PRELIMINARY CHARACTERIZATION OF POLYPHENOLIC EXTRACTS FROM GRAPE SEEDS

CARACTERIZAREA PRELIMINARĂ A UNOR EXTRACTE POLIFENOLICE OBŢINUTE DIN SEMINŢE DE STRUGURI

VASILE Ancuţa[1], COTEA V.V.[1], SAVIN C.[2], PAŞA Rodica[2], NECHITA B.[1], NICULAUA M. [1]
e-mail: ancuta.vasile@yahoo.com

Abstract. *The capacity of grapevine varieties to assemble various types of polyphenols represented a rather less studied segment of study which is currently raising more interest. These compounds may be found especially in grape skins and seeds from where they are transmitted to must and wine by macerating processes. Studies on polyphenols from grapes proved to be on the one hand essential for assessing grapevine varieties' oenological potential, and on the other hand very important in the evaluation of their beneficial effects on maintaining the metabolic balance and the health condition of the human body. In this paper we make a preliminary presentation and characterization of polyphenolic extracts from the grape seeds of seven grapevine varieties, among which four national varieties (Fetească neagră, Băbească neagră, Arcaş, Negru de Drăgăşani), two international ones Cabernet Sauvignon, Merlot) and the disease resistant Chambourcine variety.*

Key words: seed, grape, phenolic acids, resveratrol, tanins

Rezumat. *Capacitatea soiurilor de viţă de vie de a acumula diferite clase de polifenoli a constituit un segment de preocupare relativ îngust dar care se amplifică în prezent. Aceşti compuşi se regăsesc cu precădere în seminţele şi pielita boabelor de struguri de unde sunt preluaţi, în musturi şi vinuri în timpul procesului de maceraţie. Studiile privind polifenolii din struguri s-au dovedit pe de o parte esenţiale în evaluarea potenţialului oenologic al diferitelor soiuri de viţă de vie, iar pe de altă parte foarte importante în evaluarea proprietăţilor benefice ale acestora în menţinerea echilibrului metabolic şi a stării de sănătate a organismului uman. În lucrare sunt prezentate şi caracterizate preliminar extractele polifenolice obţinute din seminţele de strugurii a şapte soiuri de viţă de vie, dintre care patru soiuri autohtone (Fetească neagră, Băbească neagră, Arcaş, Negru de Drăgăşani), două din sortimentul internaţional (Cabernet Sauvignon, Merlot) şi soiul cu rezistenţă sporită Chambourcine.*

Cuvinte cheie: seminţe, struguri, acizi fenolici, resveratrol, taninuri

INTRODUCTION

Using plant extracts as a source of remedies for preventing or healing several diseases captured people's attention since the oldest times. A great part of the active biologic composites of vegetal extracts are the polyphenols, that represent a class of over 8000 composites of which the majority have been

[1] University of Agricultural Sciences and Veterinary Medicine Iasi, Romania
[2] Research and Development Station for Viticulture and Vinification Iasi, Romania

identified in different anatomic segments of plants (Georgescu C. et. al, 2005).

Among the total of superior plants, *Vitis vinifera* species contains significant quantities of almost all the classes of composites with known phenolic structure. Moreover, black grapes contain complex mixtures of composites with polyphenolic structure easy to be extracted (Tudose Irina, 2002). These composites are usually found in seeds and grape skins where from they are absorbed in musts and wines, during the maceration process. In the context of the most frequent national and international use of the natural products, the research in the present work aims to achieve a preliminary characterization of some polyphenolic extracts, obtained from grape seeds of some autochthonous varieties of grapes as well as from the international assortment. This study is also justified by the abundance of information in the specialty literature, which attracts the attention upon the anti-anaemic, anti-inflammatory, antioxidant, cardiovascular, immunomodulator and even upon the oncochimiotherapeutic effects of polyphenolic extracts (Mittal A. et al., 2003; Vitseva O. et al., 2005; Višnja Katalinic et. al., 2010).

