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Effects of partial dehydration techniques on the metabolite composition in 'Refošk' grape berries and wine*

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Abstract: The aim of the study was to investigate the impact of the on-vine double maturation raisonnée (DMR) and off-vine berry partial dehydration in the chamber (PDC) on the chemical characteristics of the grape and wine grapevine cultivar 'Refošk'. A liquid chromatography-mass spectrometry (LC-MS) system was used for the identification and quantification of phenolics and the HPLC for sugars and organic acids. Berries subjected to DMR partial dehydration reached technological maturity (18.5 °Brix) within 14 days of cane cutting and those under PDC in 22 days upon harvest. The greatest decline in berry weight was recorded in the PDC treatment (14%), followed by the DMR technique (approx. 8%). Both dehydrations significantly increased titratable and total acidity in berry and wine, while the pH level was significantly lower in DMR treated berries. Compared to PDC, DMR significantly increased the content of total flavonols and anthocyanins in berries and wine, as well as the total flavanols in berries and stilbenoids in wine. DMR treated berries contained 1.7-fold higher content of total anthocyanins, up to 2-fold higher content of total flavonols, and 1.6-fold higher total phenolic content in the skin compared to those from PDC treatment. Moreover, it increased the content of total anthocyanins, stilbenoids, and flavonol glycosides in wine. On the other hand, PDC treatment showed greater impact on phenolics in wine, especially with an increase of total hydroxycinnamic acids, flavanols, and total phenolics but with a decrease in anthocyanins contents. The study suggests that berries subjected to DMR respond differently to partial dehydration than those subjected to PDC, which was evident from the accumulation of secondary metabolites. Undoubtedly, DMR showed interesting results regarding berry and wine composition and therefore it could be introduced in the vineyard for production of dry wine as well.

Key words: Dehydration, anthocyanins, organic acids, phenolics, 'Refošk', sugars

1. Introduction

Grape quality is reflected in a balanced composition of primary and secondary metabolites, which constitute different parts of the fresh berries during their development and ripening. The accumulation of these metabolites within the berries is affected by genetic characteristics, cultivation practices, and climate conditions (Jackson and Lombard, 1993).

The phenolic compounds in the skins of red berry grapevine varieties are mostly anthocyanins, hydroxycinnamic acids, stilbenoids, flavanols, and flavonols, which are also the building blocks of the grapes' and wines' sensorial properties (Jackson and Lombard, 1993; Gonzalez-Alvarez et al., 2013). In red grapes, anthocyanins are responsible for the red color of the skins and consequently define the color and quality of the must and wine (Liang et al., 2008). Other polyphenols, such as flavonols and flavanols, mostly contribute to wine flavor and astringency (Li et al., 2009). Phenolic compounds are biosynthesized through the phenylpropanoid and flavonoid pathways and originate from the amino acid precursor, phenylalanine. These pathways are among the most studied and thoroughly described secondary metabolic pathways in plants (Yang et al., 2012). Polyphenolic compounds play an important role in the sensorial quality of grapes and wines. Their transformation during the vinification process directly or indirectly influences the quality of the wine: its structure and sensorial properties (Cheynier, 2005; Noguerol-Pato et al., 2013).

In order to change the composition of fresh berries and improve the quality of the wines produced from these, different canopy techniques, especially training systems, bunch thinning, defoliation, dehydration etc., have been introduced in viticulture (Kyraleou et al., 2015; Liu et al., 2015). Grape dehydration, used for the production of late harvest, botrytized, and ice wines, represents a measure applied for the production of sweeter wines in general, in which the contents of soluble solids, phenolics, and aroma

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compounds are usually altered in respect to the dry wine (Figueiredo-Gonzalez et al., 2013).

Mencarelli and Tonutti (2013) have summarized common on- and off-vine grape (partial dehydration in chamber (PDC)) refinement techniques, usually used for dehydration and their significant impacts on grape composition. Sun-drying is the oldest used technique for off-vine grape dehydration, which increases the content of soluble solids and phenolics in grape berries, especially hydroxycinnamic acids (caftaric, coutaric, and fertaric acid), flavanols (catechin, epicatechin, and procyanidins) and flavonols (kaempferol and quercetin glycosides) (Mencarelli and Tonutti, 2013).

The double maturation raisonnée (DMR) or double reasoned maturation first reported by Cargnello et al. (1996) and later by Carbonneau and Murisier (2009) was introduced as an on-vine berry partial dehydration technique for the production of dry wines. The same authors claim that DMR accelerates wine aging especially regarding phenolics synthesis. Double reasoned maturation proposes the cutting (wounding) of bearing canes during ripening when the berries have reached the determined composition of soluble solids. After the cutting, the berries were left for partial dehydration on vines until technological ripeness is reached. Until today, studies of the DMR technique have demonstrated the method's capability of altering soluble solids, organic acids, and phenolic compounds in berries and wines (Bonghi et al., 2012; Corso et al., 2013). DMR is still considered a less-known viticultural technique although it might replace expensive grape partial dehydration in chambers.

'Refošk' (*Vitis vinifera* L.) is a high-yielding red grapevine cultivar traditionally cultivated in the neighboring wine regions of three countries, namely Friuli-Venezia Giulia (Italy), Primorska (Slovenia), and Hrvatska Istra (Croatia), where denominations 'Teran' and 'Terrano d'Istria' are also used for the cultivar 'Refošk'. Generally, 'Refošk' produces soft berries with a low individual berry weight (about 3 g) according to OIV descriptor 503 (OIV descriptors, 2001), colorless flesh with a moderate must yield (up to 75%), and a thin blackish-blue berry skin (average thickness up to 150 μm), which is rather susceptible to sun burns. Furthermore, high contents of phenolics are accumulated in the skins of this cultivar, especially anthocyanins and stilbenoids. Therefore, the wines produced from 'Refošk' are often promoted as abundant in resveratrol, which is supposed to have a positive impact on human health (de la Lastral and Villegas, 2007).

The aim of the present study was to investigate the effect of these two different partial dehydrations techniques, especially the DMR method, on the berry maturation of the high-yielding grapevine cultivar 'Refošk'. Since such a study has never been performed to date, the experiment was conducted with a view to determine (i) the detailed phenolic fingerprint of the 'Refošk' berry skin and wine, (ii) the dynamics of phenol synthesis during berry ripening, and (iii) the specific responses of the primary and secondary metabolites in the berry skins to different techniques of partial dehydration, aimed to improve grape and wine quality.

