M. Baldus, T. Kunz and F.-J. Methner

The Analysis of Dimethyl Sulfoxide in Malt, Wort and Beer using Headspace Gas Chromatography combined with Pulsed Flame Photometric Detection (HS-GC-PFPD) – Methodology and Applications

Besides S-methyl methionine (SMM) as the thermal precursor for the unpleasant aroma compound dimethyl sulfide (DMS) in the brewhouse dimethyl sulfoxide (DMSO) is a further potent precursor during fermentation. In this paper a short and sensitive method for its analysis is introduced. In the first step DMSO is reduced to DMS by sodium metabisulfite and the generated free DMS is subsequentially analysed employing HS-GC-PFPD. In comparison to other substances sodium metabisulfite emerged as an efficient reducing agent whereby SMM and other potential precursors of volatile sulfuric compounds are not contributing to DMS under the applied conditions. In saturation of sodium metabisulfite the reduction of DMSO is exposed to be independent of its initial concentration and the recovery rate is constantly above 95 % in its usual wort- and beer concentration range. In first applications of the developed method DMSO and DMS levels are tracked during fermentation. The DMSO reduction was about 11 %, which is consistent with the accordant literature. Additionally, a non enzymatic pattern of DMS formation by reduction of DMSO with sulfite is proofed and discussed. The described method is easy to integrate to standard DMS analyses. Thereby breweries and related sectors have the possibility to upgrade their existing system without high financial and technical effort.

Descriptors: DMSO, DMSO analysis, DMS, S-methyl methionine, DMS precursor, sulfite, wine, SO_2, thiols, fermentation, oxidation, reduction

1 Introduction

Off-flavor in beer induced by dimethyl sulfide (DMS) is often considered as avoidable by efficient wort boiling or heating step and evaporation prior fermentation. Furthermore DMS decreases generally during fermentation owing to the stripping effect of the exhausting CO_2. However, several yeast strains are able to produce DMS by enzymatic reduction of DMSO [2, 3, 11, 12, 13, 14]. Unfortunately many breweries don't have the possibility to judge the potential risk of this source because a quick and exact method for the analysis of DMSO is still missing. In general DMS is generated from two precursors, S-methyl methionine (SMM) and DMSO. Primarily DMS originates from the thermal decomposition of the malt non protein amino acid SMM. This pattern plays a superior role in DMS production during malt kilning and in the brewhouse where operation temperatures of more than 70 °C are achieved [2, 18, 31]. Besides the temperature the pH value is the next limiting factor of the SMM degradation. In solution for example, at 98 °C (pH 6) the first order rate constant k of the SMM degradation is $2.43 \cdot 10^{-4} \cdot s^{-1}$, whereas at 60 °C (pH 4), k is $0 \cdot s^{-1}$ [18]. The oxidation products of DMS are DMSO and $DMSO_2$ which are additionally formed during malt kilning [2, 4, 29]. A new method for the SMM determination according to *Scherb* et al. [25] employs stable isotope dilution and LC tandem MS for highly sensitive measurements of SMM. This method is hardly to engage for daily brewery routine analysis due to the high acquisition and operation costs. In consequence the method of *White* and *Wainwright* [26] keeps the widest accepted method for SMM determination in the brewing industry up to now. The analytical procedure is done by heat-alkaline boiling of the sample and the measurement of DMS before and after treatment. The method was critical discussed a few times owing to the affirmed potential of DMS oxidation in alkaline solution during the measurement and the accompanied falsification of the SMM content [9, 27, 28]. In consequence *De Rouck* et al. [9] developed a non alkaline thermal degradation procedure, in which a SMM recovery of about 98 % was achieved.

In the brewing process after wort cooling the amount of SMM can be assumed as constant, when no further heat treatment is applied. From this point DMSO remains as potent precursor owing

Authors

Dipl.-Ing. Matthias Baldus, Dipl.-Ing. Thomas Kunz, Prof. Dr.-Ing. Frank-Jürgen Methner, Technische Universität Berlin, Institute of Biotechnology, Chair of Brewing Science;
corresponding author: matthias.baldus@tu-berlin.de

to the yeast specific enzymatic reduction mentioned before. In this context $DMSO_2$ is regarded as resistant and does not produce DMS via this pattern [4]. By reason of the high boiling point of DMSO (189 °C) and its thermal stability its concentration is assumed as being constant over the conventional brewhouse process, when an efficient removal of DMS after its evolution is practiced [2]. During fermentation it is known that DMSO reductase activity of several yeasts strains is able to reduce DMSO up to 21 % [3]. In dependence on fermentation parameters like the DMS desorption rate of the CO_2, fermentation temperature and especially the DMSO content in the pitching wort, this pattern may be responsible for relative higher DMS levels in the finished product compared to the pitching wort. According to the literature malt as the main source for DMSO in the pitching wort contains between 0–9.3 µg/g [4, 13, 29]. Relatively high DMSO levels up to 1230 ppb were also found in wine. In this connection DMSO was pronounced as one of the most abundant natural occurring sulfuric compound in wine [8]. A much higher – not necessarily natural – abundant sulfuric substance in wine is sulfur dioxide (SO_2), which is commonly added to wine as antioxidant up to 400 ppm (specific wine) [17]. *De Mora* et al. [8] added DMSO and Cysteine to young wine and found an enhanced DMS formation during storage and pointed out that DMSO remains as potent precursor even after fermentation.

