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On the Behaviour of Dimethyl Sulfoxide in the Brewing Process and its Role as Dimethyl Sulfide Precursor in Beer

Dimethyl sulfide (DMS) has a considerable impact on the aroma of beer and may lead to undesirable flavour impressions. The undoubdted role of S-methyl methionine (SMM), as the thermal precursor of DMS has been elaborately elucidated in the literature. DMS can also be generated via reduction of dimethyl sulfoxide (DMSO) during fermentation. However, there are uncertainties regarding the role of DMSO as DMS precursor and its contribution to final DMS levels in beer. The behaviour of DMSO in the brewing process has not been investigated in detail. Also, the extent to which different yeast strains reduce DMSO is mostly unknown. In this work the behaviour of DMS and its precursor SMM and DMSO was investigated throughout the brewing process. The main focus was to ascertain DMSO reduction during fermentation by lager and top-fermenting yeast. During mashing, SMM and DMSO were extracted rapidly owing to their high water solubility, whereas SMM was extracted faster. In the further course of mashing SMM and DMSO levels remained approximately constant. DMS was found to be evaporated steadily in open mashing systems and was not subject of significant oxidation to DMSO, even in a closed mashing sytem. During wort boiling SMM was degraded in a 1st order mechanism (k = 0.021 min^{-1}) whereas the generated DMS was evaporated subsequently. The levels of DMSO increased linearly with increasing evaporation of water but where not affected when boiling was conducted with a rectification column. During wort heat holding in hermetically closed systems ~15 % of the accumulated DMS was oxidized to DMSO. During fermentation significant DMS formation was observed. DMSO reduction was higher in top fermenting *Saccharomyces cerevisiae* yeast (TUM 149) than in *Saccharomyces pastorianus* lager yeast (TUM 34/70) but was not correlated with genetic yeast diversity (domestication clusters). This work demonstrates that DMSO reduction during fermentation significantly contributed to the levels of DMS in beer. Therefore we suggest that DMSO should be recognized and assessed as DMS precursor by maltsters and brewers.

Descriptors: dimethyl sulfide, dimethyl sulfoxide, S-methyl methionine, DMS precursor, fermentation, *Saccharomyces pastorianus*/cerevisiae

1 Introduction

Dimethyl sulfide (DMS) is probably the most investigated volatile sulfuric aroma compound in beer.

At concentrations above its flavour threshold (30–100 µg/L), DMS can induce specific off-flavours, which are often ascribed as ’cooked-vegetable’-like [1, 2]. However, subthreshold-levels of DMS were reported to have a positive effect on beer flavour 1. The impact of DMS on beer flavour is highly affected by the beer type [2] and masking effects of other beer aroma compounds [3].

DMS primarily originates from the non-protein amino acid S-methyl methionine (SMM), which is synthesized during germination of brewing cereals [4, 5]. SMM is heat-labile and decomposes during malt kilning to DMS and L-homoserine [6–8]. Relatively high amounts of the generated DMS diffuse out of the grain into the ventilation air. In dependence of the kilning technology, DMS is also oxidized to DMSO and minor amounts of dimethyl sulfone ($DMSO_2$) [8, 9]. In the final malt SMM levels of 0–14.5 µg/g [5, 8, 10] as well as DMSO levels of 0–10 µg/g [8, 9, 11] were reported.

Besides DMS itself, SMM and DMSO are introduced into the brewing process acting as DMS precursors. SMM generates DMS mostly during wort boiling and hot trub separation whereas DMSO can be reduced to DMS during fermentation. A main reason for the expensive wort boiling process is to hydrolyse SMM and ssubsequently evaporate the generated DMS and other undesirable volatile aroma compounds. There is a general agreement about the reaction mechanism of SMM decomposition and its contribution to DMS formation in the literature. Thermal SMM degradation follows a 1st order mechanism and is favoured at high temperatures and high pH values. Rate constants were reported to vary between 0.018–0.03 min^{-1} (pH 5.3–5.5, T = 98.5 °C–100 °C) [6, 7, 12, 13].

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In contrast to SMM, DMSO is heat stable. It reveals a high water solubility owing to its polar sulfoxide group. Its high entropy of vaporisation makes DMSO unlikely to be evaporated during wort production and its entirety probably remains in the pitching wort [2]. However, the actual pattern and the behaviour of DMSO during wort production has not been investigated in detail.

DMSO itself has no impact on beer flavour but it acts as a secondary DMS precursor during fermentation as it can be reduced by yeast [6, 9, 14–17]. Thioredoxin dependent methionine sulfoxide reductases (MSRA) reduce the sulfoxide group thereby forming DMS as a side reaction of the methionine sulfoxide reduction [18, 19]. SMM cannot be metabolized to DMS by the yeast [14, 20].

The relationship of the DMSO concentration in the pitching wort (400–800 µg/L) and the flavour threshold of DMS (30–100 µg/L), indicate that even minor DMSO reduction during fermentation may contribute to significant DMS formation, thus potentially affecting beer flavour.

DMSO reduction during fermentation was reported to be elevated at low fermentation temperatures [14, 20]. Low fermentation temperatures likewise diminish the diffusion rate of CO_2 bubbles, by which DMS is partly removed from the green beer [21].

It is also known that the species *Saccharomyces cerevisiae* reduces more DMSO than *Saccharomyces uvarum* [14, 20]. It should be mentioned that the taxonomic name of the lager yeast is now *Saccharomyces pastorianus* var. *carlsbergensis* instead of *Saccharomyces uvarum*, when the cited publications were released. Other factors promoting DMSO reduction are high pitching wort pH [2, 22], low free amino nitrogen levels [23] and high original gravity [14, 20].

Still, there are discrepancies concerning the role of DMSO as DMS precursor in beer. *Annes* [14] reported that lager yeasts were able to reduce DMSO up to 21 % and that the reduction was accompanied by significant DMS formation. By the addition of labelled DMSO to pitching wort it was shown that substantial amounts of DMS in the finished beers were originating from DMSO reduction [16, 17]. *Leemans* et al. [17] even stated that 80 percent of the total DMS present in beer was generated from spiked deuterated DMSO. On the contrary, *Dickenson* [20] reported that yeast is able of DMSO reduction, whereas the reduction has no big influence on final DMS levels in beer.

Bacterial contaminations of the family *Enterobacteriaceae* can also be a source of DMS in beer [2]. Some species, like *Escherichia coli* or *Rhodobacter sphaeroides* possess DMSO reductases using DMSO as electron acceptors in anaerobic growth [24], which may lead to a much higher DMS formation than by yeast. However, these species are rather unlikely to occur as fermentation contaminants.

This work will solely focus on DMSO reduction by beer yeast, which is controversially discussed regarding its relevance to final DMS levels in beer. The fate and behaviour of DMSO in the brewing process has not been investigated in detail. Regarding rising oil prices and ecological aspects, the brewing industry intends to reduce the amount of primary energy by partial substitution of the wort boiling process, mostly by wort heat holding and separated evaporation of DMS and other undesired volatiles [25, 26].

In comparison to the wort boiling process, DMS accumulates in the hot wort before an upfollowing desorption process is applied for its removal. There is no information available if DMS may be subject to oxidation in wort. DMS oxidation and accompanied DMSO formation would increase the risk of DMS formation during fermentation. Also, the extent to which different globally applied yeast strains are capable of DMSO reduction is mostly unknown.

The aim of this work was to track the levels of DMS and its precursors SMM and DMSO throughout the brewing process to obtain insights into potential interconversion reactions among these substances. Specifically, the effect of wort composition as well as wort boling and wort heat holding techniques were investigated. As mentioned before, top-fermenting yeast reduces more DMSO than bottom-fermenting yeast. It is likely that diverse genetic evolution during domestication of the yeast is a possible explanation. Recently, *Gonçalves* et al. [27] showed that top-fermenting yeast strains possess distinctive genomic signatures related to their domestication and that most top-fermenting *Saccharomyces cerevisiae* brewing strains belong to a major brewing strain cluster. Interestingly, some matched to other genetic clusters like the bread-, wine- or sake strain cluster. In this study we specifically selected top-fermenting strains heterogenously spread over different clusters to investigate their capability of DMSO reduction and DMS formation. The results of this work should serve for a better understanding of the role of DMSO on DMS formation and its potential contribution to the levels of DMS in beer.

