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# Study of phenolic and volatile composition of white wine during fermentation and a short time of storage

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## Summary

**The phenolic composition including hydroxybenzoic acids, hydroxycinnamic acids and flavan-3-ols was identified and quantified in all studied samples by using a reversed-phase high-performance liquid chromatography (HPLC) system coupled with diode array detection. Gallic, protocatechuic, *p*-coumaric and vanillic acids were the major phenolic substances in grape juice, whereas caffeic acid was the most abundant phenolic acid in the wine after a short time of storage. For more reliable results, the antioxidant activity of grape juice and wine was measured by β-carotene bleaching (BCB) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods.**

**The content changes of volatile compounds in the grape juice and wine were determined by using headspace solid phase microextraction (HS-SPME) coupled with gas chromatography (GC/FID and GC/MS). Hexanal, (*E*)-2-hexenal, 2-ethyl-1-hexanol, 1-hexanol, (*Z*)-nerol-oxide and linalool were the most representative compounds determined in grape juice, whereas ethyl esters of hexanoic, octanoic, decanoic and dodecanoic acids, hexyl acetate, isoamyl acetate, as well as isobutanol, isoamyl alcohol and 1-hexanol were identified as the main compounds**.

K e y w o r d s : antioxidant activity, BCB, DPPH, fermentation, polyphenols, volatiles, wine.

## Introduction

Aroma and polyphenolic compounds are important constituents of wine as they contribute to the quality of the final product. The combination of different aroma compounds such as alcohols, esters, organic acids, aldehydes, ketones and terpenes forms the character of wine and differentiates one wine from another (Demyttenaere *et al*. 2003). Wine is also an excellent source of various classes of polyphenols, which are responsible for the sensory characteristics, particularly color, astringency and bitterness (Robichaud and Noble 1990). White wine contains significantly lower amounts of total polyphenols compared with red wines, mainly hydroxycinnamic acids (HCA), hydroxybenzoic acids (HBA) and flavan-3-ols (Makris *et al*. 2003). The total phenol content of white wines, vinified with a minimal skin contact, is in the range between 100 and 250 mg·l$^{-1}$. Approximately 30 mg·l$^{-1}$ of this amount is accounted by flavonoid phenols, mainly flavan-3-ol (-)-epicatechin and (+)-catechin and its dimers, oligomers and polymers (Fisher and Noble 1994, Noble 1990). Phenolic acids in grape berries are located primarily in the skin, whereas catechins and procyanidins are located in solid parts of the berry, particularly in the seeds (Simon *et al*. 1992). Skin contact may greatly increase both the total hydroxycinnamate and flavanol concentration, but decreases in total hydroxycinnamate content may be observed during fermentation (Baranowski and Nagel 1981). In contrast to the general practise to determine the phenolic compounds in red wine grape varieties (Larrauri *et al*. 1999, Lee *et al*. 2003), it seems that little attention has been paid to the non-colored phenolic acids in white varietal grapes (Ramos *et al.* 1999). Thus, this work examines the content changes of several phenolic acids and flavan-3-ols as well as of aroma compounds in white wine sampled during fermentation and after a short time of storage. Recently numerous research studies have associated the consumption of foods rich in polyphenols, including wine, with the prevention of cardiovascular diseases, certain types of cancer and other diseases related to aging, thanks to their antioxidant properties (Rice-Evans and Packer 1998, Visioli *et al*. 2000, Greenwald *et al*. 2001). The antioxidant activity of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule strengthened by steric hindrance (Rice-Evans *et al.* 1997). During fermentation and ageing of wine, various reactions take place, in which HCA and HBA acids change their form and content (Somers *et al*. 1987). In order to obtain more reliable results, the antioxidant activity in the present study by using DPPH and BCB methods was measured.

In the case of wine, the aroma properties have a direct influence on the acceptance or rejection of the product (Marti *et al*. 2003). The aroma of wine is influenced by the action of several different compounds on the sensory organs. These volatile aromatic compounds are produced through metabolic pathways during ripening and harvest of grapes, during their fermentation and/or also during the storage of wine. Wine aroma contains hundreds of components that belong to very heterogeneous groups such as alcohols, aldehydes, ketones, esters, acids, terpenes, etc. (Marti *et al*. 2003). It is known that several volatile compounds are responsible for the fermentation aroma in wines such as ethyl esters of C6, C8 and C10 fatty acids and ace-

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tates of higher alcohols, which enhance the fruity and floral character of white wines, whereas large amounts of higher alcohols and volatile acids may degrade the wine aroma (KARAGIANNIS *et al*. 2000). This complexity and the low levels of compounds, ranging from several mg $l^{-1}$ to a few ng $l^{-1}$, require the use of extraction and also concentration techniques. In the last years the effective SPME technique is frequently used. Its main advantages are simplicity and little sample manipulation (MARTI *et al*. 2003). However, the SPME method has been shown to be very sensitive to the operating conditions and any variation of the experimental parameters markedly affects the distribution and the adsorption of the analytes (GUADARRAMA *et al*. 2001). Therefore the use of an SPME autosampler is strongly recommended.

