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Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods

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Abstract: Wines and grapes contain a large array of phenolic compounds belonging to non-flavonoids and flavonoids. This study evaluates the polyphenolic contents of six commercial red and white Macedonian wines and four grape varieties. Spectrophotometric methods were applied for the determination of the total phenolics, the total flavonoids, the total anthocyanins and the total catechins. The efficiency of acetone/water (80/20) and methanol/water (80/20) solutions for the extraction of polyphenols from grape pulp, seeds and skins were compared. The best extraction efficiency was achieved using acetone/water. The obtained results showed that Macedonian grapes are rich in polyphenols, whereby the highest concentration of total phenolics was found for Vranec grapes. The analyzed wines contained high contents of polyphenol; the highest contents were found for Disan wine produced from the Vranec variety of grapes (1515 mg/L total phenolics, 1103 mg/L total flavonoids, 237 mg/L total anthocyanins and 845 mg/L total catechins). Principal component analysis was employed to check possible groupings of the studied red and white wine samples. A clear separation of white wines from red ones was observed.

Keywords: wine; grape; polyphenols; spectrophotometry; berry extraction.

INTRODUCTION

Wines and grapes contain a number of polyphenolic constituents classified as flavonoids and non-flavonoids that play a major role in enology. They contribute to the sensory characteristics of wine, especially colour, flavour and astringency and, therefore, to the differences between red and white wines.[1] The family of wine flavonoids includes flavonols, flavanols and anthocyanins, whereby

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the non-flavonoids include phenolic acids (hydroxybenzoic and hydroxycinnamic acids and their derivatives) and stilbenes. Red wines contain all the above phenolics, while white wines contain mainly phenolic acids and flavanols.

Grape anthocyanins are red pigments, located in the first external layers of the hypodermal tissue and mainly in the vacuoles,[2] as well as in special structures called anthocyanoplasts,[3] while the teinturier varieties contain anthocyanins also in the pulp cells. The most important grape anthocyanins are the 3-glucoside forms of cyanidin, peonidin, petunidin, delphinidin and malvidin.[4] Flavonols are located in the solid parts of grapes, particularly in the skin and herbaceous parts and are mainly present as the 3-glycosides and 3-glucuronides of quercetin and myricetin, the 3-glucosides of kaempferol and isorhamnetin, and laricitrin and syringetin, predominantly found as 3-glucosides.[5,6] Catechins are located mainly in the seeds and skins.[7–10] The major monomers are (+)-catechin, (–)-epicatechin and (–)-epicatechin-3-*O*-gallate.

Many authors have studied the phenolic compounds in grapes and wines using HPLC as the most suitable analytical technique.[4,11–18] However, this technique is not available in wineries for routine analyses, whereas spectrophotometric methods, as more affordable techniques with lower expenses, lower reagent consumption and rapid measurements, can be used for wine and grape analyses to follow the changes in the polyphenol contents during grape ripening and their changes during the wine-making process. The most commonly used are methods for the determination of the total phenolics,[19–21] anthocyanins,[22–24] flavan-3--ols[21,25,26] and flavonoids.[27–30]

The FYR Macedonia is characterized by a distinctive habitat, a sub-Mediterranean climate and a very long tradition of grape growing and high quality wine making. Due to the deficiency of data for Macedonian wines and grapes, the purpose of this study was to establish a preliminary database for the polyphenolic contents of selected Macedonian wines and grapes from the Tikveš vineyard area by measuring the content of total phenolics, total anthocyanins, total flavonoids and total catechins, as well as the colour intensity and tint of the red wines.

EXPERIMENTAL

Grape samples

The content of phenolic compounds was determined for the following grape varieties: Vranec, Cabernet Sauvignon, Muscat Hamburg and Riesling (vintage 2006). Grape berries used for this study were grown at the vineyards of the Institute of Agriculture in Skopje. Samples from the cultivars were harvested in their technological ripening stage (22.5, 20.6, 21.1 and 18.5 °Brix, for Vranec, Cabernet Sauvignon, Muscat Hamburg and Riesling, respectively). The sampling was randomly made by picking berries from the top, central, and bottom parts of the clusters. The Vranec samples were collected from an 8-year-old vineyard (13.4 ha); the M. Hamburg and Riesling samples were collected from 18-year-old vineyards (3.1 and 2.5 ha, respectively) and the Cabernet Sauvignon samples from a 7-year-old vineyard (0.1 ha). The distance between the rows was 2.8 m and distance between the vines was 1.2 m. The

samples (10 kg from 30–40 plants were sampled and then reduced to 1 kg for each variety) were kept frozen before analysis. All the determinations were performed in triplicate.

