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Analysis of nutritional composition and antioxidant activity of sweet potato (*Ipomoea batatas* L.) leaf and petiole

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Summary

Two cultivars of sweet potatoes, Pumpkin and Chestnut, were planted in the didactic field of the Faculty of Horticulture, Craiova, and biochemical determinations were carried out from the blades and petioles of the leaves. This study highlights the antioxidant properties of these species, which are not well known in Romania, to encourage consumption of the vegetative parts of the plant, as well as use of the leaves in the future to develop natural antioxidants. The chlorophyll and carotene content in the blade and petiole were determined, highlighting the large amounts of petiole, depending on the cultivar. Catalase (CAT), peroxidase (POX), and vitamin C content, as well as total dry matter (TDM), reducing sugars, phenolic compounds, and antioxidant activity, were also measured. The petioles exhibited high antioxidant activity, with 62.186 µmol TE/g FW in Pumpkin and 95.168 µmol TE/g FW in Chestnut. The cultivars also exhibited differences at the blade level, with high values recorded for the Chestnut cultivar. Positive correlations between total polyphenols and reducing sugars (r = 0.89), antioxidant activity and reducing sugars (r = 0.48), and antioxidant activity and phenolic compounds (r = 0.53) were found. This study demonstrates that sweet potato leaves are a potential, inexpensive and beneficial source of natural antioxidants.

Key words: *Ipomoea batatas* L., catalase, peroxidase, polyphenols

Introduction

Regular consumption of vegetables and fruits reduces the risk of cardiovascular diseases (OTAKI et al., 2009). In vegetables and fruits, the main constituents with antioxidant properties are polyphenols, which have a beneficial effect in contributing to the prevention of these diseases. Several studies have shown that polyphenols from various foods, such as red wine, green tea, and chocolate, can reduce bad cholesterol levels (KALKAN et al., 2004). Antioxidants reduce bad cholesterol levels and stimulate the production of good cholesterol. Thus, vitamin C and flavonoids such as beta carotene or lycopene, which are found in vegetables and fruits, play an especially important role.

Different varieties of sweet potatoes are consumed worldwide. In Japan, the tuberous roots are commonly eaten and used to make spirits. A Brazilian variety called "Simon No. 1" is popularly known as a drug (NAGAI et al., 2011). They are also an important source of starch, sugar, and alcohol.

The sweet potato is one of the top 15 agricultural crops and can be used as raw material for many industrialized products, given its composition and agricultural potential. In addition, the young leaves can be consumed (ANTONIO et al., 2011). The leaves of sweet potatoes are consumed as vegetables in tropical areas, especially in Southeast Asia (VILLAREAL et al., 1982). Because the plants grow quickly in moist conditions, these leaves can be harvested several times a year. Various research studies have recently been carried out to improve the production of leaves and petioles in the varieties known as "Elegant Summer" and "Suioh" (NAGAI et al., 2011).

Several studies have demonstrated that sweet potato leaves inhibit HIV replication, mutagenicity, diabetes, and cancer cell proliferation (YOSHIMOTO et al., 2002).

In Romania, as in other European countries, consumption of sweet potato leaves is not common because this species is cultivated in small spaces. According to ŠLOSÁR et al. (2016), the main European producers of sweet potatoes are Spain and Italy.

Because they can be harvested several times a year, the annual yield of sweet potatoes is much higher than that of many other vegetables grown for the leaves. They can be prepared alone or mixed with other species, consumed fresh or dried, and stored for later use. Their nutritional content differs according to the environmental, harvest, and storage conditions, as well as the cultivar.

This study aims to highlight the nutrient-rich content of the leaf blade and petiole in the Pumpkin and Chestnut sweet potato cultivars, as well as to make the consumer aware of their beneficial role in human health. Due to their diverse biochemical composition and tolerance to diseases and pests (the plants do not require much care), consumption should certainly be encouraged for people of all ages.

Material and methods

The experiment was conducted in the didactic field of the Faculty of Horticulture, University of Craiova, Romania (latitude 44°19' north latitude and longitude 23°48' east), on reddish-brown preluvosoil with a pH of 7.5 and a humus content of 1.9%. The biological material consisted of two cultivars of sweet potato originating in South Korea: Pumpkin and Chestnut.