For these reasons it is helpful to determine the DMSO content in the malt and pitching wort or yet in must to assess the potential of DMS formation caused by DMSO reduction. In general DMSO is analysed and quantified by its initial reduction and subsequent analysis of the generated free DMS by gas chromatography (GC). Thereby several reducing agents, like stannous (II) chloride or titanium (III) chloride, are applied for DMSO reduction by acidic boiling [1, 5, 15, 29]. These types of methodologies require a high time and cost intensive operation expense. Whereby evolving acidic fumes are harmful to a series of GC capillary columns. Moreover the risk of thermal degradation of SMM to DMS and Homoserine cannot be excluded. Sodium borohydrid, as a further reducing agent, is only applicable under a high mechanistic build-up procedure using inert gas stripping for the removal and analysis of DMS [1]. In solution the substance is highly alkaline and might cause an extended breakdown of SMM in wort at higher temperatures. In consequence this method is unsuitable for the brewing industry. *Dickenson* [10] describes the reduction of DMSO in wort and beer in saturated sodium metabisulfite and the analysis by GC with flame ionization detection (FID) of free DMS before and after treatment. He achieved a reduction rate of about 90 % but further details of the procedure and GC settings are lacking in this paper. Dickenson [14] also reports that reduction of DMSO with L-cysteine represents an alternative for sodium metabisulfite. The reducing activity of sulfite is known to depend on the pH value [16]. The main reducing activity of sulphite in solution is referred to the content of available sulfuric acid (HSO_3^-), which is the quantitative present form at pH values from around 3.5–5.5, similar to beer and wort pH. The FID used by Dickenson [10] was found to be less sensitive for sulfuric compounds and is generally not applied anymore for the detection of DMS. In contrast the Flame Photometric Detector (FPD) has been proved to be highly sensitive for the detection of sulfur compounds. *Annes* [5] reduced DMSO with stannous (II) chloride and hydrochloric acid (HCl) by boiling treatment and detected DMS before and after treatment using GC-FPD. In this context the stannous chloride contained impurities of DMS when it was treated with HCl. Furthermore harmful acidic fumes emerge to the GC column as described previously. Another disadvantage was that the thermal degradation of SMM influenced the measurement noticeably when it was not removed prior DMSO reduction. *Yang* and *Schwarz* [28] extended the latter method and availed this circumstance to measure SMM and DMSO in malt extracts back-to-back by GC-FPD. Free DMS was purged out of the sample with nitrogen stripping after its analysis. SMM was then determined according to White and Wainwright [26]. A second step of nitrogen purging removed the newly built free DMS. DMSO was then reduced by stannous chloride as described before [5]. Before injecting an equilibrated headspace volume into the GC port, the acidic fumes had to be neutralized by a further step where sodium hydroxide is transferred through the vials crimp sealed teflon septa. The SMM determination is obligatory for the further DMSO determination. Summarised this method needs at least 2h plus sample preparation to gain the DMSO concentration of a single sample. Additionally the authors declare that sodium metabisulfite as used according to Dickenson [10, 13] could not be applied under their described conditions because an unidentified peak covered the DMS peak. The purpose of this study was the development of a more efficient and sensitive analytical method for DMSO based on suitable DMSO reducing agents, which can be used at lower temperatures to exclude SMM breakdown and to reduce oxidative reactions. Especially the method of Dickenson [10] is focussed to be optimized and extended by pulsed flame photometric detection (PFPD) for the DMSO determination in malt, wort and beer. The new method should acquire low technical effort, less operation time and has to be easily adopted by breweries and related sectors.

2 Materials and methods

2.1 Chemicals

Materials including dimethyl sulfoxide (DMSO; C_2H_6OS; >99 %), dimethyl sulphide (DMS; $(CH_3)_2S$; >98 %), dimethyl sulfone ($DMSO_2$; $C_2H_6O_2S$; 99 %), DL-methionine sulfonium chloride (SMM; $C_6H_{14}C\,ClNO_2$; >99 %), L-cysteine (L-Cys; $C_2H_{12}N_2O_4S_2$; ≥99 %), L-cystine ($C_6H_{12}N_2O_4S_2$; 98 %), trans-ferulic acid ($C_{10}H_{10}O_4$; 99 %), gallic acid ($C_7H_6O_5$; 97.5–102.5 %) and sodium sulfide (Na_2S; –) were purchased from Sigma Aldrich (St. Louis, USA). Ethyl methyl sulphide (EMS; C_2H_8S; >97 %) and L-glutathione (L-GSH; 97 %) were purchased from Alfa Aesar (Karlsruhe, Germany). Sodium metabisulfite ($Na_2S_2O_5$; >97 %) and L-ascorbic acid ($C_6H_8O_6$; 99 %) were obtained from Merck Chemicals (Darmstadt, Germany).

2.2 Screening for reducing substances

To figure out the most suitable reducing substance for the DMSO determination, 2 g of the sulfuric compounds sodium metabisulfite, L-Cys, L-GSH, the reductone ascorbic acid and the phenolic compounds gallic and ferulic acid were added to 5 ml of 0.1 M sodium acetate buffer (pH 4.5), respectively. The buffer was spiked with DMSO to reach concentrations of 76, 134, 190 and 288 ppb. The reduction and determination of the evolved DMS was carried out as depicted in sample preparation.

2.3 Impact of potential precursor substances on the DMSO results

To test the potential of potent precursors of volatile sulfuric compounds the following substances were added to a 0.1 M sodium acetate buffer as DMS equivalent amounts: SMM (1500 ppb), $DMSO_2$ (500 ppb), L-cystine (3000 ppb), GSH (3000 ppb), DL-methionine sulfoxide (2000 ppb). H_2S was generated by adding sodium sulfide to a nitrogen purged beer with a pH value of 4.3 (1000 ppb). All samples were then analysed as described in sample preparation and GC section.

2.4 Malt extraction

The malt extraction was carried out according to MEBAK [22] with exception of some deviations. 5 g instead of 10 g fine ground malts were extracted in 100 ml bidestilled water in closed round flasks for 30 min. The mixture was centrifuged afterwards at 7000 rpm for 10 min. The supernatant was then further processed as described in sample preparation.

2.5 Fermentation trial

Fermentation was carried out in 1 l Schott flasks at 12 °C. The yeast strain RH was used as representative bottom fermenting strain with a pitching rate of 2.5 x 10^7 cells/ml. The pitching wort (pw) was produced from 100 % pilsner malt with a final gravity of 11.2 °P in the pitching wort. Gentle stirring of the media was applied in a range just enough to prevent extended yeast settling and to compensate the missing convection in the flasks.

