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Performance of *Saccharomyces cerevisiae* Strains to Ferment Sugarcane Juice

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In the present study, four *Saccharomyces cerevisiae* strains *S. cerevisiae* (NCIM 3200), *S. cerevisiae* (NCIM 3045), *S. cerevisiae* (baker′s yeast) and *S. cerevisiae* (EC1118) have been used and compared for their capability to ferment sugars from the juice of sugarcane (of variety CO 86032) for production of sugarcane wine. The growth pattern of each strain was studied followed by the fermentation at optimized conditions such as pH and temperature. The strains′ potential to produce sugarcane wine has been compared in terms of their sugar consumption, alcohol production, titrable acidity and volatile acidity production with respect to permissible amounts given by Indian Regulations. *Saccharomyces cerevisiae* (EC1118) performed better in fermentation among other compared *Saccharomyces* strains at the optimum temperature of 28 ºC, optimum pH 5, total soluble solids of 18 ºBrix and total sugar content of 185 g/L. Analysis of sugarcane wine fermented by *Saccharomyces cerevisiae* (EC1118) has pH, 3.57, total alcohol content, 13.55 ± 1.77 %, titrable acidity, 8.30 ± 0.01 g/L and volatile acidity, 0.84 ± 0.00 g/L. The overall acceptability from sensory analysis supports the above physico-chemical analysis results of sugarcane wine.

Keywords: Sugarcane, *Saccharomyces cerevisiae*, Sugarcane wine, Sensory analysis.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a popular commercially important crop in the world and India contributes to be the second largest sugarcane producers after Brazil, achieving 330 MMT (million metric tons) of sugarcane in the year 2017-18 (Directorate of Economic and Statistics, Ministry of Agriculture, India). World's 50 % of total production occurs in Brazil and India together [1,2]. In India, Maharashtra and Uttar Pradesh states are the largest sugarcane cultivator states and playing a key role in the national economy. The sugarcane juice is favorably consumed as a refreshing drink and an effective sports drink [3]. Additionally, it is consumed for sugar and jaggery production locally. Essentially, matured sugarcanes are supposed to be processed instantaneously in sugar mills, the delaying of which would otherwise result in an undesirable drying of excess sugarcane as well as low sugar recovery due to evaporation of sugarcane juice. To avoid such post-harvest losses of excess sugarcane, microbial treatment (fermentation) of sugarcane juice might be employed to prepare value-added beverage like wine [4,5], which is more stable and hence longer shelf life. Additionally, the consumption of wine increased globally, which has stimulated the attention on the different substrate for wine production.

Due to the high content of sucrose, there is an increasing interest in using sugarcane juice as a substrate for fermentation [6]. The high moisture content of sugarcane juice eases the homogenization of media in the fermentation of juice to produce wine [7]. Low titrable acidity of sugarcane juice increases, the ethanol yield during fermentation and yeast metabolism [8]. The nutritive value of sugarcane juice is related to its high sugar content. Sugarcane juice is an excellent raw material and best alternative to grape for the production of alcoholic beverage with acceptable characteristics [9,10]. The chemical composition of alcoholic beverage or juice may vary because of climate, varieties of raw material, harvest time and methods used for clarification, extraction [11].

Wine is an alcoholic beverage produced from different carbon sources such as grapes, other fruits, vegetables and crops. It is a complex mixture of different compounds which are formed during processing of substrate, fermentation and maturation [12-14]. The wine fermentation process is a combination of different complex reactions comprising different components, techniques, and yeast [15]. *Saccharomyces cerevisiae* is mostly used popular yeast for wine making [16]. This yeast consumes sucrose, fructose, maltose, maltotriose and glucose as carbon sources [17]. During fermentation, yeasts perform biotransformation to give alcohol, carbon dioxide and other products including phenolic, volatile compounds, esters and organic acids contributing taste, flavour and aroma of the wine. Therefore, yeast plays a vital role in quality wine production. Several authors have reported the importance of yeast in wine production by fermentation [18,19]. The selection of yeasts for fermentation is an important concern that depends on multiple factors such as the ability of yeast strain for alcohol production, fermentation rate, alcohol tolerance, fermentation parameters including optimum temperature and optimum pH and the production of higher alcohols [18,20].