The crop was formed from cuttings obtained by forcing tuberous roots in a hot greenhouse. The cuttings were then root-planted by turn on May 25, 2016, and May 15, 2017, in 30 cm high beds. The distance between rows was 70 cm, and the distance between plants was 40 cm, resulting in 33.333 pl/ha. The plants were placed in three randomized blocks, with 30 plants per block.

It should be noted that, in addition to basic fertilization with 20 t/ha of compost, fertilization was performed in different phases. To control the weeds, the field was hoed at the beginning of the vegetation phase, after which the vegetative growth of the sweet potato covered the soil between the plants, and no further intervention was needed. Irrigation was carried out in 10 watering sessions, with 300-350 m^3/ha. From each cultivar, 10 leaf petioles were harvested in three repetitions for chemical analysis.

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Analytical methods

Several analyses were carried out: chlorophyll **a** and **b** content, carotenes, enzymatic activity (catalase and peroxidase), vitamin C content, total dry matter (TDM), reducing sugars, polyphenols, and antioxidant activity.

Determination of total chlorophylls and carotenes

The weighed samples, having been placed separately in 95% acetone (50 mL per gram), were homogenized with Braun MR 404 Plus for 1 minute. The homogenate was filtered and centrifuged using a Hettich Universal 320/320R centrifuge at 2500 rpm for 10 minutes. The supernatant was separated, and the absorbencies at 400-700 nm were read on a Cary 50 spectrophotometer. Chlorophyll **a** showed maximum absorbance at 662 nm, chlorophyll **b** at 645 nm, and total carotene at 470 nm. The amounts of these pigments were calculated according to the following formulas (Nagata and Yamashita, 1992):

Ca = 11.75 A662 - 2.350 A645; Cb = 18.61 A645 - 3.960 A662

Cx + c = 1000 A470 - 2.270 Ca - 81.4 Cb/227

Ca = Chlorophyll a; Cb = Chlorophyll b; Cx + c = Total carotene

Determination of vitamin C content

A sample consisting of 5-10 g of the blades and petioles of sweet potato leaves, previously ground with quartz sand, was placed in a 100 mL 2% hydrochloric acid solution. It was stirred, and after sedimenting, it was filtered into a dry glass. A 10-mL aliquot was added to a glass Berzelius beaker containing 30 mL of distilled water, 5 mL of 1% potassium iodate, and a 1-mL solution of starch. It was then titrated with potassium iodate N/250 and stirred until it turned bluish, using the method described in Elgailani et al. (2017).

The ascorbic acid concentration was calculated using the following equation:

Vitamin C mg % = 352. n.f / G, where:

n = mL used for titration; f = the factor of the potassium iodate N/250; G = sample weight in grams.

Determination of TDM

The TDM was determined by oven drying at 105 °C to constant mass (ISO 751: 2000).

Enzyme assays

Fresh tissue was homogenated with 0.1 M phosphate buffer, pH 7.0 (1:20 w/v), containing 0.1 mM EDTA. Homogenates were centrifuged for 15 minutes at 10,000 rpm, and the supernatants were used for enzyme assay. Total soluble peroxidase (POX) activity was assayed by measuring the increase in A436 due to guaiacol oxidation, and activity was expressed as ΔA/min/g fresh weight (FW). Catalase (CAT) activity was assayed through the colorimetric method at 570 nm, and the results were expressed as mmol H_2O_2/min/g at 25 °C (Băbeanu et al., 2010).

Reducing sugars

Using its the method described by Miller (1959), reducing sugars were extracted in distilled water (1:50 w/v) for 60 minutes at 60 °C. A colorimetric assay was then performed with 3,5-dinitrosalicylic acid, using glucose as the standard. Absorbance was recorded at 540 nm using a Thermo Scientific Evolution 600 UV-Vis spectrophotometer with VISION pro software.

