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# Volatile Sulfur Compounds in Hops and Residual Concentrations in Beer—A Review

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## ABSTRACT

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This review covers most of the published literature on sulfur compounds in hops, especially thioesters, thiophenes, polysulfides, terpens, and thiols.

Keywords: Beer flavor, Gas chromatography-olfactometry, Hop aroma

## RESUMEN

### Compuestos Volátiles de Azufre de Lúpulo y las Concentraciones Residuales en la Cerveza—Una Revisión

Esta revisión cubre la mayoría de publicaciones literarias en compuestos de azufre de lupulo, especialmente *thioesters*, *thiophenes*, *polysulfides*, *terpens*, y *thiols*.

Palabras claves: Sabor de cerveza, Cromatografía-olfatómica de gas, Aroma de lúpulo

For many years, investigators have attempted to determine which hop-derived compounds influence the organoleptic properties of beer (13,14,16,17,25). In a recent study, by using gas chromatography-olfactometry (GCO), Lermusieau et al (12) clearly showed that sulfur compounds afford the most detrimental hop flavors for the quality of fresh beers. Therefore, the aim of this review is to summarize all data currently available in the published literature concerning the occurrence of sulfur compounds in hops and the beers derived from them.

## CHEMICAL STRUCTURES OF SULFUR COMPOUNDS FOUND IN HOPS

From flame ionization detector (FID) and flame photometric detector (FPD) analyses, many researchers have shown that hops may contain various sulfur compounds (10,21,23) (Table I).

Methyl thioesters are prominent in the FPD chromatograms of all steam-distilled hop oils (10,14,20). They are not artifacts of the boil used in the extraction procedure since they are present in cold extracts of hops. Levels of thioesters in hops appear to be determined by variety and local growing conditions, as well as the kilning treatment for drying the cones. Synthesized by combinatorial chemistry, some thioesters exhibited hop odor at the gas chromatography (GC)-sniffing port (1). *S*-Methylthiomethyl thioesters represent a new class. They have not been isolated from any other natural source (20).

By analogy to *S*-methylthioacetate biosynthesis in yeast (20), a plausible route for the thioester biogenesis in hops could be thiolysis of acyl coenzyme A (CoA) by methanethiol coming from methionine degradation (Fig. 1). The source of methylthio-

methanethioesters in hops is still unclear. The biosynthetic pathway to these unusual thioesters could coincide in part with the route to 2,3,5-trithiahexane (found in hops, Japanese mushrooms, cooked brassicas, and some cheeses [20]).

The occurrence of 3-alkylthiophenes in the oil distilled from occasional batches of hops can be traced to abnormally high residual sulfur levels (23). Among them, 3-methylthiophene, in confirmation of its terpenoid origin, is not found with the usual 2-methylthiophene isomer. 3-(4-Methyl-3-pentenyl)thiophene is known as thioperillene in analogy with the hop oil furan derivative, perillene.

Peppard (18) and Laws et al (10) reported the presence of dimethyltrisulfide in certain steam-distilled hop oils. It was shown to originate from a labile precursor, almost certainly *S*-methylcysteine sulfoxide, during steam distillation of hops at 100°C. Dimethyltrisulfide occurred at the highest levels in oils obtained from hops that had been kilned in the absence of sulfur dioxide or stored for an extended period of time after harvest. It also occurred in significantly higher levels in hop oils, which contain high levels of residual elemental sulfur. The corresponding tetrasulfide can also occur in certain hop oils. Since heat is required for formation of both tri- and tetrasulfides, it seems likely that traces might survive into finished beer when hop is added as a late addition.

Besides dimethyltrisulfide, many other methylsulfides have been identified. The level of 3,3-dimethylallyl methyl sulfide is enhanced in hop oil by heat treatment. Methylthiohumulene was not fully identified. The humulene skeleton was suggested from mass spectroscopic fragmentation data and synthetic studies, the most likely substitution point being the doubly allylic C-3 (24).

