### Chapter 9 CORRELATION BETWEEN ANTHOCYANINS PIGMENTS FROM PURPLE POTATO LEAVES AND TUBER

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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 [5].

Currently potato is the fourth food culture of the world, after corn, wheat and rice, with a production of 329 million tonnes per year [6]. 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 [7]. Genotypes of potato with peel and pulp intensely colored (red, purple, and 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, breederøs efforts intensified to get new potato genotypes in different versions: blue peel and pulp [8-10].

Data acquisition systems that collect information about the anthocyanin pigment can be embedded in compact portable measuring devices or can be connected to a computer by cable or wireless. Different types of sensors can be integrated together in a Wireless Sensor Network so that to monitor the entire measurement process [11].

The main objective of this paper is to find a relation between the anthocyanins from purple potato leaves and tuber that will be useful to identify, directly in the field, the potato variety rich in these pigments.

#### 2. Material and Methods

## 2.1. 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:

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

One wavelength falls within the anthocyanin absorbance range, while the infrared band serves to compensate sample thickness.



Fig. 2. Absorbance band for anthocyanin [12]

The instrument measures the transmittance of both wavelengths and calculates an ACI (anthocyanin content index) value [12].

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).

#### 2.2. Anthocyanins extraction

Potato leaves or tuber (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  $45^{\circ}$ C. The extraction procedure applied for anthocyanin is schematically presented in Figure 5.





(A) (B) **Fig. 3.** Purple potato variety: A – Blue Congo [13] and B – Albastru-Violet de Galanesti



Fig. 4. Image of purple potato leaves



Fig. 5. Extraction procedure applied for anthocyanins

#### 2.3. 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:

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

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

The semnifications of symbols used in these relations are: %w/w ó percentage weight/weight (grams of solute in 100 grams of solution) A ó Absorbance

ó Molar extinction coefficient (26900 L/mol cm) L ó Path length MW ó Molecular weight (449.2 g/mol for cyanidin 3-glucoside) DF ó Dilution factor V ó Volume Wt ó 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.

ACI	AV1	AV2	AV3	AV4	AV5
	5.63	5.93	5.53	5.03	8.20
	±0.35	±0.55	±0.85	±0.25	±0.44
	<b>BC1</b>	<b>BC2</b>	BC3	<b>BC</b> 4	BC5
	6.80	7.30	7.25	7.00	7.47
	±1.03	±0.36	±1.02	±0.67	±0.51
	<i>C1</i>	<i>C2</i>	С3	<i>C4</i>	<i>C</i> 5
	7.90	8.23	6.87	7.10	6.83
	±0.75	±0.32	±0.71	±0.75	±0.61
	<i>R1</i>	<i>R2</i>	<i>R3</i>	<i>R4</i>	<i>R5</i>
	5.68	5.53	5.53	5.94	5.90
	±1.04	±0.45	±0.21	±0.38	±0.17

Table 1. Anthocyanin content index

In Table 2 are presented the results for the total anthocyanin content from potato leaves determined by the pH differential method.

TAC (mg/100g FW)	AV1	AV2	AV3	AV4	AV5
	22.23	25.86	12.20	13.90	45.37
	±0.43	±1.05	±1.23	±0.96	±2.54
	<b>BC1</b>	BC2	BC3	<b>BC</b> 4	BC5
	20.09	11.13	11.70	19.02	10.36
	±1.40	±2.35	±0.87	±1.35	±0.85
	<b>C</b> 1	<i>C2</i>	С3	<i>C4</i>	<i>C</i> 5
	13.47	12.63	6.06	5.65	5.26
	±1.68	±1.54	±0.94	±1.26	±0.82
	<i>R1</i>	<i>R2</i>	<i>R3</i>	<b>R</b> 4	<i>R5</i>
	0.00	0.00	0.00	0.00	0.00

*Table 2.* The content of anthocyanin pigments in potato leaves

In Table 3 are presented the results for the total anthocyanin content from potato tuber determined by the pH differential method.

	-	AV1	AV2	AV3	AV4	AV5
TAC	(M)	152.32	168.54	148.36	143.65	175.43
	0g H	±8.65	±10.24	±5.32	±7.65	±9.47
	01/2	<b>BC1</b>	BC2	BC3	BC4	BC5
	Gm)	101.32	75.86	87.32	98.65	56.32
		±6.32	±8.56	±10.35	±12.36	±9.62

**Table 3.** The content of anthocyanin pigments in potato tuber

As can be seen in Figure 6, the highest content of anthocyanin pigments was found in Albastru-Violet de Galanesti variety (45.37 mg / 100 g FW) - purple potato and the lowest in Roclas variety (0.00 mg / 100 g FW) - white potato.



Fig. 6. Content of anthocyanin pigments depending on the potato variety



*Fig.* 7. Correlation between anthocyanin pigment content from tuber and leaves of Albastru-Violet de Galanesti variety

Correlation between anthocyanin pigments content determined through pH differential method from potato leaves and tuber is presented in Figure 7 and Figure 8.



*Fig. 8.* Correlation between anthocyanin pigment content from tuber and leaves of Blue Congo variety

#### 4. Conclusions

For the purple variety the anthocyanin content from leaves is in accordance with the anthocyanin content from tuber. 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 the lowest content of anthocyanin has found in Roclas variety.

#### Acknowledgments:

This work was partially supported by National Institute of Research and Development for Potato and Sugar Beet and by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, PN-II-PT-PCCA-2013-4-1629 (225/2014).

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