A Role of Reductones in the Monitoring of Sulphur Dioxide Content in Wines during their Maturation and Storage

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Abstract

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The content of free SO_2 (1.3–37.9 mg/l and 5.3–19.7 mg/l for 2009 and 2010) and total SO_2 (38.3–272.0 mg/l and 52.0–102.1 mg/l for 2009 and 2010) for individual wines was in a broad interval. Results of free SO_2 found using the commercial SO_2 reagent were higher than our results since the reagent determined not only free SO_2 but also reductones. The content of titrated acids was constant with a very moderate time decrease and the values were on average about 9.1–5.3 g/l (2009) and 13.1–7.9 g/l (2010). Similarly, the content of tartaric acid was nearly constant and the values were on average about 2.37–1.47 g/l (2009) and 6.65–2.69 g/l (2010). The percentage ratio of tartaric acid in the content of titrated acids is also constant (average 24.39%–38.6% for individual wines).

Keywords: sulphuration; titrated acids; tartaric acid

Due to the maturation and aging of wine, its chemical composition changes with the time passing. To reach optimum quality of wine during longer maturation in barrels or bottles, it should be subjected to organoleptic and chemical monitoring. Regarding characteristic taste and overall condition of wine, chemical analysis and its results play a key role in the selection of wine treatment. During maturation, manifold processes occur in wine: oxidation and reduction, decrease of wine acidity, formation of cream of tartar and biological degradation of acids.

The addition of various forms of sulphur dioxide into crushed grapes, must, or wine is called wine sulphuration. Sulphur dioxide acts as a reduction, stabilisation and preservative agent (Farkaš 1980). Moreover, it also exerts a positive effect on bouquet formation and its easy oxidation results in reduction effects; thus, sulphur dioxide protects oxidable substances in wine against oxidation. Sulphur dioxide demonstrates the ability to bind oxygen, hereby protecting wine against enzymatic or non-enzymatic oxidation. It also maintains the colour of wine and prevents the loss of aromatic components and other characteristic qualities of wine. Furthermore, it decelerates the activity of bacteria responsible for biological degradation of acids (Farkaš 1980; Vercellotti *et al.* 1994; Caminati & Grabow 2006).

Must and wine contain two forms of sulphur dioxide: free and bound substance. In aqueous solution, sulphur dioxide is converted into sulphurous acid (H_2SO_3). After addition of sulphur dioxide into wine, sulphurous acid binds especially to aldehydes and saccharides. Further oxidation of sulphurous acid leads to the formation of sulphuric acid (H_2SO_4) that forms potassium sulphate (K_2SO_4) with potassium ions (Vercellotti *et al.* 1994). A reduction effect is shown solely by free sulphurous acid; it reacts with acetaldehyde to produce a compound, aldehydesulphurous acid (Vercellotti *et al.* 1994; Caminati & Grabow 2006).

In wine and must, the content of total, bound and free sulphur dioxide is detected. Even if it occurs

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in wine bound in various compounds, its amount is always specified as sulphur dioxide content. The levels of sulphurous acid, sulphites, hydrogensulphites, and gaseous sulphur dioxide are declared as the content of free sulphur dioxide in wine. The highest percentage of sulphur dioxide is represented by bound sulphur dioxide, whose largest portion, 70–90%, binds with acetaldehyde (Vercellotti *et al.* 1994; Caminati & Grabow 2006).

During wine storage, the content and forms of sulphur dioxide undergo changes. The share of free and bound sulphur dioxide varies. Wine handling such as filtration or turning barrels represents operations that cause the most significant decrease of free sulphur dioxide content (Vercellotti *et al.* 1994; Caminati & Grabow 2006). During storage, the sulphur dioxide content differs throughout the wine barrels. Wine in the upper parts of barrels, more prone to oxidation, shows a lower amount of sulphur dioxide when compared to wine in lower parts; thus wine samples are always taken from the central part of wine barrels. In old wines, sulphur dioxide acts for a shorter time due to its linkage to higher amounts of aldehydes; in young wines, sulphur dioxide affects wine-making processes for a longer time because of lower amounts of aldehydes. We observed the content of sulphur dioxide and other wine reductones (sometimes called reductants), represented namely by ascorbic acid.

Acids show a crucial influence on wine flavour and stability of its components. Acid content and degree of acidity exercise a decisive influence on the processes occurring during formation and maturation of wine. Acids contribute to the overall harmony of wine qualities and, to a certain extent, they also act as conservation agents. Wines containing higher amounts of acids are more stable due to their better resistance to oxidation. On the other hand, the concentration of malic acid above 2 g/l in wine rapidly increases the risk of bacterial infection. Higher levels of acids show a positive influence on the processes occurring during purification, clarification, and stabilisation of wine. Tartaric, lactic and malic acids are the most abundant wine acids; wine also contains smaller amounts of succinic, oxalic, and citric acids.

Tartaric acid is considered as one of the strongest wine acids; its acidity contributes considerably to sensory acidity of wine. Specifically, tartaric acid is found in grapes, cherries, and currants; it does not occur in other fruits in remarkable amounts (Farkaš 1980). It is regarded a significant acid of must and wine. Tartaric acid and its salts influence wine flavour; moreover, they even exert remarkable effects on biochemical processes occurring in wine-making (Vercellotti *et al.* 1994; Caminati & Grabow 2006).

