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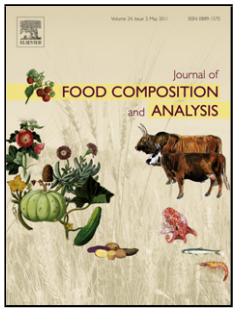

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Application of non-supervised pattern recognition techniques to classify Cabernet Sauvignon wines from the Balkan region based on individual phenolic compounds

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Highlights

- Phenolic compound composition of 16 Cabernet Sauvignon wines from the Balkan region analysed
- HPLC, spectroscopy as well as statistical analysis used to differentiate red wines
- Phenolic content depends on agricultural practices, geography, winemaking technique
- Multivariate statistical techniques applied to evaluate spatial variations in wines
- Similar agro-climatic characteristics showed shorter clustering distance

Abstract

Phenolic compounds in sixteen Cabernet Sauvignon wines from different wine-growing sub-regions in the Balkan region were investigated using HPLC with DAD and fluorescence detector and spectroscopic analysis, as well as statistical PC/F and cluster analysis. The HPLC analysis of investigated red wines showed that the content of total hydroxybenzoic acids, detected at 280 nm, was the highest in wines from Tikveš wine-growing subregion, Macedonia (127–140 mg L^{-1}). Total hydroxycinnamic acids, detected at 320 nm, were the highest in wines from Župa wine-growing subregion, Serbia (43–45 mg L^{-1}). The concentration of total flavonoids (flavan-3-ols, flavonols, flavons and flavanon), detected at 280, 360 and 322/275 nm, respectively, was the highest in wine from Katarzyna Estate wine-growing subregion, Bulgaria (167 mg L^{-1}). Finally, the concentration of total anthocyanins, detected at 520 nm, was the highest in wine from Šumadija wine-growing subregion, Serbia (1463 mg L^{-1}). The results of PCA and cluster analysis together confirmed that the content of phenolic compounds in Cabernet Sauvignon wines depends on agro-climatic factors, oenological practice in different wineries and the growing season in the Balkan regions that were investigated. The areas in the Balkan regions in this study with similar agro-climatic

characteristics showed shorter clustering distance, indicating similar phenol profiling in the red wines tested.

Keywords: Cabernet Sauvignon wines; Red wine; Phenolic compounds; Balkan subregions; Statistical analysis; Principal component analysis; Food analysis; Food composition; Wine consumers

1 Introduction

It seems that the biochemical activity of grape berries and wines depends on their phenolic compounds. However, the phenolic content and composition of grapes and wine in turn depend on the grape variety, geographical origin, vineyard location, cultivation system, climate, soil type, vine cultivation practices, harvesting time, vintage, production processes and ageing (Atanacković et al., 2013; Baydar et al., 2011; Ferreira-Lima et al., 2013; Gambelli et al., 2004; Jaitz et al., 2010; Jiang & Zhang, 2012; Kallithraka et al., 2006; Klenar et al., 2004; Radovanović et al., 2010; Rastija et al., 2009; Villano et al., 2006; Zimman et al., 2002). Wine consists of different phenolic compounds, and thus the antioxidant and biological activities of wine are connected with a synergy among these compounds.

In red wines, tannins and anthocyanins are the most important phenolic classes. Tannins contribute to the mouthfeel of wines, but they also form pigmented polymers in association with the anthocyanins (Kennedy, 2008). Recent studies indicate that consumption of small amounts of red wine on a regular basis reduces the risk of coronary heart disease and atherosclerosis, and this benefit is ascribed to the antioxidant properties of polyphenolic compounds (Fernandez-Sola, 2015). Therefore it is very important to determine which group of phenolic compounds most influences these properties of wine. In both *in vitro* and *in vivo* research trials, anthocyanins have demonstrated a noticeable ability to reduce cancer cell

proliferation and to inhibit tumour formation (Lila, 2004). In addition, if higher concentration of phenolic compounds in wines is present, their antioxidant capacity and antimicrobial activity will be higher (De Beer et al., 2003; Hou, 2003; Radovanović et al., 2008). There are many studies in the phenolic content of Cabernet Sauvignon wines from different parts of the world, such as Chile (similarities and dissimilarities in phenolic contents were determined between wines of different costs) (Caceres et al., 2012; Caceres-Mella et al., 2014), China (Bai et al., 2013; Jiang & Zhang, 2012; Zhu et al., 2014), Canada (Cliff et al., 2007), South Africa (Rossouw & Marais, 2004), Brazil (Gris et al., 2013), Slovenia (Klenar et al., 2004), France (Lorrain et al., 2013), Serbia (Atanacković et al., 2013; Radovanović et al., 2008, 2010), Croatia (Rastija et al., 2009), Greece (Kallithraka et al., 2006), Turkey (Baydar et al., 2011), Romania (Stoica et al., 2011), and Italy (Gambelli & Santaroni, 2004).

In this study, multivariate statistical techniques, such as principal component analysis, factor analysis and cluster analysis, were applied for the evaluation of spatial variations in the distribution of different compounds in wines and for the interpretation of an obtained data set. Based on a multivariate statistical approach, useful data were obtained regarding the similarities or differences among the wines that were analysed. This study aimed to analyse the differences in phenolic compounds in Cabernet Sauvignon wines from different wine-growing regions from the Balkan region, using HPLC and spectroscopic methods as well as statistical analysis, which made it easier for us to monitor the quality of these wines. To the best of our knowledge, this is the first time wines have been analysed in this way. The results from this investigation could be useful both to wine producers for the formation of market price of their products, and to wine consumers in choosing good quality Cabernet Sauvignon wines produced from particular wine areas and by specific producers.

2 Materials and methods

2.1 Chemicals and wines samples

Acetonitrile and formic acid (HPLC-grade) were obtained from Merck (Darmstadt, Germany); HPLC-grade methanol was purchased from Carlo Erba Reagent (Milan, Italy); gallic acid, caffeic acid, *p*-coumaric acid, ferulic acid, vanillic acid, syringic acid, ellagic acid, (+)-catechin, procyanidin B2, (-)-epicatechin, epigallo-catechin gallate, quercetin, morin, rutin, naringin, kaempherol, luteolin, apigenin, quercetin-3-glucoside, malvidin-3-glucoside and cyanidin-3-glucoside were supplied from Sigma Chemical Co. (St. Louis, MO, USA). The reagents were of analytical quality. Red wines selected for this study were commercial wines from the market: CS1, CS2, CS3, CS4a, CS4b, CS5a, CS5b, CS6b, CS7, CS8, CS9b, CS10, CS11, and CS12 (detailed data on investigated wines, wineries, years of production, latitudes, longitudes, and altitudes of places of productions are available in Table 1). Samples were stored at room temperature prior to analyses.

