STUDY OF SOME POLYPHENOLIC EXTRACTS OBTAINED FROM PRESSED GRAPE SKINS

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Abstract

Due to their biologically active properties, polyphenols convert waste into attractive sources for the medical and pharmaceutical products, their traditional management being varied towards other technological processes. The identification of active principles derived from the polyphenolic extracts obtained from pressed grape marc can help create an alternative use and recovery of by-products of wine, fact which increases their economic value. It also helps assess the oenological potential of vine varieties and thus to assess their beneficial properties in maintaining metabolic balance and health of the human body. In this context, the research in this paper aim to characterise the extracts obtained from pressed grape marc by identifying and quantifying the major polyphenolic compounds in their composition. To study the influence of the maceration technology on the content of polyphenolic compounds from by-products of the winemaking process, grape samples of Fetească neagră, Băbească neagră, Cabernet Sauvignon, Merlot and Arcaş grape varieties were processed using classical maceration, thermo-maceration, microwave maceration and rotary tanks maceration (ROTO-tanks). The obtained polyphenolic extracts were analyzed by high performance liquid chromatography (HPLC), several phenolic acids, stilbenes (trans-resveratrol) non-hydrolysable tannins (catechin and epicatechin), some flavones (rutin and quercitin), and a number of anthocyan compounds being identified and quantified. The results of the preliminary characterization showed quite similar values in the content of total polyphenols and tannoid matters index. The maceration variants did not affect the amount of hydroxybenzoic and hydroxycinnamic acids. The other polyphenolic compounds that were identified varied depending on the grape variety and maceration technology used. The obtained data justifies the use of pressed grape marc as raw material to obtain polyphenolic extracts, recommending further research on its biologically active properties (antiradical, antioxidant, cytotoxic and cytostatic).

Key words: grapes, macerationa, pressed grape marc, extracts, polyphenols

The use of plant extract as remedy source for different diseases has been an issue since the beginning of human medicine.

The results of numerous studies show that the biggest part of active biologic compounds are polyphenols, a class of over 8000 compounds, out of which the majority have been identified in different anatomic segments of plants (C. Georgescu et al., 2005).

Certain plants have the capacity to accumulate polyphenolic compounds belonging to one or two classes, as hydroxycinnamic derivates and anthocyans in cherries (C. F. Timberlake, 1981; B. Möller et al., 1983). On the other hand, grapes store complexes mixtures of polyphenolic compounds, the distribution of the various polyphenolic compounds in chemical classes being almost complete (Wilfred Vermerris et al., 2006).

In the specific literture, studies concerning the biological activity of some polyphenolic compounds of different species are found, in tests in pure form or as fractionate or global extracts (L.H. Yao et al., 2004; A. Mittal et al., 2003; O.Vitseva et al., 2005; C. Savin et al., 2009; V. Katalinic et al., 2010).

The multitude of data in literature regarding this theme is shadowed by the difficulty in efficient systematisation, because of the extremely varied experimental methodology, sources and methods of obtaining polyphenolic extracts and last but not least, their concentration and stability in time (Maria Escribano – Bailon et al., 2003).

In the case of grapes, the polyphenolic compounds are mostly found in the seeds and skins of the berries, from where they reach the must and wine, following maceration (C. Țardea et al., 2010; P. Ribereau-Gayon et al., 2006). Among other by-products of the wine-making process, the pressed

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grape marc, due to its physical-chemical composition, represents an important source of polyphenolic compounds (L.M. De Campos et al., 2008; D. Kammerer et al. 2004; G. Ruberto et al., 2007).

The pressed grape marc is the fermented or non-fermented residue obtained after pressing fresh grapes, its chemical composition being similar to that of the grapes, with some differences. The fresh (unfermented) grape marc contains 37 - 39 % skins, 1,0 - 1,2 % stems; 0,20 - 0,25 % green masses (leaves, shoots); 30 - 32 % pulp, 28 - 29 % seeds, while the wet fermented pressed grape marc has 25 % skins, 13 % stems; 12 % seeds (N.I. Razuvaev, 1980). The present study aims at creating an alternative mean of use for the pressed grape marc, as polyphenolic extracts, upgrading thus its economic value.

