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INFLUENCE OF DIFFERENT PH LEVELS ON WINE PREPARED FROM NAGPUR MANDARIN USING *SACCHAROMYCES CEREVISIAE*

Anjali M. Gaharwar, R. M. Shinde* and D. L. Wasule
Vasantrao Naik College of Agricultural Biotechnology, Yavatmal – 445001, India
*Corresponding author E-mail: roshan.agricos@gmail.com ; roshanshinde@pdkv.ac.in
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ABSTRACT

The aim of the study was to prepare quality wine from indigenous Nagpur mandarin as influenced by different pH levels using yeast *Saccharomyces cerevisiae*. The musts were obtained from Nagpur mandarin botanically known as *Citrus reticulata* were used as a fermentation raw material. Fruit juice extracted from Nagpur Mandarin was taken for the physicochemical analysis with an objective to prepare wine at five different levels of pH that is pH 3.0, 3.5, 4.0, 4.5 and 5.0 to get quality wine at optimum pH. All the juice samples adjusted at 25°B and placed at 20 °C to 22°C. Must juice samples were subjected to fermentation by adding 5% yeast inoculum of the *Saccharomyces cerevisiae*. After 30 days of fermentation, it was observed that the pH, of wines were found gradually decreased along with reduction in TSS, titrable acidity, ascorbic acid, and reducing sugar with increased in alcohol content of wine. The must pH 4.5 gave the maximum pH (4.2) i.e., less acidic wine. At the same pH the wine produced contained the maximum total soluble solids (8.28), minimum reducing sugar (6.42), maximum total sugar (8.30) and alcohol (9.08). The organoleptic evaluation of wine, reported that Nagpur Mandarin wine prepared at pH 4.5, scored 8.03 points for overall acceptability categorized as like very much wine and thus found acceptable for winery. Wine prepared at pH level 4.0 stood next to pH 4.5 with the acceptable wine pH (3.76), preferable TSS (8.19), titrable acidity (0.72), ascorbic acid (29.19), reducing sugar (6.69) and alcohol content (8.96). This wine achieved organoleptic score 7.88, categorized as like moderately wine and also found suitable for winery next to wine prepared with must pH at 4.5.

Keywords: *Saccharomyces cerevisiae, Citrus reticulata*, titrable acidity, ascorbic acid, reducing sugar.

Introduction

Wine is a complex product obtained by biological and biochemical transformations by wine microorganisms and during wine aging, (Aakriti Guleria, 2014). Fruits are nature's marvelous gift to the human kind as they possess life-prolonging and protecting components. Fruits provide energy, vitamins, minerals, and phytochemicals; their regular consumption improves the physiological functions and reduces the risk of various diseases, (Chhikara *et al*., 2018a; Chhikara *et al*., 2018b and Kaur *et al*., 2019). Health-enhancing functional foods such as fruit wines recently increased public interest for the well-being of life. Nagpur mandarin a unique identity for peculiar acid: sugar blend rich in vitamin C content belongs to genus Citrus. Botanically citrus fruit is known as *Citrus reticulata belongs to family Rutaceae.* India reported 1003 thousand ha area with 12546 thousand MT and 12.0 MT/ha of production and productivity respectively during 2018-19, Anonymous, 2019. *Mandarin is a nutrient rich fruit which provides* about 50 calories of energy and meet a whole day requirement of vitamin C. Mandarins are rich in flavonoids, antioxidants like naringin, hesperetin, vitamin-A, carotenes, xanthins, and lutein. Medicinal properties of mandarin, put the fruit juice drink in the topmost three instant Energizer drink available fresh as well as in ready to serve form. Rich availability of potassium, an electrolyte mineral in mandarins takes care of cardiovascular functions and control blood pressure of human body. Mandarin an excellent source

of vitamin C and antioxidants restricted the formation of free radicals reduces the risk of cancer. Verma and Joshi in 2001 reported about 30% post-harvest losses in mandarin in India. Year-round requirement of specific fruit can be meet through different value-added products that not only satisfy consumers demand but provide nutrition also. Wine a fermented beverage contains certain constituents like alcohols, acids, sugars, tannins, aldehydes, esters, vitamins, minerals, anthocyanins and flavonids is an important commercial value-added product (Amerine *et al*., 1980.)

