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Effect of recycled yeast inoculum on fermentation and quality of red wine produced from Punjab purple (Syn H516) grapes

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Fresh and recycled yeast (*Saccharomyces cerevisiae* MTCC 11815) inoculums were compared for fermentation of Punjab purple (Syn H516) grapes juice for preparation of red wine. An inoculum size of 10% of recycled yeast was optimized for an ethanol production of 9.5% (v/v) at a fermentation efficiency of 74.53% with stored juice and the same was consistent for up to two recycling lots. The prepared wine was allowed to sediment for 7 days at 4°C, siphoned and filtered and then matured for 6 months in amber colored bottles. A significant decrease in ethanol, total phenols and yeast count was observed in wines prepared from both fresh and recycled yeast inoculum during storage. Both the type of wines scored 65.9±8.01 and 63.3±8.52 on sensory analysis for fresh and recycled inoculum, respectively and were significantly better than a commercial red wine. The red wines were also found to be rich in 10 amino acids.

Keywords: Recycling, Red wine, Fermentation, Sensory analysis, Phenols, Amino acids.

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Introduction

Grapes are the foremost substrate used worldwide for wine production. In India, the wine production is still in its infancy. Out of 60 odd wineries located in the country, 33 are in Maharashtra, 17 in Karnataka and rest are distributed in Goa, Delhi, Uttrakhand, Madhya Pradesh, Haryana and Jammu and Kashmir with no winery in Punjab[1]. 'Perlette' is the only grape cultivar grown on commercial scale in Punjab. The latter being a table purpose variety, with bunches and low Brix, is not suitable for wine production[2]. As an alternative, Punjab purple (Syn H516) with berries having dark purple juice and seeds, has been found to be suitable for red wine production in Punjab[3].

The commercial ethanolic fermentation is normally batch type and requires preparation of fresh inoculum for every new batch as a result of which an initial lag phase is observed during fermentation. This lag may be reduced by recycling yeast from previous fermentation lots[4]. Recycling of yeast inoculum has been considered as an important parameter in lowering wine production costs with improved fermentation performance[5]. Hence, the present study was carried out to evaluate different concentrations of spent yeast inoculum on fermentation and quality of red wine production from Punjab purple grapes.

Materials and Methods

Raw material and fermenting yeast strain

Grapes (var. Punjab purple Syn. H516) were procured from Department of Fruit Science, Punjab Agricultural University, Ludhiana. The berries were destemmed, washed in lukewarm water (containing 0.1% potassium metabisulphite) and then crushed for juice extraction. The fermenting yeast employed in the study was our own isolate from distillery waste which was identified and characterized as *Saccharomyces cerevisiae* MTCC 11815.

Fermentation of grape juice and analytical methods

The juice of Punjab purple grape berries was pretreated by skin fermentation (skin weight 0.25 kg/L, Temperature 20°C, Time of contact 16 h) and enzymatic treatment with combination of enzymes (amylase; 25 units/100 mL + cellulase; 25 units/100 mL + pectinase; 50 units/100 mL)[6]. The juice so pretreated was chaptalized with sterile sugar to make it 20 °B and then was subjected to fermentation as 300 mL lots in 500 mL capacity glass bottles under optimized conditions[7] (25°C, 5% v/v fresh yeast inoculum having ~10^8 cells/mL)[8] with the supplementation of

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150 mg/100 mL of diammonium hydrogen phosphate (DAHP)[1]. Thereafter, the fermented ‘must’ from this first lot was partially removed after cessation of fermentation to form 5-20% of the inoculum (15 mL to 60 mL/300 mL to report four different inoculum levels i.e. 5, 10, 15 and 20 % v/v) with direct microscopic counts ranging from 3×10^9 to 1.2×10^{10} cells/mL[9, 10]. The spent yeast inoculum was used to inoculate fresh ‘must’ under optimized fermentation conditions for three sequential fermentation lots (lot 1 to 3) for inoculum recycling study. Periodic samples were taken and analyzed for ethanol content[8], pH (pH meter Hanna HI96107) and Brix (Refractrometer, Erma type, Japan). After the completion of every fermentation lot, post fermentative treatments i.e. storage and sensory analyses (by ten semi trained panelists) were carried out for wines prepared from both fresh and recycled yeast inoculums in comparison to a commercial wine. The qualitative amino acid analysis was also carried out by Thin Layer Chromatography (TLC) using butanol: acetic acid: water:: 40:20:10 as solvent system with stationary phase consisting of silica gel impregnated with 0.2% ninhydrin[9].

