

Factors Influencing the Levels of Polysulfides in Beer¹

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ABSTRACT

This study was done to identify the factors that influence the concentrations of dimethyl polysulfides, which are important contributors to the sulfury character of some Canadian beers. The brewing process was monitored using established methodology, and malt was found to be the major source of these compounds in wort. Yeast was important in reducing their concentrations during fermentation to low levels that did not change during normal maturation. Packaged beer stored for prolonged periods at room temperature or for a few days at 45°C showed marked increases in the trisulfide and tetrasulfide levels. Headspace air and, particularly, sulfur dioxide had influence on the formation of the higher sulfides. Pilot scale trials have shown that, as the level of antioxidant (sodium metabisulfite added during maturation) is increased, the formation of polysulfides also increases. The residual sulfur dioxide in the malt, probably due to the sulfuring of malt during the kilning process, and the polysulfide levels found in worts are directly related. The interaction of sulfur dioxide (or bisulfite) with wort and beer constituents is a major factor in the formation of dimethyl polysulfides. These compounds may include *S*-methylmethionine, which is a precursor for dimethyl sulfide, and methionine.

Key words: *Beer antioxidant, Beer flavor, Dimethyl polysulfides, Malt, Sulfur dioxide*

Besides the familiar dimethyl sulfide (DMS), several other organo sulfur compounds are present at much lower concentrations in Canadian beers. These include the higher sulfides, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and dimethyl tetrasulfide (DMTetraS) (14). Quantitative data indicate that DMTS and sometimes DMTetraS have a high potential for contributing sulfury characters to beers (14). Factors that influence the levels of higher sulfides in beers are therefore clearly of interest.

Recently, much attention has been directed at hops as a source of

sulfury off-odors in beers; dimethyl polysulfides are among the many compounds identified (6,9). DMTS, in particular, has been recognized as one of the most flavor-potent hop volatiles (8). Peppard proposed that, as with many vegetables evolving DMTS during cooking (4), hops may contain *S*-methyl cysteine sulfoxide as the DMTS precursor (5). At elevated temperatures such as those used to boil wort, *S*-methyl cysteine sulfoxide may eliminate unstable methanesulfenic acid, CH₃SOH, which may react with hydrogen sulfide (either from hops or from other wort sources) to yield DMTS. By itself, methanesulfenic acid can also cause DMDS to form (4).

This pertains only to unsulfured or aged sulfured hops; freshly sulfured hops apparently yield little or no DMTS. This occurs because sulfoxides are reduced to sulfides by aqueous sulfur dioxide or bisulfite at acid pH (3), and the means for producing the essential methanesulfenic acid are thereby destroyed (5).

Despite this role for hops in influencing beer polysulfides levels, previous studies on Canadian products indicated that malt is the prime source of these in worts, in which concentrations appeared much higher than in finished beers; and that their levels in bottled beers could apparently fluctuate during storage (14). Studies were made in an attempt to gain better understanding of this facet of beer flavor.

EXPERIMENTAL

Analysis of Dimethyl Polysulfides in Wort and Beer

The method used was that described earlier (14).

Influence of Air/Sulfur Dioxide (SO₂) Levels on DMTS Production in Bottled Beer

Samples of freshly bottled and pasteurized lager were treated as follows. As obtained, a 340-ml (12-oz) bottle of beer contained 10 μg of SO₂/ml and 0.7 ml of headspace air. Headspace air in several bottles was reduced by uncapping, fobbing, and immediately recapping. In other bottles, headspace air was increased by uncapping, inserting an inverted 2-ml air tube, fobbing, and recrowning. Additionally, 10 μg of SO₂/ml was added to some of the reduced-air samples as bisulfite salt. The DMTS

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level was established for the untreated (control) beer, then all samples were held at 45°C for 48 hr before DMTS was measured.

