Evaluation of Fermentation Efficiency of Yeast Strains and their Effect on Quality of Young Wines

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Abstract The yeast has important role in fermentation of wine grapes and wine quality. The fermentation of wine grapes affect by efficiency of particular yeast strain, sugar content, pH, available temperature, etc. To evaluate the efficiency of yeast strains (Premier Cuvee, RS-1, RS-2, RS-3 and natural), present study was conducted on two wine grape varieties viz.; Sauvignon Blanc (White) and Cabernet Sauvignon (Red). Efficiency of yeast strains was evaluated in terms of conversion rate of sugar into alcohol. As per recorded data, strain RS-3 (*Pichia kudriavzevii*) was found more efficient than other strains in fermentation of Cabernet Sauvignon with efficiency of 84.4 per cent but in case of Sauvignon Blanc, the commercial culture Premier Cuvee was found superior over RS-3. The quality parameters of young wines of both the varieties were also affected by the used strains. Considering the efficiency and impact on various parameters of wines, local strain, i.e., RS-3 was found at par with commercial culture (Premier Cuvee). The RS-3 strain has potential to produce quality wines. However, studies on effects of RS-3 strain on some specific quality parameters of wines like varietal aroma compounds, flavours etc. are needed.

Keywords Fermentation efficiency · Yeast strains · Cabernet Sauvignon · Sauvignon Blanc · Wine quality

Wine is an alcoholic beverage produces from grape juice. During fermentation process yeast consumes the sugars found in the grapes and converts them into alcohol. Microorganisms have a prominent role in determining the

chemical composition and hence the quality of wine. They consume grape sugars and other components and convert them into ethanol, CO₂ and hundreds of secondary end-products that, collectively, contribute to the subtlety and individuality of wine character [1, 2].

Selection of proper yeast strains is one of the major factor in production of good quality wine [3]. The ability of producing alcohol from sugar varies differentially depending upon the yeast strains. This depends upon the several characters of the strain like: alcohol tolerance, optimum pH and temperature, ability to ferment sugar, etc. The criteria for selection of yeast strains assist in the choice of yeasts that are able to improve the quality and consistency of wine. The selection process of yeast strains depends on their oenological characteristics, such as fermentative rate, tolerance to ethanol and SO₂, flocculent characteristics, the presence of killer factors, acetic acid production, H₂S, malic acid metabolism, higher alcohol production, alcohol yield, glycerol production, and extra cellular enzyme production [4].

The alcoholic fermentation usually occurs one of two ways. Firstly, yeast naturally present on grapes or on winery surfaces can conduct the fermentation. This is referred to as a “natural”, “native”, or “spontaneous” fermentation. Typically, non-*Saccharomyces* species such as *Candida*, *Kloeckera*, *Kluyveromyces*, and *Hanseniaspora* grow during the early stages of alcoholic fermentation, but their viability rapidly declines due to the lack of oxygen and increasing ethanol levels. Therefore, it is very important to select a proper yeast strain that can ferment the grapes more efficiently. The importance of each yeast source in the vineyard and winery may vary greatly, depending on a large variety of factors, such as climatic conditions, including temperature and rainfall in the region/site, the geographic location of the vineyard, the

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harvest technique, grape variety, the age of the vineyard and the soil type [5]. Keeping in view the importance of local yeast, present study was conducted to evaluate fermentation efficiency of locally identified yeast strains with commercial culture.

Present study was conducted during fruiting season of 2010 at National Research Centre for Grapes, Pune (India). The bunches of varieties viz.; Cabernet Sauvignon and Sauvignon Blanc, attaining proper ripening with desired total soluble solids (TSS) i.e. more than 23°Brix were collected from vineyards. The harvesting was done in the morning hours. Just after harvesting, bunches were placed at low temperature to remove the field heat. De-stemming was done manually. Then, grape berries were crushed in a crusher. Immediately after crushing 100 ppm potassium metabisulphite (Merck) was added in must/juice to suppress the development of natural micro flora. The must/juice used for spontaneous fermentation, was not treated with potassium metabisulphite.

