Standardization of conditions for fermentation and maturation of wine from Amla (*Emblica officinalis* Gaertn.)

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Abstract

All the useful natural components of amla, Indian gooseberry, *Emblica officinalis Gaertn.*, with therapeutic value, can be easily extracted in water after dispensing the berries in hot water. Ameliorating the extract, with the sugar made it a good medium for the growth of *Saccharomyces cerevisiae* and fermenting the sugar into ethanol to make wine. The wine was found similar to any other wine in terms of its composition, taste and aroma. The conditions for achieving the highest alcohol content and improving the sensory qualities have been standardized by evaluating the effect of addition of various exogenous nutrients, environmental conditions, fermentation technology and by maturing the wine. The supplementation of ammonium sulphate, potassium dihydrogen phosphate, proline and biotin to the hot water extract of amla proved to be best nutritional factors for highest alcohol production (12%) during the fermentation of the amla based medium with a new strain of *S. cerevisiae* in a batch fermentation. The alcohol content was further improved to 16.1% in a fed batch fermentation involving the repeated feeding of sugar for 2 cycles after an interval of 3 days each in a batch where the initial TSS was maintained at 20% and the feeding was done when the original TSS fell to 10% at each of two stages. Further, the storage of wine in oak wood barrel for a month improved its quality and led to the reduction in undesirable components such as n-propanol, n-butanol, iso-butanol, isoamyl alcohols and an increase in desirable components including ethyl acetate, phenolics, etc.

Keywords: Amla, Indian gooseberry, *Emblica officinalis*, Wine, Fermentation conditions, Maturation.

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Introduction

Wine is one of the functional fermented foods and has many health benefits. These include anti-ageing effects in red grape skins, improvement of lung function from antioxidants in white wine, reduction in coronary heart disease, development of healthier blood vessels in elderly people, reduction in ulcer-causing bacteria, destruction of cancer cells by protein present in red grape skins, prevention of stroke by keeping the arteries clean by polyphenols in red grape skins, decreasing ovarian cancer risk in women and making the bones stronger. Many wines are made from fruits having medicinal value and such wines have many additional benefits.

The exact composition of a herbal product is influenced by the method of extraction. A hot water extract is generally rich in polar components because water is a polar solvent. Oil on the other hand is a non-polar solvent and it absorbs non-polar compounds. Alcohol lies somewhere in between the polar and non-polar compounds. Herbal wines include the alcoholic extract of herbs; usually with an ethanol percentage of 12-38⁹. The ancient biomolecular and archaeological evidence for plant additives in fermented beverages dates from the early Neolithic period in China⁵ and the Middle East⁶, besides from Monte Verde in Chile, around 13,000 B.P⁷.

Amla, also known as Indian gooseberry (*Emblica officinalis Gaertn.*), is one of the useful fruit⁶. It is consumed as a fresh fruit or in the form of food products like preserve. The fruit also forms an important constituent of many Ayurvedic preparations such as *chyvanprash* and *triphala* and is regarded as “one of the best rejuvenating” herbs preparation of wine using the fruits of amla would be useful for imparting healthful properties to the wine. Production of good quality wines is a
complex biochemical process involving the sequential appearance of different microbial species including *S. cerevisiae*, lactic acid and acetic acid bacteria whose development is affected by various intrinsic and extrinsic factors. For a successful fermentation, the nutritional requirement of the microorganisms for their growth must be met by the substrate being fermented. Present paper deals with the standardization of various cultural, environmental and fermentation conditions for the improvements in amla wine fermentation and its sensory characteristics.

**Materials and Methods**

**Collection of fruits**

Fresh amla fruits, measuring about 2.5cm in diam were collected from the local market of Chandigarh city. These were washed in clean water and dried in air.

**Microorganism**

A new thermotolerant, osmotolerant and alcohol tolerant strain of *S. cerevisiae* was isolated from decaying grape berries, collected from the local market. The yeast was capable of growing up to a temperature of 45°C in presence of 30% sugar and 18% ethyl alcohol.

**Preparation of hot water extract**

One kg of intact amla fruits and 500g of cane sugar were dispensed in 2.5 litres of boiled water in a steel container and stirred the contents for 5 minutes. The container along with the contents was left undisturbed after covering it with a metal lid till the contents reached the ambient temperature. The extract was then taken out and analyzed for total soluble solids (TSS), total sugar content and pH. The TSS was adjusted to 20% with cane sugar and the pH was adjusted to 5.0, using citric acid.

