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Influence of the Date of Harvest on the Sulphur Compounds of the "Special Flavour Hop" Varieties Cascade, Mandarina Bavaria, Hallertau Blanc, Huell Melon and Polaris

Sulphur compounds have very low odour thresholds and play a relevant role in the "Special Flavour Hops". In this work, it has first been investigated by means of a flame photometric detector as to which sulphur compounds occur in the varieties Cascade, Mandarina Bavaria, Hallertau Blanc, and Huell Melon. The substances dimethyldisulphide, S-methylthioisovalerate, and S-methylthioisohexanoate have been quantitatively determined, using commercially available standards. The composition and content of the sulphur compounds are clearly dependent on the variety. At late dates of harvest, the content of the sulphur compounds once again strongly increases. It has also been confirmed analytically, that late-harvested hops have onion-like and garlic-like aroma notes.

Descriptors: sulphur compounds, special flavour hops, date of harvest

1 Introduction

The Craft Brewer movement has changed the world of hop- and beer in a sustainable way. The Craft Brewers wish to produce tastefully and skilfully brewed beers again. As a result much more is now being talked about beer again and hop varieties as well as growing regions are again perceived in a much more differentiated manner. As regards the hop ingredients the focus is now again more on the aroma compounds than on the bitter substances.

The term "Special Flavour Hops" emerged, which is now an internationally established term. It is not clear who invented this expression, but it is no longer exactly comprehensible. "Special Flavour Hops" are hops, that attract attention because of their unusual aroma nuances. Usually, these are fruity, citrus and flowery aromas, in contrast to the spicy, herbal, and woody flavours of the traditional hops.

In the case of dry hopping, hops are added to the beer on the basis of the oil content. According to literature [1, 2], the hop oil consists of 300–400 single compounds (Figure 1).

The proportion of sulphur compounds in the total oil is very low, at less than 1 %. However, sulphur compounds play an important role in the "Special Flavour Hops", since they have very low odour thresholds and contribute decisively to flavour. They can be selectively determined with a flame photometric detector (FPD), since sulphur atoms emit light with a wavelength of 394 nm upon combustion. In this thesis, the investigations will first be carried out to determine which sulphur compounds are present at all in hops. The second objective will be to find out whether there are differences in the varieties and whether the harvesting time exerts an influence. Substances for which standards are available will also be quantitatively determined.

It depends, of course, on brewing technology and the use of hops, as to whether sulphur compounds pass into the beer. The easily-volatile sulphur compounds are lost during wort boiling. During the fermentation, the yeasts can convert thioesters to ethylesters [3]. But with the dry hopping technique, sulphur compounds can be transferred into beer.

https://doi.org/10.23763/BrSc17-12Kammhuber

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2 What is known about sulphur compounds in hops up to now and what do they contribute to the aroma ?

Good reviews on sulphur compounds in hops and their importance can be found in the articles by *A. Suggett*, *M. Moir*, *J.C. Seaton* [4], *T.L. Peppard*, [5], and by *G. Lermusieau*, *S. Collin* [6]. *Prof. Narziss* writes about the behaviour of sulphur-containing aromatic compounds in the brewing process [7]. Sulphur compounds influence the odour of the hop both positively and negatively. The sulphur compounds of the hop can be divided into four groups:

- Alkylsulphides and polysulphides
- Thioester

- Sulphur-containing terpenoids
- Polyfunctional thiols

The descriptions of the substances found and their properties are summarized under Point 4. Polyfunctional thiols could only be analytically considered in recent years, since their concentrations in hops are very low, and the analytical methods were not yet sensitive enough. Sulphur-containing terpenoids can be formed when hops are treated directly with elemental sulphur. This is no longer made nowadays, so that these compounds are hardly detectable.

3 Material and methods

3.1 Hop samples

All hop samples come from the breeding yard of the Research Institute in Hüll. They were harvested as normal hops, dried at 60 °C. for 9 hours, vacuum-packed, and stored at 2 °C. Table 1 shows the harvest dates of 2015.