MATERIAL AND METHOD

The polyphenolic extracts were obtained from pressed grape marc from the processing of Feteasca neagră, Băbeasca neagră, Cabernet Sauvignon, Merlot and Arcaş grape varieties. In order to study the influence of the maceration technology on the content of polyphenolic compounds, classical maceration, thermo-maceration, microwave maceration and ROTO-tanks maceration were used on the grape samples. Because of insufficient quantity, the Arcaş grapes were processed only by classical maceration technology. 17 samples of pressed grape marc were obtained (one sample for Arcaş and four samples each for each grape variety, according to the used maceration procedures). After drying and grinding (1-2 mm) the vegetal materials were treated with ethylic ether. The extraction procedure was done in a continuous system by using the Soxhlet device, having as solvent ethanol ratio 1/10 (vegetal material (g)/solvent (mL). The time span of the process varied according to the necessary time for draining of the vegetal material (48 – 72 hours), with a constant temperature of 78°C.

In order to characterise the phenol extracts, the total polyphenols were determined using the Folin-Ciocalteau method as well as the tannoid matters index (I.M.T) using the method established by Bourzex. HPLC analysis (high performance liquid chromatography) identified and quantified a series of pehnolic acids, stilbens (trans-resveratrol), non-hydrolisable tannins (catechin and epicatechin), some flavones (rutin and quercitin), as well as a series of anthocyan compounds.

RESULTS AND DISCUSSIONS

The data obtained during the process of the characterisation of the studied polyphenolic extracts is presented in *table 1*. The analysis of the results shows relatively close values of the total polyphenolic content and tannoid matters index, indifferent of the grape variety. Within the same variety, a reduced influence is that of the maceration technology of the polyphenolic content.

Therefore, the thermo-maceration and microwave maceration extract the lowest quantity of polyphenols in wine, the pressed grape marc being thus richer in polyphenolic compounds.

Table 1

Preliminary characterisation of polyphenolic extracts obtained from pressed grape marc

Grape variety/ maceration variant	Total polyphenols, g equiv. gallic acid /L	Anthocyans, mg/L	Tannoid matter index
Feteasca neagra /thermo-maceration	0.4604	16.625	9.06
Feteasca neagra/ microwaves	0.4644	4.375	8.53
Feteasca neagra/ classical maceration	0.4046	1.750	8.17
Feteasca neagra /roto-tanks maceration	0.3708	-	9.24
Merlot / thermo-maceration	0.4644	2.265	9.20
Merlot / microwaves	0.4644	1.750	8.34
Merlot / classical maceration	0.5220	1.750	9.08
Merlot / roto-tanks maceration	0.4968	0.875	9.12
Cabernet Sauvignon/ thermo-maceration	0.3652	6.125	9.92
Cabernet Sauvignon / microwaves	0.4156	11.375	9.11
Cabernet Sauvignon / classical maceration	0.3844	-	9.03
Cabernet Sauvignon / roto-tanks maceration	0.3724	-	8.81
Băbeasca neagra / thermo-maceration	0.5616	4.375	9.04
Băbeasca neagra / microwaves	0.5728	2.625	9.12
Băbeasca neagra / classical maceration	0.3976	-	8.95
Băbeasca neagra / roto-tanks maceration	0.4489	-	9.04
Arcaş / classical maceration	0.4448	1.750	9.17

The polyphenols content varies from 0.3652 to 0.5750 g equivalent gallic acid /L, the same tendency being registered in the case of the tannoid matter index (TMI), the values being between 8.17 and 9.20.

The anthocyans were below the detection limit in the samples obtained by classical maceration and ROTO-tanks maceration (example

Cabernet Sauvignon and Băbească neagră samples). For the other extracts, the anthocyans content was reduced, varying from 0.875 to 11.375 mg/L. The extracts from Merlot showed low quantities of anthocyans, in all maceration methods. This can be because of the low content of anthocyans of the grape samples used in the experiment.

