

Phenolic contents, antioxidant activity and colour density of Slovak Pinot Noir wines

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Abstract: Recent studies show that wine contains more than thousand different compounds that could come from grapes, or could be formed in the process of winemaking and maturing. The most abundant compounds in wines are polyphenols, which affect sensory properties such as colour, taste and aroma, but also has antioxidant properties. The aim of this study was to determine total polyphenol and total anthocyanin contents, and to evaluate antioxidant effects and wine colour density of red wines 'Pinot Noir' produced in Slovakia. Thirteen analysed, bottled, quality dry 'Pinot Noir' wines with origin in various Slovak wine regions were purchased in retail network, to provide that analysed samples of wine would have the same properties as wines that are consumed by common consumers. The content of total polyphenols in analysed 'Pinot Noir' wines ranged from 1458 to 3324 mg GAE l⁻¹, while contents of total anthocyanins ranged from 43.6 to 279.6 mg l⁻¹. Antioxidant activities ranged from 80.2 % to 85.3 % inhibition of DPPH and wine colour density ranged from 0.679 to 1.495. The highest total polyphenol content, total anthocyanin content, and wine colour density was determined in wines from the south Slovakia winegrowing region, while the highest antioxidant activity in wines from Nitra winegrowing region. Results did not show significant differences among studied parameters in wines from different winegrowing regions. Results showed that Slovakian 'Pinot Noir' wines have characteristics comparable with 'Pinot Noir' wines from other countries.

Key words: wine; polyphenols; antioxidant activity; anthocyanins; 'Pinot Noir'

Vsebnost fenolov, antioksidacijska aktivnost in obarvanost slovaških vin iz sorte Pinot Noir

Izvleček: Sedanje raziskave kažejo, da vsebujejo vina več kot tisoč različnih spojin, ki izvirajo iz grozdja ali pa se lahko tvorijo v procesu pridelave in zorenja vina. Najpogostejše spojine v vinu so polifenoli, ki vplivajo na senzorične lastnosti vina kot so barva, okus in aroma, imajo tudi antioksidativne lastnosti. Namen raziskave je bil določiti celokupno vsebnost polifenolov in antocijaninov in ovrednostiti obarvanost rdečih vin, ki se pridelujejo na Slovaškem iz sorte Pinot Noir. Analizirano je bilo trinajst ustekleničenih kakovostnih suhih vin črnega pinoja, ki so izvirala iz različnih vinorodnih območij Slovaške, pridobljenih iz prodaje na drobno, da bi se zagotovili vzorci vina z enakimi lastnostmi kot jih ima vino v splošni porabi. Vsebnost celokupnih polifenolov v analiziranih vzorcih črnega pinoja je bila v območju od 1458 do 3324 mg GAE l⁻¹, medtem, ko je bila vsebnost celokupnih antocijaninov v območju od 43,6 do 279,6 mg l⁻¹. Antioksidacijska aktivnost je bila v območju od 80,2 % do 85,3 % inhibicije DPPH, obarvanost vina pa je bila v območju od 0,679 do 1,495. Največje vsebnosti celokupnih polifenolov in antocijaninov in največja obarvanost so bile določene v vzorcih vina iz južne Slovaške, največja antioksidacijska aktivnost pa v vinih iz vinorodnih območij Nitre. Izsledki niso pokazali značilnih razlik v preučevanih parametrih vin iz različnih vinorodnih območij, pokazali pa so, da so lastnosti slovaškega črnega pinoja primerljivi s črnimi pinoji iz drugih dežel.

Ključne besede: vino; polifenoli; antioksidacijska aktivnost; antocijanini; 'Pinot Noir'

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1 INTRODUCTION

The wine contains a number of polyphenolic substances that can affect its important sensory properties, such as colour, taste, bitterness and astringency (Ivanova-Petropulos et al., 2015). Phenolic substances are involved mainly in the colour changes of grapes, and play a key role in determining the quality of the wine. Antioxidant properties of phenolic compounds have positive impact on the wine stability. Their concentration in wine is affected by temperature and time of maceration, presence of SO₂, pH, and process of micro-oxygenation (Mulero et al., 2015). Main phenolic compounds in red wines are tannins, anthocyanidins, flavonols, flavan-3-ols, and stilbenes (Moreno and Peinado, 2012).