Locally identified yeast strains RS-1 and RS-2 and RS-3 have been identified as *Pichia kudriavzevii* based on sequence of D1/D2 region (GenBank Accession Number: BankIt1456460 seq1 JN566063) and (Gen- Bank Accession Number: BankIt1456460 seq2 JN566064), respectively a commercial culture (Premier Cuvee) and natural microflora (spontaneous fermentation) were used for fermentation. The juice/must was inoculated with viable cell count, i.e., 1.06×10^8 /ml of each strain. The inoculated juice of Sauvignon Blanc was placed at 18–20°C and inoculated must of Cabernet Sauvignon was placed at 20–22°C for fermentation. During fermentation process, fermenting materials were mixed twice everyday considering the non-transfer of yeast culture from one vessel to another. The material like skin, seed and yeast lees was separated from fermented material i.e. wine on 11th day after inoculation.

The wine samples were collected on the 11th day after inoculation to study the fermentation efficiency of used strains. The prepared wines were placed at low temperature for clarification. The samples for analysis of young wines were collected after 30 days storage of wines at low temperature and one racking. These samples were centrifuged at 5000 rpm for 10 min before further studies.

Different analyses were done using standard procedures. The fermentation efficiency of strains was calculated on the basis of the relationship between the sugar consumed and alcohol produced following the fermentation stoichiometry, where 1 g of total reducing sugar produces 0.461 g ethyl alcohol [6]. The pH and acidity were deduced using wine analyzing system of Metrohm. The reducing sugar was estimated by the DNSA method [7], and glucose stock solution (Merck) was used as a standard. Absorbance was taken at 540 nm using the Pharma Spac 1700 UV spectrophotometer (SHIMADZU: UV–Visible Spectrophotometer).

The titration method was used for the determination of total SO₂ and free SO₂ [8]. The wine samples were diluted 1:10 and colour intensity was measured at 420, 520 and 620 nm. Singleton and Rossi method [9] was used to estimate total phenols in the wines. Absorbances were taken at 765 nm with a UV spectrophotometer. Concentration of monomeric anthocyanins was analyzed by using pH differential method [10]. Ferric ion-reducing antioxidant power (FRAP) was estimated following the method of Benzie and Strain [11]. Quercetin standards of different concentrations were taken directly with the UV spectrophotometer immediately after the addition of 0.9 ml of FRAP solution. A standard curve was prepared for the quercetin solutions and the amount of antioxidant in the samples was estimated from the curve. For the estimation of free radical scavenging activity by the DPPH assay, the method suggested by Arnous et al. [12] was adopted. The readings were taken with a UV spectrophotometer at 515 nm. A standard curve was prepared using Trolox solutions and the amount of free radical in the samples was estimated from the curve. Alcohol content in wines was estimated by using ebulliometer.

During fermentation of Cabernet Sauvignon, maximum efficiency (84.49%) was recorded in RS-3 followed by RS-2, RS-1 and PC with values of 81.80, 80.31 and 75.36 per cent, respectively. While in case of Sauvignon Blanc, PC found with better fermentation efficiency i.e. 97.68 per cent was closely followed by RS-3 with value of 96.99 per cent. However, non-significant differences were noted among PC, RS-3 and spontaneous fermentation (natural microbes). Local yeast strain RS-1 was found least efficient in fermentation of both varieties (Fig. 1).

Data presented in Table 1 shows the effect of yeast strains on quality of young wines made from Sauvignon Blanc. Significant differences were noted in all studied parameters among the yeast strains. The pH values of wines produced from different yeast strains were ranged from 3.29 to 3.51. The pH values of all wines were within

Fig. 1 Fermentation efficiency of yeast strains in Cabernet Sauvignon and Sauvignon Blanc

Table 1 Biochemical parameters of white wines (Sauvignon Blanc)