HPLC analsysis determined a series of phenolic acids in the studied extracts, such as hydroxybenzoic and hydroxycinnamic acids. One notices that vanillic acid is predominant (*figure 1*), with lowest values in Babeasca neagra extract (0.816 mg/L) and Cabernet Sauvignon extract (0.857 mg/L), and highest in Merlot (2.240 mg/L) and Arcaş extract (2.174 mg/L). The most frequent hydroxybenzoic acid, gallic acid, did not vary significantly regarding the grape variety, having values from 0.345 mg/L (Cabernet Sauvignon) to 0.663 mg/L (Arcaş).

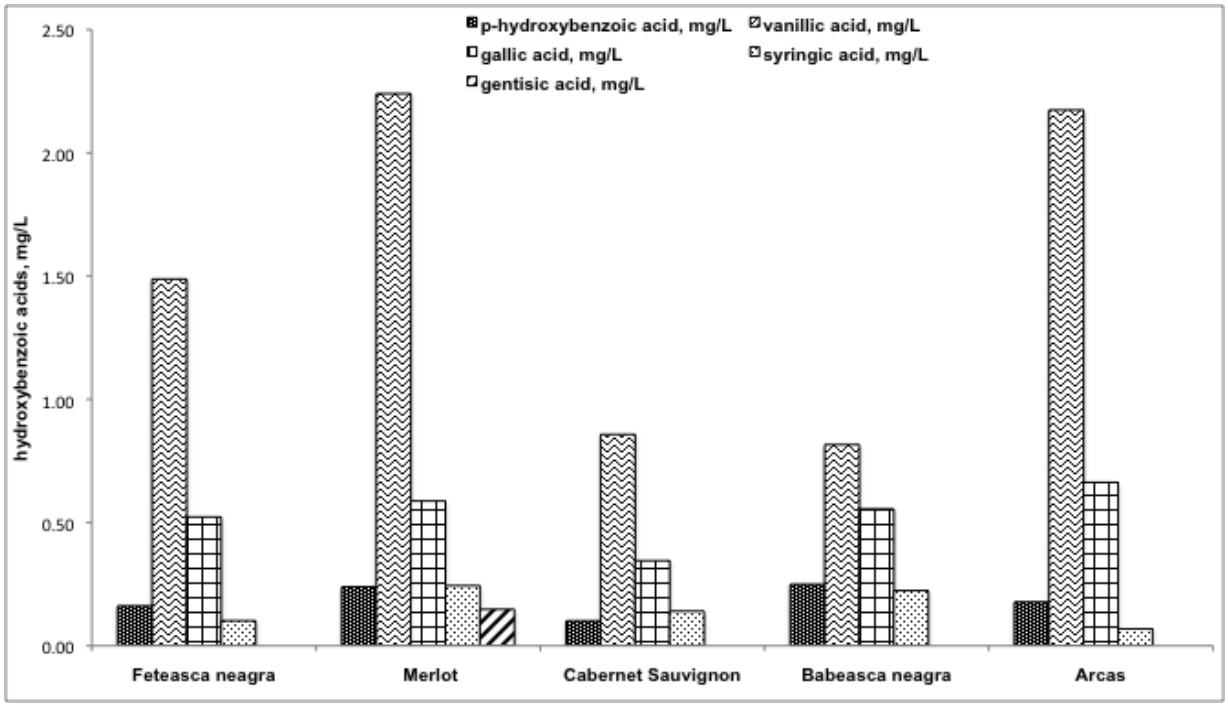

Figure 1 **Variation of the hydroxybenzoic acids' content identified in the polyphenolic extracts obtained from the pressed grape marc**

Comparing the extracts obtained from seeds and skins and the extracts obtained from pressed grape marc, in the latter the m-hydroxybenzoic acid was not identified, being probably degraded during the maceration process.

The salicylic acid could not be well evidenced, because of tannin interference. Gentisic acid, formed during the fermentation process, was found only in the extracts from Merlot.

Regarding the maceration methods, it was registered that they do not influence the quantity of the hydoxybenzoic acids from the polyphenolic extracts. So, the predominant hydroxybenzoic acid in the case of all the samples obtained by the four maceration methods was the vanillic acid (*figure 2*).

From the hydroxycinnamic acids, only the p-cumaric, ferulic and clorogenic acid were identified in the polyphenolic extracts (*table 2*).

