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INTERACTION OF POLYPHENOLS AND WINE ANTIOXIDANTS WITH ITS SULFUR DIOXIDE PRESERVATIVE

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ABSTRACT

Wine is considered to be a significant alcoholic beverage, which is the result of fermentation of grape must or mash. Wine is a must when the substances contained in it play a major role, which are essential inhibiting water, carbohydrates, acids, minerals, nitrates, polyphenols and aromatics. These biochemical components are an important tracking element in wine evaluation in terms of chemical analyzes. An important parameter of monitoring is polyphenolic substances. Polyphenol substances are identified in plant materials as several thousand pieces with a very diverse structure. However, they have a common feature up to one or more aromatic rings substituted with hydroxyl groups. These substances may be present in plant material in a small or large amount. The total daily intake of polyphenols is estimated at 1 g. This is a higher intake than antioxidant vitamin intakes and it is confirmed that their antioxidant activity is higher than that of antioxidant vitamins. When monitoring the content of all polyphenols (TPC) in selected samples using a spectrophotometric method, a higher TPC content of red wines against white white wines can be observed. Total antioxidant activity is introduced to compare antioxidant effects of different mixtures and is based on the ability to eliminate radicals. Antioxidant activity and effects of polyphenols can be inhibited by the addition of preservatives to wine. The preservative is sulfur dioxide (SO_2), which has antimicrobial and antioxidant effects. This compound is not harmless because it is a strong allergen, blocks bacteria in the digestive tract and prevents the conversion of sugars and alcohol derivatives in the liver by blocking vitamin B. In the normal life, SO_2 is consumed under the E 220 mark. The aim of this work is to monitor the change in the total polyphenols content related to free and bound sulfur dioxide (SO_2) content using accredited OIV-MA-AS323-O4B: R, 2009 samples in wine samples. Comparison of organic wines and wines produced by classical, it was found that organic wine have a higher content of biologically active substances and have a strong correlation factor TAA - total SO_2 ($r = 0.77$ to 0.91), depending on the wine variety.

