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BIOACTIVE CONSTITUENTS AND SHELF-LIFE OF SWEET POTATO (*IPOMOEA BATATAS* L.) LEAVES

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ABSTRACT
We aimed to evaluate the green biomass' of sweet potato (*Ipomoea batatas* L.) quality, through quantitative analysis of microelements, colour characteristics, and UHPLC-MS screening of bioactive constituents. The shelf life examination included sealed raw sweet potato leaves in plastic packs stored at 6 °C and 12 °C and the microbiological characteristics were monitored for 2 weeks, through enumeration of mesophilic total plate count, total fungi count, *Enterobacteriaceae* and mesophilic aerobic spores. We found, that the sweet potato leaves can be considered as the source of calcium, magnesium and phosphorus among the minerals, of which calcium is the most abundant. We identified 17 types of amino acids, 7 vitamins, mainly vitamins belonging to the Vitamin B family. Furthermore, it contained carboxylic acids, flavonoids, polyphenols and aromatic compounds. The sweet potato leaves stored at 6 °C were of satisfactory microbiological quality on day 14. Our data suggest that the sweet potato leaves could be a valuable source for healthy nutrition.

Keywords: sweet potato leaves, HPLC-MS, storage, bioactive substance, inorganic element

INTRODUCTION

Sweet potato leaves (SPLs) are the main by-product of sweet potato tuber production, however SPLs can be harvested several times during the year. Their yields are much greater than those of green leafy vegetables (AN ET AL., 2003). Compared to green leafy vegetables, sweet potato leaves are more tolerant to diseases, pests, and high moisture conditions. Sweet potato leaves contain a large amount of phenolic compounds, of which more than 70% are chlorogenic acids and their derivatives and 10% to 20% are flavonoid compounds. The polyphenol content of SPLs from 40 cultivars was two to three times higher than some common vegetables (e.g. spinach, kale) (XI ET AL., 2015). These compounds have a number of biological activity including antioxidant or antidiabetic activity (MU ET AL., 2017), or has potential in leukemia (HL-60) cancer prevention (KURATA ET AL., 2007). SPLs could potentially be upcycled into new functional food ingredients, thereby achieving an effective valorisation process with pharmacological and health protecting or food industrial significance, e.g. SPLs pectin achieved better water holding capacity and emulsion stability index than the commercially available oat beta-glucan (TOY ET AL., 2022). SPLs are an alternative source of green leafy vegetables during their off-season and could potentially alleviate food shortages. SPLs are commonly used as human foodstuff in some African and Asian countries (Japan), and preferred as food for the sustainable farming (Tufts University, Boston, USA). No scientific evidence is available about the quality variation of raw SPLs during a possible storage. MS screening examinations show that the usage of SPLs as an edible plant must be considered with caution, due to the essential oil substances which has cytotoxic effect (MARQUES ET AL., 2021). Scientific knowledge about the chemical composition and biological properties of these leaves is useful for proper usage of these specific components of this plant.

We investigated the chemical composition analysis and the storage stability of minimally processed SPLs originated from Hungarian cultivation. The purpose of the bioactive substance studies was to find out which other compounds can be found in the SPLs in addition to the known ones. SPLs have the potential to contribute to finding new solutions for the present changes in food supply and demand.

MATERIALS AND METHODS

Plant material

SPLs were sufficiently developed (*Figure 1*), free from disease, dark, greenish (length (cm): 4-15; width (cm): 3-11; colour coordinates: L*=43.01, a*= 0.26, b*= 3.31; moisture content: 76,4%). Approx. 10 kg sweet potato leaves obtained from 4 varieties (Bonita, Beauregard, Burgundy, Bellevue) were provided by Nyírségi Édesburgonya Zrt. originated from its own cultivation, in July 2020, immediately (in 4 hour) after collecting. Equal quantity of SPLs from each variety was mixed and prepared for examinations.

Figure 1. Sweet potato leaves (left), and packed samples for storage experiment (right).

Sample Preparation

For mineral element analysis, SPLs were dried in drying oven at 50 °C (Binder GmbH., Germany) until constant weight, then were ground in ball mill (MM 200, Retsch GmbH, Germany), and kept in plastic container at 6 °C until analysis.