MATERIAL AND METHOD

Polyphenolic extracts have been obtained from grape seeds of some autochthonous varieties of grapes as well as from the international varieties (Fetească neagră, Băbească neagră, Arcaş, Negru de Drăgăşani, Cabernet Sauvignon, Merlot and Chambourcine). After breaking up at dimensions of 1-2 mm, the vegetal materials have been degreased with ethylic ether. The procedure of extraction has been done in a continuous system in the Soxhlet device, using the ethylic alcohol as solvent in a proportion of 1/10 (vegetal material, g/solvent, mL).

For the preliminary characterization of polyphenolic extracts there were determined the total polyphenols through Folin-Ciocâlteu method and the coefficient of tannoid substance (I.T.S) through the method established by Bourzex. Also, through HPLC analysis (chromatography of high performance liquids) there have been identified and quantified a series of phenolic acids, stilbene (trans-resveratrol) as well as some non-hydrolysable tannins (catechin and epicatechin).

RESULTS AND DISCUSSIONS

In order to assess the beneficial properties of the polyphenolic extracts obtained from grape seeds in maintaining the metabolic equilibrium and the wellness of the human organism, these have been subjected to a process of preliminary characterization.

Therefore, in figure 1 there are presented data regarding the contents in total polyphenols of the extracts obtained in a continuous system from the varieties taken into account in this study. In the graphical representation there was noticed the vegetal extract from grape seeds belonging to the variety Fetească neagră, with a concentration in total polyphenols of 3,0 g GAE/L (equivalent grams of gallic acid). As regards the extracts obtained from seeds belonging to the varieties Chambourcine, Cabernet Sauvignon, Merlot and Arcaş there was noticed that these ones represent relatively closed values of the concentration in total

polyphenols (figure 1), respectively between 2,53 and 2,75 g GAE/L.

The same tendency was noticed in the case of the index of tannoid substances, the values being of 41,6 for the extract obtained from the seeds of the Chambourcine variety, of 41,2 for Cabernet Sauvignon, 40,4 for Merlot, respectively 38,4 for Arcaş (figure 2).

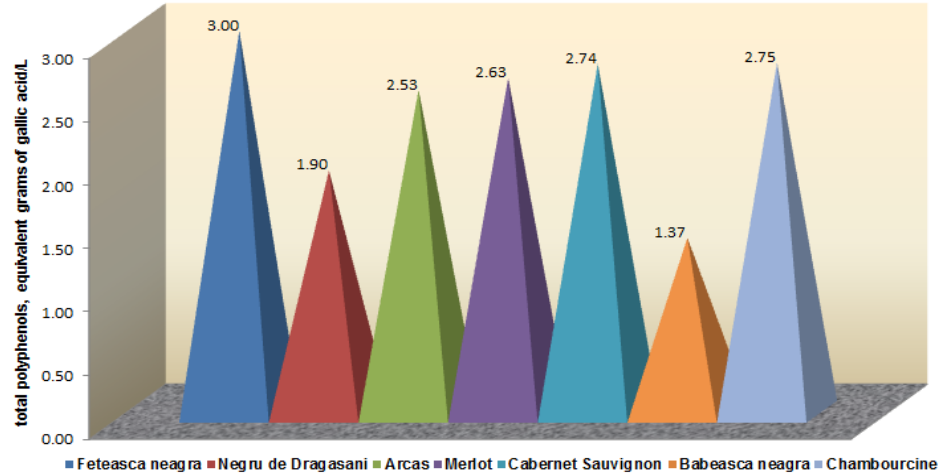

Fig. 1 – The content in total polyphenols of the polyphenolic extracts obtained from the seeds of the varieties taken into account in the present study

The extracts obtained from the varieties Negru de Drăgăşani and Băbească neagră indicated the smallest values both for the coefficient of tannoid substances (31,6 and 24,8) and for the concentration in total polyphenols (1,90 and 1, 37 g GAE/L).

