

## FERMENTED FRUIT JUICE PRODUCTION USING UNCONVENTIONAL SEASONAL FRUITS THROUGH BATCH FERMENTATION

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### ABSTRACT

The attempt was made in the present work to ferment the juice of unconventional fruits. Jamun (*Syzygium cumini* L.), pomegranate, Cocoa (*Theobroma cacao* L.) were chosen for the study. Fruits were collected from local market and farmers. Juice was extracted from the fruits and initial sugar was maintained between 13 to 26 °Brix. Fermentation was carried out using *Saccharomyces cerevisiae* at room temperature. Fixed acidity estimated in terms of tartaric acid equivalent was determined in the range of 4.2 to 6.9g/L. Radical scavenging activity of the fermented juice was between the range of 1.42 to 1.96 mmol TE/L. Metals such as Cd, Cu, Mn, Ni, Pb and Zn were estimated. Residual sugar was within 3mg/ml. Ethanol concentration was estimated in the range of 5.25 to 10.67% (v/v).

**Keywords:** Jamun, Pomegranate, *Saccharomyces cerevisiae*, Ethanol

### INTRODUCTION

Jamun (*Syzygium cumini* L.) is an evergreen tropical tree in the flowering plant family *Myrtaceae*, native to India and Indonesia. The other common names of Jamun are java plum, blackplum, jambul and Indian blackberry (Chowdhury *et al.*, 2007). India ranks second in production of Jamun in the world. The Jamun tree starts flowering in March-April. This is followed by fruiting (a berry) which appears in May- June. The berry is oblong, ovoid and shining crimson black (rich in anthocyanin pigment, an anti-oxidant) when fully ripe. The fruits are a good source of iron and are used as an effective medicine against diabetes, problems related to heart, bleeding piles and liver trouble (Satkar *et al.*, 2016). The fruit and its juice and the seed contain a biochemical called 'jamboline' which is believed to check the pathological conversion of starch into sugar in case of increased production of glucose (Chowdhury *et al.*, 2007). Ripe Jamun contain approximately 83 % water with almost solids 14 % containing a mixture of fermentable sugar. The pulp of Jamun contains appreciable amounts of fermentable sugar, which can be used for alcoholic fermentation. In India alone wastage of Jamun is 0.5 MT due to rainfall, high temperature, humidity fluctuations, improper handling, inadequate storage facilities, inconvenient transport and microbial infections (Jagtap *et al.*, 2015). The food industry uses a variety of preservation and processing methods to extend the shelf life of fruits such that they can be consumed year round, and transported safely to consumers all over the world, not only those living near the growing region (Barret and Lloyd, 2011).

The cultivation of pomegranate (Reddy *et al.*, 2007) is mainly extended to the Mediterranean basin, and in regions of Southern Asia, India, North and South America, where the high temperature allows the optimal fruit ripening. The pomegranate fruit has been used in folk medicine from ancient times as antimicrobial and as natural astringent for the treatment of diarrhea and harmful internal parasites. Nowadays, the research interest on pomegranate fruit is increased as a consequence of reports establishing its benefits on human health (Faria and Calhau, 2011). Ripe fruits are traditionally consumed fresh, also as topping in yogurt and salads, dried, as spice or acidic agent for the manufacture of chutney and curry, or processed. Pomegranate juice is consumed throughout the world because of its pleasant and unique aroma, flavour and color (Ferrara *et al.*, 2011). Besides sensory properties, pomegranate shows interesting nutritional and health-promoting features (Viuda-Martos *et al.*, 2010). In particular, the antioxidant properties of the fruit (Seeram *et al.*, 2005), which contains anti-carcinogenic (Bell and Hawthorne, 2008), antimicrobial (Reddy *et al.*, 2007), antiviral (Kotwal, 2008), anti-inflammatory (Giménez-Bastida *et al.*, 2012). In

