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Research Article

Processing a New Functional Beer with Added Yeast Isolated from Northwestern Himalayas

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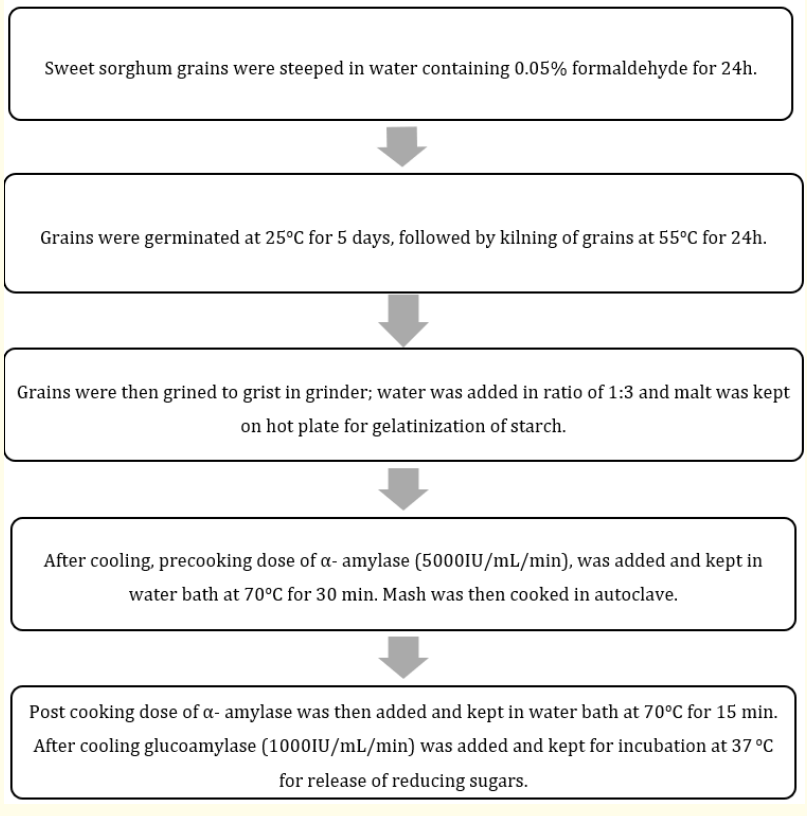
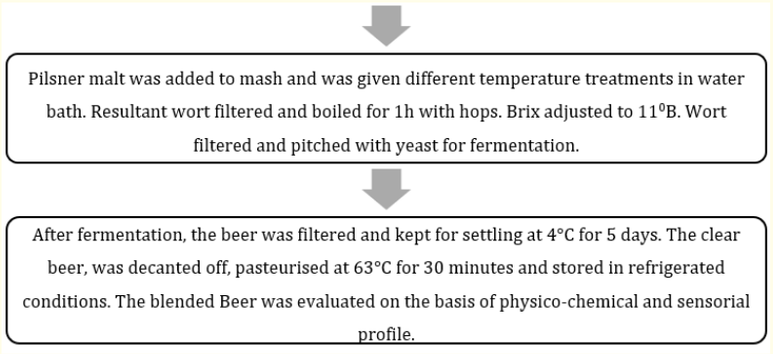
Abstract

Barley is considered as the potential substrate for beer production but in tropical countries, barley needs to be imported from temperate countries. Hence, there is a need to search for alternate substrates to meet the increasing demand of beer and to reduce its cost. In the present work, fifteen varieties of sweet sorghum grains were evaluated for their potential of beer production. Beer was produced using sweet sorghum and pilsner malt blend in the ratio of 40:60 respectively. Two *Saccharomyces cerevisiae* strains, GP4 isolated from traditional fermented beverage (*Gudanji*) of North-Western Himalayas (Bharmour region) and MTCC 11815 were compared for their fermentative capability of wort. Out of all the sweet sorghum varieties assessed, beer made from CSV 24SS using *S. cerevisiae* (GP4) had highest alcohol content of 5.8% (v/v) and the highest sensorial acceptance. The CSV 24SS variety was then used for upscaled production (20 litres) of beer using indigenous GP4 yeast tstrain. The beer produced was pale gold in colour and had a pH of 4.2 and titrable acidity of 0.36%. The beer was evaluated by a panel of judges for its sensorial profile and received a mean score of 7.8 out of 9 on the Hedonic scale, which indicates its consumer acceptance same as that of commercial malt beer.