Humulene and caryophyllene episulfides are formed when sulfur and the appropriate sesquiterpene are stored together with exposure to light or heated together in boiling water (19) (Table II). It is interesting to note that the 4,5-double bond in humulene is more reactive than the 8,9-double bond is toward sulfur, while the reverse is true for epoxidation. Myrcene is another of the major hop oil constituents, which reacts with sulfur under mild conditions (19). It can form a series of at least 10 sulfur adducts, of which up to three are often prominent in hop oil obtained by steam distillation at 100°C (Fig. 2). The major adduct that has been detected in steam-distilled hop oils at levels up to 1,000 mg/L has been chemically characterized as 4-(4-methylpent-3-enyl)-3,6-dihydro-1,2-dithiine (Fig. 2I). While no experimental work has been carried out using  $\beta$ -farnesene, this compound, like myrcene, is an alkenyl-substituted 1,3-diene and would, therefore, be expected to form compounds analogous to those formed by myrcene.

## INFLUENCE OF VARIETY AND GROWING AREA

The total thioester content of any hop oil is largely independent of the variety of hop. According to Seaton and Moir (20), growing location and cultural conditions have the most profound effect on the levels of thioesters and sulfur compounds in general. Table III illustrates the influence of the growing location

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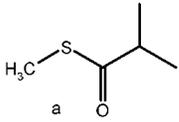
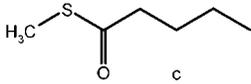
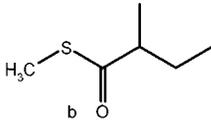
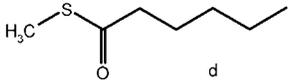
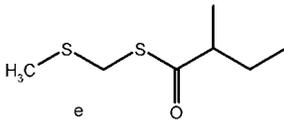
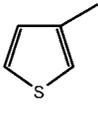
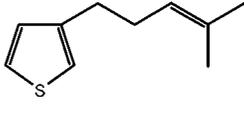
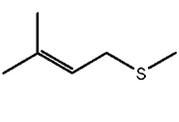
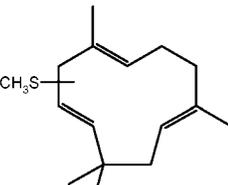
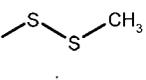
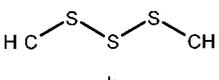
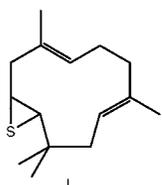
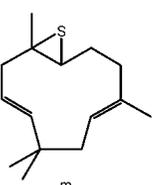
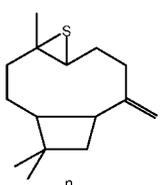
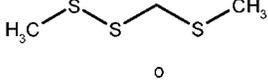
for the varieties Northern Brewer and Brewers Gold from England (Hereford) or West Germany (Hallertau). The differences are rather striking.

Very recently, Lermusieau et al (12) have shown that strong differences could also exist between varieties. For instance, compared with Saaz, Challenger was very rich in *S*-methylthioacetate, dimethyldisulfide, 4-methoxy-2-methylbutanethiol-2, diethyldisulfide, *S*-methyl-3-methylthiobutanoate, dimethyltrisulfide, dimethyltetrasulfide, and *S*-methylthiodecanoate. Many other unidentified sulfur compounds were still easily detected by GCO in the Challenger variety. By using a specific detector for sulfur compounds, Lermusieau et al (12) have shown large differences between the sulfur chemiluminescence detector profiles of six hop varieties (11). Among them, Saaz was characterized by a poor chromatogram.

## INFLUENCE OF SULFUR SPRAYS ON THE CROP

In their experience, Suggett et al (23) examined more than 30 different growths from eight varieties. Of those with a growing history that could be well established, the hops, which had been sulfur sprayed against powdery mildew, showed much higher levels of lower mercaptans, caryophyllene and humulene episulfides, dimethyltrisulfide, and the thiophene derivatives. Wye Challenger provides an interesting comparison in that, up to and including the 1976 crop, it was thought to be powdery mildew-resistant, but from 1977 onward, this property was questioned, and in general, sulfur treatments were applied. A GC comparison of a 1976 and a 1977 Challenger shows that there is no evidence for the levels of thioesters being dependent upon the extent of sulfur treatment on the crop. It illustrates, however, the typical enhancement of levels

