

EFFECT OF VARIOUS SULPHUR DIOXIDE ADDITIONS ON AMOUNT OF DISSOLVED OXYGEN, TOTAL ANTIOXIDANT CAPACITY AND SENSORY PROPERTIES OF WHITE WINES

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This study focused on the effects of various additions of sulphur dioxide (SO₂) added to bottled white wines. The article discusses technological aspects and characterizes the use of SO₂ in wine production. Twelve pairs of white wines containing various amounts of SO₂ were examined. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) photometric assay was employed to monitor total antioxidant capacity; other factors like SO₂ levels and contents of dissolved oxygen were also monitored. Differing qualities of the samples were evaluated by triangle sensory test. The goal of the study was to determine whether different amounts of SO₂ in bottles are able to show substantial effects on certain qualitative indicators such as content of dissolved oxygen and SO₂, total antioxidant capacity and sensory properties.

Keywords: white wines, sulphur dioxide, antioxidants, dissolved oxygen

Einfluss unterschiedlicher Zugaben von Schwefeldioxid auf die Menge an gelöstem Sauerstoff, antioxidative Kapazität und sensorische Eigenschaften von Weißweinen. Diese Studie behandelt die Auswirkungen verschiedener Zugaben von Schwefeldioxid (SO₂) zu abgefüllten Weißweinen. Technologische Aspekte werden diskutiert und die Verwendung von SO₂ in der Weinproduktion beschrieben. Zwölf Paare von Weißweinen, denen unterschiedliche Mengen an SO₂ zugesetzt worden waren, wurden untersucht. DPPH (2,2-Diphenyl-1-picrylhydrazyl)-Photometric Assay wurde eingesetzt, um die gesamte antioxidative Kapazität zu messen; andere Faktoren, wie SO₂-Werte und Gehalte an gelöstem Sauerstoff, wurden ebenfalls gemessen. Unterschiedliche Eigenschaften der Proben wurden mittels Dreieckstest bewertet. Das Ziel der Studie war es festzustellen, ob unterschiedliche Mengen an SO₂ in abgefüllten Weinen in der Lage sind, erhebliche Auswirkungen auf bestimmte qualitative Indikatoren wie Gehalt an gelöstem Sauerstoff und SO₂, antioxidative Kapazität und sensorische Eigenschaften zu zeigen.

Schlagwörter: Weißweine, Schwefeldioxid, Antioxidantien, gelöster Sauerstoff

Nowadays, winemakers consider sulphur dioxide (SO_2) to be essential for preserving a certain quality of wine; it is employed as an antimicrobial and antioxidant agent. Sulphur dioxide also contributes to the taste of wine and it preserves freshness in its aroma. Nevertheless, if used improperly, it can exert negative effects. The antioxidant effect of SO_2 consists in its ability to bind molecular oxygen, hereby disabling various chemical and enzymatic reactions (FURDÍKOVÁ and MALÍK, 2009; HENDERSON, 2009). Generally speaking, preservability of bottled white wines is diminished by the occurrence of progressive oxidation processes that lead to the loss of flower and fruit fragrances in wine aroma. Moreover, wine gets brown due to oxidation processes and undesirable foreign smells arise in it (FERREIRA et al., 2002).

Concerning health effects, the use of low SO_2 amounts is not risky. On the contrary, high levels of SO_2 can endanger human health causing symptoms such as headaches, nausea, gastric irritation, and breathing difficulties in asthma patients (SANTOS et al., 2012). Due to the above, SO_2 content plays an important role in wine quality control; thus, regulations specifying maximum acceptable concentrations of SO_2 have been introduced (GOMES et al., 1996; LAHO and MINÁRIK, 1970).

Sulphur dioxide has recently been ranked among allergens and its occurrence must be declared on wine labels. Winemakers use it in all technological phases of grape processing; they employ it also in treatments during fermentation and in storage containers, storage and technological rooms and auxiliary equipment and in juice fermentation, wine production and aging. From the technological point of view, it plays an unsubstitutable role in winemaking; no better substance with such a manifold use has been found up to now (KOVÁČ et al., 1990). Most of the SO_2 is added exogenously and just a part is produced by the yeast in the process of wine juice fermentation depending on the yeast strain applied (PAVLOUŠEK, 2010). The levels of endogenous SO_2 used to be lower than 10 mg/l; nowadays, they range between 30 and 50 mg/l. Special yeast produces more than 100 mg/l of SO_2 . Based on the above, SO_2 cannot be excluded from wine production, even if modern manufacturing procedures pioneer minimization of its amounts (MICHLOVSKÝ, 2012).

The goal of this study was to determine whether different amounts of SO_2 in bottles can show substantial effects on certain qualitative indicators such as content of dissolved oxygen and SO_2 , total antioxidant capacity, and sensory properties.

