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Antioxidant activity, nutritional, and phenolic composition of sweet potato leaves as affected by harvesting period

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ABSTRACT

In the present study, the nutritional and phenolic composition as well as the antioxidant activity of sweet potato leaves (SPL) harvested in 3 different periods were determined and compared. Furthermore, gray relational analysis was used to compare the comprehensive nutritional value. Results showed SPL HP1 had the highest protein value (30.8 ± 0.4 g/100 g dw), while SPL HP3 had the highest content of vitamin C (104.6 ± 4.9 mg/100 g dw), vitamin E (5.8 ± 0.4 mg/100 g dw), total polyphenol content (9.1 ± 0.3 g/100 g dw), antioxidant activity (DPPH: 7.4 ± 0.1 g VcE/100 g dw; ABTS: 10.6 ± 0.7 g VcE/100 g dw; FRAP: 0.617 ± 0.005 μ mol TroloxE/100 g dw), and comprehensive nutritional value (weighted gray relational grade 0.8336). The individual phenolic composition showed the presence of six caffeoylquinic acids, caffeic acid, and two flavonoids (quercetin and isoquercetin), which were significantly different among different harvest periods. In conclusion, HP3 was an optimal period for harvesting SPL.

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KEYWORDS


Sweet potato leaves; harvest period; nutritional composition; phenolic composition; antioxidant activity

Introduction

China has the highest vegetable production^[1] and owns large amounts of consumers in the world. Within this production, China is the leading producer of sweet potato, had an annual production of 72 million tons (63.6% of the world's production) in 2017.^[2] Sweet potato leaves (SPL), the main byproduct of sweet potato production, are rich in polyphenols, proteins, vitamins, minerals, and some functional microcomponents.^[3] As a crop, it is more tolerant of diseases, pests, and high moisture than many other leafy vegetables grown in the tropics.^[4,5] SPL can be harvested several times a year, but most of the SPL in China have been discarded or used as feed, causing serious environmental pollution and waste of resources.^[6] The annual yield of SPL is therefore ultimately much higher than that of other green vegetables.^[7]

Although the tuberous root of the plant is most commonly consumed in the Western part of the world, the SPL are increasingly presenting a growing interest for human consumption due to its high nutritional value. Previous studies^[8] have evaluated the nutritional and bioactive components of leaves from 40 sweet potato cultivars and reached the conclusion that SPL are good sources of protein, fiber, polyphenols, and minerals, and the polyphenols therein are important antioxidants. Also, it has been reported that the content of polyphenols in SPL was much higher than that in the

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whole root, flesh tissue, and peel of sweet potato, as well as other most common commercial vegetables.^[9]

Pharmaceutical studies revealed that SPL polyphenols exhibited various health-promoting biological activities, such as antioxidant activity, anti-mutagenicity, anti-cancer, anti-hypertensive, anti-microbial, anti-diabetes, anti-inflammation.^[10–13] Specifically, the bioactive compounds of SPL have been studied in humans and animals.^[14] However, these previous studies provided no information regarding the effect of leaf harvest period on phenolic composition and antioxidant properties of SPL. The leaves at a particular harvest period may have more antioxidant potential and consuming leaves at that stage may be more beneficial for human health. The aim of this study was to determine the nutritional and phenolic compounds of SPL harvested at different period (August 22, September 6, and September 21) and evaluate the antioxidant activity in order to decide the best period for harvesting the SPL.

Materials and methods

Plant materials

Fresh SPL (cultivar Shangshu No. 19) were obtained from the Academy of Agricultural and Forestry Sciences in Hebei Province, China. Cultivar Shangshu No. 19 is used for starch processing, and the rest is used for other food processing, e.g., dried fruit, juice, and chips. The leaves were collected on August 22 (harvest period No. 1), September 6 (harvest period No. 2) and September 21 (harvest period No. 3). Prior to harvest, the leaves were collected, washed, and freeze-dried. All samples were ground in a commercial grinder and stored at 4°C in sealed aluminum bags.

Proximate composition

Moisture, ash, crude fat, and crude protein contents were determined by AOAC methods.^[15] Crude protein was assessed by the Kjeldahl method, with nitrogen to protein conversion factor of 6.25 (AOAC method 976.05). Total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) were determined by digesting the sample with α -amylase, amyloglucosidase, and protease, respectively (AOAC method 991.43).

