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ORIGINAL ARTICLE

Bacterial characterization of fermented sweet potato leaves by high-throughput sequencing and their impact on the nutritional and bioactive composition

Santiago Emmanuel Suárez[1,2,3,4] | Hongnan Sun[1,2] | Taihua Mu[1,2] | María Cristina Añón[3,4]

[1]Laboratory of Food Chemistry and Nutrition Science, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing, China

[2]Key Laboratory of Agro-Products Processing, Ministry of Agriculture and Rural Affairs, Beijing, China

[3]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), La Plata, Argentina

[4]Facultad de Ciencias Exactas, Universidad Nacional de La Plata. CCT, La Plata, CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas). CIC (Comisión de Investigaciones Científicas de la Provincia de Buenos Aires), La Plata, Argentina

Correspondence
Hongnan Sun and Taihua Mu, Laboratory of Food Chemistry and Nutrition Science, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, No. 2 Yuan Ming Yuan West Road, Haidian District, P.O. Box 5109, Beijing, 100193, China.
Email: honey0329@163.com (H. S.) and mutaihua@126.com (T. M.)

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Abstract

Bacterial, nutritional, and bioactive compositions of fermented sweet potato leaves (SPL) were investigated. Three samples were utilized: control (C), natural fermentation (NF), and probiotic fermentation (PF). Bacterial composition was assessed by high-throughput sequencing. *Lactobacillus plantarum* and *Enterococcus durans* were identified in NF. *Lactobacillus plantarum* was also found in PF, showing a relative abundance of 90.2%. Polyphenol composition determined by HPLC changed considerably during NF decreasing its concentration to a value of 6.69 mg/g dw compared to the value found in C (42.77 mg/g dw). PF maintained the polyphenol concentration (38.64 mg/g dw) but some interconversion between polyphenols seems to occur. ABTS radical scavenging capacity value was similar in C and PF, demonstrating no changes in antioxidant activity. During PF antioxidant activity was maintained, and angiotensin-converting enzyme inhibitory activity was even improved. Fermentation was a good process for SPL. According to the parameters assessed, PF was better than NF.

Novelty impact statement: The effect of natural and probiotic fermentations on the bacterial composition, nutritional, and bioactive composition of sweet potato leaves was compared. Results showed that fermentation was a good process for sweet potato leaves. According to the parameters assessed, probiotic fermentation was better than natural fermentation.

1 | INTRODUCTION

Sweet potato leaves (SPL) are the main by-products of sweet potato production. Several studies have been conducted utilizing fresh SPL indicating that the fresh leaves are a good source of nutrients and polyphenols (Ishida et al., 2000). Many studies focused on the antioxidant activity and polyphenol composition in SPL (Fu et al., 2016; Islam, 2006; Sun et al., 2018; Truong et al., 2007).

Fermentation of plant-based foods has attracted increasing interest from food scientists owing to improved nutritional quality and health benefits of fermented foods (Gan et al., 2016). Fermentation is presented as a good alternative for SPL preservation

and conservation, additionally for improving the nutritional, functional, bioactive, textural, and sensory quality of the food (Aregbe et al., 2019; He et al., 2017). Fermentation could be natural; it occurs spontaneously and is stored or left without appropriate conditions of conservation. Fermentative activity is developed on the substrate by the microorganisms naturally present. In recent years, controlled fermentation using "starters" has gotten attention. These starters ensure a constant food sensory quality and improve safety. Starters could be used for many purposes like the synthesis of bioactive molecules including antioxidants, enhancing of the bioavailability of nutritive compounds, degradation of toxic and antinutritional substances, and contributing to the probiotic effect of foods (Tamang et al., 2016).

In the present research, the high-throughput sequencing (HTS) technique was used as a tool to identify the bacterial composition of three SPL samples: control (C), natural fermentation (NF), and probiotic fermentation (PF). The effect of fermentation was studied by analyzing nutritional and phenolic composition as well as antioxidant and antihypertensive activity in vitro. The work aimed to elucidate whether probiotic or natural fermentation constitutes a suitable alternative for processing and preservation of SPL.

