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PROCEDURE TO REDUCE SULPHITE IN WINE WITH ANION-EXCHANGE RESIN

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Abstract

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The aim of this experiment was to eliminate SO_2 ions present in wine using the anion-exchanger resins. To compare the effectiveness, 2 following strongly basic anion-exchange resin were used. When activated, the sodium bicarbonate solution (activation solution I) is used to prevent parallel reduction of sulphites, tartates and malates, so the anion-exchange resins were activated in two-step activation. In the second step, it was immersed into a mixture of malic acid and tartaric acid (1:1). After the application of anex into wine, the content of total SO_2 was reduced to 97–201 $mg.L^{-1}$ (depending on the amount of anex added into the wine sample). According to our expectations, the variants with anion-exchange resin activated only with bicarbonate solution, the tartrates and malates were significantly reduced. If the anion-exchange resin was activated with a two-steps activation, the tartaric acid and malic acid were reduced in the range of ± 0.13 $g.L^{-1}$. This phenomenon was strongly reflected at the anion-exchanger Aqua Osmotic 02. The changes in antioxidant content were not affected by the type of anion-exchange resin, the method of activation, or an amount of used anion-exchanger. The color parameters of wine, expressed by the L * a * b *, were not significantly affected by the effects of anion-exchange resin use.

sulphites, sulfur dioxide, anion exchanger, wine

Sulphur dioxide plays an important role in the wine-making technology. To eliminate changes caused by oxidative processes and an eventual re-fermentation of wine, it is necessary to stabilise it with some preservative preparations. Sulphur dioxide is one of them because it is capable to bind labile oxygen molecules dissolved in the solution; this means that it shows a reductive action and prevents the occurrence of both enzymatic and non-ezymatic oxidation (FARKAŠ, 1983; STEIDL, 2002). Therefore it acts as an antioxidant in wine (RIBÉREAU-GAYON *et al.*, 2000).

In wine, sulphur dioxide not only plays an reductive role but it also inhibits activities of yeasts (above all of those that are not able to synthetisize thiamin, which is split by SO_2 to inefficient compounds), bacteria and other microorganisms (VELÍŠEK, 2002). In wine, SO_2 can be present in two forms, viz. as free and bound sulphur. In wine, sulphurous acid (H_2SO_3) originates from a reaction of sulphur dioxide with water. Under the effect of wine pH, sulphurous acid is dissociated to sulphurous (SO_3^{2-}) and hydrogen sulphurous (HSO_3^-) ions, which then represent the free form of SO_2 in wine. In an acid environment (i.e. with pH 3–4), hydrogen sulphites are predominating (TOIT *et al.*, 2005).

The remaining SO_2 is bound to acetaldehyde, pyruvic acid, ketoglutaric acid, sugars, quinone, anthocyanins and other compounds (STEIDL, 2002; VELÍŠEK, 2002; OUGH, AMERINE, 1988).

However, an antiseptic effect shows only a part of free SO_2 and this is usually denoted as "active sulphur dioxide" (FARKAŠ, 1983).

When applying SO_2 to wine it is necessary to count with a certain amount of SO_2 which is produced by yeast during the fermentation, this amount does not usually exceed the value of 10 $mg.L^{-1}$ (RIBÉREAU-GAYON *et al.*, 2000). If the amount of SO_2 applied to wine during the fermentation is too high, it can lead to the suppression or discontinuation of fermentation and subsequently to wine flavor deterioration, or to the allergy symptoms in sensitive people (AGARD *et al.*, 1998).

Sulphites, as allergenic compounds, are subjected to hygienic control and must be declared on the label only in case that their concentration in the product exceeds the level of 10 mg.L^{-1}. In the Czech Republic, the content of total SO_2 in wine is specified in the Council Regulation (EC) No. 479/2008 and the Commission Regulation (EC) No. 606/2009. In white and rosé wines, the admissible maximum is 200 mg.L^{-1} while in red quality and cabinet wines with the content of residual sugar up to 5 g.l^{-1}, the approved maximum is 150 mg.L^{-1}. As far as other kinds of wine are concerned, the maximum admissible limits of SO_2 are defined in special regulations.

