

Effect of the Winemaking Practices and Aging on Phenolic Content of Smederevka and Chardonnay Wines

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Abstract Changes of the polyphenolic content, including total phenolics (TP), total flavonoids (TF), and total flavan-3-ols (TF_{3-ols}) determined by spectrophotometric methods, have been studied in Macedonian white wines from *Vitis vinifera* L. cv, Smederevka and Chardonnay, obtained under different vinifications and analyzed during 16 months of aging in bottles. Winemaking treatments for both varieties included an addition of two doses of SO₂ (50 and 100 mg/L) and two commercial yeasts for fermentation, Vinalco and Levuline, both *Saccharomyces cerevisiae* species, in order to study the influence of SO₂ and yeasts on the phenolic content of the wines. Comparing the two varieties, Chardonnay wines contained higher levels of total phenolics, flavonoids, and flavan-3-ols, probably as a result of the cultivar differences. Sulfur dioxide protected the phenolic oxidation, resulting in higher phenolic content in the wines with higher SO₂ dose, while the yeast did not show significant influence on the total phenolics. During aging, the TP, TF, and TF_{3-ols} in the wines from both

varieties decreased significantly up to the second month of storage and remained stable till the sixth month. The Student–Newman–Keuls test has been applied to ascertain possible significant differences between the studied wines, and a principal component analysis has been employed, showing separation and grouping of the wines according to the cultivar and time of aging.

Keywords Total phenolics · Total flavonoids · Total flavan-3-ols · Spectrophotometry · Smederevka · Chardonnay · Winemaking · SO₂ · Yeast

Introduction

Phenolic compounds are considered as the main factors responsible for the quality of grapes and consequently for the corresponding wines. Wine phenolics belong to two main groups: nonflavonoids and flavonoids. The major nonflavonoid phenolic compounds in white wines are hydroxycinnamic acid derivatives (Lee and Jaworski 1987) which are easily oxidized components contributing to the browning of wines during aging (Cheynier et al. 1990). The most common flavonoids are flavan-3-ols and flavonols (and anthocyanins in red wines). Flavan-3-ols, mainly catechins and catechin–gallate polymers also produce a number of oxidation products that could be regarded as browning agents in white wines (Singleton and Cilliers 1995). Regarding the group of flavonols, quercetin derivatives are the main components present in the white wines (De Beer et al. 2005).

The flavonoid composition of white wines depends on the grape composition, on their extraction into the grape juice, and also, on the subsequent reactions occurring during the vinification, postfermentation treatments, and wine aging (Rayne et al. 2008). Usually, white wines are

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made without aeration in order to avoid extensive contact with oxygen, which, if present, may cause evolution effects during aging (Nevares et al. 2009) as browning of the wine and deterioration of the overall quality. Thus, for white wine production, maceration is kept to a minimum and seldom lasts more than a few hours. Usually, the grape juice runs freely from the crushed grapes followed by the immediate addition of SO₂ in order to protect enzymatic oxidation. Controlled low temperatures (14–18 °C) are usually applied for white wine production.

While there are reports in the literature about the concentration of polyphenols in white wines, mainly obtained using HPLC-DAD (Okamura and Watanabe 1981; Hurtado et al. 1997; Budić-Leto and Lovrić 2002; Jeffery et al. 2008), still there are no data on phenolics in white wines from the Republic of Macedonia. Therefore, it was considered that determination of total phenolics, total flavonoids, and total flavan-3-ols in white wines from the Smederevka and Chardonnay varieties, produced with two doses of SO₂ and two yeasts would be of great importance for obtaining a general overview of their phenolic content and their changes during aging. For that purpose, spectrophotometric methods, being the most convenient, have been used for quantification since they require minimal sample preparation and analysis time. On the other hand, the applied spectrophotometric methods are readily available and applicable for routine quality control of wine during its production and storage.