2 | MATERIALS AND METHODS

2.1 | Plant material

Fresh SPL (cultivar Simon No.1) was obtained from Haileda Food Co., Ltd., Beijing, China, in the middle of August 2018. After harvest, the leaves were collected, washed, and freeze-dried. All samples were ground in a commercial grinder (BJ-300G, Deqing Baijie Electrical Equipment Co., Ltd, Huzhou, China) and stored at 4°C in sealed aluminum bags until used.

2.2 | Sample preparation

Five percent (w/v) of freeze-dried SPL powder was resuspended in distilled water prior to fermentation. The fermentation process was performed using a probiotic starter provided by DuPont China (Shanghai, China) in a concentration of 0.018% w/v.

Based on preliminary studies conducted in our laboratory, NF was performed for 24 h at 37°C and PF for 8 h at 37°C prior to sterilization of SPL. After this time, the pH plateau close to 4 was reached. After fermentation, samples were frozen at −40°C and freeze-dried. Freeze-dried SPL powder was used as control (C) without any fermentation process.

2.3 | Sample characterization

2.3.1 | High-throughput sequencing analysis

Fermented freeze-dried samples (C, NF, PF) were sent to Biomarker Technologies (Beijing, China) for HTS analysis. Total DNA was extracted using OMEGA DNA isolation kit (Omega, D5625-01, USA) according to the manufacturer's protocol. For the identification of bacteria present in the samples, the 16S rDNA and ITS region were amplified respectively utilizing the hypervariable regions V3 + V4. Polymerase chain reaction (PCR) was made out with the following conditions: 2 min denaturation at 98°C and then 25 cycles at 98°C for 30 s, 50°C for 30 s and 72°C for 1 min for 16S rDNA, and 30 cycles for ITS region. The amplification ended with 7 min extension at 72°C.

PCR products were tested by Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA) and sequenced paired-end with Illumina Highseq platform (Illumina Inc, SanDiego, CA, USA) at Biomarker Technologies (Beijing, China). Bioinformatic analyses were conducted to merge, filter, and then clustered the raw reads based on the similarity of 97% into different operational taxonomic units (OTUs). For the previous purpose, UCLUST of QIIME software (version 1.8.0) was used, and OTUs were aligned with the Silva (http://www.arb-silva.de/) and Unite (http://unite.ut.ee/index.php) reference gene database for bacterial classification.

2.3.2 | pH and color

The pH was measured with a pH meter (OHAUS, Starter 3100, Greifensee, Switzerland). The samples (5 g) were homogenized with 100 ml of distilled water, and the pH was tested initially and during the fermentation process. Color measurement was done by transferring 3 ml of samples into a transparent cuvette, and then an instrumental colorimeter (DigiEye System, VeriVide, Leicester, UK) was used to measure the tristimulus color coordinates (L^*, a^*, b^*) based on the reflectance spectra. The total color difference (ΔE^*) was calculated as $\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{½}$.

2.3.3 | Proximate composition

Moisture, ash, crude fat, and crude protein contents were determined by AOAC methods (AOAC [Association of Analytical Chemists], 2000). Crude protein was assessed by the Kjeldahl method, with nitrogen to protein conversion factor of 6.25 (AOAC method 976.05). Ash content was determined by heat treatment at 550°C for 12 h (AOAC method 940.26). The crude fat content was determined by the Soxhlet extraction (AOAC method 920.39).

2.3.4 | Mineral analysis

Mineral contents were determined by inductively coupled plasma atomic emission spectrometry (ICP-MS, 7700X; Agilent, Santa Clara, CA, USA) and expressed as mg mineral/ 100 g dw sample.

2.4 | Polyphenol extraction and quantification

Extraction of polyphenols from freeze-dried samples (C, NF, PF) was carried out according to the method described by Sun, Mu, Xi,

Zhang, and Chen (2014). Briefly, 10 g of leaf powder was extracted with 200 ml of 70% (v/v) ethanol for 30 min at 50°C and subjected to ultrasonic wave treatment (53 kHz). Following centrifugation at 5000*g* for 10 min at 4°C, the residue was reextracted twice with 70% ethanol as described above. The supernatants were pooled, concentrated in a rotary evaporator at 45°C, and freeze-dried to obtain a crude polyphenol extract.