Wine samples

Four red (vintage 2003) and four white wines (vintage 2003) from the Bovin Winery, located in the Tikveš vineyard area, were analyzed. The wines were obtained from the most widespread red cultivars (Vranec, Merlot and Cabernet Sauvignon) and white grape cultivars (Riesling, Smederevka, Sauvignon Blanc and Temjanika).

Instrumentation and reagents

Analysis of the polyphenols was performed with an HP 8452 Agilent UV–Vis spectrophotometer. The reagent *p*-(dimethylamino)cinnamaldehyde (*p*-DMACA), gallic acid and (+)-catechin were purchased from Fluka (Switzerland), and the Folin–Ciocalteu reagent was from Merck (Germany). All the other employed reagents were of analytical grade purity.

Preparation of the grape skins, seeds and pulps for analyses

The pedicels were removed and the berries were manually skinned. The seeds were separated from the pulp, washed with distilled water and then blotted on paper. Skins were blotted on paper towels to remove any residual pulp. The skins and seeds were ground and the pulp was blended. The skins (1 g), seeds (1 g) and pulp (1 g) were extracted twice for 15 min with 10 mL acetone/water (80/20, v/v) containing HCl (0.1/10, v/v) to prevent oxidation of the polyphenols in an ultrasonic bath at room temperature and then stirred for 30 min on a magnetic agitator. After centrifugation (3000 rpm for 10 min), the supernatants from both extractions were combined and made up to a final volume of 25 mL with distilled H_2O. The extracts were filtered through 0.45 μm membrane filters (Iso-Disc Filter, PTFE, Supelco) before spectrophotometric determination of the total phenolics, total anthocyanins, total flavonoids and total catechins.

Total phenolics assay

The Folin–Ciocalteu method[20] was used for the determination of the total phenolics. In brief, an aliquot (1 mL) of the appropriate diluted extracts was added to a 10 mL volumetric flask, containing 5 mL of distilled water. Then, 0.5 mL of Folin-Ciocalteu reagent was added and the contents mixed. After 3 min, 1.5 mL Na_2CO_3 solution of concentration 5 g/L was added and made up to a total volume of 10 mL distilled water. After keeping the samples at 50 °C (water bath) for 16 min in sealed flasks and subsequent cooling, their absorbances were read at 765 nm against distilled water as the blank. A calibration curve was constructed using gallic acid standard solutions (0–100 mg/L). The concentration of total phenolics is expressed as the gallic acid equivalent (GAE) per 1 g of fresh sample. All samples were prepared in triplicate.

Total flavonoids assay

Total flavonoid content was evaluated according to a colorimetric assay with aluminium chloride.[27] A 1 mL aliquot of wine sample or grape extract (appropriately diluted) was added to a 10 mL volumetric flask containing 4 mL of distilled water, followed by the addition of 0.3 mL of solution of $NaNO_2$ (0.5 g/L). After 5 min, 0.3 mL of a 1 g/L solution of $AlCl_3$ was added and 6 min later, 2 mL of NaOH (1 mol/L) was added to the mixture. The total volume was made up to 10 mL with distilled water, the solution was mixed and the absorbance was measured at 510 nm against a water blank. Catechin was used as the standard for the construction of a calibration curve and the concentrations are expressed as catechin equivalents (mg/g CE).

Total anthocyanins assay

The determination of the total anthocyanins was realised by the method proposed by Di Stefano *et al.*[21] The samples were diluted with a solution consisting of 70/30/1 (v/v/v)ethanol/water/HCl (concentrated) and the absorbance was measured at 540 nm. Due to the lack of a malvidin-3-glucoside standard, the total anthocyanic contents are expressed as malvidin-3-glucoside equivalents and calculated using the following equation purposed by Di Stefano, *et al.*:[21]

$$TA_{540\ nm}\,(\text{mg/L}) = A_{540\ nm}16.7d$$

where $A_{540\ nm}$ is the absorbance at 540 nm and d is the dilution.