The aim of this study was to monitor changes in several factors such as titratable acidity, content of tartaric acid, levels of reductones and sulphur dioxide for three years (October 2009–October 2012) throughout wine-making, maturation and storage of wine. The white wines of the following four varieties Müller-Thurgau, Pinot Blanc, Sauvignon, and Muscat Ottonel and the red wines Dornfelder and blue Frankish were subjected to research.

MATERIAL AND METHODS

Chemicals and instruments. NaOH at $c = 0.1$ mol/l; $C_2H_2O_4{\cdot}2H_2O$ (oxalic acid dihydrate); acetate buffer solution (20 ml of concentrated acetic acid and 80 ml of 270 g/l sodium acetate diluted up to 250 ml with distilled water); reagent solution (5 g NH_4VO_3 dissolved in 75 ml NaOH (1 mol/l) and after addition of 100 ml 270 g/l sodium acetate diluted up to 250 ml with distilled water); tartaric acid ($c_m = 10$ g/l); charcoal; I_2 at $c = 0.01$ mol/l; 16% H_2SO_4; NaOH at $c = 2$ mol/l; 1% formaldehyde; H_2O; Na_2EDTA at $c_m = 30$ g/l; starch solution; As_2O_3 (all Pliva-Lachema, Brno, Czech Republic); SO_2-Reagenz (# 8211, Filtra Önologie Lallemand Specialities GmbH, Guntramsdorf, Austria).

We employed a Level 1 pH-meter with combined pH electrode (WTW GmbH, Weilheim, Germany). Regular calibration was done using a series of buffers with pH 4.01, 7.00 and 9.23. All the spectrophotometric analyses were implemented using a Helios Delta spectrophotometer (Spectronic Unicam, Cambridge, UK). Quartz cuvettes with optical length of 10 mm were used.

The following white wines were subjected to research: Müller-Thurgau (MT), Pinot Blanc (Rulandské bílé in Czech – RB), Sauvignon (Sg), and Muscat Ottonel (Muškát Ottonel in Czech – MO). The grapes were grown in the Bzenec wine-growing village, namely in the Maršálky and Grefty (MO) vineyards. Two red wines were studied: Dornfelder (Df) and blue Frankish (Frankovka in Czech, Fr), both of them grown in the Maršálky vineyard, Bzenec. Bzenec is located in the wine-growing subregion Slovácko found in the Moravian wine producing region, Czech Republic (Jančářová *et al.* 2013).

Determination procedures. Six 2009 vintage wines (MO, MT, RB, Sg, Df, and Fr) were subjected to 3-year monitoring of several key components. Total titratable acidity, contents of tartaric acid and sulphur dioxide (levels of free, bound and total sulphur dioxide) were controlled. To compare with the quality of the 2009 harvest products, all the above parameters were also determined in the 2010 vintage wines. Due to bad weather and small harvest yield in 2010, only blue Frankish and Pinot Blanc wines were analysed; grapes of the other varieties were used to produce wine mixtures that could not be subjected to analysis.

All the 2009 and 2010 vintage wines were stored for three and two years, respectively; Muscat Ottonel, Müller-Thurgau, Pinot Blanc, and Sauvignon wines aged in glass demijohns and Dornfelder and blue Frankish wines in wooden barrels. Individual wines were sulphured according to the need in the intervals of one to three months. Samples of all the monitored wines were taken from storage containers gradually on specified days (Figures 1–2, 4–5, and 7) and analysed subsequently.

Determination of titratable acidity in wine. Determination of titratable acidity (expressed as g/l of tartaric acid) was implemented by means of alkalimetric titration with potentiometric indication of the equivalence point (Mato *et al.* 2005).

Determination of tartaric acid content in wine according to the Rebelein method. Determination of tartaric acid content was done spectrophotometrically according to the Rebelein method (Mato *et al.* 2005; Jančářová *et al.* 2013) which is based on monitoring of the colour complex it forms with ammonium metavanadate.

Determination of SO_2 content in wine. The content of sulphur dioxide was determined by iodometric titration using the standard volumetric method and commercial SO_2 reagent (CSR). Free and total sulphur dioxide (without and with correction for reductones) and reductone contents were detected (Vercellotti *et al.* 1994; Caminati & Grabow 2006) in wine. The volumetric solution of iodine was standardised against arsenious oxide.

Determination of free SO_2 content (FSC). A wine sample (50 ml) was pipetted into an Erlenmeyer flask and 16% H_2SO_4 (10 ml) and starch solution (5 ml) were added then using a graduated cylinder. The sample solution was titrated quickly with standard volumetric I_2 solution [c (I_2) = 0.01 mol/l] until blue colour persisting for 30 s was observed against the white background. Based on the consumption of the volumetric I_2 solution, the c_m concentration (in mg/l) of free sulphur dioxide in wine was calculated; total content of free sulphur dioxide and other reductones was detected. Accuracy of the method was verified by a volumetric method using commercial SO_2 reagent based on a similar procedure. The analyses were performed at 20°C. A wine sample was pipetted into a calibrated test tube up to the mark (10 ml) and the SO_2-reagent was added dropwise until blue colour persisting at least for 30 s was observed. One millilitre of the reagent corresponds to 10 mg/l of SO_2.