Materials and Methods

Chemicals

The standard chemicals, (+)-catechin and gallic acid, and the reagent *p*-(dimethylamino)cinnamaldehyde (*p*-DMACA), were purchased from Fluka (Switzerland) and the Folin-Ciocalteu reagent was from Merck (Germany). All the other used reagents were of analytical purity grade.

Vinification Protocol

Grapes from the Smederevka (Sm) and Chardonnay (Ch) varieties were harvested at ~20 and ~22°Brix, respectively, and transported to the experimental cellar of the Department for Enology, Institute of Agriculture, Skopje, Republic of Macedonia. Grapes of both varieties were divided into four lots (15 kg for each lot) and were processed separately using a mechanical crusher/destemmer. Two doses of aqueous solution of potassium metabisulfite were immediately added to the Smederevka grape mashes and were mixed to give two lots with 50 mg/L (Sm50) and two lots with 100 mg/L SO₂ (Sm100). The same procedure was applied on the grape mashes of the Chardonnay variety, yielding also two lots

Ch50 and two lots Ch100. Musts were separated from the pomace by free running at 14–16 °C, allowing minimum skin contact (about 5 min), and collected in 25-L plastic fermentation tanks. The grape juices were then inoculated with two yeasts (*Saccharomyces cerevisiae*) to start the fermentation of the musts: Vinalco (Bitola, Republic of Macedonia) and Levuline CHP (Bordeaux, France). The yeasts were rehydrated (20 g/L for Vinalco and 30 g/L for Levuline) in water (30 °C for 15 min), and the Macedonian yeast, Vinalco (Mac), was applied to two lots of both varieties containing 50 mg/L SO₂ (Sm50-Mac and Ch50-Mac) and to two other lots with 100 mg/L SO₂ (Sm100-Mac and Ch100-Mac). The French yeast, Levuline (Fr), was also applied to the remaining lots containing 50 mg/L SO₂ (Sm50-Fr and Ch50-Fr) and 100 mg/L SO₂ (Sm100-Fr and Ch100-Fr).

After finishing the fermentation, the wines were treated with 1 g/L bentonite and cold stabilized at –4 °C for a period of 2 weeks before bottling. The bottles were stored in a cellar, at a temperature between 12 and 15 °C. In order to follow the changes of polyphenols during aging, the wines were analyzed after fermentation (after 10 days for Smederevka wines and after 7 days for Chardonnay wines), after 2, 6, and 16 months of aging in bottles. The labels of wines are presented in Table 1.

Determination of Total Phenolics, Flavonoids, and Flavan-3-ols

The total phenolic (TP) content of the wines has been determined using the Folin-Ciocalteu method (Slinkard and Singleton 1977; Ivanova et al. 2010). The total flavonoid (TF) content was evaluated according to a colorimetric assay with aluminum chloride (Zhishen et al. 1999). The content of total flavan-3-ols (TF_{3-ols}) was measured using the *p*-DMACA method (Di Stefano et al. 1989).

Statistical Methods

Each wine was analyzed in triplicate. A statistical analysis including calculation of means, standard deviations, and principal component analysis (PCA) was performed using the TANAGRA 1.4.28 (Lyon, France) software. The Student–Newman–Keuls test of multiple comparison of the mean values for TP, TF, and TF_{3-ols} was applied in order to ascertain possible significant differences between the studied white wines.