2.6 Test on DMSO reduction by sulfite in lower concentration ranges

To test the impact of DMSO reduction by sulfite sodium in lower concentrations acetic buffer solution (pH 4.5) was spiked with DMSO to gain 2 ppm, sodium metabisulfite to 50 ppm (ratio 1:25). The study was carried out by the addition of 5 mL buffer/DMSO/ sodium metabisulfite into a 25 mL headspace vial. The buffer and the headspace vial were purged with nitrogen before to reduce the oxygen content. DMS evolution was tested according to MEBAK [22] after 2, 14, 23 and 28 days of storage in the dark at room temperature.

2.7 Gaschromatographic Analysis

2.7.1 Calibration

In the first step 0.1 M sodium acetate/ acetic acid buffer solution was adjusted to a pH value of 5.5. Besides this bidestilled water was boiled and purged with nitrogen to reduce the oxygen content. Afterwards the buffer solution was spiked with a DMSO/bid.-water solution ($1.104 \cdot 10^4$ ppb) to adjust DMSO concentrations of 38, 76, 134, 190 and 288 ppb. For beer the calibration is treated the same way, but the pH was adjusted to 4.5 and EtOH was added to 5 vol/vol%. The measurement was carried out as described in the sample preparation and GC section. To transform the exponential response of the PFPD into linear response, the calibration graphs of the detected DMS were constructed by plotting the log (DMS/EMS) peak area ratios against the log (DMS/EMS) concentration ratios. The calculation of the DMS concentration was carried out according to MEBAK guidelines [22].

2.7.2 Sample preparation

Free DMS was purged out of the sample using nitrogen stripping as previously described by Yang and Schwarz [28]. After treatment the sample was cooled down to 1 °C. 3.85 g of sodium metabisulfite, (20 % over saturation) was added into a headspace crimp top vial (Supelco, Sigma-Aldrich, St. Louis), which was purged with nitrogen before to reduce the oxygen content. Afterwards the vial was sealed and immediately cooled down to 20 °C before a quantity of 5 g of each ice-cold sample (1 °C) was added. Subsequently 50 µL of an ethanol-ethyl methyl sulfide solution ($2.53 \cdot 10^5$ ppb) was injected and directly hermetically closed using an aluminum crimp cap with a PTFE/silicone septum (Supelco, Sigma-Aldrich, St. Louis). The accelerated reduction of DMSO was carried out for 10 min in a water bath at 60 °C under shaking (95 rpm). Thereafter the sample was cooled down to 4 °C before the headspace vial was incubated at 50 °C in a water bath for a period of 30 min. The sample preparation for free DMS was done according to the MEBAK guidelines [22] with exception that the headspace vials were purged with nitrogen before to reduce the oxygen content.

2.7.3 Gas chromatography (GC)

200 µL of the headspace gas was injected into a HP 5890 Series II GC equipped with a pulsed flame photometric detector (PFPD 5380, OI Analytical, Texas, USA). The column used was a HP-5 model (39.0 m x 0.32 mm) from Agilent Technologies. Nitrogen was used as carrier gas with a flow rate of 2.1 ml/min. Injection was done in split mode (split ratio 1:37) at 230 °C with a split flow of 78.5 ml/min. The applied temperature program started with 1 min at 35 °C and was followed by a 7 °C/min temperature gradient to reach 55 °C and a subsequent 60 °C/min gradient to 150 °C. The PFPD was set at 250 °C, 500 V, 20 ms gate width, 6 msec delay and a trigger level of 300 mV. The detector gas flow rates were 16.0 ml hydrogen and 22.0 ml synthetic air. The obtained data were sent to a HP ChemStation (Agilent Technologies, Germany), where they were integrated and processed.

3 Results and Discussion

3.1 Optimization of the DMSO reduction with sodium metabisulfite

In the first step of the method development sodium metabisulfite was used for the reduction of DMSO. The chromatograms of the reduced solutions showed up overlapped signals of DMS and a further component, which was reported previously by Yang and Schwarz [28] using the GC oven program for DMS according to MEBAK [22]. This program was optimized in a series of trials leading to the program described in the material and methods section. Applying the optimized temperature gradient and the determined flow rates an exact separation of the two peaks could be achieved under the described GC conditions as illustrated in figure 1.

