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ORIGINAL ARTICLE

Sweet Sorghum and Pilsner Malt blended Beer Production Using Brewing Yeasts

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ABSTRACT
Four yeast strains viz. Cyberlindnera fabianii (P1), Clavispora lusitaniae (P2), Saccharomyces cerevisiae (Sc01) and Saccharomyces cerevisiae (MTCC-11815) were evaluated for beer production from sweet sorghum (SSV-84). Sc01 and P1 were assessed as fast growing strains. 11815 showed maximum alcohol production 8.3% (v/v)) and tolerance (10.7% v/v). All the yeasts were medium flocculators. The fermentation efficiency was in the range of 48-85%. Wort was fermented at 30±2 ℃. Maximum alcohol was produced by Sc01 (4.4% v/v) in 100% sweet sorghum wort. Titrable acidity and pH of the beer produced was in the range of 0.123 – 0.254% and 3.2-3.6, respectively. Color of all the beer samples was light yellow. Beer produced by Sc01 from sweet sorghum: pilsner malt (40:60) was considered best on the basis of sensory evaluation followed by P2. The results of the study infer that Sc01 can be further used for scale up of beer production.
KEYWORDS: *sweet sorghum; beer; phaff; North Western Himalayas; Clavispora lusitaniae*

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INTRODUCTION

Beer is known to exhibit some health benefits. It has been evident that it increases HDL (high density lipoprotein) so that the risk of cardiovascular disease can be reduced as HDL is scavenger of cholesterol and also lowers risk of diabetes, improves eye health, strengthens bones and reduces kidney stones [6]. Earlier it was used as a medicine for cough, cold, skin blemishes, loss of appetite etc. The common substrates used for production of beer are barley, corns, rice, sorghum, oats etc. The increasing demand of alcoholic beverages has forced brewers to search for less expensive and easily available substrates, which could benefit them with sufficient yield and less expensive technology [28]. Generally barley is used as a substrate for production of beer however, sweet sorghum is also used in certain countries viz. China [17], Brazil [25], Africa [19], USA [10] as an alternate source because of its several advantages over barley such as: it diminishes the cost of production, adapted very well to subtropical and semiarid conditions [1, 26]. Sorghum grains with higher sugar content is tolerant to various abiotic stresses, perennial growth, less water requirement along with high fermentation rate and yield, is found to be a promising substrate for alcohol production [28, 12]. Sorghum is the major cereal crop used for manufacturing traditional beers in Africa [19]. Sudan is third leading sorghum producer in Africa [12]. The concentration of minerals and macronutrients is very high in sweet sorghum [27], rich in phosphorus, iron and magnesium [21]. Sweet sorghum beers are rich in calories, thiamine, folic acid, essential amino acid lysine, nicotinic acid and riboflavin [8].

Traditionally home brewing is in practice and the beers produced in this way have short life, poor quality and unstable (organoleptically) as compared to commercial beers [8]. Usually indigenous microflora is involved in its production and is unable to make consistency in flavor, aroma, color etc. Hence, to maintain the consistency specific microflora associated, is needed to be explored, as the source of inoculum in home brewed beers is not very specific. Scientific and technological advances, regarding substrate varieties and fermentations have led to an increase in the efficiency of brewing process which

could be more amplified by the selection of new brewing yeast strains better adapted for various fermentation parameters such as alcohol production, alcohol tolerance, killer activity, flocculation properties etc. [5, 15]. It has been shown that in alcoholic beverages viz., beer, wine, whisky, brandy, rum etc quality is affected by the strain of yeast used. Fermentation ends in ethanol and CO_2 as major products along with a variety of organoleptic compounds such as aldehydes, ketones, higher alcohols, esters, organic acids which provide a characteristics flavor to beer [24]. Hence, the selection of a particular strain of yeast is very essential to preserve the sensorial profile of beer.

Phaff, a traditional inoculum of North Western Himalayas is mostly applied for the preparation of cereal based traditional alcoholic beverages for years. It is available in the form of dried white balls of rice husk weighing around 13-14g in the Himalayan region. This inoculum is a rich source of fermentative microflora and there is a huge possibility of presence of brewing yeast strains with high potential which are needed to be explored.

