THE ANTHOCYANIN COMPOSITION OF A RED GRAPE POMACE IN RELATION WITH THE WINE INDUSTRY BY-PRODUCTS VALORIZATION IN ANIMAL FEED

COMPOZIȚIA ÎN ANTOCIANI A TESCOVINEI REZULTATE DE LA OBȚINEREA VINULUI ROŞU ÎN VEDEREA VALORIFICĂRII REZIDUURILOR INDUSTRIEI VINICOLE ÎN HRANA ANIMALĂ

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Abstract. *Grape pomace (GP), by-product of wine making, contains a large part of antioxidant polyphenols originating from the input material, the grapes. High production volumes, environmental impact and nutritional content of grape pomace makes it an important subject for careful valorisation. In order to valorize it, it is necessary to determine its chemical composition in bioactive molecules. In this study the anthocyanins were determined by LC-MS from a dried red grape pomace from Valea Călugărească, Romania. Based on their retention times, UV-VIS and MS spectra using standard compounds one anthocyan and three anthocyanidins were identified and quantified from GP acetone extract: peonidin 3-O-glucoside, delphinidin, cyanidin and malvidin. The most abundant anthocyanidin is delphinidin (23.93 ±0.12 µg/mL acetone extract) followed by malvidin (10.01 ±0.06 µg/mL acetone extract), the anthocyan peonidin 3-O-glucoside (4.78 ±0.04 µg/mL acetone extract) and cyanidin (2.63 ±0.06 µg/mL acetone extract). The results show that GP contains anthocyans and anthocyanidins, which possess anti-inflammatory and anti-carcinogenic activity, cardiovascular disease prevention, obesity control, and diabetes alleviation properties, all of which are more or less associated with their potent antioxidant property.*
Key words: *grape pomace, anthocyanins, anthocyanidins, LC-MS*

Rezumat. *Tescovina, un reziduu al industriei vinicole conține o cantitate importantă de polifenoli provenind de la strugurii din care a fost obținută. Cantitățile mari rezultate, impactul asupra mediului precum şi proprietățile nutritive ale tescovinei, fac din acest material rezidual subiectul unei valorificări atente. În vederea valorificării acesteia este necesară determinarea compoziției chimice. În acest studiu compoziția în antociani şi antocianidine a unei tescovine rezultate de la obținerea vinului roşu din centru viticol Valea Călugărească, România, a fost determinată prin tehnica LC-MS. Pe baza timpilor de retenție, a spectrelor UV-VIS şi a spectrelor de masă, folosind compuşii standard corespunzători, au fost identificați şi cuantificați un antocian şi trei antocianidine: peonidina-3-O-glucozida, delfinidina, cianidina şi malvidina. Delfinidina a fost găsită ca fiind cea mai abundentă antocianidină*

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(23.93 ±0.12 μg/mL extract acetonă), urmat de malvidină (10.01 ±0.06 μg/mL extract acetonă), peonidină-3-O-glucozidă (4.78 ±0.04 μg/mL extract acetonă) şi cianidină (2.63 ±0.06 μg/mL extract acetonă). Rezultatele indică faptul că tescovina conține antociani şi antocianidine, compuşi ce au activitate anti-inflamatorie şi anticancerigenă, de prevenire a bolilor cardiovasculare, de control al obezității şi a diabetului, care toate sunt mai mult sau mai puțin asociate cu activitatea lor antioxidantă.

Cuvinte cheie: *tescovină, antociani, antocianidine, LC-MS*

INTRODUCTION

Europe produced in 2012, 16209965 tones of wine (Faostat, 2015). Romania ranks in 2012 on the 12th place in Europe and 21st place in the world in terms of wine production, with 123450 tones, resulting a large amount of waste that needs to be managed (Faostat, 2015).

Wine industry wastes account for almost 30% of the grapes used for wine production (Rondeau *et al.,* 2013). Grape pomace, a remnant of the winemaking process, is one of the most important residues of the wine industry. It consists of different amounts of grape, skin, pulp, seeds and stems if not previously removed (Fontana *et al.,* 2013; Yu and Ahmedna, 2013).

These waste materials contain biodegradable organic matter; however, their disposal generates huge amounts of industrial waste and creates serious environmental problems (Gonzalez-Paramas *et al.,* 2004). The waste loads at the processing plants could be significantly reduced through by-product usage (Bordiga et al., 2015). Grape pomace, still contain a significant amount of phenolic compounds with beneficial health-related effects (Torres *et al.,* 2002; Laufenberg *et al.,* 2003; Sagdic *et al.,* 2011).

Within the grape pomace the grape skin brings the highest concentration of anthocyans. The most abundant of these compounds in red grapes are anthocyans, mainly 3-glycosides, 3-acetylglycosides and 3-p-coumaroylglycosides of malvidin (Mv), peonidin (Pn), delphinidin (Dp), petunidin (Pt) and cyanidin (Cy) (Wulf and Nagel, 1978).

Anthocyans are specific compounds of red grapes, located mainly in the skin of the grapes. The flavylium cation from their structure includes two benzene rings, linked by an oxygenated cationic unsaturated heterocycle, derived from the 2-phenyl-benzopyrylium nucleus (Lorrain *et al.,* 2013). They are glycosylated derivatives of five aglycones or anthocyanidins: cyanidin, peonidin, petunidin, delphinidin and malvidin (Makris and Kefalas, 2013; Ky *et al.,* 2014).