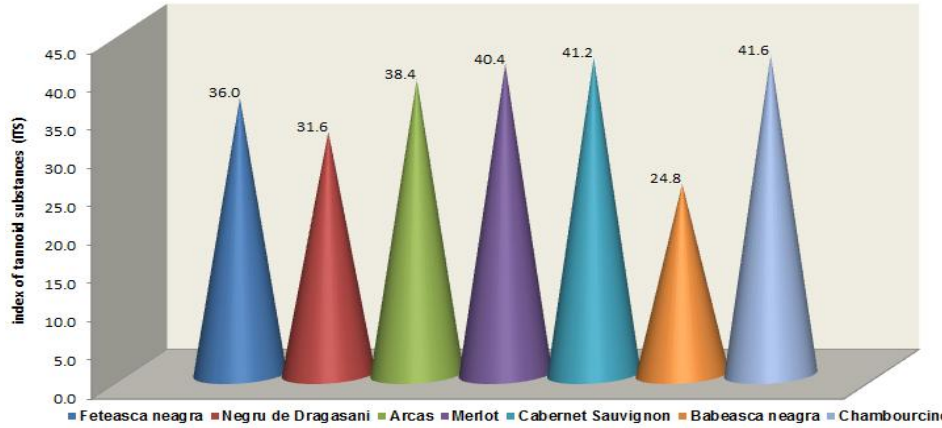

Fig. 2 - The coefficient of tannoid substances (I.M.T.) of the polyphenolic extracts obtained from the seeds of the varieties taken into account in the present study

Among the polyphenolic acids, the phenolic acids are the most common for the vegetal extracts, these ones being noticed as mixtures between the hydroxbenzoic and hydroxicinamic acids (table 1 and 2). Both categories of acids

present an interest in the characterization of the polyphenolic extracts due to the antiseptic, diuretic and stimulative action of the biliary secretion.

In the case of the analyzed extracts, the content of the hydroxibenzoic acids varied within large limits (table 1). The most important hydroxibenzoic acid, the gallic acid, presented values between 7,794 mg/L in the case of Negru de Drăgăşani and 12,452 mg/L in the case of Arcaş. As regards the salicylic acid, although in the specialty literature there is noticed the fact that it forms during the process of alcoholic fermentation, it has been identified in significant quantities in the polyphenolic extracts obtained from seeds, respectively between 42,845 mg/L at Băbească neagră and 96,639 mg/L at Cabernet Sauvignon. In relatively large quantities was found the syringic acid, with variation limits between 32,537 mg/L and 114,046 mg/L. In the analyzed polyphenolic extracts there were also identified other hydroxibenzoic acids, such as the p-hydroxibenzoic acid, m-hydroxibenzoic acid and the vanilic acid.

Table 1

Hidroxybenzoic acids identified in the polyphenolic extracts obtained from the seeds of the varies taken into account in the present study

Varieties	p-hydroxibenzoic acid, mg/L	m-hydroxibenzoic acid, mg/L	salicylic acid, mg/L	vanilic acid, mg/L	gallic acid, mg/L	syringic acid, mg/L
Fetească neagră	0.946	0.401	92.863	0.446	10.083	114.046
Negru de Drăgăşani	1.045	0.206	56.124	1.008	7.794	32.537
Arcaş	3.448	2.287	52.280	2.214	12.452	49.646
Merlot	3.049	0.200	89.675	0.320	10.786	85.451
Cabernet Sauvignon	3.145	0.403	96.639	0.655	9.960	81.482
Băbească neagră	3.295	0.502	42.845	0.585	10.016	57.682
Chambourcine	3.498	0.341	70.146	0.428	11.066	50.826

Through the HPLC analysis of the polyphenolic extracts there was also identified a series of hydroxicinamic acids, respectively the caffeic, p-coumaric, ferulic and sinapic acids, the obtained results being presented in table 2.