Graphical Abstract





Keywords: Sweet Sorghum; Pilsner Malt; Beer; Indigenous Yeast; Northwestern Himalayas

Introduction

Beer is a widely consumed fermented beverage made from cereal grains and is probably the oldest alcoholic beverage [1]. Beer is generally prepared from malted barley but now crops like corn, oats, cassava, rice and sorghum are also being used. Apart from satisfying the taste buds, beer has various health benefits which have led to its increased consumption and research viewpoint. Moderate consumption of beer has shown to reduce the chance of cardiac arrest by lowering the levels of LDL and increasing HDL levels. It also helps to reduce risk of type 2 diabetes, kidney stones and osteoporosis. Moreover, it can help to decrease obesity and is used to treat cough, cold and loss of appetite [2,3]. Barley is used for number of purposes and hence, its production is not sufficient to meet the needs of expanding brewing industry around the globe. To meet the increased demand and to improve profitability, brewers are searching for alternate substrates which could benefit them financially.

Sweet sorghum is an excellent substrate for beer production, the grains are rich in carbohydrates and have a tremendous potential to serve as a source of malt [4]. Sweet sorghum is a type of sorghum which accumulates high amount of sugars in stalks forming 70-80% of total biomass [5] and due to its high sugar content, it is proved to be a good substrate for beer production. Sorghum can be used either as an adjunct in brewing or can be used as malt. Beer prepared from sorghum malt and barley adjunct shows superior composition characters like improved colour units and increased free alpha amino nitrogen compared to the 100% sorghum malt beer [6].

Traditionally beer has been prepared in different parts of world with different substrates. Sweet sorghum is rich in phosphorous, magnesium, iron and minerals. The beers processed from sorghum are a rich source of folic acid, essential amino acids and calories. However, due to the poor hygienic quality, organoleptic differences and short shelf life it is less popular compared to barley malt beers. The use of home brewing techniques along with indigenous microflora limits the stability of these beers [7]. Apart from the yeast strain used, the composition of wort greatly affects the final aroma of beer. As yeasts utilize nutrients from the wort for growth and release the byproducts in it; changes in wort composition directly affect the flavour of beer [8]. Indigenous yeast strains isolated from traditional fermented beverages or foods can be exploited for beer production. Specific yeast strain and substrate should be selected for producing beer with consistent characteristics. In the present research sweet sorghum blended with pilsner malt and fermented using indigenous yeast strain of Northwestern Himalayas to produce a stable beer with improved organoleptic properties.

Materials and Methods

Varieties of sweet sorghum selected for study and the yeast strains

Fifteen varieties of sweet sorghum viz., SPV 2402, SPV 2530, SPV 2596, SPV 2597, SPV 2599, SPV 2600, SPV 2601, SPV 2603, SPV 2606, SPV 2610, SPV 2611, CSV 19 SS, CSV 24SS, CSH 22SS and SSV 84 were used. The grains were obtained from Department of Plant Breeding and Genetics, PAU, Ludhiana. Two *Saccharomyces cerevisiae* yeast strains, GP4 isolated from traditional fermented alcoholic beverage (*Gudanji*) of North-Western Himalayas (Bharmour region) and MTCC 11815 were compared for their fermentative capability of wort.