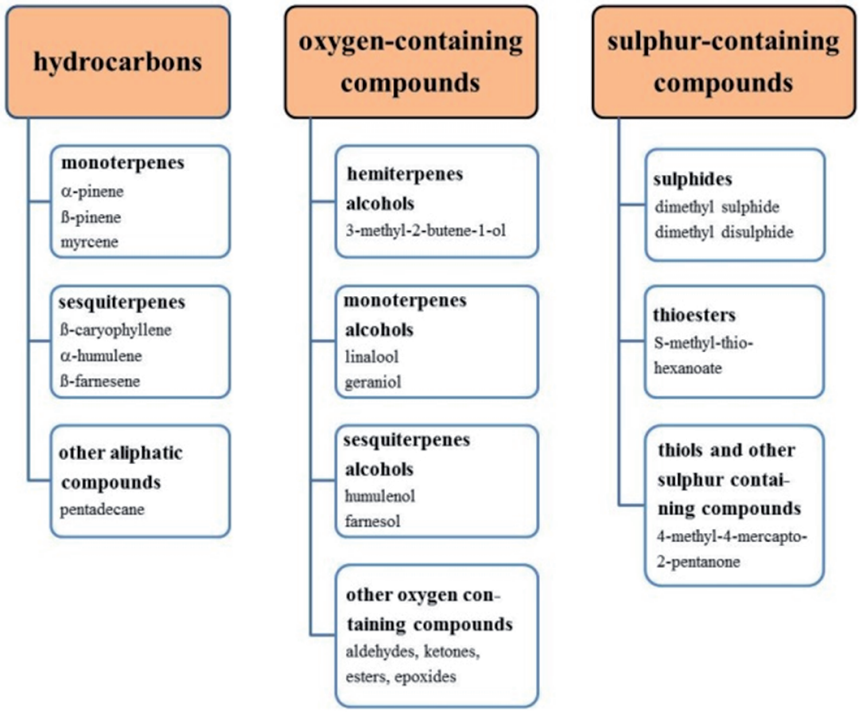


Fig. 1 The essential oils of hops

Table 1 Dates of harvest in the year 2015

variety	T1	T2	T3	T4	T5	T6
Cascade CA	18.08.	25.08.	01.09.	08.09.	15.09.	22.09.
Mandarina Bavaria MB		25.08.	01.09.	08.09.	15.09.	22.09.
Hallertau Blanc HC		25.08.	01.09.	08.09.	15.09.	22.09.
Huell Melon HN		25.08.	01.09.	08.09.	15.09.	22.09.
Polaris PA		25.08.	01.09.	08.09.	15.09.	22.09.

3.2 Analytics

3.2.1 Sample preparation

The static headspace technique was used as the sample preparation method. This has the advantage of a simple and fast sample preparation with high accuracy. 1 g of ground hops is conditioned to 80 °C. for 60 minutes, and then 3 ml of the headspace volume is injected into the gas chromatograph.

3.2.2 Static Headspace -Method

Equipment: Agilent Headspace Sampler 7697 A
settings:

- purge gas: He 5.0
- loop volume: 3 ml
- transfer line diameter: 0.53 mm
- transfer line length: 1.00 m
- **incubation time: 60 Min.**
- **incubation temperature: 80 °C**

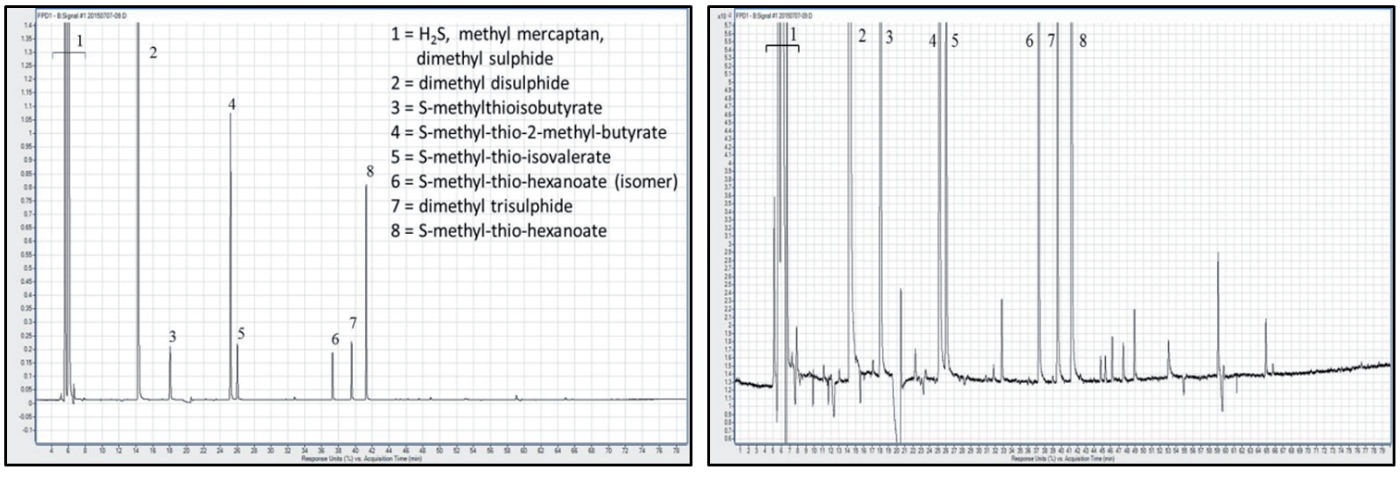


Fig. 2 Chromatogram of the variety Polaris measured with a flame photometric detector and the same chromatogram in higher resolution

