Cercetări Agronomice în Moldova
Vol. XLIV , No. 1 (145) / 2011

STUDY ON THE ANTHOCYANIN CONTENT OF SOME SOUR CHERRY VARIETIES GROWN IN IAŞI AREA, ROMANIA

R.V. FILIMON[*1], D. BECEANU[1] , M. NICULAUA[2], Cristina ARION[1],

[1]*University of Agricultural Sciences and Veterinary Medicine, Iaşi, Romania*
[2]*Oenological Research Center, Romanian Academy –Iaşi Branch, Romania*

Received October 19, 2010

ABSTRACT - The purpose of this study is to evaluate the anthocyanin (AC) and phenolic compounds (TPC) content, and the description of anthocyanin profile obtained by HPLC-DAD technique (High-Performance Liquid Chromatography - Diode Array Detector), from hydroalcoholic extracts of four varieties of sour cherry (*Prunus cerasus* L.) grown in experimental field of Research-Development Station for Fruit growing Iași, Miroslava area. Were also examined some physical-chemical properties of fruits, variety Mocăneşti 16 showing the highest moisture content (87.98%), titratable acidity (1.32 g malic/100g acid) and ascorbic acid (12 mg / 100g). AC, determined by pH differential method, had the highest value at Engleze timpurii variety (176.2 ± 0.97 mg/100g) and TPC, determined by the Folin-Ciocâlteu colorimetric method, had the maximum value at Mocăneşti 16 variety (446.89 ± 0.70 mg GAE/100g). Based on the chromatograms obtained, were identified four anthocyanins: cyanidin (cy)-3-glucoside, cy-3-rutinoside, cy-3-sophoroside, cy-3-glucosylrutinoside, expressed as a percentage of anthocyanins area. Anthocyanin profile obtained was not similar in varieties examined, and the ratio between anthocyanins differed from one variety to another. The data obtained confirm previous results on the sour cherries anthocyanin profile and can be used in food and pharmaceutical industry (functional foods) and as a basis of comparison for future studies.

Key words : Sour cherries, Anthocyanins, Phenols, HPLC-DAD.

REZUMAT – Studiu privind conținutul în antociani al unor soiuri de vișin, cultivate în zona Iași, România. Scopul acestui studiu este evaluarea conținutului în antociani (CA) şi compuşi fenolici (CFT), precum şi descrierea profilului antocianic, obținut prin tehnica HPLC–DAD (Cromatografie Lichidă de Înaltă Performanță – Diode de Detecție*),* din extractele hidroalcoolice a patru soiuri de vişin (*Prunus cerasus* L.), cultivate în câmpul experimental al Stațiunii de Cercetare-Dezvoltare pentru Pomicultură Iași, zona Miroslava. Au fost examinate şi o serie de proprietăți fizico-chimice ale fructelor, soiul Mocăneşti 16 prezentând cel

[*] E-mail: razvan_f80@yahoo.com

mai ridicat conținut în umiditate (87,98 %), aciditate titrabilă (1,32 g acid malic/100g) şi acid ascorbic (12 mg/100g). CA, determinat prin metoda diferenței de pH, a avut valoarea cea mai ridicată la soiul Engleze timpurii (176,2 ± 0,97 mg/100g), iar CFT, determinat prin metoda colorimetrică Folin-Ciocâlteu, a avut valoarea maximă la soiul Mocăneşti 16 (446,89 ± 0,70 mg GAE/100g). Pe baza cromatogramelor obținute au fost identificați patru antociani: cianidină (cy)-3-glucozid, cy-3-rutinozid, cy-3-soforozid, cy-3-glucozilrutinozid, exprimați în procente din aria corespunzătoare antocianilor, determinată pentru fiecare soi. Profilul antocianic obținut nu a fost similar la toate soiurile analizate, iar raportul dintre antociani a variat de la un soi la altul. Valorile obținute confirmă rezultatele anterioare, referitoare la profilul antocianic al vişinelor, şi vor putea fi utilizate în industria alimentară şi farmaceutică (alimente funcționale) şi ca bază de comparație pentru studii viitoare.

