Transilvania University of Brasov
FACULTY OF MECHANICAL ENGINEERING

ICMS 2019 & COMEC 2019
Brasov, ROMANIA, 21-22 November 2019

DETERMINATION OF ANTHOCYANIN PIGMENTS IN SWEET POTATO LEAVES USING SPECIFIC CONTACT SENSORS

C. L. Bădărău[1], M. Cioloca[2]
[1] Transilvania University from Brașov, Brașov, ROMANIA, carmen.badarau@unitbv.ro
[2] National Institute of Research and Development for Potato and Sugar Beet, Brașov, ROMANIA, mihaela.cioloca@potato.ro

Abstract: *In the last years the interest of the researchers regarding the natural anthocyanins increased. These pigments obtained from fruits and vegetable can be used as food dye, having attractive color and bioactive properties (with implications for human health). Selection of biological material with high content of anthocyanin is a priority. This is the reason because is important to identify quick and cheap methods used directly in the field during vegetation. This paper presents preliminary results on the determination of anthocyanin in sweet potato leaves (Hayanmi, KSP1, KSC1, Yulmi Juhwangmi) by two different methods. The anthocyanin content was determined using ACM-200-plus (Anthocyanin Content Meter) and in the laboratory using the extracts in 1% acidified methanol spectrophotometrically by the pH differential method. ACM-200-plus is an instrument designed for the rapid, non-destructive, determination of anthocyanin content in intact leaf samples.*
Keywords: *sweet potato leaves, anthocyanin pigments, Anthocyanin Content Meter Sensor.*

1. INTRODUCTION

There is a remarkable global interest to identify antioxidant compounds from plants, which may be a drug potential for use in preventive medicine and in animal and human feed [1].
Among the diseases that can be treated with herbs there is a large group of diseases associated with oxidative stress, such as cardiovascular and gastrointestinal diseases, inflammatory processes, neurodegenerative diseases, cancer, fertility disorders and diabetes, etc. [2-4].
Anthocyanin pigments are powerful antioxidants that protect cells from various forms of cancer. According to nutritionists, modern man who lives "assaulted" by pollution conditions and unhealthy foods needs to eat foods rich in antioxidant compounds [2-4].
Currently, sweet potato is grown on large areas not only in South and Central America, Africa and Oceania, but also in China, India, Japan, the Philippines, and the USA. Data on the distribution of sweet potato cultivation globally show the most pronounced concentration is in China, which owns 65% of the cultivated area with this species and achieving 90% of world production [5].
In addition to sweet potato varieties with white, yellow or orange flesh, there are some with purple flesh. The anthocyanins present in the sweet potato with the purple flesh (peonidine and cyanidine) have important antioxidant and anti-inflammatory properties. When they go through the digestive tract they can reduce the risk of illness due to the presence of heavy metals and free radicals. Cyanidins and peonidines are concentrated in the central part of the core and are found in greater quantity in the flesh than in the skin. Also, sweet potato storage proteins, called sporamines, have antioxidant effects [6].
The paper presents preliminary studies on the correlation between anthocyanin pigment content determined by ACM-200-plus equipment and anthocyanin pigment content determined by analytical methods [7]. This method could be useful in the production of new sweet potato varieties in order to identify early varieties rich in anthocyanin pigments.The main objective of this paper was to estimate the anthocyanins content from sweet potato leaves using two different methods: a specific contact sensors and analytical methods.

2. MATERIAL AND METHODS

2.1. Biological material

The sweet potato varieties Hayanmi, KSP1, KSC1, Yulmi Juhwangmi, were tested in the middle of July in National Institute of research and Development for Potato and Sugar Beet Brașov, Romania.

