M. Wurzbacher, O. Franz and W. Back

Control of sulphite formation of lager yeast

On the basis of existing literature, the influences of yeast and pitching parameters on sulphite formation were evaluated in several laboratory, pilot and industrial scale fermentations. The sulphite formation was strongly influenced by the predisposition of the yeast. Additional it was dependent on parameters affecting yeast growth during fermentation such as the physiological state of the yeast and the availability of nutrients and oxygen. The supply of the brewing yeast with nutrients and oxygen was altered by the pitching parameters. It was demonstrated, that the sulphite formation could be controlled by the pitching rate and the aeration rate. Based on the fermentation trials, a model that describes the influence of the pitching parameters using the key figures "extract per yeast cell" and "oxygen per yeast cell" is introduced. It allows the prediction of the effect of variations of aeration, pitching rate and original gravity on sulphite formation during fermentation.

Descriptors: yeast, sulphite, SO_2, flavour stability, pitching parameter

1 Introduction

The flavour stability of lager beers is profoundly dependent on the natural sulphite content, if no antioxidants are added before bottling. Sulphite is a strong reducing agent. It is formed by the brewing yeast during the main fermentation. As long as the beer contains an adequate amount of sulphite, oxidation reactions, which are thought to be the main cause for the development of stale flavour, are delayed. Accessorily sulphite can bind aroma active carbonyls (1, 2, 3).

Sulphite is an intermediate of the synthesis of sulphur containing amino acids (Figure 1). Sulphate from wort is assimilated by the yeast cell and transformed to an active state. Then the activated sulphate is reduced in several steps to sulphite and then to sulphide, thus being used to synthesize homocysteine and all the needed sulphur containing amino acids in the following steps (3, 4, 5). The cell also stores a stock of sulphur containing amino acids as glutathione (6, 7, 8). The excess amount of sulphite is excreted by the cell because of its toxicity, mostly bound to acetaldehyde (5, 9).

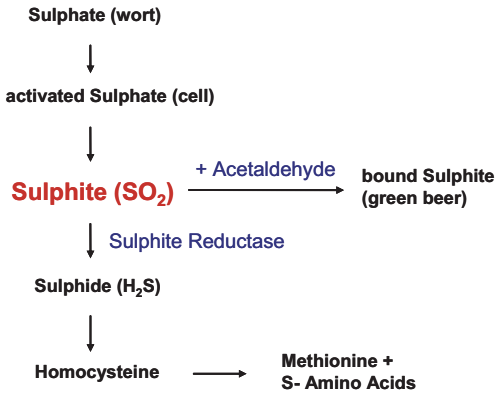

Fig. 1 Sulphite as an intermediate of the synthesis of sulphur containing amino acids (5, 14, 20)

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Immediately after pitching, the yeast starts to take up oxygen at a constant rate, initially using the cells glycogen reserves as energy supply (10, 11). Depending on the primary concentration, all the oxygen is consumed within a few hours. The cell needs elemental oxygen for its synthesis of lipids, this being essential for the membrane transport of metabolites and cell growth. The synthesis of lipids starts before the assimilation of glucose is initiated (10, 11). In the absence of oxygen, the cell is able to use wort lipids only to a certain degree to promote membrane functions and cell growth (11, 12). Thus cell growth is highly dependent on the cells reservoirs of glycogen and lipids and on the availability of oxygen. Under brewery fermentation conditions, the availability of oxygen is the restricting factor of cell growth (11).

In the first stage of fermentation with a high growth rate, the cells' demand of amino acids is high. In the beginning it is satisfied by amino acids of the wort and the cells' stock. Then, the amino acid synthesis is activated, including the sulphate assimilation pathway and the synthesis of homocysteine. Nearly all the sulphite produced is still used by the cell. With the decrease of the growth rate at the end of the growth phase, the demand for sulphur containing amino acids decreases and the reduction of sulphite to sulphide is inhibited. The cell starts excreting the accumulating sulphite. For reasons we still do not understand, the process of sulphate assimilation and the excretion of sulphite continues until the end of fermentation (13, 14).

Sulphite accumulates in the beer, if the yeast's growth rate decreases after a phase with high sulphate assimilation (14). The cellular acetaldehyde level may influence the excretion of sulphite as well, as sulphite is transferred out of the cell mostly bound to acetaldehyde. For this reason the increase of the cellular acetaldehyde level in case of osmotic stress will also increase the sulphite excretion (5, 15). Accordingly, all parameters increasing sulphate assimilation, decreasing cell growth or increasing acetaldehyde level will increase the accumulation of sulphite in the beer (14, 15).