Among the many factors which influence the fermentation process and wine quality, pH is critical. This is probably because pH determines the effectiveness of sulphur dioxide (SO_2) as an antimicrobial agent, influences microbial and colour stability, taste and ageing potential, Jackson 2008. The pH, total acidity and volatile acidity in addition to residual sugar and alcohol contents are specified in any professional wine tasting exercise, Ribéreau-Gayon *et al*., 2006. This underscores the importance attached to pH and acidity as quality parameters of wine. The acidity of must has an effect on the final acidity of wine. Nutritional and medicinal properties of mandarin may mostly be exploited through preparation of RTS and beverages which leads us to undertake the current study on preparation of quality wine from Nagpur mandarin. Since pH plays a critical role in fermentation and final wine quality, the present study was carried out to assess the effect of pH on Nagpur mandarin wine quality using yeast *Saccharomyces cerevisiae.*

Material and Methods

Preparation of must for fermentation

Mature fresh fruits of Nagpur mandarin of uniform size, fully ripened were procured from Yavatmal (Maharashtra) market during January, 2019. The glassware was washed and sterilized in the oven at 160^{o}C for 1 hour. The juice was extracted and filtered through muslin cloth and collected in jars till further use. Physiochemical analysis of mandarin fruit juice was carried out for further comparative study for wine.

Preparation of yeast culture

Yeast extract of *Saccharomyces cerevisiae*was dissolved in lukewarm water to obtained 5% inoculum as reported by Romano *et al*., 2003. Stirring was done to dissolve the yeast strain and left for 15 minutes to activate the yeast. Activated starter culture of yeast was used for fermentation (Aakriti Guleria, 2014.)

Preparation and inoculation of Nagpur mandarin must

Juice samples were maintained at five different pH levels i.e. 3.0, 3.5, 4.0, 4.5 and 5.0 by adding sodium bicarbonate and citric acid in must. TSS of juice samples were maintained to 25^{o}B. The must juice samples were inoculated with the activated yeast. The conical flasks were plugged with rubber cork having a tube at one end in flask dipped in wine sample and one end dipped in flask with water to avoid the head spaces and placed at room temperature for fermentation.

Fermentation and aging of wine

The flask was stirred for removing of CO_2 traces to avoid cloudiness with sterile glass rod and owed for fermentation. Racking was done 3-4 times at weekly interval during the fermentation process. After completion of fermentation, the wine samples were siphoned off and filtered through a clean sterilized muslin cloth and collected in sterile glass jars. Bentonite at the rate of 0.1 per cent was added in each jar and the sample jars were left undisturbed for 4 days. After clarification, the supernatant wine was siphoned off and transferred into fresh sterile bottles and corked. During maturation the wine was racked regularly.

Physicochemical analysis of fermented Nagpur mandarin wine

The wine samples ready after 30 days of fermentation were analyzed for physicochemical properties as per methods suggested by A.O.A.C. 2000. The pH was measured by digital pH meter. The alcohol estimation though specific gravity, titrable acidity, ascorbic acid, sugar contents were analyzed as suggested by Ranganna, 1977. Wines after maturation were also evaluated organoleptically to determine the effect of different levels of pH on wine quality by the semi-trained panel of ten judges keeping grape wine as a standard (1: 1 dilution). The organoleptic evaluation was performed using 9 points hedonic scale.

Results and Discussion

Physico-chemical analysis of fruit juice

The suitable pH of fruit juice for wine preparation ranged between 3-4 as suggested by BIS 2005. From the data presented in table 1, it was observed that, pH value of fruit juice ranged between 3-4.2 and also the other parameters were found desired. That meant, fruits of mrig bahar were suitable for wine production. The physicochemical characterization of mandarin fruit juice was made and presented in table 1.

Table 1: Physicochemical characterization of mandarin fruit juice

S.No.	Parameters	Fruit juice
1	Average Fruit weight	135 g
2	Juice Recovery	46.75%
3	TSS	9.80 ^{o}B
4	pH	3.88
5	colour	Deep Orange
6	Titrable Acidity (as % anhydrous citric acid)	0.74
7	Ascorbic Acid (%)	38.0
8	Reducing sugar (mg/100ml)	4.89
9	Non- Reducing sugar (%)	2.79
10	Total Sugar (%)	8.04

Physicochemical characterization of mandarin wine:

The pH of the fruit juice of Nagpur mandarin were maintained at different pH ranging from 3 to 5, to study its effect on fermentation and other physicochemical parameters of wine and thereby to develop quality wine.