Table 2

Hydroxicinamic acids identified in the polyphenolic extracts obtained from the seeds of the varieties taken into account in the present study

Varieties	caffeic acid, mg/L	p-coumaric acid, mg/L	ferulic acid, mg/L	sinapic acid, mg/L
Fetească neagră	0.525	4.576	0.967	0.355
Negru de Drăgăşani	0.409	4.562	0.988	0.329
Arcaş	0.447	4.563	0.961	0.366
Merlot	0.485	4.569	0.981	0.378
Cabernet Sauvignon	0.487	4.580	0.970	0.337
Băbească neagră	0.447	4.639	0.990	0.358
Chambourcine	0.503	4.575	0.973	0.375

Analyzing the data, one may notice the caffeic, ferulic and sinapic acids present reduced concentrations. Moreover, it can also be noticed that no matter the variety of which were obtained the extracts, the content in hydroxicinamic acids does not indicate significant variations, the values being very close one to

another.

Except for the phenolic acids in the extracts obtained from the seeds of the varieties taken into account in the present study, there was also identified the trans-resveratrol. This functions as an anti-inflammatory agent, conferring the extracts from grape seeds the capacity of reducing the values of the triglycerides and of the cholesterol in the blood. Analyzing the graphical representation of the obtained data (figure 3) one might notice a small variation of the concentration of trans-resveratrol between 2,4198 and 2,4540 mg/L, as well as its absence in the extract obtained from the seeds of Negru de Drăgăşani.

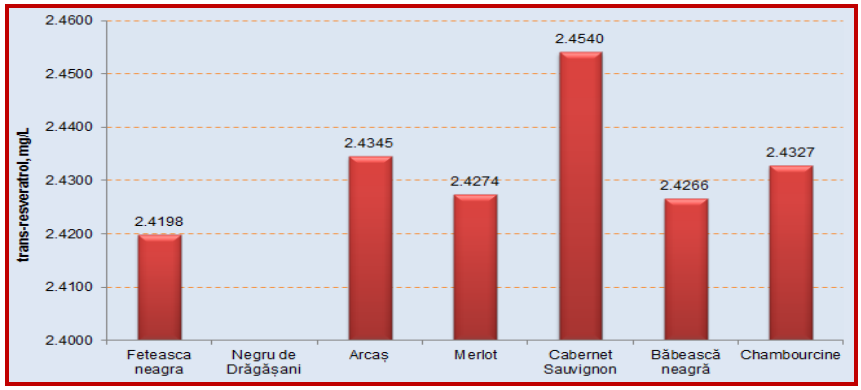

Fig. 3 - The content in trans-resveratrol of polyphenolic extracts obtained from the seeds of the varieties taken into account in the present study

Through the analysis of the polyphenolic extracts obtained from seeds there was noticed the presence of some non-hydrolysable tannins (condensed), respectively the catechin and the epicatechin (figure 4).

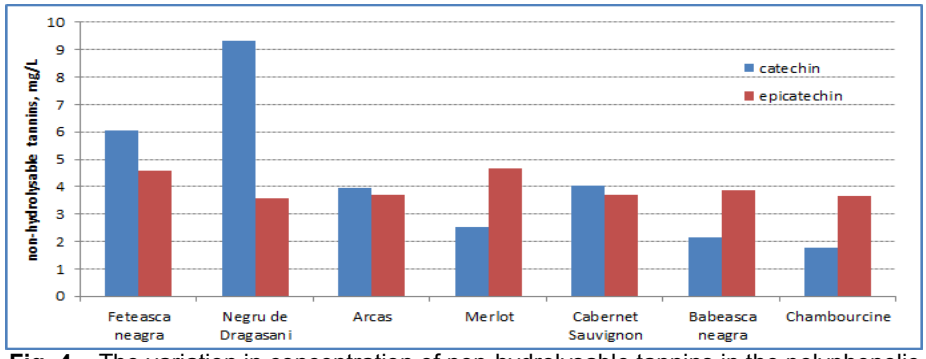

Fig. 4 – The variation in concentration of non-hydrolysable tannins in the polyphenolic extracts obtained from the varieties taken into account in the present study