MATERIAL AND METHODS

Materials

SSV 84 variety of sweet sorghum was obtained from Department of Plant Breeding and Genetics, Punjab Agricultural University Ludhiana. Pilsner malt was obtained from a commercial facility, Underdogs brewery and kitchen, Gurdevnagar, Ludhiana. Autoclaved water was used as the mashing liquor. The commercial enzymes α-amylase and glucoamylase used for liquefaction and saccharification, respectively.

Enzyme units

Throughout this paper the enzyme amounts are expressed as units of individual enzyme activity per gram of grist. 5000 IU/mL/min of pre-cooking and post-cooking dose of α-amylase is used in this study. Whereas, 1000 IU/mL/min saccharifying dose of glucoamylase is used for saccharification.

Methods

Three grist combinations are assessed in this study:

A. 100% malted sweet sorghum
B. 60% malted sweet sorghum + 40% pilsner malt
C. 40% malted sweet sorghum + 60% pilsner malt

Different enzyme amount was assessed for different grist combinations:

1. 2-11mL in 40% sweet sorghum malt, 14-16 mL in 60% sweet sorghum malt and 24-27 mL in 100% sweet sorghum malt for precooking and post cooking treatments.
2. 20-22 mL in 40% sweet sorghum malt, 30- 32 mL in 60% sweet sorghum malt and 50-54 mL in 100% sweet sorghum malt for saccharification.

Screening and selection of different yeast cultures for beer production

Yeasts isolated from *phaff*viz. P1 and P2, reference strain 11815 (*Saccharomyces cerevisiae*) and Sc01 (*Saccharomyces cerevisiae*) obtained from Department of Microbiology, PAU, Ludhiana and Department of Microbiology, HPAU, Palampur, respectively were screened on the basis of brewing traits studied viz. alcohol production, alcohol tolerance, attenuation, fermentation efficiency, flocculation and killer activity.

Preparation of the substrate for beer production

The sweet sorghum grains of variety SSV 84 were soaked in autoclaved water for different soaking periods (steeping) and germinated subsequently for 7-10 days. The germinated grains were kilned at 55°C and ground into grist. Mashing was then done by a two step mashing process using commercial α-amylase for precooking and post cooking (liquefying) at 70 ˚C for 30 min and 15 min, respectively and glucoamylase (saccharifying) enzymes for 30 min at 37 ˚C hydrolysis of fermentable sugars.

Pilsner malt addition

Pilsner malt was added to the sweet sorghum mash in the ratio of 100:0, 60:40 and 40:60 sweet sorghum mash: pilsner malt respectively. It was then given different temperature treatments in hot water bath for complete mashing i.e. 35 ˚C for 30 min (hydration of malt), 45 ˚C for 20-30 min (proteolytic enzyme activity), 60 ˚C for 30 min (β-amylase activity) and 70 ˚C (increase in β-amylase activity). After mashing, wort was filtered out using a muslin cloth and was boiled for 1 hour. After 30 minutes of boiling, bitter and aroma hops were added, procured from Underdogs brewery and kitchen, Ludhiana. Sugar was added as an adjunct to make up the brix to 11°B. The wort was cooled, filtered using a muslin cloth and pH was measured. The wort was then pitched with different yeast strains with an inoculum size of 6.5% and kept for fermentation at 30 ˚C.

Standardization of fermentation period

The standardized yeast inoculum size (6.5%) of selected yeast strains was used for inoculation of wort and kept for fermentation at three different fermentation periods i.e. 5, 7 and 10 days. Samples were analyzed for pH, alcohol content and residual reducing sugars.

Production of beer adopting all the standardized parameters

The standardized parameters obtained from the previous experiments were adopted for the beer production, wherein unfermented wort was used, that was divided in equal proportions in nine glucose bottles with each having 100:0, 60:40, 40:60, sweet sorghum : pilsner malt, respectively. The wort was inoculated with selected yeast strains and was kept at 30˚C for fermentation. After the completion of fermentation pH, alcohol content and residual reducing sugars were estimated. The beer was filtered and allowed for settling for 5 days at 4°C. The clear supernatant was decanted in new bottles and pasteurized at 65°C for 30 minutes. The bottles were stored at 4°C. The beer samples were further evaluated for their sensory analysis by a panel of judges.

