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Improving Phenolic Bioactive-Linked Functional Qualities of Sweet Potatoes Using Beneficial Lactic Acid Bacteria-Based Biotransformation Strategy

Pradeepika Chintha ^{1,2}, Dipayan Sarkar ¹, Kenneth Pecota ³, Munevver Dogramaci ⁴ and Kalidas Shetty ^{1,*}

¹ Department of Plant Sciences, North Dakota State University, Fargo, ND 58108, USA; pradeepika.chintha@icar.gov.in (P.C.); dipayan.sarkar@ndsu.edu (D.S.)

² ICAR-Central Tuber Crop Research Institute, Thiruvananthapuram 695017, Kerala, India

³ Horticultural Science, North Carolina State University, Raleigh, NC 27695, USA; kypocota@ncsu.edu

⁴ Agricultural Research Service, Sugarbeet and Potato Research Unit, Edward T. Schafer Agricultural Research Center, United States Department of Agriculture, Fargo, ND 58102, USA; munevver.dogramaci@usda.gov

* Correspondence: kalidas.shetty@ndsu.edu; Tel.: +1-(701)-231-5058

Abstract: Beneficial lactic acid bacteria (LAB) based fermentation is an effective biotransformation strategy to preserve and improve the human health-supporting functional qualities of plant-based food substrates. In this study, a food grade strain of *Lactiplantibacillus plantarum* was recruited to improve the retention, stability, and bioavailability of phenolic bioactives to enhance the antioxidant, anti-hyperglycemic, and anti-hypertensive functional qualities of three flesh-colored sweet potato varieties, Murasaki (off-white-fleshed), Evangeline (orange-fleshed), and NIC-413 (purple-fleshed). Liquid (cold water) extracts of the sweet potatoes, which are relevant for food grade applications, were fermented for 72 h at 37 °C. Total soluble phenolic content, phenolic profile, antioxidant, anti-hyperglycemic, and anti-hypertensive benefits relevant functional properties of fermented and unfermented sweet potato extracts were evaluated at 0, 24, 48, and 72 h time points using in vitro assay models. Overall, high total soluble phenolic content and total antioxidant activity were observed at 24 h, retaining this high level even after 72 h of fermentation. Additionally, moderate to high α -amylase, α -glucosidase, and angiotensin-I-converting enzyme inhibitory activities were observed in the fermented sweet potato extracts. The results suggested that LAB-based fermentation is an effective post-harvest processing strategy for a higher retention of phenolic bioactives and concurrently improves the human health protective bioactive functional qualities of sweet potatoes.

Keywords: antioxidant; anti-hyperglycemia; fermentation; in vitro study; phenolic bioactives; lactic acid bacteria



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1. Introduction

Sweet potato, an important tuber crop, is resilient to a wide range of environmental and soil conditions, which makes it an ideal vegetable crop choice for climate change adaptation-linked food security solutions globally [1]. In addition to its wide adaptability to emerging climate change, it offers nutritional benefits due to its rich profiles of diverse bioactive compounds, essential minerals, and dietary fibers [2]. The overall production, market, and consumer demand for sweet potatoes is increasing rapidly, due to the potential food quality and economic advantages, especially opportunities to improve livelihood and incomes of marginal and small-scale farmers worldwide [3]. However, the consumer demand and overall market value of the sweet potato largely depend on its nutritional profile and related high-value food applications, which must be improved by advancing effective post-harvest processing tools. The specific nutritional and health benefits of sweet potato are largely associated with its carbohydrate composition (resistant starch), dietary fiber profile, mineral content, and higher concentration of human health relevant phenolic bioactives [4]. Due to its rich source of dietary fiber and indigestible sugars such as

raffinose, verbascose, and stachyose [5], the sweet potato is also a suitable substrate that can support the growth of beneficial bacteria in the food matrix at postharvest processing stages. Therefore, sweet potatoes and sweet potato-based foods and ingredients are relevant for developing value-added functional foods and beverages with probiotic and other human health benefits with improved post-harvest strategies.

Advancing biotransformation by utilizing beneficial microorganisms, such as lactic acid bacteria (LAB)-based fermentation to improve nutritional and health-targeted attributes coupled with the organoleptic characteristics of sweet potatoes, has significant merit. Beneficial microorganism-based biotransformation strategies improve the shelf-life, nutritional qualities, sensory qualities, and organoleptic properties of appropriately fermented plant-based foods and beverages [6]. These fermented foods and beverages are especially important for improving human gut health due to their potential probiotic benefits. Therefore, controlled fermentation processes using widely available and edible LAB strains such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactiplantibacillus plantarum*, and *Bifidobacterium longum* can improve human health relevant functional qualities in plant-based foods such as the sweet potato, while also maintaining quality at the post-harvest processing stage.

Among different beneficial LAB strains, *Lactiplantibacillus plantarum* is widely found in natural plant and plant-based food matrixes and can be targeted to develop high-value fermented foods and beverages with probiotic and other human health benefit associated nutritional qualities [6]. Previously, *L. plantarum*-based fermentation was used to improve the physicochemical properties of sweet potato starch and quality of noodles derived from fermented sweet potato [7]. Similarly, Yuliana et al. [8] reported an overall improvement of the physicochemical properties of sweet potato flour after fermentation with beneficial LAB. Similarly, Wu et al. [9] found higher antioxidant activity, higher organic acid content, and anthocyanin content in purple-fleshed sweet potato fermented milk with different strains of LABs such as *L. acidophilus*, *L. gasseri*, and *L. delbrueckii* subsp. *lactis*. These beneficial bacterial strains are known for releasing bioactive peptides, which are believed to be effective angiotensin-I-converting enzyme (ACE) inhibitors, a key dietary and therapeutic target for managing chronic hypertension [10]. Previous studies have also found the presence of gamma aminobutyric acid (GABA) in LAB fermented food products, which is being considered an important food biochemical with anti-hypertensive functions [11]. Additionally, LAB strains can act as strong biotransforming microbes by releasing health promoting compounds that are generally bound in the food matrix through the fermentation process. Previous studies have shown the biotransformation of phenolic compounds, such as chlorogenic acid to caffeic acid by enzymes released from *L. acidophilus* [12]. During the biotransformation process, extracellular hydrolytic and cellulolytic enzymes such as ferulic acid esterases (FAEs) impact the de-esterification of bound phenolics which are ester-linked to polysaccharides, lipids, organic acids, and proteins in the cell wall matrix [13]. Therefore, LAB-based fermentation is an effective liquid-state biotransformation strategy to improve the stability, retention, and human health protective functional qualities of bioactive compounds and the associated health benefits of plant-based foods and beverages.

An exciting strategy pursued in this study was to harness the potential role of beneficial LAB-based fermentation to improve the phenolic bioactive-linked health benefits of fermented sweet potatoes, especially antioxidant, anti-hyperglycemic, and anti-hypertensive properties targeting major public health needs associated with non-communicable chronic disease (NCD). Therefore, the main objective of this study was to advance and optimize the beneficial LAB (*L. plantarum*)-based fermentation strategy to improve phenolic bioactive-linked antioxidant, anti-hyperglycemic, and anti-hypertensive properties of three different (off-white, orange, and purple) flesh colored sweet potato cultivars, which was previously screened for higher phenolic bioactive linked anti-diabetic properties. The wider goal of this study was to provide the key biochemical rationale to develop sweet potato-based health-targeted functional foods, such as fries, and to add them as ingredients in soups,

breads, fermented foods, and functional beverages with health benefits targeting overall NCD and digestive health challenges.

