ASSESSMENT OF BIOACTIVE POLYPHENOLIC COMPOUNDS FROM OENOLOGICAL EXHAUSTED MATERIAL

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Abstract. Horticultural products are irreplaceable sources for obtaining natural compounds, with a nutraceutical and bioactive role (functional foods). Also, the use of waste from their processing has increased significantly in recent years. Pomace from red and black grapes, resulting from the technological process of wine making (after maceration) was subjected to drying processes under natural conditions and then to the extraction of polyphenolic compounds. By analysis of the resulting fractions, obtained through repeated extractions, was determined total polyphenolic index (IPT or D_{280}) and the total content of anthocyanins (CA), of the dried pomace obtained from 15 local varieties of vines.
Unlike the established nutrient classes (proteins, vitamins, minerals), phenolic compounds are not considered vital for survival, but instead, they have properties that promote optimum human health.

INTRODUCTION

Anthocyanins are generally accepted as the largest and most important group of water-soluble pigments in nature (Horbowicz, M. *et al*., 2008).

The widely publicized French paradox made millions aware that French consumers of red wine had reduced incidence of coronary heart disease (Wrolstad, R. E., 2004).

Because of their bioactive properties and beneficial effects on human health, particular attention was given to attract new sources of valuable phenolic compounds (tannins, phenolic acids, anthocyanins etc), and developing methods of extraction, isolation and characterization of them.

Phenolic compounds form one of the most important classes of compounds in all plant secondary metabolites. Their role in both, plants and humans, is evident by active character in the biological, physiological and environmental field.

Grapes who reached the full maturity have a very complex chemically composition. This gradually changes with the processing, winemaking process, which they are subject to (Pomohaci, N., Stoian,V. *et al*., 2000). Polyphenolic compounds (anthocyanins, tannins, phenolic acids, etc) were identified in seeds (4-6%), skins (1-5%), cluster (1-3%) and pulp (trace) (Fregoni, 1998).

Anthocyanins play an important role in current research in oenology and industry of obtaining natural food colorants (Beceanu, D, 2008). Usually located in the skin of black grapes, anthocyanins are extracted only partially (30-40%) by the winemaking process, so pomace resulting from the production of red wines contains significant amounts of these phenolic compounds (Câmpeanu *et al*., 1989). They are glycosides of anthocyanidins (cyanidin, delphinidin, peonidin, petunidin, malvidin) (Cotea, D.V. *et al.*, 2010).

MATERIALS AND METHODS

This study aims to determine total polyphenolic content (TPC) and anthocyanins content (AC) of the dried pomace obtained from 15 local black grape varieties, of which 7 old grape varieties (Fetească neagră, Băbească neagră, Bătută neagră, Busuioacă de Bohotin, Negru de Căuşani, Negru vârtos, Vulpe) and 8 new *V. vinifera* creations (Amurg, Arcaş, Balada, Codană, Negru aromat, Negru de Drăgăşani, Novac and Roz de Miniş).

Grape samples was harvested on September 2009, from the Ampelographic Collection of UASVM, Iaşi, “V. Adamachi” farm, wine center Copou.

Wine technology applied was classic with destemming and crushing. Maceration was carried out in static plastic pots for 72 hours, followed by pressing (pneumatic press). Sugars content in the must, determined with refractometer, took an average of 18.7°Bx (175,5 g/l) (table 1).

After 3 weeks at room temperature, grapes pomace was considered to be dry enough to prepare for storage (average moisture was 7.5%, determined at air oven drying, 105°C for 4 hours) (Afusoae, 1988).

After 40 days of cold storage, there were three stages of extraction of phenolic compounds, to depletion of plant material, performed in the Laboratory of Oenology Research Center of the Romanian Academy, Iasi branch.

Dried pomace (seeds and skins) was ground, achieving a high degree of comminution of plant material (particles less than 0.5 mm).

Table 2

Moisture content of samples after drying and sugars content in the must

(the transformation of sugar quantity from °Bx to g/l, was made according to Otopeanu *et al.*, 1967).