Total phenolic content

The amount of total phenolic compounds in the leaf blade and petiole of sweet potato extract was determined colorimetrically with Folin-Ciocalteu reagent using the method described by Singleton and Rossi (1965), with some modifications. Briefly, each probe was prepared like this: to 0.1 mL extract (1 g FW + 10 mL 60% methanol) add 0.9 mL ultrapure water and 5 mL reactive Folin-Ciocalteu (diluted 1:10 with ultrapure water). After two minutes, 4 mL of a 7.5% sodium carbonate solution were added, and the samples were kept in the incubator at room temperature for two hours. After that, the absorbance of probes was measured at 765 nm by using an Evolution 600 double beam scanning, UV-visible spectrophotometer, PC controlled, using VISION pro software. A standard curve was prepared by using 50, 100, 150, 200, and 250 mg/L solutions of gallic acid in methanol and water (60:40 v/v). Gallic acid was used as the reference standard, and the results (total phenolic content) were expressed as gallic acid equivalents (GAE) and as mg/g FW.

Antioxidant activity

Antioxidant activity was measured in the ethanolic extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Ethanol (Merck, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Germany), and Trolox (6-hydroxi-2,3,7,8-tetramethylchroman-2-carboxylic acid) (Merck, Germany) were employed. The samples were extracted according to the same protocol described for total phenolic content. The free-radical scavenging ability of the extracts against the DPPH free radical was evaluated as described by Oliveira et al. (2008), with some modifications. Each ethanol extract of sweet potato leaf and petiole (50 mL) was mixed with 3 mL of a 0.004% (v/v) DPPH methanolic solution. The mixture was incubated for 30 minutes at room temperature in the dark, and the absorbance was measured at 517 nm on a Varian Cary 50 UV-Vis spectrophotometer. The DPPH free-radical scavenging ability was calculated in reference to Trolox, which was used as a standard reference to convert the inhibition capacity of each extract solution to the mmol Trolox equivalent (TE) antioxidant activity/L. The radical was freshly prepared and protected from light. A blank control of methanol/water was used in each assay. All assays were conducted in triplicate, and results were expressed in mmol Trolox kg^{-1} dw.

Statistical analysis

The statistical significance of differences between variants was determined with variance analysis using ANOVA and the Statgraphics Centurion XVI program (Statpoint Technologies, Warrenton, VA, USA) and by calculating the limit differences, LSD ≤ 0.05% (LSD = least significant difference). In addition, correlation coefficients (r) between chemical compounds analyzed in sweet potato leaves were calculated.

Results and Discussion

The variable climate in southwest Romania, where the summers are dry and warm, offers favourable conditions for the cultivation of sweet potatoes. The sandy or sandy loam soil allows good ventilation for the roots, which need oxygen and do not tolerate excessive humidity (Dinu et al., 2015). During the sweet potato's vegetation period, the average temperature is 20.33 °C, and the average precipitation is 269.38 (Diaconu et al., 2016). In Romania, sweet potatoes are beginning to be cultivated, and they could be a good addition to the range of less widely cultivated species. Depending on the cultivar and the environmental conditions under which the crop was planted, the nutrient content of sweet potato leaves is comparable to that of spinach.

Sweet potato leaves are an excellent source of chlorophylls and carotenes. As Tab. 1 indicates, fresh sweet potato leaves of the Pumpkin cultivar had a chlorophyll **a** content of 8.22 mg/100 g FW in the blade and 10.8 mg/100 g FW in the petiole. In the Chestnut cultivar, the chlorophyll content was 4.66 mg/100 g FW in the blade and 6.78 mg/100 g FW in the petiole. The chlorophyll content in the petiole was significantly higher than in the blade. Differences were also found between cultivars.

Tab. 1: Chlorophyll and total carotene content in sweet potato blade and petiole.

Cultivar	Specification	Chlorophyll **a** (mg/100 g FW)	Chlorophyll **b** (mg/100 g FW)	Carotene (mg/100 g FW)
Pumpkin	Blade	8.22b	8.32b	25.05a
	Petiole	10.8a	10.35a	16.39d
Chestnut	Blade	4.66d	6.82d	23.80b
	Petiole	6.78c	7.73c	16.60c
LSD ≤ 0.5		0.48	0.16	0.18

Note: Different letters within the same row indicate significant differences (P ≤ 0.05) between cultivars.