Preparation of substrate for beer production

The sweet sorghum grains were soaked in sterilized water with 0.05% formaldehyde and kept for 24 h at 30°C [9]. The grains were then germinated for 3-5 days at 25°C and were kilned in oven (Yorco YSI 431) at 55°C for 24h. The kilned grains were grounded into grist using a grinder. Water was added at the ratio of (3:1) to the malt and kept on hot plate for gelatinization of starch for 20 minutes. After cooling, precooking dose of α-amylase (5000 IU/

mL/min) was added at the rate of 11 mL for 20 g grains and mash was kept in water bath (70°C, 30 min). The mash was then cooked in autoclave for 30 minutes. After cooling, post cooking dose of α-amylase (11 mL) was added and mash was kept in water bath for a period of 15 minutes at 70°C for hydrolysis of starch. For saccharification, after cooling glucoamylase (1000 IU/mL/min) was added (22 mL) and the mash was incubated at 37°C for 48h. The yeast inoculum for fermentation was prepared by adding a loopful of culture from slant culture in 100 mL of GYE broth and kept for incubation at 28±2°C for 24 hours in a shaking BOD incubator.

Pilsner malt blending

The sweet sorghum mash obtained was then mixed with pilsner malt in the proportion of 40:60 [9]. The blend was then given different temperature treatments which were necessary for hydration of malt and improving enzyme activity. The mash was then kept in water bath for hydration of malt at 35°C for 30 min, for proteolytic enzyme activity at 45°C for 20-30 min, for β-amylase activity at 60°C for 30 min and for increase in β-amylase activity at 70°C for 30 min. After completion of mashing, wort was filtered out using a muslin cloth and boiled along with hops for 1h. Aroma (Galena, 11.8%) and bitter hops (Mosaic, 12%) obtained from Underdoggs Brewery and Kitchen, Ludhiana were used. The brix of the wort was adjusted to 11°Bby addition of sugar. The wort was then filtered again using muslin cloth and kept for cooling. Parameters like pH, reducing sugars, total sugars, Brix and titrable acidity of the wort were evaluated and wort was kept for fermentation at 25°C using inoculum size of 6.5% (v/v).

Beer production

Fermentation of wort was done until the °Brix become constant (5 days) at 25°C.The samples were withdrawn at various intervals for evaluating different physico-chemical parameters. On completion of fermentation, the beer was filtered and kept for settling at 4°C for 5 days. The clear beer, free of yeast cells was decanted off and stored in bottles. Beer was then pasteurised at 63°C for 30 minutes and stored in refrigerated conditions. The blended Beer was evaluated on the basis of physico-chemical and sensorial profile.

Comparative analysis of different beers

The beers produced from fifteen varieties of sweet sorghum were then compared based on their sensory analysis and physico-chemical characters. The beer was evaluated on the basis of appearance, flavour, colour, mouthfeel and overall acceptability for consumer acceptance on a nine-point scale called "Hedonic scale" [10]. The physico chemical parameters like pH, reducing sugars [11], total sugars [12], ethanol content [13], and total soluble solids and titrable acidity [14] were evaluated. The beer having highest acceptance on the basis of taste and other characters was selected for upscaled production. As taste is the greatest factor in acceptance main focus was given on sensorial comparison of beers.

Upscaling

On the basis of physico-chemical and organoleptic properties of the beers, sweet sorghum variety CSV 24SS was selected for upscaling of beer production using *S. cerevisiae* GP4 strain. Twenty litres of beer was produced using sweet sorghum and pilsner malt blend (40:60). The physico chemical analysis of beer was done by evaluating pH, titrable acidity, ethanol content, reducing sugars, total sugars, free amino nitrogen [15], tannin content [16], antioxidant activity [17] and bitterness. The beer was evaluated for sensorial profile by panelists who were familiar with the flavours of beer due to occasional or regular consumption of beer and by head brewer of Underdoggs Brewery and Kitchen, Ludhiana.