2. Material and methods

2.1. Experiment

The experiment was carried out in 2013 on 12-year-old 'Refošk' grapevines (*Vitis vinifera* L.), flourishing in terra rossa soil (the "red earth"; a type of red clay soil formed by the weathering of limestone), located in a nonirrigated vineyard in the sub-Mediterranean part of Slovenia, the winegrowing district (45°08′N; 13°75′E). The vines were grafted on SO4 rootstocks, trained on a double guyot, and planted on a distance of 1.0 m × 2.3 m. Three treatments were established: (i) C, control (common harvest practice in which the grapes were harvested when soluble solids reached around 18.5 °Brix; grapes of the control treatment reached technological maturity on 10 October); (ii) DMR, double maturation raisonnée (bearing shoots were cut when soluble solids reached 17.0 °Brix; bunches were left on the vines for partial dehydration until soluble solids reached the same content as in the control and PDC, around 18.5 °Brix, then the DMR grapes were harvested); and (iii) PDC, partial dehydration in chamber (the bunches were harvested when soluble solids reached 17.0 °Brix and placed in a drying chamber with air circulation until soluble solids reached the exact content as in the control and DMR, around 18.5 °Brix). The experiment was conducted in five blocks, each randomly built up of three treatments. Each treatment was composed of ten consecutive vines per block. Irrespective of the treatment, the berries reached 17.0 °Brix on 23 September (1st sampling), when the grapes for the PDC treatment were harvested, placed in perforate cases, transported to the drying chamber, and partially dehydrated with air circulation in darkness. Ambient air temperature was controlled using a thermostat in the range of 22–23 °C while outdoor air was pumped in by ventilators, retaining a relative humidity in the chamber of around 60%. On the same day (23 September) DMR was implemented, the 1-year old canes were cut, whereupon bunches were left on the vines for partial dehydration. The ripening of the control and DMR grapes had been exposed to the same environmental conditions (Figure 1). The second sampling in all treatments was performed when the berries reached technological maturity commonly defined for the 'Refošk' cultivar, meaning the content of the soluble solids reaching around 18.5 °Brix. The berries of the control treatment reached technological maturity on 10 October, the DMR grapes on 7 October (14 days

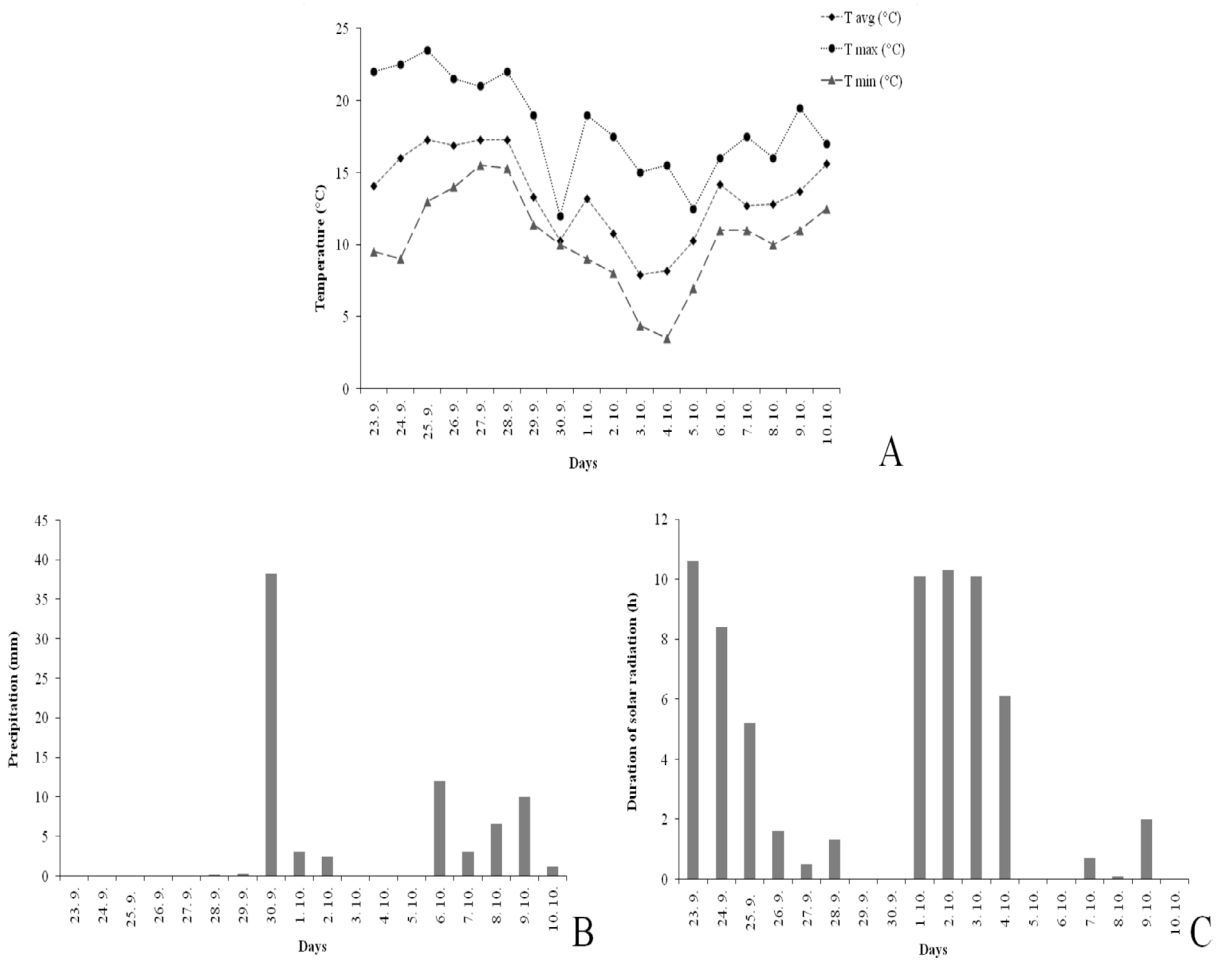

Figure 1. Meteorological data of the weather station Godnje during the experimental period from 23 September to 10 October 2013 (A): Average, maximal and minimal daily temperature (°C). (B): Average daily precipitation (mm). (C): Average daily duration of solar radiation (h).

after cane cutting, partial dehydration), and the PDC grapes on 15 October (22 days after harvest and partial dehydration in the drying chamber). The berries, sampled in five replications per treatment, were stored in PVC bags at –80 °C until further analyses. Furthermore, a respective number of berry skins per treatment were also dried at 105 °C for 72 h to determine water loss and dry weight content (Skupien, 2006).

The harvested grapes, from each treatment, were divided into four equal parts and then vinificated under the same conditions. The grapes were first stemmed; then berries were crushed and the maceration lasted for 10 days. At the beginning of the maceration, 20 g h L^{-1} of rehydrated yeast *Saccharomyces cerevisiae* Mycoferm ROUGE was added to each vessel, according to the producer instruction (INRA, Bordeaux, France). During the maceration, punching down the cap of grape marc was manually done twice per day, and at the end of maceration they were pressed separately per treatment and replicate. Obtained semifermented must was decanted into individual 30-L tanks, where the wine fermentation continued for the next 14 days. After that, the wine was decanted and 25 mL h L^{-1} of 5% sulfuric acid was added to the individual tank to prevent wine oxidation. Wine samples were taken from each tank (n = 4 per treatment) for further analysis.

2.2. Berry characteristics and wine analysis

Firstly, the weight of 100 berries was recorded in five replicates per sample. The content of soluble solids was assessed with a digital refractometer (ATAGO PAL87S) and expressed in °Brix. The initial pH value of the grape juice was recorded before measuring titratable acidity according to the method by Ough and Amerine (1988). A solution of 0.1 M NaOH was added to the sample with a semiautomatic titrator until a pH value of 7.0 was reached, while the required volume was recorded to calculate the

titratable acidity, which was expressed in grams of tartaric acid per liter. To determine alcohol content in the wine, pH, total acidity, total extract, lactic, malic, and tartaric content, a sample of wine (50 mL) was picked from each tank. Chemical analyses were carried out using a WineScan FT120 (Foss, Denmark).

