Investigation of the Relationship between the SO_2 Production of Different Yeast Strains and Thiamine Concentration

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Key words: amino acid, a-keto glutaric, pyruvic, acetic acid content, acetic aldehyde amount, free SO_2 content, thiamine addition, HPLC measurements

Summary: Our general conclusion was that the thiamine amounts of 0,6 and 3,0 mg/l added prior to wine fermentation, resulted in higher free-SO_2 level in comparison to the control. Furthermore, among the yeast strains examined in our experiments, Uvaferm BC strain produced the highest free-SO_2 content under the conditions mentioned before. As regards the dynamics of reductone and SO_2 production, the concentration of the previous one was practically identical in the middle and at the end of the fermentation while the SO_2 content – both free- and total – was lower at the end. Thiamine addition did not cause any difference in the organoleptic properties of wines as proved by sensory analysis.

Introduction

Minimisation of sulphurisation is an important question in Hungary, especially because our country is considered as a joining member of the EU and also because WHO and other health care societies put pressure to the wine producers aiming to reduce the concentration of SO_2 during vinification. According to our present knowledge, however, its application even in the modern wine producing technologies can not be avoided in well defined phases and quantities.

Four effects of sulphurous-acid are known in the practice of wine production:

- Antiseptic: The non-dissociated part of free sulphurous-acid especially shows inhibiting effect on the microorganisms. Bacteria (*eg.* lactic-acid bacteria) are particularly sensitive to it.
- Reducing (antioxidant) effect: It defends wines against both enzymatic and non-enzymatic oxidations.
- Taste and bouquet protecting effect: It binds the free acetaldehyde content, preventing the ageing of the wine and – as the consequence of it – the development of the unwanted rancidancient taste.
- Colour stabilizing effect: It binds the anthocyanins, whereby protecting them from oxidation and polimerization.

Sulphurous-acid the hydrate of sulphur dioxide, reacts with many chemicals present in the wine, therefore complex oxido-reductive conditions are developed. A part of sulphur dioxide will be oxidised to sulphuric-acid, another part forms addition complexes with different substances. Portion of sulphurous-acid that is linked to different compounds is called „*bound sulphurous-acid*", while the free, unlinked portiont is named „*free sulphurous-acid*" .

The objective of the up-to-date sulphurisation technology is setting the optimal *free sulphurous acid* level of the wines by sparing sulphurous-acid consumption.

Our work aimed at the main purposes as follows:

- Musts pressed from healthy, intact berries of Rhine-Riesling grape variety were fermented by yeast strains of different types after the addition of thiamine in different concentrations. $SO_{2,total}$–, $SO_{2,free}$– and reductone content as the function of thiamine concentration added were measured at the start, in the middle and at the end of fermentation. Relationships between the thiamine concentrations and the different fermenting yeast strains have been studied by variance-analysis.
- The wines were evaluated sensorically, by testing the effect of thiamine added and the probable SO_2 production of the different yeast strains.

Theoretical

There are several sulphur dioxide substituting procedures developed for decreasing the need for adding SO_2.

1. One of the sulphur dioxide substituting procedures is called „*SOLFURO*" when hydrogen-sulphide is introduced into the must in the prefermentative periode, aiming to reduce the H_2S production of yeast cells. (H_2S, the metabolic product of the yeast cells will repress its production.) The thyrosinase enzyme activity is also inhibited by the introduced H_2S therefore the browning process will be reduced, too, why both the colour intensity and the tint can be saved. In sensory tests Ubigli et al. (1982) could not find any difference in the wines prepared with and without H_2S introduction.
2. The following procedure for substitution of sulphur dioxide is the „*Hyperoxidation*", when O_2 is introduced into the must. Among others Guerzoni et al. (1977) substituting primarily the antioxidative effect conducted experiments of hyperoxidation by removing the oxidisable polyphenols. It has been established that this technique could replace sulphurization only partly.
3. „*Reduction of the concentration of sulphur binding substances*": If SO_2 is bound to other chemicals, this fixed sulphur dioxide also possesses antiseptic effect but in smaller scale than the free SO_2 . Musts contains sugar in a rather high amount. A significant part of it is not bound to SO_2 while the other is. During fermentation, however, yeasts form sulphur dioxide binding substances. Among them pyruvic-acid, a-keto-glutaric acid and acetaldehyde are outstanding, though acetaldehyde is of the greatest importance, undoubtedly. The formation of the above acceptors depends unambiguously on the medium, beside the sigmificant role of the fermentation conditions and the yeast strains applied.
4. „*Presence of SO_2- producing strains* ": Some authors found that certain yeasts might produce even 100 mg/l SO_2. This concentration is supposed to play a role in stabilizing the white wines, too. The SO_2 concentration formed during fermentation depends on the type of the yeast strain applied and on the medium. It is of primary importance that SO_2 content produced during fermentation be taken into account when legal sulphurization limits are to be kept.

