Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

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

Alice Fujita[a,b], Dipayan Sarkar[b], Maria Ines Genovese[a], Kalidas Shetty[b,*]

[a] Laboratory of Food Bioactive Compounds, Food and Experimental Nutrition Department, FCF, University of São Paulo, 05508-900 São Paulo, SP, Brazil
[b] Department of Plant Science, North Dakota State University, Fargo, ND 58102, USA

ARTICLE INFO

*Keywords:*
Antioxidant
α-Amylase
α-Glucosidase
Anti-diabetes
Hypertension
Phenolics
Soymilk

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

* Corresponding author. Present address: 214 Quentin Burdick Building, 1320 Albrecht Blvd., Department of Plant Sciences, North Dakota State University, Fargo, ND 58102, USA.
*E-mail address:* kalidas.shetty@ndsu.edu (A. Fujita).

http://dx.doi.org/10.1016/j.procbio.2017.05.017
Received 28 February 2017; Received in revised form 3 May 2017; Accepted 11 May 2017

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

incubation, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. After additional10 min incubation the reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then placed in boiling water (100 °C water bath for 10 min) and later cooled down to room temperature. The reaction mixture was then diluted to optimize the reading of control (only enzyme and substrate) to 1.0 at 540 nm absorbance. Absorbance of all samples (without dilution, half and one-fifth dilution) at 540 nm was recorded using a Genesys UV–vis spectrophotometer (Genesys UV/Visible, ThermoFisher,Waltham, MA, USA). The readings were compared with the controls, containing buffer instead of sample extract. The results were expressed as percent α-amylase inhibition and calculated as follows

$$\alpha - Amylase\ \ Inhibition\ \ (\%) = \left(\frac{Abs\ \ control - (Abs\ \ sample - Abs\ \ Sample\ \ Blank)}{Abs\ \ Control}\right) \times 100$$

### 2.9. α-Glucosidase inhibitory activity

The α-glucosidase enzyme inhibitory assay was performed according to the *Worthington Enzyme Manual* [17]. A total of 50 µL of fermented camu–camu extracts was added in a 96-well microplate and mixed with 100 µL of α-glucosidase enzyme (1unit/ mL) prepared in 0.1 M phosphate buffer (pH 6.9) and incubated at 25 °C for 10 min. After 10 min incubation, 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution prepared in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were then incubated at 25 °C for 5 min. Absorbance readings were recorded at 405 nm before and after 5 min incubation by using microplate reader (Spectra Max 190, Molecular Device Co., Sunnyvale, CA, USA) and compared to a control that had 50 µL of buffer solution instead of the sample extract. The results were expressed as percent of α-glucosidase inhibition and calculated as follows

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

### 2.10. Angiotensin-I-converting enzyme (ACE) inhibitory activity

Angiotensin-I converting enzyme inhibitory activity was determined according to the method modified of Kwon et al. [15]. The substrate hippuryl-histidylleucine (HHL) and ACE-I enzyme from rabbit lung (1 unit produces 1.0 µmol of hippuric acid from HHL per minute in 50 mM HEPES and 300 mM NaCl at pH 8.3 at 37 °C) were used for this assay. Fifty µL of water soluble supernatant of sample extracts was mixed and incubated with 200 µL of 2 units of ACE-I enzyme solution prepared in 1 M NaCl-borate buffer (pH 8.3) at 37 °C for 10 min. After pre-incubation, 100 µL of a 5 mM substrate (HHL) solution was added to the reaction mixture. Test solutions were then incubated at 37 °C for 1 h in a water bath. After 1 h incubation the reaction was stopped by adding 150 µL of 0.5 N HCl. The formed hippuric acid was then detected and quantified by high-performance liquid chromatography (HPLC). Five µL of the sample was injected using an Agilent ALS 1100 autosampler into an Agilent 1260 series HPLC (Agilent Technologies, Palo Alto, CA, USA) equipped with a DAD1100 diode array detector. The solvents used for the gradient were (1) 10 mM phosphoric acid (pH 2.5) and (2) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% for next 5 min and then was decreased to 0% for the next 5 min (18 min total run time). The analytical column used was a Nucleosil 100-5 C18, 250 × 4.6 mm i.d., with packing material of 5 $\mu$m particle size at a flow rate of 1 mL/min at ambient temperature. During each run, the absorbance was recorded at 228 nm and the related chromatogram was integrated using Agilent Chemstation (Agilent Technologies) enhanced integrator for detection of liberated hippuric acid (A). Pure hippuric acid (purchased from Sigma Chemical Co.) was used to calibrate the standard curve and retention time. The percent inhibition was calculated according to

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

### 2.11. Statistical analysis

All biochemical analyses were run in triplicate and entire experiment was repeated twice. Results were expressed as mean ± standard deviation (SD). For statistical analysis, the statistical software package version 11.0 (StatSoft, Inc., Tulsa, OK, USA) was used. Differences between means were first analyzed by ANOVA test and then Tukey test ($p < 0.05$).

## 3. Results and discussion

### 3.1. Determination of pH and viable LAB cells counts

In this study we have chosen 2 LAB strains for biotransformation of camu and soymilk that also have probiotic potential. Higher viability of potential probiotic bacterial cells in fermented food is critical for both successful fermentation and for improving human health relevant to nutritional quality. Physiochemical properties including pH significantly affect the viability of the bacterial strains, including LAB [18]. It has been observed in previous published study that lower phenolic concentration could promote growth of probiotic bacteria [19]. In another study, low concentration of tannins (0.1 or 0.2 mg/mL) did not inhibit *L. plantarum* growth; however at higher concentration (1 mg/mL) of same extract bacterial growth was inhibited [20]. Similarly, cranberry and lemon juices inhibited the growth of bacterial cells due to high acidity [21]. Therefore in this study the viability of the two targeted bacterial strains, *L. plantarum* and *L. helveticus* in soymilk combined with different concentrations (control, 0.5% and 1%) of freeze-dried and highly acidic camu–camu powder was evaluated (Table 1).

The reduction of pH values of fermented camu–camu and soymilk combination was more rapid with *L. plantarum* when compared to sample fermented with *L. helveticus* (Table 1). Yoon et al. [22] observed a similar trend in tomato and concluded that it could be due to the ability of *L. plantarum* to produce more lactic acid than other LAB species (*L. acidophilus*, *L. casei* and *L. delbrueckii*). Overall, addition of camu–camu powder decreased pH values due to the inherent high acidity of the fruit (Table 1). In this study difference in type of camu–camu powders (freeze and spray dried) did not have any significant effects on pH ($p < 0.05$) during fermentation (Table 1).

