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Flavour profiling of red wine with respect to different strains of yeast

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ABSTRACT

Twenty-three Saccharomyces cerevisiae strains isolated from traditional alcoholic beverages of Bharmour, Lahaul & Spiti and Bada Bhangal region of North Western Himalayas were used to prepare wine from red grapes cv. Zinfandel. The juice was fermented by S. cerevisiae strains under optimized conditions viz. 5% (v/v) inoculum size and 50 mg/100 ml DAHP supplemented at 28 °C. The prepared wine samples were compared based on their alcoholic content and fermentation efficiency. Maximum alcohol content (11.03% v/v) and fermentation efficiency (86.16%) were recorded in the wine produced by the 'ABB' S. cerevisiae (MTCC 13018) strain isolated from Bada Bhangal region. Wines were further analyzed for their ester profile using GC-MS technique. Mainly two esters, ethyl acetate and isoamyl acetate, were found to be predominant in all the wines with varying concentrations. After analysis, the selected wine samples with different alcohol and ester concentrations were subjected to sensory evaluation. MTCC 13018 strain produced enough esters and alcohol to provide a suitable bouquet to the wine, which led it to score maximum points (7) on the "Hedonic scale" and was considered best among all other strains. These findings suggest that the 'ABB' S. cerevisiae strain (MTCC 13018) can be exploited commercially, especially in micro wineries to improve indigenous wines.

Keywords: Ester profile, North Western Himalayas, Red wine, *Saccharomyces cerevisiae,* Zinfandel

INTRODUCTION

Red wine is consumed world-wide and is one of the most popular alcoholic beverages (Joshi *et al.,* 5). It is produced by anaerobic fermentation of grape sugar to ethanol by the action of yeasts. Yeasts are the prime organisms associated with fermentation processes and by exploiting the indigenous yeast diversity in the specific area or of particular product, noble brewing yeast strain can be obtained. The dominating genus is *Saccharomyces cerevisiae* among the various yeast isolates that is primarily involved in fermentation process of various traditional fermented foods and beverages.

The fundamental flavour compounds in product bouquet are secondary metabolites i.e. acetate esters, ethyl esters, volatile fatty acids, carbonyls, and fusel alcohols, produced by diverse range of micro biota. In winemaking, different yeast species from grapes and equipments can be involved in fermentation, contributing to wine flavour. As the ethanol concentration increases, the species diversity of the ecosystem gets diminished making wine fermentation as a highly selective environment, resulting in predominance of the wine yeast, *Saccharomyces cerevisiae.* The type of yeast strain chosen for fermentation determines the presence of most aroma-active compounds. During fermentation processes, wide range of aroma-active substances especially volatile esters are produced by yeast cells, which affect the flavour of alcoholic beverages. Yeast strains also determine the amount of alcohol produced during fermentation which in turn plays a major role in overall wine bouquet. Wine flavor and aroma profiles generated through different strains of *Saccharomyces cerevisiae* can be detected by wine professionals and trained panel of judges and by wine consumers.

The flavor- active compounds synthesized can be divided into six main categories: carbonyl compounds, organic compounds, Sulphur-containing compounds, phenolic compounds, higher alcohols, and volatile esters (Saerens *et al.,* 11). The yeast strain selected for fermentation directly influences the production of aroma active compounds. Alcohol acetyltransferases are the enzymes involved in ester synthesis and are encoded by *ATF* genes (Verstrepen *et al.,* 14; Keshani *et al.*, 6). Esters are trace elements as compared to other yeast metabolites but are most predominant aroma element produced by yeast (Pires *et al.,* 8), which can be detected through GC-MS analysis. North Western Himalayan region has enormous amount of microbial diversity (Keshani *et al.*, 6) and this manuscript deals with understanding and determining the range flavour phenotypes (esters) exhibited by wine yeasts of North Western Himalayas.

MATERIALS AND METHODS

The yeast strains were isolated from traditional fermented beverages of Bharmour, Lahaul & Spiti and

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Bada Bhangal region of North-Western Himalayas. The red grape variety used for study was Zinfandel which was procured from Sula Vineyards, Nashik. Healthy ripened grape berries were selected for juice extraction. The extracted juice was supplemented with 0.01% KMS and was subjected to skin treatment by using 80% grape skin weight at 10˚C for 16h to improve the color intensity of the juice.