Similar differences in chlorophyll **b** content exist between the blade and the petiole, as well as between cultivars. In Pumpkin, the chlorophyll **b** concentration was 8.32 mg/100 g FW in the blade and 10.35 mg/100 g FW in the petiole. The Chestnut cultivar recorded 6.82 mg/100 g FW of chlorophyll **b** in the blade and 7.73 mg/100 g FW in the petiole.

Not much is understood about chlorophyll's effect on health. It is known only that it is a primary photosynthetic pigment that determines the green colour of plants. Some reports suggest that it protects organisms against some cancers linked to mutant DNA by preventing proliferation of the DNA. Because these chlorophylls are found at different concentrations in many edible plants, they can be associated with certain protective effects observed in diets rich in green vegetables (Fahey et al., 2005). Furthermore, chlorophyll is present in those plants at much higher concentrations than other „phytochemicals", similar with determinations reported by Zikalala (2014) on baby spinach.

The carotene content in the blade varied from 25.05 g/100 g FW in the Pumpkin cultivar to 23.8 g/100 g FW in the Chestnut cultivar. The content in the petiole was 16.39 g/100 g FW in Pumpkin and 16.6 g/100 g FW in Chestnut. The high carotene content in the blade helps to capture the luminous energy that is transferred to the plant during photosynthesis. Additionally, the carotene from the sweet potato leaves supplies a variety of nutrients that cannot be supplied by other vegetables. The amounts of carotenes found in this study were significantly higher than those found by Odongo et al. (2015), who reported a beta carotene content of 0.53 ± 0.03 mg/100 g dry weight for sweet potato leaves, SPK 004 variety. These results demonstrate that the carotene concentrations in the blade and petiole of sweet potato leaves are higher than in the sweet potato's tuberous root. Ellong et al. (2014), in a study of eight sweet potato cultivars, observed that the doses of carotenes in sweet potato roots had values ranging from 0.50 to 1.47 mg/100 g.

This study also determined the enzymatic activity of catalase (CAT) and peroxidase (POX). This enzymatic antioxidant system plays a key role in the scavenging and regulation of the reactive oxygen species (ROS) generated in aerobic metabolic processes.

The enzymatic leaf activity caused by CAT and POX had different values between cultivars and is presented in Tab. 2. In the Pumpkin cultivar, activity due to CAT was 3.94 mmol H_2O_2/g FW/min, while activity in the Chestnut cultivar was low at only 1.15 mmol H_2O_2/g FW/min. Since both cultivars were obtained under the same crop conditions and from the same area, this difference can be attributed to the cultivar.

Tab. 2: Enzymatic activity and vitamin C content of sweet potato leaves.

No.	Cultivar	CAT[1] (mmol H_2O_2/ g FW/min)	POX[2] (ΔA/min/g FW)	Vitamin C (mg/100 g FW)
1.	Pumpkin	3.94a	1680ns	5.96a
2.	Chestnut	1.15b	1665ns	3.56b
LSD ≤ 0.5		0.17	24.81	0.20

Note: Different letters within the same row indicate significant differences (P ≤ 0.05) between cultivars. [1]CAT = catalase, [2]POX = peroxidase.

CAT catalysis is the reduction of H_2O_2 generated during photosynthesis to H_2O and molecular oxygen. CAT and POX have different affinities for ROS and appear to have different cellular roles in H_2O_2 scavenging. CAT does not need a reductant to scavenge H_2O_2, making it reducing power-free, whereas POX needs a reductant. CAT has a lower affinity for H_2O_2 (in the mM range) than POX (in the μM range) (Cruz de carvahlo, 2008). Enzymatic activity caused by POX did not vary greatly from one cultivar to another, measuring1.680 ΔA/min/g FW in Pumpkin and 1.665 ΔA/min/g FW in Chestnut.

