Title page:
ANTIOXIDANT POTENTIAL, TOTAL VITAMIN C, PHENOLIC AND FLAVONOIDS CONTENT OF SWEET POTATO LEAVES

Short running title:
SWEET POTATO LEAVES – SOURCE OF ANTIOXIDANTS

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Abstract

Sweet potato leaves are a valuable source of bioactive nutrients such chlorophyll, reducing sugars, dietary fibers, vitamins, minerals and phenolic compounds. The present study evaluated the vitamin C content, total phenolic and flavonoids contents in sampled mature leaves from five sweet potato genotypes (’Hayanmi’, ’KSP1’, ’KSC1’, ’Yulmi’, ’Juhwangmi’) grown in green house conditions. The highest values for all chemical compounds analysed were found in leaves sampled from ‘KSP1’ and ‘Yulmi’ cultivars. Positive significantly correlation between the antioxidant activity estimated by DPPH radical scavenging activity and vitamin C content (r^2 = 0.892), total polyphenol content (r^2 = 0.878) and total flavonoid content (r^2 = 0.936) were found. The results obtained in this study highlights the nutritional value and the antioxidant potential of this vegetative parte of the plant, an inexpensive source of natural antioxidants beneficial for the humans health.

ORIGINAL RESEARCH ARTICLE

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Introduction

Sweet potato leaves represents a source of antioxidants unexploited enough in the world and almost at all, until now, in Romania. Even though many species produce polyphenols and vitamin C, availability and the cost of certain materials vegetal origin limited commercial exploitation of these natural sources of antioxidants only for a few species (CHUN & al [4]). The reasons that led to the choice of this subject were: the special bioavailability of this plant, economic considerations (lower price compared to other sources of vitamin C and polyphenols), and the presence of valuable vitamins (Thiamin, Riboflavin and vitamin B6) as described by BARRERA and PICHA [2].

Recent studies on sweet potato leaves composition revealed that they contain important phytochemicals that may prevent the development of diabetes and cardiovascular diseases, having favourable impacts on cardio-metabolic health, including lowering blood pressure, improving lipid profiles and decreasing markers of inflammation (DUTHIE & al [7]; Otaki & al., 2009 [16]; Kalkan & al., 2004 [15]). Several studies have demonstrated that sweet potato leaves inhibit HIV replication and cancer cell proliferation (Yoshimoto & al., 2002 [21]).

Vitamin C is a powerful antioxidant. Humans have lose ability to synthesize this compound and depend on the diet to acquire the necessary amounts required to maintain good health. According to nutritionists, modern people who lives "assaulted" by pollution conditions and unhealthy foods needs to eat foods rich in antioxidant compounds (Andre & al., 2010 [1]; Carocho & al., 2018 [3]). Sweet potato leaves are considered a good source of vitamin C and a moderate source of certain B vitamins in human diets (Barrera, 2014 [2]).

Sweet potato leaves are predominantly consumed in Africa and Asian countries (Johnson & al., 2010 [13]). They can be prepared alone or mixed with other species, consumed fresh or dried and stored for later use (Dinu & al., 2018 [6]). The leaves can be harvested several times a year because the plants grow quickly. The annual yield of sweet potato is much higher than that of many other vegetables grown for the leaves.

In Romania, consumption of sweet potato leaves is not common because this plant is cultivated on small areas.

The aim of this study was to quantify the levels of vitamin C, polyphenol and flavonoid contents in sweet potato mature leaves from 5 cultivars grown in greenhouse conditions. Another objective of this study was to determine the antioxidant potential of this biological material using the DPPH assay.

Sweet potato leaves with high content of vitamin C, polyphenols and flavonoids could have an important impact on human health and therefore, would be of interest to consumers and producers.

Material and methods

Biological material and sampling procedure. The sweet potato varieties ’Hayanmi’, ’KSP1’, ’KSC1’, ’Yulmi’’ and ’Juhwangmi’, were tested in the

middle of July at National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania. The tissue included 10 mature leaves harvested in three repetitions.