2. Materials and Methods

2.1. Sample Preparation

The three cultivars of sweet potato selected for this study were Murasaki (off-white-fleshed cultivar), Evangeline (orange-fleshed cultivar), and NIC-413 (purple-fleshed cultivar). These three cultivars were selected from our previous cultivar screening study, based on their high baseline phenolic content and associated health benefits (in vitro assay-based antioxidant and anti-diabetic properties). The beneficial LAB strain (*Lactiplantibacillus plantarum*) was targeted to ferment aqueous extracts of selected sweet potato cultivars. For fermentation, four healthy tubers were randomly selected per cultivar, thoroughly cleaned, peeled, and chopped into small pieces. Approximately 40 g samples were weighed, added to 100 mL cold water and blended (Waring, commercial blender, CT, USA) for 5 min at low speed. The liquid extracts were centrifuged (Sorvall Biofuge Primo centrifuge, Thermo Scientific, Waltham, MA, USA) at 8000 rpm for 20 min twice and the supernatant was collected and stored in the refrigerator before fermentation.

2.2. Agar Media and MRS (Man, Rogosa, Sharpe) Broth Preparation

Agar media was prepared in a 500 mL Erlenmeyer flask to prevent overflowing of 17.5 g agar dissolved in 250 mL distilled water. Flasks were sealed tightly with aluminum foil and mixed thoroughly on a 200 °C preheated hot plate using magnetic bar for 15 min. The samples were then incubated at 100 °C for 10 min before autoclaving. After autoclaving the flasks, the agar media were maintained at 55 °C for bacterial growth plate preparation. MRS Broth preparation was carried out in a beaker, where 8.25 g MRS was added to 150 mL distilled water and mixed well. Then, 10 mL broth was added to the test tubes and covered with steel cap. The test tubes containing broth were then autoclaved.

2.3. Lactic Acid Bacteria Strain and Inoculum Preparation

Fermentation was conducted in tightly sealed Erlenmeyer flasks (covered with aluminum foil) containing 45 mL of sweet potato extracts. Initially lactic acid bacterial strain (*L. plantarum*) was inoculated into test tubes containing 10 mL Difco Lactobacilli MRS Broth (Becton, Dickson and Company Soarks, MD, USA) and then incubated for 24 h at 37 °C. This was followed by the re-inoculation of 100 µL of the grown strain with 10 mL MRS broth for another 24 h at 37 °C. Then, 5 mL bacterial culture after 48 h growth was added to each 45 mL sweet potato extract and incubated for 72 h at 37 °C. A control (only sweet potato extract) without bacterial culture was also used to compare with the fermented sample. Samples were collected (from fermented and control flasks) for biochemical analysis at 0, 24, 48, and 72 h time points during fermentation.

2.4. Viable Cell Counts

Fermented sweet potato extracts were serially diluted to 10^{-5} followed by 100 µL aliquots of each dilution plated on duplicated MRS agar (Difco) plates by the spread plate method. Different serial dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5}) were made using 100 µL of grown strain from broth and sterile water to determine the bacterial growth. Later, these plates were incubated in BD GasPak jars (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with anaerobe sachets (produces less than 1% of oxygen) for 48 h at 37 °C. Plates containing viable cells of *L. plantarum* at 0, 24, 48, and 72 h incubation were recorded as Log CFU (colony forming units)/mL.

2.5. Chemical Used

Porcine pancreatic alpha-amylase (EC 3.2.1.1), rat intestinal alpha-glucosidase (EC 3.2.1.20), 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-Diphenyl-1-

picrylhydrazyl (DPPH), and Trolox, 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH)). All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.6. Total Soluble Phenolic Content

The total soluble phenolic (TSP) content of the unfermented and LAB fermented sweet potato samples was determined based on the protocol described by Shetty et al. [14]. The assay was carried out by adding 0.5 mL of fermented and unfermented sweet potato extract to 10 mL test tubes and diluted two times by adding 0.5 mL distilled water. For the control tubes, 0.5 mL distilled water was added instead of a sweet potato extract. Each test tube also received 1 mL of 95% ethanol, 5 mL of distilled water, and 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent. This was followed by the addition of 1 mL 5% sodium carbonate and then mixed thoroughly using a vortex mixer (Digital vortex mixer, Fisher Scientific, Pittsburgh, PA, USA) and incubated for 60 min in the dark. After incubation, samples were remixed before reading the absorbance using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, Waltham, MA, USA) set at 725 nm. The absorbance values were converted to total soluble phenolic content and expressed in milligrams gallic acid equivalent (GAE) per gram of fresh weight (FW) based on a standard curve that was established using a gallic acid concentration gradient prepared using 95% ethanol as solvent.

2.7. HPLC Analysis of Phenolic Acid Profiles

First, 2 mL of the fermented and unfermented (control) sweet potato samples was centrifuged for 5 min and passed through 0.45 μ m syringe filter before 5 μ L of the sample was injected using Agilent ALS 1100 auto sampler into Agilent 1260 series HPLC equipped with DAD 1100 diode array detector (Agilent Technologies, Palo Alto, CA, USA). The solvents used for gradient elution were 10 mM phosphoric acid (pH 2.5) and 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% over the next 7 min, then decreased to 0% for the next 3 min, and maintained at same rate for the next 7 min (total run time, 25 min). The analytical column used was Agilent Zorbax SB-C18 250 $\times$ 4.6 mm i.d., with packing material of 5 μ m particle size at a flow rate of 0.7 mL/min at an ambient temperature. During each run, the chromatogram was recorded at 306 nm, 333 nm, 540 nm, and 580 nm and integrated using Agilent Chem station enhanced integrator. Pure standards of gallic acid, protocatechuic acid, chlorogenic acid, catechin, and dihydroxybenzoic acid (Sigma Chemical Co., St. Louis, MO, USA) in 100% methanol were used to calibrate the standard curve and retention times.

2.8. Total Antioxidant Activity

The total antioxidant activities of unfermented and LAB fermented sweet potato samples were measured using two different assays: 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay, and 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation assay. In the DPPH assay, described by Kwon et al. [15], 0.25 mL sample was added 1.5 mL centrifuge tubes followed by the addition of 1.25 mL 60 mM DPPH (in 95% ethanol) and mixing with a vortex mixer (Digital vortex mixer, Fisher Scientific, Pittsburgh, PA, USA) to ensure proper mixing prior to incubating for 4 min. After incubation, the mixtures were centrifuged for 1 min at 13,000 rpm to pelletize the precipitate. The absorbance of the supernatant was measured at 517 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, Waltham, MA, USA). Each sample had a corresponding control, which contained 0.25 mL 95% ethanol instead of the sample. For the ABTS assay [16], the same procedure was followed except that 1 mL of ABTS (matured stock adjusted with 95% ethanol) was added to 0.05 mL of fermented and unfermented sweet potato samples and the absorbance was measured at 734 nm using a UV-visible spectrophotometer (Genesys 10S UV- VIS spectrophotometer, Thermo Scientific, Waltham, MA, USA). A control using 95% ethanol instead of the sample

was also used. Based on the absorbance readings, the inhibition percentages for both DPPH and ABTS radicals were calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance extract}}{\text{Absorbance control}} \times 100 \quad (1)$$

The inhibition percentage obtained from the DPPH and ABTS radical scavenging assays were expressed as mM Trolox equivalents (TE) per gram of sample based on the Trolox standard curve.

