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Fermentative Production of Alcohol from *Madhuca indica* Flower and its Cake by *Kluyveromyces marxianus*

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Madhuca indica **flowers are well-known for their sugar and nutrients content due to which they are used widely for alcohol production. After alcohol production the leftover cake has no economic utilization. Present study first time reports the alcohol production potential of** ***Madhuca indica*** **flower's cake (6.2%) compared to** ***Madhuca indica*** **flowers (10.9%). Submerged fermentation was carried out in laboratory shake flask culture using** ***Kluyveromyces marxianus*** **which was isolated from curd, screened on the basis of alcohol production and identified by 16SrRNA technique. Fermentation was carried out using** ***Madhuca indica*** **flowers or its cake as substrate in fermentation medium for 36 hours at 30°C temperature in shake flask culture at 100 rpm. The fermented broths were analyzed for alcohol, total sugars, total acidity, volatile acid, and tannin content. CO_2 content was estimated from collecting solution of 3N NaOH. Results show that after fermentation 10.9 & 6.2% alcohol, 117.2 & 109.15 μg/mL total sugars, 6.8 & 14.6 meq/L total acids, 4.1 & 1.1 g/L total volatile acids, 2.9 and 2.6g carbon dioxide, and 5.95 & 8.0 (μg) tannin were produced in** ***Madhuca indica*** **flowers and** ***Madhuca indica*** **flower's cake fermentation, respectively.**

Key words: Microbial Fermentation, Submerged Fermentation, Yeast, Mahua.

Madhuca indica, commonly known as Mahua, is a tropical tree cultivated in warm and humid regions of Bihar, Chhattisgarh, Gujarat, Jharkhand, Kerala, Madhya Pradesh, Maharashtra, Orissa and Uttar Pradesh. The bark, flowers, leaves etc. of this tree has potential uses in various industries including ethanol production. This ethanol can also be blended with gasoline to be used as vehicle fuel. Many studies reported successful production of alcohol from flowers of *Madhuca indica* plant using various species of yeast[1,2,3,4] and bacteria[5] but no study has made to find out the potential of flower cake for alcohol production.

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Present study is aimed to find out the fermentative ethanol production potential of *Madhuca indica* flower and its cake by isolated yeast species.

MATERIALS AND METHODS

Isolation of yeast species

The yeast species were isolated from curd. Curd was diluted two times with distilled water in a test tube and after proper mixing on vortex; one mL of this suspension was inoculated aseptically in 100mL GYE (Glucose Yeast Extract) broth and incubated in static conditions for 48 hrs at room temperature. After incubation a loopful culture from this broth was streaked on GYE agar plates to obtain isolated colonies. Plates were incubated for 48 hrs at room temperature.

Screening and identification of potent alcohol producing strain

Five different isolated colonies were selected from the incubated plates and were further streaked individually on GYE agar plates for purification. A potent yeast species was screened out on the basis of its potential to produce alcohol in submerged fermentation. For this all the five purified colonies of yeast species were checked for their alcohol production using 100mL fermentation medium [composition (g/100ml)- yeast extract: 0.3, Bacterial peptone: 1.0, Pottasium dihydrogen phosphate: 0.6, *Madhuca indica* flower: 6.0 and pH: 6.0]. The autoclaved medium was inoculated with 6×10^6 CFU/mL yeast cells and incubated at 30°C and 100 rpm for 36 hrs[6]. After incubation the alcohol content in broth was find out by standard method[7].

Identification of potent alcohol producing yeast strain

Potent alcohol producing yeast strain was identified by 16SrRNA sequencing method. The identification was carried out in Gujarat State Biotechnology Mission (GSBTM), Gandhinagar, Gujarat.

Fermentative production of alcohol from *Madhuca indica* flower and its cake using potent yeast strain

Submerged fermentation was done in four glass bottles of 250 mL capacity each. Two bottles contain 100 mL fermentation medium of above stated composition whereas in another two bottles *Madhuca indica* flower were replaced with *Madhuca indica* flower cake (6.0g/100mL medium) in above composition. The bottles were sterilized at 15 lb/in^2 for 10 min. Now they were inoculated with 6×10^6 CFU/mL yeast cells of *Kluyveromyces marxianus* and incubated at 30°C and 100 rpm for 36 hrs[6]. To collect carbon dioxide these bottles were connected with 100 mL of 3N NaOH containing conical flasks through sterilized rubber tubes.