Sensory evaluation

The sensorial evaluation of beer was done on the basis of appearance, color, flavor, mouth feel and overall acceptability by a panel of judges. Consumer acceptance for the product was evaluated on a nine point "Hedonic scale" [2].The prepared sample and a commercial beer sample (standard) were used for sensory evaluation.

Statistical Analysis

The experimental results were statistically analysed as per the methods [23].

RESULTS AND DISCUSSION

Two morphologically different yeast isolates coded as P1 and P2, were obtained from *phaff,* a traditional inoculum collected from Palampur region of North Western Himalayas. Different sugar fermentation patterns were noticed for all the yeast isolates and the production of acid and gas were observed in majority of the strains. However, P2 was unable to ferment fructose and lactose and 11815 was unable to ferment fructose. On the basis of morphological, biochemical and growth characteristics yeast isolates were characterized using the traditional characterization techniques as described by [4, 16, 18].

The yeast isolates were identified using ITS region sequencing with the help of an outsource facility, Xcelris Labs Ltd., Ahmedabad, India. ITS (ITS1-5.8S-ITS2) region was amplified using ITS1 and ITS4 universal primers and a product size of approximately 589 and 346 was obtained for P1 and P2, respectively. After analysis of the retrieved sequences using the NCBI, USA, BLASTn program, yeast isolates were identified as *Cyberlindnera fabianii* (P1) and *Clavispora lusitaniae*(P2),respectively.

Brewing traits

Growth kinetics study of the yeast isolates depicted that P2 and Sc01 exhibited maximum turbidity in the broth due to their high growth rate (Fig 1a, 1b, 1c, 1d).