Mineral and vitamin content

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. Vitamin B₁ (thiamin) was determined according to AOAC method 942.23. Vitamin B₂ (riboflavin) was determined using AOAC method 970.65. Vitamin B₃ (niacin) analysis was assessed according to the spectro-photometric AACC method (No: 86-50A) (AACC, 2000). Vitamin E was analyzed according to AOAC method 969.40. The vitamins content was expressed as mg/100 g dw sample.

Vitamin C was determined according to the method of Chebrolu et al.^[16] with some modifications. One g of freeze-dried SPL was mixed with 10 mL of 4.5% w/v metaphosphoric acid (MPA) for 10 min with stirring, centrifuged at 4000 g for 15 min at 20°C, then 3 mL supernatant was mixed with 3 mL 5mM of Tris 2-caboxyethyl phosphine hydrochloride (TCEP) and place for 30 min at room temperature protected from light and finally filtered through a 0.22 μ m syringe filter. The vitamin C was analyzed on a Shimadzu LC-20A HPLC (Tokyo, Japan) with the UV wavelength detector set at 254 nm. L-ascorbic acid was used as a standard. Total ascorbic acid was expressed as milligrams per 100 grams of sample on a dry weight (mg/100 g dw).

Amino acid content

The amino acid content was measured by the method of Dong et al.^[17] with some modifications. Briefly, 200 mg sample was hydrolyzed with 10 mL HCl (6 mol L⁻¹) in acid hydrolysis tubes with air elimination by nitrogen-blowing at 110°C for 24 h. After cooling, filtering and washing, the hydrolyzate was diluted, dried with nitrogen flow and dissolved with 5 mL HCl (0.02 mol L⁻¹). The amino acid compositions of the samples were measured using an L-8900 Amino Acids Automatic Analyzer (Hitachi Ltd., Tokyo, Japan). Amino acid score (AAS) was used for determination of leaf protein quality according to.^[18] Amino acid composition appropriate for a pre-school child (age 2–5 years old)^[19] was used as standard for AAS estimation.

Extraction of polyphenols

Extraction of polyphenols from SPL was carried out according to the method described by Sun et al.^[8] using 70% (v/v) ethanol as a solvent.

Total polyphenol content

Total polyphenol content (TPC) was measured by the Folin–Ciocalteu method.^[5] A calibration curve consisting of chlorogenic acid standards, ranging from 0.02 to 0.10 mg/mL, was prepared. TPC was expressed as chlorogenic acid equivalent (CAE) on a dry weight (dw) basis.

Antioxidant activity

DPPH radical (DPPH·) scavenging activity. The procedure used was essentially the one described by.^[20] Briefly, 2.0 mL of diluted sample solution was mixed with 2.0 mL of DPPH solution (0.066 mM in 95% ethanol). After 30 min incubation, the absorbance was measured for sample (A1), positive control (A1) as well as blank control (A2) at 517 nm. The DPPH· scavenging activity (DPPH·) was calculated as below:

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

Instead of sample solutions, 2.0 mL ascorbic acid was used as a positive control at different concentrations (from 1.0 to 10.0 µg/mL). All results were expressed as g of ascorbic acid equivalents per 100 g of dried SPL material (g VcE/100g dw).

Ferric reducing antioxidant power (FRAP) was assayed according to Benzie and Strain.^[21] Appropriately diluted samples (150 µL) were mixed with 2850 µL of FRAP solution. The mixtures were incubated at room temperature for 30 min in the dark. The ferrous tripyridyltriazine complex (colored product) was measured by reading the absorbance at 593 nm. The standard curve was prepared using Trolox ranging from 10 to 200 µg/mL. The activity was expressed as µmol Trolox equivalents (TE)/100 g dw.

ABTS radical scavenging capacity assay

The ABTS radical scavenging activity of samples was measured using the methodology described by Estiarte et al.^[22] Briefly, ABTS⁺ working solution was prepared by the reaction of 7 mM ABTS stock solution with 2.45 mM potassium persulfate and then it was incubated in the dark for 12–16 h. Prior to perform the analysis, this was diluted in ethanol until the absorbance at 734 nm was 0.70 ± 0.02. Next, 30 µL of the sample was added to a test tube, along with 3 mL of dilute ABTS solution. After being incubated for 25 min at 30°C, the absorbance was read. The ascorbic acid standard curve was prepared at the concentrations to range from 5 to 100 mg/L. The results were expressed as g of ascorbic acid equivalents per 100 g of dried SPL material (g VcE/100g dw).

