Food Bioscience 5 (2014) 101–107

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/fbio

# Application of native yeast from Garcinia (*Garcinia xanthochumus*) for the preparation of fermented beverage: Changes in biochemical and antioxidant properties

*Amit Kumar Rai*[1], *K.A. Anu Appaiah*[*]

*Department of Food Microbiology, CSIR – Central Food Technological Research Institute, Mysore 570 020, Karnataka, India*

ARTICLE INFO

*Article history:*
Received 19 July 2013
Received in revised form
20 November 2013
Accepted 25 November 2013

*Keywords:*
Garcinia
Native yeast
Beverage
Fermentation
Organic acids
Antioxidant activities

ABSTRACT

Garcinia beverage was prepared by fermentation of ameliorated must of *Garcinia xanthochymus* using native yeast isolated from naturally fermented Garcinia must. The native yeast was identified as *Hanseniaspora* sp. (Accession no. U826968) based on biochemical and molecular tests. Low alcoholic Garcinia beverages were prepared at four different must concentration (5%, 10%, 20%, and 30% w/v). Fermentation using *Hansiniaspora* sp. was carried out for 28 days and the fermented must were analyzed for changes in physical, biochemical and sensory characteristics. The present study mainly focused on changes in organic acid profile during fermentation, in particular, degradation of oxalic acid and antioxidant properties of the fermented beverage. Results showed utilization of citric acid and 100% degradation of oxalic acid (antinutritional factor) with the synthesis of aspartic acid. Antioxidant activity (DPPH radical scavenging activity) was significantly higher in beverage with higher must concentration along with dose dependency. Upon sensory analysis, the beverage prepared with 5% and 10% must were the only acceptable ones.



## 1. Introduction

The biological process during alcoholic fermentation results in a series of biochemical changes by the action of various enzymes produced by microorganisms, especially yeast (Rai, Prakash, & Anu Appaiah, 2010). Until recently, *Saccharomyces cerevisiae* had been largely used as a starter culture for wine fermentation and little attention has been given to yeast, such as *Hansiniaspora* and *Schizosaccharomyces* spp (Jolly, Augustyn, & Pretorius 2006). However, some of the studies have shown that mixed-culture fermentation by certain *non-Saccharomyces* species with *S. cerevisiae* might positively contribute to the taste and flavor of wines (Albergaria, Torrao, Hogg, & Girio, 2003; Ciani & Ferraro, 1998; Romano, Suzzi, Comi, Zironi, & Maifreni, 1997). Among the non-*Saccharomyces* yeast involved in grape must fermentation, species of Genus *Hansiniaspora* have been reported in the early phase of the fermentation process and researchers have focused on evaluating the effects of apiculate yeast on the quality of the final product (Comi, Romano, Cocolin, & Fiore, 2001; Albergaria, Torrao, Hogg, & Girio, 2003). Fermentation of grape must using *Hansiniaspora* species has shown to produce an alcoholic beverage up to a maximum alcohol content of 5% (Fleet & Heard, 1993).

[*]*Corresponding author*. Tel.: +91 821 241 6538; fax.: +91 821 251 7233.
*E-mail addresses:* amitraikvs@gmail.com (A.K. Rai), anuapps@yahoo.com (K.A. Anu Appaiah).
[1]Present address: Regional Center of Institute of Bioresource and Sustainable Development (RCIBSD), DBT, Gangtok, Sikkim, India.

2212-4292/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fbio.2013.11.008

*Garcinia xanthochymus* belonging to the family *Clusiaceas*, is native to Asia, Polynesia, Australia and Africa where the fruits are used in the production of beverages, jams, preservatives and of vinegar (Bagget, Protiva, & Mazzola, 2005). Many *Garcinia* sp. have been reported as rich sources of secondary metabolites, such as flavonoids and phenolic acids, having therapeutic properties (Deachathai, Mahabusarakam, Phongpaichit, & Taylor, 2005). Guttiferane and gambogenone are the two benzophenones reported in fruits of *G. xanthochymus* to possess antioxidant activity and the ability to induce apoptosis in cancer cell line (Bagget et al., 2005). Low alcoholic beverages from this fruit are expected to possess similar therapeutic properties even after fermentation.

Quality of a fermented beverage is closely related to their microbial ecology (Fleet & Heard, 1993) where the extent to, which the individual species grow during fermentation process determine the types and concentration of the metabolites (Gil, Mateo, Jimenez, Pastor, & Huerta, 1996). Acids and esters produced during fermentation are quantitatively dominant and are important for wine aroma (Stashenko, Macku, & Takayuki, 1992), which affects the sensory properties of the wine. The objectives of the study was to select the yeast strain that can ferment Garcinia must with the maximum decrease in oxalic acid level and formulation of wine or beverage with better antioxidant and sensory characteristics.