For UHPLC-MS analysis, a hydro-alcoholic extract of 0.5 g SPL powder was prepared with 25 mL methanol:water (70:30) solution. The mixture was stirred at 150 rpm for 2 h at room temperature. The hydro-alcoholic extracts were filtered using a 0.22 μm PTFE syringe filter.

Analysis of Mineral Elements

0.5 g SPLs powder, 5 ml cc. HNO_3 and 5 ml H_2O_2 were added to the sample container of CEM MARS Express microwave digestion system. Ramp=35 min; temperature=200 °C; hold: 50 min; 1700 watt. The digested mixture was completed to 25 ml with distilled water, then after a fivefold-dilution, the sample was injected to PE Optima 8300 ICP-OES instrument (Perkin Elmer Inc., USA). Results are given in mg/kg powder.

Qualitative Analysis of Phytochemicals

Phytochemical analyses were performed using UHPLC-ESI-ORBITRAP-MS/MS with a Dionex Ultimate 3000RS UHPLC system (Thermo Fisher, USA) coupled to a Thermo Q Exactive Orbitrap hybrid mass spectrometer equipped with a Thermo Accucore C18 analytical column (2.1 mm × 100 mm, 2.6 μm particle size). The flow rate was maintained at 0.2 mL/min and the column oven temperature was set to 25 °C ± 1 °C. The mobile phase consisted of methanol (A) and water (B) (both acidified with 0.1% formic acid). The gradient program was as follows: 0–3 min, 95% B; 3–43 min, 0% B; 43–61 min, 0% B; 61–62 min, 95% B; and 62–70 min, 95% B. The injection volume was 2 μL. A Thermo Q Exactive Orbitrap hybrid mass spectrometer (Thermo Fisher, USA) was equipped with an ESI source. The samples were measured in both positive and negative ionization modes separately. The capillary temperature

was 320 °C and spray voltages were 4.0 kV in positive ionization mode and 3.8 kV in negative ionization mode, respectively. The resolution was 35,000 for MS1 scans and 17,500 for MS2 scans. The scanned mass interval was 100–1500 m/z. For the tandem MS (MS/MS) scans, the collision energy was set to 30 nominal collision energy units. The difference between measured and calculated molecular ion masses was less than 5 ppm in each case. The data were acquired and processed using Thermo Trace Finder 2.1 software based on own and internet databases (Metlin, Mass Bank of North America, m/z Cloud). The results were manually checked using Thermo Xcalibur 4.0 software (ThermoFisher, Waltham, MA, USA)

Storage Experiment of SPL

SPLs were rinsed in tap water and dried thoroughly by manual shaking, then the leaves were sealed tightly in plastic tray. The air was removed manually from the container and closed with adhesive tape to avoid oxygenation. The packs were stored for 14 days at 6 °C and 12 °C. Samples were taken on day 0, 7 and 14 for analysis of microbiological and physical parameters, as the followings:

For microbiological analysis, traditional culturing methods were applied. 10 g raw SPLs were mixed well with 100 ml using sterile pepton water 0.1% (w/v), then a 10-fold serial dilution were prepared. From the dilution tubes, aliquot volume (0.1 ml) was spread onto the surface of culture media in two parallel Petri-dishes. Total mesophilic aerobic microbial count was determined on Total Plate Count (TPC) agar after incubation at 30 °C for 48 hours. Total count of fungi (TFC) was determined on Rose-Bengal-Chloramphenicol agar incubated at 25 °C for 5 d. Total count of aerobic spore-forming bacteria (AS) was determined from serial dilution tubes formerly pasteurized at 80 °C for 30 min, by spread plate method on TPC agar incubated at 37 °C for 48 h. *Enterobacteriaceae* (EBac) were determined on violet red bile glucose (VRBG) agar after a 24-hour incubation at 37 °C. Results are given in lgN Cfu/g.

For measurement of physical parameters, moisture was determined by air oven method at 103 °C to constant weight. Colour coordinates (CIE Lab) were measured with Colorlite sph860 type (model CL150 Z) spectrophotometer (Colorlite GmbH., Germany), at D65 illumination and 10° observation angle. **L***: lightness (values between 0-100); **a*** (values: 0-60): red, if positive; green, if value is negative; **b*** (values between: 0-60): yellow, if value is positive, blue, if negative. Average values were calculated from 9 measurements.