Total catechins assay

The concentration of total catechins was measured using the *p*-(dimethylamino)cinamaldehyde (*p*-DMACA) method.[22] The contents of catechins in the wines are expressed as catechin equivalents (CE mg/L). An aliquot (1 mL) of an appropriately diluted sample was added to a 10 mL volumetric flask followed by the addition of 3 drops of glycerol and 5 mL *p*-DMACA reagent. The total volume was made up to 10 mL with methanol and after 7 min, the absorbance was read at 640 nm against a methanol blank. The DMACA reagent was prepared immediately before use, and contained 1 % (w/v) DMACA in a cold mixture of methanol and HCl (4:1).

Colour intensity, hue, colour composition and brilliance of the wines

The colour intensity is determined by the content and structure of the anthocyanins present in a wine and is defined as the sum of the absorbances at 420, 520 and 620 nm.[30] The absorbance of a wine was directly measured at 420, 520 and 620 nm using a 2 mm optical path and the colour intensity (*CI*), hue or tint (*T*), proportion of red colour (% *Rd*), proportion of blue colour (% *Bl*), proportion of yellow colour (% *Ye*) and the brilliance of the wine (*dA*) were calculated.[30] The tint of a wine is defined as the ratio A_{420}/A_{520}, and gives a measure of the "tint" or redness of the wine.[30] The colour composition of the wines, expressed as percentage, was calculated according to the following equations:

$$\%\ Ye \text{ or } \%\ Rd \text{ or } \%\ Bl = 100(A_{\lambda}/CI)$$

where: % *Ye* is the percentage of yellow colour (λ = 420 nm) in the overall colour, % *Rd* is the percentage of red colour (λ = 620 nm) and % *Bl* is the percentage of blue colour (λ = 520 nm) in the overall wine colour. The brilliance of a wine was calculated by the expression:

$$dA\ (\%) = (1 - (A_{420} + A_{620}/2A_{520}))\times 100$$

Statistical analysis

Statistical treatment, including means, standard deviations and Principal Component Analyses, was performed using the PC software package TANAGRA 1.4.28 (Lyon, France). The test of Student–Newman–Keul of multiple comparisons of the mean values was applied to the results of the concentrations of the different phenolics to ascertain possible significant differences between the studied wines and grape varieties.

RESULTS AND DISCUSSION

Calibration and accuracy tests for total phenolics, total flavonoids and total catechins methods

The common spectrophotometric method for the determination of the total phenolics content using the Folin–Ciocalteu reagent has been widely used in the area of enology and viticulture. This method is based on oxidation–reduction reactions in which phenolics are oxidised and show maximum absorbance in the wavelength region between 725 and 765 nm. In this study, a procedure based on the reported method[20] was used with some modifications based on testing the effects of temperature and time of the reaction between Folin–Ciocalteu reagent and standard solutions of gallic acid.

The results obtained from the test, for two concentration levels, at ambient temperature and at 50 °C, are presented in Table I as standard deviation (*SD*) and relative error (in %) with respect to the theoretical concentration.

TABLE I. Standard deviations (*SD*) and relative errors (e_r) for different time of reaction at ambient temperature and 50 °C

Time, h (ambient temp.)	Gallic acid (100 mg/L)		Gallic acid (150 mg/L)	
	Concentration found[a], mg/L	e_r / %	Concentration found[a], mg/L	e_r / %
1	98.2 ± 0.64	–1.80	143 ± 0.80	–4.66
1.5	101 ± 1.31	1.00	151 ± 0.98	0.66
2	119 ± 1.32	19.0	161 ± 0.90	7.33
2.5	134 ± 0.69	34.0	166 ± 1.06	10.66
3	147 ± 1.17	47.0	182 ± 0.84	21.33
Time, min (50 °C)	Concentration found[a], mg/L	e_r / %	Concentration found[a], mg/L	e_r / %
10	90.5 ± 0.81	–9.50	137 ± 0.90	–8.66
15	96.4 ± 1.00	–3.60	139 ± 0.96	–7.33
16	101 ± 1.04	1.00	153 ± 1.57	2.00
17	102 ± 0.91	2.00	155 ± 1.41	3.33
18	105 ± 1.07	5.00	161 ± 1.59	7.33
20	105 ± 1.30	5.00	166 ± 1.17	11.0