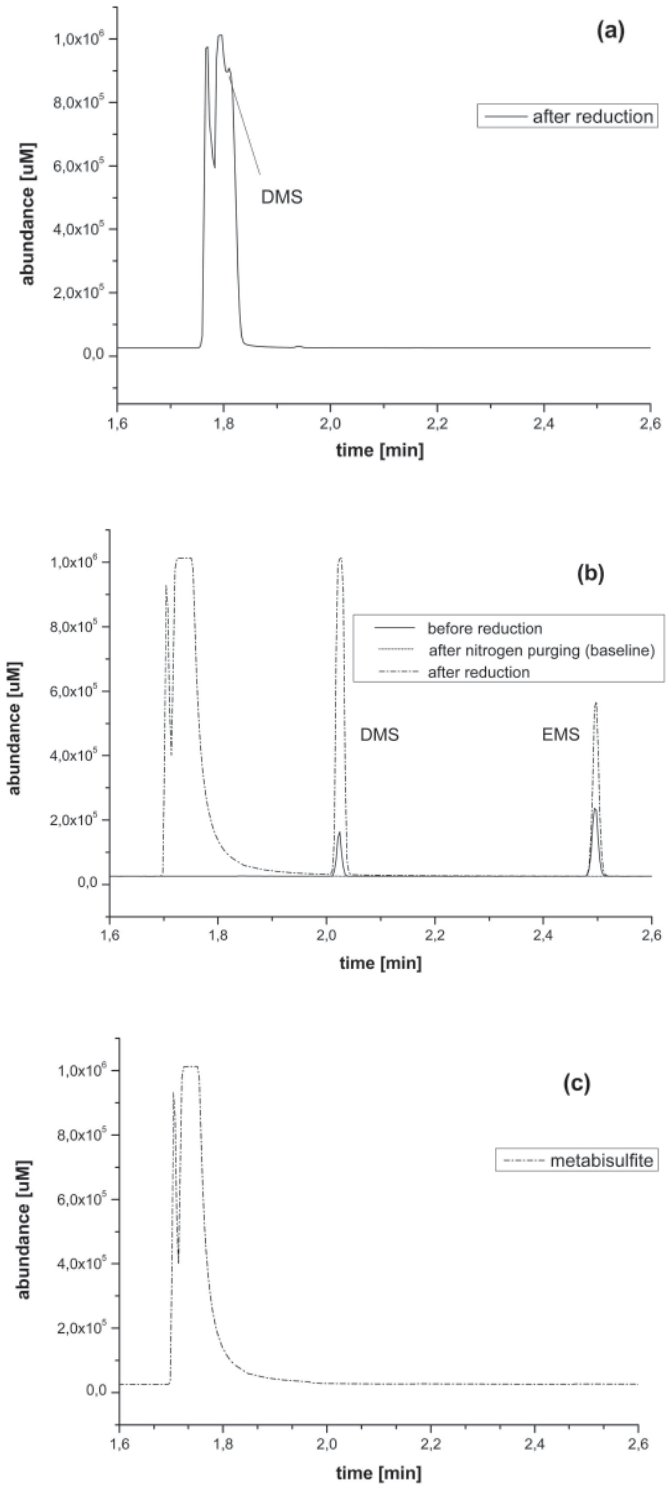

Fig. 1 chromatograms of sulfite, DMS and EMS signals:
(a) conventional method,
(b) optimized method,
(c) conventional with sodium metabisulfite exclusively

For the identification of the first peak which was declared as unknown before [28] aqueous solutions of sodium metabisulfite were investigated under the described optimized analytical conditions. Heating up aqueous HSO_3^- it dissociates to free SO_2. In case of the used calibration buffer solution (pH 4.5) only containing sodium metabisulfite the first peak was detected exclusively (Fig. 1 (c)), which verifies the assumption that the first peak orginates from the generated SO_2 during the analyses. The double signal nature of this peak might be lead back to a mixture of SO_2 and HSO_3^-. This assumption remains to be investigated.

3.2 Screening for DMSO reducing agents

Since SO_2 is a natural reducing substance of beer it was of interest to investigate the DMSO reducing ability of other 'beer-own' reducing substances like ascorbic acid, phenols, and other sulfuric compounds like L-cysteine (L-Cys) and L-glutathione (L-GSH). For the qualitative comparison of their reducing behaviour against DMSO aquivalent mass fractions of all substances were used, as described in the methods section. In figure 2 the added DMSO concentration is plotted against the resulting DMS peak area caused by DMSO reduction.

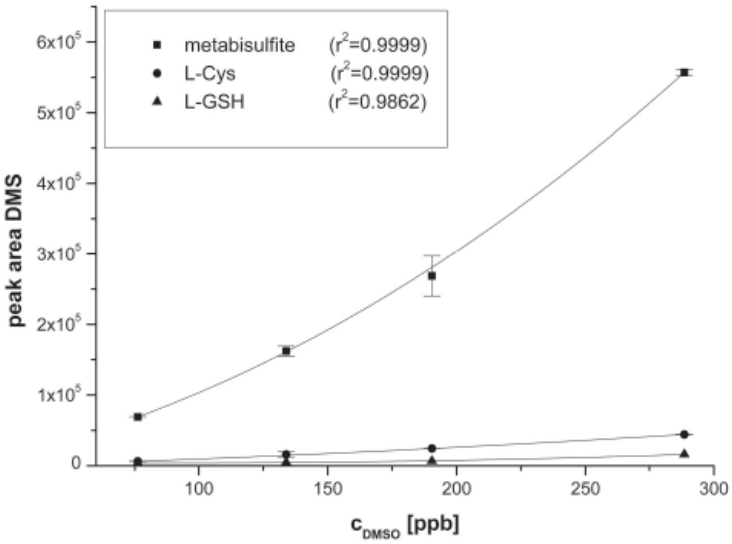

Fig. 2 DMSO calibration with different reducing agents (mean of duplicate trials, error bars: ± standard deviation(s.d.))

Figure 2 demonstrates that all tested sulfuric substances (sodium metabisulfite, L-Cys, L-GSH) are able to reduce DMSO resulting in a high correlating component specific increase of the DMS peak area. Outstandingly, sodium metabisulfite exhibits the highest capacity for the DMSO reduction in the tested DMSO concentration range. Ascorbic acid, ferulic acid and gallic acid did not produce any signal at the retention time of DMS, indicating that no reduction of DMSO occurred at the chosen measuring conditions (not shown). Moreover the solubilities of the substances were different at sample preparation conditions in order of sodium metabisulfite, L-GSH, L-Cys. Simultanously, reduced solubility diminishes the functionality. Thereby the unproportional minor difference between L-Cys and L-GSH might be explained.

L-Cys represents a limited suitability for the DMSO reduction under the used conditions due to its considerable small peak area increases. The peak areas of DMS produced by the reduction with sodium metabisulfite were in average around 10 times higher than of L-Cys, for example.

Owing to this background the sample preparation and dosage for sodium metabisulfite was further optimized resulting in the procedure described in material and methods. The calibration was

carried out in triplicate every week. The correlation coefficient was $r^2 = 0.9996 \pm 0.0004$ (n = 10; ± s.d.). In comparison to the conventional DMS calibration, the sample preparation and calibration of DMSO is easier to handle due to its high water solubility and its non volatile nature.