Results and Discussion

Influence of the Variety, SO₂, and Yeast

Comparing the wines from both varieties, Smederevka and Chardonnay, produced under the same vinification con-

Table 1 Labels for Smederevka and Chardonnay wine samples prepared under different vinifications (SO₂ doses and yeast) and analyzed during aging in bottles

Fermentation with Macedonian yeast				
Phases of analysis	50 mg/L SO ₂		100 mg/L SO ₂	
	Smederevka	Chardonnay	Smederevka	Chardonnay
After fermentation	Sm50-Mac-end fermentation	Ch50-Mac-end fermentation	Sm100-Mac-end fermentation	Ch100-Mac-end fermentation
After 2 months	Sm50-Mac-2m	Ch50-Mac-2m	Sm100-Mac-2m	Ch100-Mac-2m
After 6 months	Sm50-Mac-6m	Ch50-Mac-6m	Sm100-Mac-6m	Ch100-Mac-6m
After 16 months	Sm50-Mac-16m	Ch50-Mac-16m	Sm100-Mac-16m	Ch100-Mac-16m
Fermentation with French yeast				
Phases of analysis	50 mg/L SO ₂		100 mg/L SO ₂	
	Smederevka	Chardonnay	Smederevka	Chardonnay
After fermentation	Sm50-Fr-end fermentation	Ch50-Fr-end fermentation	Sm100-Fr-end fermentation	Ch100-Fr-end fermentation
After 2 months	Sm50-Fr-2m	Ch50-Fr-2m	Sm100-Fr-2m	Ch100-Fr-2m
After 6 months	Sm50-Fr-6m	Ch50-Fr-6m	Sm100-Fr-6m	Ch100-Fr-6m
After 16 months	Sm50-Fr-16m	Ch50-Fr-16m	Sm100-Fr-16m	Ch100-Fr-16m

2m 2 months, 6m 6 months, 16m 16 months

ditions, in general, higher levels of TP, TF, and TF_{3-ols} were determined in Chardonnay wines after the fermentation (Table 2). An analysis of variance revealed statistically significant differences in the content of total phenolics ($p < 0.001$), flavonoids ($p < 0.001$), and flavan-3-ols ($p < 0.001$) between the wines from both varieties, which could be attributed to the cultivar-related differences. Furthermore, agricultural and environmental factors could also influence the content of TP, TF, and TF_{3-ols} in the wines. The results were in accordance with previous studies where the Chardonnay wine contained higher levels of phenolics compared to the Smederevka (Mitić et al. 2010). In addition, the results showed that Macedonian wines Smederevka and Chardonnay contained higher phenolic content compared to the other white varieties (Salacha et al. 2008; Katalinic et al. 2004; Chamkha et al. 2003; Singleton et al. 1999).

Sulfur dioxide acts as an effective antioxidant and prevents the oxidation of phenolics, resulting in a higher

content of phenolic compounds in the wines with a higher dose of sulfur dioxide, as it was observed in this study too. Comparing the Smederevka wines fermented with the same yeast, but containing different SO₂ doses (Table 2), generally a higher content of total phenolics, flavonoids, and flavan-3-ols could be seen in the wines containing a higher dose of SO₂, likely because this agent prevented the phenolics oxidation. A similar trend was observed for TP, TF, and TF_{3-ols} of Chardonnay wines, except for the ones fermented with Macedonian yeast, for which a higher content of TFs and TF_{3-ols} was found in the wines with a lower SO₂ dose. This means that the addition of a higher amount of sulfur dioxide did not lead to a higher content of TF and TF_{3-ols}, as already found by Mayén et al. (1997).

With regard to the yeast influence, little is known about the ability of commercial *Saccharomyces cerevisiae* strains to affect the phenolic content of white wines during the fermentation process and wine aging. A current research

Table 2 Content of total phenolics, flavonoids, and flavan-3-ols of Smederevka and Chardonnay wines, produced under two SO₂ doses (50 and 100 mg/L) and two yeast strains (Vinalco and Levuline) analyzed after fermentation

Vinification conditions	50-Mac		100-Mac		50-Fr		100-Fr	
	Sm	Ch	Sm	Ch	Sm	Ch	Sm	Ch
TP (mg/L)	417a±2.22	855b±8.01	445c±5.83	889d±6.91	349a±3.81	848b±6.99	533c±3.09	975d±6.75
TF (mg/L)	23.8±2.17	321±2.61	31.5±3.85	126±1.83	48.4±1.47	244±4.57	64.7±1.91	303±3.03
TF _{3-ols} (mg/L)	4.03b±0.40	39.6±2.42	7.11c±0.18	24.7d±1.88	6.18b±0.56	25.0a±1.74	10.6 c±0.97	23.3a, d±2.37