Chemical and sensory properties of wine depend on yeast species used for fermentation and their metabolism [21-23]. A most significant factor in the quality of the wine is alcohol [24]. The source of yeast may affect alcohol content, types and amount of fusel oil in wine [25]. Along with yeast many different factors important in the quality of wine such as fermentation temperature, pH and sugar content of substrate [16,26-28]. The pH greatly impacts the growth of yeast and alcohol production [28a]. The temperature influences the rate of fermentation [29], growth [30-32] and the biochemical reaction of *Saccharomyces cerevisiae* [33,34].

Although a study reports use non-*Saccharomyces* yeast for production sugarcane-derived wine [35], it is reported in all other studies the use of *Saccharomyces cerevisiae* and its variants for production of sugarcane wine [5,7,9,10,11,15,36-44]. So current study reports the ability of four *Saccharomyces cerevisiae* strains to produce sugarcane juice derived fermented wine.

EXPERIMENTAL

Yeast strains: Sugarcane juice was fermented using four strains of *Saccharomyces cerevisiae viz. S. cerevisiae* (NCIM 3200), *S. cerevisiae* (NCIM 3045), *S. cerevisiae* (baker′s yeast) and a gifted strain *Saccharomyces cerevisiae* (EC 1118) from Sawarde Valley Food Foundation Winery, Chiplun, Ratnagiri, India.

Sugarcane juice as fermentation medium and its pretreatment: The sugarcane variety CO 86032 was collected from a local farm at Kolhapur, India. Sugarcane was crushed to obtain its juice which was then filtered and pasteurized for further process.

Inoculum preparation: A loopful of each strain was inoculated in pretreated sugarcane juice and kept for incubation at 26 ºC for 48 h. The number of viable cells was determined using hemocytometer.

Fermentation: Fermentation was carried out for each strain at their respective optimum pH and optimum temperature followed by incubation upto 7 days. Post incubation, broth was filtered and analyzed for alcohol content, sugar utilization and a number of viable cells.

Optimized pH and temperature: To know the optimum pH, sugarcane juice (pretreated), at an initial total soluble solid content of 18 °Brix and total sugar content of 185 g/l, was inoculated with seed culture (5 % v/v) of each strain separately. The incubation was carried out at different pH ranging from 3.6 to 9.2 at 28 ºC up to 7 days. After the purification of fermented wine, alcohol content (%) was determined by potassium dichromate method [45].

Pretreated sugarcane juice with an initial total soluble solid content of 18 ºBrix and total sugar content of 185 g/L was inoculated with seed culture (5 % v/v) of four *Saccharomyces cerevisiae* strains and incubated at different temperatures ranging from 25 ºC to 40 ºC for 7 days at respective optimum pH. After filtration of fermented broth, the alcohol content (%) of wine was determined by potassium dichromate method [45].

Analyses: Determination of the total sugars in fresh and fermented juice was done by the phenol-sulphuric acid method [46]. Volatile acidity (VA) is a measure of wine's volatile acids estimated by the method as reported by Haddad [47] and titrable acidity (TA) was analyzed by AOAC 2003 protocols [15].

Sensory analysis: Sensory analysis [48] of sugarcane wine was performed by semi-trained panelists including staff members and students (male and female) above 18 years at Department of Food Technology, Shivaji University, Kolhapur. A 20 mL of wine served in transparent glass in the white coloured room in a single session. The panel members assessed the wine, based on a nine-point hedonic scale for different attributes such as colour, aroma, flavour, taste, clarity and overall acceptability.

RESULTS AND DISCUSSION

Optimization study: At total soluble solid content of 18 º Brix, temperature 28 ºC and total sugar content of 185 g/L, the optimum pH for *Saccharomyces cerevisiae* NCIM 3045, *Saccharomyces cerevisiae* (Baker's yeast) and *Saccharomyces cerevisiae* EC 1118 was found to be pH 5 and 4.6 as optimum pH for *Saccharomyces cerevisiae* NCIM 3200 as shown in Fig. 1.