3.3 Impact of potential precursor substances on the DMSO results

As described in the material and methods section the reduction of DMSO is done at 60 °C. After addition of sodium metabisulfite to wort or beer a pH value of 3.9 is achieved. To make sure that SMM does not contribute to the DMSO results, the thermal stability of SMM was proofed by the treatment of aqueous SMM solutions under the reduction conditions. Besides SMM also other potential precursors for volatile sulfuric compounds were tested. Table 1 gives an overview about the impact of chosen sulfuric compounds ($DMSO_2$, DL-methionine sulfoxide, H_2S, L-Cys, L-cystine and L-GSH) on the DMS formation during the DMSO measurement.

Table 1 **Impact of potential precursors of volatile sulfuric compounds on the DMSO measurement (SMM check was carried out six times, all other substance checks three times)**

compound	signal
SMM	n.d.
$DMSO_2$	d.
L-cystine	n.d.
L-Cys	n.d.
L-GSH	n.d.
DL-methionine sulfoxide	d.
H_2S*	n.d.

n.d. no signal detectable
s.d. signal detectable / not interfering with DMS signal
d. signal detectable / interfering with DMS signal
*prepared by sodium sulfide addition to beer of pH 4.4

In case of SMM no DMS signal was detected, which is in line with the results of *Kovatscheva* [18]. Hence SMM does not contribute to the DMSO levels at the used pH-value (3.8–3.9) and temperature (60 °C) conditions it is to conclude that SMM is stable during the DMSO determination. In the case of $DMSO_2$ and DL-methionine sulfoxide minor peaks were observed at the retention time of DMS but in both cases the areas were below the detection limit. Processing of aqueous solutions of both substances without sodium metabisulfite addition minor DMS peaks occurred likewise. Behind this observation the DMS signals might be lead back to impurities of containing DMSO or DMS in the commercially obtained substances. Additionally, it has to be mentioned that the samples for DMSO determination are generally dilutet 1 : 5. In this concentration range, only the base line was detected for $DMSO_2$ and DL-methionine sulfoxide. All other tested substances like H_2S, L-Cys and L-cystine were found to not produce any DMS signal under the applied conditions.

3.4 DMSO and DMS levels in malt, wort and beer

In this step malts, wort and beer were screened for DMSO levels. Table 2 gives an overview about the detected DMSO levels in commercially used malt types, a pitching wort and different german beers. All determinations were carried out in triplicate except of the pitching wort and the pilsner type 1 beer. The measurements of DMSO in these cases were done 8 times to increase the accuracy of the reference value for the determination of the recovery rates in the next step.

Tab. 2 **DMS and DMSO concentrations of malt, wort and beer types**

		DMS	DMSO
malt [a, c]	pilsner malt	2.63 ± 0.14	4.44 ± 0.09
	munich malt type I	3.19 ± 0.09	7.16 ± 0.04
	munich malt type II	1.64 ± 0.04	10.31 ± 0.15
	roasted barley malt	n.d.	n.d.
	wheat malt	1.20 ± 0.02	2.1 ± 0.1
wort [b,**]	all malt pitching wort[b]	44.8 ± 2.8	588 ± 3.6[b]
beer**	pb 1	28.3 ± 0.5[c]	542 ± 0.6[b]
	pb 2[c]	21.1 ± 0.14[c]	515 ± 0.4[c]
	pb 3[c]	25.0 ± 0.6[c]	501 ± 1.7[c]
	wb 1[c]	26.3 ± 1.9[c]	388 ± 1.7[c]
	wb 2[c]	27.5 ± 2.0[c]	435.7 ± 3.2[c]

** – concentration in µg per kg DMS equivalents
a – concentration in µg per g
b – mean value (n = 8) ± standard deviation (s.d.)
c – mean value (n = 3) ± standard deviation (s.d.)
pb – pilsner beer
wb – wheat beer

The DMSO distribution especially in several types of malt shows noticeable differences. The wheat malt contained the lowest amount of DMSO followed by pilsner malt and a remarkable increase in munich malt type I and type II. In this connection it is to point out that the levels of DMS and DMSO in roasted barley malt were too low to detect. Owing to missing details on the malting conditions a direct comparison between the DMSO levels of the samples cannot be accomplished reasonable. For the roasted barley malt it can be suggested that a decelerated germination produced less amount of SMM resulting in a reduced DMS formation and its oxidation to DMSO during kilning. On the other hand the levels of $DMSO_2$ have not been analyzed in these trials, thus the further oxidation of DMSO caused by the high roasting temperatures of roasted barley malt cannot be excluded. Corresponding to the wheat malt also lower DMSO levels were detected in the analyzed wheat beer types, which were around 390 ppb. The pilsner type beers contained DMSO levels between 501 and 542 ppb. In comparison to the all pilsner malt pitching wort, the levels in beer were all lower, which might be explained by the yeast reducing activity [2, 3, 4, 13, 14].

3.5 Test on DMSO recovery in wort and beer

The pitching wort and the pilsner beer type 1 were taken further for the determination of the recovery rates as illustrated in figure 3.

In both cases the recovery rates were generally above 95 %. For wort the recovery rate was 96.5 ± 5.0 %, for beer 99.0 ± 4.9 % over the shown range of concentration steps (n = 12, ±s.d.). The

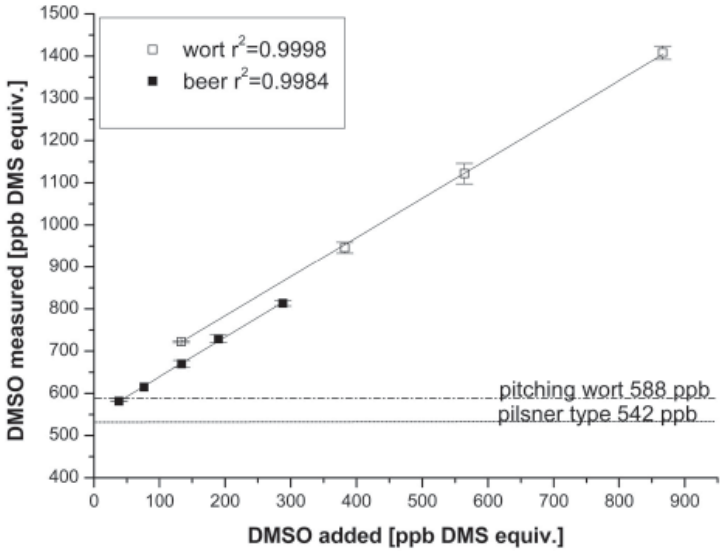

Fig. 3 **recovery rates of DMSO in wort and beer (means of triplicate trial, error bars: ± s.d.)**