2.3. Chemicals

For the quantification of sugars, organic acids, and phenolic compounds the following standards were used: glucose, fructose, tartaric acid, *p*-coumaric acid, caffeic acid, procyanidin B1, catechin, epicatechin, kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, cyanidin-3-*O*-galactoside, and myricetin-3-*O*-rhamnoside from Fluka Chemie GmBH (Buchs, Switzerland); citric acid, quercetin-3-*O*-rutinoside, quercetin-3-*O*-xyloside, peonidin-3-*O*-glucoside, resveratrol, caftaric acid, and cyanidin-3-*O*-glucoside from Sigma-Aldrich (St. Louis, MO, USA); malic acid and gallic acid from Merck KGaA (Darmstadt, Germany); and isorhamnetin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and peonidin-3-*O*-glucoside from Extrasynthese (Genay Cedex, France). Moreover, 1% (w/v) butylated hydroxyl-toluene (BHT) from Sigma-Aldrich GmBH was used to prevent the oxidation of individual phenolics. To determine total phenolic content, Folin–Ciocalteu reagent from Merck KGaA (Darmstadt, Germany) was used. Methanol was from Sigma-Aldrich GmBH. The chemicals used for the mobile phases were acetonitrile HPLC-MS grade and formic acid from Sigma-Aldrich GmBH (St. Louis, MO, USA). Water was purified and twice distilled with a Milli-Q-system (Millipore, Bedford, MA, USA).

2.4. Extraction and determination of sugars and organic acids

The extraction of sugars and organic acids followed the method reported by Rusjan et al. (2008). For each replication per treatment, 20 berries were crushed and 1 mL of obtained grape juice was diluted with double distilled water (1:10, v/v). Diluted grape juice was centrifuged and filtrated. The separation of sugars and organic acids was done according to the HPLC method described by Mikulic-Petkovsek et al. (2012a). The contents of all analyzed sugars and organic acids were expressed in g L^{-1} and summed up and presented as total sugar and acid contents in g L^{-1}, respectively.

2.5. Extraction of phenolic compounds

The extraction of phenolic compounds from the dry berry skins was performed as described by Mikulic-Petkovsek et al. (2012b, 2015). Berry skins (0.1 g) were extracted with 5 mL of methanol containing 3% (v/v) formic acid and 1% (w/v) 2.6-di-tert-butyl-4-methylphenol (BHT). Extraction was performed in five repetitions per treatment.

2.6. Determination of individual phenolic compounds using HPLC-DAD-MS^n analysis

Phenolic compounds were analyzed on a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA, USA) with a diode array detector (DAD) at 280 nm (flavanols, hydroxycinnamic acid derivatives), 350 nm (flavonols), and 530 nm (anthocyanins) according to the chromatographic conditions as it was previously described in the study by Mikulic-Petkovsek et al. (2012b, 2015). All phenolic compounds were identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionization (ESI) operating in negative and positive (for anthocyanins) ion mode. Contents of phenolics were expressed in mg 100 g^{-1} dry weight (DW) of berry skin and mg L^{-1} of wine. For phenolics missing standards, quantification was carried out using similar compounds as standards.

2.7. Determination of total phenolic content

The same extraction procedure of berry skins used for individual phenolics, but without BHT, were used to prepare extracts for total phenolics content analysis. The total phenolic content (TPC) of the extracts was assessed by the Folin–Ciocalteu phenol reagent protocol (Singleton et al., 1999). Total phenolic content was expressed as gallic acid equivalents (GAE) in mg 100 g^{-1} dry weight (DW) of berry skin and in mg L^{-1} of wine.

2.8. Statistical analysis

The data were analyzed with the Statgraphics Plus 4.0 program (Manugistics, Inc.) using one-way analysis of variance (ANOVA) and some data of the produced wine were analyzed by principal component analysis (PCA). The significance of the treatment on the content of primary and secondary compounds was analyzed separately for each sampling, using the LSD multiple range test with a significance level of $P \leq 0.05$. Means and standard errors are presented (mean ± SE) and statistical differences among treatments are denoted by different letters.

3. Results

3.1. Berry and wine characteristics, and sugar and organic acid contents

The meteorological data depict increased solar radiation on most days during the experimental period (as high as 10 h of sunny weather per day) and only 4 days of cloudy weather (Figure 1). Additionally, intense daily temperature oscillations, which potentially caused changes in the berry metabolism, were recorded.

All berries were characterized by comparable soluble solids contents, approx. 18.5 °Brix, which is common for the technologically mature berries of the high-yielding grapevine cultivar 'Refošk'. Single berry weight, one of the main indicative factors of grape ripeness, increased 11%

from the first sampling until harvest in control plants. However, berry weight decreased by 8% in DMR and by 13% in PDC treatment (Table 1). In a detailed study on individual sugars' accumulation, no significant impact of partial dehydrations was recorded (Table 1). Glucose (from 86.8 to 88.1 g L^{-1}) and fructose (from 83.3 to 84.9 g L^{-1}) represented the main sugars in the examined grape berries (approx. 98% of the total sugars). Sucrose was only detected in small amounts (from 2.7 to 5.3 g L^{-1}) (Table 1). The highest rate of sugar increase was measured in DMR treated berries (0.93 g L^{-1} per day), followed by PDC (0.64 g L^{-1} per day) and control berries (0.62 g L^{-1} per day).

The titratable acidity of DMR and PDC treated berries increased by 12% and 6% by the second sampling, respectively; however, an average decrease of 0.08 g L^{-1} per day was observed in the control, with an average increase of 0.14 g L^{-1} per day in DMR and 0.10 g L^{-1} per day in PDC treatments. The most abundant organic acid in 'Refošk' grapes was malic acid. In the technologically mature DMR and PDC berries a significantly higher content of malic acid was measured in comparison to the control. The same response to partial dehydration was observed in terms of tartaric acid; its content was 1.2- to 1.4-fold higher in DMR and PDC compared to the control, respectively. Furthermore, the content of tartaric acid decreased by 18% and malic acid by 5% from the first to the second sampling. Fumaric, shikimic, and citric acids were only detected in traces (less than 0.5 g L^{-1}). However, the partial dehydration of the berries resulted in a significantly higher content of total organic acids in DMR (27% increase) and PDC (30% increase) berries compared to the control. When it comes to the pH value of the analyzed berries, the most interesting response was observed since the significantly lowest pH level was measured in DMR in respect to the

Table 1. Grapevine cultivar 'Refošk' (*Vitis vinifera* L.) characteristics treated with different dehydration techniques.