2 Material and methods

2.1 Fate of DMS, SMM and DMSO during wort production

2.1.1 Closed mashing system

Since it is not clear if interconversion reactions of DMS and its precursors, for example DMS oxidation to DMSO, take place during mashing, the procedure was conducted in closed 2 L Duran® bottles (Schott AG, Mainz, Germany) to avoid DMS evaporation. The bottles were closed with open screw caps containing silicone/PTFE seals. The seals were perforated with a closable hole (diameter 7 mm) for sampling. 1800 g of double distilled ultrapure water (Milli-Q®) was introduced into the bottle. The bottle was heated to 63 °C in a water bath, which was mounted on a magnetic stirring device (3581200 C-MAG HS 7, IKA®-Werke GmbH & CO. KG, Staufen, Germany). 514.2 g of fine grist from Pilsner malt (Ireks GmbH, Kulmbach, Germany) were introduced into the bottle under magnetic stirring. The grist:water ratio was 1:3.5 and the headspace volume was minimized in order to prevent extensive DMS desorption. After dispersion, the mash was heated (1.5 °C/min) to 66 °C were it was incubated for 30 minutes. Afterwards, the temperature was increased to 72 °C, where a holding period of 20 minutes was carried out. For mashing-out, the temperature was increased to 78° C. After mashing-in (~5 minutes) as well as at the end of mashing samples were taken for the quantification of DMS, SMM and DMSO. ~ 10 g of mash were withdrawn using a 10 mL pipette, to which a tip with enlarged diameter (4 mm) was attached. The content was added into 40 mL of ice-cold ultrapure water in a Falcon® tube (50 mL), which was subsequently closed. The screwing thread of the tube was covered with 2 layers of PARAFILM®. The sample weight was recorded and the tubes were centrifuged at 1 °C and 7340 × g for 10 minutes. DMS, SMM and DMSO were analysed from the supernatant.

2.1.2 Open mashing system

Mashing in the brewing industry can be regarded as an open system as DMS is able to diffuse out of the mash into the atmosphere. Therefore we conducted mashing in a mashing bath (Bender & Hobein GmbH, Munich, Germany) with stainless steel beakers on which stainless steal caps were attached. The caps contained a hole (diameter ~ 10 mm) through which the stirrer (diameter ~ 4 mm) was connected to the stirring motor of the mashing bath. 350 g of double distilled ultrapure water were filled into the the beakers, which were heated to 63 °C. 100 g of fine grist (100 % Pilsner malt, Ireks GmbH, Kulmbach, Germany) were introduced under stirring. The mashing regime used was the same as decribed for the closed mashing system. Samples were taken as described before.

2.1.3 Wort boiling, rectification and wort heat holding

Wort boiling was carried out in an open 2L Duran® bottle. The bottle was filled with 1800 g of wort (original gravity of 12 °P), which was produced via the open mashing regime as described before. The bottle was covered with 2 layers of aluminium foil before it was placed on a combined heating / magnetic stirring device (3581200 C-MAG HS 7, IKA®-Werke GmbH & CO. KG, Staufen, Germany). At the beginning of boiling crushed hop pellets (Bravo, 15.3 % α-acids, Simon H. Steiner Hopfen GmbH, Mainburg, Germany) were added to acchieve 25 bitter units in the resulting cast-out wort. Samples were taken at the beginning of boiling, after 30-, 60-, 90- and 120 minutes. For sampling, 20 mL of wort were injected into 30 mL of ice-cold double-distilled ultrapure water. Evaporation was determined gravimetrically and via assessment of the original gravity at each sampling step.

2.1.4 Rectification

To minimize the effect of water evaporation, the same boiling procedure was conducted with a 450 mm long DURAN® Vigreux column (VWR GmbH, Darmstadt, Germany), which was mounted onto the neck of the bottle. Samples were taken at the beginning and after 90 minutes of boiling as described under wort boiling.

2.1.5 Wort heat holding

To exclude water as well as DMS evaporation, 50 g of kettle-full wort (produced as described under wort boiling) were separated into 50 mL DURAN® bottles (Schott AG, Mainz, Germany) and were tightly closed using screw caps with PTFE coated silicone seals. The bottles were placed in a water bath, which was adjusted to 99.9 °C. After reaching of 98 °C inside the bottles, the heat holding time was started. Samples were taken after 0-, 30-, 90- and 120 minutes. The bottles were cooled down stepwise with tap water (2 minutes) and subsequently ice-cold water before they were analysed for DMS, SMM and DMSO.

2.1.6 Fermentation

Wort was produced in an open mashing system. After mashing, the wort was cooled down to 20 °C and was filtered over cellulose filters (Whatman, grade 597 ½, GE healthcare, Buckinghamshire, Great Britain). The original gravity of the wort was adjusted to 12 °P with double distilled ultrapure water. Boiling was conducted for 60 minutes as described before. After boiling, the wort was stirred gently and the bottles were placed in a water bath (95 °C) for 20 minutes. After sedimentation of the hot trub the wort was decanted into sterile 2 L Duran® bottles and was cooled down to 20 °C, were the extract was adjusted again to 12 °P. 3 drops of autoclaved, diluted (8 % (v/v)) antifoam reagent (Antifoam A Concentrate, Sigma Aldrich, St. Louis, USA) were added to the wort. The wort was then stripped with sterile air for 30 minutes in order to achieve a dissolved O_2 concentration of ~8 mg/L. Furthermore the air stripping quantitatively removed the DMS from the pitching wort. We used this procedure to ascertain the yeast-related formation of DMS during fermentation. After stripping, 40 g of pitching wort were filled into 50 mL Falcon® tubes. The screwing thread of the tubes were covered with 2 layers of PARAFILM®. The respective yeast suspension was added to acchieve a final cell count of 2.5×10^7 cells/mL. Fermentation locks containing 15 mL of ethanol solution (70 % (v/v)) were installed onto the tubes. Fermentation was carried in the Falcon® tubes at whether 12 °C for lager yeast or 18 °C for top-fermenting yeast in an incubator. Fermentation was stopped after achieving the yeast specific final degree of attenuation. The fermentation locks were removed and the tubes were tightly closed with screw caps before they were centriguged as described before. The supernatant was used for beer analysis (extract, pH, ethanol conentration) as well as for the determination of the DMS- and DMSO concentration.

2.1.7 Ultrasonic treatment of yeast

After fermentation and centrifugation the yeast pellet was resuspended in 10 g of sterile double-distilled ultrapure water. The mixture was incubated for 20 minutes in an unltrasonic bath (Sonorex Digital DK 1028 P, Bandelin electronic GmbH und Co. KG, Berlin, Germany) maintaining a temperature of 4 °C. After the treatment, the supension was centrifuged and the concentration of DMSO was analysed in the supernatant.

2.1.8 Impact of wort composition on DMSO reduction during fermentation

The impact of different wort composition was investigated by varying the grist composition with different malt types. The grist compositions were as follows: 100 % Pilsner Malt (A), 80 % Pilsner malt + 20 % Melanoidin malt (Weyermann® Malzfabrik, Bamberg, Germany) (B), 80 % Pilsner malt + 20 % CARAFA® Type II malt (C) (Weyermann® Malzfabrik, Bamberg, Germany). Furthermore 70 % Pilsner malt was combined with 30 % of unmalted barley (Quench) (D). Wort production and fermentation was carried out as described under fermentation. Fermentation temperature was 12 °C.

2.1.9 Screening of top-fermenting yeast strains on DMSO reduction during fermentation

In this section the extent to which top fermenting yeast strains are capable of DMSO reduction and DMS formation was aimed to be investigated. Yeast strains were selected based on their genetic diversity as shown by whole genome sequencing by *Gonçalves* et al. [27]. The strains chosen from the main beer cluster were TUM 149 (German wheat beer yeast), TUM 177 (Altbier / Kölsch yeast) and TUM 210 (English ale yeast). Furthermore TUM 511 (American ale yeast from the wine cluster), TUM 480 (African beer yeast from the bread cluster) and TUM 68 (German wheat beer yeast from the sake cluster) were selected. Wort production and fermentation were carried out as described before, whereas the fermentation temperature was set to 18 °C in this trial.

