



Biochemical Changes in Wine Production from *Emblca officinalis*

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ABSTRACT

Indian gooseberry (*Emblca officinalis*), is one of the useful fruit. Wine is one of the functional fermented foods that have many health benefits. Commercially, wine is manufactured by the fermentation of yeast which involves the conversion of sugar to alcohol. The natural fermentation was done in both aerobic and anaerobic condition. During fermentation process, pH, titrable acidity, polyphenols, reducing sugar, ascorbic acid, moisture content were analyzed. The ascorbic acid was slowly reduced and utilized in the production of wine. The reducing sugar of amla juice was 9.8% in 10 days of fermentation. It was rapidly raised to 12.6% in the end of anaerobic fermentation. The amount of ethanol was found to be 4.6% in anaerobic and 2.4% in aerobic condition.

Keywords: *Emblca officinalis*, Reducing sugars, ascorbic acid, fermentation.

INTRODUCTION

In India, there are so many variety of soft drinks include aerated soft drinks, beverages containing fruit juice etc. The juice based beverage is manufactured in small scale when compared to synthetic carbonated drinks. The advantage of fruit based beverage showed high amount of nutritional factors over the synthetic aerated water^{1,2}.

Antioxidants are the vital substances which protect the body from damage caused by free radical induced oxidative stress. There are many free radical scavenging antioxidants exist within body. Most of them are derived from dietary sources of fruits, vegetables and teas³. In healthy persons, the productions of free radicals are balanced by oxidative defense system. The oxidative stress is produced by generation of free radicals which lead to the depletion of antioxidants. This free radical may cause DNA mutation and target cell damage. This results in cell senescence and death⁴. The improper functioning of free radical defense mechanisms lead to atherosclerosis, ischemic heart disease, ageing, diabetes, immunosuppressant and neurodegenerative diseases.

Herbal medicines remain important resource in developing countries and cure diseases. In world population, 62-80% of people still rely on traditional medicines for the treatment of disease. Currently there is a demand for taking natural antioxidants and natural antimicrobials of herbal resources⁵.

Indian gooseberry is highly nutritious and is an important dietary source of vitamin C, minerals and amino acids. The high amount of calcium, phosphorus, iron, carotene, thiamine, riboflavin and niacin.

The fruit juice contains vitamin C which is highly stable due to the presence of tannins and polyphenols^{6,7}. The antioxidant effect of amla extract are mainly due to the

presence of phenolic compounds such as flavonoids, phenolic acid, tannins and phenolic diterpenes⁸.

Amla was used to prepare ready to serve beverage⁹. Storage conditions of amla juice also play an important role in post harvest. The main objective of this study was to extract the microbial and biochemical changes in amla juice when it was subjected to aerobic and anaerobic fermentation.

MATERIALS AND METHODS

The amla fruit were collected from fruit market. Amla pulp was removed, and then seed coat was separated to get amla seed. 1.5 kg of amla was boiled in 1.5 L of water and the boiled amla juice was kept in vessel. The boiled amla juice was shifted into two different vessels, one was open to air and other one was tightly closed.

Isolation of fungi and bacteria from initial fermented juice

During the days of fermentation one green layer occurred on the top of juice in open container even in 5 days. The juice in closed sample fungi layer was appeared on 15 days of fermentation. The fungi and bacteria sample was taken from the initial layer that formed on the juice during fermentation.

Biochemical analysis of fermented juice

Total phenolic content

Total poly phenol was measured in juice samples by taking 250 mg of amla juice in 10 ml of methanol and water (70:30) in a test tube and heated on a water bath at 70°C for 10 minutes. The samples were cooled and centrifuged at 3500 rpm for 10 minutes. 0.2 ml of supernatant was made up to 10ml with distilled water. 5ml of sample was mixed with 0.5 ml of saturated sodium carbonate and 0.2 ml of folin–ciocalteau reagent. The volume was made upto 10 ml with distilled water. The



absorbance was read at 765nm after 1 hour by UV-visible spectrophotometer¹⁰.

Ascorbic acid

Sample solution was prepared and its equivalent to 0.2 mg of standard ascorbic acid in water containing 3% metaphosphoric acid. It was titrated against standard 2,6 dichloroindophenol dye. The development of pink color was an end point. The titration was repeated to get concordant value¹¹.