2.9. α -Amylase Enzyme Inhibitory Activity

The assay protocol used in this study was adapted from the Worthington Enzyme Manual [17]. Undiluted, half-diluted, and one-fifth diluted fermented sample extracts were used to determine the potential dose dependent response in α -amylase enzyme inhibitory activity. The dilutions were carried out using distilled water. The buffer used was 0.1 M sodium phosphate (pH 6.9) with 0.006 M sodium chloride added to it. Volumes of 500 μ L, 250 μ L and 100 μ L sweet potato sample extracts that were undiluted, half-diluted, and one-fifth diluted, respectively, were added to test tubes while the control tubes had 500 μ L buffer only. Additionally, each sweet potato sample extract had a corresponding sample blank tube which contained 500 μ L of the sample extract but no enzyme. Then, 500 μ L porcine pancreatic amylase (0.5 mg/ mL buffer) was added to all the tubes except for the sample blank and blank tubes and incubated for 10 min at 25 °C. After incubation, 500 μ L of 1% starch (1 g/100 mL buffer) was added to all the tubes and incubated for 10 min. The reaction was then stopped by the addition of 1 mL of 3, 5 dinitro salicylic acid (DNS) and the tubes were placed in a boiling water bath for 10 min at 100 °C. Then, the tubes were removed from water bath and cooled at room temperature. The reaction mixture in the tubes was then diluted by adding 10 mL of distilled water to adjust the absorbance of the control to 1.0 ± 0.02 and the absorbance was measured at 540 nm using a UV-VIS Genesys spectrophotometer (Genesys UV- visible, Thermo Scientific, Waltham, MA, USA). The inhibition percentage of α -amylase enzyme inhibitory activity was calculated based on the absorbance readings and using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Abs control} - (\text{Abs extract} - \text{Abs sample blank})}{\text{Abs control}} \times 100. \quad (2)$$

2.10. α -Glucosidase Enzyme Inhibitory Activity

The α -glucosidase enzyme inhibitory activity assay of unfermented and LAB fermented sweet potato samples was determined based on the protocol from the Worthington Enzyme Manual [18] with some modifications taken from McCue et al. [19]. A volume of 50 μ L (undiluted), 25 μ L (half-diluted), and 10 μ L (one-fifth diluted) of each sweet potato sample (for dose dependent response) extract was pipetted into 96 well microtiter plates. The half and one-fifth dilutions were made up to a total of 50 μ L in volume by adding 25 μ L and 40 μ L of 0.1 M potassium phosphate buffer (pH 6.9) respectively. Each fermented and unfermented sweet potato sample extract had a corresponding control with 50 μ L of phosphate buffer instead of the sample. Finally, the volume in all the wells was made up to 100 μ L by the addition of 50 μ L of phosphate buffer in each well including the control. Then, 100 μ L of 0.1 M potassium phosphate buffer (pH 6.9) containing α -glucosidase enzyme (1 U/mL) was added to each well and incubated at 25 °C for 10 min. After incubation with the enzyme, 50 μ L of 5 mM p-nitrophenyl- α -D-glucopyranoside solution (substrate) 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 taken before (0 min) and after (5 min) incubation period using a microplate reader (Thermomax, Molecular device Co., San Jose, CA, USA) set at 405 nm wavelength.

The percentage of inhibition of α -glucosidase enzyme inhibitory activity was calculated based on the absorbance readings and using the following equation:

$$\% \text{ Inhibition} = \frac{(\text{Abs control 5min} - \text{Abs control 0min}) - (\text{Abs extract 5min} - \text{Abs extract 0min})}{(\text{Abs control 5min} - \text{Abs control 0min})} \times 100 \quad (3)$$

2.11. Angiotensin-I-Converting Enzyme Inhibitory Activity

The assay protocol used in this study was adapted from the method modified by Kwon et al. [15]. The enzyme ACE-1 and substrate hippuryl-L-histidyl-L-leucine were purchased from Sigma Aldrich (St. Louis, MO, USA). A volume of 50 μ L extract sample was added to micro-centrifuge tubes and mixed with 200 μ L 2 mU of ACE enzyme solution (in 1 M NaCl-borate buffer, pH 8.3) and incubated for 10 min at 25 °C. This was followed by the addition of 100 μ L hippuryl-L-histidyl-L-leucine and placement in a 37 °C water bath for 60 min. The reaction was stopped by the addition of 150 μ L 0.5 N HCl. The ACE inhibitory activity was measured by quantifying the liberated hippuric acid using a high-performance liquid chromatography (HPLC) (Agilent 1260 series equipped with autosampler and DAD 1100 diode array detector, Agilent Technologies, Palo Alto, CA, USA) protocol. The solvents used for the gradient were 10 mM Ortho-phosphoric acid (pH 2.5) and 100% methanol. The analytical column used was Agilent Zorbax SB-C18, 250–4.6 mm i.d. with packing material of 5 μ m (column temperature 22–24 °C). The inhibition percentage was calculated using the area of liberated hippuric acid peak with the following equation:

$$\% \text{ Inhibition} = \frac{\text{AREA control} - (\text{AREA sample} - \text{AREA sample blank})}{\text{AREA control} - \text{AREA blank}} \times 100 \quad (4)$$

2.12. Data Analysis

The entire LAB-based fermentation experiment was repeated two times. Every time point from each experiment was carried out in triplicates. Means, standard errors, and standard deviations were calculated from replicates within the experiments and analyses were conducted using Microsoft Excel XP. The data were analyzed for analysis of variance (ANOVA) using Statistical Analytical Software (SAS version 9.4; SAS Institute, Cary, NC, USA). Significant statistical differences between sweet potato cultivars, fermentation treatments, and sweet potato cultivars $\times$ fermentation treatments interactions for all in-vitro assays were determined using Tukey's least square means separation at the 0.05 probability level.

3. Results and Discussion

3.1. Total Soluble Phenolic Content and Phenolic Profile

The high perishability and spoilage of vegetables is a major post-harvest challenge that hinders their effective integration as both fresh and processed food in health-focused food solution strategies. A major constrain is that in addition to high perishability occurring at storage stages, there is a deterioration of nutritional and other health relevant qualities due to the chemical changes of bioactive compounds in stored vegetables. Specifically, the composition and biological activity of phenolic compounds undergo significant changes during post-harvest handling and storage. Additionally, different food processing methods affect the content and bioactivity of phenolic compounds in processed foods. Therefore, advancing strategies to improve the stability and retention of phenolic metabolites and associated health protective food qualities of vegetables such as sweet potatoes is important for advancing high-value food applications. Fermentation using beneficial microorganism is a traditional food processing method that improves the human health relevant food qualities of plant-based food substrates and often enhances food quality and shelf-life.

In the current study, a beneficial LAB (*L. plantarum*) strain was rationally recruited to bio-transform aqueous extracts of different fleshed-colored sweet potatoes to improve their stability and retention of phenolic bioactive compounds and associated human health ben-

efits. Three different flesh-colored sweet potato cultivars, which were previously screened and optimized, were selected for LAB-based liquid state fermentation (72 h). The total soluble phenolic (TSP) content of fermented and unfermented sweet potato was determined using the Folin-Ciocalteu reagent-based method. Additionally, the impact of changes in pH during fermentation on TSP content was also determined by comparing sweet potato extracts with natural pH and after adjusting to neutral pH (6.0) at all fermentation time points.

Overall, a higher retention of total soluble phenolic (TSP) content was observed among all three sweet potato cultivars during 72 h of fermentation (Figure 1). Interestingly, the TSP content of fermented sweet potato was generally like the unfermented (control) sample even after adjusting to neutral pH. Analysis of variance showed statistically significant difference in TSP content among the three sweet potato cultivars, while the fermentation and interaction between cultivar $\times$ fermentation had no statistically significant effect. The differences in TSP content among the cultivars was possibly due to their different flesh colors, as observed earlier in our cultivar screening study.