Cuvinte cheie : vişine, antociani, fenoli, HPLC-DAD.

INTRODUCTION

Sour cherries (Fr. *cerises acides*, Eng. *sour cherries, tart cherries*) are among the first fresh fruits of the year, being appreciated for their early appearance (the first varieties are mature in May) and the nutritional and therapeutic value (Beceanu, 2002). Sour cherries are consumed in a fresh state in a smaller proportion than by industrialization, an exception being the hybrid varieties between cherry and sour cherry, that are sweeter and have a pleasant acidity (e.g. Engleze timpurii) (Beceanu, 2011). Due to their important biologic properties, there is a special interest in the commercial production of foods rich in anthocyanins (*functional foods*). The fruit juices, mainly prepared from red fruits, are the most available sources of anthocyanins and polyphenols (Fanali *et al.*, 2011).

Anthocyanins represent one of the major groups of hydrosoluble pigments belonging to the flavonoid class, that are metabolism secondary products (Davies, 2004). The interest in anthocyanins has significantly increased in recent years, due to the health benefits brought by these to the human body (Blando *et al.*, 2004). On the basis of the clinical studies carried out, it has been suggested that anthocyanins have an anti-inflammatory and anticarcinogenic action, they prevent cardiovascular diseases, they may control obesity and diminish the action of diabetes, all these actions being associated more or less to their antioxidizing capacity (He and Giusti, 2010).

The specialized literature, offers data, varying within very large limits, related to the anthocyanin content of sour cherries, this varying between 27.8 and 210 mg/100 g (Mazza and Miniati, 1993; Antal *et al.*, 2003; Blando, 2004; Beceanu, 2011), and the phenolic compounds content of sour cherries, is considered to be among the highest in fruits, 429.5 mg GAE (Gallic acid equivalent)/100g of fresh product (Marinova *et al.,* 2005).

Very few studies have been registered in the international databases, focused on the sour cherry anthocyanins, by using the HPLC method, coupled with DAD or UV-

VIS detector (Esti *et al*., 2002). The first study on the sour cherry anthocyanins, dates from 1870, and it belongs to Rochleder (Mazza and Miniati, 1993). Later on, diverse authors (Dekazos, 1970; Mazza and Miniati, 1993; Blando *et al*., 2004). isolated and identified three main anthocyanins: cy-3-glucosylrutinoside, cy-3-sophoroside and cy-3 glucoside. The appearance of different forms of anthocyanins, as well as the ratio among them differs depending on the variety and numerous factors (structure and pigment concentration, pH, temperature, co-pigment presence, metal ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products, sulphur dioxide and other factors) (Mazza and Miniati, 1993; Whiting, 1995; Davies, 2004; Gould *et al*., 2009).

MATERIAL AND METHOD

The study aims at determining the anthocyanin content (AC) and the total quantity of phenolic compounds (TPC) from the ethanolic extracts obtained from the fruits of four sour cherry varieties (*Prunus cerasus* L.): Engleze timpurii, Crişana 2, Meteor Korai, Mocăneşti 16, cultivated in the experimental field of Research Development Station for Fruit Growing Iaşi, Miroslava area, Iaşi county, Romania. The harvesting of varieties was made by varieties, at their maturity for consumption, when the fruits have developed the varietal characteristic color and optimal gustatory features (Grădinariu *et al*., 1998), in the interval 12.06.2010 – 22.06.2010.

We determined certain physical-chemical properties of fruits: the average mass of a fruit, relative density (the picnometric method), the moisture content (drying off in a drying chamber, for 4 hours at 105°C), the soluble dry substance (the refractometric method), titrating acidity (titrating with NaOH, 0.1 n), ascorbic acid content (titrating with 2.6 DI, STAS 5950-85), pH (the potentiometric method), reducing sugars (Schoorl method), activity of catalase (the gasometrical method) and peroxidase (the guaiacol method), as factors influencing the anthocyanin content.