Values with the same letter(s) within a row are not significantly different at $p > 0.05$ analyzed by the Student–Newman–Keul's test; 50–50 mg/L SO₂, 100–100 mg/L SO₂; results are presented as average from three replicates ± standard deviation

Sm Smederevka; Ch Chardonnay; Mac Macedonian yeast, Vinalco; Fr French yeast, Levuline

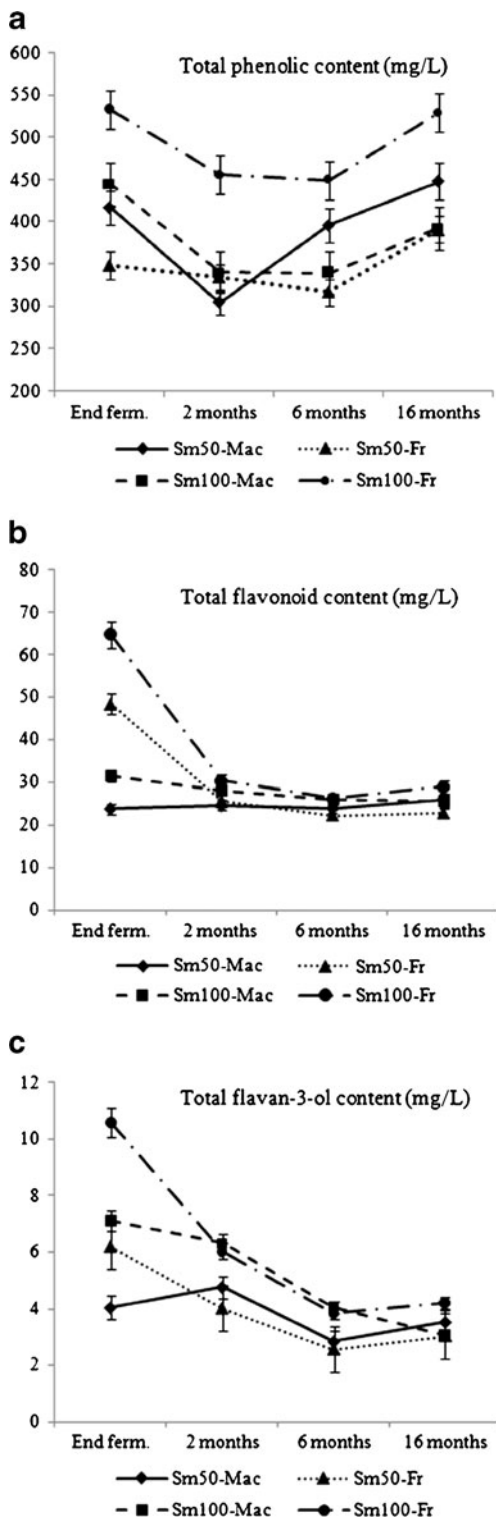


Fig. 1 Changes of **a** total phenolics, **b** total flavonoids, and **c** total flavan-3-ols in Smederevka wines fermented with Macedonian and French yeast, containing 50 and 100 mg/L of SO₂ during storage: after fermentation, after 2, 6, and 16 months. *Error bars* represent standard deviations. *Sm*, Smederevka; *Mac*, Macedonian yeast; *Fr*, French yeast; 50–50 mg/L SO₂, 100–100 mg/L SO₂