2.5 | Individual phenolic composition

Freeze-dried polyphenol extracts were weighed and then dissolved in 80% (v/v) methanol reaching a concentration of 200 μg/ml. An aliquot of the supernatant from the sample solution was filtered through a 0.22-mm membrane filter (Keyilong Lab Equipment Co., Ltd., Tianjin, China) and analyzed for individual phenolic compounds according to the method described by Sun, Mu, Xi, and Song (2014). Detected peaks were identified and quantified by comparing the retention time and peak area to that of known standards. Concentration was expressed as mg/g of dw.

2.6 | Total polyphenol content and antioxidant activity

Total polyphenol content (TPC) was measured by the Folin–Ciocalteu method described by Sun, Mu, Xi, Zhang, and Chen (2014). Chlorogenic acid was used as standard. TPC was expressed as chlorogenic acid equivalent (CAE) on a dry weight (dw) basis.

DPPH radical (DPPH·) scavenging activity was determined by the method described by Meneses et al. (2013), and calculated as below:

$$\text{DPPH}\% = (1 - \text{A1}/\text{A2}) \times 100.$$

where A1 represents the sample and positive control and A2 the blank control. All results were expressed as g of ascorbic acid equivalents per 100 g of dried SPL material (g AAE/100 g dw).

ABTS radical scavenging capacity was measured using the method described by Estiarte et al. (1999). The results were expressed as g of ascorbic acid equivalents per 100 g of dried SPL material (g AAE/100 g dw).

2.7 | ACE inhibitory activity

The angiotensin-converting enzyme (ACE) inhibition assay was performed according to Hurst and Lovell-Smith (1981) with slight modifications (Suárez & Añón, 2019). Polyphenol samples (C, NF, PF) solutions were added to a 0.2 M borate buffer solution containing 2.0 M NaCl and 5 mM hippuryl–histidyl–leucine (HHL), pH 8.3. The IC_{50} value was defined as the concentration (mg/ml) in the reaction mixture necessary to inhibit ACE activity by 50%. Captopril was used as a positive control of ACE inhibition. Total polyphenol content in the supernatant was determined using the Folin–Ciocalteu method as it was described in 2.6.

2.8 | Statistical analysis

The statistical analysis was performed with GraphPad Prism 6.0 (GraphPad Prism Inc., CA, USA). Data were expressed as the mean ± standard deviation of at least two independent experiments. The Analysis of Variance (ANOVA) was employed to compare means between groups through the Tukey test, with a significance level of $p < .05$.

3 | RESULTS AND DISCUSSIONS

3.1 | High-throughput sequencing analysis

A total of 10 bacterial genera were identified (Figure 1a). Seven genera were found in C and the relative abundance of each one was distributed more equitably compared to NF and PF. *Pantoea* and *Weissell*a were the most abundant genera in NF reaching around 60% of relative abundance. *Enterobacter* and *Enterococcus* reaching close to 30% of relative abundance were the other two genera with high representation in NF. Considering the bacterial composition of initial control samples, during NF some bacteria were able to grow in the experimental conditions imposed and the initial composition of microorganisms changed. Spontaneous fermentations typically result from the competitive activities of a variety of autochthonous and contaminating microorganisms. Those best adapted to the conditions during the fermentation process could grow and predominate (Rodríguez et al., 2008).

The sample fermented with the probiotic was the most homogeneous according to the genera composition. *Lactobacillus* represented approximately 90% of relative abundance on average.

A further identification of the bacterial composition at the species level (Figure 1b) demonstrated that the dominant species of C and NF samples remained unspecific due to the knowledge limitation. According to Figure 1b, *Methylobacterium tarhaniae* and *Sphingomonas parapaucimobilis* were identified in C samples with relative abundance values of 11.9% and 10.7%, respectively. *Lactobacillus plantarum* and *Enterococcus durans* were found in NF samples and the relative abundance was 1.19% and 14.9%, respectively. *Lactobacillus plantarum* was the most abundant bacteria in PF with a relative abundance of 90.2% on average. This result demonstrated the capacity of *L. plantarum* to grow and ferment SPL.

