DIMETHYL SULPHIDE—A REVIEW

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The sources of dimethyl sulphide (DMS) in beer and their relative significance to levels of DMS which are produced under various brewing conditions, are reviewed.

Key words: *Bacteria, dimethyl sulphide, malt, sulphur compound, review, yeast.*

Chemical and physical properties of dimethyl sulphide

Dimethyl sulphide (DMS) is a thioether with a relatively low boiling point (38°C). At concentrations below 300 mM it is soluble in water.[75] Thioethers in general are oils, with disagreeable odours at all but the lowest concentrations. Nevertheless DMS makes an important, and generally beneficial, contribution to the odour and flavour of many foodstuffs, including tea,[37] cocoa,[41] milk,[57] wines,[42] rum,[40] sweet corn,[11] and numerous cooked vegetables. DMS is a metabolic product of many biosystems, particularly those in marine habitats.[32] Its distinctive aroma is associated with shellfish[46,57] and DMS is also produced in abundance by marine algae.[32]

DMS is beneficial to the taste and aroma of lager[70] at concentrations above its flavour threshold of *ca* 30 µg/litre,[3] but below 100 µg/litre. When present in quantities above 100 µg/litre, DMS imparts a flavour which is usually described as 'cooked sweet-corn' or 'blackcurrant-like'. Ales usually contain much less than 30 µg/litre of DMS and so have none of its flavour character.

Sources of DMS

To date two main routes have been elucidated for the production of DMS in biological systems:

(a) The breakdown of sulphonium compounds (Fig. 1). Algae contain high levels of dimethyl-β-propiothetin[32] and many plants, including germinated barley,[19,34] wheat[34] and oats,[58] contain S-methyl-methionine (SMM). This compound is destroyed by heat, releasing DMS, which accounts for the large quantities of DMS in cooked vegetables. Additionally, enzymes have been isolated from fungi, bacteria and algae which specifically hydrolyse sulphonium compounds to DMS.[16,67]

(b) The reduction of dimethyl sulphoxide (DMSO), a natural constituent of several foodstuffs.[52] Conversion of DMSO to DMS has been demonstrated in cats,[22] cows,[66] agricultural crops[62] and both eukaryotic and prokaryotic micro-organisms.[77]

The known precursors of DMS in beer are SMM and DMSO, both of which originate from malt. This review describes how the levels of DMS which arise in beer are influenced by processing conditions during malting and brewing and how the amount in beer can be regulated.

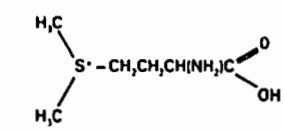
The influence of processing conditions on the level of DMS in beer

Barley variety and germination.—DMS is released quantitatively from SMM by heating in alkali and this has been used as a convenient assay for SMM in malt, adjuncts, wort and beer.[72] SMM is absent from raw barley but levels increase steadily during germination.[72] The enzyme(s) involved in this synthesis have not been studied although it seems likely that SMM is formed from S-adenosylmethionine (SAM) and methionine by a system similar to that present in wheat germ.[34]

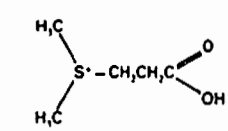
Customarily, levels of SMM in green malt which has germinated for 5 days at 16°C are of the order of 30 µg DMS equivalents/g dry weight of malt. Amounts are influenced, however, possibly by barley variety[35,49,72] and certainly by malting conditions. The concentration of SMM developed in green malt varies greatly between barley varieties germinated under identical conditions,[72] although it has been suggested that such variation is due to different rates of modification rather than to inherent differences in the capacities of individual cultivars to form SMM.[70] The higher the nitrogen content of a barley, the more SMM it will produce on malting.[20] Also the longer a barley is stored before malting the greater will be the amount of SMM formed during germination (Table I). However, sufficient SMM to supply the amounts of DMS normally found in beer develops in malt prepared from barley stored for only short periods.

The development of SMM is increased by factors which accelerate malting, *e.g.* the combination of abrasion and gibberellic acid and the use of relatively high temperatures during steeping and germination.[70] Treatment with potassium bromate markedly reduces the amount of SMM in green malt, probably by a combination of restricted root growth (rootlets contain a high concentration of SMM[71]) and inhibition of the enzyme which forms SMM during germination.

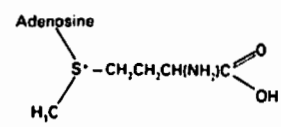
Kilning.—The SMM in green malt is the ultimate source of all the DMS found in beer. During kilning SMM breaks down releasing DMS, most of which is lost with the exhaust gases. Breakdown of SMM occurs readily during the drying period at 65°C (Fig. 2) and approximately 40% of the DMS released at this stage is retained in the malt. During curing SMM, which is slightly less stable in dried malt than in green malt,[21] decomposes at an increased rate. However the amount of free DMS in the malt alters little. This is partly due to increased volatilisation of DMS at the higher temperatures, DMS being lost at a rate similar to that at which it is released from SMM. Additionally, some DMS is oxidised to DMSO (Fig. 2).[7]



S-methylmethionine



dimethyl-β-propiothetin



S-adenosylmethionine

Fig. 1. Three major naturally occurring cationic sulphur compounds.

TABLE I. Effect of Barley Storage on Synthesis of SMM During Malting.

