Chapter 6

DETERMINATION OF ANTHOCYANIN PIGMENTS IN POTATO USING SPECIFIC CONTACT SENSORS AND ANALYTICAL METHODS

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1. Introduction

Currently, 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.

The only source for the production of anthocyanin pigments are plants.

Anthocyanins are found in: blueberries, blackberries, raspberries, cherries, cranberries, black currant, elderberry, eggplant, purple corn, black beans and purple potatoes.

Currently potato is the fourth food culture of the world, after corn, wheat and rice, with a production of 329 million tonnes per year [5]. Worldwide, in terms of harvested area potato ranks seven after wheat, rice, corn, barley, sorghum and rapeseed. In terms of consumption, potato ranks third after rice and wheat. In Romania, currently, from the total cultivated area of 8.9 million hectares, potato ranks third with a share of about 3.2% after cereals which represent 62% and oilseeds 15%.

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 [6]. Genotypes of potato with peel and pulp intensely colored (red, purple, blue) have antioxidant capacity 2-3 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, breederôs efforts intensified to get new potato genotypes in different versions: blue peel and pulp [7-9].

This 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. This method could be useful in the production of new potato varieties in order to identify early varieties rich in anthocyanin pigments. These research results can be of great benefit to breeders and brings more knowledge in the field of potato crop improvement.

2. Objectives

The main objective of this paper is to determine the anthocyanins content from purple potato leaves through two different methods: using specific contact sensors and analytical methods

Another important objective is the determination of total flavonoids content from potato leaves.

3. Material and Methods

3.1. Determination of anthocyanin content using specific contact sensors

The ACM-200 plus Anthcyanin Content Meter (Figure 1) provides a fast estimate of anthocyanin content on the intact leaves of plants and flowers. Reduce grinding or destructive assays. The measurement is rapid, nondestructive and simple to obtain.

Fig. 1. *Image of Anthocyanin Content Meter (ACM 200 plus)*

Laboratory methods for determination of anthocyanin content are both time consuming and destructive to the sample. Typically, a sample must be detached, ground up in a solvent and then assayed in 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 2).

The ACM-200 plus uses 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.

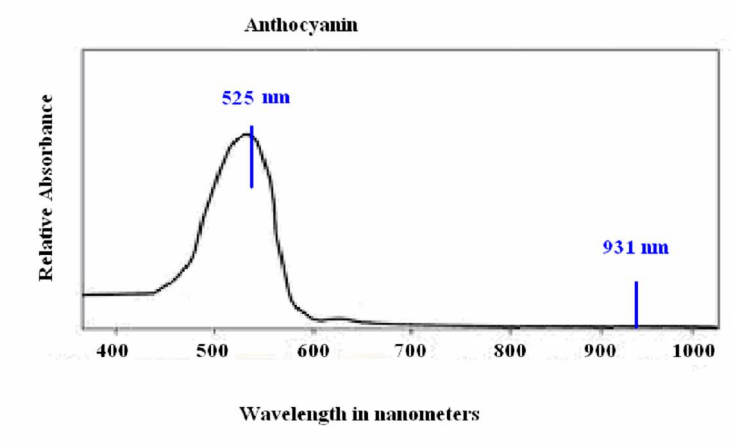

Fig. 2. *Absorbance band for anthocyanin [10]*

The instrument measures the transmittance of both wavelengths and calculates an ACI (anthocyanin content index) value [10].

The analyzes regarding the content of anthocyanin pigments with ACM 200 plus were conducted on two varieties of purple potato (Figure 3) (Albastru Violet de Galanesti - AV and Blue Congo - BC varieties) on five different plants in three repetitions. As controls were used Romanian varieties Christian - C (red peel / white pulp) and Roclas - R (white peel and pulp).

(A) *(B)*

Fig. 3. *Purple potato variety: A – Blue Congo [11] and B – Albastru-Violet de Galanesti*

3.2. Anthocyanins and flavonoids extraction

Potato leaves (Figure 4) 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 and flavonoids is schematically presented in Figure 5.