The following influences on sulphite content of green beer are mentioned in the existing literature:

Sulphite formation is highly dependent on the yeast strain (16, 17, 18). The condition of the yeast influences the sulphite formation as well. Propagated yeast will form less sulphite than cropped yeast (19). The sulphite formation of cropped yeast increases with extending starving time before pitching (13). The lipid content of pitching wort affects the sulphite formation, if there is not enough oxygen available for lipid synthesis (20). The wort's spectrum of amino acids influences the formation of sulphite via sulphate

assimilation (15). Sulphate content of the wort normally has no effect on the sulphite formation because of its excess level in the pitching wort (21). The original gravity influences the sulphite formation, most probably via the acetaldehyde level. The fermentation of worts with higher gravity will lead to increasing sulphite levels (2, 15, 21).

Lastly sulphite formation is affected by the pitching parameters. The effect of the pitching rate is still not clear. Different authors present contradictory results (2, 21, 22). The aeration affects the formation of sulphite to a high degree. The sulphite formation increases with decreasing aeration rates (2, 12, 21, 22). Sulphite formation is temperature dependent as well. Maximum sulphite formation is reached by a fermentation temperature of 16°C. It decreases intensely with fermentation temperatures higher than 20°C or lower than 10°C (2, 22). The pressure seems to have no influence on the formation of sulphite (22).

Although a lot of publications on sulphite formation are existing, technological instruments for its practical control are still missing. The goal of this investigation was to clarify the influences of the yeast and the pitching parameters on the sulphite formation. Methods to control its level were designed.

2 Material and methods

2.1 Brewing trials

Brewing trials at industrial scale (700 hl) were monitored in two breweries A and B. Both breweries worked with a yeast of strain 34, but from different yeast culture collections.

In brewery A, the bright wort (12%, 700 hl per brew) was cooled after the whirlpool stage to the pitching temperature of 9°C. The solid matter constituted between 200 and 250 mg/l. The methionine content ranged between 30 and 40 mg/l. The aeration was carried out in the wort pipe in the brewhouse with a rate of 20 l of air per 100 l of wort (= 20 l/hl = 0.2). The wort was pitched with a rate of $15 \cdot 10^6$ cells per ml. The fermentation was carried out at 9°C during 6 to 7 days. Every brew was fermented in a separate tank.

In brewery B the wort (12%, 700 hl per brew) was cooled after the whirlpool stage to 9°C. Its solid matter ranged around 300 mg/l. The wort's methionine content was similar to the one of brewery A. The wort was aerated in the brewhouse at a rate of 20 l/hl (0.2). Then the wort was pitched at a rate of $15 \cdot 10^6$ cells per ml and transferred to a flotation tank where it remained for 2 hours. After this time, the solid matter had declined to around 200 mg/l. Then the pitched brew was pumped to a large cylindroconical fermentation tank. One tank was filled with up to four brews, one every three hours.

The yeasts (fresh cropped yeast) of both breweries showed 1 to 2% of dead cells in the methylene blue test. The assimilated yeast was prepared by a special propagation process (Yeast Assimilation) in the breweries. The Assimilation Technology procedure is an aerobic propagation procedure which differs from other propagation methods by specifically implementing ideal growth conditions in the cultivation tanks (23). While during regular propagation procedures yeast cells are in more or less every kind of physiological growth phase, with the assimilation technique all cells are held in the logarithmic growth phase. Therefore it is necessary to align engineering, technology and the yeast requirements in an optimal way. Another characteristic of the Assimilation Technology is that it is used as a repeated-fed-batch process. The use of Assimilation-Yeast provides a number of advantages for beer production (high fermentative capability, steeper pH-fall, and fast diacetyl-reduction, etc.), and gives beer a fresh, and pure taste (23).

Pilot scale brewing trials (10 or 20 l containers) and trials in laboratory scale (EBC tall tubes: 2 l (24)) were carried out with similar fermentation conditions. Cooled wort was taken from brewery A and, depending on the trial, fermented with yeast A or B. For each trial, fresh cropped yeast (thick slurry) of 1st generation was obtained from the breweries (if not otherwise mentioned). The pitching rate accounted for $15 \cdot 10^6$ cells per ml. A check of the pitched wort with the counting chamber showed deviations of maximum $2 \cdot 10^6$ cells per ml. The fermentations were carried out at 9 to 10°C. If not otherwise mentioned, the brews were aerated at a rate of 0.2 (20 l/hl).