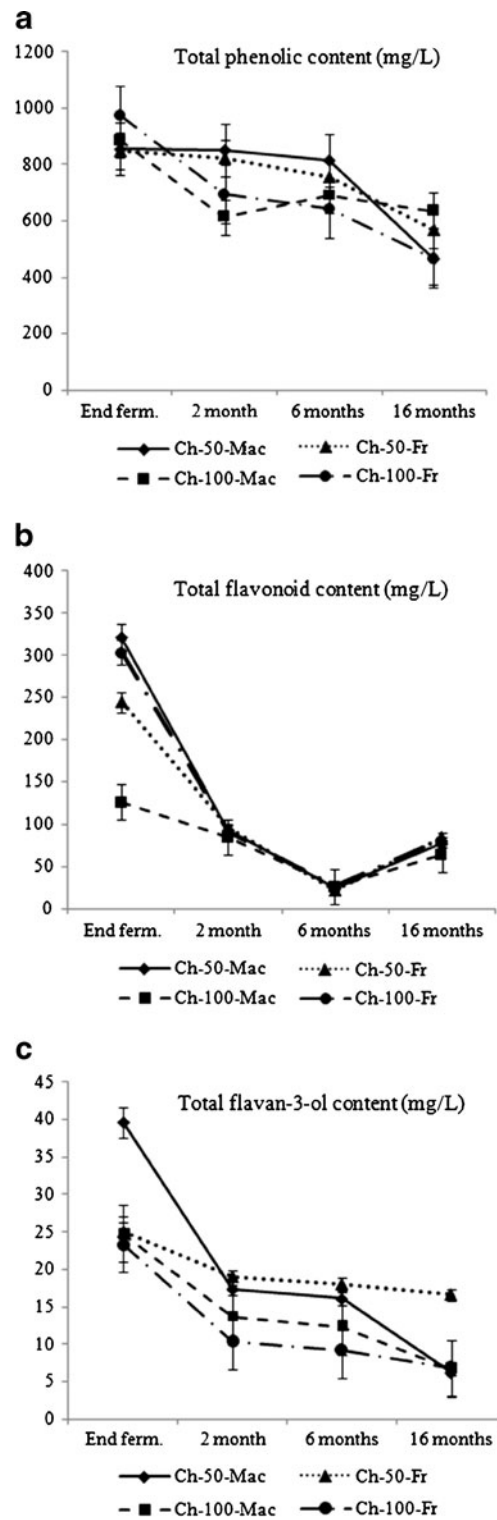
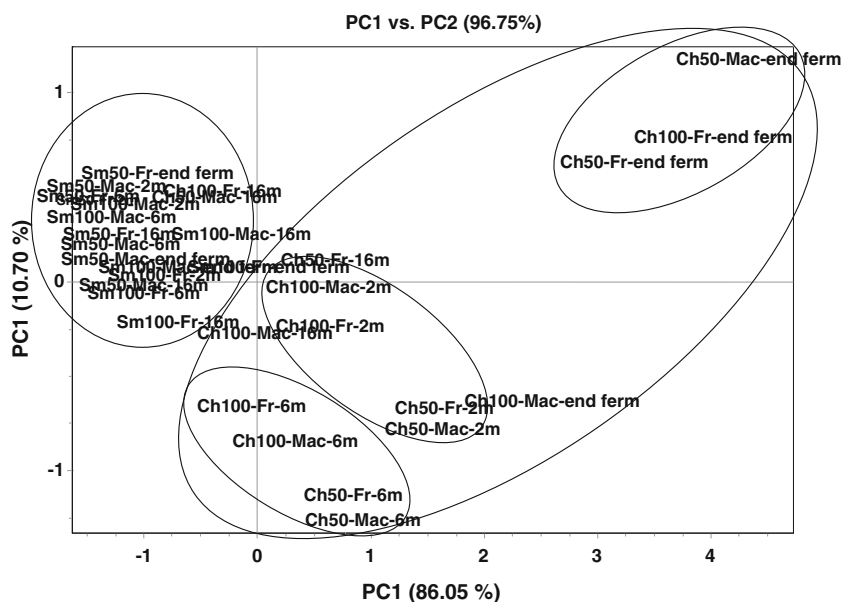


