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REDUCE THE SULPHUR DIOXIDE CONTENT OF WINE BY BIOLOGICAL PROCESS IN RELATION TO THE CONTENT OF POLYPHENOLIC SUBSTANCES

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ABSTRACT

In recent years, many papers have paid attention to the bioactive compounds, particularly to the antioxidant activity of polyphenolic compounds in food and beverages, due to their positive effect on human body. Therefore, the phenolic compounds and their antioxidant capacity become an important quality parameter, especially in niche markets concerned with health benefits.
Wine is an excellent source of various classes of polyphenols. The phenolic compounds are responsible for the sensory characteristics, particularly colour, astringency, bitterness, and aroma, too. Another very important ingredient in the wine is added the sulphur dioxide, which has the role to protect these reductive components of wines.
Sulphur dioxide has the effects of antioxidant, antimicrobial and preservative that is mutually merging. However, it is possible to increase the stability of the complex of biologically active substances (BAS), as holders of natural antioxidant capacity. The present contribution brings opportunities rather than full elimination, but a substantial reduction of the content of sulphur dioxide, which protects the wine during the entire technological process of manufacture and treatment. The dosage of sulphur dioxide can be reduced in its values from 15 to 40% of the total health of the authorized maximum sulphur dioxide content of wine.

Keywords: Polyphenols, stabilization, antioxidant capacity, sulphur dioxide

INTRODUCTION

As consumers have become more conscious of the health benefits of phenolic compounds and their antioxidant activities via the conventional media, the beverage industries have recognized new marketing opportunities for their products. Wine is a widely consumed beverage in the world, with thousands of years of tradition.
As well as phenolic compounds in red wine exhibit a board spectrum of beneficial pharmacological properties, believed to be related to their antioxidative properties. Anti-atherogenic, anti-tumour, anti-ulcer, and anti-inflammatory activities have all been demonstrated by the consumption of red wine and red wine phenolic compounds **(Kinsella *et al.*, 1993; Clifford *et al.*, 1996; Saito *et al.*, 1998; Estruch *et al.*, 2004; Stocker *et al.*, 2004; Valko *et al.*, 2007)**. As a material for winemaking, the phenolic compounds of wine grape are one of the most important aspects determining wine quality. Many published papers have focused on the essential contributions of phenolic compounds profiles to wine quality and sensory properties **(Spranger *et al.*, 2004; Vidal *et al.*, 2004)**.
The phenolic profiles in wine depend on the phenolic compounds present in the grapes, the extraction parameters, winemaking technologies as well as fermentation temperature, yeast strain, processing enzymes, cap management, and alcohol concentration **(Fang *et al.*, 2007; García-Falcón *et al.*, 2007; Fang *et al.*, 2008)**. On the other hand, phenolic compounds of grapes are affected by many factors such as agrotechnical processes, genetic variation, and maturity, climatic and geographical conditions **(Dokoozlian *et al.*, 1996; Kennedy *et al.*, 2002; Dopico-García *et al.*, 2008;)**. Other factors that influence the extent of phenolic extraction are the molecular weight, size and type of phenolic molecules, the surface area, the concentration gradient, other temperature treatments including grape and must freezing and thermo-vinification, and factors that affect cell permeability, such as pectolytic enzyme selection **(Romero-Cascales *et al.*, 2005)**. Also, the environmental condition (temperature, annual precipitation levels, altitude, and geochemical characteristics) can affect the vine grapes maturation and consequently the concentration of their phenolic compounds.
Sulphur dioxide is already after a century yet indispensable additive component of the wines, but also of other beverages, even some of the dishes, especially fruit origin. It has the effects of antioxidant, antimicrobial and preservative that is mutually merging. Replacement of it within the framework throughout his effect is not possible so far. However, it is possible to increase the stability of the complex of biologically active substances (BAS), as holders of natural antioxidant capacity. During the entire technological process of the production and care of wine either escape or remain with their antioxidant values to a large extent in the residual biomass. The present contribution brings opportunities rather than full elimination, but a substantial reduction of the content of sulphur dioxide, which protects the wine during the entire technological process of manufacture and treatment. The dosage of sulphur dioxide can be reduced in its values from 15 to 40 % of the total health of the authorized maximum sulphur dioxide content of wine. For 20-25 % of the adult population are even allowed doses of sulphur dioxide a strong allergen and wine or other drinks or some fruit dishes it treated to become unacceptable.
Sulphur dioxide is the main antioxidant, antiseptic and complexed with other agents, preservatives agent. It is necessary to know its various forms occur throughout oenological technological process. Application of SO_2 in relation to its various forms to various compounds of wine is a prerequisite to prevent undesired organoleptic changes **(Salaha *et al.*, 2008)**. Sulphur dioxide is highly soluble in cold water and in wine, but with increased temperature decreases its solubility. Generally, at 20 ºC in 1 dm^3 of water was dissolved 39 dm^3 SO_2. Solubility is half compared to 0 ºC but twice the temperature of 40 ºC **(Poláček *et al.*, 2003; Michlovský, 2012)**. Created sulphurous acid dissociates in aqueous solution to the substance delivery of free SO_2 and SO_2 parts, which is bonded with other substances in the wine and a so-called bounded sulphur dioxide. The total content of SO_2 as sum of both forms is very important parameter of quality of wines supervised by the government legislation. Undissociated H_2SO_3 is present in negligible scale, which must constitute at max. 3 $mg.L^{-1}$ and at this concentration is not inhibitive for microorganisms **(Farkaš, 1983; Záhorský, 2013)**. Free SO_2 consists of three substances - molecular (active), hydrogen sulphite ions and sulphite ions. The amount of the individual substances depends on the dissociation constants and the pH of the environment **(Záhorský, 2013)**. Molecular SO_2 is very important form due to its antiseptic properties. Slightly pass by simple diffusion through the cell membrane of microorganisms and thereby inhibiting the activity of enzymes and proteins in the cell. Molecular SO_2 most active in the pH range 0-2. With increasing pH, amount of molecular SO_2 decreases, so its value is needed