## Material and Methods

S a m p l e s : Wine was made from native grape varieties (*Vitis vinifera* L.) grown on the location Gornja Voća in the subregion Zagorje (winery of Branko Kos) and harvested at the technological state of ripeness in September 2005. After harvesting, blending of grapes ('Rhine Riesling' 80 %, 'Šipon' 15 % and 5 % mixed grapes) was performed. The technical production procedure consisted of the following steps: after pressing, the obtained must was placed in stainless steel tanks; then, 20 g $hl^{-1}$ of potassium-metabisulphite was added followed by sedimentation at 12 °C for 48 h. Pure must was decanted. The culture of multiplied selected yeast *Saccharomyces cerevisiae,* under the commercial name VIN13 (Anchor Biotechnologies, South Africa), was added in a quantity of 20 $g \cdot hl^{-1}$. The fermentation temperature was kept at 14 °C. After fermentation (sugar content below 2.5 $g \cdot l^{-1}$), the wine was decanted. The wine was kept under controlled conditions and was regularly analysed for the level of free $SO_2$. The description of samples regarding their sampling time is included in Tab. 1.

C h e m i c a l s : Vanillic and *p*-coumaric acids were obtained from Fluka (Buchs, Switzerland). Ferulic acid, gallic acid, protocatechuic acid, (+)-catechin, (-)-epicatechin and (-)-epicatechin gallate were obtained from Sigma (Sigma-Aldrich Chemie, Steinheim, Germany). Caffeic acid and HPLC grade methanol were obtained from Merck, (Darmstadt, Germany). Formic acid and Folin-Ciocalteu were of analytical grade and supplied by Kemika (Zagreb, Croatia). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and linoleic acid were obtained from Aldrich (Sigma-Aldrich Chemie, Steinheim, Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl) and Tween 40 were purchased from Fluka (Buchs, Switzerland). β-carotene was obtained from Sigma (Sigma-Aldrich Chemie, Steinheim, Germany). All standards of aroma compounds, except 1-hexanol and 3-decanol, were purchased from Sigma. 1-hexanol and 3-decanol were obtained from Fluka and ABCR GmbH & Co (Karlsruhe, Germany), respectively.

T o t a l p h e n o l i c c o n t e n t ( T P C ) : The total phenol content in selected samples was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (SINGLETON and ROSSI 1965). The measurements were calibrated against gallic acid standards and the results were expressed as $mg \cdot l^{-1}$ of gallic acid equivalents (GAE). The data represent the average of three measurements.

A n t i o x i d a n t a c t i v i t y

D e t e r m i n a t i o n o f a n t i o x i d a n t a c t i v i t y w i t h t h e D P P H r a d i c a l s c a v e n g i n g m e t h o d : The samples were analyzed according to the technique reported by BRAND-WILLIAMS *et al.* (1995). An aliquote of 20 µl was added to a volume of 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.094 mM in methanol up to completing 1 ml. The free radical scavenging activity using the free radical DPPH reaction was evaluated by measuring the absorbance at 515 nm after 60 min of reaction at 20 °C in a water bath. The reaction was carried out in closed Eppendorf tubes shaken at 20 °C. The results were expressed as mmol $l^{-1}$ Trolox equivalents, a vitamin E analogue (YAMAGUCHI *et al.* 1998). All determinations were performed in triplicate.

D e t e r m i n a t i o n o f a n t i o x i d a n t a c t i v i t y w i t h t h e β - c a r o t e n e b l e a c h i n g m e t h o d : The antioxidant activity with the BCB method was measured using the procedure of Von Gadow *et al.* (1997). 200 µl of undiluted grape juice or wine sample was added to the reaction mixtures. Readings were taken immediately (t = 0) and at 15 min intervals for 2 h (t = 120 min) on a spectrophotometer UV-VIS Unicam, Helios β spectrophotometer, at 470 nm. The vials were placed in a water bath at 50 °C between measurements.

The antioxidant activity coefficient (AAC) was calculated from the data according to the formula (MALLET *et al.* 1994):

$$AAC = [(A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)})] \times 1000$$

where $A_{A(120)}$ is the absorbance of the antioxidant at t = 120 min, $A_{C(120)}$ is the absorbance of the control at t = 120 min and $A_{C(0)}$ is the absorbance of control at t = 0 min. All determinations were also performed in triplicate.

H P L C a n a l y s i s : The samples were filtered through a 0.45 µm filter (Nylon Membranes, Supelco, Bellefonte, USA) before HPLC analysis. 20 µl of each sample were injected for HPLC analysis using a Varian Pro Star Solvent Delivery System 230 (Varian, Walnut Creek, USA) and a Photodiode Array detector Varian Pro Star 330 (Varian, Walnut Creek, USA) by using a reversed-phase column Pinnacle II C-18 column (Restek, USA) (250 x 4.6 mm, 5 µm i.d.). The solvents employed were water plus 3 % formic acid (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 $ml \cdot min^{-1}$. The elution was performed with a gradient starting at 2 % B to reach 32 % B at 20 min, 40 % B at 30 min and 95 % B at 40 min, and became isocratic for 5 min. Chromatograms were recorded at 278 nm.

Detection was performed with a Photodiode Array Detector by scanning between 200-400 nm, with a resolution of 1.2 nm. Phenolic compounds were identified by