2.2 Determination of total phenols, hydroxycinnamoyl tartaric acids and flavonols

Total phenol, hydroxycinnamoyl tartaric acid and flavonol contents in selected red wine samples were determined spectrophotometrically, as already reported (Mazza et al., 1999; Radovanović et al., 2010). The absorbances (A) at 280, 320 and 360 nm were recorded using Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA); $A_{280\ nm}$ was used to estimate total phenol content, using gallic acid as the standard compound; $A_{320\ nm}$ was used to estimate hydroxycinnamoyl tartaric acid content using caffeic acid as the standard compound; and $A_{360\ nm}$ was used to estimate total flavonol content using quercetin as the standard compound.

2.3 High performance liquid chromatography analysis (HPLC)

Phenolic compounds contents were determined using HPLC by direct injection of each wine sample (previously filtered through a 0.45 μm pore size membrane filter) into an Agilent 1200 chromatographic system-photodiode array detector (DAD) with radiofrequency identification tracking technology for flow cells, and fluorescence detector for multiwavelength detection, with online acquisition of excitation (Ex) and emission (Em) spectra, and Chem-Station software. Elution was carried out in gradient mode using two solvent mixtures: (A) formic acid/water (5:95 v/v) and (B) acetonitrile/formic acid/water (80:5:15 v/v). The elution profile was as follows: from 0 to 28 min, 0-10.0% B, from 28 to 35 min, 10-25% B, from 35 to 40 min, 25-50% B, from 40 to 45 min, 50-80% B, and for the last 10 min again 0% B. Aliquots of 5 μL were injected into 4.6×250 mm RPC-18 column (Zorbax Eclipse XDB-C18) (Agilent Technologies, Santa Clara, CA, USA) with 5 μm particle size and t=30 °C. The flow rate was 0.8 mL min^{-1} (Radovanović et al., 2010). The detection wavelengths were 280, 320, and 360 nm for UV, and 275/322 nm ($\lambda_{Ex}/\lambda_{Em}$) for fluorescence detection. Identification and quantification of various phenolic compounds were made using calibration curves obtained with the solutions of standards: gallic acid, caffeic acid, *p*-coumaric acid, ferulic acid, vanillic acid, syringic acid, ellagic acid, (+)-catechin, procyanidin B2, (-)-epicatechin, epigallo-catechin gallate, quercetin, morin, rutin, naringin, kaempherol, luteolin, apigenin, quercetin-3-glucoside, malvidin-3-glucoside and cyanidin-3-glucoside, under the same conditions as wine samples. The results are expressed in mg per L of the sample (mg L^{-1}).

2.4 Determination of pH value

The pH values of selected Cabernet Sauvignon wines were determined using a pH meter (Hanna Instruments, Woonsocket, Rhode Island, USA).

2.5 Alcohol content

The alcohol content is given according to the data provided by the wine producers, since only commercial wines were investigated.

2.6 Statistical analysis

Analysis of variance (ANOVA) was used to determine the significance ($p \leq 0.05$) of the data obtained in all experiments. All results were determined to be within 95% confidence level for reproducibility. The values represent a mean value of at least three replications.

Principal component analysis was used as a statistical tool. It is used with the aim to evaluate the dataset, reducing its dimension and preserving most of the statistical information. PCA permits establishing the relationships among variables. The analysis was performed using data analysis and statistical application available for Microsoft Excel® (XLSTAT 2014.2.03, Addinsoft SARL, Paris, France). Finally, HCA (hierarchical cluster analysis) of standardized variables, using the Ward method as an amalgamation rule and squared Euclidean distance as a measure of the proximity between the samples, was performed.

3 Results and discussion

3.1 Contents of phenolic compounds in Cabernet Sauvignon wines

Phenolic profile of wine (determined by relative proportions of different phenolic compounds) is a characteristic for each corresponding grape variety according to environmental conditions. In addition, during the process of wine preparation, significant changes take place in the composition and content of phenolic compounds, as a result of fruit disintegration as well as wine fermentation and aging (Baydar et al., 2011; Ferreira-Lima et al., 2013; Jaitz et al., 2010; Jiang & Zhang, 2012; Kallithraka et al., 2006; Radovanović et al., 2010; Rastija et al., 2009; Villano et al., 2006; Zimman et al., 2002).

The contents of phenolic compounds of specific classes (total phenols, hydroxycinnamoyl tartaric acids and flavonols) in investigated Cabernet Sauvignon wines are provided in the online Supplementary material, Table S1.

The spectroscopic analysis of the Cabernet Sauvignon wines investigated has shown that total phenolic content ranged from 997 to 1968 mgGAE L^{-1}; hydroxycinnamoyl tartaric acid content ranged from 200 to 353 mgCAE L^{-1}; and flavonol content ranged from 110 to 209 mgQE L^{-1}. The concentrations of phenolic compounds are comparable to the levels in the literature for wines produced in some neighboring countries (Gambelli et al., 2004; Kallithraka et al., 2006; Klenar et al., 2004; Rastija et al., 2009). Total phenols, hydroxycinnamoyl tartaric acid and total flavonol concentrations were the lowest in Cabernet Sauvignon, 2008 (CS8) produced by Cevin winery, Niš wine-growing subregion, Serbia; and the highest in Cabernet Sauvignon "Oplenac", 2009 (CS4b) produced by Kraljevski vinogradi, Šumadija wine-growing subregion, Serbia. There are differences among the wines produced from the same type of grape variety by various winemaking techniques from different producers (Lachman et al., 2007; Radovanović et al., 2008, 2010; Villano et al., 2006).

Table S2 in the Supplementary Material gives the concentrations of four hydroxybenzoic acids (gallic, vanillic, syringic, and ellagic acid) and five hydroxycinnamic acids (*t*-caftaric, *t*-coutaric, caffeic, chlorogenic, and *p*-coumaric acid) in Cabernet Sauvignon wine samples, determined at 280 and 320 nm by HPLC.

