ASSESSMENT ON THE POLYPHENOLIC BIOACTIVE POTENTIAL IN FRUITS OF SOME BLACKCURRANT (*RIBES NIGRUM L.*) VARIETIES GROWN IN IASSY AREA, NE OF ROMANIA

EVALUAREA POTENȚIALULUI BIOACTIV POLIFENOLIC AL FRUCTELOR UNOR SOIURI DE COACĂZ NEGRU (*RIBES NIGRUM* L.) CULTIVATE ÎN AREALUL MUNICIPIULUI IAŞI, NE ROMÂNIEI

FILIMON V. R.[1], NICULAUA M.[2], COȚOVANU Roxana[1], ARION Cristina[1], BĂETU Alina[1]
e-mail: razvan_f80@yahoo.com

Abstract. *In recent years a large number of epidemiological studies have suggested remarkable benefits to human health by an high intake of fresh or processed (juice, jam etc.) blackcurrants. Fruits of Ribes nigrum L. are a important source of biologically active compounds, mostly belonging to the class of polyphenols, especially anthocyanins, which give the red-violet color of the fruits epicarp. The purpose of the study is the determination the total amount of anthocyanins and polyphenols, identification of the main anthocyanins (HPLC-DAD) and the participation percentage of each representative to the anthocyanin profile of ethanolic extracts obtained from fruits of two blackcurrant varieties, grown in the NE area of Romania. Measurements have revealed an important anthocyanin content in both analyzed varieties (319.97±1.89 mg cy-3-gl/100 g fruit, at Ronix variety and 286.41±1.19 mg cy-3-gl/100 g fruit, at Abanos variety), being identified four major anthocyanins, delphinidin and cyanidin with their glycosidic forms, glucoside and rutinoside.*
Key words: blackcurrant, anthocyanin, polyphenolic, Iassy area, HPLC

Rezumat. *În ultimii ani un număr mare de studii epidemiologice au sugerat beneficii remarcabile aduse sănătății umane printr-un consum ridicat de coacăze negre în stare proaspătă sau prelucrate (sucuri, gem etc). Fructele speciei Ribes nigrum L. reprezintă o sursă importantă de compuşi biologic activi, aparținând clasei polifenolilor, în special antociani, ce conferă culoarea roşie-violet a fructelor. Scopul acestui studiu este determinarea cantităților totale de antociani şi polifenoli, identificarea principalilor compuşi antocianici (HPLC-DAD) şi a procentului de participare a fiecărui reprezentant la profilul antocianic al extractelor etanolice obținute din fructele a două soiuri de coacăz negru, cultivate în zona de NE a României. Determinările au relevat un conținut important în antociani a celor două soiuri analizate (319,97±1,89 mg cy-3-gl/100 g fruct la soiul Ronix şi 286,41±1,19 mg cy-3-gl/100 g fruct la soiul Abanos), fiind identificați patru antociani majoritari, reprezentați de delfinidină şi cianidină cu formele lor glicozidice, glucozid şi rutinozid.*
Cuvinte cheie: coacăze negre, antociani, polifenoli, arealul Iaşi, HPLC

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

INTRODUCTION

Early european traditional medicine mentions the use of black currant fruit to treat arthritis, gout, inflammation, stomach pains, diseases of the kidneys, as a diuretic and remedy for fatigue (Matsumoto et al., 2005). Although black currants contain a large number of nutrients, including fiber, minerals, essential fatty acids and vitamins (ascorbic acid), another class of bioactive compounds is considered the main responsible for numerous health benefits suggested by the studies conducted in the last decade, including the prevention of cancers, of some cardiovascular and inflammatory diseases,vision disorders, and this is the class of phenolic compounds (Bishayeea et al., 2010).

Anthocyanins are the largest group of water soluble vegetable pigments, belonging to the flavonoid class, chemicallybeing derivatives of flavylium salt (2-phenylbenzopyrilium)(Ghosh and Konishi, 2007).

