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Effect of different levels of yeast inoculum and pH on wine prepared from Ambia bahar fruits of Nagpur Mandarin

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Abstract
The present investigation entitled 'Studies on preparation of wine from Nagpur mandarin' was conducted at Post-harvest Technology Laboratory, Department of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during the years 2015-16. The experiment consisted of two different factors *viz*., levels of yeast (*Saccharomyces cerevisiae* var. *ellipsoideus*) inoculum and levels of pH with three replications using Factorial Completely Randomised Design (FCRD). The biochemical composition of wine prepared from ambia bahar fruits of Nagpur mandarin indicated that the levels of wine yeast (*Saccharomyces cerevisiae* var. *ellipsoideus*) inoculum used as 3, 6 and 9 per cent and levels of pH 3.0, 3.5, 4.0, 4.5 and 5.0 affected the quality. The wine prepared with 6 per cent yeast inoculum and 4.0 of must yielded higher alcohol content followed by wine prepared with 6 per cent yeast inoculum and 3.5 pH of must.

Keywords: Nagpur mandarin, *Citrus reticulata*, *Saccharomyces cerevisiae*, wine, inoculum level, pH

Introduction
Mandarin is a citrus fruit of the species *Citrus reticulata*. It is distinguished from other citrus species by the relatively loose skin of the fruits, the relative ease with which the segments can be separated, and (in most cultivars) the green cotyledons (Anon., 2009) [3]. It is the highest valued citrus fruit.

Mandarin fruit is antispasmodic, sedative, cytophylactic, and digestive. Fresh mandarin calms the intestines and aids in digestion. It is tonic to the liver and its gentle action is suitable for treating hiccups. Mandarin fruit promotes cell generation and its aroma is inspiring and strengthening (Watson, 1994) [16].

Among mandarins, Nagpur mandarin (Central India), Kinnow mandarin (North–West India), Coorg mandarin (South India) and Khasi mandarin (North-East India) are the commercial cultivars of India. Fruits of Nagpur mandarin are yellowish green to orange, oblate, rind thin, fine texture and good flavour and taste. Size is medium and the skin is easily peelable (Anon., 2009) [3].

Nagpur mandarin is the only cultivar of mandarin grown in Vidarbha for last 200 years, on around 100.7 thousand ha area. 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 ambia bahar fruits of Nagpur mandarin have less juice content, TSS and ascorbic acid content, along with more acidity (Bhatnagar *et al.*, 2012) [4]. Hence, ambia bahar fruits of Nagpur mandarin are less liked by the consumers than mrig bahar fruits. With a view to solve these problems and for value addition of the fruit, diversification of the produce towards food processing industry is the present need. This can be achieved by converting the production into various value added products like unfermented and fermented beverages. Among the unfermented and fermented beverages juice and wine are considered as the most important products, respectively.

Many physico-chemical conditions play an important role in ethanol content of wine (Kumar *et al.*, 2009) [8]. 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) [11].

Keeping in view the above facts and in order to produce good quality wine from ambia 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 ambia bahar fruits of Nagpur mandarin.

Material and Methods

Fully matured and well ripened ambia bahar fruits of Nagpur mandarin were procured during November 2015-16 from local market of Akola, Maharashtra. The trial was carried out at Post-harvest Technology Laboratory, Department of Horticulture, Dr. PDKV, Akola.

The fruits procured for experiment were washed thoroughly, wiped, dried in air and then used for experiment. The fruit weight was recorded using an electronic balance. The fruits were cut into halves perpendicular to the fruit axis. Seeds were removed from fruit segments with the help of pointed knife and the juice was extracted using citrus juice extractor. The physico-chemical characteristics of the fruits such as juice recovery, colour, TSS, acidity, TSS: acidity ratio, pH, total sugars, reducing sugars, non-reducing sugars and ascorbic acid were evaluated on the same day, using standard methods.