Table 1

Antioxidative activity and contents of total phenols, hydroxybenzoic acids, hydoxycinnamic acids and flavan-3-ols

| No. | day | tpc | dpph | bcb | ga | pro | van | syr | caf | cou | fer | cat | epi | gal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 228 | 0.478 | 278.61 | 1.19 | 1.94 | 7.36 | 2.02 | 2.48 | 2.63 | 2.16 | 2.63 | 4.68 | 4.22 |
| 2 | 6 | 260 | 1.051 | 288.21 | 4.16 | 5.00 | 8.35 | 0.48 | 2.45 | 1.71 | 2.87 | 3.03 | 1.42 | 9.95 |
| 3 | 8 | 246 | 0.525 | 303.00 | 2.02 | 2.69 | 5.17 | 0.25 | 2.05 | 1.85 | 2.02 | 1.84 | 0.67 | 10.15 |
| 4 | 10 | 235 | 0.474 | 233.27 | 2.29 | 7.55 | 10.19 | 0.29 | 2.27 | 0.27 | 2.05 | 1.96 | 2.27 | 10.39 |
| 5 | 15 | 237 | 0.655 | 238.27 | 2.55 | 8.95 | 10.46 | 0.31 | 4.15 | 0.34 | 2.49 | 2.28 | 2.69 | 11.45 |
| 6 | 21 | 242 | 0.674 | 273.92 | 2.84 | 10.09 | 12.17 | 0.36 | 4.76 | 0.36 | 2.59 | 2.84 | 2.49 | 11.62 |
| 7 | 26 | 286 | 1.098 | 309.93 | 3.04 | 8.47 | 11.94 | 0.37 | 4.89 | 0.42 | 2.65 | 2.73 | 2.02 | 11.68 |
| 8 | 33 | 342 | 1.208 | 325.17 | 3.23 | 8.31 | 12.29 | 0.37 | 5.94 | 0.66 | 2.80 | 2.69 | 1.89 | 11.82 |
| 9 | 63 | 325 | 1.165 | 314.87 | 3.06 | 8.03 | 10.31 | 0.47 | 6.21 | 1.35 | 2.83 | 2.54 | 1.64 | 12.04 |
| 10 | 94 | 347 | 1.238 | 335.02 | 2.89 | 7.73 | 12.78 | 0.97 | 6.90 | 1.69 | 2.98 | 2.52 | 1.59 | 12.24 |
| 11 | 125 | 345 | 1.230 | 334.64 | 2.63 | 6.62 | 12.39 | 2.48 | 8.22 | 1.88 | 3.20 | 2.23 | 1.52 | 13.40 |

Day 0 - date of harvesting; day 6 - start of fermentation; tpc - total phenol content in mg $GAE^{-1}$; dpph - 2,2-diphenyl-1-picrylhydrazyl content in mmol Trolox $l^{-1}$; bcb - β-carotene bleaching in AAC; ga- gallic acid; pro - protocatechuic acid; van - vanillic acid; syr - syringic acid; caf - caffeic acid: cou - coumaric acid; fer - ferulic acid; cat - catechin; epi - epicatechin; gal - epicatechin gallate. Values are expressed as mean values in mg $l^{-1}$ sample (n = 3). The standard error depends on the compound and is around 10 %.

comparing the retention times and spectral data with those of authentic standards. Quantitative determinations were performed using standard curves. The data acquisition and treatment were conducted using the Star Chromatography Workstation Version 5 software. All analyses were repeated three times, and the results were expressed as mean values in milligrams per liter of wine.

Headspace-SPME: The wine volatiles were sampled by HS-SPME with a 100 µm PDMS fiber (Supelco) using an MPS 2 autosampler from Gerstel GmbH, Germany. To the sample of wine (200 ml) the internal standard 3-decanol was added resulting in a concentration of 0.1 ppm (v/v). An aliquot of 10 ml was placed into a 20-ml headspace vial containing solid NaCl p.a. (3 g) and capped with a crimp cap and teflon-lined septum. Equilibration time before absorption was 10 min at 35 °C and shaking (300 rpm). The fiber was exposed to the wine headspace for 15 min at 35 ºC with further shaking. Thermal desorption followed for 2 min in the injector (splitless mode) at 250 ºC and additional thermal cleaning (3 min at 250 ºC, split ratio 1:10).

Gas chromatographic analyses by GC/FID and GC/MS: The analyses were performed with an Agilent Technologies 6890 gas chromatograph equipped with a flame ionization detector (FID). Compounds were separated on a polar column HP INNOWax, 0.25 mm ID x 30 m length x 0.5 µm film thickness. The FID temperature was 250 ºC. Helium was used as a carrier gas with a column flow rate of 1.1 ml $min^{-1}$. The temperature program was the following: 40 ºC (3 min), from 40 to 200 ºC at 3 K $min^{-1}$ and 15 min at 200 ºC.

For identification, the same GC with an Agilent 5973 MSD in the electron impact ionization mode (70 eV) was used. GC run parameters were the same as described above. Compounds were identified using the Wiley 138, NIST02 and HPCH 1607 (Allured Corp., USA) libraries of mass spectra or by comparison with the mass spectrum and retention index of authentic references. Results were expressed as relative content of aroma compounds calculated on the basis of the peak area of the individual compounds in relation to the internal standard.

Statistical analysis: The statistical treatment of the data was performed by the software Statistica 7.1 (StatSoft, Inc., Tulsa, OK, USA).

## Results and Discussion

Blending of wines of different grape varieties has a long tradition in Croatian wine growing regions (Kovacevic Ganic *et al*. 2003). The wine studied in this paper presents one of the most often prepared blended wine in Croatian subregion Zagorje. Blending of 'Šipon', 'Riesling' and Muscat grape varieties leads to an improvement in the sensory quality of wine, which is the result of an increased refinedness and complexity of the aroma of blended wine.