to monitor and regulate them by adding SO_2. SO_2 reacts with many different substances in wine and molecular SO_2 is only a small percentage of free SO_2. In the field of wine pH 3-4 is only 1-6 % in molecular form **(Henderson, 2009; Divol *et al.*, 2012)**. To protect the wine from oxidation and microbial activities are recommended values of molecular SO_2 in ranges 0.6-0.9 mg.L^{-1} for antioxidative effect. Antimicrobial effects have 0.8-1.3mg molecular SO_2 and 1.5 mg.L^{-1} molecular SO_2 is suitable for wines with residual sugar content. The concept of free and molecular sulphur dioxide is important to consider at the same time in relation to the microbiological stability and the ability to absorb oxygen. The concentration of free SO_2 should be regulated according to the required protection against oxidation. Molecular SO_2 concentration is adjusted according to the desired antiseptic protection **(Michlovský, 2012)**. Acetaldehyde is a by-product of alcoholic fermentation. Its content decreases with the termination of fermentation since the acetaldehyde may be transported back into yeast cells and metabolized to ethanol. Free acetaldehyde gives the wine an undesirable odour after staleness **(Jackson, 2008)**. The sulphur dioxide added to the must before fermentation period causes the formation of acetaldehyde. Bounded acetaldehyde cannot be degraded during the fermentation due to its high affinity with SO_2 **(Michlovský, 2014a)**. Aldehyde compounds may contribute to the character of the wine flavour, aroma of various herbs or weathered apple. Combining with sulphur dioxide is transformed into additive compound acetaldehyde-sulphur dioxide which prevents stale taste. In practice at this observation must be added the monitored low dose of SO_2 to produce a slight excess of free SO_2 relative to acetaldehyde. This character staleness appears in wine of Madeira type, which is desirable (Table1) **(Michlovský, 2014b)**.

Table 1 Content of SO_2 in different types of wine produced conventional and organic (BIO) technology.

Type of wine	Conventional	BIO
	Total cont. SO_2 [mg.L^{-1}]	Total cont. SO_2 [mg.L^{-1}]
White and rose below 2 g.L^{-1} of sugar	200	150
White and rose from 5 g.L^{-1} of sugar	250	220
Red wine below 2 g.L^{-1} of sugar	150	100
Red wine from 5 g.L^{-1} of sugar	200	170
Late harvest	300	270
Grape's selection	350	320
Berry selection, Cibeba selection, Ice wine, Straw wine	400	370
Liqueur wine with sugar content below 5 g.L^{-1}	150	120
Liqueur wine with sugar content from 5 g.L^{-1}	200	170
Quality sparkling wine	185	155
Sparkling wine other	235	205

Many papers dealing with phenolic compounds of wine and grapes and their total antioxidant capacity have been published. However, little attention has been paid to comparison on phenolic compounds of wine grapes from different origin in Moravian wine, as well as on comparison of phenolic contents and antioxidant activities of phenolic compounds. Flavonoids, phenolic acids, flavonols and resveratrol and other groups of compounds could be key agents of the antioxidant action on the human metabolism pathway, the reason why we wanted to qualify the wines from a nutritional point of view.

The aim of this study was to determine the total content of phenolic compounds, to identify and quantify individual phenolic compounds and determination of the total antioxidant activity in relation to various forms of sulphur dioxide in wine samples collected from different geographical regions of Austria and the Czech Republic.