The pigment extracted from black currant juice, administered to human subjects, favored rapid visual adaptation to darkness (Davies , 2004). Delphinidin and cyanidin, two of the main aglycones of black currants, have been identified as the most effective inhibitor of low density lipoprotein oxidation (Cacace and Mazza, 2003), indicating the important functional value of *Ribes nigrum L.* anthocyanins.In black currants, anthocyanins content identified by various authors fall within the range of 80-810 mg/100 g fruits, anthocyanin profile being composed from four main anthocyanins, glycosides of delphinidin and cyanidin (Mazza and Miniati, 1993, Slimestad and Solheim, 2002, Gould et al., 2009).

MATERIAL AND METHOD

Anthocyanin content (ACY)and total phenolic compounds (CFT) of ethanolic extracts obtained from fruits of two varieties of black currant (*Ribes nigrum L.*), Abanos and Ronix, created in 1999 and 2000 respectively, by the Research Institute for Fruit Growing Piteşti-Mărăcineni, was measured. Fruits were harvested from the experimental field of the University of Agronomical Sciences and Veterinary Medicine (UASVM) Iassy, V. Adamachi Farm,at fruits maturation, when the last berry from the clusterturned its color to purple, approx. 60 days after flowering phenophase (Radu and Boboescu, 2007), on 26.06.2010 for Abanos and 01.07.2010 for Ronix variety.

Were determined several physico-chemical properties of the fruits: the average mass of a berry, moisture content, soluble solids (°Bx), titratable acidity, ascorbic acid, juice pH, reducing sugars (Schoorl method).Fruits were stored at -18±2°C until extraction (5 days), anthocyanin transformation being minimal for fruits kept in a frozen state (Mazza and Miniati, 1993).

A quantity of 5 g skin was treated initially with 50 mL extraction solution, C_2H_5OH-HCl-H_2O (96:1:3) and kept at room temperature (18±2°C) overnight. Subsequently two more washes of plant material were made with 30 mL and 20 mL of solvent, resulting a final report between plant material and extract of 1:20. Before the third filtering was applied an ultrasound treatment on the sample containers, as a means of increasing the property transfer process and desorption. The three fractions were cumulated and stored extraction at 4±1°C, in the dark.

Determination of total monomeric anthocyanins content (ACY) was performed by the pH differential method:

$$A \text{ (absorbance)} = (A_{520\ nm} - A_{700\ nm})\ pH\ 0.68 - (A_{520\ nm} - A_{700\ nm})\ pH\ 3{,}56$$

Results were expressed as mg equivalent cyanidin-3-glucoside /100 g fruit:

$$ACY\ (mg\ cy\text{-}3\text{-}gl, mg/L) = (A \times MW \times DF \times 10^3) / (\varepsilon \times l);$$

where: ACY - total anthocyanin content (cyanidin-3-glucoside equivalents(cy-3-gl), mg/L), A - absorbance, MW - molecular weight (449.2 g/mol forcy-3- gl); DF - dilution factor; l -optical pathlength in cm (1 cm); Ɛ- molar extinction coefficient for cy-3-gl(26 900L×mol^{-1}× cm^{-1}); 10^3- factor for conversion from g to mg(J. AOAC Int., 2005).

To obtain the total phenolic compounds content (TPC) was used Folin-Ciocalteu photocolorimetric method (FC), by measuring absorbance at λmax (765 nm) (Singleton and Rossi, 1965), results were expressed in grams of gallic acid equivalent (g GAE)/100g fresh weight (FW). Measurements were made using a UV-VIS spectrophotometer, Analytik Jena Specord 200,the results represent the average of three determinations, having calculated standard deviation (±) and coefficient of variability of data, statistical analysis beeing performed with application ANOVA: Single factor from Microsoft Excel, Data Analysis Tools.

Using a Shimadzu LC 20 liquid chromatograph, with a Hypersil ODS C18 separation column (25 cm length) at 20 °C, was performed the separation of anthocyanins. Elution was achieved at a flow rate of 1.2 mL/min. As eluent A was used H_2O:HCOOH:CH_3CN (87:10:3) and as eluent B, H_2O:HCOOH:CH_3CN (40:10:50), increasing eluent B from 6% to 60%. Anthocyanin compounds were individualized with a diode array detector (DAD) Shimadzu at λ 518 nm, and their identification was made according to Slimestad et al., 2002 and Oszmiański et al., 2009.

