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Development of a New Quantitation Method for Polyfunctional Thiols and Its Application for Investigation of the Characteristic Aroma of “Flavor Hops”.

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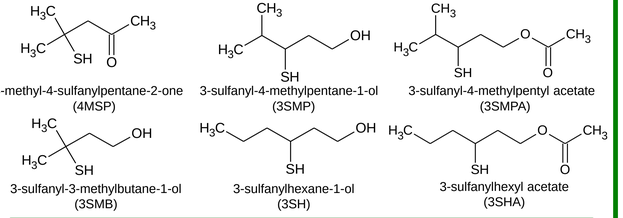
Introduction

Polyfunctional thiols are important compounds for the characteristic aroma of hops. Many researchers are interested in polyfunctional thiols, because of their characteristic flavors and very low thresholds. However, the thiol contents in beer are extremely low, and it is very difficult to analyze such low-level thiols. Moreover the most conventional analytical method uses a harmful reagent that contains a mercury compound. In this study, we developed a new method for quantitation of polyfunctional thiols without using mercury compounds and applied the method to investigating their contribution to the characteristic aroma of “flavor hops”.



Development of a New Method for Thiols

Problems of existing method

- ✓ The most conventional thiol specific extraction method uses a harmful reagent that contains a mercury compound.
- ✓ There are some derivazation methods, but they can’t be used with olfactometry.

We developed a new thiol specific extraction method using silver ion fixed SPE cartridge.

A Newly Developed Thiol Specific Extraction Method

Extract with dichloromethane

Solid-phase extraction with silver ion fixed SPE cartridge

Agilent 7000B triple quadrapole mass spectrometer

Solid-phase extraction was performed by ASPEC GX-274 (GILSON).

The method allows us to analyze eight samples per day without using mercury compounds.

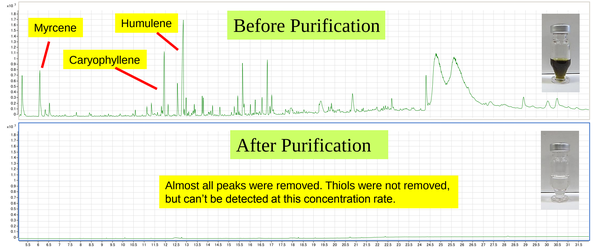


Figure 1. Total ion chromatogram of Hallertauer Tradition hop extract before or after solid-phase extraction with silver ion fixed SPE cartridge. Both extracts were ten times concentrated.

Table 1. Recovery and Repeatability in Hops

	Precursor (m/z)	Product (m/z)	CE (V)	Recovery (%)	CV (%)
4MSP	132	89	6	74	6.3
3SMP	134	100	0	93	4.1
3SH	134	82	2	97	5.3
3SMPA	116	88	4	95	2.8
3SHA	116	88	4	100	3.2
3SMB	120	71	10	83	5.0

Recovery and repeatability were evaluated using spiked Hallertauer Tradition (n=6). The spiked concentration was 250 μg/kg for 3SMP, 25 μg/kg for the others.

Figure 2. SRM chromatogram of non-spiked or spiked Pilsner beer.

Table 2. Limit of Detection and Calibration of Beer

	Odor threshold [a] (ng/L)	Limit of detection [b] (ng/L)	Cariblation rage (ng/L)	R^2
4MSP	1.5 (1)	1.4	0-100	0.998
3SMP	70 (2)	7.1	0-1000	0.993
3SH	55 (1)	2.1	0-250	0.996
3SMPA	160 (2)	2.9	0-100	0.997
3SHA	4 (3)	3.7	0-100	0.997
3SMB	1500 (4)	18.8	0-1000	0.999

[a] Odor thresholds are in beer except for 3SMB in model wine.
[b] Limit of detection was evaluated using non-spiked or spiked (5ng/L or 25ng/L) Pilsner beer and defined as three times the S/N.

