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Effect of different levels of wine yeast inoculum and PH of must on wines prepared from *Mrig Bahar* fruits of Nagpur mandarin

SY Kadu, VU Raut, PK Nagre, DM Panchbhai and SG Bharad

Abstract

The present investigation on effect of different levels of wine yeast inoculum and pH of must on wines prepared from *Mrig Bahar* fruits of Nagpur mandarin was conducted at PHT Laboratory, Department of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during the year 2016-17. The experiment consisted of two different factors *viz.*, levels of wine yeast (*Saccharomyces cerevisiae* var. Ellipsoideus) inoculum and levels of pH of must with three replications using Factorial Completely Randomised Design. The biochemical analyses of the composition of wines prepared indicated that the different levels of wine yeast inoculum as 3, 6 and 9 per cent and levels of pH of must as 3.0, 3.5, 4.0, 4.5 and 5.0 affected the quality of wines prepared. The wine prepared from *Mrig Bahar* fruits of Nagpur mandarin using 6 per cent wine yeast inoculum and 4.0 pH of must yielded highest alcohol.

Keywords: Nagpur mandarin wine, *Citrus reticulata*, *Mrig Bahar*, *Saccharomyces cerevisiae*, wine yeast inoculum, must pH

Introduction

Mandarin (*Citrus reticulata*) fruit contains moisture 82.6-90.2 g, protein 0.61-0.215 g, calories 32-45 Kcal, calcium 25-46.8 mg, phosphorus 11.7-23.4 mg, iron 0.17-0.62 mg, thiamine 0.048-0.128 mg, riboflavin 0.014-0.041 mg, niacin 0.199-0.38 mg, ascorbic acid 13.3-54.4 mg and carotene 0.013-0.175 mg per 100 g of edible portion (Morton, 1987) ^[14]. It is antispasmodic, sedative, Cytophylactic, and digestive. Fresh mandarin calms the intestines and aids in digestion. Mandarin fruit promotes cell generation and its aroma is inspiring and strengthening (Watson, 1994) ^[20].

Mandarin juice has a poor shelf-life and faces problem of post-harvest losses. Along with these, about 25 per cent fruits of Nagpur mandarin remain undersized, which are locally called as "*Choora*" and gain less price in market. Studies on seasonal variations in Nagpur mandarin revealed that *Mrig Bahar* fruits of Nagpur mandarin have more juice content, TSS and ascorbic acid content, along with less acidity (Bhatnagar *et al.*, 2012) ^[3]. Nagpur mandarin is one of the major citrus fruit crops of India, which suffers from post-harvest losses during the glut period. With a view of value addition of the fruit, diversification of the produce towards food processing industry is the need of the day. The easy availability, comparatively low cost, high nutritive value and good sugar content of mandarin, together make it a suitable alternative substrate for wine production.

Many Physico-chemical conditions play an important role in ethanol content of wine (Kumar *et al.*, 2009) ^[11]. Several researchers have reported that many factors, including fermentation temperature, pH, inoculum size, sugar concentration, type of fermentation can significantly influence the ethanol content of fruit wine. Similarly, pH of juice / must is an important parameter for the successful progress of fermentation because of two possible reasons that is retarding the growth of harmful bacteria by acidic solution and promoting the growth of yeast which grows well in acidic conditions (Mathewson, 1980) ^[12].

Keeping in view the above facts and in order to produce good quality wine from *Mrig Bahar* fruits Nagpur mandarin, present investigation was undertaken to study the influence of different levels of yeast inoculum and pH of must on chemical composition of wine prepared from *Mrig Bahar* fruits of Nagpur mandarin.

Material and Methods

Fully matured and well ripened Mrig Bahar fruits of Nagpur mandarin were procured during

April 2016-17 from local market of Akola, (MS) India. The trial was carried out at Post-harvest Technology Laboratory, Department of Horticulture, Dr. PDKV, Akola. The entire process of preparation of wine from *Mrig Bahar* fruits of Nagpur mandarin is shown diagrammatically in Fig. 1.



Fig 1: Preparation of wine from *Mrig Bahar* fruits of Nagpur mandarin

Various biochemical components of wines of *Mrig Bahar* fruits of Nagpur mandarin *viz.*, alcohol, residual sugars, acidity, volatile acidity, ascorbic acid and non-enzymatic browning were analysed by using methods suggested by FSSAI (2015) ^[6], Sadasivam and Manickam (1996) ^[17], Ranganna (2000) ^[15], Amerine *et al.* (1980) ^[1], Mazumdar and Majumdar (2003) ^[13], and Ranganna (2000) ^[15], respectively. The pH of wine was measured by using Perkin Elmer pH meter at 30 °C temperature.