The yeast inoculum was prepared by inoculating loopful of yeast culture to the 100 ml yeast extract peptone dextrose (YEPD) broth in separate 250ml Erlenmeyer flasks. All the flasks were incubated on rotary shaker at 100 rpm at 28 ˚C for 24 h. The Starter culture was prepared from seed inoculum by adjusting the grape juice to 9˚Brix (by diluting with sterile water). 2% of the seed inoculum was inoculated in pasteurized grape juice and incubated at 28 ˚C for 24h on shaking (100 rpm). After skin treatment, the grape juice (350 ml) was taken in fermentation flasks. The juice was supplemented with Diammonium hydrogen ortho phosphate (DAHP) (50 mg/100ml). The juice in each flask was inoculated with 5% (v/v) starter cultures of 23 different strains. The flasks with inoculated juice were incubated at 28 ˚C (Nikhanj and Kocher, 9). The fermenting juice was analyzed till constant values of ˚Brix were obtained and reducing sugars (g/100ml), total sugars (g/100ml), pH, titrable acidity and alcohol content (% v/v) were determined (Table 1).

Glass Brix Hydrometers (0-10 ˚B, 10-20 ˚B) and Erma refractometer of 0-32 ˚B were used to analyze the total soluble solids (% TSS). A pocket-sized pH meter (Hanna HI96107) was used to determine the pH of juice. Method of Dubois *et al.* (3) was used to estimate the total sugars of juice and periodic samples. Similarly reducing sugars of the juice and periodic samples were analyzed by the method of Miller (7). Ethanol was estimated by using chemical oxidation method of Caputi *et al.* (2).The fermentation efficiency of different experiment treatments was calculated as:

$$\frac{\text{Actual ethanol recovery } (\%\frac{v}{v})}{\text{Theoretical ethanol recovery } (\%\frac{v}{v})} \text{ X } 100$$

Table 1. Analysis of pH, acidity, total sugars, alcohol content and fermentation efficiency.

S. No.	Yeast Cultures	pH	Acidity	Total sugars (g/100mL)	Alcohol % (v/v)	Fermentation efficiency (%)
1.	GP2	3.465	0.431	1.2433^{defg}	9.54^{defgh}	74.5^{defgh}
2.	GP4	3.435	0.434	1.3167^{abc}	9.43^{efgh}	73.63^{efgh}
3.	ABB	3.4	0.436	1.1067^{k}	11.03^{a}	86.16^{a}
4.	X4	3.405	0.434	1.24^{defg}	10.06^{bcdef}	78.63^{bcdef}
5.	SMSC	3.43	0.433	1.1367^{jk}	9.91^{cdefg}	77.43^{cdefg}
6.	Sc24	3.4	0.435	1.34^{ab}	9.27^{gh}	72.43^{gh}
7.	GP6	3.42	0.433	1.1867^{ghij}	10.2^{bcd}	79.63^{bcd}
8.	SM2	3.42	0.433	1.1567^{hijk}	10.23^{bcd}	79.9^{bcd}
9.	SB	3.42	0.433	1.15^{ijk}	10.7^{ab}	83.53^{ab}
10.	SMLC	3.46	0.432	1.36^{a}	9.16^{h}	71.56^{h}
11.	Sc01	3.415	0.435	1.1567^{hijk}	10.1^{bcde}	78.86^{bcde}
12.	CHK-9	3.395	0.434	1.16^{hijk}	10.31^{abc}	80.56^{abc}
13.	P-4	3.465	0.431	1.2567^{cdef}	9.33^{fgh}	72.86^{fgh}
14.	P-7	3.445	0.432	1.23^{defg}	10.04^{bcdef}	78.43^{bcdef}
15.	B-8	3.455	0.433	1.3167^{abc}	9.66^{cdefgh}	75.5^{cdefgh}
16.	Sc02	3.405	0.435	1.27^{bcd}	10.26^{bcd}	80.16^{bcd}
17.	Sc06	3.43	0.431	1.1967^{fghij}	9.733^{cdefgh}	76.0^{cdefgh}
18.	Sc08	3.425	0.436	1.2133^{efghi}	10.14^{bcde}	79.16^{bcde}
19.	Sc09	3.45	0.434	1.2267^{defg}	9.59^{cdefgh}	74.86^{cdefgh}
20.	Sc10	3.425	0.432	1.14^{jk}	9.86^{cdefgh}	77.03^{cdefgh}
21.	Sc20	3.43	0.432	1.2167^{defgh}	10.05^{bcdef}	78.53^{bcdef}
22.	Sc04	3.45	0.434	1.27^{cde}	9.58^{cdefgh}	74.85^{cdefgh}
23.	CHK-4	3.43	0.434	1.2267^{defg}	10.09^{bcde}	78.85^{bcde}

