Potato Juice Fermented with *Lactobacillus casei* as a Probiotic Functional Beverage

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Abstract This study was conducted to evaluate probiotic potato juice as a potential substrate for the production of *Lactobacillus casei*, and the change in the functionality of potato juice was monitored during fermentation. *L. casei* grew well in potato juice without nutrient supplementation, and lactic acid bacteria of fermented ‘Haryoung’ juice reached $1.7 \times 10^9$ CFU/mL after a 48 h fermentation. DPPH radical scavenging activities of the potato juices decreased after a 72 h fermentation, but fermented colored potato juice still maintained >50% radical scavenging activity. The survival rate of *L. casei* fermented in ‘Haryoung’ juice was 89.0% after exposure to an acidic condition, and *L. casei* in all fermented potato juice samples showed the ability (50-85%) to survive in the presence of bile. These results suggest that fermented potato juice might serve as a probiotic functional beverage for vegetarians or consumers who are allergic to dairy products.

Keywords: potato, probiotic beverage, *Lactobacillus casei*, antioxidant activity, lactic fermentation

Introduction

Probiotics are live microorganisms that confer a beneficial effect on the host when administered in proper amounts (1). Multiple reports have described their health benefits on gastrointestinal infections, antimicrobial activity, improved lactose metabolism, reduction in serum cholesterol, anticarcinogenic properties, antiarrheal properties, and improvement in inflammatory bowel disease (2,3). Probiotic products are usually marketed in the form of fermented milks and yoghurts; however, there is also a demand for the vegetarian probiotic products with the increase in consumer vegetarianism. Furthermore, lactose intolerance and cholesterol content are two major drawbacks related to fermented dairy products (4).

Consumer demand for nondairy-based probiotic products has increased in recent years, and numerous lactic acid bacteria (LAB) such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Bifidobacterium longum*, and *Bifidobacterium lactis* have been used in fruits to produce probiotic beverages (5,6). Fruits and vegetables are healthy foods, because they are rich in antioxidants, vitamins, dietary fibers, and minerals. Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent their use by certain segments of the population (7).

Potatoes (*Solanum tuberosum* L.) are currently the 4th most important food crop worldwide after maize, wheat, and rice, with production of more than 323 million tons. Due to their low cost, low fat content, and good source of carbohydrate, high quality protein, fiber, and vitamins, potatoes play an important role in human nutrition (8). Colored potato cultivars, in which the skin or flesh of the stem tubers is red, purple, blue, or orange, are a recent improvement over white fleshed potato varieties. These colored potatoes are rich in certain antioxidants such as polyphenolics and contain high amounts of nutrients compared to white fleshed potato varieties (9). Colored potato cultivars, in which the skin or flesh of the stem tubers is red, purple, blue, or orange, are a recent improvement over white fleshed potato varieties. These colored potatoes are rich in certain antioxidants such as polyphenolics and contain high amounts of nutrients compared to white fleshed potato varieties (9).

Colored potatoes contain 2-fold higher levels of phenolic acids compared to yellow-fleshed potatoes (11). The antioxidant capacity of red or blue colored potatoes is 2-3 times higher compared with that in potatoes with white/yellow flesh;
therefore, these potatoes represent a possibility to enhance antioxidants (12).

The objective of this study was to determine the suitability of potato juice for producing probiotic juice by LAB, to analyze the functionality of probiotic potato juice, to estimate the survival rate of LAB from probiotic potato juice in gastric juice and bile salts, and to study the viability of probiotic organisms in the product over a proposed shelf life of 4 weeks at 4°C.

Materials and Methods

Samples Potatoes were purchased from Pyeongchang, Korea (seed potatoes). A general potato type (‘Superior’) and 3 colored potatoes (‘Haryoung’, ‘Hongyoung’, and ‘Jayoung’) were used for experiments. The colors of the potato flesh were white (‘Superior’), yellow (‘Haryoung’), red (‘Hongyoung’), and purple (‘Jayoung’), respectively.