Time of storage before malting (days)	Malt hot water extract (L°/kg)		S-methyl methionine (μg DMS equiv/g dry weight malt)
	Miag setting 7	Fine-coarse difference	
15	283	16	10·1
36	297	5	14·9
64	297	4	15·4
97	293	8	17·1
132	297	6	20·5
160	298	6	19·3

Maris Otter-type barley, germinative energy on receipt 98%. Steeping conditions: 8 h wet, 16 h dry, 24 h wet at 16°C (no additions). Five days germination at 16°C. Dried at 65°C for 24 h and de-rooted before analysis.

All malt contains DMS, DMSO and SMM. The chief factors influencing the levels in malt of these three compounds are the quantity of SMM in green malt and the kilning schedule employed. Clearly more SMM survives at lower kilning temperatures and the DMS which is released is not oxidised to DMSO. Only malts which have been subjected to kilning temperatures in excess of 60°C give worts from which yeast can produce DMS.[50] It was originally thought that the SMM in malt formed part of a peptide which, at these higher temperatures, became activated to a form metabolisable by yeast.[73] Later studies showed that SMM is not peptide-bound in either green or kilned malt.[12] The precursor formed at these higher temperatures is DMSO.[7]

Nevertheless, an initial drying of green malt at low temperature, appears to influence DMSO formation significantly. If the moisture content of green malt is reduced to about 4% by gently drying before advancing the temperature to 65°C, then the resultant malt contains significant quantities of a substance which can be converted to DMS by yeast (*i.e.* DMSO).[3] Additionally, more SMM survives. If, however, the green malt is kilned at higher temperatures from the outset, the production of DMSO is significantly diminished.[3] The DMSO content of malt is increased at the expense of SMM, by curing lightly kilned malt at 80°–85°C.

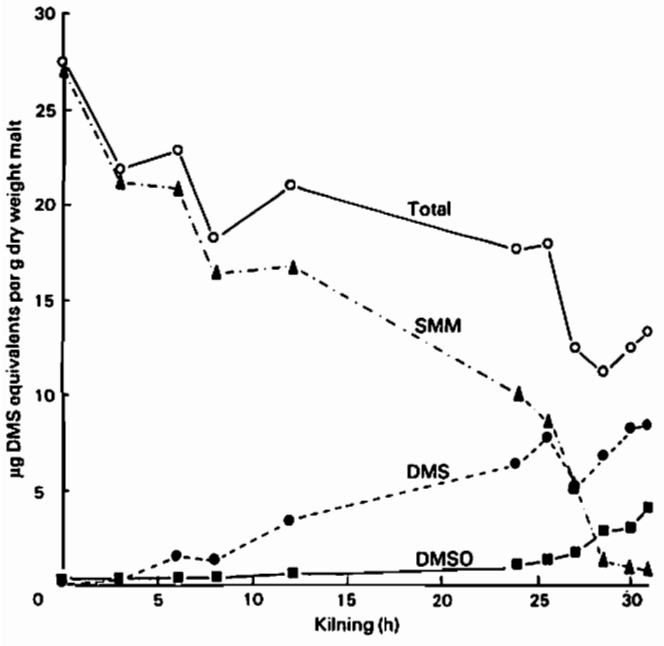


Fig. 2. The effect of kilning on the levels of SMM, DMS and DMSO in malt. Kilning schedule: 24 h at 65°C; 3 h at 85°C; 3 h at 95°C; 1 h at 105°C. ▲ SMM; ● DMS; ○ SMM + DMSO; ■ DMSO.

The kiln design also influences SMM breakdown. An increased depth of grain lowers the amount of SMM which survives kilning whereas raising the rate of air flow through the grain has the opposite effect.[30]

Mashing and wort boiling.—Early work by Anderson *et al*[3] established that alterations in the mashing regime have little effect on DMS levels in beer. Free DMS in ground malt is readily liberated upon addition of water.[72] Most of this DMS is lost during mashing and in the early stages of wort boiling. More important with regard to the DMS arising in beer, are the quantities of DMSO and SMM extracted into sweet wort.

DMSO is very soluble in water and is therefore easily extracted during mashing. Furthermore it is heat-stable and non-volatile (b.p. 189°C) and so is not lost during the boiling of wort. It is possible that some DMSO is reduced to DMS by sulphydryl compounds during wort boiling[35] and that DMS itself may be oxidised to DMSO at this stage,[7] but overall, these effects either balance each other or are insignificant. The small amounts of DMSO which have been tentatively identified in hops[60] are insufficient to affect significantly the quantity of DMSO in pitching wort.

Like DMSO, SMM is readily dissolved from malt at all mashing temperatures and little breakdown occurs during infusion mashing. Boiling part of the mash as employed in decoction procedures, will cause slightly greater decomposition of SMM.

Most significant with regard to DMS levels in beer, is the thermal decomposition of SMM during wort boiling. This breakdown was found by Wilson & Booer to be a first order reaction, SMM having a half-life of about 35 min at pH 5·4.[74] Similar results were obtained by Dickenson.[18] The half-life of SMM doubles for each 6°C reduction in temperature. Using this information and from a knowledge of the process conditions, Dickenson constructed equations to predict the free DMS level in pitching wort from the SMM level in malt.[18] He assumed that free DMS initially present in the sweet wort and that formed by decomposition of SMM during the wort boil would all be lost by evaporation and hence he was able to predict the quantity of DMS formed in wort during residence in the hot wort receiver. Several workers have now confirmed that DMS present in the sweet wort or formed during the wort boil is readily driven off but that the breakdown of SMM continues during the period between boiling of wort and its subsequent cooling in the Paraflow. The DMS released in this time is not lost.[36,64,74] Thus the extent of breakdown of SMM and of DMS retention will depend on the design of the brewery plant, the temperature of the wort after boiling and the period of time involved. SMM continues to break down during the transfer of boiling wort (101°C) to the whirlpool and throughout the residence time.[18] In a well insulated whirlpool the temperature of the wort will not fall below 90°C before transfer to the

TABLE II. The Reduction of DMSO in Glucose-Salts and Ale Wort (1·040) During Fermentation by *S. cerevisiae* NCYC 240 at 8°C.