The HPLC analysis of the Cabernet Sauvignon wines investigated has shown that the concentration of total hydroxybenzoic acids was the highest in Cabernet Sauvignon "Alexandria", 2008 (CS9b) produced by Tikveš wine-growing subregion in Macedonia with 140 mg L^{-1}; and Cabernet Sauvignon "Čardak", 2009 (CS10) from Lozar, Povardarie wine-

growing subregion, Macedonia (127 mg L^{-1}). In selected Cabernet Sauvignon wine samples, gallic acid was the predominant acid compound; its content (shown as the percentage of total acid content in Table S2 in the Supplementary Material) reached 88.58% in Cabernet Sauvignon "Čardak".

The concentration of total hydroxycinnamic acids was the highest in CS5a, Cabernet Sauvignon "Terra Lazarica", 2008, Rubin, Župa wine-growing subregion, Serbia (45 mg L^{-1}). *Trans*-caftaric acid was the predominant acid (79.85%) in selected Cabernet Sauvignon wine samples, such as "Oplenac", 2008 (CS4a) produced by Kraljevski vinogradi, Šumadija wine-growing subregion, Serbia; this agrees with published data for other wines (Favre et al., 2014; Ferreira-Lima et al., 2013; Gris et al., 2013; Menković et al., 2014; Singleton et al., 1978).

In Table S3 in the Supplementary Material, the concentrations of four flavan-3-ols are shown ((+)-catechin, procyanidin B_2, (-)-epicatechin and (-)-epigallocatechin gallate), six flavonols (quercetin –3-glucoside, rutin, myricetin, morin, quercetin and kaempferol**)**, two flavons (luteolin and apigenin) and flavanon-naringin in Cabernet Sauvignon wine samples, determined at 275/322 and 360 nm using HPLC-method.

The HPLC analysis of investigated Cabernet Sauvignon wines has shown that the concentration of total flavan-3-ols (Supplementary Material, Table S3) was the highest in Cabernet Sauvignon, 2007 (CS12) produced by Katarzyna Estate, Mezzek wine-growing subregion, Bulgaria (144 mg L^{-1}). In selected Cabernet Sauvignon wine samples, (+)-catechin was the predominant flavan-3-ol compound, as high as 49.03% in Cabernet Sauvignon "Čardak", 2009 (CS10) produced by Lozar, Povardarie wine-growing subregion, Macedonia. The concentration of total flavonols was the highest in Cabernet Sauvignon, 2007 (CS3) produced by Plantaža, Podgorica wine-growing subregion, Montenegro (35 mg L^{-1}). Quercetin-3-glucoside was predominant flavonol compound (45.80% in selected Cabernet

Sauvignon wines, such as "Čardak", 2009 (CS10)). The concentration of total flavonoids, detected at 280, 360 and 322/275 nm, was the highest in Cabernet Sauvignon wine produced by Katarzyna Estate, Mezzek wine-growing subregion, Bulgaria (167 mg L^{-1}), followed by Cabernet Sauvignon "Oplenac", 2008 (134 mg L^{-1}) produced by Kraljevski vinogradi, Šumadija wine-growing subregion, Serbia and Cabernet Sauvignon "Alexandria", 2008 produced by Tikveš, Tikveš sub-growing subregion, Macedonia (118 mg L^{-1}).

In the Supplementary Material Table S4, the concentrations of fifteen monomeric anthocyanins, namely delphinidin-3-*O*-glucoside (Dp-3gl), cyanidin-3-*O*-glucoside (Cy-3gl), petunidin-3-*O*-glicoside (Pt-3gl), peonidin-3-*O*-glucoside (Pn-3gl), malvidin-3-*O*-glucoside (Mv-3gl) and their 3-acetyl and 3-coumaroylglucoside derivatives in Cabernet Sauvignon wine samples, determined at 520 nm using HPLC, are shown. The HPLC analysis of investigated Cabernet Sauvignon wines has shown that the concentration of total anthocyanins was the highest in Cabernet Sauvignon "Oplenac", 2009 (CS4b) produced by Kraljevski vinogradi, Šumadija wine-growing subregion, Serbia (1463 mg L^{-1}). Malvidin-3-glucoside and its derivatives were the predominant anthocyanins (86.47%) in Cabernet Sauvignon, 2009 produced by Rubin, Župa wine-growing subregion, Serbia.

3.2 Statistical analysis

In the first step of the statistical evaluation, Kolmogorov-Smirnov test (the significance level α was 0.05) was preliminary used to test the normality of concentration distribution with each investigated compound. This test revealed whether the original data set was normally distributed or not (total phenolics, esters of wine acid and flavonols; hydroxybenzoic acids; hydroxycinnamic acids; flavan-3-ols; monomeric anthocyanins in the form of 3-acetyl glycosides), after which the data were ln-transformed to show normal distribution (flavonols; luteolin and apigenin; main monomeric anthocyanins in the form of glycosides; anthocyanins

in the form of 3-*p*-cumaroylglycosides and malvidin-vinylglucoside). Thereafter the original data in the first case and ln-transformed data (data equal to zero were removed from further analysis because the ln function is not defined for zero) in the second case were used for further analyses.

3.2.1 Principal component analysis/Factor analysis (PCA/FA)

In order to reveal the relations between selected compounds, the monitoring data sets that were obtained were subjected to PCA/FA. Before applying PCA modelling, one should test the data matrix in order to detect outliers. Application of Grubb's test to experimental data resulted in the detection of no outliers in datasets for total phenols, esters of wine acid and flavonols and hydroxycinnamic acids (see Supplementary Material, Table S5). Outliers were detected in datasets of hydroxybenzoic acids, flavan-3-ols, flavonols, luteolin and apigenin, main monomeric anthocyanins in the form of glycosides, monomeric anthocyanins in the form of 3-acetyl glycosides and anthocyanins in the form of 3-*p*-cumaroyl glycosides and malvidin-vinylglucosides (Grubbs, 1969). Outliers were discarded from PCA analysis (Supplementary Material, Table S5).