To achieve comparability with the aeration technology of the studied breweries and to achieve exact aeration rates, a special method was designed: In the trials the aeration was done by the mixing of definite volumes of wort and air in the closed fermentation containers by shaking for a certain time. According to Henry's law the solubility of oxygen at these conditions is a function of temperature, pressure and the volumetric ratio of air and wort. A constant pressure (here: 1 bar) and temperature (here: 10°C) assumed, the solubility of oxygen is only affected by the volumetric ratio of air and wort. Example given, an aeration rate of 0.2 was achieved by the mixing of 3.35 litres of air and 16.65 litres of wort in a 20 l container. After the aeration procedure all containers of one trial were adjusted to the same liquid level. To avoid an uncontrolled uptake of oxygen of the wort before the aeration procedure, all vessels used had to be rinsed with CO_2 or N_2. To prevent a diffusion of oxygen into the pitched wort after aeration, the headspace was rinsed with CO_2 after the aeration.

2.2 Analysis

Sulphite (sulphur dioxide) was determined with the distillation method according to MEBAK. The beer sample was acidified with phosphoric acid and boiled. By this procedure the reversible bound sulphite was released and all sulphite was converted to sulphur dioxide, which was distilled into a recipient. Then the sulphuric acid formed was titrated with sodium hydroxide. The results were calculated as mg SO_2/l (25). The method has a good reproducibility, as shown in table 1. By this reason we quoted the results with higher precision as specified by MEBAK. It is known that the titration can additionally detect other acidic substances than sulphuric acid, so that the findings could range little higher than the accurate sulphite contents. We could not observe this effect by the measurement of samples of sulphite-free beer spiked with known quantities of sulphite.

Table 1 Reproducibility of the sulphite content of one sample of beer

measurement	1	2	3	4	5	6	7
sulphite [mg SO_2/l]	2,1	2,0	2,2	1,9	2,1	2,1	2,2

The determination of oxygen in the pitching wort was carried out with an electrochemical sensor (portable analyzer 3650, Orbisphere, Genf, Switzerland). To avoid deviations caused by the yeast's oxygen uptake during measurement, the content of dissolved oxygen in the wort was determined in separate containers aerated without yeast.

The alcohol content of the green beer was detected by NIR spectrometry (beer alcolyzer, Anton Paar, Graz, Austria) and the extract

content was measured by an oscillating u-tube (densitymeter DMA 4500, Anton Paar, Graz, Austria).

The total yeast counts were determined using a counting chamber (hemocytometer).

2.3 Solubility of oxygen in small scale trials

The content of dissolved oxygen of the pitching wort could be altered easily by the aeration rate, as illustrated in Figure 2.

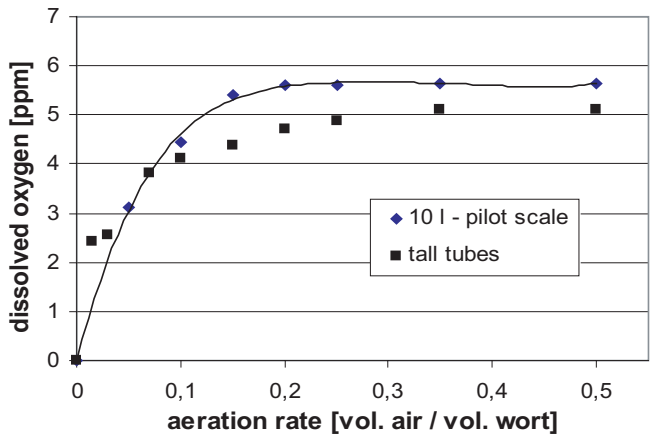

Fig: 2 Dissolved oxygen depending on the aeration rate in different containers (10°C)

In the range of low aeration rates (< 0.1) the dissolved oxygen increased strongly with an increasing aeration. Aeration rates higher than 0.3 did not alter the content of the dissolved oxygen as saturation was reached. For unaerated wort, an unverifiable low oxygen content was assumed. The characteristics of dissolved oxygen in 10l-containers could be approximated using a polynomial equation of grade 6 (x: aeration rate [vol. air / vol. wort], y: dissolved oxygen [ppm], $R^2 = 0{,}9985$):

$$y = 1{,}2629 \cdot 10^3 \cdot x^5 - 2{,}0951 \cdot 10^3 \cdot x^4 + 1{,}3975 \cdot 10^3 \cdot x^3 - 4{,}7292 \cdot 10^2 x^2 + 8{,}1331 \cdot 10^1 \cdot x$$

The dissolved oxygen in the tall tubes did not fit in a simple algebraic model.