After screening of fruit juice, powdered cane sugar was added to raise the TSS to 24°B. After setting the TSS to 24°B, it was divided into five equal parts. The pH of each part of the juice was set to five different treatments of pH 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), by using citric acid or calcium carbonate as per requirement. These musts were supplemented with 0.03 per cent diammonium hydrogen phosphate and 150 ppm potassium metabisulphite. Each of the musts of five pH treatments were subdivided into three different 1000 mL sterilised reagent bottles to 750 mL each and inoculated with either 30 mL, 60 mL or 90 mL of already prepared 48-h old inoculum of *Saccharomyces cerevisiae* var. *ellipsoideus* and the volume of each reagent bottle was made up to 1000 mL with the musts of respective pH, resulting in adjustment of inoculum concentration to three different levels of yeast inoculum as S_1 (3%), S_2 (6%) and S_3 (9%), respectively. These 15 treatment combinations of levels of inoculum and pH were triplicated as per the experimental design. An air lock was put in the mouth of bottles to prevent external air contact.

Fermentation was allowed to continue up to 14 days at 28± 2°C. After completion of the fermentation process, siphoning was done to separate wine from the sediment. After Siphoning, the liquid was clarified using 0.008% bentonite to recover wine of crystal clear quality finish. The crystal clear wine was supplemented with 100 ppm KMS to inhibit the wine yeast. The wine was clarified again by decantation for two times after a sedimentation period of 7 days each.The clarified wine was filled in fresh sterile glass bottles and sealed air-tight with crown caps, keeping approximately 0.7 cm head-space. These wines were pasteurised in hot water at a temperature of 65°C for 20 minutes. The entire process of fermentation is shown diagrammatically in Fig. 1.

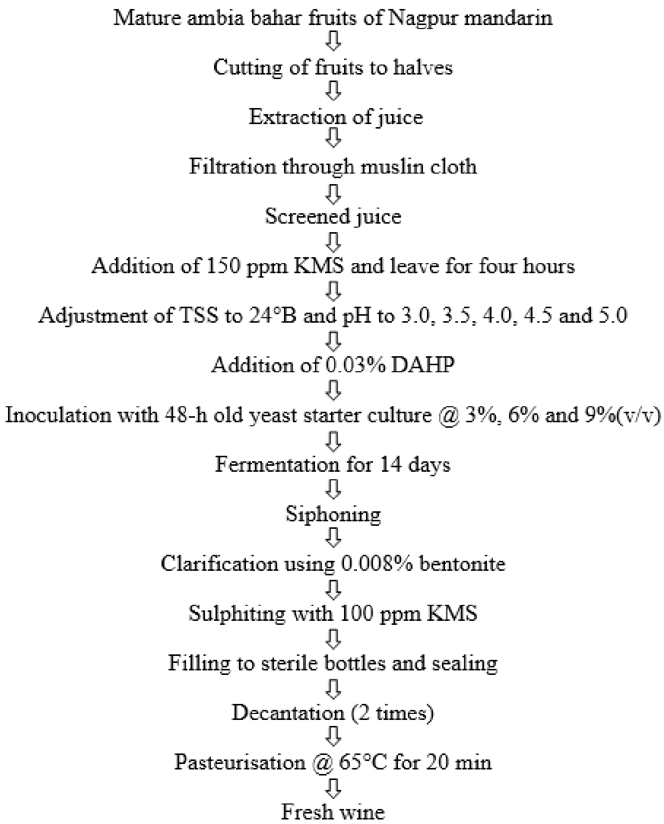

Fig 1: Flow chart for preparation of wine from fruits of Nagpur mandarin

The Nagpur mandarin wines prepared by using different treatment combinations, as mentioned above were analysed fresh for alcohol, residual sugars, titratabie acidity, volatile acidity, ascorbic acid and non-enzymatic browning by using methods suggested by FSSAI (2015) [6], Sadasivam and Manickam (1996) [15], Ranganna (2000) [13], Amerine *et al.*