Strong positive correlations ($r>0.7$) were observed between ln-transformed data of quercetin-3-glucoside and kaempferol (r=0.933), morin and kaempferol (r=0.929), ln-transformed data of apigenin and naringin (r=0.988), Pt-3-ac-gl and Pn-3-ac-gl (r=0.857) and ln-transformed data of Pn-3-*p*-coum-gl and Mv-3-vinylphenolgl (r=0.972); positive strong correlations were also observed between data of total phenols and esters of wine acid (r=0.882), total phenols and total flavonols (r=0.762), and esters of wine acid and total flavonols (r=0.859); moderate positive correlations ($0.3<r<0.7$) were between caffeic acid and *p*-coumaric acid (r=0.683), and procyanidin B_2 and (-)-epicatechin (r=0.767) (Shrestha & Kazama, 2007; Varol et al., 2012).

Before proceeding with PCA/FA in all cases, the suitability of the data for factor analysis and rightness of its implementation was checked (Table 2).

From the shape of the scree plot, (Supplementary Material, Fig. S1), the number of important components that will be used in further calculations can be observed. The description of the PCA results obtained for different groups of compounds under study is summarized in Table 3.

Correlations and similarities between the variables are represented in Fig. S2 (see Supplementary Material). Variables with low loadings have no significant impact on the structure of data, while the elements with high loadings have the most influence on grouping and separation of samples. High correlations were observed between the data of total flavonols and esters of wine acid, and esters of wine acid and total phenols, quercetin-3-glucoside and kaempferol, apigenin and naringin, ln-transformed data of Pt-3-gl and Mv-3-gl, Pt-3-ac-gl and Pn-3-ac-gl and ln-transformed data of Pn-3-*p*-coum-gl and Mv-3-vinylphenolgl. Medium loading was observed between procyanidin B_2 and (-)-epicatechin. Observation plots based on the contents of components are represented in Fig. 1. From Fig. 1a, it is visible that high content of total phenols is present in samples on the right side of the plot, and low total phenol content is on the left side of the plot. Also, it can be concluded that high content of esters of wine acid is present in samples in the upper half of the plot, while low content is represented on the opposite side of the plot. Similar comments can be made for the other plots.

Statistical analysis showed that contents of hydroxybenzoic acids do not depend on the geographic region. The same conclusion is valid for flavan-3-ols, flavonols, luteolin and apigenin, monomeric anthocyanins in the form of glycosides, anthocyanins in the form of 3-

acetyl glycosides, and anthocyanins in the form of 3-*p*-cumaroylglycosides and malvidin-vinylglucosides.

PCA and cluster analysis together gave useful information on investigated wines. For example, cluster I in Figure 2i was constructed using samples CS2 (Sekulovic, Bosnia and Herzegovina, 2008) and CS4a (Kraljevski vinogradi, Oplenac, Serbia, 2008), which show high content of Pt-3-*p*-coum-gl, and high content of Pn-3-*p*-coum-gl. This information enables us to monitor content of these just two compounds of one of the wines in order to predict the quality of wines from these regions.

3.3 Cluster analysis

The dendrogram of the clusters obtained from analyzing Cabernet Sauvignon samples is presented in Fig. 2.

The dendrogram in Figure 2a shows that all monitoring samples can be grouped into three main clusters. Cluster I is formed by samples CS1, CS5a, CS7, and CS8; cluster II is formed by samples CS2, CS3, CS9a, CS9b, CS10, CS11, and CS12; and cluster III is formed by samples CS4a, CS4b, CS5b, and CS6a. Similarly, dendrogram in Figure 2b shows that all monitoring samples can be grouped into four main clusters. Cluster I is formed by samples CS1, CS3, and CS7; cluster II is formed by samples CS2, CS5b, CS9a, CS10, and CS11; cluster III is formed by samples CS5a, CS8, and CS12; and cluster IV by samples CS6a and CS6b. The dendrogram in Figure 2c shows that all monitoring samples can be grouped into three main clusters: cluster I is formed by samples CS1, CS3, CS5a, CS5b, CS6a and CS6b; cluster II is formed by samples CS2, CS8, and CS10; and cluster III is formed by samples CS4a, CS4b, CS7, CS9a, CS9b and CS12. The dendrogram in Figure 2d shows that all monitoring samples can be grouped into three main clusters-cluster I is formed by samples CS2, CS3, CS5a, CS6b, CS9a, and CS11; cluster II is formed by samples CS4a and CS8; and

cluster III is formed by samples CS5b, CS7, and CS9b. The dendrogram in Figure 2e shows that all monitoring samples can be grouped into three main clusters. Cluster I is formed by sample CS6a; cluster II is formed by samples CS6b and CS8; and cluster III is formed by sample CS10. The dendrogram in Figure 2f shows that all monitoring samples can be grouped into three main clusters. Cluster I is formed by sample CS3 and CS10; cluster II is formed by sample CS4b; and cluster III is formed by sample CS8. The dendrogram in Figure 2g shows that all monitoring samples can be grouped into three main clusters. Cluster I is formed by sample CS2; cluster II is formed by sample CS3 and CS4a; and cluster III is formed by sample CS4b. The dendrogram in Figure 2h shows that all monitoring samples can be grouped into three main clusters. Cluster I is formed by samples CS1, CS3, CS8, CS9a, and CS9b; cluster II is formed by samples CS2, CS7, and CS12; and cluster III is formed by sample CS5a. The dendrogram in Figure 2i shows that all monitoring samples can be grouped into three main clusters. Cluster I is formed by samples CS2 and CS4a; cluster II is formed by samples CS4b; and cluster III is formed by samples CS9a and CS12.

4 Conclusion

This study has shown that Cabernet Sauvignon wines are rich sources of numerous phenolic compounds. The wines that were evaluated could not be differentiated due to place of production; the reason for this could be the regional originality of Balkan Cabernet Sauvignon wines. PCA and cluster analysis together gave useful information on the investigated wines. It enabled us to monitor the content of several compounds from the cluster of just one of the wines in order to predict the quality of wines from these regions. Results from this investigation can be useful both to wine producers for the formation of market price of their products, and to wine consumers in choosing good quality Cabernet Sauvignon wines produced from particular wine growing region and producer.