The measurements of vitamin C content in sweet potato leaves shows levels of 5.96 mg/100 g FW in Pumpkin and 3.56 mg/100 g FW in Chestnut. The difference between cultivars may be because the Pumpkin cultivar's leaves are more intensely green than those of the Chestnut cultivar. Research has demonstrated that spinach leaves with an intensely green color have higher vitamin C concentrations than light-colored spinach leaves (Edenharder et al., 2001). The Zikalala study (2014) indicated that vitamin C concentration was 4.46 mg/g in spinach, suggesting that sweet potato leaves can replace spinach in various preparations. According to Ishiguro et al. (2004), the average vitamin C content of sweet potato leaves was 7.2 mg/100 g FW, higher than the values recorded in this study.

The total dry matter for the blade, presented in Tab. 3, ranged from 16.0% for Pumpkin to 18.5% for Chestnut; for the petiole, it ranged from 12.3% for Pumpkin to 16.0% for Chestnut. High values were observed in both cultivars.

In comparison with other vegetables, this study found that the TDM content in sweet potato leaves was higher than the 10.0% to 12.8% values in some vegetable species reported by Asaolu (2012). This indicates that sweet potato leaves contain more dry matter and, therefore, more nutrients, and should be considered for human consumption.

Reducing sugars in the blade ranged from 23.40% in the Pumpkin cultivar to 25.68% in Chestnut. In the petiole, the sugars were higher than those in the blade, at 43.07% for Pumpkin and 51.91% for Chestnut. The differences between the values in the blade and the petiole are significant both within the same cultivar and between the two cultivars. The sweet potato is high in reducing sugars due to the high levels of sugars that appear in the leaves during photosynthesis.

Polyphenol content. Sweet potato leaves are a source of polyphenolic compounds. These biologically active compounds have multiple actions, including antioxidation, antimutagenicity, antiinflammation, and anticarcinogenesis. Sweet potato leaves contain more polyphenols than any other commercial vegetables, such as spinach (Islam, 2007).

Polyphenol content in the blade was 2.598 mg/g FW for the Pumpkin cultivar and 2.250 mg/g FW for the Chestnut cultivar. There were

Tab. 3: Biochemical composition of sweet potato leaves.

No.	Specification	TDM (%)	Reducing sugars (%)	Total polyphenols (mg/g FW)	Antioxidant activity (µmol TE/g FW) (DPPH)
1.	Blade	16.0b	23.80b	2.598c	42.290d
	Petiole	12.3c	43.07a	5.384a	62.186c
2.	Blade	18.5a	25.68b	2.250d	62.688b
	Petiole	6.0b	51.91a	4.736b	95.168a
LSD ≤ 0.5		0.92	16.09	0.17	0.46

Note: Different letters within the same row indicate significant differences (P ≤ 0.05) between cultivars.

no significant differences between cultivars. This study found higher polyphenol levels than those obtained by Ghasemzadeh et al. (2012) on the leaves of six cultivars in Malaysia. The quantities in the petiole were double those accumulated in the blade, but again, no significant differences were observed between cultivars.

Truong et al. (2007), in a study of three sweet potato cultivars, claimed that the phenols from the leaves of this species were approximately 8, 16, and 18 times higher, respectively, than those found in the root coat, in the entire root, and in the root pulp. They also reported that the phenolic-compound content ranged between 1,123.6 and 1,298.1 mg chlorogenic acid/100 g FW; equivalent values expressed in gallic acid are 587 mg GAE/100 g FW and 623 mg GAE/100 g FW, respectively.

The total polyphenolic content in the leaves of Pumpkin and Chestnut was 2.5 mg/g FW and 2.2 mg/g FW. According to Islam et al. (2002), who evaluated 1,389 genotypes for total phenol content, these varieties can be classified as average polyphenolic accumulators, which have a content of less than 5 g. Those with a content greater than 9 g are high content accumulators (ChA equivalent per 100 g dry matter).

The sweet potato leaves used in this study (as well as the petioles) had a higher total phenol content than the Iranian olive pulp studied by Hajimahmoodi et al., (2008). They argued that this pulp is a source of natural antioxidants with the highest level of total polyphenols, approximately 2.997 ± 0.361 g GAE/100 g. However, the Chestnut petiole had a polyphenol content of 4.736 mg /g FW, and the Pumpkin had a content of 5.384 mg/g FW.