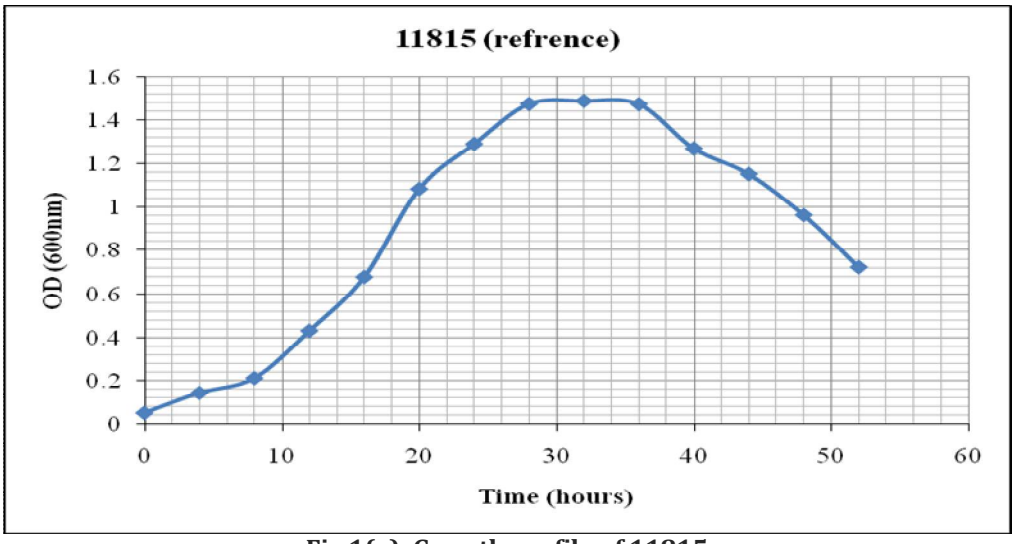

Fig 1(a). Growth profile of 11815

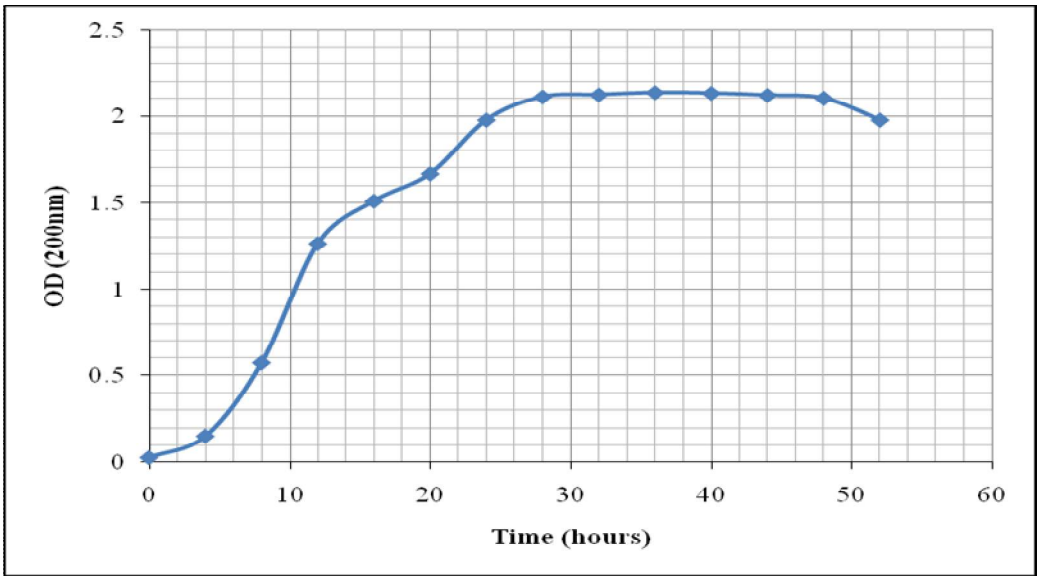

Fig 1(b). Growth profile of P1

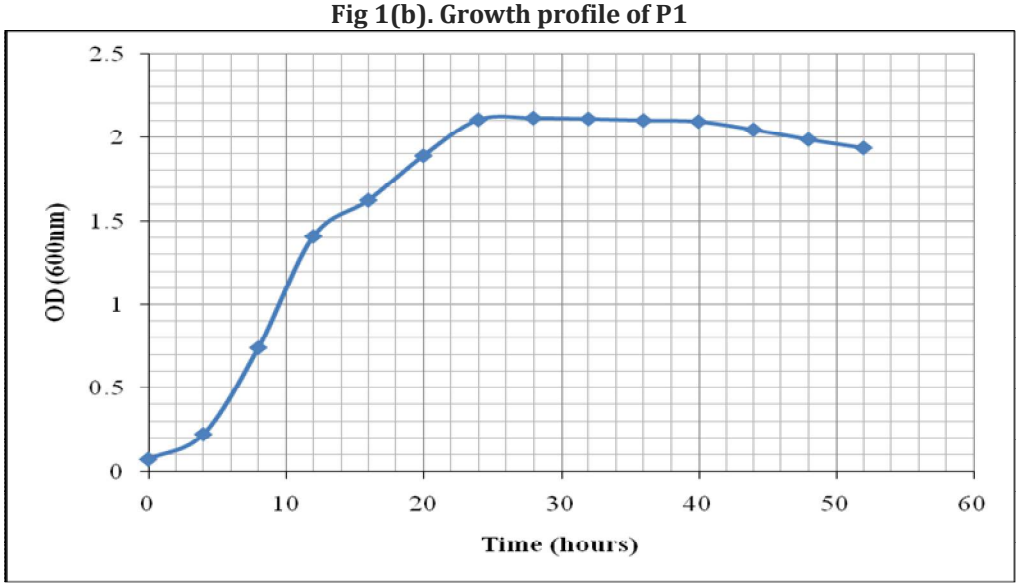

Fig 1(c). Growth profile of P2

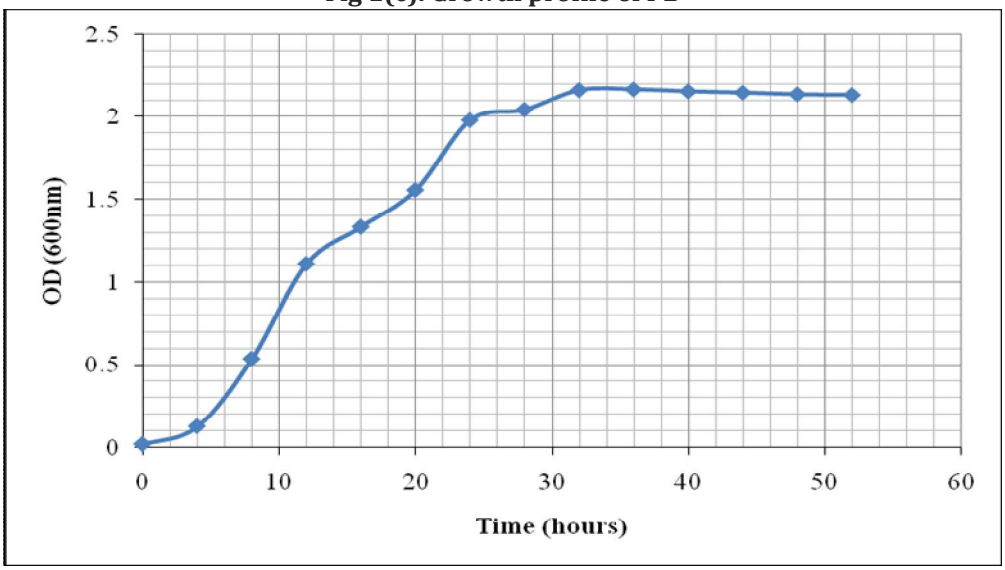

Fig 1(d). Growth profile of Sc01

Production of alcohol in high concentration along with alcohol tolerance by a yeast strain is of substantial significance in the brewing industry [11]. Hence, it was pertinent to test high alcohol producing yeast strains and also their ability of alcohol tolerance. 11815 and P1 were at par for their alcohol production ability and were found to produce highest alcohol content i.e. 8.3 % (v/v) and 8.1% (v/v), respectively. However, Sc01 and P2 showed 7.9 % (v/v) and 7.7 % (v/v) of alcohol production, respectively (Table 1).

Table 1. Fermentation of sugarcane juice by yeast isolates for evaluation of their brewing potential

Cultures	Days	Brix (°B)	Total sugars (g/100mL)	Reducing sugars (g/100mL)	Attenuation	Alcohol (% v/v)	Fermentation efficiency (%)	Ethanol yield (g/g)
11815	0	16	14.8	13.6	0	8.3^{a}	81.1^{a}	0.45^{a}
	1	4.5	3.6	2.2	62.7			
	2	0	0.2	0	75.4			
P1	0	16	14.8	13.6	0	8.1^{ab}	79.1^{a}	0.43^{a}
	1	13.7	12.2	10.9	14.5			
	2	2	1.24	0.3	67.8			
	3	0	0.03	0	71.6			
P2	0	16	14.8	13.6	0	7.7^{c}	75.2^{b}	0.43^{c}
	1	13.9	12.5	11.1	12.2			
	2	2.2	1.4	0.2	61.7			