The experiment was laid in two factor Completely Randomised Design. First factor, wine yeast inoculum had three different levels as S_1 (3%), S_2 (6%), and S_3 (9%) whereas, second factor, pH of must had five different levels as P_1 (3.0 pH), P_2 (3.5 pH), P_3 (4.0 pH) P_4 (4.5 pH), and P_5 (5.0 pH). All the observations were taken in triplicate and results were the mean of the triplicate readings. The data collected on various observations during the course of investigation was subjected to statistical analysis applying statistical package

for agricultural workers developed by CCSHAU, Hisar.

Results and Discussion

From the data presented in table 1, it can be observed that the fruits of *Mrig Bahar* of Nagpur mandarin used for the experiment recorded juice recovery of 49.72%, colour orange, TSS 11.10°B, acidity 0.79%, TSS: acidity ratio 14.00 and pH 4.06. It had 8.34% total sugars, 5.40% reducing sugars, 2.94% non-reducing sugars and 40.69 mg ascorbic acid per 100 mL of fruit juice. On the basis of readings of different Physicochemical parameters of the fruit it can be stated that the fruits have been procured at proper stage of maturity and have desirable characteristics for conversion into wine. Further, on the basis of TSS and total sugars of juice it can be concluded that the juice of Nagpur mandarin needs amelioration with sugar for preparation of wine.

Table	1: Physico-chemical characteristics of Mrig	Bahar	fruits of
	Nagpur mandarin		

Sr. No.	Characteristics	Readings
1	Juice recovery (%)	49.72
2	Colour	Orange
3	TSS (°B)	11.10
4	Acidity* (%)	0.79
5	TSS: acid ratio	14.00
6	pH	4.06
7	Total sugars (%)	8.34
8	Reducing sugars (%)	5.40
9	Non-reducing sugars (%)	2.94
10	Ascorbic acid (mg100 mL ⁻¹)	40.69

*as citric acid

Biochemical parameters of wine

Various biochemical parameters of wine prepared from *Mrig Bahar* fruits of Nagpur mandarin, such as alcohol, acidity, pH, residual sugars, volatile acidity, ascorbic acid and non-enzymatic browning were analysed.

Alcohol

The data regarding alcohol content of wine prepared from *Mrig Bahar* fruits of Nagpur mandarin, as influenced by different levels of yeast inoculum and pH of must, for fresh, and 3, 6, and 9 months ageing is presented in Table 2. The data presented reveals that alcohol content of wine was significantly affected by different levels of yeast inoculum and pH of must individually, as well as by the interaction of these two factors.

 Table 2: Effect of different levels of yeast inoculum and pH on the alcohol content of wines

Alcohol (%)								
Treatment	S 1	S	S_2		3	Mean		
P ₁	8.90 (17.36)	9.45 (17.90)	9.39 (17.85)	9.25 (17.70)		
P ₂	9.12 (17.57)	9.54 (17.99)	9.12 (17.57)	9.26 (17.71)		
P3	9.09 (17.55)	9.81 (18.25)	9.26 (17.71)	9.39 (17.84)		
P 4	9.29 (17.74)	9.48 (17.93)	9.37 (17.82)	9.38 (17.83)		
P5	8.79 (17.24)	8.93 (17.39)	9.26 (17.72)	8.99 (17.45)		
Mean	9.04 (17.49)	9.44 (17.89)	9.28 (17.73)			
	S	S		Р		S X P		
'F' test	Sig		Sig		Sig			
SE (m)±	0.013	0.013		0.017		0.029		
CD at 5%	0.038		0.049			0.084		

(Figures in parentheses indicate arc sine transformed values)

The maximum alcohol content as 9.44 per cent recorded in

treatment S_2 (*Saccharomyces cerevisiae* var. *ellipsoideus* inoculated at 6%) was found significantly superior to other yeast inoculum treatments. While minimum alcohol content of 9.04 per cent was recorded in treatment S_1 (*Saccharomyces cerevisiae* var. *ellipsoideus* inoculated at 3%).

A higher amount of alcohol content in wine of 6 per cent inoculum level in this experiment is closer to the results of Honde and Adsule (1998)^[9], who studied the effect of inoculum levels on the chemical composition of sapota wine and recorded higher (7.27%) alcohol content at 5 per cent inoculum level than at 2 per cent inoculum level (7.03%). Similarly, Khandelwal *et al.* (2006)^[10] also showed that, use of 5 per cent inoculum of *Saccharomyces cerevisiae* for preparation of pure and blended Kinnow wine contributed to the highest ethanol production.