2.1.10 Time course of DMSO and DMS during fermentation

The fermentation was carried out as described in the screening trial. The yeast strains selected for the demonstration of DMSO reduction and DMS formation were TUM 149 (top-fermenting, 18 °C) and TUM 34/70 (bottom-fermenting, 12 °C). On each fermentation day, sample tubes were analysed for Extract, DMS and DMSO.

2.1.11 Quantification of DMS, SMM and DMSO

Quantification of DMS and DMSO was conducted using headspace gas chromatography combined with pulsed flame photometric detection (HS-GC-PFPD) according to a previously described method [11]. The detection limit for DMS of the applied system was 6 µg/L. SMM was quantified according to *White* and *Wainwright* [10]. The data of SMM and DMSO are presented in DMS equivalents (SMM*, DMSO*).

2.1.12 Wort and beer analysis

Extract (2.19.3), color (3.1.2), pH (3.1.3), apparent degree of attenuation (2.9.5), free amino nitrogen (2.8.4.1.1) and ethanol (2.10.5) were analysed according to MEBAK guidelines [28].

2.1.13 Quantification of amino acids

Amino acids (alanine, glycine, valine, leucine, isoleucine, threonine, serine, proline, aspartic acid, glutamic acid, methionine, phenyl alanine, lysine, histidine, tyrosine) were analysed in the pitching worts as described by *Wietstock* et al. [29].

2.1.14 Microbiological analyses

To exclude that microbial contaminations are responsible for DMSO reduction and DMS formation during fermentation, we analysed yeast and beer after fermentation on contaminations by bacteria and wild yeast according to MEBAK guidelines (10.12.2) [30]. After centrifugation of the green beer, approximately 2–3 g of yeast remained in the tube, which was resuspended in 10 mL autoclaved, double distilled, ultrapure water. Abundance of *Lactobacilli* and *Pediococci* was tested using VLB-S7S-Agar. Lysin-Agar was used to test the presence of non-*Saccharomyces* yeast. VRBD-Agar was used to assure that *Enterobacteriaceae* were not present in wort (anaerobic incubation for 24 h at 28 °C).

2.2.15 Statistics and data analysis

All experiments of this work have been carried out in triplicate. The mean values (n = 3) of the DMSO concentrations were compared using the Tukey Honest Significance Difference (HSD) test at a confidence level of 95 %. The analyses were conducted with Microsoft XLstat (version 2014.5.03, Addinsoft, USA).

3 Results and discussion

During malt kilning, SMM is largely degraded to L-homoserine and DMS, whereas the latter is partially oxidized to DMSO. Substantial amounts of DMS and its precursors remain in the malt and are introduced into the brewing process via mashing. Yet, it is not entirely clear to which extent interconversion reactions of DMS and its precursors, especially DMSO, take place during wort production. We initially investigated the behaviour of DMS, SMM and DMSO in a closed mashing system in order to prevent DMS desorption. We used this approach to more precisely balance the amounts of DMS and its precursors before and after mashing (Fig. 1).

In the open mashing system (Fig. 1 a) the DMS concentration was

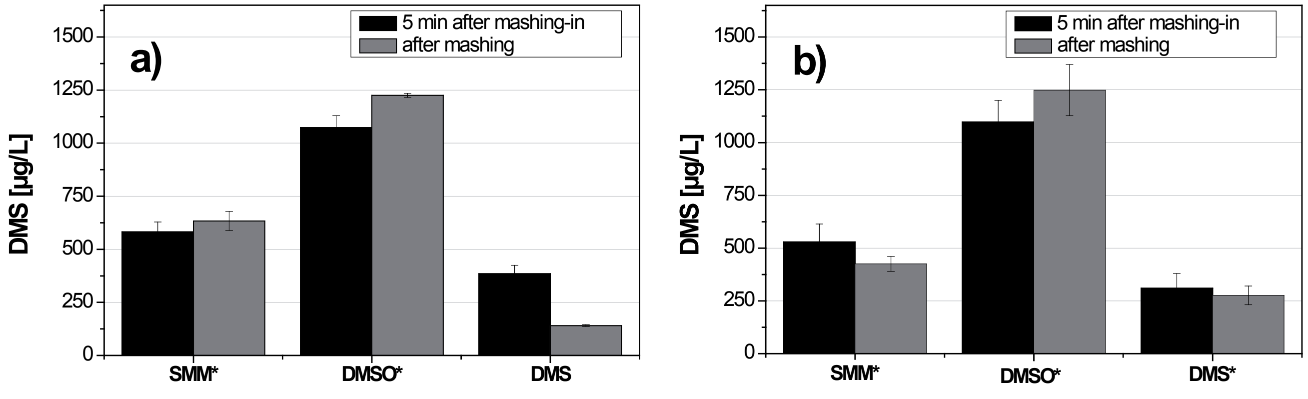

Fig. 1 **Levels of SMM, DMSO and DMS before and after mashing in an open mashing system (a) and in a closed mashing system (b). Results are presented as mean values (n = 3) ± standard deviation**

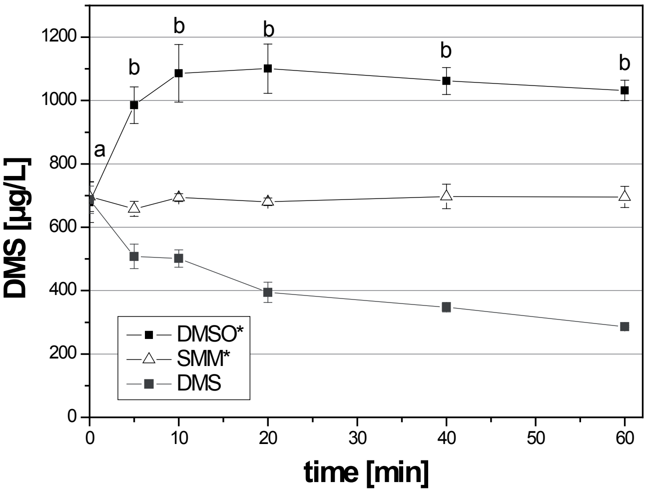

Fig. 2 Levels of SMM, DMSO and DMS during isothermal mashing at 62 °C in an open mashing system. Results are presented as mean values (n = 3) ± standard deviation. Different letters above the DMSO data indicate significant differences according to the Tukey HSD test

significantly reduced (~63 %), probably as a result of evaporation. In the closed mashing system (Fig. 1 b) no significant change of the DMS concentration was observed. A similar observation was made by *Scheuren* et al. [31].

Even though higher levels of DMS were accumulated in the mash of the closed system, the DMSO increase was similar to the open mashing system. As the concentration of SMM remained unchanged during mashing, we propose that DMS was not subject to significant oxidation to DMSO in the course of the mashing process. The abundance of reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radicals or fatty acid hydroperoxides during mashing is well known [32]. Recently, it was reported, that hydrogen peroxide and hydroxyl radicals are capable of DMS oxidation and DMSO formation [33]. However, DMS seemed to be not affected by potentially abundant ROS in this trial. Mash consists of miscellaneous biomolecules, which are susceptible to oxidation. Therefore, it is likely that for example fatty acids, polyphenols or proteins may have been preferably oxidized over DMS, probably also related to their higher water solubility and accompanied higher availability for ROS. Still, from the present results, we cannot exlude that any kind of red-ox reactions of DMS-DMSO did occur, even though they have not been observable within the experimental data. In the next experiment, we monitored the course of DMS and its precursors in an open mashing system at 62 °C.