After having made the physical-chemical determinations, registered on the harvest date, the fruits were kept at the temperature of -18±2°C. Anthocyanins transformation is considered as minimal for the sour cherries kept in a frozen state (Mazza and Miniati, 1993). After three days of keeping, we carried out the hydroalcoholic extracts at the laboratory of Oenological Research Center, Romanian Academy – Iaşi Branch, Romania. The vegetal material was prepared, weighed (5 g of sample), ground with extraction solution (100 mL) and stored overnight, in dark colour containers, with a cap screw, at room temperature (22±2°C). The higher temperature (with a maximum at 30-35°C) favours the anthocyanins extraction, by increasing their solubility and the diffusion coefficient (Cacace and Mazza, 2003).

The separation of the solid fraction was made by filtering the samples through qualitative filter paper. The second and third extraction stage, were carried out at the same time interval, by resumtion of the solid part with extraction solution, at the same ratio (1:20 g/mL), until depletion of the vegetal material. The extraction yield of the phenolic

compounds may be augmented by an increase of the ratio between solvent and the solid material (Cacace and Mazza, 2003). Before the third filtering, we applied an ultrasound treatment to the containers containing the sample analyzed. The ultrasound device used was the Digital Ultrasonic Cleaner ProsKit SS-802, and the ultrasound time was 480 seconds, for each sample. The use of ultrasounds, as a means of intensification of the processes for property transfer (mass, heat) and desorption, has been signaled in the specialized literature ever since 1970's. They demonstrated that ultrasounds may dislocate the organic matter adsorbed on a certain area of sediments, and by applying ultrasounds, aggregation and agglomeration of particles is reduced (Isopescu, 2007).

The extraction was carried out with ethanol-HCl-water (96:1:3) system, and there resulted a pH=1.5 ± 0.1, a value where the chemical composition of anthocyanins is considered stable. Acids are very important in maintaining the stability of anthocyanins, and they are necessary in the formation of flavilium cation (at pH 1.5-2) and, also, to improve the extraction efficiency (Socaciu, 2008).

Since we need to obtain extracts for alimentary use, that must not contain toxic reagents (methanol, diethilic ether, acetone) (Baerle and Guțanu, 2003), we preferred the extraction system with ethanol, although recoveries are not as important as those obtained by methanol extraction (methanol is by 20 % more efficient than ethanol and by 73% than water) (Socaciu, 2008).

The three extraction fractions were cumulated, and the containers were stored at low temperatures and in the dark (6±1°C), to avoid the hydrolysis of anthocyanins, that occurs in a weakly acid and warm environment, when anthocyanidines are formed and the sugar part is released (Cercasov *et al.*, 2005).

To obtain the value of the total content in phenolic compounds (TPC), we used the colorimetric method Folin-Ciocâlteu (FCI). The phenolic compounds contained in the extract are oxidized by the Folin-Ciocâlteu reagent. This reagent is made of a mixture of phosphowolframic acid ($H_3PW_{12}O_{40}$) and phosphomolibdic acid ($H_3PMo_{12}O_{40}$) which, after the oxidization of phenols, is reduced to a mixture of blue tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) oxides. The blue coloring occurred has a maximum absorption in the area 750-765 nm and it is proportional to the total quantity of phenolic compounds initially present. Determinations were made using a UV-VIS Analytik Jena Specord 200 spectrometer, as follows: we measured the absorbance λ=765 nm, in a quartz tank of 1 cm, as compared to the distilled water. This method is reliable, reproducible and it may be used for any type of alcoholic extract. To express the content of phenolic compounds in Gallic acid equivalent (GAE), we drew a calibration curve, with the linear equation: $y = 1.3238 \cdot x - 0.0014$.

The pH differential method and HPLC technique are the most used methods for monomeric anthocyanins analysis (Lee *et al.*, 2008). Chromatography is the most recommended technique for the separation of complex mixtures (Mondello *et al.*, 2002), so HPLC becomes an irreplaceable instrument in the identification and quantification of different individual anthocyanins in a sample (Davis, 2004; Lee *et al.,* 2008). In an acid environment, there is a balance between the coloured and the colorless forms of anthocyanins. This balance depends on the pH. We chose pH = 0.6 and pH = 3.5 and we measured

absorbance (optic density) for λ = 520 and 700 nm, both for the sample under analysis and the blank test, using a glass tank with 1 cm optic line, as compared to the distilled water. The variation of the coloring intensity between those two pH values is proportional to the anthocyanin content. By this variation, the phenolic function is not affected and they admit that other phenolic compounds (tannins) do not interfere with the determination. The calculation formula was: $A = (A_{max} - A_{700})_{pH\ 0.68} - (A_{max} - A_{700})_{pH\ 3.5}$.