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Figure Captions

Fig. 1. Principal component score plot (F1 and F2) of the studied Cabernet Sauvignon samples based on the content of particular class of compounds: a) phenols, esters of wine acid and flavonols; b) hydroxybenzoic acids; c) hydroxycinnamic acids; d) flavan-3-ols; e) flavonols; f) luteolin and apigenin; g) main monomeric anthocyanins in the form of glycosides; h) monomeric anthocyanins in the form of 3-acetyl glycosides; and i) anthocyanins in the form of 3-*p*-cumaroylglycosides and malvidin-vinylglucosides.

Fig. 2. Dendrogram of the Cabernet Sauvignon samples represented in PCA plots obtained in cluster analysis based on Ward Linkage and Euclidean Distance: a) the contents of total phenols, esters of wine acid and flavonols; b) the contents of hydroxybenzoic acids; c) the contents of hydroxycinnamic acids; d) the contents of flavan-3-ols; e) the contents of flavonols; f) the contents of luteolin and apigenin; g) the contents of main monomeric anthocyanins in the form of glycosides; h) the contents of monomeric anthocyanins in the form of 3-acetyl glycosides; and i) the contents of anthocyanins in the form of 3-*p*-cumaroylglycosides and malvidin-vinylglucosides.

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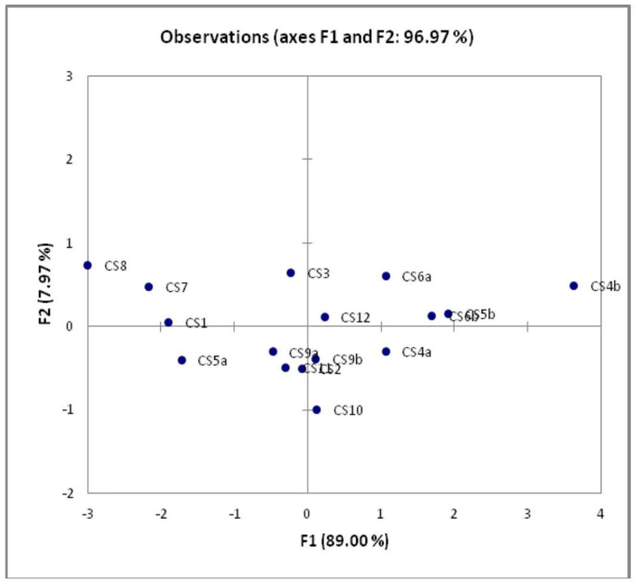
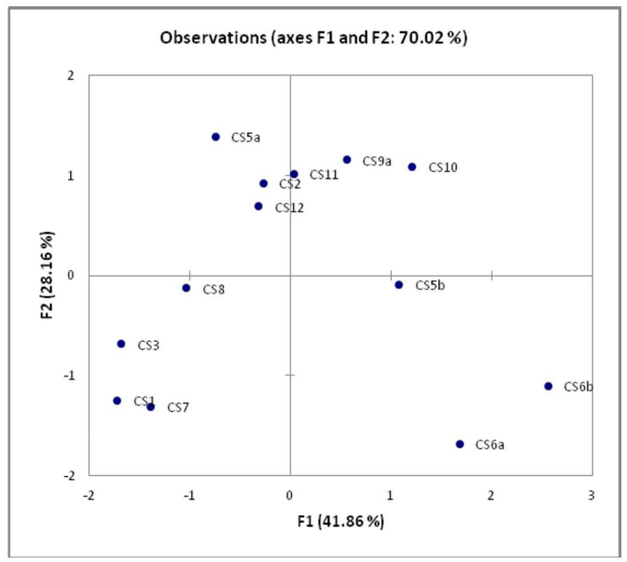


b)



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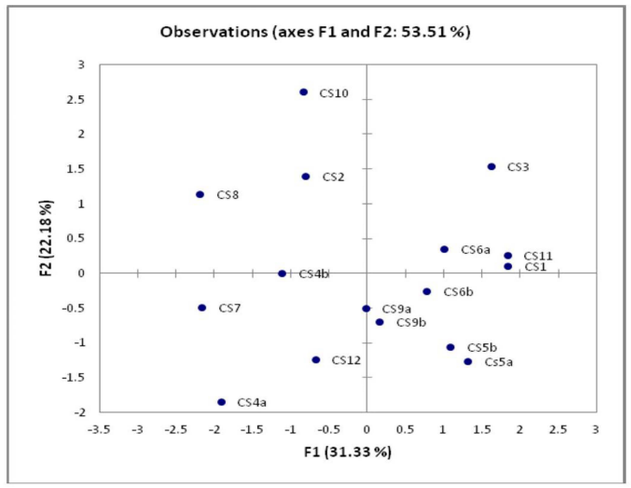

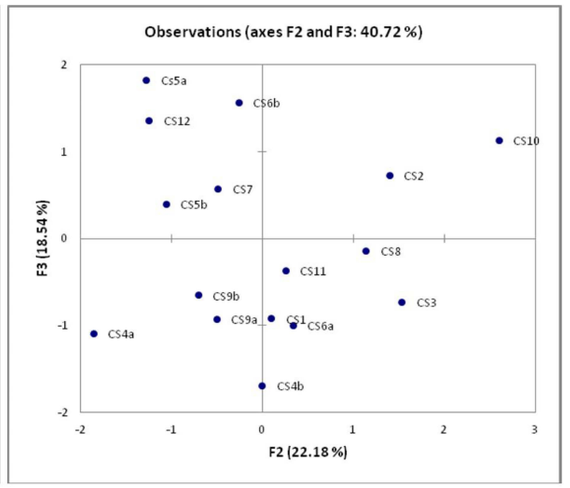

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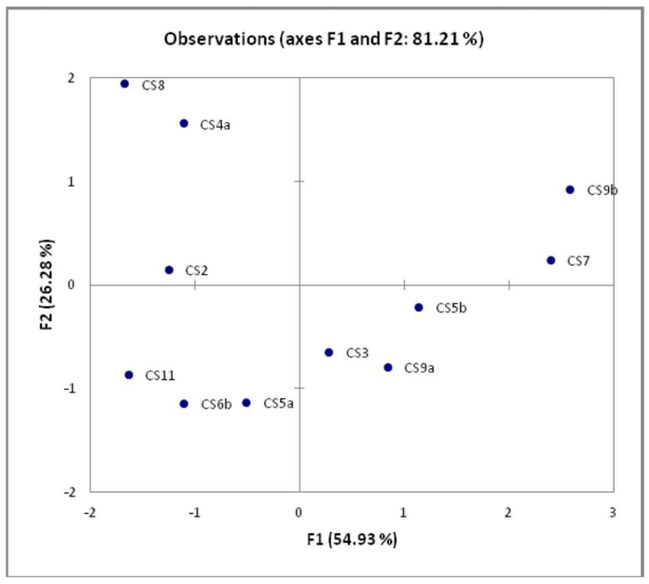