RESULTS AND DISCUSSIONS

Immediately after harvest, were determined several physical and chemical properties of fresh fruits, presented in table 1.

Table 1

The physico-chemical properties of black currant fruits

Variety	M. fr.* (g)	M.* (%)	T. ac.* (g m. a.)	Asc.ac.* (mg %)	Rd. sg.* (gl. %)	pH (units)	SDS* (°Bx)	TDS* (%)
Abanos	**1.24±0.05**	**81.01±1.04**	**2.68±0.01**	122.18±1.16	8.92±1.02	3.08±0.24	13.91±0.19	18.99±1.04
Ronix	1.10±0.07	80.11±0.98	2.62±0.09	**157.12±1.56**	**10.52±1.04**	**3.10±0.32**	**14.92±1.02**	**19.89±0.98**

Legend:*M. fr. - average mass of fruit; M (%) - moisture; T. ac. (g m.a.) - titratable acidity (g malicacid/100g); Asc. ac. (mg %) - ascorbic acid (mg/100 g); Rd. sg. (gl. %) - reducing sugars (mg glucose/100 g fruits); SDS (°Bx) - soluble dry substance (°Brix); TDS (%) - total dry substance (%);CV<10; *p-value*>0,05.

VarietyAbanos presented a content in moisture and titratable acidity higher than Ronix variety, containing a more significant quantity of ascorbic acid (157.12 mg/100 g), however values are lower than those presented in literature for this species (160-200 mg ascorbic acid/100 g).

After extraction of numerical data, obtained by analyzing the absorption spectra (fig. 1) were determined the total quantities of monomeric anthocyanins (ACY). ACY values at Ronix variety (table 2), increased up to a maximum of 319.97±1.89 mg g cy-3-O-gl equivalent/100g FW, compared to the variety Abanos, with only 286.41±1.19 mg cy-3-gl/100 g FW. Anthocyanins being the main phenolic compounds that give colorto extract, differences between quantities

determined from the two species can be also observed through visual examination of extracts color (fig. 2).

Table 2

ACY and TPC content of black currant fruit extracts

Variety	ACY (mg cy-3-gl/100 g)	TPC (g GAE/100g)	ACYofTPC (%)
Abanos	286.41±1.19	1.99±1.31	**33.49**
Ronix	**319.97±1.89**	**2.24±1.05**	33.31

CV<10; *p-value*>0.05

TPC content was, the same like for ACY, higher at Ronix variety (2.24 g GAE/100g FW), the percentage of anthocyanins from total phenolic compounds exceeding 33% in both varieties.

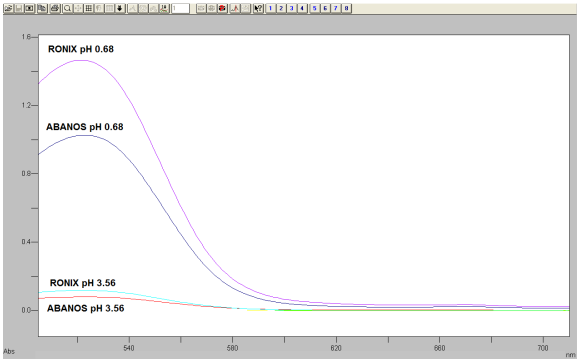


Fig. 1- Overlay of absorbance spectra at λ 520 nm, pH 0.68 and 3.56

Fig. 2-Color of ethanolic extracts obtained from black currant fruits

It is well known that the anthocyanin decomposition is accelerated by the presence of ascorbic acid.Direct condensation between anthocyanins and ascorbic acid has been postulated as a mechanism for anthocyanin degradation (Marti et al., 2002). It may be noted that Ronix variety, containing higher amount of ascorbic acid, had the higher quantity of anthocyanins and phenolic compounds.