Grape characteristics	1st sampling§	2nd sampling		
	23 Sep.	10 Oct.	7 Oct.	15 Oct.
		Control	DMR	PDC
Weight of 100 berries (g)	205.7 ± 7.5	227.1 ± 5.5 c*	190.5 ± 2.0 b	178.4 ± 6.5 a
Soluble solids (°Brix)	17.6 ± 0.4	18.4 ± 0.2 a	18.8 ± 0.2 a	18.6 ± 0.2 a
Titratable acidity (g L^{-1})	11.6 ± 0.5	10.1 ± 0.1 a	13.1 ± 0.4 b	12.3 ± 0.2 b
pH level	2.52 ± 0.06	2.96 ± 0.05 b	2.54 ± 0.04 a	2.74 ± 0.03 b
Fructose (g L^{-1})	79.3 ± 1.9	84.9 ± 0.7 a	83.3 ± 1.0 a	84.9 ± 1.9 a
Glucose (g L^{-1})	81.7 ± 1.1	86.9 ± 0.7 a	88.1 ± 0.6 a	87.8 ± 1.5 a
Sucrose (g L^{-1})	2.69 ± 0.51	2.75 ± 0.86 a	5.32 ± 0.18 b	5.22 ± 0.19 b
Total sugars (g L^{-1})	163.8 ± 2.08	174.5 ± 1.7 a	176.7 ± 0.8 a	177.9 ± 2.7 a
Tartaric acid (g L^{-1})	4.91 ± 0.40	4.04 ± 0.14 a	5.13 ± 0.32 b	5.46 ± 0.46 b
Malic acid (g L^{-1})	7.87 ± 0.54	7.48 ± 0.12 a	9.56 ± 0.73 b	9.61 ± 0.46 b
Total acidity (g L^{-1})	13.2 ± 0.9	11.9 ± 0.1a	15.2 ± 0.9 b	15.5 ± 0.9 b
WINES				
Alcohol (vol %)		10.3 ± 0.03a	10.5 ± 0.04a	10.4 ± 0.02a
pH		3.03 ± 0.01a	3.00 ± 0.05a	3.13 ± 0.07a
Lactic acid		0.075 ± 0.03a	-	0.66 ± 0.02b
Malic acid		5.25 ± 0.03a	6.23 ± 0.08c	5.52 ± 0.08b
Tartaric acid		4.93 ± 0.04b	5.40 ± 0.05c	3.70 ± 0.09a
Total acidity		10.65 ± 0.09a	11.70 ± 0.5b	12.47 ± 0.3b
Total extract		27.35 ± 0.06a	28.37 ± 0.08b	30.02 ± 0.18c

*Different letters in rows of the 2nd sampling of berry skin or among different types of wine denote significant differences (LSD test, $P < 0.05$) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).
§ at first sampling no significant differences were observed in analyzed characteristics among treatments; therefore an average with standard error of all treatments is given.

control and PDC treatment. The data suggest that the pH level of partially dehydrated grape berries subjected to DMR remains stable.

Wine composition undoubtedly reflects the contents of primary and secondary metabolites in grape berries. Regarding the soluble solids content in berries, insignificant differences in alcohol contents of wines among treatments were expected and they ranged between 10.3 and 10.5 vol % (Table 1). Significantly lower pH in grape from DMR treatment did not influence the pH in wine. The measured contents of organic acids in wines reflected the grape total acidity, where a wine from PDC berries had the significantly highest content and total acidity (for 17%), followed by DMR (for 9.5%) compared to the control wine. The total acidity of the produced wines could be primarily attributed to the content of malic and tartaric acid. The content of malic acid prevailed, especially in DMR wine. According to the measured content of lactic acid, we can observe that the spontaneous malolactic fermentation did not expire in the produced wine. Partial dehydration of berries also resulted in significantly higher content of total extract in wines; the significantly highest was measured in PDC treatment, followed by DMR, with the lowest in the control wine (Table 1).

3.2. Phenolic compounds in berry skins

Anthocyanins were the most abundant phenolic compounds in berry skins (approx. 93% of the total analyzed phenolic compounds in 'Refošk'), followed by flavonols, flavanols, and stilbenoids (Table 2; Figure 2). Irrespective of treatment, malvidin glycosides represented 48%–52%, delphinidin glycosides 17%–18%, petunidin glycosides 14%–18%, peonidin glycosides 10%–12%, and cyanidin glycosides 3%–5% of the total analyzed anthocyanins (TAA) in berry skins. Malvidin-3-glucoside was the major anthocyanin from the group of malvidin derivatives, determined in the analyzed berry skins, followed by malvidin-3-acetylglucoside and malvidin-3-coumaroylglucoside.

Table 2. Anthocyanins (mg 100 g^{-1} DW) in berry skin of grapevine cultivar 'Refošk' (*Vitis vinifera* L.) treated with different dehydration techniques.

	1st sampling§		2nd sampling					
Phenolics	23 Sep.		10 Oct.		7 Oct.		15 Oct.	
		%‡	Control	%	DMR	%	PDC	%
Cyanidin-3-glucoside	158.2 ± 17.8	2.3	158.9 ± 14.1 b*	2.8	99.0 ± 8.3 a	1.3	109.9 ± 9.9 a	2.4
Cyanidin-3-acetylglucoside	52.7 ± 5.2	0.7	45.01 ± 4.51 ab	0.8	51.5 ± 4.5 b	0.7	36.7 ± 3.3 a	0.7
Delphinidin-3-glucoside	858.1 ± 62.2	12.2	739.8 ± 34.0 b	13.1	875.3 ± 38.5 c	11.3	453.2 ± 25.5 a	10.0
Delphinidin-3-acetylglucoside	201.2 ± 20.3	2.7	114.7 ± 7.3 a	2.0	203.8 ± 16.3 b	2.6	116.5 ± 11.4 a	2.5
Delphinidin-3-coumaroylglucoside	201.1 ± 20.1	2.8	176.9 ± 16.4 a	3.1	242.1 ± 19.3 b	3.1	148.1 ± 13.0 a	3.2
Malvidin-3-glucoside	2328 ± 247	35.4	2065 ± 92 a	36.5	2778 ± 269 b	35.1	1661.2 ± 93.4 a	37.3
Malvidin-3-acetylgalactoside	9.59 ± 0.93	0.1	6.91 ± 0.84 a	0.1	11.80 ± 1.00 b	0.2	8.44 ± 0.94 ab	0.2
Malvidin-3-acetylglucoside	531.9 ± 42.0	7.9	376.1 ± 36.3 a	6.6	678.3 ± 45.9 b	8.7	408.7 ± 42.5 a	8.7
Malvidin-3-coumaroylglucoside	499.1 ± 39.1	7.7	378.9 ± 39.5 a	6.6	671.1 ± 73.5 b	8.3	337.9 ± 31.6 a	7.3
Petunidin-3-glucoside	738.5 ± 62.1	10.9	683.0 ± 66.7 ab	11.9	875.4 ± 43.2 b	11.3	582.2 ± 66.4 a	12.6
Petunidin-3-acetylglucoside	142.9 ± 15.7	2.0	108.2 ± 12.9 a	1.9	162.1 ± 8.3 b	2.1	80.0 ± 7.3 a	1.8
Petunidin-3-coumaroylglucoside	175.7 ± 12.5	2.5	146.4 ± 14.3 a	2.6	222.0 ± 21.0 b	2.8	117.2 ± 21.6 a	2.6
Peonidin-3-glucoside	630.5 ± 72.5	9.4	529.1 ± 44.2 a	9.2	755.2 ± 82.1 b	9.5	355.4 ± 32.7 a	7.8
Peonidin-3-acetylglucoside	83.4 ± 9.2	1.2	58.9 ± 5.7 a	1.0	97.8 ± 10.2 b	1.2	50.3 ± 6.4 a	1.1
Peonidin-3-coumaroylglucoside	122.93 ± 13.2	1.9	93.3 ± 9.7 a	1.6	145.3 ± 16.5 b	1.8	67.8 ± 6.3 a	1.5
Total anthocyanins	6734 ± 528	86.2	5682 ± 358 a	90.2	7869 ± 600 b	89.4	4533 ± 294 a	88.6

*Different letters in rows of the 2nd sampling denote significant differences (LSD test, $P < 0.05$) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).

§ at first sampling no significant differences were observed in analyzed characteristics among treatments; therefore an average with standard error of all treatments is given.

‡ % - percentage of each phenolic compound according to total content of associated phenolic group within individual treatment and percentage of each phenolic group according to total analyzed phenolics within individual treatment.