[a]Values are the average from 3 replicates ± *SD*

As can be seen from Table I, the optimal results, *i.e.*, the lowest relative errors of 1.00 and 0.66 % for both analyzed concentration levels, were obtained when the solutions were allowed to stand for one hour and thirty minutes at ambient temperature. Shorter and longer reaction times caused higher relative errors. A significant reduction of the analysis time, with a very low relative error, was achieved when the prepared solutions were allowed to stand at 50 °C. The best results with lowest relative errors (1.00 and 2.00 %), were obtained when the solution was kept at 50 °C for 16 min, hence this procedure was used for the calibration and for the analysis of the wine and grape skin, seed and pulp samples. The calculated linear dependence of absorbance (A) on the mass concen-

tration (γ) of gallic acid in a series of standard solutions, with correlation coefficient 0.9997, was the following:

$$A(\mathrm{G}) = 0.005385\gamma(\mathrm{G})\ [\mathrm{mg/L}] - 0.007261$$

The accuracy of the procedure was checked using the standard additions method on an actual red wine sample and satisfactory results (Recovery = 102 – – 106 %, Table II) confirmed that the method is accurate and convenient for quantitative analysis.

TABLE II. Accuracy of the standardized methods for the determination of the total phenolics, total flavonoids and total catechins in wine

Standard addition γ / mg L^{-1}	Calculated γ / mg L^{-1}	Experimentally found[a] γ / mg L^{-1}	*SD*	Recovery, %
Total phenolics				
–	–	1118	24.14	–
250	1368	1454	62.08	106
625	1743	1782	36.53	102
1000	2118	2215	44.3	104
Total Flavonoids				
–	–	697	21.95	–
100	797	836	21.98	105
250	945	945	28.82	99.8
400	1097	1037	65.74	94.5
Total Catechins				
–	–	8.3	0.47	–
4	12.3	11.8	0.83	96.3
10	18.3	18.6	1.16	102
16	24.3	23.8	1.26	98.0

[a]Values are the average from 3 replicates

For the determination of the total flavonoids, a chlorometric method using $AlCl_3$ was applied for the analysis of the wines and grape extracts. This method is based on the formation of stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols, which exhibit maximum absorbance at 510 nm. On the other hand, the determination of catechins by the *p*-DMACA assay is based on the formation of coloured products in reaction of this aldehyde reagent with tannins.[25] The absorbances of the resulting products of this reaction involving monomeric flavan-3-ols ((+)-catechin and (-)-epicatechin) are read at 640 nm. For the determination of the total flavonoids and catechins in the analysed wine and grape extracts, calibration diagrams with five concentration points of catechin as the standard were constructed. The obtained linear dependences of the absorption of catechin, A(C) and the mass concentration of catechin, γ(C) in pure solutions, with correlation coefficients 0.9979 for flavonoids (1) and 0.9993 for catechins (2), were the following:

$$A(\mathrm{C}) = 0.002889\,\gamma(\mathrm{C})\ [\mathrm{mg/L}] - 0.03774 \quad (1)$$
$$A(\mathrm{C}) = 0.025183\,\gamma(\mathrm{C})\ [\mathrm{mg/L}] + 0.000627 \quad (2)$$

The accuracy of these spectrophotometric methods was also checked by the standard additions method in red wine (Recovery = 94.5 – 105 % for flavonoids and Recovery = 96.3 – 102 % for catechins) (Table II).

Grape extraction procedures

Most of the procedures for determination of polyphenols in grapes use aqueous methanol or acetone for extraction. Marinova *et al.*[31] used 80 % aqueous methanol for extraction on an ultrasonic bath. Kennedy *et al.*[32] extracted the phenolics from skins with 66 % aqueous acetone during 24 h at 20 °C and evaporated the solvent after filtration of the extract. Montealegre *et al.*[33] used methanol, water and formic acid for extraction of the phenolic compounds from lyophilized skins and seeds. In this work, methanol and acetone (80 % aqueous solutions, v/v with 0.1 % HCl) were tested for the extraction of the phenolic components and the obtained results were compared. The efficiency of extraction was checked by re-extraction of the residue using the same procedure. It was noticed that one extraction step was not enough for the total removal of the analyzed components from the skins, seeds and pulp. Second and third extractions of the residue were performed, whereby no detectable amounts of polyphenols were determined in the third extract. Therefore, the first two extracts were combined and then analyzed. The standard deviations and the sum of concentrations for the total phenolics, total flavonoids, total anthocyanins and total catechins obtained with two extraction steps with methanol/water (80/20) and acetone/water (80/20) are shown in Table III. The measurements were averaged and the results are given as the mean with the standard deviation. It was found that an overall slightly better extraction efficiency of the phenolics from the skins and seeds was achieved using acetone. This was mainly evident for the extraction of catechins from skins and the extraction of flavonoids from the seeds, which can probably be attributed