In practice, it can sometimes happen that this allowed limit is exceeded. In such a case, thre are several methods how to remove the excessive SO_2 from wine. The simplest method is to mix the over-sulphurised wine with another that does not contain SO_2 (or only a low level of this compound). The bubbling of over-sulphurised wine with gaseous oxygen can release a part of SO_2 into the atmosphere; this treatement, however, is associated with a risk of occurrence of negative oxidative processes. This method, however, enables to remove free SO_2 and only a minimum amount of its bound form. Another method of removal of sulphites from wine represents the vacuum heating of wine in a special apparatus so that the excessive SO_2 is volatilised. In this case, however, there is a risk of the loss of aromatic compounds and of ethanol (FARKAŠ, 1983).

The application of resins used in the food industry represents another possibility how to decrease the content of excessive SO_2 in wine. As mentioned above, the anex is an anion-exchange resin. This high-molecular-weight substance is a polymer showing a sufficient porosity and most frequently is based on styrenes, polyacrylates, phenolformaldehyde resins etc. The basic anex skeleton bears functional (charged) groups that can be dissociated in aqueous medium (MARHOL, 1976). Anions remaining in the solution must be balanced with those that are released from it. In case that the amount of anex is exhausted, it can be regenerated by means of adding the solution of another anion (JELÍNEK *et al.*, 2009). However, when applying the aforementioned resins into wine, not only a decrease in the content of sulphites but also of acids and other qualitative components of wine can take place. The ratio of reduced anions is influenced above all by their concentration in wine and by their affinity to the applied resin (HÜBNER *et al.*, 2006).

MATERIAL AND METHODS

Preparation of anion-exchange resin

The capacity to decrease the content of total SO_2 was studied with two resins that are normally used in the food industry. The first one was a strongly basic anion-exchange resin IMAC HP555 with quartenary ammonium groups; the divinylbenzene and styrene copolymers of this resin were of the type I (hereinafter mentioned only as HP555) while the second one was a part of the fill of the anion-exchanger Aqua Osmotic 02 that is used for making of demineralised water by means of the system catex-anex (hereinafter mentioned only as Aqua Osmotic 02).

Resins were acitivated in a two-step system. Before activating anion exchangers were washed 3 times with distilled water. In the first step, resins were activated by means of immersion into a 10-percent sodium bicarbonate solution (the activation solution I) for a period of 24 hours. Thereafter, they were carefully rinsed with distilled water untill the moment when pH of the mixture of anex and disitlled water reached the value 7.0. In the second step, it was immersed into the activation solution II for a period of another 24 hours. The activation solution II was a mixture of malic acid and tartaric acid (1:1); the total concentration of both acids in the solution was 50 g.L^{-1}. In the final stage of the activation process, resins were immersed into distilled water so that the residues of the activation solution II could be washed away from the anex surface. The last rinsing was performed only once because we did not want to wash away those anions of both acids that were bound on the anexfunctional groups. To be able to carry out a mutual comparison of results, anexes activated in the same manner were used (i.e. by means of their immersion into a 10-percent solution of sodium bicarbonate for a period of 24 hours. Each sample anion exchange resin was investigated in 3 repetitions, each sample analysis was performed 3 times.

Treatment of wine

In this experiment, the grapewine variety Pinot Blanc was used; the wine sample was mixed with potassium disulphite (K_2S2O_5) so that the content of total SO_2 was 563 mg.L^{-1} and content of free SO_2 was 387 mg.L^{-1}.Resins activated in this way were applied into wine samples (containing 563 mg.L^{-1} of total SO_2) in volumes of 15, 30 and 40 g.L^{-1}. All experimental variants were performed in three replications. Wine with anex was placed into a shaker and the sample was shaken for a period of 60 minutes. Thereafter, the resin was removed from wine and the sample was analysed.

