EFFECT OF INITIAL TOTAL SOLUBLE SOLIDS ON PHYSICO-CHEMICAL, ANTIOXIDANT AND SENSORY PROPERTIES OF MULBERRY (MORUS INDICA L.) WINE

UTICAJ INICIJALNIH UKUPNO RASTVORLJIVIH MATERIJA NA FIZIČKO-HEMIJSKA, ANTIOKSIDATIVNA I SENZORNJA SVOJSTVA VINA OD PLODA DUDA (MORUS INDICA L.)

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ABSTRACT

Influence of initial total soluble solids (TSS) levels on physico-chemical, antioxidant and sensory attributes of mulberry fruit wine was investigated and is reported here. Mulberry fruits contained medium acid (Titratable acidity: 0.73 ± 0.16 % as malic acid), medium sugar (TSS: 15.60 ± 0.20 °Bx) but were having good ascorbic acid (103.00 ± 0.24 mg/100 g) as compared to grapes which are otherwise considered excellent fruits for wine production. Thus, table sugar was added to the mulberry pulp to raise the TSS to three different levels that is 22, 24 and 26 °Bx before the start of the fermentation process. Among three musts, the rate of fermentation (RF) and fermentation efficiency (FE) of treatment T3 (26 °Bx) was the highest. Further, the prepared wine was analyzed for various physico-chemical, antioxidant and sensory properties. With the increase in TSS of the mulberry pulp subjected to fermentation (22 ºBx to 26 ºBx), different quality factors for example reducing sugars, total sugars, titratable acidity, volatile acidity, ethanol content, higher alcohols and total phenols increased whereas, pH decreased after the completion of fermentation process. The antioxidant in different treatments such as ascorbic acid ranged in between 67.58 - 74.45 mg/100 mL, anthocyanin in between 48.23 - 67.58 mg/100 mL and total phenols in between 345.5 - 379.0 mg/ L. The wine prepared from the treatment of T3 (26 ºBx) must recorded the highest sensory quality and was adjudged to be the best wine in our study.

Key words: mulberry fruit, wine, rate of fermentation, alcohol, phenols.

INTRODUCTION

The mulberry (Morus indica L.) belongs to the Morus genus of the Moraceae family. Plants of the genus Morus are known to be a rich source of flavonoids including quercetin 3-malonylglucoside, rutin, isoquercetin (Katsube et al., 2006), cyanidin 3-rutinoside and cyanidin 3-glucoside (Chen et al., 2006; Kang et al., 2006). A high content of total phenolics in white, red, and black mulberry fruits have been reported by Erçil and Orhan (2007). The fruit generally remains unutilized despite of its high physico-chemical and antioxidant properties. Therefore, the utilization of the fruit in wine preparation can give a new promising product to the market. Alcoholic beverage wine is prepared by fermenting the fruit pulps with appropriate processing and additions (Amerine et al., 1980). The basic process of wine making involves the fermentation of grape pulp or any other pulp or pulp by Saccharomyces cerevisiae var. Ellipsoideus (Amerine et al., 1980; Joshi et al., 2014; Abrol and Joshi, 2011). Therefore, an attempt has been made to utilize the fruit to make mulberry fruit wine by raising the total soluble solids of the mulberry pulp by addition of the table sugar to the level of 22, 24 and 26 °Bx before starting the fermentation process.

MATERIAL AND METHOD

Materials

The berries of mulberry were procured from the College of Horticulture of VCSG Uttarakhand University of Horticulture and Forestry, Bharsar, India. The berries were converted into pulp by hot pulping method and used for the preparation of mulberry wine. Sucrose, the common sugar was procured from...
the local market for the preparation of mulberry wine. The pectin esterase enzyme used in the studies was manufactured by M/S Triton Chemicals, Mysore, India under the brand name “Pectinol”.

Preparation of wine

Yeast culture: The yeast culture viz. Saccharomyces cerevisiae var. *Ellipsoideus*, (UCD 595) used in the study was originally obtained from Department of Enology and Viticulture, California, Davis, USA. It was maintained on yeast malt extract agar (YMEA) medium and re-cultured after every three months or whenever needed from the stock yeast culture.

Preparation of must: For conducting the planned experiment and to prepare must, 0.1 % diammonium hydrogen phosphate (DAHP) as nitrogen source and 0.5 % pectinase enzyme for clarification were added to the pulp. The total soluble solids (TSS) of must was raised with sugar to 22 °Bx, 24 °Bx and 26 °Bx and named as T1, T2 and T3. The respective must were inoculated with 5 % of activated culture of *Saccharomyces cerevisiae* var. *Ellipsoideus*. The fermentation of each treatment was carried out in five liters capacity narrow mouth glass carboys, which was filled up to 75 % of their capacity.