Recently, addition of thiamine (Vitamin-B_1) as the component of mineral and vitamin supplement in must fermentation is becoming more and more frequent and also as a separate additive . German wine law permits its use in a concentration of 0.6 mg/l. In Hungary, in the cases of thiamine depleted Botrytised wines, thiamine completion (in the form of thiamine-hydrochloride) is widely used, which is allowed by the Hungarian wine law. By the thiamine fortification alcoholic yield increases, but the main result is the decrease of sulpurous acid demand, that can be reduced to even 50 mg/l on this way. Thiamine addition raises the rate of fermentation and the specific sugar consumption, which will result higher fermentation temperature that might cause losses of volatiles.

Both the results achieved and the demands of enological practice both stimulated our further investigations in this topic. The sulphurous acid level of base wine and must is of great significance, because it might cause troubles during manufacturing, *e.g.* sparkling wine production. (The sulphurous acid content of sparkling wine must be low).

Thiamine (aneurine) is a water soluble vitamin, which influences the growth and fermentation rate and other important features of the yeast strains. Healthy grapes and their musts are rather rich in thiamine (160–450 mg/l). Thiamine (in the form of pyrophosphate) is the coenzym of yeasts decarboxylase enzyme and plays a key role in the oxydative decarboxylation of 2-ketoacids. It is essential for the function of several enzymes (*e.g.* pyruvic-acid-decarboxylase, 2-ketoglutaric-acid-decarboxylase) in fermentations and biosynthesis. In the case of thiamine depletion, its substrates, the ketoacids will be enriched in the must. Since sulphurous acid is strongly bound by ketoacids, their formation during fermentation should be avoided (*Scholten,* 1995).

Material and method

Yeast strains

In our experiments Uvaferm SC; Uvaferm 228 and Uvaferm BC wine yeast strains were used:

Their main characteristics are detailed below.

UVAFERM SC: Dried yeast. *Saccharomyces cerevisiae* strain SC 4416. It was selected in Cognac.

Fermentation behaviour: Short prefermentation period, fast and complete fermentation of sugars. The strain carries killer-activity. Optimal fermentation temperature range is between 15 and 30 °C. It is active above 10 °C.

Foam formation: It forms little foam because of its pectolytic-activity.

Application and dosage: Uvaferm SC is a significant aroma producing strain. Its ester-production is extremely high, but its fusel-alcohol formation is rather low (that is advantageous in the production of spirits).

	Dosage (g/hl)
Musts of white wines	20–30
Mashes of blue grape	20–30
Mashes of spirits	15–30

Fusel-oil production: Comparative analyses show that Uvaferm SC produces 8–15% less fusel alcohol than other yeast strains.

Ester production: Comparative investigations prove that this strain formed by *ca.* 12% more ethyl acetate than the control one. The amount of iso-amyl-acetate (typical characteristic of banana) has also been increased drastically by Uvaferm SC. Hexyl-acetate content (a compound characteristic to pear) has been raised to a smaller extent. 2-phenyl-ethyl-acetate (characteristic to rose) has been produced by this strain in great amount as well.

UVAFERM 228: Suggested for the production of white wines of aromatic type.

