

PHENOLIC COMPOUNDS IN MERLOT WINES OBTAINED THROUGH DIFFERENT TECHNOLOGIES IN IAȘI VINEYARD, ROMANIA

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ABSTRACT. Obtaining red quality wines depends on the raw matter composition and also on the extraction technology, used in the processing of grape and must. Thus, two methods of maceration-fermentation on lees (classical and in rotating tanks), two thermal methods (thermomaceration and microwave maceration) and two methods rarely used industrially in wine-making (cryomaceration and ultrasound maceration) were experimented. Even in the years with less than favorable climatic conditions, from Merlot grape variety one obtained for the most part, legally speaking, quality wines (with the exception of microwave macerated and cryomacerated wines, which had only 21–22 g/L non-reducing extract. In regard to alcohol content, all obtained samples had more than 11% vol. The obtained Merlot wines were rich in glycerol (8–9 g/L), fact that favorably influenced their organoleptic traits. Total phenolic content had values between 1,97 and 2,86 g/L for the Merlot wines obtained through maceration-fermentation and thermomaceration.

Ultrasound maceration did not favor phenolic extraction from grape skins and the obtained wines were poor in anthocyanins and tannins (0,94–1,1 g/L). In regard to the maceration technology used, free anthocyanins were found in variable proportions in wines, between 77 and 91%. The sum of acylated anthocyanins participation percentages was between 8,8 and 22,7%, and the ratio between the acetylated and cumarilated participation percentages registered small values, variety-specific, between 1,1 and 2,8.

Key words: Merlot; Maceration; Polyphenols; Anthocyanins.

REZUMAT. Compușii fenolici din vinurile Merlot, obținute prin diferite procedee în podgoria Iași. Obținerea vinurilor roșii de calitate depinde atât de compoziția materiei prime, cât și de tehnologia de extracție, aplicată la prelucrarea strugurilor și a musturilor. În acest scop, s-au experimentat două metode

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de macerare-fermentare pe boștină (clasică și în cisterne rotative), două metode termice (macerarea cu microunde și termomacerarea) și două metode neimplementate pe scară largă în producție (criomacerarea și macerarea cu ultrasunete). Chiar și în anii cu condiții climatice mai puțin favorabile, din soiul Merlot s-au obținut vinuri de calitate, care întrunesc, în mare parte, condițiile prevăzute în legislație, cu excepția celor obținute prin macerare cu microunde și ultrasunete, care conțin numai 21–22 g/L extract sec nereducător. Sub aspectul conținutului în alcool, la toate variantele analizate s-au realizat mai mult de 11% vol. Vinurile obținute din soiul Merlot au fost bogate în glicerol (8–9 g/L), ceea ce a influențat favorabil însușirile organoleptice ale acestora. Conținutul în polifenoli totali a avut valori cuprinse între 1,97 și 2,86 g/L la vinurile Merlot, obținute prin macerare-fermentare și termomacerare. Macerarea cu ultrasunete nu a favorizat extragerea compușilor fenolici din pielea boabelor, iar vinurile obținute au fost sărace în antociani și taninuri (0,94–1,1 g/L). În funcție de modalitatea de macerare, antocianii liberi s-au regăsit în vinuri în proporții diferite, variabile între 77 și 91%. Suma dintre proporțiile de participare ale antocianilor acilați s-a situat între 8,8 și 22,7%, iar raportul dintre procentele de participare ale antocianilor acetilați și cumarilați a înregistrat valori mici, specifice soiului, situate în intervalul 1,1–2,8.

Cuvinte cheie: Merlot; macerare; polifenoli; antociani.

INTRODUCTION

Internationally, Romanian red wines are appreciated and solicited more than the white ones because of the four trademark varieties: Fetească neagră, Merlot, Cabernet Sauvignon and Pinot Noir. Because of the high phenolic compound content, red

wines are less fragile, having higher redox stability, in comparison to the white wines (Mihalca *et al.*, 2010). This study was conducted for the purpose of increasing the quality of red wine obtained from Merlot grapes, in regard to varietal potential and the extraction technology of phenolic compounds from grape skins.

In enology, phenolic compounds hold an important place, because they influence the color and the taste qualities of wines, like astringency, hardness and flavor. Phenolic compounds found in grapes and wines are: phenolic acids, tannins and most of the coloring substances (Cotea *et al.*, 2009).