Keywords: total polyphenol; antioxidant aktivity; organic; red wine; white wine

INTRODUCTION

Grape vines and, above all, their product of production - wine is considered to be a significant alcoholic beverage resulting from the fermentation of grape musts or grapes, which has retained its popularity for many millennia **(McIntyre et al. 2015)**. Production is divided into several technological steps, including grape harvesting, cropping and crushing, pressing, fermentation, apple fermentation, training and bottling. Wines are characterized by the high content and essential role of inhibiting water, carbohydrates, acids, minerals, nitrogenous substances, polyphenols and aromatics. For example, aromatic substances can be classified into aromatic substances from grapes resulting from fermentation and occurring during wine maturation. They play a significant and notable part in the choice of wine by the consumer, as this is the first impression the wine customer will acquire. Important biologically active substances of the wine are polyphenols **(Del Pino-García et al. 2016)**. These are mostly contained in red wines, to a lesser extent in white wines **(Bajčan et al. 2016)**. For example, the content of significant polyphenol resveratrol is reported in white wines ranging from 0.2 – 0.8 mg.L^{-1}, on the other hand in red wines 2 – 6 mg.L^{-1} **(Kyseláková et al. 2003)**. Red wine contains many bioactive polyphenols such as resveratrol, anthocyanins, catechins, and tannins that do not originate in grapes, but in oak barrels, where red wines often mature **(Panchal et al., 2013)**. Polyphenolic substances, especially reseratrol, in cooperation with other components of wine (alcohol, etc.) are attributed to a positive effect on human health. Some studies show positive effects on cardiovascular system, oxidative stress, cholesterol, and others **(Gea et al., 2014; Karadeniz et al. 2014)**. This research is carried out using spectrophotometric techniques using for example the DPPH method, the reaction of the test substance with a stable free radical of 1,1-diphenyl-2-picrylhydrazyl, for antioxidant capacity **(Bajčan et al. 2017)**. An important component of the wine is the preservative and inhibiting agent sulfur dioxide (SO_2) or E220. In wine we can find it both in the form of endogenous, which is created during fermentation, and above all as exogenous. Exogenous, non-bound SO_2 is added in various technological operations. Bonded sulfur dioxide is formed by the enzymatic transformation of sulfur compounds (sulfur amino acids - cysteine, cystine, methionine, glutathione, free elemental sulfur, etc.) by the action of Saccharomyces, in addition to a number of other metabolites, in the unsaturated grape juice itself, but mainly during the alcoholic fermentation cerevisiae. SO_2 has both strong antimicrobial effects but also primarily reductive (antioxidant) effects. Most antimicrobial and antioxidant effects are usually attributed to free SO_2. Sulfur dioxide is mainly used in the form of gas, but also an aqueous solution of sulfuric acid or hydrogen sulphide or a powder. Due to its properties it is very well soluble in water. At 20 °C, 39 liters of SO_2 are dissolved in 1 liter **(Valášek et al. 2014)**. The formation and development of bound sulfur dioxide depends on a number of factors (formation during fermentation of wine) **(Romano et al., 1993)** and may range from several mg. L^{-1} to 30 mg. L^{-1} in extreme conditions, bound sulfur dioxide may occur at concentrations up to 100 mg. L^{-1} **(Rankine et al., 1969; Eschenbruch 1974; Dott et al., 1976; Suzzi et al., 1985)**. Concentration of bound SO_2 along with free SO_2 produced microorganisms during alcoholic fermentation is often critical to the course of malolactic fermentation **(Henick-Kling et al. 1994)**. Endogenous sulfur dioxide is present mainly in the form of bound but in small amounts also free sulfur dioxide **(Wells et al., 2011)**. The presence of both forms should be taken into account when exogenous sulfur dioxide is dosed. By classical iodometric titration using accredited methods OIV-MA-AS323-O4B: R, 2009 **(OIV, 1990)** the content of free and bound sulfur dioxide in wine was monitored. At the same time corrections were made for the presence of reducing agents (reducers).

Scientific hypothesis

Wine is a very popular alcoholic beverage spread throughout the world. The aim of this research was to present the results of analyzes in monitoring the interaction of sulfur dioxide and biologically active substances contained in wine. In the experiment were used three varieties of white wine and red wine standard or made oraganic form in the wine region of Moravia, Czech Republic.

MATERIAL AND METHODOLOGY

Samples of wine

Samples of used white and red wines come from different wineries from the wine region of the Moravian, Mikulov, Slovácko and Velkopavlovice subregions, which includes more than 13 000 hectares of vineyards. There are approximately 18,000 small, recreational or professional growers here. The average annual temperature is 9.42 °C, the annual precipitation diameter is 510 mm and the average annual sunshine is 2244 hours according to the 78-year average found at the Winery Brewery in Velké Pavlovice. The climate is transient with an incline towards the inland, with occasional invasions of humid Atlantic air or even ice from the inland. The growing season is a bit shorter than in Western Europe. White wines produced according to classic methods of Riesling, Pinot Blanc and Veltliner. Red wines of Pinot Noir, André and Frankovka. In addition, organic wines were used, namely white wines of the Veltliner, Pinot Blanc and Riesling wines, red wines of the Pinot Noir, André and Frankovka varieties. All of these wines were produced and are produced in the year 2016. Five samples of an identical batch of wine were collected and analyzed from each wine variety. In total, sixty samples of classical and organic wines were analyzed.

Chemicals and laboratory equipments

Standardization of iodine solution

Sulfuric acid (H_2SO_4), starch (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic), Potassium iodide (KI), Potassium dichromate ($K_2Cr_2O_7$), and Sodium thiosulfate ($Na_2S_2O_3$) (Ing. Petr Lukeš, Uherský Brod, Czech Republic)

Determination of SO_2 by OIV-MA-AS323-04B : R 2009

Sulfuric acid (H_2SO_4), starch (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic), Sodium hydroxide (NaOH), EDTA 3, Acetaldehyde, Iodine (I_2) (Ing. Petr Lukeš, Uherský Brod, Czech Republic), ordinary laboratory glassware and equipment, stopwatch, 25 mL burette digital, lamp.

