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Preparation and evaluation of antioxidant capacity of Jackfruit (*Artocarpus heterophyllus* Lam.) wine and its protective role against radiation induced DNA damage

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ABSTRACT

Jackfruit is an underutilized edible fruit in the tropics and subtropics. The aim of this study was to produce wine from jackfruit pulp and to evaluate the total phenolic and flavonoid contents and antioxidant properties of the wine. The ability of scavenging free radicals was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant assay (FRAP), N, N-dimethyl-p-phenylendiamine (DMPD) and nitric oxide (NO) scavenging assays. Experimental results indicated that jackfruit wine was effective in DPPH radical scavenging (69.44 ± 0.34%), FRAP (0.358 optical density value, O.D.), DMPD (78.45 ± 0.05%) and NO (62.46 ± 0.45%) capacity. By the analysis of the high performance liquid chromatography coupled to diode array detector (HPLC-DAD), two phenolic compounds namely gallic acid and protocatechuic acid were identified. The jackfruit wine was also able to protect H_2O_2 + UV radiation and γ-radiation (100 Gy) induced DNA damage in *pBR322* plasmid DNA. The antioxidant and DNA damage protecting properties of jackfruit wine confirmed health benefits when consumed and could become a valuable source of antioxidant rich neutraceuticals. Additionally, the wine could be a commercially valuable by-product for the jackfruit growers.

1. Introduction

Fruits are a supplementary diet having dietary sources of various antioxidant phyto-compounds. The fruits have gained increasing interest among nutrition specialists, food scientists and consumers since frequent consumption of fruits contributed to reduce the risks of certain cardiovascular diseases and cancers (Liu, 2003). In the recent years, demand for fresh and processed fruits has been substantial and this trend is likely to continue in future. The current research focus is to assess the potential of under-utilized tropical fruit species which have been explored by the food industry in order to meet the growing needs of the ever increasing consumer market for several by-products including wine.

The jackfruit (*Artocarpus heterophyllus* Lam., Family – Moraceae) tree is a wild plant bearing the largest known edible fruit (up to 35 kg) and distributed throughout the tropics and subtropics. Young fruits and seeds are used as vegetables. The pulp of ripe fruit is eaten fresh and also used in fruit salads for its high nutritive value. Every 100 g of ripe jackfruit pulp contains carbohydrate 18.9 g, protein 1.9 g, fat 0.1 g, moisture 77%, fiber 1.1 g, total mineral matter 0.8 g, calcium 20 mg, phosphorus 30 mg, iron 500 mg, vitamin-A 540 I.U., thiamin 30 mg and caloric value 84 calories. Ripe fruits are canned in syrup. The pulp is processed, dehydrated and sold as dried pulp, juice, biscuits, chutney, jam, jelly, toffee and pastes. The jackfruit is a rich source of phenolics and flavonoids having good antioxidant properties (Jagtap et al., 2010; Soong and Barlow, 2004). The plant is commonly referred as 'poor man's food' for its availability, low cost and abundance during the season. The versatile tree has served the needs of rural communities considerably by providing food, nutrition and many other traditional medicines to the people of the South-East Asia, Indonesia, Western part of Java and India (Jagtap and Bapat, 2010). However, following maturity and harvest, large quantities of ripe jackfruits rapidly deteriorate and are usually wasted as a result of poor handling and inade-

Abbreviations: DMPD, N, N-dimethyl-p-phenylendiamine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant assay; HPLC, high pressure liquid chromatography; JFW, jackfruit wine; NO, nitric oxide; RSC, radical scavenging capacity; SNP, sodium nitroprusside.

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quate storage facilities. Thus, it becomes essential to develop new methodologies for processing ripe jackfruits and tropical fruits, and search for new applications to minimize post harvest and production losses to generate more profits and promote the sustainable use of biomes (Duarte et al., 2010). Winemaking is one of the most ancient technologies and the most commercially prosperous biotechnological product (Moreno-Arribas and Polo, 2005). Tropical fruits have been used as substrates for the production of wines (Okunowo et al., 2005). Therefore, use of this technique for fermenting ripe fruits or its juice is considered to be an attractive means of utilizing surplus and over-ripe jackfruit. Using this approach, several studies have been carried out on the preparation of fruit wines namely kiwi (Soufleros et al., 2001), cajà (Dias et al., 2003), banana (Akubor et al., 2003) mango (Reddy and Reddy, 2005), cocoa (Dias et al., 2007) orange (Selli et al., 2008), gabiroba (Duarte et al., 2009) and pineapple (Pino and Queris, 2010).

Previously, physiochemical characteristics and sensory evaluation of fermented jackfruit have been studied (Asquieri et al., 2008); however, not much information is available in the literature concerning the production of beverages using ripe jackfruit. Therefore, the objective of the present work was to produce and evaluate the quality of wine prepared from jackfruit juice for the development of functional beverages with health beneficial properties comprising a systemic study on the neutraceuticals, phytochemicals and bioactivity of the jackfruit wine. This would enhance commercialization and utilization of the fruit. In the present investigation, total phenolic and flavonoid contents, antioxidant and DNA damage protecting activity of jackfruit wine have been evaluated.