EXPERIMENTAL

MATERIAL AND METHODS

Instrumentation

For measurement of antioxidant activity was used diode array spectrometer Biochrom Libra S6 (Biochrom Ltd, Cambridge, UK). For HPLC analysis was used UltiMate® 3000 HPLC system consisted of UltiMate 3000 RS pump, UltiMate 3000 RS autosampler, UltiMate 3000 RS column compartment and UltiMate 3000 RS diode array detector (Varian Inc., Santa Clara, CA, USA). Chromatographic separation was carried out on Supelcosil LC-18-DB column (250x4.6 mm, 5 µm, Supelco, USA) at 30 °C by gradient elution with a mobile phase containing solvent A (5% v/v aqueous acetonitrile acidified with 0.35 mL trifluoroacetic anhydride (TFAA) and solvent B (50% v/v aqueous acetonitrile acidified with 0.25 mL TFAA). Run time was 30 min and the flow rate was 0.5 mL.min^{-1}. For HPLC-MS analysis was used combination of HPLC system described above with Bruker Daltonics AmaZon X HCT (High-Capacity Trap) MS system with 3D ion trap technology.

Chemicals

Folin-Ciocalteau reagent, gallic acid, 2,4,6-tris-(-2-pyridyl)-s-triazine (TPTZ) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were obtained from Sigma-Aldrich (Steinheim, Germany). A standard solution of DPPH c=0.20 mol.L^{-1} was prepared in methanol. Working DPPH solution was prepared at c=100 µmol.L^{-1} containing acetate buffer of pH 4.3 in the ratio 1:2 (DPPH:buffer). Tannin was obtained from Merck KGaA (Darmstadt, Germany). Phenolic reference standards including gallic acid, catechin, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, rutin, cinnamic acid, quercetin and resveratrol were purchased from Aldrich (Zwijndrecht, Belgium). Lab-Scan acetonitrile (ACN) was obtained from POCH A.S. (Gliwice, Poland). Other chemicals and reagents were purchased from Penta, Chrudim and/or Lachema, Brno, (both Czech Republic). All solutions were prepared with deionised (DI) water (Aquaosmotic, Tišnov, Czech Republic).

Analysed wine samples

We analysed the wine samples of 2019 vintage; identical technological process was used in their production. To compare qualities of wine, BIO wines from the same vineyards grown in common ways were analysed, too. During fermentation, the content of SO_2 was maintained between 25 to 30 mg.L^{-1} to prevent undesirable oxidation processes. Shortly before bottling (5–7 days), the sulphurisation was implemented via the addition of potassium metabisulphite $K_2S_2O_5$. The amount of added SO_2 depended on its level in wine, on concentration of oxygen and on pH of the wine sample. The total content of SO_2 is listed in Table 2. The SO_2 levels shown in the table are average values calculated from three determinations. The detailed classification and descriptions of the analysed samples are shown in Table 2. About 1500 bottles were produced from each sample.

Methods

Iodometric determination of sulphur dioxide content

The determination of SO_2 levels was implemented based on and in accordance with the following references and regulations:

1. O. I. V. – Compendium of international methods of wine and must analysis, 2011, the OIV-MA-AS323-04B:R2009 method,
2. List and description of analytical methods according to art. 120g of the first subparagraph of the NR/(ES) n. 1234/2007, NK(EHS) n. 2676/90 regulation from September 17, 1990, that specifies EU methods used for wine analyses.

Sulphur dioxide content was determined immediately after opening the bottle with the tested wine.

Determination of free sulphur dioxide:

- A tested sample (50 mL) was pipetted into a conical 500 mL flask
- A H_2SO_4 solution (1:10 V/V, 3 mL), Chelaton III (30g.L^{-1} EDTA, 1 mL) and starch solution (0,5 %; 5 mL) were added then
- The solution was immediately titrated with standard 0.01M iodine solution until the blue-violet color of the solution persisted for 15 seconds at least. The volume of iodine solution employed in the titration was labelled V_1.

Determination of total sulphur dioxide:

- Immediately after determination of free SO_2, NaOH (4 M, 8 mL) was added to the previously titrated solution, stoppered, and stirred and left then standing on the lab bench for 5 minutes
- Under permanent agitation, H_2SO_4 (1:10 V/V, 10 mL) was added from a cylinder and the solution was titrated with 0.01 M iodine solution until blue violet colour persisting for 15 seconds appeared. The volume of iodine solution used in the titration was labelled V_2

- Sodium hydroxide solution (4 M, 20 mL) was added then; the solution was stirred afterwards and left standing on the lab bench for 5 minutes
- Then cold distilled water (200 mL) was supplemented, the solution was stirred and H_2SO_4 (1:10 V/V, 30 mL) was put into the solution from a cylinder and the mixture was immediately titrated with standard 0.01 M iodine solution. The volume of iodine solution consumed was labelled V_3.