TABLE III. Polyphenolic contents determined in the methanol and acetone skins and seeds extracts and percent difference between both extracts (*TP*: total phenolics, *TF*: total flavonoids, *TA*: total anthocyanins, *TC*: total catechins)

Vranec grapes	Methanol extracts[a], mg/g	Acetone extracts[a], mg/g	Difference, %
Skins/*TP*	48.1 ± 1.13	48.4 ± 1.13	0.62
Skins/*TF*	6.90 ± 0.42	7.01 ± 0.29	1.59
Skins/*TA*	11.5 ± 0.68	11.9 ± 0.65	3.48
Skins/*TC*	2.24 ± 0.18	2.71 ± 0.28	20.98
Seeds/*TP*	162 ± 5.65	166 ± 5.66	2.47
Seeds/*TF*	15.3 ± 0.75	18.9 ± 0.60	23.53
Seeds/*TC*	24.6 ± 1.15	25.3 ± 1.69	2.85

[a]Values are the average from 3 replicates

to the more efficient dissolution of the external lipidic layer of the seeds by acetone, which is less polar and thus a better solvent for lipids than methanol, yielding the largest amounts of polyphenols.[34]

Composition of grape phenolic

The developed spectrophotometric methods for the determination of the total phenolics, total anthocyanins, total flavonoids and total catechins in grape extracts of skin, seed and pulp were applied. The results obtained for Vranec, Cabernet Sauvignon, Muscat Hamburg and Riesling grapes varieties are presented in Table IV.

TABLE IV. Phenolic composition (mg/g, fresh weight) of grapes varieties Vranec, Cabernet Sauvignon, Muscat Hamburg and Riesling (*TP*: total phenolics, *TF*: total flavonoids, *TA*: total anthocyanins, *TC*: total catechins)

Grape variety		*TP*[a] / mg L^{-1}	*TF*[a] / mg L^{-1}	*TA*[a] / mg L^{-1}	*TC*[a] / mg L^{-1}
Vranec	Pulp	2.58 ± 0.14	0.77a ± 0.11	0.15 ± 0.01	0.15 ± 0.02
	Seed	166 ± 1.61	18.9 ± 0.68	–	25.3d ± 0.96
	Skin	48.4 ± 1.94	7.01 ± 0.36	11.9 ± 0.83	2.71e ± 0.33
Cabernet	Pulp	1.65 ± 0.11	0.65ab ± 0.06	0.02a ± 0.003	0.08ab ± 0.12
Sauvignon	Seed	113 ± 0.70	7.35 ± 0.43	–	17.2 ± 0.44
	Skin	31.5 ± 1.41	23.3 ± 0.97	5.67 ± 0.17	2.62e ± 0.13
Muscat	Pulp	1.14a ± 0.16	0.39bc ± 0.05	0.03a ± 0.003	0.05ac ± 0.01
Hamburg	Seed	135 ± 2.33	9.4 ± 0.71	–	24.2d ± 0.52
	Skin	37.6 ± 1.30	2.94d ± 0.22	2.74 ± 0.09	4.08 ± 0.31
Riesling	Pulp	1.06a ± 0.12	0.50c ± 0.05	–	0.05bc ± 0.01
	Seed	126 ± 1.55	15.8 ± 0.22	–	22.1 ± 0.34
	Skin	10.2 ± 0.84	3.65d ± 0.23	–	1.55 ± 0.07

[a]Values with the same letter(s) within a column are not significantly different at $p < 0.05$ by the Student–Newman–Keul's test

The polyphenolic compounds were mainly located in the grape seeds and skins, whereas the pulp contained a very low concentration of these components. From the data obtained in this study, it was observed that the seeds contained the highest contents of total phenolics (*TP*), total flavonoids (*TF*) and total catechins (*TC*), whereas anthocyanins (*TA*) were mainly located in the skins. It was found that the seeds from the Vranec variety contained the highest amounts of total phenolics, total flavonoids and total catechins among the analysed varieties, which was in concordance with previously published data for this variety from other regions of the Balkan Peninsula.[35] As expected, the highest concentration of total anthocyanins was also found in the skin extracts of the Vranec variety. It should be emphasized here that the Vranec variety dominates in the Macedonian vineyards; it is the autochthonous variety for Montenegro and a regionally well-known variety grown in Serbia and Croatia, traditionally used for the production of high quality wines, such as the analysed Vranec and Disan (discussed below),

which are characterized by an intense dark red colour. Hamburg grapes, a traditional table grape variety, also contained a high level of polyphenolic components and are thus recommended for regular human consumption.