From temperature optimization studies, it was observed that the optimum temperature for *Saccharomyces cerevisiae* NCIM 3200 and *Saccharomyces cerevisiae* (Baker's yeast) to be 35 ºC while for *Saccharomyces cerevisiae* NCIM 3045 and *Saccharomyces cerevisiae* EC 1118 to be 25 and 28 ºC, respectively as shown in Fig. 2.

Growth kinetics of *Saccharomyces* strains

Growth pattern of *Saccharomyces cerevisiae* NCIM 3045: Pretreated sugarcane juice when fermented at optimum temperature (25 ºC), total soluble solids 18 ºBrix and pH 5 for 7 days exhibited following yields with respect to alcohol content (%) as given in Table-1.

Growth pattern of *Saccharomyces cerevisiae* NCIM 3200: Pretreated sugarcane juice when fermented at optimum temperature (35 ºC), total soluble solids, 18 ºBrix and pH 4.6 for 7 days exhibited following yields with respect to alcohol content (%) (Table-1).

Growth pattern of *Saccharomyces cerevisiae* (Baker's yeast): Pretreated sugarcane juice when fermented at optimum

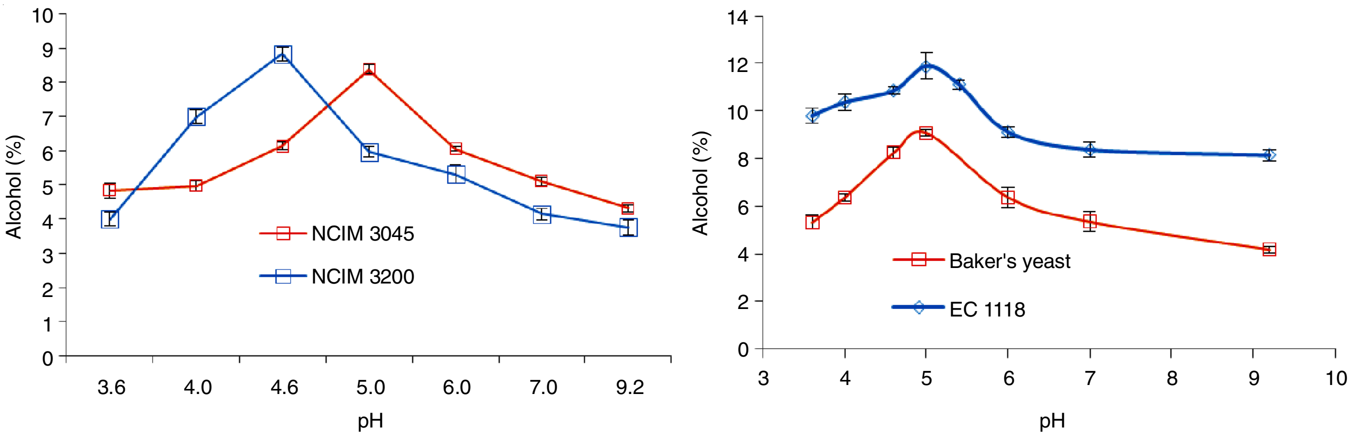

Fig. 1. Study of pH optimization for NCIM 3045, NCIM 3200, Baker's yeast and EC 1118