**TABLE I**  
Sulfur Compounds Found in Hops<sup>a</sup>

Thioesters		
<i>S</i> -Methyl-2-methylthiopropionate (a)		
<i>S</i> -Methyl-2-methylthiobutanoate (b)		
<i>S</i> -Methyl-3-methylthiobutanoate		
<i>S</i> -Methylthiopentanoate (c)		
<i>S</i> -Methyl-4-methylthiopentanoate		
<i>S</i> -Methylthiohexanoate (d)		
<i>S</i> -Methyl-5-methylthiohexanoate		
<i>S</i> -Methylthioheptanoate		
<i>S</i> -Methylthiomethyl-2-methylthiopropionate (e)		
<i>S</i> -Methylthiomethyl-2-methylthiobutanoate		
<i>S</i> -Methylthiomethyl-3-methylthiobutanoate		
<i>S</i> -Methylthiomethyl-4-methylpentanoate		
<i>S</i> -Methylthiomethylthiohexanoate		
Thiophenes		
3-Methylthiophene (f)		
3-(4-Methylpent-3-enyl)-thiophene (g)		
Methylsulfides		
Dimethylsulfide (DMS)		
3,3-Dimethylallyl methyl sulfide (h)		
Methylthiohumulene (i)		
Polysulfides		
Dimethyldisulfide (DMDS) (j)		
Dimethyltrisulfide (DMTS) (k)		
Episulfides		
1,2-Epithiohumulene (l)		
4,5-Epithiohumulene (m)		
4,5-Epithiocaryophyllene (n)		
Others		
Methanethiol		
Dimethyl sulfoxide (DMSO)		
2,3,5-Trithiahexane (o)		

<sup>a</sup> Seaton and Moir (21) and Takabe et al (25).

of methyl mercaptan and dimethyltrisulfide and the emergence of thiophenes and caryophyllene and humulene episulfides (23).

### INFLUENCE OF KILNING AND SULFURING IN THE KILN

Concentrations of thioesters in Wye Challenger hops increased an average of 40% during kilning (20). However, in experiments with Wye Challenger and Wye Northdown hops, no predictable or consistent differences were found between thioesters levels in oils from SO<sub>2</sub>-sulfured and unsulfured hops (20) (Table IV).

### INFLUENCE OF BOILING AND FERMENTATION

At the end of a 60-min copper boil, no purely hop-derived sulfur volatiles are detectable. On the other hand, after late hopping, three sulfide peaks (dimethylsulfide [DMS], dimethyl disulfide [DMDS], and dimethyltrisulfide [DMTS]) are enhanced, and in fact, most of the sulfur compounds previously identified in hop oil are now apparent in the wort (23). During fermentation, there is a small general reduction in the level of these volatiles (Table V). According to Suggett et al (23), dimethyltrisulfide and some of the higher thioesters could be quantitatively removed. A number of sulfur compounds could, however, survive through maturation and final packaging. On occasion, dimethyltrisulfide reappears in maturation or packaging from methanesulfenic acid (18) or methional and methionol (5). Levels of thioesters, 3-methylthiophene, and dimethyltrisulfide found in such a beer in Table V can be compared with the thresholds given in Table VI. Of the thioesters, only the *S*-methyl-2-methylthiobutanoate (odor described as “truffle” for the pure compound according to Gijs et al [6]) was found in the final beers at levels above its determined threshold and only for certain hop growths. In dry-hopped beers, the *S*-methyl-2-methylthiobutanoate and the *S*-methylthiohexanoate (odor described as “pineapple” for the pure compound according to Berger et al [1]) can occur at significant concentrations. Their flavor profiles are shown in Table VII.

By GCO, Lermusieau et al (12) recently identified in late-hopped beers many other sulfur compounds derived from hops. Even in low concentrations (sometimes no FID peak), they can impart very strong typical flavors to the final beer. Among them, 2-methyl-3-furanethiol (“roasted meat”) has been stronger when the Saaz variety was used. On the contrary, dimethyldisulfide, diethyldisulfide, or *S*-methylthioisovalerate (= *S*-methyl-3-

methylthiobutanoate) were more persistent with the Challenger cultivar. Dimethyltrisulfide was another organoleptically active sulfur compound found in beers, whatever the variety used.