(1980) [2], Mazumdar and Majumdar (2003) [12], and Ranganna (2000) [13], 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. 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 ambia bahar of Nagpur mandarin used for the experiment recorded juice recovery of 48.29%, colour orange, TSS 9.96°B, acidity 0.82%, TSS: acidity ratio 12.10 and pH 3.83. It had 7.39% total sugars, 4.81% reducing sugars, 2.58% non-reducing sugars and 36.33 mg ascorbic acid per 100 mL of fruit juice. On the basis of readings of different physico-chemical 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. The pH of juice is within the range of 3.0 to 4.0 as suggested by BIS (2005) [5]. 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 fruits

Sr. No.	Characteristics	Readings
1	Juice recovery (%)	48.29
2	Colour	Orange
3	TSS (°B)	9.96
4	Titratable acidity* (%)	0.82
5	TSS: acid ratio	12.10
6	pH	3.83
7	Total sugars (%)	7.39
8	Reducing sugars (%)	4.81
9	Non-reducing sugars (%)	2.58
10	Ascorbic acid (mg 100 mL^{-1})	36.33

*as citric acid

Biochemical parameters of wine

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

Alcohol

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. In this, wine prepared by using treatment S_2 (*Saccharomyces cerevisiae* var. *ellipsoideus* inoculum at 6%) recorded maximum alcohol (8.87%) whereas, minimum alcohol (8.47%) was found in treatment S_1 (*Saccharomyces cerevisiae* var. *ellipsoideus* inoculum at 3%). Production of significantly higher amount of alcohol content in wine prepared with 6 per cent inoculum as recorded in this experiment is nearer to the findings of Khandelwal *et al*. (2006) [7], who conducted a trial on preparation of wine with 5, 7 and 9 per cent inoculum of *Saccharomyces cerevisiae* and observed that use of 5 per cent inoculum for preparation of pure and blended Kinnow wine contributed to the highest ethanol production.

Alcohol content of wine prepared with treatment P_3 (pH 4.0) was significantly higher (8.77%) than the readings of alcohol content of other pH treatments. On the other hand, wine prepared by treatment P_1 (pH 3.0) had minimum alcohol (8.52%). In respect of interaction effect, significantly higher amount of alcohol production (9.20%) was recorded in treatment combination S_2P_3 (i.e. yeast inoculum at 6% with 4.0 pH of must), which was followed by treatment combination S_2P_2 (i.e. yeast inoculum at 6% with 3.5 pH of must) which produced 8.96% alcohol. On the other hand, treatment combination S_1P_1 (i.e. yeast inoculum at 3% with 3.0 pH of must) recorded minimum alcohol (8.38%).

All the readings of alcohol content of wine of present investigation fall within the range of 8 to 15.5 per cent, as stated in Indian Standard Table Wines – Specification (BIS, 2005) [5]. The results of present investigation are in close conformity with the findings of Kumbhar *et al.* (2002) [9] in respect of pomegranate wine.

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.

In present investigation, minimum residual sugars in wine (3.77%) was recorded in treatment S_2, in which 6 per cent wine yeast inoculum was used. This reading was significantly lower than the readings of residual sugars of other two treatments of yeast inoculum. In respect of effect of pH of must on residual sugars content in wine, treatment P_3 (i.e. 4.0 pH) was associated with minimum reading as 3.81 per cent, which was significantly lower than the readings of residual sugars content in wines prepared from other treatments of pH of must. As a function of interaction of levels of yeast inoculum and pH, minimum residual sugars content of 3.11 per cent was found in treatment combination S_2P_3 (i.e. 6% yeast inoculum with 4.0 pH), which was significantly lower than the readings of all other treatment combinations. Thus, on the basis of significantly lower residual sugars content of wine, treatments S_2 (6% inoculum of *Saccharomyces cerevisiae* var. *ellipsoideus*) and P_3 (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. It can be confirmed from significantly higher values of alcohol content of the treatments S_2 and P_3; and the treatment combination S_2P_3.