Table 2: Effect of varying levels of pH on physicochemical characteristics of mandarin wine

Different level of pH of juice must	pH of wine	TSS (%)	Titrable Acidity (%)	Ascorbic acid (100mg/ml)
pH-3.0	2.77	7.86	1.15	28.80
pH-3.5	3.24	8.10	0.85	28.93
pH-4.0	3.76	8.19	0.72	29.19
pH-4.5	4.20	8.28	0.60	29.15
pH- 5.0	4.58	8.21	0.52	28.98

pH : From the data recorded on the changes in the pH of fermented wine of mandarin prepared at different pH levels showed variation in the pH level obtained at 30 days of fermentation. Among different wine samples, wine prepared at pH 4 and 3.5 level were found suitable as recorded the acceptable pH for winery. However, less acidic pH 4.2 was obtained at must pH 4.5 (Table 2) was also found suitable for winery. The pH of the wines was found decreased against the pH of juice must after fermentation. During fermentation yeast produces enzymes which bring about various biochemical transformations. These enzymes are protein in nature, and without the requisite pH, temperature and ionic strength may be denatured. Enzymatic activities and metabolism is very sensitive to pH changes. Mathapathi *et al*., 2004, Sonnleitner, 1999 and Gaharwar *et al.,* 2017 also reported the similar trend in wines. However, gradual increased trend of pH of wine were found during maturation of wine. The pH of wine was found in increasing trend with the must pH that determined its influence on wine quality. This might be due to precipitation of organic acids and formation of alcohol in wine. The results are in accordance with the findings of Gautam, and Chundawat, 1998; Kumar *et al*. 2009; Lokesh *et al*., 2014 and Saha Jayata, 2016.

TSS: Fresh extracted mandarin juice samples having TSS 9.80ºB were maintained at 25ºB, when placed for fermentation at different pH levels 3.0, 3.5, 4.0, 4.5 and 5.0. It was reported that, the TSS from all the wine samples were found to be decreased following the fermentation period (Table 2). It is due to utilization of sugar for fermentation activities by yeast the same results are obtained by Joshi *et al*. 1997, Sharma and Joshi 2003, Joshi *et al*. 2014, Joshi *et al*. 2015 and Rachana *et al*. 2021. Wine prepared at 4.5 pH recorded maximum TSS 8.28ºBrix followed by wine at pH 4.0 and 3.5. The minimum TSS 7.86 was found in mandarin wine prepared at pH 3.0. It means that, must pH influence the TSS of wine and stated that, increased in pH of wine, increased the wine TSS and vice versa. The results are strongly in confirmation with the results reported by Patharkar *et al.,* 2017 who reported that pH 4.5 was found most suitable for production of mandarin wine. Khandelwal *et al.* 2006 reported that, the maximum TSS reduction during the fermentation of mandarin juice was found with 5% level of inoculum and strain MTCC 180. Sharma and Joshi 2003 in aonla wine and Panda *et al*. 2014 in bael wine reported decreased in TSS as advanced in storage period of wine. The results are in agreement with the findings of Chaudhary *et al*. 2014.

Titratable Acidity: From the data, it was observed that, pH levels of wine increased with increased must pH levels from 2.77 to 4.58, There found decreased in acidity of wines from 1.15 to 0.52. It showed that, pH and acidity are irreversibly correlate to each other. The pH of wine after fermentation may increase as a result of acid precipitation or yeast and bacterial metabolism, Jackson, 2008. The minimum titratable acidity 0.52% of mandarin wine was found at pH 5.0 and the maximum titratable acidity 1.15% was found with pH 3.0 (Table 2). The acidity ranged from 0.52% to 0.85%was suitable for winery as in prescribe range and supported by Snell FD, Ettre ISL. 1974. The decreasing trend of titratable acidity in wine with increased in alcohol percentage during fermentation. the results are supported by the research work carries out by Joshi *et al*. 2012 and Lokesh *et al*. 2014 in jamun wine and Rachna *et al*. 2021 in bael wine.

Ascorbic acid: It was reported that, ascorbic acid content of the wine was found to be decreased at 30 days of fermentation of wine. The minimum ascorbic acid content 28.80 mg/ml was found at wine prepared at pH 3.0 and the maximum ascorbic acid content 29.19 mg/ml was obtained with wine prepared at 4.0 pH (Table 2). Khandelwal *et al*. 2006 and Malav *et al*. 2014 also reported decreased in ascorbic acid content during fermentation than the original juice samples may be due to production of organic acids during the fermentation.