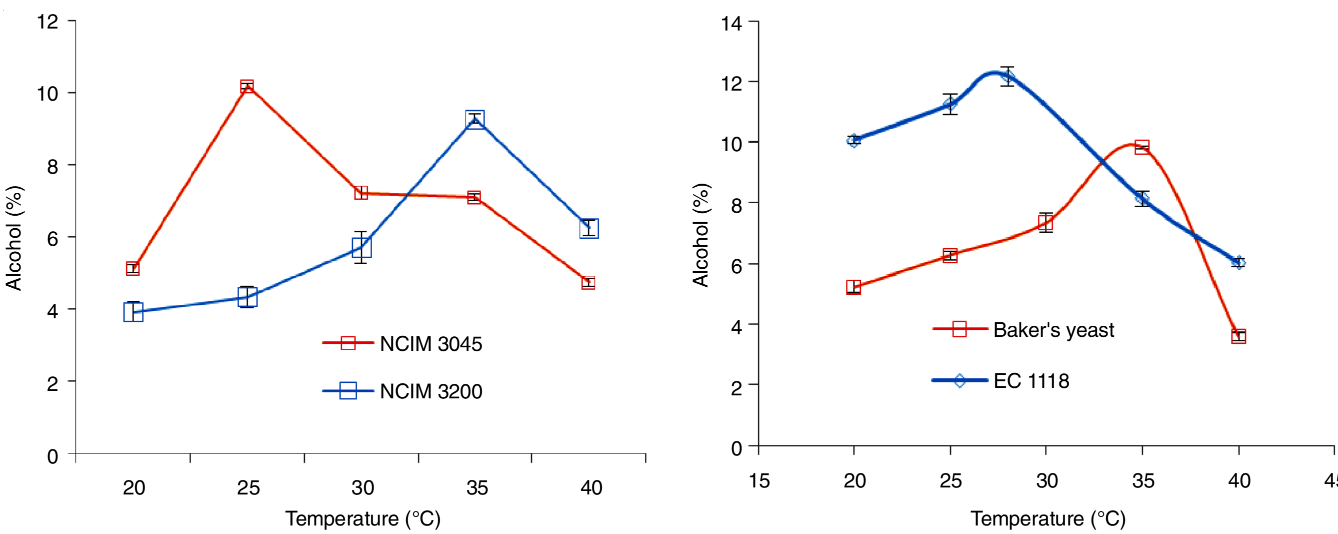

Fig. 2. Study of temperature optimization for NCIM 3045, NCIM 3200, Baker's yeast and EC 1118

TABLE-1
GROWTH PATTERN OF FOUR *Saccharomyces* STRAINS

Time (days)	No. of cells (mL × 10^6)	Sugar conc. (g/L)	Alcohol (%)	No. of cells (mL × 10^6)	Sugar conc. (g/L)	Alcohol (%)
	Saccharomyces cerevisiae NCIM 3045			*Saccharomyces cerevisiae* NCIM 3200		
0	004.66 ± 0.51	180.33 ± 4.51	0.00 ± 0.00	04.13 ± 0.611	182.33 ± 2.52	0.00 ± 0.00
1	006.70 ± 1.21	126.03 ± 06.71	02.60 ± 0.03	006.53 ± 0.64	128.13 ± 07.02	04.45 ± 00.22
2	017.33 ± 5.03	84.37 ± 04.13	04.31 ± 0.16	014.33 ± 3.51	98.33 ± 02.52	07.66 ± 00.05
3	164.33 ± 4.04	78.63 ± 06.58	06.05 ± 0.13	248.00 ± 2.64	85.73 ± 03.20	08.43 ± 00.12
4	185.00 ± 5.00	69.27 ± 05.49	09.21 ± 00.57	335.66 ± 9.81	80.31 ± 02.64	08.49 ± 00.47
5	700.33 ± 2.51	64.97 ± 05.96	10.94 ± 00.28	575.00 ± 5.00	48.50 ± 02.65	09.19 ± 00.17
6	723.33 ± 5.77	59.17 ± 08.60	10.74 ± 00.36	595.33 ± 5.03	43.53 ± 01.53	09.38 ± 00.20
7	569.33 ± 10.06	57.57 ± 08.64	11.19 ± 00.43	517.33 ± 6.42	42.66 ± 02.08	09.34 ± 00.12
	Saccharomyces cerevisiae (Baker's yeast)			*Saccharomyces cerevisiae* EC 1118		
0	007.50 ± 0.2	178.67 ± 7.77	0.00 ± 0.00	012.5 ± 0.00	156.35 ± 2.99	00.00 ± 00.00
1	006.43 ± 0.40	118.53 ± 08.05	04.93 ± 00.09	130.33 ± 5.50	101.53 ± 02.90	01.02 ± 00.02
2	032.93 ± 2.75	95.35 ± 05.13	06.68 ± 00.30	107.33 ± 6.42	34.38 ± 03.95	01.91 ± 00.07
3	042.16 ± 3.32	78.97 ± 05.22	07.76 ± 00.22	122.67 ± 2.51	14.24 ± 03.00	02.78 ± 00.11
4	159.33 ± 9.01	68.19 ± 04.93	08.37 ± 00.34	127.33 ± 4.04	02.99 ± 00.71	05.62 ± 00.21
5	236.66 ± 7.63	46.20 ± 02.23	09.94 ± 00.13	79.333 ± 4.04	02.48 ± 00.23	09.97 ± 00.21
6	146.00 ± 5.29	38.83 ± 06.20	10.01 ± 00.18	016.00 ± 1.73	00.65 ± 00.52	13.55 ± 01.17
7	143.00 ± 4.35	37.16 ± 06.24	10.14 ± 00.32	–	–	–