Anthocyanins are red pigments found in grapes, especially in the skins. They are responsible for the color of red and rose wines and represent 38% of the total polyphenolic content of wines (Vivar-Quintana *et al.*, 2002). The anthocyanin content of young red wines is between 200 and 300 mg/L; this amount is reduced to half after the first year, stabilizing itself at around 200 mg/L (Țârdea *et al.*, 2001).

MATERIALS AND METHODS

The material used for this study consisted in Merlot grapes, harvested from Iași-Copou, Romania, vineyard in the year 2008.

With the aim of knowing the different red wine-making technologies and their influence on the composition and quality of wines, one experimented the maceration-fermentation methods

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(classical and in rotating tanks), and also other methods like thermomaceration, microwave maceration, cryomaceration and ultrasound maceration. All experiments were conducted between 2008 and 2010 in the Enology Laboratory of the Horticulture Faculty of University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iași, Romania.

In order to obtain the wines, the following procedures were used:

Classical maceration-fermentation (M-V1)

After grape crushing and destemming, the marc treated with cu 3–4 g/hL SO₂, in order to prevent oxidation and spontaneous fermentation, was placed in 100 L static tanks, with the addition of pectolytic enzymes (Zymorouge G[®]), that enhance the extraction of polyphenols from skins to must (Gherghină *et al.*, 2009).

After 3–4 hours, the mark was inoculated with selected yeasts (*Saccharomyces oviformis* from Research and Development Station for Viticulture and Winemaking Iași, Romania collection, isolated from Copou vineyard), with non-foaming properties, alcohol proficient and temperature resilient. Marc homogenization was done 3–4 times/day and the fermentation temperature never exceeded 25°C. The fermenting must was separated from the lees by using a hydraulic press when the alcohol content reached 7–9%. The remainder of the fermentation process was carried-out in standard fermentation vessels, also constantly reducing the upper gas space.

By creating an anaerobiosis environment in the presence of carbon dioxide, one insured the favorable conditions for the stimulation and propagation of malo-lactic bacteria from the spontaneous microflora. Thus, the

malolactic fermentation took place in autumn, right after the end of the alcoholic fermentation. After the end of the biological activities, the wine was decanted; SO₂ was added, along with the appropriate amount of gelatin to clarify the wine. The young wine was kept at 8–10°C in full tanks. After 3 months, the SO₂ quantity was corrected, the wine was filtered, bottled and analyzed.

Maceration-fermentation in rotating tanks (M-V2)

After crushing and destemming, 3–4 g/hL SO₂ was added to the marc, which was placed in a rotating micro-tank, along with pectolytic enzymes. After 3–4 hours, the marc was inoculated with active yeast leaven (the same one used at the classical method). The rotating micro-tank offered the possibility of easy marc homogenization; the maceration duration was 2–3 days and the rotating speed was six rotations/minute, working for 5 minutes every hour, alternating the direction. After the maceration process ended, the marc was pressed and the obtained wine sustained identical treatments as in the previous case (malolactic fermentation, sulphitation, gelatin clearing, filtering, conditioning, bottling and analysis).

In the first two experimental variants, the extraction of polyphenolic compounds from skins and seeds to must was conducted simultaneously with the alcoholic fermentation process, fact that beneficially influenced the maceration process.

Microwave maceration (M-V3)

After grape crushing and destemming, the partially decanted and lightly sulphitated marc was placed in a microwave oven (modified for the purpose of this experiment) and maintained for 15 minutes at 650 W.

The radiation heated the small water quantities inside the cells, transforming it into vapors, which led to an increase in intracellular pressure and ultimately to the destruction of cellular walls and membranes, thus releasing cellular constituents (and also the polyphenolic compounds) in must (Mandal *et al.*, 2007, Niculaua *et al.*, 2008; Tudose-Sandu-Ville *et al.*, 2009, 2010).

Immediately after the microwave treatment, the marc was pressed and the must was cooled-down to 18–20°C, mixed with the untreated one and placed in fermentation tanks. The must was inoculated with yeasts (*Saccharomyces oviformis* from Research and Development Station for Viticulture and Winemaking Iași collection) and fermented. Because all the endogenous bacteria was destroyed by the radiation, in order to start and conduct the malolactic fermentation, wine containing these bacteria was used (1,5–2% of the total wine volume). After the end of the malolactic fermentation, the wine underwent all of the treatments previously mentioned in the first two technological variants.