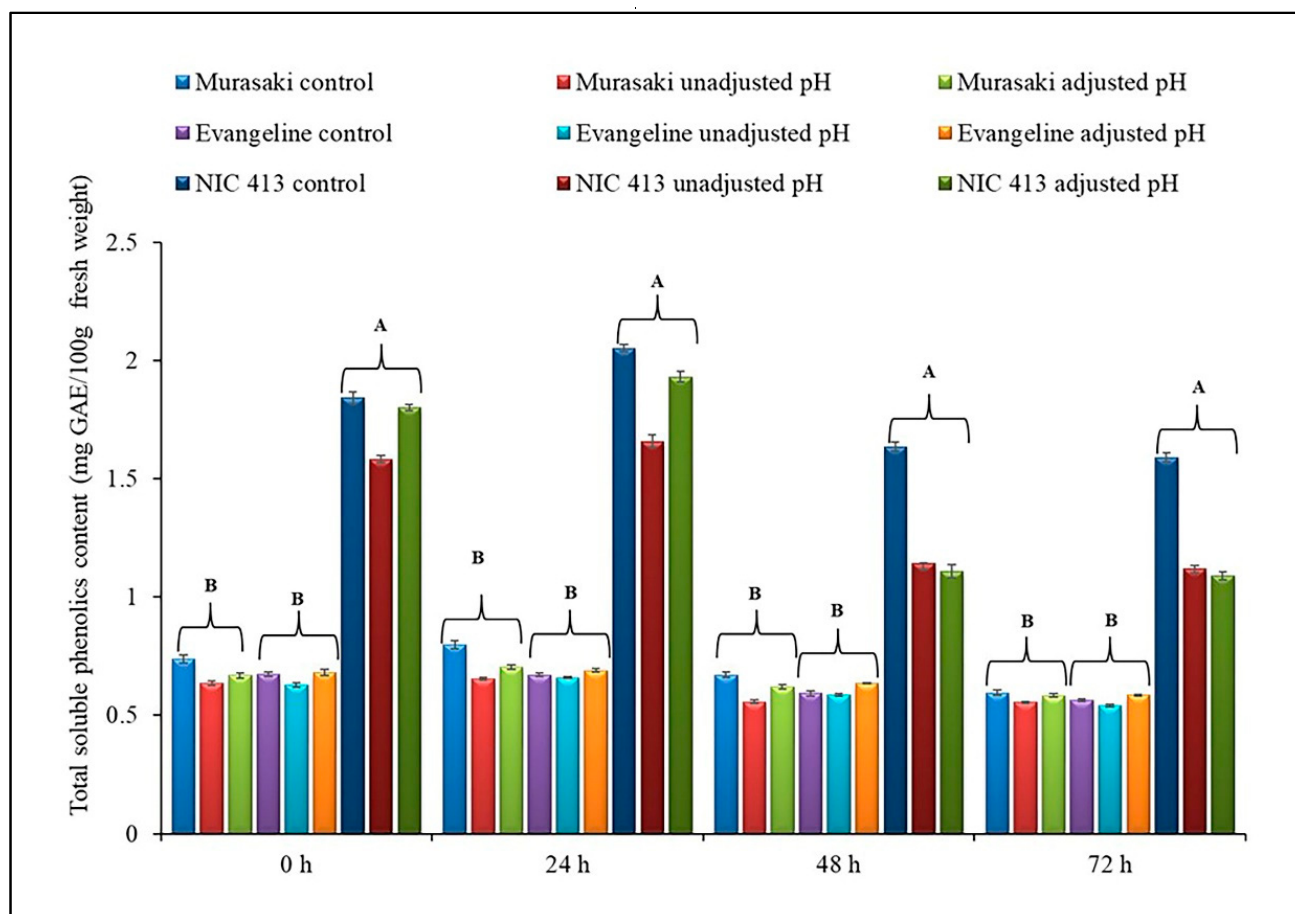


Figure 1. Total soluble phenolic content (mg GAE/100 g fresh weight) of unfermented (control) and fermented (pH unadjusted and adjusted) sample of three sweet potato cultivars at 0, 24, 48, and 72 h fermentation time points. Different uppercase letters (A, B) indicate statistically significant ($p < 0.05$) differences between sweet potato cultivars at each fermentation time point.

Among the different sweet potato cultivars, the purple fleshed NIC-413 had a significantly ($p < 0.05$) higher TSP content across all fermentation time points. A slight improvement of TSP content was observed in purple-fleshed cultivar after 24 h fermentation and then it gradually reduced from 24–72 h. However, the reduction of TSP content was not statistically different when compared to the results of 0 h and unfermented sample.

In a previous study, Wu et al. [9] reported an increased phenolic acid content in sweet potato (purple-fleshed) fermented with milk LAB cultures. In another study, an increase in phenolic content was reported for fermented sweet potato, when compared to the unfermented raw and boiled samples [12]. However, the changes in phenolic content in these studies might be related to the changes in phenolic composition, specifically the release of some bound fractions after fermentation. The current TSP content results and those from previous studies suggest that LAB-based fermentation is an effective strategy to improve the retention and stability of phenolics and that this biotransformation strategy can be targeted to design sweet potato-based functional foods and beverages with probiotic benefits. In addition to TSP content, it is important to understand the effect of fermentation on the composition of phenolic compounds, as this biotransformation process changes the mobility and bioavailability of specific phenolics in food matrices.

In this study, catechin and gallic acid were found in all three sweet potato cultivars irrespective of fermentation treatment and time points (Table 1 and Supplementary File). In addition, protocatechuic acid was observed in purple-fleshed NIC-413, while chlorogenic acid was detected in orange-fleshed Evangeline and purple-fleshed NIC-413, specifically in the unfermented and fermented (with pH adjusted) samples. Previously, Ateea et al. [20] and Padma and Picha [21] also reported chlorogenic acid as major phenolic acid found in different sweet potato cultivars. In another study, Shen et al. [12] reported ferulic acid and p-coumaric acid in LAB fermented sweet potato, while it was not present in unfermented sweet potato extracts. However, in the current study, ferulic acid and p-coumaric acid were not found, which might be due to use of food grade (cold water) extraction and the different HPLC analytical protocol. Additionally, dihydroxybenzoic acid was only found in the unfermented and 0 h fermented samples of off-white fleshed Murasaki and the 0 h unfermented sample of Evangeline. Significant changes in the contents of individual phenolic compounds during fermentation were also observed. Enhanced catechin content was found in fermented sample of all three sweet potato cultivars at 24, 48, and 72 h fermentation time points, and it was statistically significant when compared with unfermented samples. Similarly, the chlorogenic acid content of NIC-413 also increased at 24 and 48 h fermentation time points, specifically in fermented and pH-adjusted samples. Overall, a lower gallic acid content was found in fermented sweet potato samples when compared to unfermented extracts. The improvement in catechin and chlorogenic acid contents in select sweet potato cultivars with LAB fermentation has potential relevance in terms of its human health protective benefits, as both phenolic compounds are considered as potent dietary antioxidants with diverse functional benefits. Significant anti-inflammatory and redox protective functionalities of isolated and purified catechin [22,23] and chlorogenic acid [24,25] were previously reported. Additionally, anti-diabetic and anti-hypertensive properties were also found in plant foods that are rich in catechin and chlorogenic acid [26–28].

Therefore, it is also important to investigate the effect of the LAB-based fermentation of sweet potatoes rich in different phenolics with human health relevant functional qualities such as antioxidant, anti-hyperglycemic, and anti-hypertensive properties.