2. Materials and methods

2.1. Chemicals and reagents

All the reagents and solvents used during experiment were of analytical grades and the highest purity purchased from various commercial suppliers. DPPH (2,2-diphenyl-1-picrylhydrazyl) and gallic acid from Sigma (St. Louis, MO, USA); protocatechuic acid from BDH (Poole, England); HPLC grade methanol from Merck (India); 2,4,6-tripyridyl-s-triazine (TPTZ), N, N-dimethyl-p-phenylendiamine (DMPD), sodium nitroprusside (SNP), pectinase from Himedia, Mumbai, India and sulphanilamide and N-(1-naphtyl)ethylenediamine dihydrochloride were from S.D. Fine Chemicals Ltd., Mumbai, India. $CsCl_2$ purified plasmid DNA of *pBR322* was procured from Bangalore Genei Pvt. Ltd., Bangalore, India. The remaining chemicals not listed above were from Himedia, Mumbai, India and S.D. Fine Chemicals Ltd., Mumbai, India.

2.2. Plant material

The fully ripened fruits of *A. heterophyllus* were collected between April and June from Radhanagari locality of Western Ghats, India. The fruits were cleaned and separated into pulp and seeds. The yellow colored pulp having sweet taste was stored in polystyrene bags in deep-freezer (−20 °C) until further analysis.

2.3. Must preparation

For the preparation of fermenting must, jackfruit pulp was defrosted at room temperature, cut into small pieces and ground to uniform slurry using the mechanical grinder. The pulp slurry was further diluted with distilled water to reduce turbidity which has pH of 5.5 and sugar content of 11°Brix. The viscous pulp slurry was further clarified using pectinolytic enzyme (Pectinase). Potassium metabisulphate was added (200 mg L^{-1}) as a source of sulphur dioxide to inhibit the bacterial growth (Dias et al., 2007; Duarte et al., 2009). The pulp slurry was then pasteurized (80 °C, 15 min) and stored at 10 ± 2 °C until required.

Table 1
General composition of jackfruit juice and wine.

Juice composition	
Soluble solids (°Brix)	11.0 ± 0.00
pH	5.53 ± 0.04
Wine composition	
Alcohol (% v/v)	4.33 ± 0.12
pH	4.37 ± 0.03
Total phenolic compounds (mg GAE mL^{-1})	0.053 ± 0.00
Total flavonoids compounds (mg Rutin equivalent mL^{-1})	0.016 ± 0.00

Data shown as mean ± SD, $n = 3$.

2.4. Preparation of Yeast culture

The Yeast strain *Saccharomyces cerevisiae* (NCIM 3282) procured from National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune, India was maintained on a medium containing glucose (5.0 g L^{-1}), peptone (5.0 g L^{-1}), yeast extract (3.0 g L^{-1}) solidified with agar (2.0%). Inoculum of the selected strain was prepared by inoculating a single colony of *S. cerevisiae* into 50 mL jackfruit pulp slurry and incubated in darkness on a gyratory shaker with 100 rpm for 48 h at 28 ± 2 °C.

2.5. Wine preparation

The fermentation process was initiated by transferring the 48 h grown yeast inoculum to a conical flask (1 L) containing jackfruit pulp slurry and the flask was incubated at 28 ± 2 °C in darkness for 12 days. At the end of fermentation, the broth was centrifuged at 5000 rpm for 20 min to separate out the yeast cells, and then the flask containing wine was stored at 8 °C to aid the sedimentation of solid material. After 10 days, the wine was transferred to another flask having aeration and aqueous solution of bentonite (10 g L^{-1}) was added to facilitate the sedimentation of non-fermentable solids. The mixture was homogenized and incubated at 8 °C for 48 h for the sedimentation of flocculent material. Later, the two phases of wine (liquid and solid) were separated by filtration to obtain a clear wine. The filtered wine was stored at 4 °C in airtight glass container to avoid oxygen contact. All assays were carried out in triplicate.

2.6. Standard chemical analyses of jackfruit must and wine

The standard chemical parameters of jackfruit must (°Brix, pH) and wine (pH, alcohol) were determined according to the standard methods (AOAC, 1997). The general composition of must and wine is presented in Table 1.

2.7. Determination of total phenols content

The total phenol content of jackfruit wine (JFW) was determined spectrophotometrically according to Folin-Ciocalteu method as described by Singleton and Rossi (1965). The Jackfruit wine (1000 μL) was mixed with 1800 μL Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and incubated at room temperature for 5 min followed by the addition of 1200 μL of sodium carbonate (15%, w/v). The mixture was incubated for 90 min at room temperature and absorbance was recorded at 765 nm using an UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The total phenols content of the samples was compared with the standard curve of gallic acid and expressed as milligrams of gallic acid equivalents per mL (mg GAE mL^{-1}).