Based on the chromatograms obtained (fig. 3) were identified two main anthocyanidines, cyanidin and delphinidin, with the glycosidic forms: 3-O-glucoside and 3-O-rutinoside (fig. 5).The main anthocyanin present in the analyzed extracts,conform to peak area, was the delphinidin-3-O-rutinoside (dp-3-rut), with a participation percentage of 69.55% from the total anthocyanins area, at the Abanos variety and 49.63%, for Ronix variety. At the opposite pole, was identified cyanidin-3-O-glucoside (cy-3-gl), with a participation rate of 2.73% and 0.80%, at Abanos and respectively Ronix variety. Anthocyanin cyanidin-3-O-rutinoside (cy-3-rut), had a different behavior in this case, thus at the variety Abanos held a percentage of 19.02% of total area, while at the variety Ronix, the percentage was more than double41.06%. This reflects in the color extracts, cyanidin (E163 a), according to *Codex Alimentarius*, gives deep red color to

solvent in which is dissolved, while delphinidin (E163 b) shows shades of blue, which can be observed also by visual examination of extracts color (fig. 2).

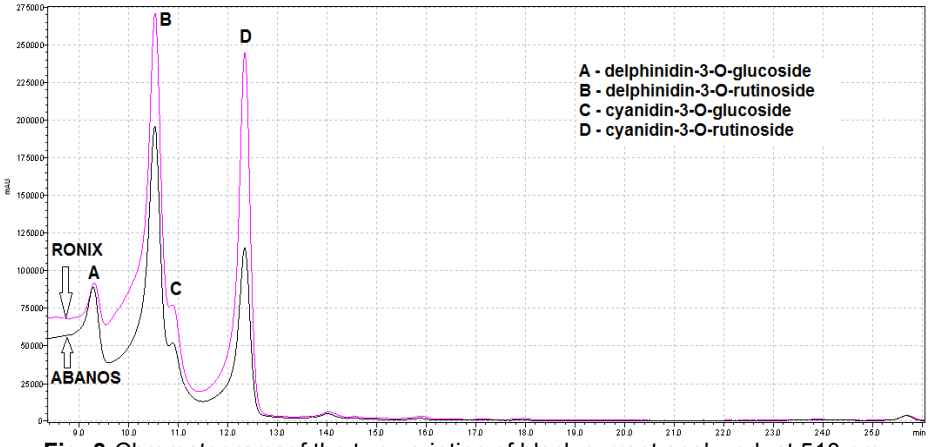
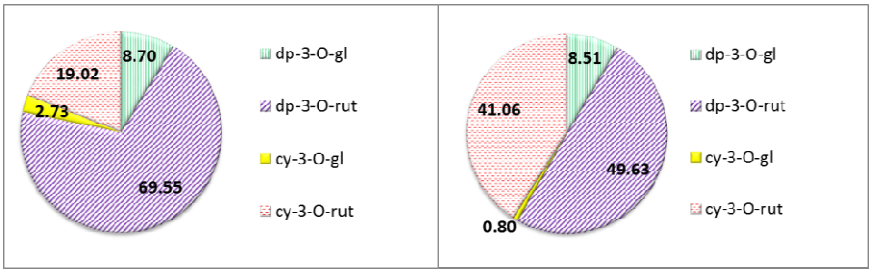


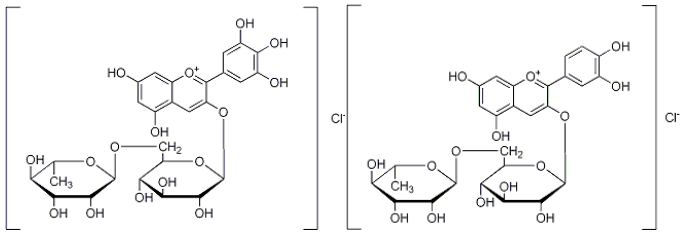
Fig. 3-Chromatogramsof the two varieties of black currant analyzed, at 510 nm



Abanos variety *Ronix variety*

Fig. 4 -Area percentages (%) corresponding to the identified anthocyanins

Dp-3-glwas the most constant anthocyanin in the experiment, showing very similar values in the profileof both varieties, 8.70% at Abanos variety and 8.51% at Ronix variety (fig.4).



delphinidin-3-O-rutinoside cyanidin-3-O-rutinoside

Fig. 5-Chemical structure of main anthocyanins identified in black currants

CONCLUSIONS

1. Ascorbic acid content was determined to be lower than values offered by the literature for *Ribes nigrum L*. fruits, in both varieties analyzed.Variety with a higher content of ascorbic acid, also had the highest amount of anthocyanins.