Results and Discussion

Sweet sorghum malt is used for beer production in different parts of Africa and is also used as adjunct in other parts of the world. ICAR-Directorate of Seed Research with ICRISAT and central or state agricultural universities is working continuously on developing high yielding varieties with resistance to various pests. Final physico-chemical parameters of beers produced using different sweet sorghum varieties are presented in Table 1. The varieties used for beer production were different from each other with respect to variations in carbohydrate content, grain size, germination capacity and storage time. Based on the evaluation (Table 1), it is evident that the indigenous *S. cerevisiae* yeast strain (GP4) produced better quality beers compared to MTCC-11815. The beer produced from sweet sorghum variety CSV 24SS had the highest alcohol content (5.8% v/v) whereas beer produced from SPV 2530 and SPV 2611had the lowest alcohol content (1.4% v/v). The traditional sorghum beers made in Africa are generally cloudy and have suspended solids in them [7]; however, in our study the beer produced was clear due to the high flocculation capacity of the GP4 yeast strain used. Our results are in concordance with the alcohol content of pilsner malt beers with an average range of 4.2-5.8% (v/v) [22] may be because of 60 % portion of pilsner malt in the substrate. In a study conducted by.

Alcohol is the most important and abundant volatile compound found in beer and contribute to its sensory attributes. Thus, yeast strains producing ethanol in high concentration play significant role in brewing industry [18]. *S. cerevisiae* GP4 strain was isolated from *Gudanji,* a traditional fermented beverage of North-Western Himalayan region. This yeast strain produced higher alcohol content compared to MTCC-11815 in each variety of beer with fermentation efficiency of 82.38%. The CSV 24SS beer had lower residual reducing sugars and total sugars accounting about 700mg/L and

4000mg/L respectively. Among the different physico-chemical attributes of beer; pH and titrable acidity are responsible for the taste and colour. They also provide preservative effect to beer by affecting the redox potential and microbial growth [19]. In this study the pH of different beers ranged from 3.7-4.7 with CSV 24SS having pH 4.2 and the titrable acidity of the sweet sorghum blended beers was in range of 0.12 to 0.36%, which is in the palatable range (Table 1). The acidity in beer is contributed by metabolic by products of yeasts, hops and malt which include organic and carbonic acid [20].

Sweet sorghum variety	Yeast strain	Alcohol content (%v/v)	CD_2 (5%)	TSS (°B)	CD_2 (5%)	pH	CD_2 (5%)	Acidity (%)	CD_2 (5%)	Reducing sugars (mg/L)	CD_2 (5%)	Total sugars (mg/L)	CD_2 (5%)
SPV 2402	GP4	4.9	0.485	0	0.176	4	NS	0.21	0.028	800	0.015	5000	0.139
	11815	2.5		2		4.2		0.24		1500		15000	
SPV 2530	GP4	2.4	0.245	2	0.318	4.2	NS	0.12	0.031	1500	0.027	14000	0.215
	11815	1.4		3		4		0.33		2700		20000	
SPV 2596	GP4	4.6	0.427	0	0.309	4	NS	0.28	0.035	1000	0.028	6000	0.21
	11815	1.5		3.5		3.7		0.28		3000		23000	
SPV 2597	GP4	3.9	0.368	1	0.279	4	NS	0.33	0.036	2000	0.035	10000	0.213
	11815	1.5		3		4.1		0.24		3400		22000	
SPV 2599	GP4	4.5	0.427	0	0.176	4.6	NS	0.19	0.02	800	0.017	6000	0.159
	11815	1.8		2		4.4		0.12		1800		17000	
SPV 2600	GP4	4.4	0.413	1	0.197	4	NS	0.24	0.033	900	0.025	8000	0.182
	11815	1.6		2		3.9		0.28		2700		19000	
SPV 2601	GP4	3.7	0.352	0	0.176	4.2	NS	0.33	0.038	900	0.023	8000	0.166
	11815	1.5		2		4.2		0.28		2500		17000	
SPV 2603	GP4	4.4	0.438	0	0.088	4.2	NS	0.12	0.027	800	0.014	7500	0.148
SPV 2606	11815	2.3	**0.501**	1	**0.088**	4	**NS**	0.28	**0.038**	1400	**0.013**	15000	**0.126**
SPV 2610	GP4	5	0.363	0	0.197	4.4	NS	0.28	0.038	900	0.021	6000	0.185
	11815	2.7		1		4.2		0.33		1200		13000	
SPV 2611	GP4	3.8	0.483	1	0.176	3.9	NS	0.28	0.04	800	0.019	9000	0.163
CSV 19SS	11815	1.6	0.466	2	0.176	3.9	0.544	0.33	0.033	2300	0.019	19000	0.154
CSV 24SS	GP4	5.3	0.553	0	0.088	4.2	NS	0.36	0.036	900	0.014	4000	0.12
CSH 22SS	11815	1.4	0.272	2	0.197	4	NS	0.28	0.037	2000	0.023	18000	0.183
SSV 84	GP4	5	0.442	0	0.088	4.7	NS	0.24	0.036	900	0.013	7000	0.139
CD_1 (5%)	11815	1.7		2		4		0.28		2000		16000	
	GP4	5.8		0		4.2		0.33		700		4000	
	11815	2.4		1		3.8		0.24		1400		13000	
	GP4	2.5		1		4.4		0.21		1000		12000	
	11815	1.8		2		4.2		0.36		2400		17000	
	GP4	4.4		0		3.7		0.24		800		5000	
	11815	2.4		1		3.7		0.33		1200		15000	
	GP4	0.404		0.067		0.384		0.024		0.009		0.073	
	11815	0.176		0.193		0.369		0.026		0.02		0.16	