Wine phenolic composition and colour variables of the wines

The results for *TP*, *TF*, *TA* and *TC* for the analyzed red and white wines are presented in Table V. The highest concentrations of phenolic compounds, flavonoids and catechins were found in the Disan wine and the values for the total anthocyanins were very close to those obtained for the Vranec wine. Disan and Vranec wines contain 1515 and 1382 mg/L total phenolics, respectively, which are in agreement with some previous results (1470–1684 mg/L total phenols) for several types of dessert wines made from Vranec cultivars.[36] Both wines were produced from the Vranec grape variety grown in a distinctive region rich with minerals and micronutrients. Disan is traditionally made from later harvested more senescent Vranec grapes, after reduction of the crop before blooming. According to Magarino and San-José,[37] the phenolic content increases throughout the ripening of the grape and it is expected that wines made from later harvested grapes would contain higher contents of phenolics, as was observed from the obtained results for the Disan wine. However, the concentrations of the phenolic families in wines depend not only on the grape variety, but also on additional factors, such as the edaphoclimatic conditions, the enological practices, the storage conditions, *etc*.[13,38–40] During bottle aging of wine, modifications in the polyphenolic composition occur as a result of different transformations, such as oxidation processes, condensation and polymerization reactions including direct reactions between anthocyanins and flavanols or reactions between anthocyanins and flavanols through ethyl bridges,[41–43] whereby stable pigments are formed

TABLE V. Concentration (average from 3 replicates) of total phenolics, total flavonoids, total anthocyanins and total catechins in the analysed red and white wines (*TP*: total phenolics, *TF*: total flavonoids, *TA*: total anthocyanins, *TC*: total catechins)

Wine	Colour	Vintage year	TP^a / mg L^{-1}	TF^a / mg L^{-1}	TA^a / mg L^{-1}	TC^a / mg L^{-1}
Vranec	Red	2003	1382 ± 38.2	922a ± 12.0	239ab ± 14.8	834a ± 28.3
Disan (Vranec)	Red	2003	1515 ± 27.6	1104 ± 70.7	237ac± 9.89	846a ± 27.5
Cabernet Sauvignon	Red	2003	1185 ± 50.2	910a ± 22.6	258d ± 13.4	755 ± 25.4
Merlot	Red	2003	1119 ± 28.9	686 ± 17.7	267bcd ± 16.9	566 ± 27.5
Riesling	White	2003	205ab ± 11.3	70.8b ± 1.27	–	11.9b ± 1.09
Smederevka	White	2003	230c ± 12.0	69.7b ± 1.13	–	20.7 ± 0.85
Sauvignon Blanc	White	2003	218ac ± 16.3	52.4 ± 2.97	–	11.1b ± 0.30
Temjanika (Muscat de Frontignan)	White	2003	185b ± 9.89	61.3 ± 2.68	–	9.37 ± 0.87

[a]Values with the same letter(s) within a column are not significantly different at $p < 0.05$ by the Student–Newman–Keul's test

which stabilize the wine colour. All these reactions are related to changes in the colour and sensorial characteristics, such as the flavour, bitterness and astringency of the final wine. White wines contain lower contents of polyphenols compared to red wines. Among the analysed white wines, the highest phenolic content was measured for the Smederevka wine, the most widely cultivated white grape variety in Macedonia, and the lowest value was observed for the Temjanika wine.

The data for the total flavonoid contents showed that all the analyzed red wines had high flavonoid levels, comparable to the published results for other world wines.[21] The highest flavonoid content was found in the Disan wine made from the Vranec grape variety. The total flavonoid values for the analysed Smederevka and Riesling wines were similar and not statistically different.

