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Bio-processing and analysis of mixed fruit wine manufactured using *Aegle marmelos* and *Phoenix dactylifer*

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Abstract
Mixed fruit wine (*Aegle marmelos* and *Phoenix dactylifer*) was produced using *Saccharomyces cerevisiae*. Fermentation of the fruits lasted for 21 days respectively, during which aliquot samples analysis of pH, titrable acidity, specific gravity, total soluble solids, vitamin C and reducing sugar were carried out using standard procedures. Specific gravity of the wine was observed to reduce significantly (0.44 mg/ml) as the fermentation progresses. The pH of the fruit must during the period of fermentation decreased to 4.1. During the fermentation period, reduced (TSS 4.3˚ Brix) and increased vitamin C content (49 µg/g) was observed. At the end of 21st day of fermentation, the alcohol content was observed to be 7.9% v/v. The titrable acidity of the wine was found to show steady trend (0.662%) with time throughout the period of fermentation. Organoleptic analysis further showed significant acceptance of the wine by consumers. Hence, the present study exhibited that acceptable wine could be produced from mixed fruits of *Aegle marmelos* (bael) and *Phoenix dactylifer* (date).

Keywords: Wine, bael, date, fermentation, must, yeast

1. Introduction
Wine is among the most credited high value added artifacts from fruits [1]. It is an alcoholic beverage made from juices of mixture of fruits by fermentative action of microbes either impulsively or seeding by a specific strain mostly belonging to yeast species [2]. Most commercially produced wines are habitually made from fermented grapes (by adding different species of yeast to the crushed grapes). In the European countries, wine is legally considered as the fermented juice of grapes only [3]. From time to time wines are produced from different types of fruits such as guavas orange, blackberry, Paw-Paw, mango, Pineapple, Banana, Lemon, Watermelon etc. thus, one could easily prepare wine from nearly any plant matters that could be fermented into alcohol [1]. Most fruits, vegetables, spices, herbs, berries etc have the potential to produce variety of wine [4]. Wine manufacturing generally involves the use of yeast to ferment the must/ juice of a preferred fruit or mixture of fruits for a number of days, depending on the objective of the wine maker [5]. *Saccharomyces cerevisiae* has the capability of converting any carbohydrate source into an alcoholic compound for the production of different types of wines [6].
The nutritional worth of date palm (*Phoenix dactylifer*; family: *Arecaceae*) is indispensable source of healthy living [7]. Previous research findings have exhibited that date fruits possess vital nutrients that prevent cardiac diseases and contains minerals that aids improvement in eye sights and other medicinal properties for the welfare of the individual when ingested [8]. The key components of dates are the 78% carbohydrates, dietary fiber 6.4% to 11.5%, proteins 1% to 3%, vitamins and minerals 0.1 to 916 mg/100 g of date flesh a readily available source of energy to the humans [9].
Bael (*Aegle marmelos* L.; family: Rutaceae) is a tropical seasonal fruit, whose pulp is highly mucilaginous with 10 to 15 seeds and is rich in β-carotene [10]. The bioactive compounds present in bael fruits are marmelosin, luvangetin, aurapten, psoralen, marmelide and tannin [11]. Bael is reported to contain a number of coumarins, alkaloids, sterols and essential oils such as palmitic, oleic and linoleic acid [11]. With an excellent flavor, nutritive and therapeutic values of bael fruit there is also commercial potentiality for processing it for herbal medicines and food products [10]. The current article deals with the research related to the preparation of mixed fruit wine from pulp of Bael fruit and Date fruit by fermenting with the wine yeast, *Saccharomyces cerevisiae*, and evaluation of the wine with reference to nutrients and sensory attributes.

2. Methodology

2.1 Sample Collection: Ripe bael fruits (Aegle marmelos) and date fruits (Phoenix dactylifer) were obtained from the garden of Om Sterling Global University (OSGU), Hisar, Haryana. The fruits were brought fresh to the fermentation laboratory of Department of chemistry, OSGU for processing. The fruits were washed thoroughly with sterile water and rinsed with deionized water containing 0.1% formaldehyde.