**Preparation of inoculum**

100ml of sterilized GYE broth (yeast extract, 0.3%, malt extract, 0.3%, peptone, 0.5% and glucose, 1.0%, pH 4.5), dispensed in 250ml Erlenmeyer flask was inoculated with a loopful of 24h old actively growing culture of *S. cerevisiae*. The flask was inoculated at 30°C on a rotary shaker (150 rpm) for overnight and the cells were separated by centrifugation at 10000 rpm (4°C, 15 min). These were washed twice with distilled water and re-suspended in distilled water to give a concentration of 10^6cells/ml which was used as a pre-inoculum. Pre-inoculum (30ml) was added to 300 ml of the hot water extract of amla with a TSS content of 10% and 100 ml of the same was then dispensed in each of the three 250ml Erlenmeyer flasks. These were incubated at 30°C on rotary shaker (150 rpm) for overnight and the contents of the three flasks were then, mixed and used as an inoculum for the fermentation.

**Fermentation of hot water extract of amla**

The amla containing medium having a TSS of 20%, pH 4.5, prepared as hot water extract was subjected to batch fermentation. The medium containing intact amla berries, was taken in Erlenmeyer flask (5 l), inoculated with 10% v/v yeast inoculum, supplemented with 100 ppm sodium metabisulphite and incubated in a stationery state after plugging with cotton wool in a Biochemical Oxygen Demand (BOD) incubator at 37°C. The contents of the flask were mixed 2-3 times a day daily and the progress in fermentation was noted by analyzing TSS with the help of a hand refractometer.

After completion of the fermentation, the contents were siphoned using a rubber tubing to separate the amla berries that rose to the top and the yeast cells, which settled at the bottom. The wine was clarified, by repeated siphoning for 3-4 times after a sedimentation period of 3 days each and analyzed for various constituents.

**Effect of nutritional and environmental factors on alcohol production**

The amla wine production was standardized by taking 50g amla berries in different sets of 250 ml Erlenmeyer flasks and adding 125 ml boiling water in each. Cane sugar was added to adjust the TSS at 20% and maintaining the pH at 4.5 with citric acid. The effect of nutritional factors was studied by supplementing the production medium, separately, with various nitrogen sources (0.5% w/v) including ammonium sulphate, urea, diammonium hydrogen phosphate, malt extract, yeast extract, soyabean meal, corn steep liquor, peptone, metal salts (0.1% w/v) including magnesium sulphate, calcium chloride, sodium chloride, potassium chloride, zinc sulphate, potassium dihydrogenorthophosphate, amino acids (0.01% w/v) including alanine, phenyl alanine, tyrosine, tryptophan, ornithine, threonine, aspartic acid, proline, serine, valine, arginine, leucine, lysine, glutamic acid,
glycine, methionine, histidine, cystine and vitamins (0.01% w/v) including thiamine, riboflavin, nicotinic acid, pantothenate, biotin, niacin, pyridoxine, folic acid and B-complex.

The effect of temperature was studied by incubating the amla based production media, after inoculation, at different temperatures including 25, 30, 35, 40 and 45°C. The effect of TSS was studied by adjusting the solid content in the production media at 10, 15, 20, 25, 30 and 35% with cane sugar.

**Wine production with repeated fed-batch fermentation**

This was studied by taking 1kg of amla berries and dispensing in 2.5 l of boiling water and adjusting the initial TSS of the production medium at 20%. The batch was repeatedly fed twice with a sugar syrup (70% TSS) after the TSS of the medium reached 10% after 3 and 6 days of fermentation, respectively, to readjust the TSS to 20% at each of the two stages.

**Maturation of amla wine**

Ageing of amla wine was done for 30 days in two types of containers, the glass bottles and oak wood barrel (2.5 l), procured from M/S Jagatjit Industries Limited, Hamira, Punjab (India). The containers were filled up to the brim and analyzed for various components after 30 days of ageing.