The data in respect of alcohol content of Nagpur mandarin wine, as affected by different pH levels of must, showed significant differences. Treatment P_3 i.e. pH 4.0 was associated with maximum alcohol content (9.39%), which was at par with treatment P_4 i.e., 4.5 pH (9.38%). During this trial, treatment P_5 i.e., 5.0 pH recorded minimum alcohol content as 8.99 per cent.

In respect of different treatment combinations, S_2P_3 recorded significantly higher alcohol content with a reading of 9.81 per cent. This treatment combination was followed by treatment combination S_2P_2 i.e., 6 per cent *Saccharomyces cerevisiae* var. *ellipsoideus* inoculum with 3.5 pH, which recorded 9.54 per cent alcohol. It can be also observed that, treatment combination of *Saccharomyces cerevisiae* var. *ellipsoideus* inoculum at 3 per cent with 5.0 pH (S₁P₅) was associated with the minimum values of alcohol content as 8.79.

All the readings of alcohol content of wine of present investigation fall within the range of 8 to 15.5 per cent by volume as per Indian Standard Table Wines – Specification (BIS, 2005), and in the range of 7 to 16 per cent of the total volume of wine as stated by Vilanova *et al.* (2007) ^[19]. Similar findings regarding alcohol content of wine in the range of 6.6 to 11.53 per cent have been reported by different researchers as 11.53 per cent in bael wine by Chauhan *et al.* (2016) ^[5]; and 6.6 to 7.5 per cent in jamun wine by Gaikwad *et al.* (2016) ^[7].

The wines prepared from treatment combinations S_2P_3 and S_2P_2 can be considered superior in respect of various biochemical constituents as they had higher levels of alcohol content and most of the nutrients present in fruit juice of Nagpur mandarin, along with other compounds synthesised by the fermenting yeast, as stated by Bhutani *et al.* (1989) ^[3].

Titratable acidity

 Table 3: Effect of different levels of yeast inoculum and pH on the acidity of wines

Acidity* (%)									
Treatment	S_1	S	2	S	3	Mean			
P ₁	1.21 (6.31)	1.21 (6.32)		1.23 (6.37)		1.22 (6.33)			
P ₂	0.94 (5.57)	0.93 ((5.53)	0.94 (5.55)		0.94 (5.55)			
P3	0.76 (4.99)	0.76 (4.99)		0.77 (5.04)		0.76 (5.01)			
P_4	0.64 (4.60)	0.65 ((4.61)	0.64 (4.59)		0.64 (4.60)			
P5	0.53 (4.19)	0.54 ((4.23)	1.23) 0.54 (4.20)		0.54 (4.20)			
Mean	0.82 (5.13)	0.82 ((5.14)	0.82 ((5.15)				
	S	H		P		S X P			
'F' test	NS	Si		ig		NS			
SE (m)±	0.020	0.020		0.026		0.046			
CD at 5%	-		0.076			-			

*as citric acid

(Figures in parentheses indicate arc sine transformed values)

In present investigation, the individual effect of different levels of yeast inoculum, and the interaction effect of different levels of yeast inoculum and pH of must on the acidity of Nagpur mandarin wine was non-significant. Whereas, effect of the different levels of pH of must on the acidity of the wines prepared from *mrig bahar* fruits of Nagpur mandarin was found to be significant.

Significantly higher acidity of wine (1.22%) was recorded in treatment P₁ (3.0 pH). On the other hand, the treatment P₅ (5.0 pH) recorded minimum acidity as 0.54 per cent. This difference in the acidity of wine amongst the various pH treatments is because of adjustment of pH of must at 3.0, 3.5, 4.0, 4.5, and 5.0 for treatment P₁, P₂, P₃, P₄, and P₅, respectively, as a part of experimental methodology, and as a general principle, the acidity of a solution is inversely proportional to its pH.

All the readings of acidityof wines of the treatments P_2 (i.e., 3.5 pH), P_3 (i.e., 4.0 pH), P_4 (i.e., 4.5 pH) and P_5 (i.e., 5.0 pH) fall in between 0.5 to 1.0%, which is considered as the suitable range for acidity of the wine, as reported by Snell and Ettre (1974) ^[18]. Contrarily, all the readings of acidity of the treatment P_1 (i.e., 3.0 pH) were more than 1.0%, suggesting that, wine prepared from treatment P_1 may not be preferred.

pН

The data presented in Table 4 is related with the effect of different levels of yeast inoculum and pH of must on pH of Nagpur mandarin wines.