The content of total phenols, determined by the Folin-Ciocalteu method, in grape juice and wine samples is given in Tab. 1. These values varied from 228 to 347 $mg \cdot l^{-1}$ gallic acid equivalents (GAE). As can be seen all samples tested in this study showed an evident antioxidant effect. Because the methods used to measure the antioxidant activity are extremely dependent on the reaction conditions and the substrates or products, all methods do not yield the same values for the activity (Fukumoto and Mazza 2000). Therefore, Frankel *et al.* (1993) and Warner (1997) suggested using more than one method to measure the antioxidant activity, by detecting the primary and secondary oxidation products, and using tests that detect specific substrates or products. The antioxidant activity of grape juice and wine was measured by β-carotene bleaching (BCB) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods. Upon comparing the total phenol content and antioxidant activity of samples, it can be observed that

the content of total phenols in wine is higher than in grape juice, as well as the values of the antioxidant activity measured by the DPPH and β-carotene bleaching methods. The results obtained by Brand-Wiliams *et al.* (1995) demonstrated that the number of DPPH radical molecules reduced by the phenolic acids decreased in the order caffeic acid > protocatechuic acid > ferulic acid > vanillic acid > *p*-coumaric acid. From this sequence it can be concluded that cinnamic acid derivatives are better antioxidants than their benzoic acid counterparts. This can be explained in terms of the CH = CHCOOH group, which participates in stabilizing the radicals of cinnamic acid derivatives by resonance. Further, caffeic acid exhibited stronger antioxidant effect compared with both catechin and epicatechin, using β–carotene and DPPH methods (Fukumoto and Mazza 2000). A previous study (Makris *et al*. 2003) demonstrated that the antioxidant activity of grape juices and wine is not a property of single phytochemical compounds, but is widely distributed among the phenolic constituents and it is dependent on both the total polyphenolic content and the relative amounts of individual polyphenols. Tab. 1 summarizes the content of identified hydroxybenzoic acids in the studied samples. Vanillic acid was detected as the major hydroxybenzoic acid in grape juice, followed by syringic, protocatechuic and gallic acids. In the final phenolic composition of wine the content of protocatechuic and gallic acids was higher than the content of syringic acid. With the value of 12.39 $mg \cdot l^{-1}$ (Tab. 1) vanillic acid was the dominant hydroxybenzoic acid in wine. The identified hydroxybenzoic acids represent 5.5 and 7.0 % of the total phenols in grape juice and wine, respectively. Hydroxycinnamic acids in wine originate from hydroxycinnamic tartaric esters' hydrolysis during fermentation (Cheynier *et al*. 1986). These acids, as secondary metabolites of phenylalanine, are well known in grapes representing potential browning and oxidation substrates, as well as contributing to the bitterness of wines and juices (Baranowski and Nagel 1981). As can be seen (Tab. 1) the content of caffeic acid, as the major compound of this group, increased from 2.48 $mg \cdot l^{-1}$ in grape juice to 8.22 $mg \cdot l^{-1}$ in wine. The content of *p*-coumaric and ferulic acids amounted to 2.63 $mg \cdot l^{-1}$ and 2.16 mg $l^{-1}$ in grape juice as well as to 1.88 and 3.20 $mg \cdot l^{-1}$ in wine, respectively. The content of all identified hydroxycinnamic acids was the lowest during the fermentation, especially in the first few days. This evolution of *p*-coumaric acid can be related to the activity of some of the microorganisms present in grape, which can metabolize the free phenolic acids into volatile phenols (4-vinyl and 4-ethyl derivatives). The volatile phenols contribute to the aroma of wine. In light of this, still Smit *et al*. (2003) offered very interesting prospects for the development of wine yeast starter strains with optimized decarboxylation activity on phenolic acids and the improvement of wine aroma in the future.

The most important group of phenolic compounds causing bitterness in red and white wines is flavan-3-ols. These phenols are extracted from the skins, stems and seeds of grapes in the course of vinification. (+)-Catechin, (-)-epicatechin and (-)-epicatechin gallate were identified from this group (Tab. 1). The lowest content of (+)-catechin (1.84 $mg \cdot l^{-1}$) and (-)-epicatechin (0.67 $mg \cdot l^{-1}$) was determined on the second day of fermentation when the fermentation reactions were the strongest. Generally, the content of (+)-catechin content varied from 1.84 to 3.03 $mg \cdot l^{-1}$ (mean 2.26 $mg \cdot l^{-1}$). The content of (-)-epicatechin ranged from 0.67 to 1.68 $mg \cdot l^{-1}$ (mean 2.10 $mg \cdot l^{-1}$) and the mean content of (-)-epicatechin gallate was 10.86 $m \cdot l^{-1}$. The identified flavan-3-ols represent 5.7 % of the total phenols in wine. This is less than in typical red wines but in accordance with the values expected for white wines with no maceration (Goldberg *et al*. 1999; Baderschneider and Winterhalter 2001). These compounds also possess potent antioxidant, anticarcinogenic and anti-inflammatory properties (Karagiannis *et al*. 2000). Among the tested samples, significant differences were observed in the content of caffeic acid and epicatechin gallate, the content of which steadily increased from grape juice to the last wine sample. A direct correlation between the antioxidant effectiveness of grape juices and their total phenol content was demonstrated by a correlation analysis (Tab. 1). The obtained results showed a good correlation between the total polyphenol content of wine and their antioxidant activity measured by the DPPH (0.92) and the BCB (0.84) methods, as well as between the results of both used methods (0.81). In the past few years, an increasing interest in plant polyphenols, which are frequent components of the human diet, has been manifested. The supplementation of natural antioxidants contained in the food including wine through a balanced diet could be more effective and also more economical than the supplementation of an individual antioxidant in protecting the body against oxidative damage under different conditions (Rapisarda *et al*. 1999).

Aroma substances are important in wine as they contribute to the quality of the final product, form the character of wine and differentiate one wine from another (Demyttenaere *et al*. 2003).