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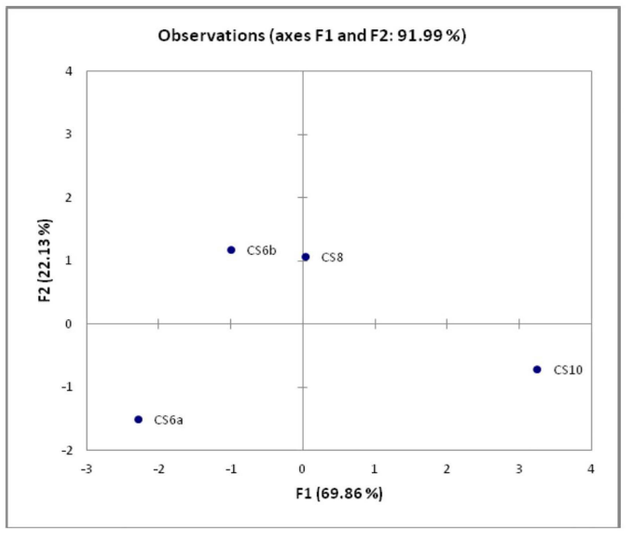

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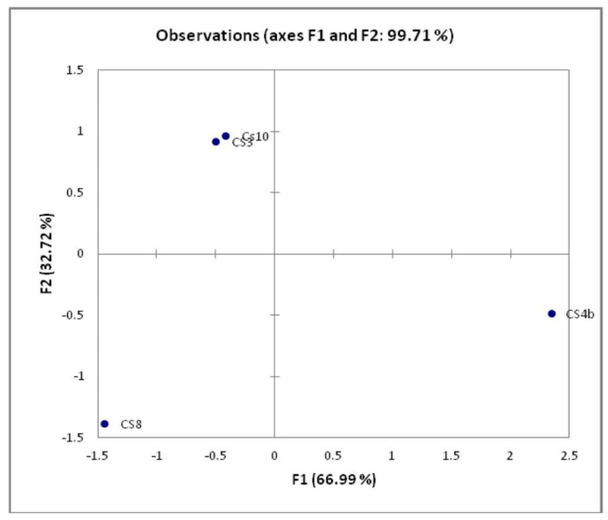

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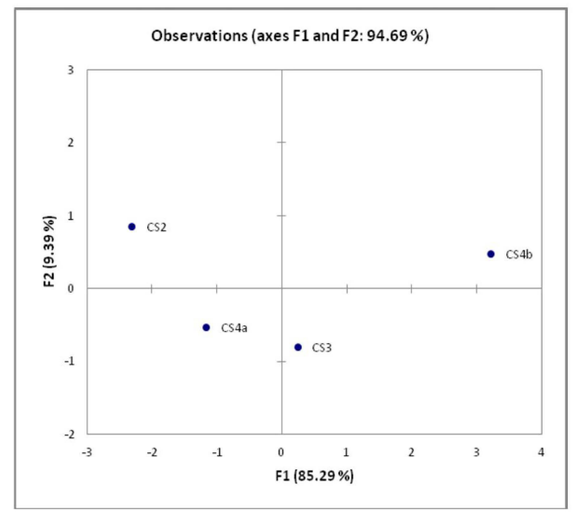

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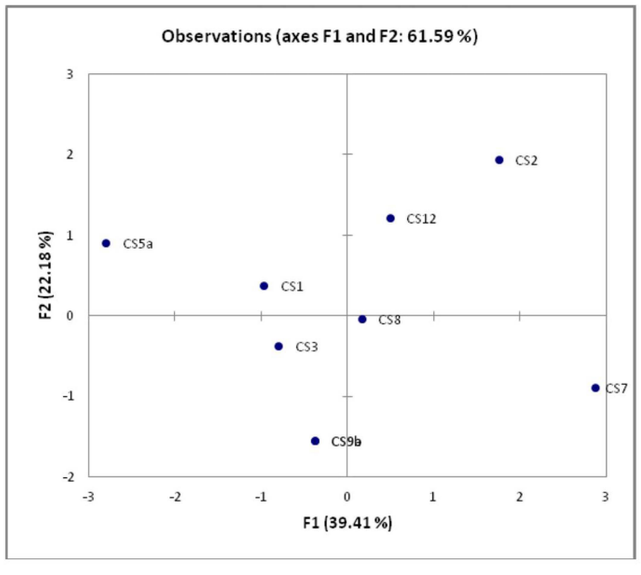

