YEASTS AND NATURAL PRODUCTION OF SULPHITES

Maik WERNER[1], Doris RAUHUT[1], Philippe COTTEREAU[2]
[1] State Research Institute Geisenheim, Germany; [2] IFV Rodilhan, France

Extract from Research Results in Code of Best Practice for Organic Winemaking, produced under the EU FP6 STREP project ORWINE

Production of sulphites (SO_2) by yeast during alcoholic

During alcoholic fermentation yeasts naturally produce sulphur dioxide (SO_2) as a metabolic intermediate of the sulphate reduction pathway (Romano and Suzzi (1993), Ribéreau-Gayon et al., (2006)). Yeast strains can be categorized into ***low SO_2 producers*** i.e. *Saccharomyces cerevisiae* var*. ellipsoideus* and ***high SO_2 producers*** i.e *Saccharomyces bayanus* Sacardo. Certain yeast strains can produce up to 300 mg/L of sulphite during fermentation. Dott and Trüper (1976) described that the sulphite reductase of the sulphite-producing yeast strains might be altered. As a consequence sulphite (SO_2) will be accumulated in the cell and finally be released into the must. Former assumptions about mutations being the cause of the sulphite production could not be confirmed. Today producers of commercial dried yeast consider this important property of the yeast during the selection process. It is only when wine-makers wish to induce a spontaneous fermentation that the properties of the fermenting yeast strains cannot be guaranteed.

The majority of today's commercial yeast strains are considered to be low SO_2 producers, showing a production up to 20 mg/L of total SO_2. Only few yeast strains appear to have a higher production (up to 80 mg/L SO_2).

Fig. 1: Production of SO_2 by 22 commercial yeast strains during fermentation. Mean value of the triplicate. Bars show the standard deviation.

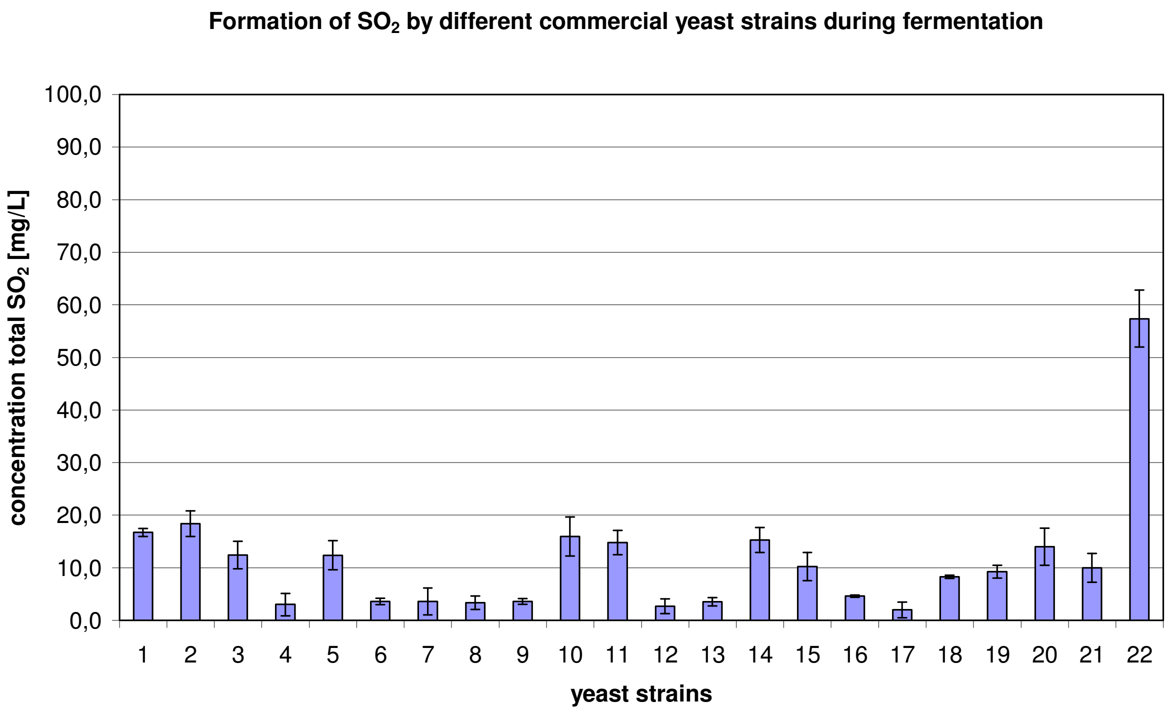


Figure 1 shows the SO_2 production of 22 commercial yeast strains used in Europe.
No. 1 to 21 were recommended by the yeast producers as low SO_2 producers. No. 22 is a reference strain with a high SO_2 production. The fermentations were performed with 2007 Riesling must, which was pasteurised in order to eliminate any undesired micro-organisms. The