Quantification of phenolic compounds by RP-HPLC

SPL samples were accurately weighed and then dissolved in 80% (v/v) methanol to prepare a sample solution (200 µg/mL). An aliquot of the supernatant was filtered and analyzed for individual phenolic compounds according to the method described by Sun et al.^[23] Peaks were detected at 326 nm using a prominence RID-20A UV/VIS detector. Quercetin, Myricetin, Apigenin, Isoquercetin, Tiliroside, Astragaln, Kaempferol, caffeic acid, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, and 3,4,5-tri-*O*-caffeoylquinic acid (Sigma Aldrich, Inc.) were used as standards. Detected peaks were identified and quantified by comparing the retention time and peak area to that of known standards.

Gray relational analysis

Gray relational analysis (GRA), deduced by the gray system theory^[24], has been broadly applied for evaluating the intrinsic interrelationships between multiple variables. Each harvest period was regarded as one of the factors of the system. The perfect cultivar, which had all optimum parameters, was established as a reference. Then, the weighted gray relational grade (WGRG) was calculated to evaluate the degree of association between the ideal cultivar and different samples. The cultivar with the highest WGRG value should have the best nutritional value.

The process of the GRA was performed as follows: The protein, TDF, lipid, TPC, FRAP, ABTS, DPPH, vitamin C, E, B₁, B₂, B₃, minerals, and AAS (%) were chosen as positive correlation indicators, while the contents of water and toxic mineral elements were chosen as a negative indicator. The positive correlation indicators of ideal cultivars were 5% higher than the maximum, and the negative correlation indicators were 5% lower than the minimum. The normalization of original data and weighted gray relational grade (WGRG) calculation was done using the mathematical procedure described by.^[25] WGRG was used to evaluate the degree of association between the ideal cultivar and different samples. The cultivar with the highest WGRG value should have the best nutritional value.

Statistical analysis

The statistical analysis was performed with the 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 Least Significant Difference (LSD) test, with a significance level of 0.05.

Results and discussion

Nutritional value of sweet potato leaves harvested in three different periods

The effect of harvest period on proximate composition of SPL is shown in Table 1. According to the results, the content of moisture, ash, and protein was highest in SPL HP1 (87.70 ± 0.05 g/100 g fw, 11.30 ± 0.05 and 30.8 ± 0.4 g/100 g dw, respectively). The content of fat in SPL HP1 and HP2 showed no significant difference (3.6 ± 0.4 and 3.7 ± 0.2 g/100 g dw, respectively), and much higher than SPL HP3 (2.91 ± 0.07 g/100g dw). The total dietary fiber and soluble dietary fiber contents were similar in the three period of harvest but the insoluble dietary fiber in HP2 and HP3 (45.07 ± 0.09, 43.8 ± 1.1 g/100 g dw, respectively) was significantly higher than HP1 (42.8 ± 0.5 g/100 g dw). The moisture, fat, protein and ash contents obtained in this study were similar to those reported by Sun et al.^[8], where they assessed the nutritional composition of leaves from 40 sweet potato cultivars. The gradual decrease in protein and fat content measured throughout the different harvesting periods were reported by.^[26,27] Dietary fiber content (TDF, IDF, and SDF) was higher than previous report from SPL. For example, Ishida et al.^[3] found the following values: 45.7–45.9, 39.1–39.9, 5.77–6.83 g/100 dw for TDF, IDF, and SDF, respectively. They worked with

Table 1. Moisture^a, ash, fat, crude protein, total dietary fiber (TDF), insoluble dietary fiber (ISF) and soluble dietary fiber (SDF) contents (g/100 g dw)^b of sweet potato leaves harvested at different periods (HP1, HP2, HP3)^c

Component	HP1	HP2	HP3
Moisture ^b	87.70 ± 0.05a	86.95 ± 0.06b	86.05 ± 0.04c
Ash	11.30 ± 0.05a	10.67 ± 0.04b	10.39 ± 0.06c
Fat	3.6 ± 0.4a	3.7 ± 0.2a	2.9 ± 0.1b
Protein	30.8 ± 0.4a	29.2 ± 0.2b	26.2 ± 0.2c
TDF	49.8 ± 0.5a	51.0 ± 1.7a	51.8 ± 1.8a
IDF	42.8 ± 0.5a	45.1 ± 0.1b	43.8 ± 1.1b
SDF	7.3 ± 0.8a	8.1 ± 0.3a	8.1 ± 1.3a

^aMoisture content was expressed in g/100 g fw.

^bData are means ± SD (n = 3). Values within the same line with different letters are significantly different ($p < 0.05$).