No.	Variety	Moisture, after drying (%)	Sugars in must (°Bx)	Sugars in must (g/l)
1	Amurg	8,20	18,0	171
2	Arcaş	6,25	22,0	195
3	Balada	7,40	20,0	193
4	Băbească neagră	8,85	18,0	171
5	Bătută neagră	**10,94**	15,0	138
6	Busuioacă de Bohotin	6,50	20,5	194
7	Codană	6,00	15,5	143
8	Fetească neagră	6,10	20,0	193
9	Negru aromat	6,30	23,0	196
10	Negru de Căuşani	7,00	20,0	193
11	Negru de Drăgăşani	8,00	19,0	182
12	Negru vârtos	7,95	15,0	138
13	Novac	6,60	20,5	198
14	Roz de Miniş	9,50	12,0	105
15	Vulpe	7,15	**22,5**	**221**

The solution used for the extractions was ethanol, purity 96%, acidified with 1 % hydrochloric acid, thus resulted a pH = 1 - 1.5. Rapport plant material / solvent was 1:10 (g/v), and extraction time was approximately 72 hours.

In grapes skin, predominate anthocyanins without acyl group, which can be removed very quickly, while acyl anthocyanins can be removed gradually (Ţârdea, C., 2007).

Just at Vulpe variety, extraction took place in seven stages, up to a theoretical level of 99.5%, proved to be the richest variety in anthocyanin pigments, among those examined.

Before last filter was applied a ultrasound treatment on vessels containing pomace treated with extraction solution, 480 seconds, for each sample, as a means of enhancing the process of transfer of property and desorption, operation reported in the literature since 1970.

Total polyphenolic index or D_{280} index express total content of phenolic compounds (phenolic acids, tannins and coloring substances) existing in the samples analyzed (Ţârdea, C., 2007), with values in the literature range between 20 and 100 (Cotea, D. V. *et al*., 2010).

The method principle for determining the index D_{280} is that, benzene nuclei, characteristic of phenolic compounds, strongly absorb ultraviolet light, with a maximum around wavelength λ =275-280 nm (Zamfir, C., 2009).

Measurements were made using a UV-VIS spectrometer Analytik Jena Specord 200, as follows: absorbance was measured at λ = 280 nm, in 1 cm quartz cell, in comparison with distilled water, the result being index D_{280}. For expressing the content of phenolic compounds in grams gallic acid equivalent (GAE), reported at gram of plant material, it was drew a calibration curve, using gallic acid solutions of different concentrations (Fig. 1).

It was necessary to determine the Folin-Ciocâlteu index (IFC) to obtain values of phenolic compounds at λ = 750 nm and applying formulas. This method is reliable, reproducible and can be used to any wine or alcoholic extract. Analysis is based on reducing phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic acid mixture ($H_3PMo_{12}O_{40}$), to tungsten and molybdenum blue oxides in alkaline medium, in the presence of phenols. IFC is specific only to phenolic compounds with reducing properties and has values between 16 and 75 in red wines.

According to Ribereau-Gayon et al., 1972, for red wines D280/Fc value ratio, falls within the range 1.2 - 1.3.

In acidic medium, there is a balance between colored and colorless forms of *anthocyanins*. This balance is a function of pH (Davies, K., 2004). It was chosen pH 0.6 and pH 3.5 and measured the absorbance (optical density) at λ = 520 nm for both, sample and blank, using 1 cm optical path glass cell, compared with distilled water.

Coloring intensity variation between pH values is proportional to the anthocyanins content. With this variation, phenolic function is not affected and it is recognized that other phenolic compounds (tannins) do not interfere the determination (Zamfir,C., 2009).

