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, Maharashtra, India. The present investigation entitled ‘Studies on preparation of wine from Nagpur mandarin’ was conducted

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, cytophilactic, 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 mirg 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 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...
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.

The Nagpur mandarin wines prepared by using different treatment combinations, as mentioned above were analysed fresh for alcohol, residual sugars, titratable acidity, volatile acidity, ascorbic acid and non-enzymatic browning by using methods suggested by FSSAI (2015)\(^{(10)}\), Sadasivam and Manickam (1996)\(^{(13)}\), Ranganna (2000)\(^{(13)}\), Amerine et al.
(1980) [9], 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) [9]. 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.

| 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, titratable 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 S₁ (Saccharomyces cerevisiae var. ellipsoideus inoculum at 6%) recorded maximum alcohol (8.87%) whereas, minimum alcohol (8.47%) was found in treatment S₅ (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 P₁ (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 P₁ (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 S₃P₃ (i.e. yeast inoculum at 6% with 4.0 pH of must), which was followed by treatment combination S₄P₃ (i.e. yeast inoculum at 6% with 3.5 pH of must) which produced 8.96% alcohol. On the other hand, treatment combination S₅P₁ (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) [9]. 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 S₃, 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 P₁ (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 S₃P₃ (i.e. 6% yeast inoculum at 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 S₂ (6% inoculum of Saccharomyces cerevisiae var. ellipsoideus) and P₁ (4.0 pH); and treatment combination S₃P₃ (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 S₂ and P₁; and the treatment combination S₃P₃.

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 P₁ (i.e. 3.0 pH) to P₃ (i.e. 5.0 pH). On the basis of specifications given by BIS (2005) [9] for dry as well as sweet table wines, both acidity and pH readings of two treatments of pH of must viz., P₁ (i.e.3.5 pH) and P₃ (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.

Table 1: Physico-chemical characteristics of fruits

| Characteristics | Readings |
|-----------------|----------|
| Juice recovery (%) | 48.29    |
| Colour          | Orange   |
| TSS (°B)        | 9.96     |
| Titratable acidity* (%) | 0.82     |
| TSS: acid ratio | 12.10    |
| pH              | 3.83     |
| Total sugars (%) | 7.39     |
| Reducing sugars (%) | 4.81     |
| Non-reducing sugars (%) | 2.58     |
| Ascorbic acid (mg 100 mL⁻¹) | 36.33   |

*as citric acid
Voluntary 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.

Voluntary 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 S3 (i.e., 9% yeast inoculum) and maximum reading (0.019%) was recorded in treatment S1 (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 P1 (3.0 pH) to P3 (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 S3P1, whereas maximum 0.021 per cent volatile acidity was found in S5P5.

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).

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    |             |                     |                        |     |
| S1 - (3% yeast inoculum)| 8.47 (16.92)| 4.32 (11.99)        | 0.76 (4.95)            | 3.80|
| S2 - (6% yeast inoculum)| 8.87 (17.33)| 3.77 (11.17)        | 0.77 (4.97)            | 3.79|
| S3 - (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                |             |                     |                        |     |
| P1 - (3.0 pH)           | 8.52 (16.97)| 4.30 (11.97)        | 1.17 (6.20)            | 2.81|
| P2 - (3.5 pH)           | 8.67 (17.13)| 4.00 (11.53)        | 0.89 (5.40)            | 3.26|
| P3 - (4.0 pH)           | 8.77 (17.23)| 3.81 (11.23)        | 0.71 (4.82)            | 3.75|
| P4 - (4.5 pH)           | 8.65 (17.10)| 4.04 (11.58)        | 0.59 (4.40)            | 4.27|
| P5 - (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)       |             |                     |                        |     |
| S1P1 - (3% yeast inoculum and 3.0 pH)| 8.38 (16.83)| 4.47 (12.20)        | 1.13 (6.11)            | 2.85|
| S1P2 - (3% yeast inoculum and 3.5 pH)| 8.49 (16.94)| 4.30 (11.96)        | 0.90 (5.43)            | 3.23|
| S1P3 - (3% yeast inoculum and 4.0 pH)| 8.55 (17.00)| 4.20 (11.82)        | 0.71 (4.82)            | 3.83|
| S2P1 - (3% yeast inoculum and 4.5 pH)| 8.52 (16.97)| 4.21 (11.84)        | 0.59 (4.40)            | 4.25|
| S2P2 - (3% yeast inoculum and 5.0 pH)| 8.41 (16.86)| 4.41 (12.12)        | 0.48 (3.97)            | 4.82|
| S2P3 - (6% yeast inoculum and 3.0 pH)| 8.60 (17.05)| 4.32 (11.99)        | 1.18 (6.24)            | 2.76|
| S2P4 - (6% yeast inoculum and 3.5 pH)| 8.96 (17.41)| 3.56 (10.87)        | 0.87 (5.35)            | 3.29|
| S2P5 - (6% yeast inoculum and 4.0 pH)| 9.20 (17.66)| 3.11 (10.16)        | 0.70 (4.81)            | 3.73|
| S2P6 - (6% yeast inoculum and 4.5 pH)| 8.87 (17.33)| 3.76 (11.19)        | 0.59 (4.39)            | 4.29|
| S2P7 - (6% yeast inoculum and 5.0 pH)| 8.73 (17.19)| 4.07 (11.64)        | 0.50 (4.07)            | 4.87|
| S3P1 - (9% yeast inoculum and 3.0 pH)| 8.57 (17.03)| 4.12 (11.71)        | 1.18 (6.24)            | 2.82|
| S3P2 - (9% yeast inoculum and 3.5 pH)| 8.57 (17.03)| 4.14 (11.74)        | 0.89 (5.41)            | 3.27|
| S3P3 - (9% yeast inoculum and 4.0 pH)| 8.57 (17.03)| 4.12 (11.71)        | 0.71 (4.82)            | 3.71|
| S3P4 - (9% yeast inoculum and 4.5 pH)| 8.55 (17.00)| 4.13 (11.73)        | 0.59 (4.40)            | 4.28|
| S3P5 - (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-enzymatic Browning*** |
|-------------------|----------------------|-----------------------------|--------------------------|
| Yeast inoculum level |                      |                             |                          |
| S1 - (3% yeast inoculum) | 0.018               | 25.15                       | 0.015                    |
| S2 - (6% yeast inoculum) | 0.017               | 26.44                       | 0.014                    |
| S3 - (9% yeast inoculum) | 0.019               | 27.16                       | 0.015                    |
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.

**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. *ellipsoideus* with 3.5 pH of must.

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