Fermentation: Fermentation for all the treatments was carried out at room temperature (22-25°C). When a stable TSS was reached, the fermentation was considered completed. Air locks were fitted in the mouth of glass carboys near the end of fermentation.

Siphoning/racking: When fermentation was complete, siphoning/racking was done after 15 days and then after one month.

Physico-chemical analysis

The weight of 10 berries was taken with the help of physical balance in gram. Total soluble solids (TSS) were measured using an Erma hand refractometer (0 to 32 °B) and the results were expressed as degree Brix (°B). The readings were corrected by incorporating the appropriate correction factor for temperature variation (AOAC, 1980). Titratable acidity was estimated by titrating a known aliquot of the sample against NaOH solution using phenolphthalein as an indicator (AOAC, 1980) and expressed as per cent malic acid. The total and reducing sugars of fruit and wine were estimated by Lane and Eynon volumetric method (AOAC, 1980) by titrating the sample against Fehling A and Fehling solutions. ELTOP-3030 pH meter was used to measure pH. The volatile acidity of mulberry wine was determined by the standard method (Amerine et al., 1980). Quantity of ethanol in the wine was estimated by the spectrophotometric method (Caputi et al., 1968) whereas, higher alcohols in wine was estimated by the method given by Giammon and Nakagiri (1982).

Antioxidant analysis

Ascorbic acid content was determined as per standard method (AOAC, 1980) using 2, 6-dichlorophenol-indophenol dye. The total phenols content in different wines were determined by Folin-Ciocalteu calorimetric procedure given by Singleton and Rossi (1965). Anthocyanins in wines were measured by a method given by Harborne (1973). One mL of wine was taken in 16 mL of acidic methanol containing 1% hydrochloric acid. The contents were allowed to stay overnight at 4°C. Thereafter, absorbance was recorded at 530 nm.

Sensory analysis

Sensory analysis was performed on a prescribed proforma given by Amerine et al. (1980).

### Statistical analysis

Statistical analysis of the quantitative data of chemical parameters was done by Completely Randomized Design (CRD) Factorial (Cochran and Cox, 1963).

### RESULTS AND DISCUSSION

#### Physico-chemical Characteristics of fruit

Physico-chemical characteristics of mulberry fruit are shown in Table 1. The reducing sugars and total sugars were found to be 9.93 ± 0.19 % and 12.26 ± 0.13 %, respectively. pH value of the pulp was estimated to be 3.57 ± 0.01. Anthocyanin content was recorded to be 122.50 ± 0.18 mg/100 g. The original TSS of the mulberry pulp used for the preparation of wine was slightly less (15.60 °Bx) and therefore, a need was felt to raise the TSS by the addition of table sugar to the must ranging from 22 to 26.00 °Bx.

#### Table 1. Physico-chemical characteristics of fresh mulberry fruit

<table>
<thead>
<tr>
<th>Physico-chemical characteristics</th>
<th>Mean ± SD (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit weight (g)</td>
<td>6.40 ± 0.32</td>
</tr>
<tr>
<td>TSS (°Bx)</td>
<td>15.60 ± 0.20</td>
</tr>
<tr>
<td>Titratable acidity (as % malic acid)</td>
<td>0.73 ± 0.16</td>
</tr>
<tr>
<td>pH</td>
<td>3.57 ± 0.01</td>
</tr>
<tr>
<td>Ascorbic acid (mg/ 100 g)</td>
<td>103.00 ± 0.24</td>
</tr>
<tr>
<td>Reducing sugars (%)</td>
<td>9.93 ± 0.19</td>
</tr>
<tr>
<td>Total sugars (%)</td>
<td>12.26 ± 0.13</td>
</tr>
<tr>
<td>Anthocyanin (mg/ 100 g)</td>
<td>122.50 ± 0.18</td>
</tr>
</tbody>
</table>

SD: Standard deviation

#### Fermentation behaviour of must

The results (Figure 1) depicted the fermentation behaviour of the mulberry must prepared at different initial TSS (22, 24 and 26 °Bx) levels. After completion of the fermentation (336 h), the lowest TSS (7.8 °Bx) was observed in T1 (22 °Bx), which was attributed due to the lower initial sugar content than that of T3 (26 °Bx) mulberry must (8.8 °Bx). The higher decrease in TSS during initial fermentation is attributed to the higher fermentability of musts of different treatments (described earlier) because of more availability of sugar and less ethyl alcohol in the medium. With increase in time however, the ethanol content increased exerting inhibitory effect on the fermentability (Mota et al., 1984; Nishino et al., 1985; Sharma and Joshi, 1996; Abrol and Joshi, 2011). Therefore, the trend of ethanol increase or TSS fall during fermentation is natural as discussed earlier in other wines also (Amerine et al., 1980; Joshi and Bhutani, 1990; Sharma and Joshi, 1996; Joshi et al., 1999; Abrol and Joshi, 2011).