### LIGHTSTRUCK FLAVOR

3-Methyl-2-butene-1-thiol (MBT), also known as prenyl mercaptan, is the major impact character in lightstruck beer. The pure compound has a pungent leek-like odor, which becomes

TABLE II  
Levels of Sesquiterpene Episulfides in Various Hop Oils<sup>a</sup>

Hop Oil	Temperature of Production (°C)	1,2-Epithiohumulene Level (mg/L)	4,5-Epicaryophyllene Level (mg/L)
Commercial A	100	<10	<10
Commercial B	100	360	370
Bullion	100	350	290
	25	<10	<10
Wye Northdown	100	<10	<10
	25	<10	<10
Goldings <sup>b</sup>	100	9,300	3,360
	25	2,520	1,470
Fuggles	100	50	110
Wye Target	100	<10	<10

<sup>a</sup> Peppard and Laws (20).

<sup>b</sup> This hop was known to contain a high level of residual elemental sulfur (5,000 mg/kg).

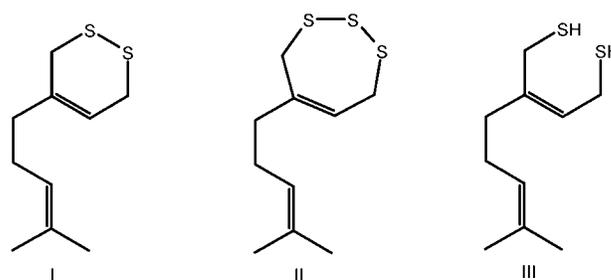


Fig. 2. Sulfur compounds derived from myrcene (20): 4-(4-methylpent-3-enyl)-3,6-dihydro-1,2-dithiine (I), cyclic trisulfide composed in other adducts only partially characterized (II), and dithiol derived from the reduction by yeast during fermentation of compound I (III).

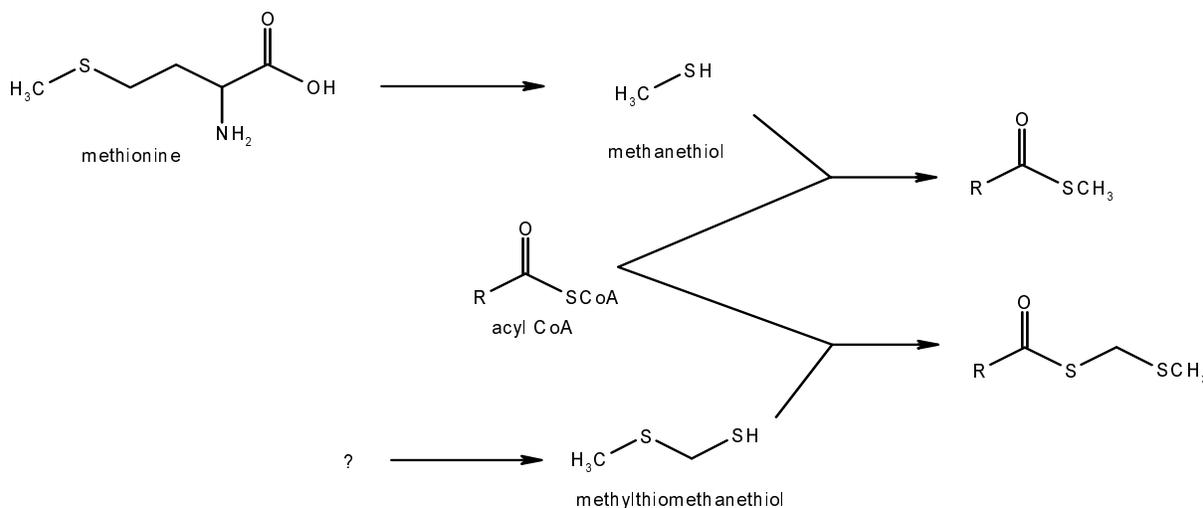


Fig. 1. Possible pathways for the production of thioesters in hop oil (22).