The results of viable LAB cell counts indicated that both LAB strains could grow well in soymilk with camu–camu powder, however, *L. helveticus* had better growth and stability ($p < 0.05$) when compared to *L. plantarum* after 72 h of fermentation (Table 1). Between these two LAB strains, *L. helveticus* is well known as protease producer and *L. plantarum* as tannase producer. Therefore, soymilk protein potentially contributed to the higher growth of *L. helveticus*. Addition of 0.5% and 1% of camu–camu powder did not result in differences in growth of *L. plantarum* ($p < 0.05$). In contrast, *L. helveticus* growth was inhibited with increasing concentration of camu-camu, especially when compared to the control.

Furthermore soymilk combined with spray-dried camu–camu powders and with different concentrations of gum arabic (6%, 12% and 18%) was used as substrate for *L. helveticus* fermentation. In previous studies, prebiotic properties have been observed in gum arabic and therefore have potential to provide stable substrate for growth of LAB and probiotic microorganisms [23]. Although, the addition of 0.5% of camu–camu powders did not show any significant difference

**Table 1**
pH measurement and LAB growth (Log CFU/mL) of samples of camu–camu powders in soymilk after fermentation with *Lactobacillus plantarum* and *Lactobacillus helveticus* at 0, 24, 48, and 72 h.

| Test | Microorg. | Conc. of powder | Powder | pH | | | | Log CFU/mL | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Time (h) | | | | Time (h) | | | |
| | | | | 0 h | 24 h | 48 h | 72 h | 0 h | 24 h | 48 h | 72 h |
| 1 | *L. plantarum* | 0% | w/o powder | 6.16 ± 0.02$^{a}$ | 4.15 ± 0.04$^{d}$ | 4.03 ± 0.07$^{de}$ | 4.03 ± 0.05$^{de}$ | 6.30 ± 0.05$^{bc}$ | 6.49 ± 0.14$^{a}$ | 6.29 ± 0.03$^{bc}$ | 5.01 ± 0.08$^{d}$ |
| | | 0.5% | Freeze-dried | 5.65 ± 0.07$^{b}$ | 3.94 ± 0.04$^{ef}$ | 3.82 ± 0.03$^{fg}$ | 3.80 ± 0.01$^{fg}$ | 6.40 ± 0.12$^{ab}$ | 6.48 ± 0.05$^{a}$ | 6.17 ± 0.09$^{c}$ | 4.92 ± 0.09$^{d}$ |
| | | 1% | Freeze-dried | 5.26 ± 0.06$^{c}$ | 3.85 ± 0.08$^{fg}$ | 3.75 ± 0.02$^{g}$ | 3.73 ± 0.02$^{g}$ | 6.30 ± 0.06$^{bc}$ | 6.52 ± 0.02$^{a}$ | 6.26 ± 0.07$^{c}$ | 4.98 ± 0.10$^{d}$ |
| 2 | *L. helveticus* | 0% | w/o powder | 6.42 ± 0.08$^{a}$ | 6.23 ± 0.02$^{b}$ | 5.86 ± 0.09$^{c}$ | 5.21 ± 0.04$^{e}$ | 5.82 ± 0.07$^{c}$ | 5.28 ± 0.09$^{de}$ | 6.49 ± 0.12$^{b}$ | 6.78 ± 0.08$^{a}$ |
| | | 0.5% | Freeze-dried | 5.83 ± 0.06$^{c}$ | 5.43 ± 0.07$^{d}$ | 4.02 ± 0.06$^{g}$ | 3.92 ± 0.02$^{g}$ | 5.80 ± 0.03$^{c}$ | 4.95 ± 0.05$^{f}$ | 5.10 ± 0.07$^{ef}$ | 5.85 ± 0.09$^{c}$ |
| | | 1% | Freeze-dried | 5.36 ± 0.05$^{de}$ | 4.51 ± 0.09$^{f}$ | 4.04 ± 0.05$^{g}$ | 3.98 ± 0.03$^{g}$ | 5.81 ± 0.04$^{c}$ | 4.41 ± 0.11$^{g}$ | 4.38 ± 0.10$^{g}$ | 5.38 ± 0.07$^{d}$ |
| 3 | *L. helveticus* | 0% | w/o powder | 6.48 ± 0.06$^{a}$ | 6.27 ± 0.01$^{ab}$ | 5.94 ± 0.08$^{bc}$ | 5.28 ± 0.11$^{de}$ | 5.82 ± 0.07$^{d}$ | 5.28 ± 0.09$^{g}$ | 6.49 ± 0.12$^{b}$ | 6.78 ± 0.08$^{a}$ |
| | | 0.5% | Freeze-dried | 5.91 ± 0.08$^{cd}$ | 5.47 ± 0.01$^{d}$ | 5.05 ± 0.02$^{ef}$ | 4.30 ± 0.09$^{g}$ | 5.80 ± 0.03$^{de}$ | 4.95 ± 0.05$^{h}$ | 5.10 ± 0.07$^{gh}$ | 5.85 ± 0.09$^{cd}$ |
| | | 0.5% | 6% GA | 6.10 ± 0.08$^{ab}$ | 5.51 ± 0.02$^{d}$ | 5.26 ± 0.06$^{de}$ | 5.06 ± 0.06$^{ef}$ | 5.80 ± 0.09$^{de}$ | 4.38 ± 0.08$^{i}$ | 5.59 ± 0.05$^{ef}$ | 5.98 ± 0.07$^{cd}$ |
| | | 0.5% | 12% GA | 6.18 ± 0.07$^{ab}$ | 5.58 ± 0.02$^{cd}$ | 5.03 ± 0.01$^{ef}$ | 5.00 ± 0.12$^{ef}$ | 5.85 ± 0.03$^{cd}$ | 5.52 ± 0.04$^{f}$ | 5.84 ± 0.07$^{d}$ | 6.06 ± 0.08$^{c}$ |
| | | 0.5% | 18% GA | 6.24 ± 0.07$^{ab}$ | 5.46 ± 0.07$^{d}$ | 5.02 ± 0.08$^{ef}$ | 4.73 ± 0.11$^{f}$ | 5.90 ± 0.02$^{cd}$ | 5.91 ± 0.03$^{cd}$ | 5.89 ± 0.04$^{cd}$ | 5.81 ± 0.09$^{d}$ |
| 4 | *L. helveticus* | 0% | w/o powder | 6.04 ± 0.01$^{a}$ | 6.22 ± 0.03$^{a}$ | 5.71 ± 0.02$^{b}$ | 5.31 ± 0.03$^{de}$ | 5.82 ± 0.07$^{c}$ | 5.28 ± 0.09$^{de}$ | 6.49 ± 0.12$^{b}$ | 6.78 ± 0.08$^{a}$ |
| | | 1% | Freeze-dried | 5.19 ± 0.06$^{e}$ | 4.99 ± 0.05$^{f}$ | 3.74 ± 0.04$^{ij}$ | 3.74 ± 0.02$^{ij}$ | 5.81 ± 0.04$^{c}$ | 4.41 ± 0.11$^{h}$ | 4.38 ± 0.10$^{h}$ | 5.38 ± 0.07$^{d}$ |
| | | 1% | 6% GA | 5.47 ± 0.01$^{cd}$ | 4.50 ± 0.13$^{h}$ | 3.74 ± 0.10$^{ij}$ | 3.64 ± 0.09$^{j}$ | 5.96 ± 0.07$^{c}$ | 4.82 ± 0.15$^{g}$ | 5.15 ± 0.11$^{def}$ | 5.80 ± 0.05$^{c}$ |
| | | 1% | 12% GA | 5.65 ± 0.02$^{bc}$ | 5.12 ± 0.11$^{ef}$ | 3.88 ± 0.12$^{i}$ | 3.61 ± 0.04$^{j}$ | 5.98 ± 0.04$^{c}$ | 4.92 ± 0.11$^{fg}$ | 5.10 ± 0.06$^{ef}$ | 5.88 ± 0.09$^{c}$ |
| | | 1% | 18% GA | 5.83 ± 0.01$^{b}$ | 5.36 ± 0.06$^{de}$ | 4.99 ± 0.03$^{f}$ | 4.76 ± 0.06$^{g}$ | 5.88 ± 0.09$^{c}$ | 5.10 ± 0.06$^{ef}$ | 5.14 ± 0.12$^{def}$ | 5.80 ± 0.07$^{c}$ |