fermentation temperature was 18℃, the inoculation dosage was 30 g/hl pure dried yeast. Rehydration was done by water (35℃) for 25 minutes. The results show predominantly two groups of yeast strains. One group produces under 10 mg/L total SO_2, the other group produces between 10 and 20 mg/L total SO_2. Only one yeast strain reaches a concentration of 57 mg/L of total SO_2.

Fig. 2: Production of SO_2 by one commercial yeast strain during fermentation in must from different grape varieties. Mean value of the triplicate. Bars show the standard deviation.

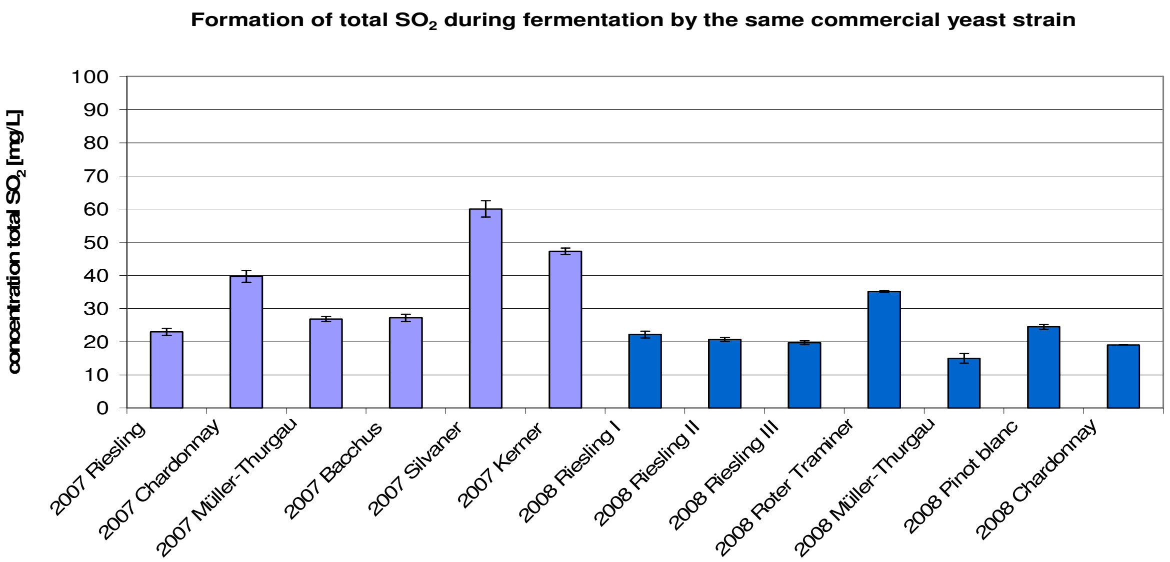


Figure 2 shows the concentration of SO_2 after the alcoholic fermentation by the same commercial yeast strain in must from different grape varieties (vintage 2007 and 2008). Fermentation conditions were the same as for the comparison of yeast strains. All the different grape juices were pasteurised, in order to eliminate any undesired micro-organisms. The results show that the formation of SO_2 during fermentation depends also on the yeast variety and the composition of the grape juice. The grape juices in figure 2 were all fermented with the same commercial yeast strain, but the concentration of total SO_2 varies from 15 to 60 mg/L after the alcoholic fermentation. This indicates that even a yeast strain that is considered as a low SO_2 producer can produce higher concentrations in certain grape juices in certain years.