The results reported in this manuscript showed for the first time the bacterial composition of fermented SPL. Lactobacillus, Weissella, and Enterococcus are a representative genus of lactic acid bacteria (LAB). LAB was previously identified in different fermented products.

Different research has demonstrated the presence of LAB found in this study in fermented products from other sources. Fessard et al. (2016) isolated from papaya, tomato, or sliced cabbage samples

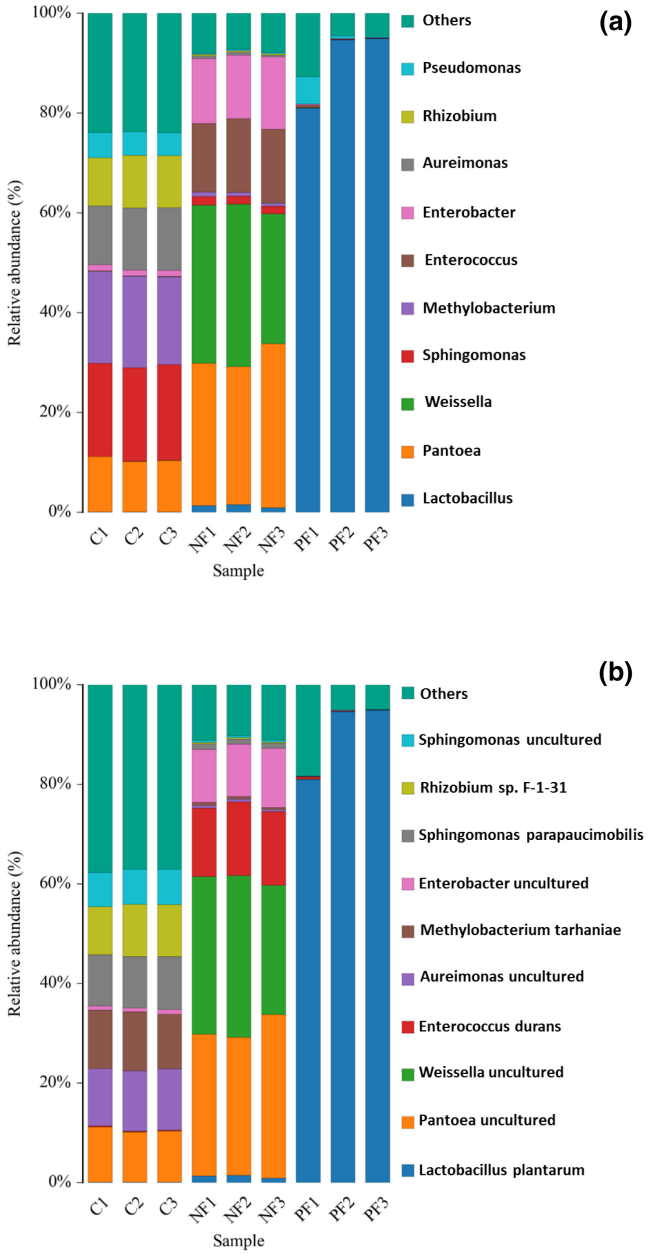

FIGURE 1 Bacterial composition at genus level (a) and species level (b) of different sweet potato leaf samples. C, control; NF, natural fermentation; PF, probiotic fermentation; Numbers (1,2,3) indicate replicates of each sample.

LAB and revealed that *Weissella cibaria* can act as a powerful acidifier among other results. Wang et al. (2019) isolated and selected four LAB (*Lactobacillus plantarum*, *Lactobacillus farciminis*, *Lactococcus lactis*, *Weissella thailandensis*) from Moringa oleifera leaves silage and these strains were utilized as inoculants.

3.2 | Physicochemical and nutritional characterization of fermented samples

The pH was tested along the fermentation processes for both NF and PF. NF showed an initial pH of 5.54 while PF showed a pH of 5.45. Table 1 shows the pH of the two fermented samples after the fermentation process, no significant difference was found reaching values of 4.13 and 3.92 for NF and PF, respectively. Microorganisms presented in NF had the same ability to *Lactobacillus plantarum*, mainly presented in PF, for the acidification of the medium.

