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Comparing product stability of probiotic beverages using litchi juice treated by high hydrostatic pressure and heat as substrates

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ABSTRACT

The aim of this study was to evaluate the use of litchi juice treated by high hydrostatic pressure (HHP) as substrates for producing a probiotic beverage by *Lactobacillus casei*. The quality attributes and product stability of fermented heat- and HHP-treated litchi juice by *L. casei* were compared. Compared with fermented heat-treated litchi juice, fermented HHP-treated litchi juice showed a better color, flavor and overall acceptance, and also retained more total phenolics and antioxidant capacity. Both viability counts of *L. casei* were more 8.0 log CFU/mL in heat- and HHP-treated litchi juice after 4 weeks of storage at 4 °C. Besides, some quality attributes in fermented heat- and HHP-treated litchi juice showed the tendency to slow decrease during storage at 4 °C, but both scores of overall acceptance of fermented heat- and HHP-treated litchi juice showed no reduction after the storage of 4 weeks at 4 °C. Overall, the application of HHP treatment could be an ideal alternative of heat treatment to ensure the microbial safety, consistent sensory and nutritional quality of fermented litchi juice prior to fermentation.

Industrial relevance: The study is relevant to fermentation of litchi juice by probiotic *L. casei*. In this study, results shown the application of high hydrostatic pressure (HHP) treatment could be an ideal alternative of heat treatment to ensure the microbial safety, consistent sensory and nutritional quality of fermented litchi juice prior to fermentation.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a subtropical to tropical fruit of high commercial value in international trade. Apart from being consumed freshly, litchi fruit is also processed into juice, canned litchi and dried fruit. Litchi juice has an attractive color, good aroma and sweetsour mouthfeel, and it is preferably used as a natural antioxidant drink (Yu, Wu, et al., 2013). Its consumption rate could be increased even further by increasing its health benefits with probiotics produced by fermentation. Probiotics represent probably the archetypal functional food, and are defined as a living microbial supplement, which beneficially affect the host by improving its intestinal microbial balance. Microorganisms most commonly used as probiotics belong to the heterogeneous group of lactic acid bacteria (*Lactobacillus*, *Enterococcus*,...) and to the genus *Bifidobacterium*, and which have been added to yogurt and other fermented dairy products (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013).

However, the demand for non-dairy probiotic products increased because of the problems associated with the high cholesterol content, allergy risks, and others negative factors associated with the consumption of dairy products. These concerns have led to the development of probiotic products from various food matrices including fruits and vegetables (Ankolekar, Pinto, Greene, & Shetty, 2012; Mousavi, Mousavi, Razavi, Emam-Djomeh, & Kiani, 2011). Moreover, the fermentation of fruit juice by probiotic lactic acid bacteria is an ideal way for consuming sugars to produce organic acids, and also has been considered as a simple and valuable biotechnology for maintaining and (or) improving the safety, nutritional, sensory and shelf-life properties of fruit juice (Prado, Parada, Pandey, & Soccol, 2008). The spontaneous fermentation of fruit juice typically results from a variety of autochthonous and contaminating microorganisms which may lead to high risk for failure. From hygiene and safety point of views, the use of pasteurization treatment combined with selected starter cultures is recommended in probiotic fermentation of fruit juice, as it would lead to a rapid inactivation and inhibition of spoilage and pathogenic bacteria, and further ensure the fermented products with the microbial safety, consistent sensory and nutritional quality (Rodríguez et al., 2009).

The juices are generally heated to more than 72 °C in a conventional pasteurization treatment, and it seriously affects the sensory and nutritional quality of juices (Prado et al., 2008; Tan, 2011). At present, non-thermal processing technologies for food preservation and safety are gaining widespread acceptance throughout the food industry because of consumer demand for high quality food. Among these technologies, high hydrostatic pressure (HHP) processing is a good alternative to

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pasteurization due to its limited effects on covalent bonds resulting in minimal modifications in nutritional and sensory quality (Oey, Lille, Van Loey, & Hendrickx, 2008). Up to now, numerous studies had demonstrated that HHP treatment is efficient enough to destroy microorganisms in fruit juices with little effects on sensory properties and nutritional value, and also had investigated the changes of some endogenous enzymes' activity related to food quality during HHP treatment (Cao et al., 2011; Krebbers et al., 2003; Oey et al., 2008). However, the use of HHP-treated fruit juices as substrates for probiotic microorganisms has not been evaluated.

The aim of the present study was to evaluate the hypothesis that HHP treatment could be applied in litchi juices prior to fermentation. Thus, the quality attributes and product stability of fermented heat- and HHP-treated litchi juice by probiotic lactic acid bacteria were compared herein.

2. Materials and methods

2.1. Lactobacillus casei cultures

A probiotic *L. casei* strain was presented by the Guangdong Culture Collection Center of China. It was activated to stationary phase in MRS broth (Guangzhou HuanKai Microbiological Technology Co. Ltd., Guangdong, China) and used to inoculate litchi juice.

2.2. HHP and heat treatment of litchi juice

Litchi fruit (cv. Huaizhi) at 95% maturation was harvested from a commercial orchard in Guangzhou, China. In order to obtain fresh litchi juice, the litchi fruit was peeled, destoned, processed with a pulper (Midea Co., Guangdong, China), and then passed through a filter cloth (100 mesh). In heat treatment, the fresh litchi juice with pH 4.56 was thermally processed (95 °C, 1 min) in a tubular heat exchanger (Shanghai Pilotech Equipment Co., Ltd., China). After heating, the juice was immediately cooled down to 25 °C by cooled water. Meanwhile, HHP processing was carried out in a 3.0 L capacity unit (RLGY-600, Wenzhou Beinuo Machinery Co., Zhejiang, China) with water as the pressure transmitting medium. Before HHP treatment, fresh litchi juice with pH 4.56 was placed into aluminum-coated polyethylene bags. Samples were subjected to pressures of 500 MPa for 2 min. The pressure vessel was at ambient temperature (~25 °C). The pressurization rate was about 120–150 MPa/min and the depressurization was immediate (<3 s). The treatment time reported in this study did not include the pressure-increase time and pressure-release time.