Tab. 2 summarizes all identified aroma compounds determined in studied samples. From an enological point of view, these compounds could be divided into two groups: one including compounds of fermentation origin and the second group including compounds, which have varietal or pre-fermentative origin and are scarcely affected by the fermantation process (Bueno *et al*. 2003). As can be seen, hexanal, (*E*)-2-hexenal, 1-hexanol, 2-ethyl-1-hexanol, (*Z*)-neroloxide and linalool were the most abundant aroma compounds in grape juice. The contribution of (*E*)-2-hexenal to the aroma is the greatest of these compounds, which supports the observation that the hexenols formed enzymatically when the grapes are crushed and oxidized during the aging process (Chisholm *et al*. 1995). Terpene compounds form an important part of the grape bouquet (Demyttenaera *et al*. 2003). These compounds do not change during the alcoholic fermentation and they are present in both the grape juice and wine. In accordance with the literature data, the higher alcohols, fatty acids and esters are the most important groups of the yeast-synthesised aroma substances of the fermentation bouquet. The content of aroma compounds increase after fermentation. Ethyl esters of hexanoic, octanoic, decanoic and dodecanoic acids are the most represented aroma compounds of studied wine. Ethyl esters hydrolyze more slowly than acetates, so their fruit

Table 2

Peak area ratio of selected aroma compounds

| No. | Compounds / day | 0 | 6 | 8 | 10 | 15 | 21 | 26 | 33 | 63 | 94 | 125 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a1 | ethyl acetate | 0.00 | 0.05 | 0.43 | 0.58 | 0.87 | 0.95 | 1.05 | 1.02 | 0.81 | 0.74 | 0.71 |
| a2 | ethyl butanoate | 0.00 | 0.00 | 0.05 | 0.07 | 0.10 | 0.09 | 0.11 | 0.13 | 0.09 | 0.07 | 0.07 |
| a3 | hexanal | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| a4 | isobutanol | 0.00 | 0.00 | 0.07 | 0.08 | 0.26 | 0.32 | 0.39 | 0.32 | 0.23 | 0.17 | 0.09 |
| a5 | isoamyl acetate | 0.01 | 0.01 | 1.72 | 2.61 | 3.41 | 3.21 | 3.18 | 3.15 | 2.76 | 2.06 | 1.39 |
| a6 | isoamyl alcohol | 0.01 | 0.02 | 1.22 | 2.59 | 3.95 | 4.11 | 3.82 | 3.63 | 3.64 | 3.48 | 3.36 |
| a7 | (*E*)-2-hexenal | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| a8 | ethyl hexanoate | 0.00 | 0.01 | 3.78 | 5.10 | 7.06 | 6.74 | 5.26 | 5.30 | 3.39 | 3.09 | 1.38 |
| a9 | hexyl acetate | 0.01 | 0.03 | 2.05 | 1.99 | 1.93 | 1.72 | 1.64 | 1.57 | 1.32 | 0.68 | 0.43 |
| a10 | terpinolen | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.59 | 0.01 | 0.01 |
| a11 | 3-hydroxy-2-butanaone | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.02 | 0.04 | 0.04 | 0.02 | 0.02 | 0.01 |
| a12 | (*Z*)-3-hexen-1-ol acetate | 0.01 | 0.00 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.02 | 0.01 |
| a13 | 1-hexanol | 0.12 | 0.15 | 0.15 | 0.16 | 0.20 | 0.18 | 0.21 | 0.17 | 0.16 | 0.13 | 0.05 |
| a14 | (*Z*)-3-hexen-1-ol | 0.00 | 0.00 | 0.04 | 0.04 | 0.05 | 0.06 | 0.06 | 0.04 | 0.03 | 0.03 | 0.03 |
| a15 | ethyl octanoate | 0.00 | 0.04 | 19.02 | 34.99 | 62.10 | 64.92 | 64.18 | 58.39 | 44.06 | 28.19 | 20.61 |
| a16 | (*Z*)-neroloxide | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.03 | 0.02 | 0.02 |
| a17 | 2-ethyl-1-hexanol | 0.01 | 0.05 | 0.06 | 0.09 | 0.02 | 0.03 | 0.11 | 0.11 | 0.12 | 0.05 | 0.02 |
| a18 | linalool | 0.04 | 0.03 | 0.05 | 0.08 | 0.09 | 0.09 | 0.07 | 0.07 | 0.07 | 0.04 | 0.03 |
| a19 | 1-octanol | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 |
| a20 | methyl decanoate | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.00 | 0.00 |
| a21 | ethyl decanoate | 0.00 | 0.02 | 6.21 | 7.86 | 29.22 | 30.13 | 27.99 | 25.11 | 26.75 | 23.41 | 13.18 |
| a22 | 3-methylbutyl octanoate | 0.00 | 0.00 | 0.26 | 0.34 | 0.40 | 0.39 | 0.41 | 0.22 | 0.13 | 0.08 | 0.07 |
| a23 | diethyl succinate | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| a24 | ethyl 9-decanoate | 0.00 | 0.00 | 0.03 | 0.04 | 0.07 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 |
| a25 | α-terpineol | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 | 0.00 | 0.01 |
| a26 | citronellol | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 |
| a27 | nerol | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 | 0.00 |
| a28 | 2-phenylethyl acetate | 0.04 | 0.05 | 0.14 | 0.16 | 0.17 | 0.17 | 0.19 | 0.19 | 0.17 | 0.14 | 0.11 |
| a29 | β-damascenone | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 |
| a30 | ethyl dodecanoate | 0.00 | 0.00 | 1.15 | 1.54 | 2.26 | 2.62 | 2.83 | 1.84 | 0.89 | 0.40 | 0.40 |
| a31 | geraniol | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| a32 | 3-methylbutyl decanoate | 0.00 | 0.00 | 0.04 | 0.07 | 0.19 | 0.17 | 0.18 | 0.04 | 0.03 | 0.02 | 0.02 |
| a33 | 2-phenylethanol | 0.00 | 0.01 | 0.11 | 0.24 | 0.53 | 0.39 | 0.32 | 0.35 | 0.30 | 0.11 | 0.14 |
| a34 | octanoic acid | 0.01 | 0.04 | 0.44 | 0.44 | 0.90 | 0.65 | 0.66 | 0.54 | 0.48 | 0.49 | 0.37 |
| a35 | decanoic acid | 0.03 | 0.07 | 0.50 | 0.43 | 0.47 | 0.52 | 0.48 | 0.46 | 0.84 | 0.75 | 0.56 |