The content of free (X_1) and total (X_2) SO_2 expressed in $mg.L^{-1}$ was calculated according to the formulae below:

$$X_1 = 12{,}8 \,.\, V_1 \,.\, f$$
$$X_2 = 12{,}8 \,.\, (V_1 + V_2 + V_3) \,.\, f$$

where ……..V_1 means the volume of standard iodine solution used to determine free SO_2
where ……. V_2 and V_3 mean the volumes of standard iodine solution used in titration of total SO_2
where …….. f means the factor of the standard 0.01M I_2 solution.
The results were expressed in $mg.L^{-1}$ as average values calculated from three determinations.

Determination of oxygen dissolved in wine

The analysis is based on the flow of wine through a polarographic sensor with a diffusion membrane. Under given voltage, the oxygen on the cathode/electrolyte interface layer enables passage of electric current that is directly proportional to its concentration in the analysed mixture of gasses.
Wine in each bottle was thoroughly stirred before each analysis using a stirring device. The bottle was fixed into a 29971 Sampler stand. The cork stopper was perforated with a lever and a needle connected to the delivery system was placed inside. By nitrogen from a pressure bottle placed next to the sampler, the wine was gradually delivered into the polarographic oxygen sensor connected to the evaluation unit of a Micro Logger 3650 analyser. The device directly read the amount of dissolved oxygen in ppm. The wine flow was set to the value of 300 $mL.min^{-1}$. The authors monitored the O_2 content every 60 seconds directly on the instrument display. For each sample, determination of dissolved oxygen level took two minutes **(Denwel, 1998)**. The measurement was implemented in triplicate immediately after stoppering the bottle and then after 150 days of storage, again in triplicate.

Folin-Ciocalteau method

The TPC was determined according to the Folin-Ciocalteau method (**Rastija *et al.*, 2009**). Briefly, 0.025 mL of sample was mixed with 1 mL of 10-fold diluted Folin-Ciocalteau reagent and allowed to stand for 3 min. Then 5 mL of 200 $g.L^{-1}$ sodium carbonate (Na_2CO_3) was added, and final volume was made up to 50 mL with DI water. Each sample was measured spectrophotometrically at 765 nm after 30 min of standing against blank. Five-point calibration was strictly linear (R^2>0.9999) in the concentration range 0-250 $mg.L^{-1}$ tannin as the standard. The determined values were expressed as tannin equivalents (TE, $mg.L^{-1}$). All samples were analysed as triplicates. Highly repeatable results for standards and samples were obtained.

DPPH radical scavenging activity (Price *et al.*, 1995; Rodrígues-Delgado *et al.*, 2001)

A mixture of undiluted sample (0.1 mL) with 10 mL working DPPH solution was measured immediately at 515 nm against a methanol blank (AC(0)). The mixture was then incubated at room temperature and dark for 30 minutes and it has been again measured spectrophotometrically at 515 nm (AA(t)). The gallic acid (GA) calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The measurement was compared to the standard calibration curve, and the free radical scavenging activities were expressed as millimoles of gallic acid equivalents (GAE) per milliliter of sample ($\mu mol.mL^{-1}$). The calibration curve was strictly linear (A=855.59 c - 16.015, R^2=0.9980, where A is absorbance value, c is concentration of gallic acid in standard solutions) in the concentration interval 0.02–0.08 $\mu mol.mL^{-1}$ gallic acid. The $\mu mol.mL^{-1}$ inhibition of DPPH radical caused by a wine samples were determined according to the following formula: (AC(0) – AA(t))/AC(0)×100, where AC(0) is the absorbance of the sample at t=0 min and AA(t) is the absorbance of sample at t=30 min). All samples were analysed as triplicates.

Antioxidant capacity by the DPPH test assay

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay was conducted according to the method of **Thaipong *et al.* (2006)**. This test is based on the reduction of DPPH. In its radical form, DPPH* absorbs light at 515 nm, but upon reduction by an antioxidant or a radical species, the absorption disappears.
The stock solution was prepared by dissolving DPPH (24 mg) in methanol (100 mL) and then stored at -20 °C until needed. The working solution was obtained by mixing the stock solution (10 mL) with methanol (45 mL) to obtain the absorbance of 1.1±0.02 units at 515 nm using a LIBRA S6 spectrophotometer (Biochrom Ltd., Cambridge, the UK). Wine samples (150 µL) were allowed to react with the DPPH solution (2,850 µL) for 24 hours in the dark. Then the absorbance was taken at 515 nm.
The results of absorbance were converted using a calibration curve of the standard and expressed in ascorbic acid equivalents in $mg.L^{-1}$ (AAE) **(Rupasinghe *et al.*, 2006)**.