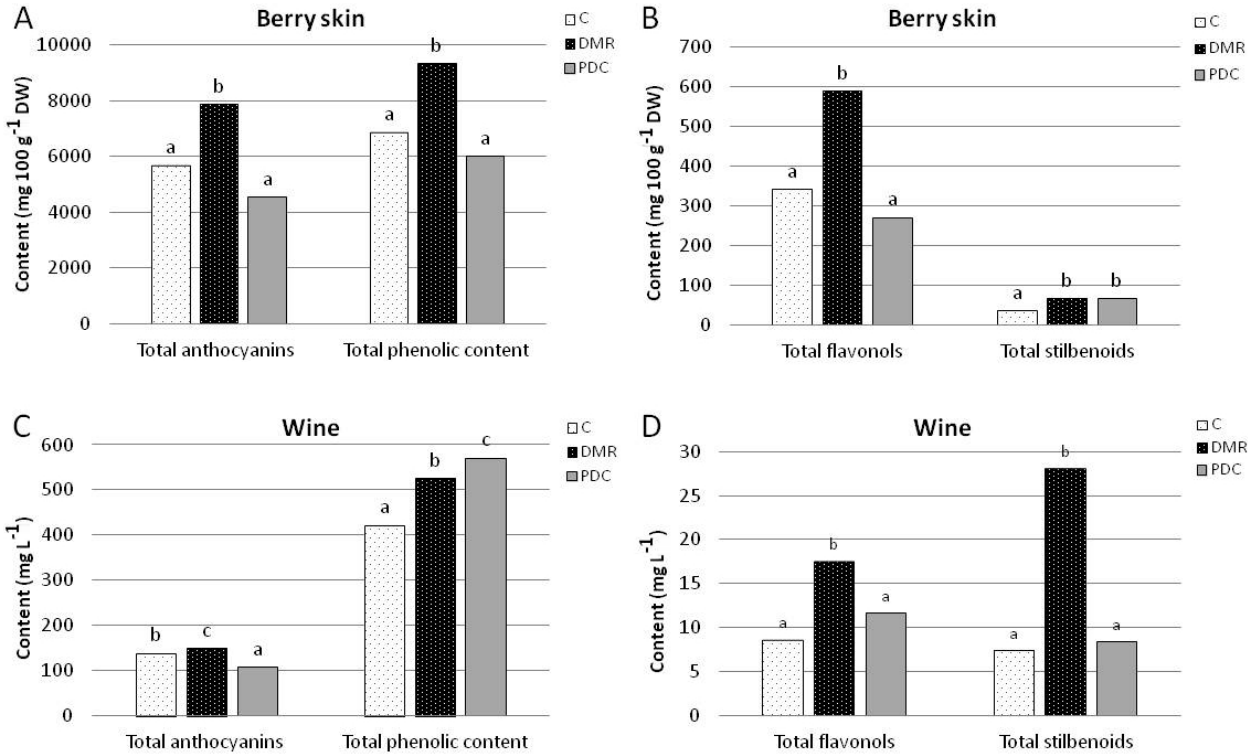

Figure 2. Content of phenolics from different phenolic groups in berry skins of grapevine cultivar 'Refošk' (*Vitis vinifera* L.) (mg 100 g^{-1} DW) (A, B) and refošk wine (mg L^{-1}) (C, D) treated with different dehydration techniques.

The malvidin aglycones prevailed in wine where 15 anthocyanins were identified and quantified (Table 3). However, malvidin-3-glucoside with cyanidin-3-glucoside and petunidin-3-glucoside represented 50%–60% of TAA in refošk wine. Moreover, several anthocyanins, which were not present in berry skin, have been identified in wine. Total anthocyanin content of the refošk wine ranged from 106.8 to 148.7 mg L^{-1}.

DMR treatment significantly increased the total anthocyanin content in the skins compared to the control and PDC treatment, reaching 38% higher levels in comparison to the control and 73% higher than PDC berries (Table 2; Figure 2). Based on the anthocyanin content in the berry skins we can assume that 'Refošk' grapes reached their optimal phenolic maturity on the day of the first sampling but in order to increase the berries' sugar content the bunches were left on the vines for an additional 17 days for the control treatment, 14 days for DMR, and 22 days for PDC treated berries.

A 50% increase in delphinidin-, malvidin-, petunidin-, and peonidin-3-acetylglucoside, and malvidin- and petunidin-3-coumaroylglucoside content was measured in DMR berries compared to the control treatment at the second sampling. In contrarst, the content of cyanidin-3-glucoside significantly decreased in DMR berries (Table 2). Interestingly, the content of individual and total anthocyanin was not significantly different between the control and PDC treatment, except in terms of cyanidin- and delphinidin-3-glucoside contents.

Significant differences in the contents of individual anthocyanins were also observed among wines of different treatments (Table 3). Wine prepared from DMR berries contained significantly higher anthocyanins contents when compared to the wine of other treatments. DMR wine contained 7% higher content of TAA in comparison to the control wine. Moreover, PDC wine was characterized by 23% lower content of TAA compared to the wine prepared from control berries (Figure 2).

The highest share of total analyzed flavonols (TAF) in the berry skins of 'Refošk' was represented by myricetin glycosides (45%), followed by quercetin glycosides (35%), syringetin glycosides (10%), kaempferol glycosides (5%), and isorhamnetin glycosides (5%) (Table 4). The major flavonols were myricetin-3-glucoside and quercetin-3-glucoside, combined representing 3/4 of TAF. The content of all individual flavonols decreased in range from 36% to 69% from the 1st to the 2nd sampling in the control berries (Table 4). 'Refošk' berry skin in the control treatment contained 340.3 mg 100 g^{-1} DW TAF at harvest. In comparison to that, approximately 1.7-fold higher levels were measured in the berry skins of the DMR treatment and 13% lower TAF levels in PDC treatment (Figure 2).

Table 3. Anthocyanins (mg L^{-1}) in refošk wine prepared from grapes of different dehydration techniques.

Phenolics	Control	%‡	DMR	%	PDC	%
Cyanidin-3-glucoside	34.02 ± 1.32b*	24.6	25.74 ± 1.62a	17.3	25.83 ± 1.43a	24.2
Malvinidin-3-acetylglucoside	2.25 ± 0.07a	1.6	2.69 ± 0.17b	1.8	1.93 ± 0.11a	1.8
Malvinidin-3,6-*p*-coumaroyl-glucoside	8.92 ± 0.32b	6.5	11.82 ± 0.17c	8.0	7.62 ± 0.12a	7.2
Malvinidin-3-glucose-ethyl(epi)catechin 1	1.08 ± 0.17a	0.8	3.07 ± 0.22b	2.1	0.96 ± 0.11a	0.9
Malvinidin-3-glucose-ethyl(epi)catechin 2	11.94 ± 0.41b	8.7	16.12 ± 0.48c	10.9	8.11 ± 0.67a	7.6
Malvinidin-3-glucoside	18.62 ± 1.19a	13.5	25.98 ± 1.80b	17.5	15.99 ± 1.28a	15.0
Peonidin-3-acetylglucoside	3.29 ± 0.09b	2.4	3.78 ± 0.12c	2.5	2.31 ± 0.12a	2.2
Peonidin-3,6-*p*-coumaroyl-glucoside	2.07 ± 0.07a	1.5	2.41 ± 0.18ab	1.6	2.86 ± 0.18b	2.7
Peonidin-3-glucose-ethyl(epi)catechin	0.87 ± 0.26ab	0.6	1.48 ± 0.21b	1.0	0.35 ± 0.13a	0.3
Peonidin-3-glucoside	13.65 ± 0.56b	9.9	18.36 ± 1.15c	12.1	10.78 ± 0.08a	10.1
Petunidin-3-acetylglucoside	5.60 ± 0.12b	4.1	5.03 ± 0.10a	3.4	5.23 ± 0.16ab	4.9
Petunidin-3-glucoside	30.58 ± 1.30c	22.2	26.36 ± 0.77b	17.7	20.73 ± 0.37a	19.4
Pyrano-malvidin-3-glucoside (vitisin B)	2.51 ± 0.31b	1.8	2.46 ± 0.06b	1.7	1.33 ± 0.17a	1.2
Pyrano-malvidin-3-*p*-coumaroylglucoside	1.08 ± 0.15a	0.8	2.11 ± 0.29b	1.4	1.68 ± 0.13b	1.6
Coumaroylvitisin A	1.47 ± 0.037b	1.1	1.58 ± 0.11b	1.1	1.07 ± 0.04a	1.0
Total anthocyanins	137.98 ± 1.25b	32.8	148.70 ± 4.17c	56.8	106.82 ± 2.40a	23.1