	3	0	0.6	0	67.5			
Sc01	0	16	14.8	13.6	0	7.9^{bc}	77.1^{ab}	0.446^{c}
	1	4.5	2.5	1.4	61.2			
	2	0	0.7	0	70.8			
CD 5%		0.94	0.85	0.77	4.72	0.243	5.50	0.001

Results are shown as mean of three replications, different letters denote significant differences among values of various traits (P<0.05)

Mostly, tolerance to alcohol is determined by the yeast survivability in the presence of alcohol as described in the studies conducted by [20] and not by the production of alcohol in presence of high ethanol concentration as recommended by [14].The maximum alcohol content tolerated by yeasts without losing their alcohol production ability was found out to be 10.7 % (v/v) in case of 11815, and the lowest alcohol tolerance was at par in case of Sc01 and P2 i.e. 9.2 % (v/v) and 9.0 % (v/v), respectively (Table 2) without loosing their alcohol production ability. Color change was observed in the fermented sugarcane juice produced after completion of fermentation, in which different concentration of ethanol was added in sugarcane juice before pitching. The yeast isolates viz. 11815, P1, P2 and Sc01 exhibited attenuation in the range of 50-80 %. P2 exhibited attenuation of 76.8 % indicates this strain as good attenuator. The fermentation efficiency of these yeast isolates was in the range of 48-85 % (Table 1) depicting the wide range of their brewing potential.