Values are expressed as means ± SD. $^{a-j}$Different letters in each Test denote significant differences for pH and log CFU/mL, respectively ($p < 0.05$).

($p < 0.05$), but higher preservation of *L. helveticus* was observed when 1% spray-dried camu–camu powder was added in comparison with freeze-dried powder ($p < 0.05$).

### 3.2. Total soluble phenolic content

Total soluble phenolic content (TSP) of fermented camu–camu soymilk combinations was determined using Folin-Ciocalteau method at 0, 24, 48 and 72 h. Phenolic content increased when camu–camu powder was added in soymilk (Fig. 1A and B). Overall, total phenolic contents did not change and remained stable after 72 h of fermentation. Both LAB strains had similar trends, and did not show any significant differences ($p < 0.05$) at 0, 48, and 72 h. However, at 24 h a decrease of 30% of TSP content was observed with both LAB strains. These results suggested a potential initial utilization and potential rearrangement of phenolics to polymeric forms at 24 h, and then further release of the soluble free phenolic compounds due to fermentation at 48 h. Apostolidis et al. [24] found similar decrease in phenolic content when cranberry was fermented. Similarly, McCue et al. [25] suggested that phenolics and other polymeric forms like lignans were prone to polymerization and metabolic rearrangement due to high antioxidant activity during early stages of fermentation. Besides that, production of peroxidase, β-glucosidase and laccase were also observed after 24 h of fermentation [26] that contribute to less soluble forms of phenolics. Especially *L. plantarum* can produce tannase after 24 h of growth [27].

As previously observed, soluble phenolic contents of fermented samples with spray-dried camu–camu powders were lower than freeze-dried powders which were mainly due to the loss in the drying process, especially due to microencapsulation with gum arabic ([9], Fig. 1C and D). Adding 0.5% of spray-dried camu–camu powders (m/v) with gum arabic (6%, 12% and 18%) did not result in any significant differences in TSP content ($p < 0.05$) during the fermentation (Fig. 1C). On the other hand, when 1% of spray-dried powder was added the TSP content decreased, with exception in camu–camu powder with 18% gum arabic after 72 h fermentation (Fig. 1D). Two probable mechanisms were considered for the increased phenolic contents in camu–camu powder with 18% gum arabic at later stages of fermentation. Gum arabic is polysaccharide and the sugars linkages are D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and 4-O-methyl glucuronic acid [23]. Probiotic LAB strains potentially released the sugars, which reacted with the reagent Folin-Ciocalteau and overestimated the soluble phenolic content. Another possibility is that gum arabic stimulated the LAB to produce more enzymes responsible for depolymerization of phenolic compounds with higher molecular weight and released more soluble phenolics. Overall, the maintenance of higher level of soluble phenolic content in camu–camu and soymilk combination during fermentation has significant relevance for designing new functional ingredients and probiotic beverages using LAB strains. Such strategy has diverse benefits such as improving nutritional quality, enhancing shelf-life, and improving food safety parameters.

### 3.3. Antioxidant activity by DPPH free radical scavenging assay

Total antioxidant activity of fermented camu–camu and soymilk combination was determined using DPPH free radical scavenging assay (Fig. 1A and B). Previous studies have found high antioxidant activity in soy fermented products [28]. In this study soymilk without camu–camu powders had lower antioxidant activity after 72 h of fermentation (33.5% and 40.6% DPPH inhibition for *L. plantarum* and *L. helveticus*, respectively) (Fig. 1A and B). In contrast, soymilk with camu–camu powders (1%) had significantly higher antioxidant activity (around 90% DPPH-inhibition) even after 72 h fermentation (Fig. 1). Both freeze-dried and spray-dried camu–camu powder with soymilk had high antioxidant activity even after 72 h fermentation. The maintenance of high antioxidant activity during the fermentation is probably due to the conversion of glycosides to aglycone forms by enzymes