The measurement of the dissolved oxygen in the aerated wort revealed clear differences between containers of different scale. The oxygen saturation level in the tall tubes was clearly lower than in the pilot scale containers. A possible explanation is that the wort in the tall tubes unintentionally got warmed during the aeration procedure (room temperature) and that the static pressure in the tall tubes was lower than in the containers. At industrial scale the pressure in the wort pipe and the static pressure caused by the liquid level of the pitching tank could cause even higher saturation levels. This coherence must be considered when comparing results of trials carried out in different scales. Additional other values as the cells in suspension and the fermentation duration pointed out clear differences in the fermentation performance of small scale containers of different geometric design.

2.4 Influence of the yeast quality in the trials

To check the reproducibility of the fermentation trials some trials were rerun after a certain time with equal conditions. Table 2 presents the sulphite contents of four beers produced in different fermentation trials in 10l-containers with yeast A and an aeration of 0.2.

It can be seen, that the sulphite contents produced in the different trials could deviate. Figure 3 shows the same effect in industrial scale trials. As new yeast was obtained from the breweries for each trial and the experiments were carried out over a long period, we assume that the natural quality fluctuations of the breweries' yeasts influenced the findings. This fact must be regarded at the evaluation of the findings. Example given, the fermentation trials described in Figures 5, 8, 9 and 10 were carried out some months later than the other trials, leading to generally higher sulphite contents than the earlier trials. Most probably the yeast quality had changed over this period.

Table 2 SO_2-formation in fermentation trials at equal conditions

trial Nr.	1	2	3	4
sulphite [mg/l]	10.4	10.5	11.5	12.5

3 Results

3.1 Sulphite formation and yeast

The sulphite formation of cropped yeast from 1st generation was tracked in the two breweries A and B (Figure 3).

The formation of sulphite started in both breweries on the second day and went on until the completion of fermentation (5th day). Between the 5th and 8th day, when all extract was consumed by the yeast, no changes in the sulphite level could be observed. The sulphite formation of the two yeasts in the two breweries clearly was on different levels. This seemed to be caused by different formation rates after the second day of fermentation.

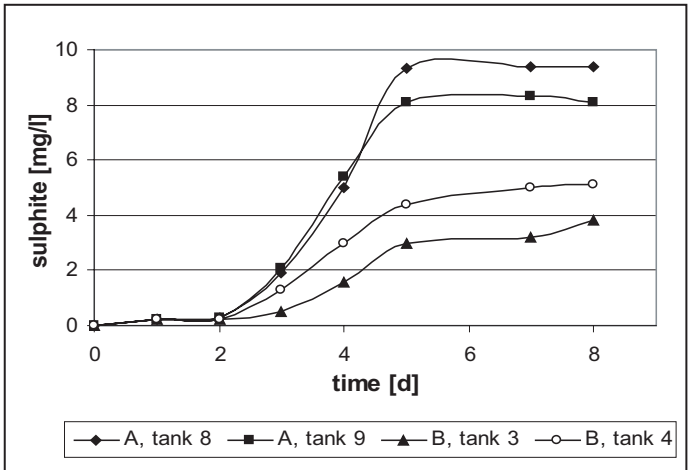

Fig. 3 Formation of sulphite during main fermentation in different tanks in the breweries A and B

To check the different behaviour of the two yeasts, the yeasts were exchanged. One brew of brewery A was fermented in brewery A with yeast B and one brew of brewery B was fermented in brewery B with yeast A. The results are presented in table 3, indicating that the different SO_2-levels of the two breweries were caused by the yeast's predisposition and not by wort quality or technical influences.

Table 3 SO_2-formation [mg/l] of the yeasts A and B in the breweries A and B

	yeast A	yeast B
brewery A / wort A	8,8	4,3
brewery B / wort B	9,4	4,5

The fermentations of the two yeasts were followed until the 5th generation. We observed that the SO_2-content of the resulting beers increased with ascending generation number (table 4).

Table 4 SO_2-formation of succeeding yeast generations on breweries conditions

	2nd generation	3rd generation	4th generation	5th generation
yeast A	9,4	10,4	12,2	14,1
yeast B	4,3	4,3	4,3	5,6

In contrast, assimilated yeast (yeast A) formed only about 1 mg/l SO2 in several pilot scale trials, even when the brew was pitched without aeration. In the industrial scale the sulphite formation of assimilated yeast (A) during 1st generation was around 4 mg/l according to brewery A.

To check the behaviour of a blend of assimilated yeast and cropped yeast, the yeasts were mixed in several ratios and pilot scale fermentation trials were carried out. Figure 4 shows the results of two trials of fermentations with blended yeasts (yeast B).