Table 1: Physico chemical characteristics of beers produced using different sweet sorghum varieties.

CD_1 (5%) is the statistical difference between sweet sorghum varieties for each yeast.

CD_2 (5%) is the statistical difference between two yeasts for each sweet sorghum variety.

The visual characteristics affecting beer for consumer acceptance include colour, clarity and foam characteristics. The colour of different beers produced in the present research was pale gold in colour. The beers produced from sweet sorghum are generally dark coloured and the beers produced from pilsner malt are generally light coloured [21,22]. The blending of these two substrates results in pale gold colour of our beer. However, since the sweet sorghum was used in less proportion compared to barley the dark colour of the sweet sorghum malt was weakened. The colour of beer develops from the products formed in grains due to Maillard reaction during the malting process. The kilning of grains gives major input to beer colour and the boiling of wort with hops also contributes towards it. The colour of beer can be altered after fermentation with the help of roasted malt extract or caramel colour [23].

Sensory evaluation involves the use of human senses (i.e., smell, taste) for analysis of the product prepared for commercialization or on a lab scale. The sensory characteristics viz., colour, aroma and mouthfeel are very closely linked to the quality of beer. In the present work, all the fifteen beer samples were evaluated by the Head brewer of Underdogss Brewery and Kitchen, Ludhiana for their sensory attributes. The beers were evaluated on the basis of appearance, flavour, mouth feel and overall acceptability. The scoring method followed was nine point 'Hedonic scale' [10]. All the beers received a score above 5.25 out of total score of 9. However, the highest score of 7.75 points was received by CSV 24SS beer (Figure 1). Based on the sensory score and the physico chemical parameters, CSV 24SS sweet sorghum blended beer was selected for upscaled production. The sweet sorghum blended beer was highly acceptable and was comparable in taste with the commercial barley beer.

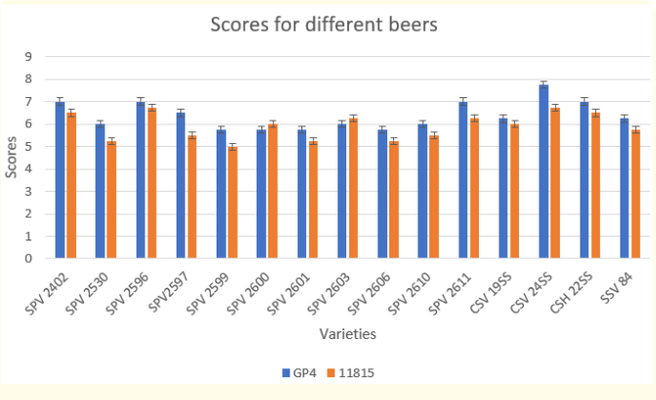


Figure 1: Sensory score analysis of beers produced from different sweet sorghum varieties using indigenous GP4 and MTCC 11815 *Saccharomyces cerevisiae* strains.