Determination of total polyphenol compounds (TPC)

Distilled water, Folin–Ciocalteu reagent (FCR) (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic), Sodium carbonate (Na_2CO_3) (Ing. Petr Lukeš, Uherský Brod, Czech Republic).

Determination of total antioxidant ativity by DPPH metod

Methanol, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic)

Spekctrophotometric methods

Spectrophotometric measurements were performed on a Lambda 25 UV-VIS spectrophotometer (PerkinElmer, USA) in 10 mm optical quartz cuvettes.

Methods

Determination of free SO_2

50 mL of wine sample is pipetted Into a 500 mL volumetric flask, we add 3 mL of 16% H_2SO_4, 1 mL EDTA 3 solution having a concentration of 1%, 5 mL of starch solution is titrated against a white background I_2 solution having a concentration of 0.02 mol.L^{-1} to blue color. The obtained power consumption is used in the final calculation (V1).

Determination of total SO_2

After titration of free SO_2 we add to a sample 8 mL NaOH solution at a concentration of 4 $mol.L^{-1}$, after 5 minutes we add 10 mL of 16% H_2SO_4 solution titrated with iodine. We use final consumption to calculate (V2). Then we add 20 mL of NaOH, and 200 mL of distilled water after 5 minutes, 30 mL of 16% H_2SO_4 solution and titrate with iodine to a blue color. We get V3 consumption.

Correction for reductones

We measure out 50 mL wine sample, 1 mL of 1% formaldehyde, and after 30 minutes add 3 mL of 16% H_2SO_4, 1 mL EDTA 3 solution having a concentration of 1%, 5 mL of starch solution and titrate against a white background I_2 solution having a concentration of 0.02 $mol.L^{-1}$ to blue color. With this step, we get V4 consumption.

Calculation concentration SO_2 ($mg.L^{-1}$)
Concentration of free SO_2 c = (V1-V2).f.12,8
Concentration of total SO_2 c = (V1+V2+V3-V2).f.12,8
12,8 – coefficient for conversion to SO_2 when used 0,025 M I_2

Determination of total polyphenol content (TPC)

To determine the total content of phenolic compounds (TPC), a spectrophotometric method using Folin-Ciocalteau reagent based on to reduce the phosphomolybdate-tungsten complex by phenolic substances in an alkaline medium. The modified method of **Singleton and Rossi (1965)** according to **Sumczynski et al. (2015)** was used. Determination was performed at a wavelength of 765 nm after a 30 min incubation. The total content of phenolic substances was expressed as gallon (GAE) in $mg.L^{-1}$. The repeatability of the assay was verified on 10 parallel determinations for cm = 0.5 $g.L^{-1}$ of tannin. The calibration dependence A = f (cm) was constructed using six calibration solutions. For the preparation of calibration solutions, we dispense approximately 20 mL of distilled water into four 50 mL volumetric flasks and pipette 0.4; 0.6; 0.8; 1.0 mL of standard solution, add 1 mL of Folin-Ciocalteau and mix. After 3 minutes add 5 mL of 20% Na_2CO_3 solution, make up to the mark with distilled water and mix. After 60 minutes, we measure the intensity of the staining in a 10 mm cuvette at 765 nm against the blank spectrophotometrically. In the same way, determine the absorbance of the samples. According to the regression curve equation we calculate the polyphenol content expressed as mg gallic acid (GAE).L^{-1}.

Determination of total antioxidant ativity by DPPH metod

Total antioxidant activity was assessed by modification method of **Rop et al. (2010)**. First, a stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of metanol. A working solution is then prepared from the prepared stock solution by mixing 10 mL of the stock solution and 45 mL of methanol. Subsequently, the working solution thus prepared is spectrophotometrically measured at a wavelength of 515 nm against methanol as blank. A sample of 450 µL of wine was pipetted into a test tube and then 8.55 mL DPPH working solution was added. After 60 minutes of incubation in the dark, the sample was measured spectrophotometrically at said wavelength. Absorption loss was recalculated using the linear regression equation to equivalent Trolox (TE).L^{-1}.

