ANTHOCYANIN PIGMENT CONTENT OF SOME CHERRY VARIETIES GROWN IN IASI AREA, ROMANIA

CONŢINUTUL ÎN PIGMENŢI ANTOCIANICI AL UNOR SOIURI DE CIREŞ CULTIVATE ÎN ZONA IAŞI, ROMÂNIA

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Abstract. *The purpose of the study was the determination of the total monomeric anthocyanins content (AC) by pH differential method and anthocyanin profile, obtained by HPLC-DAD technique, from fruits of six local varieties of sweet cherry (Prunus avium), grown in experimental field of RDSF (Research-Development Station for Fruit growing), Iasi. AC ranged between 46.84±1.86 mg/100g, at Van variety and 443.72±1.54 mg/100g, at Amar Maxut variety. Based on the chromatograms obtained were identified 4 anthocyanins: cyanidin (cy)-3-glucoside, cy-3-rutinoside, pelargonidin (pg)-3-rutinoside, peonidin (pn)-3-rutinoside, expressed as percentage of area (%). Cy-3-rutinoside was found in the highest proportion in all varieties examined (over 91 % of total anthocyanins area), the ratio of the pigments identified was specific to each variety.*

Key words: cherries, anthocyanins, phenolics, HPLC-DAD

Rezumat. *Scopul studiului a fost determinarea conţinutului total în antociani monomerici (CA) prin metoda diferenţei de pH şi obţinerea profilului antocianic, prin tehnica HPLC-DAD, din fructele a 6 soiuri autohtone de cireş (Prunus avium), cultivate în câmpul experimental al SCDP (Staţiunea de Cercetare - Dezvoltare pentru Pomicultură), Iaşi. CA a avut valori cuprinse în intervalul 46,84±1,86 mg/100g, la soiul Van şi 443.72±1.54 mg/100g, la soiul Amar Maxut. Pe baza cromatogramelor obţinute au fost identificaţi 4 antociani: cianidină (cy)-3-glucozid, cy-3-rutinozid, pelargonidină (pg)-3-rutinozid, peonidină (pn)-3-rutinozid, exprimarea valorilor fiind în procente de arie (%). Antocianul cy-3-rutinozid, se găseşte în proporţia cea mai ridicată în toate soiurile analizate (peste 91 % din totalul ariei corespunzătoare antocianilor), raportul dintre pigmenţii identificaţi fiind specific fiecărui soi.*

Cuvinte cheie: cireşe, antociani, fenoli, HPLC-DAD

INTRODUCTION

Cherries (fr. *cerises douces*, eng. *sweet cherries*), are fruits appreciated for their early appearance (early varieties, are mature in May) and the nutritional value (Beceanu D., 2002). Cherries are required both as fresh fruit and for industrialization (compotes, jams, syrups, juices etc.). In Romania, cherry tree finds optimal conditions for expression of their agrobiological potential (Budan S.

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and Grădinariu G, 2000), cherry production in Romania was constant in recent years (2007-2009), 65-68 thousand tons (Beceanu D., 2011).

Anthocyanins represent one of the major groups of hydrosoluble pigments belonging to the flavonoid class, which are metabolism secondary products (Davies, K., 2004). On the basis of the studies carried out on the cell line in animals and clinical in humans, it has been suggested that anthocyanins have an anti-inflammatory and anticarcinogenic action, they prevent cardiovascular diseases, all these actions being associated more or less to their antioxidizing capacity (He J., and Giusti Monica, 2010).

Anthocyanins are the second large class of phenolic compounds in cherries (after hydroxycinamates), with a rate of about 26 % of total phenolic compounds (Kelley D. S., 2006). The specialized literature, offers data, varying within very large limits, related to the anthocyanin content of cherries, between 2 and 450 mg/100 g (Coşofreţ S. *et al.,* 2006; Horbowicz M. *et al.,* 2008; Gould K., 2009).