Ester profile of wines was analyzed by using gas chromatography and mass spectrometry (GC-MS) by SIGMA tests & Research Centre, Delhi.

Wine was bottled and studied for different physico-chemical parameters. The selected wine samples on the basis of alcohol and esters content were further subjected to sensory evaluation by a panel of judges. Product was evaluated for consumer acceptance on a nine point "Hedonic scale" (Amerine *et al.,* 1). The wine samples were evaluated by a panel of 4 judges, who were acquainted with wine either occasionally or frequently. The most suitable brewing yeast strain was deposited in MTCC, Chandigarh.

RESULTS AND DISCUSSION

The physicochemical analysis of fresh grape juice showed 20 ˚B TSS when measured with hydrometer, pH 3.51, 0.408 acidity, 18.5 g/100ml total sugars and 16.2 g/100ml reducing sugars. Alcohol is the most important and abundant volatile compound found in wine and contribute to its sensory attributes. Thus, yeast strains producing ethanol in high concentration play significant role in brewing industry (Goldammer, 4). The strains used in present study were found to produce alcohol content ranged from 9.16% (v/v) to 11.03% (v/v) (Table 1). Maximum alcohol content was observed in strain ABB whereas SMLC produced minimum alcohol content. During the fermentation process, total soluble solids (TSS) of the juice were reduced from 20˚B to 0 ˚B by all strains used. Fermentation efficiency of twenty-three yeast strains was found in the range of 71.56% - 86.16% (Table 1). Yeast isolate coded as ABB showed maximum fermentation efficiency of 86.16% whereas SMLC showed minimum i.e. 71.56%. The decrease in pH and sugar content but slightly increase in titrable acidity was also noticed (Table 1).

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

Various molecules from different classes of compounds such as alcohols, aldehydes, and esters were detected after GC-MS analysis. Ethyl acetate, isoamyl acetate, phenyl ethyl acetate, ethyl caprylate and ethyl caproate were detected in all wine samples. 2-Methyl-1-butanol was analyzed in four wine samples while 3-Methyl-1-butanol and 2-Methoxy butane were found in all samples except one, 2-Methyl-1-propanol and Phenylethyl alcohol were detected in three and six wine samples, respectively. Isobutane, 2-Methoxy-2-methyl propane, hexane and 2-Methoxy pentane were detected in one wine sample only. Most predominant esters detected were ethyl acetate and isoamyl acetate (Table 2).

GC-MS is the most used technique to detect volatile organic compounds produced by the action of yeasts in wine. Volatile or semi- volatile compounds can be separated by the application of gas chromatography whereas their detection can be done by mass spectrometry (Sneddon *et al.,* 12).

Among all esters, ethyl acetate is most important for flavor and aroma profile of wine. Usually it gives dry fruity musty pineapple flavor but when its amount exceeds 200 mg/l, it slightly gives flavor of acetic acid (Rodriguez-Bencomo *et al.,* 10). Isoamyl acetate provides fruity flavor more likely banana (Torres *et al.,* 13). In present study, higher alcohols in all the wine samples were detected within the limit. Mainly two esters viz., ethyl acetate and isoamyl acetate were detected in all wines with varied concentration. In some wines, ethyl acetate concentration exceeds 200 mg/l which results in slight change in organoleptic characteristics.