Extraction. One gram was weight into a centrifuge tube (50 ml) and 50 ml of aqueous 96% ethanol was added. The mixture was homogenized for 5 min. (Vortex), centrifuged at 10,000 rpm for 10 min. and filtered through Whatman (Number 400) filter paper.

Total phenolic content (TPC) analysis. The TPC was determined spectrophotometrically by Folin Ciocâlteu method (Singleton & al., 1999 [17]) with several modifications (Dinu & al., 2018 [6]). 50 µl of leaves extracts were mixed with 100 µl distilated water in a 96 well flat bottom assay plate (NUNC, Denmark). 50 µl Folin Ciocâlteu reagent were added and mixed for 1 min. in the plate reader (TecanSun Rise, softwere Magellan). After 5 min., 80 µl of a 20% solution (w/v) of Na_2CO_3 were added and mixed with a pipette; the microplates were shaken for 5 min. in the plate reader. After that, the plates were incubated at room temperature in the dark, agitating at 150 rpm on a MicroPlate Shaker (Biosan PST-60HL-4, Latvia) for 90 min. The absorbance of the samples was determined at 725 nm (TecanSun Rise, softwere Magellan). Gallic acid was used as standard and total phenolic content was expressed as milligrams GAE (Gallic acid equivalents) per gram of fresh weight (FW) materials.

Total flavonoids content (TFC) analysis. It is based on the nitration of any aromatic ring bearing a catechol group (two contiguous hydroxyls in the aromatic ring) with its three or four positions unsubstituted or not sterically blocked, followed by the formation of an aluminium complex which turns to red in basic medium (Kalita & al., 2014 [14]). Aliquots of 150 µl of extracts were also transferred to 1.5 ml tubes. Volumes of 600 µl distillated water and 45 µl of a 7.5% solution sodium nitrite were added to each tube, mixed by inversion and left to react for 5 min. A volume of 45 µl aluminium chloride 10% solution was pipetted onto the tubes, mixed by inversion and allowed to react for 1min. Finally, 300 µl of a 1N sodium hydroxide solution and 360 µl distillated water were added and the tubes vortex mixed. The absorbance of every solution was measured at 510 nm against the blank using a spectrophotometer DR2800 (Hach Lange, USA). Values of the absorbance samples were interpolated into a minimum squares regression equation (a 5-point calibration curve with an r^2 value of 0.998), which was calculated with the absorbance and the corresponding concentration of each quercetin standard. Quercetin hydrate in ethanol was used as the standard. Final results were calculated taking into account sample weight, extraction volumes and dilution factors applied and were expressed as mg quercetin equivalents (QE) per gram of fresh weight (FW) of sample (Damsa & al., 2016 [5]).

Extraction and analysis of vitamin C was carried out by following steps (indicated by the manufacturer instructions - Megazyme Ltd., Sigma Aldrich, USA, cod product MAK074): adding 10 ml of 6% (w/v) aqueous solution of

meta phosphoric acid to one gram of sweetpotato leaves; vortex this mixture for 1 min.; adjust to pH 3.5-4; quantitatively transferring to a 20 ml volumetric flask; centrifuge at 13.684 g (14.000 rot/min.) for 5 min. (a part of the mixture was taken in 1.5 ml tubes). Analysis of the L ascorbic acid content was carried out using the supernatant and an enzymatic method (L-ascorbic acid test kit, Megazyme Ltd., Sigma Aldrich, USA, cod product MAK074) following the instructions of the manufacturer. This method is based on the color change of the absorption caused by the reduction of 3-(4.5-dimethylthiazol-2)-2.5-diphenyltetrazolium bromide before and after ascorbic oxides is added, the concentration of L ascorbic acid being proportional to this change. The absorbance was measured at 570 nm (Tecan SunRise micro plate reader, software Magellan).