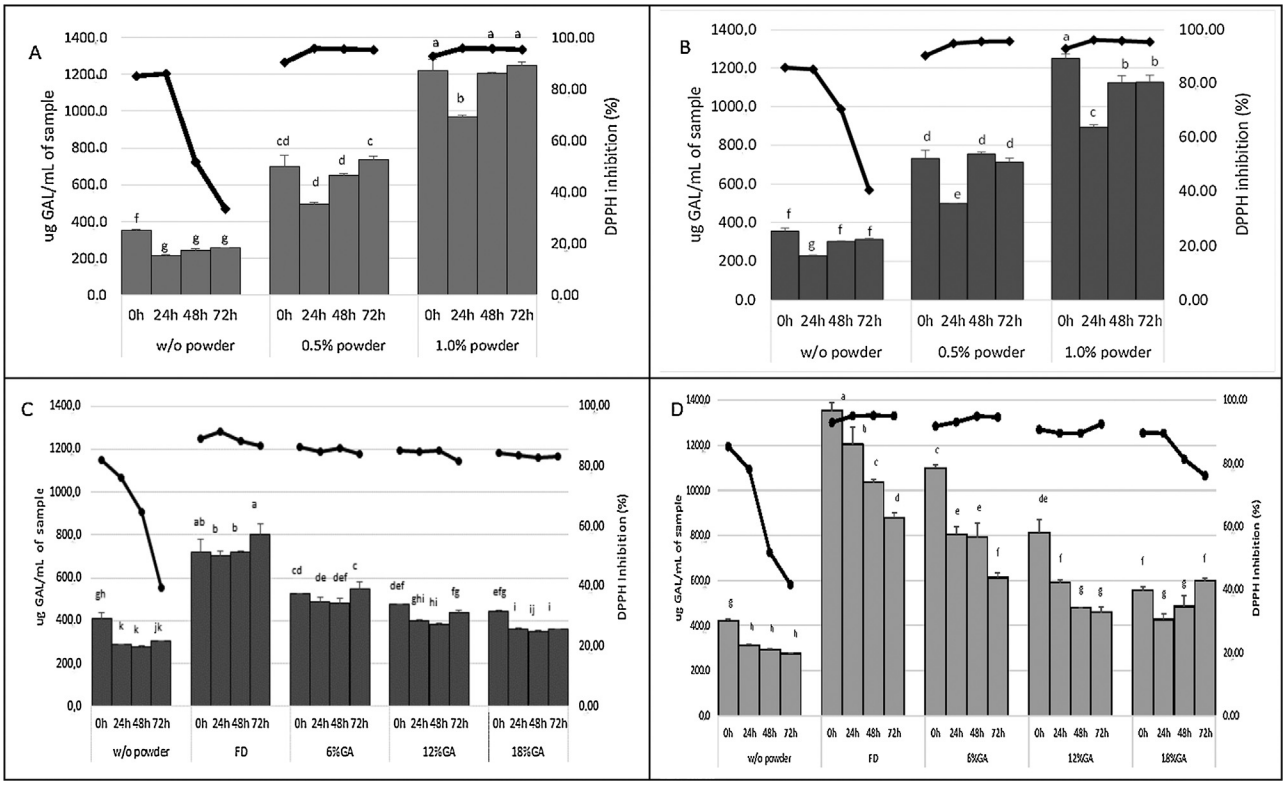


**Fig. 1.** Total phenolics contents (μg GAE/mL of sample) and DPPH inhibition (%) of freeze-dried camu–camu powder in soymilk (0.5% and 1.0%) during fermentation by *Lactobacillus plantarum* (A) *Lactobacillus helveticus* (B) at 0, 24, 48 and 72 h. Total phenolic content and DPPH inhibition (%) of freeze-dried (FD) and spray-dried camu–camu powders (0.5%) (C) and (1.0%) (D) with 6%, 12% and 18% of gum arabic after fermentation with *Lactobacillus helveticus*.

produced through fermentation process. Besides this, synergism between phenolic compounds and other bioactive compounds may have contributed to this result of high antioxidant activity. Therefore, combination of soymilk with camu–camu powders improved the total antioxidant activity and this was sustained over the fermentation period of 72 h. This high antioxidant enrichment approach has significant relevance to target chronic oxidative stress associated with T2D and chronic inflammation associated with other NCDs.

### *3.4. Anti-diabetic properties through α-amylase, α-glucosidase enzyme inhibitory activity*

Plant-based foods rich in phenolics have shown potential to inhibit α-amylase and α-glucosidase enzymes, which are key targets for pharmaceutical drugs to improve glucose metabolism [5,29]. Inhibiting these enzymes slows down the breakdown of polysaccharide and starch in the gut and subsequently reduces the rate of absorption of glucose in the small intestine which in turn helps to control postprandial hyperglycemia linked to T2D. In this study, α-amylase and α-glucosidase inhibitory activities of LAB fermented soymilk with and without freeze and spray-dried camu–camu powder (0.5% and 1%, m/v) were evaluated after 0, 24, 48 and 72 h using model *in vitro* assays.

Previous results have shown very high α-glucosidase and moderate α-amylase inhibitory activity in aqueous extract of camu–camu [7]. Therefore camu–camu with high anti-hyperglycemic properties is ideal target to design functional food ingredients for countering early stages of T2D [7]. The aim of this study was to improve and evaluate the changes in the inhibitory activities of these key T2D targeted enzymes during LAB fermentation, which could offer alternative use of camu–camu powder as probiotic beverages for human health relevant applications. Two LAB strains used in this study resulted in very different trends for α-amylase inhibitory activity (Fig. 2). For *L. plantarum* (Fig. 2A) the highest α-amylase enzyme inhibitory activity was observed in soymilk with 1% of camu–camu powder after 24 and 48 h of fermentation. On the contrary, for *L. helveticus* the highest α-amylase enzyme inhibitory activity was found after 72 h of fermentation in soymilk with 1% of camu–camu powder (Fig. 2B).

In this study, fermented sample of freeze-dried camu–camu powder combined with soymilk had higher α-amylase enzyme inhibitory activity when compared with fermented sample of spray dried camu–camu powder combined in soymilk. When 0.5% of camu–camu powder was added in soymilk and then fermented with LAB, 2 times greater α-amylase enzyme inhibitory activity was observed in freeze-dried powder after 48 h and 4 times greater after 72 h of fermentation when compared to the control (Fig. 2C). Further, addition of 1% of camu–camu powder also enhanced α-amylase enzyme inhibitory activity and highest inhibitory activity was observed in freeze-dried camu soymilk combination (Fig. 2D, $p < 0.05$). The higher α-amylase enzyme inhibitory activity in freeze-dried camu–camu powder was also positively correlated with higher TSP content of freeze-dried camu-camu.

In previous study, aqueous extracts of camu–camu powders had very high α-glucosidase inhibitory activity [7]. The addition of higher concentrations of camu–camu powders in soymilk significantly enhanced α-glucosidase inhibitory activity even after 72 h of fermentation with LAB (Fig. 3C and D). Overall, the α-glucosidase inhibitory activity remained very high during the entire period of fermentation (at 24, 48, and 72 h).

