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Sulphate uptake and sulphite formation related to the methionine and/or cysteine content of grape must during the fermentation by strains of Saccharomyces cerevisiae

by

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Sulfataufnahme und Sulfitbildung in Beziehung zum Methionin- und/oder Cystein-Gehalt von Traubenmost während der Vergärung durch einige Stämme von Saccharomyces cerevisiae

Zusammenfassung. — Es konnte gezeigt werden, daß sich normale und sog. SO_2-bildende Hefestämme hinsichtlich der Sulfatabsorption beträchtlich unterscheiden, d. h. SO_2-bildende Stämme verbrauchen durchschnittlich die doppelte Sulfatmenge. Die Zugabe steigender Konzentrationen von Methionin und/oder Cystein zu Traubenmost reduziert bei allen Stämmen die Aufnahme von Sulfat und die Bildung von Sulfit während der Gärung. Da Cystein ähnliche Veränderungen verursacht wie Methionin, wird angenommen, daß es ebenfalls die Aufnahme und Aktivierung von Sulfat regelt.

Introduction

Recent investigations showed that the so-called SO_2-forming yeast strains are able to produce H_2S during the fermentation of grape juice in quantities similar to those of normal strains (Eschenbruch 1972 a). These findings were contradictory to those of Dittrich and Staudenmayer (1968) who postulated that SO_2-forming yeasts

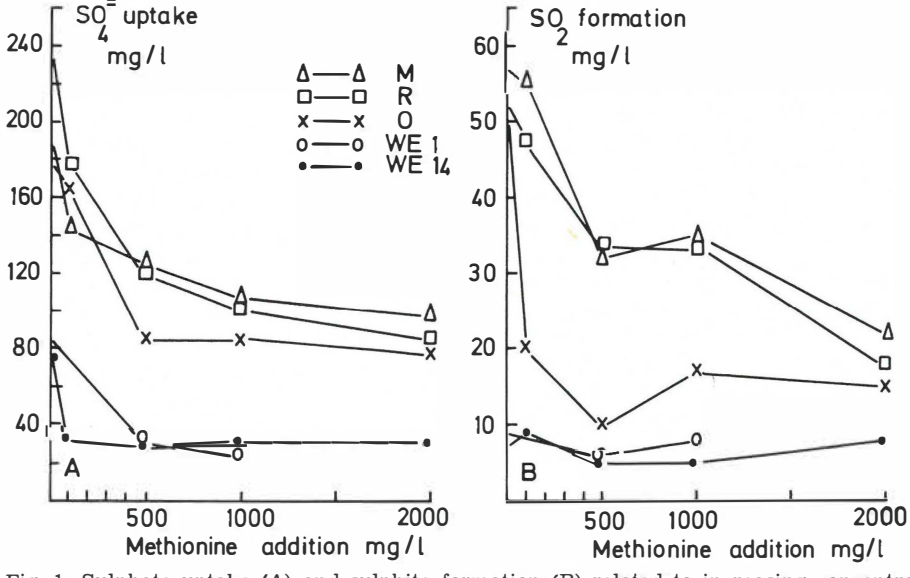


Fig. 1: Sulphate uptake (A) and sulphite formation (B) related to increasing concentrations of methionine added to Steen must, vintage 1971.

were defective mutants, unable, on account of a genetical blockage, to reduce the sulphite, deriving from the sulphate of the must, to H_2S. Consequently SO_2 would accumulate.

On the other hand we demonstrated, that the concentration of both methionine and cysteine in the must can decisively participate in the regulation of SO_2 formation (Eschenbruch 1972 b). Sufficiently high levels of these two sulphur containing amino acids decreased the sulphite synthesis to less than 10 mg/l, not only with normal but also with SO_2-forming strains.

Hence the question arose whether differences existed in the absorption of sulphate from the must by normal and SO_2-forming strains and to what extent this uptake could be influenced by methionine and cysteine. The influence of these two amino acids was already demonstrated by Kleinzeller *et al.* (1959) and Maw (1963, 1965) and could possibly explain our observation in that SO_2-forming strains produce only 20 mg SO_2/l in some musts and 80 mg/l in others. Würdig and Schlotter (1971) also reported that considerable variations in the SO_2 synthesis occur when different fruit juices were fermented with one and the same strain.