Only few studies have been registered in the international databases, focused on the cherries and sour cherries anthocyanins, by using the HPLC method, coupled with DAD or UV-VIS detector (Esti M. *et al.,* 2002). Content in the main anthocyanidins in cherries, is dominated by cyanidin (cy), followed, in much smaller quantities, by peonidin (pn) and pelargonidin (pg) (Horbowicz, M. *et al.*, 2008). Major anthocyanins from cherries, were identified as: cy-3-glucoside, cy-3-rutinoside, cy-3-sophorozide, pn-3-glucoside, pn-3-rutinoside (Mazza G., Miniati E., 1993). The occurrence of these anthocyanins and the ratio between them is specific to each variety (Davies K., 2004, Mozetič B., Trebše P., 2004).

MATERIAL AND METHOD

Was measured the anthocyanin content (AC) and total quantity of phenolic compounds (TPC) of ethanolic extracts from fruits of six varieties of cherry (*Prunus avium* L.), three of sweet cherry: *Van, Stella, Maria*, two hybrid elites proposed for patent application by RDSF (Research-Development Station for Fruit growing) Iasi: *Oana (HC-840 860)*, *Radu (HC-840 836)* and a variety of bitter cherry: *Amar Maxut*, all grown in experimental field of RDSF Iaşi, Miroslava area, Romania. The harvesting of samples was made at their maturity of consumption, when the fruits have developed the varietal characteristic color and optimal gustatory features (Grădinariu G. *et al.,* 1998), in the interval 09.06.2010 – 16.06 2010.

We determined certain physico-chemical properties of fruits: the average mass of a fruit, the moisture content (drying off in a drying chamber, 4 hours at 105°C), the soluble dry substance (the refractometric method), titratable acidity, pH (the potentiometric method), reducing sugars (Schoorl method), activity of catalase (the gasometrical method) and peroxidase, as factors influencing the anthocyanin content.

Fruits were stored at the temperature of -18±2°C*,* anthocyanins transformation being considered as minimal for cherries kept in a frozen state (Mazza G., Miniati E., 1993). Ethanolic extracts, were obtained with a ratio between plant material and solvent of 1:20 (5g fruit/100 mL extraction solution). The efficiency of phenolic compounds extraction can be enhanced by an increase of the ratio between the solvent and solid (Cacace J.E., Mazza G., 2003). The containers were left in the dark at room temperature (18±2°C), overnight. Before the third filtering, we applied an

ultrasound treatment to the samples, to reduce aggregation and agglomeration of particles.

Being necessary to obtain extracts for alimentary use, which should not contain toxic reagents, extraction was performed with ethanol-HCl-water system (96:1:3) (pH 1.5±0.1). Acids are very important in maintaining stability of anthocyanins, are necessary in the formation of flavylium cation, the most stable form (at pH 1.5 - 2) (Socaciu Carmen, 2008). The three extraction fractions were cumulated, and stored at low temperatures (6±1°C) and in the dark, to avoid acidic hydrolysis, which takes place in a weak acid warm medium, when anthocyanidins are formed and the glucidic part is released (Cercasov Cornelia *et al.*, 2005).

pH differential method, is based on the following principle: in the acidic environment, there is a balance between the colored and colorless forms of anthocyanins (Lee J. *et al.*, 2005). This balance is in function of pH (Lee J. *et al.*, 2008). Calculation formula: A (absorbance) = $(A_{520} - A_{700})_{pH\ 0.68} - (A_{520} - A_{700})_{pH\ 3.5}$. Coloring intensity variation between these two pH values is proportional to the anthocyanin content (mg anthocyanins /100 g fruit). A_{700}, is reduced, due to the presence of other phenolic compounds (Horbowicz M. *et al.*, 2008).

To obtain the total phenolic compounds content (TPC), was used Folin-Ciocâlteu colorimetric method. Measurements were made using a UV-VIS Analytik Jena Specord 200 spectrometer, at wavelength (λ) 765 nm. Expression of content in phenolic compounds was made in grams gallic acid equivalent (g GAE)/100 g fruit.