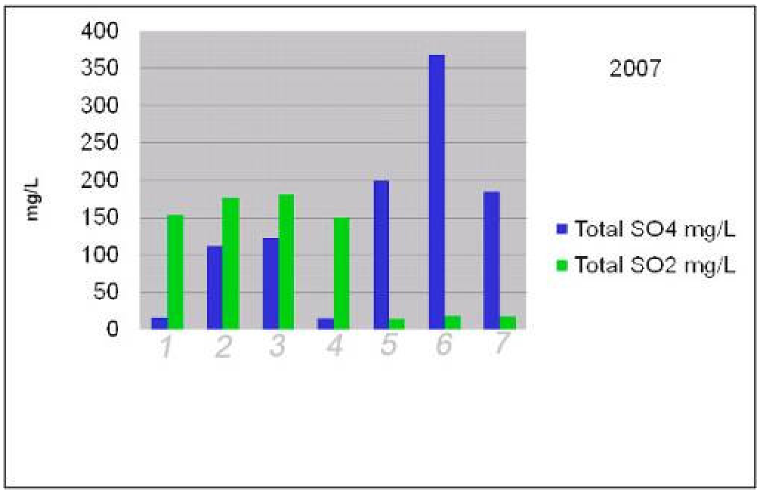


Fig. 3: Production of SO_2 by two different commercial yeast strains during alcoholic fermentation in Chardonnay must with the addition of ammonium sulphate and ammonium phosphate.

Variant 1-4: yeast strain 1; variant 5-7: yeast strain 2; variant 1 and 5: control; variant 2, 3 and 6: addition of ammonium sulphate, variant 4 and 7: addition of ammonium phosphate. Source: partner IFV.

Figure 3, shows that the concentration of sulphate plays an important role in SO_2 production during the alcoholic fermentation. Sulphate is present in the natural must or it can be introduced by the addition of ammonium sulphate, a nutrient. Alternatively ammonium can be added as ammonium phosphate. As the results in figure 3 show, not every yeast strain has the same ability to produce SO_2 on the basis of SO_4. Yeast strain 2 does not use sulphate, neither the natural nor the added sulphate in a relevant amount. This explains why this yeast strain can be considered as a low SO_2 producer. The yeast strain 1 shows a high ability to produce SO_2 on the basis of SO_4, even if it is only naturally present in the must. This yeast strain can be considered as a high producer of SO_2.These results were only obtained in white and rosé wines.

The sulphur dioxide produced by the yeast will be bound to SO_2 binding compounds. Thus it will be included in the estimate of the amount of total SO_2 in the wine, which is limited by regulations, but it will not be available as active free SO_2. The final requirement for SO_2 by the specific wine is determined by many wine compounds, such as acetaldehyde, 2-keto-glutarate and pyruvate, but also the amount of sugar. Only by adding an adequate amount of sulphur dioxide will the wine be finally protected by a certain amount of active free SO_2.

Influence of nutrients on the production of SO_2–binding compounds by yeasts

During alcoholic fermentation yeasts are able to produce certain by-products which bind to sulphur dioxide (SO_2). Acetaldehyde is probably the best known substance because its presence in a free form significantly influences the sensory character of a wine. If it is present in the free form, it causes an “oxidative note” which is often considered as an off-flavour. Only for specific wine types is it appreciated.

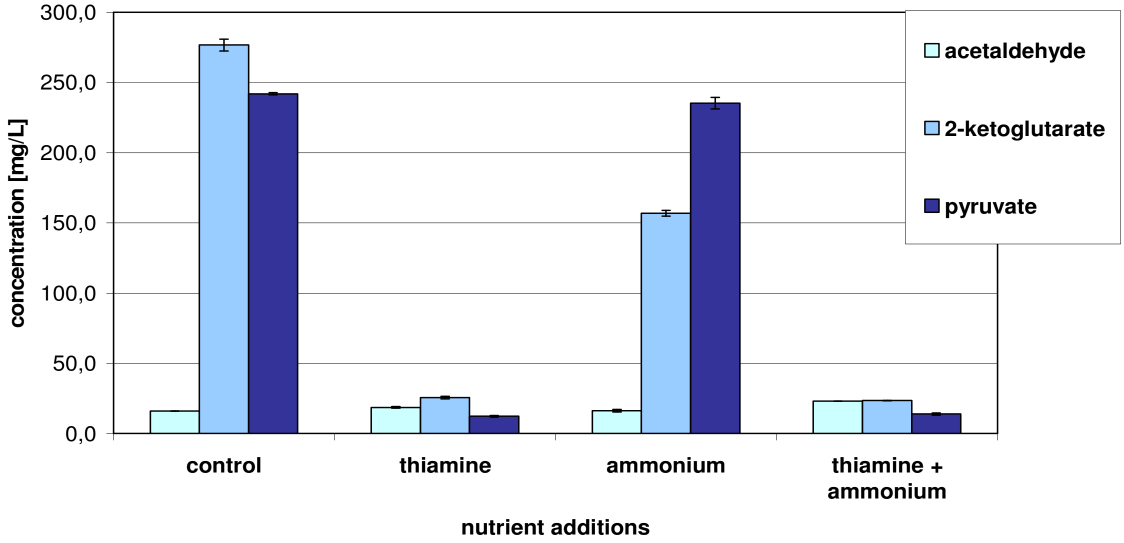
In addition to acetaldehyde there are many other carbonyl compounds which can act as binding partners for SO_2 in the wine. The higher the total concentration of binding compounds the lower the amount of active free SO_2 in the final wine at a given addition of sulphur dioxide.