**TABLE III**  
**Influence of the Growing Location on the Concentrations (mg/kg) of Thioesters Found in Hops<sup>a</sup>**

Thioester	Northern Brewers		Brewers Gold	
	Hallertau	Hereford	Hallertau	Hereford
Methyl				
S-Methyl-2-methylthiopropionate	41	294	37	372
S-Methyl-2-methylthiobutanoate	194	836	79	590
S-Methylthiopentanoate	14	126	...	28
S-Methylthiohexanoate	354	1,725	207	690
Methylthiomethyl				
S-Methylthiomethyl-2-methylthiobutanoate	67	418	67	203

<sup>a</sup> Seaton et al (22).

**TABLE IV**  
**Influence of Kilning and Sulfuring in the Kiln on the Concentrations (mg/kg) of Thioesters Found in Hops<sup>a</sup>**

Thioester	Wye Challenger			Wye Northdown	
	Green	Kilned		Kilned	
		SO <sub>2</sub> Free	+ SO <sub>2</sub>	SO <sub>2</sub> Free	+ SO <sub>2</sub>
Methyl					
S-Methyl-2-methylthiopropionate	92	126	126	76	64
S-Methyl-2-methylthiobutanoate	462	726	660	290	312
S-Methylthiopentanoate	140	238	210	36	45
S-Methylthiohexanoate	1,725	2,323	2,140	626	1,086
Methylthiomethyl					
S-Methylthiomethyl-2-methylbutanoate	296	370	296	166	166

<sup>a</sup> Seaton et al (22).

**TABLE V**  
**Levels of Free Sulfur in Hops and Selected Sulfur Compounds in Hop Oils and Late-Hopped Lagers<sup>a</sup>**

Hop	Free Sulfur (mg/kg)	3-Methylthiophene		Total Thioesters		Dimethyltrisulfide	
		Hop Oil (mg/kg)	Beer (µg/L)	Hop Oil (mg/kg)	Beer (µg/L)	Hop Oil (mg/kg)	Beer (µg/L)
A	1,700	340	5.0	4,500	1.8	480	0.18
B	200	90	1.5	4,000	1.8	200	0.11
C	0	0	0	800	0.5	0	0.12

<sup>a</sup> Seaton et al (22).

**TABLE VI**  
**Flavor Descriptions and Threshold Values in Beer for Selected Hop Oil Sulfur Compounds<sup>a</sup>**

Compound	Flavor Description	Threshold (µg/L)
Methyl thioesters		
S-Methyl-2-methylthiopropionate	Cheesy, estery, cooked vegetable	5
S-Methyl-3-methylthiobutanoate	Cheesy, hop, cooked vegetable	50
S-Methyl-2-methylthiobutanoate	Truffle	1
S-Methylthiohexanoate	Cooked vegetable, sulfury, soapy	1
Methylthiomethyl thioesters		
S-Methylthiomethyl-2-methylbutanethiolate	Onion, garlic, astringent	8
Sulfides		
Dimethylsulfide (DMS)	Cooked vegetable, onion, rubbery	7.5
Dimethyltrisulfide (DMTS)	Cooked vegetable, onion, sulfury	0.1
CH <sub>3</sub> SCH <sub>2</sub> SSCH <sub>3</sub> (2,3,5-trithiahexane)	Burnt, rubber	4
(CH <sub>3</sub> ) <sub>2</sub> C=CHCH <sub>2</sub> SCH <sub>3</sub> (dimethylallyl methyl sulfide)	Garlic, rubbery, onion	0.2
Sulfur adducts		
3-Methylthiophene	Burnt, astringent	500
Myrcene disulfide	Fragrant, grassy	10
1,2-Epithiohumulene	Musty, sulfury, cardboard	200

<sup>a</sup> Peppard and Laws (20) and Seaton et al (22).

**TABLE VII**  
**Levels of S-Methyl-2-Methylthiobutanoate and S-Methylthiohexanoate in Hop Oils and Corresponding Beers and a Comparison with Their Aromatic Profiles<sup>a</sup>**

Beer	Hop	S-Methyl-2-Methyl Thiobutanoate		S-Methyl Thiohexanoate		Profile Scores <sup>a</sup>		
		Oil (mg/kg)	Beer (µg/L)	Oil (mg/kg)	Beer (µg/L)	Cheesy	Cabbage	Burned Rubber
A	Challenger	600	1.1	2,100	2.5	++	++	+
B	Northern Brewer	110	0.2	250	0.3	+	+	
C	Wye Northdown	500	1.0	2,000	3.0	++	+++	++
D	Target	120	0.2	250	0.3	+	++	

<sup>a</sup> Suggett et al (24).