Reducing Sugar

The reducing sugar was determined by DNS method. The different stages of fermented juice were taken as samples. 0.1 ml of sample react with 3,5 Dinitro Salicylic acid to get yellow which read colorimetrically at 540nm.¹²

Sensory analysis

The Sensory analysis of color, texture, flavor, taste and appearance were monitored regular intervals of fermentation in aerobic and anaerobic state.

RESULTS AND DISCUSSION

Extracted amla juice was subjected to aerobic and anaerobic fermentation for 60 days. The physical and chemical properties of fermented juice were summarized

in Table 1 and 2. The titrable acidity was gradually increased from 2.0% to 4.2 % in anaerobic fermentation. The acidity was maintained in juice due to fermentation of sugars. The acidity was rapidly raised from 2.0% to 9.28 in aerobic fermentation. The initial pH of the amla juice was 4.0. During anaerobic state, the pH was gradually decreased to 3.61 in 60 days of fermentation. The pH of the juice was slightly decline in aerobic conditions. The changes in temperature and pH enhance the acidity nature of amla juice.

Vitamin C, a natural antioxidant rich in Indian gooseberry. The amla juice contained 416 mg/100 ml of ascorbic acid. The reduction of ascorbic acid was found to be higher in aerobic state when compared with anaerobic condition. The ascorbic acid was slowly reduced and utilized in the production of wine¹³.

The reducing sugar of amla juice was 9.8% in 10 days of fermentation. It was rapidly raised to 12.6% in the end of anaerobic fermentation.

The doubling the amount of reducing sugar (24.6%) was found in 60 days of aerobic fermentation. Hussain reported that acidity and reducing sugars were increased with decrease in pH and ascorbic acid were similarly observed in mango juice during two months storage¹⁴.

Table 1: Biochemical analysis of amla juice in anaerobic fermentation

Days of storage	pH	Titrable acidity(%)	Ascorbic acid(mg/100ml)	Reducing sugars(%)	Polyphenol (mg/100ml)	Protein(mg/100ml)
0	4.0	2.0	416.0	7.8	170.1	0.8
10	4.3	2.1	387.5	9.8	168.4	0.75
20	4.15	2.7	358.1	10.2	167.3	0.82
30	3.95	3.4	336.4	10.7	164.2	0.88
40	3.82	3.98	298.3	11.8	162.1	0.91
50	3.70	4.0	256.4	11.8	156.3	0.83
60	3.61	4.2	225.3	12.6	150.3	0.81

Table 2: Biochemical analysis of amla juice in aerobic fermentation

Days of storage	pH	Titrable acidity(%)	Ascorbic acid(mg/100ml)	Reducing sugars%	Polyphenol	Protein(mg/100ml)
0	4.0	2.0	416.0	7.8	170.1	0.8
10	4.35	2.95	365.9	8.7	169.2	0.81
20	4.39	3.86	355.7	10.5	168.8	0.84
30	4.29	4.40	295.6	13.5	168.1	0.88
40	4.82	6.95	245.1	14.6	167.8	0.91
50	4.20	8.40	238.0	19.3	167.2	0.92
60	3.86	9.28	204.9	24.6	167.0	0.95

The total phenolic contents were found to be higher in juices and residues than seed¹⁵. The initial polyphenol of amla juice was 170.1 mg/100ml. The phenols were gradually declined in both conditions. Poonam mishra reported that different processing of amla lead to change in ascorbic acid content, 70% of antioxidant activities as

percentage of inhibition of oxidation in amla fruits which correlated positively with total phenols¹⁶

Microbes play an important role in fermentation. The sterilized amla juice was further subjected to be aerobic and anaerobic conditions¹⁷. The yeast and molds were appeared on 10th day of anaerobic and 20th day of aerobic



fermentation. This was identified by potato dextrose agar (Fig 1).