According to our previous experiments, thermal pasteurization at 95 °C for 1 min was near with HHP (500 MPa/2 min at 25 °C) to obtained an equivalent inactivation of microorganisms. Therefore, the litchi juice samples in this study were processed using the two treatments.

2.3. Fermentation of litchi juice

The litchi juices (500 mL) were immediately inoculated with *L. casei* to yield an initial concentration of 5.0 log CFU/mL after heat and HHP treatment, and then incubated at 30 °C for 18 h. Before inoculated with *L. casei*, the pH of litchi juice was adjusted to 5.6 by adding sterilized NaOH solution (4 mol/L). After fermented by *L. casei*, the fermented heat- and HHP-treated litchi juice was then distributed into 50-mL aseptic PET bottles under hygienic conditions and stored at 4 °C. Samples were taken out at a week intervals and used for further assay.

2.4. Microbial analysis

Each sample was serially diluted with sterile 0.85% NaCl solution, and then the dilution was used for microbial enumeration by pour plate methods. The Plate Count Agar (Guangzhou HuanKai Microbiological Technology Co. Ltd., Guangdong, China) was used for detecting the viable cells of total aerobic bacteria. The Plate Count Agar plates were incubated at 37 °C for 2 days. The Rose Bengal Agar (Guangzhou HuanKai Microbiological Technology Co. Ltd., Guangdong, China) was used for detecting the viable cells of yeast and molds. The Rose Bengal Agar plates were incubated at 28 °C for 3–4 days. The Violet Red Bile Agar (Guangzhou HuanKai Microbiological Technology Co. Ltd., Guangdong, China) was used for detecting the viable cells of *Enterobacter*. The Violet Red Bile Agar plates were incubated at 37 °C for 1 day. The MRS Agar (Guangzhou HuanKai Microbiological Technology Co. Ltd., Guangdong, China) was used for detecting the viable cells of lactic acid bacteria. The MRS Agar plates were incubated in an anaerobic incubator (Shanghai Yiheng Instruments Co., Ltd., China) at 30 °C for 2–3 days. Each test was performed in duplicate and results were expressed as colony-forming units (CFU) per milliliter.

In order to investigate the survivals in the litchi juice treated by HHP and heat, the microbial colonies were randomly selected from plates above with 30–300 colonies. When <20 colonies were present on a plate, all of the colonies were selected for further examination and identification. All the selected colonies were streaked onto correspondent agar plates for further purification. Then the purified bacteria colonies were identified using 16S ribosomal DNA (rDNA)-based methods and the detail of 16S ribosomal DNA (rDNA)-based methods had been demonstrated in previous studies (Yu, Wu, et al., 2013).

2.5. Determination of pH

The pH of the fermented litchi juice samples (20 ± 1 °C) was measured using a Metrohm744 pH meter with a glass electrode (Metrohm Co. Ltd., Switzerland).

2.6. HPLC analysis of sugars, organic acids, and ascorbic acid

Sugars, organic acids, and ascorbic acid were analyzed by HPLC in an Agilent system. The volume of the injected sample for each run was 20 μL, while quantification was performed by external calibration based on peak area. Sugars were quantified by a RI detector and separated on a Shodex Asahipak NH2P-50 4E (250 × 4.6 mm) column using 75% acetonitrile aqueous solution as the mobile phase at a flow rate of 1 mL/min and 30 °C. Organic acids were separated on an Agilent ZORBAX SB-Aq (4.6 × 250 mm) column at 30 °C using 0.02 mol/L $(NH_4)_2HPO_4$ aqueous solution (pH 2.7) as the elution at a flow rate of 0.8 mol/L, and quantified with a UV–VIS detector at 210 nm. Ascorbic acid was separated on an Agilent ZORBAX SB-Aq (4.6 × 250 mm) column using 0.02 mol/L $(NH_4)_2HPO_4$ aqueous solution (pH 2.7) as the mobile phase at a flow rate of 1 mL/min and 30 °C, and quantified with a UV–VIS detector at 254 nm.

2.7. Determination of total phenolics

Total phenolics of fermented litchi juice were analyzed according to the method reported by Ross, Beta, and Arntfield (2009). Each litchi juice sample (2 mL) was mixed with 8 mL methanol containing 1% (v/v) HCl. Suspension was kept for ultrasonication (10 min) at 30 °C. Extract was centrifuged at 5000 ×*g* (5 min), and then the total phenolic content of the supernatant was determined using the Folin–Ciocalteu method (Saura-Calixto, 1998) and expressed as gallic acid equivalent (GAE) g/L of juice.

2.8. Determination of antioxidant capacity

The antioxidant capacity of the fermented litchi juice sample was evaluated by oxygen radical absorbance capacity (ORAC). ORAC assay was performed according to Ou, Hampsch-Woodill, and Prior (2001) using the Infinite M200 microplate reader (Tecan Group Ltd., Switzerland). 80 μL of freshly prepared sodium fluorescein solution (1.25 μM/L in 75 mM/L phosphate buffer, pH 7.4) and 20 μL litchi juice