In viticulture, polyphenolic compounds play a very important role, because they affect the character, quality, taste, and colour of red wines (Li et al., 2009). The main source of polyphenols in wines are grape berries. They are in skin, pulp, seeds, and grape juice (Jackson, 2008). The final composition of polyphenolic compound in wine depends mainly on their content in grapes, which depends on many factors, such as climatic conditions, extraction, as well as winemaking technologies, and chemical reactions during the aging of wine (Atanacković et al., 2012).

Colour is one of the most important properties of red wines. Main cause of the red colour of wine are anthocyanins and their derivatives, which are formed during the fermentation process. Colour of red wine is influenced by many factors, including type and content of anthocyanins, pH, free SO₂ content, and extent of polymerization and co-pigmentation (Versari et al., 2008). During the first two years of wine maturation, monomeric anthocyanins go through a wide series of reactions, in which new pigments, important for colour stability, are formed. Although anthocyanins are odourless and almost tasteless, they can interact with other aromatic substances, and thus affect the taste of wine. Anthocyanins are water soluble flavonoid pigments, which contribute to the red, violet, or blue colour of the grapes, depending on the pH (He et al., 2012). Monomeric forms of anthocyanins are responsible for the red colour of young wines, and contribute to the development of red polymer pigments during the wine maturation (Versari et al., 2008). Main monomeric anthocyanins of red wines are 3-O-monoglucosides, which include delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside (Jackson, 2008).

The antioxidant activity of anthocyanins is considered to be one of their most important physiological functions (Yang et al., 2009). Intake of anthocyanins

has been linked to a number of human health benefits. They have strong antioxidant properties, and act as protective agents against many chronic diseases (Welch et al., 2008).

'Pinot Noir' is intended mainly for the production of quality varietal wines in the category of the late harvest to grape selection. It has a genetically lower content of anthocyanins. The usual alcohol content in these wines is about 13 vol. %. The wines are lighter brick colour and their aroma is distinctly fruity reminiscent of cherries, plums, and forest fruits. Wines made from this variety are usually extractive with a pleasant taste of tannins and are suitable for archiving (Pavloušek, 2007). In ordinary vintages, it provides soft, velvety, alcoholic, full-bodied wines with a delicious almond bouquet. They reach their peak at the bottle maturity that, according to the year and quality, sometimes appears only after several years (Malík et al., 2005). According to the Vineyard Register of the Slovak Republic (2020), the total area of bearing vineyards as of 31.7.2020 was 11090 ha. Red grapevine varieties represent 3226 ha, 'Pinot Noir' represents 223 ha. In the last decade, there has been a decrease of the total vineyard area by 23.2 %. According to OIV (2020), wine production in Slovakia in 2020 was cca 300000 hl, with Pinot Noir representing less than 1 % of it.

The aim of this study was to determine and evaluate properties and their mutual correlations in Slovak wines Pinot Noir, and to compare them with other Slovak red wines.

2 MATERIALS AND METHODS

2.1 SAMPLES

Analysed, bottled, quality dry Pinot Noir (PN) wines and their characteristics are mentioned in Table 1. Wine samples with origin in various Slovak winegrowing regions (WR) were purchased in retail network, to provide that analysed samples of wine would have the same properties as wines that are consumed by common consumers.

2.2 CHEMICALS AND INSTRUMENTS

The chemicals used for all analysis were: Folin-Ciocalteu reagent, monohydrate of gallic acid p. a., anhydrous sodium carbonate p. a., citric acid p. a., disodium hydrogenphosphate dodecahydrate, 35 % hydrochloric acid p. a., ethanol p. a., methanol p. a., 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical p. a., Trolox (pure).