The bacterial colonies were also observed in open fermented medium using nutrient agar (Fig2). The amount of ethanol was found to be 4.6% in anaerobic and 2.4% in aerobic condition. The color of amla juice was changed from green to light yellow on 30 days of fermentation and finally reached dark brown in anaerobic state. The taste was modified from sweet sour to beer in matured stage of fermentation. The initial appearance of solution was found to be clear and colloidal nature at 40 days of fermentation and reached opalescent in mature stage. The difference in color was due to the browning reactions between sugars and amino acids accelerated by changes in pH and temperature.



Figure 1: Fungal layer and its identification using potato dextrose agar



Figure 2: Bacterial layer and its identification using nutrient agar media

CONCLUSION

Amla juice is a healthy natural antioxidant and therapeutic agent. The natural wine production from amla juice is recommended to take daily to maintain good health without any side effects. Thus natural energy producing and antioxidant rich wine was produced and recommended for maintaining health in adults with beer taste.

REFERENCES

1. Akhtar, SS. Mahmood, S. Naz, M. Nasir and MT. Sultan. Sensory evaluation of mangoes (*Mangifera indica.L*) grown in different regions of Pakistan. *Pakistan journal of Botany*, 41(6), 2009, 2821-2829.
2. Abbo ES, Odeyemi G and Glurius TO, Studies on the storage stability of Soursop juice, *African J. Microbiol*, 21(2), 2006, 197-214.
3. Effat S and Hassan F Antioxidant activity of some furanocoumarins isolated from *Heraclurn persicum*, *Pharmaceutical Biology*, 42, 2004, 396-399.
4. Young ,IS and Woodside JV Antioxidant in health and diseases, *J.Clin. Pathol*, 54, 2001, 176-186.
5. Pourmorad Antioxidant activity, phenol and flavonoid of some selected Iranian medicinal plants, *Afr. J. Biotech*, 5, 2006, 1142-1145.
6. Srinivasan. Vitamin C in plants, Indian gooseberry *phyllanthus embillica*. *Nature*; 153, 1994, 684-94.
7. Dhale DA. Pharmacognostic evaluation of *phyllanthus embillica* linn . *Inter. J. Pharma and biosciences*, July, 3(3), 2012, 210-7.
8. Dewanjee S, Maiti A, Majunder R and Majunder A, Evaluation of antimicrobial activity of hydro alcoholic extract of *Schma wallichii park*, *Pharmacology online*, 1, 2008, 523-528.
9. Deka BC, Sethi V, Prasad R, Batra PK. Application of mixtures methodology for beverages from mixed fruit juice/pulp. *J. Food Sci. Technol.*, 38(6), 2001, 615-618.
10. Jayaprakash GK, Singh RP, Sakariah KK., Antioxidant activity of grapes seed extracts on peroxidation models. *Food. Chem*, 73, 2001, 285-90.
11. AOAC. (2002). Official Methods of Analysis. (17th Edn). Association of Official Analytical Chemists. Washington, USA.
12. Miller GL, Method of reducing sugars, *Anal.chem*, 31, 1972, 426.
13. Sarvesh R, and P Kumar. To Study the Storage Analysis of Developed Amla Mango Blended. *Adv. Biores.* 4(2), 2013, 109-117.
14. Hussain, S, S. Rehman, MA. Randhawa and M. Iqbal. Studies on Physico-chemical, microbiological and sensory evaluation of mango pulp storage with chemical preservatives. *J. Res. (Sci.)*, *BZ Uni., Multan, Pak.*, 14, 2003, 01-09.
15. S.Anbuselvi, Manas Jha, "Phytochemical and antimicrobial activity of *Emblca officinals* seed, *World Journal of pharmaceutical Research*, 4(8), 2015, 1336-1341.
16. Poonam mishra, Vijeyta srivastava, Deepmalaverma and GK Rai. Phytochemical properties of chakiya variety of amla and its effect of different dehydration methods., *Afri.J.Food.Sci.*; 3(10), 2009, 303-6.
17. Swetha Desaroju and Krishna mohan Gottumukkala, Current trends in research of *Emblca officinalis* A-Pharmacological perspective, *Int. J. Pharm.Sci.Rev.Res*, 24(2), 2014, 150-159.
18. Ranaurd OMGC, Baron A, Guyats Drilleau JF. Interactions between apple cell walls and native apple polyphenols. Quantification and some consequences . *Inter.J.Bio.Macromol*, 29, 2001, 115-125.

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