Cafeic acid was identified only in Arcas extract (0.256 mg/L). The hydroxycinnamic acids have subunit values, the maceration variants as well as the grape variety having a non significant influence on their content.

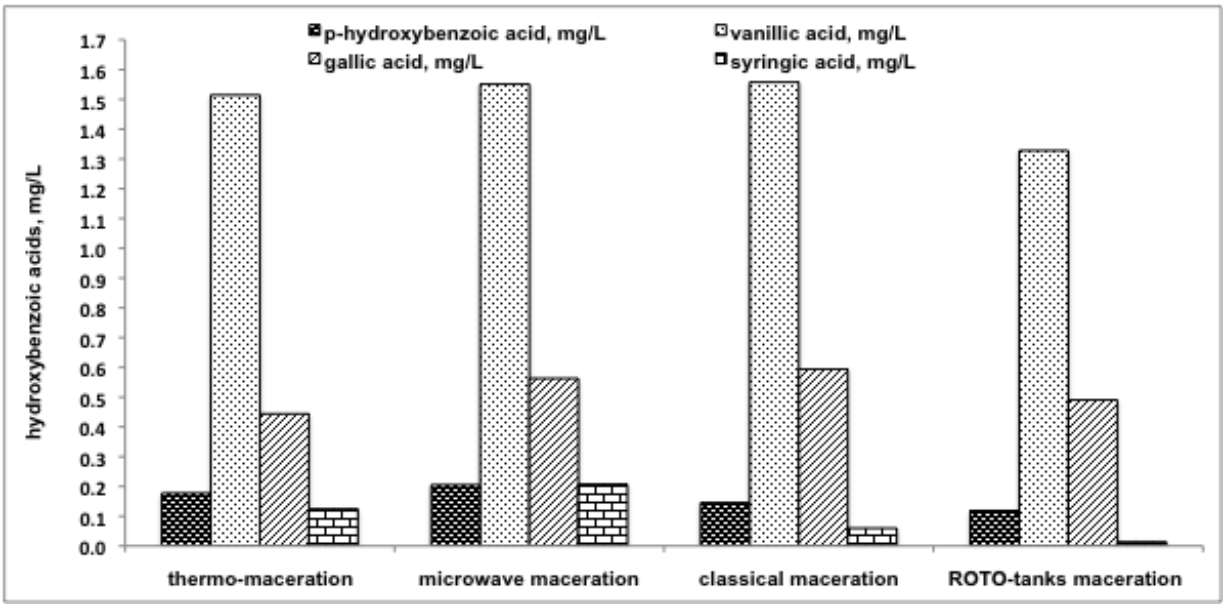

Figure 2 **Variation of the hydroxybenzoic acids' content identified in the polyphenolic extracts obtained from the pressed grape marc of Fetească neagră grape variety according to maceration variants**

Table 2

Hydroxycinnamic acids identified in the polyphenolic extracts obtained from the pressed grape marc

Grape variety / maceration variant	p-cumaric acid, mg/L	ferulic acid, mg/L	clorogenic acid, mg/L
Feteasca neagra /thermomaceration	1.228	0.015	0.149
Feteasca neagra/ microwaves	0.925	-	0.054
Feteasca neagra/ classical maceration	0.567	0.000	0.047
Feteasca neagra /roto-tanks maceration	0.372	0.018	0.033
Merlot / thermomaceration	0.958	0.166	0.063
Merlot / microwaves	0.150	0.050	0.076
Merlot / classical maceration	0.834	-	-
Merlot / roto-tanks maceration	0.079	0.007	
Cabernet Sauvignon/ thermomaceration	0.114	0.153	0.157
Cabernet Sauvignon / microwaves	0.244	0.065	0.037
Cabernet Sauvignon / classical maceration	0.653	0.010	0.020
Cabernet Sauvignon / roto-tanks maceration	0.562	-	0.023
Babeasca neagra / thermomaceration	0.431	0.011	-
Babeasca neagra / microwaves	1.279	0.018	-
Babeasca neagra / classical maceration	0.884	-	0.032
Babeasca neagra / roto-tanks maceration	0.776	0.013	-
Arcas / classical maceration	0.561	-	0.047

Besides the phenolic acids, in the polyphenolic extracts, trans-resveratrol was analysed as well (*figure 3*). It is one of the main active principles, determining the bioactive values of the extracts.