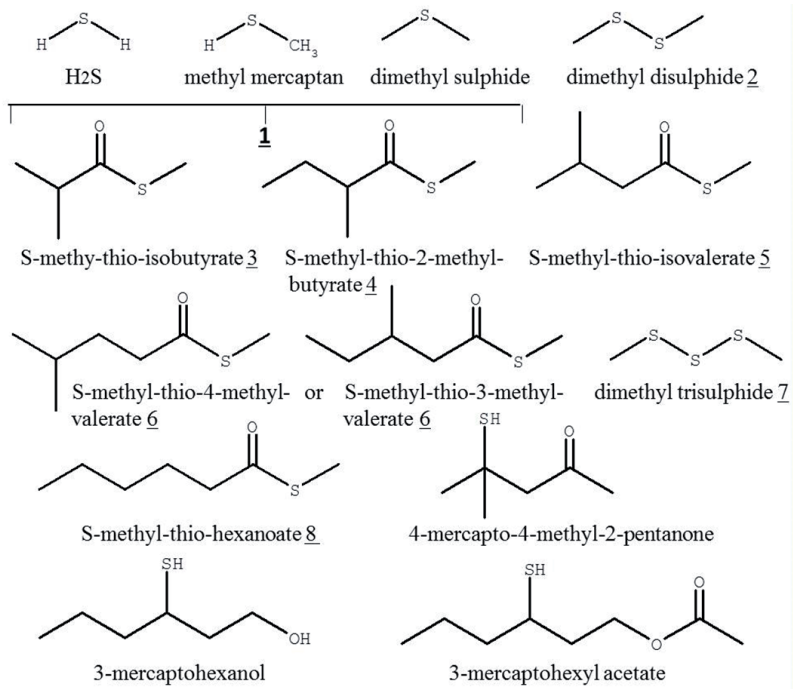


Fig. 3 Chemical structures of the identified sulphur compounds

Table 2 Significant masses of the selected sulphur-containing compounds

Substance	Masses (m/z)	Masses used for quantification
dimethyl disulphide	94, 79, 45	94
S-methyl-thio-isovalerate	132, 85, 57, 41	132
4-mercapto-4-methyl-pentano-ne (4-MMP)	132, 55, 43	132
S-methyl-thio-hexanoate	131, 99, 71, 43	131
3-mercaptohexanol	55, 41, 57, 61, 47,100, 67	134
3-mercaptohexylacetate	43, 55, 41, 88, 83, 73, 116	116

- injektion time: 30 Sek.
- loop filling pressure: 0.8 bar
- loop pressure: 0.7 bar

3.2.3 Gaschromatography

Equipment: Agilent Gaschromatograph 7890 B
settings:

- oven temperature program: 60 °C (5 Min.)
  1 °C/Min. up to 75 °C,
  2 °C/Min. up to 150 °C
  1 °C/Min. up to 160 °C
  4 °C//Min. up to 230 °C
  230 °C (5 Min.)
- injector temperature: 200 °C
- carrier gas: He 5.0
- split 3:1
- split flow 3.0 ml/Min.
- column: 60 m x 0,25 mm, Polyethyleneglycol, fused silica cross-bonded (Macherey and Nagel)
- flame photometric detector FPD
- heater transfer line: 200 °C
- emissions block: 150 °C
- air flow: 100ml/Min.
- H_2 Flow: 75 ml/Min.
- makeup flow He: 60 ml/Min.

3.2.4 Mass spectrometer

Equipment: Agilent 5977A
settings:

- ion source temperature: 250 °C
- quadrupole temperature: 150 °C
- measurement mode: SIM/Scan

3.3 Standards

S-methylthio-isovalerate, S-methylthiohexanoate, 4-mercapto-4-methyl-2-pentanone (4-MMP), 3-mercaptohexanol, 3-mercaptohexyl acetate were purchased from Chemos Regenstauf, and dimethyl disulphide from Merck. The purity of all substances is greater than 99 %.

3.4 Identification of the sulphur compounds using the flame photometric detector and mass spectrometer

The flame photometry detector was used to investigate which sulphur compounds at all occur in hops (Figure 2). All main sulphur compounds could be identified by comparison of the mass spectra with the spectra in the NIST (National Institute of Standards and Technology) MASS Spectral Library and with pure reference standards, if they were available (Figure 3).

In particular, the higher resolution (Figure 2) shows that some other sulphur compounds still occur in smaller concentrations in the hops. Prof. S. Collin has found about 41 thiols in hops [8, 9, 10].