As can be seen, an appreciable amount of sulphite was only formed with ratios of cropped yeast higher than 50%. The differences of the sulphite levels among the two trials were most probably due to a different yeast quality. The fact that the sulphite production did not match linearly to the blending ratio is most probably due to the higher growth rate of the assimilated yeast. As we know from several pilot scale trials, assimilated yeast always shows high growth rates during fermentation, even in case of pitching without aeration. Thus we designed an experiment to test a blend of yeast at extreme conditions. Wort was pitched with 60% of cropped yeast and 40% of assimilated yeast at a minimal aeration rate of only 0.026 (2.6 l/hl). At the end of fermentation, the total yeast counts reached $50 \cdot 10^6$ cells/ml and the sulphite content rose to 4.9 mg/l (Table 5). The fermentation duration accorded to the brew pitched with cropped yeast at an aeration rate of 0.2.

This shows that it is possible to pitch blends of assimilated and cropped yeast at low aeration rates. The assimilated yeast promotes cell growth in spite of a deficiency of oxygen and the cropped yeast highly accumulates sulphite.

Table 5 Formation of sulphite in relation to the pitching rate (pilot scale)

yeast	aeration ratio	sulphite [mg/l]
cropped yeast	0.2	5.4
blended yeast	0.026	4.9

3.2 Sulphite formation and pitching parameters

Besides the influence of the yeast and the yeasts physiological condition, the formation of sulphite was influenced by the pitching parameters.

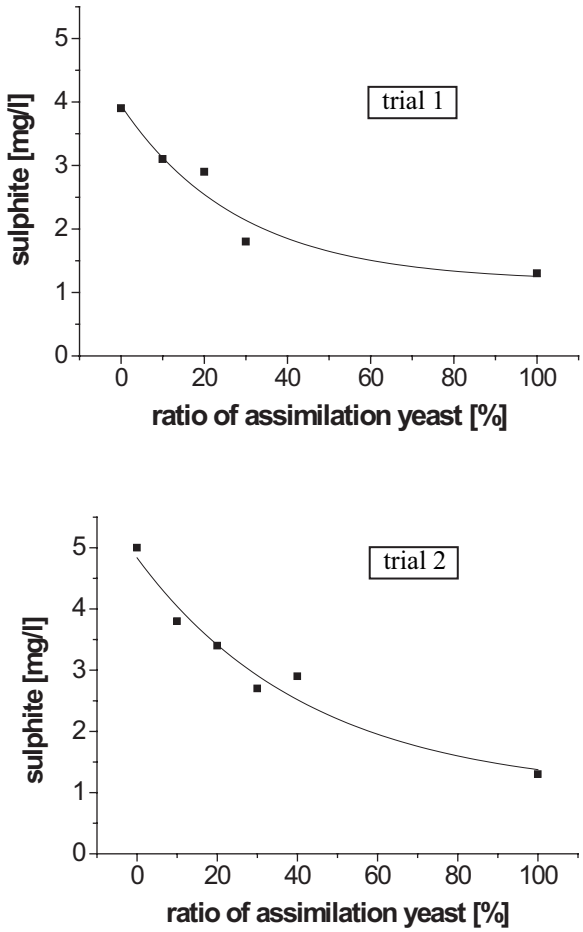

Fig. 4 Sulphite formation in fermentation trials pitched with blends of cropped and assimilated yeast (pitching rate: 15·106/ml; aeration: 0.2)

3.2.1 Pitching rate

Pilot scale fermentation trials with differing pitching rates were carried out with yeast B (Table 6). In the trials the aeration was adapted to the pitching rate to realize an approximately constant oxygen uptake per yeast cell. Thus all the brews were aerated with a rate of 1l air per 100 g yeast slurry.

It can be seen, that the sulphite formation slightly increased with a decreasing pitching rate.

In trials with different pitching rates and constant aeration rates no clear results could be obtained.

3.2.2 Aeration

To analyse the effect of aeration on sulphite formation, two brews were pitched in pilot scale with yeast A at different aeration rates

Table 6 Formation of sulphite in relation to the pitching rate

pitching rate [g/l]	sulphite [mg/l]
5,3	7,4
10,5	5,3
25,0	4,8

(0.2 and 0.05). The sulphite content was investigated during fermentation (Figure 5).