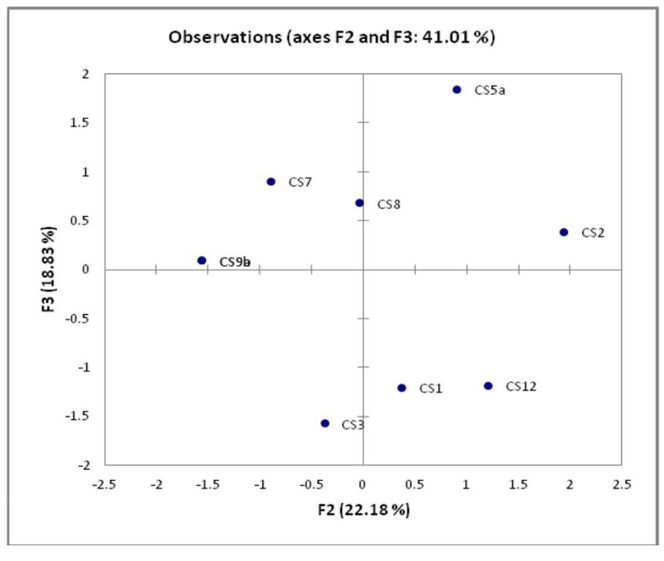

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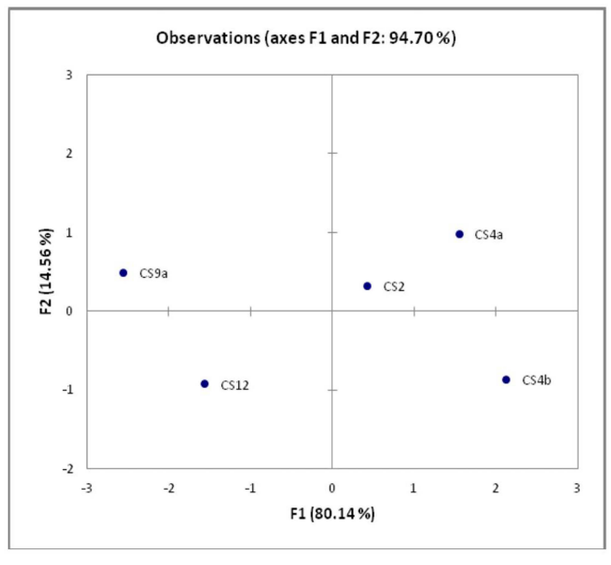
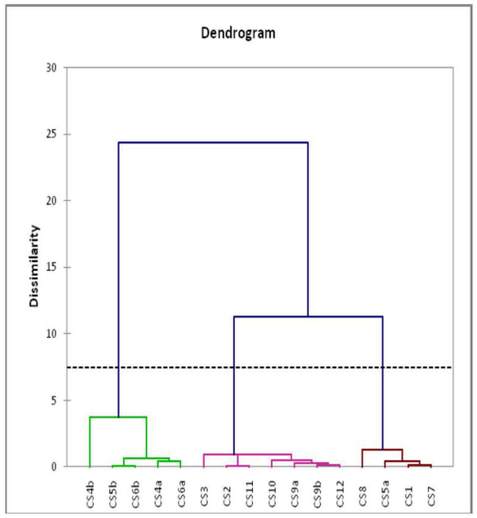


FIG. 1

a)



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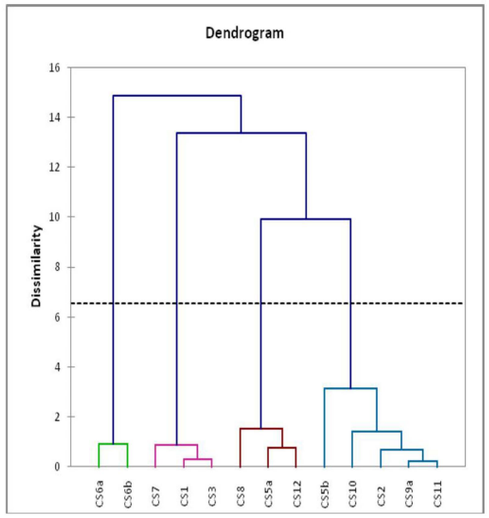

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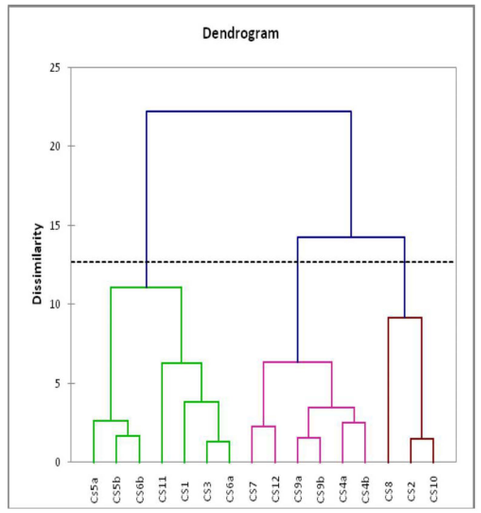

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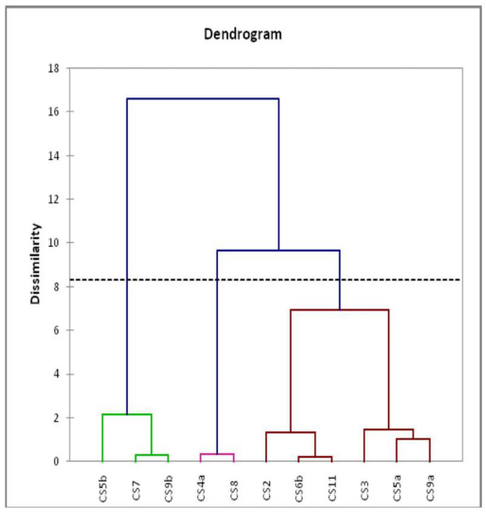

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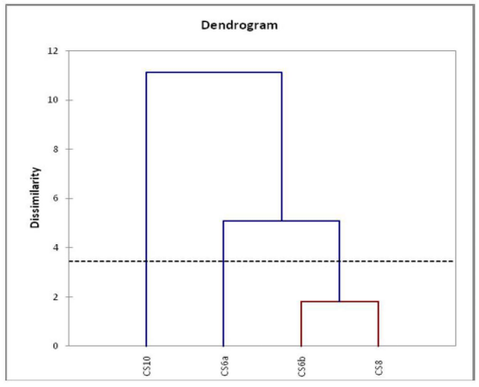

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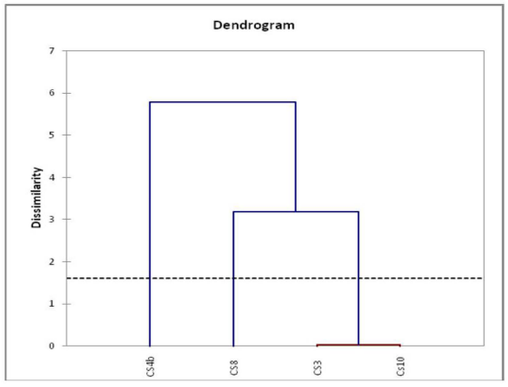

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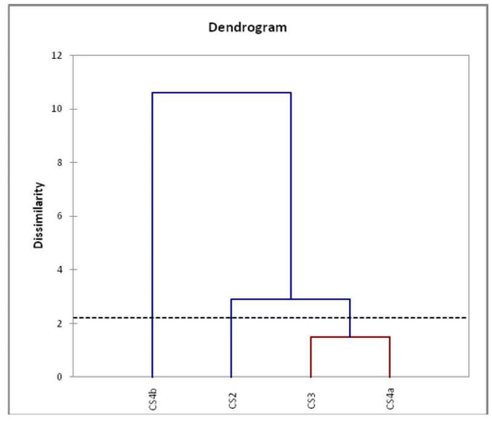

