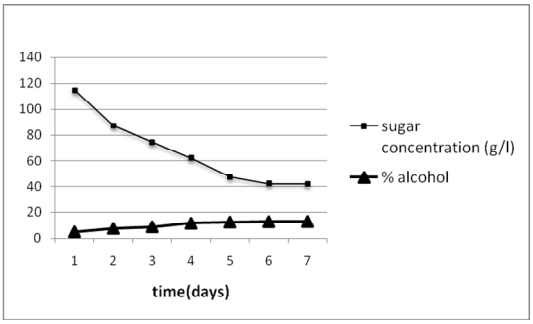

Figure 3: growth kinetics of *S.cerevisiae* for fermentation of sugar cane juice

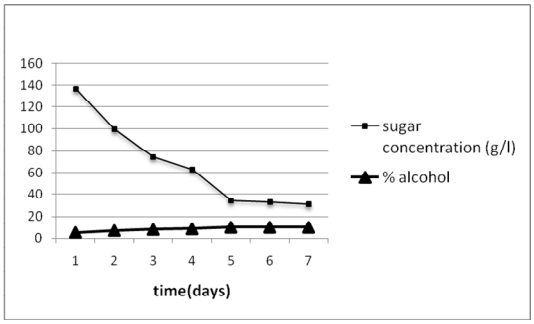

Figure 4: Effect of guava leaves on alcohol production

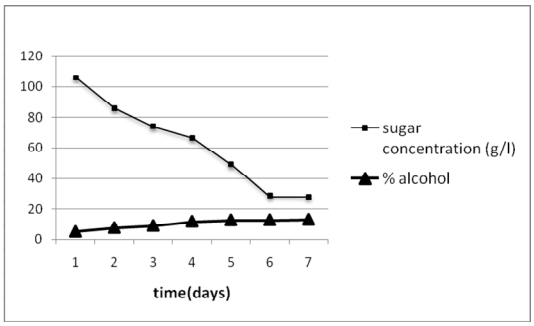

Figure 5: Effect of biotin on alcohol production

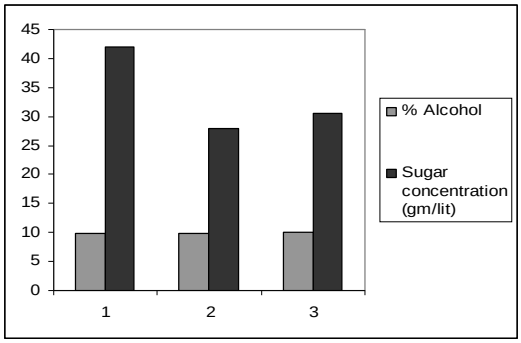

Figure 6: Effect of additives on alcohol production and sugar utilization(1-wine without additives, 2-Wine with biotin, 3-wine with guava leaves)