Titratable acidity and pH

Titratable acidity and pH of wine are negatively correlated, wherein gradual increase in one parameter results in corresponding decrease in other parameter, and vice-versa. Hence, both, titratable acidity and pH of wine, were found to be significantly affected by the treatments of different levels of pH of must. A gradual decrease in acidity of wine from 1.17 to 0.49% and corresponding increase in pH of wine from 2.81 to 4.83 was recorded as an effect of change in pH level from treatment P_1 (i.e. 3.0 pH) to P_5 (i.e. 5.0 pH). On the basis of specifications given by BIS (2005) [5] for dry as well as sweet table wines, both acidity and pH readings of two treatments of pH of must *viz.*, P_2 (i.e.3.5 pH) and P_3 (i.e.4.0 pH), were found to be in prescribed range.

In present investigation, effect of levels of yeast inoculum as well as interaction effect of levels of yeast inoculum and pH of must on titratable acidity and pH of Nagpur mandarin wine, was not significant. The increase in pH after conversion of must into wine might be due to precipitation of acids during fermentation. These results are in accordance with the results obtained by Amerine *et al.* (1972) [1].

All the readings of acidity and pH of wines, as a function of effect of different levels of yeast inoculum, were found to fall within the specifications of Indian standard table wines (BIS, 2005) [5]. Similarly, on the basis of specifications for acidity and pH of dry and sweet table wines, six treatment combinations *viz.*, S_1P_2, S_1P_3, S_2P_2, S_2P_3, S_3P_2 and S_3P_3 were found suitable for wine making from ambia bahar fruits of Nagpur mandarin.

Volatile acidity

On perusal of data of table 3, it can be observed that individual effect of different levels of wine yeast inoculum and pH of must, as well as interaction effect of these two factors on ascorbic acid content of Nagpur mandarin wine was significant.

Volatile acidity of wine prepared from ambia bahar fruits of Nagpur mandarin was significantly affected by different levels of yeast inoculum and pH, as well as by interaction effect of these two treatment factors.

In respect of individual effect of yeast inoculum on volatile acidity, minimum reading (0.017%) was recorded in treatment S_2 (i.e. 6% yeast inoculum) and maximum reading (0.019%) was recorded in treatment S_3 (i.e. 9% yeast inoculum). Gradual increase in volatile acidity from 0.015 per cent to 0.020 per cent was observed with increase in pH of must from treatment P_1 (3.0 pH) to P_5 (5.0 pH). This might be due to conversion of more sugar into acetic acid by bacteria at higher pH. Minimum 0.013 per cent volatile acidity was recorded in treatment combination S_2P_1, whereas maximum 0.021 per cent volatile acidity was found in S_1P_5.

All the readings of volatile acidity of fresh wine in this experiment are much below the established limits of volatile acid contents in fruit wine in different countries as 1 to 1.5 gL^{-1} (equivalent to 0.100% to 0.150%), as stated by Lonvaud–Funel (1995) [10].

Table 2: Alcohol, residual sugars, titratable acidity and pH of wine prepared from ambia bahar fruits of Nagpur mandarin as influenced by different levels of yeast inoculum and pH