Table 1: Characteristics of analysed wines

Sample	Producer	Winegrowing region	Quality	Vintage
PN-LC1	Karpatská Perla, Šenkvice	Little Carpathian	Grape selection	2011
PN-LC2	Mrva a Stanko, s. r. o., Trnava	Little Carpathian	Grape selection	2012
PN-LC3	VPS, s. r. o., Pezinok	Little Carpathian	Grape selection	2013
PN-LC4	Lacko & Majtán, Malacky	Little Carpathian	Quality	2012
PN-SS1	Villa Víno Rača, a. s., Bratislava	South Slovak	Late harvest	2013
PN-SS2	Víno Matyšák, s. r. o., Pezinok	South Slovak	Grape selection	2012
PN-SS3	Vinárske závody Topoľčianky, s. r. o.	South Slovak	Quality	2013
PN-SS4	Vinárske závody Topoľčianky, s. r. o.	South Slovak	Late harvest	2013
PN-SS5	Víno Nitra / Ch. Modra, Trnava	South Slovak	Grape selection	2012
PN-N1	Muráni-Víno Čajkov, s. r. o., Čajkov	Nitra	Cabinet	2010
PN-N2	Agropest, s. r. o., Veľký Cetín	Nitra	Grape selection	2012
PN-N3	Pivnica Radošina, s.r.o. Trnava	Nitra	Grape selection	2012
PN-N4	PD Mojmírovce	Nitra	Grape selection	2012

PN – Pinot Noir, LC – Little Carpathian winegrowing region, SS – South Slovakia winegrowing region, N – Nitra winegrowing region

All analysed parameters – total polyphenol content, total anthocyanin content, antioxidant activity and wine colour density in wines were analysed by UV/VIS spectrophotometry (spectrophotometer Shimadzu UV/VIS – 1240, Shimadzu, Japan).

2.3 WINE ANALYSIS

2.3.1 Determination of total polyphenol content

Total polyphenol content (TPC) was assessed by the modified method of Singleton & Rossi (1965) using of 20 % solution of Na_2CO_3 , Folin-Ciocalteu reagent and distilled water. 1 ml of wine sample was pipetted into 50 ml volumetric flask and diluted with 25 ml of distilled water. Then, 2.5 ml Folin-Ciocalteu reagent was added to dilute the mixture, and after 3 minutes, 1.5 ml of 20 % solution of Na_2CO_3 was added. Then, the sample was filled with distilled water to volume 50 ml, and after mixing, left at the laboratory temperature for 2 hours. The blank and calibration solutions of gallic acid were prepared by the same procedure. The absorbance of samples solutions was measured against blank solution at 765 nm. The content of total polyphenols in wines was calculated as the amount of gallic acid equivalent (GAE) in mg per 1 litre of wine (mg GAE.l^{-1}).

2.3.2 Determination of antioxidant activity

Antioxidant activity (AA) was assessed by the method of Brand-Williams et al. (1995) using of DPPH

(2, 2-diphenyl-1-picrylhydrazyl) radical. Exactly, 3.9 ml of DPPH solution was pipetted into cuvette. The absorbance of DPPH solution was measured at 515.6 nm. Then, 0.1 ml of wine sample was added, stirred, and left for 10 minutes. After 10 minutes, absorbance at 515.6 nm was measured, and antioxidant activity was expressed as % inhibition of DPPH, and also as Trolox equivalent calculated from the calibration curve (TE l^{-1})

2.3.3 Determination of total anthocyanin content

Total anthocyanin content (TAC) was assessed by the modified pH differential method of Lapornik et al. (2005), based on the transformation of all anthocyanins to red coloured flavylum cation by reduction of the pH of wine samples with HCl solution to values 0.5-0.8. The total anthocyanin content was calculated from the difference of absorbance values of both solutions (diluted original and a sample of wine acidified with HCl) and expressed as the amount of malvidin-3-monoglucoside in mg l^{-1} of wine.

2.3.4 Determination of wine colour density

Wine colour density (WCD) was measured by the method of Sudrand (1958) as the sum of the absorbance at 420 nm and 520 nm. The absorbance of the wine samples was measured in 0.2 cm path length glass cells. WCD was also expressed in the absorbance unit (AU)

considering dilution factor ($R = 5$), for obtaining better comparison with other authors.

All chemical analyses were performed as four parallels. Results were expressed by average \pm standard deviation.

2.4 STATISTICAL ANALYSIS

MS Excel 2016 and XLSTAT were used to perform statistical analysis. To obtain statistically significant information about the differences between the tested samples, nonparametric Kruskal-Wallis test was conducted (Addinsoft, 2014).

