STUDY OF SOME POLYPHENOLIC EXTRACTS OBTAINED FROM LEES OF WINE

STUDIUL UNOR EXTRACTE POLIFENOLICE OBȚINUTE DIN DROJDIA DE VIN

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Abstract. *The identification of active principles derived from the polyphenolic extracts obtained from lees of wine can help create an alternative use and recovery of by-products of wine, fact which increases their economic value. The research in this paper aim to characterise the extracts obtained from lees of wine by identifying and quantifying the major polyphenolic compounds in their composition. 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 lees of wine as raw material to obtain polyphenolic extracts, recommending further research on its biologically active properties.*
Key words: *lees of wine, maceration, polyphenols.*

Rezumat. *Identificarea principiilor active ale extractelor polifenolice obținute din drojdia de vin poate contribui la crearea unei alternative de utilizare şi valorificare a subproduselor din vinificație, fapt ce măreşte valoarea lor economică. Cercetările din prezenta lucrare urmăresc să realizeze caracterizarea unor extracte obținute din drojdia de vin prin identificarea şi cuantificarea celor mai importanți compuşi polifenolici din compoziția acestora. Rezultatele procesului de caracterizare preliminară au evidențiat valori relativ apropiate ale conținutului de polifenoli totali şi al indicelui de materii tanoide. Variantele de macerare nu au influențat cantitatea de acizi hidroxibenzoici şi hidroxicinamici. Ceilalți compuşi polifenolici identificați au variat atât în funcție de soi cât şi de varianta de macerare. Datele obținute justifică utilizarea drojdiei de vin ca materie primă pentru obținerea unor extracte polifenolice, recomandându-se continuarea cercetărilor cu privire la proprietățile lor biologic active.*
Cuvinte cheie*: drojdie de vin, maceratie, polifenoli.*

INTRODUCTION

The grapes store complex mixes of polyphenolic compounds, mostly found in seeds and skins of the berry, from where they are transferred in to must and then wine through maceration (Țardea et. al., 2010; Ribereau-Gayon et. al., 2006).

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Among the by-products of the winemaking process, the lees of wine is considered an important source of polyphenolic compounds due to its physical-chemical composition (Pérez-Serradilla et. al., 2011; Yi Chun et. al., 2009; Cheng et. al., 2011). It can be used as raw and cheap matter for producing natural chemical compounds (Braga et. al., 2002; Bustamante et. al., 2008; Naziri et. al., 2012) as well as from an environmental point of view, in dealing with management of waste.

The present study aims at creating an alternative for the use of the lees of wine resulted in the winemaking process as polyphenolic extracts.

MATERIAL AND METHOD

The polyphenolic extracts were obtained from the lees of wine of Fetească neagră, Băbească neagră, Cabernet Sauvignon Merlot and Arcaş wine-making process. In order to study the influence of the maceration technology on the content of polyphenolic compounds, the grapes were processed using classic maceration, thermo-maceration, microwave maceration and rotating tanks maceration.

After drying and crushing, the obtained lees of wine was degreased with ethylic alcohol in the following ratio 1 g plant material: 20 mL solvent for removing lipophilic substances. The extraction process was done in a continuous manner with the Soxhlet device, using ethylic alcohol in a ration of 1/10 (plant material (g)/solvent (mL). The time span of the extraction varied according to depletion time of plant material (48 – 72 hours), with a constant temperature of 78°C.

For a preliminary characterisation of polyphenolic extracts, total polyphenols were determined, using the spectrophotometric method Singleton V. And tannoid matter indices (I.M.T), method established by Bourzeix et al., 1986.

A HPLC analysis (high performance liquid chromatography) identified and quantified a series of phenolic acids, stilbens (trans-resveratrol), non-hydrolysable tannins (catechin and epicatechin), as well as flavons (rutin and quercitin).

RESULTS AND DISCUSSIONS

The data obtained in the preliminary characterisation process of the analysed polyphenolic extracts are presented in table 1. Analysing the results, one can observe similar values regarding the content of total polyphenolic compounds and tannoid matter index, disregarding the grape variety used.

Within the same grape variety, a reduced influence of used technology on the polyphenolic content is noticed. Therefore, the wine variants obtained through classic maceration and rotating tanks maceration have a lower concentration of polyphenolic compounds, the lees of wine being richer in polyhenolic compounds.

The extracts obtained from lees of wine of Cabernet Sauvignon have mean values of anthocyanin content of 25.219 mg/L. No matter of the maceration technique, extracts from the lees of wine of Băbească neagră wine has small values of anthocyans. An explanation could be the fact that the used grapes have a small content of anthocyans.