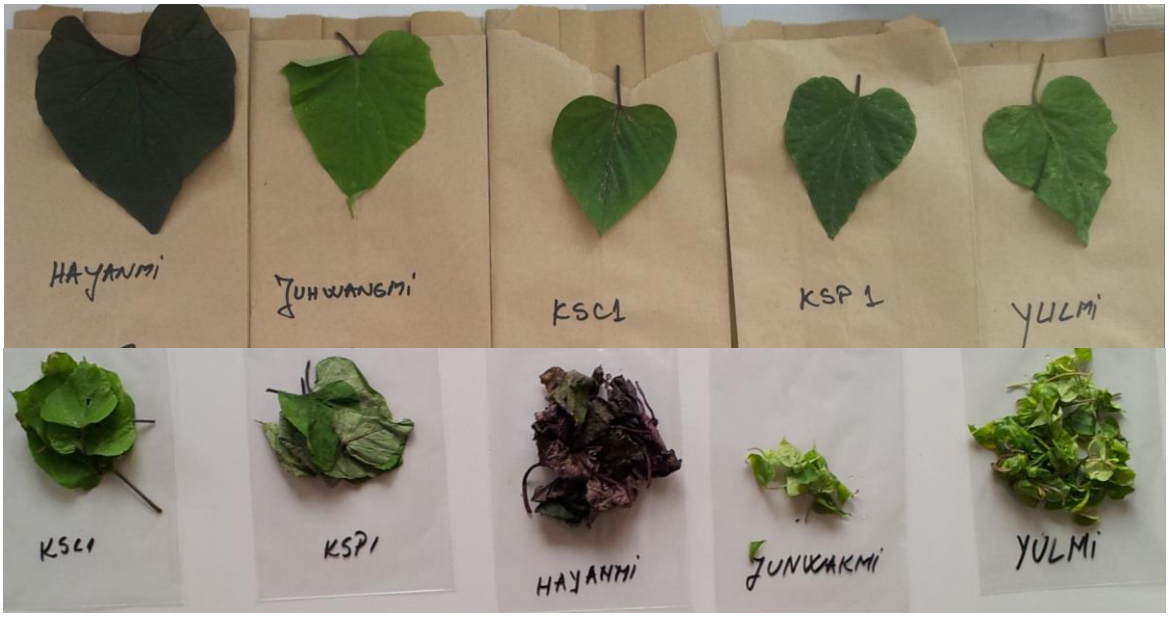

Figure 1: Samples of sweet potato varieties prepared for testing. In our research work the variety KSC1 was not tested for antocianin pigments.

2.2. Determination of anthocyanin content using specific contact sensors

The ACM-200-plus Anthocyanin Content Meter (Figure 2A) provides a fast estimate of anthocyanin content on the intact leaves of plants and flowers. It reduce grinding or destructive assays. The measurement is rapid, non destructive and simple.

A B

Figure 2: Anthocyanin Content Meter (ACM-200-plus Anthocyanin Meter) (A). Absorbance band for anthocyanin [8] (B).

Laboratory methods for determination of anthocyanin content are both time consuming and destructive to the sample. Usually, a sample must be detached, ground up in a solvent and evaluate the absorbance of the sample using a spectrophotometer.

Anthocyanin has distinct optical absorbance characteristics that the ACM-200-plus exploits in order to determine relative anthocyanin concentration. A strong absorbance band is present in the green range (Figure 2B). The ACM-200-plus uses the transmittance to estimate the anthocyanin content in leaf tissue according to the formula:

$$ACI = \frac{Transmi\tan ta\ (931nm)}{Transmi\tan ta\ (525nm)} \quad (1)$$

One wavelength falls within the anthocyanin absorbance range, while the infrared band serves to compensate sample thickness. The instrument measures the transmittance of both wavelengths and calculates an ACI (anthocyanin content index) value [8].

2.3. Anthocyanins extraction

Sweet potato leaves (Figure 1) in amount of ~0.5 g was homogenized for 30 min in 1% acidified methanol (40 ml in portions of 10 ml). Extracts were centrifuged (10000 rpm, 15 min) and concentrated at 45°C. The extraction procedure applied for anthocyanin pigments is schematically presented in Figure 3.

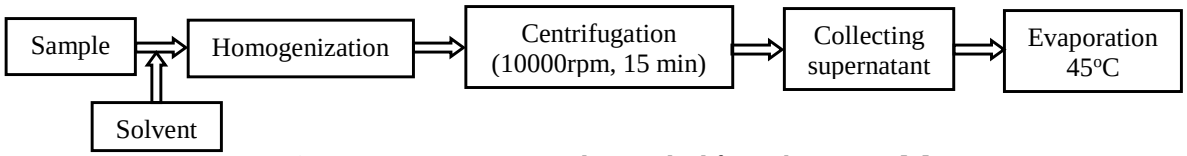

Figure 3: Extraction procedure applied for anthocyanins [9].