DPPH assay. Volumes 20 μl of the leaves extracts were added to 20 μl of distillated water in a 96 –well bottom microplate. 200 μl of 120 mg/l DPPH radical solution (using ethanol as a solvent) was then added and mixed thoroughly. The absorbance was measured using a plate reader (TecanSun Rise, software Magellan) at 515 nm after keeping the plates in the dark for 30 min. A control with 20 μl of ethanol (no extract) was also included in each plate. The DPPH radical scavenging activity was calculated with the formula:

DPPH radical inhibition (%) = [(A control – A sample)/ A control] x 100 (%)

where A is the absorbance at 515 nm.

Statistical interpretation. The experiments were carried out in triplicates and statistical analysis was performed by one way analysis of variance (ANOVA). Also, Duncan's multiple range test was used. A linear regression analysis was performed to evaluate the correlation coefficient.

Results and discussions

Total vitamin C content (TVCC)

The results regarding the total vitamin C content in sweet potato leaves (table 1) show levels ranged from 2.50± 0.53 mg/100 g FW ('Hayanmi' cv.) to 12.16 ± 0.73 mg/100 g FW ('KSP1' cv.). High content in vitamin C was observed in another leaves intensely green from 'Yulmi' cultivar (8.99±0.60 mg/100 g FW). The differences between the varieties tested could be because the intensity of the leaves colour.

Several researches had demonstrated that spinach leaves with intensely green colour have higher vitamin C content than light - coloured spinach leaves (Edenharder & al., 2001 [8]). According to Zikalala & al. (2014) [22] the ascorbic acid content in spinach was 4.46 mg/100 g FW, suggesting that sweet potato leaves could replace spinach in different food dishes. Ishiguro & al. (2004) [10] studies indicated that the average of vitamin C content of sweet potato leaves was 7.2 mg/100 g FW, higher than the average of the values recorded in this study (6.71 mg/100 g FW). Regarding the amount of vitamin C, in comparison with the results presented by Ishiguro & al. (2004) [10] and Dinu & al. (2018 [6]), some of the samples analysed in this study (especially

those sampled from ‘Yulmi’ and ‘KSP1’ cv.) had higher total vitamin C content (table 1).

Table 1. Total vitamin C, total polyphenols and total flavonoids content of the sweet potato leaves (mean values ± standard deviation)

Cultivar	Vitamin C (mg/100 g FW)	TPC (mg GAE/100 g FW)	TFC (μg QE/100 g FW)
’Hayanmi’	2.50±0.53 d	43.82±2.59 c	6.04±0.89 c
’Juhwangmi’	4.53±0.43 c	40.13±2.62 c	6.84±1.43 c
’KSC1’	5.38±0.47 c	53.49±13.83 c	8.70±1.01 b
’KSP1’	12.16±0.73 a	120.53±16.22 a	15.65±0.70 a
’Yulmi’	8.99±0.60 b	83.54±9.54 b	14.07±0.81 a
LSD	*0.458*	*8.632*	*0.817*

Values not followed by the same letter in the same row are significantly different ($P<0.05$) between cultivars according to Duncan’s test.
Abbreviations: FW fresh weight; TPC= total polyphenol content; TFC= total flavonoids content; GAE= Gallic acid equivalent; QE= quercetin equivalent.

Total polyphenol content (TPC)

The total polyphenol content of the sweet potato leaves analysed is presented in table 1. There were significantly differences between the genotypes, samples from ‘KSP1’ cv. and ‘Yulmi’ cv. having the highest TPC values (120.53 mg GAE/100 g FW respectively 83.54 mg GAE/100 g FW). The lowest TPC was found for ‘Juhwangmi’ cv. (40.13 mg GAE/100 g FW).

The TPC values reported in our study are higher than those reported by Ghasemzadeh & al.(2012) [9] on the leaves of six cultivars grown in Malaysia. Truong et al. (2007) [19] in a study of three sweet potato genotypes reported that TPC ranged between 1,123.6 and 1,298.1 mg chlorogenic acid/100g FW (equivalent values expressed in gallic acid were 587 mg GAE/100 g FW and 623 mg /100 g FW).

