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BIOACTIVE COMPOUNDS CONTENT AND ANTIOXIDANT ACTIVITY IN THE LEAVES OF SOME SWEET POTATO CULTIVARS (*IPOMOEA BATATAS* L.)

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Abstract

This study was aimed to determine the content of bioactive compounds and the antioxidant activity of sweet potato leaves, to encourage the consumption of the vegetative organs of this plant (leaves) and to identify new sources of natural antioxidants. The studied biological material was represented by six sweet potato cultivars cultivated in the Didactic Field (2018) of the Faculty of Horticulture in Craiova. The total dry matter content (DMC), the content of phenolic compounds and flavonoids, the enzymatic activity of catalase and peroxidase and the antioxidant activity (DPPH and ABTS) were determined. Cultivar 3 with the highest enzymatic activity of catalase (6.6 mM H_2O_2/g/min fm) and peroxidase (615.8 ΔA/min/g fm) and cultivar 6 with the highest content of phenolic compounds (10.237 mg GAE/g fm) were noted and flavonoids (9.73 mg QE/g fm) as well as the highest antioxidant activity (72.18 µM TE/g fm by DPPH method and 75.22 µM TE/g fm by ABTS method). The results suggest that the leaves of this species should be used as valuable food to cope with future changes in food supply and demand, especially in developing countries.

Key words*: peroxidase, catalase, phenols, flavonoids, antioxidant activity.*

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) has become a research topic in recent years due to its unique nutraceutical and functional properties. It is the sixth most important food crop in the world, with an annual production of tuberous roots of over 178 million tons in 2014 (FAOSTAT, 2017). This culture has tolerance to diseases and pests, to water and heat stress (Dinu et al., 2015). The unique composition of the species has various benefits for human health such as: antioxidant, anti-inflammatory, antitumor, antidiabetic, antimicrobial, anti-obesity effects. Sweet potato is characterized by a production represented by the tuberous root, shoots, petiole and leaf. The daily consumption of tuberous roots was estimated at 17.70 g/day in the Korean population, and that of petiole was represented at a small amount of 0.42 g/day (KHIDI, 2014). Recent studies have shown that sweet potatoes contain many functional components such as phenols, flavonoids, dietary fiber that improve the health of the population.

It should be noted that in addition to the significant amount of tuberous roots, this species annually produces over 70 million tons of biomass, which is mostly discarded (Yang, et al., 2019). Young shoots and leaves are rich in nutrients and are widely used as healthy plant foods (Dinu et al., 2018; Sun et al., 2014), but mature shoots and leaves that account for most of the sweet potato biomass are partly ignored or end up, in the happiest case, fodder for animals. Truong et al. (2007) also found that the leaves have the highest phenolic acid content compared to the peel and root of three varieties of American sweet potatoes. These indicate that sweet potato leaves can be a valuable source of antioxidants. However, a large amount of leaves generated by the

production of sweet potatoes of 3 million tons in 2014 (FAOSTAT, 2017) was discarded in South Korea, a large consumer of this species. It is very important to know the value and use of the shoots and ripe sweet potato leaves, to improve the economic value of the crop and reduce environmental waste.

Ishida et al. (2000) showed that the content of total phenolic compounds in leaves, petioles, shoots and roots is 90, 45, 90 and 180 mg/100 g in the 'Koganesengan' cultivar and 356, 126, 197 and 154 mg/100 g in the 'Beniazuma' cultivar. Islam (2006) and Jeng et al. (2015) identified several anthocyanins, flavonoids, phenolic extracts from sweet potato stems and leaves. Extracts of sweet potato stems and leaves have been shown to have multiple physiological and health functions (Jeng et al., 2015) and potential uses in the food and medicine industries (Yuan et al., 2015; Matins et al., 2017).

Peroxidase (Hamid, 2009) has been widely used in several industries. It is said to be the key enzyme in the enzyme-linked immunosorbent assay and has been used as a biosensor to accurately detect physical, chemical, and biological signals (Zhang et al., 2018; Fojtíková et al., 2017; Fatibello-Filho et al., 2007). Peroxidase can be used in many manufacturing processes, such as adhesives, computer chips, auto parts and drum and box liners. In the food industry, peroxidase is an excellent food additive for whitening flour. Like polyphenol oxidase, peroxidases can catalyze the transformation of catechins into theaflavins and can improve the quality of black tea (Kusano et al., 2015; Stodt et al., 2014). Peroxidase can also be used to treat industrial wastewater and remove toxic pollutants by oxidation and polymerization catalyzed by enzymes or by converting toxic materials into less harmful substances such as phenols, polycyclic aromatic hydrocarbons and aromatic amines (Kotchey et al., 2013; Durán and Esposito, 2000).