Analytical methods

Contents of free and total SO_2 in original wine samples and those with added anex doses were estimated by means of iodometry (ZOECKLEIN, 1990), determination of total antioxidative capacity by method DPPH (BRAND-WILLIAMS *et al.*, 1995), contents of total polyphenols were determined by means of the Jerumanis method (1972) and expressed in mg.L^{-1} of gallic acid. For the estimation of malic and tartaric acids, the method of high-performance liquid chromatography (HPLC) was used with the column Polymer IEX H form 8 µm 250 × 8 mm (mobil phase 1 mM sulphuric acid, flow rate 0.7 ml.min^{-1}at the temperature of 50°C, high-pressure pump Chrom SDS 150, and the detector Spectra system UV6000LP at the wavelengt 210 nm) (PAVLOUŠEK, KUMŠTA, 2011). The colors parameters of wine samples in the CIE Lab colour space was estimated using the colorimeter LOVIBOND RT850i. The calibration was performed using deionized water as standard. Measurements were performed in a 2-mm absorption cell. The method was performed according to the manual device.

Statistical analysis

Analysed data were determined in three separate solutions. Analysis of variance (ANOVA) was carried out using the programm STATISTICA 8. Significant differences were calculated according to the Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

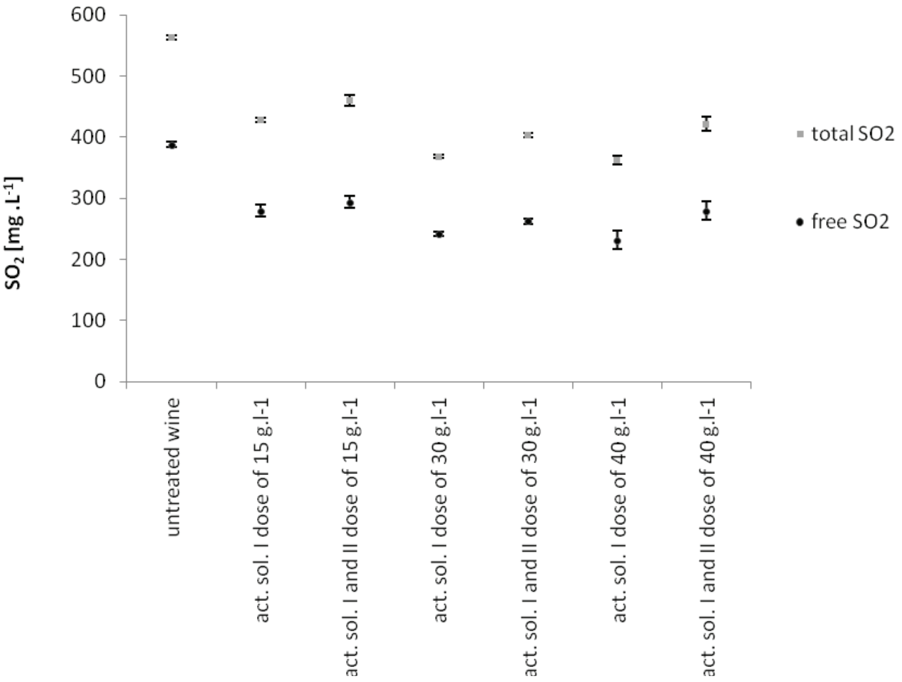
When using resins for the reduction of SO_2 content in wine (and with the minimum possible losses of organic acids), it is necessary to minimalise the binding of tartaric and malic acid anions on the active surface of the resin. If these anions are bond on functional groups of the resin already within the process of anex activation, these are exchanged with anions present in wine immediately after the anex application into wine samples so that the content of both acids in wine is not decreased. The estimation of total and free SO_2 in wine treated by means of resins revealed the existence of statistically significant differences between and among individual experimental variants. Effects of the anex itself, method of its activation and the dose added into wine were corroborated. The anex Aqua Osmotic 02, activated quite normally (i.e. only by means of immersion into the activation solution I) showed to be the most efficient method of reduction of the total SO_2 content. The dose of 40 g.L^{-1} of resin caused a reduction of total SO_2 by 201 mg.L^{-1} (the content of free SO_2 was decreased by 156 mg.L^{-1}). On the other hand, however, the lowest effect on the reduction of SO_2 content was observed after the application of HP555 in the dose of 15 g.L^{-1} (activated also only with activation solution I; in this variant, contents of total and free SO_2 were decreased by 97 mg.L^{-1} and 52 mg.L^{-1}, respectively). Individual experimental variants and their effects on the decrease of SO_2 contents in wine are presented in Figs. 1 and 2.