Determination of total SO_2 content. A wine sample (50 ml) was placed with a pipette touching the bottom into an Erlenmeyer flask containing NaOH solution (12 ml; c = 2 mol/l). The flask was sealed and its content was mixed. After 15 min, 16% H_2SO_4 (15 ml) and starch solution (5 ml) were added using a graduated cylinder. The solution was titrated with volumetric I_2 solution [c (I_2) = 0.01 mol/l] until blue colour persisting for 30 s was observed against the white background. Based on the consumption of the standard I_2 solution, the c_m concentration (in mg/l) of total sulphur dioxide in wine was calculated; total sulphur dioxide and other reductone contents were detected.

Reductone correction (RC). A wine sample (50 ml) was pipetted into an Erlenmeyer flask and 1% formaldehyde (1 ml) was added. The flask was sealed and its content was mixed. After 30 min, 16% H_2SO_4 (10 ml), Na_2EDTA solution (1 ml) and starch solution (5 ml) were added using a graduated cylinder. The sample solution was titrated quickly with volumetric I_2 solution [c (I_2) = 0.01 mol/l] until blue colour persisting for 30 s was observed against the white background. The amount of standard I_2 solution consumed in titration of reductones should be deducted in the calculation of molarity of free or total SO_2 in wine. Reductone content was expressed as mg/l of ascorbic acid, or as mg/l of sulphur dioxide for the purpose of comparison with sulphur dioxide levels determined with a commercially available agent.

Mathematical and statistical methods of evaluation

Titratable acids in wines. Always 3 parallel titrations were carried out for each of the wines on all dates of analyses during 3 years (wines of the year 2009) or 2 years (wines of the year 2010). The first and the second derivative of titration curve ($\delta pH/\delta V$ ev. $\delta^2 pH/\delta V^2 = f(V)$) was used for exact determination of equivalence points using the POTIK program in Microsoft Excel. The average values of concentrations c_m, respective standard deviations and confidence intervals $L_{1,2}$ (α = 0.05) were calculated (Figure 2) from found values.

Repeatability of determination (expressed as the relative standard deviation s_r – always from 10 parallel determinations) for all wines of the year 2009 and 2010 was determined in the time of wine-making –

s_r(10/2009) or s_r(10/2010) and then in October of the next years.

Tartaric acid in wines. Parameters of calibration curves (slope – b, intercept – a, standard deviation of regression – $s_{y,x}$, power of correlation coefficient – r^2, determination limit – $c_{\lim}$) for five standard solutions of freshly prepared tartaric acid for c_m = 1, 2, 3, 4, and 5 g/l, and concentrations of tartaric acid in all samples of wines at all times of analyses during 3 years (wines of the year 2009) or 2 years (wines of the year 2010) were determined using the SKAZA program in Microsoft Excel.

Repeatability of determination (expressed as the relative standard deviation s_r) was calculated using 10 parallel determinations at two levels of concentrations of tartaric acid (low – c_m = 1 g/l and high – c_m = 4 g/l) in January 2010 (wines of the year 2009) – s_r (01/2010, low), s_r (01/2010, high) or in October 2010 (wines of the year 2010) – s_r (10/2010, low), s_r (10/2010, high) and then in October of the next years.

Always 3 parallel determinations of individual wine samples were carried out for each of the wines on all dates of analyses during 3 years (wines of the year 2009) or 2 years (wines of the year 2010). The average values of concentrations c_m, respective standard deviations and confidence intervals $L_{1,2}$ (α = 0.05) were calculated (Figure 5) from found values.

SO_2 in wines. Always 3 parallel titrations (separately for free SO_2, for total SO_2 and for reductones) were carried out for each of the wines on all dates of analyses during 3 years (wines of the year 2009) or 2 years (wines of the year 2010). The average values of concentrations c_m, respective standard deviations and confidence intervals $L_{1,2}$ (α = 0.05) using the JODIK program in Microsoft Excel were calculated (Figure 7) from found values of consumptions of the volumetric I_2 solution. Accuracy of the method was verified by a volumetric method using commercial SO_2 reagent based on a similar procedure.

Repeatability of determination (expressed as the relative standard deviation s_r – always from 10 parallel determinations) for free SO_2, total SO_2 and reductone determination, for all wines of the year 2009 and 2010 was determined in the time of winemaking – s_r (10/2009) or s_r (10/2010) and then in October of the next years.

RESULTS AND DISCUSSION

Determination of titratable acidity in wine

Repeatabilities of determinations of titratable acidity in individual wines of the year 2009 and 2010 are given in Table 1.