temperature (35 ºC), total soluble solids, 18 ºBrix and pH 5 for 7 days exhibited following yields with respect to alcohol content (%) as shown in Table-1.

Growth pattern of *Saccharomyces cerevisiae* EC 1118: Pretreated sugarcane juice, when fermented at optimum temperature (28 ºC), total soluble solids, 18 ºBrix and pH 5 for 6 days exhibited following yields with respect to the alcohol content (%) (Table-1).

Comparison of growth by four strains of *Saccharomyces cerevisiae*: The growth pattern of the selected *Saccharomyces* strains is shown in Fig. 3. It may be observed that *Saccharomyces cerevisiae* NCIM 3045, *Saccharomyces cerevisiae* NCIM 3200 and *Saccharomyces cerevisiae* (Baker's yeast) initiate their log phase at the end of 2[nd] day whereas the commercial strain *Saccharomyces cerevisiae* EC 1118 exhibits its log phase immediately on the 1[st] day itself.

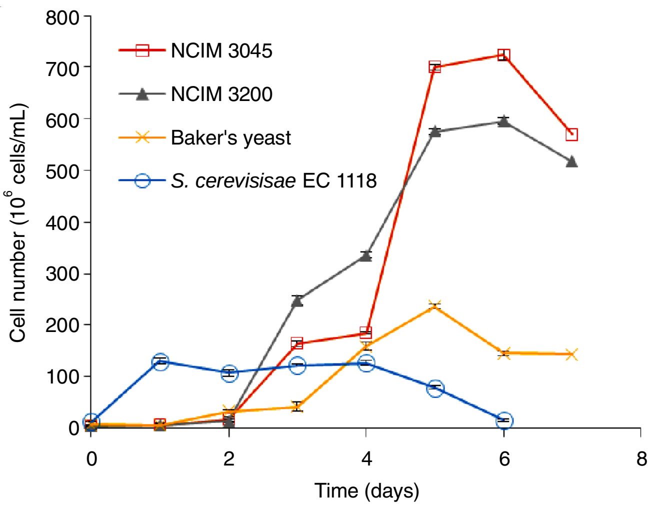

Fig. 3. Growth pattern of four *Saccharomyces* strains

As per as cell count of all strains is concerned, *Saccharomyces cerevisiae* NCIM 3045 tops among other chosen strains and *Saccharomyces cerevisiae* EC 1118 stands lowest. Despite its lower cell count up to 6[th] day, the performance of *Saccharomyces cerevisiae* EC 1118 is considerable in terms of its sugar consumptions and alcohol production compared to other *Saccharomyces* strains under study.

Fermentation of sugarcane juice by four strains and its comparative analyses: Fermentation of sugarcane juice was performed by four *Saccharomyces* cerevisiae strains at their respective optimized conditions and compared for their alcohol content (%), total soluble solids (ºBrix), total acidity (g L^{-1}), pH and volatile acidity (g L^{-1}).