Medium	DMSO addition (μg/litre)	DMS produced (μg/litre)	% Reduction of DMSO addition
Glucose-salts	0	0	—
	500	52	13·0
	1000	98	12·3
	5000	508	12·8
Ale wort	0	22	—
	500	42	5·1
	1000	59	4·8
	5000	172	3·8

Fermentations were carried out as previously described.[7]

Paraflow cooler. In a poorly insulated whirlpool the wort temperatures may fall further to 80°–85°C resulting in slower decomposition of the SMM.[18] Residence time of the wort in the whirlpool normally varies between 30 and 60 min, though if transfer to the cooler is slower and requires, for example, a further 2 h, some wort may be subjected to a maximum 3 h residence in the whirlpool. Wilson & Booer[74] found that, in a situation where DMS in the beer was arising both from breakdown of SMM in hot wort and by yeast metabolism, the final DMS level could be controlled by adjusting the length of the boil. However, if the levels of SMM in malt are high enough (3–8 μg DMS equivalents/g malt) and more than 50 μg DMS equivalents/litre survive boiling, then adjustment to the length of time for which the boiled wort remains in the whirlpool should enable the DMS in the pitching wort, and subsequently in the beer, to be regulated. For example, in one brewery, the extension of the time spent in the whirlpool by 60 min resulted in an increase of about 50 μg/litre (*i.e.* to 93–115 μg DMS/litre) in the pitching wort.[18]

Fermentation and conditioning.—All pitching worts normally contain DMS, DMSO and SMM. The levels of SMM in the wort can vary considerably but this compound plays no further part in the production of beer DMS because yeast does not release DMS from it.[5,71] SMM is rapidly absorbed by the yeast during fermentation[48,72] and is presumably metabolised to methionine by a methyl transferase. Material that gives DMS on heating in alkali can still be found in the wort during the later stages of fermentation.[13] This is attributed to the release of S-adenosyl methionine (SAM) from the yeast.[7,39] However, SAM can be disregarded as a precursor of DMS as it will not readily break down to release DMS during the pasteurisation or storage of beer.

Commercial worts, ale or lager, generally contain 200–400 μg DMSO/litre.[6] Unlike SMM, DMSO is converted by yeast to DMS.[7,8,9] Yeast normally reduces less than 25% DMSO during fermentations,[5] although the precise extent of reduction depends on many factors, including yeast strain, temperature of fermentation, pH, composition of the medium and the nature of the fermentation vessel.[5,10] However, when all other factors are constant, alteration of the level of DMSO in the medium does not affect the proportion which is reduced during fermentation (Table II). In glucose-salts-DMSO medium approximately 13% reduction occurs at all DMSO concentrations, whilst in ale wort only 4–5% of the DMSO is converted. Plainly, although the proportion of DMSO which is reduced may be low, the amount of DMS produced will be significant if DMSO levels in wort are high.

Yeast strain: Strains of *Saccharomyces uvarum* are generally less efficient in reducing DMSO than are strains of *S. cerevisiae*.[5,71] The strain *S. cerevisiae* NCYC 240 has a higher capacity to reduce DMSO than has any other strain investigated at BRF.[5]

Temperature: DMS formation during fermentation depends heavily on the temperature.[5] Laboratory-scale fermentations of an ale wort of high gravity resulted in five-times more DMS being produced at 8°C than at 25°C.[5] This is due to increased DMS production by yeast at the lower temperature rather than decreased volatilisation of DMS. Hence, the low temperatures normally employed for lager fermentations will favour DMS formation by yeast whereas the high temperatures used for ale fermentations restrict DMS production by this pathway.

Specific gravity: Yeast produces disproportionately more DMS from high gravity worts than from those of lower gravity.[5] The yield of DMS during fermentation at 8°C of an ale wort (SG 1·033) was only one-fifth of that achieved when the wort gravity was raised to 1·060 with glucose or sucrose.[5] Slightly less DMS was produced when the wort gravity was increased with maltose, fructose or hydrolysed maize starch, though the amount was still significantly more than in the control wort of SG 1·033. This effect can also be demonstrated, albeit to a lesser extent, in glucose-salts-DMSO medium.

The effect of specific gravity of pitching wort on DMS levels in bottled beers produced in a pilot brewery is shown in Figs. 3 and 4. Increasing the specific gravity in the range of 1·036 to 1·084 leads to a disproportionate increase in the levels of DMS in beer for both ale and lager worts fermented with *S. cerevisiae* NCYC 240 and with *S. uvarum* NCYC 1324. This is due to DMS produced during fermentation rather than to free DMS remaining from the pitching wort. There have been many reports of the effects of high gravity brewing on flavour characteristics of beer, but as yet no mention has been made of extra DMS or flavours due to it caused by fermenting high gravity worts.

TABLE III. Effect of Wort pH on the Production of DMS by Yeast.