Unsurprisingly, DMS continuously decreased in the course of isothermal mashing at 62 °C, which is most likely a result of the high relative volatility of DMS [34] and its low boiling point (37 °C). In the first 5 minutes the biggest drop of the DMS concentration was detected. This observation was probably evoked by the DMS concentration gradient and the rapid diffusion of DMS out of the mash into the headspace of the mashing beaker. This process is supported by the evaporation of water. The headspace volume accounted for ~20 % of the total beaker volume. The further smaller decline of DMS during mashing may be explained by water saturation and decreasing DMS concentration gradient in the headspace. Diffusion of DMS into the atmosphere was probably decelerated by the small hole diameter of the beaker cap, through which also the stirring staff was inserted. However, at the end of mashing ~58 % of the total DMS had vanished from the system. The DMSO concentration was significantly increased after mashing-in (1 min) and reached its maximum concentration after a mashing time of 10 minutes. In the further course of mashing the DMSO concentration remained approximately constant. Refering to the aforementioned diffusion behaviour of DMS, the increase of DMSO during mashing is most likely related to an extraction process from the grist particles into the aqueous mash phase. The observed slower extraction behaviour, compared to SMM, can potentially be explained by the amphiphilicity of DMSO, which makes it capable of interacting with proteins [35] or amylose helices [36]. It is also likely that DMSO interacts with hemicelluloses. In such case DMSO extraction behaviour would probably correlate with malt modifcation.

3.1 Wort boiling, rectification and heat holding

The behaviour of DMS, SMM and DMSO in wort boiling and -heat holding is illustrated in figure 3. During atmospheric wort boiling, DMS was evaporated below the detection limit within 30 minutes, most certainly because of its high relative volatility [34]. SMM was thermally degraded following an exponential decline (dotted line,

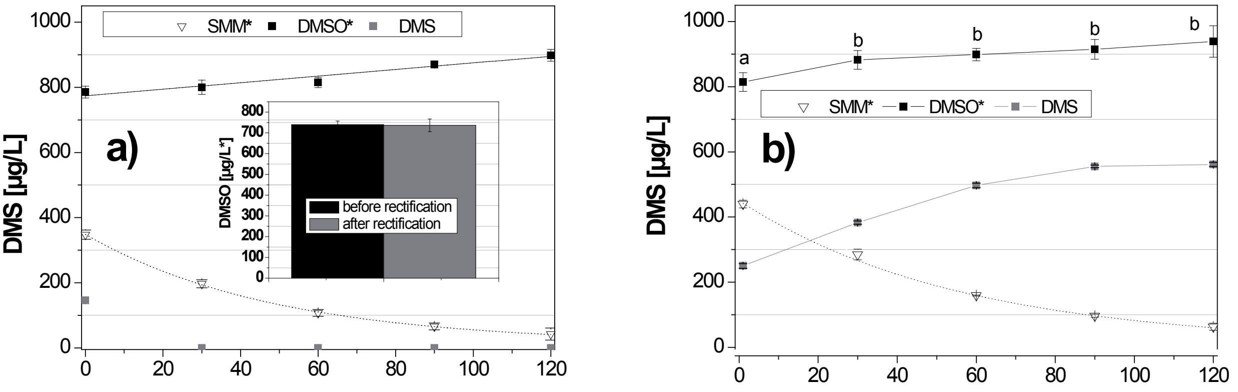

Fig. 3 Behaviour of DMS, SMM and DMSO during atmospheric wort boiling (a) inserted graphic DMSO concentration before- and after boiling with a rectification column) and wort heat holding (b). Results are presented as mean values (n = 3) ± standard deviation. Different letters in b) indicate significant differences according to the Tukey HSD test

R^2: 0.999). From the slope of the exponential fit, the 1st order rate constant was determined to be $k = 0.021\ min^{-1}$, which is consistent with the half-life of SMM under similar conditions determined by *Dickenson* [6]. The DMSO concentration increased linearly with boiling time. As the DMSO levels were still rising while no DMS was detectable anymore, DMS oxidation is very unlikely to be the source of the DMSO increase. The total evaporation of water was ~16 %. As the boiling point of DMSO (189 °C) is relatively high, it is supposed that DMSO was concentrated in the progression of wort boiling as a result of the loss of water. To test this assumption, a further wort boiling experiment was conducted in which the evaporation was minimised to 0.2 % by the installation of a Vigreux-column (rectification boiling). The inserted diagram in figure 3 a shows that the concenctration of DMSO before and after rectification boiling did not change, which verifies the previous assumption. From the wort boiling trials we conclude that DMSO was not subject of significant biochemical alteration, whether in an oxidative nor reductive way.

During wort heat holding, the residence time of DMS in the hot wort is elevated as a result of the missing evaporation of water. To test the extent to which DMS oxidation occurs during heat holding, wort was separated into closed Duran® bottles which were incubated at 99.9 °C (Fig. 3 b). Again, an exponential decline of SMM was observed ($R^2 = 0.998$). The rate constant was determined to $0.017\ min^{-1}$, which is smaller than the rate constant in the atmospheric boiling trial. This was probably related to the fact, that the wort temperature in the bottles did not exceed 98 °C. As expected, the DMS concentration increased during heat holding as a result of the missing evaporation. In this context the DMSO concentration increased significantly within 30 minutes, whereas no significant increase was observed in the further progress of wort heat holding. It is supposed that the DMSO increase was evoked by DMS oxidation. Though, after 120 minutes the DMSO increase was solely ~15 %, which suggest a certain stability of DMS against oxidation under the applied conditions. According to Henry's law, the solubility of oxygen above 95 °C is below 1 mg/L. The fact that significant DMS oxidation only took place in the first period of heat holding may have been related to the higher solubility of oxygen at lower temperatures. During heating of the wort to ~98 °C the dissolved oxygen may have been reduced to ROS, such as hydrogen peroxide or hydroxyl radicals, for example. Recently, it was reported that molecular oxygen was not capable of DMS oxidation [33], therefore we proposed that ROS formation was responsible for DMS oxidation. Again, the relative low amount of DMSO formation can probably explained by the fact that ROS likewise reacts with miscellaneous wort components. As discussed before, these reactions would have competed with DMS for ROS and eventually proceed at much higher reaction rates. Again, the low water solubility of DMS may additionaly have diminished its availabity for ROS and concomitant DMSO formation.

Table 1 **Analytical data of pitching worts (A: 100 % Pilsner malt; B: 80 % Pilsner malt, 20 % Melanoidin malt; C: 80 % Pilsner malt, 20 % Carafa II malt; 70 %; D: Pilsner malt, 30 % barley)**

wort	A	B	C	D
FAN [mg/L]	166	116	129	184
Amino acids [mg/L]	954	817	474	665
Methionine [mg/L]	21	15	8	11

Table 2 **Analytical data of beer produced from different pitching worts (12 °P) (A: 100 % Pilsner malt; B: 80 % Pilsner malt, 20 % Melanoidin malt; C: 80 % Pilsner malt, 20 % Carafa II malt; 70 %; D: Pilsner malt, 30 % barley)**

	Apparent degree of Attenuation	pH	Ethanol (v/v)
	[%]	–	[%]
A	77.4	4.27	5.7
B	74.8	4.24	5.5
C	80.2	4.31	6.0
D	74.8	4.21	5.5

3.2 DMSO reduction during fermentation

In all fermentation trials of this work, we did not find any bacteria or wild yeast contaminations relevant for DMS formation, which was the premise for the analyses of DMS and DMSO. Also, as a consequence of the wort stripping described in the material and methods section, there was no DMS detectable in the pitching worts. This was also crucial to evaluate the contribution of the yeast to DMS formation. To assess the DMSO reduction by yeast during fermentation and to test if this pathway influences the DMS levels in beer, a 100 % Pilsner malt wort was fermented with lager strain TUM 34/70 at 12 °C. Additionally, worts with different grist compositions (A: 100 % Pilsner malt; B: 80 % Pilsner malt, 20 % Melanoidin malt; C: 80 % Pilsner malt, 20 % Carafa II malt; D: 70 % Pilsner malt, 30 % barley) were fermented under the same conditions to examine the impact of wort composition on DMS formation during fermentation.

The levels of FAN and amino acids, especially methionine, were analysed in the respective pitching worts as they are known to have a decisive impact on the reduction of DMSO by yeast [22, 23]. The data are presented in table 1.

Fermentation was stopped after 8 days of fermentation as the apparent final degree of attenuation of TUM 34/70 (>74 %) was reached for all fermentations. Some parameters of the respective beer analysis are presented in table 2. In figure 4 a, the levels of DMSO before (after pitching) and after fermentation are illustrated. Noticable is the significant difference of the DMSO concentration among the pitching worts. These differences can be explained by the different DMSO concentrations in the malt types, from which the worts were produced from.