Results and Discussion

Effect of recycled yeast

The results presented in the Table 1 revealed increase in ethanol production with 10% (v/v) inoculums while higher inoculum sizes reported lower ethanol production which is attributed to the fact that higher biomass required more sugars for its growth[4]. The same was also reflected in the fermentation efficiencies of different inoculum levels. It was found that among the lots, the second recycled lot (Lot 2)

Table 1—Effect of recycled yeast on ethanolic fermentation of pretreated juice from Punjab Purple grapes

*LOT no.	Time (days)	Inoculum level (% v/v) 5			10			15			20		
		pH	Brix (R.S)	%Eth (F.E)[1]	pH	Brix (R.S)	% Eth (F.E)[1]	pH	Brix (R.S)	% Eth (F.E)[1]	pH	Brix (R.S)	%Eth (F.E)[1]
1	1	3.5	12(10.8)	2.55	3.45	10.5 (8.75)	4.6	3.5	11(8.51)	3.2	3.4	9.5 (6.1)	3.47
	2	3.45	6.75(3.65)	5.1	3.4	3.25 (1.9)	8	3.4	4(2.15)	5.6	3.35	3.25 (1.45)	4.95
	3	3.35	3.25(1.35)	8.92 (69.68)	3.45	2.25 (0.43)	9.54 (74.53)	3.4	3.75(1.65)	8.76 (68.43)	3.3	3 (1.09)	9.5 (74.21)
2	1	3.5	13.5(11.77)	3.95	3.5	12 (10.55)	4.7	3.5	8(5.56)	5.3	3.5	6 (4.56)	5.5
	2	3.45	7.75(5.74)	5.45	3.35	5.5 (3.93)	6.3	3.35	5(3.81)	5.65	3.5	4 (2.50)	6.5
	3	3.35	4.25(1.12)	8.4 (65.62)	3.25	3.5 (1.11)	8.14 (63.59)	3.25	3.25(0.95)	7.36 (57.5)	3.35	3 (0.40)	8.3 (64.84)
3	1	3.5	10(8.69)	3.25	3.5	11 (9.76)	3.25	3.5	9.5(7.4)	2.50	3.5	10.5 (7.43)	2.86
	2	3.45	5(1.85)	5.55	3.25	5 (1.76)	6.50	3.35	4.5(1.68)	4.60	3.35	4 (1.47)	4.95
	3	3.35	3(0.71)	9.67 (75.54)	3.2	2 (0.44)	9.97 (77.89)	3.3	2.5(0.53)	8.53 (66.64)	3.25	3.5 (0.57)	6.85 (53.51)
F.E. (%)		70.28 ±4.98			71.9 ± 7.48			64.18 ± 5.84			64.18 ± 10.36		
CD (5%)		Ethanol -0.388											

* Recycled lots; Initial number of cells- 2×10^8 cells/mL Fermentation conditions: % Eth-Ethanol (% v/v); R.S – reducing sugars (%w/v).
Actual ethanol produced: Scale of fermentation: 300 mL.
**[1]Fermentation Efficiency (F.E.) % (v/v) = × 100 Initial Brix : 20°B (in all lots)
Theoretical ethanol produced Temperature : 25°C
Theoretical Ethanol % (v/v) = available sugar × 0.64 Inoculum size : 5% (v/v) (fresh)

reflected a decrease in ethanol from first lot (Lot 1) but third recycled lot (Lot 3) produced statistically similar ethanol levels as that of first lot. However, the ethanol level of 12.10% (v/v), (Table 2) which was achieved, earlier with fresh juice using fresh yeast inoculum could not be obtained. This decrease in % ethanol level by fresh yeast inoculum may be due to the reason that stored grapes (which were treated with KMS prior to storage) were used for fermentation. Literature also suggests that fermentation is prevented by one of the following oenological procedures: sulphiting or addition of carbon dioxide (carbonation of the must or by sorbic acid). The use of untreated or fresh grapes may further improve the final ethanol levels[10].

Further in literature, we didn't come across any report on recycling of inoculum for grapes. But, there were reports on other substrates which reported decrease in fermentation efficiency with yeast cell recycling and also suggested that intermittent addition of nutrients may improve ethanol production during yeast recycling[11]. Similar report was also observed that there is decrease in ethanol yield from beet molasses with increase in inoculum size and the authors have also reported that the recycling of *Saccharomyces cerevisae* for fermentation of beet molasses was successful for 4 fermentation cycles where ethanol yield decreased after 2nd lot[12]. In the present study, there was no decrease in number of fermentation days for ethanol production among recycled lots, however, the reducing sugars presented a decreasing trend after successive lots.