Table 2. Alcohol tolerance studies of yeast isolates

Cultures	Alcohol (% v/v)	Alcohol Added	Fermentation efficiency (%)	Ethanol yield (g/g)	Alcohol tolerance	Attenuation
11815	8.3^{a}	4%	81.1^{a}	0.45^{a}	10.7^{a}	75.4^{a}
P1	8.1^{ab}	4%	79.1^{a}	0.43^{a}	9.3^{b}	71.6^{a}
P2	7.7^{c}	4%	75.2^{b}	0.43^{c}	9.0^{c}	67.5^{b}
Sc01	7.9^{bc}	4%	77.1^{ab}	0.45^{c}	9.2^{bc}	70.8^{ab}
CD 5%	0.243		5.50	0.001	0.071	0.14

Results are shown as mean of three replications, different letters denote significant differences among values of various traits (P<0.05)

The yeast isolates used in the study were found out to be medium flocculators in comparison to MTCC 170 reference strain, a high flocculator (Fig 1b). Flocculation is a characteristic unique to brewer's yeast. It is important and desirable for yeast to be flocculating, used in brewing industry. It is a property that allows yeast to come together and drop at the bottom of a fermenter [13].

Beer production

Three yeast isolates viz. 11815, P2 and Sc01 were used for beer production using sweet sorghum grains as a substrate. Usually lager beer is produced and preferred around the globe and with the alcohol content variation of 3-7%. P2 was found suitable to brew the lager beer as it produces 3-5% of alcohol on the basis of its brewing traits studied. Sweet sorghum variety SSV-84 was steeped at different time intervals in autoclaved water, and the optimum steeping period of 24 h was observed. Sweet sorghum grains were germinated after 7 days at 25 ˚C showing the development of endosperm and production of natural amylase. After sprouting, the grains were kilned at 55 ˚C. The grains were then grounded into grist with the help of grinder and were made ready for the mashing process. Mashing is a process in which the enzymes present in malt, breakdown the starch into sugars, resulting in liquid called wort.

Insufficient diastatic activity in the grains requires addition of exogenous enzymes [12]. Natural enzymes released during germination i.e modification of the endosperm are not sufficient for mashing process. In this study, exogenous enzymes were added. The precooking and post cooking liquefying enzyme (α-amylase) amount was optimized as 11 mL in 40% sorghum malt, 16 mL in 60% sorghum malt and 27 mL in 100% sorghum malt. Amount of the saccharifying enzyme (glucoamylase) was standardized as, 22 ml in 40% sorghum, 32 ml in 60% sorghum and 54 ml in 100% sorghum. Different mashing temperatures were used to release sufficient reducing sugars and 60 ˚C temperature for 30 min was found optimum for their release viz. 4.5 mg/g in 40% sorghum malt, 3.8 mg/g in 60% sorghum malt and 2.4 mg/g in 100% sorghum malt. The wort produced was separated using muslin cloth and brix was adjusted to 11˚B, then the wort was boiled for 1 h, after boiling the wort for half hour, aroma (fuggle, 5%) and bitter (Columbus, 12.5%) hops were added and boiled further for next half hour, after cooling the wort, again the volume makeup was done with autoclaved water as the volume of the wort decreased after boiling and then the wort was distributed equally in 9 glucose bottles, 3 of each grist combinations, pitched with yeast isolates Sc01, 11815 and P2. Optimum temperature for beer production was found out to be 28-30 ˚C. The maximum alcohol was produced at 7th day of fermentation by yeast strain Sc01 viz. 4.4% (v/v), 11815 produced 3.9% v/v alcohol in 100:0 ratio and 3.2% v/v alcohol production was observed by P2 (having sweet sorghum: pilsner malt ratio 100:0). There was no decrease in brix observed after this period. The least alcohol production was seen in 60:40 sweet sorghum: pilsner malt ratio by P2. Reducing sugars were 0.2 mg/g at the 7th day of fermentation and were not reduced to 0 mg/g on prolonging the fermentation period to ten days. The fermentation efficiency, attenuation, alcohol content, titrable acidity and pH of the beer produced ranged from 30-65 %, 35-73 %, 2.3% – 4.4% (v/v) (Table 3, 0.123 – 0.254 % and 3.2-3.6, respectively. Color of all the nine beer samples produced was light yellow.