Though, no significant reduction of the DMSO concentration was found for each of the fermented worts, implying that there was also no direct correlation between DMSO reduction and the levels of FAN and methionine in the pitching wort. The levels of DMS after fermentation are shown in Figure 4 b. As described in the material & methods section, we stripped the pitching wort with sterile air for the quantitative removal of DMS, therefore the data depict the formation of DMS during fermentation. In all fermentations, significant formation of DMS took place. The increase of DMS varied between 8–14 µg/L whereas no significant difference was found among the wort compositions. In this study, we did not find

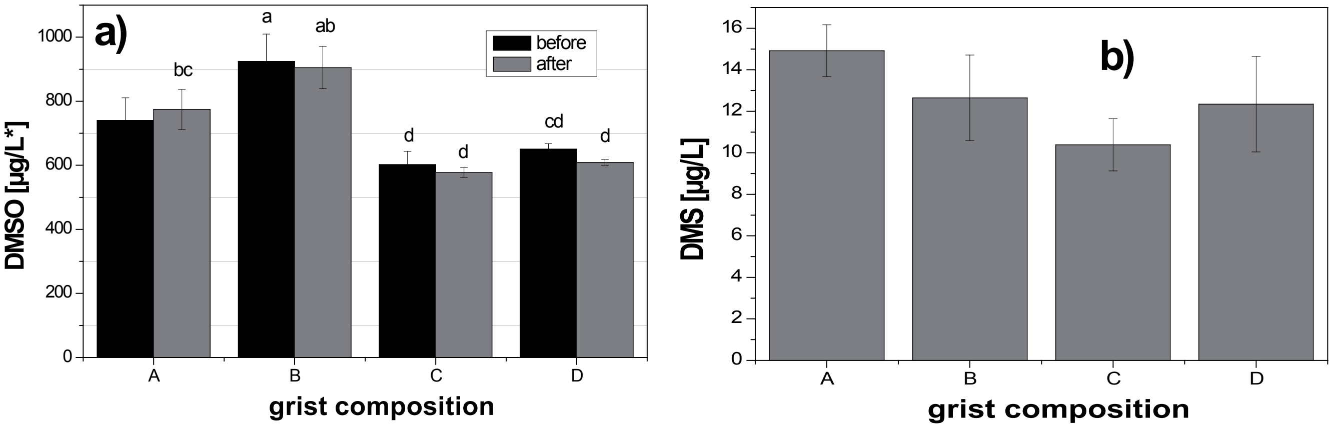

Fig. 4 Levels of DMSO before and after fermentation (a) and DMS increase during fermentation (b) with TUM 34/70 at 12 °C. (A: 100 % Pilsner malt; B: 80 % Pilsner malt, 20 % Melanoidin malt; C: 80 % Pilsner malt, 20 % Carafa II malt; 70 %; D: Pilsner malt, 30 % barley). Results are presented as mean values (n = 3) ± standard deviation. Different letters in a) indicate significant differences according to the Tukey HSD test.

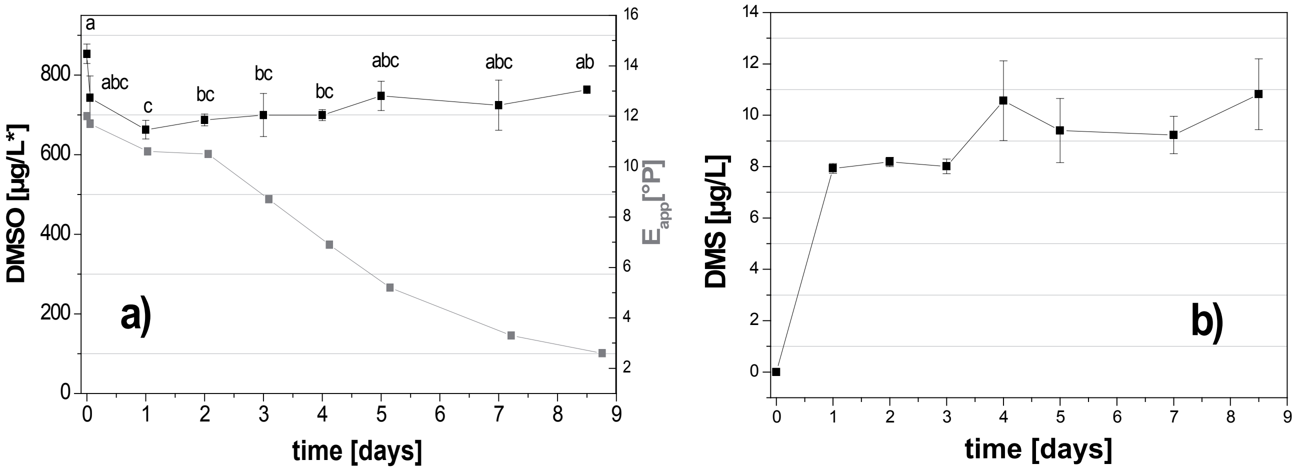

Fig. 5 Behaviour DMSO and apparent extract (a) and DMS increase during fermentation (b) with TUM 34/70 at 12 °C. Results are presented as mean values (n = 3) ± standard deviation. Different letters in a) indicate significant differences according to the Tukey HSD test

correlations between FAN, DMSO reduction and DMS formation. *Gibson* et al. [23] reported that DMSO reduction by lager yeast was declined from ~70 % to ~20–30 %, when FAN was increased from 70 mg/L to 3.5 g/L. In our study the levels of FAN varied between 116–184 mg/L, which is representative for pitching worts. Though, the relative small differences compared to the investigations of *Gibson* et al. [23] were probably insufficient to account for different rates of DMSO reduction.

The worts with 20 % Carafa malt proportion (wort C) and 30 % barley proportion (wort D) produced similar levels of DMS even though the initial DMSO concentrations in these worts were lower. In this case, a possible explanation may be found in the lower levels of amino acids, especially methionine (Table 1). It is also likely that other inhibitors, especially methionine sulfoxide (MetSO) [23] have affected the DMS formation in this investigation. It was reported that the inhibitory effect of MetSO is even higher than the effect of methionine [37]. Unfortunately, whether from this study or others, there are no data available on the concentration of MetSO in different malt types and the corresponding pitching worts. From the results of figure 4 DMSO can only be assumed to be the source of DMS in the resulting beers.

To ascertain the period of DMS formation, the fate of DMSO (a) and DMS (b) were recorded daily during fermentation of wort A with TUM 34/70 at 12 °C (Fig. 5). The first data point in the diagram presents the concentration of DMSO in the pitching wort. After yeast addition and centrifugation, the DMSO concentration was significantly decreased (~13 %). The processing time until DMSO analysis was approximately 30 minutes. These data suggest that a part of DMSO was quickly absorbed by the yeast cells after pitching. In the further course of fermentation the DMSO concentration tended to increase slightly until the end of fermentation. At the end of fermentation, the apparent degree of attenuation was 78.5 % and ~11 % of the DMSO content was reduced.

A significant DMS increase was observed in the first period of fermentation, whereas the main DMS formation of ~8 µg/L took place during the 1st day of fermentation. The DMS concentration remained approximately constant for another 2 days, before it was slightly increased to 9–12 µg/L, which ended up to be final DMS concentration in the green beer. On fermentation day 4, we removed all of the supernatant from the centrifuged sample and resuspended the remaining yeast pellet in sterile, ultrapure water. The suspension was exposed to an ultrasonic treatment for cell