 Table 4: Effect of different levels of yeast inoculum and pH on the pH of wines

рН								
Treatment	S ₁	S_2		S ₃		Mean		
P ₁	2.82	2.	.81	2.7	75	2.79		
P2	3.27	3.	.30	3.2	24	3.27		
P ₃	3.83	3.75		3.74		3.77		
P4	4.21	4.21		4.28		4.23		
P ₅	4.75	4.	71	4.79		4.75		
Mean	3.77	3.	76	3.76				
	S		F			S X P		
'F' test	NS		Sig			NS		
SE (m)±	0.025		0.032			0.056		
CD at 5%	-		0.093			-		

Based on data available, it can be observed that, pH of must significantly affected the pH of the wine, whereas, the individual effect of yeast inoculum on pH of wine as well as interaction effect of yeast inoculum and pH of must on pH of wine was non-significant.

A perusal of the data of pH of wines reveals that, there was a significant gradual increase in pH of wine from treatment P_1 to P_5 . This significant gradual increase in the pH of wine amongst various pH treatments is because of adjustment of pH of must at 3.0, 3.5, 4.0, 4.5, and 5.0 for treatment P_1 , P_2 , P_3 , P_4 , and P_5 , respectively, as a part of experimental methodology, which has been ultimately reflected in the wines prepared with these specific pH treatments.

Residual sugars

The residual sugar content of wine depends upon the initial sugar content of must and the degree of fermentation. Thus, a wine having minimum residual sugars might have a history of higher degrees of fermentation, and vice-versa.

Residual sugars (%)								
Treatment	S1	S2		S3		Mean		
P ₁	3.57 (10.89)	2.66 ((9.38)	2.58	(9.24)	2.94 (9.84)		
P ₂	3.08 (10.11)	2.44 ((8.96)	3.17 (10.25)	2.90 (9.77)		
P3	3.20 (10.30)	1.91 ((7.93)	2.82 ((9.67)	2.64 (9.30)		
P4	2.80 (9.64)	2.55 ((9.19)	2.65 (9.35)		2.67 (9.39)		
P5	3.76 (11.18)	3.65 (11.02)	2.93 (9.85)		3.45 (10.68)		
Mean	3.28 (10.42)	2.64 ((9.30)	30) 2.83 (9.67				
	S		Р			S X P		
'F' test	Sig		Sig			Sig		
SE (m)±	0.087		0.112		0.194			
CD at 5%	0.251		0.324			0.562		

Table 5: Effect of different levels of yeast inoculum and pH on the residual sugars of wines

(Figures in parentheses indicate arc sine transformed values)

From the data of this trial, it can be observed that, minimum readings for residual sugars as 2.64 per cent was associated with S_2 i.e., 6 per cent yeast inoculum level. Whereas, in respect of different pH levels, treatment P_3 i.e., 4.0 pH recorded minimum residual sugars of wine (2.64%) which was at par with P_4 i.e., 4.5 pH (2.67%). As a function of interaction of different levels of yeast inoculum and pH, significantly lower value for residual sugars (1.91%) was recorded by S_2P_3 i.e., the treatment combination of 6 per cent wine yeast inoculum with 4.0 pH.

Thus, on the basis of significantly lower residual sugars content of wine, treatments S_2 (6% inoculum of *Saccharomyces cerevisiae* var. *ellipsoideus*), and P₃ (4.0 pH); and treatment combination S_2P_3 (6% inoculum of *Saccharomyces cerevisiae* var. *ellipsoideus* with 4.0 pH) can be considered superior which might have undergone higher degrees of fermentation.

Volatile acidity

According to Henick-Kling (1995)^[8], the presence of volatile acids in very small quantity is inevitable in the final matured wines and this does not necessarily mean that, they indicate at the beginning of deterioration.

Table 6: Effect of different levels of yeast inoculum and pH on the volatile acidity of wines

Volatile acidity** (%)								
Treatment	S1 S2		2	S 3		Mean		
P1	0.018	0.0)16	0.0	19	0.018		
P2	0.017	0.0)20	0.0	24	0.020		
P ₃	0.024	0.02		0.02		0.024		
P 4	0.025	5 0.0		0.024		0.025		
P5	0.026	0.0	0.025		25	0.025		
Mean	0.022	0.021		0.0	23			
	S		Р		S X P			
'F' test	Sig		Sig			Sig		
SE (m)±	0.000		0.001		0.001			
CD at 5%	0.001		0.002		0.003			

**as acetic acid

In respect of individual effect of yeast inoculum on volatile acidity of wine, significantly lower reading as 0.021 per cent was recorded for treatment S_2 (i.e. 6% yeast inoculum). Similarly, in respect of effect of pH of must on volatile acidity of wine, minimum volatile acidity of wine was recorded in treatment P₁ i.e., 3.0 pH (0.018%), wherein treatment P₂ i.e., 3.5 pH was at par with P₁ with volatile acidity 0.020 per cent.