The data in figure 3 show that the thermo-maceration sample has the highest content of trans – resveratrol in the case of the polyphenolic extracts obtained from the pressed grape marc of the Fetească neagră, Cabernet Sauvignon and Băbească neagră grape varieties. The extract obtained from Merlot and Arcaş, trans – resveratrol is predominant in the classical maceration samples. The HPLC analysis of the polyphenolic extracts showed the presence of non-hydrolysable tannins, such as catechin and epicatechin.

The catechin content from the pressed grape marc extracts varies between 0.8 mg/L in the Cabernet Sauvignon extract, to 15.2 mg/L in the Merlot extract. These values represent the same maceration technology, the classical one.

In the case of epicatechin, higher quantities were identified in the extract from Fetească neagră and Merlot (*figure 4*).

Other identified active principles from the polyphenolic extracts of the pressed grape marc were rutin and quercitin (*table 3*).

The extracts obtained from the Feteasca neagră pressed grape mark are registered, with an average content of 6.54 mg/L rutin and 2.52 mg/L quercitin as well as that of Arcaş with 5.58 mg/L rutin and 2.05 mg/L quercitin.

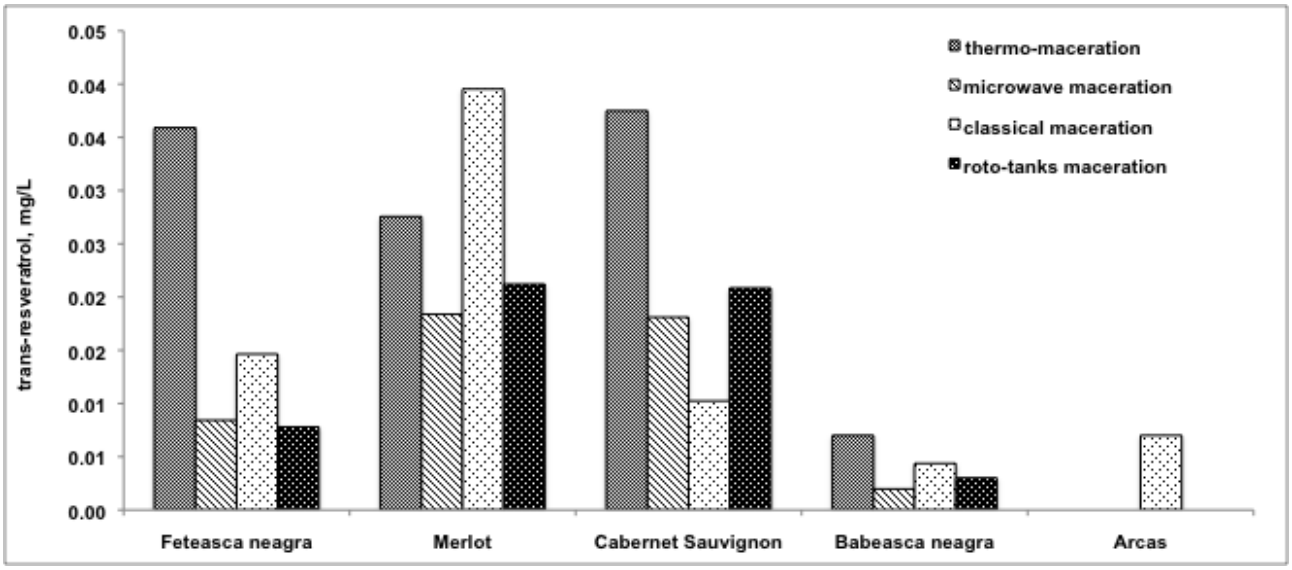

Figure 3 **Variation of the trans-resveratrol content in the polyphenolic extracts obtained from the pressed grape marc according to the maceration variant**