No significant differences in α-glucosidase inhibitory activity were observed between types of camu–camu powders used in this study ($p < 0.05$). Addition of 0.5% and 1% of camu–camu powder in soymilk significantly increased α-glucosidase inhibitory activity after 48 and 72 h LAB fermentation. Alpha-glucosidase inhibitory activity increased 2 times with addition of 0.5% freeze dried camu powder in soymilk after 48 h of LAB fermentation when compared to the control (fermented soymilk without camu-camu) (Fig. 3C). When 1% of freeze-dried powder was added the α-glucosidase inhibitory activity increased 2 times compared to control at 24 h after fermentation and reached the highest α-glucosidase inhibitory activity (around 90%) after 48 h of

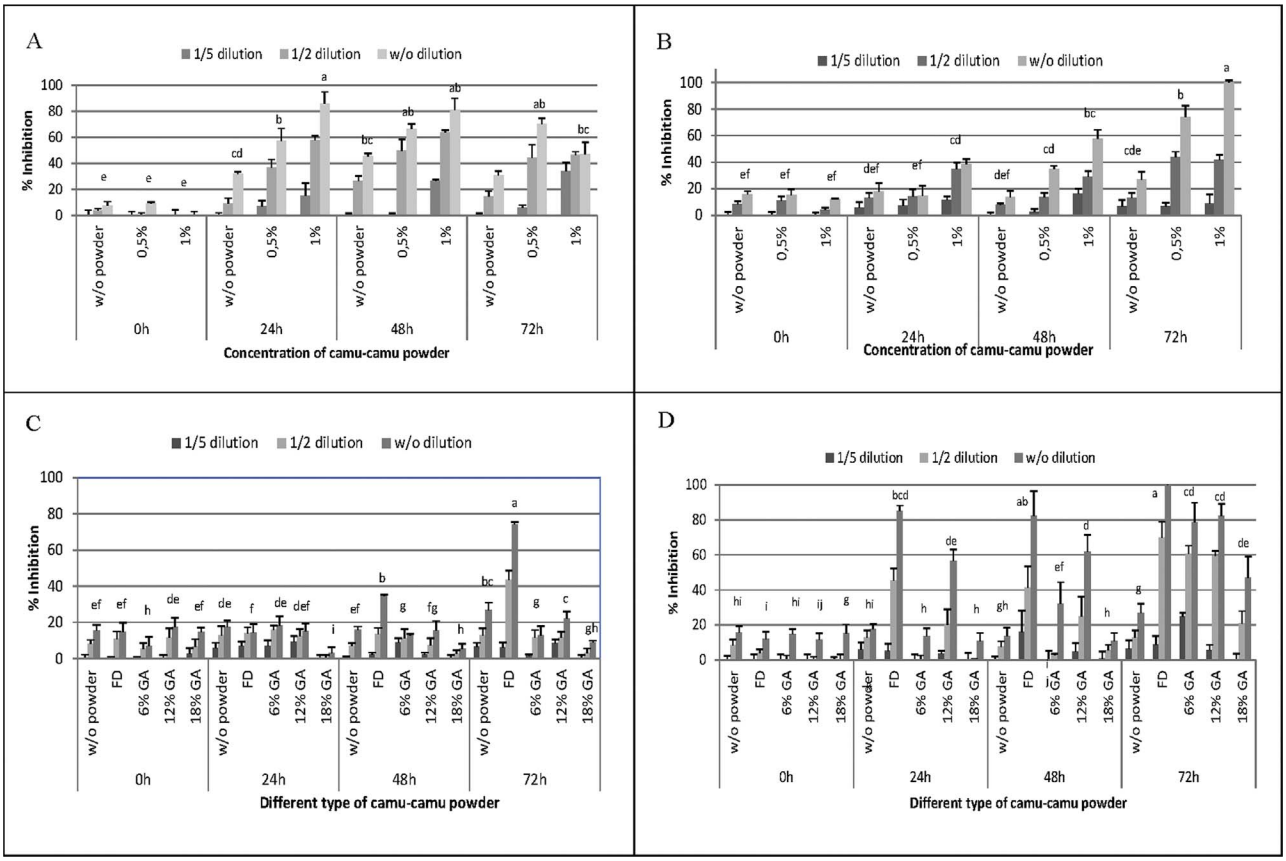


**Fig. 2.** α-Amylase inhibitory activity of freeze-dried camu–camu powder in soymilk (0.5% and 1.0%) during fermentation by *Lactobacillus plantarum* (A) *Lactobacillus helveticus* (B) at 0, 24, 48 and 72 h α −amylase inhibitory activity of freeze-dried (FD) and spray-dried camu–camu powders(0.5%) (C) and (1.0%) (D) with 6%, 12% and 18% of gum arabic after fermentation with *Lactobacillus helveticus*.

fermentation (Fig. 3D).

Phenolic compounds of plant-based foods including fruits have been reported as major contributors for inhibition of these anti-hyperglycemia-linked enzymes in previous studies [30,31]. Major phenolic compounds detected in camu–camu were ellagic acid, ellagitannins, anthocyanins and quercetin [32,7]. Degradation and/or mobilization of these specific phenolic compounds from camu–camu after LAB fermentation potentially contributed to the variations in α-amylase and α-glucosidase inhibitory activities. For example, tannins are effective against α-amylase enzyme inhibitory activity in a previous study [33]. Therefore, production of tannase by LAB fermentation potentially yield tannins and may have contributed to moderate α-amylase inhibitory activity of camu–camu and soymilk combination. On the other hand, anthocyanins, ellagic acid, and quercetin exhibited significant α-glucosidase inhibitory activity in previous study [34]. Increased concentration of aglycones by β-glucosidase activity of LAB may have resulted in higher α-glucosidase inhibitory activity of LAB fermented camu–camu and soymilk combination. Overall the results of this study shows significant potential for using safe LAB-based fermentation strategy to develop anti-hyperglycemia relevant probiotic beverage from camu–camu and soymilk combination.

### *3.5. Anti-hypertensive properties through angiotensin-I-converting enzyme (ACE) inhibitory activity*

Hypertension is a common risk factor associated with T2D. Inhibition of ACE is an important target for pharmaceutical drugs to control hypertension [31]. Phenolics from plant based food have shown ACE inhibitory activity and have potential to prevent risk for developing high blood pressure commonly associated with T2D [35]. Quercetin and cyanidin-3-*O*-glucoside have shown significant ACE enzyme inhibitory activity in previous studies [36]. Although, ellagic acid did not show any ACE enzyme inhibitory activity [15], isoflavones, especially genistein, have been reported to have anti-hypertensive properties in *in vivo* models [37].