Initial wort pH	Final beer pH	Initial DMS (μg/litre)	Final DMS μg/litre)	Apparent DMS production (μg/litre)
4·78	3·91	57·6	65·0	7·4
4·95	3·96	53·5	76·8	23·3
5·10	4·00	47·0	84·6	37·6
5·30	4·09	49·9	98·6	48·7
5·46	4·12	58·3	98·8	40·5
5·60	4·20	49·2	94·2	45·0
5·75	4·25	49·5	138·0	88·5

1·060 ale wort was adjusted to pHs ranging from 4·8 – 6·0 before autoclaving. The worts were fermented at 8°C with *S. cerevisiae* NCYC 240.

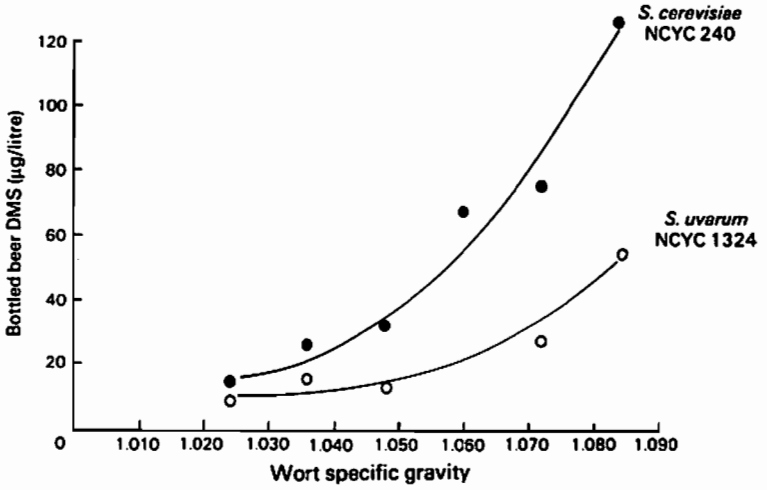


Fig. 3. The effect of wort gravity on beer DMS levels after fermentation of an all-malt ale wort. (C. D. Booer & R. J. H. Wilson, unpublished data). Fermentation was performed at 15°C in cylindroconical vessels.

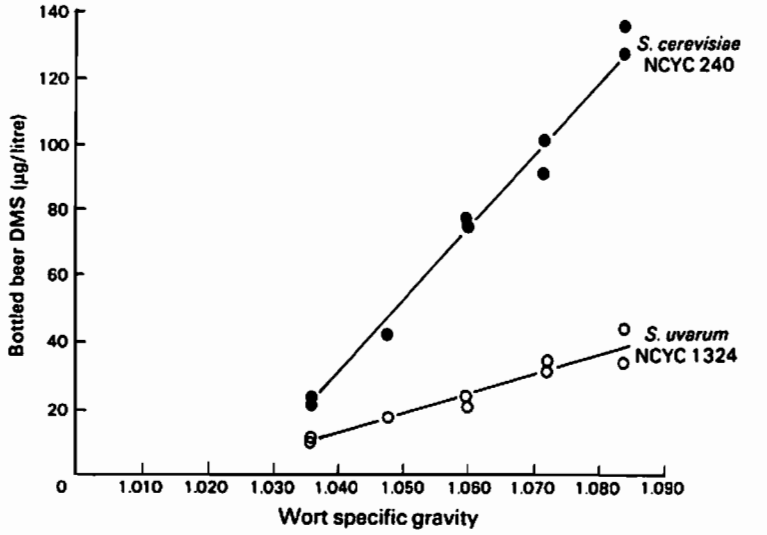


Fig. 4. The effect of wort gravity on beer DMS levels after fermentation of an all-malt larger wort. (C. D. Booer & R. J. H. Wilson, unpublished data). Fermentation was performed at 15°C in cylindroconical vessels.

pH: The fermentation of worts of increased pH leads to higher DMS levels (Table III). The amount of yeast growth was unaffected. This effect of pH on DMS synthesis may explain, at least in part, why some commercial worts favour DMS production by yeast while others do not. In particular, lager worts are usually of pH 5·4–5·7[44] compared with ale worts which are typically *ca* pH 5·1.[44]

Fermentation vessel: Anderson *et al.*[3] demonstrated that fermentation in open vessels leads to much lower values for DMS in the finished beers compared with those fermented in closed vessels. This was in a situation where much of the DMS was produced during fermentation. Similarly Booer & Wilson[13] showed that fermentation of the same lager wort in an enclosed conical vessel results in three times as much DMS as in an open-topped vessel. Deep fermentors also favour higher DMS levels in the beer.[33]

Volatility of DMS: Most lager pitching worts will already contain substantial quantities of free DMS. The use of lightly kilned malts containing high levels of SMM, together with extended periods of hot wort settling, can result in levels of DMS in the pitching wort well above the flavour threshold level. Early in fermentation there is often a marked fall in DMS levels due to the removal of DMS with the fermentation gases and to the general volatility of DMS in solution. It might be expected that DMS is completely lost from beer during the course of fermentation. However its concentration subsides to a constant but finite value[21] and frequently slight increases in the DMS concentration will be observed during the later stages of primary fermentation. These are due to the reduction of DMSO by yeast. Many workers have all too readily dismissed this route for DMS production as being insignificant in comparison with the DMS carried over from the pitching wort. Thus, Szlavko & Anderson[64] discounted DMSO for their ale and lager fermentations, although their results demonstrate a 30% increase in free DMS towards the end of an ale fermentation. Quantities of DMS produced during fermentation are often masked if high levels of DMS are already present in pitching wort. In one experiment DMS was added to wort prior to pitching with *S. cerevisiae* NCYC 240 (Table IV). Whilst production of DMS could clearly be witnessed in the control to which no extra DMS had been added, such production was not readily discernible when levels of DMS in the pitching wort were raised.