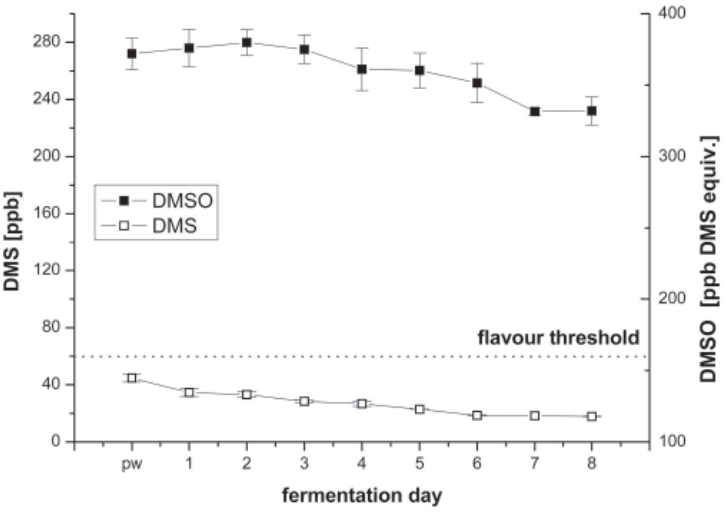

Fig. 4 **course of DMSO and DMS during fermentation (means of duplicate trial, error bars: ± s.d.)**

results demonstrate that the method is able to deliver exact DMSO measurements and proves to be applicable for routine analysis.

3.6 DMSO and DMS during fermentation

In an additional application the levels of DMS and DMSO were tracked during fermentation. Figure 4 demonstrates the course of DMS and DMSO during fermentation.

In the first 3 days of fermentation the level of DMSO remained approximately constant, whereas DMS was steadily reduced up to about 40 % on the third day Followed by a considerable DMSO concentration drop of about 4 % during the fourth fermentation day, in correlation the decline of DMS was slightly reduced. A similar behaviour with a DMSO reduction of about 6 % could be observed from sixth to the seventh fermentation day. In this case the DMS decline was almost stopped and remained constant until the end of fermentation. The DMSO content did not change in the last day of fermentation. The observation from the last day might be explained by the reduced yeast activity in that period because the attenuation degree of 78 % was already achieved after seven days.

In general no direct correlation between the net DMSO reduction of about 11 % and the overall DMS reduction of about 60 % could be observed. For the explanation two important aspects have to be considered. As described in literature [2, 3, 4, 13, 14, 23] the interplay between DMSO and DMS during fermentation is mainly attributed by DMS desorption caused by evolving CO_2 bubbles and newly generated DMS by DMSO reductase.

3.7 Test on DMSO reduction by sulfite in lower concentration ranges

Since the reduction of DMSO to free DMS is done by high concentration of sodium metabisulfite in the introduced analytical method, it was of further interest to evaluate a potential contribution of the endogenous sulfite in beer or wine to rising DMS levels during storage. In this connection additional model trials with reduced amounts of DMSO and sulfite in buffer solutions (pH 4.5) were carried out. The DMS formation in the used sodium acetate buffer containing around 2 ppm DMSO and 50 ppm sulfite over a storage time of 28 days is presented in table 3.

Tab. 3 **DMS evolution in a buffer solution (pH 4.5) containing 2 ppm DMSO and 50 ppm sufite (means of duplicate trial ± s.d.)**

d	DMS [ppb]
2	n.d.
14	30.8 ± 0.04
23	36.5 ± 0.32
28	43.2 ± 1.3

After two days at room temperature in the dark no significant DMS formation was observed. Whereas after 14 days of incubation the solution contained already around 31 ppb DMS followed by a further increase to 36.5 ppb after 23 days and 43.2 ppb after 28 days which represents a total DMSO reduction of around 2.2 %. At every day of measurement the reference buffer solution which only contained DMSO showed no signal at the retention time of DMS. The results give a clear advice that the reduction of DMSO by sulfite can also take place in low concentration ranges.

4 General discussion

The results have clearly shown that specific beer own reducing agents like ascorbic acid, ferulic acid and gallic acid are unsuitable for DMSO analyses owing to their missing DMSO reducing ability under the applied conditions. Additional investigated thiols like L-Cys and L-GSH exhibit only slightly linear DMSO reducing properties with increasing DMSO concentration. With a big distance sodium metabisulfite demonstrated the remarkable highest sensitivities for DMSO reduction and best solubility during the applied sample processing. In this connection it has to be noted, that the reducing ability of the single substances were compared only at constant parameters (pH, temperature, time) and the conducted reduction procedure was not suitable for the determination of the quantitative substances endogenous DMSO reducing ability, therefore the substances where just qualitatively compared via the generated DMS signals. Additionally, it is not to exclude that

the investigated substances can change their reducing properties by deviating conditions. In this context the slightly linear reducing properties of L-Cys under the applied conditions could not verify L-Cys as alternative reducing agent for sodium metabisulfite as declared according to Dickenson [14].

Behind all these observations the focus was set on sodium metabisulfite owing to its demonstrated remarkable highest sensitivity for DMSO reduction and better solubility during the applied sample processing and a reliable method for DMSO determination was generated. Moreover, sodium metabisulfite offers further benefits like easy handling, comparable high solubility in water and low costs.