The fermentation pitched with a lower aeration rate, clearly reached a higher sulphite content. The sulphite formation of both brews during fermentation appeared remarkably similar. The formation rates matched very well. The sulphite formation of the lower aerated brew started earlier (during 1st day of fermentation) in contrast to the brew with the higher aeration rate, which resulted in the higher sulphite level at completion of fermentation. To investigate the

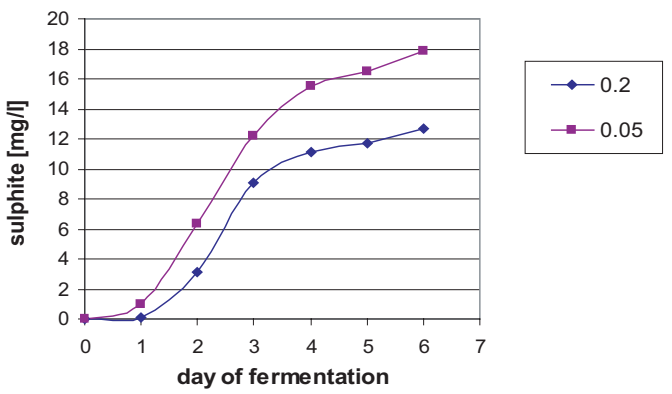

Fig. 5 Sulphite formation during fermentation of two brews pitched with different aeration rates (yeast A)

correlation between the aeration rate and the sulphite level of green beer several brews were pitched in pilot scale with the yeasts A and B using altering aeration rates (Figure 6).

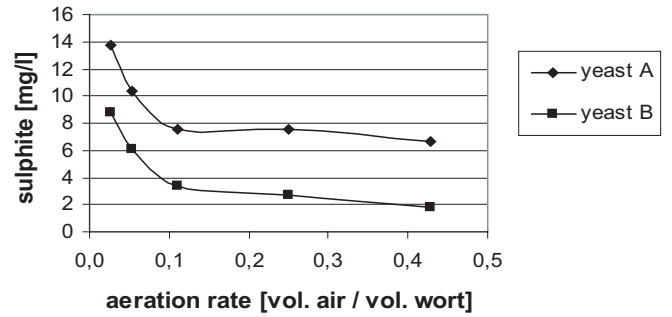

Fig. 6 Sulphite formation of two yeasts dependent on the aeration rate (26)

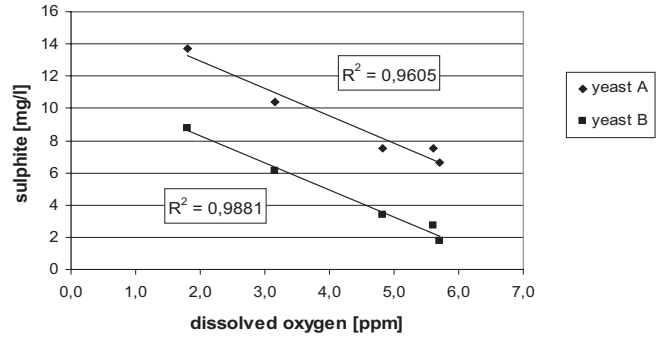

Fig. 7 Sulphite formation of two yeasts dependent on the dissolved oxygen

Both yeasts showed the same behaviour concerning the aeration rate. Sulphite formation strongly increased with aeration rates lower than 0.1. In the domain of aeration rates higher than 0.1, the sulphite formation increased only very slightly with decreasing aeration rates. In Figure 7 the sulphite contents of the two fermentation trials were correlated with the dissolved oxygen. As it was not possible to measure the oxygen in the pitched brews, the oxygen concentrations were calculated accordant to Figure 2.

The correlation between the sulphite formation and the initial oxygen content of the pitching yeast seemed to be linear. To consolidate this, a fermentation trial with yeast A at laboratory scale was carried out (Figure 8). In conjunction to every fermentation tube, a second tall tube was aerated without yeast to determine the dissolved oxygen.

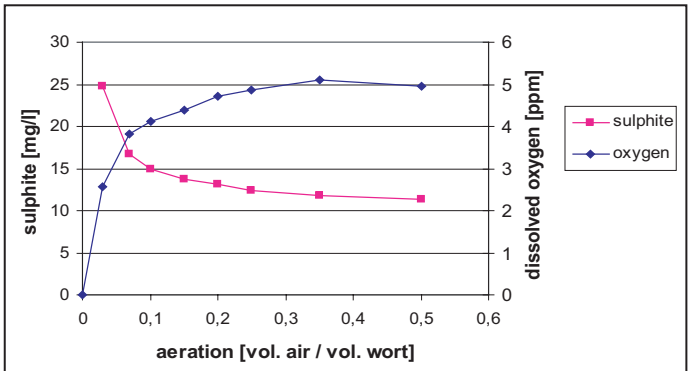

Fig. 8 Sulphite formation of yeast A and dissolved oxygen in a laboratory scale trial dependent on the aeration rate

These results supported the assumption that the sulphite formation of yeast A was linear correlated to the content of dissolved oxygen of the pitching wort (Figure 9).