The amount of DMS present in beer represents that which is present in pitching wort and which survives fermentation

TABLE IV. The Effect of Added DMS on the Level of DMS in the finished Beer.

	Initial DMS (μg/litre)	Final DMS (μg/litre)
Control	<10	42
'Low DMS'	45	51
'High DMS'	172	102

1·040 ale wort fermented at 8°C with *S. cerevisiae* NCYC 240.

and that which is formed by yeast action. Only in extremely unusual circumstances will no DMS be formed during fermentation.

The pathways by which DMS can be produced are summarised in Fig. 5.

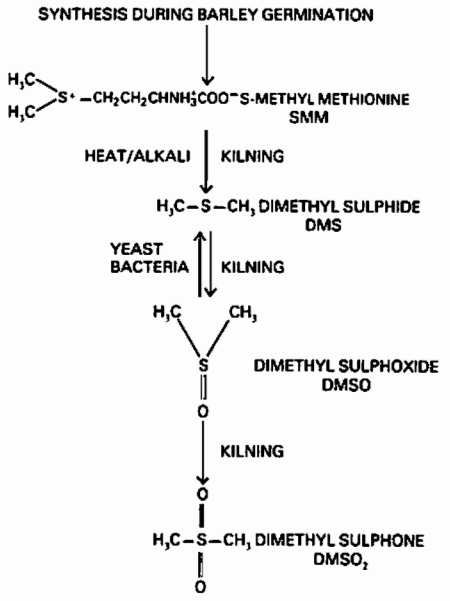


Fig. 5. Pathways of DMS formation and conversion.

Commercial trials

Levels of SMM, DMS and DMSO have been determined throughout the course of a lager brew in a commercial brewery (Table V). Although the quantities of DMS in pitching wort and beer after primary fermentation are similar, the knowledge that much DMS will have been lost through evaporation makes it apparent that a significant proportion of the DMS in beer has arisen through metabolism by yeast. The assay for DMSO[6] is not precise enough to enable minor variations in the level of DMSO in the medium to be followed accurately. It has already been noted that yeast reduces only small proportions of DMSO.

It is clear from Table V that DMS is produced during cold conditioning. This is perhaps surprising as growth of yeast is normally complete by this stage and the low temperatures employed should restrict both yeast metabolism and the volatilisation of DMS. However, there has been one previous report of significant quantities of DMS being produced through metabolism by yeast under such conditions[18] and in this case DMS levels increased from 31 to 60 μg/litre over 10 days at 0°C. Booer & Wilson[13] also found slight increases in beer DMS levels between racking and bottling 4 weeks later. This occurred for ale and lager brews that had been fermented in both conical and open fermentation vessels at 8°C.

The enzymology of DMSO reduction by yeast

Washed cell suspensions of *S. cerevisiae* NCYC 240 grown on glucose-salts, MYGP, or hopped wort media reduce DMSO to DMS.[7] Although the addition of DMSO, methionine sulphoxide (MetSO) or biotin sulphoxide to glucose-salts growth medium does not increase the amount of DMSO reductase in suspensions[9] (*i.e.* the enzyme is not inducible), the activity is profoundly influenced by the nature of the basal growth medium. Activities are of the order of 300, 100 and 20–60 ng DMS formed/min/g wet weight of yeast grown on glucose-salts, MYGP and wort respectively. There appears to be present in wort, and perhaps to a lesser extent in MYGP, some factor which inhibits the reduction of DMSO by yeast, or alternatively a factor needed for DMSO reduction is less available during growth of yeast on more complex media.

Cell-free extracts of *S. cerevisiae* reduce DMSO if NADPH is available as electron donor. NADH cannot replace NADPH. Evidence suggests that DMSO reduction in yeast is catalysed by MetSO reductase.[8,10] Extracts of yeast can be separated into fractions which upon recombination restore both DMSO reductase and MetSO reductase.[8] MetSO competitively inhibits DMSO reductase.[9,10] Indeed the affinity of the enzyme for MetSO is far higher than for DMSO[9,10] and MetSO is reduced *ca* 640 times faster than is DMSO.[8] It seems that DMSO reduction is probably a fortuitous event catalysed by an enzyme whose true function in yeast is the reduction of MetSO.

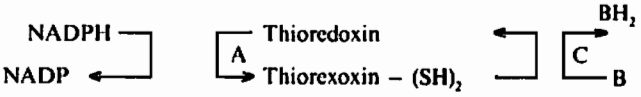
MetSO reductase has been purified from yeast and shown to be an enzyme with three protein components.[53] One of these is MetSO-reducing protein *per se*. The other two components comprise an electron transfer system which delivers electrons from NADPH to MetSO reductase. These proteins are thioredoxin and thioredoxin reductase and they have attracted extensive study in recent years due to their involvement in a wide range of metabolic events in most types of orgamism.[26,27] A summary of the demands on the thioredoxin system which have been so far demonstrated is

TABLE V. Levels of DMS, SMM and DMSO in Samples of Lager Wort and Beer from a Commercial Brewery.