^cHP1, HP2, and HP3 means the sweet potato leaf samples harvested on August 22, September 6, and September 21, respectively.

Table 2. Mineral and vitamin composition (mg/100 g dw) of sweet potato leaves harvested in different periods (HP1, HP2, HP3).

Component	HP1	HP2	HP3
Na	69.1 ± 4.6a	24.6 ± 0.2b	34.7 ± 0.7b
Ca	1334.0 ± 70.5a	1407.4 ± 15.4a	1318.8 ± 22.3a
K	3321 ± 188a	1963.8 ± 61.7b	3341.7 ± 104a
P	153.3 ± 0.8a	142.4 ± 0.8b	137.7 ± 1.5c
Fe	19.9 ± 1.2a	13.9 ± 0.2b	11.1 ± 0.6b
Zn	3.3 ± 0.2a	3.3 ± 0.1a	3.2 ± 0.0a
Cu	1.49 ± 0.08a	1.35 ± 0.05a	1.52 ± 0.01a
Mn	9.9 ± 0.4a	6.31 ± 0.08b	7.43 ± 0.08c
Mg	513 ± 19a	458 ± 1b	455 ± 7b
Se*	9.01 ± 0.02a	8.78 ± 0.05b	8.48 ± 0.02c
Pb	<0.04	<0.04	<0.04
Hg	<0.01	<0.01	<0.01
As	0.11 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00b
B1	0.62 ± 0.01a	0.53 ± 0.00b	0.49 ± 0.00c
B2	6.36 ± 0.04a	5.69 ± 0.06b	4.45 ± 0.01c
B3	0.54 ± 0.04a	0.50 ± 0.02a	0.45 ± 0.01a
C	21.9 ± 0.3a	54 ± 1b	104 ± 5c
E	3.24 ± 0.02a	3.3 ± 0.2a	5.8 ± 0.4b

Data are means ± SD (n = 3). Values within the same line with different letters are significantly different ($p < 0.05$).

*(µg/100g)

HP1, HP2, and HP3 means the sweet potato leaf samples harvested on August 22, September 6, and September 21, respectively.

two Japanese varieties, Koganesengan (KS) and Beniazuma (BA). Different factors contribute to the discrepancy in dietary fiber including genotype, maturity, and nutritional composition, also the methodology used could influence the final result.

Table 2 shows the mineral content of SPL. SPL HP1 presented the highest values for Na, P, Fe, Mn, Mg, and Se. The three-harvest periods assessed showed similar concentration of Ca, Zn and Cu, and K presented the highest value on HP3. The most abundant macroelement was K in agreement with previous reports.^[8] The content of toxic metals such as As, Pb, and Hg in the three-harvest periods were below to the maximum level according to the Codex Alimentarius (0.5, 0.3, and 0.1 mg/Kg for As, Pb, and Hg, respectively). Pace et al.^[28] evaluated Ca, Fe, and Zn contents of a total of 64 “Jewel” SPL samples collected at the end of the growing season and compare the content in younger (initial 10-cm portion from the tip) and older leaves (second 10-cm portion from the tip). The mineral contents varied between 836 ± 42–1.144 ± 43 mg/100g, 10.9 ± 0.4–13.4 ± 0.7 mg/100g and 2.5 ± 0.1–2.9 ± 0.1 mg/100g for Ca, Fe, and Zn, respectively. Generally, older material contained the highest concentration of Ca and Fe and lowest Zn content. In our study, the concentration of these minerals was a little bit higher according to the results showed in Table 2 and we only found difference on Fe content being this value higher in SPL HP1.

Vitamin contents in the samples are shown in Table 2. Different vitamin distribution was found in function of harvest period. Vitamin B₁ and B₂ content were highest in HP1, no difference was found on B₃ and HP3 showed the highest value for vitamin C and E. The vitamin E content obtained in HP1 and HP2 was also similar to the amounts found by Ishida et al.^[3] in the cultivar KS. The vitamin C content obtained expressed in fresh weight were 2.69, 7.06, 14.59 mg/100 g fw for HP1, HP2, and HP3, respectively. Different ranges of vitamin C have been reported in the literature. Our results are similar to the results obtained by Sui et al.^[29] but less than the content published by Ishida et al.^[3] and Barrera and Picha.^[30]