Enzymatic systems may combat oxidative stress in the human body by maintaining a balance between oxidants and antioxidants. This is particularly important because under oxidative stress excessive formation of reactive oxygen species (ROS) can damage biomolecules, such as DNA, proteins, lipids and carbohydrates, and lead to numerous disease conditions. The enzyme superoxide dismutase (SOD) catalyzes the scavenging of (O−2) to H_2O_2, whereas peroxidase (POX) and catalase (CAT) are involved in reduction of H_2O_2 from cells (Băbeanu et al., 2017).

The screening carried out in this study aimed to highlight the major content of bioactive compounds and the antioxidant activity existing in leaves of six sweet potato cultivars cultivated in south-western Romania.

MATERIALS AND METHODS

The research was located in the teaching field of the Faculty of Horticulture in Craiova, in southwestern Romania. Six sweet potato genotypes were studied. The culture was established by cuttings obtained by forcing the tuberous roots. The cuttings were planted in the first decade of May 2018, on 30 cm high layers. The distance between rows was 70 cm, and between plants in a row of 40 cm, resulting in 35,714 pl/ha. Two months after planting, leaves were harvested from all 6 cultivars and brought to the laboratory for chemical analysis.

Biochemical analysis. Methanol used for the extraction was from Sigma-Aldrich. Gallic acid, ascorbic acid, 1.1-diphenyl-2-picrylhydrazyl, 6-hydroxy -2,5,7,8 - tetramethylchromon 2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich, Germany. Quercetin was purchased from Carl Roth. Folin-Ciocalteu reagent was obtained from Merck, Germany. All the other used chemicals were of analytical grade.

Dry matter content (DMC) (%) was determined gravimetrically by drying 25 g leaf fresh tissue to a constant weight at 105°C.

For antioxidant enzymes extraction, fresh tissue was homogenised with 0,1M phosphate buffer, pH 7.5 (1:20 w/v),) containing 0,1mM EDTA. The homogenates were centrifuged for 20 min at 6000 rpm and the supernatants were used for enzyme assays.

Total soluble peroxidase (guaiacol-type E.C.1.11.1.7) activity (POX) was assayed by measuring the increase in A_{470} due to guaiacol oxidation to tetraguaiacol on addition of H_2O_2 (Dinu et al., 2018) and their activity was expressed as ΔA/min/g fm (fresh matter).

Catalase activity (E.C.1.11.1.6) CAT activity was assayed through the colorimetric method at 570 nm using $K_2Cr_2O_7$/acetic acid reagent, and the results were expressed as mmol H_2O_2/min/g fm at 25°C (Dinu et al., 2018).

The extracts for the determination of total phenolic content, total flavonoids content and antioxidant activity were prepared into 80% aqueous methanol (1:20 w/v) in "Fungilab" ultrasonic bath at 24°C for 70 minutes. The resulting slurries were centrifuged at 4000 g for 5 min and the supernatants were collected.

Determination of total phenolic content (TPC). Each extract was mixed with Folin-Ciocalteu reagent and saturated sodium carbonate (Na_2CO_3) solution (Băbeanu et al., 2020). The mixture was allowed to stand at room temperature for 60 min and then the absorbance was recorded at 765 nm. The total phenolic content (TPC) was calculated using a standard curve prepared using gallic acid and expressed as mg of gallic acid equivalents (GAE) per gram.

The total flavonoids content was determined by colorimetric method with 10% $Al(NO_3)_3$ and 5% sodium nitrite ($NaNO_2$) in alkaline medium (Băbeanu et al., 2020). The absorbance was measured at 500 nm and the results were calculated from quercetin calibration curve and expressed as mg QE/100 g fm.

*DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay***:** A 0.075 mM (final concentration) DPPH solution in methanol was mixed with sample extracts and vortexed thoroughly. The absorbance of the mixtures was recorded after 20 min. The absorbance of the remaining DPPH radicals was measured at 517 nm (Dinu et al., 2018). A blank reagent was used to study stability of DPPH over the test time. The scavenging activity of extracts was evaluated according to the formula: % scavenging = $[A_0-(A_1–A_S)]/ A_0]100$, where A_0 is the absorbance of DPPH alone, A_1 is the absorbance of DPPH + extract and A_S is the absorbance of the extract only.