It is extremely difficult to identify these substances since the mass spectrometer is too insensitive. By comparison with pure standards, 4-MMP, 3-mercaptohexanol, and 3-mercaptohexyl acetate could be identified. An enrichment method for sample preparation is currently being worked out to improve the sensitivity and to enable a quantitative determination.

3.5 Quantification using the standard addition

All quantitative determinations were performed with the mass spectrometer. For the quantitative analysis, the substances dimethyl disulphide 2, S-methylthio-isovalerate 5, and S-methylthiohexanoate 8 were selected, since standards exist for these substances. Table 2 shows the significant masses of the identified compounds and the masses used for quantitative determination. The method of

standard addition was used for quantification. Each sample is divided into four aliquots. One sample each is the test sample. The substances to be measured are added to the other three aliquots in ascending concentrations.

Figure 4 illustrates the procedure of the standard addition. To determine the concentration, a straight line is placed through the measuring points. The intercept point of the line with the x-axis is the concentration of the searched substance. The regression coefficient was in nearly every case higher than 0.99, which means a high linearity.

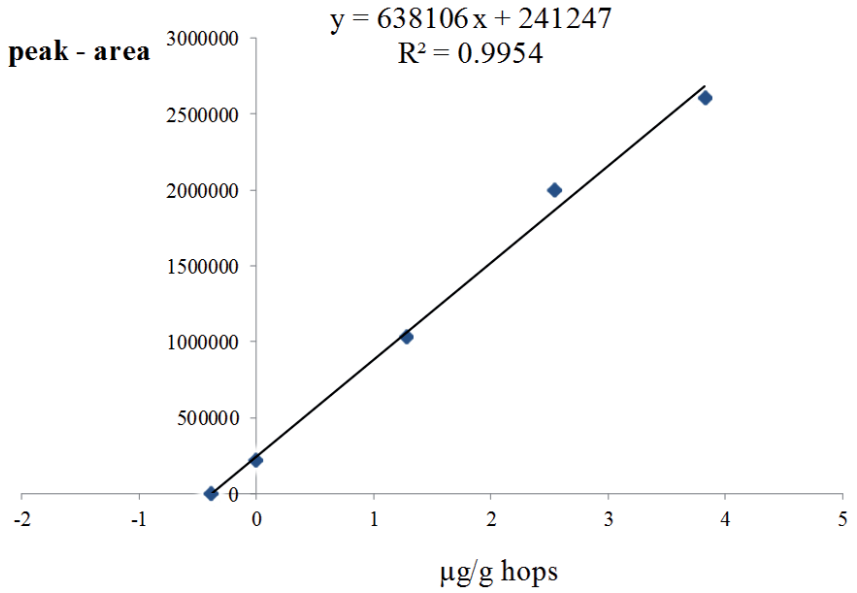


Fig. 4 Quantitative determination of dimethyl disulphide by standard addition

4 Results and discussion

4.1 Properties and biosynthesis pathways of the identified substances

4.1.1 Alkylsulphides and polysulphides

Alkylsulphides and polysulphides are not specific for hops; they are produced by the degradation of proteins (sulphur-containing amino acids, such as methionine and cysteine), and their smell is sulphurous, carbbage-like, onion-like, and reminds one of boiled vegetables. Alkyl- and polysulphides are rather negative and hops should contain small amounts. Figure 5 shows the formation of alkyl sulphides and table 3 shows their properties. These compounds also come via the malt into the beer. During the wort boiling, they are evaporated.