Amax, represents absorbance for the maximum wave length (e.g. 520 nm), and the value A700, is subtracted due to the presence of other phenolic compounds (Horbowicz *et al.,* 2008). To express the results in mg/100 g, we used a calibrating curve, with the linear equation: y (mg/L) = 386.15 · A - 1.3713.

We made up an anthocyanin profile, by using the HPLC-DAD technique, and we identified the main representatives of this class of compounds in the analysed varieties, and also the values of the ratios between the main anthocyanins, in area proportions (% aria), evaluated on the basis of chromatograms obtained for every variety. By means of a liquid chromatograph Shimadzu LC 20 type, using a column Hypersil ODS C18 type (with particle diameter of 5 μm and 25 cm long), we may carry out, the separation of the mixture obtained by extraction at a temperature of 25°C. Elution was carried out by a concentration gradient method, for a debit of 1.2 mL/min. As eluent A, we used water:formic acid:acetonitrile 87:10:3, and as eluent B, water:formic acid:acetonitrile 40:10:50, with increasing gradient of eluent B, from 6 % up to 60 %. Both solutions, and the samples subject to analysis, were filtered by tangential filters of 0.45 μm. The anthocyanin compounds were individualized by means of a Shimadzu DAD device, at the wave length 518 nm. The reagents, necessary for the extraction and determination of the analyzed compounds were purchased from Chimopar, Bucharest and Merck Romania, Bucharest.

RESULTS AND DISCUSSION

Sour cherries are non-climacteric highly perishable fruits (Beceanu, 2007).

The physical-chemical properties of the sour cherry varieties under study, are presented in *Table 1*, values being different from one variety to another. The Crişana 2 variety had the fruit with the highest average mass (6.2 g), and Mocăneşti 16 was the variety with the highest moisture content (87.98%), titrating acidity (1.32 g malic acid /100g) and ascorbic acid (12 mg/100g). Meteor Korai variety registered the highest content of reducing sugars (7.81 mg glucose/100 g), and the content in soluble dry substance (°Bx) was maximum for Engleze timpurii variety (16.5°Bx), this being a variety obtained by inter-specific hybridization, between cherry and sour cherry.

The activity of catalase was best highlighted for Mocăneşti 16 breed (1.8 cm^3 O_2/g/h), and the peroxidase activity, as a factor influencing the anthocyanin content of fruits, was considered as reduced, underlined by an extremely poor coloration of guaiacol and hydrogen peroxide solution.

Table 1 – Physical-chemical characteristics of the sour cherry varieties under study

Variety	M. fr. (g)	Rel. d. (g/cm^3)	M. (%)	T. ac. (g m.a.)	As. ac. (mg%)	Rd. sg. (gs. %)	pH	SDS (°Bx)	Cat. act. $cm^3O_2/g/h$
Engleze timpurii	4.9	1.0716	81.81	0.73	10.3	5.70	**3.6**	**16.5**	0.7
Meteor Korai	5.3	1.0527	85.87	0.80	11.1	**7.81**	3.3	15.1	1.6
Mocăneşti 16	4.8	1.0558	**87.98**	**1.32**	**12.0**	7.36	3.2	10.9	**1.8**
Crişana 2	**6.2**	1.0592	84.89	1.28	11.7	6.78	3.2	14.7	1.4

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

Following the interpretation of the absoption specters obtained for the wavelength specific to every analyzed compound and application of the calculation formulas, we obtained the results for the anthocyanin quantity (AC) and the total content in phenolic compounds (TPC), calculated by means of Folin-Ciocâlteu index (FCI). The data obtained represent the average of three determinations and they have calculated the standard deviation (*Table 2*).