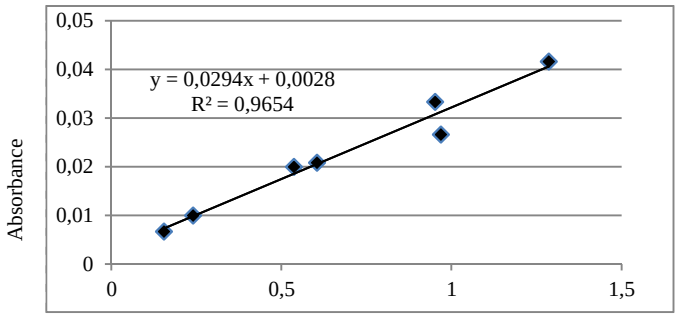


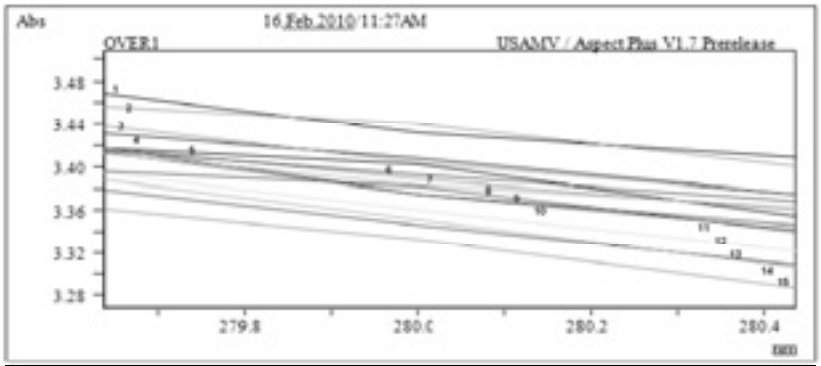
Fig. 1 Calibration curve (g GAE), for the index D_{280}

RESULTS AND DISCUSSION

Observations during the drying of pomace showed that yield samples at drying was approximately 20 %, from1 kg of fresh pomace resulted 200 g dry pomace (seeds, skins and small parts of rachides).

Was found that grape varieties with lighter colour were more susceptible to mold, than the intensely colored ones.

By the analysis of absorption spectra obtained, can be observed very close values of the index D_{280}, at specific 280 nm wavelength (Fig. 2). Each variety is represented in the spectrogram with numbers, for easy identification, and can be found in the chart legend.



1. Negru de Drăgăşani	6. Negru vârtos	11. Balada
2. Novac	7. Fetească neagră	12. Busuioacă de Bohotin
3. Negru de Căuşani	8. Vulpe	13. Arcaş
4. Codană	9. Roz de Miniş	14. Bătută neagră
5. Amurg	10. Negru aromat	15. Băbească neagră

Fig. 2 Absorption spectra of the samples analyzed, for index D_{280}

Following interpretation of the absorption spectra obtained at the specific wavelength of each compound analyzed and applying formulas, were obtained the results for the total polyphenolic compounds and anthocyanins content, at the samples under study.

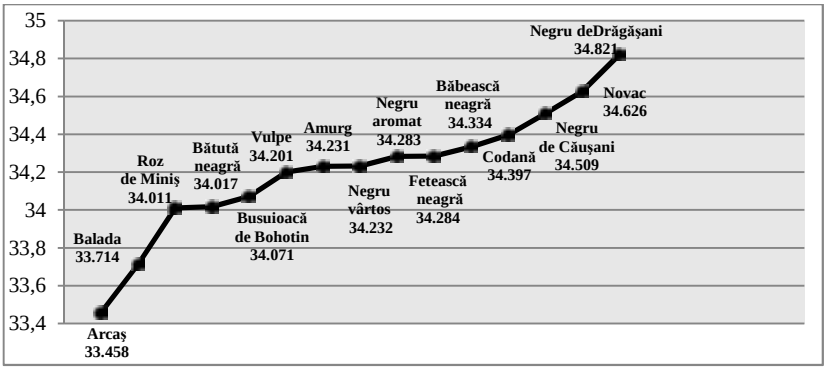


Fig. 3 Graphical representation of the D_{280} index values at the studied varieties

Values of D_{280} index were expressed with 4 decimal to highlight very small differences that occur at the total amount of polyphenols in the studied varieties, values which fall within the theoretical limits of this parameter. Can be observed the location of this index, close to the theoretical lower limit, but still with significant values ranging between 33.458 (Arcaş) and 34.821 (Negru de Dragăşani) (Fig. 3).

Regarding the content of total phenolic compounds (Fig. 4), determined quantitatively, it is noted that it has very similar values, except Vulpe variety with a higher value, 0.6851 g/g, phenolic compounds expressed as gallic acid equivalent (GAE).