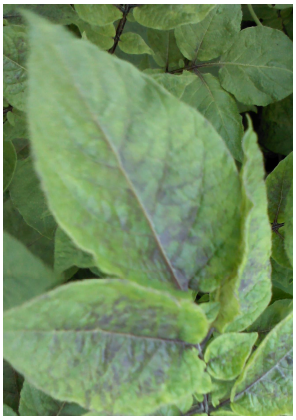

Fig. 4. *Image of purple potato leaves*

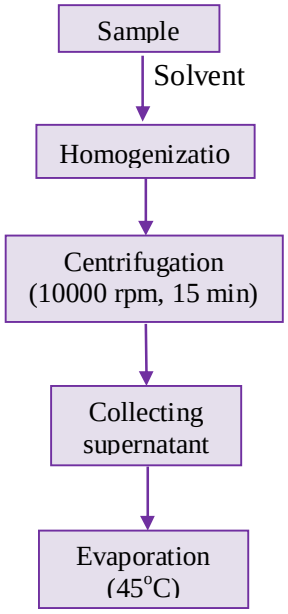

Fig. 5. *Extraction procedure applied for anthocyanins and flavonoids*

3.3. Determination of total flavonoid content

The total flavonoids content (TFC) of different extracts was determined using the aluminium chloride assay [12]. The extracts were taken in different test tubes and diluted with distilled water followed by the addition of 150µl of sodium nitrite (5% $NaNO_2$, w/v). After 6 min the mixture was treated with 150µl of aluminium chloride (10% $AlCl_3$, w/v) and incubated for 6 min. Later 2 ml of sodium hydroxide (NAOH 1N) was added and volume was made up to 5 ml with distilled water. The absorbance was measured at 510nm after 15 min using a spectrophotometer. Distilled water was used as blank. The TFC was expressed in mg quercetine equivalents for 100g of fresh material (FW).

Concentration values of extracts were obtained from Quercetin standard curve (Figure 6) [13], by interpolating to the X- axis.

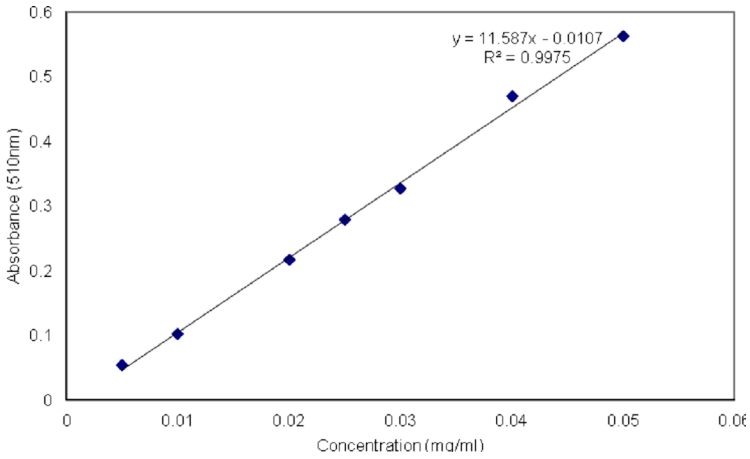

Fig. 6. *Calibration curve of quercetine [13]*

TFC was calculated by using the following formula:

$$TFC = \frac{R \cdot DF \cdot V \cdot 100}{W} \qquad (2)$$

The semnifications of symbols used in this relation are:

R - Result obtained from the standard curve

D.F - Dilution factor

V - Volume of stock solution

W - Weight of plant used in the experiment

3.4. Determination of anthocyanin content

The total anthocyanins content were determined by the differential pH method [14] 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:

$$\%\, w/w = \frac{A}{\varepsilon L}\ MW\ \ DF\ \ \frac{V}{W_t}\ 100 \tag{3}$$

$$A = \left(A_{510nm} - A_{700nm}\right)_{pH=1} - \left(A_{510nm} - A_{700nm}\right)_{pH=4.5} \tag{4}$$

The semnifications of symbols used in these relations are:

%w/w ï percentage weight/weight (grams of solute in 100 grams of solution)

A ï Absorbance

Ůï Molar extinction coefficient (26900 L/mol cm)

L ï Path length

MW ï Molecular weight (449.2 g/mol for cyanidin 3-glucoside)

DF ï Dilution factor

V ï Volume

W_t ï sample weight

4. 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.

Anthocyanin content index Table 1

	AV1	AV2	AV3	AV4	AV5	BC1	BC2	BC3	BC4	BC5
ACI	5.63 ±0.35	5.93 ±0.55	5.53 ±0.85	5.03 ±0.25	8.20 ±0.44	7.67 ±1.03	7.20 ±0.36	8.33 ±1.02	6.43 ±0.67	6.73 ±0.51
	C1	C2	C3	C4	C5	R1	R2	R3	R4	R5
ACI	7.90 ±0.75	8.23 ±0.32	6.87 ±0.71	7.10 ±0.75	6.83 ±0.61	6.20 ±1.04	5.53 ±0.45	5.53 ±0.21	6.07 ±0.38	5.90 ±0.17

In Table 2 are presented the results for the total anthocyanin content determined by the pH differential method and in table 3 for total flavonoids content determined by aluminium chloride assay.