Amino acid composition and the amino acid score in comparison with the standard amino acid pattern (age: 2–5 years old) of WHO^[19] were evaluated. Table 3 shows the amino acid composition for the three-harvest periods and the amino acid score for essential amino acids. For non-essential amino acids HP1 and HP2 showed the highest content. According to essential amino acids, Cys, Met, Tyr, and His showed the same values for the three-harvest periods. The content of the rest essential amino acids was highest in HP1 and HP3. In the three-harvest periods the first limiting amino acid was “Cys-Met”, and the amino acids score were 18.6 ± 2.9 , 17.5 ± 4.0 and 10.6 ± 1.2 from HP1 to HP3, respectively. According to the study done by Ishida et al.^[3], Lys was the first limiting amino acid with an amino acid scores of 76.9% and 83.9% for KS and BA cultivars, respectively. In our study Lys was the second limiting amino acid with amino acids scores of 76.9 ± 0.8 , 77.1 ± 0.8 and 65.3 ± 2.0 for HP1, HP2, and HP3, respectively. The values found for^[3] matched with the results showed but the discrepancy in the AAS order could be explain for the differences in the amino acid concentrations. The Lys/(Met+Cys) relation reported for^[3] was 1.58 and in this study was 11.4 (average for the 3 harvest periods), which means 7.2 times higher.

Total polyphenol content and antioxidant activity

The Folin–Ciocalteu method was used to determine the total polyphenol content of SPL. The results are shown in Figure 1(a). SPL HP3 showed the highest content (9.1 ± 0.4 g/100g dw), followed by HP2 (7.0 ± 0.3 g/100g dw) and finally HP1 (6.0 ± 0.3 g/100g dw). These results

Table 3. Amino acid contents (mg/g dw)^a and amino acid score (AAS)^b (%) of sweet potato leaf protein.

Amino acid	HP1		HP2		HP3	
	AA content	AAS	AA content	AAS	AA content	AAS
<i>Non-essential amino acids</i>						
Asp	$32.3 \pm 1.7a$	-	$27.8 \pm 0.7b$	-	$28.7 \pm 2.0b$	-
Ser	$8.5 \pm 0.4a$	-	$8.2 \pm 0.2a$	-	$7.3 \pm 0.5a$	-
Glu	$28.3 \pm 1.4a$	-	$25.8 \pm 0.7b$	-	$21.1 \pm 1.4c$	-
Gly	$10.8 \pm 0.6a$	-	$10.5 \pm 0.3a$	-	$8.3 \pm 0.6b$	-
Arg	$11.1 \pm 0.3a$	-	$11.3 \pm 0.0a$	-	$9.2 \pm 0.7b$	-
Ala	$11.8 \pm 1.0a$	-	$11.3 \pm 0.1a$	-	$9.3 \pm 0.6b$	-
Pro	$8.6 \pm 0.7a$	-	$8.3 \pm 0.2a$	-	$6.1 \pm 0.4b$	-
<i>Essential amino acids</i>						
Thr	$8.9 \pm 0.5a$	$85 \pm 2a,b$	$8.7 \pm 0.2a,b$	$87 \pm 1a$	$7.0 \pm 0.5b$	$79 \pm 3b$
Cys ^c	$0.4 \pm 0.3a$	$18 \pm 3a$	$0.5 \pm 0.6a$	$17 \pm 4a$	$0.1 \pm 0.0a$	$10 \pm 1b$
Met	$0.9 \pm 0.2a$	-	$0.8 \pm 0.0a$	-	$0.6 \pm 0.0a$	-
Val	$11.4 \pm 0.9a$	$111 \pm 0a$	$10.8 \pm 0.1a$	$106 \pm 1a$	$8.8 \pm 0.5b$	$96 \pm b$
Ile	$8.8 \pm 0.5a$	$107 \pm 1a$	$8.4 \pm 0.2a,b$	$102 \pm 1a$	$6.7 \pm 0.4b$	$91 \pm 3b$
Leu	$16.1 \pm 0.9a$	$83 \pm 1a$	$12.9 \pm 4.1b$	$71 \pm 13b$	$11.9 \pm 0.7b$	$68 \pm 2b$
Tyr	$5.9 \pm 0.4a$	$92 \pm 1a$	$6.4 \pm 0.1a$	$94 \pm 1a$	$4.7 \pm 0.2a$	$81 \pm 2b$
Phe	$11.1 \pm 0.6a$	-	$10.9 \pm 0.3a$	-	$8.8 \pm 0.5b$	-
Lys	$13.1 \pm 0.7a$	$77 \pm 1a$	$13.1 \pm 0.3a$	$77 \pm 1a$	$10.0 \pm 0.6b$	$65 \pm 2b$
His	$5.0 \pm 0.2a$	$85 \pm 1a$	$5.1 \pm 0.1a$	$91 \pm 1a$	$4.4 \pm 0.2a$	$88 \pm 2a$

^aData are means \pm SD (n = 3). Values within the same line with different letters are significantly different ($p < 0.05$).