Table 1. Alcohol production by different isolates

Isolates	Alcohol content (%)
A	6.48 ± 0.13
B	5.55 ± 0.39
C	4.63 ± 0.21
D	10.18 ± 0.21
E	7.56 ±.028

Madhuca indica flower and its cake were also analyzed initially before fermentation for their reducing sugar and total sugar contents following standard methods[9].

Physico-chemical analysis of fermented broth and CO_2 estimation

After 36 hrs fermentation the broth was analyzed for its ethanol[7]; total acids and total volatile acids[8]; and total sugar and tannin contents[9]. Simultaneously carbon dioxide content in 3N NaOH was determined titrimetrically.

Statistical Analysis

The experiment was performed in duplication and obtained data are analyzed statistically[10]. Average data are presented here along with standard error of mean.

RESULTS AND DISCUSSION

Among different isolates obtained, isolate-D produces maximum alcohol content of 10.18% (Table 1). This isolate was sequenced and identified as *Kluyveromyces marxianus*. Before fermentation total sugars content in *Madhuca indica* flowers (20400 µg/mL) and its cake (1670 µg/mL) were found higher than their reducing sugar contents (4950 and 800 µg/mL), respectively (Fig 1).

After fermentation total sugar contents of *Madhuca indica* flowers and its cake were reduced by 99.42 and 93.46%, respectively (Fig. 2). The rapid decrease in the total sugar content was due to a rapid multiplication of yeast cells and rapid conversion of the sugars into alcohol via glucose metabolism[11, 12]. Alcohol content in fermentation broth followed the same trend of total sugar content and found higher in *Madhuca indica* flowers than its cake (Fig. 3). During fermentation

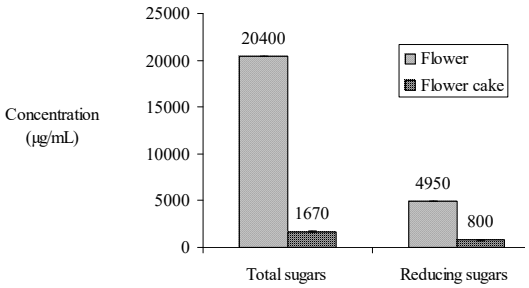

Fig. 1. Total sugar and reducing sugar (µg/mL) of *Madhuca indica* flowers and flower's cake

sucrose is being converted into fructose and glucose which are then converted into alcohol[13].

Total acids production was higher in *Madhuca indica* flowers cake than flowers (Fig. 4) whereas total volatile acids were found higher in *Madhuca indica* flowers (Fig. 5). Total acid production follows the pathway of lactic acid fermentation; on the other hand total volatile acids are produced via acetic acid fermentation phase. Ethanol is produced by acetic acid fermentation process thatswhy its content was found higher in *Madhuca indica* flowers fermentation (Fig. 3). Further, volatile acids are being produced by fermentation of sugars, so higher sugar level in *Madhuca indica* flowers may result in higher volatiles in this treatment[1].

During fermentation sugars are being utilized by yeast and carbon dioxide is produced as end product[14]. Production of carbon dioxide had a direct relationship with the rate of substrate degradation and ultimately with rate of alcoholic fermentation. Although no major difference is

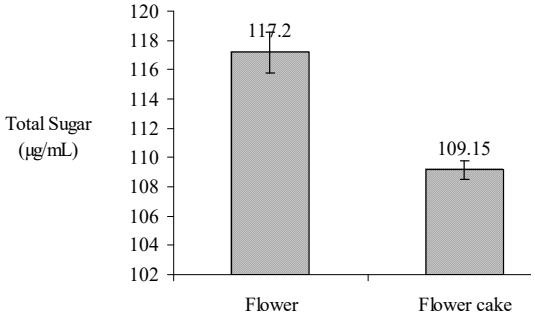

Fig. 2. Total sugar content (µg/mL) of *Madhuca indica* flowers and flower's cake