In this study, high ACE enzyme inhibitory activity was observed (around 90%) for all LAB soymilk fermented sample (Table 2), even without addition of camu–camu powders. Therefore, isoflavones and peptides of soymilk substrate could have contributed to the high ACE enzyme inhibitory activity in LAB fermented soymilk with and without addition of camu-camu. Both LAB strains had similar trend, as fermentation progressed over time ACE enzyme inhibitory activity increased after 72 h when 0.5% and 1% of camu–camu powders were added. However, there were no significant differences ($p < 0.05$) when 0.5% of different types of camu–camu powders were added. When 1% of powders were added, spray-dried powders (with 6% and 12% of gum arabic) had slightly higher ACE enzyme inhibitory activity than control. Since camu–camu powder did not exhibit ACE inhibition [7], those results suggested synergism of compounds present in camu–camu and soymilk combination. But irrespective of the specific roles of phenolic compounds to induce ACE enzyme inhibitory activity, the results of this study have shown significant promises to target LAB fermented camu–camu and soymilk combinations for targeting hypertension, commonly associated with T2D.

## 4. Conclusions

LAB mediated fermentation strategy was strategically used to improve human health relevant bioactive profiles and associated anti-hyperglycemic and anti-hypertensive properties in camu–camu and soymilk combinations integrating the benefits of both plant sources. Lactic acid bacteria fermentation of camu–camu (especially freeze dried

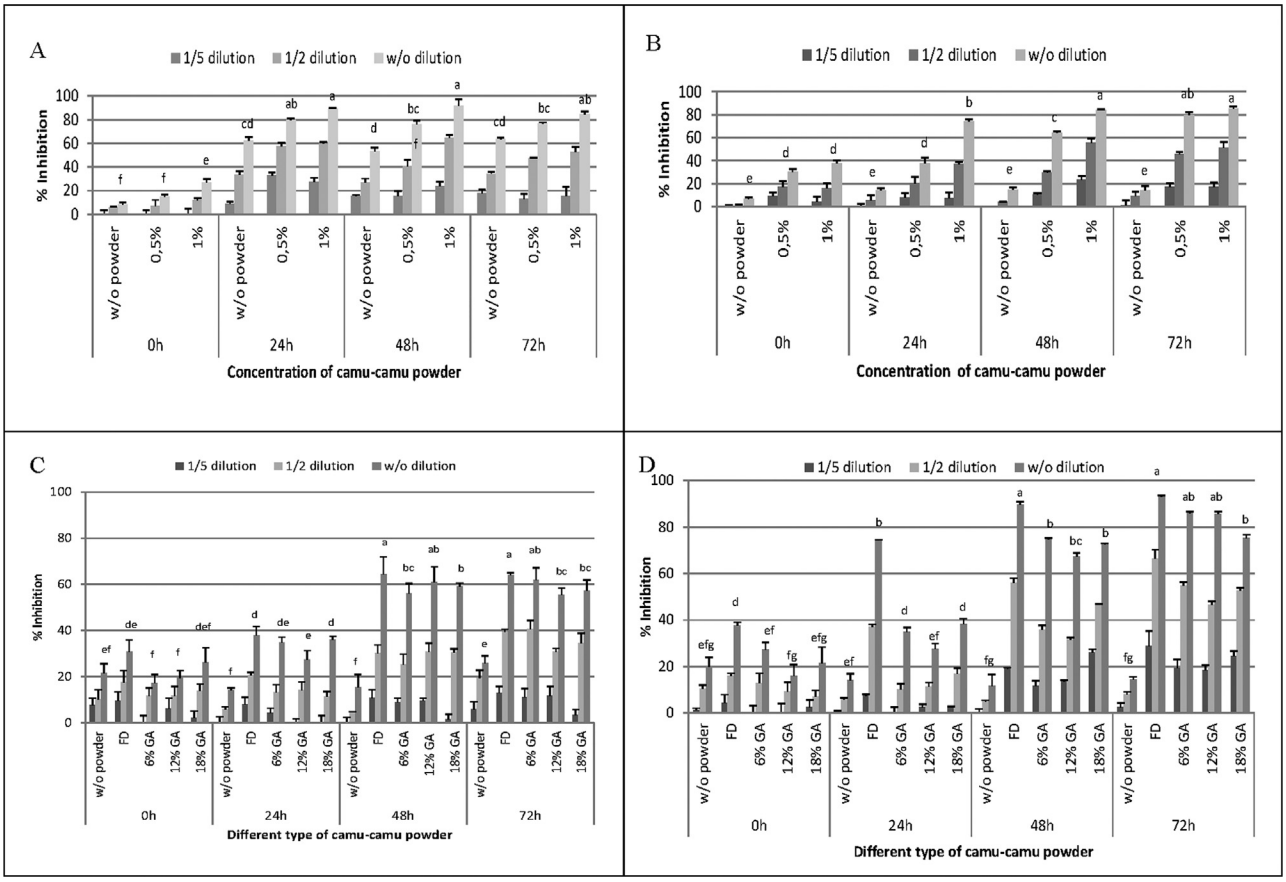


**Fig. 3.** α −Glucosidase inhibitory activity of freeze-dried camu–camu powder in soymilk (0.5% and 1.0%) during fermentation by *Lactobacillus plantarum* (A) *Lactobacillus helveticus* (B) at 0, 24, 48 and 72 h α −glucosidase inhibitory activity of freeze-dried (FD) and spray-dried camu–camu powders(0.5%) (C) and (1.0%) (D) with 6%, 12% and 18% of gum arabic after fermentation with *Lactobacillus helveticus*.

form) in soymilk resulted in high α-amylase and α-glucosidase inhibitory activity *in vitro*, which has significant relevance to target hyperglycemia-linked oxidative stresses. Furthermore LAB fermentation of camu–camu and soymilk combination with *L. plantarum* and *L. helveticus* did not change the soluble phenolic content even after 72 h. The presence of soymilk also resulted in high ACE enzyme inhibitory activity and it even improved with *L. plantarum* fermentation and thus camu–camu and soymilk combinations could be an ideal design to develop functional probiotic beverage for targeting hypertension, which is a common risk factor associated with T2D. This *in vitro* study provides significant insights and scientific rationale for designing probiotic beverages from phenolic bioactive enriched camu–camu using LAB

**Table 2**
Angiotensin I-converting enzyme (ACE) inhibitory activity of fermented camu–camu mixed in soymilk by *Lactobacillus plantarum* and *Lactobacillus helveticus* at 0, 24, 48, and 72 h.