3 RESULTS AND DISCUSSION

All studied parameters – total polyphenols content (TPC), total anthocyanins content (TAC), antioxidant activity (AA) and wine colour density (WCD) of the Slovak Pinot Noir wines are described in Table 2.

Total polyphenols content (TPC) in analysed wines ranged from 1458 to 3324 mg GAE l⁻¹, reaching an average TPC 2334 mg GAE.l⁻¹. TPC in Slovak Pinot

Noir wine was about the same as TPC in Argentinian Pinot Noir wines (2319 mg GAE l⁻¹), higher than TPC in Croatian (1825 mg GAE l⁻¹), Italian (2029 mg GAE l⁻¹), French (2062 mg GAE l⁻¹) and Chilean Pinot Noir wines (1759 mg GAE l⁻¹), but lower than TPC in Czech (8714 mg GAE l⁻¹) and French Pinot Noir wines (3545 mg GAE.l⁻¹) (Landrault et al., 2001; Mlček et al., 2019; Šeruga et al., 2011; Van Leeuw et al., 2014). Previous studies of Slovak red wines showed about the same TPC in Blaufränkisch wines – 2003 mg GAE.l⁻¹, Saint Laurent wines – 2297 mg GAE l⁻¹, Cabernet Sauvignon wines – 2424 mg GAE l⁻¹, and higher TPC in Slovak Alibernet wines – 3057 mg GAE l⁻¹ (Bajčan et al., 2012; Bajčan et al., 2015; Bajčan et al., 2016).

According to the average TPC, wines from SSWR reached the highest content (2543 mg GAE l⁻¹), followed by wines from LCWR (2418 mg GAE l⁻¹) and wines from NWR (1990 mg GAE l⁻¹). However, the results did not show significant differences in TPC among Pinot Noir wines from different vineyard areas in Slovakia, as shown in Figure 1.

Total anthocyanins content (TAC) in analysed wines ranged from 43.6 to 279.2 mg l⁻¹, reaching an average TAC 153.3 mg l⁻¹. TAC in Slovak Pinot Noir wines was higher than TAC in Uruguayan Pinot Noir wines

Table 2: Total polyphenols content, total anthocyanins content, antioxidant activity and wine colour density in analysed Pinot Noir wines from Slovakia

Sample	TPC (mg GAE l ⁻¹)	TAC (mg l ⁻¹)	AA (%)	AA (mmol TE l ⁻¹)	WCD _{0.2}	WCD _{1.0} (AU)
PN-LC1	3138 \pm 52	128.5 \pm 1.4	81.9 \pm 0.3	1.015 \pm 0.004	0.956 \pm 0.011	4.78 \pm 0.055
PN-LC2	3039 \pm 26	167.4 \pm 1.4	80.5 \pm 0.5	0.992 \pm 0.006	1.049 \pm 0.008	5.245 \pm 0.04
PN-LC3	2035 \pm 26	229.6 \pm 1.8	83.9 \pm 0.5	1.050 \pm 0.006	0.771 \pm 0.015	3.855 \pm 0.075
PN-LC4	1458 \pm 25	82.5 \pm 1.2	84.5 \pm 0.4	1.061 \pm 0.006	0.918 \pm 0.014	4.59 \pm 0.07
Average LCWR	2418 \pm 816 ^a	152 \pm 71.4 ^a	82.7 \pm 1.9 ^a	1.030 \pm 0.034 ^a	0.924 \pm 0.135 ^a	4.618 \pm 0.675 ^a
PN-SS1	2604 \pm 13	271.3 \pm 9.8	80.7 \pm 0.5	0.995 \pm 0.006	1.495 \pm 0.021	7.475 \pm 0.105
PN-SS2	2690 \pm 24	69.8 \pm 1.4	85.3 \pm 0.4	1.074 \pm 0.005	0.679 \pm 0.006	3.395 \pm 0.03
PN-SS3	1777 \pm 24	279.2 \pm 1.4	83.8 \pm 0.3	1.048 \pm 0.004	0.959 \pm 0.008	4.795 \pm 0.04
PN-SS4	3324 \pm 26	159.4 \pm 2.1	81.0 \pm 0.4	1.000 \pm 0.005	1.113 \pm 0.016	5.565 \pm 0.08
PN-SS5	2318 \pm 25	101.5 \pm 1.6	82.7 \pm 0.4	1.029 \pm 0.006	1.045 \pm 0.009	5.225 \pm 0.045
Average SSWR	2543 \pm 751 ^a	176.2 \pm 101.7 ^a	82.7 \pm 2.2 ^a	1.029 \pm 0.036 ^a	1.058 \pm 0.396 ^a	5.291 \pm 1.98 ^a
PN-N1	1995 \pm 25	43.6 \pm 2.1	84.3 \pm 0.6	1.057 \pm 0.007	0.832 \pm 0.007	4.16 \pm 0.035
PN-N2	1943 \pm 12	87.6 \pm 2.8	80.2 \pm 0.5	0.987 \pm 0.006	1.156 \pm 0.016	5.78 \pm 0.08
PN-N3	1895 \pm 24	199.5 \pm 1.4	84.1 \pm 0.4	1.053 \pm 0.005	0.779 \pm 0.01	3.895 \pm 0.05
PN-N4	2125 \pm 25	173.6 \pm 2.1	83.6 \pm 0.6	1.042 \pm 0.007	0.923 \pm 0.015	4.615 \pm 0.075
Average NWR	1990 \pm 112 ^a	126.1 \pm 75.Z ^a	83.1 \pm 2.0 ^a	1.035 \pm 0.034 ^a	0.923 \pm 0.183 ^a	4.613 \pm 0.915 ^a
Total average	2334 \pm 577	153.3 \pm 76.4	82.8 \pm 1.7	1.031 \pm 0.030	0.975 \pm 0.210	4.875 \pm 1.05