The values of relative standard deviations (1.24% to 3.27%) show a satisfactory repeatability of the method of determination of titratable acidity (Table 1). Figure 1 illustrates titratable acidity found in Muscat Ottonel, Müller-Thurgau, Pinot Blanc, Sauvignon, Dornfelder, and blue Frankish wines between October 2009 and October 2012. The respective confidence intervals of the 2009 vintage wines of Pinot Blanc and blue Frankish are shown in Figure 2. The highest titratable acidity ranging between 9.1 and 6.7 g/l was found in Pinot Blanc; the lowest titratable acidity ranging between 8.4 and 5.3 g/l was detected in Sauvignon. During the 3-year monitoring, the highest decrease of titratable acidity (by 3.1 g/l) was revealed in Sauvignon and its lowest decrease (by 1.1 g/l) was detected in Muscat Ottonel.

Titratable acidity with respective confidence intervals of 2010 Pinot Blanc and blue Frankish found in October 2010–October 2012 is depicted in Figure 2. Due to unfavourable weather in August 2010–October 2010, titratable acidity in 2010 wines was 2 g/l higher than in 2009 products (Figure 2).

A decrease of titratable acidity depends on the length of storage. Except for Dornfelder, the 2009 vintage wines showed the highest drop of titratable

Table 1. Repeatability s_r (%) (as relative standard deviations) of determinations of titratable acidity for all wines of the year 2009 and 2010

Wine	2009				2010		
	10/2009	10/2010	10/2011	10/2012	10/2010	10/2011	10/2012
MO	2.68	2.14	2.74	1.94	–	–	–
MT	3.02	2.49	2.89	2.04	–	–	–
RB	2.36	1.88	1.79	1.24	2.77	2.44	1.79
Sg	2.85	2.18	2.24	2.42	–	–	–
Df	3.27	2.89	2.48	2.98	–	–	–
Fr	2.64	2.91	2.14	2.78	2.88	1.99	1.42

MO – Muscat Ottonel; MT – Müller-Thurgau; RB – Pinot Blanc; Sg – Sauvignon; Df – Dornfelder; Fr – blue Frankish

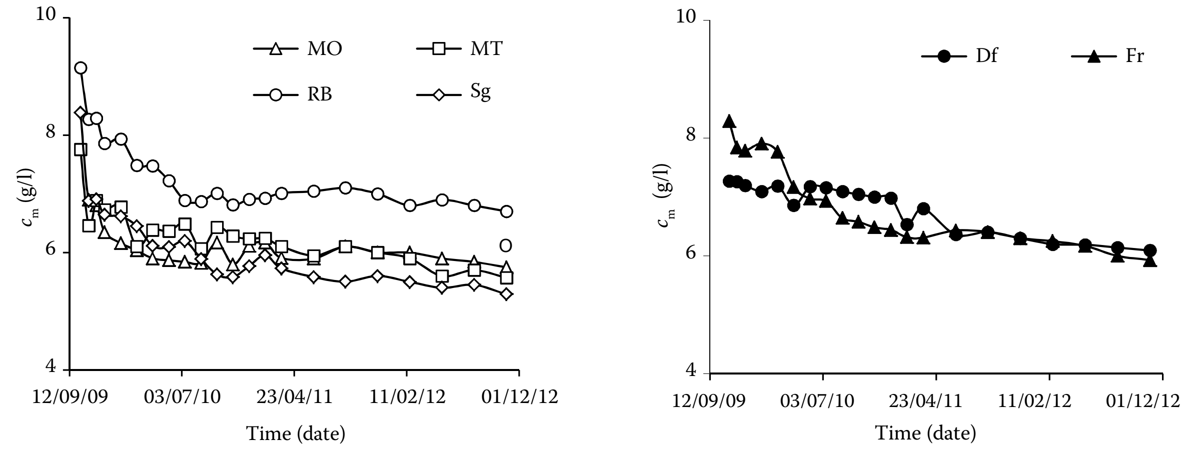

Figure 1. Changes in titratable acidity in 2009 vintage wines (MO – Muscat Ottonel; MT – Müller-Thurgau; RB – Pinot Blanc; Sg – Sauvignon; Df – Dornfelder; Fr – blue Frankish) in 2009–2012

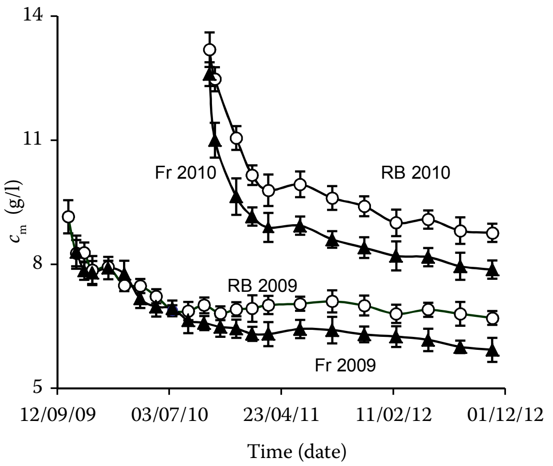

Figure 2. Changes in titratable acidity in 2009 and 2010 Pinot Blanc (RB) and blue Frankish (Fr) in 2009–2012

acidity (68–89%) during the first year of storage; its decrease in the second and third years of archiving was insignificant. Similarly, the 2010 vintage wines also demonstrated the highest drop of titratable acidity during the first year (85–89%). The percentage decrease of titratable acidity in 2009 and 2010 wines throughout individual years of storage is shown in Figure 3.