Sugar contents in wine (Reducing sugar, non-reducing sugar and total content): The data recorded on changes in sugar contents of mandarin wines prepared at different pH levels is presented in table 3. The data, showed considerable variations in sugar content at different levels of pH. It was observed that, the reducing sugar was found decreased at 30 days of fermentation. It might be due to utilization of sugar for the processes of fermentation. similar trend was reported by Bardiya *et al*.1974, Gaharwar *et al*. 2018, Idolo Ifi *et al*. 2012 and Panda *et al*. 2014. Maximum reducing sugar were reported at pH 3.0 (6.90) and the minimum was found with pH 5 (6.38) followed by 4.5 (6.42), that indicates maximum sugar was utilized by yeast for its conversion into alcohol. Increased in level of pH thus showed a positive impact of pH over wine fermentation.

Table 3: Effect of varying levels of pH on Sugar and alcohol contents of Nagpur mandarin wine

Different level of pH of juice must	Reducing sugar (%)	Non-reducing sugar (%)	Total sugar (%)	Alcohol (%)
pH-3.0	6.90	0.98	7.88	8.66
pH-3.5	6.80	1.29	8.09	8.91
pH-4.0	6.69	1.48	8.17	8.96
pH-4.5	6.42	1.88	8.30	9.08
pH-5.0	6.38	1.53	7.91	8.65

Alcohol: The data recorded on changes in alcohol (%) of fermented wine produced from Nagpur mandarin at various level of pH are presented in table no.3. From, the data, it was observed that, the alcohol content of wine was found varied at 30 days of fermentation as affected by different levels of pH of must. The maximum alcohol content of wine was reported at must pH 4.5 when kept for fermentation. During the aging, the alcohol level was found to be increased which might be due to fermentation and conversion of sugar into alcohol as supported by Ribéreau-Gayon in 2006. Thus, it is strongly emphasized that, % yeast inoculam and must pH have significant impact on the fermentation quality of wine. Patharkar *et al*. 2017 who reported recovery of maximum alcoholic percentage (ethanol) in mandarin fruit wine prepared at pH 4.5 at temperature 27^{o}C. The highest ethanol production was reported using 5 per cent inoculum of *Saccharomyces cerevisiae* in Kinnow wine. the results are supported by Khandelwal *et al*. 2006, Sapana *et al*. 2002 and Reddy and Reddy, 2009. Joshi *et al.* 1997 reported 8.57% of alcohol content in Kinnow wine.

Organoleptic evaluation of wines: The data of organoleptic evaluation for wine samples prepared at different pH (Table 4). All the wine samples after 30 days of fermentation were evaluated for organoleptic parameters viz., colour, flavor, taste, appearance and overall acceptance. Parameters were decided as per 9 points hedonic scale; where points score 5 stood for neither like nor dislike, 6 for like slightly, 7 for like moderately, 8 for like very much and 9 for like extremely. the scale was also stated by Sukanya and Michael 2014. The wines were served to panel of 10 members and the scores were averaged and finalized the mean. Based on the final score, the quality and overall acceptability of wine was determined. Wine prepared at pH 4.5 followed by pH 4, showed maximum overall acceptability score tends to like very much (Fig 1.) However, overall mean points calculated on average of various parameters scores at particular must pH wine stated that must pH 4.5 was best for preparation of quality wine.