Thermomaceration (M-V4)

The partially decanted and sulphitated marc (2–4 g/hL SO₂) was placed in a thermo-winemaker, heated to 65–70°C in the core and maintained for 30 minutes; the heating agent used was water. After thermomaceration, the marc was pressed and the resulted must was assembled with the free-run unheated must. Favorable conditions for the biological processes to take place were created (alcoholic and malolactic fermentations), like in the case of the previous proposed experimental variant.

Cryomaceration (M-V5)

In order to extract the phenolic compounds from skins through

cryomaceration, whole grapes were used; these were slowly frozen to favor the forming of big ice crystals inside the plant cells, which, in turn, perforate the cellular walls, releasing their content in the intercellular medium (Roșca, 2007). Cryomaceration took place at –25°C for 24 hours. After this process, the frozen grapes were crushed, destemmed, thawed and heated to 10 °C, sulphitated (3–4 g/hL SO₂) and pressed. The resulting must was heated to 18–20°C, placed in fermentation tanks and inoculated with the same yeasts as in the previous cases. Also, the malolactic fermentation and treatments were conducted like in the first two variants.

Ultrasound maceration (M-V6)

After crushing and destemming, the sulphitated marc (3–4 g/hL SO₂) was placed in an ultrasound vat (2000 W), in order to destroy the cellular walls and to extract the cellular contents into must (at 35 KHz). After 15 minutes of ultrasound maceration, the marc was pressed and the resulted must was processed like in the previous cases.

All wine samples were subjected to physico-chemical analyses (density, alcoholic content, total and volatile acidity, free and total SO₂, reducing sugars, total dry extract, non-reducing extract, glycerol content and pH). Also, several specific spectrophotometric analyses (Folin-Ciocalteu index, total polyphenolic index and total anthocyan content) and chromatographic analyses (anthocyan profile) were conducted.

RESULTS AND DISCUSSION

In order to obtain the wines proposed by this experiment, homogenous grape raw matter was used, harvested in the same day and from the same lot. The differences

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between the analyzed components of wines appeared due only to the initial winemaking procedures and treatments applied. These components

essentially determine the particularities of the wines obtained by the proposed technological variants (*Table 1*).

Table 1 – Main compositional characteristics of Merlot wines, 2008

Characteristic	Experimental variants					
	M-V1	M-V2	M-V3	M-V4	M-V5	M-V6
Alcohol (% vol)	11,8	12,1	12,0	11,9	12,2	11,8
Reducing sugars (g/L)	1,22	1,36	0,78	0,92	1,16	1,55
Total acidity (g/L C ₄ H ₆ O ₆)	6,08	6,73	6,34	6,85	5,44	6,37
Volatile acidity (g/L CH ₃ COOH)	0,46	0,44	0,48	0,44	0,52	0,56
pH	3,66	3,51	3,63	3,55	3,78	3,61
Free SO ₂ (mg/L)	26	18	25	27	15	20
Bound SO ₂ (mg/L)	79	68	81	73	53	77
Total SO ₂ (mg/L)	105	86	106	100	68	97
Total dry extract (g/L)	25,42	26,36	22,08	24,42	26,16	23,75
Non-reducing extract (g/L)	24,2	25,0	21,3	23,5	25,0	22,2
Glycerol (g/L)	8,1	8,9	8,3	8,7	9,2	8,3

From the 2008 harvest one obtained dry red Merlot wines with alcoholic contents between 11,8 and 12,2% vol., according to the technology used in grape processing. The 0,4% vol. difference is due to the alcohol losses that took place during the tumultuous fermentation stage when, with the release of carbon dioxide, small quantities of alcohol were volatilized.

All the wine samples were fermented until most of the sugars were depleted; thus, the samples are red dry wines, with only 0,78–1,55 g/L remnant sugars.

The acidity of the obtained wines registered normal values in all cases, between 5,44 and 6,73 g/L C₄H₆O₆. Because at harvest the grapes had a total acidity of 8,2 g/L C₄H₆O₆, favorable conditions for the malolactic fermentation were created, thus

obtaining wines with a balanced acidity.

The real acidity of wine (pH) registered values between 3,5 and 3,78, in regard to the applied winemaking technique. By using cryomaceration in grape processing, bigger quantities of tartaric acid were neutralized, thus leading to the lowering of total acidity to 5,44 g/L C₄H₆O₆ and to an increase in pH to 3,78.