^bAAS (%) calculated for essential amino acids.

^cThe AAS for Met-Cys and Tyr-Phe was calculated together.

HP1, HP2, and HP3 means the sweet potato leaf samples harvested on August 22, September 6, and September 21, respectively.

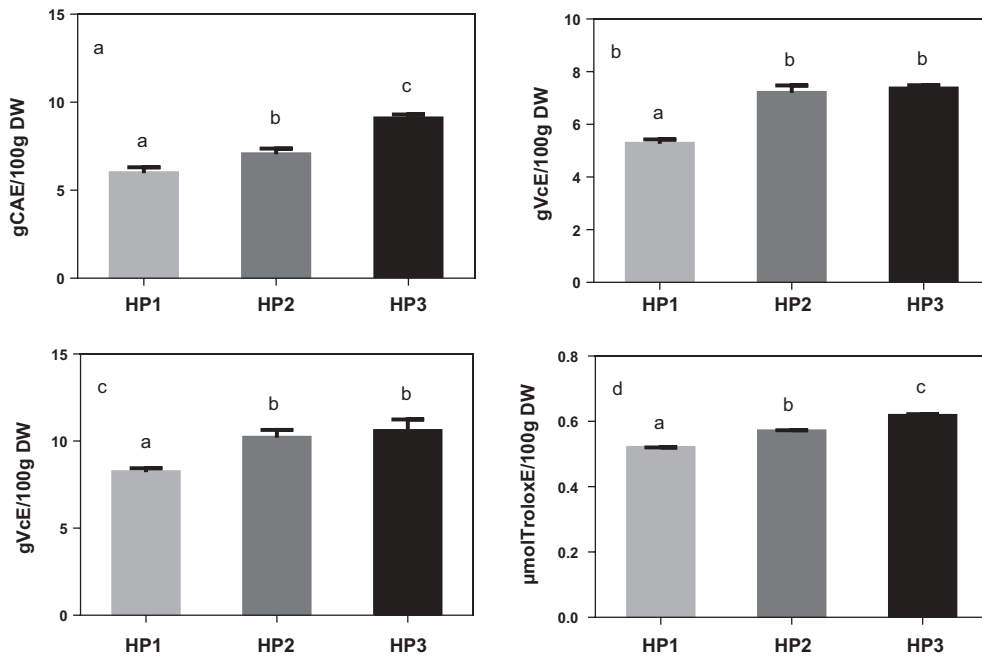


Figure 1. Effect of different harvest period on total polyphenol content (A), DPPH (B), ABTS (C) and FRAP (D) of SPL. Values are means \pm SD of three determinations. Harvest period that were not significantly different are assigned the same letter ($p > .05$).

corroborated the previous literature reports. Sun et al.^[8] determined the TPC of 40 sweet potato cultivars finding a range of 2.73–12.46 g/100 g dw, which was similar to the findings reported for^[7] (1.42–17.1 g/100 g dw). It was noted that there is an increase in the production of phenolic compounds over time. Padda and Pichia^[31] revealed TPC varies with the age of SPL tissue. In their study the concentration of TPC in young leaves (8.73 g/100g dw) was significantly higher than in mature (2.78 g/100 DW) and old leaves (2.15 g/100g dw). The differences found with our study may be due to the fact that this author worked with leaves obtained from vines at three different locations: top (young), middle (mature) and lower part (old) of the vine harvested at the same time. However, young leaves do not always contain higher concentrations of phenolics than mature leaves, especially if individual compounds are examined^[32] and not all secondary compounds follow the same pattern during leaf development.^[33] At the present, there are insufficient studies on this subject from SPL. Although the influence of leaf age has been described for other species, there is no general conclusion applicable to all plant species.^[34,35] For example, Blum-Silva et al.^[36] revealed no significant differences in levels of phenolic compounds comparing leaves of *Ilex paraguariensis* harvested at one and 6 months.