Sample	Specific gravity	DMS (μg/litre)	SMM (μg DMS equiv/litre)	DMSO (μg/litre)
Sweet wort	1·073	1275	663	938
Ex-paraflow, first wort	1·076	87	66	1004
Ex-paraflow, second wort	1·028	26	28	425
Pitching wort†	1·045	46	35	590
Beer, after primary fermentation	1·008	47	61*	591
Beer, after conditioning	1·006	55	55*	532

*This is probably *S*-adenosyl methionine (SAM).
Results are the average of determinations on three consecutive lager brews. Average values for the all-malt grist were 4·0 μg DMS, 2·0 μg DMS equivalent of SMM and 3·9 μg DMSO per g malt.
†Calculated from ex-paraflow, first wort and ex-paraflow, second wort.

TABLE VI. Metabolic Roles for the Thioredoxin Reductase/Thioredoxin System.



A Thioredoxin reductase (E.C. 1.6.4.5.)
B Acceptor molecule
C Specific reductase

C	B	Organism	Reference
MetSO reductase (EC 1.6.99.–)	MetSO DMSO	*Saccharomyces*	8,53
Sulphate reductase (EC 1.8.1.–)	PAPS	*Saccharomyces*	53
Sulphite reductase (EC 1.8.1.2)	sulphite	*Saccharomyces*	68
Ribonucleotide reductase (EC 1.17.4.1.)	nucleoside diphosphates	Various	65
Protein disulphide reductase	proteins	bacteria, yeast, mammals	29

Other roles of thioredoxin system

Role	Organism	Reference
Degradation of insulin	mammal	26
Subunit of DNA polymerase	bacteriophage T7	45
Regulation of enzyme activity	plants	15
	bacteria	47
Reduction of non-disulphides *e.g.* Cu^{2+}, alloxan	*E. coli*	25,28
Reduction of other sulphoxides	rat liver	1

given in Table VI. This list is not exhaustive and it seems likely that in future further enzymes and functions will be added. Thioredoxin is evidently of great significance in control of intermediary metabolism. Clearly the reduction of sulphoxides is only one of several drains on the intracellular supply of reduced thioredoxin. At present little is known of how the cell regulates the distribution of such reducing power between the various functions or of the precise role of sulphoxide reductases.

Sulphoxides are readily formed from their sulphides by peroxidation.[63] In an aerobic environment many sulphides are present as their sulphoxides and the possession of sulphoxide reductase activity would confer an advantage on organisms able to scavenge such oxidised substances. MetSO is a natural component of the blowfly[43] and may be a normal constituent of proteins as it is converted to methionine during the acid hydrolysis used in determination of the primary structure of proteins. The enzyme, MetSO reductase could therefore have a role in protein turnover. MetSO competes metabolically with glutamic acid.[14] In this context, MetSO reductase may serve the role of preventing the accumulation of an undesirable material. Another possible function for sulphoxide reductase is as a metabolic 'sink' involved in the control of thioredoxin levels.[75] Under conditions where reduced thioredoxin accumulates it could conceivably be 'drained away' in the otherwise meaningless reduction of sulphoxide to sulphide.

From a knowledge of the properties of DMSO reductase, hypotheses can be drawn on the rationale for the influence of temperature and wort composition on the amount of DMS produced by yeast. The initial rate of DMS formation by *S. cerevisiae* NCYC 240 growing on ale wort (SG 1·065), increases with fermentation temperature from 100 ng/litre/h at 8°C to 790 ng/litre/h at 25°C.[5,9] Nonetheless, the overall yield of DMS at the end of fermentation is inversely proportional to growth temperature.[5] The phenomenon is due to some characteristic of yeast grown in wort as the maximum level of DMS produced by *S. cerevisiae* NCYC 240 growing on glucose-salts-DMSO medium was similar at all temperatures. Furthermore, no such temperature effect occurs with *Enterobacter cloacae* grown on wort. There are several possible explanations for this phenomenon:

1. Yeast DMSO reductase depends on a supply of reduced thioredoxin for the conversion of DMSO to DMS during fermentation.[8] Thioredoxin is also the reducing agent for other enzymes. In the presence of substrates for other enzymes, reduced thioredoxin will be channelled away from DMSO reductase resulting in less DMS production. It is likely that as the growth temperature is raised there is a greater demand on reduced thioredoxin by other enzymes, particularly those involved in cell division, *viz* ribonucleotide reductase and protein-disulphide reductase (see Table VI). The rate of growth of yeast in the much poorer glucose-salts-DMSO medium is less influenced by temperature and, consequently, such effects are masked.
2. DMSO reduction by yeast is less efficient in wort than in glucose-salts media. The activity of the reductase toward DMSO in washed yeast cell suspensions is higher in cells grown on glucose-salts than in cells grown on wort.[10] When cells grown on wort are transferred to glucose-salts medium they soon develop a higher level of DMSO reductase activity. Conversely, yeast transferred from glucose-salts to wort exhibits the characteristic lower activity.[10] Cells grown in wort at 8°C have more DMSO reductase than those grown at 25°C.[10] Hence it would appear that there is an inhibitor of DMSO reductase activity present in wort and it has greater access to the enzyme at 25°C than at 8°C.