Results are expressed as relative concentrations calculated on the basis of peak area of the individual compounds in relation to the peak area of an internal standard. The values are mean values of three replications. The standard error of concentration depends on the compound and is around 10 %.

aroma (apple, banana) would not be lost as fast. Other important esters of wine were isoamyl acetate, hexyl acetate, ethyl acetate and 2-phenylethyl acetate. Acetates, which are initially produced enzymatically, are slowly hydrolyzed during storage until equilibrium is reached with the corresponding acids and alcohols, increasing the concentration of acetic acid. A decrease in acetate concentration could be responsible for a loss in fruitiness while an increase in acetic acid concentration could be detected if other aging processes such as oxidation have raised the acetic acid level to above threshold concentrations (Chisholm *et al*. 1995). In very low content, ethyl acetate has a pleasant odor that contributes to the olfactory complexity and has a significant influence on the quality of wine. But the content of ethyl acetate contributes significantly to the volatile character of "acetic nose" and levels of 150 to 200 $mg \cdot l^{-1}$ impart spoilage character to wine. (Ribereau-Gayon *et al*. 1999). Among the alcohols isoamyl alcohol, 1-hexanol, 2-ethyl-1-hexanol, isobutanol and 2-phenylethylethanol were the most representative compounds. The isoamyl alcohol contributes to the chemical and harsh odor, whereas 1-hexanol and (*Z*)-3-hexenol resemble the green, grassy odor (Komes *et al*. 2005). The presence of 2-phenylethyl alcohol as well as the presence of β-damascenone in wine can result in a rose-like flavor (Demyttenaere *et al*. 2003).

The principal component analysis (PCA) and the correlation analysis reveal a lot of significant interactions between the 34 estimated parameters (Figs 1, 2 and Tab. 3). The highest correlation coefficients between the sum parameter methods (TPC, DPPH, BCB) and single phenols appear between DPPH and ferulic acid (0.92), DPPH and caffeic acid (0.80) as well as between TPC and caffeic

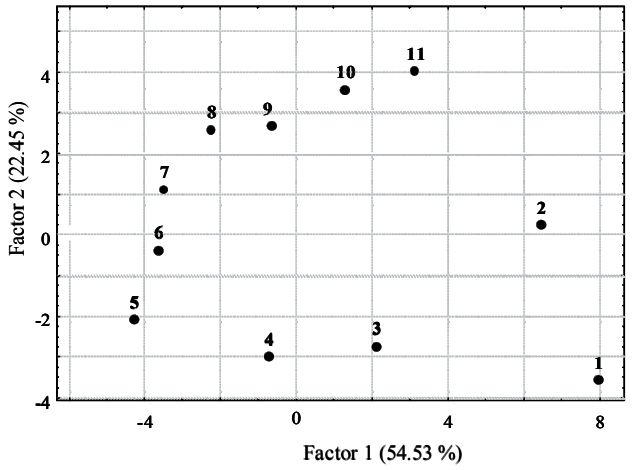


Fig. 1: PCA-Plot of factor 1 vs. factor 2 for samples (cases).

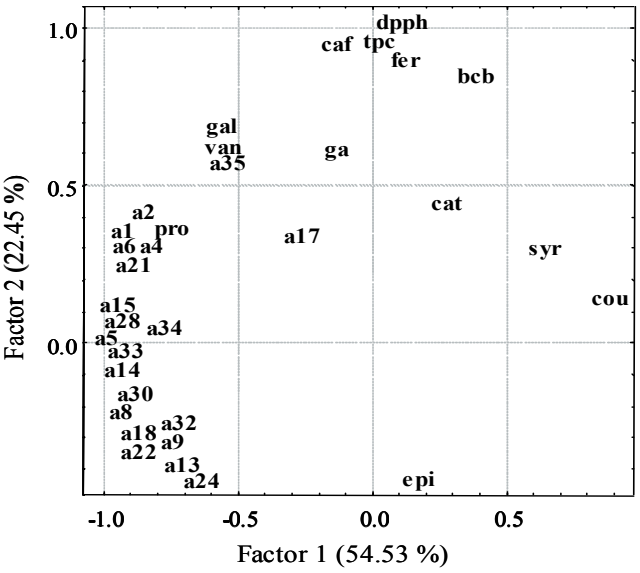


Fig. 2: PCA-Plot of factor 1 vs. factor 2 for parameters.

acid (0.88). The obtained results showed a good correlation between the total polyphenol content of samples and antioxidant activity measured by the DPPH (0.91) and the BCB (0.85) methods, as well as between the results of both used methods (0.80). The numerous relationships exist between phenols and volatiles. The most significant correlations were determined between protocatechuic acid (pro) and eight volatiles: ethyl acetate (0.86), isobutanol (0.81), isoamyl acetate (0.83), isoamyl alcohol (0.90), ethyl octanoate (0.86), ethyl decanoate (0.87), diethyl succinate (0.84) and 2-phenylethanol (0.81). The higest correlation was determined also between vanillic acid and β-damascenone as well as between caffeic acid and geraniol. On the other hand between coumaric acid (cou) and volatiles exist almost all negative correlations, except between this acid and hexanal (0.64) and (*E*)-2-hexenal (0.64).