Table 1 shown the color parameters measured after fermentation, compared with the control group whose lightness value (L^*) was 43.25 according to Luo et al. (2020), both fermented samples, NF (24.93) and PF (29.62) showed lower values of L^*, resulting in a darker appearance. This decrease was more pronounced for NF. The a^* values increase after NF and PF, demonstrating less greenness compared to the control which showed a negative value. No significant difference was found between NF and PF. NF and PF showed lower b^* values than the control sample presenting a less yellowish tone than this. The magnitude of the color difference was assessed by calculating the ΔE^*. ΔE^* value of NF and PF decreased compared to the control. Color is a critical point for the consumer's preference, for that reason, future studies need to be done to assess whether the difference's magnitude shown before is acceptable for consumers or if it is necessary the utilization of additives (García et al., 2017).

Proximate composition was shown in Table 1. Control SPL was similar to previous studies published about different sweet potato cultivars (Ishida et al., 2000; Suárez et al., 2020; Sun, Mu, Xi, & Song, 2014). Ash, fat, and protein of SPL did not significantly change by NF or PF.

Mineral composition was shown in Table 1. Calcium (Ca), Potassium (K), and Phosphorus (P) were found as the main minerals. The content of K was higher in PF and the content of P decreased after NF and PF. No significant difference was found in Na, Ca, Fe, Zn, Cu, Mn, Mg, and Se.

3.3 | Bioactive value of fermented sweet potato leaves

3.3.1 | Individual phenolic composition

Individual phenolic composition chromatographic patterns obtained by RP-HPLC were shown in Figure S1 and the polyphenols identified for C, NF, and PF were listed in Table 2. All polyphenol concentrations (No.2 to No.10, Table 2) diminished during NF with one exception, 5-*O*-caffeoylquinic acid whose concentration is maintained. The sum of individual polyphenols concentrations in NF was 6.69 mg/g dw, significantly lower than C sample (42.77 mg/g dw). The changes experienced during PF were not as pronounced as NF. Individual polyphenol concentration sum slightly decreases to a value of 38.64 mg/g dw in PF. 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, and 3-*O*-caffeoylquinic acid significantly increase their concentration compared to the control (72%, 32%, and 17%, respectively) and 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid decrease their concentration significantly (22%, 78%, and 11%, respectively). Interconversion

TABLE 1 Color information, pH, proximate and mineral composition of sweet potato leaves samples

Treatment	Control	Natural fermentation	Probiotic fermentation
pH	–	4.13 ± 0.04[a]	3.92 ± 0.11[a]
*L**	43.25 ± 045[a1]	24.93 ± 0.14[c]	29.62 ± 0.04[b]
*a**	−10.20 ± 0.13[c]	1.91 ± 0.03[a]	1.71 ± 0.07[b]
*b**	17.50 ± 0.18[a]	10.74 ± 0.14[c]	15.99 ± 0.17[b]
Δ*E*	47.75 ± 0.25[a]	32.28 ± 0.10[c]	37.70 ± 0.09[b]
Proximate composition (g/ 100 dw)			
Ash	12.98 ± 0.22[a]	12.56 ± 0.69[a]	12.87 ± 0.28[a]
Fat	2.75 ± 0.52[a]	3.69 ± 0.37[a]	3.42 ± 0.27[a]
Protein	34.77 ± 0.23[a]	34.77 ± 0.13[a]	34.81 ± 0.54[a]
Mineral composition (mg/ 100 dw)			
Na	50.9 ± 0.3[a]	49.6 ± 0.6[a]	56.1 ± 0.1[a]
Ca	728 ± 0[a]	762 ± 2[a,b]	721 ± 3[a,b]
K	6134 ± 90[b]	5642 ± 0[c]	6171 ± 22[a]
P	444 ± 3[a]	389 ± 0[b]	408 ± 1[b]
Fe	14.5 ± 0[a]	13.3 ± 0.1[a]	13.2 ± 0.1[a]
Zn	3.1 ± 0[a]	3.0 ± 0.0[a]	3.2 ± 0.0[a]
Cu	1.2 ± 0.1[a]	1.3 ± 0.0[a]	1.2 ± 0.0[a]
Mn	15.4 ± 0.2[a]	14.8 ± 0.1[a]	16.9 ± 0.0[a]
Mg	297 ± 0[a]	282 ± 2[a]	299 ± 0[a]
Se[2]	0.005 ± 0.000[a]	0.005 ± 0.004[a]	0.005 ± 0.000[a]
Pb	0.008 ± 0.000[a]	0.027 ± 0.001[a]	0.009 ± 0.00[a]
Hg	<0.001[a]	<0.001[a]	<0.001[a]
As	0.016 ± 0.000[a]	0.016 ± 0.000[a]	0.016 ± 0.000[a]

