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Improving anti-hyperglycemic and anti-hypertensive properties of camu-camu (*Myriciaria dubia* Mc. Vaugh) using lactic acid bacterial fermentation

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ABSTRACT

Camu-camu (*Myriciaria dubia* Mc. Vaugh) is a tropical fruit rich in phenolic antioxidants with diverse human health benefits. The aim of this study was to improve phenolic antioxidant–linked functionalities of camu–camu relevant for dietary management of early stages of type 2 diabetes (T2D) and associated hypertension using lactic acid bacterial (LAB) fermentation. Dried camu–camu powder combined with soymilk was fermented using two LAB strains, *Lactobacillus plantarum* & *Lactobacillus helveticus* individually and evaluated for total soluble phenolic content, total antioxidant activity, α-amylase, α-glucosidase, and angiotensin-I-converting enzyme (ACE) inhibitory activities using *in vitro* assay models. Overall, fermentation of camu–camu and soymilk combination with both LAB strains resulted in higher α-amylase, and α-glucosidase inhibitory activities, while total soluble phenolic content and antioxidant activity did not change significantly with fermentation. Improvement of ACE enzyme inhibitory activity was also observed when camu–camu (0.5 & 1%) and soymilk combination was fermented with *L. plantarum*. Therefore such safe and value added fermentation strategy with LAB can be used to improve human health relevant phenolic antioxidant profile in camu–camu and has relevance for designing innovative probiotic beverage to target improved food designs for dietary support for T2D and associated hypertension management.

1. Introduction

The health benefits of camu–camu was rationally improved as a food-based beverage in a soymilk substrate design for type 2 diabetes and hypertension targets using lactic acid fermentation. The rapid rise in prevalence and occurrence of non-communicable chronic diseases (NCDs), such as type 2 diabetes (T2D) and associated hypertension is in part related to recent global changes in lifestyle and dietary patterns to which individual genetic make-up is responding in different ecologies. These diet and lifestyle related changes include higher consumption of calorie-dense hyper-processed foods, sedentary lifestyle, and increasing stress in everyday-life [1]. The number of individuals with T2D is increasing exponentially, and it is estimated to reach over 592 million by year 2035 globally [2]. The social and economic burden of NCDs including T2D is enormous with direct individual annual costs for diabetes treatment being around $11,917 in the United States [3]. It is important to develop complementary food-based therapeutic dietary support strategies which are safe, sustainable, and cost-effective along with pharmaceutical drug based interventions. Nutritionally balanced food designs with higher proportion of dietary fiber that support the microbiome and phytochemical enrichment targeted to counter the chronic disease pathways is one such cost effective strategy which can contribute significantly to reduce the overall risk of T2D and its associated complications [4]. It has been suggested with some empirical evidence that higher consumption of whole grains, berries, vegetables, and fruits rich in phenolic bioactives with high antioxidant activity is associated with lower risk of T2D and its complications [5].

Therefore such food-based preventative strategies are potentially more effective during early-stages of the disease development and can play significant role to counter micro and macro-vascular complications associated with T2D [6]. Previously T2D relevant bioactive benefits were observed in camu-camu [7], which has been further developed in this study. Camu-camu is a fruit from tropical Amazon with high health relevant phenolic bioactive profile. Previous studies have found very high vitamin C and ellagic acid in camu–camu when compared to others fruits [8]. High antioxidant activity, antimicrobial, and anti-diabetic properties, especially very high α-glucosidase inhibitory activity was also observed in camu–camu [7]. However, this fruit is highly perishable, which makes transportation and storage very expensive. The sensory property, mostly high acidity is also another limiting factor

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for lower consumption of camu–camu as a fresh fruit. Rapidly processing camu–camu into a well preserved powder and further using fermentation based and low energy requiring processing is one potential strategy to extend shelf-life, to improve sensory qualities, and to increase functionality of previously established human health relevant bioactive compounds [7,9]. Such fermentation strategy can advance the value added consumption coupled to new market opportunities, and can harness the potential human health benefits of this bioactive enriched fruit.

Traditional fermentation strategy, including LAB based fermentation has long been used in dairy and plant-based foods to improve food quality and food safety parameters [10]. Fermentation also helps to improve shelf-life and nutritional quality of perishable foods [11]. Therefore fermenting fruit ingredients in a synergistic nutrient base with LAB with probiotic potential offers excellent strategy to design new functional foods, beverages and food ingredients with potential human health benefits, such as improved intestinal microflora regulation, enhanced anticarcinogenic, anti-inflammatory, and anti-diabetic properties [12]. Further, targeted phenolic bioactives in fermented fruits also has potential to exert dual functions as antimicrobial against pathogenic microorganisms, while promoting the growth of good bacteria in the gut [13]. Maintenance of good microbiome in the gut is essential to improve overall metabolism including glucose metabolism, which can potentially be part of prevention and management of T2D. Further improvement in phenolic antioxidant activity and α-glucosidase enzyme inhibitory activity was also observed in LAB fermented fruit substrate [12]. Therefore camucamu fruit containing high phenolic antioxidant content and high anti-diabetic properties is an excellent target for designing new probiotic beverage integrating LAB fermentation. However prior to effective beverage design with camucamu, it is important to optimize the ideal fruit concentrations in an effective protein nutrient carrier substrate like soymilk which can be rationally bio-transformed by LAB with probiotic potential thereby enhancing health benefits.