Different letters indicate significant differences ($p < 0.05$) among different winegrowing regions.

(78.1 mg l⁻¹), and lower than TAC in Australian Pinot Noir wines (190 mg l⁻¹, 232 mg l⁻¹) (Carew et al., 2013; Piccardo et al., 2019; Song et al., 2014).

Previous studies of Slovak red wines showed higher TAC in Blaufränkisch wines – 266.1 mg l⁻¹, Saint Laurent wines – 264.0 mg l⁻¹, Cabernet Sauvignon wines – 220.6 mg l⁻¹, and Alibernet wines – 403.0 mg l⁻¹. (Bajčan et al., 2012; Bajčan et al., 2015; Bajčan et al., 2016).

According to the average TAC, wines from SSWR reached the highest content (176.2 mg l⁻¹), followed by wines from LCWR (152 mg l⁻¹) and wines from NWR (126 mg l⁻¹). However, the results did not show significant differences in TAC among Pinot Noir wines from different vineyard areas in Slovakia, as shown in Figure 1.

Antioxidant activity (AA) in analysed wines ranged from 80.2 % (0.987 mmol TE l⁻¹) to 85.3 % (1.074 mmol TE l⁻¹), reaching an average AA 82.8 % (1.031 mmol TE l⁻¹). AA in Slovak Pinot Noir wines was higher than AA in South American Pinot Noir wines (47.93 - 66.70 %), but lower than AA in Croatian Pinot Noir wines (4.3 mmol TE l⁻¹) (Granato et al., 2011; Šeruga et al., 2011). Previous studies of Slovak red wines showed about the same AA in Blaufränkisch wines – 83.8 %, Saint Laurent wines – 81.2 %, Cabernet Sauvignon wines – 78.8 %, and lower average AA in Slovak Alibernet wines –

74.5 % (Bajčan et al., 2012; Bajčan et al., 2015; Bajčan et al., 2016).

According to the average AA, wines from NWR reached the highest content (83.1 %), followed by wines from LCWR (82.7 %) and wines from SSWR (82.7 %). However, the results did not show significant differences in TAC among Pinot Noir wines from different vineyard areas in Slovakia, as shown in Figure 1.