2.4. Determination of total anthocyanin content

The total anthocyanins content (TAC) were determined by the differential pH method [10-11] based on the property of anthocyanin pigments to change the color with pH. Two dilutions of the same sample were prepared, the first one in potassium chloride buffer (0.025 M, pH 1.0) and the second one in sodium acetate buffer (0.4 M, pH 4.5), pH being adjusted with HCl 0.2N. After equilibration at room temperature for 15 min, the absorbance of two dilutions was read at 510 nm and 700 nm. Total monomeric anthocyanins (mg cyanidin 3-glucoside equivalent/ 100 g Fresh Weight) were calculated as follows:

$$\%\, w/w = \frac{A}{\varepsilon L}\ MW\ \ DF\ \ \frac{V}{W_t}\ \ 100 \qquad (2)$$

$$A = \left(A_{510nm} - A_{700nm}\right)_{pH=1} - \left(A_{510nm} - A_{700nm}\right)_{pH=4.5} \qquad (3)$$

A – Absorbance; ε – Molar extinction coefficient (26900 L/mol cm); L – Path length ; MW – Molecular weight (449.2 g/mol for cyanidin 3-glucoside); DF – Dilution factor; V – Volume; W_t – sample weight

3. RESULTS AND DISCUSSIONS

Results regarding anthocyanin pigment content determined by ACM-200-plus equipment are presented in Table 1. Values represent the mean of three repetitions.

Table 1: Anthocyanin content index (ACI) of the leaves samples (SD =standard deviation)

Variety / plant sample	S1	S2	S3	S4	S5	S6	**Mean ± SD**
Hayanmi	19,5	21,4	18,6	18,8	15,1	23,1	19.4 ± 2.727
KSP1	8,2	7,9	10,0	10,6	9,8	6,3	8.8 ± 1.618
Yulmi	10,0	8,8	9,1	11,0	10,1	10,7	9.95 ± 0.864
Juhwangmi	5,1	5,0	5,4	5,8	5,4	5,7	5.4 ± 0.316

The results for the total anthocyanin content (TAC) determined by the pH differential method are presented in Table 2. The results are presented in mg galic acid equivalent for 100 g fresh weight.

Table 2: Total anthocyanin pigments content (TAC) of the leaves samples (mg GAE/100g FW)

Variety / plant sample	S1	S2	S3	S4	S5	S6	**Mean ± SD**
Hayanmi	27.03 ±0.83	37.86 ±1.05	22.50 ±1.33	23.50 ±0.196	10.47 ±1.54	41,5 ±0.824	27.676 ±6.029
KSP1	15.47 ±1.68	12.63 ±1.54	24.13 ±2.35	27.70 ±0.87	21.20 ±1.71	10.00 ±0.96	18.52 ± 8.047
Yulmi	21.02 ±1.35	10.36± 0.85	13.47 ±1.68	32.63 ±1.54	21.70 ±0.87	28.02 ±1.35	21.867 ± 6.758
Juhwangmi	9.65 ±1.26	9.26 ±0.82	15.32 ±0.35	19.65 ±0.58	15.98 ±0.49	18.58 ±0.87	14.74 ± 4.391

The highest content of anthocyanin pigments was found Hayanmi variety (21.676±4.029 mg GAE/100g FW) and the lowest in Juhwangmi variety (6.24 ± 1.721mg/100g FW) (figure 4 and table 2).