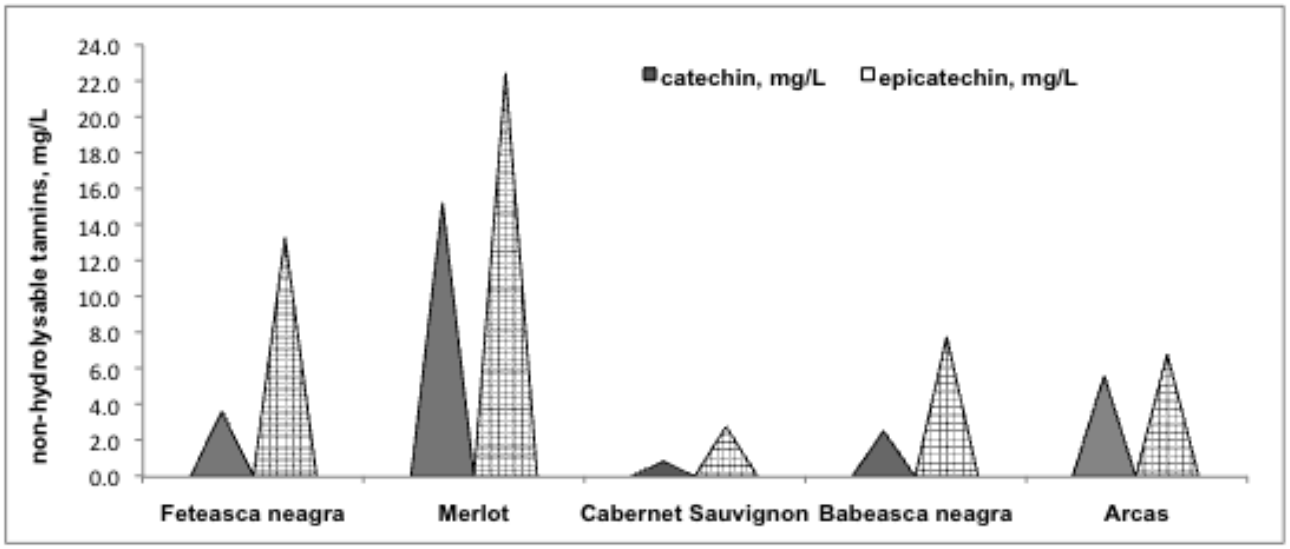

Figure 4 **Variation of the concentration of non-hydrolysable tannins in the polyphenolic extracts obtained from the pressed grape marc of the studied grape varieties**

Table 3

Flavones identified in the polyphenolic extracts obtained from the pressed grape marc

Grape variety / maceration variant	rutin, mg/L	quercitin, mg/L
Feteasca neagra /thermomaceration	6.510	3.452
Feteasca neagra/ microwaves	5.222	1.923
Feteasca neagra/ classical maceration	4.963	2.006
Feteasca neagra /roto-tanks maceration	9.467	2.716
Merlot / thermomaceration	3.420	0.220
Merlot / microwaves	4.374	0.237
Merlot / classical maceration	4.791	1.841
Merlot / roto-tanks maceration	11.564	1.614
Cabernet Sauvignon/ thermomaceration	5.347	5.781
Cabernet Sauvignon / microwaves	4.709	4.638
Cabernet Sauvignon / classical maceration	5.256	5.252
Cabernet Sauvignon / roto-tanks maceration	1.773	4.071
Babeasca neagra / thermomaceration	3.120	0.425
Babeasca neagra / microwaves	2.941	0.596
Babeasca neagra / classical maceration	1.870	0.084
Babeasca neagra / roto-tanks maceration	3.646	1.528
Arcas / classical maceration	5.575	2.045

CONCLUSIONS

The study of the extracts obtained from pressed grape marc underlined the presence of non-hydrolysable tannins (catechin, epicatechin) and of flavones (rutin) in important quantities.

The phenolic acids and the trans – resveratrol were identified in low quantities, having subunit values, except vanillic acid.

The maceration variants used (classical maceration, thermo-maceration, microwave maceration and ROTO-tanks maceration) does not significantly influence the polyphenolic compounds content' from the pressed grape marc.

The identified active principles stand proof for the use of pressed grape marc for obtaining polyphenolic extracts. An alternative use of the by-products of the wine-making process is created, increasing their economic value.

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