Effect on sugar utility during fermentation: As the fermentation progresses, the sugar is consumed by *Saccharomyces* sp. In sugarcane juice fermentation, the sugar consumption was found to be fastest by *Saccharomyces cerevisiae* EC 1118 strain among all the other strains under study and slowest by *Saccharomyces cerevisiae* NCIM 3045 as shown in Fig. 4.

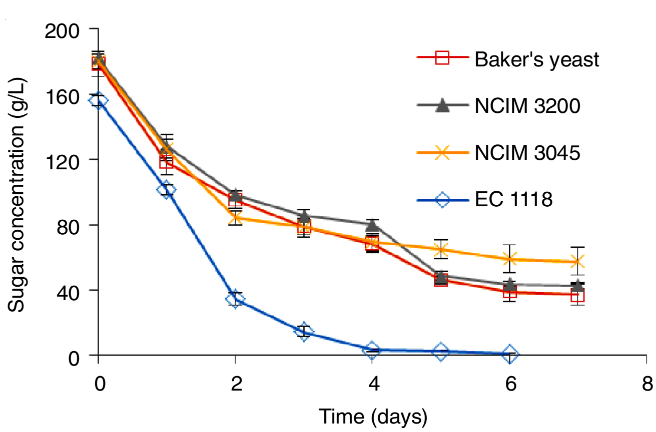

Fig. 4. Effect on sugar consumption by four strains

Effect on alcohol production of fermentation: The amount of total alcohol content (%) produced by four strains has shown in Fig. 5. All the four *Saccharomyces* strains were analyzed for their alcohol production for up to 7 days. However, *Saccharomyces cerevisiae* EC 1118 has shown its maximum production of alcohol on the 6th day of fermentation which is in line with its fastest consumption of sugar resulted up to 6[th] day. The same strain gives higher production on the 6[th] day compared to other *Saccharomyces* strains which yield less alcohol production in even up to the 7[th] day of fermentation.

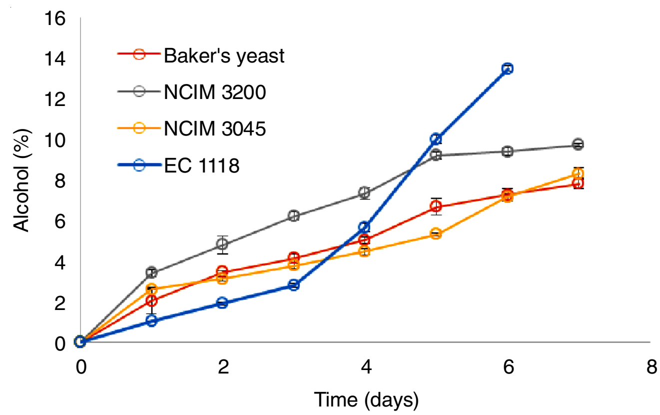

Fig. 5. Study of alcohol production by four strains

Overall performance of *Saccharomyces cerevisiae* strains: Based on the results obtained in Table-2, it can be observed that *Saccharomyces cerevisiae* EC 1118 exhibited good overall performance in all concerns. Moreover, as per as volatile acidity is concerned, only a wine prepared by *S. cerevisiae* EC 1118 is falling in the standards mentioned by Indian Regulations (Food Safety and Standards (Alcoholic Beverages Standards) Regulations, 2016) compared to other strains. Such a low volatile acidity thus obtained in this wine is a desirable one as suggested by Oliveira *et al.* [7].

Titrable acidity is a substantial element for determining the quality of fermented wine [7] and expected to be in a standard range. In current studies, all the sugarcanes wines produced by four strains exhibited the titrable acidity under given standard value. Similarly, pH of the sugarcane wines prepared by all the strains appeared to be within the standard range of pH 3.3 to pH 3.6 (Table-2). The physico-chemical analysis recommends that sugarcane juice can be used as an alternate substrate for wine production.

