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Augmentation of chemical and organoleptic properties in *Syzygium cumini* wine by incorporation of grape seeds during vinification

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ABSTRACT

The role of grape seed tannins on improving organoleptic properties and its involvement in color stabilization in red wine are well established. The addition of grape seeds as the source of condensed tannins in fruit wine may provide a solution for its color instability and improvement of sensory attributes. *Syzygium cumini* is traditionally known for its therapeutic properties. In the current study, the influence of yeasts and grape seed addition during fermentation on the chromatic, phenolic and sensory attributes of the wine was accessed. Grape seed addition improved the color characteristics of wine and increased overall phenolic composition. Analysis by HPLC revealed 6 major anthocyanins, among which 3, 5-diglucoside form of delphidin and petunidin was found to be the major components. Cluster and PLSR analysis explained the impact of seed addition on the yeasts, as well as on the perception of panelists, with bitterness and astringency as the dominating attributes.

1. Introduction

Jamun or *jambolan* (*Syzygium cumini* L) is a seasonal perishable berry belonging to the family Myrtaceae, and it is an underutilized tropical fruit which grows widely in different climatic conditions. The fruit has delicate astringent taste and is known for curative and therapeutic (stomachic, carminative, diuretic and digestive) properties (Vijayanand, Jagan Mohan Rao, & Narasimham, 2001). Since the *jamun* fruits are highly perishable and cannot be transported over a long distance, processing of fruits into various non-alcoholic and alcoholic beverages has helped in the preservation of fruit.

Fruit wines are gaining importance because of differences in taste and nutritive values and attempts are being made to improve the quality of fruit wines using various methods (Jagtap & Bapat, 2015; Rai, Prakash, & Anu-Appaiah, 2010). A noteworthy amount of work on wine making from minor fruit such as *jamun* is in progress because of their therapeutic values (Chowdhury & Ray, 2007; Joshi, Sharma, Girdher, & Abrol, 2012). Although there is extensive work on the preparation of wines from various fruits, very few studies have been focused on improvement of organoleptic properties and color stability. *Jamun* fruits and wines are known to be astringent, but there is a need to enhance the organoleptic properties by balancing it with bitterness (Venugopal & Anu-Appaiah, 2017). The evidence has shown that the use of non-*Saccharomyces*/indigenous yeasts impart distinct regional characters, and its effect on the overall perception of *jamun* wine needs to be evaluated (Jolly, Augustyn, & Pretorius, 2006). Apart from this, another important aspect in fruit wines is its long term storage with respect to color stability, where they tend to loose color over a period of time.

Phenolics and their interaction with various cofactors play a significant role in improving organoleptic properties and are also involved in long-term color stability (Bimpilas, Panagopoulou, Tsimogiannis, & Oreopoulou, 2016; Moreno-Arribas, Polo, & biochemistry. Springer: NY, 2009). Unlike grapes, *jamun* anthocyanins are present majorly as di-glucosides (Jampani, Naik, & Raghavarao, 2014) and its interaction with cofactors are barely studied. The addition of cofactors such as proanthocyanidins (PAs) may enhance the organoleptic properties, as well as improve color stability. Grape seeds are one of the major sources for PAs, which are known to contain these phenolics with a lower mean degree of polymerization (mDP) (Vidal et al., 2010). PAs derived from grape are well studied for its contribution towards astringency and bitterness in wines along with their role in color stability by forming polymeric pigments (He, Pan, Shi, & Duan, 2008; Oliveira et al., 2014).

Various attempts have been made in order to improve the quality of the *jamun* wine by thermo-vinification process, by varying the dilution of the pulp to get a more stable wine and value addition of *jamun* to form red wine (Chowdhury & Ray, 2007; Holegar, Suresha, Jagadeesh, & Kalagudi, 2015; Joshi et al., 2012). Our earlier studies indicated a negative impact on the overall acceptance of wine by the incorporation of *jamun* seeds during vinification. The impact was mainly due to increase in astringency, bitterness along with its negative effect on color stability (Venugopal & Anu-Appaiah, 2017). Hence, a novel approach of incorporating grape seeds during the *jamun* wine fermentation was made in order to balance astringency and

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enhance the organoleptic attributes of the wine. This we hope would bring in a stabilized *jamun* wine with acceptable sensory attributes and bring down the short comings of the product.

2. Material and methods

2.1. Chemicals

All the chemicals used were of analytical reagent grade. Yeast extract peptone dextrose agar (YEPDA) was procured from Hi-Media (Mumbai, India). HPLC grade acetonitrile and formic acid were purchased from Merck (USA).

2.2. Wine preparation

2.2.1. Inoculum preparation

Saccharomyces cerevisiae (AAV2) (KF551990) isolated from fruit sample and *Pichia gummiguttae* (Strain C) (MCC 1273) isolated from fermented *Garcinia* juice were used in the present study. Both the yeasts used in the study were maintained in YEPDA slants. The inoculum was prepared as described by Rai and Anu-Appaiah (2014). Briefly, the mother culture of the yeasts was inoculated in YEPD broth and incubated for 24 h at 25 ± 1 °C. Following the incubation period, respective yeast cells were harvested, washed and suspended in sterile physiological saline.