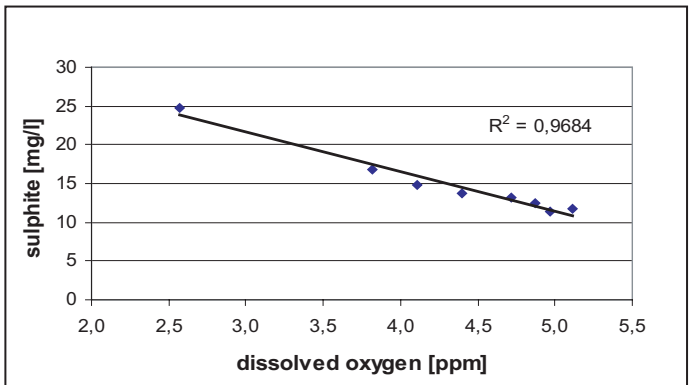

Fig. 9 Sulphite formation of yeast A in a laboratory scale trial dependent on the dissolved oxygen

3.2.3 Extract

Laboratory scale fermentations were carried out with yeast A (in this case cropped yeast of second generation). To realize different original gravities an unhopped wort extract was blended with distilled water in the required ratios. The brews were aerated at a rate of 0.3. Figure 10 illustrates the sulphite formation during the fermentation versus the original gravity of the pitching wort.

It can be seen, that the sulphite formation was highly dependent on the original gravity. The correlation seemed to be linear. In

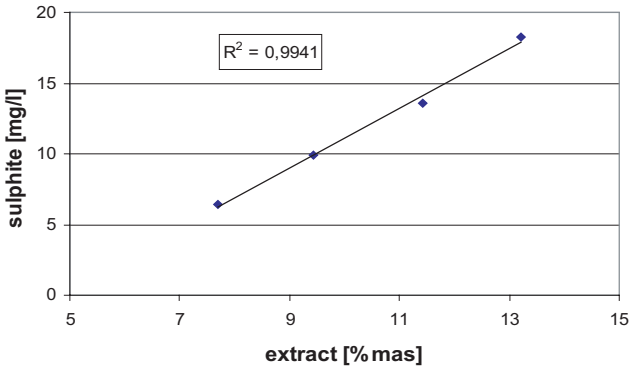

Fig. 10 Sulphite formation of yeast A in laboratory scale dependent on the original gravity

this case, an enhancement of the original gravity of 1% raised the sulphite formation about 2 mg/l. The brews with higher original gravity reached higher sulphite contents, although the aeration rate of 0.3 was relatively high.

4 Discussion

The sulphite formation is influenced by numerous parameters. According to the literature it starts when the growth rate diminishes and continues until the end of fermentation. Thus it is consequential that most of these parameters are closely correlated to yeast growth as will be pointed out in the following. Beside these parameters concerning the cell growth, the predisposition of the yeast got a strong impact on the sulphite formation.

In our experiments, the two yeasts A and B showed different sulphite formation at the same fermentation conditions. The different sulphite levels at completion of fermentation were caused by differing formation rates. The exchange of the yeasts between the breweries showed that this was due to the yeasts predisposition and not to wort quality or technical influences. This was remarkable as both yeasts belonged to the same strain but were purchased from different yeast libraries.

The condition of the pitching yeast had a pronounced effect on the sulphite formation. As we observed, the sulphite formation increased with acceding generation number. A possible explanation for this behaviour is that the cells are not able to regenerate their stock of lipids in brewery fermentation conditions because there is not enough oxygen available (compare to (11, 12)). Assimilated yeast hardly formed any sulphite. This result indicated the good condition of the assimilated yeast. The assimilated yeast was still in the growth phase and the aerobic metabolism has been running for a long time so that the cells lipid composition and oxygen supply was optimal. For this reason, pitching with assimilated yeast caused long growth periods, requiring a very late inhibition of cell growth and accordingly a very late initiation of sulphite formation.

When pitching was carried out with a blend of assimilated and cropped yeast, the sulphite formation decreased above average with an increasing amount of assimilated yeast. This behaviour was most probably caused by the higher growth rate of the assimilated yeast, leading to a misaligned blending ratio during fermentation (after the growth phase). To achieve an appreciable amount of sulphite after fermentation, we propose ratios of assimilated yeast of 30% or lower. Due to the good condition of the assimilated yeast, pitching with low aeration rates was possible. Using this method we reached comparable sulphite contents as with the use of cropped yeast. Accordingly a continual import of highly vital yeast into the production process is achieved.

Besides the condition of the pitching yeast, the pitching parameters influenced the formation of sulphite to a high degree. Only assimilated yeast still being in the growth-phase at pitching time was hardly influenced by the pitching parameters for the reasons discussed.