Ferric ion reducing antioxidant power (FRAP)

The reducing activity of the samples was determined by FRAP method **(Peng *et al.*, 2002)**. A 0.1 $\mu mol.L^{-1}$ standard solution of gallic acid (GA) was prepared in H_2O. The oxidant in the FRAP assay was prepared by mixing 5 mL of 10 $mmol.L^{-1}$ 2,4,6-tripyridyl-s-triazine (TPTZ) in water, 50 mL of acetate buffer pH 3.6, and 5 mL of $FeCl_3.H_2O$ (20 $mmol.L^{-1}$). Sample (0.025 mL) was added to 4 mL reagent and absorbance was measured spectrophotometrically at 593 nm (A0min). Then sample solution has been allowed to stand at room temperature and in dark for 10 min and measured again at 593 nm (A10min). The difference of absorbances (ΔA=A10min-A0min) of the reaction mixture was calculated and related to ΔA of a Fe(II) standard solution. The difference in absorbance ΔA was linearly proportional to the concentration of antioxidant and indicated increased reducing power. The measurement was compared to a calibration curve of prepared gallic acid solution, and then final results expressed as micromoles of gallic acid equivalents (GAE) per millilitre of the sample (µmol/mL). The calibration curve was strictly linear (A=1.0800 c + 0.0072, R^2=0.9999, where A is absorbance value, c is concentration of gallic acid in standard solutions) in the concentration interval 0.02–0.1 $\mu mol.mL^{-1}$ gallic acid. All samples were analysed as triplicates.

HPLC/MS analysis of phenolic composition

The individual phenolic compounds were quantified using a HPLC method using gradient elution with the mobile phase containing solvent A (5% V/V aqueous ACN acidified with 0.35 mL trifluoroacetic anhydride (TFAA) and solvent B (50% V/V aqueous ACN acidified with 0.25 mL TFAA). The UV detector was set at 205, 210, 275 and 375 nm. Wine sample was filtered using 0.45 µm pore size Nylon membrane filter 13 mm (FFNN1345-100, Gronus, UK) using filter devices (Millipore, Bedford, MA, USA) before injecting. Injection volume was 20 µL. Individual phenolic compounds were identified by comparing retention times and UV-VIS spectra of the corresponding standard compounds and data were quantified using the corresponding calibration curves of the individual standard compound.
For identification and final confirmation of some compounds was used MS system Amazon X, which representing one from the latest developments in ion trap technology. With greatly enhanced sensitivity, MS/MS speed and „Zero-Delay Alternating“ polarity switching, the Amazon X is very suitable for the analysis of complex samples when more in depth and detailed analysis of molecular structure is needed. This instrument supported by spectral MS^n libraries is very effective mass spectrometer for MS/MS based multi-compound screening.

RESULTS AND DISCUSSION

In this study, two sets of wines were analysed. In first were 32 wine samples including 16 white and 16 red wines, which were made from grapes of Grüner Veltliner (Veltlin green) and Zweigelt varieties, were selected for determination of total phenolic contents (TPC) and total antioxidant activity (TAA). Grüner Veltliner is a variety of white wine grape grown primarily in Austria and in Czech Republic. Zweigelt is a red wine grape variety that is the most widely grown in Austria nowadays.
Second set represent the wine samples of 2019 vintage; identical technological process was used in their production. To compare qualities of wine, BIO wines from the same vineyards grown in common ways were analysed, too (Table 2).

Total contents of phenolics

The different variations of red and white wine samples were tested for total content of phenolic compounds in four sets of analyses. The total phenolic contents varied from 218 to 328 $mg.L^{-1}$, averaging 263 $mg.L^{-1}$, for the four white wine samples SGV and from 1182 to 1232 $mg.L^{-1}$, averaging 1216 $mg.L^{-1}$, for the four red wine samples SZW. The total phenolic contents ranged from 268 to 283 $mg.L^{-1}$, averaging 274 $mg.L^{-1}$ for PGV samples and from 564 to 729 $mg.L^{-1}$, averaging 651 $mg.L^{-1}$ for red wine samples PZW. Samples PGV-3 and PZW-3 have high content of total phenolic; the same as SGV-3 and SZW-3. Probably, the high content of phenolic content of grape samples depends on growing part of vineyard, shelter place from wind, intensity of sunlight radiation as well as shaded or non-shaded clusters and other factors (Figure 1 and 2).

The effect of SO_2 levels on the content of dissolved oxygen and antioxidant capacity

The determined levels of free and total SO_2 served for basic evaluation of influence of reduced SO_2 amounts added at bottling on the content of antioxidants and oxygen in wine. The results of the analyses enabled gaining overall survey of the problems studied.