“foxy” or “skunky” upon dissolution in water (8). Odor and flavor difference thresholds lie in the range of 0.2 to 0.4 ng of MBT per L in water (24). In beer, MBT is slightly less flavor-active, with reported difference thresholds of 1.25–2.5 ng/L in the beer (7).

Although MBT is derived from hops, there is only one confirmation that it could actually occur in hops. By applying GCO on Saaz and Challenger dichloromethane extracts, Lermusieau et al (12) detected its typical spicy odor at the expected Kovats index.

Brenner et al (2) found that tasters cannot detect lightstruck character in some beer samples at close proximity but have no problem detecting it in the same sample at a distance of 6–10 feet away. They believe that it is possible to exceed the maximum capacity of the odor registering system when testing samples are close to the nose.

Due to the high flavor activity of MBT, the demands on any technique used to measure it are considerable. High sensitivity and good selectivity are required. Goldstein et al (7) used large samples (4.3 L) and gas extraction followed by collection on glass wool impregnated with mercury cyanide. The thiols were then freed with acid and extracted into dichloromethane. A portion of this dichloromethane thiol solution was directly injected onto the GC column. Detection limits of less than 1 ng/L could be obtained using mass spectrometric detection (7).

Although brewers regard MBT as an undesirable character in beer, no studies have been reported concerning the hedonic characteristics of this compound. Singleton (22) notes that lightstruck character cannot be wholly unacceptable to consumers of beer since several beers of considerable sales volume display this character today. Lermusieau et al (12) identified it using GCO as the major impact compound of a fresh beer, even when protection against light was ensured. On the other hand, this odor was not detected at all at the sniffing port if no hop was added in the boiling kettle.

Goldstein et al (7) found that most beers contain small quantities (1–5 ng/L) of MBT before they are exposed to light. They propose that it is formed during wort boiling but do not suggest how. This suggestion is intriguing since it indicates that the presence of lightstruck character in beer does not necessarily imply that beer has been exposed to light. Actually, it is currently believed that the presence of iso- $\alpha$ -acids is essential for the development of lightstruck character. The involvement of the isopentenyl side-chain on the iso- $\alpha$ -acid is indicated by the fact that closely related compounds, which do not possess this group (e.g., commercially reduced or hydrogenated hop bitter acids or both) fail to generate lightstruck flavor in beer. According to De Keukeleire (4), upon exposure to ultraviolet light, iso- $\alpha$ -acid, which has an absorbance maximum approximately 276 nm, can absorb light to form a singlet excited state that undergoes intersystem crossing to produce the triplet excited state. The formation of the triplet state of the delocalized  $\beta$ -triketochromophore is followed by intramolecular energy transfer to the localized  $\alpha$ -hydroxyketone moiety (3). In this state, a Norrish type I  $\alpha$ -cleavage can occur to yield the 3-methyl-2-butenyl radical, which is a precursor to MBT (3; D. Hastings, M. J. McGarrity, L. Bordeleau, and D. J. Thompson, unpublished data). In the presence of visible light, one or more photosensitizers are involved in the process. They absorb visible light (e.g.,  $\lambda_{\max}$  of riboflavin = 445 nm) and may transfer some of this energy to iso- $\alpha$ -acids. Apart from immediate consumption or storing beer in light-proof containers (dark glass or cans), the photosensitivity can be circumvented by reduction of the iso- $\alpha$ -acids so that the deleterious photochemical process is prohibited (3). Antioxidants, in particular those that react with free radicals, can also be used to control the development of lightstruck character in beer. Compounds that contain 1,8 ether linkages, such as 1,8-cineole, reduce the sensitivity of beer to light (15).

The source of the sulfur atom in MBT has not yet been clearly identified. Experiments in which beer was dialyzed against water in cellophane membranes indicated that the donor molecules are of both high and low molecular weight, thus proteins,

polypeptides, and free amino acids may all take part in the lightstruck reaction to some degree (9).

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