Among the reports on other substrates, 20% inoculum level for recycling sugarcane fermentation batches was also reported[3]. The lower efficiencies observed at 15% and 20% inoculum levels may be attributed to sustain the high initial inoculum levels. Hence the present study suggested that recycled yeast (10%, v/v) may be suitable for ethanol fermentation of grape juice as the final ethanol concentration of the successive lots was statistically similar.

Effect on quality parameters

As wine quality is an important parameter for consumer acceptability, the wines prepared from recycled lots were subjected to quality analysis. The grape wines prepared under the optimized fermentation conditions of Brix (20°B), temperature (25°C), inoculum sizes of 5% (v/v) for fresh and 10% (v/v) of recycled yeast and DAHP (0.15% w/v) supplementation were subjected to settling for 7 days at refrigerated conditions (4°C) in glass bottles. Afterwards, the bottles were stored at 15 ± 2°C and at every 15 days upto 6 months, the process of racking was repeated. During this storage period, various physiochemical and microbiological parameters were observed (Table 2).

Table 2—Effect of storage time on microbiological and physicochemical properties of wine from Punjab purple variety

Storage Time (days)	Parameters (wines prepared from fresh and recycled yeast inocula)							
	% Ethanol (v/v)		pH		Total phenols* (mg/100mL)		Total yeast count (cfu/mL)	
	Fresh	Recycled	Fresh	Recycled	Fresh	Recycled	Fresh	Recycled
0	12.10	9.97	3.4	3.5	222.6	207.1	6.2×10^6	7.3×10^6
15	11.63	9.86	3.4	3.4	210.9	201.4	1.1×10^1	2.1×10^1
30	11.49	9.64	3.4	3.4	206.2	180.6	0.1×10^1	0.3×10^1
45	11.22	9.53	3.4	3.4	187.4	175.9	0.0	0.0
60	10.89	9.36	3.3	3.4	145.8	155.7	0.0	0.0
75	10.71	9.19	3.3	3.3	138.8	141.4	0.0	0.0
90	10.66	9.10	3.3	3.3	106.6	127.2	0.0	0.0
120	10.45	8.99	3.3	3.3	102.4	112.9	0.0	0.0
150	10.42	8.91	3.3	3.3	100.2	110.4	0.0	0.0
CD (5%)	$CD_{ethanol}$ (days)-0.508 $CD_{ethanol}$ (inoculum)-0.239		NS		CD_{phenol} (days)-8.25 CD_{phenol} (inoculum)- NS		-	

*% decrease in phenols in fresh yeast inoculum wine- 55 %

*% decrease in phenols in recycled yeast inoculum wine- 47 %

Total SO_2 = 22.1 ppm

Free SO_2 = 8.2 ppm

The results revealed that fresh yeast used as inoculum was significantly better than recycled yeast though a significant decrease in % ethanol during storage was observed in both inoculums which may be attributed to chemical oxidation of the ethanol during racking. However, the decrease in ethanol became constant after about 45 days of storage till 150 days upto which storage studies were carried out. No change in pH was observed in both the treatments. The decrease in total phenols was significant with storage as total phenols decreased (fresh fresh wine) to 55 and 47% in fresh and recycled yeast inoculum, respectively. Literature also reports decrease of anthocyanins to 90% with no change in flavonol content of red wines during storage[13]. It was also observed that the decrease in phenols stabilized after 90-120 days of storage. Similar trends have been reported in white and Merlot wines. While a decrease in total phenols upto 6 months was observed elsewhere[14], a continuous decrease of phenols upto 16 months of storage was also reported in another study[15]. Another important observation was the absence of viable yeast cells after 30 days of storage in the racked wine suggesting that yeast tends to settle quickly during storage (Table 2). The free and total sulphur dioxide in the stored wine were also analyzed. The wine from fresh yeast culture was having 22.1 ppm of total SO_2 and 8.2 ppm of SO_2 at 90 days of storage which was less than the recommended values of 150-400 and 25-40 ppm, respectively for red wines[16,17]. It has been reported that sulfur dioxide levels higher than 25 ppm of free SO_2 severely bleach the color of red muscadine wine but lessen browning in high pH wines[18]. High SO_2 levels also lessen browning of wine stored at 20°C, but not at higher storage temperatures.