3.2. Total Antioxidant Activity

The total antioxidant activity of unfermented and LAB-fermented sweet potato samples was determined using two different assays: 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay and 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation assay.

Statistically significant ($p < 0.05$) differences in antioxidant activity (DPPH assay) between sweet potato cultivars, fermentation time points, and interactions between cultivars $\times$ fermentation time points were observed (Table 2). The highest total antioxidant activity (DPPH-based) was observed in the fermented and unadjusted pH sample of purple-fleshed (NIC-413) (83.82%) sweet potato followed by the unfermented (80.52%) and fermented with an adjusted pH (70.8%) samples of the same cultivar after 24 h of fermentation.

Table 1. Major phenolic compounds ($\mu\text{g/g}$ FW) ($n = 6$) in LAB fermented and unfermented sweet potatoes at 0, 24, 48, and 72 h fermentation time points.

	Sweet Potato Cultivar	Fermentation Treatments	Gallic Acid	Catechin	Protocatechuic Acid	Chlorogenic Acid	Dihydroxybenzoic Acid
0 h	Murasaki	Control	2.86	0.98	n.d. ^x	n.d.	0.66
		pH Adjusted	2.81	1.03	n.d.	n.d.	0.47
		pH Unadjusted	2.39	0.27	n.d.	n.d.	0.34
	Evangeline	Control	2.59	0.95	n.d.	1.32	0.05
		pH Adjusted	2.15	0.74	n.d.	n.d.	n.d.
		pH Unadjusted	1.20	3.53	n.d.	0.94	n.d.
	NIC-413	Control	2.77	0.30	0.08	0.99	n.d.
		pH Adjusted	3.14	4.19	0.07	n.d.	n.d.
		pH Unadjusted	1.40	5.00	0.07	n.d.	n.d.
24 h	Murasaki	Control	2.81	0.13	n.d.	n.d.	n.d.
		pH Adjusted	2.57	12.18	n.d.	n.d.	0.35
		pH Unadjusted	0.47	14.21	n.d.	n.d.	n.d.
	Evangeline	Control	3.21	1.38	n.d.	1.30	n.d.
		pH Adjusted	2.56	12.75	n.d.	0.45	n.d.
		pH Unadjusted	1.82	15.24	n.d.	0.90	n.d.
	NIC-413	Control	2.89	4.66	0.25	n.d.	n.d.
		pH Adjusted	1.32	1.46	0.19	2.12	n.d.
		pH Unadjusted	1.30	16.61	0.06	n.d.	n.d.
48 h	Murasaki	Control	3.40	0.80	n.d.	n.d.	n.d.
		pH Adjusted	1.86	14.42	n.d.	n.d.	n.d.
		pH Unadjusted	1.53	16.84	n.d.	n.d.	n.d.
	Evangeline	Control	2.29	1.18	n.d.	0.83	n.d.
		pH Adjusted	0.86	12.75	n.d.	0.38	n.d.
		pH Unadjusted	0.96	12.85	n.d.	n.d.	n.d.
	NIC-413	Control	1.47	1.34	0.13	0.08	n.d.
		pH Adjusted	1.38	13.04	0.14	1.11	n.d.
		pH Unadjusted	1.06	21.43	0.04	0.17	n.d.
72 h	Murasaki	Control	2.37	0.47	n.d.	n.d.	n.d.
		pH Adjusted	1.96	14.0	n.d.	n.d.	n.d.
		pH Unadjusted	1.53	18.03	n.d.	n.d.	n.d.
	Evangeline	Control	1.25	1.11	n.d.	0.73	n.d.
		pH Adjusted	0.99	14.40	n.d.	0.34	n.d.
		pH Unadjusted	0.83	20.31	n.d.	n.d.	n.d.
	NIC-413	Control	2.84	0.08	0.20	n.d.	n.d.
		pH Adjusted	1.05	2.14	0.15	n.d.	n.d.
		pH Unadjusted	0.96	24.58	0.11	n.d.	n.d.

^x n.d.—Not detected.

However, for the other two sweet potato cultivars, high antioxidant activity was observed in 0 h sample when compared to other fermentation time points. Interestingly, even after 72 h of fermentation, the fermented sweet potato sample of Murasaki and Evangelina showed similar antioxidant activity (DPPH-based) as the 24 h and 48 h time points. Therefore, the results of this study indicated that LAB-based fermentation is an effective strategy to retain and even improve the antioxidant activity of sweet potatoes.

Previously, many in vitro studies have found high antioxidant activities in anthocyanins rich purple sweet potatoes along with ameliorative effects and antihypertensive properties [29,30]. Wu et al. [9] reported significantly high ($p < 0.01$) free radical scavenging activity in the Chingshey purple sweet potato after fermentation with three different strains of *Lactobacillus* such as *L. gasseri*, *L. acidophilus*, and *L. delbrueckii* subsp. *lactis*. In this study, improved antioxidant activities, higher GABA (gamma-aminobutyric acid) concentration, anthocyanins, and organic acids content were observed in fermented matrix developed from the Chingshey purple sweet potato. Similarly, Zhong-hue and Jie [31] observed high superoxide anion radical scavenging activity and high Fe^{+3} ions reducing capacity in wine derived from purple fleshed sweet potato.

Table 2. Total antioxidant activity (DPPH and ABTS free radical scavenging assay) of unfermented and fermented (pH adjusted and unadjusted) sample of three sweet potato samples ($n = 12$) at 0, 24, 48, and 72 h fermentation time points.

Sweet Potato	Fermentation	0 h		24 h		48 h		72 h	
		DPPH	ABTS	DPPH	ABTS	DPPH	ABTS	DPPH	ABTS
Murasaki	Control	55.54 ± 0.52 ^{x e-k y}	99.00 ± 0.1 ^a	42.29 ± 0.52 ^{k-o}	98.71 ± 0.82 ^a	34.59 ± 1.32 ^{mno}	95.51 ± 0.16 ^{ab}	29.31 ± 0.29 ^o	71.45 ± 0.25 ^e
	pH unadjusted	46.05 ± 0.71 ^{i-o}	90.91 ± 0.13 ^{a-d}	38.08 ± 0.72 ^{k-o}	84.01 ± 0.52 ^{b-e}	30.90 ± 1.30 ^o	81.68 ± 0.25 ^{cde}	34.22 ± 0.54 ^{on}	81.22 ± 0.07 ^{de}
	pH adjusted	51.90 ± 0.38 ^{g-n}	98.86 ± 0.66 ^a	45.26 ± 0.73 ^{j-o}	98.48 ± 1.93 ^a	41.19 ± 0.56 ^{k-o}	98.24 ± 0.10 ^{ab}	40.25 ± 0.4 ^{k-o}	89.50 ± 0.14 ^{a-d}
Evangelina	Control	64.67 ± 0.39 ^{b-h}	99.10 ± 2.17 ^a	52.71 ± 0.54 ^{j-o}	99.06 ± 0.14 ^a	49.32 ± 0.60 ^{h-n}	98.72 ± 0.61 ^a	37.36 ± 0.34 ^{l-o}	98.43 ± 0.12 ^a
	pH unadjusted	60.82 ± 0.18 ^{d-j}	99.25 ± 0.11 ^a	42.92 ± 0.43 ^{m-r}	93.82 ± 0.08 ^{a-d}	44.42 ± 0.31 ^{j-o}	94.27 ± 0.38 ^{abc}	43.48 ± 0.76 ^{j-no}	89.29 ± 0.17 ^{a-d}
	pH adjusted	62.70 ± 0.45 ^{c-i}	99.30 ± 0.36 ^a	52.56 ± 0.38 ^{ikl}	99.08 ± 0.11 ^a	50.42 ± 0.90 ^{g-n}	98.74 ± 1.75 ^a	49.94 ± 0.43 ^{g-n}	98.45 ± 0.18 ^a
NIC-413	Control	76.70 ± 0.07 ^{a-d}	98.98 ± 0.22 ^a	80.52 ± 0.27 ^{abc}	98.66 ± 0.21 ^a	74.67 ± 0.12 ^{a-d}	98.07 ± 0.13 ^{ab}	74.75 ± 0.06 ^{a-d}	98.14 ± 0.09 ^{ab}
	pH unadjusted	78.30 ± 0.99 ^{ab}	98.99 ± 2.68 ^a	83.83 ± 0.32 ^a	99.04 ± 0.12 ^a	79.74 ± 0.53 ^{abc}	98.95 ± 0.13 ^a	79.81 ± 0.68 ^{abc}	98.96 ± 0.11 ^a
	pH adjusted	75.61 ± 0.12 ^{a-g}	98.97 ± 0.58 ^a	78.68 ± 0.30 ^{a-d}	98.90 ± 0.11 ^a	71.76 ± 0.05 ^{a-e}	98.47 ± 0.33 ^a	70.31 ± 0.71 ^{a-f}	98.64 ± 0.10 ^a