Table 2 - AC and TPC values for the analysed samples (in increasing order of AC)

Variety	AC (mg/100g)	TPC (mg GAE/100g)
Mocăneşti 16	107.46±0.29	**446.89±0.70**
Crişana 2	117.12±0.91	370.18±0.19
Meteor Korai	117.50±0.99	321.43±0.14
Engleze timpurii	**176.20±0.97**	325.18±0.14

The highest anthocyanin content was registered at Engleze timpurii variety (176.2±0.97 mg/100g), followed by Meteor Korai and Crişana 2 varieties that had very close values 117.50±0.99 mg/100g and, 117.12±0.91 mg/100g, respectively. The phenolic compounds were identified in larger quantities in Mocăneşti 16 variety (446.89±0.70 mg GAE/100g), followed by Crişana 2 variety (370.18±0.19 mg GAE/100g).

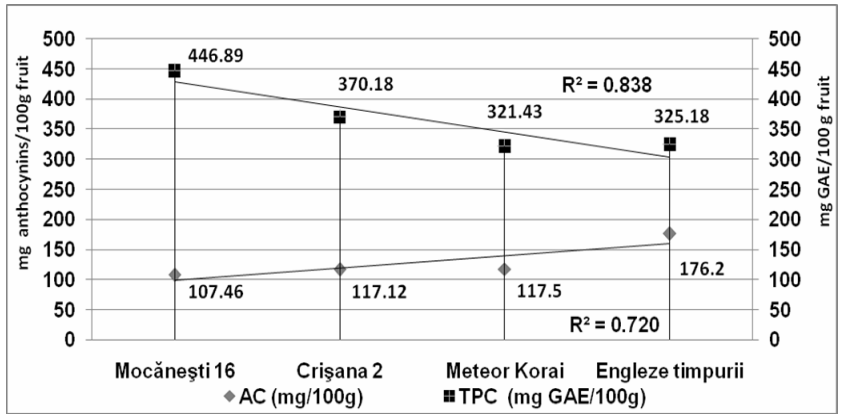

Figure 1 – Correlation of AC and TPC values

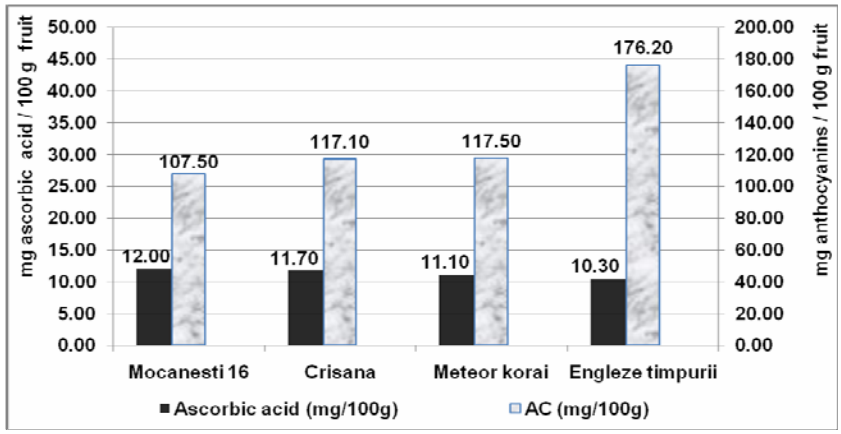

Figure 2 – Graphic representation of AC values and the quantity of ascorbic acid (mg/100g)

The increase of the phenolic substance content in extract does not correspond to the increase of the quantity of anthocyanins colorimetrically active (Baerle and Guțanu, 2003), more precisely the ratio between AC and TPC is specific to each variety *(Fig. 1),* and there is no correlation between these parameters. Mocăneşti 16 variety, though having the highest TPC value from all varieties under study (446.89±0.70 mg GAE/100g), it has the lowest AC value (107.46±0.29 mg/100g).