When using the anex HP555, no statistically significant difference between the method of activation and the amount of removed sulphites was observed. After the application of this resin, the highest decrease in contents of free and total SO_2 was recorded after the dose of 40 g.L^{-1} (151 mg.L^{-1} and 125 mg.L^{-1} of total and free SO2, respectively). The amounts of eliminated SO_2 were therafter significanly decreasing: after the resin dose of 30 g.L^{-1} contents of free and total SO_2 were decreeased by 104 mg.L^{-1} and 123 mg.L^{-1}, respectively, and the dose of 15 g.L^{-1}, contents of free and total SO_2 decreeased by 100 mg.L^{-1} and 73 mg.L^{-1}, respectively. When applying the anex Aqua Osmotic 02, the method of activation on its capacity to decrease the content of sulphites in wine was already demonstrated. The anex Aqua Osmotic 02, activated in a common manner, decreased the content of SO_2 (both free and total) more than that activated by means of a two-step activation. Again, the most intensive decrease in the content of SO_2 was recorded after the application of the highest dose of resin; however, the difference between the dose of 40 g.L^{-1} and that of a 30 g.L^{-1} was not significant. When applying resins activated by means of solutions I and II, no effect of the applied dose on the elimination of malic and tartaric acid was observed. As compared with original (control) samples of wine, changes in the content of tartaric acid ranged from –0.1 to +0.04 g.L^{-1} after the application of activated resins while those taking place in the content of malic acid fluctuated within the rage of –0,2 to –0,04 g.L^{-1}.

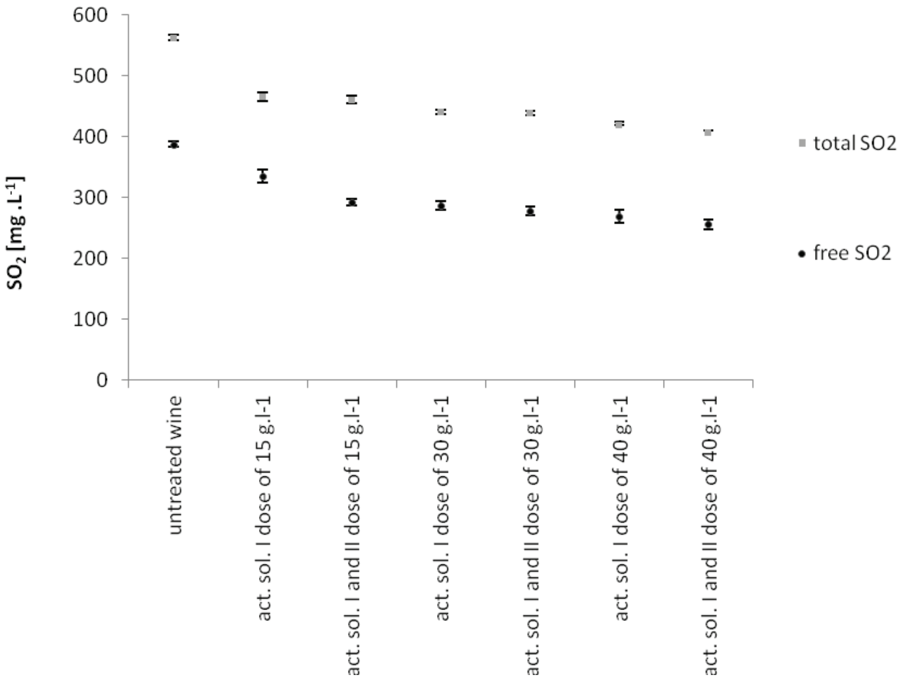
This means that it was corroborated that resins were capable to remove efficiently sulphites contained in wine. FARKAŠ (1983), who used the Czechoslovak resin MFD as a tool for the deacidification of wine, obtained similar results. When using the flow method (i.e. when the anex MFD was placed into a glass column and wine from a tank was flowing through it), the contents of total and free SO_2 in wine were decreased from 129 to 47 mg.L^{-1} and from 43 to 6 mg.L^{-1}, respectively.