Table1

Preliminary characterisation of polyphenolic extracts obtained from the lees of wine

Grape variety/ maceration variant	Total polyphenols, g equiv. gallic acid /L	Anthocyans, mg/L	Tannoid matter index
Fetească neagră /thermo-maceration	0.1312	14.875	5.92
Fetească neagră / microwaves	0.1712	14.875	5.72
Fetească neagră / classical maceration	0.2706	18.125	7.00
Fetească neagră /roto-tanks maceration	0.2728	20.125	6.48
Merlot / thermo-maceration	0.1552	22.750	6.36
Merlot / microwaves	0.2312	16.625	6.44
Merlot / classical maceration	0.1756	6.875	6.04
Merlot / roto-tanks maceration	0.1704	7.875	5.88
Cabernet Sauvignon/ thermo-maceration	0.2420	20.125	5.92
Cabernet Sauvignon / microwaves	0.2880	31.500	6.04
Cabernet Sauvignon / classical maceration	0.3066	23.875	6.48
Cabernet Sauvignon/roto-tanks maceration	0.3132	25.375	6.56
Băbească neagră / thermo-maceration	0.1936	6.125	5.60
Băbească neagră / microwaves	0.1092	3.500	5.60
Băbească neagră / classical maceration	0.1212	1.250	6.60
Băbească neagră / roto-tanks maceration	0.1204	1.750	6.04
Arcaş / classical maceration	0.1776	17.500	6.40

Through HPLC, a series of phenolic acids, respectively hydroxybenzoic and hydroxycinnamic acids were identified. Among the hydroxybenzoic acids, the vanillic and p-hydroxybenzoic acids are majorly represented, in relatively similar quantities (figure 1).

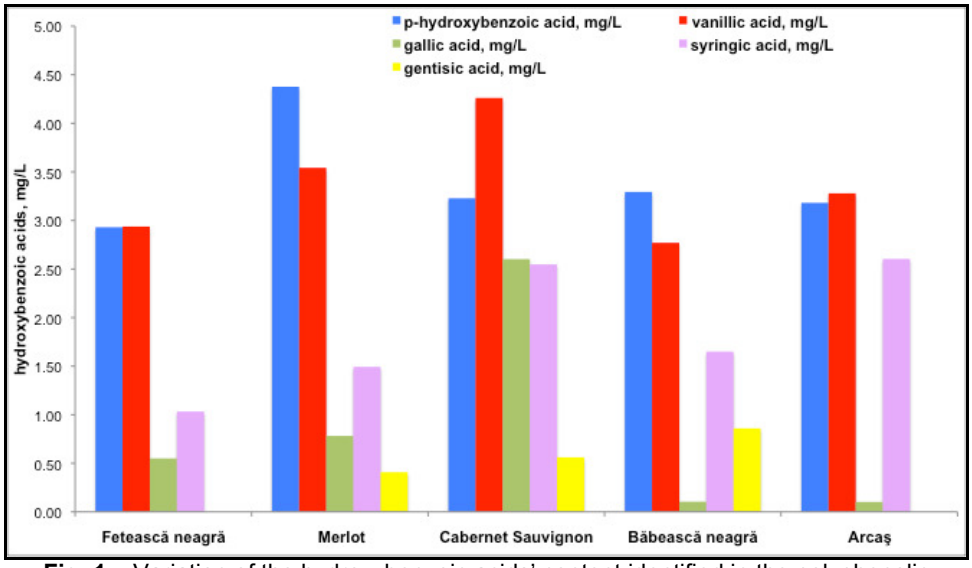

Fig. 1 – Variation of the hydroxybenzoic acids' content identified in the polyphenolic extracts obtained from the lees of wine

Gallic acid, the most frequent hydroxybenzoic acid, was identified in highest quantity in the lees of wine of Cabernet Sauvignon wine (2.602 mg/L).

Syringic acid has higher values that gallic acid. This can be justified by the creation process of syringic acid, moreover by the anthocyanidins (malvidin, oenidine) degradation during alcoholic fermentation.

Compared to the extracts obtained from seeds and skins, the extracts obtained from the lees of wine, n-hydroxybenzoic acid was not identified, having probably degraded during the maceration process.

At the same time, salicylic acid could not be well identified because of tannin interference. Gentisic acid is formed during the fermentation process and was identified only in the extracts obtained from the lees of wine of Merlot, Cabernet Sauvignon and Băbească neagră wines.

HPLC analysis identified some hydroxycinnamic acids in the alcoholic polyphenolic extracts, respectively caffeic, p-coumaric, ferullic, sinapic and clorogenic acids (table 2).

The registered data in table 2 show subunit values for all the identified hydroxycinnamic acids, regardless of the maceration variant.