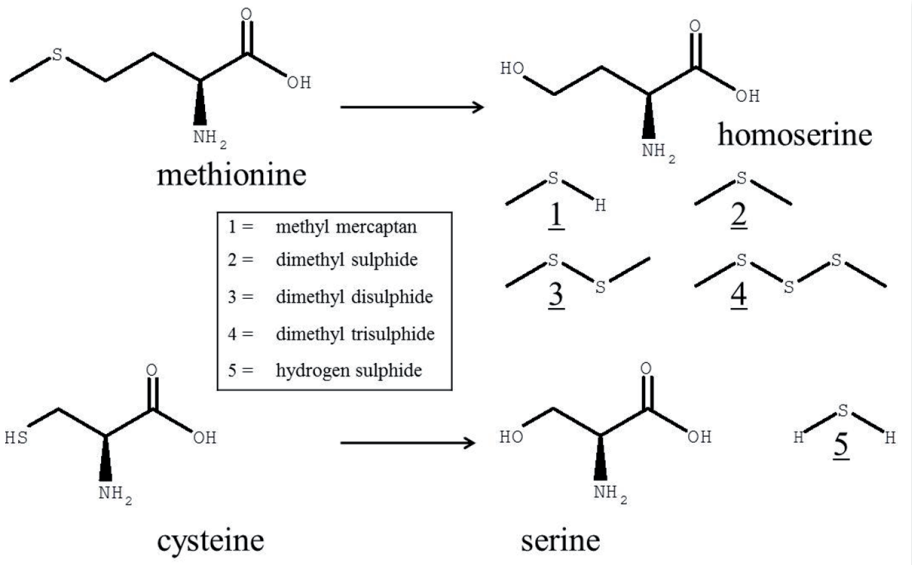


Fig. 5 Formation of alkylsulphides

Table 3 Sensory properties of alkyl and polysulphides [5, 11, 12, 13]

compound	odor threshold ppb	odor impression
hydrogen sulphide	20–100	rotten eggs
methyl mercaptan	20	rotten vegetable, unpleasant
dimethyl sulphide	25–60	cooked vegetable, onion-like, rubbery
dimethyl disulphide	3–50	cooked vegetable, onion-like, sulphurous
dimethyl trisulphide	0.1	cooked vegetable, onion-like, sulphurous
dimethyl tetrasulphide	0.2	cooked vegetable, onion-like, sulphurous

4.1.2 Thioesters

The thioesters S-methylthioisobutyrate, S-methylthio-2-methylbutyrate, and S-methylthioisovalerate are very similar to the side chains of the alpha acids. They are presumably formed by the biosynthesis pathway of the alpha acids (Figure 6). S-methyl-thiohexanoate, and S-methylthio-4-methylpentanoate are likely to result from the fatty acid metabolism.

The thioesters are also judged rather negatively. A. Suggett, M. Moir, and J.C. Seaton wrote in the Proceedings of the European Brewery Convention Congress, Berlin (West) 1979 [16] that hops with high thioester contents are not suitable for the "flavour hopping". The contents and composition of the thioesters are, in any case, specific to the particular species. The Polaris variety has a particularly high content of thioesters (in particular, S-methylthiohexanoate), which is perhaps the reason for its difficult handling. The use of dry hopping requires experienced brewers.

4.1.3 Polyfunctional thiols

Polyfunctional thiols occur only in very small amounts (ppb) in hops,

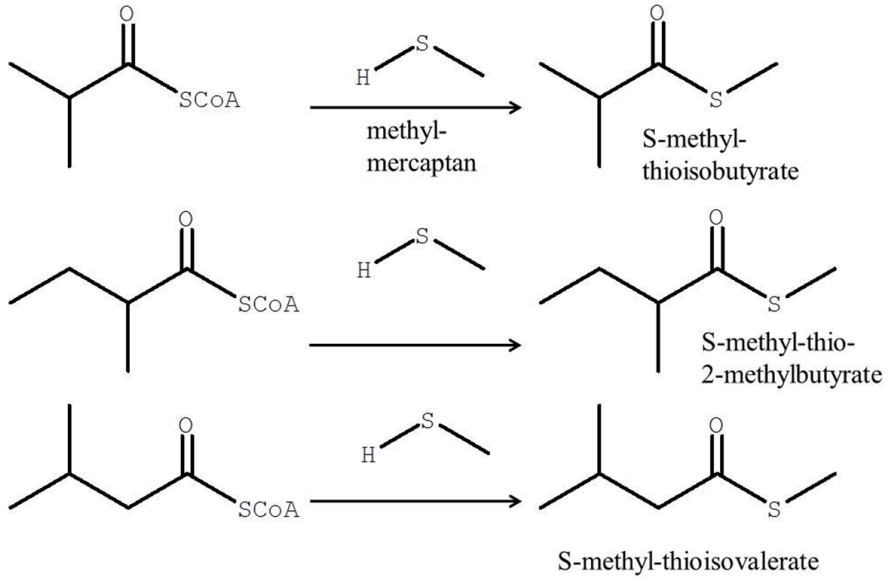


Fig. 6 Model for the biosynthesis pathway of the thioesters

Table 4 Sensory properties of thioesters [5]

Compound	Odour thres-hold ppb	Odour impression
S-methyl-thioisobutyrate	4–40	cheesy, cooked vegetable
S-methyl-thio-2-methylbutyrate	1	soapy, cooked vegetable
S-methyl-thioisovalerate	50 [14]	cheesy, cooked vegetable
S-methyl-thio-4-methylpentanoate	15	musty, cooked vegetable
S-methyl-thiohexanoate	0.3–1 [15]	soapy, cooked vegetable

Table 5 Sensory properties of polyfunctional thiols [17, 18]