| Test | Microorg. | Conc. of powder | Powder | % ACE inhibition | | | |
|---|---|---|---|---|---|---|---|
| | | | | Time (h) | | | |
| | | | | 0 h | 24 h | 48 h | 72 h |
| *1* | *L. plantarum* | 0% | w/o powder | 90.1 ± 0.6$^{b}$ | 86.5 ± 0.3$^{d}$ | 86.5 ± 0.6$^{d}$ | 87.3 ± 0.7$^{cd}$ |
| | | 0.5% | Freeze-dried | 88.9 ± 0.3$^{bc}$ | 89.2 ± 1.7$^{bc}$ | 90.6 ± 1.3$^{b}$ | 94.5 ± 0.2$^{a}$ |
| | | 1% | Freeze-dried | 89.8 ± 0.9$^{b}$ | 92.8 ± 0.3$^{a}$ | 94.5 ± 0.1$^{a}$ | 94.0 ± 1.0$^{a}$ |
| *2* | *L. helveticus* | 0% | w/o powder | 89.5 ± 1.3$^{cd}$ | 90.2 ± 0.1$^{c}$ | 90.2 ± 0.2$^{c}$ | 89.4 ± 0.2$^{cd}$ |
| | | 0.5% | Freeze-dried | 88.9 ± 1.2$^{d}$ | 92.8 ± 0.2$^{b}$ | 90.4 ± 0.4$^{c}$ | 94.3 ± 0.1$^{a}$ |
| | | 1% | Freeze-dried | 93.5 ± 1.2$^{ab}$ | 90.5 ± 0.9$^{c}$ | 93.8 ± 1.5$^{ab}$ | 93.7 ± 0.5$^{ab}$ |
| *3* | *L. helveticus* | 0% | w/o powder | 79.0 ± 0.8$^{ab}$ | 69.3 ± 0.9$^{cde}$ | 73.1 ± 0.7$^{abcde}$ | 67.2 ± 3.4$^{de}$ |
| | | 0.5% | Freeze-dried | 79.8 ± 2.3$^{a}$ | 77.0 ± 0.3$^{abcd}$ | 78.2 ± 1.0$^{abc}$ | 74.0 ± 0.5$^{abcde}$ |
| | | 0.5% | 6% GA | 66.0 ± 8.1$^{e}$ | 78.0 ± 0.6$^{abc}$ | 75.6 ± 0.6$^{abcde}$ | 68.5 ± 1.3$^{cde}$ |
| | | 0.5% | 12% GA | 78.9 ± 1.4$^{ab}$ | 76.4 ± 1.3$^{abcd}$ | 72.8 ± 0.5$^{abcde}$ | 68.5 ± 0.5$^{cde}$ |
| | | 0.5% | 18% GA | 79.4 ± 0.7$^{ab}$ | 74.9 ± 0.4$^{abcde}$ | 69.7 ± 2.2$^{bcde}$ | 68.8 ± 0.5$^{cde}$ |
| *4* | *L. helveticus* | 0% | w/o powder | 87.8 ± 0.5$^{h}$ | 93.0 ± 0.2$^{c}$ | 91.9 ± 0.2$^{e}$ | 89.8 ± 0.2$^{g}$ |
| | | 1% | Freeze-dried | 91.9 ± 1.4$^{e}$ | 93.7 ± 1.2$^{ab}$ | 94.2 ± 0.5$^{a}$ | 92.1 ± 0.4$^{de}$ |
| | | 1% | 6% GA | 93.2 ± 0.2$^{bc}$ | 92.9 ± 0.2$^{bc}$ | 90.7 ± 0.1$^{f}$ | 94.1 ± 0.3$^{a}$ |
| | | 1% | 12% GA | 92.7 ± 0.1$^{cd}$ | 93.9 ± 0.1$^{bc}$ | 90.8 ± 0.4$^{f}$ | 94.2 ± 0.3$^{a}$ |
| | | 1% | 18% GA | 92.8 ± 0.4$^{cd}$ | 93.1 ± 0.2$^{bc}$ | 91.7 ± 0.3$^{e}$ | 92.3 ± 0.1$^{de}$ |

Values are expressed as means ± SD. $^{a–h}$Different superscript letters in a test denote significant differences for ACE inhibition activity ($p < 0.05$).

fermentation in a soymilk substrate, which can then be targeted for dietary management of early stages T2D and associated hypertension risk.

## Acknowledgements

We would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing fellowships and support to carry out this research.