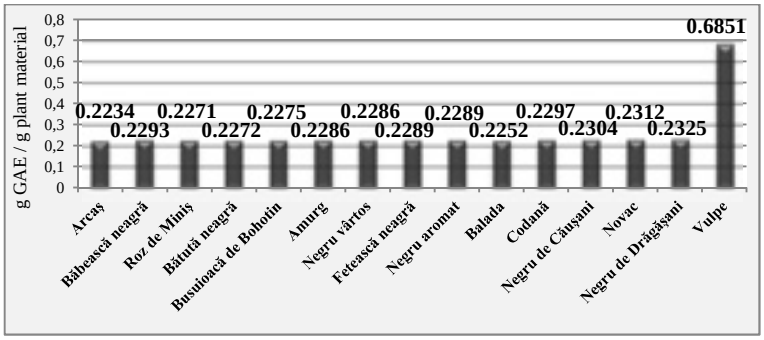


Fig. 4 Graphical representation of the values of total polyphenolic content (g GAE /g plant material) **at the studied varieties.**

From the data, we can notice the existence of local varieties of grapes with a potential for accumulation of phenolic compounds, higher than established varieties such as Fetească neagră or Băbească neagră, like Vulpe, Novac, Balada and Negru de Drăgăşani, although were not harvested at technological maturity of each, but were harvested all at once.

IFC index values fall within the theoretical range of this index and are presented in Table 2, with values of D_{280}/IFC ratio, expressing the proportion of phenolic compounds with reducing properties from the total polyphenolic compounds amount. It is recognized that Folin-Ciocâlteu reagent react also with other substances than phenolic compounds (protein, vegetable gum, etc.)

(Ikawa *et al*., 1988). Low ratio values D_{280}/IFC are explained by the presence in extracts of other compounds with reducing character, but wich do not belong to the phenols group.

Table 2

D_{280} and IFC values from samples studied (in order of increasing value D_{280}**) and the value of ratio between indexes.**

No.	Variety	D_{280}	IFC	D_{280}/IFC
1	Arcaş	**33.458**	69.48	0.48
2	Balada	33.714	88.48	0.38
3	Roz de Miniş	34.011	**98.76**	**0.34**
4	Bătută neagră	34.017	66.57	0.51
5	Busuioacă de Bohotin	34.071	56.79	0.59
6	Vulpe	34.201	75.41	0.45
7	Amurg	34.231	67.36	0.50
9	Negru vârtos	34.232	80.80	0.42
8	Negru aromat	34.283	75.03	0.45
10	Fetească neagră	34.284	69.05	0.49
11	Băbească neagră	34.334	**56.28**	**0.59**
12	Codană	34.397	57.89	0.59
13	Negru de Căuşani	34.509	62.96	0.54
14	Novac	34.626	83.99	0.41
15	Negru de Drăgăşani	**34.821**	88.33	0.39

Regarding the AC (Fig. 5) of dried pomace, obtained from considered varieties in this study, can notice Vulpe variety with high-value of AC index, 119.13 mg/g. It is noted that are local varieties with high potential for accumulation of anthocyanins, such as varieties: Novac, Amurg, Balada, Negru de Drăgăşani, Vulpe. At the opposite pole are varieties: Băbească neagră, Roz de Miniş, Negru vârtos, Bătută neagră, with low values of this parameter.

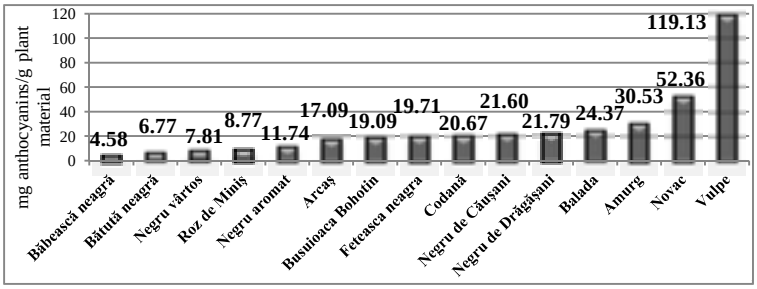


Fig. 5 Graphical representation of AC values at studied varieties (mg anthocyanins/g plant material)**.**

CONCLUSIONS

Content of polyphenolic compounds in grapes is very variable, depending on variety, thus, at the samples analyzed, the values recorded were within the range of 0.2234 g/g (Arcaş variety) and 0.6851 g/g (Vulpe variety), phenolic compounds expressed as gallic acid equivalent (GAE).