**Analytical methods**

TSS was checked using a hand refractometer (Erma). The pH was measured using digital pH meter. The total sugars (in terms of glucose), titrable acids (in terms of tartaric acid), total soluble proteins (in terms of BSA), total phenolics (in terms of gallic acid), ascorbic acid and ethyl alcohol were determined using standard protocols. The estimation of elements was done by atomic absorption spectrophotometer (Hitachi). Fermentation efficiency was calculated as:

\[
\text{Fermentation Efficiency} = \frac{\text{Alcohol content in wine}}{(F.E.)} \times 100
\]

\[
\text{Alcohol obtainable from sugars utilized}
\]

Ethyl acetate, n-propanol, n-butanol, iso-butanol and iso-amyl alcohol were detected and quantified by gas chromatograph (Hewlett Packard 5790) equipped with flame ionizing detector (FID) using a glass column (6′ × 1/4′) packed with carbowax-20M. The analyzing conditions were Gases: Nitrogen 25 ml/min, oxygen 94 ml/min and hydrogen 30 ml/min; temperature T1 40°C for 1 min, T2 200°C, injection temperature 250°C; Detector temperature 200°C; Injection volume: 1μl, 2 μl and 3 μl.

**Sensory evaluation of amla wine**

The sensory evaluation of the unmatured and matured wines was done by a panel of five judges on the basis of scoring in terms of appearance, colour, aroma, bouquet, acescent, total acid, sugar, body, flavour, astringency and general quality as per the prescribed performa.

**Results and Discussion**

**Pattern of sugar utilization and alcohol production**

The hot water extract of amla berries with the TSS of 20% proved to be a good growth medium for *S. cerevisiae* and got converted into wine with the composition as given in Table 1. The yeast strain grew well and utilized sugar at the rate of 104 mg/h producing ethanol at the rate of 53 ml/h. Quantitatively, ethyl alcohol is the most important component present in all the alcoholic beverages and is associated with stimulatory and intoxicating properties of these beverages. The ethyl alcohol content in wines generally varies with the type of the product and ranges from 10-14%, in most table wines, but in some fortified wines, alcohol obtained by distillation may be added to increase its level. Most of the sugar utilized in the present study was converted into alcohol, with a fermentation efficiency of 80% revealing an overall yield of 9.0 % (v/v) at the end of 168h of fermentation (Fig. 1). The pH of the medium revealed a gradual fall with the progress in fermentation, showing a final value of 3.4 at the end of fermentation.

**Composition of amla wine**

The fermentation of amla based medium brought the formation of various alcohols including ethyl alcohol, n-propanol, n-butanol, iso-butanol and isoamyl alcohol, the levels of which, as observed in the resulting wine were 9% v/v, 33.45, 8.9, 58.98 and 120.67mg/l respectively (Table 1). This observation corroborates the earlier findings of several workers who also observed the formation of these alcohols during fermentation of fruit juices and reported that the proportion of these alcohols vary with the type of wine and is related with yeast species. The levels of higher alcohols up to 400 mg/l improve the aroma while the
concentration higher than this level deteriorates the quality of wine. The total acidity, in terms of tartaric acid, in the present study was 2.77% with a pH of 3.3 (Table 1). The sum total of acids determine the amount of tartness a wine will deliver on the palate and the resultant low pH ranging from 3.1-3.5 may help to keep the microbiological and chemical reactions properly controlled. The fresh amla wine prepared in the present study was found to have the total phenolic compounds at a level of 1.0% (Table 1). Fruits including amla contain a major group of compounds, referred to as phenolic substances and/or tannins, accounting for 0.01-2.75% and responsible for colour, taste, mouth feel, oxidation and other chemical reactions. These compounds give characteristic colour to the wines and account for differences in flavour of red and white wines. The phenolic compounds of wine are very important, as they contribute to sensory characteristics, particularly colour, astringency and bitterness and as they are also involved in biochemical and pharmacological effects, including antimicrobial, anticarcinogenic and antioxidant properties. Esters are insensible components of wine quality as they impart the fruity flavour to the wines. The amla wine prepared in the present study also exhibited the presence of esters detected in the form of ethyl acetate at a level of 17.6mg/l (Table 1). From the winemaker’s point of view, the fruit nitrogen components are important as nutrients for the yeast fermentation, as enzymes such as phenolase (PPO) and as factor involved in haze formation, especially in white wines. The protein contents in most of the wines are negligible. The amla wine prepared in the present study also shown the presence of P, K, Ca, Mg and Fe as the major elements amounting to 80, 1030, 190, 450 and 11mg/l with traces of Cu, Zn and Cr in the range of 1.1, 0.9 and 1mg/l, respectively (Table 1). Vitamin C was detected in appreciable amounts (0.46g/100ml) (Table 1). Wine yeasts are good source of many B-vitamins and thus, account for an increase in the levels of these in wine. The yeast strain used in the present study produced 9% (v/v) ethanol from 17.6% of sugar utilized during the fermentation at 37°C. The influence of wine yeast on wine composition and quality is already well-known. The tolerance of yeast to high temperature, high solid concentration and alcohol concentration is responsible for great differences in composition.