Parameters	Values
Alcohol content	5.8% (v/v)
pH	4.2
Reducing sugars	700 mg/L
Total sugars	3000 mg/L
°Brix	0
FAN	35mg/L
Tannin content	63mg/L
Antioxidant content (AEAC)	0.440
Acidity	0.36%
Bitterness	30.2 IBU

Table 2: Physico chemical properties of CSV 24SS beer.

Upscaling is the process of increasing desired product in amount, size or production. The physico-chemical parameters of the upscaled beer are given in table 2. The beer had an alcohol content of 5.8 % (v/v), pH 4.2 and titrable acidity of 0.36%. The beer was evaluated for sensorial profile by a panel of judges who were acquainted with beer either occasionally or frequently on the basis of 'Hedonic scale' [10]. The beer received the highest score of 8.5 out of 9 by the panelists. The upscaled beer was then evaluated on the basis of two different scales by head brewer of Underdoggs Brewery and Kitchen, Ludhiana viz., Hedonic scale [10] and sixteen-point scale [24]. The beer received a score of 7.5 out of 9 and a score of 12.4 out of 16 by the head brewer. The beer was found acceptable by the head brewer of Underdoggs Brewery and Kitchen and was similar in taste with respect to commercial barley beer. Goode., *et al.* [25] also compared the sorghum and barley blended beer with commercial barley beer and found no significant differences with respect to aroma, mouthfeel, initial taste, after taste and overall acceptability. The results from this study suggest sweet sorghum variety CSV 24SS as an excellent blend source for barley malt beers. Moreover, the sweet sorghum pilsner malt blended beer is economical and had a production cost of Rs 24.18/litre which is less as compared to the commercial barley beer (Table 3).

Conclusion

Sweet sorghum variety CSV 24SS is a potential substrate for brewing industry. The blending of sweet sorghum with barley for beer production has shown to produce beer with palatable sensorial profile. Blending with sweet sorghum will help to reduce the over dependence on barley malt for beer production. The health benefits of sorghum also make it a good source for beer production. Among the fifteen varieties of sweet sorghum evaluated, sweet sorghum variety CSV 24SS proved to be best among others for beer production. Indigenous *Saccharomyces cerevisiae* GP4 yeast strain demonstrated better fermentative behavior and produced supe-

Parameters	Sweet sorghum-pilsner malt blended beer
Raw materials	Sweet sorghum @ Rs 50/kg (800 grams Rs 40/-) Pilsner malt @ Rs 100/kg (1.2 Kg Rs 120/-)
Hops	Aroma hops @ Rs 4/gram (12 grams Rs 48/-) Bitter hops @ Rs 4/gram (12 grams Rs 48/-)
Sugar	@ Rs 43/kg (530 grams Rs 23/-)
Enzymes	Alpha amylase @ Rs 100/litre (Rs 3.5/-) Glucoamylase @ Rs 100/litre (Rs 3.5/-)
Bottle and stickers	@ Rs 4/- (750 mL Rs 88/-)
Miscellaneous costs (Pasteurisation, electricity, water etc.)	Rs 25/-
Recovery	16.5 litres (82.5%)
Total cost	Rs 399/-
Cost per 750 mL bottle (/litre)	Rs 18.13/bottle (Rs 24.18/litre)

Table 3: Economics of sweet sorghum and pilsner malt blend beer (20 litres).

rior quality beer over *S. cerevisiae* MTCC 11815 yeast strain. The sensorial profile of sweet sorghum-pilsner malt blended beer was comparable to commercial beer and can be used as an alternate to the 100 % barley malt beer.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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