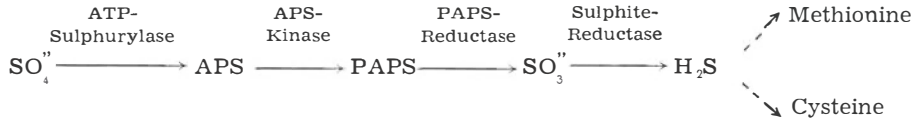
Therefore sulphate uptake and sulphite synthesis had to be investigated with regard to increasing concentrations of methionine and/or cysteine not only with normal but also with SO_2-forming yeasts, without addition of sulphite to the must before or during the fermentation.

Materials and Methods

A must of the grape variety Steen, vintage 1971, with a sugar content of 21.3° Brix, 6.8 g total acid/l, 260 mg sulphate/l (as K_2SO_4) and with a pH 3.6 was used for the tests where either methionine or cysteine was added. For the simultaneous addition of these two amino acids a Steen must, vintage 1972, with 20° Brix, 6.7 g total acid/l, 230 mg sulphate/l (as K_2SO_4) and with a pH 3.3 was used. No sulphite was added to the must either during or after fermentation. Aliquots of 150 ml of must were measured into 250 ml Erlenmeyer flasks sealed with fermentation caps. Methionine and cysteine were always added after sterilisation at 0.5 atm for 5 min. An inoculum was chosen in order to obtain 1500 cells per mm^3 aliquot and the fermentation temperature was 25° C. The yeast strains employed were as previously described (Eschenbruch 1972 a). After completion of fermentation, sulphite and sulphate were determined according to Paul (1958) and Rebelein (1969) respectively. The fermentation rate was determined in terms of daily loss in weight, and yeast growth by cell count checks. In the sulphate determination all samples were sterile filtered, allowed to stand for 10 min after the addition of $BaCl_2$, centrifuged for 10 min at 3000 rpm and weighed after cooling for 30 min in a dessicator. Instead of platinum, porcelain crucibles were used. Sulphate was determined in triplicate, SO_2 singly and mean values for three parallel fermentations were calculated.

Results

Figs. 1A and B demonstrate the sulphate uptake and the SO_2 formation of the two normal strains WE 1 and WE 14 (two pure cultured yeasts which are supplied to wineries) as well as the three socalled SO_2-forming strains R, O and M. With increasing concentrations of methionine the uptake of sulphate decreases with all five yeasts; with strain M, for instance, from 197 mg/l to 98 mg/l and with WE 14 from 76 mg/l to 30 mg/l after 2000 mg methionine per litre had been added. The unfermented must contained 260 mg sulphate per litre.



Scheme 1: Sulphate reduction sequence.

The formation of SO_2 is reduced simultaneously. With strain M, for instance, SO_2 decreases from 56 to 22 mg/l. The yeasts R and O show the same tendency. However, the SO_2 synthesis of WE 1 and WE 14 is not influenced.

Two facts become evident. Firstly, yeast strains which form anomalously high amounts of sulphite (R, O, M) absorb much more sulphate than normal strains (WE 1, WE 14). Secondly, methionine decreases the uptake of sulphate by the yeast cell. This automatically causes less SO_2 formation, since sulphite is an intermediate of the reduction sequence of sulphate to sulphide (see Scheme 1).

The addition of cysteine instead of methionine yields data shown in Figs. 2A and B. Again, with all five strains, increasing concentrations of cysteine decrease the uptake of sulphate considerably. Similarly the formation of sulphite by the strains R, O and M is clearly reduced, to as low as 10 mg/l. Again, WE 1 and WE 14 are not influenced. It should however be noted that the effect of cysteine on the uptake of sulphate and on the formation of SO_2 seems to be more pronounced than that of methionine.