The values of total anthocyanins in the analysed wines were very similar and were in concordance with published results for 10 red wines most spread in the region of former Yugoslavia, whereas the Vranec and Cabernet Sauvignon were found to have the highest amounts of anthocyanins.[35] All the analyzed red wines contained high levels of catechins with the Disan and Vranec wines containing, as expected, the highest contents. These results are in agreement with published data for these types of wines from other regions.[44]

The contents of catechins in the white wines were lower compared to the red wines, with the highest concentrations being observed for the Smederevka wine.

Katalinić *et al.*[45] measured the phenolic content of ten wines and obtained results in ranges 2402–3183 and 292–308 mg/L for the total phenolics in red and white wines, respectively. The analyzed wines contained 69.7–398 mg/L total anthocyanins, the flavonoid content, expressed as mg/L gallic acid, ranged from 1941–2893 mg/L, and the content of catechins was 0.25–12.7 mg/L. The anthocyanic content measured for Cabernet Sauvignon wines made from grapes treated with different irradiation were 320–402 mg/L.[46] The obtained results for the analysed Macedonian wines were also in agreement with the data published by Savova *et al.*[47] for 21 Bulgarian wines and the found total phenolics and total anthocyanic contents were 921–1821 and 22–274 mg/L, respectively.

Red and white wines have a different phenolic composition, which is characteristic for each variety. The polyphenolic content of the final wine depends not only on the grape variety, but also on the different winemaking procedures applied for production. Red wine production includes the procedure of maceration, which is not applied for white wine production, *i.e.*, white wines are produced without grape mash, having no contact with the grape skins. Therefore, white wines (Table V) contained lower amounts of polyphenols.

The results for the colour variables of the analyzed samples are presented in Table VI, from which it can be seen that the values of the colour intensity were between 0.98 for Vranec to 1.45 for Cabernet Sauvignon and it varied from one

variety to another. With regard to tint, low values (0.5–0.7) are characteristic for young red wines, which increase throughout aging. In this study, the tint values for the wines were relatively high, ranging from 0.71 (for Vranec and Disan) to 0.85 (for Merlot). The values for the brilliance of the wine, *dA*%, were below 40 %, which showed that the colour of the red wine was dark and atypical.[30] It can be seen that the lowest value for this parameter was found for the Merlot wine (46.6 %), which had the highest yellow proportion (41.5 %). The Vranec and Disan had *dA* % values greater than 50 %, which shows that the red colour was dominant in these wines. The obtained results for the analysed wines were in agreement with previously published data.[21,38,47]

TABLE VI. Colour composition of the analyzed red wines (the values are average from 3 replicates); *CI*: colour intensity, *T*: tint, *Ye* %: percentage of yellow colour contribution, *Rd* %: percentage of red colour contribution, *Bl* %: percentage of blue colour contribution in the overall colour, *dA* %: brilliance of the wine

Wine	Vintage year	*CI*	*T*	*Ye* %	*Rd* %	*Bl* %	*dA* %
Vranec	2003	0.98	0.71	37.0	51.9	11.0	53.8
Disan (Vranec)	2003	1.24	0.71	36.9	52.2	10.9	54.2
Cabernet Sauvignon	2003	1.45	0.81	39.7	48.7	11.6	47.3
Merlot	2003	1.14	0.85	41.5	48.4	10.2	46.6

The obtained results suggest that the analysed Macedonian red and white wines and grapes did not significantly differ in terms of phenolic contents from other varieties in the world. The Vranec wines, compared to the analysed Merlot and Cabernet Sauvignon wines, possessed the highest phenolic potential, as shown by the highest results for the concentrations of the compounds from different phenolic groups.

Principal component analysis

Principal component analysis (PCA) was applied in order to investigate the possible grouping of red and white wines samples according to the content of total phenolics, total flavonoids, and total catechins. From Table VII and the correlation score plot in Fig. 1, it can be seen that the first principal component (PC1), had the dominant influence, accounting for 99.61 % of the variability and the second principal component (PC2) accounted for 0.23 % of the variability, *i.e.*, together, PC1 and PC2 account for 99.84 % of the total variance.

A clear separation was noticed between the red and white wines: the white wines were located in the second principal component and red wines in the first principal component. A further distinction was made in the white wine group according to PC2: the Temjanika and Riesling were located in the positive part of PC2 and separated from Smederevka and Sauvignon Blanc, which were located in the negative part of PC2. The red wines were also further divided, whereby the Vranec and Disan were grouped and located in the negative part of PC1, while

the Merlot was located very low in the negative part of PC1 and Cabernet Sauvignon, very high in the positive part of PC1.