As regards the content of catechin it can be noticed the large interval of variation, from 1,785 mg/L at Băbească neagră to 9,319 mg/L at Negru de Drăgăşani. In the case of the epicatechin, higher values were noticed at the varieties Fetească neagră (4,583 mg/L) and Merlot (4,679 mg/L), in the case of the other varieties the values being close one to another.

CONCLUSIONS

1. The polyphenolic extracts obtained from grape seeds belonging to the varieties Chambourcine, Cabernet Sauvignon, Merlot and Arcaş indicated relatively close values of the concentration in total polyphenols of the coefficient of tannoid substances. The extracts that were obtained from Negru de Drăgăşani and Băbească neagră characterized through low values, both for the coefficient of tannoid substances (31,6 şi 24, 8) and for the concentration in total polyphenols. (1,90 şi 1,37 g GAE/L).

2. As regards the phenolic acids, it was noticed that the gallic acid indicated values between 7,794 mg/L at Negru de Drăgăşani and 12,452 mg/L at Arcaş. The salicylic and siringic acids were identified in significant quantities, with variation limits between 42,845 mg/L and 96,639 mg/L, respectively 32,537 mg/L and 114,046 mg/L. No matter the variety from which were obtained the extracts, the content in hydroxicinamic acids (caffeic, p-coumaric, ferulic, sinapic) did not present significant variations, the values being very close one to another.

3. There was noticed a small variation of the concentration of trans-resveratrol between 2,4198 and 2,4540 mg/L, as well as its absence from the extract obtained from the seeds of Negru de Drăgăşani.

4. The preliminary characterization of the polyphenolic extracts obtained from the seeds of different varieties of grapes contributes to the assessment of their oenological potential and implicitly at the assessment of the beneficial properties in maintaining the metabolic equilibrium and the wellness of the human organism.

Acknowledgments: *This work was cofinanced from the European Social Fund through Sectoral Operational Programme Human Resources Development 2007-2013, project number POSDRU/I.89/1.5/S62371 "Postdoctoral Schole in Agriculture and Veterinary Medicine area".*

REFERENCES

1. Georgescu C., Bratu I., Tămaş M., 2005 - *Studiul unor polifenoli din Rhododendron kotskyi*. Revista de Chimie, Bucureşti, vol. 56, 7, pp. 779 – 783.

2. Mittal A, Elmets CA, Katiyar SK, 2003 - *Dietary feeding of proanthocyanidins from grape seeds prevents photocarcinogenesis in SKH-1 hairless mice: relationship to decreased fat and lipid peroxidation*. Carcinogenesis vol. 24, pp. 1379–1388.

3. Tudose Irina, 2002 – *Biomasa vegetală ca sursă de produse cu potenţial biologic activ*. „Progrese în biotehnologie", Editura Sedcom Libris, Iaşi, pp. 26-45

4. Vitseva O., Varghese S., Chakrabarti S., Folts J.D., Freedman, J.E., 2005 - *Grape seed and skin extracts inhibit platelet function and release of reactive oxygen intermediates*. Journal of Cardiovascular Pharmacology, vol. 46, pp. 445–451.

5. Višnja Katalinic, Sonja Smole Možina, Danijela Skroza, Ivana Generalic, Helena Abramovic, Mladen Miloš, Ivica Ljubenkov, Saša Piskernik, Ivan Pezo, Petra Terpinc, Mladen Boban, 2010 - *Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in Dalmatia (Croatia)*. Food Chemistry vol 119, pp. 715–723