Table 3. Beer production from sweet sorghum and pilsner malt by using different yeast strains (11815, P2 & Sc01).

Yeast isolate	Blending (sorghum wort : Pilsner Malt)	Days	Final ˚Brix	Final Specific gravity (g/ml)	Attenuation	Alcohol (% v/v)	Fermentation efficiency (%)	Ethanol (g/g)
11815	100:0	3	0	14	67.5^{ab}	3.9^{b}	55.4^{b}	0.28^{b}
	60:40	5	0	19	55.0^{d}	3.0^{c}	42.6^{c}	0.22^{d}
	40:60	3	1.6	22	47.5^{de}	2.8^{c}	39.8^{c}	0.24^{d}
P2	100:0	6	0.3	15	65.0^{ac}	3.6^{b}	51.1^{b}	0.27^{b}
	60:40	6	0.9	24	42.5^{e}	2.4^{d}	34.1^{d}	0.19^{f}
	40:60	6	2.3	27	35.0^{e}	2.3^{d}	32.7^{d}	0.21^{e}
SC01	100:0	5	0	12	72.5^{ab}	4.4^{ab}	62.5^{a}	0.32^{a}
	60:40	6	0.5	15	65.0^{ac}	3.6^{b}	51.1^{b}	0.27^{b}
	40:60	5	2.2	18	57.5^{cd}	3.1^{c}	44.1^{c}	0.28^{b}
CD 5%			0.12	0.45	8.62	0.31	4.42	0.02

*Intial brix – 11 *pH-5 *initial specific gravity- 41 g/mL *Acidity- 0.4 % (w/v)

Results are shown as mean of three replications, different letters denote significant differences among values of various traits (P<0.05)

Nine beer samples were put to sensory analysis to find out the acceptability by a commercial facility, Underdogs brewery and kitchen, Gurdevnagar, Ludhiana. Three beer samples produced by using Sc01, P2 and 11815 yeast isolates having 40:60 sweet sorghum: pilsner malt ratio were considered suitable for further sensory evaluation by a panel of judges in Punjab Agricultural University, Ludhiana. These beer samples were subjected to evaluation by a panel of eight judges on a 9 point 'Hedonic scale. Beer

produced by using Sc01 (*Saccharomyces cerevisiae*) and P2 (*Clavispora lusitaniae*) strain having 40:60 ratio of sweet sorghum: pilsner malt was considered the best on the basis of sensory profile.

CONCLUSION

Beer being most popular drink is used worldwide. Beer produced from sweet sorghum grains usually give a medicinal taste hence, there is a need to enhance its taste to increase the consumer demand by blending it with malt. In this study, pilsner malt and sweet sorghum combination is used to enhance the flavor. Traditional yeast isolates MTCC-11815 (*Saccharomyces cerevisiae*), Sc01 (*Saccharomyces cerevisiae*) and P2 (*Clavispora lusitaniae*) were used for beer production. The most acceptable beer was obtained using 40:60 ratio of sweet sorghum: pilsner malt combination by using Sc01 yeast strain. This combination can gain wide consumer acceptance due to its taste acceptability and therefore can be commercialized.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Authors have no conflict of interest.

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CONTRIBUTION

All the authors contributed in designing and carrying out the research as well in preparation of manuscript.

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