Compound	Odour thres-hold ppt	Odour impression
4-mercapto-4-methyl-2-pentanone	0.8	black currant, box tree
3-mercaptohexanol	4.2	black currant, passion fruit
3-mercaptohexylacetate	60	grapefruit, passion fruit

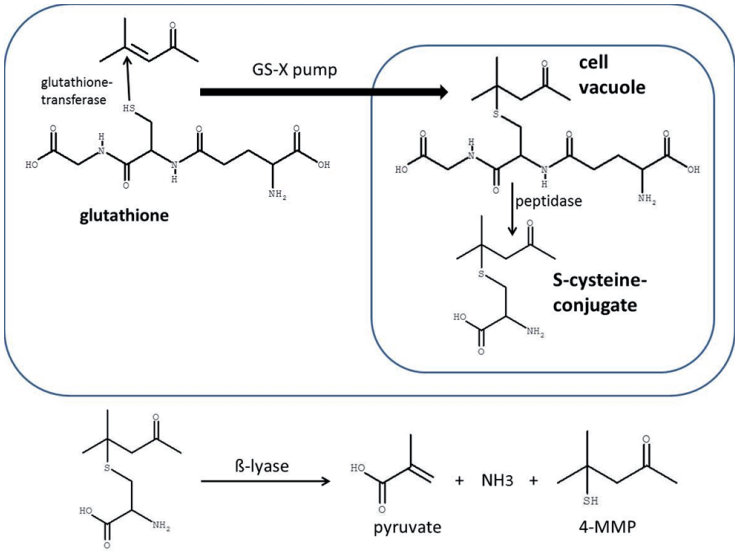


Fig. 7 Biosynthesis pathway of the polyfunctional thiols, according to M. Wüst [17]

but they have extremely low odor thresholds and so contribute to the aroma of hops (Figure 3). Table 5 describes the sensory properties.

4-mercapto-4-methyl-2-pentanone (4-MMP): 4-MMP was identified as the substance which causes a black currant or muscat-like bouquet in Sauvignon Blanc or Cabernet Sauvignon wines. In higher concentrations, 4-MMP is very foul-smelling and smells like cat urine, but in very low concentrations the 4-MMP mediates the typical black currant aroma [18]. Steinhaus was the first who identified 4-MMP as a key aroma compound in hops [19]. Investigations of *Kishimoto* et al. [20, 21] show that the variety Cascade cultivated in the USA has more 4-MMP than the variety cultivated in the Hallertau. This is associated with the higher amounts of copper that are applied in the Hallertau. Cu2 + ions can bind thiols. Today, however, only 4 kg of copper per hectare and year are permitted. Nor can it be directly deduced that copper alone is responsible for this. The different environmental conditions, too, can certainly play a role.

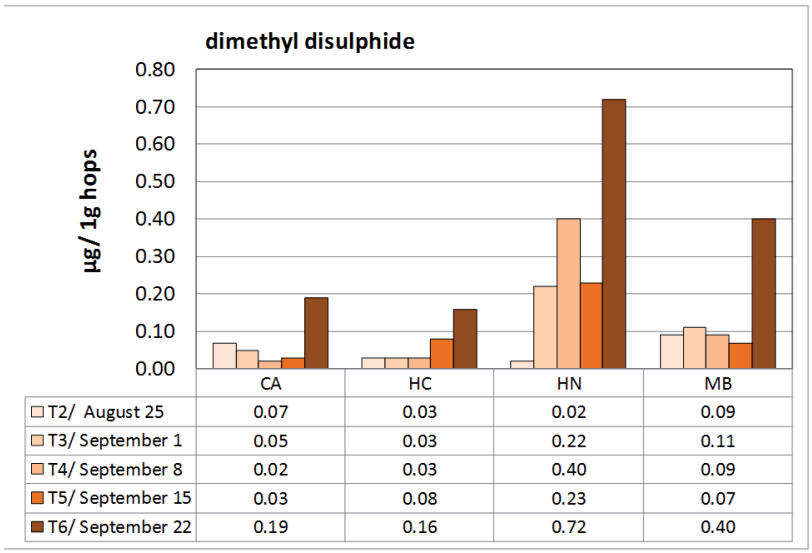
Of the polyfunctional thiols of the hops, 10–20 % are bound to cysteine. The biosynthetic pathway, according to *M. Wüst* [17], is shown in Figure 7. First, a precursor (in this case mesityloxide) binds to glutathione. This compound is stored in the cell vacuoles. The cysteine conjugate of the corresponding thiol then cleaves. In the brewing process, the polyfunctional thiols are released by the enzymes of the yeast (β-lyase).