Wine colour density (WCD) in analysed wines ranged from 0.679 (3.395 AU) to 1.459 (7.475 AU), reaching an average WCD 0.975 (4.875 AU). Song et al., (2014) reported about the same average WCD in Australian Pinot Noir wines (3.61 - 8.47 AU). WCD in Slovak Pinot Noir wines was higher than WCD in Australian Pinot Noir wines (2.4 - 3.7 AU) (Carew et al., 2013). Previous studies of Slovak red wines showed higher WCD in Cabernet Sauvignon wines – 1.399 and Alibernet wines – 2.317 (Bajčan et al., 2015; Bajčan et al., 2016).

According to the average WCD, wines from SSWR reached the highest content (1.058), followed by wines from LCWR (0.924) and wines from NWR (0.923). However, the results did not show significant differences in WCD among Pinot Noir wines from different vineyard areas in Slovakia, as shown in Figure 1.

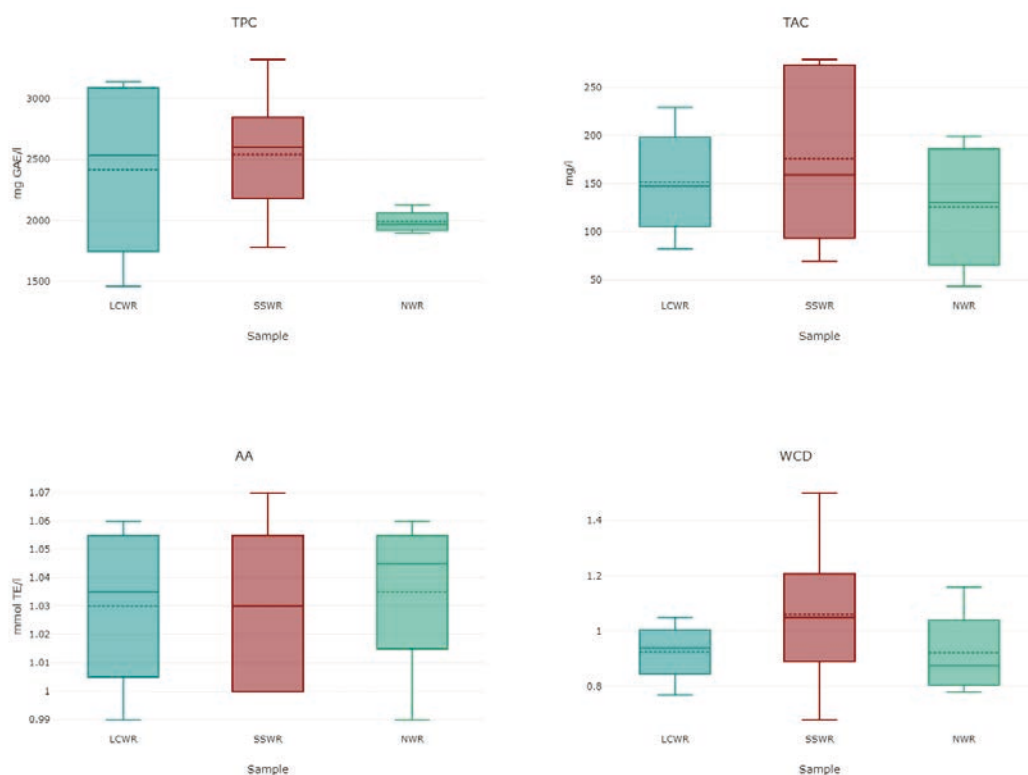


Figure 1: Differences among individual properties of wines from different winegrowing regions

In order to examine the mutual relations among analysed parameters, the linear regressions were made. Results are shown in Figure 2. The statistical evaluation of the obtained results confirmed strong negative linear correlation between AA and WCD ($r = -0.825$), which is in accordance with previous reports by Bajčan et al. (2016) for Slovak Cabernet Sauvignon wines and for Slovak Alibernet wines (Bajčan et al., 2015). Furthermore, there were not confirmed correlations between TPC and TAC ($r = 0.01$), between TPC and AA ($r = -0.052$), between TPC and WCD ($r = 0.277$), between TAC and AA ($r = -0.171$), and between TAC and WCD ($r = 0.038$). Bajčan et al. (2015) and Bajčan et al. (2016) reported moderate positive correlations between TPC and TAC ($r = 0.542$), TAC and WCD ($r = 0.600$), and TPC and WCD ($r = 0.697$) in Slovak Cabernet Sauvignon wines and moderate positive correlation between TPC and TAC ($r = 0.447$), TAC and WCD ($r = 0.660$), moderate negative correlation between TAC and AA ($r = -0.532$), and strong positive correlation between WCD and TPC ($r = 0.887$), and strong negative correlation between TPC and AA ($r = -0.917$) in Slovak Alibernet wines. Based on our results, it can be stated that there are no strong correlations between the individual monitored properties of wines, except for AA and WCD. These correlations are unusual and in disagreement with other authors. Granato et al. (2011) reported moderate positive correlation between TPC and AA ($r = 0.59$) in Australian Pinot Noir wines. Šeruga et al. (2011) reported strong positive correlation between TPC and AA in Croatian Pinot Noir wines ($r = 0.9885$).