It was demonstrated that in the variant with the most intensive decrease in SO_2 content the decrease in contents of tartaric and malic acid was also very marked. On the other side, however, the lowest changes in contents of both acids (as compared with the original wine) were observed after the application of the resin Aqua Osmotic 02, reactivated by means of a two-step activation in solutions I and II (Figs. 3 and 4).

When using the DPPH method for the estimation of antioxidant capacity, no statistically significant differences between individual variants were observed (i.e. as dependent on the type of resin, appllied dose, and method of activation). The content of total polyphenols (JERUMANIS, 1972) was decreased in a similar manner after the application of both resins, regardless to the method of their activation. After the application of Aqua Osmotic 02 and HP555, the initial content of gallic acid in wine (17.3 mg.L^{-1}) was reduced to 10.2 mg.L^{-1} and 11.0 mg.L^{-1}, respectively. There was also a positive correlation between the decrease in the content of total polyphenols and the applied dose of anex ($r = 0{,}973$). The most intensive reduction was observed after the application of the dose of 40 g.L^{-1} (i.e. 9.5 mg.L^{-1}; and 8.5 mg.L^{-1} after the application of HP555 and Aqua Osmotic 02, respectively) while after the dose of 15 g.L^{-1}, the lowet changes



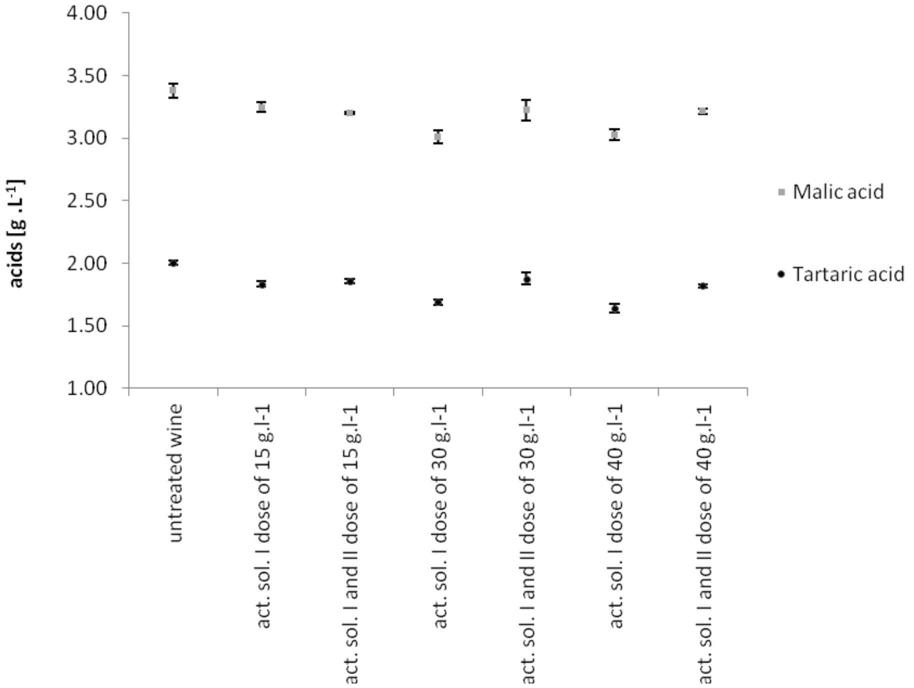
1: *Amounts of removed SO$_2$ after the application of anion-exchange resin Aqua Osmotic 02 (n = 5; P ≤ 0.05)*