In comparison with another commercial vegetable, such as spinach, sweet potato leaves contain more polyphenol compounds (Islam & al., 2002; Islam, 2007 [11-12]).

Total flavonoid content (TFC)

The results regarding TFC of the sweet potato leaves tested are presented in table 1. There were observed significant differences ($p<0.05$) between the samples. The ’KSP1’ cultivar had the highest value of TFC (15.65±0.70 μg QE/g DW), whereas ’Hayanmi’ cultvar had the lowest value (6.04±0.89 μg QE/g DW). The mean values of TFC obtained for the material tested were in the following order: ’KSP1’ > ’Yulmi’ > ’KSC1’ > ’Juhwangmi’ > ‘Hayahnmi’. These values could explain the significantly differences between the antioxidant potential of the leaves from cultivars analysed.

Antioxidant activity DPPH radical-scavenging activity

The reduction capability of DPPH radical was determined by decrease in absorbance at 515 nm induced by antioxidants (included in the samples

studied). The results are presented in figure 1. As shown in this figure, the cultivar KSP1 had the highest level of DPPH inhibition percentage (2.97%±0.09), followed by 'Yulmi' (2.56%±0.11), 'KSC1' (1.93%±0.06), 'Hayanmi' (1.70%±0.08) and 'Juhwangmi'(1.62%±0.10). The values of DPPH inhibition percentage were significantly different between the genotypes tested ($P<0.05$) (figure 1).