^x ± Standard error; ^y Different lowercase letters represent statistically significant differences in antioxidant activity between sweet potato sample × fermentation time points interactions at 95% probability level separately for DPPH and ABTS antioxidant assay.

Like the results of TSP content and DPPH-based antioxidant activity, statistically significant ($p < 0.05$) differences in antioxidant activity (ABTS-based assay) were observed between sweet potato cultivars $\times$ fermentation time points (Table 2). In general, even after 72 h fermentation, high antioxidant activity was observed in purple-fleshed (NIC-413) and orange-fleshed (Evangeline) sweet potato samples, while mean antioxidant activity was reduced in the off-white-fleshed (Murasaki) sweet potato sample. However, the fermented sample of Murasaki (pH adjusted) had significantly higher antioxidant activity when compared to the unfermented control sample after 72 h fermentation. Therefore, the results of this study indicated that LAB based fermentation has a positive effect on retaining and improving the antioxidant activity of sweet potatoes; thus, this strategy can be targeted for designing sweet potato-based functional foods and beverages that are particularly relevant for their antioxidant-linked anti-inflammatory benefits. Managing chronic oxidative stress is key for preventing and halting the progression of type 2 diabetes pathogenesis. Furthermore, it is also important to find and determine other functional benefits like anti-hyperglycemic and anti-hypertensive properties in order to design functional foods and ingredients from fermented and bioprocessed sweet potatoes targeting wider anti-diabetic benefits.

To understand the potential anti-hyperglycemic property of fermented sweet potato samples, α -amylase enzyme inhibitory activity was determined using an in vitro assay model. Moderate to high α -amylase enzyme inhibitory activity (ranging from 67.76% to 89.93%) was observed in the fermented and unfermented sweet potato samples of three cultivars with different flesh colors (Table 3).

A significant ($p < 0.05$) dose-dependent response [undiluted, half-diluted, and one-fifth diluted] in α -amylase enzyme inhibitory activity was also observed in fermented and unfermented samples [results of undiluted and half-diluted sample are presented in Table 3 and a one-fifth dilution that had no inhibition was not included]. Overall, cultivar differences, fermentation, and the pH of the sample had statistically significant effect on α -amylase enzyme inhibitory activity ($p < 0.05$); however, no statistically significant difference was observed between cultivars $\times$ fermentation time point interactions.

The α -amylase enzyme inhibitory activity gradually decreased from 0 to 72 h in most samples. The rate of reduction was significantly higher for the purple-fleshed sweet potato cultivar (NIC-413). The fermented sweet potato sample with natural pH had higher α -amylase enzyme inhibitory activity at most fermentation time points, and this was primarily due the interference of acidic condition. Among three sweet potato cultivars, the fermented sample of Murasaki had higher α -amylase enzyme inhibitory activity even after 48 and 72 h of fermentation. Therefore, for anti-hyperglycemic benefits, LAB-based fermentation can only be targeted for select sweet potato cultivars.

Previously, a reduction in α -amylase enzyme inhibitory activity was observed in fermented yam [32]. Aligning these studies, the higher phenolic-linked antioxidant activity and moderate α -amylase enzyme inhibitory activity of fermented, purple-fleshed sweet potato (NIC-413) have potential relevance in health focused food applications, especially for improvements in overall dietary interventions to counter chronic oxidative stress and chronic hyperglycemia in the early stages of type 2 diabetes.

The potential inhibitory activity of another key anti-hyperglycemic relevant enzyme in fermented and unfermented sweet potato samples, α -glucosidase, was also determined using an in vitro assay model. In this study, low to moderate (13–55%) α -glucosidase enzyme inhibitory activity was observed in fermented and unfermented sweet potato samples (Table 4). Additionally, a dose-dependent [undiluted, half-diluted, and one-fifth diluted] response in α -glucosidase enzyme inhibitory activity was also found in LAB-fermented and unfermented sweet potato sample. The results of undiluted and half diluted samples are presented in Table 4 and the one-fifth dilution, which had no inhibition, was not included. Interestingly, the α -glucosidase enzyme inhibitory activity of Murasaki (off-white-fleshed) sweet potato improved after 72 h fermentation, while for orange-fleshed (Evangeline) and purple-fleshed (NIC-413) cultivars, it remained statistically at par between 0 to 72 h

fermentation time points. Overall, the results of α -glucosidase enzyme inhibitory activity showed statistically significant ($p < 0.05$) differences among cultivars, between fermentation time points, and cultivars $\times$ fermentation time points interactions in undiluted samples. The moderate α -amylase and α -glucosidase enzyme inhibitory activities in the fermented Murasaki sweet potato sample after 72 h of fermentation have potential relevance for using LAB fermentation to improve the anti-hyperglycemic functional benefits of off-white sweet potato cultivars. However, future studies with different white and off-white sweet potato cultivars are needed to further confirm the potential anti-hyperglycemic benefits of LAB-based fermentation strategy.

Table 3. α -Amylase enzyme inhibitory (%) activity (undiluted and half diluted) of unfermented and fermented (pH adjusted and unadjusted) sweet potato samples ($n = 12$) at 0, 24, 48, and 72 h fermentation time points.

Sweet Potato	Fermentation	0 h		24 h		48 h		72 h	
		Undiluted	Half-Diluted	Undiluted	Half-Diluted	Undiluted	Half-Diluted	Undiluted	Half-Diluted
Murasaki	Control	88.39 ^{ab}	2.71 ^{abc}	76.21 ^{abc}	26.70 ^{ab}	81.90 ^{abc}	6.95 ^{abc}	72.44 ^{ab}	4.73 ^{abc}
	pH unadjusted	77.31 ^{abc}	1.79 ^{abc}	80.06 ^{abc}	19.4 ^{ab}	94.87 ^a	28.29 ^a	47.85 ^{abc}	32.68 ^a
	pH adjusted	88.84 ^{ab}	10.35 ^{abc}	55.03 ^{abc}	14.88 ^{ab}	89.89 ^{ab}	4.87 ^{abc}	46.84 ^{abc}	4.61 ^{abc}
Evangeline	Control	95.33 ^a	6.26 ^{abc}	76.55 ^{abc}	31.48 ^a	84.31 ^{abc}	12.89 ^{ab}	53.23 ^{abc}	17.18 ^{ab}
	pH unadjusted	83.95 ^{abc}	6.29 ^{abc}	69.38 ^{abc}	18.64 ^{ab}	88.17 ^{ab}	22.31 ^{ab}	78.05 ^{ab}	22.42 ^{ab}
	pH adjusted	59.32 ^{abc}	13.09 ^{ab}	63.01 ^{abc}	6.35 ^{abc}	47.09 ^{abc}	8.02 ^{abc}	21.48 ^{bc}	8.45 ^{abc}
NIC-413	Control	83.79 ^{abc}	9.67 ^{abc}	82.88 ^{abc}	37.18 ^a	65.11 ^{abc}	15.67 ^{ab}	80.38 ^{ab}	21.76 ^{ab}
	pH unadjusted	66.01 ^{abc}	3.56 ^{abc}	67.22 ^{abc}	23.28 ^{ab}	73.28 ^{abc}	42.81 ^a	69.51 ^{abc}	17.44 ^{ab}
	pH adjusted	78.01 ^{abc}	10.05 ^{abc}	14.26 ^c	10.89 ^{abc}	26.86 ^{abc}	10.97 ^{abc}	28.87 ^{abc}	10.77 ^{abc}