The data points of the eleven samples in Fig. 1 represent the development of the totality of the 48 parameters from must to the wine at day 125.

The present study provides new insights into the changes of polyphenolic and aroma compositions during the fermentation and a short time of storage. Therefore, in order to obtain the complete information regarding these changes during aging of wine, this research will be continued.

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Table 3

Correlation matrix including 49 parameters*

| | tpc | dpph | bcb | ga | pro | van | syr | caf | cou | fer | cat | epi | gal | a1 | a2 | a3 | a4 | a5 | a6 | a7 | a8 | a9 | a10 | a11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| tpc | 1.00 | **0.91** | **0.85** | 0.41 | 0.30 | **0.61** | 0.26 | **0.88** | 0.12 | **0.81** | 0.14 | -0.45 | **0.62** | 0.44 | 0.41 | -0.40 | 0.26 | 0.15 | 0.45 | -0.40 | -0.13 | -0.22 | 0.30 | 0.21 |
| dpph | **0.91** | 1.00 | **0.80** | **0.68** | 0.37 | **0.62** | 0.15 | **0.80** | 0.05 | **0.92** | 0.43 | -0.46 | **0.62** | 0.39 | 0.34 | -0.35 | 0.32 | 0.10 | 0.38 | -0.34 | -0.17 | -0.32 | 0.28 | 0.27 |
| bcb | **0.85** | **0.80** | 1.00 | 0.28 | -0.06 | 0.28 | 0.34 | **0.68** | 0.43 | **0.66** | 0.21 | -0.44 | 0.36 | 0.20 | 0.15 | -0.16 | 0.12 | -0.12 | 0.14 | -0.16 | -0.35 | -0.35 | 0.19 | 0.05 |
| ga | 0.41 | **0.68** | 0.28 | 1.00 | 0.48 | 0.40 | -0.40 | 0.28 | -0.31 | **0.66** | 0.56 | -0.57 | 0.59 | 0.26 | 0.22 | -0.43 | 0.30 | 0.14 | 0.22 | -0.39 | 0.04 | -0.09 | 0.15 | 0.31 |
| pro | 0.30 | 0.37 | -0.06 | 0.48 | 1.00 | **0.84** | -0.40 | 0.52 | **-0.82** | 0.42 | 0.23 | -0.16 | **0.73** | **0.86** | **0.79** | **-0.69** | **0.81** | **0.83** | **0.90** | **-0.70** | **0.74** | 0.46 | 0.16 | **0.68** |
| van | **0.61** | **0.62** | 0.28 | 0.40 | **0.84** | 1.00 | 0.07 | **0.79** | -0.50 | **0.68** | 0.32 | -0.05 | **0.66** | **0.74** | **0.64** | -0.48 | **0.64** | 0.55 | **0.81** | -0.49 | 0.38 | 0.05 | 0.00 | 0.56 |
| syr | 0.26 | 0.15 | 0.34 | -0.40 | -0.40 | 0.07 | 1.00 | 0.38 | **0.67** | 0.31 | -0.02 | 0.36 | -0.24 | -0.35 | -0.41 | 0.48 | -0.46 | -0.57 | -0.23 | 0.47 | **-0.65** | **-0.71** | -0.13 | -0.52 |
| caf | **0.88** | **0.80** | **0.68** | 0.28 | 0.52 | **0.79** | 0.38 | 1.00 | -0.03 | **0.84** | 0.15 | -0.24 | **0.68** | **0.61** | 0.53 | -0.44 | 0.42 | 0.32 | **0.69** | -0.45 | 0.07 | -0.14 | 0.26 | 0.22 |
| cou | 0.12 | 0.05 | 0.43 | -0.31 | **-0.82** | -0.50 | **0.67** | -0.03 | 1.00 | 0.07 | -0.01 | 0.11 | -0.49 | **-0.73** | -0.74 | **0.64** | **-0.74** | **-0.86** | **-0.69** | **0.64** | **-0.89** | **-0.76** | 0.06 | **-0.78** |
| fer | **0.81** | **0.92** | **0.66** | **0.66** | 0.42 | **0.68** | 0.31 | **0.84** | 0.07 | 1.00 | 0.48 | -0.34 | **0.62** | 0.33 | 0.23 | -0.29 | 0.24 | 0.02 | 0.39 | -0.27 | -0.20 | -0.44 | 0.19 | 0.09 |
| cat | 0.14 | 0.43 | 0.21 | 0.56 | 0.23 | 0.32 | -0.02 | 0.15 | -0.01 | 0.48 | 1.00 | 0.24 | -0.08 | -0.01 | -0.07 | 0.31 | 0.28 | -0.14 | -0.04 | 0.33 | -0.19 | -0.48 | 0.05 | 0.23 |
| epi | -0.45 | -0.46 | -0.44 | -0.57 | -0.16 | -0.05 | 0.36 | -0.24 | 0.11 | -0.34 | 0.24 | 1.00 | **-0.73** | -0.27 | -0.25 | **0.74** | -0.07 | -0.18 | -0.20 | **0.72** | -0.09 | -0.27 | -0.14 | -0.06 |
| gal | **0.62** | **0.62** | 0.36 | 0.59 | **0.73** | **0.66** | -0.24 | **0.68** | -0.49 | **0.62** | -0.08 | **-0.73** | 1.00 | **0.75** | **0.69** | **-0.93** | 0.53 | **0.61** | **0.77** | **-0.92** | 0.46 | 0.37 | 0.17 | 0.40 |