general, fruit wines are processed in the same way as wine made from grapes and significant compositional changes take part during winemaking (Ginjom, D'Arcy, Caffin & Gidley, 2011; Heinonen, Lehtonen, & Hopia, 1998). Both fermentations and ageing entail the transformation of native substances into secondary metabolites able to have an impact on the quality of the final product (Duarte *et al.*, 2010; Ginjom, D'Arcy, Caffin, & Gidley, 2011; Yae *et al.*, 2007). Furthermore, cultivars can also affect wine composition and not all may be useful for optimal fermentation purposes (Towantakanit, Park, & Gorinstein, 2011). Thus, taking into account the great diversity existing among the sensorial and phytochemical properties of pomegranate cultivars (Mena, Garcia-Viguera *et al.*, 2011), differences in the final quality of pomegranate varietal wines should be expected. Despite these considerations and the fact that some work on pomegranate fermented juices has already been performed (Yae *et al.*, 2007; Zhuang *et al.*, 2011), there is no substantial information on pomegranate wine composition, covering all stages of the pomegranate winemaking process.

Cocoa (*Theobroma cacao* L.) is world-wide known for its beans used in the manufacture of chocolate. For a long time the production and commercialisation of cocoa has been the basis for the economy of some Brazilian states, mainly Bahia. The cocoa pulp is a substrate rich in nutrients, which can be used in industrial processes for by-product manufacture (Schwan & Wheals, 2004). Cocoa can be readily fermented by yeasts such as *Saccharomyces cerevisiae*, producing an alcoholic beverage. *Saccharomyces cerevisiae* has been used in fermentative processes for thousands of years, according to the first historical stories of the production of beer and wine (Rose, 1977; Demain, 2000; Ostergaard *et al.*, 2000). Because of commercial importance of this microorganism, strains with good fermentative characteristics have been selected and commercialised in dehydrated form and/or lyophilised to be employed in breweries, wineries and other industries (Fleet & Heard, 1993; Colagrande *et al.*, 1994; Cappello *et al.*, 2004). A range of environmental factors influences the production of metabolites and survival of yeasts during industrial fermentations. The main factors are temperature, pH, sugars concentration and acidity of fruit juice (substrate). In case of yeasts, temperature and tolerance of ethanol have an important influence on their performance (Heard & Fleet, 1988). Winemaking is a good example of the biotechnological evolution in the beverage production, passing from art to science-based technology.

Traditionally, cacao seed fermentation is a noncontrolled process initiated by microorganisms naturally occurring at fermentation sites, including yeasts, lactic and acetic bacteria, Bacilli and filamentous fungi. The diversity of yeasts associated with cacao seed fermentation is heterogenous, varying in terms of

location, producing country, climate conditions and fermentation method and duration.

In view of these many medicinal and therapeutic properties of Jamun, Pomegranate, cocoa and because of its short availability period, an attempt has been made in this study to preserve the unconventional seasonal fruit by fermenting its juice through batch fermentation using *Saccharomyces cerevisiae*.

## MATERIAL AND METHODS

### Mash preparation

#### Jamun

Jamun was collected from local city market of Mangaluru situated in Dakshina Kannada district, Karnataka State, India. Fresh jamun fruits collected were washed and peeled, extracting seeds. Extracted seeds were kept for drying in hot air oven at 71°C for 1-2 hours. The pulp of the fruit along with its skin was crushed by mortar and pestle and kept in freezer to avoid contamination. The dried seeds were also crushed by mortar and pestle. The pulp, seeds and the skin were pasteurized at 70-80°C for 15 minutes along with 300mL of water in a flask. The boiled mash was plugged with cotton and allowed to cool.

#### Pomegranate

Bhagwa variety of pomegranate was collected from Hosadurga taluk situated in Karnataka state, India. The peels of pomegranate were removed and the seeds were excised from the pulp. The pulp was macerated using mortar and pestle. The initial sugar concentration in the mash was measured using the hydrometer and found to be 26 °Brix and the pH was adjusted suitable for the growth of yeast.

#### Cocoa

The cocoa was collected from plantation situated in Peruvai village of Dakshina Kannada district, Karnataka State, India. The cocoa seeds were cut into small pieces and ground to uniform slurry by pestle mortar. The slurry was further diluted with distilled water to reduce the turbidity (Kocher et al., 2011). The pH and the sugar content are adjusted before fermentation. The crushed mixture was pasteurized in a conical flask at 70-80° C for 15 minutes along with 400mL of water.