Sample	pH	TTA (g/l)	Volatile acids (g/l)	Total SO ₂ (mg/l)	Free SO ₂ (mg/l)	Total sugar (g/l)	Reducing sugar (g/l)	Alcohol (% v/v)	Phenols (g/l)	FRAP (mg/ml)	DPPH (mM)
RS-1	3.32	6.98	0.152	41.95	9.70	17.49	16.42	10.9	0.1265	0.000540	0.306
RS-2	3.29	7.09	0.147	44.90	12.90	2.60	1.55	13.3	0.1229	0.000810	0.273
RS-3	3.31	7.29	0.255	28.95	17.50	2.21	1.48	13.5	0.1193	0.000350	0.243
PC	3.51	6.05	0.097	41.20	14.60	11.28	10.20	13.1	0.1401	0.000808	0.340
NT	3.42	7.31	0.136	35.45	9.50	10.59	9.53	13.0	0.1074	0.000660	0.200
SEM±	0.011	0.0133	0.011	0.2903	0.1347	0.2445	0.186	0.077	0.000764	0.0000066	0.0118
CD at 5%	0.044	0.0521	0.0374	1.1353	0.526	0.7969	0.608	0.252	0.00249	0.0000261	0.0385

acceptable limit of good wines. Total titratable acidity content of wines ranged between 6.05 and 7.31 g/l. The wine made from PC was recorded with minimum titratable acidity and maximum value was recorded in wine made from spontaneous fermentation. Wine made from RS-3 contained maximum volatile acidity with the value of 0.255 g/l followed by RS-1, RS-2, NT and PC with values of 0.152, 0.147, 0.136 and 0.097 g/l, respectively. Total SO₂ content of wines also varied according to the yeast strains. Maximum concentration (44.90 mg/l) was reported in RS-2 followed by RS-1, PC, NT and least (28.95 mg/l) was in RS-3. However, maximum free SO₂ content was found in RS-3 followed by PC. Sugar content of these wines showed the fermentation efficiency of particular strain. The wines obtained from the inoculation of RS-1 contained maximum total sugars and reducing sugar. Minimum reducing sugar i.e. 1.48 g/l was observed in RS-3 followed by RS-2 (1.55 g/l). Wine produced by the inoculation of RS-3 recorded maximum alcohol i.e. 13.5 percent followed by RS-2. Whereas, wine made from RS-1 contained minimum alcohol (10.9%). The antioxidant capacity of young wines was also affected by the used yeast strains. The antioxidant capacity was reflected through the values of phenolic content, FRAP and DPPH

assay. Wines produced from spontaneous fermentation was found with minimum value of phenolics i.e. 0.107 g/l while maximum concentration was reported in wine made from RS-1 with the value of 0.126 g/l. The values of FRAP and DPPH were maximum in wines obtained from Premier Cuvee (Table 2).

The yeast strains significantly affected various parameters of young wines. The pH value of young wines of Cabernet Sauvignon were slightly higher than wines made from Sauvignon Blanc. The values of pH were ranged from 3.55 to 3.65 and non-significant differences were noted among wines prepared from different yeast strains. The TTA values were found between the limit of 6.74 and 6.98 g/l which were significantly affected by the yeast strain. Wine from RS-3 contained higher TTA and lowest TTA was recorded in PC. But the maximum volatile acidity was found in wine made from PC and minimum was in RS-2. The SO₂ (free and total) values of these wines were lower than wines produced from Sauvignon Blanc. Wines made from inoculation of yeast strains contained less sugars and reducing sugars than spontaneous fermentation. Minimum sugars and reducing sugars were recorded in wines made from RS-3 followed by RS-2. The strains of RS series were found superior over commercial culture in

Table 2 Biochemical parameters of red wines (Cabernet Sauvignon)

Sample	pH	TTA (g/l)	Volatile acids (g/l)	Total SO ₂ (mg/l)	Free SO ₂ (mg/l)	Total sugar (g/l)	Reducing sugar (g/l)	Phenols (g/l)	FRAP (mg/ml)	DPPH (mM)	Monomeric anthocyanins (mg/l)	Colour intensity	Alcohol (% v/v)
RS-1	3.55	6.95	0.164	7.21	3.58	5.28	3.69	1.98	0.0247	0.530	353.34	18.77	10.7
RS-2	3.65	6.76	0.144	7.38	4.36	5.25	3.66	2.05	0.0212	0.536	359.21	20.37	11.3
RS-3	3.56	6.98	0.168	9.78	4.19	4.74	2.83	2.07	0.0211	0.535	345.51	23.16	11.3
PC	3.64	6.74	0.424	5.50	4.17	5.94	4.61	2.26	0.0300	0.549	421.85	21.25	12.0
NT	3.57	6.97	0.184	5.79	4.85	6.53	5.86	2.48	0.0336	0.545	384.66	20.13	11.7
SEM±	0.0344	0.0095	0.0259	0.0082	0.0198	0.212	0.116	0.0264	0.00757	0.00344	17.534	0.898	0.059
CD at 5%	NS	0.0372	0.0844	0.0323	0.0775	0.693	0.379	0.0862	NS	0.0112	57.151	2.928	0.192