Figs. 3A and 3B show the results obtained after the simultaneous addition of both methionine and cysteine to the must which contained 230 mg sulphate per litre. In contrast to the results shown in Figs. 1 and 2, a distinct influence on the SO_2 formation of the strains WE 1 and WE 14 is evident. Under these conditions they produce 50% less sulphite, i. e. the SO_2 synthesis is reduced from 8 to 4 mg/l. The

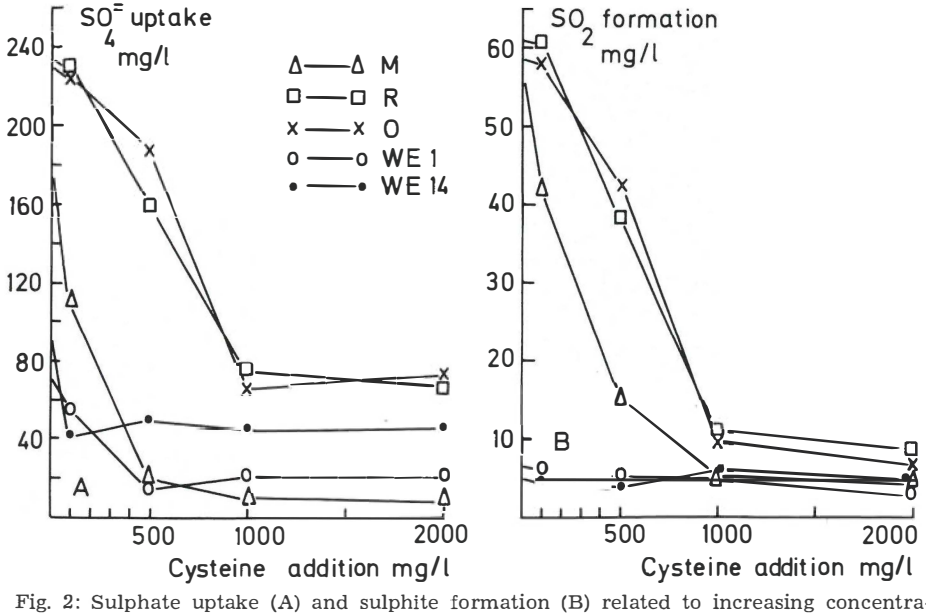


Fig. 2: Sulphate uptake (A) and sulphite formation (B) related to increasing concentrations of cysteine added to Steen must, vintage 1971.

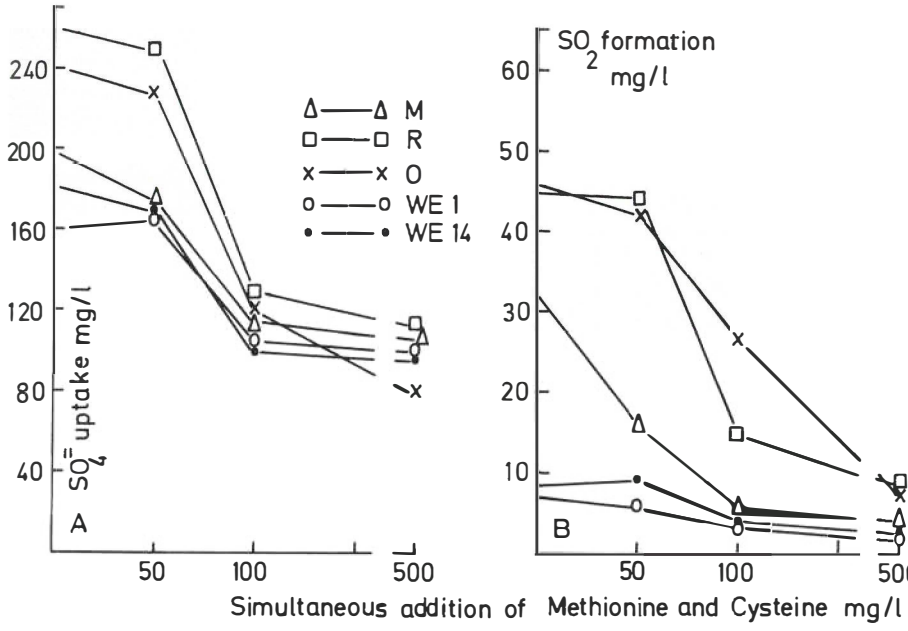


Fig. 3: Sulphate uptake (A) and sulphite formation (B) related to increasing concentrations of both methionine and cysteine added to Steen must, vintage 1972. Methionine and cysteine were each added at the shown concentrations, e. g. 50 indicates that 50 mg of each was added.

effect of the simultaneous addition of 100 mg/l of each of both amino acids is more pronounced than in the case where they have been added separately. These differences can not be ascribed to growth or fermentation differences since these were not influenced by the addition of these two amino acids.