Table 2

Hydroxycinnamic acids identified in the polyphenolic extracts obtained from the lees of wine

Grape variety / maceration variant	caffeic acid, mg/L	p-coumaric acid, mg/L	ferullic acid, mg/L	sinapic acid, mg/L	clorogenic acid, mg/L
Fetească neagră /thermo-maceration	-	0.272	0.078	0.015	0.037
Fetească neagră / microwaves	0.171	0.403	0.017	0.012	0.052
Fetească neagră / classical maceration	0.222	0.433	0.061	0.015	0.064
Fetească neagră /roto-tanks maceration	0.495	0.624	0.089	0.018	0.103
Merlot / thermo-maceration	0.230	0.682	0.163	0.009	0.423
Merlot / microwaves	0.134	0.178	0.026	0.008	0.440
Merlot / classical maceration	0.252	0.434	0.030	0.046	0.334
Merlot / roto-tanks maceration	0.370	0.691	0.033	0.084	0.228
Cabernet Sauvignon/ thermo-maceration	0.080	0.414	0.013	0.112	0.283
Cabernet Sauvignon / microwaves	0.065	0.982	0.095	0.086	0.173
Cabernet Sauvignon / classical maceration	0.139	0.503	0.043	0.071	0.218
Cabernet Sauvignon/roto-tanks maceration	0.272	0.113	0.020	0.015	0.197
Băbească neagră / thermo-maceration	0.053	0.632	0.047	0.016	0.050
Băbească neagră / microwaves	0.042	0.276	0.072	-	0.030
Băbească neagră / classical maceration	0.085	0.563	0.049	0.008	0.140
Băbească neagră / roto-tanks maceration	0.117	0.495	0.050	-	0.230
Arcaş / classical maceration	0.197	0.692	0.161	0.036	0.910

Beside phenolic acids, in the analysed polyphenolic extracts trans-resveratrol was identified (figure 2). It is one of the most important active principles and determines the bioactive values of the extracts.

The trans-resveratrol content varied from 0.06 mg/L at Merlot wine obtained by thermo-maceration to 0.45 mg/L in Cabernet Sauvignon obtained through the same process.

Analysing the data in figure 2, a correlation between the maceration variants and the trans-resveratrol content can be drawn.

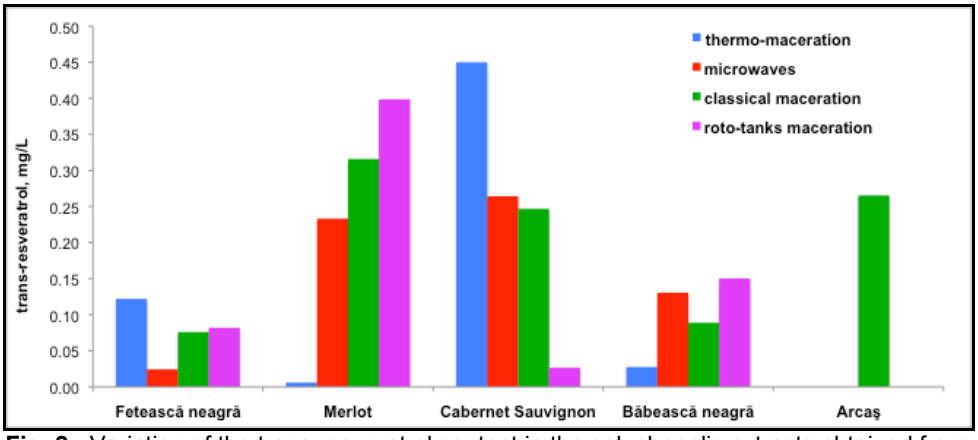

Fig. 2 - Variation of the trans-resveratrol content in the polyphenolic extracts obtained from the lees of wine according to the maceration variant

A HPLC analysis of the polyphenolic extracts obtained from the lees of wine identifies non-hydrolysable tannins: catechin and epicatechin, as well as some flavons, like rutin and quercitin (table 3). They have similar values to those of catechin and epicatechin content. In the extracts obtained from the lees of wine of Cabernet Sauvignon, the catechin and epicatechin mean values are 4.84 mg/L, respectively 3.76 mg/L.