Product, strain: Dried yeast. Saccharomyces cerevisiae strain D51 228. It was selected in Danstar (Germany), and also known as Steinberger strain. It was recommended by the Research Institute for Viticulture and Enology of the Hungarian Ministry of Agriculture, Eger, Hungary.

Fermentation behaviour: This strain has a very short prefermentative phase (2–6 hours). Wines fermented are outstandingly rich in aroma because of the strong β-glucosidase activity and are clarified extremely well.

Alcohol resistance: The strain tolerates alcohol content up to 16% (v/v).

Foam forming: It forms no foam.

Temperature demand: Its fermentation activity starts at 6–7 C°. The optimum fermentation range is between 15 and 25 °C. From 18 to 20 °C the strain performs the maximal aroma production. At a lower fermentation temperature, the bouquet of the wine will be less intensive but more elegant.

Application, dosage: The strain influences the organoleptic features positively, in the case of cold fermentation (below 15 °C) it significantly enhances the typical character of wine.

Its use can be advised especially in that cases, when terpene alcohols play an important role in the formation of the aroma character (e.g. *Muscat* varieties, *Chardonnay, Irsai Olivér, Cserszegi Fűszeres, Tramini, Leányka, Királyleányka.etc.*). The strain releases the characteristic aromas even from the *Furmint* wine, which is poor in primary volatile compounds.

	Dosage (g/hl)
Musts of white wines	20–30
Mashes of blue grape	20–30
In case of delayed or stuck fermentation	15–30

UVAFERM BC: Suggested for sparkling wines fermented in tanks or for restarting fermentation.

Product, strain: Dried yeast strain, selected in France (Institute Pasteur, Paris). Saccharomyces bayanus No. 1–877.

Fermentation behaviour: The strain is extremely active even at high sugar and alcohol contents. The lag-phase is relatively long, fermentation is slow and perpetual therefore the final extract content will be high. From 11–12 °C it ferments very well.

Alcohol tolerance: It shows outstanding alcohol tolerance up to 21% v/v. It has the best alcohol tolerance among the UVAFERM strains studied.

Fermentation capacity: 16.8 g/l sugar content yields 1% v/v alcohol.

SO_2- production: low (*ca.* 20 mg/l).

Application, dosage: UVAFERM BC is a universally applicable yeast strain. It can be used for the fermentation of both must and sparkling wine. It can be applied for repeated fermentation and in the case of stuck fermentation, if the reason of stop was not the low temperature (below 10 °C). In the case of sparkling wines fermented in tank, it gives full, bodied product. This strain is advised for the fermentation of autolysis-tasting full-bodied sparkling wines kept on yeast-bed. Its autolysis starts faster than that of the PM strain. This dried yeast is excellent for the fermentation of mashes of spirits as well.

	Dosage (g/hl)
Musts of white wines	20–30
Mashes of red wines	20–30
Sparkling wine fermentation	20–40
Mashes of spirits	15–30
In the case of stuck fermentation	30–50

Must samples derived from the Etyek-Buda District and were produced from healthy, intact berries of the *Rhine-Riesling* grape variety.

Fermentation experiments were carried out under controlled temperature conditions in the Perbal cellars of Nyakashegy Ltd. in microvinification scale (glass balloons of 5 litre capacity). Musts were not sulphurized. Tiamine was added into the experimental samples in four different concentrations: 0 (control), 0.6; 3.0; and 6.0 mg/l. The first set of sampels was fermented by Uvaferm SC , the second by Uvaferm 228, the third by Uvaferm BC strains and the fourth one was fermented spontaneously.

The yeasts were rehydrated by adding ten fold quantity of water and must mixture at 35–40 °C (e.g. 1 kg yeasts in 10 l liquid) and suspended by continuous mixing. After approximately 15 minutes, rehydrated yeasts suspension was added to the must to be fermented.

Total and free SO_2 concentrations were measured in accordance with the Hungarian Standard. Reductone content was also determined by iodometry *(Paul* 1963).

Results

Our experimental work started in September, 2000. Musts of perfectly healthy and intact berries of *Rhine-Riesling* grape variety were used only. In the middle and at the end of fermentation the free- and total-SO_2 as well as reductone contents were determined as the function of thiamine concentration and yeast strains added. The averages of the measured values in the samples are shown in column diagrams.