*Different letters in rows denote significant differences (LSD test, $P < 0.05$) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).
‡ % - percentage of each phenolic compound according to total content of associated phenolic group within individual treatment and percentage of each phenolic group according to total analyzed phenolics within individual treatment.

In contrarst to the berry skin, the contents of TAF in wine were relatively low, ranging from 8.65 to 17.48 mg L^{-1} (Table 5). Wine produced from DMR berries contained 2-fold higher TAF than the control wine. However, no significant differences in TAF content were determined between wines produced from PDC and control berries (Figure 2).

From the group of hydroxycinnamic acids (HCA) only *p*-coumaric acid hexose was detected in the berry skins of 'Refošk' (Table 4). Grape skin contained from 0.72 to 1.48 mg 100 g^{-1} DW of *p*-coumaric acid hexose and its content generally decreased from the first to the second sampling. However, the lowest content of *p*-coumaric acid hexose was measured in PDC treated berries.

However, seven hydroxycinnamic acid derivatives were identified in refošk wine (Table 5). Irrespective of the treatment, the most abundant was caftaric acid, which ranged from 11.43 to 20.68 mg L^{-1}. The remaining hydroxycinnamic acid derivatives were detected in contents below 4.13 mg L^{-1}. Wine prepared from DMR berries contained significantly lower contents of *p*-coumaric acid derivative 1 and 2, *trans*-fertaric acid, and caftaric acid compared to the control and PDC wine. On the other hand, wines prepared from PDC berries were characterized by 1.1–1.8 higher contents of hydroxycinnamic acid derivatives than the control.

Procyanidin dimer was the only phenol from the group of flavanols identified in berry skin. Its content ranged from 215.7 to 244.6 mg 100 g^{-1} DW on the second sampling (Table 4). A significantly higher procyanidin dimer content was quantified in DMR berries compared to the control. On the other hand, PDC treatment did not impact the content of the identified flavanol. Six flavanols were identified in wine refošk and the most abundant were catechin and procyanidin trimer (Table 5). The results suggested that DMR significantly decreased the total flavanol content and most individual flavanols in wines, which cannot be affirmed for epicatechin. On the other hand, a significant increase in total flavanols was observed in wine produced from PDC berries, especially of epicatechin, procyanidin tertramer, and trimer.

Two resveratrol hexosides were identified and quantified from the group of stilbenoids in the berry skins, which were termed resveratrol hexosides 1 and 2, according to their retention (elution) time characteristics. The fragmentation patterns suggest these compounds to be *cis*- and *trans*-resveratrol-3-glucosides previously reported in berry skins by Chiva-Blanch et al. (2011). The contents of both identified stilbenoids decreased during grape maturation (Table 4), which is in accordance with

Table 4. Phenolic compounds (mg 100 g^{-1} DW) in berry skins of grapevine cultivar 'Refošk' (*Vitis vinifera* L.) treated with different dehydration techniques.

	1st sampling§				2nd sampling			
Phenolics	23 Sep.		10 Oct.		7 Oct.		15 Oct.	
		%‡	control	%	DMR	%	PDC	%
Procyanidin dimer	300.0 ± 21.8	4.4	244.6 ± 17.4 a*	3.6	288.0 ± 20.7 b	3.1	215.7 ± 15.4 a	3.6
p-coumaric acid derivative	1.48 ± 0.16	0.02	1.30 ± 0.14 b	0.02	1.08 ± 0.17 ab	0.01	0.72 ± 0.08 a	0.01
Myricetin-3-galactoside	30.7 ± 2.8	4.3	13.9 ± 1.4 a	4.1	25.0 ± 2.4 b	4.3	17.6 ± 1.3 ab	5.9
Myricetin-3-glucoside	247.8 ± 23.5	36.0	144.8 ± 14.4 a	42.5	210.9 ± 17.8 b	36.1	117.8 ± 6.8 a	39.7
Myricetin-3-glucuronide	5.85 ± 0.52	0.9	3.42 ± 0.33 a	1.0	6.21 ± 0.67 b	1.1	4.16 ± 0.52 ab	1.4
Quercetin-3-galactoside	27.5 ± 2.9	3.9	8.50 ± 0.95 a	2.4	15.7 ± 1.8 b	2.7	11.9 ± 1.8 ab	4.0
Quercetin-3-glucoside	182.9 ± 17.6	25.3	73.7 ± 5.5 a	22.1	106.7 ± 12.2 b	18.5	57.6 ± 4.1 a	19.8
Quercetin-3-glucuronide	56.1 ± 4.2	9.5	25.9 ± 2.7 a	7.4	74.8 ± 8.1 b	12.5	22.4 ± 3.6 a	7.5
Quercetin-3-rutinoside	9.30 ± 0.87	1.4	5.43 ± 0.53 a	1.6	9.41 ± 1.21 b	1.6	5.42 ± 0.93 a	1.8
Kaempferol-3-galactoside	4.16 ± 0.35	0.7	1.91 ± 0.24 a	0.6	6.15 ± 0.93 b	1.0	4.53 ± 0.72 b	1.5
Kaempferol-3-glucoside	8.03 ± 0.95	1.2	3.17 ± 0.43 a	0.9	7.52 ± 0.85 b	1.3	4.61 ± 0.54 a	1.5
Kaempferol-3-coumaroylglucoside	17.4 ± 1.8	2.6	11.0 ± 1.3 a	3.2	19.0 ± 1.7 b	3.2	12.0 ± 1.2 a	4.0
Isorhamnetin-3-glucoside	6.82 ± 0 .72	1.0	3.72 ± 0.46 a	1.1	6.87 ± 5.07 b	1.2	3.57 ± 0.43 a	1.2
Isorhamnetin-3-rutinoside	24.5 ± 1.4	3.8	14.3 ± 1.4 a	4.2	26.6 ± 2.8 b	4.5	10.2 ± 1.2 a	1.4
Syringetin-3-glucoside	45.4 ± 4.2	6.7	17.9 ± 2.4 a	5.2	38.6 ± 3.9 b	6.4	14.7 ± 3.1 a	5.0
Syringetin-3-rutinoside	19.0 ± 1.7	2.8	12.3 ± 1.6 a	12.3	32.9 ± 4.5 b	5.7	10.4 ± 1.3 a	3.4
Total flavonols	685.7 ± 71.5	8.4	340.3 ± 26.7 a	5.4	586.6 ± 47.9 b	6.6	296.9 ± 16.0 a	5.8
Resveratrol-hexoside 1	38.4 ± 2.8	63.3	20.9 ± 2.6 a	60.8	35.7 ± 4.6 b	56.0	47.4 ± 3.8 c	74.7
Resveratrol-hexoside 2	19.6 ± 1.7	36.7	13.7 ± 1.8 a	39.2	28.1 ± 3.2 b	44.0	17.4 ± 1.9 ab	25.3
Total stilbenoids	58.1 ± 5.9	0.7	34.7 ± 4.4 a	0.5	63.9 ± 7.8 b	0.7	65.0 ± 7.4 b	1.3