Determination of tartaric acid content in wine. The confidence intervals $L_{1,2}$ (α = 0.05) of parameters of calibration curves and repeatability (relative standard deviations) of determinations of tartaric acid content are documented in Table 2.

The calibration curves have a satisfactory range of linearity [$L_{1,2}(s_{y,x})$ = 0.010–0.014 (2009) and 0.009–0.015 (2010), $L_{1,2}(r^2)$ = 0.990–0.995 (2009) and 0.991–0.995 (2010)] and the values of relative standard deviations

Table 2. Confidence intervals of parameters of calibration curves and repeatability (as relative standard deviations) of determinations of tartaric acid content

	$L_{1,2}$ (b)	$L_{1,2}$ (a)	$L_{1,2}$ ($s_{y,x}$)	$L_{1,2}$ (r^2)	$L_{1,2}$(c_{lim}) (g/l)
Parameters of calibration curves[a]					
2009	0.062–0.068	–0.017–0.007	0.010–0.014	0.990–0.995	0.109–0.119
2010	0.060–0.067	–0.016–0.004	0.009–0.015	0.991–0.995	0.110–0.123
Repeatability – s_r (%)[b]					
2009		01/2010	10/2010	10/2011	10/2012
	low	4.85	4.44	3.90	3.12
	high	2.66	2.24	3.16	2.96
2010		–	10/2010	10/2011	10/2012
	low	–	4.42	3.36	2.03
	high	–	2.93	3.86	2.08

[a]calculated from all calibration curves prepared on individual dates of analyses; [b]calculated from 10 parallel determinations at two levels of concentrations (low and high); b – slope; a – intercept; $s_{y,x}$ – standard deviation of regression; r^2 – power of correlation coefficient; c_{lim} – determination limit

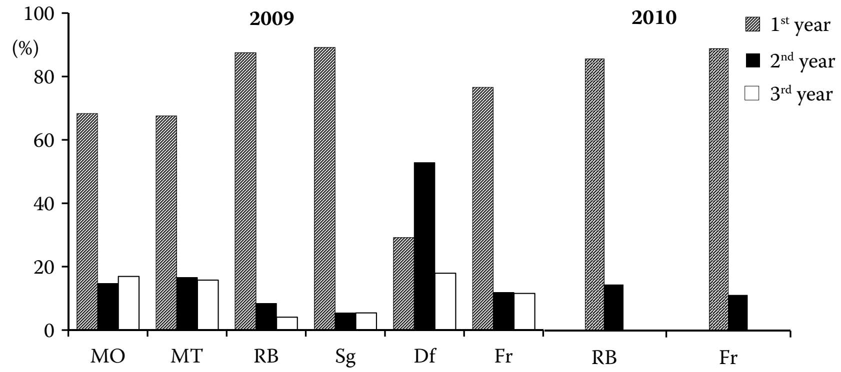

Figure 3. Percentage decrease of titratable acidity in 2009 and 2010 vintage wines (MO – Muscat Ottonel; MT – Müller-Thurgau; RB – Pinot Blanc; Sg – Sauvignon; Df – Dornfelder; Fr – blue Frankish) and 2010 vintage wines (RB – Pinot Blanc; Fr – blue Frankish) during storage in 2009–2012

[(2.24%–4.85% (2009) and 2.08%–4.42% (2010)] show a good repeatability of the method of determination (Table 2).

The content of tartaric acid in 2009 Müller-Thurgau, Pinot Blanc, Sauvignon, Muscat Ottonel, Dornfelder and blue Frankish wines was monitored from January 2010 to October 2012. Figure 4 illustrates tartaric acid content in individual wines produced in 2009. The respective confidence intervals of the 2009 vintage wines of Pinot Blanc and blue Frankish are shown in Figure 5.

As apparent, tartaric acid content ranging between 1.47 and 2.42 g/l in individual wines remained almost unchanged throughout monitoring; the lowest tartaric acid content was found in Dornfelder wine (average value of 1.65 g/l) and the highest one was detected in Müller-Thurgau wine (average value of 2.27 g/l). The highest change of tartaric acid content reaching 0.87 g/l was found in blue Frankish wine.

Similarly to titratable acidity, tartaric acid content was monitored in Pinot Blanc and blue Frankish wines on specified days between October 2010 and October 2012. The results of the tartaric acid content with respective confidence intervals of the above 2010 wine analyses are shown in Figure 5.

As apparent in Figure 5, at first tartaric acid contents in individual wines decreased significantly and then, after wine formation, they remained almost unchanged throughout the monitoring and showed just an insignificant decrease. Like titratable acidity, the tartaric acid content in 2010 vintage wines grew high; it namely reached the values by 1.2 g/l higher when compared to the tartaric acid content in 2009 vintage wines (Figure 5). Figure 6 illustrates the percentage of titratable acidity attributable to tartaric acid in individual 2009 and 2010 vintage wines.