These differences in the activity of DMSO reductase in whole cells are not due to altered levels of the enzyme itself[10] and are unlikely to be due to different levels of

other thioredoxin-linked enzymes as DMSO reductase activity in cell suspensions normally alters little through all phases of yeast growth. It is possible that the inhibitor in wort is methionine sulphoxide. This compound inhibits DMS production by yeast in both glucose-salts-DMSO[7] and wort[5] through its competitive inhibition of DMSO reductase.[8] Methionine sulphoxide is present in lager wort at concentrations of 10–30 μM. Similar quantities are present in ale wort. As the K_i of DMSO reductase for methionine sulphoxide is *ca* 50 μM,[9] it seems that these low concentrations in wort are nonetheless sufficient to cause substantial inhibition, if as seems likely, the inhibitor is concentrated by yeast.

3. Less DMS is produced during fermentation at 25°C of glucose-salts-DMSO medium if 10 mM-L-methionine is present. This is due to inhibition of DMSO reductase. Whilst methionine itself is not inhibitory, *S*-adenosylmethionine can be demonstrated to reduce the activity of this enzyme (Table VII). At 8°C less inhibition of DMSO reduction in yeast is seen, possibly because at lower temperatures there is less uptake of methionine, or alternatively *S*-adenosylmethionine synthetase may be temperature sensitive. Methionine levels in lager wort are approximately 140–190 μM, although the concentration of *S*-adenosylmethionine in yeast grown on hopped wort at 25°C is only approximately 0·3 μmol/g wet weight. This might suggest that this compound is only one of the inhibitors of DMSO reduction by yeast.
4. A previously unconsidered possibility, is that DMS is further metabolised by yeast. At all growth temperatures the level of DMS produced by yeast declines after reaching a maximum.[5] Remarkably, an enzyme, apparently a hydroxylase, which converts DMS to methyl mercaptan and formaldehyde, has been found in the bacterium *Hyphomicrobium S.*[12] It is conceivable that there is an equilibrium in fermentations between DMS formation from DMSO, further metabolism of DMS and its loss by volatilisation. The enzymes which might catalyse DMS conversion would be faster-acting at increased temperatures, with the result that less DMS is released into the medium.

As has already been mentioned, yeast normally converts less than 25% of the available DMSO to DMS. If yeast could reduce DMSO as efficiently as *E. cloacae* (see later) then the finished beer would probably be rendered unpalatable by the high levels of free DMS. Nevertheless it is possible to grow yeast under conditions when the proportion of DMSO reduced is greatly increased. Under nitrogen limitation in glucose-salts-DMSO medium, *S. cerevisiae* NCYC 240 will reduce up to 70% of the DMSO available (Table VIII). As nitrogen (in the form of NH_4^+) is restored to the medium the amount of DMS produced in fermentation falls. This phenomenon is probably connected with the amount of growth permitted by the medium and at low concentrations of NH_4^+, cell division and, in turn, protein disulphide reductase activity, may be limited. Reduced thioredoxin is consequently released for other reactions, including the reduction of DMSO. Such rate-limiting conditions are not obtained in wort and consequently less thioredoxin is likely to be available for the reduction of DMSO during growth of yeast on this medium.

TABLE VII. Inhibition of DMSO Reductase by *S*-Adenosylmethionine.

Concentration of SAM (mM)	DMSO reductase ng DMS. min^{-1}. (mg protein)$^{-1}$
0	0·87
1	0·76
2	0·67
3	0·62
4	0·50
5	0·45
20	0·25
No extract	0
No NADPH	0·17
No DMSO	0·11

Each assay contained 3·3 mg protein from an extract of *S. cerevisiae* NCYC 240 grown on MYGP medium. Other conditions were as previously described[8]

TABLE VIII. Effect of N in Medium on Yeast Growth and DMSO Reduction.

Medium N (mg/litre)	Final yeast weight (g wet wt/litre medium)	Final DMS (μg/litre)
500	21·48	2510
300	20·48	2352
200	21·40	2358
100	16·44	3168
50	11·28	5600
25	9·72	7220

S. cerevisiae NCYC 240 (2·5 g wet wt/litre) was grown in glucose/salts/DMSO (DMS potential 10 mg/litre) at 25°C for 6 days.

DMS FORMATION BY SPOILAGE OR ORGANISMS

Bacteria are capable of producing DMS from DMSO. Ando *et al.*[4] found that bacterial DMSO reduction was largely confined to the *Enterobacteriaceae.* However subsequent work has shown that many prokaryotic micro-organisms can reduce DMSO.[77] No other sulphur volatiles are produced from DMSO by these organisms and the more stable $DMSO_2$ is not reduced. Organisms have been discovered that are capable of utilising DMSO as an electron acceptor to support anaerobic growth[76] and other organisms have been grown on DMSO[12] and DMS[61] as sole sources of carbon.

All bacteria which reduce DMSO have been shown to possess a DMSO reductase which differs from the corresponding enzyme in yeast. The bacterial system was first studied by Zinder & Brock, who demonstrated an NADH-linked DMSO reductase in *E. coli.*[77] Rates were three-fold lower when NADPH was electron donor. Cells grown anaerobically had higher levels of DMSO reductase than had aerobically-grown cells but the presence of DMSO did not effect the amount of enzyme present, *i.e.* the enzyme develops in response to the lack of oxygen rather than to the presence of DMSO. DMSO reductase has been demonstrated in the brewery spoilage organisms *E. cloacae* and *Obesumbacterium proteus* as well as *E. coli.*[8] NADH was the optimum electron donor in each case. Although activity was detected in both soluble and membrane fractions isolated from *E. cloacae,* the rate was stimulated by combination of the two fractions, suggesting that proteins from both are needed for full activity. Association of DMSO reductase with membrane fractions would be expected if its role was as part of an electron transport chain for anaerobic growth.