- *Results obtained in the middle of the fermentation with Uvaferm BC (see Figure 1):* Total SO_2 content was the

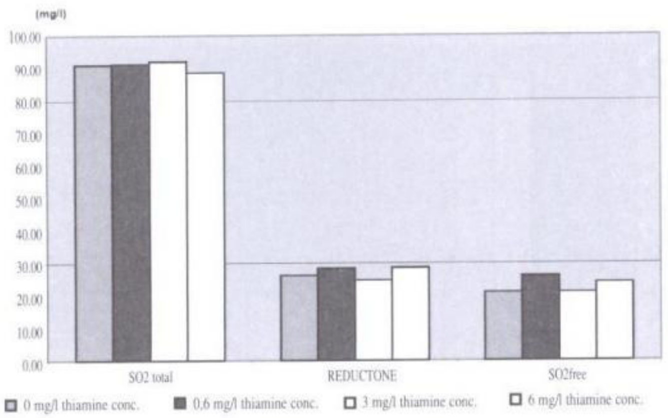

Figure 1 Rhine-riesling fermented by Uvaferm BC strain (the middle of fermentation)

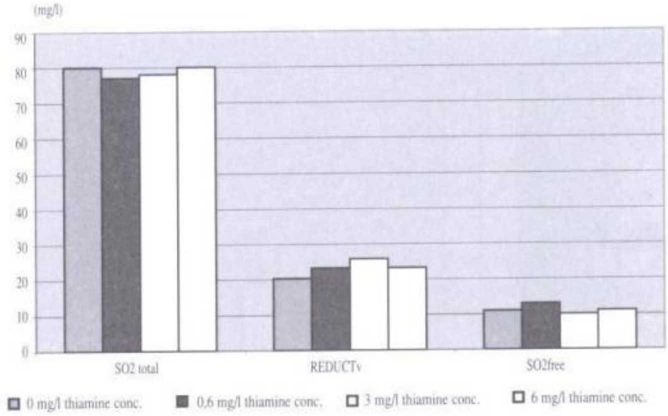

Figure 2 Rhine-riesling fermented by Uvaferm BC strain (end of fermentation)

highest at 3.0 mg/l thiamine concentration. The highest free SO_2 content was measured at 0.6 mg/l thiamine concentration, while the highest reductone content was obtained at 0.6 and 6.0 mg/l thiamine concentrations. Significant difference was found in the case of reductone and free SO_2 contents measured as the function of different thiamine concentrations, while the changes of the total SO_2 contents were not significant.

- *Results obtained at the end of the fermentation with Uvaferm BC*: (see *Figure 2*)*:* Total-SO_2 content was the highest at 0 and 6.0 mg/l, while the free SO_2 content at 0.6 mg/l and the reductone content at 3.0 mg/l thiamine concentrations. Differences in the reductone, total and free SO_2 contents were significant at different thiamine concentrations.
- *Results obtained in the middle of the fermentation in the case of spontaneous wine fermentation* (see *Figure 3*)*:* Total SO_2 content at 0.6 and 3.0 mg/l free SO_2 content at 0.6 mg/l and the reductone content at 0 and 3.0 mg/l thiamine concentrations were the highest. The 0.6 mg/l thiamine concentration resulted the highest averages of the total and free SO_2 and reductone contents. Reducton and total SO_2 contents did not differ significantly as the function of thiamine concentrations, while differences in the free SO_2 and thiamine concentrations were significant.
- *Results obtained at the end of fermentation in the case of spontaneous wine fermentation* (*see Figure 4*)*:* Total SO_2 content at 0.6 mg/l, free SO_2 content at 0 and 0.6 mg/l and the reductone content at 3.0 mg/l thiamine concentrations were the highest. Thiamine concentration of 0.6 mg/l resulted the highest value when the free and total SO_2 and reductone contents were evaluated. Reductone and free SO_2 contents differed significantly in the function of thiamine concentration, while the total SO_2 and thiamine contents did not.
- *Results obtained in the middle of the fermentation with Uvaferm 228* (see *Figure 5*)*:* Total SO_2 content at 6.0 mg/l, free-SO_2 content at 0.6 mg/l and reductone content at 3.0 mg/l thiamine concentrations were the highest. Evaluating the averages of free-and total-SO_2 and reductone contents, the 3.0 mg/l thiamine concentration resulted the highest values. Reductone, total SO_2 and free SO_2 values did not differ as the function of thiamine concentration.