### Table 1: Composition of fresh and matured amla wines

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Fresh wine</th>
<th>Aged in glass bottle</th>
<th>Aged in oak barrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Pale yellow</td>
<td>Pale yellow</td>
<td>Pale yellowish orange</td>
</tr>
<tr>
<td>TSS (%)</td>
<td>4.6</td>
<td>4.6</td>
<td>4.8</td>
</tr>
<tr>
<td>Total Acids (g/100 ml)</td>
<td>2.77</td>
<td>2.05</td>
<td>1.60</td>
</tr>
<tr>
<td>pH</td>
<td>3.3</td>
<td>3.4</td>
<td>3.52</td>
</tr>
<tr>
<td>Soluble proteins (g/100 ml)</td>
<td>2.0</td>
<td>1.82</td>
<td>1.75</td>
</tr>
<tr>
<td>Vitamin C (g/100 ml)</td>
<td>0.46</td>
<td>0.40</td>
<td>0.39</td>
</tr>
<tr>
<td>Ethanol (% v/v)</td>
<td>9.0</td>
<td>8.95</td>
<td>8.9</td>
</tr>
<tr>
<td>Phenolics (g/100 ml)</td>
<td>1.0</td>
<td>0.95</td>
<td>1.75</td>
</tr>
<tr>
<td>Sugar (g/100 ml)</td>
<td>0.6</td>
<td>0.58</td>
<td>0.54</td>
</tr>
<tr>
<td>Ethyl acetate (mg/l)</td>
<td>17.6</td>
<td>59.76</td>
<td>128.67</td>
</tr>
<tr>
<td>n-propanol (mg/l)</td>
<td>33.45</td>
<td>7.76</td>
<td>ND</td>
</tr>
<tr>
<td>n-butanol (mg/l)</td>
<td>8.9</td>
<td>6.5</td>
<td>ND</td>
</tr>
<tr>
<td>Isobutanol (mg/l)</td>
<td>58.98</td>
<td>45.46</td>
<td>11.23</td>
</tr>
<tr>
<td>Isoamyl alcohol (mg/l)</td>
<td>120.67</td>
<td>74.86</td>
<td>40.12</td>
</tr>
<tr>
<td>Mineral/Trace elements (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Zn</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>P</td>
<td>80</td>
<td>79</td>
<td>81</td>
</tr>
<tr>
<td>K</td>
<td>1030</td>
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<td>1045</td>
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<tr>
<td>Ca</td>
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<td>Mg</td>
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<td>458</td>
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<tr>
<td>Fe</td>
<td>11</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Cr</td>
<td>1</td>
<td>1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**Optimization of nutritional and environmental factors**

Of the various nitrogenous compounds evaluated, ammonium
sulphate induced the maximum alcohol level of 11.5% (v/v) exhibiting a fermentation efficiency of 91% (Fig. 2). This corroborates the earlier findings\(^2\) which have indicated that fermentations are benefited by the addition of ammonium salts. Of the various metal salts supplemented, potassium dihydrogen phosphate and MgSO\(_4\) produced the high alcoholic yields of 11 and 10.7% v/v, respectively revealing fermentation efficiencies of 87 and 86% (Fig. 3). These results are in agreement with the earlier studies, which suggest that potassium and magnesium are required for optimum growth of yeast though growth and fermentation were possible on unsupplemented molasses\(^2\). Magnesium and potassium are regarded as bulk cations establishing the required ionic environment of the yeast cell\(^2\).

Of the various amino acids, the supplementation of proline brought out the highest fermentation efficiency producing the maximum alcohol content of 10.8% (Fig. 4), whereas among various exogenous vitamins, biotin proved to be most favourable for the production of highest alcohol yield of 11.2 % with a fermentation efficiency of 89% (Fig. 5). Yeasts have no known direct requirement for amino acids. However, fermentation requires amino acids as a catalyst in synthesizing nitrogen into the free ammonium state which is required by yeasts. The yeast strain used in the present study though produced highest alcohol content of (10%) at temperatures of 25 and 30°C showing 83% efficiency, it also worked very well at elevated temperatures of 35 and 40°C, where it produced 9% alcohol with 75% fermentation efficiency (Fig. 6). Most of
the yeast strains grow best at a temperature less than 35°C, the species capable of growing at a temperature more than 37°C are very rare and are very important for alcohol production as the temperature of the fermentation tanks exceeds 40°C during summers because of the exothermic nature of fermentation process\(^3\). Changes in membrane fluidity of the mesophilic yeast lead to a retarded or no growth higher temperatures. The yeast strains able to adapt to these changes are able to survive at higher temperatures and are thermotolerant\(^4\).