RESULTS

Regarding the mineral element content, SPLs are considered to be a source of Calcium (9800 mg/kg) with its largest amount, as well as Magnesium (2800 mg/kg) and Phosphorus (3500 mg/kg) among minerals. Moreover, it contained 350 mg/kg Iron, 310 mg/kg Sodium, 170 mg/kg Manganese, 71 mg/kg Strontium, 38 mg/kg Boron, 36 mg/kg Zinc, 27 mg/kg Barium, 16 mg/kg Copper and 3,1 mg/kg Nickel. The SPLs did not contain heavy metals: Arsenic, Lead (<5 mg/kg) and Cadmium (<1 mg/kg). Cobalt, Chromium and Molybdenum were under detection limit (<1 mg/kg), too. Potassium was not measured.

Through UHPLC-Ms analysis, a total of 51 compounds were identified in the hydro-alcoholic extract of SPLs: amino acids (AAs; 17), vitamins (7), carboxylic acids (7), coumarins (5), polyphenols (4), flavonoids (7), lignan (1), aldehydes (2), and terpenoid (1). Essential and semi-essential AAs were found: Arg, Lys, His, Thr, Val, Met, Phe, Trp, and Ile or Leu. Cystein was not detected. Various other AAs were found: Arg, Ser, Asn, Asp, Gln, Glu, Pro, Tyr, N-acetylphenylalanine. Vitamins, mainly belonging to the Vitamin B family: riboflavin, nicotinic acid (niacin), adenine, pantotenic acid, pyridoxin, furthermore pyridoxal and nicotinamid were identified. Our measurement confirmed the presence of simple coumarins: esculin (12.32 min), esculetin (14.23 min), scopoletin (18.61 min) along with scopoletin-7-O-glucoside (14.48 min),

and isofraxidin (17.94 min). Of the chlorogenic acids quinic acid (1.24 min), ferulic acid (19.38 min) and caffeic acid(14.49 min) were present, and coumaroyl-quinic acid (Rt: 19.2, m/z: 337.09235 in negative ionization) beside the known components: O-ferruloyl-quinic acid isomers (m/z: 367.10291, at 3 different retention times: 14.57; 18.02; 18.45 min.). Cis- and trans-aconitic acid were identified at 1.33 and 1.65 min. Shikimic acid (at 1.35 min in negative ionization with m/z 173.04500*)*, abscisic acid (at 25.4 min in negative ionization at m/z 263.12834) and matairesinol (at 22.37 min in positive ionization with m/z 359.14947) were detected. Furthermore, various other phytochemicals were screened, which are presented in *Table 1*.

Microbiological and physical parameters of stored packed SPLs are shown in *Table 2*. Moisture content and colour have changed during storage in relation to time and temperature. Moisture content and colour coordinates were higher and more stable at lower storage temperature. At 12 °C the variation was more significant. The storage experiment was finished after 14 days because we experienced a food safety risk, visibly, mold appeared in the packs when the leaf was stored at 6 °C and in case of the storage at 12 °C – we experienced the same after 7 days.

DISCUSSION

Regarding the mineral element content, along with other researchers, we found that the SPLs are low in Sodium, abundant in Calcium and the Ca:P ratio was close to optimal (2:1), which is important for bone health. SPL were rich in polyphenols, aminoacids, vitamins and some functional microcomponents which is compliant with the findings of the cited researchers e.g. ISHIDA ET AL. (2000). Among the known chlorogenic acids, coumaroyl-quinic acid, which is mostly present in coffee, has not yet been detected in SPL (MU ET AL., 2017). Our measurement confirmed the presence of simple coumarins, just as in green biomass wastes of Jerusalem Artichoke (KASZÁS ET AL., 2020). Some of the simple coumarins are known as phytoalexins which can contribute to keeping the green biomass healthy and microbiologically safe. At the same time, esculin, esculetin and scopoletin have also shown health promoting potential e.g. antiadipogenic activity against the preadipocyte cell line in *in vitro* assay systems (VENUGOPALA ET AL., 2013). Oxalate was not present in our sample, which is in contrast with results by TSAI ET AL. (2005) who measured 48 mg/100 g oxalate in fresh SPL. Matairesinol (polyphenol/lignan) confers anti-allergic effects in an allergic dermatitis mouse model. Higher matairesinol intake were associated with lower vascular inflammation and endothelial dysfunction, which could have some implications in cardiovascular disease prevention (PELLEGRINI ET AL., 2010). Shikimic acid is a precursor of tryptophane synthesis and many alkaloids, aromatic metabolites. Tryptophan is the precursor of serotonin, which is involved in the regulation of mood and anxiety. Abscisic acid is a potent insulin-sensitizing compound and has the potential against pre-diabetes, type 2 diabetes and metabolic syndrome (LEBER ET AL., 2020).