The most extractive wines were obtained by using the lees maceration-fermentation techniques (M-V1 and M-V2) and cryomaceration (M-V5); in these cases, the non-reducing extract content varied between 24–25 g/L. Wines obtained through microwave maceration and ultrasound maceration had lower non-reducing extract concentrations (21–22 g/L),

fact that prevents them to be classified as DOC wines.

Wine polyphenols are represented mainly by anthocyanins and tannins, with a decisive influence on wine's phenolic character and traits.

The evaluation of wine's polyphenolic content is done by determining the total polyphenolic index (T.P.I.), the Folin-Ciocalteu index (F.C.I) and total anthocyan concentration; the obtained results are presented in *Table 2*.

Table 2 – F.C.I., total polyphenolic and anthocyan contents of Merlot wines, 2008

Maceration variant	F.C.I.	Total polyphenols (mg/L)	Anthocyanins (mg/L)
M-V1 (classic)	43,82	2750	301,2
M-V2 (rotating tanks)	43,28	2860	301,9
M-V3 (microwave)	27,53	1820	298,4
M-V4 (thermomaceration)	32,17	1970	290,2
M-V5 (cryomaceration)	22,81	1510	166,1
M-V6 (ultrasound)	13,20	1030	127,8

The Folin-Ciocalteu index is specific only to phenolic compounds with reducing properties; this index registered values that varied between 13,2 (M-V6) and 43,28 (M-V2); the lowest results were registered in the case of the wines obtained through cryomaceration (M-V5) – 22,81 and through ultrasound maceration (M-V6) – 13,2.

The total polyphenolic compounds quantities reflected the fact that the maceration process used played a defining role in the quality of the red wines. The samples obtained through classic maceration (M-V1), rotating tanks maceration (M-V2) and thermomaceration (M-V4) registered the highest values in this regard, varying between 1970 mg/L (M-V4) and 2860 mg/L (M-V2). Through cryomaceration (M-V5) the obtained wines were atypical in regard to grape variety and vineyard, with only 1510 mg/L total phenolic compounds.

When the maceration process took place simultaneously with the fermentation process (M-V1 and M-V2), anthocyan extraction was enhanced by the beneficial influences of alcohol, temperature, marc homogenization, pH variation etc. For the first to proposed variants, the anthocyan content of wines were normal, between 301,2 mg/L (M-V1) – 301,9 mg/L (M-V2), considering the fact that the harvest year presented less-than-favorable climatic conditions, leading to smaller quantities of polyphenolic compounds being accumulated in grape skins.

By using cryomaceration (M-V5), the wine contains significantly smaller quantities of anthocyanins (166 mg/L), in comparison to the wines obtained by using other proposed technologies (for instance, this concentration is only 55% of the quantity extracted through classic means, which is 301 mg/L).

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Ultrasound maceration did not favor anthocyan extraction from grape skins and the obtained wines had only 127,2 mg/L (M-V6). One can say that this experimental technique does not exploit the full potential of the variety, but can be regarded as a good technology in obtaining rose wines, when only a moderate extraction of phenolic compounds is required (Tudose-Sandu-Ville, 2012).

By knowing wine's anthocyan profile, one can determine the variety of the grapes used in obtaining that particular wine and can also confirm its authenticity and tipicity. The analyses were conducted through HPLC, the anthocyan being individualized with the help of an

ultraviolet detector. For each chromatogram, the participation percentages of each anthocyan were identified and calculated: delphinidin-3-monoglicoside (Dp), cyanidin-3-monoglicoside (Ci), petunidin-3-monoglicoside (Pt), peonidin-3-monoglicoside (Po), malvidin-3-monoglicoside (Mv), peonidin-3-monoglicoside-acetilate (Po-a), malvidin-3-monoglicoside-acetilate (Mv-a), peonidin-3-monoglicoside-cumarilate (Po-c) and malvidin-3-monoglicoside-cumarilate (Mv-c).

Tables 3 and 4 contain the participation percentages of the main free and acylated anthocyan in the 2008 Merlot wines.