Table 1: Simplified general overview about relevant SO_2-binding carbonyl compounds present in wine and specialty wine. Under practical conditions their concentration varies from very low to high depending on the metabolic activity of yeast or other micro-organisms.

Carbonyl Compound	**Impact on SO_2 binding**	**Origin**
Acetaldehyde	High	Yeast metabolism
Pyruvate	High	Yeast metabolism
2-Ketoglutarate	High	Yeast metabolism
Reducing Sugars (Glucose, Fructose, …)	High, depending on concentration	Grape origin or addition
Gluconic acid	High	Microbial activity on grapes
5-Ketofructose	High	Microbial activity on grapes
Xyloson	High	Microbial activity on grapes
Propanal	Low	Microbial activity
Butanal	Low	Microbial activity
Glycerolaldehyde	Low	Microbial activity
Isobutylaldehyde	Low	Microbial activity
Diacetyl	Low	Microbial activity

Research trials have shown that the natural production of the three SO_2-binding compounds acetaldehyde, pyruvate and 2-ketoglutarate depend on the yeast strain and on the composition of the natural must. With regards to the nutritional composition of the must, thiamine plays a key role in the formation of SO_2-binding compounds. Thiamine acts as co-enzyme of pyruvate decarboxylase which lowers the concentration of the last intermediates in the sugar depletion pathway of the yeast. Certain factors like heat treatment of the must or *Botrytis* activity on the grapes can lower the natural concentration of thiamine in the must. Figure 4 shows the effect of the addition of nutrients (ammonium and thiamine) on the concentration of SO_2-binding compounds in a pasteurised Riesling must after alcoholic fermentation.

Fig. 4: Effect of the addition of di-ammonium-hydrogenphosphate (0.5 g/L) and thiamine (0.6 mg/L) on the concentration of acetaldehyde, pyruvate and 2-ketoglutarate in the final wine. Fermentation was performed by *Saccharomyces cerevisiae* in a pasteurised Riesling must. Mean value of the triplicate. Bars show standard deviation. Source: SRIG



The high concentration of the SO_2-binding compounds in the control wine can be explained by the pasteurisation of the juice, which was necessary to eliminate any undesired micro-organisms. The positive effect of ammonium and thiamine on the reduction of the SO_2–binding compounds can be demonstrated very clearly. The concentration of the substances could be reduced very much, even though the SO_2–binding substances could not be eliminated. Additionally the fermentation activity of the yeast could also be increased by both substances.
According to the different concentrations of carbonyl compounds in the wine, each wine has a different "need" for SO_2 in order to guarantee consistent quality and stabilisation. Reducing sugars, such as glucose and fructose, which are present in sweet style wines, increase the binding potential significantly. Furthermore the pH-value and the temperature of the wine play an important role regarding the balance of free and bound sulphur dioxide, which is further described in the chapter about SO_2 management.

References:

Dott, W. and Trüper, H. G. (1976): Sulphite Formation by Wine Yeasts, III. Properties of Sulphite Reductase, Archives of Microbiology 108, Springer Verlag, p. 99-104

Romano, P. and Suzzi, G. (1993): Sulphur dioxide and wine micro organisms. In: Wine Microbiology and Biotechnology. Edited by Fleet, G., Harwood Academic Publishers GmbH, Chur, Switzerland, p. 373-393

Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D. (2006) Handbook of Enology, Volume 2, John Wiley and Sons, England, p. 264

http://www.vignevin.com/outils-en-ligne/fiches-levures/levures-a-production-moyenne-a-elevee-de-so2.html

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. (2006) Handbook of Enology, Volume 1, John Wiley and Sons, England

Wucherpfennig, K. (1985) Die schwefelige Säure im Wein – önologische und toxikologische Aspekte, Deutsches Weinbau Jahrbuch, 213-241

ACKNOWLEDGEMENT
The authors gratefully acknowledge from the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Specific Targeted Research Project "ORWINE" SSPE-CT-2006-022769.

DISCLAIMER

The views expressed in this publication are the sole responsibility of the author(s) and do not necessarily reflect the views of the European Commission.
Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of the information contained herein.