Note: Values within the same line with different letters are significantly different ($p < .05$).

[1]Value obtained from Luo et al. (2020).

[2]Se content was expressed in μg/100 g.

TABLE 2 Content of individual phenolic compounds in sweet potato leaves. Concentration was expressed as mg/g of dw

Peak	Identity	Control	Natural fermentation	Probiotic fermentation
1	5-*O*-caffeoylquinic acid	2.88 ± 0.26[b]	3.09 ± 0.06[b]	10.47 ± 0.02[a]
2	4-*O*-caffeoylquinic acid	0.32 ± 0.02[a]	NF	0.47 ± 0.01[a]
3	3-*O*-caffeoylquinic acid	12.98 ± 0.23[b]	0.64 ± 0.04[c]	15.73 ± 0.15[a]
4	Caffeic Acid	0.68 ± 0.04[a]	0.35 ± 0.03[b]	0.18 ± 0.00[b]
5	Isoquercetin	1.41 ± 0.03[a]	0.86 ± 0.01[b]	NF
6	3,4-di-*O*-caffeoylquinic acid	7.08 ± 0.02[a]	0.45 ± 0.00[c]	5.53 ± 0.03[b]
7	3,5-di-*O*-caffeoylquinic acid	12.19 ± 0.23[a]	0.66 ± 0.02[c]	2.64 ± 0.02[b]
8	4,5-di-*O*-caffeoylquinic acid	3.33 ± 0.01[a]	0.21 ± 0.02[c]	2.96 ± 0.01[b]
9	3,4,5-tri-*O*-caffeoylquinic acid	0.61 ± 0.00[a]	0.43 ± 0.00[b]	0.45 ± 0.01[a,b]
10	Quercetin	1.29 ± 0.18[a]	NF	0.32 ± 0.02[b]

Note: Values that were significantly different are assigned different letter ($p < .05$). NF: not found.

between di-caffeoylquinic acid into mono-caffeoylquinic acid occurs. Evidently during PF *Lactobacillus plantarum* was able to break down the established bond between caffeic acid and quinic acid, the two constituent monomers of these polyphenols (Islam et al., 2002). The union's position of these two molecules did not seem to be an impediment to the breakdown bond, due to occur in the three di-caffeoylquinic acids. At the same time, caffeic acid decreased its concentration (74%) despite the release that it has been suggested by di-caffeoylquinic acids. Isoquercetin was not found it and quercetin decreased its concentration to a value of 0.32 mg/g dw in PF.

Some literature has demonstrated the ability of LAB to deglycosylate, de-esterify, de-carboxylate, and de-methylate dietary

phenolic compounds (Hervert-Hernández & Goñi, 2011). Bel-Rhlid et al. (2013) have demonstrated the capability of *Lactobacillus johnsonii* to transform chlorogenic acids from green coffee extract into 4-vinylcatechol. Rodríguez et al. (2008) also indicated that *Lactobacillus plantarum* could degrade some hydroxycinnamic acids, including caffeic acid among others and the reaction involved in their metabolism were decarboxylation and reduction of the phenolic acids.

3.3.2 | Total phenolic content and antioxidant activity

Figure 2a exhibited the total polyphenol value of 19.45 g CAE/100 g dw for SPL C. NF and PF demonstrated a reduction of TPC in different magnitudes. The reduction in NF was 34.8% and for PF was 5.7%. As we observed, the decrease was more pronounced in NF than PF, where a slight loss was detected. These results are in concordance with the individual phenolic pattern shown in Table 2 as we discussed in 3.3.1.