Influence of Sodium Metabisulfite Added During Maturation on the Levels of Dimethyl Polysulfides in Stored Bottled Beer

Three 40-L samples of ale were collected, with exclusion of oxygen, in sterilized stainless steel pressure vessels from a single brewery fermentor at dropping. One was given an addition of sodium metabisulfite equivalent to 10 µg of SO₂/ml, the second was given twice this level, and the third was untreated. All three were conditioned regularly in the pilot brewery and, after hand bottling and pasteurization, were stored at room temperature (20°C) for periodic polysulfides analyses and taste evaluations.

Taste Evaluations

Ranking of the three beers according to their perceived sulfury attributes was performed by a trained, eight-member panel that twice challenged each set of three (total of 16 verdicts) at monthly intervals. At different sittings, the same products were also ranked

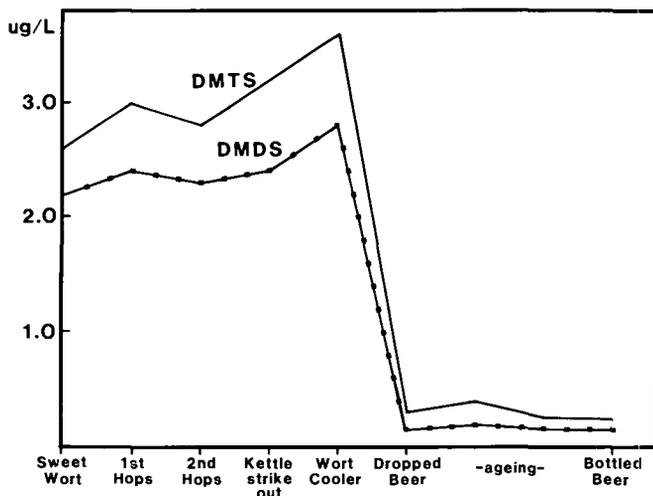


Fig. 1. Typical concentration profiles for dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) during commercial production of Canadian beers.

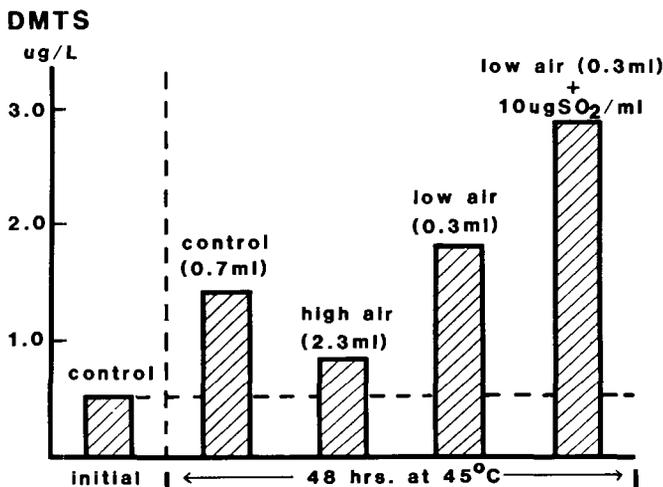


Fig. 2. Influence of levels of headspace air (in parentheses) and sulfur dioxide (SO₂) on formation of dimethyl trisulfide (DMTS) during punishment of bottled beer.

for degree of (cardboardy) staleness by an eight-member panel selected on the basis of acuity to this characteristic.

Influence of Residual SO₂ on Malt

Malts containing 0, 11, and 19 µg of SO₂/g were each used to produce all-malt, 12°P worts in triplicate, using a previously described minibrewery (1). The cooled worts were examined for polysulfides.

Additionally, the 0 and 19-µg SO₂/g malts were utilized on the 60-L pilot brewery scale to prepare all-malt (12°P original gravity) beers. The polysulfide levels were determined on the finished products at bottling and during subsequent storage.

Influence of SO₂ on Decomposition of S-Methylmethionine (SMM) and Methionine

A solution (340 ml) buffered at pH 5.2 (phthalate) and containing 10 µg of SMM/ml and 10 µg of SO₂/ml (as sodium metabisulfate) was refluxed 1½ hr, cooled, and analyzed for the expected DMS and any higher sulfides. SMM was also treated similarly in the absence of the metabisulfite. Methionine was likewise treated, except that DMS was neither anticipated nor observed to be produced.