In the application of the optimized method the main precursor SMM was stable against thermal decomposition and did not generate DMS during the sample preparation at a comparative low temperature of 60 °C and a pH of 3.8. Also the higher DMS oxidation product $DMSO_2$ and other precursors for further volatile sulfuric compounds were not contributing to the DMSO results. $DMSO_2$ is much more stable against reduction than DMSO and might thereby not be reduced by the reducing agent sodium metabisulfite. Apart from that the levels of $DMSO_2$ in malt and beer are negligible low and most yeast strains are not able to reduce this substance leading to DMS [4]. Moreover the further tested precursors like DL-methionine sulfoxide, L-Cys and L-cystine are not influencing the DMSO results.

Addditonally, compared to the method of Yang and Schwarz [28] the introduced method requires less operation time and expense. Furthermore it is possible to eliminate the influences of the obligatory determination of SMM and the retention time for DMSO reduction at 100 °C in highly acidic solutions on the redox behavior of DMS and DMSO. Supplementary the practical application of the new method involves the minimization of oxidative reactions by comparative low temperatures and nitrogen purging of the sample vials and the samples themselves. On this way a potential DMSO generation during the sample preparation was minimized or inhibited. The tested recovery rates of DMSO in wort and beer were above 95 % indicating the accuracy of the developed method in the solution matrix of wort and of beer.

In the application of the method different malt types, wort and beers were screened for DMS and DMSO levels. Although remarkable differences especially in the malt types were observed, direct conclusions could not be carried out owing to the missing production information like germination and kilning conditions. Nevertheless the malt DMSO levels of this work lie in a range of 0–10.3 µg/g, which is in line with the accordant literature [4, 13, 29].

In an additional application DMSO and DMS were tracked over the course of fermentation. It was shown that the DMSO reduction had a slightly undesirable influence on the DMS decline during fermentation. Neverthless the DMS desorption caused by evolving CO_2 bubbles had the predominant influence and is responsible for the decreased DMS content in the beer in compared to the pitching wort. Apart from that the conducted stirring mechanism could have enhanced the DMS reduction even it was very gently just enough to keep the yeast in despense. The average DMSO reduction of 11 % in this study is significant but compared to the literature [3, 2, 13] where DMSO reductions up to 21 % are reported it is rather low. The low impact of DMSO reduction to DMS increases was reported previously by *Dickenson* and *Anderson* [13]. They concluded in their investigations on DMS formation during fermentation, that the DMS formation is not significantly contributing to the DMS levels in beer when the pitching wort contains less than 500 ppb of DMSO. In line with these findings, it has to be pointed out the detected DMSO decrease was from around 380 ppb in the pitching wort to around 330 ppb after fermentation.

Regarding the non enzymatic DMSO reduction at lower concentrations compared to the applied reduction procedure for the DMSO analysis further investigations showed that this pattern also deserves some attention. The applied concentrations were not as low as average beer values for these substances, nevertheless not as far away (both around 5–6 times higher). The overall DMSO reduction after 28 days of incubation time of around 2.2 % seems to be low at first side. Considering the DMS flavour threshold of 50–60 ppb [2, 24] and average DMSO contents in beer of about 500 ppb a reduction of around 2.2 % after 4 weeks would increase the DMS level significantly and might be responsible for DMS off-flavors in the finished beer and would be dependent on the initial DMSO concentration after bottling, for example. In case of wine, this beverage contains much higher levels of sulfite and conditionally also of DMSO [8, 17]. The DMS increase by the addition of DMSO and Cysteine to wine detected by De Mora et al. [8] could be corresponding to DMSO reduction by sulfite. In their study data of the sulfite content in the wines were not available. On the other hand L-cysteine and other thiols might have had an impact on DMS formation in these wines which would be in line with the results of the present work. Further investigations on the impact of the non enzymatic reduction of DMSO by sulfite have to be carried out owing to the bulk of reactions in which sulfite is involved in beer or in wine, for example consumption by oxygen [19, 20, 21] or complexation reactions with aldehydes [6, 7].

5 Conclusion

Taking all results of this study into account it can be concluded that the developed method proofed to be a sufficient tool for the determination of DMSO in the brewing industry and related sectors. Moreover new insights were generated indicating that the reduction of DMSO by specific beer own reducing substances like sulphite may contribute to DMS formation during fermentation and storage of beer and wine. Current investigations at the Technische Universität Berlin, Institute of Biotechnology, Chair of Brewing Science are dealing with the kinetics of DMSO reduction by sulphites and thiols to evaluate these pathways on their influence on off flavour induced by DMS.

Acknowledgment

Grateful acknowledgements go to Victoria Schiwek[1] for her interest and technical support during the method development. The colleague Torsten Seewald[1] is kindly acknowledged for the helpful and interesting discussions.

[1]Technische Universität Berlin, Institute of Biotechnology, Chair of Brewing Science.