4 CONCLUSIONS

Total phenolic contents, total anthocyanin contents, antioxidant activities and wine colour densities of Pinot Noir wines from three vineyard regions of Slovakia was determined in this study. Studied Pinot Noir wines showed high antioxidant activity, content of polyphenols and anthocyanins, the substances that contribute to the various health benefits.

The highest total polyphenol content, total anthocyanin content, and wine colour density was determined in wines from South Slovakia winegrowing region, while the highest antioxidant activity in wines from Nitra winegrowing region. At the end, Slovak Pinot Noir wines showed lower wine colour density in comparison to other Slovak wines. Results did not show significant differences among studied parameters in wines from different winegrowing regions. Based on statistical evaluation, strong negative correlation between antioxidant activity and wine colour density was determined.

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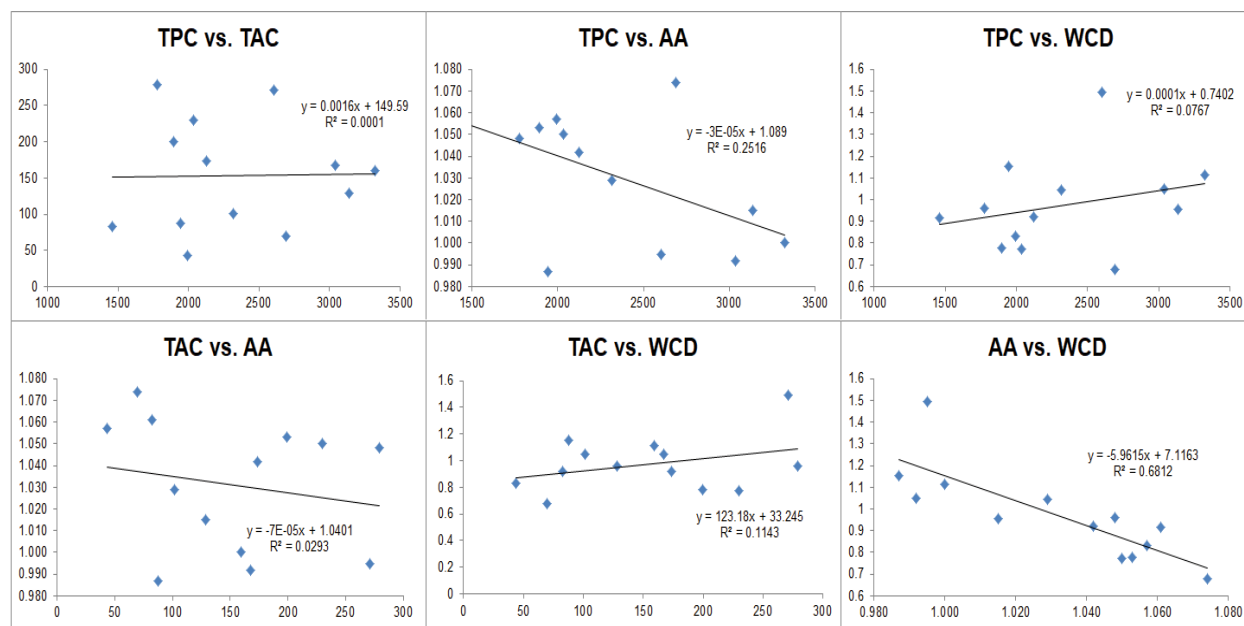


Figure 2: Correlations among analysed parameters

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