RESULTS AND DISCUSSION

Samples taken throughout the commercial process yielded concentration profiles for DMDS and DMTS such as the typical profiles shown in Fig. 1. These confirmed observations (14) on random wort samples that the major part of both compounds was present before hops were introduced. Some concentration increases did occur during hopping and kettle boiling, but were more pronounced between kettle strikeout and emergence of wort from the cooler. Although on very reduced scales, these resembled earlier findings (10) that DMS accumulated in the hot-wort tank because of continued thermally induced precursor breakdown. Also similar to DMS, the polysulfides concentrations fell dramatically during fermentation to levels generally found in freshly bottled products. The potential for CO₂ to purge these much higher-boiling (>110°C) compounds out of the fermenting wort might be anticipated to be much less than DMS (b.p. 38°C), and assimilation by yeast might well be responsible for reductions in their concentrations; however this has not been investigated.

With this general perspective on the process, attention was directed at factors influencing polysulfides in the packaged product, because the DMTS level increased markedly, and DMTEtraS started to appear in heat-punished beers (14). The elevated temperature was clearly a factor and, at times, some DMTS increases were observed in beers as a result of pasteurization, but these were inconsistent among batches, indicating that other agents had influence also. In this regard, analytical data on some beers subjected to heat punishment suggested that levels of headspace air or SO₂ might be linked to increases in DMTS levels.

To investigate, several bottles of freshly pasteurized lager were selected. Some were given additional headspace air, whereas others were given reduced headspace air. Of the latter samples, half also received an addition of SO₂. All samples were then subjected to 45°C. After 48 hr, measurements revealed that DMTS levels were elevated in all samples, but to markedly different degrees (Fig. 2). Compared with the untreated control, the high air level apparently suppressed DMTS production, whereas the reduced air level enhanced its formation.

SO₂ as bisulfite salt is commonly added to beer to impart some protection against the flavor deterioration that occurs as development of "cardboardy" characteristics. One of its more important roles in this regard is its capacity to deplete available free oxygen in the package, bisulfite being oxidized to sulfate in the process. When headspace air levels are perceived to cause available sulfite to oxidize to different degrees, then SO₂ (or bisulfite) can be

considered to somehow facilitate DMTS formation. Indeed, the DMTS level found in the low air sample having additional SO₂ reinforces this.

To examine this apparent effect under normal conditions, samples of a brewery-fermented ale treated with 0, 10, and 20 µg of SO₂/ml at dropping were finished in the pilot brewery. Ale was chosen because *Saccharomyces cerevisiae* strains produce marginal levels of SO₂ compared with *S. carlsbergensis* strains (11) and because a low level of native SO₂ in the control beer was desired. After bottling, the three products were analyzed for sulfides and subjected to comparative taste evaluations. They were then held at 20°C for monthly scrutiny. Results are shown in Table I.

The added SO₂ was found to influence the initial levels of sulfides in the beers, with the double-addition level product having considerably increased DMTS concentration and even measurable DMTEtraS. During the six months that the products were held at 20°C, DMDS levels in each tended to gradually decrease, whereas those for both the tri- and tetrasulfides increased markedly in the first month and advanced marginally to peak at the second. The added SO₂ also appeared to magnify the concentration increases attained by both at this time. After two months, their levels had declined; thus, the fact that SO₂ has dropped to very low analytical levels over this time span may not be entirely coincidental.