DPPH radical scavenging activity (Figure 1(b)) of SPL was highest on HP2 and HP3 without any significant difference between this two-harvest periods. Similar results were obtained when the antioxidant activity was assessed using the ABTS radical scavenging activity (Figure 1(c)). The ferric reducing antioxidant power confirmed that the SPL HP1 showed the lowest antioxidant activity and we founded difference between HP2 and HP3 being the antioxidant activity highest in SPL HP3 (Figure 1(d)). The results found, regarding antioxidant activity, were in agreement with previous research carry out with SPL.^[23,37]

According to the individual phenolic composition explained in the next section SPL HP3 showed the highest concentration of caffeic acid, 4,5-di-*O*-caffeoylquinic acid and 3,4,5-tri-*O*-caffeoylquinic acid (Table 4). Previous research conducting with individual phenolic compounds from SPL indicated that caffeic acid showed the highest antioxidant activity.^[6] Islam et al. (2003) found

Table 4. Content of individual phenolic compounds in sweet potato leaves harvested at different periods (mg/g of dw).

Peak	Identity	HP1	HP2	HP3
1	5- <i>O</i> -caffeoylquinic acid	3.801 ± 0.036a	5.122 ± 0.003b	4.285 ± 0.002c
2	3- <i>O</i> -caffeoylquinic acid	8.882 ± 0.059a	13.074 ± 0.053b	12.437 ± 0.027c
3	Caffeic Acid	1.337 ± 0.007a	1.251 ± 0.005b	1.441 ± 0.006c
4	Isoquercetin	4.095 ± 0.010a	5.797 ± 0.015b	4.674 ± 0.005c
5	3,4-di- <i>O</i> -caffeoylquinic acid	6.924 ± 0.008a	12.622 ± 0.022b	11.272 ± 0.005c
6	3,5-di- <i>O</i> -caffeoylquinic acid	14.712 ± 0.018a	22.487 ± 0.028b	20.989 ± 0.004c
7	4,5-di- <i>O</i> -caffeoylquinic acid	2.565 ± 0.002a	3.885 ± 0.008b	4.788 ± 0.005c
8	3,4,5-tri- <i>O</i> -caffeoylquinic acid	0.686 ± 0.008a	0.872 ± 0.002b	0.964 ± 0.002c
9	Quercetin	1.738 ± 0.016a	2.342 ± 0.015b	2.322 ± 0.065b

Values are means ± SD of three determinations. Harvest period that were significantly different are assigned different letter ($p < 0.05$). HP1, HP2, and HP3 means the sweet potato leaf samples harvested on August 22, September 6, and September 21, respectively.

3-*O*-caffeoylquinic acid as the highest DPPH activity followed by 3,4,5-triCQA and caffeic acid. Although caffeic acid and 3,4,5-tri-*O*-caffeoylquinic acid are in low concentration in SPL, apparently these compounds are the main contributors to the antioxidant activity.

Individual phenolic composition

HPLC chromatographic pattern of the phenolic compounds of SPL is shown in Figure 2 and the identification results for the polyphenols are shown in Table 4. The HPLC profiles of the three-harvest periods tested showed peaks at the same retention time, there being no qualitative difference among. SPL was mainly composed of six caffeoylquinic acids, a certain amount of caffeic acid and two flavonoids, consistent with previous report.^[7,23,38,39] The results suggest that the main phenolic compound in SPL was 3,5-di-*O*-caffeoylquinic acid and the concentration was higher in SPL HP2. 5-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, isoquercetin and 3,4-di-*O*-caffeoylquinic acid also showed the higher concentration in SPL HP2. The content of caffeic acid, 4,5-di-*O*-caffeoylquinic acid and 3,4,5-tri-*O*-caffeoylquinic acid were higher in SPL HP3. Quercetin showed the higher value both in SPL HP2 and HP3. A sum of individual phenolic compounds detected by HPLC of 44.71, 67.45 and 63.17 mg/g dw were obtaining for HP1, HP2, and HP3, respectively. This data was lower than that estimated by using the Folin–Ciocalteu method (59.73, 70.40, and 90.79 mg/g dw respectively). One possible reason could be the different interferences in the sample which react with the Folin reagent or some unknown individual phenolic peaks in the HPLC chromatograms profile not identified.^[40]

Gray relational analysis

According to the GRA, the weighted gray relational grade (WGRG) for SPL was 0.8124, 0.7885, and 0.8336 for HP1, HP2, and HP3, respectively. Which means SPL HP3 was the harvest period closer to the perfect cultivar defined according to this methodology. This result could be explained by the best performance find in this HP according to the TPC, antioxidant activity and content of vitamin C and E, among others nutrients. As we explain in discussion section, the first harvest period (HP1) shows the highest content of nutritional components and as the time passes to realize the harvest, phenolic component increase, such we can see in HP3 and the content of nutritional components decrease.