### Inoculum development and fermentation

The standardization of inoculum size is important as sugar consumption is balanced between biomass development and ethanol production (Kocher, 2011). Inoculation of the yeast culture into the mash depends on the time at which the yeast enters log phase which is determined in the growth kinetics study. Initial sugar concentration was estimated by hydrometer and pH by pH meter. Yeast from master culture was inoculated into MGYD liquid media from slant culture. 10 ml of MGYD media having culture yeast *Saccharomyces cerevisiae* was pitched into the flask containing mash after 7 hours. The cotton plugged flask was kept in incubator shaker at a temperature of 31°C, 100 rpm for a span of 3 to 4 days for batch fermentation. Once the concentration of sugar decreased to less than 1g/litre, the fermentation was stopped. The fermented sample was clarified by centrifugation at 7000 rpm for 15 minutes (Kocher, 2011). The clarified sample was taken for analysis of parameters like pH, acidity, residual sugar, ethanol and metal concentration.

### Analysis

#### pH

The initial pH of the sample is checked and adjusted to 3.5. The pH is also determined after fermentation by pH meter.

#### Soluble solid (sugar)

Hydrometer was used for the determination of initial sugar. The concentration was expressed in terms of °Brix. To bring the sugar concentration to required value chaptalization was carried out (Reddy et al., 2009; Kocher et al., 2011).

#### Titrateable Acids

Titrateable acidity (TA) was determined by titration of a strong base i.e 0.5N NaOH against 25 ml of sample to an end point of pH 8.2 using potentiometric titration (Jacobson, 2006).

### Volatile acid

Fermented fruit juice was distilled at 118 °C in order to determine the amount of Volatile acid (VA). 5 ml of distillate was titrated against 0.5N NaOH using phenolphthalein as indicator to determine volatile acid content (Moura et al., 2010).

### Determination of antioxidant activity

Fermented fruit juice was investigated for antioxidant activity by the 2, 2-diphenylpicrylhydrazyl (DPPH) method (Seruga et al., 2011). 120µL of methanolic DPPH solution (1 mmol L) was mixed with 50 µL of fermented fruit juice sample and 1880µL of methanol. Incubation of the mixture was carried out in dark at room temperature for 15 min and at 517 nm the absorbance of this mixture ( $A_{\text{Sample}}$ ) was measured against the blank sample (50µL of fermented fruit juice, 2000µL of methanol). The blank DPPH solution was freshly prepared (120µL of 1 mmol L DPPH, 1930 µL of methanol) and absorbance at 517 nm ( $A_{\text{DPPH}}$ ) was measured. The calibration curve for Trolox, constructed by linear regression of absorbance value ( $A_{\text{Trolox}}$ ) vs. Trolox concentration, was used to calculate the antioxidant activity of wine sample and to express their anti oxidant value in mmol of Trolox equivalents (mmol TE/L). Trolox standards with final concentration 0 – 2550 µmol/L in methanol were assayed under the same conditions as those used for the samples; i.e. 50µL of Trolox was mixed with 120µL of methanolic 1 mmol/L DPPH solution and 1880 µL of methanol. After 15 min, the absorbance at 517 nm ( $A_{\text{Trolox}}$ ) against the prepared blank sample was measured. (Seruga et al., 2011)

### Metal analysis by AAS

Metal analysis was carried out by Atomic absorption Spectroscopy (GBC Avanta) after carrying out acid digestion of the sample. 3 ml of the sample was taken in 250 ml digestion flask, mixed with freshly prepared 3 ml of nitric acid and 3 ml of Hydrochloric acid, incubated at 60°C. Heating was continued until the solution becomes clear and colourless (Woldemariam et al., 2011). The cooled digest was made up to 100 ml, by adding deionised ultra-pure water, stored in refrigerator for further analysis (Nikolakaki et al., 2002). Standard metal solution of five different concentrations was prepared for calibration.

### Residual sugar estimation

The concentration of residual sugars was estimated by the using the UV Vis spectrophotometer at 540 nm with 3, 5- DNSA reagent (Miller, 1959).