* Different letters in rows of the 2nd sampling denote significant differences (LSD test, $P < 0.05$) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).
§ at first sampling no significant differences were observed in analyzed characteristics among treatments; therefore an average with standard error of all treatments is given.
‡ % - percentage of each phenolic compound according to total content of associated phenolic group within individual treatment and percentage of each phenolic group according to total analyzed phenolics within individual treatment.

the reports by Moreno et al. (2008). The results suggested that the DMR and PDC treatments significantly increased the content of resveratrol hexosides approximately 1.3 to 2.3 times in comparison to the control. DMR berries contained particularly high levels of resveratrol-hexoside 1. On the other hand, PDC berries were characterized by high contents of resveratrol-hexoside 2. However, the wine produced from DMR berries contained significantly higher content of total stilbenoids compared to the wine prepared from control and PDC berries (3.8- and 3.3-fold, respectively) (Figure 2).

3.3. Total phenolic content in berry skin and wine

The total phenolic content (TPC) is in close connection to the levels of analyzed individual phenolics and thus higher levels of each phenolic compound result in a higher TPC in plant tissues (Mikulic-Petkovsek et al., 2013). In general, the berry skins of DMR treated grapes contained significantly higher levels of total phenolics (9334 mg GAE 100 g^{-1} DW) compared to PDC and control treatment (Table 4). Moreover, PDC berry skins contained approx. 13% less total phenolics compared to the control berries, but the difference between them was insignificant. The wine made from PDC/DMR berries was characterized by 1.35- and 1.24-fold higher total phenolic content in comparison with the control wine, respectively (Figure 2).

3.4. Principal component analysis

Principal component analysis (Figure 3) performed on total acidity, total extract, total hydroxycinnamic acids,

Table 5. Phenolic compounds (mg L^{-1}) in refošk wine prepared from grapes of different dehydration techniques.

Phenolics	Control	%‡	DMR	%	PDC	%
p-coumaric acid derivative 1	2.78 ± 0.14b*	10.5	1.09 ± 0.07a	5.7	3.23 ± 0.10c	8.9
p-coumaric acid derivative 2	2.64 ± 0.12b	9.9	1.44 ± 0.02a	7.6	2.59 ± 0.04b	7.1
p-coumaric acid derivative 3	0.087 ± 0.002a	0.3	0.122 ± 0.002b	0.6	0.152 ± 0.001c	0.4
cis-coutaric acid	1.25 ± 0.03a	4.7	1.74 ± 0.04b	9.1	2.19 ± 0.02c	6.0
trans-coutaric acid	2.24 ± 0.07a	8.4	2.00 ± 0.13a	10.4	3.45 ± 0.06b	9.5
trans-fertaric acid	3.23 ± 0.04b	12.2	1.29 ± 0.02a	6.8	4.13 ± 0.33c	11.4
Caftaric acid	14.36 ± 0.34b	54.0	11.43 ± 0.49a	59.7	20.68 ± 1.05c	56.7
Total hydroxicinamic acid	26.61 ± 0.62b	6.3	19.14 ± 0.71a	7.3	36.44 ± 0.81c	7.9
Catechin	74.37 ± 1.60b	31.0	29.3 ± 0.04a	6.1	87.14 ± 7.51b	29.1
Epicatechin	0.99 ± 0.02a	0.4	1.18 ± 0.03b	2.4	1.72 ± 0.04c	0.6
Procyanidin dimer 1	50.78 ± 0.23b	21.2	25.35 ± 0.23a	52.3	60.82 ± 1.32c	20.4
Procyanidin dimer 2	13.81 ± 0.29b	5.8	7.25 ± 0.05a	15.0	17.33 ± 1.11c	5.8
Procyanidin trimer	73.20 ± 1.58b	30.5	28.8 ± 0.04a	6.0	93.38 ± 3.56c	31.3
Procyanidin tetramer	26.88 ± 0.79b	11.2	8.87 ± 0.74a	18.3	37.95 ± 3.90c	12.8
Total flavanols	240.05 ± 5.01b	57.0	48.48 ± 0.79a	18.5	498.36 ± 3.09c	64.6
Resveratrol hexoside 1	1.78 ± 0.09a	23.8	7.07 ± 1.07b	24.9	1.96 ± 0.18a	24.0
Resveratrol hexoside 2	3.26 ± 0.21a	43.4	12.35 ± 0.77b	44.0	2.53 ± 0.35a	30.2
Resveratrol hexoside 3	2.45 ± 0.42a	32.8	8.70 ± 0.55b	31.1	3.93 ± 0.78a	45.7
Total stilbenoids	7.49 ± 0.33a	1.9	28.13 ± 2.25b	10.7	8.44 ± 1.29a	1.8
Laricitrin-3-glucoside	5.41 ± 0.35a	62.3	14.58 ± 1.96b	83.0	8.41 ± 1.35a	70.9
Quercetin-3-glucuronide	0.96 ± 0.06b	11.0	0.53 ± 0.03a	3.1	1.16 ± 0.06c	10.2
Syringetin-3-glucoside	2.29 ± 0.01b	26.7	2.39 ± 0.06b	13.9	2.09 ± 0.06a	18.8
Total flavonols	8.65 ± 0.41a	2.1	17.48 ± 2.05b	6.7	11.66 ± 1.35a	2.5

*Different letters in rows denote significant differences (LSD test, $P < 0.05$) among treatments: control (common ripening), DMR (Double Maturation Raisonnée – on-vine partial dehydration) and PDC (partial dehydration in chamber).
‡ % - percentage of each phenolic compound according to total content of associated phenolic group within individual treatment and percentage of each phenolic group according to total analyzed phenolics within individual treatment.

total flavanols, total stilbenoids, total flavanols, total anthocyanins, and total phenolic content of refošk wine showed two principal components; the first explained 61.75% and the second 38.24% of the data variability. The analysis showed (data not shown) that the variables with the highest contributions to the first component were total acidity, total extract, total flavanols, total hydroxycinnamic acids, and total phenolic content. The highest contribution in relation to the second component was obtained by total anthocyanins, total flavonols, and total stilbenoids.