2.2 Wine yeast: The wine yeast was obtained from the Microbiological Laboratory of OSGU. This yeast culture has earlier been employed for wine making from different fruits and other substrates [12, 13].

2.3 Must Fermentation: The date and bael fruits were washed carefully with 0.1% sodium metabisulphite solution. The fruits were incised, manually deseeded; pulp was separated, blended (with water 1:1 w/v ratio) and filtered to obtain the must. Aliquots of the must were obtained and used for pH, temperature, titrable acidity and reducing sugar analysis. The must was poured into a sterile 500 ml glass fermenter. This was followed by the addition of 100mg/ml Sodium metabisulphite (anti-microbial), 100g of granulated sugar (for fortification), 0.1% Ammonium sulphate 0.15% potassium dihydrogen for yeast supplementation. The juice was inoculated with yeast obtained and was kept for period of 21 days to ferment, with daily analysis of pH, temperature, reducing sugar and titrable acidity parameters. (Figure 1).

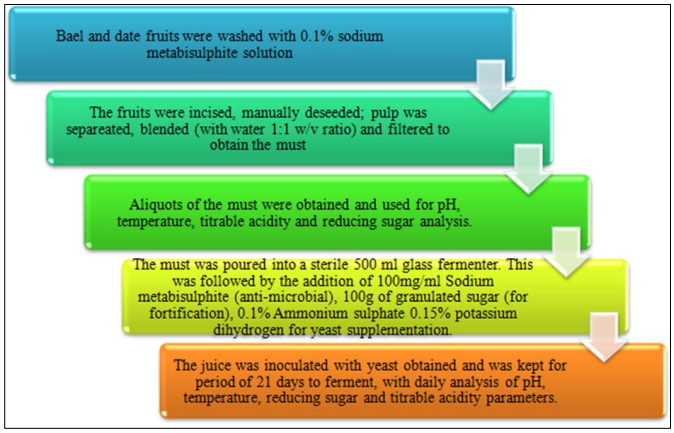

Fig 1: Bio-processing of Mixed fruit Wine of *Aegle marmelos* and *Phoenix dactylifer*

2.4 Racking and bottling: Initial racking was carried out at ambient temperature. Racking of the bael wine was carried out when total soluble solids (TSS) reached around 3.5˚ Brix. 0.035% Bentonite was added before the final racking to remove the last remaining residues. After final racking, 100 mg/ ml of sodium metabisulphite was again added as preservative before bottling. The bottles were filled full with wine, corked and sealed with bee wax.

2.5 Bio-chemical analysis

2.5.1 pH Determination: 10 ml of the "must" was put into a sterile beaker, and the pH of the must determine using a pH a digital pH meter [14].

2.5.2 Determination of Reducing Sugar: 1 ml of 3, 5-Dinitrosalicyclic acid (DNS) was poured to 1 ml of supernatant of sample, in a test tube and the mixture was then heated in water bath for 10 min. The test tube was then cooled rapidly under tap water and the final volume was adjusted to 12 ml with distilled water. The optical density of the sample was read against the blank in the spectrophotometer around 540 nm absorbance. The concentration of reducing sugar in the supernatant was estimated from the glucose standard curve [15].

2.5.3 Determination of Specific Gravity: 50 ml specific gravity bottle was thoroughly cleaned and dried. The weight of the dried bottle (W_1) was recorded. It is then filled with deionized water and surface of the bottle was cleaned with a cotton wool and weighed as (W_2). The bottle was empty and cleaned twice with 10ml of the must. Thereafter, the bottle was filled to the brim with the "must" and the bottle cleaned with cotton wool and weighed as (W_3). The specific gravity (S.G) was calculated as follows:

$$S.G = S/W$$

Where,

$S = (W_3-W_1)$; $W = (W_2-W_1)$

Other values that were estimated include fermentative capacity (VC), AFD (Apparent Fermentation Degree and, Fermentation velocity (FV) [16].