In *Fig. 2* we present the correlation between AC and the quantity of ascorbic acid, of fruits under study. A reverse correlation was noticed between AC and the ascorbic acid content, thus, the sour cherry varieties having a low content of ascorbic acid, registered higher

anthocyanin content, than the varieties richer in vitamin C. The mechanism of ascorbic acid oxidization produces peroxide, a compound known as an anthocyanin inhibitor (Delgado-Vargas and Paredes-López, 2003). The oxygen and hydrogen peroxide may easily oxidize anthocyanins, and most of the times, this process is accelerated by the presence of ascorbic acid (Horbowicz *et al.,* 2008).

We could not establish a correlation between AC and the average mass of the fruit, the ratio between these terms being characteristic to each variety. Engleze timpurii variety, though having a medium size fruit (4.9 g), it had the highest value of AC.

The chromatograms obtained by HPLC technique coupled with DAD are given in *Fig. 3*, for every variety. Following their interpretation, we identified four anthocyanins: cyanidin (cy)-3-sophoroside, cy-3-glucosylrutinoside, cy-3-glucoside and cy-3-rutinoside, compounds confirmed by the specialized literature, too. Their expression was made in area percentages, the ratio between the anthocyanins indentified being characteristic to each variety *(Fig. 4)*.

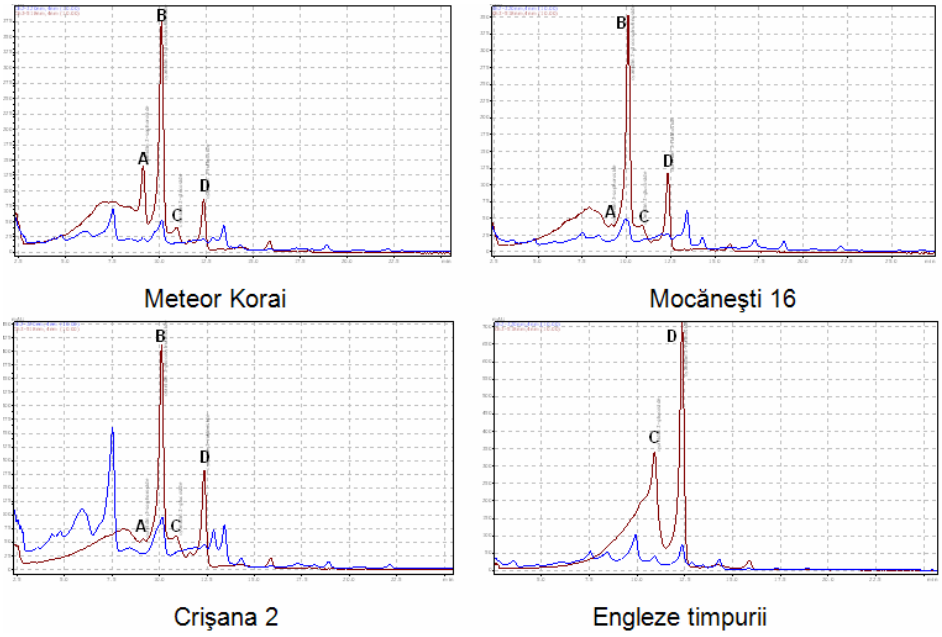

Figure 3 – Chromatograms obtained by HPLC-DAD for the four sour cherry varieties
A – cy-3-sophoroside; B – cy-3-glucosylrutinoside; C – cy-3-glucoside; D – cy-3-rutinoside

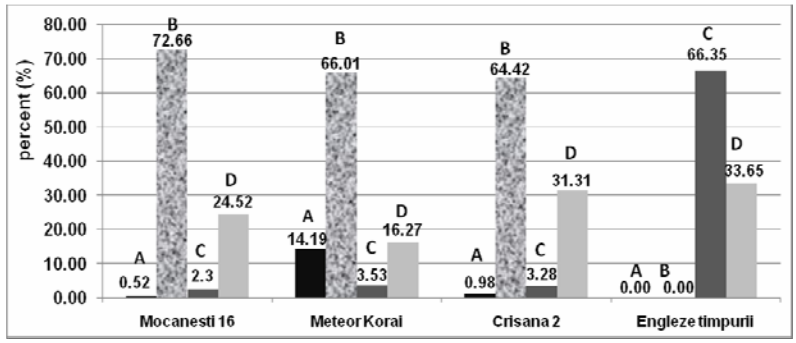

Figure 4 – Area percentages corresponding to the four anthocyanins identified
A – cy-3-sophoroside; B – cy-3-glucosylrutinoside; C – cy-3-glucoside; D – cy-3-rutinoside