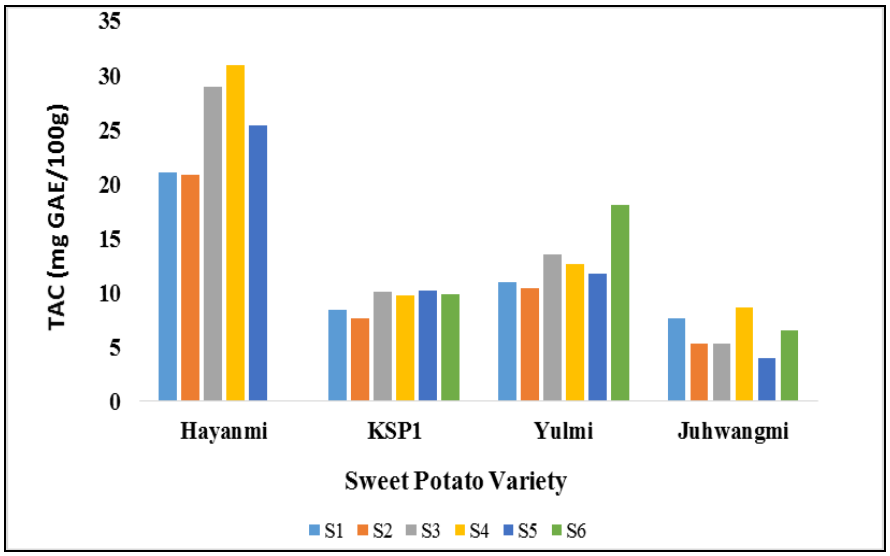

Figure 4: Content of anthocyanin pigments depending on the potato variety.

Correlation between anthocyanin pigments content determined through pH differential method and using the ACM-200-plus equipment is presented in Figure 5 A&B&C&D.

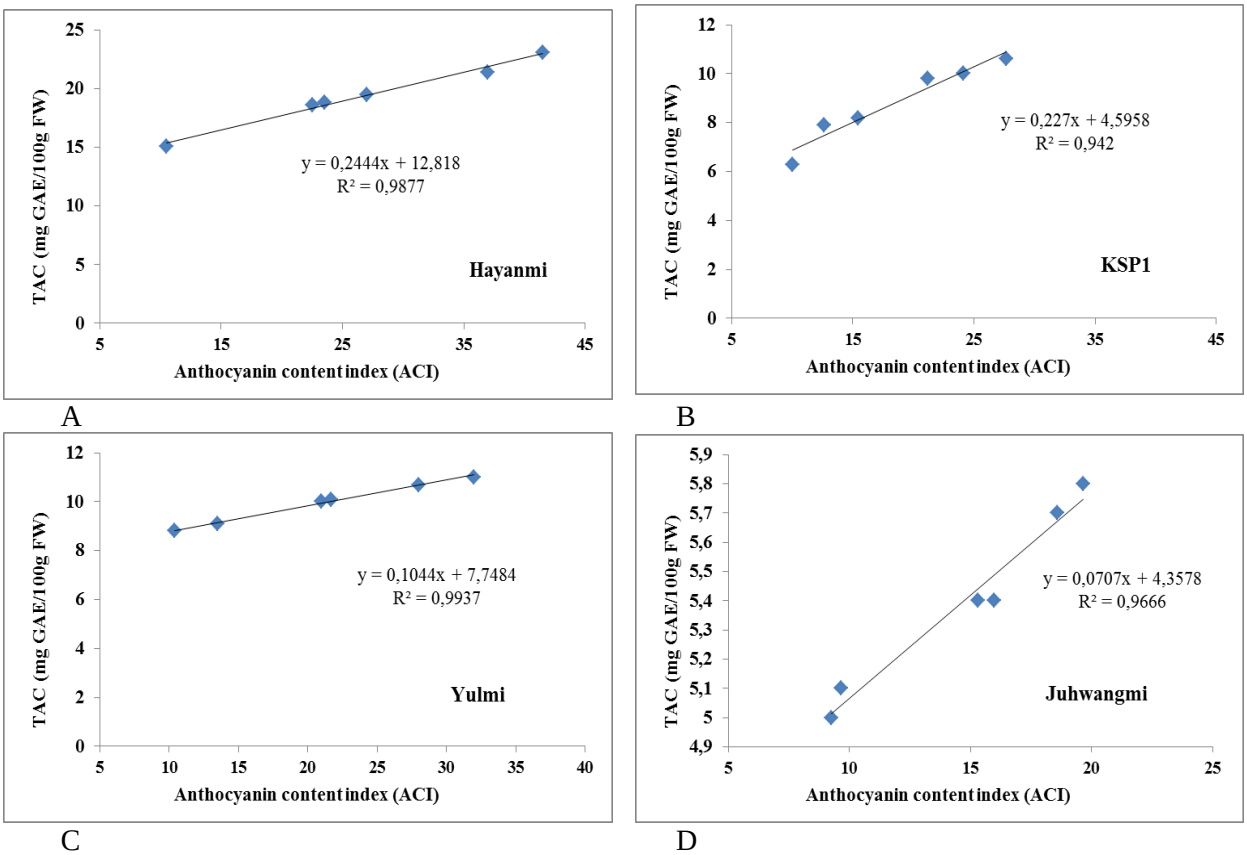

Figure 5: Correlation between anthocyanin pigment content determined by the two methods for the sweet potato varieties tested: Hayanmi (A), KSP1(B), Yulmi (C) and Juhwangmi (D).