2.5.4 Estimation of Titrable Acidity: Around 200ml of distilled water was poured to a sterile 500ml conical flask and then boiled. 1ml of 1% aqueous alcoholic phenolphthalein indicator solution was added drop by drop. It was then titrated with 0.1M NaOH solution to obtain a faint pink colour. Now, 5ml of the "must" was pipetted and poured to the boiling neutralized solution and titrated again to the end point using the same 0.1M NaOH solution [17]. The titrable acidity was expressed as tartaric acid and was calculated thus:

$$\text{Tartaric acid (g/100 ml)} = (V \times M \times 75 \times 100)/ 100 \times V_m$$

Where,

V= Volume of NaOH (final –initial reading); M= Molarity of NaOH; V_m= Volume of must.

2.5.5 Total soluble solids (TSS): 50mL of the sample was poured into a measuring cylinder at 20˚C; and a brix thermo-hydrometer was dipped into it. It was expressed as percent acidity [18].

2.5.6 Vitamin C content: Samples were determined for their Vitamin C content as per [19].

2.5.7 Organoleptic assay: Sensory and non sensory attributes of wine such as (taste, aroma, flavor, overall acceptance, appearance, color, relative sweetness, alcohol content, effervescence, acidity/alkalinity) were evaluated using a 9-point Hedonic scale by 20 panelists (10 men and 10 women, aged 20–35 years) selected from staff and faculty members of OSGU, Hisar, who were familiar with wine consumption. Samples were served in clean transparent glasses. Questionnaires and water for mouth rinsing between each tasting were provided. The panelists were asked to read through the questionnaires and the meaning of each attribute (taste, aroma, flavor, color/appearance and after taste) was

explained to the panelists to avoid any mis-interpretation [20]. The wine produced was presented to the selected panel for sensory analysis. Another set of wine sample was evaluated as a second replication the following day. The sensory evaluation data were presented as the means of the panelist's score. A standard test was used to test the statistical significance of the difference observed between scores of the two sets.

2.6 Statistical method: All analysis were performed using statistical software SPSS (SPSS Software for Windows release 17.0 SPSS Inc., Chicago, IL, USA).

3. Result and Discussion

Generally, wine is prepared from the fresh ripened fruits. The composition of must and wine of bael fruit is presented in Table 1.

3.1 pH Determination: Throughout the period of fermentation, pH of the must was within the acidic range. This was irrespective of the yeast strain used for fermentation. A decrease in pH (4.1) of the mixed fruit wine was found in the present study, which was probably due to the accumulation of organic acids that result in reduction of spoilage by harmful pathogens.

Table 1: Comparison of biochemical properties of Bael fruit, Date fruit and mixed fruit wine

Parameters	Mean value ± Standard deviation (n=10)		
	Bael wine	Date wine	Mixed fruit wine
pH	4.6±0.02	4.8±0.01	4.1±0.01
Reducing Sugars (mg/ml)	0.45±0.00	0.99±0.03	0.44±0.00
Specific gravity (kgm^{-3})	1.11±0.01	1.055±0.00	1.05±0.01
Titrable Acidity (%)	0.16±0.04	0.562±0.02	0.662±0.00
Total Soluble Solids (TSS) (^{0} Brix)	6.90±0.00	12 ±0.2	4.3±0.00
Vitamin C content (µg/g)	80.00 ±0.01	55±0.5	49±0.02

3.2 Determination of Reducing Sugar: The reducing sugar content was found to be 0.44 mg/ml. The rate of sugar consumption was concluded to be related to the rate of yeast growth [21]. A lower amount of reducing sugar content was recorded for mixed fruit wine. This implies that less amount of reduced sugar in the samples is proportional to the alcohol produced by the yeast. At the end of the 21st day, of the fermentation, the concentration of alcohol in the mixed fruit wine was observed to be 7.9% v/v.