With the implication of SO₂ in protection against flavor staling, panels evaluating the three products were asked to rank them according to sulfury attributes and degree of staleness. The development of sulfury characteristics might be considered strictly an aspect of staling, but the panel was specifically trained to use the more usual "cardboardy" character as the benchmark for this. Initially, the double-addition level beer, having much enhanced DMTS concentration, was considered significantly more sulfury than the other two. After one month at 20°C, the beer with 10 µg of SO₂/ml had much increased DMTS and DMTEtraS concentrations and was also significantly more sulfury than the control (which had no added SO₂); it was not, however, as sulfury as the product with twice the level of added SO₂. At this time, the control was perceived to be significantly staled. These rankings also held after four months. Only after six months did the single-addition level product appear stale compared with its still sulfury double-addition counterpart.

Overall, good correlation was shown between the sulfide analytical data and panel pronouncements on sulfury attributes. The latter might well have masked the early stages of cardboard flavor development in the treated samples. Nevertheless, the panel findings indicated that the apparent advantages of using metabisulfite for protection against flavor staling must be weighed against its increased potential for causing sulfury characteristics to develop in beers.

Because of an apparent link between SO₂ and formation of higher sulfides in finished beers, which was clearly enhanced at elevated temperatures, SO₂ could be anticipated to influence their production in wort. Furthermore, with malt normally being sulfured at kilning, examining worts produced from malts having differing residual SO₂ levels seemed logical. Sweet worts were prepared in triplicate from three such malts on a minibrewery (1.5-L) scale (1) and analyzed for higher sulfides. Levels were found to be quite low compared with production worts, reflecting both extraction efficiency and boil characteristics of the minibrewery. Nevertheless, the results (Table II) indeed indicated that residual SO₂ on the malt did influence wort concentrations of DMDS and DMTS. The effect of SO₂ could also be demonstrated by adding sodium metabisulfite to the mash water. When malts having both 0 and 19 µg of SO₂/g were used to produce all malt beers on the pilot-brewery (60-L) scale, the influence apparently carried through to the finished products, as indicated by their DMTS levels, determined as 0.3 and 0.9 µg/L, respectively.

These observations indicated an alternative route for DMTS to enter beers, aside from the degradation of S-methyl cysteine sulfoxide—the precursor in hops that was inhibited by SO₂.

However, the role of SO₂ (or bisulfite) in promoting the formation of DMTS or the other polysulfides is not readily explained. Indeed, the opposite might well have been predicted on the basis of bisulfite causing rupture of disulfide linkages (7). Obviously the matter could not be viewed this simplistically, particularly because several potential reactions for SO₂ in the complex brewing environment have long been recognized. With limited SO₂, importance of the reactions will be governed by the prevailing pH, the relative concentrations of potential reactants, and the energetics of the interactions. Because detailed knowledge in this area is lacking, any rationale for SO₂ involvement must be highly speculative.

Nevertheless, because malt is implicated, the sulfur amino acids must also be involved, particularly methionine, because the polysulfides are methylated. Also, the dimethyl polysulfides levels encountered in worts or beers indicate a very low yielding reaction or, more likely, a sequence of reactions. Such might be the decomposition of methionine in solution, reported to occur under a variety of conditions (12). Wainwright et al (13) obtained low yields of methanethiol and DMDS when methionine was incubated for 2 hr at 24°C with metabisulfite in the presence of ferrous ions. Because H₂S in wort and beer is probably available, particularly at elevated temperatures, the potential may exist for such methionine degradation to yield very low levels of higher

TABLE I
Effect of Adding SO₂ (Sodium Metabisulfite) During Maturation on Stored Finished Ale Properties

SO ₂ Added	Months at 20°C	Concentration (µ/L) ^a			Taste Evaluation ^b	
		DMDS	DMTS	DMTEtraS	Sulfury	Staled
Nil	0	0.9	0.3	0
	1	0.9	0.7	0	...	*
	2	0.5	1.0	trace	NT	...
	3	0.6	0.6	0	...	**
	6	NT	NT
	10 µg/ml	0	1.2	0.5	0	...
1	1.1	1.5	0.3	*
2	0.9	1.8	0.6	NT	NT	NT
3	0.8	1.5	0.2	*
6	0.6	0.7	0	**
20 µg/ml	0	1.2	1.2	0.2	**	...
	1	1.2	2.6	1.2	**	...
	2	0.7	2.9	1.5	NT	NT
	3	0.7	2.1	0.9	**	...
	6	1.2	1.5	0.2	**	...