The standards calibration curves (Trolox-T and ascorbic acid- AsA) were plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as µmol TE/g fm and µmol AsA/g fm.

ABTS radical cation scavenging activity: ABTS was dissolved in water to a 7 mmol/l concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mmol/l potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS radical cation solution was diluted with methanol to an absorbance of 0.70 at 734 nm. 0.1 ml sample extract was mixed with 2.9 ml of diluted ABTS radical cation solution. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The standards calibration curves (Trolox and ascorbic acid) were plotted as a function of the percentage of *ABTS radical cation* scavenging activity. The final results were expressed as µmol TE/g fm and µmol AsA/g fm.

All the spectrophotometric measurements were carried out with Evolution 600 UV-Vis spectrophotometer, Thermo Scientific, England, with VISION PRO software.

Statistical analysis. The statistical significance of differences betwee 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 differrence). In addition, the correlation coefficient (R^2) between the antioxidant activity (expressed Trolox and ascorbic acid) and the total polyphenol content was calculated.

RESULTS AND DISCUSSIONS

The results of this study, on the total *dry matter content* (DMC) demonstrate a variation from 18.42% for cultivar 6 to 14.3% for cultivar 2 (Table 1). High content was recorded in cultivar 5 (18.20%) but also in cultivars 1, 3 and 4 with values over 16%. In a previous study conducted by us in 2018, on two sweet potato cultivars grown in the same area, DMC values were obtained in the leaf blade of 16.00% in Pumpkin and 18.5% in Chestnut (Dinu et al., 2018). The DMC content recorded in the sweet potato leaf is higher than that reported by Asaolu (2012) of 10.0-12.8% in different vegetable species.

Phenolic compounds showed cultivar 6 with 10.237 mg GAE/g fm, followed by cultivar 1 with 7.188 mg GAE/g fm and cultivar 3 with 6.071 mg GAE/g fm (Table 1). The lowest content was recorded in cultivar 4 with only

1.747 mg GAE/g fm, 5.8 times lower than in cultivar 6. Our results are consistent with those reported by (Sun et al., 2014) in a study on 40 sweet potato cultivars, and in some cultivars, they are even higher than these values (values from 2.73 g ChA/100 g DW and 12.46 g ChA/ 100 g DW, equivalent to 1.579 mg GAE/g fm – 7.286 mg GAE/g fm). Hue et al. (2012) determined a content of total polyphenols in sweet potato leaves between 2.78 and 5.35 g GAE/100 g DW, and Islam et al. (2002) reported a content in phenols ranging from 1.42 to 17.1 g/100 g dry matter. Values similar to our results have been reported in other studies evaluating the content of phenolic compounds, one in 116 varieties of sweet potatoes grown in China (Xu et al., 2010) or another study on three sweet potato cultivars grown in Kansas State University (Su et al., 2019).

The content of polyphenols in leaves is influenced by the interaction of several factors: genotype, environmental conditions, applied crop technology, harvest period. In our study the investigated cultivars being grown in the same environmental conditions and respecting the same technology differences observed in content in total polyphenols are due to the cultivar.

Truong et al. (2007) stated that leaf polyphenols recorded much higher values than those from tuberous root, pulp or bark as well as from other common vegetable species. Over 20 phenolic acids have been identified in sweet potato leaves, of which chlorogenic acid is the majority, followed by caffeic, quinic and protocatechuic acids (Islam et al., 2002).

The values for *flavonoid compounds* ranged from 2.71 mg QE/100 g fm in cultivar 2 to 9.73 mg QE/100 g fm in cultivar 6 (Table 1). Cultivars 1, 3, 5 and 6 are also highlighted for this biocompound, cultivars that also had a high content of phenolic compounds. The presence of these flavonoid compounds in sweet potato leaves has been investigated in several studies: Hue et al., (2012) on six sweet potato varieties identified a content that ranged from 96 μg catechin equivalents/g to 263.5 catechin equivalents μg/g (determined by the vanillin-HCl test), and Fu et al. (2016) reported a value of 3.4 mg QE/g dry matter, in ethanol extract of 70%.