Amongst various concentrations of sugar in various batches used for wine production, the batch with 10% sugar revealed the highest fermentation efficiency of 90% followed by 88% observed in the batch with 15% initial sugar concentration. The efficiency of alcohol production decreased with an increase in initial sugar concentration probably due to increased osmotic pressure of the medium or overloading of the cells because of high concentration of substrate. The maximum alcohol concentration which could be achieved in the study was 12% in the batch having 25% initial sugar concentration (Fig. 7). High substrate concentrations inhibit the growth of yeast cells as a result of high osmotic pressure and low water activity leading to the dehydration of the yeast cells. On the other hand yeasts able to adapt to these changes in osmotic pressure are osmotolerant\(^5\).

**Fed batch fermentation for amla wine production**

The amla extract medium was fed with sugar syrup (70% TSS) to re-adjust the TSS to 20% every time. Fed-batch
fermentation is a production technique in between batch and continuous fermentation. A proper feed rate, with the right component constitution is required during the process. This system worked very well by eliminating the effect of high sugar concentration, at the start of fermentation and produced an alcohol content of 16.6% v/v at the end of 9 days of fermentation. The rate of utilization of substrate and alcohol production increased immediately after feeding the batch with fresh dosing of sugar (Fig. 8).

Maturation of amla wine

The ageing of the wine improved the wine characteristics in terms of clarity and proved to be very useful in bringing significant reduction in undesirable alcohols like n-propanol, n-butanol, isobutanol, isoamyl alcohol as well as total acids. On the other hand the desirable components including ethyl acetate increased with the ageing in both the containers, however, the degrees of improvements were more in wooden barrel than in glass bottles. The storage in oak wood also brought about an improvement in phenolics, aroma, taste and stabilization of the wine colour (Table 1). The wine matured in oak wood barrel was also adjusted to be the better after tasting by a panel of five judges (Table 2). Oak is the most commonly used wood for barrels used for ageing quality wines and spirits known to improve wine characteristics and colour stability.

Conclusion

It is concluded that amla berries can be used as a valuable ingredient for the production of an herbal wine with all the important properties of wine having
Table 2: Evaluation card of amla wines matured in glass bottles and oak wood barrels by five tasters

| Characteristics | Max. score | Score by tasters | I | II | III | IV | V |
|-----------------|------------|------------------|--|--|--|--|--|--|--|--|--|--|---|
|                 | G | O | G | O | G | O | G | O | G | O | G | O |
| Colour          | 2.0 | 1.0 | 2.0 | 1.0 | 1.5 | 1.0 | 2.0 | 1.5 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Aroma           | 2.0 | 1.0 | 2.0 | 1.0 | 1.5 | 1.0 | 2.0 | 1.5 | 2.0 | 1.0 | 2.0 | 1.0 | 2.0 |
| Bouquet         | 2.0 | 0.5 | 2.0 | 1.0 | 1.5 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Acescent        | 2.0 | 0.5 | 2.0 | 1.0 | 1.5 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Total acid      | 2.0 | 0.5 | 2.0 | 1.0 | 1.5 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Sugar           | 1.0 | 1.0 | 0.5 | 1.0 | 1.0 | 1.0 | 0.5 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Body            | 1.0 | 1.0 | 1.0 | 0.5 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Flavour         | 2.0 | 1.0 | 2.0 | 1.0 | 1.5 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Astringency     | 2.0 | 1.0 | 2.0 | 0.5 | 2.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| General quality | 2.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Total           | 20.0 | 10.0 | 19.5 | 10.0 | 17.5 | 11.0 | 19.0 | 12.5 | 19.0 | 10.5 | 17.0 |

G: Wine matured in glass bottles
O: Wine matured in oak wood barrel

...medicinal characteristics of amla fruits. This can thus prove to be a good herbal drink with alcohol as a stimulant, phenolics and vitamin C as antioxidants. The alcohol content achieved in the wine was 16.1% in a fed batch fermentation involving the repeated feeding of sugar for 2 cycles. Further, the storage in oak wood barrel for a month improved the quality of wine and led to the reduction in undesirable components such as n-propanol, n-butanol, isobutanol, isoamyl alcohols and an increase in desirable components including ethyl acetate, phenolics, etc.

References