Cy-3-glucosylrutinoside was the main anthocyanin in the fruits of three studied varieties, registering the highest percentage in Mocăneşti 16 variety (72.66 %), followed by cy-3-rutinoside, who appeared in all varieties, and had the highest share in Engleze timpurii variety (33.65 %). Mention must be made of the fact that anthocyanins cy-3-sophoroside and cy-3-glucosylrutinoside could not be identified in Engleze timpurii variety, this having the main anthocyanin cy-3-glucoside (66.35 %). This aspect was attributed to the fact that Engleze timpurii is a variety coming from the hybridization between cherry and sour cherry, cy-3-glucoside being one of the main anthocyanins in cherries (Mazza and Miniati, 1993; Mozetič and Trebše, 2004).

CONCLUSIONS

The four sour cherry varieties studied registered a series of physico-chemical characteristics that differ from one variety to another. The total content of anthocyanins had the highest value in Engleze timpurii variety (176.2±0.97 mg/100g), and the lowest in Mocăneşti 16 variety (107.46±0.29 mg/100g). As for the content of phenolic compounds, this registered the maximum value in Mocăneşti 16 variety (446.89±0.70 mg GAE/100g), and the minimum value in Meteor Korai variety (321.43±0.14mg GAE/100g).

We could not establish a correlation between AC and TPC, Mocăneşti 16 variety, though having the highest value of TPC (446.89±0.70 mg GAE/100g) among the varieties under study, it had the lowest AC value (107.46±0.29 mg/100g). We identified an inverse ratio between the ascorbic acid content and AC, thus the sour cherry varieties having a higher content of vitamin C (Mocăneşti 16, Crişana 2), had a lower anthocyanin content. In these varieties the activity of enzymes was more important, at Mocăneşti 16 variety was registered the highest intensity of catalase activity, namely 1.8 $cm^3O_2/g/h$.

Through the analysis of the chromatograms obtained by HPLC technique coupled with DAD, we identified four anthocyanins: cy-3-sophoroside, cy-3-glucosylrutinoside, cy-3-glucoside and cy-3-rutinoside, expressed in area percentages, their ratio being specific to each variety. Cy-3-glucosylrutinoside, was the main anthocyanin in the fruits of three varieties studied (over 64 %), only in Engleze timpurii variety the main anthocyanin was cy-3-glucoside (66.35%).

The data obtained, fall into the values given by the specialized literature regarding the physico-chemical properties, the total content of anthocyanins and phenolic compounds and the anthocyanins profile of sour cherries, and they will be used in the food and pharmaceutical industry (functional foods) and as a comparison basis for the future studies.

REFERENCES

Antal Simona-Diana, Gabriela Garban, Z. Garban, 2003 – *The anthocyans: biologically-active substances of food and pharmaceutic interest*. The Annals of the University Dunărea de Jos of Galați - Food Technology. Fascicle VI, p. 106-115.

Baerle A., V. Guțanu, 2003 – *Optimizarea extracției antocianilor din fructe de scoruşe negre (Optimization of anthocyanins extraction from black mountain ash fruits).* Analele Ştiințifice ale U.S.M. Seria "Ştiințe chimicobiologice", Chişinău, p. 346-350.

Beceanu D, **2007** – *European criteria to apreciate the cherries qualities*. Revista Cercetări Agronomice în Moldova vol. 1 (129), Iaşi, p. 39-45.

Beceanu D., 2011 – *Tehnologia produselor horticole (Horticultural products technology), vol. 2.* Editura PIM, Iaşi.

Beceanu D., A.Chira, I. Paşca, **2002** – *Fructe, legume şi flori* (*Fruits, vegetables and flowers*). Edit. MAST, Bucureşti.