Table 3 – Participation percentages of free anthocyan in Merlot 2008 wines

Maceration variant	Free anthocyan (participation, %)				
	Dp	Ci	Pt	Po	Mv
M-V1 (classic)	11,32	2,24	10,48	12,30	40,92
M-V2 (rotating tanks)	9,71	2,89	10,20	13,39	41,55
M-V3 (microwave)	10,95	1,57	11,58	10,41	42,99
M-V4 (thermomaceration)	7,40	0,64	10,45	6,33	63,53
M-V5 (cryomaceration)	0,74	0,22	2,43	7,54	80,27
M-V6 (ultrasound)	8,13	1,30	9,77	11,30	58,77

Table 4 – Participation percentages of acylated derivates of peonidin and malvidin in 2008 Merlot wines

Maceration variant	3-gl-a		3-gl-c		Σ (%a + %c)	$\Sigma\%a / \Sigma\%c$
	Po	Mv	Po	Mv		
M-V1 (classic)	4,08	10,78	2,92	4,86	22,74	1,92
M-V2 (rotating tanks)	4,56	11,40	2,34	3,96	22,26	2,54
M-V3 (microwave)	4,76	10,25	2,67	4,82	22,50	2,01
M-V4 (thermomaceration)	1,59	4,56	1,19	4,31	11,65	1,12
M-V5 (cryomaceration)	0,82	5,72	0,55	1,71	8,80	2,89
M-V6 (ultrasound)	2,33	5,55	0,65	2,20	10,73	2,76

From the anthocyan profile analysis, one can conclude that in the color composition malvidin was predominant (40,9–80,2%), in various percentages, in regard to the technology used.

The cryomaceration process produced wines with different anthocyan profile than the other ones; thus, the participation percentages of the free anthocyan were: 80% malvidin, 7,5% peonidin, 2,4% petunidin, and delphinidin and cyanidin had subunitary values. The results obtained for the other variants were more similar. Except for the wines obtained through cryomaceration, one can conclude that the free anthocyan profile of Merlot wine consists in: malvidin (40,9–63,5%), peonidin (6,3–12,3%), petunidin (9,7–11,5%), delphinidin (7,4–11,3%) and cyanidin (1,3–2,9%).

By using ultrasound maceration, the obtained red wines contain a small quantity of anthocyan (127 mg/L), but the anthocyan profile is similar to the ones of the other obtained wines, thus keeping the overall allure of Merlot variety. Out of the total 89,2% free anthocyan, malvidin comprised of 58,8%, peonidin 11,3%, petunidin 9,7%, delphinidin 8,1% and cyanidin 1,3%.

The results analysis can reflect that the Merlot wines contain a higher quantity of acetylated anthocyan, in comparison to cumarilated anthocyan, fact observed in their ratios, between 1,1 and 2,89.

The sum of acylated anthocyan varied between 8,8 and 22,7%,

according to the maceration technique. The wines obtained through classical maceration (M-V1), rotating tank maceration (M-V2) and microwave maceration (M-V3) contained the highest proportions of acylated anthocyan, consisting in 23% of the overall anthocyan profile; the other three proposed variants (thermomaceration, cryomaceration and ultrasound maceration) produced wines with decreased acylated anthocyan content, (8,8–11,6% of total).

By analyzing the anthocyan fingerprint, one can state that Merlot wines had a higher percentage of acylated anthocyan (8,8–22,7%), fact that grants them a better resistance and a higher color stability during the maturation and aging processes.

CONCLUSIONS

Even in the years with less-than-favorable climatic conditions, from Merlot grape variety one can produce quality wines by using maceration-fermentation technologies (classical and in rotating tanks), thermomaceration and cryomaceration, according to the current legislation on wine quality levels.

The anthocyan content in wines was different, according to the maceration technology used; the results varied greatly, between 127,8 mg/L, when ultrasound maceration was used, and 301,9 mg/L, when the wines were obtained through rotating

tank maceration-fermentation. Ultrasound maceration did not favor anthocyan extraction from grape skins and the overall polyphenolic content in these wines is specific to rose vines.

In regard to grape variety and maceration method used, free anthocyanins were found in wines in different proportions, between 77 and 91%. In all the wine samples, malvidin was found in the highest proportion, followed, in order, by peonidin, petunidin and delphinidin.

The acylated anthocyan contents (acetylated and cumarilated) varied in regard to the maceration variant employed. The existing ratio between acetylated and cumarilated anthocyanins has a small value for Merlot wines (between 1,1 and 2,8). By knowing this parameter, one can determine the grape variety used in making red wines, because it doesn't vary according to vineyard or harvest year.

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