Biocatalysis and Agricultural Biotechnology 13 (2018) 198–203

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

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

Potential of snake fruit (*Salacca zalacca* (Gaerth.) Voss) for the development of a beverage through fermentation with the Kombucha consortium

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ARTICLE INFO

Keywords:
Snake fruit
Kombucha consortium
Antioxidant
Antibacterial
Bioactive compounds
Sensory properties

ABSTRACT

Sugared snake fruit juices were fermented for 14 days with the Kombucha consortium containing yeasts and acetic acid bacteria. Five Indonesian cultivars (*Salak Doyong, Salak Madu, Salak Pondoh, Salak Segaran* and *Salak Suwaru*) of the fruit were studied. The physicochemical and sensory properties of the fermented products revealed the snake fruit Kombucha from the *Salak Suwaru* cultivar as the most acceptable with color indices L* and b* respectively 30.5 and 13.2; total sugar, 7.54%; total soluble solids, 11.3%; total acidity, 1.65%; pH, 3.15; and sensory scores (out of 5) of 3.90 for color, 3.70 for taste and 3.80 for aroma. The fermentation enhanced antioxidant activity of the snake fruit Kombucha, as assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and consistent with increases in phenolics, tannins and flavonoids. Acetic acid was the major organic acid of the fermented product that showed an enhanced antibacterial activity by inhibiting Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Snake fruits can be used to develop functional food beverages and foods through the Kombucha fermentation.

1. Introduction

Snake fruit (*Salacca zalacca* (Gaerth.) Voss) is a tropical fruit widely found in South East Asia. It is known by various names such as *Salak* (Indonesia, Malaysia and Philippines), *Rakam* (Thailand), *Sa Laka* and *She Pi Guo Zong* (China), *Schlagenfrucht* and *Zalak* (Germany), *Sarakka Yashi* (Japan), *Salaca* (Span) and*Yingan* (Myanmar) (Mohd Zaini et al., 2013). It has a white firm pulp with a sweet, slight acidic and astringent taste. Snake fruit is a good source of vitamins, minerals, dietary fiber, and bioactive compounds with antioxidant activities (Aralas et al., 2009; Suica-Bunghez et al., 2016). There are many snake fruit cultivars or genotypes worldwide, and each cultivar has unique physical, chemical and sensory characteristics. The ripe fruit is mostly eaten fresh, but it can also be processed into canned fruits, juices, dried fruits, pickles, syrups, and fermented products. Some studies revealed the suitability of sugared snake fruit juice for wine and vinegar fermentations, as it is a good medium for relevant microorganisms (Gunam et al., 2009; Zubaidah et al., 2017). This favourable fermentability property of the sugared snake fruit can be explored for other fermented products with functional properties.

Kombucha (tea fungus) is a refreshing health-promoting beverage of sugared tea infusion fermented by a symbiotic consortium of yeast species and acetic acid bacteria. Various yeasts (e.g. *Pichia*, *Candida*, *Zygosaccharomyces*, *Brettanomyces*, and *Saccharomyces* species) and *Acetobacter xylinum* have been identified in Kombucha fermentation (Jayabalan et al., 2014). The beverage possesses functional properties such as antimicrobial, antioxidant, anticancer, and antidiabetic, and is beneficial in treating gastric ulcer (Jayabalan et al., 2011; Aloulou et al., 2012; Bhattacharya et al., 2013; Banerjee et al., 2011; Chakravorty et al., 2016). Specifically, Kombucha is reported (Sreeramalu et al., 2000; Jayabalan et al., 2014) to inhibit a broad spectrum of Gram-positive (e.g. *Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacteria (e.g. *Escherichia coli* and *Pseudomonas aeruginosa*). Moreover, total phenolic compounds and radical scavenging activities of Kombucha increase with fermentation time (Jayabalan et al., 2014). In view of the functional properties of Kombucha, substrates other than tea had been studied, including Jerusalem artichoke, echinacea, mentha, eucalyptus, sour cherry, grape, orange, and blackcurrant (Yavari et al., 2010, 2011; Jayabalan et al., 2014; Gamboa-Gómez et al., 2016; Lobanova et al., 2016; Ayed et al., 2017).

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https://doi.org/10.1016/j.bcab.2017.12.012
Received 27 September 2017; Received in revised form 20 December 2017; Accepted 24 December 2017
Available online 26 December 2017
1878-8181/

However, we are unaware of the fermentation of snake fruit juices with the Kombucha consortium, and in view of the global availability of snake fruits, a study along these lines would provide another product to health-conscious consumers particularly in areas where snake fruits are abundant. Therefore, using different cultivars of snake fruit to understand cultivar effects, the objectives of this research were to:

(a) Investigate fermentation of snake fruit with the Kombucha consortium.
(b) Evaluate physicochemical and sensory properties of the fermented products.
(c) Assess antioxidant and antibacterial activities of the most promising cultivar, with its bioactive compounds.

2. Materials and methods

2.1. Materials

Snake fruits of commercial maturity were obtained from plantations in Malang, Jombang and Kediri, East Java, Indonesia, and were of cultivars *Salak Doyong, Salak Madu, Salak Pondoh, Salak Segaran,*and *Salak Suwaru*. Commercial Kombucha starter was purchased from a local distributor, while cane sugar was bought from a local supermarket.