Different letters represent statistically significant differences in α -amylase enzyme inhibitory activity between fermented and unfermented sweet potato samples at a 95% probability level (separate for both dilutions) at each fermentation time point.

Table 4. α -Glucosidase enzyme inhibitory (%) activity (undiluted and half diluted) of unfermented and fermented (pH adjusted and unadjusted) sweet potato samples ($n = 12$) at 0, 24, 48, and 72 h fermentation time points.

Sweet Potato	Fermentation	0 h		24 h		48 h		72 h	
		Undiluted	Half-Diluted	Undiluted	Half-Diluted	Undiluted	Half-Diluted	Undiluted	Half-Diluted
Murasaki	Control	42.35 ^{f-i}	28.12 ^{a-d}	41.19 ^{g-j}	30.93 ^a	43.77 ^{e-i}	31.89 ^a	48.90 ^{a-f}	32.63 ^a
	pH unadjusted	44.37 ^{e-i}	29.22 ^{a-d}	50.48 ^{a-e}	32.11 ^a	55.62 ^{abc}	34.67 ^a	50.17 ^{b-e}	30.30 ^{ab}
	pH adjusted	34.55 ^{j-m}	18.32 ^{gh}	29.53 ^m	27.21 ^{a-e}	30.04 ^{lm}	19.53 ^{e-h}	55.07 ^{abc}	33.86 ^a
Evangeline	Control	48.22 ^{c-g}	32.30 ^a	47.03 ^{d-h}	33.55 ^a	47.39 ^{d-g}	34.84 ^a	47.18 ^{d-fg}	30.55 ^a
	pH unadjusted	44.92 ⁱ	30.11 ^{ab}	44.65 ^{e-i}	27.92 ^{a-d}	48.59 ^{c-g}	31.12 ^a	56.22 ^{ab}	32.92 ^a
	pH adjusted	37.76 ^{i-l}	26.94 ^{a-f}	34.08 ^{j-m}	21.61 ^{d-h}	34.80 ^{klm}	22.64 ^{b-g}	32.66 ^{klm}	18.97 ^{fgh}
NIC-413	Control	48.37 ^{c-g}	32.56 ^a	47.58 ^{d-g}	30.53 ^a	48.68 ^{c-f}	33.71 ^a	48.82 ^{b-f}	32.37 ^a
	pH unadjusted	48.38 ^{c-g}	31.21 ^a	53.64 ^{a-d}	29.97 ^{abc}	55.66 ^{abc}	13.91 ^h	57.88 ^a	30.52 ^a
	pH adjusted	38.81 ^{ijk}	26.92 ^{a-f}	39.65 ^{kl}	22.05 ^{c-g}	28.02 ^m	13.74 ^h	30.04 ^m	14.94 ^{gh}

Different letters represent statistically significant differences in α -glucosidase enzyme inhibitory activity between cultivars $\times$ fermentation time points interactions at 95% probability level.

The higher retention of α -glucosidase enzyme inhibitory activity along with the high phenolic-linked antioxidant activity in select sweet potatoes after 48 and 72 h LAB-based fermentation are the key findings of this study. The higher retention and stability of phenolic content may be directly linked, and relevant to retaining and improving α -glucosidase enzyme inhibitory activity in fermented sweet potato samples. Previously, the presence of specific phenolic compounds such as 6-O-caffeoylsophorose in combination with the acylation of phenolics to sugar in fermented sweet potato were found to be associated with reduced postprandial glucose levels in rat models [33]. Therefore, the total phenolic content and changes in the composition of phenolic acids during fermentation through the release of bound phenolics might have significant impact, potentially determining the anti-hyperglycemic and other human health relevant functional qualities of fermented plant-based foods.

Overall, the results of the present study suggest that LAB-based fermentation can be targeted to improve the stability and retention of phenolic bioactive linked antioxidant and anti-hyperglycemic functional qualities in select sweet potato cultivars, especially for the off-white-fleshed sweet potato cultivar (Murasaki).

3.3. Anti-Hypertensive Functionality

Like anti-hyperglycemia, managing chronic hypertension is also key to counter vascular complications commonly associated with type 2 diabetes and other non-communicable chronic diseases [34]. The renin-angiotensin-aldosterone system, targeting the inhibition of angiotensin-I-converting enzyme (ACE) is key therapeutic strategy to reduce morbidity and mortality associated with chronic hypertension [35]. Foods and food ingredients derived from bioactive-enriched plant food sources have previously shown ACE inhibitory activity [36,37]. Ishiguro et al. [38] reported high ACE inhibitory property in peptides isolated from sweet potatoes. Similarly, a significant ACE inhibitory property was observed with sweet potato protein hydrolysates [39]. However, the ACE inhibitory property of food grade sweet potato extracts before and after fermentation was not explored previously.

In this present in vitro assay model-based study, moderate to high ACE inhibitory activity was found in all three sweet potato cultivars and with and without LAB fermentation (Table 5). Among the three sweet potato cultivars, higher mean ACE inhibitory activity was found in purple fleshed NIC-413 (58–95% inhibition), followed by orange-fleshed Evangeline, and then off-white fleshed Murasaki. Interestingly, higher ACE inhibitory activity was observed after 72 h incubation both in LAB fermented and unfermented (control) samples, when compared with 0 h sweet potato samples. Additionally, in orange-fleshed Evangeline, significantly high ACE inhibitory activity was observed in LAB fermented and pH adjusted sample at the 48 and 72 h fermentation time points. A similar trend in the improvement of ACE inhibitory activity was also observed in the LAB fermented and pH adjusted NIC-413 sample at the 48 h fermentation time point. Therefore, the results of the current study indicate that LAB fermented sweet potatoes are a good dietary target to counter chronic hypertension. The high antioxidant and high ACE inhibitory activities found in phenolic enriched purple-fleshed NIC-413 have significant health benefit relevance. Fermented (LAB-based) and unfermented NIC-413 can be integrated in dietary and therapeutic interventions to improve macro and microvascular complications, which are relevant for reducing type 2 diabetes associated health risks.

Table 5. Angiotensin-I-converting enzyme (ACE) inhibitory (%) activity (undiluted) of unfermented and fermented (pH adjusted and unadjusted) sweet potato samples ($n = 12$) at 0, 24, 48, and 72 h fermentation time points.