The chemical composition of the grape marc has to be determined prior to its utilization as dietary feedstuff for farm animals. In our study, the anthocyanins composition of a red grape pomace from Valea Călugărească winery, Romania, was determined by High Performance Liquid Chromatography-Photo Diode Array coupled with Mass Spectroscopy (HPLC-PDA-MS).

MATERIAL AND METHOD

Anthocyan extraction

The red grape pomace was provided by a local producer and derived from Valea Calugareasca, a Romanian winery. The anthocyanins from the dried grape pomace were extracted in acetone 80% (ratio, sample: solvent being 1:7 w/v) for 20 hours at 37^{o}C with continuous shaking. After the extraction the liquid phase was collected, representing the GP acetone extract.

High Performance Liquid Chromatography-Photo Diode Array coupled with Mass Spectroscopy (HPLC-PDA-MS) anthocyanin composition analysis for the grape pomace

The chromatographic measurements were performed using a complete HPLC SHIMADZU system using a C18 column. The HPLC system was coupled to a mass spectroscopy detector, LCMS-2010, using an electrospray ionization interface ESI. Due to the specificity of the studied components, we used the positive ionization mode. The analytical mobile phase consisted of 5% formic acid in water (solvent A) and 5% formic acid in methanol (solvent B). The compounds were separated using gradient elution with 0.15 mL/min flow. The experiments were conducted at 40^{0}C constant column temperature; one analytical run took 47 minutes.

The calibration curve and the correlation coefficient were calculated, which allowed us to determine the range of response linearity. Calibration curves were produced for 7 different points, the measurements being done in triplicates.

RESULTS AND DISCUSSIONS

HPLC-MS method was used for the qualitative and quantitative analysis of GP anthocyanins and anthocyanidins (Fig. 1). Table 1 shows the obtained values.

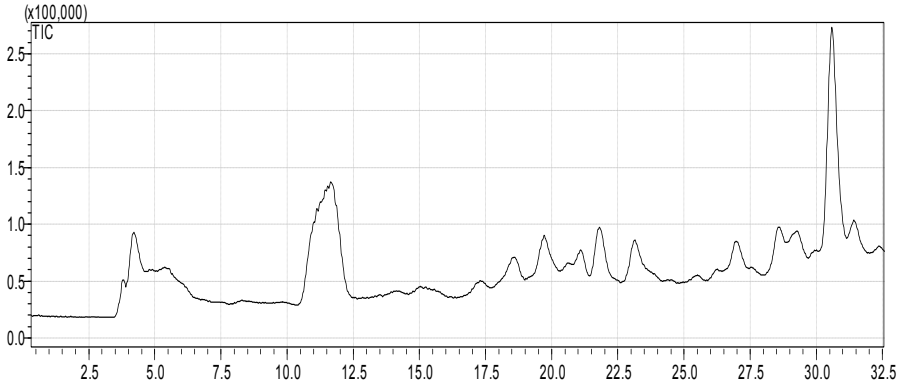

Fig. 1 - HPLC-MS chromatogram of the dry grape pomace acetone extract (GP*;* delphinidin $[M+H]^+$=303, malvidin $[M+H]^+$=331, cyanidin $[M+H]^+$=287, peonidin 3-O-glucoside $[M+H]^+$=463).

Table 1

Some performance characteristics of HPLC-MS method and the results concerning the anthocyanidins and anthocyanins

Analyte	t_R minutes	$[M+H]^+$	Equation of the standardization curve A:aria; C: conc. (µg/mL)	R	LoD (µg/mL)	Range of response linearity (µg/mL)	GP content (µg/mL)
Delphinidin	20±0.4	303	A=616931.5×C+349797.1	0.9996	0.04	0.5-50	23.93±0.12
Peonidin 3-O-glucoside	19.6±0.2	463	A=756370.8×C+516791.1	0.9996	0.07		4.78±0.04
Cyanidin	20.9±0.1	287	A=1651123×C+873662.6	0.9996	0.11		2.63±0.06
Malvidin	27.1±0.3	331	A=1109632×C+619282.7	0.9996	0.12		10.01±0.06

The equations of the calibration curves show a good linearity between the peak area and the concentration of the analyte, over a 1.5 (μg/mL) range of concentrations. The performance characteristics of HPLC-MS method for 4 anthocyanins and the analytical results are shown in Table 1.

Based on the retention time, of the UV-VIS spectra and on the mass spectra, using the proper standard compounds, we identified and quantified 1 anthocyans, peonidin 3-O-glucoside and three anthocyanidins: delphinidin, cyanidin and malvidin. The most abundant anthocyanidin is delphinidin (23.93 ±0.12 μg/mL acetone extract), followed by malvidin (10.01 ±0.06 μg/mL acetone extract), peonidin 3-O-glucoside (4.78 ±0.04 μg/mL acetone extract) and cyanidin (2.63 ±0.06 μg/mL acetone extract).

These results show that the grape pomace is rich in anthocyans and anthocyanidins, compounds with anti-inflammatory and anticancerogenic effects, compounds which may prevent cardiovascular diseases, may control obesity and diabetes in human patients. These effects are more or less associated to the antioxidant activity of these anthocyans.

CONCLUSIONS

1. The experimental results show that the red grape pomace, by-product from Valea Călugărească winery, contains the following anthocyanins and anthocyanidins: delphinidin, malvidin, peonidin 3-O-glucoside and cyanidin.

2. Delphinidin is the most abundant anthocyanidin.

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