Blando Federica, Carmela Gerardi, Isabella Nicoletti, 2004 - *Sour cherry (Prunus cerasus L) anthocyanins as ingredients for functional foods*. J Biomed Biotechnol, vol. 5, p. 253–258.

Cacace J. E., G. Mazza, 2003 – *Optimization of extraction of anthocyanins from black currants with aqueous ethanol.* Journal of Food Science, Vol. 68, Nr. 1, p. 240-248.

Cercasov Cornelia, Valentina-Claudia, 2005 – *Compuşi naturali cu acțiune terapeutică (Natural compounds with therapeutic action)*. Edit. Universității din Bucureşti.

Davies K., **2004** – *Plant pigments and their manipulation*, *Annual plant reviews*, Volume 14, CRC Press, Boca Raton, Florida, USA.

Dekazos E. D., **1970** – *Anthocyanin pigments in red tart cherries*. J. Food Sci., vol 35, p. 237.

Delgado-Vargas F., O. Paredes-López, 2003 - *Natural colorants for food and nutraceutical uses.* CRC Press, Taylor & Francis Group, BoAC Raton, USA.

Esti M., L. Cinquanta, F. **Sinesio**, **E. Moneta**, **M. Di Matteo, 2002** - *Physicochemical and sensory fruit characteristics of two sweet cherry cultivars after cool storage*. Food Chemistry, Vol. 76, Nr. 4, Elsevier, UK. p. 399–405.

Fanali Chiara, Laura Dugo, G. D'Orazio, Melania Lirangi, Marina Dachà, Paola Dugo, L. Mondello, 2011 – *Analysis of anthocyanins in commercial fruit juices by using nano-liquid chromatography*

electrospray - mass spectrometry and high performance liquid chromatography with UV-vis detector. J. Sep. Sci., vol. 34. Wiley, Weinheim. p. 150–159.

Gould K., K. Davies, C. Winefield C., **2009** – *Anthocyanins - Biosynthesis, Functions and Applications*. Springer Science & Business Media, LLC, New York, USA.

Grădinariu G., M. Istrate, M. Dascălu, 1998 – *Pomicultură (Fruit growing)*. Edit. Moldova, Iaşi.

He J., Monica Giusti, **2010** - *Anthocyanins: natural colorants with health-promoting properties*. Annual review of food science and technology. Vol. 1, p. 163-187.

Horbowicz M., R. Kosson, Anna Grzesiuk, H. Dębski, 2008 – *Anthocyanins of fruits and vegetables - their occurrence, analysis and role in human nutrition*. Vegetable crops research bulletin, vol. 68, p. 5-22.

Isopescu Raluca, 2007 - *Modificarea distribuției dimensiunilor particulelor de carbonat de calciu precipitat, prin utilizarea câmpului ultrasonic (Change of particles size distribution of precipitated calcium carbonate, using ultrasonic field).* Rev. Chim. Bucureşti, vol. 58, Nr. 2, p. 246-250.

Lee J., Ch. Rennaker, R. E. Wrolstad, 2008 - *Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods*. Food Chemistry, vol. 110. Edit. Elsevier, p. 782–786.

Marinova D., F. Ribarova, M. Atanassova, 2005 – *Total phenolics and total flavonoids in bulgarian fruits and vegetables*. Journal of the University of Chemical Technology and Metallurgy, 40, 3, p. 255-260.

Mazza G., E. Miniati, 1993 – *Anthocyanins in fruits, vegetables, and grains*. Edit. CRC Press, Boca Raton, USA.

Mondello L., A. Lewis, K. Bartle, 2002 - *Multidimensional Chromatography.* John Wiley & Sons, Ltd., Baffins Lane, Chichester, West Sussex, UK.

Mozetič Branka, **Polonca Trebše, 2004** - *Identification of sweet cherry anthocyanins and hydroxycinnamic acids using HPLC coupled with DAD and MS detector Acta Chim. Slov.*, vol. *51*, p. 151-158.

Socaciu Carmen, 2008 – Food colorants, chemical and functional properties*, CRC Press, Boca Raton, USA.*

Whiting P.W., **1959** – *The anthocyanin pigments of plants*. Ed. J. M. Dent and sons Ltd., Toronto.