The data reported in the literature are difficult to compare due to differences in extraction methods, solvents used and test methods. Two important flavonoids have been identified in the composition of sweet potato leaves: quercetin and isoquercetin (Suarez et al., 2020). Flavonoid compounds exert a strong biological activity with beneficial effects on human health: it suppresses the reactive formation of oxygen, chelates trace elements involved in the production of free radicals and regulates and protects antioxidant defense.

Table 1. Biochemical compounds and enzymatic activity in sweet potato cultivars

The cultivar	DMC (%)	Phenolic compounds (mg GAE/g fm)	Flavonoid compounds (mg QE/100 g fm)	Peroxidase (ΔA/min/g fm)	Catalase (mM H_2O_2/g/min fm)
1	16.97^{b}	7.188^{b}	7.66^{c}	615.8^{a}	3^{c}
2	14.3^{c}	2.395^{e}	2.71^{e}	212.2^{f}	4.9^{b}
3	16.8^{b}	6.071^{c}	8.23b	320.0^{c}	6.6^{a}
4	16.38^{b}	1.747^{f}	3.18^{d}	346.4^{b}	3.0^{c}
5	18.2^{a}	4.369^{d}	8.64^{b}	296.0^{d}	6.2^{a}
6	18.42^{a}	10.237^{a}	9.73^{a}	246.6^{e}	4.9^{b}
LSD 5%	**1.01**	**0.21**	**0.41**	**19.83**	**0.47**

Note: Different letters within the same row indicate significant differences ($P \leq 0.05$) between cultivars

Enzymatic antioxidant activity

Catalase catalyzes the dismutation of hydrogen peroxide generated during photosynthesis in water and molecular oxygen and peroxidase breaks down H_2O_2 by oxidizing substrates. In addition to their role in treatment systems, peroxidases have been recognized as being involved in several cellular processes, inclu-

ding growth control, lignification, pathogen defense, and growth regulator catabolism. The enzymatic activities and the antioxidant activity vary depending on the analyzed species and cultivar (Băbeanu et al., 2017; Soare et al., 2017; Korus, 2011). In a study of different species of *Brassica*, superoxide dismutase activity recorded high levels in kale, peroxidase recorded high values in red cabbage, highest value for catalase activity was determined in broccoli, and highest value for antioxidant activity in kale (Soare et al., 2017).

Peroxidase activity ranged from 615.8 ΔA/min/g fm at cultivar 3 to 212.2 ΔA/min/g fm at cultivar 1 while catalase activity ranged from 6.6 mM H_2O_2/g/min fm at cultivar 3 and 3.0 mM H_2O_2/g/min fm in cultivars 1 and 4. Cultivator 3 with high enzymatic antioxidant activity is highlighted followed by cultivar 5, 6 and 2.

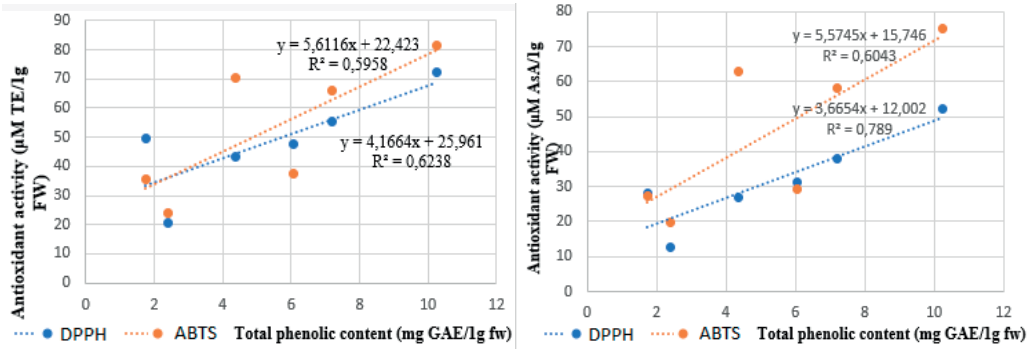
Kim et al. (2009) investigating the activity of antioxidant enzymes during the development of sweet potato leaves found significant increases in peroxidase activity during the late stage of development while catalase activity increases during the early stage of leaf development. It was observed that in sweet potato there is a major isoform of catalase that has the highest protein content and the highest enzymatic activity in mature leaves compared to young and completely yellow leaves at senescence (Afiyanti and Chen., 2014; Chen et al., 2011).