Based on the storage experiment results, the microbiological condition of the SPLs stored at 6 °C was significantly better than that of the sample kept at 12 °C. The SPLs stored at 6 °C were of adequate quality for 14 days, so it could be stored without risk for 14 days. After 7 days of storage, the quality of the SPLs was inadequate when stored at 12 °C. The change in the colour and moisture content of the SPLs predicts a decrease in its quality. Cooled storage is needed to preserve SPLs quality.

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Table 1. Some phytochemicals of Alcoholic Extract of SPLs. RT-retention time (min); Frag.-fragment. ●Number in order of retention time.

No.●	Name	Formula	RT (min)	Measured Mass (m/z)		Frag.1	Frag.2	Frag.3	Frag.4	Frag.5
				$[M+H]^+$	$[M-H]^-$					
32	Naringenin-6,8-di-C-glucoside	$C_{27}H_{32}O_{15}$	17.01		595.16630	505.1346	475.1255	415.1035	385.0935	355.0830
33	Syringaldehyde (3,5-Dimethoxy-4-hydroxybenzaldehyde)	$C_9H_{10}O_4$	17.41	183.06574		155.0706	140.0473	123.0445	95.0499	
40	Kaempferol-3,7-di-O-glucoside	$C_{27}H_{30}O_{16}$	18.70		609.14556	447.0939	446.0851	285.0410	283.0256	255.0306
41	Isorhamnetin-di-O-glucoside	$C_{28}H_{32}O_{17}$	19.04		639.15613	477.1037	315.0515	314.0435		
42	Indole-4-carbaldehyde	C_9H_7NO	19.07	146.06004		118.0653	117.0574	91.0546		
43	Coumaroylquinic acid	$C_{16}H_{18}O_8$	19.20		337.09235	191.0554	173.0445	163.0389	119.0488	93.0330
46	Hyperoside (Quercetin-3-O-galactoside, Hyperin	$C_{21}H_{20}O_{12}$	22.73		463.08765	301.0348	300.0277	271.0246	255.0301	151.0020
47	Isoquercitrin (Hirsutrin, Quercetin-3-O-glucoside)	$C_{21}H_{20}O_{12}$	22.99		463.08765	301.0343	300.0279	271.0254	255.0299	151.0023
48	Astragalin (Kaempferol-3-O-glucoside)	$C_{21}H_{20}O_{11}$	24.75		447.09274	285.0422	284.0333	255.0301	227.0349	
49	Isorhamnetin-3-O-glucoside	$C_{22}H_{22}O_{12}$	24.97		477.10330	315.0519	314.0437	299.0213	271.0250	243.0296
51	12-Oxo phytodienoic acid	$C_{18}H_{28}O_3$	39.42		291.19603	273.1871	247.2075	165.1271		

Table 2. Microbiological and physical characterization of raw sweet potato leaves during storage. n.m- not measured. ● data in lgN Cfu/g.

temp.	day	TPC●	TFC●	AS●	EBac●	moisture (%)	L*	a*	b*
	0	1,3	1	<1	<1	76,43	43,01	0,26	3,31
6 °C	7	1,3	<1	<1	<1	72,77	42,95	0,29	3,33
	14	1,9	1,2	<1	<1	72,92	42,82	0,27	3,38
12 °C	7	2,9	1,7	<1	<1	65,19	40,92	1,08	2,36
	14	n.m.	visible mold		n.m.	n.m.	n.m.	n.m.	n.m.

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