The content of anthocyanin pigments in potato leaves Table 2

	AV1	AV2	AV3	AV4	AV5	BC1	BC2	BC3	BC4	BC5
TAC (mg/100g FW)	22.23	25.86	12.20	13.90	45.37	20.09	11.13	11.70	19.02	10.36
	C1	C2	C3	C4	C5	R1	R2	R3	R4	R5
TAC (mg/100g FW)	13.47	12.63	6.06	5.65	5.26	0.00	0.00	0.00	0.00	0.00

The content of flavonoids in potato leaves Table 3

	AV1	AV2	AV3	AV4	AV5	BC1	BC2	BC3	BC4	BC5
(mg/100g FW)	818.07	639.91	532.77	642.89	945.88	627.57	525.69	758.43	464.91	533.06
	C1	C2	C3	C4	C5	R1	R2	R3	R4	R5
(mg/100g FW)	285.36	336.95	473.24	367.34	578.89	706.58	786.17	759.18	883.50	811.00

As you can see in figure 7, the highest content of anthocyanin pigments was found in Albastru-Violet de Galanesti variety (45.37 mg/100g FW) - purple potato and the lowest in Roclas variety (0.00mg/100g FW) - white potato.

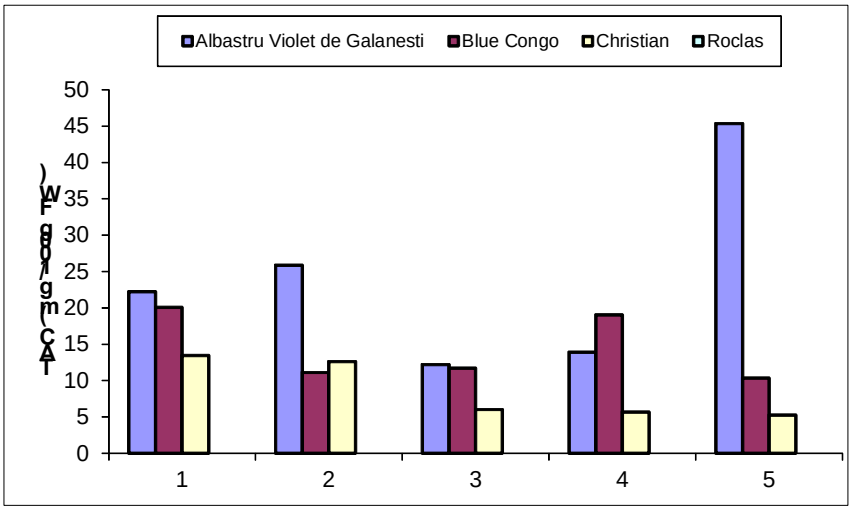

Fig. 7. *The image represents the content of anthocyanin pigments depending on the potato variety*

Also, as you can see in Figure 8, the highest content of flavonoids was found in Albastru-Violet de Galanesti variety (945.88 mg/100g FW) - purple potato and the lowest in Christian variety (285.36 mg/100g FW) ï red potato.

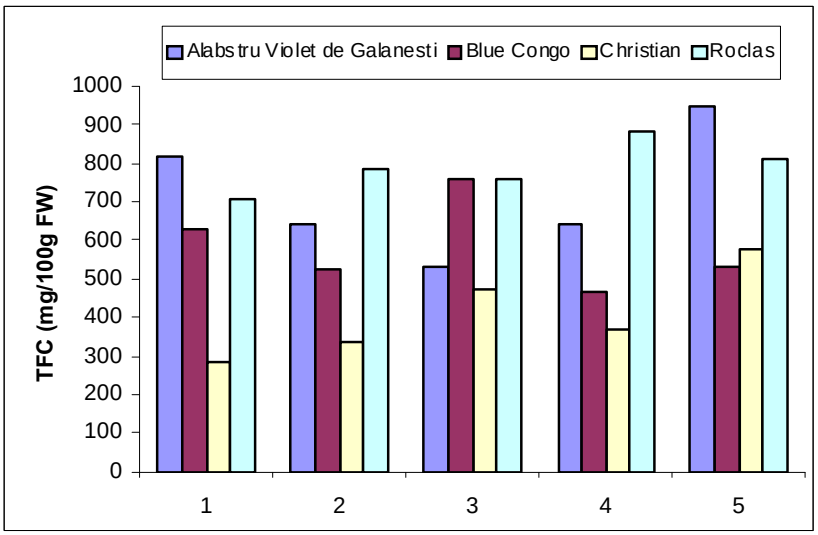

Fig. 8. *The image represents the content of flavonoids depending on the potato variety*

Correlation between anthocyanin pigments content determined through pH differential method and by ACM 200 plus equipment is presented in Figure 9, Figure 10 and Figure 11.