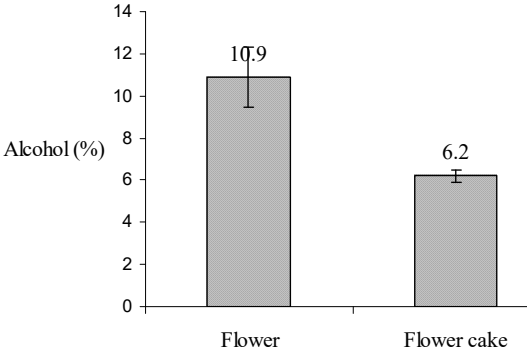

Fig. 3. Alcohol content (%) of *Madhuca indica* flowers and flower's cake

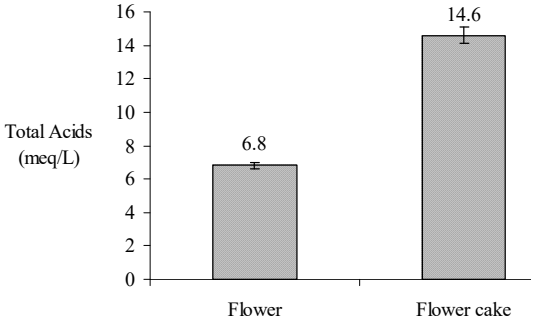

Fig. 4. Total acids (meq/L) content of *Madhuca indica* flowers and flower's cake

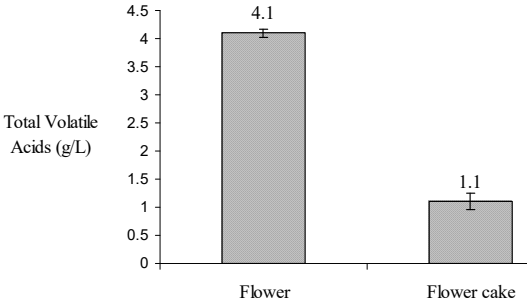

Fig. 5. Total volatile acid (g/L) content of *Madhuca indica* flowers and flower's cake

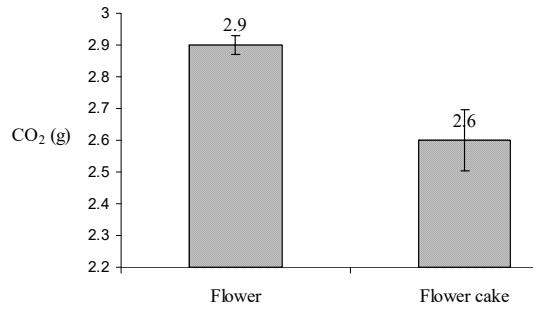

Fig. 6. CO_2 content (g) of *Madhuca indica* flowers and flower's cake

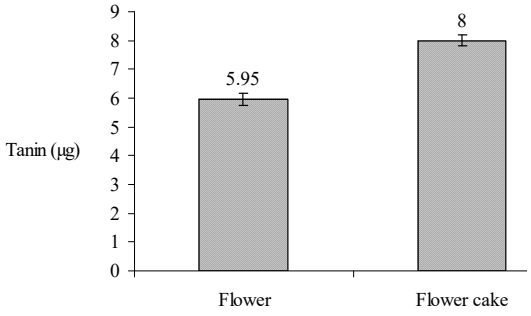

Fig. 7. Tanin content (µg) of *Madhuca indica* flowers and flower's cake

found in amount of carbon dioxide production in fermentations of *Madhuca indica* flowers and its cake (Fig. 6) but its content was slightly higher in *Madhuca indica* flowers which suggest higher alcoholic fermentation efficiency of *Madhuca indica* flowers than its cake.

Tannins are the polyphenolic compounds remains attached with skin and in stalk and seed. They are extracted by the reaction with acids. More total acids content in *Madhuca indica* flower's cake (Fig. 4) may extract more tannin (Fig. 7).

CONCLUSION

From the result, it could be concluded that *Madhuca indica* flowers and its cake could be potential feed stock for alcohol production using *Kluyveromyces marxianus*.

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