Sweet Potato	Fermentation	0 h	24 h	48 h	72 h
Murasaki	Control	70.19	70.23	77.16	87.66
	pH unadjusted	80.23	53.74	66.98	68.67
	pH adjusted	57.96	81.13	75.71	75.32
Evangeline	Control	60.10	54.28	63.71	87.96
	pH unadjusted	51.84	44.94	41.37	85.67
	pH adjusted	52.57	63.35	92.09	93.15
NIC-413	Control	74.80	93.01	88.97	95.84
	pH unadjusted	67.28	59.44	58.50	94.28
	pH adjusted	86.71	86.15	96.58	95.82

3.4. Viable Cell Count of *Lactiplantibacillus plantarum* in Fermented Sweet Potatoes

The presence of active LAB counts in fermented food and beverage sample is important for their potential probiotic and gut health benefits. In this present study, viable cell counts of *L. plantarum* in fermented sweet potato sample were determined at different time points (0, 24, 48, and 72 h during fermentation). Maximum counts of *L. plantarum* were observed during the initial stages of fermentation (0 and 24 h) when compared to later

periods (48 and 72 h), which could be due to the presence of more nutrients at the initial fermentation stage (Table 6 and Figure 2).

Table 6. Viable cell count (Log CFU/mL) of *Lactiplantibacillus plantarum* with three different dilutions (10^{-3} , 10^{-4} , 10^{-5}) in fermented sweet potato samples at 0, 24, 48, and 72 h fermentation time points.

Sweet Potato	0 h			24 h			48 h			72 h		
	10^{-3}	10^{-4}	10^{-5}	10^{-3}	10^{-4}	10^{-5}	10^{-3}	10^{-4}	10^{-5}	10^{-3}	10^{-4}	10^{-5}
Murasaki	5.89 ± 0.12	5.26 ± 0.34	0	6.68 ± 0.76	6.47 ± 0.44	6.12 ± 0.74	4.1 ± 0.23	3.94 ± 0.66	3.21 ± 0.18	3.78 ± 0.26	3.22 ± 0.31	2.96 ± 0.11
Evangelina	2.21 ± 0.31	2.02 ± 0.54	0	4.28 ± 0.69	3.95 ± 0.29	3.34 ± 0.43	2.45 ± 0.14	1.91 ± 0.21	0	2.71 ± 0.34	1.92 ± 0.15	1.04 ± 0.08
NIC-413	6.23 ± 0.45	5.49 ± 0.23	0	6.72 ± 0.87	6.15 ± 0.76	5.9 ± 0.24	4.62 ± 0.36	3.27 ± 0.62	0	3.60 ± 0.45	2.67 ± 0.19	1.64 ± 0.16

Overall, there was a reduction in colony forming units of LAB during fermentation from 24 to 72 h. The decrease in the viable cell counts might be due to the differences in pH or due to the declining growth of lactic acid bacteria after consuming all available nutrients that are required to sustain their growth. Significant differences in the viable cell counts of *L. plantarum* due to different sweet potato substrate (cultivar differences) sources were observed, as Murasaki and NIC-413 supported higher growth (Table 6) at the initial fermentation stages. Therefore, for probiotic application, 24 h fermentation of sweet potato and the selection of the right cultivar like Murasaki might be optimum, while a longer period of LAB fermentation can be targeted for other health-focused food and therapeutic applications. Furthermore, other plant-based foods with different fiber, oligosaccharide, and bioactive profiles like whole grains, beans, and root vegetables (parsnip, rutabaga) can also be integrated with fermented sweet potato to improve the growth of LAB and to enhance the probiotic and human gut health benefits. This fermentation strategy and food synergies can be targeted to design novel sweet potato-based fermented foods (bio-transformed flours for fries and whole fermented sweet potato foods and beverages) and as functional ingredients to add into breads, soups, and commercial baby foods for wider health benefits.

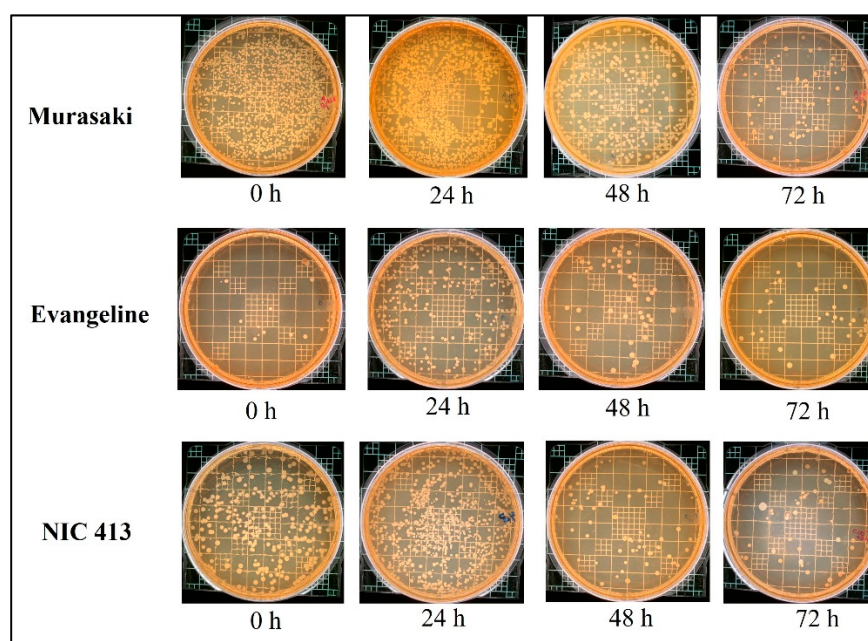


Figure 2. Viable cell count of *Lactiplantibacillus plantarum* (at 10^{-3} dilution) in LAB fermented sweet potato cultivars; Murasaki, Evangelina, and NIC-413 at 0, 24, 48, 72 h incubation (37 °C).

4. Conclusions

In the present study, a higher retention of phenolic bioactive-linked antioxidant and anti-hyperglycemic functional qualities was observed in the LAB fermented sweet potato samples. High total soluble phenolic (TSP) content and high total antioxidant activity were observed at 24 h and remained statistically similar even after 72 h of fermentation. Moderate to high α -amylase and α -glucosidase enzyme inhibitory activities were also observed in unfermented and fermented sweet potato extracts. Among the three sweet potato cultivars, the fermented and unfermented extracts of purple-fleshed NIC-413 had higher phenolic contents and associated antioxidant activities, while off-white fleshed colored Murasaki showed higher α -amylase and α -glucosidase enzyme inhibitory activities, which are relevant for their anti-hyperglycemic benefits. Additionally, moderate to high anti-hypertensive property relevant ACE inhibitory activity was also observed in the fermented sweet potato samples. Therefore, LAB based fermentation can be targeted for improving the retention of phenolic bioactives and associated antioxidant and anti-hypertensive functional qualities as part of developing sweet potato based functional foods and ingredients to add into traditional and modern foods such as breads, soups, fries, and baby foods. The LAB-fermented sweet potato can be integrated into health-focused dietary solution strategies, especially to mitigate chronic oxidative stress-linked early stages of type 2 diabetes and improve digestive health. Improving the bioavailability and mobilization of soluble phenolics of sweet potato using LAB-based fermentation is an exciting approach for the development of functional food ingredients and value-added sweet potato-based foods that can provide improved nutritional and health benefits.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae7100367/s1>. Figure S1. Chromatogram (High Performance Liquid Chromatography Analysis) of unfermented sweet potato (Murasaki) extracts at 0 h fermentation time point. Detected phenolics were gallic acid, catechin, and dihydroxybenzoic acid; Figure S2. Chromatogram (High Performance Liquid Chromatography Analysis) of unfermented sweet potato (NIC-413) extracts at 0 h fermentation time point. Detected phenolics were chlorogenic acid and protocatechuic acid.

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