Therefore, the major aim of this study was to improve and evaluate phenolic bioactive-linked antioxidative, anti-hyperglycemic, and anti-hypertensive properties of camu–camu combined with soymilk by fermenting with two separate LAB strains; *L. helveticus* and *L. plantarum*. Further different concentrations of freeze and spray-dried camu–camu powder in soymilk with gum arabic as a carrier agent for substrate stabilization were compared to find optimum formulation for LAB fermentation.

2. Materials and methods

2.1. Materials

Freeze-dried and spray-dried of camu–camu pulp (*Myrciaria dubia* Mc. Vaugh) powders were collected from Sao Paulo, Brazil. Plain soymilk was purchased from a local supermarket (Hornbachers, Fargo, ND, USA). The bacterial strains used in this study were *L. helveticus* (ATCC 12046) and *L. plantarum* (NCDO 1193) provided by Rosell Institute Inc., Montreal, Canada.

2.2. Spray-drying

Spray-drying of camu powder was performed in a pilot scale spray-dryer (Labmaq, SD 5.0, Brazil). The pulp was injected by a peristaltic pump at a fixed rate of 44 mL/min and was spray-dried at inlet air temperatures (120 °C) using different concentrations of gum arabic (6, 12 and 18%) as a carrier agent (Nexira Brazil Com. Ltd., Brazil).

2.3. Freeze-drying

Two kilograms of frozen pulp were lyophilized in a Pironi 501 freeze-drier (Thermo Electron Corporation, New York, USA) at −80 °C and 100 *m*Torr for 120 h.

2.4. Fermentation with soymilk

Initially, 100 μL of frozen *L. helveticus* and *L. plantarum* stock were inoculated separately and individually into 10 mL MRS broth (Difco, Becton, Dickinson and Co., Franklin Lakes, New Jersey, USA) for 18 h at 37 °C. After that, 100 μL of the grown strain was re-inoculated into 10 mL MRS broth for 18 h at 37 °C. Then 150 mL of soymilk were placed into each 250 mL Erlenmeyer flask. Different concentration of camu–camu powders (0, 0.5%, and 1%) were then added and mixed in soymilk. Camu-camu and soymilk combinations were then inoculated with 1.5 mL of freshly grown LAB strain (6.30 Log CFU/mL *L. plantarum* and 5.82 Log CFU/mL *L. helveticus*). Fermentation was carried out at 37 °C in a closed incubator (VWR), and 30 mL of samples were taken out at 0, 24, 48 and 72 h for biochemical assays. The samples were centrifuged at 15000*g* for 15 min prior to carrying out *in vitro* assays.

2.5. Growth of bacteria by colony counts assay

The concentration of LAB (CFU/mL) in fermenting medium was determined at 0, 24, 48 and 72 h, by pipetting 100 μL of the sample, serially diluting, and plating on MRS medium. The plates were incubated anaerobically in BBL GasPak jars (Becton, Dickinson and Co., Franklin Lakes, New Jersey, USA) with BD GasPak EZ anaerobe container system sachets (Becton, Dickinson and Co. Franklin Lakes, New Jersey, USA) at 37 °C for 24 h, and individual colonies were counted. The pH of the samples was also measured at 0, 24, 48 and 72 h.

2.6. Total soluble phenolic content

Total soluble phenolic content of LAB fermented camu–camu and soymilk combinations was determined by Folin-Ciocalteau method as described by Shetty et al. [14]. Briefly, 0.5 mL of sample extract was added to a test tube and mixed with 0.5 mL of distilled water (2 times dilution), 1 mL of 95% ethanol, and 5 mL of distilled water. To each sample, 0.5 mL of 50% (vol/vol) Folin-Ciocalteau reagent and 1 mL of Na_2CO_3 (5%) was added and mixed using a vortex. The combined mixture was then incubated in dark for 1 h at room temperature (26 °C). After 1 h incubation the absorbance of the sample was recorded at 725 nm using a UV–vis spectrophotometer (Genesys UV/Visible, ThermoFisher, Waltham, MA, USA). Results were expressed as μg of gallic acid equivalent/mL of sample.

2.7. Antioxidant activity by DPPH free radical scavenging assay

Total antioxidant activity was determined using DPPH (1,1-Diphenyl-2-picryl-hydrazyl) free radical scavenging assay described by Kwon et al. [15]. A total of 250 μL aliquot of the sample extract was mixed with 1250 μL of DPPH stock solution (60 μM in ethanol). Control was carried out with 250 μL of 95% ethanol instead of sample. After 5 min incubation at room temperature absorbance was measured at 517 nm using a UV/VIS spectrophotometer (Genesys UV/Visible, ThermoFisher,Waltham, MA, USA) The inhibition percentage was calculated as follows

$$DPPH\ \ Inhibition\ \ (\%) = \frac{(Abs\ \ control - Abs\ \ sample)}{Abs\ \ control} \times 100$$

2.8. α-Amylase inhibitory activity

The α-amylase inhibitory activity was determined using an assay modified from the *Worthington Enzyme Manual* [16]. A total of 500 μL of sample extract was mixed with 500 μL of α-amylase enzyme solution (0.5 mg/mL in 0.02 M sodium phosphate buffer, pH 6.9 with 0.006 M NaCl) and was incubated at 25 °C for 10 min. After 10 min pre

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