If the aeration rate was adapted to the pitching rate achieving an approximately constant oxygen uptake per cell, the sulphite formation increased with a decreasing pitching rate. A possible explanation is that the lower pitching rate caused a higher metabolic turnover per cell after the growth phase, thus leading to the higher sulphite formation. This could not be observed in comparable trials with a constant aeration rate. Further investigations are necessary to clarify the effect of the pitching rate on the fermentation performance and the sulphite formation.

The aeration influenced the sulphite formation most probably via the length of the yeast's growth phase, as sulphite formation starts when cell growth diminishes (13, 14) and yeast growth is oxygen dependent (10, 11). Higher aeration rates led to a longer growth phase and thus to a later initiation of sulphite formation. As saturation was easily reached with higher aeration rates, a good correlation to the sulphite content of the green beer could only be found in the range of aeration rates of 0.2 or lower. When comparing the sulphite content of the green beer with the dissolved oxygen of the pitching wort, we found an apparently linear correlation.

The original gravity had a strong influence on the yeast's sulphite formation. The sulphite content of the green beer increased markedly with increasing gravity of the pitching wort, most probably caused by the increasing metabolic turnover per cell. In addition the osmotic stress could have led to higher sulphite formation.

The results indicated that the pitching parameters did influence the sulphite formation not by the formation rate, but via the ratio of aerobic (growth phase) and anaerobic (fermentation phase) metabolic turnover. This caused differences in the point of time when sulphite formation is initiated. To interpret this, we designed a simple model of the effect of the pitching parameters on the sulphite formation: If wort and yeast quality and the technical circumstances (e.g. tank geometry) are constant, the fermentation is mainly influenced by aeration, pitching rate and temperature. A constant temperature assumed, the fermentation is characterised by the proportions of aeration and pitching rate. If we regard a single yeast cell, it is only influenced by the absolute amount of available oxygen (per cell) and the absolute amount of fermentable sugars (per cell). We adopted the key figures "oxygen per cell", and "extract per cell" to characterise the course of fermentation. The higher is the oxygen per cell (a constant extract per cell assumed), the longer is the growth phase, the later the sulphite formation is initiated, the lower is the final sulphite content. The higher is the extract per cell (at constant oxygen per cell), the longer is the anaerobic fermentation phase after the growth phase, the higher is the final sulphite content. The oxygen per cell is controlled by the aeration rate and the pitching rate. The extract per cell is controlled by the pitching rate and is influenced by the growth rate. The parameter extract per cell also allows the consideration of varying original gravities. Figure 11 illustrates the coherence of the different parameters.

A variation of the aeration will alter the oxygen per cell ratio. A variation of the original gravity will alter the extract per cell ratio. A variation of the pitching rate will alter both the oxygen per cell and the extract per cell. As oxygen per cell and extract per cell

are parameters of contradictory effects on sulphite formation, this model could also explain why no clear effect of the pitching rate on sulphite formation could be found yet. We assume, that each of the parameters can dominate, dependent on the fermentation conditions.

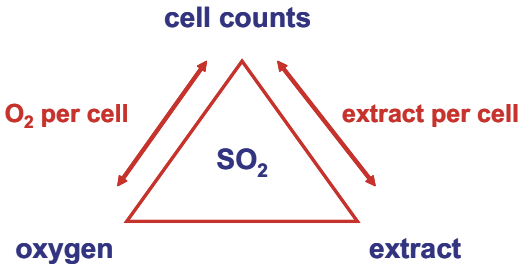

Fig. 11 Coherences of the key figures of sulphite formation

As a result of the contradictory interaction of the pitching rate and the aeration on the oxygen per cell we propose both to increase the sulphite formation, a slight decrease of the pitching rate in combination with a careful decrease of the aeration. High gravity brewing will always cause a higher sulphite formation.

5 Conclusion

The sulphite formation rate is dependent on the yeasts predisposition. The time of initiation of sulphite formation in the course of fermentation can be controlled by the pitching parameters. Based on this result, we invented a model that describes the relation between the pitching parameters and the sulphite formation. It supports the results above according to the literature. We are now able to predict the consequences on the sulphite formation of technological actions concerning the pitching parameters. It must be noticed, that this is a very simple model, which does not define all the effects of the mentioned factors, e.g. the influence of osmotic stress and cellular acetaldehyde level on sulphite formation. We are currently checking this model in continuative fermentation trials.

This investigation as well presents an example of, how to evaluate influences on fermentation by-products, whose formation is closely correlated to the yeast's growth-phases.

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