### Quantitative estimation of ethanol

Ethanol was determined using potassium dichromate method (Fletcher et al., 2003). 25 ml of centrifuged sample was distilled at 80°C. The distillate was used for ethanol estimation.

## RESULTS AND DISCUSSION

### Acidity

#### Volatile acidity (VA)

Amount of acetic acid present in fermented fruit juice is expressed as VA. The result reveals the presence of acetic acid in the fermented fruit juice. In the current investigation the volatile acidity estimated was 0.6 g/L, 0.1g/L and 0.3g/L of acetic acid, respectively for jamun, pomegranate and cocoa fermented fruit juice. VA of pomegranate wine was reported between 0.26 to 0.36 g/L acetic acid (Mena et al., 2012)

#### Titrateable acidity (TA)

$$TA \text{ (g/L)} = 75 \times N \times (T/S)$$

(1)

Where N is the normality of NaOH, T is the titer volume (in ml), S is the sample volume (in ml), and 75 is a constant. Moura et al., (2010) reported TA of 7.05 to 9.83 g/L tartaric acid. Vahl et al., (2013) reported TA in the range of 4.80 to 6.24 g/L tartaric acid.

Current investigated revealed the presence of higher TA of 7.5 g/L in jamun fermented juice compared to 6.70 to 6.80g/L reported by Arekar and Lee, (2015).

#### Fixed acidity (FA)

$$\text{Fixed acidity} = \text{Titrateable acidity} - \text{Volatile acidity}$$

(2)

As per the Organisation of Vine and Wine (OIV) norms FA should not be less than 5g/L.

From the current investigation Jamun, cocoa fermented fruit juice meets the criteria.

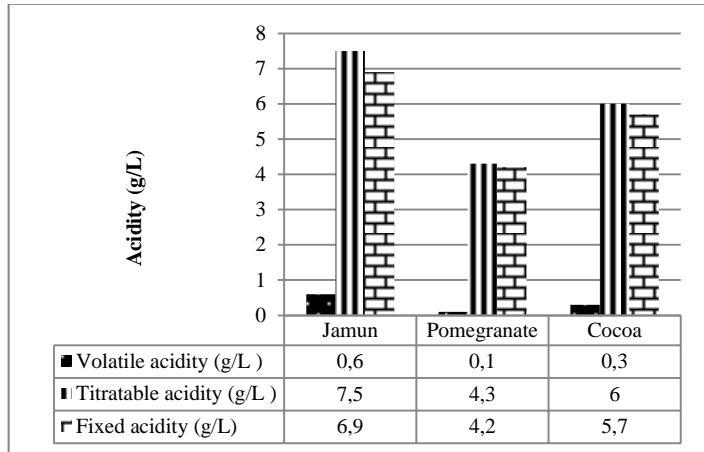


Figure 1 Acidity of fermented fruit juices

**Antioxidant assay**

Cocoa fermented fruit juice exhibited strong antioxidant activity of 1.96 mmol TE/L. Least antioxidant activity revealed by Jamun. Radical scavenging activity of 80.56% to 81.90% was reported by Arekar C. and S.S. Lee, (2015) in jamun microvinification.