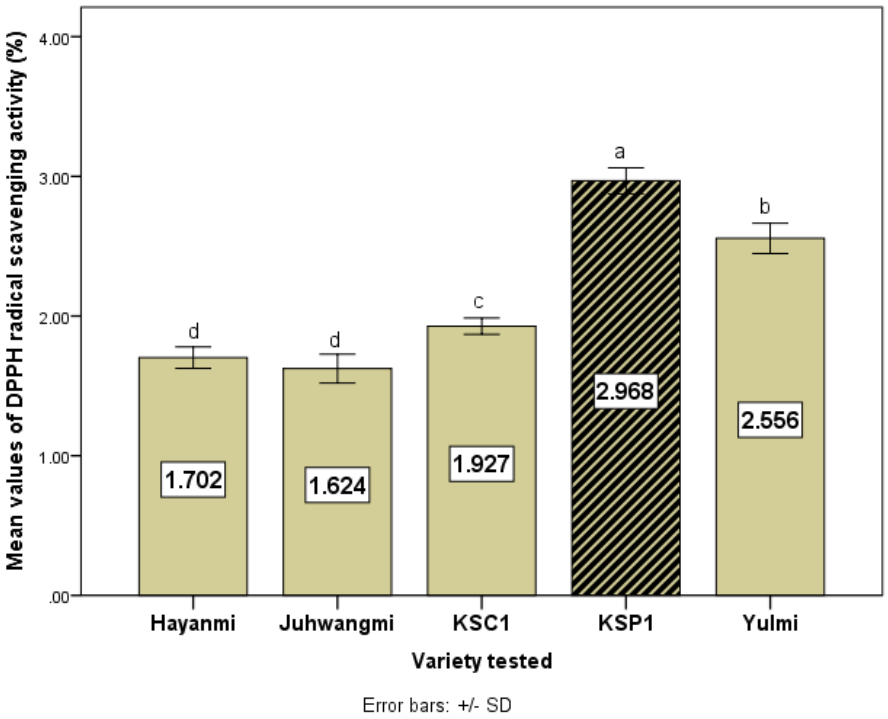


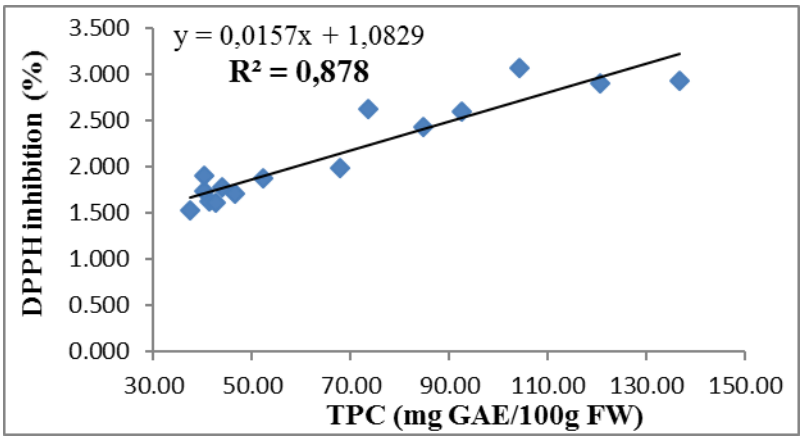
Figure 1. Antioxidant activity of sweet potato leaves by DPPH assay.Values not followed by the same letter are significantly different (P<=0.05) according to Duncan's test. Abbreviations: DPPH=2, 2-diphenyl-1 picrylhydrazyl; SD=standard deviation.

As in the case of TFC and TPC, the leaves from 'KSP1' and 'Yulmi' cv. had high values of DPPH inhibition %, closed to that specified by other studies. The high level of antioxidant potential of leaves sampled from sweet potato was demonstrated by Yang & al. (2005) [20]. Analysing 23 vegetable species consumed in Taiwan, Yang & al. (2005) [20] found the highest DPPH radical capture activity for *Ipomoea batatas*.

In our study, the TVCC, TPC and TFC values were strongly correlated with the antioxidant activity estimated by DPPH radical scavenging activity. The lowest value of the correlation coefficient (r^2) was obtained in case of TPC. It is possible that some differences between polyphenol content influenced the antioxidant capacity because the complex samples (vegetable extracts) could content some polyphenol non antioxidants.

Our study show that the DPPH values were strongly correlated with the TFC ($r^2 = 0.936$).

A



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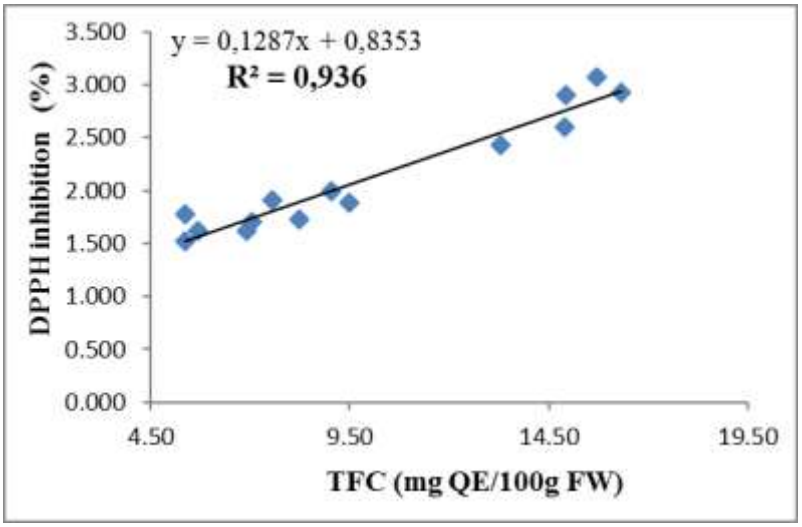