MATERIALS AND METHODS

ANALYZED WINE SAMPLES

We analyzed wine samples from vintage 2013; identical technological processes were used in their production. In order to compare qualities of wine, organic wines from the same vineyards were analyzed, too. During fermentation, the content of SO_2 was maintained between 25 to 30 mg/l to prevent undesirable oxidation processes. Shortly before bottling (5 to 7 days), the sulphurization was implemented via the addition of potassium metabisulphite $\text{K}_2\text{S}_2\text{O}_5$. 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 analyzed samples are shown in Table 1. About 1500 bottles were produced from each sample.

ANALYTICAL METHODS

IODOMETRIC DETERMINATION OF SULPHUR DIOXIDE CONTENT

The determination of SO_2 levels was implemented based on and in accordance with the Compendium of international methods of wine and must analysis (OIV, 2011) and the List and description of analytical methods (EU, 1990 and 2007). Sulphur dioxide content was determined immediately after opening the bottle.

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 (30 g/l EDTA, 1 ml) and starch solution (0.5 %; 5 ml) were added, then the solution was immediately titrated with standard 0.01 M iodine solution until the blue-violet colour 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 then left standing on the lab bench for five minutes.

Under permanent agitation, H_2SO_4 (1:10 v/v, 10 ml)

Table 1: Description of analysed wines

Number of sample	Variety	Quality category	Addition of SO ₂	Winegrowing 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	Pinot blanc	Late harvest	lower	Mikulovská	dry
12	Welschriesling	Late harvest	higher	Mikulovská	dry
13	Traminer Rot	Grapes selection	lower	Znojemská	semi-dry
14	Traminer Rot	Grapes selection	higher	Znojemská	semi-dry
15	Riesling weiss	Late harvest	lower	Mikulovská	dry
16	Riesling weiss	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	Veltliner Grün	Late harvest	lower	Mikulovská	dry
22	Veltliner Grün	Late harvest	higher	Mikulovská	dry
23	Kerner	Late harvest	lower	Mikulovská	semi-sweet
24	Kerner	Late harvest	lower	Mikulovská	semi-sweet

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 five 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 contents of free (X_1) and total (X_2) SO_2 expressed in mg/l were calculated according to the formulae below:

$$X_1 = 12.8 \times V_1 \times f$$

$$X_2 = 12.8 \times (V_1 + V_2 + V_3) \times f$$

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 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 analyzed mixture of gases. 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 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 MicroLogger 3650 analyzer. The device read directly the amount of dissolved oxygen in ppm. The wine flow was set to the value of 300 ml/min. 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 stopping the bottle and then after 150 days of storage, again in triplicate.

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY (TAC)

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay was conducted according to the method of THAIPOONG 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, 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 (AAE) (RUPASINGHE et al., 2006).

SENSORY ANALYSIS

The sensory analysis board comprised 24 members. The bottles were opened approximately 15 minutes prior to degustation. The wine samples were ranked according to sugar content from dry to semi-sweet wines. The O.I.V. degustation glasses were used and non-sparkling water was employed as a taste neutralizer. The sensory triangle test is considered the most suitable tool for comparison of individual wine samples after 150 days since bottling; it is more sensitive when contrasted to methods employing scales or to the pair reference test because it enables identification of smaller differences between compared samples (POKORNÝ et al., 1998). Twelve pair samples of wine with lower and higher addition of SO₂ were evaluated according to the BS ISO 4120:2004 (British Standards Institution, 2004) standardized method. Individual evaluations were recorded in the degustation list of the sensory triangle test that contrasted three samples out of which two were identical. Each sample was tested in duplicate.

RESULTS AND DISCUSSION

EFFECT OF SO₂ LEVELS ON CONTENTS OF DISSOLVED OXYGEN AND TAC

The determined levels of free and total SO₂ served for a

basic evaluation of the influence of reduced SO₂ amounts added at bottling on the content of antioxidants and oxygen in wine. The results of the analyses enabled gaining an overall survey of the problems studied.

Table 2 illustrates consumption of oxygen after 150 days since bottling. The oxygen content ranged from 0.231 to 0.599 ppm at bottling and from 0.004 to 0.101 ppm after 150 days of storage.

After 150 days of storage, the wines with higher SO₂ levels contained slightly less oxygen than they did at bottling, when SO₂ 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 SO₂ levels is comparable after 150 days of storage. The above conclusion is also supported by the low correlation ($R^2 = 0.12$) at bottling and after 150 days of storage ($R^2 = 0.04$), respectively.

In wines of specific varieties, higher amounts of SO₂ added prior to bottling led rather to reduced contents of SO₂ after 150 days of storage, which is in accordance with the findings of DIMKA et al. (2013). The decrease of total SO₂ amount during 150 days since bottling ranged from 1 to 36 mg/l representing thus 12 %, which corresponds to the results reported by other researchers (SKOUROUMOUNIS et al., 2005). After 150 days of storage, we found 18 to 38 mg/l of free SO₂ and 110 to 165 mg/l of total SO₂, which represents similar or slightly lower amounts than reported by other authors (BAROŇ and KUMŠTA, 2012; KALLITHRAKA et al., 2009).