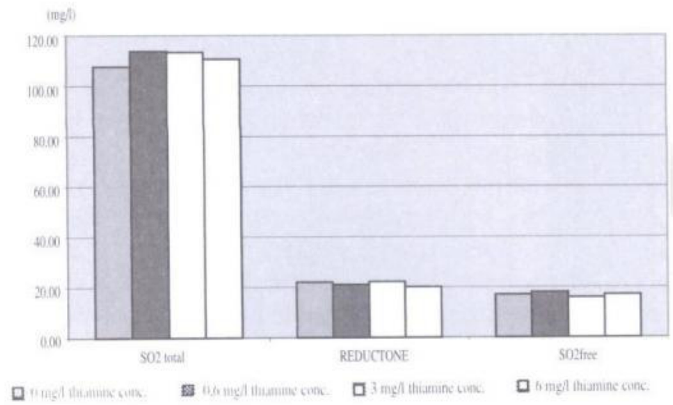

Figure 3 Rhine-riesling fermented spontaneously (the middle of fermentation)

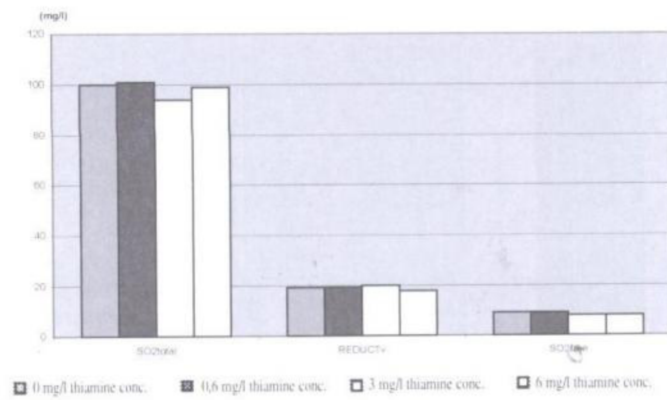

Figure 4 Rhine-riesling fermented spontaneously (end of fermentation)

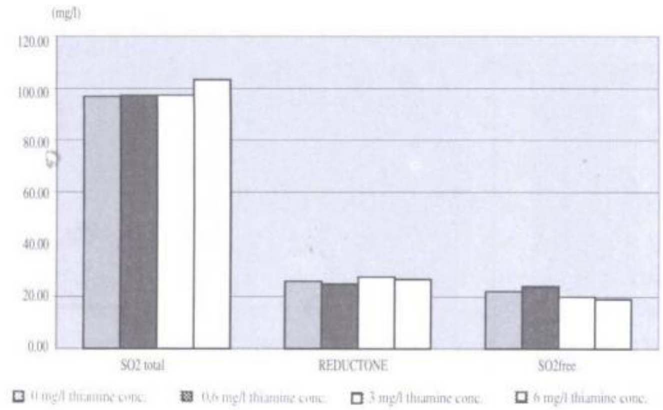

Figure 5 Rhine-riesling fermented by Uvaferm 228 strain (the middle of fermentation)

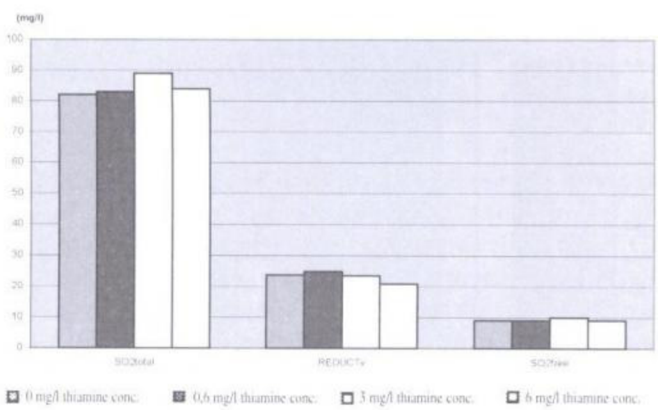

Figure 6 Rhine-riesling fermented by Uvaferm 228 strain (end of fermentation)