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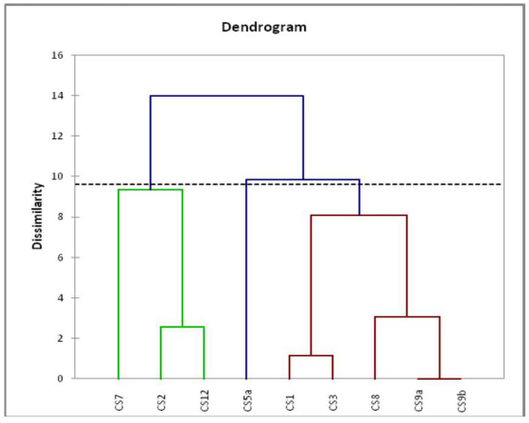

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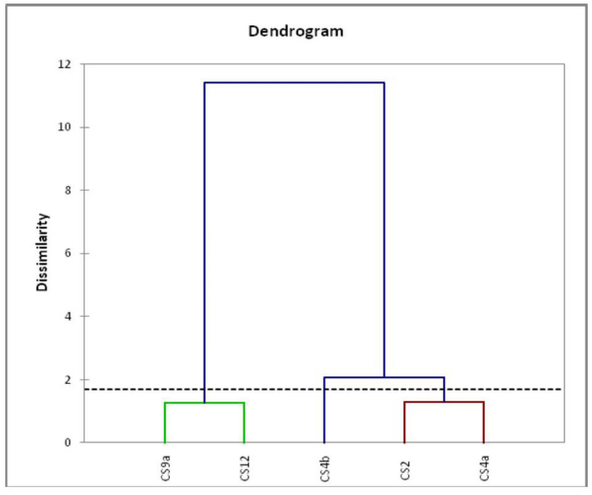

FIG. 2

Table 1

Detailed data on investigated wines, wineries, years of production, latitudes, longitudes, and altitudes of places of production

Sample	Wine	Winery	Year	Latitude of wine-growing subregion	Longitude of wine-growing subregion	Altitude of wine-growing subregion
CS1	Cabernet Sauvignon	Laguna, Poreč, Croatia	2007	45°13'38'' N	13°35'41''E	29 m
CS2	Cabernet Sauvignon	Sekulovic, Trebinje, Bosnia and Herzegovina	2008	42°42'42'' N	18°20'44'' E	275 m
CS3	Cabernet Sauvignon	Plantaža, Podgorica, Montenegro	2007	42°26'17'' N	19°15'29'' E	44.5 m
CS4a	Cabernet Sauvignon "Oplenac"	Kraljevski vinogradi, Topola, Serbia	2008	44°15'09'' N	20°40'34'' E	221 m
CS4b	Cabernet Sauvignon "Oplenac"	Kraljevski vinogradi, Topola, Serbia	2009	44°15'09'' N	20°40'34'' E	221 m
CS5a	Cabernet Sauvignon "Terra Lazarica"	Rubin, Kruševac, Serbia	2008	43°35'0'' N	21°19'36'' E	137 m
CS5b	Cabernet Sauvignon "Terra Lazarica"	Rubin, Kruševac, Serbia	2009	43°35'0'' N	21°19'36'' E	137 m
CS6a	Cabernet Sauvignon	Rubin, Kruševac, Serbia	2008	43°35'0'' N	21°19'36'' E	137 m
CS6b	Cabernet Sauvignon	Rubin, Kruševac, Serbia	2009	43°35'0'' N	21°19'36'' E	137 m
CS7	Cabernet Sauvignon	Radmilovac, Belgrade, Serbia	2008	44°49'14'' N	20°27'44'' E	116.75 m
CS8	Cabernet Sauvignon	Cevin, Nis, Serbia	2008	43°19'29'' N	21°54'11'' E	192 m
CS9a	Cabernet Sauvignon "Alexandria"	Tikveš, Kavadarci, Macedonia	2008	41°26'00'' N	22°00'00'' E	320 m
CS9b	Cabernet Sauvignon "Alexandria"	Tikveš, Kavadarci, Macedonia	2009	41°26'00'' N	22°00'00'' E	320 m
CS10	Cabernet Sauvignon "Cardak"	Lozar, Veles, Macedonia	2009	41°42'50'' N	21°46'13'' E	210 m
CS11	Cabernet Sauvignon	Pivka, Negotino, Macedonia	2007	41°28'59'' N	22°05'32'' E	155 m
CS12	Cabernet Sauvignon	Katarzyna Estate Mezzek, Svilengrad, Bulgaria	2007	41°46'00'' N	26°12'00'' E	60 m

Table 2
Suitability of data for factor analysis

Compounds	**Kaiser-Meyer-Olkun adequacy of sampling, KMO** (Kaiser, 1960)*	**Bartlett's test of sphericity, p** (Bartlett, 1954)§
Hydroxycinnamic acids	0.467	0.618
Flavan-3-ols	0.528	0.078
Monomeric anthocyanins in the form of 3-acetyl glycosides	0.416	0.571
Anthocyanins in the form of 3-*p*-cumaroylglycosides and malvidin-vinylglucoside	0.424	0.049

*Accepted value 0.6
§It should be $p<0.5$

Table 3
Principal component analysis results

Compounds	Characteristic values	Variances
Total phenols, hydroxycinnamoyl tartaric acids and flavonols	2.670	89.001% 7.965%
Hydroxybenzoic acids	1.674 1.126	41.858% 28.160%
Hydroxycinnamic acids	1.880 1.331 1.112	31.332% 22.179% 18.539%
Flavan-3-ols	2.197 1.051	54.930% 26.277%
Flavonols	4.192 1.328	69.859% 22.133%
Luteolin and apigenin	2.010	66.986% 32.720%
Main monomeric anthocyanins in the form of glycosides	4.265	85.293% 9.393%
Monomeric anthocyanins in the form of 3-acetyl glycosides	2.364 1.331 1.130	39.405% 22.180% 18.833%
3-*p*-cumaroylglycosides and malvidin-vinylglucoside	3.205	80.135% 14.560%