2.2. Snake fruit juice preparation and Kombucha fermentation

The snake fruits were peeled, washed, and cut into small sizes, from which 400 g was mixed (1:1, w/w) with water, juiced (blended) and filtered (cheese cloth). The snake fruit juices were sweetened (1:10, w/v) with the cane sugar, pasterurized (Waterbath Memmert, Germany) at 65 °C for 30 min and cooled to room temperature before storing in a sterile jar and refrigerating until used. The juices were sweetened or sugared to provide additional sugars and facilitate fermentation as reported elsewhere (Sreeramalu et al., 2000; Jayabalan et al., 2014). The sugared juices were inoculated (1:10 w/w) with the Kombucha starter and incubated for 14 days at room temperature. Samples of the fermenting broth were taken at days 0, 7 and 14 for analysis.

2.3. Media and chemicals

Culture media, nutrient agar (CM0003 Oxoid™) and potato dextrose agar (CM0139 Oxoid™) were products of Thermo Fischer Scientific, USA. Sodium hydroxide (Merck 106462, Germany), oxalic acid (Merck 100495, Germany), anthrone reagent (Merck 101468, Germany), sulphuric acid (Merck 100731, Germany), glucose (Merck 108337, Germany), gallic acid (Sigma-Aldrich, Germany), Folin-Ciocalteau phenol reagent (Sigma F9252, Sigma-Aldrich, Germany), tannic acid (Sigma-Aldrich, Germany), quercetin (Sigma-Aldrich, Germany), sodium carbonate (Merck 1063950500, Germany), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) used, were products of the indicated suppliers, and were of an analytical grade, as well as other minor reagents.

2.4. Physicochemical analysis

Color was measured by a color reader (Konica Minolta CR-10, Japan) and pH by a pH meter (Hanna, Thermo Fischer Scientific, USA). Total sugar was determined by the anthrone method (Islam et al., 2013) with modifications, whereby 1 mL of the fermented or non-fermented juices was transfered to a test tube and mixed with 5 mL of the anthrone reagent (0.05 g anthrone in 50 mL of concentrated H_2SO_4). The test tube were held at 100 °C for 12 min and cooled before measuring the absorbance at 630 nm in a spectrophotometer (Spectro 20D Plus, Labomed, USA) with a glucose solution as the standard. Total acidity was measured according to Ranggana (1977), whereby 10 mL of the fermented or non-fermented juices was mixed with 100 mL of distilled water and some drops of 1% phenolphthalein indicator, and then titrated with 0.1 M NaOH.Total soluble solids of the samples were measured by refractometry (Atago handheld refractometer N-1E, Japan). One drop of sample was placed on the prism, closed the daylight plate over the sample, then read through the focusable cushioned-rubber eyepiece.

2.5. Microbiological analysis

Yeast and bacteria counts of the Kombucha samples were done using the standard plate count procedure according to BAM-FDA protocol (Maturin and Peeler, 2001) that involved mixing 1 mL of the samples with 9 mL of sterile 0.1% peptone water (10^{-1} dilution). Aliquots of the mixture and subsequent dilutions were further diluted with the peptone water to have serial decimal dilutions from 10^{-2} to 10^{-8}. One milliliter of each dilution was transfered into duplicated petri dishes, into which 15 mL of sterile nutrient agar (50 °C) containing cycloheximide (4 g/L) to prevent yeast growths was poured, mixed immediately and left to solidify before incubation at 37 °C for 24 h., after which the growths were counted. For yeast counts, the same procedure was followed without the addition of cycloheximide, but with the potato dextrose agar as the medium and the incubation was at 30 °C for 48 h.

2.6. Antioxidant and antibacterial activities

Antioxidant activity was measured in vitro by using the DPPH radical scavenging activity method (Hatano et al., 1988), whereby 1 mL of 0.2 mM DPPH solution was mixed with 2 mL of the snake fruit Kombucha. The mixture was incubated in a dark room for 30 min and the absorbance was measured at 517 nm with the control being the sample blank. Changes in the absorbance were measured, and antioxidant activity was expressed as % DPPH radical scavenging ability.

Antibacterial activity assay against pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli* was conducted by using the agar diffusion technique of Wolf and Gibbon (1996). One milliliter of the indicator bacteria culture with 10^8 colony forming units (cfu) per mL was transfered to a sterile petri dish, into which 10 mL of sterile nutrient agar was poured, and upon solidification, six 8 mm-diameter wells were made by a sterile perforator. An appropriate volume (100 μL) of sterile snake fruit Kombucha was transferred into the well and incubated at 37 °C for 24 h. The diameter of the inhibition zone was measured with a micrometer.

2.7. Bioactive compounds analysis

Total phenolic content was determined with the Folin-Ciocalteu reagent (Yang et al., 2007) using gallic acid as the standard. One milliliter of the methanolic extract of the fermented or non-fermented juices was placed into a test tube and vortexed (Nissin mixer N-20 M, USA) for 15 s with 1.5 mL of the Folin-Ciocalteau reagent, and allowed to stand at room temperature for 5 min, before adding 1.5 mL of 0.57 M Na_2CO_3 and incubated for 90 min at room temperature. Absorbance was measured at 750 nm using the spectrophotometer, with the same mixture except the sample extract was replaced by methanol as the blank. Total phenolic content was expressed as mg GAE (Gallic Acid Equivalent)/L.

Tannin content was determined with the Vanilin-HCl method as described by Price et al. (1978) with minor modifications, whereby 1 mL of the test juice was mixed with 5 mL of Vanillin/HCl mixture in a test tube and held for 20 min at room temperature. The formed color was determined at 500 nm wavelength. The tannic acid was used as the standard, and tannin content was expressed as mg TAE (Tannic Acid Equivalent)/L.

Total flavonoids were estimated according to the method of Nabavi