Table 3

Non-hydrolysable tannins and flavones identified in the polyphenolic extracts obtained from the lees of wine

Grape variety / maceration variant	catechin, mg/L	epicatechin, mg/L	rutin, mg/L	quercitin, mg/L
Fetească neagră /thermo-maceration	0.915	-	0.712	1.082
Fetească neagră / microwaves	5.076	0.951	1.317	2.314
Fetească neagră / classical maceration	5.602	1.614	1.389	2.216
Fetească neagră /roto-tanks maceration	10.816	3.890	2.139	3.252
Merlot / thermo-maceration	2.255	1.548	1.733	3.315
Merlot / microwaves	2.321	1.108	2.570	5.585
Merlot / classical maceration	1.952	1.178	3.007	7.901
Merlot / roto-tanks maceration	1.582	1.248	3.444	10.218
Cabernet Sauvignon/ thermo-maceration	4.517	5.446	2.597	4.643
Cabernet Sauvignon / microwaves	4.089	4.682	2.201	13.783
Cabernet Sauvignon / classical maceration	4.840	3.763	2.267	11.223
Cabernet Sauvignon/roto-tanks maceration	5.914	1.160	2.003	15.244
Băbească neagră / thermo-maceration	2.704	2.240	1.047	1.645
Băbească neagră / microwaves	0.502	0.048	0.159	1.361
Băbească neagră / classical maceration	1.692	1.251	0.645	2.267
Băbească neagră / roto-tanks maceration	0.679	0.263	0.243	2.890
Arcaş / classical maceration	0.689	0.063	0.849	12.305

Concerning flavones, stands extracts obtained from the lees of wine of variety Merlot with an average of 2.69 mg/L rutin and 6.75 mg/L quercetin and extract of Cabernet Sauvignon with 2.27 mg/L rutin and 11.22 mg/L quercetin.

CONCLUSIONS

1. The study of the extracts obtained from lees of wine underlined the presence in high quantities of non-hydrolysable tannins (catechin, epicatechin) and flavons (rutin and quercitin). Phenolic acids and trans-resveratrol were identified in smaller quantities with subunit values, except some hydroxybenzoic acids (vanillic, p-hydroxybenzoic and syringic acids).

2. The maceration variants (classic maceration, thermo-maceration, microwave maceration and rotating tanks maceration) do not significantly influence the polyphenolic content of the lees of wine.

3. The identified active principles are proof for the possibility of lees of wine for obtaining polyphenolic extracts. Therefore, an economically efficient alternative for the role of winemaking by-products is created.

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REFERENCES

1. Bourzeix M, Heredia N, Kovac V, 1986 – *Les procyanidols de la grappe de raisin et du vin*. Journales Internatinal D'etude du Groupe Polifenoliques et Assemblée Generale, vol 13, p. 403 – 411.

2. Braga G. Fernando, Lencart e Silva A. Fernando, Alves Arminda, 2002 - *Recovery of Winery By-products in the Douro Demarcated Region: Production of Calcium Tartrate and Grape Pigments*. Am. J. Enol. Vitic. vol 53., p. 41-45.

3. Bustamante M.A., Moral R, Paredes C, Perez-Espinosa A, Moreno-Caselles J, Perez-Murcia MD, 2008 - *Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry*. Waste Manag, vol. 28, p. 372-380.

4. Cheng V. J., Bekhit A. E. D., McConnell M., Mros S., Zhao J. H., 2011 - *Effect of extraction solvent, waste fraction and grape variety on the antimicrobial and antioxidant activities of extracts from wine residue from cool climate*. Food Chemistry vol. 134, p. 474 – 482.

5. Naziri Eleni, Mantzouridou Fani, Tsimidou Z. Maria, 2012 - *Recovery of squalene from wine lees using ultrasound assisted extraction - a feasibility study*. Journal of agricultural and food chemistry vol. 60 issue 36, p. 9195 - 9201.

6. Pérez-Serradilla J.A., Luque de Castro M.D., 2011 - *Microwave-assisted extraction of phenolic compounds from wine lees and spray-drying of the extract*. Food Chemistry vol. 124 issue 4, p. 1652 - 1659.

7. Ribéreau-Gayon P., Dubourdieu D., Donèche B., Lonvaud A., 2006 - *Phenolic Compounds, 2nd Edition*. Handbook of Enology, The Chemistry of Wine, Vol. 2. John Wiley & Sons Ltd, Chichester, West. Sussex (England), p. 141–205.

8. Singleton V.L., Rossi J.A. jr, 1965 – *Colorimetry of total phenolics with phosphomolybdic – phosphotungsticacid reagents*. Amer J. Enol. Viticult. vol. 16., p. 144-158.

9. Ţardea C, Sârbu Gh., Ţârdea Angela, 2010 - *Tratat de vinificaţie*, Editura "Ion Ionescu de la Brad", Iaşi, 766 p.

10. Yi Chun, Shi John, Kramer John, Xue Sophia, Jiang Yueming, Zhang Mingwei, Ma Ying, Pohorly Joseph, 2009 - *Fatty acid composition and phenolic antioxidants of winemaking pomace powder*. Food Chemistry vol. 114 issue 2, p. 570-576.