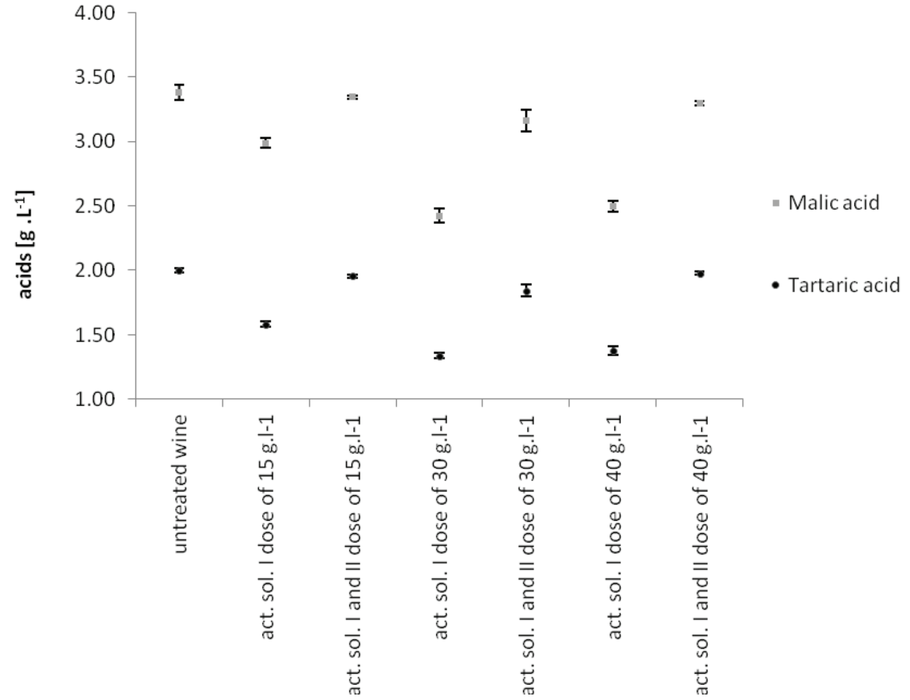
2: *Amounts of removed SO$_2$ after the application of anion-exchange resin HP555 (n = 3; P ≤ 0.05)*

in contents of polyphenols were observed (i.e. 12.6 mg.L^{-1} and 12.9 mg.L^{-1} after the application of HP555 and Aqua Osmotic 02, respectively).

It was found out during the measurement of colour parameters L * a * b * that there were a minimal reduction in all three dimensions by both used aninon-exchange resins. The largest deviations from the original sample values were detected for wine applications anion-exchange resin activated by the solution I and II at a dose of 40 g.L^{-1} as shown in Tab. I. These variations are within the color spectrum so low that without accurate measurements with a colorimeter, they are virtually with our eye unperceivable.



3: *Changes in the content of acids after the application of anion-exchange resin HP555 (n = 3; P ≤ 0.05)*



4: *Changes in the content of acids after the application of Aqua Osmotic 02 (n = 3; P ≤ 0.05)*

CONCLUSION

Anion-exchange resins can be used as a friendly method to removal of SO_2 from wine. However, anion-exchanger resins needs to be treated with malic and tartaric acid solution to avoid content reduction of these acids in wine. Total polyphenols content by Jerumanis is decreased, depending on the dose of applied anion-exchange resin, but not on the type of activation.When using the DPPH method for the estimation of antioxidant capacity, no statistically significant differences between individual variants were observed. The color parameters of wine, expressed by the L * a * b *, were not significantly affected by the effects of anion-exchange resin use.