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., color, aroma and mouthfeel are very closely linked to the quality of wine. Wine aroma is one of the major factors contributing to the wine quality and helps in differentiating a variety of wines (Yang and Lee 15). In the wine cellar, sensory evaluations by outsiders, trained panelists and wine experts help the winemakers to make decisions on winemaking practices and help them to avoid flaws resulting from their own cellar palate. Five wine samples on the basis of heterogeneity in ester profile were put for sensory analysis to find out the acceptability of wine samples. Commercial wine was used as standard for comparison. These wine samples were subjected to evaluation by a panel of four judges on a 9 point 'Hedonic scale'. Amount of ethyl acetate produced by yeast strains SB, Sc01, GP6 and GP4 are 147.69mg/l, 133.92 mg/l , 41.36 mg/l and 130 mg/l; and of isoamyl acetate are 1160 mg/l, 400mg/l, 1100mg/l and 660mg/l respectively. Although wine produced by using ABB strain (MTCC 13018) had small amount of esters (ethyl acetate 36.55mg/l and isoamyl acetate 940 mg/l) as compared to others but these were found sufficient to provide good aroma and flavor to the wine. Moreover, ABB strain produced maximum alcohol content of 11.03% among all the strains, which resulted in its more acceptability as compared to other wine samples. The wine produced by ABB strain (MTCC 13018) was considered best among all on the basis of sensory evaluation (Table 3).

Yeasts play vital role in the production of good quality wine as they are directly involved in ester as well as other volatile compounds production. This study facilitated in understanding the range of

Table 2. GC-MS analysis of ester profile of wine samples.

Yeast cultures	Ethyl acetate (mg/L)	Isoamyl acetate (mg/L)	Phenyl ethyl acetate (mg/L)	Ethyl caprylate (mg/L)	Ethyl Caproate (mg/L)
GP2	16.44	380	Within limit 0.01	Within limit 0.01	Within limit 0.01
GP4	130.59	660	Within limit 0.01	Within limit 0.01	Within limit 0.01
AB	36.55	940	Within limit 0.01	Within limit 0.01	Within limit 0.01
X4	83.44	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
SMSC	16.66	1120	Within limit 0.01	Within limit 0.01	Within limit 0.01
Sc24	103.41	1300	Within limit 0.01	Within limit 0.01	Within limit 0.01
GP6	41.36	1100	Within limit 0.01	Within limit 0.01	Within limit 0.01
SM2	256.91	1120	Within limit 0.01	Within limit 0.01	Within limit 0.01
SB	147.69	1160	Within limit 0.01	Within limit 0.01	Within limit 0.01
SMLC	31.33	900	Within limit 0.01	Within limit 0.01	Within limit 0.01
Sc01	133.92	400	Within limit 0.01	Within limit 0.01	Within limit 0.01
CHK9	108.17	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
P4	425.59	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
P7	135.89	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
B-8	305.46	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
Sc02	227.19	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
Sc06	321.05	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
Sc08	287.53	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
Sc09	95.54	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
Sc10	284.73	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
Sc20	304.82	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
CHK4	305.46	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01
Sc04	364.35	Within limit 0.01	Within limit 0.01	Within limit 0.01	Within limit 0.01

Table 3. Sensory evaluation of wine prepared by using AB, SB, Sc01, GP6 and GP4 along with commercial wine sample as standard (Std).

Sr. No.	Sample code	Sensory parameters				
		Appearance/ Color	Flavor	Mouthfeel	Overall Acceptability	Total score
1	AB	7	7	7	7	7
2	SB	7	6	6	6	6
3	Sc01	7	7	6	6	6
4	Std	8	5	5	6	6
5	GP6	7	6	5	6	6
6	GP4	7	6	6	6	6
	t- test (5%)	5.0	5.4	5.9	3.8	5.9

esters, the flavor phenotypes, which wine yeasts of North-Western Himalayas exhibit. Among all the studied strains AB strain (MTCC 13018) showed the paramount fermentation potential and flavor suggesting its prospective use in industrial sector.

AUTHORS' CONTRIBUTION

Conceptualization of research (K, GSK); Designing of the experiments (K); Contribution of experimental materials (HK, K, GSK); Execution of field/lab experiments and data collection (HK, K); Analysis of data and interpretation (HK, K, GSK); Preparation of the manuscript (HK, K)

DECLARATION

The authors have no conflict of interest to declare

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