Statisic analysis

Results are reported as mean values with standard deviation (SD). Differences between observed results were detected by t-test (Statistica, 2018, StatSoft, Inc., USA). A p <0.05 (*) and p <0.01 (**) was considered statistically significant.

RESULTS AND DISCUSSION

Free SO_2

Free sulfur dioxide (Table 1) in test samples of standard white wines ranges from 5.37 to 11.14 $mg.L^{-1}$. The smallest content was found in the white wine of the Riesling variety, on the contrary, the white wine of the Pinot Blanc variety. The content of free SO_2 in white organic wines ranged from 0.41 to 0.69 $mg.L^{-1}$. The least free SO_2 was determined in the white organic wine of the Veltliner variety, most notably Pinot Blanc. There is already a distinction between standard and organic wine with a different free SO_2 content of up to 10.73 $mg.L^{-1}$. The most significant difference can be seen (Table 1; Table 3) for the Pinot Blanc and organic Pinot Blanc varieties (p <0.01), with a difference of 10.45 $mg.L^{-1}$ free SO_2. If we compare the achieved values (Table 1) with a study of sulfur dioxide **(Valášek et al., 2014)**, which gives the value of free SO_2 in Riesling 23 $mg.L^{-1}$, Veltliner 33 $mg.L^{-1}$ and Pinot Blanc 28 $mg.L^{-1}$, our analyzed samples achieves significantly lower free SO_2 values in the same wine samples from the same wine region and sub-region. For samples of red wines (Table 2) we can observe free SO_2 content in the range of 0.83 - 32.19 $mg.L^{-1}$. Both of these values are recorded for red wine of the Pinot Noir variety, the lower of which was determined for organic wines. **Ivanova et al. (2015)** provides a comparison of free SO_2 in Pinot Noir, the conclusion of their study suggests a free SO_2 content of 11.52 $mg.L^{-1}$.

Total SO_2

Total SO_2 was determined after deduction of reductons. In standard wines (Table 1) the content ranges from 13.00 – 53.40 $mg.L^{-1}$ total SO_2. The lowest content was recorded for organic Pinot Blanc wine (13.00 $mg.L^{-1}$), on the other hand, most of the Riesling organic wine (25.10 $mg.L^{-1}$). For standard production wines, the lowest value is found for Riesling wine (29.78 $mg.L^{-1}$), most notably for Veltliner (53.40 $mg.L^{-1}$). In red vines, large differences in total SO_2 content can be seen. **Valášek et al. (2014)** shows the values of Pinot Blanc 148 $mg.L^{-1}$, Riesling 119 $mg.L^{-1}$ and Veltliner 236 $mg.L^{-1}$ as compared to the established values (Table 1). The difference between the smallest and the highest content is about 110 $mg.L^{-1}$ total SO_2. At the same time, the highest content was determined at Frankovka (135.95 $mg.L^{-1}$), at least at Frankovka organic (24.40 $mg.L^{-1}$). The maximum permitted amount of total SO_2 as laid down in Commission Regulation (EC) No. 606/2009, which sets the maximum SO_2 content in silent white wines at 200 $mg.L^{-1}$, in red wines at 150 $mg.L^{-1}$. For wines with residual sugar greater than 5 $g.L^{-1}$, the maximum value for white wine is

250 mg.L^{-1} and in red for 200 mg.L^{-1}. Therefore, if we compare all the aspects with the wine samples mentioned above, it is necessary to state that all the set values are inferior and above all organic wines, which show up to 10 times lower SO_2 than the allowed limit.