Using HPLC-DAD technique, we was drawn a profile of anthocyanins, identifying the main representatives of this class, and the ratio of the anthocyanins in each variety, expressed as a proportion of area (% area). By means of a Shimadzu LC 20 liquid chromatograph, using a Hypersil ODS C18 column type (25 cm length) at a temperature of 25°C, can be obtained the separation of the extraction mixture. Anthocyanins have been individualised, using a Shimadzu DAD, at a wavelength of 518 nm. Reagents used were purchased from Merck Romania, Bucharest. Statistical data processing was performed with Microsoft Excel software (ANOVA test).

RESULTS AND DISCUSSIONS

Physico-chemical properties of the fruits are presented in table 1, values being specific to each variety.

Table 1

Physico-chemical characteristics of the cherry varieties under study

Variety	M. fr. (g)	M. (%)	T. ac. (g m. a.)	Rd. sg. (g. %)	pH	SDS (°Bx)	Cat. act. (cm^3O_2/g/h)
Van	6,5	84,22	0,72	**10,42**	3,65	16,12	0,85
Stella	6,6	**86,41**	0,36	5,97	3,83	13,92	**1,35**
Maria	7,3	83.92	0,44	9,15	3,69	18,92	0,42
Radu	6,4	79,64	**0,73**	7,22	**3,97**	14,72	1,00
Oana	**7,4**	81,72	0,44	8,18	3,91	**17,12**	0,35
Amar Maxut	4,2	81,78	**0,73**	8,46	3,59	12,72	1,10

M. fr. – average mass of fruit, M (%) – moisture, T. ac. (g m. a.) – titratable acidity (g malic acid /100g), Rd. sg. (g. %) – reducing sugars (mg. glucose/100 g product), SDS (°Bx) – soluble dry substance (°Brix), Cat. act. – catalase activity.

Fruits moisture ranged between 79.64 % (Radu variety) and 86.41 % (Stella variety) and titratable acidity had higher values at Radu and Amar Maxut varieties (0.73 g malic/100g acid). Van variety had the highest reducing sugars content (10.42 mg glucose/100g) and Radu variety, the highest pH (3.9). Catalase activity was best highlighted at Stella variety (1.35 $cm^3O2/g/h$) and peroxidase activity, as a factor influencing the content of anthocyanins, was assessed as very low.

Following the interpretation of the absorption spectra, we obtained the results for the total content of anthocyanin (AC) and phenolic compounds (TPC) (table 2). The data obtained represent the average of three determinations and have calculated the standard deviation.

Amar Maxut variety, had the highest AC value (443.72±1.54 mg/100g) and TPC (794.62±0.08 mg GAE/100 g), this variety having the most intensely colored fruits, among the analysed varieties. A major positive correlation was found between AC and TPC values (R^2=0.957, *p-value* < 0.01 %) (fig. 1), the varieties with a high TPC, had also a high AC, the report being specific to each variety.

Table 2

AC and TPC values of the analysed varieties

No.	Variety	AC (mg/100g)	TPC (mg GAE/100g)
1	**Van**	46.84±0.88	162.62±0.33
2	**Stella**	77.34±0.45	240.46±0.26
3	**Maria**	154.19±0.90	352.17±0.21
4	**Radu**	191.26±0.64	387.56±0.19
5	**Oana**	378.93±1.02	563.42±0.42
6	**Amar Maxut**	**443.72±1.54**	**794.62±0.08**

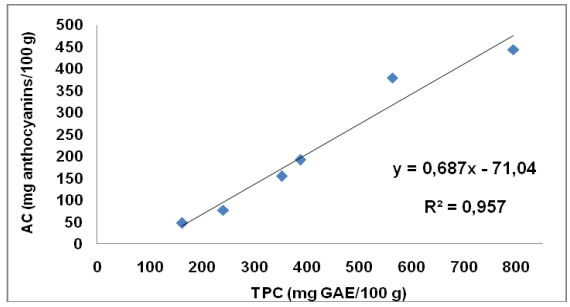

Fig. 1 - AC-TPC values corelation

HPLC chromatograms obtained were shown, for each variety (fig. 2). Following their interpretation, we have identified four anthocyanins: cy-3-glucoside, cy-3-rutinoside, pn-3-rutinoside and pg-3-rutinoside, confirmed also by the literature. Their expression was made as a percentage of area, ratio between anthocyanins identified, being specific to each variety (table 3). Cy-3-rutinoside was the main anthocyanin in all varieties examined, with the highest percentage at Stella variety (95.94 %). Anthocyanin pg-3-rutinoside was determined to have the

