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Fermentation-based biotransformation of bioactive phenolics and volatile compounds from cashew apple juice by select lactic acid bacteria

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ABSTRACT

Select lactic acid bacteria (LAB); *Lactobacillus plantarum*, *L. casei* and *L. acidophilus* were targeted for enhancing bioactives and flavor volatiles of cashew apple juice (CAJ) that is an underutilized byproduct from cashew nut processing in Tropical countries. Results indicated the vitamin C and phenolic metabolites such as condensed tannin can be increased at certain stages such as at 12 h over the 48 h fermentation period. Whereas antioxidant activity based on DPPH and ABTS radical scavenging activity generally decreased from initial unfermented stage range of (75%–95%) to consistently in the 50% range by 48 h of fermentation and this follows the decrease in viable counts. The fermentation process increased the condensed tannin contents in CAJ whereas hydrolysable tannins decreased. In this study the changes in flavor volatile types were also analyzed over the course of CAJ fermentation. The results indicated that LAB changed the flavor profiles of fermented CAJ and overall the fruity odor decreased, but the whiskey and acid odor increased. These results provide the foundation to further target the functional benefits of LAB-induced fermented CAJ for further human, animal, and plant health applications.

1. Introduction

Cashew (*Anacardium occidentale*) is very resilient and hardy tropical plant with origins in tropical Brazil whose modified fruit called cashew apple is a rich source of bioactive phytochemicals with potential for diverse biological applications. Cashew belongs to genus Anacardium of the family Anacardiaceae. Cashew apple is commonly an agricultural by-product from cashew nut production and close to 90% of this fruit is lost or underutilized [1]. Cashew apple can be consumed fresh or processed into value-added juice, jams, jelly, syrups, various beverages and other food products. Further it can be used as substrate for fermentation or other biotransformations including enzyme-based synthesis. Cashew apple is an excellent source of bioactive compounds, minerals, vitamins and unique flavors [1,2]. Cashew apple juice (CAJ) contains ascorbic acid that is 4X to 10X higher than orange and pineapple juice [3]. Additionally it contains phenolic compounds such as leucodelphinidin, anarcardic acid, cardol, tannins, and terpenoid such as carotenoids that is an antioxidant with potential health benefits [4,5]. Ascorbic acid and total phenolic compounds in particular are the two classes of bioactives that can be targeted for enhancing functional health benefits by novel microbial biotransformation. It is known that the strong radical scavenging activity of proanthocyanidins (condensed tannins) are also responsible for the typical astringent and acid taste of some fruits [6] and could also have other functional health benefits. Combining these tannin astringency cashew apples can be processed into a beverage due to its fleshy pulp, soft peel, lack of seeds and high sugar concentration. Further it has strong exotic flavor [7,8] from volatile constituents such as, esters (38–80% of total volatiles), alcohols (30% of total volatiles), acids (15% of total volatiles), aldehydes, ketones, and ethers [2]. These flavor bioactives can be targeted for further value addition through microbial bioatransformation, including both aerobic growth and fermentation using food grade bacteria [9].

Fermentation is a well-established traditional technology across the globe used to enhance the shelf-life, nutritional and organoleptic qualities and remove undesirable compounds of primary food substrates [10]. Further fermentation processes have now been targeted and developed to increase the synthesis of biologically active microbial metabolites for diverse functional benefits using suitable substrates or appropriate nutrients [1] and with the right microbial target. Among these targets lactic acid bacteria (LAB)-based fermentation clearly contributes to improvement of nutritional value and digestibility of various foods, decreasing lactose intolerance, controlling potential infections and additionally has relevance to add further functional bioactives [11]. Functional benefits of lactic acid bacteria due to their

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complex enzymes could also lead to the novel modification of flavor attributes [12,13].

Therefore the genus Lactobacillus is an excellent target for value addition of CAJ as it can grow in the pH range from mild acidic to neutral values and temperatures from 2 to 53 °C, with optimum temperature range of 30–40 °C and optimum pH range of 5.5–6.2 [14]. Among various species targets, *Lactobacillus plantarum* is the species most frequently used to ferment food products of plant origin [15]. In terms of antioxidant enrichment lactic acid bacteria with β-glucosidase activity (including *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, and *Bifidobacterium longum*) have potential based on the ability to increase isoflavone aglycone [16]. *Lactobacillus acidophilus* and *Lactobacillus casei* could produce active enzymes such as amylase, lactate dehydrogenases, peptidases and proteinase that can transform primary food matrix into functional moieties. Specifically *L. plantarum* is known to produce active enzymes such as amylase, β-glucosidase, decarboxylase, lactate dehydrogenases, peptidase, phenolic acid decarboxylases, phenol reductase, proteinase and tannase [17] and these have relevance for diverse food substrate biotransformations. Based on this rationale several fruit juice substrates such as pomegranate juice [18], blueberry, strawberry, noni juice [19], and cashew apple juice [5] were fermented using lactic acid bacteria.