Table 3: continued

| | a12 | a13 | a14 | a15 | a16 | a17 | a18 | a19 | a20 | a21 | a22 | a23 | a24 | a25 | a26 | a27 | a28 | a29 | a30 | a31 | a32 | a33 | a34 | a35 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| tpc | 0.20 | -0.39 | -0.05 | 0.11 | 0.45 | 0.33 | -0.28 | 0.36 | 0.24 | 0.37 | -0.34 | 0.34 | -0.33 | 0.11 | 0.05 | **-0.77** | 0.23 | **0.72** | -0.19 | **0.69** | -0.34 | -0.02 | 0.08 | **0.60** |
| dpph | 0.06 | -0.24 | -0.07 | 0.13 | 0.13 | 0.33 | -0.29 | 0.38 | 0.11 | 0.38 | -0.33 | 0.40 | -0.40 | -0.01 | 0.00 | **-0.73** | 0.13 | **0.75** | -0.14 | 0.59 | -0.22 | -0.01 | 0.04 | 0.44 |
| bcb | -0.06 | -0.43 | -0.20 | -0.11 | 0.20 | 0.19 | -0.52 | 0.23 | 0.20 | 0.14 | -0.47 | 0.19 | **-0.61** | 0.04 | -0.29 | **-0.66** | -0.01 | 0.55 | -0.30 | 0.50 | -0.44 | -0.31 | -0.16 | 0.40 |
| ga | 0.08 | 0.18 | 0.05 | 0.20 | -0.27 | 0.36 | 0.02 | 0.26 | 0.04 | 0.28 | -0.04 | 0.31 | -0.17 | -0.07 | 0.14 | -0.31 | 0.14 | 0.46 | 0.09 | 0.18 | 0.05 | 0.14 | 0.08 | 0.15 |
| pro | **0.62** | 0.42 | **0.74** | **0.86** | 0.18 | 0.33 | **0.69** | **0.71** | 0.21 | **0.87** | 0.59 | **0.84** | 0.35 | 0.08 | 0.57 | -0.30 | **0.75** | **0.74** | **0.68** | **0.62** | **0.66** | **0.81** | **0.76** | 0.57 |
| van | 0.32 | 0.01 | 0.45 | 0.60 | 0.23 | 0.17 | 0.29 | 0.60 | 0.06 | **0.71** | 0.22 | **0.74** | 0.00 | 0.14 | 0.46 | -0.59 | 0.49 | **0.85** | 0.37 | **0.75** | 0.34 | 0.48 | 0.48 | 0.50 |
| syr | **-0.62** | **-0.86** | -0.51 | -0.53 | -0.02 | -0.58 | **-0.65** | -0.46 | -0.50 | -0.36 | **-0.63** | -0.31 | -0.44 | -0.13 | -0.38 | -0.44 | -0.59 | -0.12 | -0.60 | 0.18 | -0.48 | -0.50 | -0.49 | -0.19 |
| caf | 0.20 | -0.38 | 0.20 | 0.33 | 0.35 | 0.07 | -0.07 | 0.41 | 0.05 | 0.59 | -0.14 | 0.56 | -0.17 | -0.02 | 0.04 | **-0.85** | 0.32 | **0.78** | 0.01 | **0.87** | -0.02 | 0.24 | 0.32 | **0.67** |
| cou | **-0.67** | **-0.71** | **-0.84** | **-0.87** | -0.07 | -0.43 | **-0.88** | **-0.68** | -0.40 | **-0.65** | **-0.87** | **-0.66** | -0.57 | -0.35 | **-0.64** | -0.19 | **-0.80** | -0.41 | **-0.88** | -0.33 | **-0.80** | **-0.85** | **-0.77** | -0.29 |
| fer | -0.12 | -0.38 | -0.08 | 0.09 | -0.05 | 0.00 | -0.28 | 0.19 | -0.20 | 0.37 | -0.36 | 0.39 | -0.35 | -0.21 | -0.08 | **-0.81** | 0.00 | **0.65** | -0.18 | 0.60 | -0.15 | 0.02 | 0.04 | 0.36 |
| cat | -0.26 | 0.19 | -0.20 | 0.08 | -0.42 | 0.00 | -0.05 | 0.11 | -0.16 | 0.19 | -0.24 | 0.33 | -0.55 | -0.51 | 0.19 | -0.01 | -0.24 | 0.38 | 0.00 | -0.04 | 0.07 | -0.03 | -0.22 | -0.28 |
| epi | -0.25 | 0.07 | -0.18 | -0.06 | -0.05 | -0.39 | 0.11 | -0.21 | -0.27 | -0.12 | -0.06 | -0.07 | -0.03 | -0.36 | 0.26 | 0.45 | -0.33 | -0.24 | -0.02 | -0.28 | 0.13 | 0.02 | -0.20 | -0.51 |
| gal | 0.47 | 0.01 | 0.56 | 0.55 | 0.18 | 0.33 | 0.26 | 0.55 | 0.22 | **0.64** | 0.33 | 0.59 | 0.23 | 0.29 | 0.09 | **-0.67** | 0.65 | 0.66 | 0.37 | 0.75 | 0.29 | 0.49 | 0.62 | 0.76 |

* Bold numbers are significant correlation coefficients.

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