3.3 Determination of Specific Gravity: In this case of the mixed fruit wine steady decline in specific gravity were observed throughout the period of fermentation. This decrease was found to be irrespective of the yeast strain and the fruits used in the wine production. Between 11th to 21st days of the fermentation, specific gravity was observed to be 1.05 kgm^{-3}. A correlation was found between fermentative capacity (VC) and AFD (Apparent Fermentation Degree) of the wine. Both parameters measure the quality and rate of sugar utilization, respectively. The VC of the samples was found out to be 13 and AFD was found to be 4.5. High values indicate good quality of sugar utilization. Fermentation velocity (FV) tends to measure the percentage or rate of sugar conversion to alcohol. In present study FV was found to be 0.679 that exhibited significant delayed fermentation, which encourages volatile acidity accumulation during fermentation.

3.4 Titrable Acidity: This was observed to show a gradual rise with time throughout the period of fermentation. At the 21st day of fermentation, acid concentration in the fruit wine was observe to increase from the initial concentration ranges 0.662%. This might be due to the increase in content of organic acids with the progression of fermentation process that enhances the acidic properties of the wine.

3.5 Total soluble solids (TSS): TSS decreased from 18˚ Brix in must to 4.3˚ Brix in wine. The decrease in total sugar content and TSS from must to wine was indicative of the consumption of the sugar sources by the wine yeast to produce ethanol. The wine was found to be moderately alcoholic (7.9% v/v).

3.6 Vitamin C content: The high-level of ascorbic acid in the wine would be beneficial to the body since ascorbic acid has the potential of carrying out stimulation of certain enzymes, collagen biosynthesis, hormonal activation, antioxidant, detoxification of histamine, phagocytic functions of leukocytes, the formation of nitrosamine, and proline hydroxylation among others [22]. In the present study level of vitamin C was raised to 49 µg/g during fermentation process indicating health benefits of present mixed fruit wine.

3.7 Organoleptic Assay: The data in table 2 revealed that on the basis of the mean score mixed fruit wine is found to be overall acceptable with moderate aroma, taste and flavor. Non sensory attributes such as color, alcohol content, alkalinity, relative sweetness and effervescence were also found adequate as per the general wine standards.

The biochemical results so obtained are useful to compare the quality of mixed fruit wine with that of other wines. The biochemical composition of the mixed fruit wine corroborated with the biochemical parameters of other tropical fruit wines [23, 24, 25, 26, 27, 28, 29].

Table 2: Organoleptic analysis of mixed fruit wine

Sensory Evaluation		Non- Sensory Evaluation	
Attributes	Mixed fruit wine	Attributes	Mixed fruit wine
Aroma	5.5	Colour	7.7 (Dark red)
Taste	8.5	Relative Sweetness	8.0 (Sweet)
Flavor	7.02	Alcohol content	7 (Natural)
Overall Acceptance	8.6	Effervescence	6.5 (Still)
Appearance	8.77	Acidity/Alkalinity	8.3 (Acidic)

n =10; Values are means of the panelist scores; 1=Dislike extremely; 2= dislike strongly; 3 =dislike moderately; 4 =dislike slightly, 5= neither like or dislike; 6= like slightly; 7 =like moderately; 8=like strongly; 9=like extremely

4. Conclusion

In the present study, mixed fruit wine of bael and date fruit was prepared and its bio-chemical and sensory attributes were evaluated. It was found to be acceptable among the consumers. Both Bael and date fruits are seasonally available in India in sufficient quantities. Variety of health promoting chemical constituents present in both the fruits further enhances the therapeutic value of mixed fruit wine. There is an abundant scope to bioprocess both the fruits (rich in bioactive compounds and nutritional attributes) into value-

added products such as wine to preserve these fruits nutrients, aroma and taste to be available round the year.

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