Dry pomace extracts, from local grape varieties of black grapes, are rich in polyphenolic compounds, with values of D_{280} index between 33.458, the Arcaş variety and 34.821 at Negru de

Drăgăşani variety, being thus a material with a real recovery potential in the sector of obtaining compounds with nutraceutical role and therefore functional food.

Reducing capacity of the plant material is given by the polyphenolic compounds and other compounds that are initially formed in the plant or during processing of grapes and at drying and storage of pomace (protein etc).

Quantities of anthocyanins, obtained after the extractions, varies in very large limits, with a maximum value at Vulpe variety, 119.13 mg/g and a minimum at Băbească neagră variety, 4.58 mg/g. This fact show that pomace, even in dry state, is a valuable source of vegetal pigments, which may have a significant utilization in food and pharmaceutical industry, and can successfully replace synthetic compounds.

Grape pomace, dried naturally, do not require expensive storage space, keeping its biologically active properties.

The high values of indices examined supports the extraction of these compounds from the considered material and for the future arise the question of using methods with lower solvent consumption and/or its recovery and recycling.

REFERENCES

Afusoae, Iulia, Savu, Maria, (1988) – Chimie şi biochimie vegetală. Lucrări practice. Institutul Agronomic „Ion Ionescu de la Brad", Iaşi. For internal use. {Book}

Beceanu, D., (2008) – *Materii prime horticole*, Editura "Ion Ionescu de la Brad", Iaşi. {Book}

Câmpeanu, R., Kontek, Adriana, Gavrilescu, I., Nanu, I., (1989) – *Cercetări privind obţinerea coloranţilor naturali din tescovina roşie.* Analele I.C.V.V. Valea Călugărească, vol XII, pag. 301-309 {Journal Article}

Cotea, D. V., Zănoagă, C., Cotea, V. V., (2010) – *Tratat de Oenologie*, Vol. I, Ed. Acad. Române, Bucureşti. {Book}

Davies, K., (2004) – *Plant pigments and their manipulation.* Annual plant reviews. Volume 14, CRC Press, Boca Raton, Florida, USA. Pag. 311. {Book}

Fregoni M., 1998 - *Viticoltura di qualità.* Stampe Grafiche Lama, Piacenza, pp. 707. {Book}

Horbowicz, M. *et al*., (2008) – *Anthcyanins of fruits and vegetables - their occurrence, analysis and role in human nutrition*. Vegetable crops research bulletin, vol. 68, pag. 5-22. DOI10.2478/v10032-008-0001-8.

Ikawa, M., Dollard, Catherine, Schaper, A. T., (1988) - *Reaction of Folin-Ciocalteau phenol reagent with purines, pyrimidines, and pteridines and its relationship to structure.* Agric. Food Chem., 1988, 36 (2), pp 309–311. DOI: 10.1021/jf00080a017.

Otopeanu, G., Stănescu, C, Anghelescu,I., (1967) – *Ghid practic pentru laboratoarele din unităţile de producţie.* Redacţia Revistelor agricole, Bucureşti. {Book}

Pomohaci N., Stoian V., *et al*., (2000) – *Oenologie. Vol. I, Prelucrarea strugurilor şi producerea vinurilor*. Ed. Ceres, Bucureşti. {Book}

Ribereau–Gayon, R., (1972) - *Evolution des composes phenolique en cours de la maturation du raisins*, Conn.Vigne Vin, 6:161-175. {Journal Article}

Ţârdea, C., (2007) – *Chimia şi analiza vinului*. Edit. Ion Ionescu de la Brad, Iaşi. {Book}

Wrolstad, R., E., 2004 – *Anthocyanin Pigments- Bioactivity and Coloring Properties* Journal of Food Science, Vol. 69, Nr. 5. DOI: 10.1111/j.1365-2621.2004.tb10709.x.

Zamfir, C., (2009) – *Studiul autenticităţii şi tipicităţii vinurilor roşii obţinute din soiuri autohtone*. PhD Thesis. UASVM, Iaşi.{PhD Thesis}.

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