Treatment Details	Alcohol (%)	Residual Sugars (%)	Titratable Acidity* (%)	pH
Yeast inoculum level				
S_1 - (3% yeast inoculum)	8.47 (16.92)	4.32 (11.99)	0.76 (4.95)	3.80
S_2 - (6% yeast inoculum)	8.87 (17.33)	3.77 (11.17)	0.77 (4.97)	3.79
S_3 - (9% yeast inoculum)	8.58 (17.03)	4.11 (11.69)	0.77 (4.98)	3.77
F Test	Sig	Sig	NS	NS
SE(m)+	0.013	0.050	0.021	0.018
CD at 5%	0.037	0.143	-	-
pH level				
P_1 - (3.0 pH)	8.52 (16.97)	4.30 (11.97)	1.17 (6.20)	2.81
P_2 - (3.5 pH)	8.67 (17.13)	4.00 (11.53)	0.89 (5.40)	3.26
P_3 - (4.0 pH)	8.77 (17.23)	3.81 (11.23)	0.71 (4.82)	3.75
P_4 - (4.5 pH)	8.65 (17.10)	4.04 (11.58)	0.59 (4.40)	4.27
P_5 - (5.0 pH)	8.59 (17.04)	4.17 (11.78)	0.49 (4.02)	4.83
F Test	Sig	Sig	Sig	Sig
SE(m)+	0.017	0.064	0.027	0.023
CD at 5%	0.048	0.185	0.077	0.067
Interaction (S×P)				
S_1P_1 - (3% yeast inoculum and 3.0 pH)	8.38 (16.83)	4.47 (12.20)	1.13 (6.11)	2.85
S_1P_2 - (3% yeast inoculum and 3.5 pH)	8.49 (16.94)	4.30 (11.96)	0.90 (5.43)	3.23
S_1P_3 - (3% yeast inoculum and 4.0 pH)	8.55 (17.00)	4.20 (11.82)	0.71 (4.82)	3.83
S_1P_4 - (3% yeast inoculum and 4.5 pH)	8.52 (16.97)	4.21 (11.84)	0.59 (4.40)	4.25
S_1P_5 - (3% yeast inoculum and 5.0 pH)	8.41 (16.86)	4.41 (12.12)	0.48 (3.97)	4.82
S_2P_1 - (6% yeast inoculum and 3.0 pH)	8.60 (17.05)	4.32 (11.99)	1.18 (6.24)	2.76
S_2P_2 - (6% yeast inoculum and 3.5 pH)	8.96 (17.41)	3.56 (10.87)	0.87 (5.35)	3.29
S_2P_3 - (6% yeast inoculum and 4.0 pH)	9.20 (17.66)	3.11 (10.16)	0.70 (4.81)	3.73
S_2P_4 - (6% yeast inoculum and 4.5 pH)	8.87 (17.33)	3.76 (11.19)	0.59 (4.39)	4.29
S_2P_5 - (6% yeast inoculum and 5.0 pH)	8.73 (17.19)	4.07 (11.64)	0.50 (4.07)	4.87
S_3P_1 - (9% yeast inoculum and 3.0 pH)	8.57 (17.03)	4.12 (11.71)	1.18 (6.24)	2.82
S_3P_2 - (9% yeast inoculum and 3.5 pH)	8.57 (17.03)	4.14 (11.74)	0.89 (5.41)	3.27
S_3P_3 - (9% yeast inoculum and 4.0 pH)	8.57 (17.03)	4.12 (11.71)	0.71 (4.82)	3.71
S_3P_4 - (9% yeast inoculum and 4.5 pH)	8.55 (17.00)	4.13 (11.73)	0.59 (4.40)	4.28
S_3P_5 - (9% yeast inoculum and 5.0 pH)	8.63 (17.08)	4.03 (11.57)	0.49 (4.03)	4.80
F Test	Sig	Sig	NS	NS
SE(m)+	0.029	0.111	0.046	0.040
CD at 5%	0.084	0.321	-	-

*as citric acid (Figures in parentheses indicate arc sine transformed values)

Table 3: Volatile acidity, ascorbic acid and non-enzymatic browning of wine prepared from ambia bahar fruits of Nagpur mandarin as influenced by different levels of yeast inoculum and pH

Treatment Details	Volatile Acidity** (%)	Ascorbic Acid (mg 100 mL^{-1})	Non-enzymztic Browning***
Yeast inoculum level			
S_1 - (3% yeast inoculum)	0.018	25.15	0.015
S_2 - (6% yeast inoculum)	0.017	26.44	0.014
S_3 - (9% yeast inoculum)	0.019	27.16	0.015