Total polyphenol

A spectrophotometric method using the Folin-Ciocalteau reagent was used to determine the total polyphenols in wine and organic wine samples, using gallic acid as the standard. For the wines tested, the higher value of the total polyphenols was predominantly for organic wines, for all their samples, both white and red. For whites of white wines, Veltliner organic predominates with 678.78 mg GAE.L^{-1}, at least Riesling 203.06 mg GAE.L^{-1}. As a result, the difference with wine with the highest total polyphenols, Veltliner and Veltliner organic, is more than 200 mg GAE.L^{-1}. Red wines have up to 2 to 3 times the white content of total polyphenols against white wines, this being determined by the production process and, above all, the biological properties of the grape vine itself. As can be seen (Table 2), the highest value was determined for the André organic sample (1349.12 mg GAE.L^{-1}), more than 400 mg GAE.L^{-1} was the lowest value of the total polyphenols determined for the Frankovka wine produced by the standard procedure. **Špakovska et al. (2012)** indicates the value of 256 mg of GAE.L^{-1} as the average content of total polyphenols in selected white wine samples. Pinot Blanc in the research reached an average value, according to the results obtained (Table 1) it is possible to observe a higher content of polyphenols in grape varieties mentioned by about 100 mg GAE.L^{-1}, Pinot Blanc organic records almost double the content. On the contrary, **Lapčíková et al. (2017)** presents the content of total polyphenols in samples Riesling (1085 mg GAE.L^{-1}) and Veltliner (732 mg GAE.L^{-1}). These very high values of total polyphenols in our research do not reach even the favored organic wines. The reason for this can be laid to the south wine region, different soil composition and meteorological conditions.

Antioxidant activity

To determine the antioxidant activity of wine samples, a DPPH method was used which is based on the reaction of the test substance with the stable 1,1-difenyl-2-picrylhydrazyl radical and the trolox standard. Results from Table 1 and Table 2 confirm the statement in the previous chapter that higher antioxidant activity is noted in

Table 1 Comparison of free, fixed and total sulfur dioxide, total polyphenols and antioxidant activity in samples of standard and organic white wines (n = 5).

White wine	Free SO_2 (mg.L^{-1})±SD	Fixed SO_2 (mg.L^{-1}) ±SD	Total SO_2 (mg.L^{-1}) ±SD	TPC (mg GAE.L^{-1}) ±SD	TAA (mg TE.L^{-1}) ±SD
Pinot Blanc	11.14 ±1.88	50.59 ±2.21	42.13 ±2.24	317.76 ±14.26	559.85 ±65.05
Pinot Blanc organic	0.69 ±0.20	18.85 ±0.38	13.00 ±0.18	405.12 ±6.07	674.55 ±6.61
Riesling	5.37 ±0.32	34.80 ±1.10	29.78 ±1.08	203.06 ±7.79	445.75 ±1.60
Riesling organic	0.63 ±0.07	28.51 ±0.62	25.10 ±1.28	319.72 ±6.62	508.50 ±3.62
Veltliner	9.33 ±3.96	58.40 ±2.58	53.40 ±2.32	445.45 ±6.53	685.13 ±15.81
Veltliner organic	0.41 ±0.02	21.19 ±0.66	15.16 ±0.66	678.78 ±18.65	806.28 ±11.65

Table 2 Comparison of free, fixed and total sulfur dioxide, total polyphenols and antioxidant activity in samples of standard and organic red wines (n = 5).

Red wine	Free SO_2 (mg.L^{-1})±SD	Fixed SO_2 (mg.L^{-1}) ±SD	Total SO_2 (mg.L^{-1}) ±SD	TPC (mg GAE.L^{-1}) ±SD	TAA (mg TE.L^{-1}) ±SD
Frankovka	28.52 ±4.98	154.73 ±3.83	135.95 ±4.90	905.21 ±3.85	2123.31 ±24.91
Frankovka organic	0.87 ±0.07	30.00 ±0.82	24.40 ±0.56	1020.52 ±14.75	2570.92 ±78.41
André	26.88 ±1.56	125.97 ±4.52	111.11 ±4.30	1130.96 ±35.37	2312.99 ± 18.17
André organic	1.04 ±0.06	35.11 ±1.28	30.01 ±1.23	1349.12 ±28.01	2529.25 ± 33.73
Pinot Noir	32.19 ±0.80	134.52 ±3.06	103.54 ±4.03	1046.30 ±57.81	1862.01 ±47.89
Pinot Noir organic	0.83 ±0.10	37.5 ±0.82	32.17 ±0.80	1300.04 ±12.89	2039.22 ±49.29

Note: Table 1 and Table 2: SO_2 – sulfur dioxide; Total SO_2 after deduction of reductons; TPC – total polyphenol content; TAA - total antioxidant ativity using DPPH - radical scavenging activity; TE – trolox equivalent; GAE - gallic acid equivalent; ±standard deviation.