## 2. Materials and methods

### 2.1. Inoculum and treatment of must

The yeast used for fermentation was isolated from naturally fermented Garcinia must and was maintained on potato dextrose agar (PDA). Inoculum was prepared by adding 50 μl of mother culture of the yeast into 5 ml of potato dextrose broth (PDB) and then the whole content was transferred to 100 ml of PDB, incubated at 37 °C for 18 h at 150 rpm on a orbital shaking incubator (M/s Tehnica, India). The yeast cells were harvested by centrifuging (C31Cooling centrifuge, Remi-India, India) at 3000*g* for 10 min, washed twice and re-suspended in sterile physiological saline (100 ml). The cell counts in the inoculum were in the range of $10^7$–$10^8$ cells/ml of physiological saline.

Fruits of *Garcinia xanthochymus* were collected from Kodagu district of Karnataka, India. Fermented Garcinia beverage was prepared according to method described by Rai et al. (2010). Garcinia fruits were washed with tap water followed by blanching in boiling water for 20 min. After cooling the fruits, the skin was peeled off, deseeded and the minced pulp was used for preparing beverage. *Garcinia* beverage was prepared using 10% (w/v) pulp of Garcinia, and brix value was adjusted using cane sugar between 19° and 20°. Natural fermentation was carried out for 21 days and yeasts were isolated from the naturally fermented must. Isolated yeasts were identified based on biochemical and molecular methods. In a second experiment, Garcinia beverages were prepared with four different must concentrations of fruit 5% (HAN1), 10% (HAN2), 20% (HAN3) and 30% (HAN4) with the native yeast (*Hanseniaspora*). The initial brix of all of the beverages were adjusted with cane sugar between 19° and 20°. The must was pasteurized at 75 °C for 20 min before adding the inoculum (5%, v/v). Fermentation was carried out in a glass container at 23–25 °C for 28 days.

The fermenting must was stirred once every day and samples were drawn after every 3 days till the final day, for analysis. After fermentation, the beverage was filtered, pasteurized at 75 °C for 20 min and clarified before bottling. Samples were analyzed in triplicate for various physical and chemical properties.

### 2.2. Biochemical and molecular characterization of isolated yeast

Biochemical identification of isolated yeast was carried out according to Barnett, Payne, and Yarrow (2000). For molecular identification, the 18s sRNA was amplified using the primers (Sigma-Aldrich, USA) 5′GGTCCGTGTTTCAAGACGG (MW-5860) as a forward primer and 5′GCATATCAATAAGCGGAGGAA (MW-7468) as reverse primer. Polymerase chain reaction (PCR) was performed in the Thermocycler Gene Amp PCR system 9700 (Applied Bio-system, Perkin-Elmer, Foster City, CA, USA) as per the standard protocol (Sambrook & Russell, 2001). Upon amplification, the PCR product was analyzed by agarose gel electrophoresis. The PCR product was eluted from the gel and purified by using HipurATM bacterial and yeast genomic DNA purification kit (Himedia, Mumbai) and sent for nucleotide sequencing at M/s Bangalore Genei. The nucleotide sequence was analyzed by the basic local alignment search tool (BLAST) on the National Center for Biotechnology Information (NCBI) database. The gene sequence now stands deposited with the accession number EU826968 in the Gen Bank database maintained by NCBI, USA (http://www.ncbi.nlm.nih.gov/Genbank).

### 2.3. Physical and chemical analysis

Brix value of the samples were measured using a Refractometer (Erma hand held Refractometer, Japan). pH measurements were accomplished by directly immersing the combined glass calomel electrode in to the sample using pH meter (CD Instrumental Pvt. Ltd., Bangalore, India). Protein content was estimated following the AOAC method (AOAC, 2000) and calorific value was calculated using the formula described by Rai et al. (2010).

Calorific value (Kcal) = 6.9 × 0.794 × alcohol% + (4 × reducing sugar) + (2.4 × total sugar).

Alcohol produced during fermentation was estimated as described by Capauti, Veda, & Brown (1968). Fermented beverages were also analyzed for total sugar (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), reducing sugar (Miller, 1959) and organic carbon (Walkley, 1947).

### 2.4. Sensory analysis

Sensory analyses of different Garcinia beverages were carried out to evaluate the aroma and their acceptance by a panel of trained judges using Quantitative Descriptive Analysis (QDA). QDA consisted of 15 cm scale anchoring at low (1.25 point) or detection threshold and high (13.75 point) or the saturation threshold (Stone & Sidel, 1998). A group of 15 panelists were