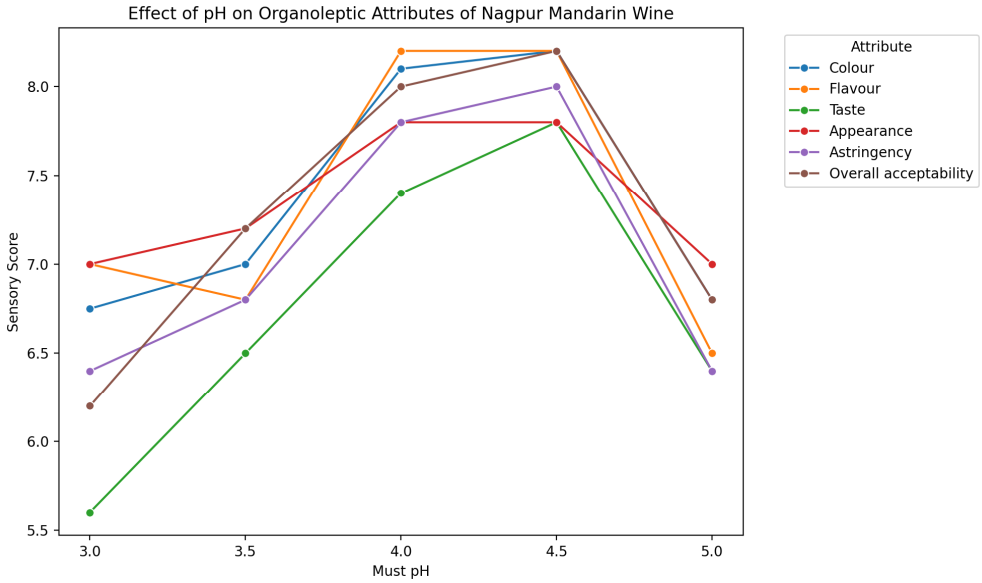

Fig. 1 : Effect of Must pH on Sensory Attributes of Nagpur Mandarin Wine

Table 4: Effect of different levels of pH on organoleptic evaluation of Nagpur mandarin wine.

Wine prepared at must pH	Colour	Flavour	Taste	Appearance	Astringency	Overall acceptability	Overall Mean
pH 3.0	6.75	7.0	5.6	7.0	6.4	6.2	6.49
pH 3.5	7.0	6.8	6.5	7.2	6.8	7.2	6.92
pH 4.0	8.1	8.2	7.4	7.8	7.8	8.0	7.88
pH 4.5	8.2	8.2	7.8	7.8	8.0	8.2	8.03
pH 5.0	6.8	6.5	6.4	7.0	6.4	6.8	6.65

To provide a more comprehensive analysis, we correlate the sensory (organoleptic) data from Table 4 with the sugar and alcohol data from Table 3. to understand how changes in pH not only affect the chemical composition but also the sensory quality of the wine. As pH increases, reducing sugar slightly decreases, non-reducing sugar increases up to pH 4.5, total sugar peaks at pH 4.5, and alcohol content also reaches its maximum at pH 4.5 before dropping at pH 5.0. In Fig 2. The blue line represents the sensory score, while the red line shows alcohol content. Both peak at pH 4.5, indicating that this pH not only produces the highest alcohol content but also the best sensory quality.

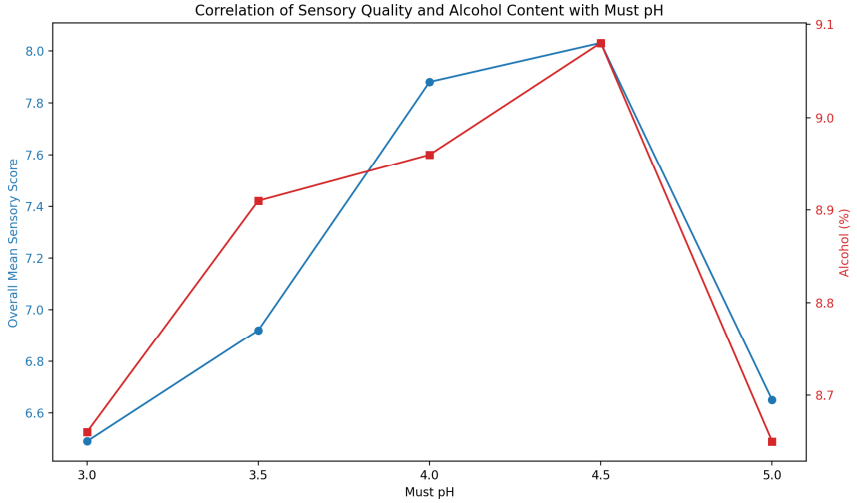

Fig. 2 : Correlation of sensory quality and alcohol content with must pH.

Conclusion

Mandarin wines prepared at five different pH on physicochemical analysis at 30 days of fermentation showed that, prescribed parameters of pH, TSS, acidity, Ascorbic acid and alcohol content were meet by wine prepared at pH 4.5 followed by 4.0. Cultivars, maturity, and climatic conditions matters the quality of wine. overall acceptability score for mandarin wine ranged from 6.2 to 8.2 showed the difference in impact of must pH over wine quality. Must pH level 4.5 produced the minimum acidic wine which was found most suitable for overall acceptability and overall mean score points. Also, wine prepared at pH 4 was found suitable as recorded the acceptable pH and overall acceptability score for like moderately wine. Wine prepared at pH 4.5 reported high total soluble solids (8.28 ^{o}B), reducing sugar, (6.42 %) total sugar (8.3%) and alcohol (9.08%). From organoleptic evaluation of wine it was concluded that Nagpur Mandarin wine prepared at pH4.5 was found the most acceptable for winery followed by at pH4.0.

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