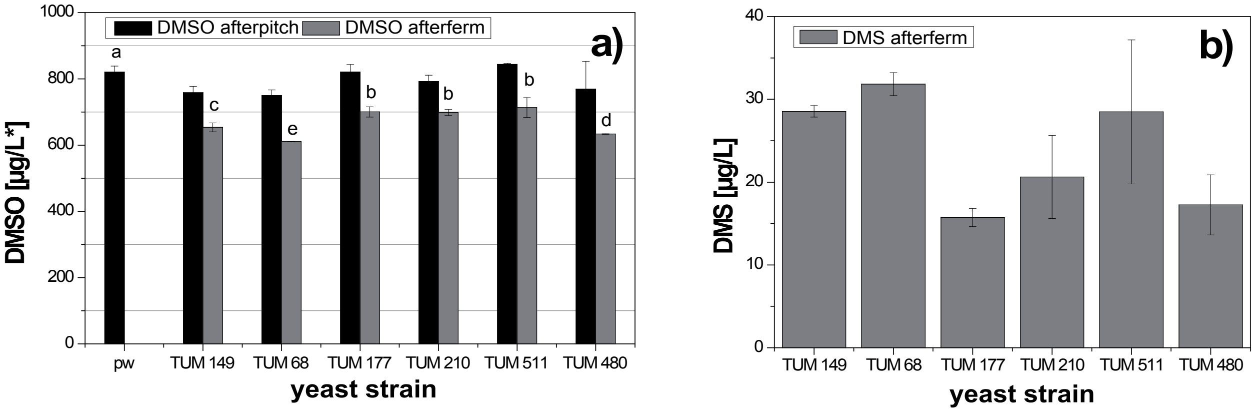

Fig. 6 **Reduction of DMSO (a) and DMS increaseduring fermentation (b) by top-fermenting yeast strains. pw: pitching wort; afterpitch: after pitching; after ferm: after fermentation. Results are presented as mean values (n = 3) ± standard deviation. Different letters above DMSO data of pitching wort and after fermentation indicate significant differences according to the Tukey HSD test**

lysis. After the treatment the dispersion was centrifuged again. The supernatant was then analysed for DMSO. The analysis revealed that 104 ± 10 µg/L of DMSO were released from the yeast by this treatment.

Leekumjorn and *Sum* [38] reported that DMSO can accumulate in the intermembrane space of phospholipid bilayers as a result of amphiphilic interactions. It is therefore likely that DMSO had accumulated in the yeast plasma membrane. The data further indicate that the small increase of DMSO in the course of fermentation may be related to yeast autolysis and concomitant release of DMSO into the green beer. The difference between the sum of DMSO in the supernatant (~700 µg/L) and the release after ultrasonic treatment to the levels of DMSO in the pitching wort (~854 µg/L) is ~50 µg/L, suggesting that also 50 µg/L of DMS have been generated. The discrepancy to the levels detected in the green beer (~10 µL) may have been removed from the green beer via CO_2 desorption [21]. Still, the results demonstrate that the amount of DMSO taken up by the yeast in the 1st period of fermentation is not strictly related to its biochemical reduction to DMS. It is also likely that yeast accumulates DMSO in the course of propagation and therefore also yeast management (propagation, re-pitching) seems to play a role in DMS formation during fermentation. In summary of this experiment we propose therefore, that a minor part DMSO was reduced to DMS by TUM 34/70, which was responsible for DMS formation. The observations may also explain the slight increases of DMSO in the previous trial (Fig. 4 a, e.g. grist composition A), where significant DMS formation was observed likewise.

As described in the introduction, top-fermenting yeast was declared to produce more DMS during fermentation than bottom-fermenting yeast. The extent to which genetically diverse top-fermenting yeasts are capable of DMSO reduction and DMS formation during fermentation of wort A is shown in figure 6.

The degree of attenuation of all fermentations was >74 % and the pH values of the resulting beers varied between 4.2–4.3.

All tested top fermenting yeast strains were able to significantly reduce the DMSO content in the course of fermentation. Highest DMSO reductions were found for TUM 68 from the sake cluster (~26 %), the African beer strain TUM 480 from the bread cluster (~23 %) and the German wheat beer strain TUM 149 from the main beer cluster (~20 %), which were significantly different from each other. The Kölsch/Alt strain TUM 177, the English ale strain TUM 210 (both ~15 %) and the American ale strain from the wine cluster TUM 511 (~13 %) reduced less DMSO, but there were no significant differences among the latter strains. Interestingly, TUM 149 and TUM 68, which are widely applied for the production of wheat beer reduced more DMSO after pitching compared to the other strains. TUM 68 reduced more DMSO than TUM 149, which is in accordance to the respective increase in the DMS concentration. Also, TUM 177, used for the production of “Alt” and “Kölsch” reduced less DMSO and consequently generated less DMS. Unfortunately, the relatively high standard deviations in the DMS concentrations, especially in the fermentations with TUM 210 and TUM 511 impeded a statistical comparison of DMS formation among the yeast strains. However, in each fermentation significant DMSO reduction was accompanied by a significant increase of DMS (Fig. 6 b). Highest levels were found for TUM 68 (~32 µg/L) and TUM 149 (~29 µg/L). TUM 210 (~21 %), TUM 177 (~16 µg/L) and TUM 480 (~17 µg/L) formed less DMS.

The DMS levels found in the beers were much lower than the overall DMSO reduction, which may, again, have been evoked by CO_2 desorption. As it was shown for the lager strain TUM 34/70 another explanation may be that the DMSO uptake by the yeast is not necessarily followed by its reduction to DMS.

The screening of the top-fermenting yeast strains shows that there was no huge difference in DMSO reduction among the tested top-fermenting strains. It is likely that MSRA is highly expressed in each strain as it is an ubiquitously abundant enzyme in pro- and eukaryotic cells, which is essential for the recovery of methionine.

The higher DMSO reduction and DMS formation by the top-fermenting strains is in agreement with the literature [2, 9]. TUM 149 was further applied to monitor the DMSO reduction and DMS formation over time (Fig. 7).

As observed by fermentation with TUM 34/70 a fast absorption of DMSO into the yeast was observed, whereas it was less pronounced in TUM 149. Significant DMSO reduction only took place in the first period of fermentation, while DMS formation reached its

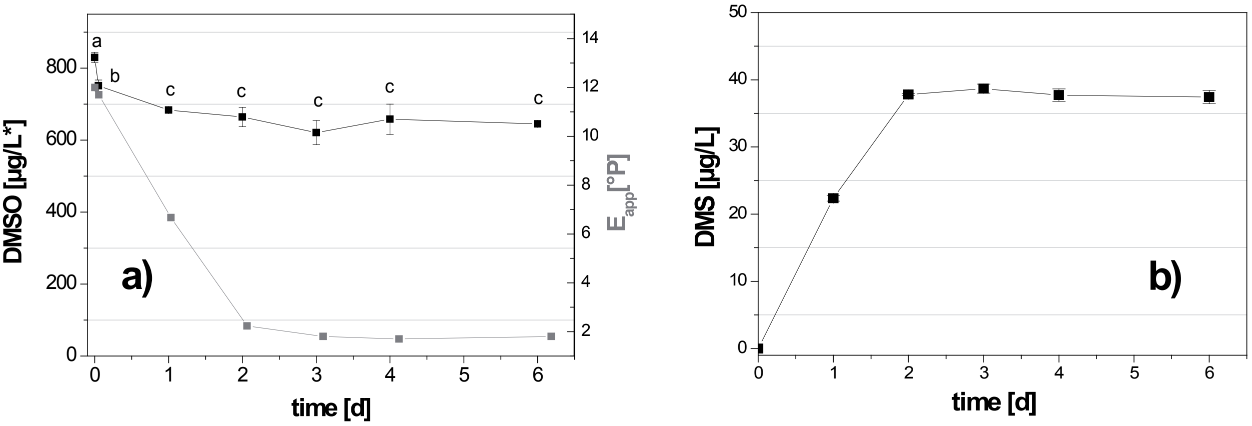

Fig. 7 **Fate of DMSO and apparent extract (a) and DMS (b) during fermentation with TUM 149 at 18 °C. Results are presented as mean values (n = 3) ± standard deviation. Different letters in a) indicate significant differences according to the Tukey HSD test**

maximum (~38 µg/L) after the 2nd day of fermentation. At the end of fermentation the apparent degree of attenuation was 85.4 % and ~19 % of the DMSO content was reduced. The experiment confirms that the top-fermenting yeast strain TUM 149 was capable of significant DMSO reduction and elevated DMS formation during fermentation.

4 Conclusion/Summary

In this work the behaviour of DMS and its precursors SMM and DMSO was investigated in the brewing process.