2. Anthocyanin profile of this species contains four main anthocyanins, confirmed by chromatographic analysis, glucoside and rutinoside forms of delphinidin and cyanidin, with dp-3-rut the most important anthocyanin.

3. Black currants, grown in the NE of Romania, belong to the group of horticultural products with an important contentof phenolic compounds, in particularly anthocyanins, which confirms the huge bioactive potential of fruits, being recommended for the use in food, pharmaceutical and cosmetic industries.

REFERENCES

1. **Bishayeea A., Mbimbaa T., Thoppila R.J., Háznagy-Radnaib E., Siposc P., Darvesha A.S., 2010** - *Anthocyanin-rich black currant (Ribes nigrum L.) extract affords chemoprevention against diethylnitrosamine-induced hepatocellular carcinogenesis in rats.* J. of Nutritional Biochemistry. Available online at www.sciencedirect.com, accessedin february 2012.
2. **Cacace J.E., Mazza G., 2003**- *Optimization of extraction of anthocyanins from black currants with aqueous ethanol.* Journal of Food Science, Vol. 68, nr. 1, p. 240-248.
3. **Davies K., 2004**-*Plant pigments and their manipulation.* Annual plant reviews.Vol. 14, C.R.C. Press, Boca Raton, Florida, USA.
4. **Ghosh D., Konishi T., 2007** - *Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function.* Asia Pac. J. Clin. Nutr., vol. 16 (2), p. 200-208.
5. **Gould K., Davies K., Winefield C., 2009** - *Anthocyanins - Biosynthesis, Functions and Applications*. Springer Science & Business Media, LLC, New York, USA.
6. **Kebler T., Jansen B., Hesse A., 2002** - *Effect of blackcurrant, cranberry and plum juice consumption on risk factors associated with kidney stone formation*. European J. of Clinical Nutrition, vol. 56, p. 1020-1023.
7. **Matsumoto H.,Takenami E., Iwasaki-Kurashige K., Osado T., Katsumura T., Hamaoka T., 2005** - *Effects of blackcurrant anthocyanin intake on peripheral muscle circulation during typing work*. Eu. J. App. Physiology, vol. 94, p. 36-45.
8. **Marti N., Perez A., Garcia C., 2002** - *Influence of storage temperature and ascorbic acid addition on pomegranate juice.* J. Sci. Food Agric.,vol. 82, p. 217-221.
9. **Mazza G., Miniati, E., 1993** - *Anthocyanins in Fruits, Vegetables, and Grains*. Edit.CRC Press, Boca Raton, USA.
10. **Oszmiański J., Wojdyło Aneta, 2009** - *Effects of Blackcurrant and Apple Mash Blending on the Phenolics Contents, Antioxidant Capacity and Colour of Juices.* Czech J. Food Sci.,vol. 27 (5), p. 338–351.
11. **Radu Gina, Boboescu I., 2007** - *Managementul tehnico-economic al culturii arbuştilor fructiferi şi procesarea fructelor.* Ed. Eurobit, Timişoara.
12. **Singleton V.L., Rossi J.A., 1965** - *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents.* Am. J. Enol. Vitic. vol. 16, p. 144-158.
13. **Slimestad R., Solheim H.J., 2002** - *Anthocyanins from Black Currants (Ribes nigrum L.).* Agric. Food Chem., vol. 50, p. 3228-3231.
14. *****, 1989**-*Class names and the international numbering system for food additives*. CAC-GL 36-89, p. 1-55. *Codex Alimentarius Commision*.
15. *****, 2005** - *Total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines*. pH Differential Method. J. AOAC Int. vol. 88, p. 1269.