The above Table 3 illustrates consumption of oxygen after 150 days since bottling. The oxygen content ranged between 0.231 to 0.599 ppm at bottling and between 0.004 to 0.101 ppm after 150-day storage.

After 150 days of storage, the wines with higher SO_2 levels contained slightly less oxygen than they did at bottling, when SO_2 amount showed almost no influence on the content of oxygen in the wine. Moreover, the loss of oxygen in wines with both low and high SO_2 levels is comparable after 150-day storage. The above conclusion is also supported by virtually no correlation (R^2=0.12) both when bottled and after 150 days of storage (R^2=0.04).

In individual varieties of wine, higher amounts of SO_2 added prior to bottling led rather to reduced content of SO_2 after 150 days of storage, which is in accordance with the findings of **Dimkou *et al.* (2013)**. The decrease of total SO_2 amount during 150 days since bottling ranged between 1 to 36 mg.L^{-1} representing thus 12 %, which corresponds to the results reported by other researchers **(Skouroumounis *et al.*, 2005)**. After 150 days of storage, we found 18–38 mg.L^{-1} of free SO_2 and 110–165 mg.L^{-1} of total SO_2, which represents similar or slightly lower amounts than reported by other authors **(Kallithraka *et al.*, 2009; Baroň & Kumšta 2012)**.

Furthermore, the oxygen levels in all the monitored wines were very low even at the bottling, which implies that SO_2 content did not play a crucial role. The low level of oxygen is not caused solely by the amount of antioxidants, since a very low correlation at bottling (R^2=0.05) and after 150 days (R^2=0.11) was proved.

The low oxygen amount was possibly caused using inert gasses, especially of nitrogen that was used already at crushing the grapes and then at schooling and racking the wine. During the preparation and filtration before bottling, the temperature of the wine was adjusted to 16 °C. By the means of filter glass, it was pumped through N_2 and CO_2 which led to release of bound oxygen. Vacuum treatment of empty bottles and filling them with gaseous nitrogen and then with wine was employed in the filling monoblock. These findings are also reported by **Brody (2011).** The application of inert gasses in the production of wine maintains a higher content of phenolic compounds **(Cáceres-Mella *et al.*, 2013)**.

The different variations of red and white wine samples were tested for total content of phenolic compounds in four sets of analyses. The total phenolic contents varied from 218 to 328 mg.L^{-1}, averaging 263 mg.L^{-1}, for the four white wine samples SGV and from 1182 to 1232 mg.L^{-1}, averaging 1216 mg.L^{-1}, for the four red wine samples SZW. The total phenolic contents ranged from 268 to 283 mg.L^{-1}, averaging 274 mg.L^{-1} for PGV samples and from 564 to 729 mg.L^{-1}, averaging 651 mg.L^{-1} for red wine samples PZW. Samples PGV-3 and PZW-3 have high content of total phenolic; the same as SGV-3 and SZW-3. Probably, the high content of phenolic content of grape samples depends on growing part of vineyard, shelter place from wind, intensity of sunlight radiation as well as shaded or non-shaded clusters and other factors (Figure 1 and 2).

Table 2 Description of analysed wines

Number of samples	Name of variety	Level of quality	Addition of SO_2	Winery sub region	Classification according to sugar content
1	Chardonnay	Late harvest	lower	Slovácká	semi-sweet
2	Chardonnay	Late harvest	higher	Slovácká	semi-sweet
3	Chardonnay BIO	Late harvest	lower	Slovácká	semi-sweet
4	Chardonnay BIO	Late harvest	higher	Slovácká	semi-sweet
5	Pinot Gris BIO	Late harvest	lower	Slovácká	dry
6	Pinot Gris BIO	Late harvest	higher	Slovácká	dry
7	Pinot Blanc	Late harvest	lower	Mikulovská	semi-dry
8	Pinot Blanc	Late harvest	higher	Mikulovská	semi-dry
9	Pinot Blanc	Late harvest	lower	Slovácká	semi-dry
10	Pinot Blanc	Late harvest	higher	Slovácká	semi-dry
11	Riesling Walnut	Late harvest	lower	Mikulovská	dry
12	Riesling Walnut	Late harvest	higher	Mikulovská	dry
13	Traminer red	*Grape's selection*	lower	Znojemská	semi-dry
14	Traminer red	*Grape's selection*	higher	Znojemská	semi-dry
15	Riesling Rhenish	Late harvest	lower	Mikulovská	dry
16	Riesling Rhenish	Late harvest	higher	Mikulovská	dry
17	Moravian muscat	Late harvest	lower	Mikulovská	semi-sweet
18	Moravian muscat	Late harvest	higher	Mikulovská	semi-sweet
19	Sauvignon	Late harvest	lower	Mikulovská	semi-dry
20	Sauvignon	Late harvest	higher	Mikulovská	semi-dry
21	Veltlin green	Late harvest	lower	Mikulovská	dry
22	Veltlin green	Late harvest	higher	Mikulovská	dry
23	Kerner	Late harvest	lower	Mikulovská	semi-sweet
24	Kerner	Late harvest	lower	Mikulovská	semi-sweet