During mashing SMM and DMSO were extracted quickly but were not subject of significant biochemical changes thereafter. ~60 % of the DMS was lost by evaporation. As expected, SMM was thermally degraded during wort boiling and wort heat holding following a first order mechanism. DMSO was concentrated during wort boiling owing to the loss of water, whereas it was not subject to significant biochemical alterations. However, during wort heat holding, when evaporation was excluded, significant amounts of DMS were oxidized to form DMSO. Though, the extent was below 20 % and the contribution of DMS oxidation to final DMSO levels in the pitching wort is rather small. These findings indicate that the wort production has only a minor influence on the levels of DMSO in the pitching wort and that more auspicious approaches for DMSO minimization are rather to be found in the malting process. However, DMSO concentrations of ~800 µg/L were quantified in the pitching worts (100 % Pilsner malt), which is around 15 times higher than the DMS flavour threshold in lager beers, for example. During fermentation TUM 34/70 generated significant amounts of DMS even though DMSO reduction was insignificant. The yeast quickly absorbed a part of the DMSO whereas it was not recovered entirely as DMS. Besides CO_2 desorption, accumulation of DMSO in the plasma membrane may further elucidate this observation.

In biology, DMSO is widely applied as cryoprotectant for enzymes and cell cultures [38, 39]. In this context Leekumjorn and Sum [38] showed that DMSO diffuses fastly into phospholipid bilayers and that significant amounts accumulate in the intermembrane space. These findings are probably transferable to the observations of the present study and may explain the DMSO release from yeast evoked by the ultrasonic treatment. The enzymes responsible for DMSO reduction (MSRA) are located in the cytosol and in the mitochondria of the yeast cell, whereas they are not present in the yeast plasma membrane [40]. It is therefore supposed that higher levels of DMSO were recovered from the intermembrane space of the yeast plasma membrane than from the cytosol or intracellular compartments. This would also explain the relative low enzymatic formation of DMS by yeast.

In in *E. coli* for example, the DMSO reductase (DMSORA) is bound to the plasma membrane [39], meaning that the bioavailability of DMSO for the enzyme is higher. This seems to be further explanation why such bacteria are capable of a much higher DMSO reduction and DMS formation than yeast.

The increase of DMS during fermentation evoked by the bottom-fermenting yeast TUM 34/70 was ~8-15 µg/L, whereas the top-fermenting yeasts produced much more DMS (~15–38 µg/L). It is also very likely that another part of the generated DMS was removed from the green beer by CO_2 desorption [21]. The DMS concentration found in the beers were below the flavour threshold, especially regarding bottom-fermented beers. The DMS increase during fermentation by the bottom fermenting TUM 34/70 does certainly not evoke an off-flavour impression. However, the contribution of DMSO reduction to the levels of DMS can be estimated as significant and thus, potentially affects beer flavour.

Even though much higher levels of DMS were found in the top-fermented beers, it is important to mention that these beers are generally less sensitive towards DMS off-flavours as a result of masking effects by other aroma compounds, like esters or phenolic aroma compounds.

However, in this study, there was no DMS detectable in the pitching worts, which is generally not the case in the brewing industry. Pitching wort DMS levels can be increased by improper wort management, for example insufficient degradation of SMM during wort boiling followed by extensive re-formation of DMS in the whirlpool. In such cases, a formation of DMS during fermentation may be of high relevance to beer flavour.

In this work DMS formation during fermentation was investigated in

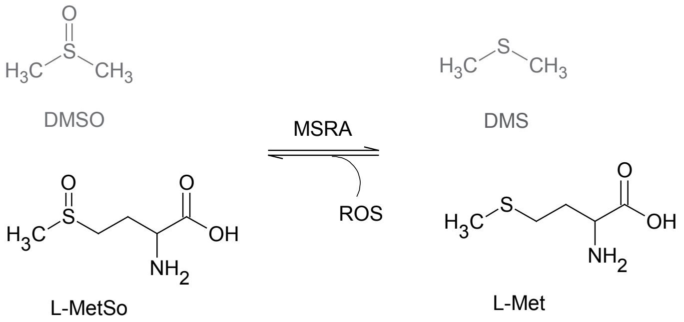

Fig. 8 Proposed mechanism of DMSO reduction and DMS formation by yeast based on literature findings [18,19]

cylindro-conical tubes in small scale fermentations. Fermentation tanks applied by the brewing industry reach heights of up to ~20 m. The upscale factor would be ~200. The biggest difference is most certainly the higher hydrostatic pressure, which leads to a higher CO_2 dissolution. This consequently diminishes DMS desorption. Therefore, DMS formation may eventually be of higher relevance in bigger tanks or even under application of pressure during fermentation. However, more investigations have to be carried out in industrial scales combined with sensory analyses to further evaluate the contribution of fermentation derived DMS formation on the beer flavour.

In this work, the differences in DMSO reduction among the top-fermenting yeasts (~13–26 %) were rather small and did not directly correlate with genetic diversity and domestication clusters. The enzyme, responsible for DMSO reduction is actually a methionine sufoxide reductase (MSRA). Such enzymes can be found ubiquitously in nature. MSRA are present among mammals, yeast and bacteria serving as a defense system against oxidative stress [41]. They can repair oxidative damage (e.g. by ROS) via reduction of the sulfoxide group. To some extent all aerobic cells are exposed to oxidative stress in their environment. We believe that MSRA is equally distributed and expressed among the different yeast strains investigated in this work as the DMSO reduction in yeast is a side-effect of the action of MSRA (Fig. 8).

The fact that DMSO reduction by yeast is controlled by FAN- and especially methionine and MetSO concentration and that MSRA are located in the yeast mitochondria indicate a correlation to mitochondrial activity, which was also shown by *Samp* et al. [37]. Mitochondrial activity as well as FAN utilization proceeds under aerobic growth conditions early during fermentation. This would explain why DMSO reduction and DMS formation in this work was also observed in the early stages of fermentation. The maximum of DMSO reduction in this work was ~26 %, meaning that relatively high amounts of DMSO are recovered in the final beer. To our knowledge, the behaviour of DMSO during beer storage is unknown and needs further research. As DMSO contributed significantly to the final DMS concentration in beer, we suggest that maltsters and brewers should consider DMSO as an additional DMS precursor.

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5 Literature

1. Anderson, R. J.; Clapperton, J. F.; Crabb, D. and Hudson, J. R.: Dimethyl Sulphide as a Feature of Lager Flavour, J. Inst. Brew., **81** (1975), no. 3, pp. 208-213.
2. Anness, B. J. and Bamforth, C. W.: Dimethyl Sulphide – a Review, J. Inst. Brew., **88** (1982), no. 4, pp. 244-252.
3. Hanke, S.; Ditz, M.; Back, W.; Krottenthaler, M. and Becker, T.: Influence of Ethyl Acetate, Isoamyl Acetate and Linalool on off-Flavour Perception in Beer, BrewingScience – Monatsschrift für Brauwissenschaft, **63** (2010), no. 7, pp. 94-99.
4. Dethier, M.; De Jaeger, B.; Barszczak, E. and Dufour, J.-P.: In Vivo and In Vitro Investigations of the Synthesis of S-Methylmethionine during Barley Germination, J. Am. Soc. Brew. Chem., **49** (1991), no. 31.
5. Hysert, D. W.; Weaver, L. and Morrison, W.: The Origin and Control of Dimethyl Sulfide and Its Precursor in Malt, MBAA TQ, **17** (1980), no. 1, pp. 34-43.
6. Dickenson, C. J.: The Relationship of Dimethyl Sulphide Levels in Malt, Wort and Beer. J. Inst. Brew., **85** (1979), no. 4, pp. 235-239.
7. Scheuren, H.; Tippmann, J.; Methner, F.-J. and Sommer, K.: Decomposition Kinetics of Dimethyl Sulphide: Decomposition Kinetics of Dimethyl Sulphide, J. Inst. Brew., **120** (2014), no. 4, pp. 474-476.
8. Yang, B.; Schwarz, P. and Horseley, R.: Factors Involved in the Formation of Two Precursors of Dimethylsulfide During Malting, J. Am. Soc. Brew. Chem., **56** (1998), no. 3, pp. 85-92.
9. Anness, B. J.; Bamforth, C. W. and Wainwright, T.: The Measurement of Dimethyl Sulphoxide in Barley and Malt and Its Reduction to Dimethyl Sulphide by Yeast, J. Inst. Brew., **85** (1979), no. 6, pp. 346-349.
10. White, F. H. and Wainwright, T.: The Measurement of Dimethyl Sulphide Precursor in Malts, Adjuncts, Worts and Beers, J. Inst. Brew., **82** (1976), no. 1, pp. 46-48.
11. Baldus, M.; Kunz, T. and Methner, F.-J.: The Analysis of Dimethyl Sulfoxide in Malt, Wort and Beer Using Headspace Gas Chromatography Combined with Pulsed Flame Photometric Detection – Methodology and Applications, BrewingScience – Monatsschrift für Brauwissenschaft, **66** (2013), no. 9/10, pp. 154-161.
12. Hysert, D. W.; Morrison, N. M. and Weaver, R. L.: Dimethyl Sulfide Precursor in Beer, J. Am. Soc. Brew. Chem., **37** (1979), pp. 169-174.
13. Wilson, R. J. H. and Booer, C. D.: Control of the Dimethyl Sulphide Content of Beer by Regulation of the Copper Boil, J. Inst. Brew., **85** (1979), no. 3, pp. 144-148.
14. Anness, B. J.: The Reduction of Dimethyl Sulphoxide to Dimethyl Sulphide during Fermentation, J. Inst. Brew., **86** (1980), no. 3, pp.134–137.
15. Bamforth, C. W.: Dimethyl Sulphoxide Reductase of Saccharomyces Spp, FEMS Microbiol. Lett., **7** (1980), pp. 55-59.
16. Gibson, R. M.; Large, P. J. and Bamforth, C. W.: The Use of Radioactive Labelling to Demonstrate the Production of Dimethyl Sulphide from Dimethyl Sulphoxide during Fermentation of Wort, J. Inst. Brew., **91** (1985), no. 6, pp. 397-400.
17. Leemans, C.; Dupire, S. and Macron, J. Y.: Relation between Wort DMSO and DMS Concentration in Beer, Eur. Brew. Conv. Proc. 24th Congr. Oslo (1993).