Preparation of potato juice Samples were washed and peeled. The potato juices were prepared using a commercial food processor (Juice Maker J 500; Samsung, Seoul, Korea). To increase the use of sugar, the potato juices were liquefied with α-amylase (Sigma-Aldrich, St. Louis, MO, USA) for 48 h at 37°C. Liquefied potato juices were sterilized for 20 min at 100°C and centrifuged to remove insoluble materials. Then, the potato juices were stored at −75°C in preparation for the fermentation experiment. Fermentation experiments were conducted in sealed test tubes, each containing 20 mL of pasteurized potato juice without supplementary nutrients or water.

Probiotic LAB L. plantarum ATCC 8014, L. casei ATCC 393, and Lactobacillus delbrueckii subsp. bulgaricus ATCC 11842 were purchased from the Korean Culture Center of Microorganisms. After preliminary experiments, L. casei was selected to prepare the probiotic potato juices. The strain was subcultured twice on a MRS (BD, Sparks, MD, USA) plate and broth prior to inoculation, according to the user’s manual instructions.

Fermentation of probiotic potato juice Fermentation experiments were conducted in sealed test tubes, each containing 20 mL pasteurized potato juice without supplementary nutrients or water. L. casei (ATCC 393) was cultured in MRS broth for 24 h at 37°C, equivalent to 1×10^6 CFU/mL, and inoculated in pasteurized potato juice. The fermentation process was performed at 37°C for 72 h. Samples were taken at 0, 24, 48, and 72 h for chemical and microbiological analyses.

Chemical and microbiological analyses Samples were taken at 24 h intervals for chemical and microbiological analyses. The pH of the fermented potato juice was measured using a pH meter (Orion 3 star Benchtop; Thermo Orion, Beverly, MA, USA). Total acidity, expressed as lactic acid, was determined by titrating with 0.1 N NaOH to pH 8.2. Viable cell counts (CFU/mL) were determined by the standard plate method with Lactobacilli MRS broth after a 48 h inoculation at 37°C. Reducing sugar content was analyzed as glucose equivalents by the 3,5-dinitrosalicylic acid (DNS) method of Miller (13).

Analysis of potato juice antioxidant activity Antioxidant activity of the potato juices was evaluated by testing their scavenging ability against DPPH and ABTS radicals. The DPPH radical scavenging effects of samples were estimated according to the method of Chen et al. (14) with some modifications. Two mL of each fermented potato juice was added to 1 mL of 0.2 mM DPPH radical solution. The mixture was shaken and allowed to stand for 30 min at 37°C, and then the absorbance was measured at 517 nm with a spectrophotometer (UV-2001; Hitachi, Tokyo, Japan). The percent inhibition activity was calculated by the following equation: DPPH radical scavenging activity (%)  =1−(ABS_{sample} at 517 nm/ABS_{control} at 517 nm)]×100

The antioxidant activity of the fermented potato juices was also measured by the ABTS radical cation decolorization assay (15). ABTS was dissolved in water to a 7.4 mM concentration. The ABTS radical cation (ABTS⁺) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Prior to assay, the solution was diluted in ethanol to give an absorbance of 0.700±0.02 at 734 nm. A reagent blank reading was taken (A₀). After adding 3 mL of diluted ABTS solution to 50 μL of sample, the absorbance was measured at 734 nm after exactly 6 min of initial mixing (A₁). The ABTS radical scavenging rate was calculated using the following formula:

\[\text{ABTS radical scavenging activity (%) } = (\frac{1 - A_{0} - A_{1}}{A_{0}}) \times 100\]

Determination of total phenolic content (TPC) TPC was determined according to the method of Al-Weshahy and Rao (8). A 0.2 mL aliquot of each of the fermented potato juices was mixed with 0.2 mL of Folin-Ciocalteu’s reagent and allowed to stand at room temperature for 3 min. Then, 0.4 mL of 10% Na₂CO₃ was added to the mixture. After standing for 60 min, the absorbance was measured with a spectrophotometer at 725 nm. The results are expressed as mg of tannic acid equivalents (TAE).