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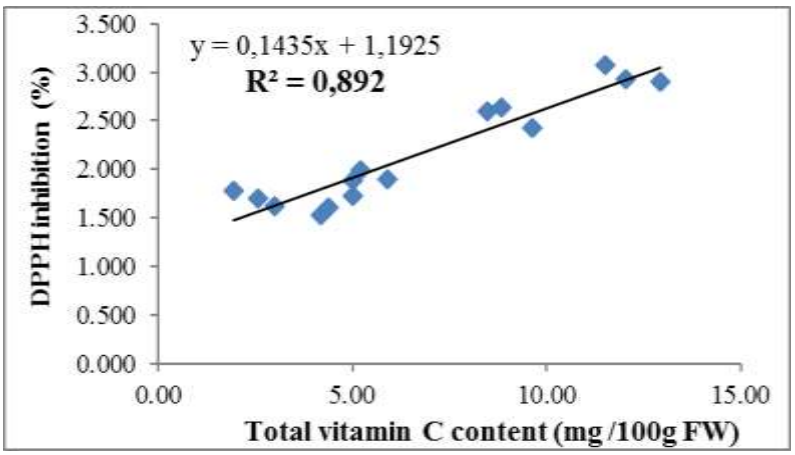

Figure 2. Relationship of total polyphenolic content (A), total flavonoid content (B) and total vitamin C content (C) with DPPH assay. Abbreviations: DPPH=2, 2-diphenyl-1-picrylhydrazyl; TPC= total polyphenol content; GAE= gallic acid equivalent; QE= quercetin equivalent; TFC= total flavonoid content.

A good correlation was found and between TPC and DPPH scavenging activity (r^2 = 0.878) and with total vitamin C content (r^2 = 0.892) (figure 2 A&B&C). Table 2 presents the Pearson correlation coefficients ($p<0.01$) between DPPH inhibition percentage of the samples and the TPC, TFC, TVCC. The results reveal a positive siginificantly correlation between the antioxidant activity estimated by DPPH radical scavenging activity and all the chemical compounds analysed.

Table 2. The Pearson correlation coefficients between chemical compounds analysed and DPPH inhibition of sweet potato leaves samples

Specification	TPC (mg GAE/100 g FW)	TFC (μg QE/100 g FW)	DPPH inhibition (%)
TVCC (mg/100 g FW)	0.921**	0.943**	0.944**
TPC	-	0.924**	0.937**
TFC	-	-	0.969**

Abbreviations: TVCC= total vitamin C content; TPC= total polyphenol content; GAE= gallic acid equivalent; QE=quercetin equivalent; TFC= total flavonoid content.

Sun & al. (2014) [18] found that the antioxidant potential was strongly correlated with TPC (r^2= 0.761) and according to Ghasemzadeh & al. (2012) [9] the correlation between the antioxidant activity and TPC had high value (r^2= 0.827). Regarding this correlation, Dinu & al. (2018 [6]) found for the varieties 'Pumpkin' and 'Chestnut', lower value of this coefficient (r^2= 0.53).

There are great differences between the price of vegetables or fruits rich in polyphenol, flavonoids and vitamin C. So, for beneficial and economic alternatives for improving health in poor communities, where the access to this source of natural antioxidants cause them to be limited, cheaper sweet potato leaves could be considered a good choice.

Conclusions

The results presented in this study highlight the level of some biochemical compounds of the leaves sampled from five sweet potato genotypes (valuable antioxidants source for human consumption).

The DPPH scavenging activity is due among others to the content in vitamin C, polyphenols and flavonoids (which were detected in significant amount in sweet potato leaves analysed). The highest mean values for these compounds were found in leaves sampled from 'KSP1' cv. (TPC=120.53±16.22 mg GAE/100 g FW; TFC=15.65±0.70 μg QE/100 g FW and total vitamin C content=12.16±0.73 mg/100 g FW) and from 'Yulmi' cv. (TPC=83.54±9.54 mg GAE/100 g FW; TFC=14.07±0.81 μg QE/100 g FW and total vitamin C content=8.99±0.60mg/100g FW). The values of DPPH scavenging activity

were significantly different between the genotypes tested ($P<0.05$), the cultivar 'KSP1' having the highest percentage of radical inhibition (2.97%±0.09).
Sweet potato leaves could contribute to the daily intake of vitamin C, polyphenols and flavonoids (phytochemicals with functional and antioxidant potential) and their consumption thereby may have positive effects on the human health.

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