lowest participation rate in five of the analysed varieties, reaching the lowest percentage at Stella variety (0.21 %) and highest at Oana variety (2.03 %).

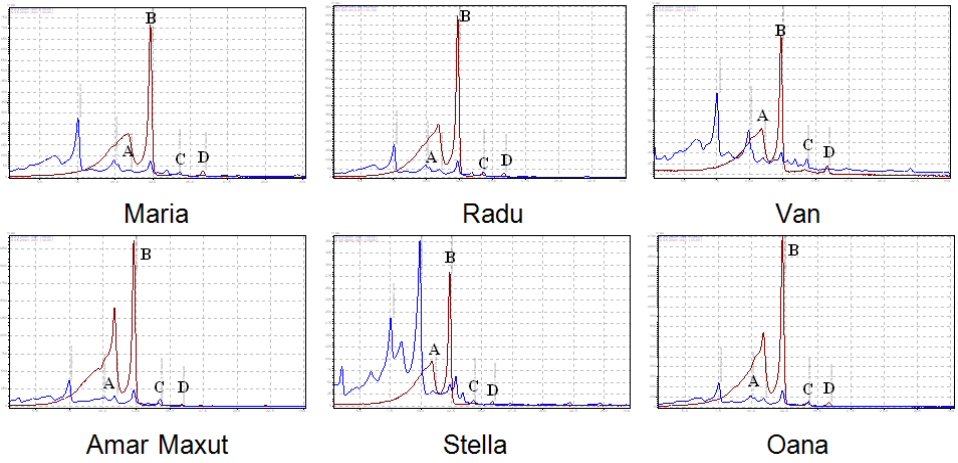

Fig. 2 - Chromatograms obtained by HPLC-DAD
A - cy-3-glucoside; B - cy-3-rutinoside; C - pg-3-rutinoside; D - pn-3-rutinoside

Table 3

Area percentages corresponding to the anthocyanins identified

Variety / Anthocyanin	cy-3-rutinoside	cy-3-glucoside	pg-3-rutinoside	pn-3-rutinoside	Total (%)
Stella	95.94	1.38	0.21	2.46	100.00
Maria	94.00	0.78	1.52	3.70	100.00
Radu	93.78	1.98	1.52	2.72	100.00
Van	93.05	1.41	0.40	5.15	100.00
Amar Maxut	92.98	5.18	1.00	0.84	100.00
Oana	91.72	4.00	2.03	2.25	100.00

CONCLUSIONS

1. The six cherry varieties analysed showed a series of physico-chemical characteristics (the average mass of a fruit, the moisture content, titratable acidity, soluble sugars, pH), with specific values for each variety.

2. Total anthocyanin and total phenolic compounds content, had the highest value at the Amar Maxut variety, 443.72±1.54 mg/100g, respectively 794.62± 0.08 mg GAE/100g, in this variety predominate cyanidin, with the two glycosidic forms identified: cy-3-rutinoside (92.98 %) and cy-3-glucoside (5.18 %).

3. Van variety, had the lowest amount of anthocyanins (46.84±0.88 mg/100g) and phenolic compounds (162.62±0.33 mg GAE/100g), at this variety, pn-3-rutinoside, being in the highest proportion (5.15 %), of all studied varieties.

4. Only Oana variety presented a percentage of pg-3-rutinoside higher than 2%, this anthocyanin being identified in the smallest proportion.

5. The data obtained, are within the range of values presented in the specialized literature, related to AC, TPC and individual anthocyanins identified, and can be used in food and pharmaceutical industry (*functional foods*).

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