4. Discussion

Grape and wine composition depends on many factors, most importantly environmental conditions and cultivation practices. Weather conditions (temperature, precipitation, and insolation) greatly affect the chemical parameters of berries while they are exposed to them on the vine. As for the control and DMR treated berry in the present study, metabolisms were altered by their environment. The greatest impact could potentially be ascribed to the intense solar radiation during the experimental period as control and DMR berries were subjected to as much as 10 h of insolation per day. On the other hand, controlled temperature and darkness in the drying chamber triggered a different berry response, which is in accordance with Mencarelli and Tonutti's (2013) findings. Generally, DMR and PDC practices dehydrate the berries, however, under different conditions. DMR berry dehydration takes place on the vine itself and is affected by specific annual weather conditions, which impact the duration of the dehydration and consequently alter the berry composition. On the other hand, PDC takes place in a drying chamber and is operated by air circulation. Generally spoken, berry dehydration increased the soluble solids content in

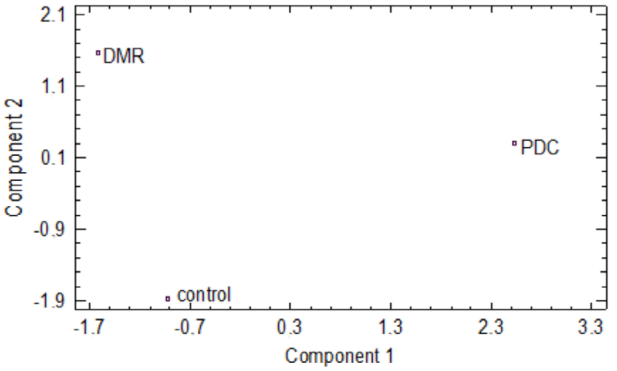

Figure 3. Principal component analysis (PCA) of the results of Refošk wine for total acidity, total extract, total hydroxycinnamic acids, total flavanols, total stilbenoids, total anthocyanins, and total phenolic content according to different dehydration techniques.

grape berries (Corso et al., 2013). Furthermore, a faster concentration or accumulation of soluble solids in DMR grapes despite the slightly slower dehydration with respect to the PDC technique could be attributed to the fact that after cane cutting the leaves on the shoots of the vigorous 'Refošk' cultivar might remained photosynthetically active for a certain period, several days, resulting in an increase in soluble solids in berries. Moreover, periods of high precipitation and lower insolation during the DMR (Figure 1) might have decelerated wilting of leaves and consequently also the photosynthesis. Corso et al. (2013) suggested that increased sugar accumulation in 'Raboso Piave' (*Vitis vinifera* L.) berries under DMR could be associated with xylem backflow and reduced phloem functionality, resulting in berry water loss.

DMR and PDC treatments of 'Refošk' berries increased the total acidity from the 1st to 2nd sampling. Greater importance should be ascribed to the modifications in the pH level due to its impact on wine chemical and microbiological stability. Zoecklein et al. (1999) reported that a grape pH level above 3.60 might negatively impact the wine's sensory attributes. In our study, a higher pH level was only measured in the berries that underwent PDC treatment. There were no differences in pH values among different types of wines. The measured alcohol content in refošk wine was in accordance with the requirements of the Rules on Wine (2000), which was expected according to the aim of the study. In accordance with reports by Cargnello et al. (2006), the PDC and DMR undoubtedly increased the content of total acidity and extract content, which makes wine more pleasant, sweet, and full bodied (Reboredo-Rodriguez et al., 2015).

In terms of the 'Refošk' cultivar slow leaf wilting was observed after cane wounding, suggesting that the leaves remain photosynthetically active for a few days, despite the xylem flow from the rootstock and trunk to the shoots being interrupted. Zsofi et al. (2014) suggested that mild to moderate water stress results in increased anthocyanin synthesis in the berry skins, which could be the case in our study as well. Wine prepared from DMR berries contained significantly higher individual and total anthocyanin contents compared to other treatments, which was expected according to their contents in the berry skin and which was also clearly demonstrated in principal component analysis (Figure 3).

The content of flavonols in 'Refošk' berry skin from DMR was almost 2-fold the content of that from the control, which was also reflected in significantly higher content of total flavonols in DMR wine. Similarly, Corso et al. (2013) detected that the application of DMR induced the accumulation of quercetin. In grape berries, flavonols play a role as UV protectants and are strongly correlated with the accumulated dose of UV-B radiation (Martinez-Luscher et al., 2014), which has also been observed in our experiment in DMR treated vines and the control treatment. Drying in chamber (PDC) resulted in 13% lower content of total flavonols in berry skin. However, the observed decrease in our study was not severe and was insignificant.

A decrease in hydroxycinnamic acid derivative *p*-coumaric acid hexoside was recorded in berry skin after drying in chamber. Similarly, this trend was observed with sun drying, which resulted in 67% loss of hydroxycinnamic acids (HCAs) in comparison to fresh grapes (Karadeniz et al., 2000), proposing that HCAs are subject to oxidative degradation by different enzymatic pathways. Interestingly, wine prepared from PDC berries contained almost 40% higher content of total HCAs compared to the control wine. Contrary to this, DMR wine was characterized by 27% lower contents of HCAs than the control wine.

DMR berries contained higher contents of procyanidin dimer compared to the control and PDC berries. Contrary to the control and PDC, DMR wine had significantly reduced flavanol concentrations. Also Bonghi et al. (2012) found that the procyanidins B1 and B2 reduction in berry skin of 'Raboso Piave' cultivar is in correlation with the DMR technique. The flavanol profile of wine was more diverse compared to the berry skin. Catechin, procyanidin dimer 1, and procyanidin trimer combined represented 65% (DMR wine) to 85% (control) of total analyzed flavanols in wine. PDC wine resulted in 2-fold higher total flavanol content in comparison with the control wine.

It has been established that the synthesis of resveratrol in grape berries and leaves is stimulated by stress such as fungal infection, injury, light penetration, and UV light exposure (Crupi et al., 2013). The results suggested that DMR and PDC treatment significantly increased the content of resveratrol hexosides from 1.3- to 2.2-fold,

compared to the control berries, especially resveratrol-hexoside 1 (PDC) and resveratrol-hexoside 2 (DMR). Possibly, increased levels of stilbenoids in 'Refošk' DMR berries can be ascribed to prolonged exposure to UV light and water deficit in canes and leaves. DMR wine in our study also contained the highest contents of stilbenoids but no significant differences were confirmed between the control and PDC wine.

TPC in the research was significantly higher in DMR berries in comparison with the control and PDC berries. The reason for this result can be primarily attributed to water deficit. Zsofi et al. (2014) observed that berries undergoing moderate water stress contained higher levels of total phenolics in comparison to well-watered plants. As opposed to DMR berries, PDC berries were dehydrated under a controlled atmosphere of the drying chamber: constant temperature and darkness. Therefore, differences in levels of phenolics between these two treatments could be ascribed to diverse dehydrating conditions. Wine prepared from PDC/DMR berries was characterized by 35%/24% higher TPC compared to the control wine, respectively. This can be ascribed to higher total extract content and lower water content of DMR and PDC berries.

The partial dehydrations of berry skin of red grapevine variety with DMR or PDC have undoubtedly different impact on grape and wine composition. PDC and DMR berries increased the content of organic acids, which affected the wine, but pH remained stable only in grapes at DMR. DMR treatment significantly increased the content of anthocyanins, flavonol glycosides, and stilbenoids in berry skin and wine. On the other hand, wine made from dried grapes in chamber (PDC) was characterized by increased contents of hydroxycinnamic acids and flavanols, as well as total phenolics. Significant differences in the levels of phenolic contents between DMR and PDC berries can potentially be ascribed to the different dehydration conditions and water status of the plant upon DMR treatment. In contrast, PDC treatment was conducted under controlled conditions in the drying chamber. It can be concluded that the application of the DMR technique is much simpler, cheaper, and faster compared to partial dehydration in a chamber. Double maturation raisonnée can potentially be utilized for the production of dry 'Refošk' wine.

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