Fig. 2 Changes of **a** total phenolics, **b** total flavonoids, and **c** total flavan-3-ols in Chardonnay wines fermented with Macedonian and French yeast, containing 50 and 100 mg/L of SO₂ during storage: after fermentation, after 2, 6, and 16 months. *Error bars* represent standard deviations. *Ch*, Chardonnay; *Mac*, Macedonian yeast; *Fr*, French yeast; 50–50 mg/L SO₂; 100–100 mg/L SO₂

Fig. 3 Principal component score plot (PC1 and PC2) of Smederevka and Chardonnay wines obtained under different vinification conditions and stored during 16 months, based on the spectrophotometric data for TP, TF, and TF_{3-ols}. *Ch*, Chardonnay; *Mac* Macedonian yeast; *Fr* French yeast; 50–50 mg/L SO₂; 100–100 mg/L SO₂



suggests adsorption of phenolic compounds to the yeast cell walls (Mazauric and Salmon 2005). Comparing the wines from both varieties fermented with the same yeast strain, no significant differences ($p > 0.05$) in the content of TP and TF_{3-ols} were found, except for Ch50 where a higher amount of TF_{3-ols} was noticed in the wine vinified with Vinalco yeast (Table 2). The applied yeasts did not show a significant influence on the TP and TF_{3-ols} probably because of their similar fermentation rates and/or their belonging to the same *Saccharomyces cerevisiae* species, which was in agreement with the previously published data for Merlot wines (Ivanova et al. 2009). On the other hand, Smederevka wines vinified with the Levuline yeast tended to contain a significantly higher amount of TF in comparison to wines fermented with Vinalco yeast (Table 2). This conclusion cannot be made for TF in the studied Chardonnay wines. Anyway, these differences in the content of TF could suggest that yeast can contribute to white wine phenolics, especially to the flavonoid level.

Influence of Aging

The amount of TP, TF, and TF_{3-ols} in the studied Smederevka and Chardonnay wines have been determined in the following four phases: after fermentation, after 2, 6, and 16 months. These results are presented in Figs. 1 and 2.

As stated in the literature (Kallithraka et al. 2009), the total phenolic content decreases during the wine aging. Thus, the content of TP for the wines from both varieties was decreasing significantly ($p < 0.001$) to the second month of storage (except for Sm50 wine), remaining stable till the sixth month of storage. Afterwards, the TP content increased in the Smederevka wines at the 16th month of

storage (Fig. 1a), although this was not observed for Chardonnay wines. The higher final amount of TP could be a result of the increased content of caffeic, *p*-coumaric, and ferulic acids due to the possible hydrolysis of the esters of tartaric acids (caftaric, coutaric, fertaric) during storage. On the other hand, the decreased total phenolic content, observed in Chardonnay wines, is presumably a result of oxidation of the phenolic compounds such as flavonoids and flavan-3-ols, which are considered as significant contributors to the overall total phenolic content, and their degradation leads to changes of their content by loss of reactive hydroxyl groups (Singleton et al. 1999). The changes of flavan-3-ol monomers, (+)-catechin and (–)-epicatechin lead to a decrease of TP with aging. Furthermore, benzoic acids, such as gallic acid, also decrease significantly during the storage period, contributing to the TP reduction. In addition, a formation of protein–tannin complexes has also been shown to contribute towards a decrease in phenolic content of white wine (Singleton et al. 1979; Betés-Saura et al. 1996).