7 References

1. Andreae, M. O.: Determination of Trace Quantities of Dimethylsulfoxide in Aqueous Solutions, Anal. Chem., **52** (1980), pp. 150-153.
2. Annes, B. J. and Bamforth, C.: Dimethyl Sulfide – A review, J. Inst. Brew. **88**, (1982), pp. 244-258.
3. Annes, B. J.: The Reduction of Dimethyl Sulfoxide during Fermentation, J. Inst. Brew., **86** (1980), pp.134-137.
4. Annes, B.J.; Bamforth, C. and Wainwright, T.: The Measurement of Dimethylsulfoxide and Its Reduction to Dimethylsulfide by Yeast, J. Inst. Brew., **85** (1979),pp. 346-349.
5. Annes; B. J.: The Determination of Dimethyl Sulfoxide in Aqueous Solution, J. Sci. Food Agric., **32** (1981), 353-358.
6. Baert J. J.; De Clippeleer J.; Hughes P. S.; De Cooman L. and Aerts G.: On the Origin of Free and Bound Staling Aldehydes in Beer. J. Agric. Food Chem., 21 (2012), no. 60(46), pp. 11449-11472.
7. Barker, R.L.; Gracey, D.E.F.; Irwin, A.J.; Pipasts, P. and Leiska E.: Liberation of staling aldehydes during storage of beer, J. Inst. Brew. **89** (1983), no. 6, pp. 411-415.
8. De Mora, S. J.; Lee, P.; Shooter, D. and Eschenbruch, R.: The Analysis and Importance of Dimethylsulfoxide in Wine, Am. J. Enol. Vitic **44** (1993), no. 3, pp. 327-332
9. De Rouck, G.; Flores-González, A.; De Clippeleer, J.; De Cock, J.; De Cooman L. and Aerts, G.: Sufficient Formation and Removal of Dimethyl Sulfide (DMS) without Classic Wort Boiling, BrewingScience – Monatsschrift für Brauwissenschaft, **63** (2010), pp. 31-40.
10. Dickenson, C.J.: Determination of dimethyl sulfoxide in malt, wort and beer, J. Inst. Brew., **87** (1980), pp. 16.
11. Dickenson, C. J.: The Relationship of Dimethyl Sulfide Levels in Malt, Wort and Beer, J. Inst. Brew, **85** (1979), pp. 235-239.
12. Dickenson, C, J.: Dimethyl Sulfide – Its Origin and Control in Brewing, J. Inst. Brew., **89** (1983), pp. 41-46.
13. Dickenson, C.J. and Anderson, R.G.: The relative importance of S-methyl methionine and Dimethyl Sulfoxide as Precursors of Dimethylsulfide in Beer, EBC Proc., **18** (1981), pp. 413-419.
14. Dickenson, C. J.: Cambridge Price Lecture Dimethyl Sulphide – Its Origin and Control In Brewing, J. Inst. Brew., **89** (1983), no. 1, pp. 41-46.
15. Gavrillin, M. V.; Sen'chukova, G. V. and Kompantseva, E. V.: Methods for the Synthesis and Analysis of Dimethyl Sulfoxide (A Review), J. Pharm. Chem., **34** (2000), no. 9, pp. 490-493.
16. Hoffmann, M. R. and Edwards J. O.: Kinetics of the oxidation of sulfite by hydrogen peroxide in acidic solution, J. Phys. Chem., **79** (1975), no. 20, pp. 2096-2098.
17. Jackowetz, N.; Li, E. and Mira de Orduña, R.: Sulfur Dioxide Content of Wines: The Role of Winemaking and Carbonyl Compounds, Research News from Cornell's Viticulture and Enology Program, Research Focus 2011-2013.
18. Kovatscheva, E. G.: Beständigkeit von S-Methylmethionin (Vitamin U) in Pufferlösungen und Pflanzensäften bei thermischer Behandlung, Die Nahrung, **22** (1978), no. 3, pp. 315-321.
19. Kunz, T.; Stephan, A. and Methner, F.-J.: Grundlegendes zur Elektronenspinresonanz-Spektroskopie (ESR) und Untersuchungen zum Zusammenhang zwischen oxidativer Bierstabilität und dem SO_2-Gehalt. Monatsschrift für Brauwissenschaft, **7** (2002), pp.140-152.
20. Kunz, T.; Müller, C. and Methner, F.-J.: EAP Determination and Beverage Antioxidative IndeX(BAX) – advantegeous tools for Evaluation of the oxidative flavour stability od beer and beverages, BrewingScience – Monatsschrift für Brauwissenschaft, **66** (2013), pp. 12-22.
21. Kunz, T.; Reinhardt, C.; Eon-Jeong, L.; Dörr, T. and Methner, F.-J.: Impact of Fermentable and Non-Fermentable Carbohydrates on the Sweetness, Improvement of Palate Fullness and SO_2-Content in Beer, Brewing Science – Monatsschrift für Brauwissenschaft, **65** (2012), pp. 140-149.
22. MEBAK e.V.- Mitteleuropäische Brautechnische Analysenkommission, Methodensammlung 1996, vol III, 3rd edition.
23. Niefind, H.J. and Spät, G.: Ein Beitrag zur Bildung flüchtiger Schwefelverbindungen, EBC Proc., **14** (1973), pp. 297-307.
24. Rübsam, H.; Becker, T. and Krottenthaler, M.: Optimierung der Sudhaustechnologie mit Dimethylsulfid als Leitkomponente, BRAUWELT, **150** (2010), pp. 534-539
25. Scherb, J.; Kreissl, J.; Haupt, S. and Schieberle, P.: Quantitation of S-methyl methionine in raw vegetables and green malt by a stable isotope dilution assay using LC-MS/MS: comparison with dimethyl sulfide formation after heat treatment., J. Agric. Food Chem., **57** (2009), no. 19, pp. 9091-9096.
26. White, F. H. and Wainwright, T.: The measurement of dimethyl sulfide precursor in malts, adjuncts, wort and beer, J. Inst. Brew., **82** (1976), no 1, pp. 46-48.
27. Vermeulen, C. and Collin, S.: Sulfur compounds: synthesis and detection; De Clerck, J.: Chair Conference Proceedings, Leuven, Belgium, 2002.
28. Yang, B. and Schwarz, P. B.: Application of Nitrogen-Purging of Malt Extracts to Measure Two Dimethylsulfide Precursors by Headspace Gas Chromatography, J. Am. Soc. Brew. Chem., **56** (1998), no. 3, pp. 81-84.
29. Yang, B.; Schwarz, P. and Horseley, R.: Factors involved in the Formation of two Precursors of Dimethyl Sulfoxide During Malting, J. Am. Soc. Brew. Chem. **56** (1998), no. 3, pp. 85-90.
30. McArdle, J.V. and Hoffmann, M.R.: Kinetics and mechanism of the oxidation of aquated sulfur dioxide by hydrogen peroxide at low pH., J. Phys. Chem., **87** (1983), no. 26, pp. 5425-5429.
31. Zürcher, C.; Gruss, R. and Kleber, K.: Technological Measures to reduce the Dimethyl Sulfide Content in Beer, EBC Proc., **17** (1979), pp. 175-188.

Received 23 August 2013, accepted 14 October 2013