NS non significant, *FRAP* ferric ion reducing antioxidant power, *DPPH* 2, 2-diphenyl picrylhydrazyl, *TTA* total titratable acidity

consuming the sugars during fermentation. The maximum phenols conc. (2.48 g/l) was noted in wine prepared from natural fermentation followed by PC and RS-3. The strain RS-1 was found with minimum phenols (1.98 g/l). Almost same trend was observed in the evaluation of antioxidant activities by FRAP and DPPH assays. Maximum FRAP value was noted in wine made from natural fermentation followed by PC. Where as, in case of DPPH assay, maximum value was recorded in PC followed by natural fermentation. Maximum value of monomeric anthocyanins was noted in wine made from inoculation of commercial culture i.e. Premier Cuvee which was followed by spontaneous fermentation whereas; RS-3 was recorded with minimum anthocyanins. But in case of colour intensity, maximum value i.e. 23.16 was noted in wine made from RS-3 followed by PC. The alcohol content of these young wines was significantly affected by the yeast strain used for must fermentation.

Fermentation efficiency is totally dependent up on the ability of yeast strain to respond over various stress conditions subjected during fermentation, viz. high ethanol concentration, nutrient availability, etc. [13]. The differences in fermentation efficiencies among yeast strains were also found. The fermentation efficiency of both white and red wines were influenced by yeast strains in present study also [6]. Joshi et al. [14] also noted the variations in fermentation efficiencies of yeast strains when worked on plum wines.

The biochemical parameters of wines produced from different yeast strains showed significant differences. These results were in confirmation with the work of Vilanova and Massneuf-Pomarede [15]. They also observed significant differences in the quality parameters of wines made from different yeast strains. These wines were highly acceptable. Differences in volatile acidity of wines made from different yeast strains have shown the ability to produce volatile compounds varies among yeast strain [2, 16]. The acidity in wines gives a taste, aroma and finally body to particular wine. Sugar content of wine also affects the taste but the acceptability of wine depends on many factors. The sugar was most efficiently utilized by yeast strain RS-3 than other strains in both the varieties. The utilization of sugars is an important parameter which decides the performance of the yeast culture. The wines made from RS-3 had maximum alcohol content, indicating that it has good potential for the efficient utilization of sugars for the production of alcohol over other used yeast strains. The specific environmental conditions in the must, viz. high osmotic pressure, the presence of SO₂, temperature and cellar hygiene, all play a role in determining which species can survive and grow in the must [17]. The effect of yeast strains was recorded in the total and free SO₂ content in wines. Yeast strains differ widely in their ability to produce

sulphite and sulphide [18]. However, in addition to strain effect, the nutrient composition of the grape juice, the concentration of sulphate, must clarification, the initial pH and temperature all affect sulphite formation by wine yeasts [19]. Wines made from different yeast strains differed in their antioxidant activities. These results confirmed the results of Caridi et al. [20]. Yeast strains significantly affected colour intensity, concentration of phenols and monomeric anthocyanins [3, 21].

From the results of present study it can be concluded that, the yeast strains have their own impact on quality of wines. Among all the used strains, RS-3 (non-*Saccharomyces*) was found to be more efficient in fermentation of both varieties. This strain is also found more suitable for the production of dry wines having least concentration of residual sugar. RS-1 was found to be least efficient in both varieties. It can be concluded that the yeast strains influence fermentation behaviour and quality parameters of wines. Among locally identified yeast strains, RS-3 is found at par with commercial culture and has potential to be utilized for obtaining quality wines.

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