- *Results obtained at the end of the fermentation with Uvaferm 228* (see *Figure 6*): Total and free SO_2 contents at 3.0 mg/l and the reductone content at 0.6 mg/l thiamine concentration were the highest. When the avareges of the free and total SO_2 and reductone contents, measured were evaluated, the 3.0 mg/l thiamine concentration resulted the highest values. Reductone, total and free SO_2 contents did not differ significantly as the function of thiamine concentration.
- *Results obtained in the middle of the fermentation with Uvaferm SC* (see *Figure 7*): Total SO_2 at 6.0 mg/l, free SO_2 content at 0 and 6.0 mg/l, reductone content at 0; 3.0 and 6.0 mg/l thiamine concentrations were the highest. The 6.0 mg/l thiamine concentration resulted the highest values when the free and total SO_2 and reductone contents were evaluated. Reductone, total and free SO_2 contents did not differ significantly as the function of thiamine concentration.
- *Results obtained with Uvaferm SC at the end of the fermentation* (see *Figure 8*): Total SO_2 content at 6.0 mg/l, free SO_2 content at 0; 3.0 and 6.0 mg/l, reductone content at 3.0 and 6.0 mg/l thiamine concentrations were the highest. Thiamine concentration of 6.0 mg/l resulted the highest values when the free and total SO_2 and reductone averages were evaluated. Reductone and free SO_2 content did not differ significantly as the function of thiamine concentration, while total SO_2 and thiamine concentrations did.

Results of sensory evaluation: Thiamine amounts added did not cause organoleptic differences of wines.

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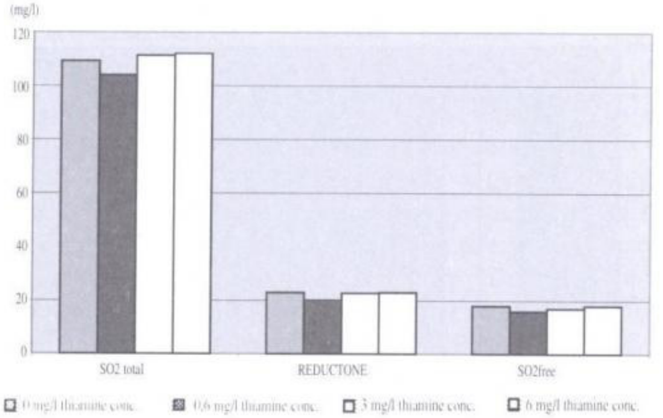

Figure 7 Rhine-riesling fermented by Uvaferm SC strain (the middle of fermentation)

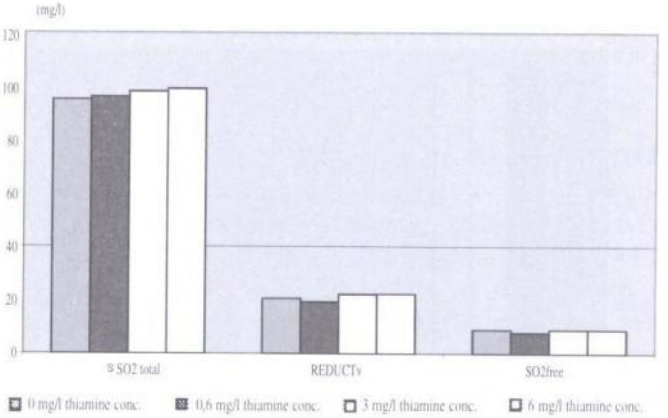

Figure 8 Rhine-riesling fermented by Uvaferm SC strain (end of fermentation)