The antioxidant activ*ity* of leaf extracts was determined by DPPH and ABTS cation radical removal test, which are the most accepted and used methods of evaluating antioxidant activity. The results were calculated using two standards: Trolox and ascobic acid. Antioxidant activity varies depending on the cultivar studied (Table 2).

Table 2. Antioxidant activity in sweet potato cultivars

The cultivar	Antioxidant activity DPPH (μM TE/g fm)	Antioxidant activity ABTS (μM TE/g fm)	Antioxidant activity DPPH (μM AsA/g fm)	Antioxidant activity ABTS (μM AsA/g fm)
1	55.16 [b]	65.93 [c]	37.94 [b]	19.92 [f]
2	20.7 [f]	23.8 [f]	12.66 [f]	27.33 [e]
3	47.88 [d]	37.4 [d]	31.26 [c]	29.29 [d]
4	49.7 [c]	35.46 [e]	28.28 [d]	58.35 [c]
5	43.5 [e]	70.29 [b]	26.92 [e]	62.79 [b]
6	72.18 [a]	81.27 [a]	52.27 [a]	75.22 [a]
LSD 5%	**1.07**	**1.04**	**0.84**	**0.82**

Note: Different letters within the same row indicate significant differences ($P \leq 0.05$) between cultivars



a. b.

Figure 1. Correlation between antioxidant capacity (expressed as Trolox and ascorbic acid) and polyphenol content

The values of *DPPH* radical scavenging activity compared to standard Trolox ranged from 20.70 μM TE/g fm (for cultivar 2) to 72.18 μM TE/g fm (for cultivar 6). High value is also found in cultivar 1 with 55.16 μM TE/g fm. The results obtained expressed in ascorbic acid ranged from 12.66 μM AsA/g fm (in cultivar 2) to 52.27 μM AsA/g fm (in cultivar 6) followed by cultivar 1 by 37.94 μM AsA/g fm. The free radical scavenging activity expressed in Trolox or ascorbic acid highlights cultivar 6 with the highest values, followed by cultivar 1 and cultivar 2 with the lowest values. The results obtained in our study are similar to those reported by Sun et al. (2014); Suarez et al., (2020); Dinu et al. (2018). Suarez et al. (2020) in a study that determines and compares the content of phenolic compounds and antioxidant activity in sweet potato leaves determined in three different periods recommends harvesting leaves in late September when they determined the highest content of total polyphenols.

The values of the *ABTS* radical scavenging activity compared to the standard Trolox show cultivar 6 with 81.27 μM TE/g fm, followed by cultivar 5 with 70.29 μM TE/g fm, cultivar 1 with 65.93 μM TE/g fm and the lower value in cultivar 2 - 23.80 μM TE/g fm. The same order is found for the activity of free radicals expressed in ascorbic acid (cultivar 6 - 75.22 μM TE/g fm, cultivar 5-62.79 μM TE/g fm, cultivar 1 with 58.35 μM TE/g fm and the smallest value in cultivar 2 with 19.22 μM TE/ g fm.

The values for antioxidant activity recorded in this study are higher than those obtained by Truong et al. (2007) and Ghasemzadeh et al. (2012). The high level of activity of capturing sweet potato leaves was also demonstrated in the study by Yang et al. (2005), in which *Ipomea batatas* had the highest DPPH radical scavenging activity of 23 vegetable species consumed in Taiwan. The content in total polyphenols correlates linearly positively with the antioxidant activity expressed Trolox or ascorbic acid (Figure 1 a and b). These correlations can be explained by the fact that antioxidant activity is largely due to the content in phenols and flavonoids that have the property to scavenge free radicals being powerful antioxidants.

CONCLUSIONS

The high content of phenolic compounds and flavonoids in 4 sweet potato cultivars.

suggests that the leaves of this species should be used as valuable food to cope with future changes in food supply and demand, especially in developing countries. The antioxidant activity determined in the extract from sweet potato leaves, explained by the presence of compounds with antioxidant properties (phenols, flavonoids, ascorbic acid, antioxidant enzymes) is responsible for the beneficial effects of eating sweet potato leaves. These antioxidants act as chemo-preventive agents against oxidative stress, caused by the excess of reactive oxygen species and which is associated with the appearance of many diseases.

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