18. Hansen, J.: Inactivation of MXR1 Abolishes Formation of Dimethyl Sulfide from Dimethyl Sulfoxide in Saccharomyces Cerevisiae, Appl. Environ. Microbiol., **65** (1999), no. 9, pp. 3915-3919.
19. Kwak, G.-H.; Choi, S.-H.; Kim, J.-R. and Kim, H.-Y.: Inhibition of Methionine Sulfoxide Reduction by Dimethyl Sulfoxide, BMB Rep., **42** (2009), no. 9, pp. 580-585.
20. Dickenson, C. J.: Cambridge Prize Lecture Dimethyl Sulfide – Its Origin And Control in Brewing, J. Inst. Brew., **89** (1983), no. 1, pp. 41-46.
21. Morrison, N. M.; Hysert, D. W. and Van Gheluwe, G.: Assay and Control of Dimethylsulfide in Brewery Carbon Dioxide, J. Am. Soc. Brew. Chem., **39** (1981), pp. 131-135.
22. Sugai, T.; Kanauchi, M. and Bamforth, C. W.: Characterization of Dimethyl Sulphoxide Reductase from Brewing Yeast: Characterization of Dimethyl Sulphoxide Reductase from Brewing Yeast, J. Inst. Brew., **123** (2017), no. 3, pp. 337-346.
23. Gibson, R. M.; Large, P. J. and Bamforth, C. W.: The Influence of Assimilable Nitrogen Compounds in Wort on the Ability of Yeast to Reduce Dimethyl Sulphoxide, J. Inst. Brew., **91** (1985), no. 6, pp. 401-405.
24. Bilous, P. T. and Weiner, J. H.: Dimethyl Sulfoxide Reductase Activity by Anaerobically Grown Escherichia Coli HB101, J. Bacteriol., **162** (1985), no. 3, pp. 1151-1155.
25. Feilner, R.; Rehmann, D.; Methner, F.-J.; Baldus, M.; Kunz, T. and Scheuren, H.: Wort Stripping - Thermodynamic Considerations on the Evaporation of Aroma Substances in Continuous Desorption Processes. BrewingScience – Monatsschrift für Brauwissenschaft, **66** (2013), no. 9/10, pp. 65-74.
26. Hertel, M. O. and Sommer, K.: Wort Boiling by Batch Rectification-Possibilities for Reducing Required Evaporation, MBAA TQ 2009.
27. Gonçalves, M.; Pontes, A.; Almeida, P.; Barbosa, R.; Serra, M.; Libkind, D.; Hutzler, M.; Gonçalves, P. and Sampaio, J. P.: Distinct Domestication Trajectories in Top-Fermenting Beer Yeasts and Wine Yeasts, Curr. Biol., **26** (2016), no. 20, pp. 2750-2761.
28. Miedaner, H.: Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission II 4 (2002).
29. Wietstock, P. C.; Baldus, M.; Öhlschläger, M. and Methner, F.-J.: Hop Constituents Suppress the Formation of 3-Methylbutanal and 2-Furfural in Wort-Like Model Solutions, J. Am. Soc. Brew. Chem., **75** (2017), no. 1, pp. 41-51.
30. Pfenninger, H.: Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission III (1996).
31. Scheuren, H.; Dillenburger, M.; Roland, F.; Tippmann, J.; Methner, F.-J. and Sommer, K.: Die Austreibung von DMS Im Sudhaus – Teil 3: Validierung und Anwendung, BRAUWELT **154** (2014), no. 14, pp. 400-403.
32. Stephenson, W. H.; Biawa, J.-P.; Miracle, R. E. and Bamforth, C. W.: Laboratory-Scale Studies of the Impact of Oxygen on Mashing, J. Inst. Brew., **109** (2003), no. 3, pp. 273-283.
33. Baldus, M.; Tsushima, S.; Xi, D.; Majetschak, S. and Methner, F.-J.: Response Surface and Kinetic Modeling of Dimethyl Sulfide Oxidation – On the Origin of Dimethyl Sulfoxide in Malt, J. Am. Soc. Brew. Chem., **76** (2018), pp. 1-9.
34. Scheuren, H.; Dillenburger; Tippmann, F.-J.; Methner, F.-J. and Sommer, K.: The Volatility of Dimethyl Sulfide Measured by Flash Distillation, BrewingScience – Monatsschrift für Brauwissenschaft, **67** (2014), no. 3, pp. 69-71.
35. Jóhannesson, H.; Denisov, V. P. and Halle, B.: Dimethyl Sulfoxide Binding to Globular Proteins: A Nuclear Magnetic Relaxation Dispersion Study, Protein Sci. Publ. Protein Soc., **6** (1997), no. 8, pp. 1756-1763.
36. Winter, W. T. and Sarko, A.: Crystal and Molecular Structure of the Amylose – DMSO Complex, Biopolymers, **13** (1974), no. 7, pp. 1461-1482.
37. Samp, E. J.; Foster, R. T.; Edelen, C. and others: Influence of Cardiolipin on Lager Beer Dimethyl Sulfide Levels: A Possible Role Involving Mitochondria?, J. Am. Soc. Brew. Chem., **68** (2010), pp. 4, pp. 204-209.
38. Leekumjorn, S. and Sum, A. K.: Molecular Study of the Diffusional Process of DMSO in Double Lipid Bilayers, Biochim. Biophys. Acta BBA – Biomembr., **1758** (2006), no. 11, pp. 1751-1758.
39. Sambasivarao, D.; Scraba, D. G.; Trieber, C. and Weiner, J. H.: Organization of Dimethyl Sulfoxide Reductase in the Plasma Membrane of Escherichia Coli, J. Bacteriol., **172** (1990), no. 10, pp. 5938-5948.
40. Kwak, G.-H.; Kim, J.-R. and Kim, H.-Y.: Expression, Subcellular Localization, and Antioxidant Role of Mammalian Methionine Sulfoxide Reductases in Saccharomyces Cerevisiae, BMB Rep., **42** (2009), no. 2, pp. 113-118.
41. Moskovitz, J.: Methionine Sulfoxide Reductases: Ubiquitous Enzymes Involved in Antioxidant Defense, Protein Regulation, and Prevention of Aging-Associated Diseases, Biochim. Biophys. Acta BBA – Proteins Proteomics, **1703** (2005), no. 2, pp. 213-219.

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