During this investigation, treatment combination S_2P_1 had minimum reading for volatile acidity (0.016%) amongst all other treatment combinations.

All the readings of volatile acidity of wine in the present investigation are lower than the maximum permissible limit as per Indian Standard Table Wines – Specification (i.e., 1 gL⁻¹ equivalent to 0.1%) for dry as well as sweet table wines (BIS, 2005)^[4].

Ascorbic acid

The data of ascorbic acid content of Nagpur mandarin wine, as influenced by different levels of wine yeast inoculum of *Saccharomyces cerevisiae* var. *ellipsoideus* and pH of must is presented in Table 7.

 Table 7: Effect of different levels of yeast inoculum and pH on the ascorbic acid of wines

Ascorbic acid (mg 100 mL ⁻¹)								
Treatment	S 1	S_2		S3		Mean		
P1	27.78	28	.81	29.	.51	28.70		
P ₂	28.51	29.41		30.	.09	29.34		
P ₃	27.98	29.40		30.52		29.30		
P4	28.23	29.05		30.62		29.30		
P5	28.38	28.	.87	30.	.33	29.19		
Mean	28.28	29.	.11	30.	.21			
	S		P			S X P		
'F' test	Sig		NS			NS		
SE (m)±	0.554		0.716			1.240		
CD at 5%	1.601		-	-		-		

A perusal of the data presented reveals that the effect of different levels of *Saccharomyces cerevisiae* var. *ellipsoideus* on ascorbic acid content of wine was significant. In this, maximum ascorbic acid content as 30.21 mg 100 mL⁻¹ was recorded in treatment S_3 . On the other hand, effect of different levels pH of must, as well as interaction effect of different levels of *Saccharomyces cerevisiae* var. *ellipsoideus* and pH of must, on ascorbic acid content of wine was non-significant.

Non-enzymatic browning

From the data of non-enzymatic browning (NEB) presented in Table 8, it can be observed that effect of different levels of yeast inoculum and pH of must, independently as well as in combination, had significant effect on non-enzymatic browning of wines prepared from *mrig bahar* fruits of Nagpur mandarin.

Non-enzymatic browning								
Treatment	S 1	S	2	S	3	Mean		
P 1	0.014	0.0)14	4 0.015		0.015		
P_2	0.017	0.0)13	3 0.0		0.015		
P3	0.015	0.0		0.014		0.014		
P 4	0.016 0.02)15	0.020		0.017		
P5	0.017	0.0)16	0.016		0.016		
Mean	0.016	0.0)15	5 0.016				
	S		Р			S X P		
'F' test	Sig		Sig			Sig		
SE (m)±	0.000		0.000			0.001		
CD at 5%	0.001		0.001			0.001		

 Table 8: Effect of different levels of yeast inoculum and pH on nonenzymatic browning of wines

All the readings of NEB of wines were within the range of 0.013 to 0.020, as measured by optical density of wine samples at 440nm. These variations in NEB of different wine samples might be due to the differences in rate of ascorbic acid degradation, caramelization (degradation of sugars), and the Maillard reaction (sugar-amino acid reaction) in these wine samples, which resulted in non-enzymatic browning (Rufian-Henares *et al.*, 2009)^[16].

Conclusion

Storage studies of wines prepared from *Mrig Bahar* fruits of Nagpur mandarin, using various treatment combinations of different levels of wine yeast inoculum and pH of must revealed that, various biochemical parameters of wines were influenced by these two factors individually as well as in combination. On the basis of findings of present investigation and specifications suggested for different chemical constituents of Indian standard wine, it can be said that a standard quality wine with higher alcohol content can be prepared from *Mrig Bahar* fruits of Nagpur mandarin by using two treatment combinations: first, 6 per cent inoculum of *Saccharomyces cerevisiae* var. *ellipsoideus* with 4.0 pH of must; and the second, 6 per cent inoculum of *Saccharomyces cerevisiae* var. *ellipsoidus* with 3.5 pH of must.

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