4.2 The influence of the harvest date on sulphur compounds

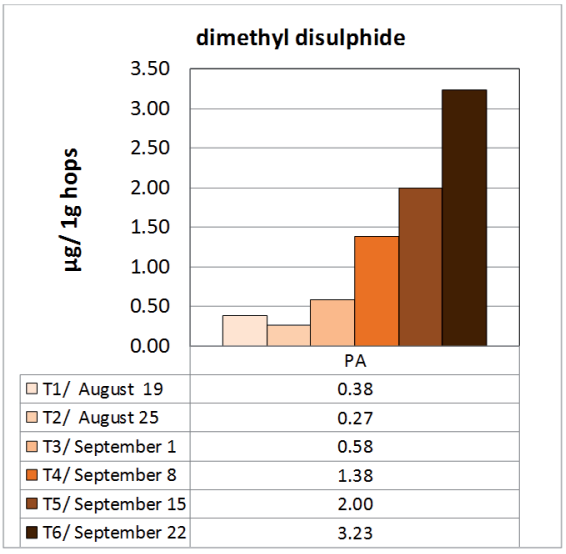
The substances dimethyldisulphide 2, S-methylthio-isovalerate 5, and S-methylthio-hexanoate 8 could be very well determined quantitatively using the standard addition. Figures 8, 9 and 10 show the dependence of these substances on the date of harvest.

4.2.1 Discussion

All sulphur compounds increase strongly at later harvesting dates. In particular, the variety Polaris has a very high content of sulphur compounds, and so, the variety is always shown individually. Late-harvested hops often have onion- and garlic-like flavours, which is certainly due to sulphur compounds. This could also be demonstrated analytically. In this work, sensory impressions should be supported by analytics. Basic knowledge should be created about which sulphur compounds occur in hops and are characteristic of certain hop species. What is to be found again in the beer would be a different question and would certainly be a suitable topic for further brewing research.

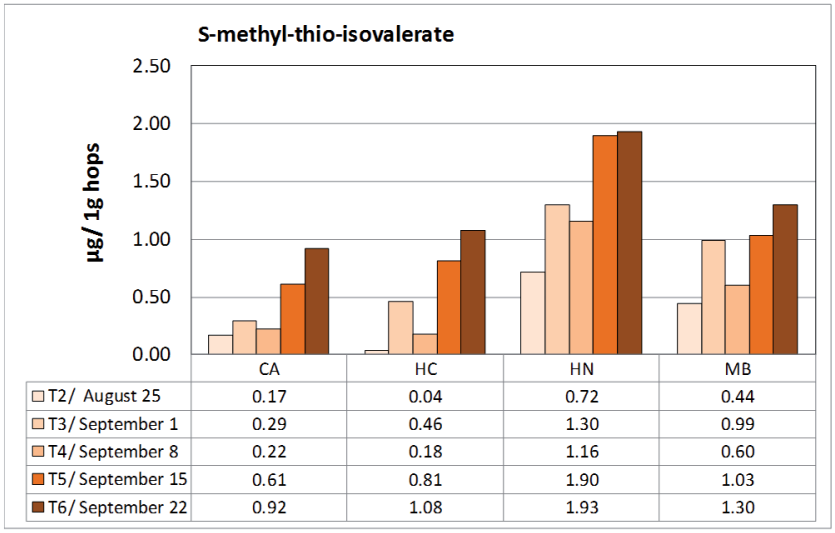


	CA	HC	HN	MB
T2/ August 25	0.07	0.03	0.02	0.09
T3/ September 1	0.05	0.03	0.22	0.11
T4/ September 8	0.02	0.03	0.40	0.09
T5/ September 15	0.03	0.08	0.23	0.07
T6/ September 22	0.19	0.16	0.72	0.40

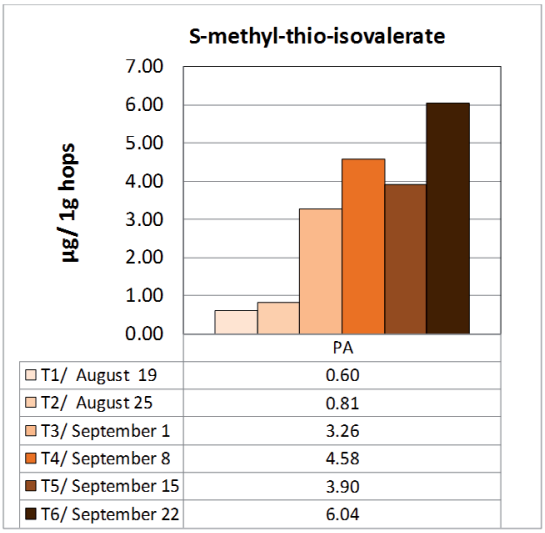


	PA
T1/ August 19	0.38
T2/ August 25	0.27
T3/ September 1	0.58
T4/ September 8	1.38
T5/ September 15	2.00
T6/ September 22	3.23