I: *Changes in values of colour parameters CIE L*a*b* in wine with anion-exchange resins activated differently and in different doses (n = 3; P ≤ 0.05)*

Method of activation anion exchange-resin	L*		a*		b*	
	HP555	Aqua Osmotic 02	HP555	Aqua Osmotic 02	HP555	Aqua Osmotic 02
untreated wine	99.17 ± 0.06	99.17 ± 0.06	–0.71 ± 0.01	–0.71 ± 0.01	3.84 ± 0.04	3.84 ± 0.04
a. s. I dose of 15 g.L^{-1}	99.15 ± 0.07	99.26 ± 0.07	–0.63 ± 0.01	–0.59 ± 0.02	3.48 ± 0.08	3.42 ± 0.02
a. s. I + II dose of 15 g.L^{-1}	99.27 ± 0.01	99.35 ± 0.02	–0.61 ± 0.01	–0.62 ± 0.01	3.44 ± 0.08	3.40 ± 0.09
a. s. I dose of 30 g.L^{-1}	99.08 ± 0.21	99.21 ± 0.10	–0.59 ± 0.02	–0.55 ± 0.02	3.23 ± 0.03	3.08 ± 0.06
a. s. I + II dose of 30 g.L^{-1}	99.05 ± 0.07	99.19 ± 0.13	–0.61 ± 0.01	–0.56 ± 0.02	3.28 ± 0.03	3.05 ± 0.03
a. s. I dose of 40 g.L^{-1}	98.86 ± 0.04	98.86 ± 0.11	–0.61 ± 0.01	–0.47 ± 0.08	3.10 ± 0.07	3.06 ± 0.14
a. s. I + II dose of 40 g.L^{-1}	99.33 ± 0.09	99.19 ± 0.11	–0.55 ± 0.02	–0.58 ± 0.02	3.13 ± 0.06	3.25 ± 0.12

SUMMARY

This paper deals with possibilities of wine desulphitation by means of a strongly basic anion-exchange resin (anex) IMAC HP555 and the resin from the ion-exchanger Aqua Osmotic 02 without a greater loss of organic acids. The anex resins bind anions contained in wine to their functional groups and thus decrease the content of sulphites.

To prevent a decrease in the content of total anions (i.e. tartates and malates) a two-step anex activation was used. In the first step, the resin was immersed into a 10-percent sodium bicarbonate solution for a period of 24 hours (in the dose of 1,000 ml of solution per 100 g of anex). In the second step, it was immersed into a mixture of malic acid and tartaric acid (1:1); the total concentration of both acids in the solution was 50 g.L^{-1} and the soaking period was also 24 hours. After the application of anex into wine, the content of total SO_2 was reduced to 97–201 mg.L^{-1} (depending on the amount of anex added into the wine sample). When using these two-step-activated anexes, contents of malic and tartaric acids ranged within the original values with deviations of ± 0.13 g.L^{-1}. Neither the type, nor the method of activation, nor the dose or amount of ion exchange resin had an impact on the changes in the content of antioxidants in the sample provided by method DPPH. The content of total polyphenols (expressed in mg of gallic acid per 1 liter) was being reduced depending on the dose of the anion-exchange. The highest decrease was observed at doses of ion exchange resin 40 g.L^{-1}. There were no significant changes at the analyzed samples in colour parameters L * a * b * when exposed to anion-exchange resins. From these results, it is evident that the application of ion exchange resin for 60 minutes has no significant effect on the change in colours parameters of treated white wine.

Based on the obtained results it can be stated that it is preferable to use the reduction of SO_2 in wines the resin from the ion-exchanger Aqua Osmotic 02, which had a greater ability to build on each other sulphite anions. This ability, however, caused a greater reduction of tartaric and malic acids. Using two-steps activation, however, prevents a significant loss of these acids.

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