F Test	Sig	Sig	Sig
SE(m)+	0.000	0.478	0.000
CD at 5%	0.001	1.381	0.001
pH level			
P_1 - (3.0 pH)	0.015	26.16	0.014
P_2 - (3.5 pH)	0.016	26.14	0.015
P_3 - (4.0 pH)	0.019	25.96	0.015
P_4 - (4.5 pH)	0.020	26.63	0.015
P_5 - (5.0 pH)	0.020	26.35	0.015
F Test	Sig	NS	Sig
SE(m)+	0.000	0.617	0.000
CD at 5%	0.001	-	0.001
Interaction (S×P)			
S_1P_1 - (3% yeast inoculum and 3.0 pH)	0.016	25.40	0.013
S_1P_2 - (3% yeast inoculum and 3.5 pH)	0.016	24.40	0.016
S_1P_3 - (3% yeast inoculum and 4.0 pH)	0.020	24.64	0.015
S_1P_4 - (3% yeast inoculum and 4.5 pH)	0.019	25.72	0.015
S_1P_5 - (3% yeast inoculum and 5.0 pH)	0.021	25.58	0.015
S_2P_1 - (6% yeast inoculum and 3.0 pH)	0.013	26.22	0.013
S_2P_2 - (6% yeast inoculum and 3.5 pH)	0.015	26.77	0.014
S_2P_3 - (6% yeast inoculum and 4.0 pH)	0.018	26.74	0.014
S_2P_4 - (6% yeast inoculum and 4.5 pH)	0.019	26.43	0.016
S_2P_5 - (6% yeast inoculum and 5.0 pH)	0.019	26.04	0.015
S_3P_1 - (9% yeast inoculum and 3.0 pH)	0.016	26.87	0.016
S_3P_2 - (9% yeast inoculum and 3.5 pH)	0.017	27.25	0.017
S_3P_3 - (9% yeast inoculum and 4.0 pH)	0.019	26.51	0.015
S_3P_4 - (9% yeast inoculum and 4.5 pH)	0.022	27.73	0.013
S_3P_5 - (9% yeast inoculum and 5.0 pH)	0.019	27.43	0.015
F Test	Sig	NS	Sig
SE(m)+	0.001	1.069	0.000
CD at 5%	0.002	-	0.001

** as ascorbic acid *** OD at 440 nm

Ascorbic acid

Perusal of data presented in Table 3 reveals that effect of different levels of *Saccharomyces cerevisiae* var. *ellipsoideus* on ascorbic acid content of wine was significant. In this, maximum 27.16 mg 100 mL^{-1} ascorbic acid content was recorded in wine prepared with 9 per cent yeast inoculum and minimum 26.44 mg 100 mL^{-1} ascorbic acid was found in wine prepared with 3 per cent yeast inoculum. On the other hand, effect of different levels pH of must, as well as interaction effect of levels of *Saccharomyces cerevisiae* var. *ellipsoideus* and pH of must, on ascorbic acid content of wine was non-significant.

Ascorbic acid content of all the wine samples was lower than the ascorbic acid content of original fruit juice of Nagpur mandarin. This might be due to reduction in relative proportion of ascorbic acid content in fruit juice because of addition of different components such as yeast nutrients, citric acid, calcium carbonate and cane sugar to the fruit juice for amelioration.

Non-enzymatic browning

From the data of non-enzymatic browning (NEB) presented in Table 3, 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 Nagpur mandarin wine. All the readings of non-enzymatic browning of wine in this experiment were in the range of 0.013 to 0.017, as measured by optical density of wine samples at 440nm. This variation in NEB of different wine samples might be due to the differences in rate of ascorbic acid degradation, caramalisation (degradation of sugars), and the Maillard reaction (sugar-amino acid reaction) in these wine samples, which result in non-enzymatic browning (Rufian-Henares *et al.*, 2009) [14].

Conclusions

Results obtained in this experiment reveal that there is significant influence of different levels of yeast inoculum and pH of must on various biochemical parameters of wine. 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 ambia 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 second, 6 per cent inoculum of *Saccharomyces cerevisiae* var. *ellipsoidus* with 3.5 pH of must.

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