The antioxidant activity of the two sweet potato cultivars varied greatly between the blade and petiole within the same cultivar, as well as between cultivars. The highest values were recorded in the leaf petiole, with 62.186 µmol TE/g FW in Pumpkin and 95.168 µmol TE/g FW in Chestnut, while the blade content was 42.290 µmol TE/g FW in Pumpkin and 62.688 µmol TE/g FW in Chestnut. It is notable that the petiole developed greater antioxidant activity than the blade. This result is also supported by Jeng et al. (2015), who found that the blade had lower antioxidant activity than the petiole of sweet potato.

The values for blade antioxidant activity recorded in this study are higher than those obtained by Truong et al. (2007) and Ghasemzadeh et al. (2012). This activity in sweet potato leaves is equivalent to that recorded by Zambrano-Moreno et al. (2015) in eggplants, in both conventional and organic culture.

The high level of capturing activity in sweet potato leaves was also demonstrated in the study by Yang et al. (2005), in which *Ipomea batatas* had the highest DPPH radical-capture activity of 23 vegetable species consumed in Taiwan.

The high antioxidant activity correlates well with the polyphenol content. In both the Pumpkin and Chestnut cultivars, polyphenol content was greater in the leaf petiole than in the blade. This higher content correlates positively with intense antioxidant activity, indicating intense activity in this part of the leaf.

Several other studies have also demonstrated the positive relationship between high levels of phenols (e.g., phenolic acid) and antioxidant activity. Bertoncelj et al. (2007) established that in seven types of honey originating in Slovenia, increased levels of phenolic compounds were correlated with high antioxidant activity. Céspedes et al. (2008), in a study of the mature fruits of *Aristotelia chilensis* (Mol) Stuntz (Elaeocarpaceae), found that high antioxidant activity was strongly correlated with total polyphenol content, and thus the fruits could be used as antioxidant, cardioprotective, and nutraceutical sources. Garcia-Alonso et al. (2004) analyzed 28 different species of fruits and found no significant correlation between antioxidant activity and flavonol content. The authors therefore argue that the antioxidant activity of the analyzed fruits cannot be attributed only to their flavonol content; it must also involve the actions of various antioxidant compounds present in fruits, as well as possible synergistic and antagonistic effects that are still unknown.

Tab. 4 presents the correlations between TDM, reducing sugars, total polyphenols, and antioxidant activity. The results reveal a positive correlation between total polyphenols and reducing sugars (r = 0.89), between reducing sugars and antioxidant activity (r = 0.84), and between antioxidant activity and total polyphenols (r = 0.53). According to Sun et al. (2014), the correlation coefficient between antioxidant activity and total polyphenol content was the highest (r = 0.76032), and Ghssemzadeh et al. (2012) found that antioxidant activity was strongly correlated with total polyphenol content (R^2 = 0.827).

Tab. 4: Correlation coefficients (r) between chemical compounds analyzed in sweet potato leaves.

Specification	Reducing sugars (%)	Total polyphenols (mg/g FW)	Antioxidant activity (µmol TE/g FW) (DPPH)
TDM (%)	- 0.49	- 0.82	0.03
Reducing sugars (%)	-	0.89**	0.84**
Total polyphenols (mg/g FW)	-	-	0.53

Note: *p 5% = 0.58, **p 1% = 0.71

Conclusions

This study demonstrates the importance of sweet potato leaves (both the blade and petiole) as a natural source of antioxidants. The two

sweet potato cultivars used in this study had the highest carotene content in the blades, with 25.05 mg/100 g FW in Pumpkin and 23.80 mg/100 g FW in Chestnut. The capacity to scavenge free radicals is due to the presence of total polyphenols, which were present in significant amounts in the leaf petiole. These polyphenols also produced high antioxidant activity in Pumpkin at 62.186 µmol TE/g FW, and an even higher activity level in Chestnut at 95.168 µmol TE/g FW. The significant correlations between carotenes and antioxidant activity and between total polyphenols and antioxidant activity suggest that the antioxidant activity of blade and leaf petiole can be attributed mainly to phenolic compounds. Because sweet potato leaves could be used in the food, medicine, and agriculture industries as a source of natural antioxidants, the research effort to confirm the antioxidant activities of *Ipomoea batatas* phenols should be intensified.

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