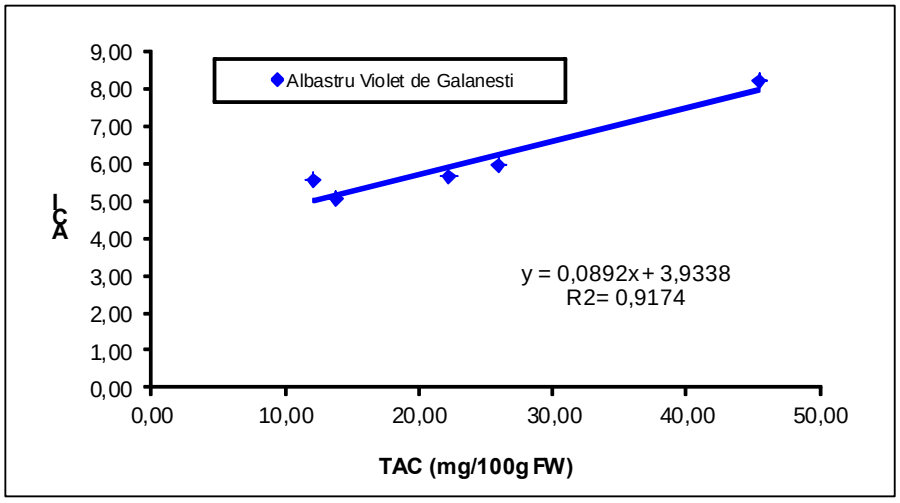

Fig. 9. *Correlation between anthocyanin pigment content determined by the two methods for Albastru-Violet de Galanesti variety*

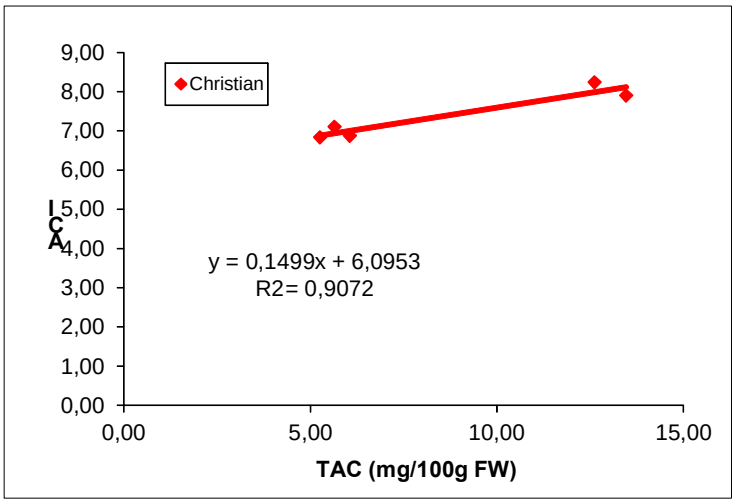

Fig. 10. *Correlation between anthocyanin pigment content determined by the two methods for Christian variety*

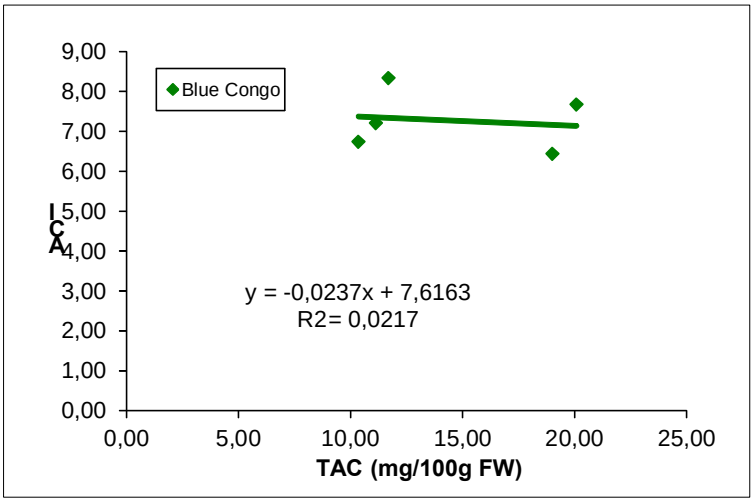

Fig. 11. *Correlation between anthocyanin pigment content determined by the two methods for Blue Congo variety*

5. Conclusions

- For Albastru Violet de Galanesti and Christian varieties, the content of anthocyanin pigments determined by the pH differential method is in accordance with data obtained with equipment ACM 200 plus.
- For Congo Blue variety was not possible a correlation between the two methods and therefore these analyzes will be repeated.

- The plants that were made the determinations were noted and after harvesting will be done the anthocyanin extraction from tubers.
- This method could be useful for determining directly in the field of potato varieties rich in anthocyanin pigments if we will find a correlation with anthocyanins content of tubers.
- Albastru-Violet de Galanesti variety has the highest amount of anthocyanin pigments and also, the highest amount of flavonoids.
- The lowest content of anthocyanin has found in Roclas variety and the lowest content of flavonoids was found in Christian variety.

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