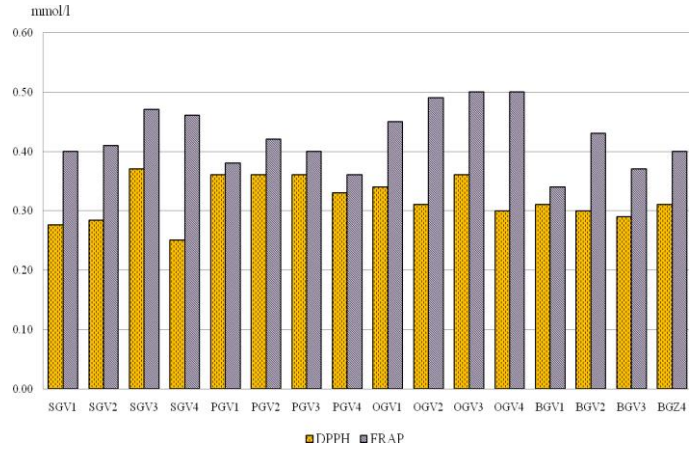

Figure 1 Total antioxidant activities in white wine samples determined by the DPPH and FRAP methods (GAE mmol.L^{-1}) **(Fišera *et al.*, 2012).**

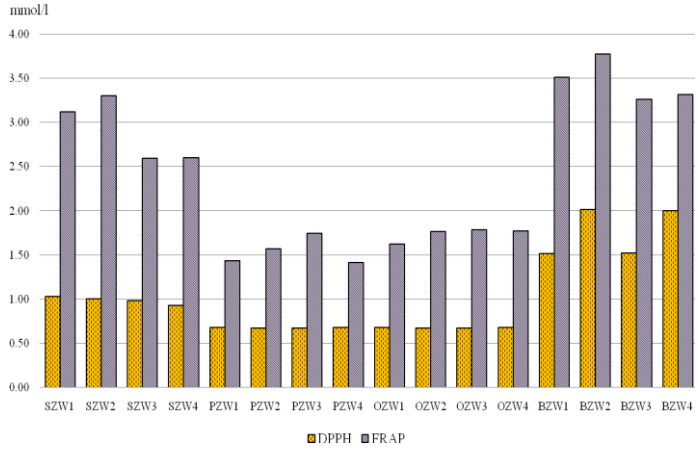

Figure 2 Total antioxidant activities in red wine samples determined by the DPPH and FRAP methods (GAE mmol.L^{-1}) **(Fišera *et al.*, 2012).**

Comparing total antioxidant capacity of individual pair samples with higher and lower SO_2 content, we found that the wines with higher SO_2 additions showed lower TAC than the wines with lower amounts of SO_2 added. At the bottling, the TAC values ranged from 92.11 mg AAE.L^{-1} to 178.19 mg AAE.L^{-1} and after 150 days, they fluctuated from 87.72 mg AAE.L^{-1} to 171.95 mg AAE.L^{-1}.

By comparing TAC concentrations in high SO_2 level wines to average TAC values determined by other researchers, the wine samples we monitored contained common levels of antioxidants which did not exceed the usual limits **(Paixão *et al.*, 2007)**. The TAC values range commonly between 50 to 350 mg.L^{-1} **(Lachman *et al.*, 2007)**. Slightly lower TAC was found in the Chardonnay BIO and Pinot Gris BIO samples, which can be caused by the fact that grapevine grown under the ecological conditions is subjected to lower stress than the grapevine produced in the common way. Lower TAC in BIO red wines was also found by **Tassoni *et al.* (2013)**.

Unlike the wines with higher SO_2 levels, all the wines with lower SO_2 content showed the values above 100 AAE mg.L^{-1}.

We can generally conclude that SO_2 acts as an antioxidant; it takes oxygen from wine destroying or suppressing hereby microorganisms including wild yeast, acetic and lactic acid bacteria that are oxygen dependent **(Pátek, 2000)**.

At the beginning of our research, we worked on the assumption that initial higher amount of SO_2 will lead to higher final concentration of TAC. The results of analyses did not prove the hypothesis, which could have been caused for example by SO_2 binding to saccharides, especially to glucose and fructose, and to acetaldehyde and other carbonyl compounds as well as to colourful or slime substances, pectin, polypeptides, pyruvic acid, and to other substances **(Farkaš, 1980; Michlovský, 2012)**. Based on the above information, slightly lowered SO_2 addition implemented before bottling can result in increase of TAC.