Sensory analysis of sugarcane wine fermented by *Saccharomyces cerevisiae* EC 1118: After physico-chemical analysis of sugarcane wine sensory analysis was carried out to understand the acceptability of the consumers. Semi-trained panel members tasted the wine samples using nine-point hedonic scale [(1) Dislike extremely, (2) Dislike very much, (3) Dislike moderately, (4) Dislike slightly, (5) Neither like nor dislike, (6) Like slightly, (7) Like moderately, (8) Like very much and (9) Like extremely)].

Table-3 represents the frequency and average notes for six attributes such as colour, aroma, flavour, taste, clarity and overall acceptability assessed by 30 semi-trained testers using nine-point hedonic scale method [35]. Based on average notes received for each attribute, Table-3 shows the highest score

TABLE-2
COMPARATIVE STUDY OF FOUR STRAINS OF *Saccharomyces cerevisiae* YIELDING SUGARCANE WINE

Parameters	*S. cerevisiae* NCIM 3045	*S. cerevisiae* NCIM 3200	*S. cerevisiae* Baker's yeast	*S. cerevisiae* EC1118	Indian regulations
Alcohol content (%)	11.19 ± 0.43	09.34 ± 0.12	10.14 ± 0.32	13.55 ± 1.77	7-14
Titrable acidity (g L^{-1})	03.68 ± 0.08	4.28 ± 0.08	04.10 ± 0.10	8.30 ± 0.01	<10
Volatile acidity (g L^{-1})	6.433 ± 0.06	4.15 ± 0.13	4.06 ± 0.55	0.84 ± 0.00	<1.2
pH	3.34 ± 0.15	3.43 ± 0.12	3.51 ± 0.10	3.57 ± 0.05	3.0 - 4.0

TABLE-3
SENSORY ANALYSIS OF SUGARCANE WINE FERMENTED BY *Saccharomyces cerevisiae* EC 1118

Attribute	Frequency and average notes									
	1	2	3	4	5	6	7	8	9	Mean
Colour	0	0	0	0	1	8	11	10	0	7.00
Aroma	0	0	0	0	5	15	6	4	0	6.30
Flavour	0	0	0	0	5	10	10	5	0	6.50
Taste	0	0	0	0	4	12	10	4	0	6.47
Clarity	0	0	0	0	0	8	15	7	0	6.97
Overall acceptability	0	0	0	0	0	11	12	6	1	6.90

(1) Dislike extremely, (2) Dislike very much, (3) Dislike moderately, (4) Dislike slightly, (5) Neither like nor dislike, (6) Like slightly, (7) Like moderately, (8) Like very much, (9) Like extremely.

for color followed by clarity, overall acceptability, flavour taste, and aroma. All the attributes score was more than six which indicates consumer's acceptance of sugarcane wine. As the testers are not familiar to sugarcane wine the score for aroma was less than other attributes [7]. The highest score (7) of colour attribute specify that consumers "moderately liked" the colour of wine. On the hedonic scale, the frequency for overall acceptability which relates to global preference was greater (12 panel members, 40 % of total panel) for point number 7 on the hedonic scale, signifying that testers "Liked moderately" the sugarcane wine.

Conclusion

The results obtained by the current study illustrates the potential of four *Saccharomyces cerevisiae* strains *viz. S. cerevisiae* (NCIM 3200), *S. cerevisiae* (NCIM 3045), *S. cerevisiae* (Baker's yeast) and *S. cerevisiae* (EC 1118) for making wine from juice of sugarcane (variety CO 86032) as a fermentation medium. All the strains could successfully produce sugarcane wine with variable alcohol content, due to the varying capability of individual strains for consuming sugars from sugarcane juice. As per as higher alcohol content, titrable and lower volatile acidity is concerned, *Saccharomyces cerevisiae* EC 1118 could yield better wine than other strains. However, it would be also interesting to reconnoiter its vitamins and minerals followed by its perception by consumers. Hitherto, the comparison between the strains suggests *Saccharomyces cerevisiae* EC 1118 be the best candidate among compared strains in making good quality sugarcane wine due to its superiority in performing fermentation and alcohol production in less time.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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