2.2.2. Must preparation

Jamun fruits were collected from local vendors in Mysuru district, Karnataka, India. Before pulping, fruits were de-stemmed and washed in running tap water. In order to maintain the homogeneity of the experiment, the fruits were processed together. Seeds were separated from the pulp which was adjusted to 23° brix using cane sugar. The grape seeds used for the present study were collected from Shiraz variety procured from Bagalkot district, Karnataka. Seeds were separated from the fruits manually and it was washed properly before use. The seeds added to the fermentation must were obtained from the equal amount of grape (35 g of grape seeds/kg of *jamun* pulp). Potassium metabisulphite as a source of sulfur dioxide was added at 30 mg L^{-1}, while nitrogen source was not added to the pulp.

2.2.3. Fermentation of jamun

Fermentation of *jamun* pulp was carried out in two sets of 2 kg each. Fermentation of *jamun* was carried out with and without the addition of grape seeds using two isolates (AAV2 and strain C). Grape seeds collected from Shiraz were added to the pulp before inoculation of the cultures. The starter inoculum of the yeast isolates was added to attain a final concentration of 10^6 colony forming units (CFU)/mL in the individual must. Fermentation was carried out at 20 ± 1 °C in glass containers for 21 days (Venugopal & Anu-Appaiah, 2017). The fermenting must was stirred once every day, and samples were collected every week (0, 7 and 14th day) for analysis along with final wine. After fermentation, the wine was filtered and clarified with bentonite clay for further studies. Samples were analyzed in triplicate for various physical and chemical properties.

2.3. Analytical method

The samples were analyzed for total sugars (g/100 mL), total acidity (g/L of malic acid) and pH according to the official methods (International Organisation of vine, 2016). Total soluble solids (TSS) was measured using hand held refractometer (Toshniwal, India), total SO_2 (mg/L) and primary amino nitrogen (mg of N/L) were assessed using Megazyme (Ireland) kit.

Alcohol content in the wine samples was quantitatively determined using Shimadzu (Japan) gas chromatography equipped with flame ionization detector (FID), with Zebron Wax plus capillary column containing polyethylene glycol. The temperature program followed was: 40 °C (1 min hold) to 70 °C at a rate of 5 °C min^{-1} and to 220 °C at a rate of 25 °C min^{-1} for 3 min. The carrier gas was nitrogen, with the flow rate of 1 ml min^{-1} (Nambiar, Venugopal, Shetty, & Appaiah, 2016).

2.4. Spectrophotometric parameters

Phenolic composition and chromatic characteristics of *jamun* wine samples were analyzed using Shimadzu UV/VIS spectrophotometer (UV-1800, Japan). The samples were centrifuged at 4000*g* for 10 min before taking it for further analysis. All the analyses were carried out in triplicates.

2.4.1. Browning parameters and characterization of color fractions

2.4.1.1. Browning index. The browning index of the *jamun* wines with and without grape seeds was determined according to Figueiredo-Gonzalez, Cancho-Grande, and Simal-Gandara (2013). In brief, the absorbance was measured at 420 and 440 nm. The color index was measured by comparing it with the initial must.

2.4.1.2. CIELab space. The color analysis of wine was investigated using CIELab space. The parameters that define the CIELAB space are red/green color component (a^*), yellow/blue color component (b^*), luminosity (L^*) and the Euclidean distance (ΔE^*). Chroma (c^*) defined as the color intensity is calculated by the equation $c^* = \sqrt{a^2 + b^2}$, and Hue (h^*) is calculated from the equation $h^* = Tan^{-1}\left(\frac{a^*}{b^*}\right)$ (International Organisation of vine & wine, 2016).

2.4.2. Determination of phenolic contents

2.4.2.1. Total polyphenol analysis (TPC). TPC of the wine samples was calculated spectrophotometrically, where the samples were diluted 100 times and OD was measured at 280 nm as described by Wine, Ari, Anzano, and Amora (2006).

2.4.2.2. Total flavonoid content (TF). The total flavonoids content of wines was determined by $AlCl_3$ method (Hosu, Cristea, & Cimpoiu, 2014). In brief, to 0.5 mL of wine, 0.4 mL $AlCl_3$ solution, 0.5 mL of 100 g/L CH_3COONa solution and 4 mL distilled water was added. After 15 min of incubation, the absorbance of the resultant mixture was measured at 430 nm and is expressed as catechin equivalent flavonoid content.

2.4.2.3. Total tannins (TT). TT was determined according to Figueiredo-Gonzalez et al. (2013). In brief, to 2 mL of wine samples taken in screw capped amber tubes (1:50), 6 mL of HCl (12 N) was added and incubated in a boiling water bath for 30 min. After incubation samples were cooled rapidly followed by addition of ethanol (1 mL) and were homogenized. The resulting mixture was measured at 550 nm (A 550). Wines were processed in another set similarly without the incubation step and the absorbance was measured at 550 nm (A^0 550). TT was expressed as TT = (A 550–A^0 550).

2.4.2.4. Total anthocyanin determination by pH differentiation method. The pH differential method as described by Lee, Durst, and Wrolstad (2005) was followed to assess the total anthocyanin content. One milliliter of wine samples were diluted in 4 mL of two different buffers; 0.025 M potassium chloride pH = 1.0 and 0.4 M sodium acetate pH = 4.5, respectively. Absorption was measured at 510 and 700 nm after 30 min of incubation at room temperature.

2.4.2.5. Copigmented, monomeric and polymeric anthocyanin content. To study the effects of SO_2 and acetaldehyde on the forms of anthocyanins, the monomeric, copigmented, and polymeric anthocyanin contents of wine samples were determined using the colorimetric method. According to this method, 2 mL of wine sample was incubated at