The tartaric acid to titratable acidity ratio remained almost unchanged in all the 2009 vintage wines throughout three years of their monitoring. The lowest percentage of tartaric acid was found in Dornfelder (average value of 24.9%); the highest percentage was detected in Müller-Thurgau (average value of 37.0%).

The proportion of tartaric acid in titratable acidity in 2010 vintage wines decreased remarkably at first. After the formation of wine, it remained almost unchanged throughout the monitoring period. The following average percentages were found in individual wines: Pinot Blanc 37.7% and blue Frankish 36.3%. The above average percentages of tartaric

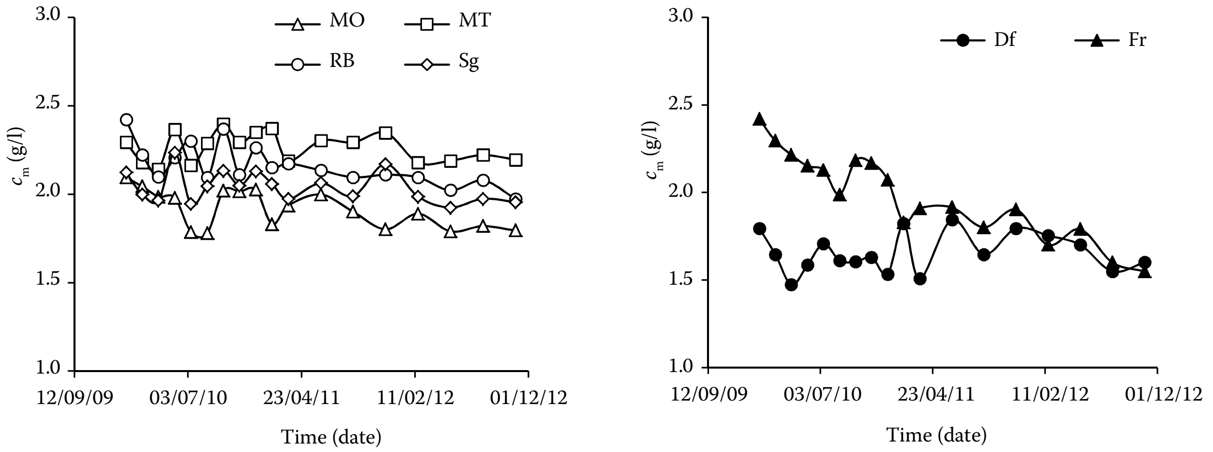

Figure 4. Changes in tartaric acid content in 2009 vintage wines (MO – Muscat Ottonel; MT – Müller-Thurgau; RB – Pinot Blanc; Sg – Sauvignon; Df – Dornfelder; Fr – blue Frankish)) in 2010–2012

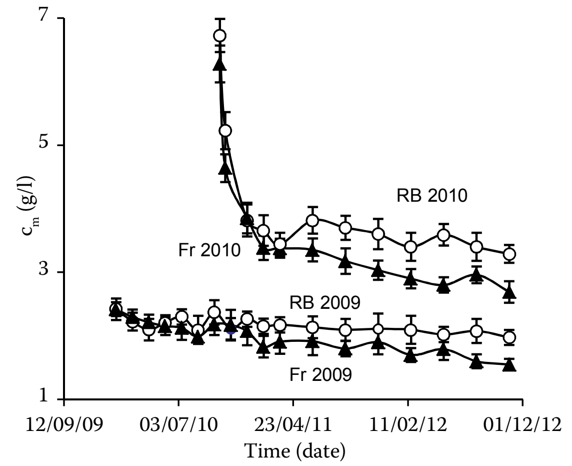

Figure 5. Changes in tartaric acid content in 2009 and 2010 Pinot Blanc (RB) and blue Frankish (Fr) in 2010–2012

acid in 2010 vintage wines are approximately by 7% higher than in 2009 vintage wines.

Determination of sulphur dioxide content in wine

Repeatabilities of determination of the sulphur dioxide and reductone content in individual wines of the year 2009 and 2010 are given in Table 3.

The values of relative standard deviations (0.84 to 3.96%) show a satisfactory repeatability of methods of determination of the sulphur dioxide and reductone content (Table 3).

Free and total sulphur dioxide was determined in all six 2009 vintage wines; the correction for reductone content was also done. To compare, free sulphur dioxide content was determined with a commercially available reagent. The results were in good agreement. Out of the 2010 vintage wines, Pinot Blanc and blue Frankish were monitored.

The range of values of the content of free SO_2, total SO_2 and reductones found in the individual 2009 vintage wines in the period of January 2010–October 2012 and in the individual 2010 vintage wines (RB and Fr) in the period of December 2010–October 2012 is given in Table 4.

For the 2009 Pinot Blanc and blue Frankish, Figure 7 shows January 2010–October 2012 comparison of the following factors: (a) free sulphur dioxide content determined by the method specified above; (b) free sulphur dioxide content detected with a standard commercially available reagent; and (c) total content of free sulphur dioxide and reductones (with respective confidence intervals) determined by the methods specified above.