#### Fig. 1. A comparison of fermentation behaviour of mulberry musts of different treatments
A comparison of rate of fermentation (RF) which is decrease in TSS was revealed that the T1 (26 °Bx) mulberry must had the highest RF (1.23). While, 22 °Bx mulberry must gave the least fermentation rate (1.01).

Physico-chemical characteristics

Table 2 summarizes the effect on total soluble solids, total sugars, reducing sugars and titratable acidity of wines of different treatments. The highest TSS was observed in T3 and the lowest in T1. The wide variation in TSS of different treatments is apparently related to the difference in sources of sugars, initial TSS as well as the fermentation behaviour of different must. The must with T1 as fermentable sugar reduced the highest TSS in all the treatments but produced less ethanol content. The wide variation in TSS is also apparently related to the difference in fermentability behaviour of the musts (Abrol and Joshi, 2011). The reducing sugars of different treatments ranged between 0.41 to 0.48 per cent. Amongst the treatments, T1 had the highest reducing sugars (0.48 %) followed by T2 mulberry wine (0.44 %) and (0.41 %) lowest in T1. With the increase in initial sugar level, reducing sugars content were increased. In different wines, total sugars ranged between 1.11 to 1.25 per cent. The highest total sugars of 1.25 per cent were recorded in T3 and lowest (1.11 %) in T1.

From the results it is clear that reducing and total sugars left in the mulberry wine are very less, which is termed as residual sugars. The behaviour is obvious after fermentation as observed earlier in plum, apricot wine and wild apricot wine and mead after completion of fermentation (Joshi and Sharma, 1993; Joshi et al., 1990, Abrol and Joshi, 2011).

The titratable acidity and pH were found to be inversely related with each others. The highest titratable acidity and lowest pH was recorded in T3. Similar findings have been reported earlier in apricot wine (Joshi and Sharma, 1994) and wild apricot wine and mead (Abrol and Joshi, 2011).

A significant difference for ethanol content was observed among the different treatments, and the highest ethanol content was recorded in T1 (12.12 %) while the lowest in T1 (10.15%). Table wine contains alcohol from 7 to 14 % (Amerine et al., 1980) and from this point of view based on ethanol content all the wines prepared in the experiment falls within the category of table wines. It is also noticeable from the data that the volatile acidity ranged in between 0.027 to 0.030 per cent (as acetic acid) among the different treatments. A sound wine generally has volatile acidity less than 0.04 % as acetic acid (Amerine et al., 1980). High volatile acidity indicates acetification of wine. The amounts of higher alcohols in different wines were significantly different from each other. With the increase in initial TSS level from 22 to 26 °Bx, increase in higher alcohols content was observed and it ranged in between 123.0 to 150.5 mg/ L.

It is clear from the perusal of data in Figure 4 that the increase in initial TSS level in the mulberry must resulted a decline in the ascorbic acid, anthocyanin and total phenols after completion of fermentation process. The ascorbic acid content was recorded significantly different and ranged from 74.45 mg/ 100 mL to 67.58 mg/ 100 mL. The highest anthocyanin content was observed in T1 (48.23 mg/ 100 mL) and lowest in T3 (67.58 mg/ 100 mL). The total phenols of wine prepared from the must of various treatments were recorded significantly different and ranged in between 345.5 mg/ L to 379.0 mg/ L. At low concentration, the higher alcohols may play an important role in sensory quality (Amerine et al., 1980). Lesser quantity of higher alcohols denotes the non-oxidative conditions of all the wines (Guymon et al., 1961) which indirectly reflects the proper conditions of wine preparation adopted in this study. The differences can be attributed to the variation in the phenol content of the fruit berries of the mulberry used for wine making.
Sensory quality

Composite scoring test was carried out for the sensory evaluation of mulberry wine of various treatments (Figure 5).

In comparison to sensory scores of mulberry wines of different treatments, wine of T3 scored the highest score for major attributes. The wine from initial TSS of 24 °Bx (T2) was also liked by the judges and awarded with a good sensory score after T3. Out of three initial TSS levels, the wine prepared from 26 °Bx must scored excellent rating because of balanced astringency, alcohol, sugars and acid content.

**CONCLUSION**

Rate of fermentation (RF) of T3 must was found higher than other must. Out of three mulberry must used for wine, the treatment T3 wine had higher TSS, total sugars content, pH, volatile acidity, ethanol, total phenols and total anthocyanin. With the increase in initial TSS level of musts, TSS, reducing sugars, total sugars, titratable acidity, volatile acidity, ethanol content, higher alcohols and total phenols increased whereas, total anthocyanin, ascorbic acid and pH decreased. On the basis of physico-chemical and sensory quality characteristics, it can be concluded that must containing initial TSS of 26 °Bx produced excellent quality mulberry wine.
REFERENCES


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