Figure 2b showed DPPH radical scavenging activity. The control sample presented a value of 8.02 g AAE/100 g dw and fermented samples showed a decrease in their capacity for radical scavenging. The reduction in the scavenging activity was 21.8% and 11.7% for NF and PF, respectively. When the antioxidant activity was assessed using ABTS assay, different results were found (Figure 2c). NF markedly reduced the ABTS value (31.8%), and in PF no changes were observed. DPPH and ABTS showed a decrease in antioxidant activity in NF, this result agrees with the individual phenolic composition and TPC found in NF.

Changes in antioxidant activity pointed out that modifications of polyphenols composition take place over fermentation. The difference found in DPPH and ABTS on PF did not establish an accurate conclusion on whether PF contributes to maintaining antioxidant activity when compared to C, but PF contributes to maintaining antioxidant activity when compared to NF. As was commented before, *Lactobacillus plantarum* contains enzyme machinery to produce high-added value compounds, such as hydroxytyrosol and pyrogallol, two powerful antioxidants. (Rodríguez et al., 2009). A common conclusion for several works is that the selection of a starter can contribute to maintaining or increasing antioxidant activity when compared to spontaneous fermentation (Septembre-Malaterre et al., 2018).

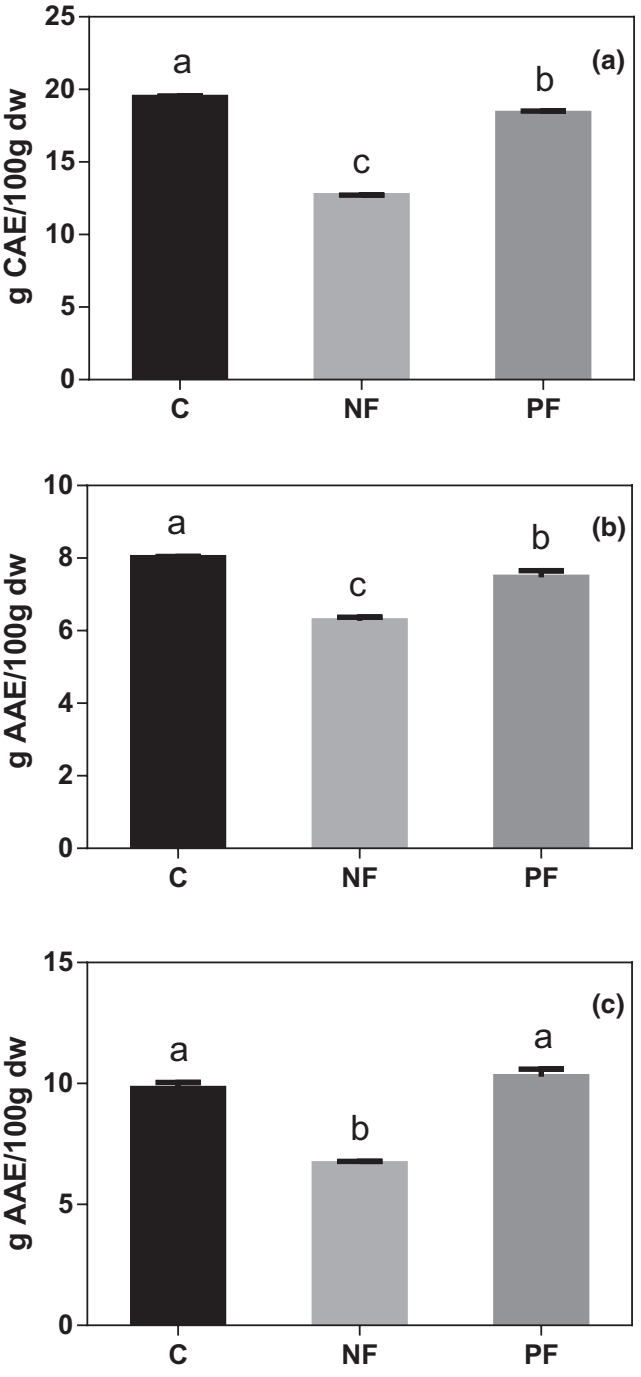

FIGURE 2 Total polyphenol content (a), DPPH (b), ABTS (c) of sweet potato leaf samples. C, control; NF, natural fermentation; PF, probiotic fermentation. Samples that were not significantly different are assigned the same letter ($p < .05$).