^aDMDS = dimethyl disulfide, DMTS = dimethyl trisulfide, DMTEtraS = dimethyl tetrasulfide.

^b* = 95% significant, ** = 99% significant, NT = not tested.

TABLE II
Influence of Malt Sulfur Dioxide (SO₂) Content on Levels of Higher Sulfides in Wort

Malt	Wort Higher Sulfides (µg/L)	
	Dimethyl Disulfide	Dimethyl Trisulfide
0 µg of SO ₂ /g	0.4	0.9
	0.4	1.1
	0.4	1.1
11 µg of SO ₂ /g	0.5	1.7
	0.6	1.6
	0.6	1.9
19 µg of SO ₂ /g	1.0	2.6
	1.4	2.6
	1.3	1.9

TABLE III
Sulfides Found in Solutions^a of Methionine or S-Methylmethionine^b
After Refluxing 90 Min With and Without Sodium Metabisulfite^b

	Sodium Metabisulfite	Amount ($\mu\text{g/L}$)		
		Dimethyl Sulfide	Dimethyl Disulfide	Dimethyl Tetrasulfide
S-Methyl- methionine	+	537	5.8	1.2
	-	526	0.6	0.4
Methionine	+	0	6.6	1.4
	-	0	3.3	0.6

^apH = 5.2.

^b10 $\mu\text{g/ml}$.

TABLE IV
Effect of Beer pH on the Levels of Polysulfides Recovered by Purging

pH	Amount ($\mu\text{g/L}$) ^a		
	DMDS	DMTS	DMTetraS
4.1	0.6	0.4	0
2.0	0.5	1.7	0.2
10.0	5.0	0.4	0

^aDMDS = dimethyl disulfide, DMTS = dimethyl trisulfide, DMTetraS = dimethyl tetrasulfide.

sulfides as the result of insertion reactions involving elemental sulfur generated by reaction of H_2S with aqueous SO_2 . This postulation could include the DMS precursor S-methylmethionine (SMM), in that bisulfite could conceivably cause some regeneration of methionine by stripping the charged methyl group from SMM.

In an investigation of the latter, sodium metabisulfite was added to a solution of SMM. The mixture was refluxed, then cooled and examined for any DMDS that might accompany the expected large quantity of DMS. Indeed, not only was a low yield of DMDS realized, but some DMTS also was observed (Table III) despite the purposeful omission of external H_2S from the system. SMM alone, under the same conditions, also yielded some DMDS and DMTS but at much lower concentrations, suggesting that bisulfide facilitated their formation. That this might well proceed through methionine was observed when the latter was similarly treated both with and without metabisulfite.

Although this may indicate a potential role for SO_2 , its real significance for the formation of polysulfides in the beer environment is quite a different matter. Speculation suggests that this may be one route through which DMS precursor disappears from stored beer at a faster rate than DMS increases, as reported by Hysert et al (2).

The probability that the situation is much more complex may be seen in Table IV, which shows how markedly adjusting a beer's pH before purging affects the levels of the polysulfides recovered.

Although these pH values were extreme, lowering the pH increased the levels of the higher sulfides, whereas at higher pH the disulfide yield apparently increased tenfold.

Clearly, more investigations need to be done before detailed understanding of this important facet of beer flavor can be established.

SUMMARY

In Canadian brewing, formation of dimethyl polysulfides appears to be most favored by the presence of SO_2 (or bisulfite) at elevated temperatures, both during wort production and in finished beers.

Malt is the major source of polysulfides entering worts, and the degree of sulfuring at kilning influences the levels produced. Yeast apparently plays an important role in reducing wort polysulfide levels during fermentation.

Although much remains to be understood in this complex area, indications suggest that S-methylmethionine can be a common precursor for the family of dimethyl sulfides in Canadian beers.

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