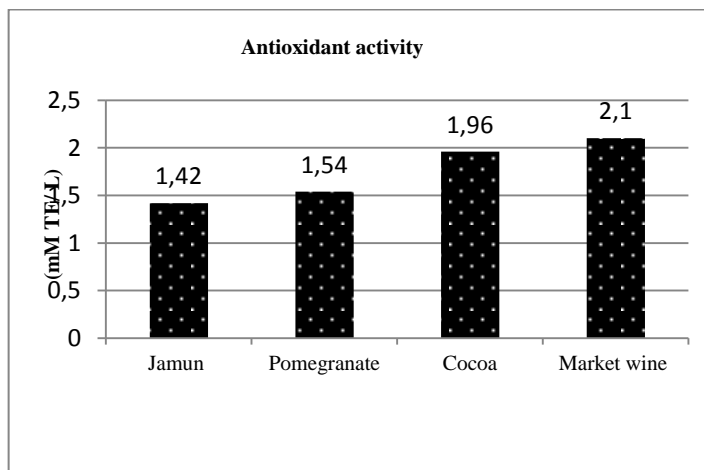


Figure 2 Radical scavenging activity of fermented fruit juice

**Metals**

Most metals are important for efficient alcoholic fermentation (Pohl 2007). Cu, Fe and Mn are responsible for changes in stability and modification of the sensory quality of concoction after bottling. Fermented fruit juice of jamun contains metals like Zn and Cd with concentration of 0.067ppm and 1.563 ppm respectively. Several other metals like Cu, Mn, Pb and Ni showed negligible concentration. Even though pomegranate fermented juice has the metals such as Cd, Cu, Mn, Ni, Pb, Zn. Only Cd is beyond the accepted range of OIV standards. Cocoa fermented fruit juice consist of Cd, Cu, Pb, Zn. Cd exceeds the acceptable range of OIV.

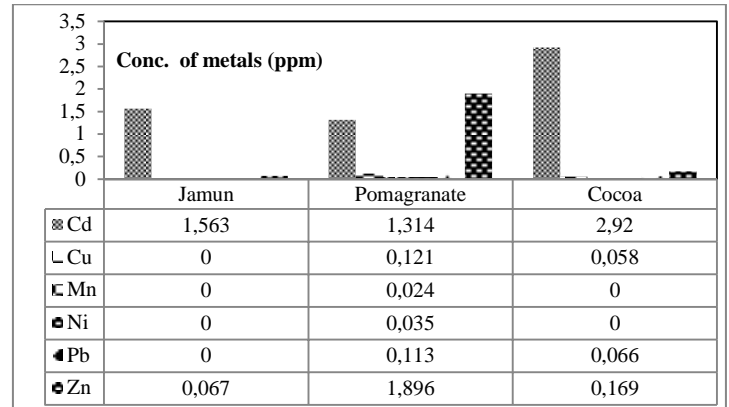


Figure 3 Concentration of metals in fermented fruit juice of Jamun, Pomegranate and Cocoa

**Residual sugar**

Amount of residual sugar present in all the fruit juice sample is within 3 mg/mL.

**Ethanol**

Ethanol concentration of various fermented fruit juices is given in Fig.4. Ethanol concentration in the range of 9.81 to 10.30 % was reported by Arekar and Lee, (2015) in jamun fermented juice. Current investigation reported 10.67% ethanol compare to rest of the fermented juice and also reported by literature. Higher ethanol concentration in pomegranate wine of 10.91 % (v/v) reported by Berenguer et al., (2015).

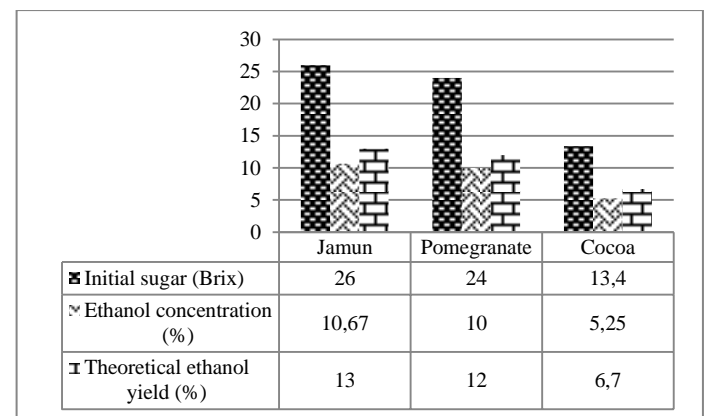


Figure 4 Theoretical and experimental ethanol concentration determined in fermented fruit juice

**CONCLUSION**

According to the study carried out it was found that the jamun pulp and seeds mash could be readily fermented by *Saccharomyces cerevisiae* thereby producing a promising alcoholic beverage. Residual sugar after fermentation was 2.1593 mg/mL. Fixed acidity was reported as 6.90 g/L which was greater than 5.5g/L therefore we could conclude that there was no contamination. The sample had an ethanol content of 10.67%.

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