With regard to the content of flavonoids in Smederevka and Chardonnay wines (Figs. 1b and 2b), a significant

Table 3 Loadings of the features in the first three principal components for the analyzed Smederevka and Chardonnay wines

	PC1	PC2	PC3
TP	0.9115	–0.3846	–0.1456
TF	0.9017	0.4152	–0.11201
TF _{3-ols}	0.9682	–0.0246	0.249
% of variance	86	11	3

decrease in TF was observed up to the second month of storage, followed by a slight decrease till the sixth month. On the 16th month of storage, no significant differences ($p > 0.05$) between the flavonoid content of the wines aged from 6 to 16 months, from both varieties, were observed, even a slight increase (not significant, $p > 0.05$) in the TF was measured in the Chardonnay wines. The decreased flavonoid content is probably a result of the oxidative degradation, precipitation, or hydrolysis of the flavonols (Zafrilla et al. 2003). The poorly soluble aglycones, formed as a result of hydrolysis, can also precipitate and contribute to the decrease of the total flavonoid content in wines.

The changes of the flavan-3-ols content followed a similar trend to the ones of the flavonoids. Thus, the content of TF_{3-ols} significantly decreased till the second month ($p < 0.001$) in the wines from both varieties. Afterwards, they remained stable during storage (only a slight increase in Smederevka wines was observed but not significant, $p > 0.05$). The reduction of flavan-3-ols in the white wines is affected by the oxidation and polymerization reactions of flavan-3-ols and procyanidins, which have a strong influence on the susceptibility of white wines to browning (Simpson 1982). Furthermore, the storage conditions affect the content of flavan-3-ols since they can undergo hydrolysis, oxidation, and complexation during aging (Zafrilla et al. 2003).

Principal Component Analysis

A principal component analysis has been applied on the results for TP, TF, and TF_{3-ols} in order to discover the similarities and/or differences between the Smederevka and Chardonnay wines, and to evaluate if the parameters: variety, SO₂, yeast, and time of aging could distinguish the studied white wines. The score plot of the first two PCs which account for 96.75% of the total variability of the wine samples shows a clear separation of Smederevka and Chardonnay wines (Fig. 3). Thus, the grouping of the samples was made mainly according to the variety: Chardonnay wines, mainly located in the positive part of PC1 (86.05%) were clearly separated from the Smederevka wines, mainly located in the negative part of the PC1. As can be seen from Table 3, all three parameters TP, TF, and TF_{3-ols} contributing positively to PC1, are responsible for the varietal characterisation and differentiation of both cultivars. In fact, Chardonnay wines contain a higher content of these phenolic compounds in the all-studied aging phases. With regard to PC2, the grouping of the Chardonnay wines was observed according to the storage period. The wines analyzed after fermentation were placed in the positive part of PC2, which were well separated from the wines aged for 2 months and for 6 months (Fig. 3). As can be seen from Table 3, TF is the value with the highest

contribution on the PC2, which means that the separation of the samples was performed according to the flavonoids in a correlation with storage time. No clear separation was observed for Smederevka wines during storage. In general, the grouping between the Smederevka and Chardonnay wines was performed as a result of the cultivar differences in TP, TF, and TF_{3-ols} content. Furthermore, the separation of the Chardonnay wines was based mainly on the differences of the flavonoid content, decreasing in the wines during storage.

Conclusions

This study presents valuable data for the phenolic content of the white wines Smederevka and Chardonnay, produced under different treatments from grape varieties grown in the Republic of Macedonia, as well as useful information for the polyphenolic changes during aging in bottles. In general, the differences between Smederevka and Chardonnay wines were found in the higher content of TP, TF, and TF_{3-ols} for Chardonnay wines probably attributed to the cultivar-related differences. The higher amount of sulfur dioxide affected the higher content of total phenolics, flavonoids, and flavan-3-ols in the wines, while the yeast did not show a significant influence on TP and TF-ols, except on the content of TF. During storage, the content of TP was highest after fermentation, followed by a gradual decrease up to the second month, and remained stable till the sixth month of storage. The evolution trend of the total flavonoids and flavan-3-ols for the wines from both varieties was similar showing a gradual decrease of the content during wine aging.

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