Furthermore, the oxygen levels in all the monitored wines were very low even at bottling, which implies that SO₂ 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 by the use of inert gases, especially of nitrogen that was used from crushing until 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 N₂ and CO₂ which caused the 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 gases in the production of wine maintains a higher content of phenolic compounds (CÁCERES-MELLA et al., 2013).

Comparing total antioxidant capacity of individual pair samples with higher and lower SO₂ content, we found

that the wines with higher SO₂ additions showed lower TAC than the wines with lower amounts of SO₂ added. At bottling, the TAC values ranged from 92.11 mg AAE/l to 178.19 mg AAE/l and after 150 days, they fluctuated from 87.72 mg AAE/l to 171.95 mg AAE/l. By comparing TAC concentrations in high SO₂ 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 from 50 to 350 mg/l (LACHMAN et al., 2007). Slightly lower TAC values were found in the Chardonnay BIO and Pinot Gris BIO samples, which can be caused by the fact that grapevines grown under organic conditions are subjected to lower stress than conventionally grown grapevines. Lower TAC in BIO red wines was also found by TASSONI et al. (2013). Unlike the wines with higher SO₂ levels, all the wines

with lower SO₂ content showed values above 100 AAE mg/l.

We can generally conclude that SO₂ 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 et al., 2000).

At the beginning of our research, we worked on the assumption that initial higher amounts of SO₂ will lead to higher final concentrations of TAC. The results of analyses did not prove the hypothesis, which could have been caused for example by SO₂ binding to saccharides, especially to glucose and fructose, and to acetaldehyde and other carbonyl compounds as well as to pigments or mucilage, pectins, polypeptides, pyruvic acid, and to other substances (MICHLOVSKÝ, 2012; FARKAŠ, 1980). Based on the above information, slightly lowered SO₂ addition implemented before bottling can result in an increase of TAC.

Table 2: Comparison of the content of SO₂, O₂ and AAE in wine at bottling and after 150 days of storage

Number of sample	Average amount of SO ₂ at bottling		Average amount of SO ₂ after 150 days of storage		Average values at bottling	Average values after 150 days	Average values at bottling	Average values after 150 days
	Free (mg/l)	Total (mg/l)	Free (mg/l)	Total (mg/l)	Average O ₂ amount at bottling (ppm)	Average O ₂ amount after 150 days (ppm)	AAE at bottling (mg/l)	AAE after 150 days (mg/l)
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

EVALUATION OF SENSORY ANALYSIS RESULTS

To implement the evaluation at the $\alpha = 0,05$ significance level, at least 13 evaluators must declare a difference. The results are listed in Table 3.

The evaluators reported a difference just in two pairs of samples when they correctly identified the samples with higher SO_2 content. The identification of the set number 12 corresponds with the highest difference in SO_2 content; according to analytical results, the set number 2 does not show any significant difference in SO_2 addi-

correlation between wine sulphurization before bottling and total TAC. In all the cases, antioxidant capacity was higher in wines with a 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 contents of dissolved oxygen, but not a significant decrease of oxygen content. The amount of oxygen dissolved in samples was very low after 150 days of storage, which is probably caused by inert gasses used in processing and during bottling.

An important aspect, whose monitoring in wine pro-

Table 3: Results of sensory analysis

Set number	Sample number	Number of correct answers	Significant difference
1	1, 2	9	NO
2	3, 4	15	YES
3	5, 6	8	NO
4	7, 8	12	NO
5	9, 10	11	NO
6	11, 12	10	NO
7	13, 14	11	NO
8	15, 16	9	NO
9	17, 18	9	NO
10	19, 20	12	NO
11	21, 22	8	NO
12	23, 24	18	YES

tion. This could be caused by the fact that the analyses were implemented five months after bottling and the evaluated wines contained 28 mg/l to 33 mg/l of free SO_2 . Such levels might not be perceived as negative and the experts might not be able to find differences in wine qualities. Generally, we can conclude that the triangle test did not identify significant differences between samples. Many authors report that SO_2 improves taste and it preserves freshness in wine aroma (FRIVIK et al., 2003). FRIVIK (2003) found that the effect of SO_2 level on the identification of differences in aroma and flavour attributes is negligible. BAKKER et al. (1998) came to the same conclusion.

CONCLUSION

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 a direct

duction is considered essential, is pH. At higher pH, the need of SO_2 increases (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 indentified correctly just in two of them.

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 unacceptable even if they are not harmful to health. Nowadays, winemaking and wine treatment cannot do without SO_2 and sulphites, because no suitable additive which could replace the sulphur compounds has been suggested up to now.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance received from the Zámecké vinařství s.r.o. company, the producer of nobitored wine samples. The research this article reports on was implemented under the support of the operational Program called Research and Development for Innovations (Výzkum a vývoj pro inovace) that is co-funded by the European Fund for Regional Development (ERDF) and also subsidized from the state budget of the Czech Republic within the Centre of Polymer Systems Project (reg. n.: CZ.1.05/2.1.00/03.0111).

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Received May, 14th, 2014