3.3.3 | ACE inhibitory activity

The ACE inhibition-concentration curves were shown in Figure 3. The IC_{50} was calculated from the curves and expressed as the concentration (mg/ml) of soluble polyphenols. The IC_{50} organized in a decreasing order was 1.09 mg CAE/ml, 0.35 mg CAE/ml and 0.12 mg CAE/ml for C, NF, and PF, respectively. The polyphenols present in NF and PF were able to increase the ACE inhibition, changes in polyphenol composition exposed in section 3.3.1 could be the cause of this behavior. Other authors have shown the antihypertensive activity of Chlorogenic acid (CQA) previously (Suzuki, Fujii, et al., 2006; Suzuki, Yamamoto, et al., 2006). More recently, Bhullar et al. (2014) have demonstrated the possible use of caffeic acid and its derivatives in the treatment of hypertension through multi-target modulation of renin–angiotensin–aldosterone system (RAAS). Recently Santos et al. (2020) used *Cuphea spp.* leaf polyphenols extracts with ethanol 40% (v/v) for the inhibition of ACE. Investigation of the

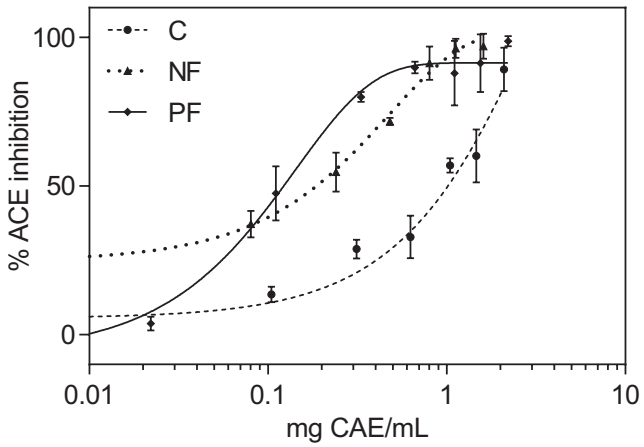

FIGURE 3 ACE inhibition curves using polyphenols extracted from sweet potato leaf samples. C (●), control; NF (▲), natural fermentation; PF (♦), probiotic fermentation as inhibitors. Continuous and non-continuous lines represent the non-linear regression using one phase decay equation for each data set.

potential inhibitor of ACE activity was performed with the 11 crude extracts and all samples showed inhibition capacity with %inhibition between 31.66% and 9.88%. The authors could not affirm the inhibition mechanism but described that some polyphenols inhibit enzyme activity by competing with the substrate for the active sites (Ojeda et al., 2010).

4 | CONCLUSION

Lactobacillus plantarum and *Enterococcus durans* were identified in NF and mainly *Lactobacillus plantarum* in PF. Changes in polyphenol composition and concentration during fermentation were found. This study revealed that *Lactobacillus plantarum* used as a starter was useful to improve the fermentation quality, maintaining the antioxidant activity and improving the ACE inhibition compared to the control SPL. Results showed the potential utilization of SPL in the elaboration of a functional beverage. This is the first approach to SPL fermentation, bacterial identification, and assessing bioactive properties; so, additional studies are necessary for further applications.

AUTHOR CONTRIBUTIONS

Santiago Suárez: Formal analysis; investigation; writing – original draft. **Hongnan Sun:** Conceptualization; methodology; supervision; writing – review and editing. **Tai-Hua Mu:** Conceptualization; methodology; supervision; writing – review and editing. **Maria Cristina Añón:** Conceptualization; methodology; writing – review and editing.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ORCID

Hongnan Sun https://orcid.org/0000-0003-0631-4775
Taihua Mu https://orcid.org/0000-0002-1308-0121

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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