Although its precursor was unidentified, high levels of DMS had previously been shown to be formed by brewery spoilage organisms.[2,38] Keevil *et al*[38] compared the growth at 20°C on sterile, aerated hopped wort (SG 1·040) of *S. cerevisiae* NCYC 1062, *Citrobacter freundii* and mixtures of these organisms. Whereas yeast was apparently unable to produce DMS, very large quantities of this substance were produced within 45 h of bacterial growth. Production of DMS was lowered ten-fold in mixed cultures. *O. proteus* (also known as *Hafnia protea* or *Flavobacterium proteus*[54]) grows especially well in competition with yeast in wort.[17] Priest & Hough[55] showed that when *O. proteus* grows in wort. DMS could attain levels of 360 μg/litre whereas formation was very much less if yeast was present. Anness

TABLE IX. Formation of DMS by Spoilage Organisms.

Lager	Concentration in beer (μg/litre)	
	DMSO	DMS
Normal	474	31
Infected	74	219

found a similar effect.[5] No explanation is forthcoming at present, but it is possible that bacteriocins produced by yeast interfere with bacterial growth.

Unlike yeast, bacteria are able to quantitatively convert DMSO in wort or artificial media to DMS, which is consistent with the existence of a different enzyme for DMSO reduction in bacteria.[5] Because of the high efficiency with which bacteria can reduce DMSO, the levels of this sulphoxide in wort are of great significance in relation to bacterial infection. This is readily apparent from the comparison of DMS and DMSO levels in uninfected and contaminated batches of the same lager (Table IX). In the absence of infection the quantities of DMSO in worts and beers are generally similar.[6]

Pasteurisation and storage

Finished lager beer contains DMS, DMSO and small quantities of *S*-adenosylmethionine, but very little SMM. There is no evidence that pasteurisation can significantly alter beer DMS levels. DMSO and *S*-adenosylmethionine will not break down under these conditions to release DMS. Small quantities of DMS may be lost from the beer during bottling but in the absence of infection by spoilage organisms these final stages of the brewing process should have little effect on the level of DMS in beer.

One recent study[31] showed that there was no increase in DMS in beers stored at room temperature with a headspace of air. However a compound designated 'DSMP', which is present in beer that released DMS when heated with alkali, did appear to be affected. Storage of bottled beer at 40°C and 60°C resulted in significant loss of this compound and slight increases in the level of DMS in the beer, although these changes did not appear to be directly related. 'DMSP' was not identified but is probably a mixture of *S*-adenosylmethionine and *S*-methyl methionine. Grigsby & Palamand[23] also reported increased levels of DMS in beer subjected to warm storage, though significant changes only occurred at temperatures above 38°C. Hence increases in the DMS level may occur under extreme conditions of storage.

The control of DMS levels in beer

The relative contribution of the two pathways for DMS formation during brewing, *viz* heat-breakdown of SMM and yeast conversion of DMSO, differs between breweries, probably due to a number of factors. Certain companies claim that the yeast-linked route is relatively unimportant.[21] For others, DMSO reduction is undoubtedly of great significance.[13] Precisely how much DMS arises by yeast metabolism is most conveniently ascertained by performing laboratory-scale fermentations of wort from which free DMS has been removed by evaporation. Alternatively DMSO reduction can be blocked by the addition to wort of methionine sulphoxide.

For reasons outlined in an earlier section, it is certain that DMS produced by yeast always makes some contribution to DMS levels in beer. When worts from a range of sources were fermented with *S. cerevisiae* NCYC 240, 2–5% of the DMSO was reduced in each case. Commercial worts containing only 220 μg DMSO/litre therefore supported the production of 4–6 μg DMS/litre. Fermentation of worts containing 500–600 μg DMSO/litre, however, gave much higher levels of DMS, and for such worts the yeast-linked pathway is highly significant in regard to DMS levels in beer. Worts containing the least DMSO were from a brewery which claims negligible yeast-linked DMS formation.

DMSO levels are highest in worts produced from malts which are cured at temperatures in excess of 75°C. However, changes in the kilning schedule to give more DMSO in the malt, *e.g.* by kilning for at least 2 h at 70–80°C, would probably cause problems with colour. For most breweries it is not practical either, to consider changing the yeast strain to one which is more adept in the reduction of DMSO. Again it is generally not feasible to alter the design of the fermentation vessels. One of the few treatments which seems at all practicable, is the alteration of the pH of the pitching wort.

The thermal breakdown of SMM is much more readily controlled. Under conditions where the yeast-related pathway is believed to be of lesser significance, DMS levels in lager depend on the content of SMM in malt, the degree of survival of SMM during wort boiling, the amount of DMS produced and retained during the period between the copper boil and the cooling of wort and the loss of DMS by volatilisation during fermentation.

Well-modified malts (*i.e.* high SMM levels), kilned at temperatures not exceeding 65°C, will contain significant amounts of SMM (3–8 μg DMS equivalents/g malt) but greatly reduced amounts of DMSO. Careful control of the wort boil will give rise to wort containing low levels of DMS but sufficient SMM (50–150 μg DMS equivalents/litre) to enable further breakdown to occur in the whirlpool. DMS formed at this stage is not readily lost and regulation of the time which elapses before hot wort is cooled enables the level of DMS in pitching wort to be controlled.

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