For all the varieties, the total content of anthocyanin pigments determined by pH differential method is in accordance with data obtained with equipment ACM-200-plus. The strongest correlation was obtained in case of variety Yulmi.

Sweet potatoes are significant source of natural antioxidants and exhibit antioxidant activity as demonstrated in recent time by many authors. Studies have indicated that these phytochemicals have high free-radical scavenging activity, which helps to reduce the risk of chronic diseases and age-related neuronal degeneration [12]. Genotypes of sweet potato with peel and pulp intensely colored have antioxidant capacity 3,2 times higher than the white / yellow genotypes, and these aliments could help to supplement the required daily doses of antioxidants in the diet. As a result, in recent years, breeder's efforts intensified to get new genotypes in different versions: blue peel and pulp.

4. CONCLUSION

The content of anthocyanin pigments determined by the pH differential method was in accordance with data obtained with equipment ACM-200-plus in case of the samples tested.
This method could be useful for determining directly in the field of sweet potato varieties rich in anthocyanin pigments if in the future a correlation with anthocyanins content of tubers will be find.
Hayanmi variety has the highest amount of anthocyanin pigments.
The lowest content of anthocyanin has found in Juhwangmi variety.

REFERENCES

[1] André C, Larondelle Y, Evers D., (2010) Dietary antioxidants and oxidative stress from a human and plant perspective: a review. Current Nutrition and Food Science 6(1):2-12.
[2] Patel, N.J., Gujarati, V.B, Gouda1, T.S., Venkat Rao, N., Nandakumar, K., Shantakumar, S.M., 2006. Antidiarrhoeal activity of alcoholic and aqueous extracts of roots of tylophora indica (wight & arn.) in rodents. Pharmacology online, 1:19-29.
[3] Kupeli E, Tatli II, Akdemir ZS, Yesilada E. (2007) Bioassay-guided isolation of anti-inflammatory and antinociceptive glycoterpenoids from the flowers of Verbascum lasianthum Boiss. ex Bentham. Journal of Ethnopharmacology, 110(3):444-50.
[4] Bucciarelli AY and Skliar MI (2007) Medicinal plants from Argentina with gastro protective activity. Ars. Pharmaceutica 48(4):361-369.
[5] Roșu A., (2006) Manual de biotehnologii vegetale aplicate în micropropagarea batatului [Ipomoea batatas L. (Lam.)]. ProEditură și Tipografie București, p. 9
[6] (Cioloca M. (2016) Cartoful dulce – o cultură de perspectivă pentru fermierii din România. Cartoful în România, vol. 25, p. 80-82.
[7] Puiu I, Olteanu G, Mărculescu A, Ghinea A, Damșa F. (2014) Soil resources and agricultural crops vegetation status monitoring by using specific and precision sensors. Editura: Bulletin of the Transilvania of Brasov - Food and Enviroment, Brasov, 2014-07-24, pg. 88-98, ISSN 978-606-19-0390-0.
[8] ACM-200-PLUS Anthocyanin Meter - User Guide.
[9] Damşa F, Woinaroschy A, Olteanu G (2015) Quantification of total flavonoid and total anthocyanin content from purple potato, Revista de chimie Vol 66.
[10] Giusti MM (2007) Analisys of anthocyanins. Food Colorants, Chemical and Functional Properties, ISBN: 978-0-8493-9357-0, 429-547
[11] Sconţa, Z.M. (2012) Extraction, purification, characterization and in vitro testing of anthocyanin-rich fractions obtained from aronia melanocarpa and vaccinium sp, PhD Thesis, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca
[12] Teow, C.C., Van-Den Truong, McFeeters, R.F., Thompson, R.L., Pecota, K.V., Yencho, G.C. (2007) Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food Chemistry. doi: 10.1016/j.foodchem.2006.09.033.