TABLE VII. Individual influence of the principal components (PC1: first principal component, PC2: second principal component, PC3: third principal component)

Influence	PC1	PC2	PC3
Eigen value	2.9884	0.0068	0.0048
Explained (%)	99.61	0.23	0.16
Cumulated (%)	99.61	99.84	100

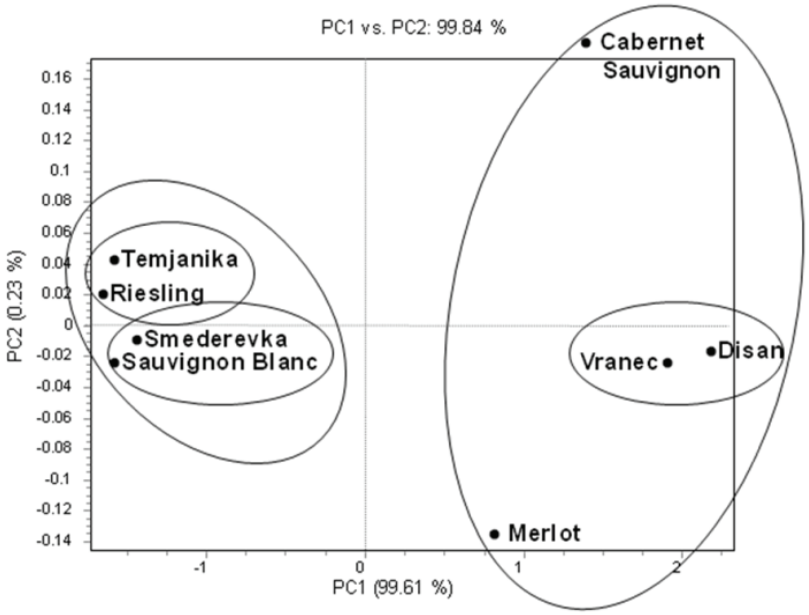

Fig. 1. Principal Component score plot (PC1 and PC2) of the studied white and red wines, based of spectrophotometric data for the total phenols, total flavonoids and total catechins.

CONCLUSIONS

The obtained results showed that the phenolic compositions of local Macedonian wines and grapes are similar to the cultivars from other countries in quantity and quality. The Vranec variety possesses the highest phenolic potential with a high content of total phenolics, total flavonoids, total anthocyanins and total catechins and the wine had a high value of the colour intensity. The accuracy of the commonly used spectrophotometric methods was checked by the standard addition method and the Folin-Ciocalteu assay was slightly modified in order to shorten the analysis time. Extraction of polyphenolic compounds from grape skin, seed and pulp was performed with acetone/water (80/20) and its efficiency checked.

Applying appropriate winemaking technologies, the Vranec grape variety can be a raw material for making high quality and premium Macedonian wines. This was supported by the results for the wines Vranec and Disan, for which the highest concentrations of total phenolics, flavonoids, anthocyanins and catechins were evidenced in this study.

ИЗВОД

ОДРЕЂИВАЊЕ САДРЖАЈА ПОЛИФЕНОЛА У МАКЕДОНСКОМ ГРОЖЂУ И ВИНУ СТАНДАРДНИМ СПЕКТРОФОТОМЕТРИЈСКИМ МЕТОДАМА

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Вино и грожђе садрже велики број флавоноида и нефлавоноидних фенолних једињења. У овој студији је одређиван полифенолни састав шест комерцијалних црвених и белих македонских вина и четири врсте грожђа. Спектрофотометријске методе су коришћене за одређивање укупних фенола, флавоноида, антоцијана и катехина. Упоређивана је ефикасност раствора ацетон/вода (80/20) и метанол/вода (80/20) у екстракцији полифенола из пулпе, коштица и љуске зрна грожђа. Ефикаснија екстракција је постигнута смешом ацетон/вода. Добијени резултати су показали да је мекедонско грожђе богато полифенолима, а највећа концентрација укупних фенола је нађена у грожђу врсте Вранец. Анализирана вина, такође, садрже доста полифенола, посебно вино Дисан од грожђа Вранец (1515 mg/L укупних фенола, 1103 mg/L укупних флавоноида, 237 mg/L укупних антоцијана и 845 mg/L укупних катехина). Анализа основних компоненти је примењена на испитиване узорке и нађена је јасна разлика између врста белих вина и црвених вина.

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