Conclusion

In this study, the effect of harvest period on nutritional and phenolic composition as well as antioxidant activity was described. Gray relational analysis (GRA) was used for the comparison of different parameters measured and try to find which is the best period for harvest the leaves. In

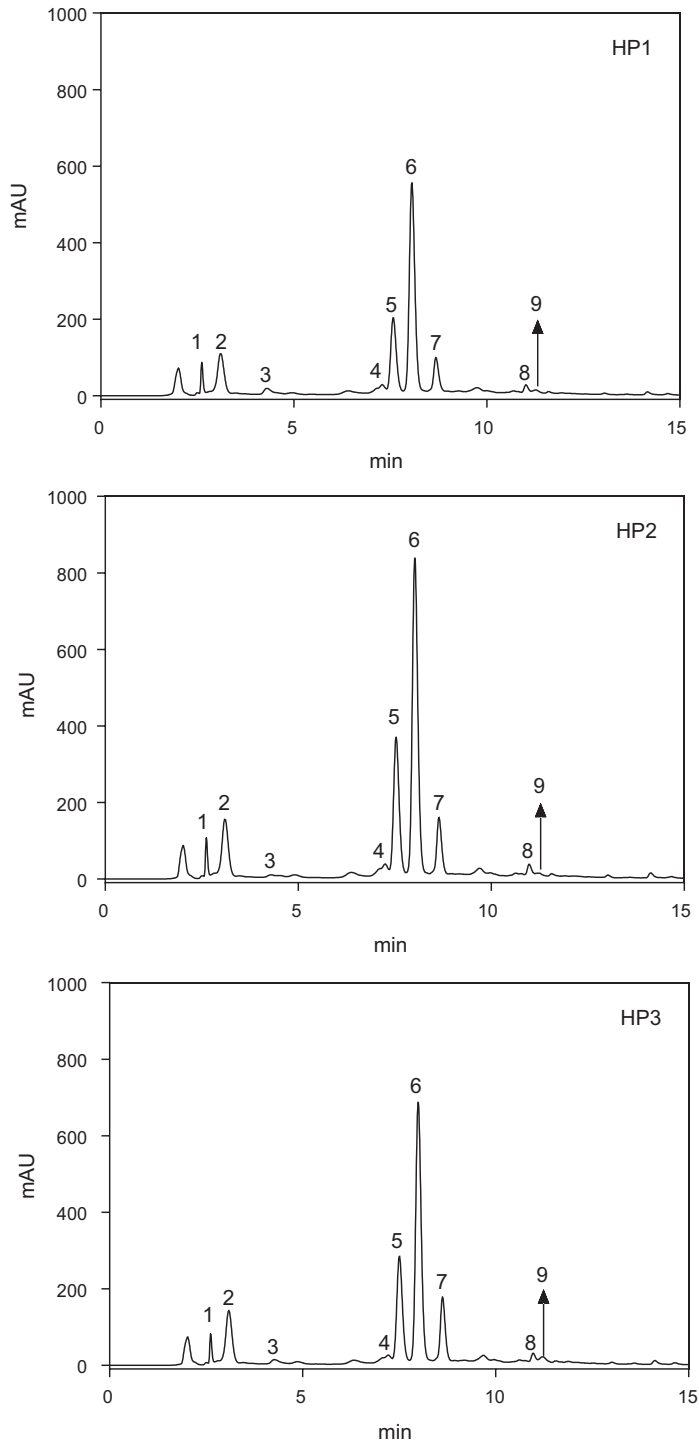


Figure 2. HPLC chromatograms of different phenolic compounds in SPL at 326 nm for different harvest period. Peaks 1 is 5-*O*-caffeoylquinic acid; peak 2 is 3-*O*-caffeoylquinic acid; peak 3 is caffeic acid; peak 4 is isoquercetin; peak 5 is 3,4-di-*O*-caffeoylquinic acid; peak 6 is 3,5-di-*O*-caffeoylquinic acid; peak 7 is 4,5-di-*O*-caffeoylquinic acid; peak 8 is 3,4,5-tri-*O*-caffeoylquinic acid; peak 9 is quercetin.

summary the results showed that SPL could be harvest in the last period (HP3) without loss in the nutritional value and phenolic compounds presents in the leaves. Even though harvesting practices may affect the concentration of certain nutrients contained in the SPL, the leaf's overall integrity as a rich source of dietary nutrients remains intact.

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