Table 3 Statistically significant differences between classic and organic wines

	Free SO_2	Fixed SO_2	Total SO_2	TPC	TAA
Pinot Blanc classic / organic	**	**	**	**	*
Riesling classic / organic	**	**	**	**	**
Veltliner classic / organic	**	**	**	**	**
Frankovka classic / organic	**	**	**	**	**
André classic / organic	**	**	**	**	**
Pinot Noir classic / organic	**	**	**	**	**

Note Table 3: *p <0.05; **p <0.01.

organic wines. Here the highest antioxidant activity was determined for white wine Veltliner organic with a value of 806.28 mg $TE.L^{-1}$, for red wine Frankovka organic 2570.92 mg $TE.L^{-1}$. The lowest antioxidant activity was recorded in Riesling white wine of 445.75 mg $TE.L^{-1}$, the highest grade of antioxidant activity in red wine André 2312.99 mg $TE.L^{-1}$, while the lowest in Pinot Noir 1862.01 mg $TE.L^{-1}$. From the above results it is evident that the antioxidant activity in red wines is generally higher up to 5 times compared to white wines. This is confirmed by the assertion used for the determination of total polyphenols. **Stratil et al. (2008)** evaluate the antioxidant activity of different wines Czech wine regions. Veltliner antioxidant activity (614 mg $TE.L^{-1}$) achieves the same results as our sample (Table 1). **Lachman et al. (2009)** gives the result of Frankovka of 1230 mg $TE.L^{-1}$ as compared to Table 2, we find more than double the antioxidant activity values.

Correlation of sulfur dioxide content of biologically active substances

These results show a strong correlation of total sulfur dioxide antioxidant activity with Pinot Blanc organic, where the correlation coefficient was r = 0.91, Veltliner r = 0.81 and Veltliner organic r = 0.77. For André organic, this correlation coefficient was the strongest of all red wines r = 0.91. A slight correlation in relation to antioxidant activity and total SO_2 achieved results for Pinot Blanc bovine varieties (r = 0.51) and Riesling (r = 0.22). A slight correlation also results in red wines and Frankovka where the correlation coefficient r = 0.36 was here. With the strong correlations we can conclude that the total SO_2 influences the antioxidant activity of the red wines. Here, however, there is a strong correlation with white wines before red wine.

CONCLUSION

The study, which examined the effect of sulfur dioxide on total polyphenols and antioxidant activity in samples of white and red grape wines produced by the strandart route and organic wines. A strong relationship between SO_2 content and antioxidant activity was observed, especially in organic wines. It is possible to see the significant difference in total amount of sulfur dioxide in white wine Pinot Noir organic and classic samples (p <0,01) and red wine Frankovka (p <0,01). The lowest amount total SO_2 was recorded in organic Pinot Blanc (13,00 $mg.L^{-1}$) and organic Frankovka (24,4 $mg.L^{-1}$). The highest total amount of SO_2 was determined by Veltliner (53,40 $mg.L^{-1}$) and Frankovka (135,95 $mg.L^{-1}$). Organic André achieved the highest content of TPC (1349.12 $mg.GAE.L^{-1}$) and Frankovka organic highest content of TAA (2570.92 $mg.TE.L^{-1}$). The fact, however, is that SO_2 in wine serves as an antioxidant and protects wine from oxidation and acts as an antimicrobial agent. Therefore, we can state, according to the results, that organic wine provides higher biologically active values and contains less allergen. However, their sensory properties may differ from the standard as well as wines made shorter shelf life and quality may decrease during storage.

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