## References

[1] S.E. Kahn, M.E. Cooper, S. Del Prato, Pathophysiology and treatment of T2D: perspectives on the past, present, and future, Lancet 383 (2014) 1068–1083.
[2] World health organization (WHO), http://www.who.int/mediacentre/factsheets/fs297/en/, (2017) (Accessed 26.02.17).
[3] T. Seuring, O. Archangelidi, M. Suhrcke, The economic costs of T2D: a global systematic review, Pharmacoeconomics 33 (8) (2015) 811–831.
[4] D.P. Belobrajdic, A.R. Bird, The potential role of phytochemicals in wholegrain cereals for the prevention of type-2 diabetes, Nutr. J. 12 (62) (2013) 1–12.
[5] F.F. Anhê, Y. Desjardins, G. Pilon, S. Dudonné, M.I. Genovese, F.M. Lajolo, A. Marette, Polyphenols and T2D: a prospective review, Pharma Nutr. 1 (4) (2013) 105–114.
[6] F. Persson, M. Lindhardt, P. Rossing, H.H. Parving, Prevention of microalbuminuria using early intervention with renin-angiotensin system inhibitors in patients with T2D: a systematic review, J. Renin Angiotensin Aldosterone Syst. 17 (3) (2016) 1–10.
[7] A. Fujita, D. Sarkar, S. Wu, E. Kennelly, K. Shetty, M.I. Genovese, Evaluation of Phenolic-linked bioactives of camu–camu (*Myrciaria dubia* Mc Vaugh) for anti-hyperglycemia, antihypertension, antimicrobial properties and cellular respiration, Food Res. Int. 77 (2015) 194–203.
[8] T. Inoue, H. Komoda, T. Uchida, K. Node, Tropical fruit camu–camu (*Myrciaria dubia*) has anti-oxidative and anti-inflammatory properties, J. Cardiol. 52 (2008) 127–132.
[9] A. Fujita, K. Borges, R. Correia, B.D.G.M. Franco, M.I. Genovese, Impact of spouted bed drying on bioactive compounds, antimicrobial and antioxidant activities of commercial frozen pulp of camu–camu (*Myrciaria dubia* Mc Vaugh), Food Res. Int. 54 (1) (2013) 495–500.
[10] K.H. Steinkraus, Nutritional significance of fermented foods, Food Res. Int. 27 (3) (1994) 259–267.
[11] X. Bian, Z. Muhammad, S.E. Evivie, G.W. Luo, M. Xu, G.C. Huo, Screening of antifungal potentials of *Lactobacillus helveticus* KLDS 1.8701 against spoilage microorganism and their effects on physicochemical properties and shelf life of fermented soybean milk during preservation, Food Control 66 (2016) 183–189.
[12] C. Ankolekar, K. Johnson, M. Pinto, D. Johnson, R.G. Labbe, D. Greene, K. Shetty, Fermentation of whole apple juice using *Lactobacillus acidophilus* for potential dietary management of hyperglycemia, hypertension, and modulation of beneficial bacterial responses, J. Food Biochem. 36 (6) (2012) 718–738.
[13] D. Hervert-Hernandez, C. Pintado, R. Rotger, I. Goni, Stimulatory role of grape pomace polyphenols on Lactobacillus acidophilus growth, Int. J. Food Microbiol. 136 (1) (2009) 119–122.
[14] K. Shetty, O.F. Curtis, R.E. Levin, R. Wikowsky, W. Ang, Prevention of verification associated with *invitro* shoot culture of oregano (*Origanum vulgare*) by Pseudomonas spp, J. Plant Physiol. 147 (1995) 447–451.
[15] Y.I. Kwon, D.A. Vattem, K. Shetty, Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension, Asia Pac. J. Clin. Nutr. 15 (2006) 107–118.
[16] Worthington Biochemical Corp, Alpha amylase, in: V. Worthington (Ed.), Worthington Enzyme Manual, Freehold, 1993, pp. 36–41.
[17] Worthington Biochemical Corp, Maltase-a-glucosidase, in: V. Worthington (Ed.), Worthington Enzyme Manual, Freehold, 1993(pp. 261).
[18] A. Patel, J.B. Prajapati, O. Holst, A. Ljungh, Determining probiotic potential of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented food products, Food Biosci. 5 (2014) 27–33.
[19] J.A. Curiel, R. Muñoz, F. López de Felipe, pH and dose-dependent effects of quercetin on the fermentation capacity of *Lactobacillus plantarum*, LWT Food Sci. Technol. 43 (6) (2010) 926–933.
[20] N. Rozès, C. Peres, Effects of phenolic compounds on the growth and the fatty acid composition of *Lactobacillus plantarum*, Appl. Microbiol. Biotechnol. 49 (1) (1998) 108–111.
[21] S. Nualkaekul, D. Charalampopoulos, Survival of *Lactobacillus plantarum* in model solutions and fruit juices, Int. J. Food Microbiol. 146 (2011) 111–117.
[22] K.Y. Yoon, E.E. Woodams, Y.D. Hang, Probiotication of tomato juice by lactic acid bacteria, J. Microbiol. 42 (2004) 315–318.
[23] G.O. Phillips, T. Ogasawara, K. Ushida, The regulatory and scientific approach to defining gum arabic (*Acacia senegal* and *Acacia seyal*) as a dietary fibre, Food Hydrocolloids 22 (1) (2008) 24–35.
[24] E. Apostolidis, Y. Kwon, R. Shinde, R. Ghaedian, K. Shetty, Inhibition of Helicobacter pylori by fermented milk and soymilk using select lactic acid bacteria and link to enrichment of lactic acid and phenolic content, Food Biotechnol. 25 (1) (2011) 58–76.
[25] P. McCue, A. Horii, K. Shetty, Solid-state bioconversion of phenolic antioxidants from defatted soybean powders by *Rhizopus oligosporus*: role of carbohydrate-cleaving enzymes, J. Food Biochem. 27 (6) (2003) 501–514.
[26] P. McCue, K. Shetty, Phenolic antioxidant mobilization during yogurt production from soymilk using Kefir cultures, Process Biochem. 40 (5) (2005) 1791–1797.
[27] H. Rodríguez, J.A. Curiel, J.M. Landete, B. de las Rivas, F. López de Felipe, C. Gómez-Cordovés, J.M. Mancheño, R. Muñoz, Food phenolics and lactic acid bacteria, Int. J. Food Microbiol. 132 (2-3) (2009) 79–90.
[28] C.H. Lin, Y.T. Wei, C.C. Chou, Enhanced antioxidative activity of soybean koji prepared with various filamentous fungi, Food Microbiol. 23 (7) (2006) 628–633.
[29] K. Hanhineva, R. Törrönen, I. Bondia-Pons, J. Pekkinen, M. Kolehmainen, H. Mykkänen, K. Poutanen, Impact of dietary polyphenols on carbohydrate metabolism, Int. J. Mol. Sci. 11 (4) (2010) 1365–1402.
[30] D. Sarkar, C. Ankolekar, M. Pinto, K. Shetty, Dietary functional benefits of Bartlett and Starkrimson pears for potential management of hyperglycemia, hypertension and ulcer bacteria Helicobacter pylori while supporting beneficial probiotic bacterial response, Food Res. Int. 69 (2015) 80–90.
[31] M.D.S. Pinto, Y.I. Kwon, E. Apostolidis, F.M. Lajolo, M.I. Genovese, K. Shetty, Functionality of bioactive compounds in Brazilian strawberry (*Fragaria x ananassa* Duch.) cultivars: evaluation of hyperglycemia and hypertension potential *using in vitro* Models, J. Agric. Food Chem. 56 (2008) 4386–4392.
[32] J.C.S. de Azevêdo, A. Fujita, E.L. de Oliveira, M.I. Genovese, Correia RTP. Dried camu–camu (*Myrciaria dubia* H.B.K. Mc Vaugh) industrial residue: a bioactive-rich Amazonian powder with functional attributes, Food Res. Int. 62 (2014) 934–940.
[33] G.J. McDougall, F. Shpiro, P. Dobson, P. Smith, A. Blake, D. Stewart, Different polyphenolic components of soft fruits inhibit α-Amylase and α-Glucosidase, J. Agric. Food Chem. 53 (7) (2005) 2760–2766.
[34] Q. You, F. Chen, X. Wang, Y. Jiang, S. Lin, Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase, LWT Food Sci. Technol. 46 (1) (2012) 164–168.
[35] C. Manach, G. Williamson, C. Morand, A. Scalbert, C. Rémésy, Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, Am. J. Clin. Nutr. 81 (2005) 230–242.
[36] N. Balasuriya, H.P. Rupasinghe, Antihypertensive properties of flavonoid-rich apple peel extract, Food Chem. 135 (4) (2012) 2320–2325.
[37] R.L. Edwards, T. Lyon, S.E. Litwin, A. Rabovsky, J.D. Symons, T. Jalili, Quercetin reduces blood pressure in hypertensive subjects, J. Nutr. 137 (11) (2007) 2405–2411.