Since cashew apple juice possess excellent bioactive potential to produce a healthy functional food with excellent flavor attributes [5,12,13,20] and further cashew tree can be grown in dry arid regions of Thailand we have targeted cashew apple juice as a substrate for LAB-based bioactive enrichment for diverse biological applications from food-derived human health to animal and plant health applications. Further we have targeted the analysis of volatile aroma constituent through this targeted LAB-based biotransformation of CAJ. Based on the above overall rationale the focused objective of this present study was to evaluate the primary functional transformations of major bioactives such as vitamin C, phenolics and aroma compounds and derived overall antioxidant potential of fermented CAJ by select and targeted *Lactobacillus* spp. (*L. acidophilus*, *L. casei*, and *L. plantarum*). This initial study is essential as the key foundation to further advance specific functional analysis and benefits targeted for human, animal and crop food applications of LAB-biotransformed CAJ.

2. Materials and Methods

2.1. Materials and cashew apple juice (CAJ)

Other than mentioned specifically, all chemicals were purchased as analytical grade from Sigma-Aldrich Chemical Co., Ltd. (Singapore). Fresh cashew apples from the Heritage Grower Corporation Ltd., located in the South of Thailand were used in this study. The cashews were harvest in the 80% mature stage at the crop season (December–March 2014). Cashew apples were cleaned with filtered water and then chopped into small pieces. The edible pieces were homogenized and blended using a Waring blender for 5 min and stored in freezer. After that stored CAJ were pasteurized for 15 min at 70 °C and 20 g of CAJ was used for each fermentation study. The initial cashew apple substrate contained 86.12 g total carbohydrate, 1.41 g of protein, 2.07 g of fat and initial pH of 4.0.

2.2. Lactic acid bacteria strains and inoculum preparation

Lactobacillus acidophilus TISTR 1338, *Lactobacillus casei* TISTR 390, and *Lactobacillus plantarum* TISTR 543 were obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. The culture were activated at 30 °C for 24 h in 125 mL Erlenmeyer flasks containing 25 mL of Difco lactobacilli deMan, Rogosa and Sharpe (MRS) broth (BD, Sparks, MD, USA). Cell cultivation was carried out statically in an incubator until the cell density reached 1.000 which corresponds to 9.18 Log colony forming units (CFU) per milliliter, using the McFarland scale [7]. Cell density was spectrophotometrically determined at 600 nm and this culture was used as inoculum in the fermentation process.

2.3. Cashew apple juice (CAJ) fermentation

Fermentation experiments were conducted in sealed Erlenmeyer flasks (covered with aluminum foil), each containing 20 g of pasteurized CAJ without supplementary nutrients or water. Two milliliters of inoculum, containing 9.18 Log CFU/mL of various LAB strains, were added to 125 mL Erlenmeyer's flasks containing pasteurized CAJ. The initial cell count in the juice was 8.18 Log CFU/mL. The fermentation process was performed at 30 °C for 48 h. Samples were taken at 0, 12, 24, and 48 h for viable cell count and analysis of targeted phytochemicals, bioactivity and volatile compounds as described below.

2.3.1. Viable cell counts

Cell viable counts of fermented CAJ were obtained by serial dilution with 0.7% NaCl solution until 10^{-6} dilutions. Aliquots of 0.1 mL of dilution were plated, in triplicate in plates containing MRS Agar (spread plate method). The plates were incubated for 36–48 h at 37 °C, and plates containing 20–350 colonies were measured or recorded as Log CFU/mL.

2.3.2. pH and total titratable acidity (%TTA)

The pH of fermented CAJ was measured during the fermentation periods using a pH meter (Mettler Toledo, Fisher Scientific, UK) calibrated with buffer solutions at pH 4.0 and 7.0 (Laboratory Chemical, New Zealand). Total titratable acidity, expressed as percentage of lactic acid, was determined by titration with 0.1 M NaOH using the method of [21].

2.3.3. Total sugar and reducing sugar

Reducing sugar contents were analyzed as glucose equivalents by Nelson-Somogyi method [22]. Total sugar contents were analyzed as glucose equivalents by the phenol sulfuric acid method of Dubois et al. [23]. Absorbance was determined on UV-Spectrophotometer (Biomate 3, Genesys 10, Thermo Electron Corporation, Germany).

2.3.4. Vitamin C contents

Vitamin C contents were determined following the AOAC method for vitamin C (ascorbic acid) in vitamin preparations and juice [21]. A total of 200 μL sample was added to 50 mL Erlenmeyer's flasks and mixed with 1.8 mL distilled water and 5 mL metaphosphoric acid-acetic acid solution. The mixture (7 mL) was titrated with 2,6-dichloroindophenol standard solution, its titration volume was recorded and used to quantify vitamin C content of the samples (mg of ascorbic acid equivalent (AAE)/L of sample). The indophenol solution was standardized by titrating 1 mg/mL ascorbic acid standard solution (2 mL ascorbic acid standard solution with 5 mL metaphosphoric acid-acetic acid solution) and sample blanks.

2.3.5. Total phenolic contents

Total phenolics of samples were determined by the Folin–Ciocalteu colorimetric method according to Huang et al. [24]. Briefly, sample (10 μL) was added to the test tube and mixed with 90 μL distilled water and 2 mL of 2% (w/v) Na_2CO_3 solution. After incubation for 3 min, 100 μL of Folin-Ciocalteu reagent was added. After standing for 30 min at room temperature, the absorbance was measured at 750 nm, using distilled water as a blank. Results were expressed as mg gallic acid equivalents (GAE)/L of sample by using gallic acid standard curve calibration (0.025–04 mg/mL).

2.3.6. Condensed tannin contents

Total tannins or condensed tannins were measured using the