Table 3. Repeatability – s_r (%) (as relative standard deviations) of determinations of sulphur dioxide and reductone content for all wines of the year 2009 and 2010

Wine	2009				2010		
	10/2009	10/2010	10/2011	10/2012	10/2010	10/2011	10/2012
Free SO_2							
MO	1.71	1.39	1.44	1.24	–	–	–
MT	2.17	1.56	1.89	2.06	–	–	–
RB	1.97	1.29	2.04	0.84	1.55	1.97	0.99
Sg	2.84	2.55	1.85	2.19	–	–	–
Df	3.96	3.24	3.29	2.62	–	–	–
Fr	2.44	2.84	2.22	2.09	2.24	2.48	1.79
Total SO_2							
MO	2.04	2.55	1.88	2.09	–	–	–
MT	2.87	2.78	2.24	2.42	–	–	–
RB	2.22	1.97	2.42	1.79	1.44	1.99	1.92
Sg	2.89	2.44	2.24	2.96	–	–	–
Df	3.88	3.04	2.97	2.92	–	–	–
Fr	2.98	2.88	2.55	2.92	2.88	2.05	2.34
Reductones							
MO	2.22	2.42	1.44	1.24	–	–	–
MT	2.08	2.99	2.77	2.04	–	–	–
RB	2.24	2.44	2.55	1.12	1.88	2.24	1.56
Sg	2.29	2.24	2.12	1.97	–	–	–
Df	3.22	3.68	3.34	2.64	–	–	–
Fr	2.77	2.68	2.91	2.88	2.98	2.92	2.55

MO – Muscat Ottonel; MT – Müller-Thurgau; RB – Pinot Blanc; Sg – Sauvignon; Df – Dornfelder; Fr – blue Frankish

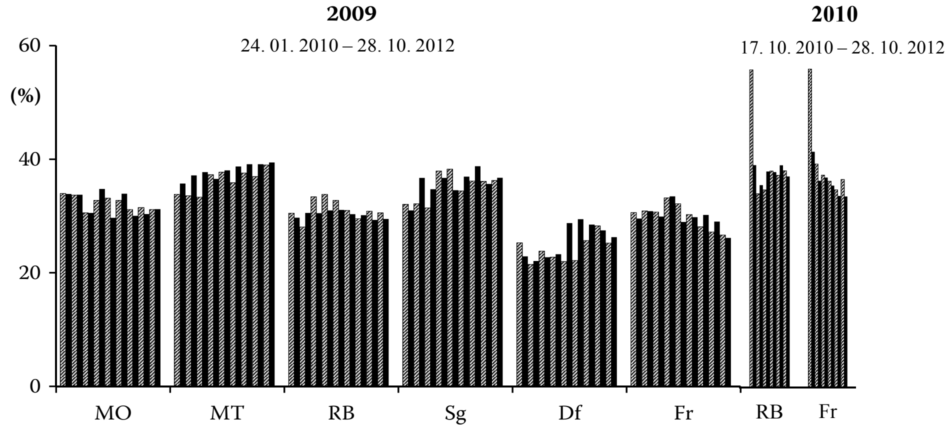

Figure 6. The percentage of tartaric acid in titratable acidity in 2009 vintage wines (MO – Muscat Ottonel; MT – Müller-Thurgau; RB – Pinot Blanc; Sg – Sauvignon; Df – Dornfelder; Fr – blue Frankish) and in 2010 vintage wines (RB – Pinot Blanc; Fr – blue Frankish)

For the 2010 Pinot Blanc and blue Frankish, Figure 7 shows the following factors monitored in December 2010–October 2012: (a) content of free sulphur dioxide determined by the method specified above; (b) content of free sulphur dioxide detected with a standard commercially available reagent; and (c) total content of free sulphur dioxide and reductones (with respective confidence intervals) determined by the methods specified above.

As apparent in Table 4 and Figure 7, sulphur dioxide content, especially free sulphur dioxide content, varies to a large extent depending on dates of