Discussion

1. The assumption of previous investigations, viz. that the concentration of the sulphur containing amino acids, methionine and cysteine, present in the must could possibly influence the amount of SO_2 being formed during fermentation via sulphate uptake or sulphate activation could be confirmed (Eschenbruch 1972 b). The extent to which increasing concentrations of methionine and/or cysteine reduce the sulphate uptake and the linked synthesis of sulphite, can be seen in the data of Figs. 1—3.

de Vito and Dreyfuss (1964) reported, that the ATP-sulphurylase of baker's yeast, which activates the sulphate of the substrate to adenosine -5'-phosphosulphate (APS), is repressed by methionine and that the sulphite-reductase, which reduces sulphite to sulphide, is repressed by cysteine. However, no inhibition of the ATP-sulphurylase either by cysteine or methionine, sulphite or thiosulphite could be demonstrated. This is elucidated in Scheme 1.

The influence of methionine on the absorption of sulphate can therefore be explained as a repression of the ATP-sulphurylase. A suppressed activation of sulphate consequently reduces the formation of sulphite (Fig. 1).

The fact that the addition of cysteine does not cause an accumulation of sulphite, as could be expected from the repression of the sulphite-reductase by cysteine, and the fact that the sulphate uptake decreases (Fig. 2), could be an indication that the activation and transport of sulphate is regulated by cysteine. With *Salmonella typhimurium*, for instance, Dreyfuss and Monty (1963) proved that the sulphate-transport-system was in fact repressed by cysteine.

The more effective influence of the simultaneous addition of both methionine and cysteine on the decrease in sulphate uptake and sulphite synthesis could result from a marked reduction in the sulphate turn-over rate (Fig. 3, Scheme 1). In effect a decreased rate of methionine and cysteine synthesis would occur. Since they are already present as ready-formed molecules, the necessity of their synthesis by means of the complex and energy-requiring sulphate reduction sequence is not critical.

2. If the extent to which the addition of 100 mg of both methionine and cysteine per litre influences the sulphite synthesis by sulphite-forming yeasts is considered (Fig. 3), one can understand why these yeasts in some musts form only 20 and in others 80 mg SO_2/l. Different concentrations of these amino acids could have caused these variations. Only very few references on the free amino acids content in musts are available which support this postulation. Rapp and Reuther (1971) found that the amount of methionine varied between 17—90 mg/l with different grape varieties. Dittrich *et al.* (1970) analysed four musts with 17—30 mg methionine per litre. Some musts of Spanish grape varieties contained only traces or no methionine (Llaguno-Marchena 1972). Furthermore, it has been emphasised by Bergner and Haller (1969) that increasing pressure during grape pressing raises the free amino acids content of the must. However, data on cysteine concentrations in grape juices are to our knowledge not available. On this basis it also appears unlikely and contrary to published work (Dittrich and Staudenmayer 1970) that sulphite formation by SO_2-forming strains is independant of the substrate.

3. The sulphate determinations resulted in another important aspect, viz. yeast strains forming little sulphite take up comparatively little sulphate (WE 1 and WE 14). On the other hand the socalled SO_2-forming strains have a high rate of SO_2 formation but also have a correspondingly high sulphate uptake. It therefore seems unlikely that in these yeasts it is the defective sulphite-sulphide reduction which causes SO_2 accumulation (Dittrich and Staudenmayer 1968). It would rather appear that the cell membrane and sulphate activation to APS and PAPS are more important in this system. When it is furthermore considered that SO_2-forming strains (R, O, M) form amounts of sulphide similar to that of normal strains (WE 1, WE 14) it will be apparent that the sulphite-sulphide blockage supposition is less acceptable (Eschenbruch 1972 a).

Considerable differences in activities of many enzymes occur during the fermentation of wort with flocculating and non-flocculating bottom yeast strains of *Saccharomyces carlsbergensis* (Piendl 1971). Similar considerations could apply to normal and sulphite forming strains of *Saccharomyces cerevisiae*.

Summary

It has been demonstrated that normal and so-called SO_2-forming yeasts differ considerably in sulphate absorption, i. e. SO_2 forming strains consume on an average twice as much sulphate. The addition of increasing concentrations of methionine and/or cysteine to the grape must reduces the uptake of sulphate and the formation

of sulphite with all strains during fermentation. Since cysteine causes changes similar to methionine it is assumed that it also regulates the uptake and activation of sulphate.

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