Sensory analysis

The wines prepared from Punjab purple under optimized conditions with fresh and recycled yeast inoculums were found to be of standard quality with mean scores of 65.9±8.01 and 63.3±8.52, respectively at 150 days of storage whereas the commercial wine used as standard was also of standard sensory quality but with a lower mean score value of 56.2±9.47 (Table 3). Hence, red wine prepared from Punjab purple grapes was significantly better than commercial wine whereas the red wines prepared from recycled yeast inoculum and fresh yeast inoculum were insignificantly different from each other.

In literature, the sensory characteristics of grape wines (white and red wines both) prepared from five different grape varieties/hybrids were studied and species specific variation in sensory characteristics of grape wines were reported[19]. Matured Amla wine was better than non-matured wine in terms of its sensory attributes[20]. The authors also reported that Amla wine matured in oak wood barrel had higher quantity of desirable components including ethyl acetate and phenolics, etc. and was better in its sensory characteristics than the wine stored in glass bottles.

Qualitative analysis

The qualitative analysis of wine with respect to amino acids carried out by TLC reflected the presence of histidine, arginine, lysine, threonine, methionine, alanine, valine, tyrosine, methionine, valine in red wine (Fig.1) on the basis of its comparison with literature for their Rf values[21,22].

Table 3—Sensory evaluation of grape wine (Punjab purple) at 150 days of storage

Sensory analysis (characters)	Maximum points	Age of wine (days)		
		~180	150	150
		Commercial wine	Fresh yeast inoculum wine	Recycled yeast inoculum wine
Appearance	16	12.2±3.73	13.8±2.27	13±2.04
Taste	24	15.8±2.48	17.9±3.44	17.6±2.28
Aroma	24	16.2±4.48	19.8±3.34	18.3±4.53
Total acidity	8	7.0±1.61	7.8±0.6	7.8±0.6
Overall feel	8	5.0±1.34	6.6±0.9	6.6±0.9
Total	80	56.2±9.47	65.9±8.01	63.3±8.52
CD (5%)			4.076	

*****The above scoring is mean ± standard deviation of evaluation by 10 panelists: Ratings: Superior 68-80); Standard (52-68); Below standard (36-52); Unacceptable/Spoiled (4-36)

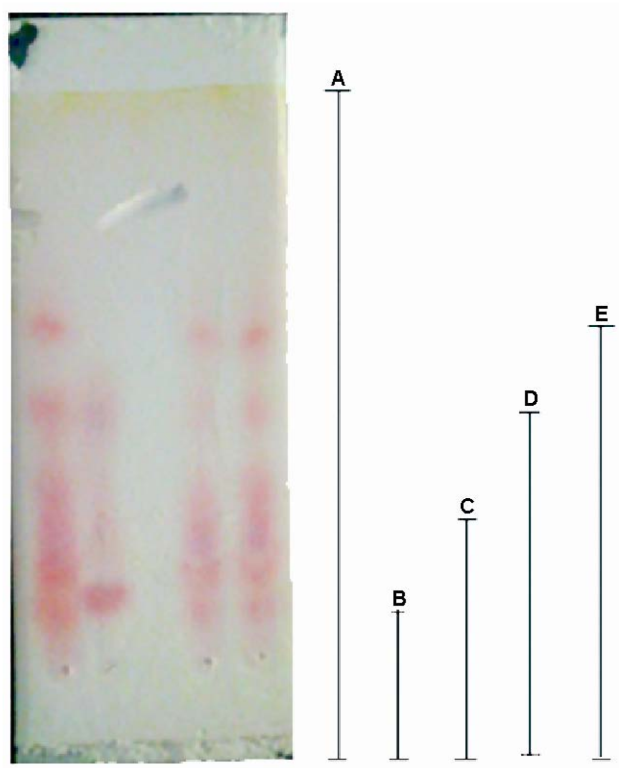

Fig. 1—Thin layer chromatogram of amino acids in red wine (Punjab purple). A: Distance travelled by solvent=15 cm; B= Histidine/ Arginine/ Lysine - 2.5 cm; C=Threonine - 4.7 cm; D=Methionine/ Alanine/ Valine/Tyrosine - 7.6 cm; E=Methionine/ Valine - 9.9 cm

Conclusion

The present study suggested that the spent/recycled yeast inoculum was suitable for preparation of red wine but there was no decrease in number of fermentation days. Moreover, the sensory quality of prepared wine by recycled yeast was found at par with fresh yeast inoculum but was superior than a commercial wine tested.

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