Table 3 Comparison of the content of SO_2, O_2 and AAE (ascorbic acid equivalent) in wine when bottling and after 150 days of storage.

Number of sample	The average from the SO_2 amount at the bottling		The average from the SO_2 amount after 150 days		The average from the values at the bottling	The average from the values after 150 days	The average from the values at the bottling	The average from the values after 150 days
	Free [mg.L^{-1}]	Total [mg.L^{-1}]	Free [mg.L^{-1}]	Total [mg.L^{-1}]	The average from the O_2 amount at the bottling [ppm]	The average from the O_2 amount after 150 days [ppm]	AAE at the bottling [mg.L^{-1}]	AAE after 150 days [mg.L^{-1}]
1	47	155	29	136	0.368	0.011	129.52	128.85
2	52	161	33	141	0.361	0.008	118.32	116.44
3	46	154	27	132	0.272	0.006	101.78	98.16
4	53	164	28	138	0.283	0.005	95.45	90.28
5	46	167	33	154	0.263	0.013	111.22	109.36
6	50	174	23	146	0.260	0.012	92.11	87.72
7	48	172	28	148	0.311	0.004	178.19	171.95
8	51	179	28	148	0.312	0.005	134.14	133.18
9	46	148	37	128	0.236	0.008	119.95	119.24
10	52	156	36	128	0.231	0.008	120.55	119.16
11	47	131	31	118	0.590	0.005	123.21	117.65
12	54	146	28	131	0.599	0.004	110.58	102.66
13	44	154	28	133	0.319	0.006	105.32	106.12
14	52	164	33	128	0.325	0.004	94.10	94.18
15	42	138	23	119	0.440	0.050	168.39	159.00
16	49	154	31	141	0.448	0.025	162.21	156.56
17	45	164	31	157	0.469	0.019	141.12	134.96
18	53	179	37	165	0.483	0.101	124.29	120.86
19	46	146	31	136	0.325	0.033	154.20	147.60
20	55	164	33	136	0.317	0.014	136.37	127.36
21	42	120	33	118	0.376	0.037	169.87	166.56
22	49	133	36	110	0.361	0.025	141.32	141.30
23	45	134	18	110	0.415	0.022	126.70	126.64
24	50	143	38	142	0.398	0.008	115.44	107.78

CONCLUSION

According to the results, following main practical significant summaries can be concluded that the geographical origin, average annual temperature, annual levels of precipitation and paedology have influence to total content of phenolic and total antioxidant activity/total antioxidant capacity and the concentration of phenolic compounds could be a marker for possible identification of wines geographical origin. In addition, the total contents of phenolic compounds significantly correlated with antioxidant activity and contents of individual phenolic compounds and gallic acid was the most abundant compound; tannic acid, caffeic acid, quercetin and rutin activities were intermediate and ferulic acid and resveratrol showed the lowest influence on the free radical-scavenging activity.

Comparing the qualities in corresponding pairs of wines, we can conclude that using the analysis of total antioxidant capacity by the DPPH method we found direct dependence of influence of wine sulphuration before bottling on total TAC. In all the cases, antioxidant capacity was higher in wines with lower addition of SO_2. Neither the SO_2 content nor antioxidant levels influenced the total amount of dissolved oxygen. After 150 days, the samples with higher initial content of SO_2 demonstrated just slightly lower content of dissolved oxygen, but not the significant decrease of oxygen content. The amount of oxygen dissolved in samples was very low after 150-day storage, which is probably caused by inert gasses used in manufacture and during bottling.

An important aspect, whose monitoring in manufacture of wine is considered essential, is pH. At higher pH, the need of SO_2 is increased (**Jacobson, 2006; Monro *et al.*, 2012**). Almost in all cases, sensory analysis did not find any significant differences in SO_2 levels between individual corresponding samples. The higher SO_2 content was identified correctly just in two of them **(Valášek *et al.*, 2014)**.

A series of components, strong reducing agents, are comprised in wine TAC. Besides other sources, fermentation also produces reducing agents. Total antioxidant capacity of the above reducing substances is not insignificant and it can make the content of SO_2 and sulphites in wine lower, which is appreciated by consumers for whom the permissible amounts of SO_2 and sulphites are inacceptable even if they are not risky for health. Nowadays, winemaking process and wine treatment cannot do without application of SO_2 and sulphites, because no suitable additive capable to replace the sulphur compounds has been suggested up to now.

The present study constitutes the first, but separate part solved problems the possibility of reducing the content sulphites in wine, but completely independently usable in practice. However, there are increased demands knowledge of basic principles of biological nature oenological technologists, because therein lies most of the methodological conditions of use.

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