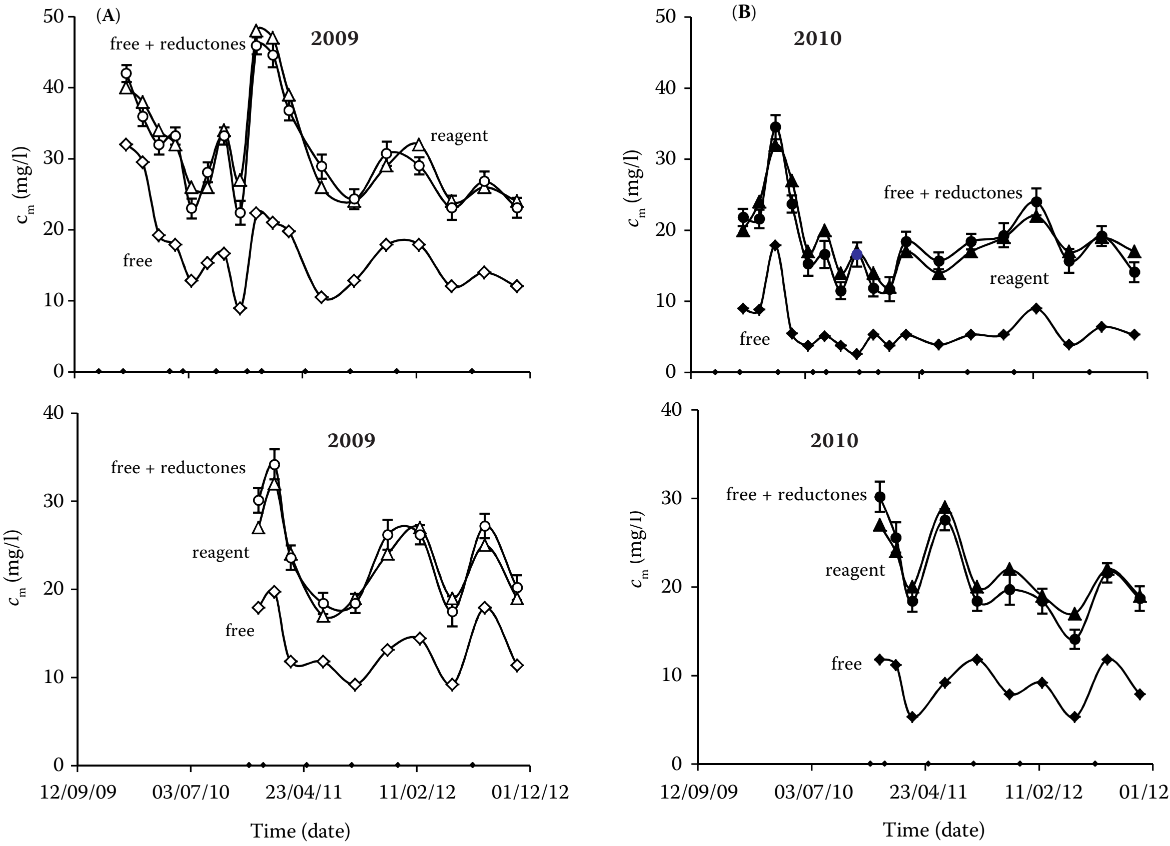

Figure 7. Comparison of free SO_2 content determined by theFSC method, free SO_2 content determined with a standard commercially available agent (CSR), and total content of free sulphur dioxide and reductones determined by the FSC and RC methods: (**A**) Pinot Blanc, and (**B**) blue Frankish in 2009 and 2010

Table 4. The content of free SO_2, total SO_2 and reductones (expressed as mg/l of ascorbic acid) in 2009 vintage wines and in 2010 vintage wines

Wine	Free SO_2	Total SO_2	Reductones
2009			
MO	13–260	805–1288	140–528
MT	13–105	817–1323	140–456
RB	89–320	907–1340	176–659
Sg	13–79	654–1392	71–385
Df	39–379	1622–2720	352–1280
Fr	26–179	383–1262	181–500
2010			
RB	9.2–19.7	52.6–84.0	18.1–39.9
Fr	5.3–11.8	52.0–102.1	18.1–50.6

MO – Muscat Ottonel; MT – Müller-Thurgau; RB – Pinot Blanc; Sg – Sauvignon; Df – Dornfelder; Fr – blue Frankish

sulphuration that are plotted as small points on the time (date) axis.

Our findings confirm a significant fact that the content of free sulphur dioxide determined with a standard commercially available reagent is remarkably higher than the levels provided by the method described above (Figure 7) because in iodometric titration, the standard commercially available reagent detects both free sulphur dioxide and reductones contained in wine. Reductone content is then mistaken for free sulphur dioxide content, which can lead to insufficient sulphuration of wine and overestimated results of SO_2.

CONCLUSION

The content of titrated acids in individual wines in the entire monitored time period was in the main constant with a very moderate time decrease (Figures 1 and 2) and the values were on average about 9.1–5.3 g/l (2009) and 13.1–7.9 g/l (2010). The content of tartaric acid in individual wines in the entire monitored time period was also in the main constant with a very moderate time decrease (Figures 4 and 5) and the values were on average about 2.37–1.47 g/l (2009) and 6.65–2.69 g/l (2010). In wines of the year 2010 (as a result of adverse weather conditions in the period of August–October 2010) the content of titrated acids and tartaric acid was about 1–2 g/l higher than in wines of the year 2009. For wines of both years the percentage ratio of tartaric acid in the content of titrated acids is in the main constant, see Figure 6 (average 24.4–38.6% for individual wines).

The content of free SO_2 and total SO_2 in dependence on the time of SO_2 addition to individual wines was in the broad interval of values (Table 4) – free: 1.3–37.9 mg/l (2009), 5.3–19.7 mg/l (2010); total: 38.3–272 mg/l (2009), 52.0–102 mg/l (2010). Results of free SO_2 obtained using the commercial SO_2 reagent were higher than our results, which corresponds with the content of reductones, because this reagent determined not only free SO_2 but also reductones (Figure 7). This fact is a reason for the frequent absence of needed free SO_2 in wines and overestimated results of SO_2 determination. There are not any great differences between individual wines, and between white wines and red wines.

This work confirmed also some regularities in the chemical composition of wines in dependence on their formation published in the literature (Farkaš 1980; Vercellotti *et al.* 1994; Caminati & Grabow 2006; Jančářová *et al.* 2013).

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