Fig. 8 Dimethyl disulphide concentrations of the varieties CA, HC, HN, MB, and PA, depending on the date of harvest

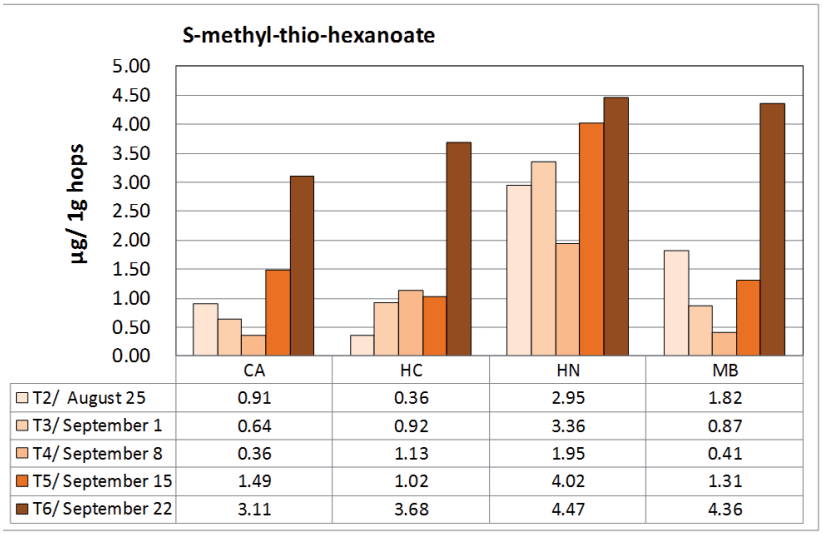


	CA	HC	HN	MB
T2/ August 25	0.17	0.04	0.72	0.44
T3/ September 1	0.29	0.46	1.30	0.99
T4/ September 8	0.22	0.18	1.16	0.60
T5/ September 15	0.61	0.81	1.90	1.03
T6/ September 22	0.92	1.08	1.93	1.30

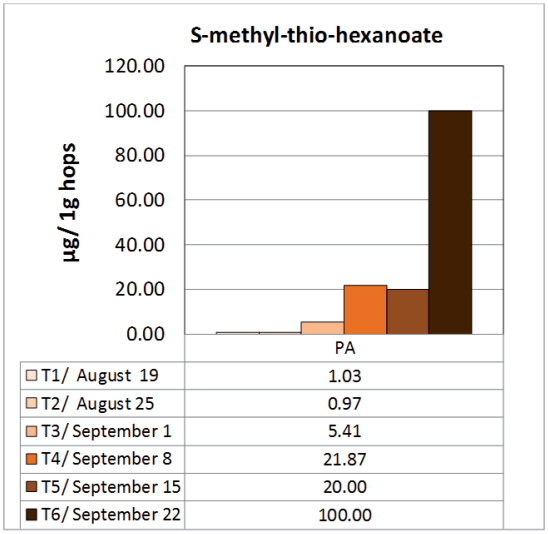


	PA
T1/ August 19	0.60
T2/ August 25	0.81
T3/ September 1	3.26
T4/ September 8	4.58
T5/ September 15	3.90
T6/ September 22	6.04

Fig. 9 S-methyl-thio-isovalerate concentrations of the varieties CA, HC, HN, MB, and PA, depending on the date of harvest



	CA	HC	HN	MB
T2/ August 25	0.91	0.36	2.95	1.82
T3/ September 1	0.64	0.92	3.36	0.87
T4/ September 8	0.36	1.13	1.95	0.41
T5/ September 15	1.49	1.02	4.02	1.31
T6/ September 22	3.11	3.68	4.47	4.36



	PA
T1/ August 19	1.03
T2/ August 25	0.97
T3/ September 1	5.41
T4/ September 8	21.87
T5/ September 15	20.00
T6/ September 22	100.00

Fig. 10 S-methyl-thio-hexanoate concentrations of the varieties CA, HC, HN, MB, and PA, depending on the date of harvest

5 Conclusion/summary

All main sulphur compounds could be identified by comparison of the mass spectra and with pure substances. The content and composition of the sulphur compounds are clearly dependent on the variety. The variety Polaris has the highest content of sulphur compounds from the previously measured varieties. The substances dimethyldisulphide 2, S-methylthio-isovalerate 5, and S-methylthio-hexanoate 8 could be determined quantitatively with the aid of the standard addition. All sulphur compounds strongly increase at later harvest dates and the onion- and garlic-like flavour impressions of late harvested hops have also been confirmed analytically.

6 Literature

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Received 18 July 2017, accepted 2 October 2017