Results of Hop and Beer Analysis

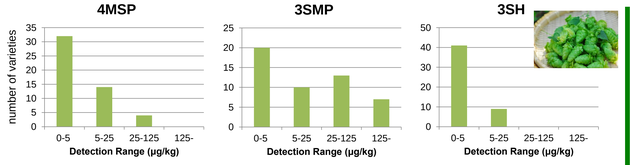


Figure 3. Detection range of polyfunctional thiol in 50 varieties of hops

Table 3. Result of Typical Hop Analysis (μg/kg)

Variety	4MSP	3SMP	3SH	3SMPA	3SHA	3SMB
Hallertauer Tradition	<1	1	1	<1	<1	5
Hallertau Blanc	2	432	18	89	<1	11
Mandarina Bavaria	1	53	8	9	<1	7
Huell Melon	<1	<1	<1	<1	<1	5
Citra	67	52	18	2	<1	51
Mosaic	49	205	15	1	<1	26
Equinox	11	175	4	4	<1	25
Simocoe	23	88	10	1	<1	10
Cascade	17	18	5	1	<1	13
Nelson Sauvin	31	492	9	11	<1	64
Galaxy	21	34	8	1	<1	17

Test beers were made with late-hopping in our pilot-scale brewery. Hallertauer Tradition, Citra and Nelson Sauvin were used for flavoring at 1.6 g/L. Polyfuncitonal thiols were analyzed by the newly developed method. Other compounds were analyzed by SPME-GC-MS according to previously described methods [(5)].

Table 4. Result of Beer Analysis

	Hallertauer Tradition	Citra	Nelson Sauvin		Hallertauer Tradition	Citra	Nelson Sauvin
Polyfunctional thiols (ng/l)							
4MSP	ND	48	28	3SH	94	119	91
3SMP	11	80	578	3SHA	6	7	7
3SMPA	ND	8	26	3SMB (μg/l)	1.02	1.51	1.51
Other compounds (μg/L)							
linalool	128	219	74	ethyl 2-methylpropanoate	2.9	3.9	7.0
geraniol	1.8	12.0	6.8	ethyl 2-methylbutanoate	0.8	0.8	1.1
citronellol	3.6	25.0	12.3	ethyl 3-methylbutanoate	0.9	1.6	2.6
myrcene	9.3	7.1	1.1	ethyl 4-pentanoate	0.6	0.3	0.7
1-hexanol	27.9	33.4	31.6	2-methylpropyl butylate	13.1	4.5	13.1
cis-3-hexenol	5.3	3.8	3.4	2-methylbutyl butylate	37	44	96
				3-methylbutyl butylate	2.0	6.8	5.6

Except for 3SH, 3SHA and 3SMB, 16 componuds were evaluated by sensory test.

Sensory Test

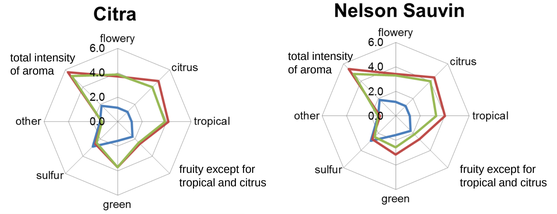


Figure 4. Result of recombination test

Reference beer
Pilsner beer
(linalool level 3.1 μg/L)

Recombination beer
16 compounds were spiked to the reference beer to simulate the composition of test -beer

Test beer
Late-hopped with Citra or Nelson Sauvin

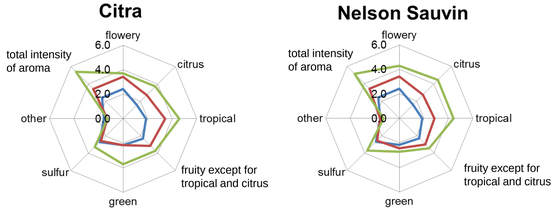


Figure 5. Result of omission test

Reference beer
Pilsner beer
(linalool level 4.5 μg/L)

Recombination beer
16 compounds were spiked to the reference beer to simulate the composition of test beer

Thiol omission beer
Omitted 4MSP, 3SMP and 3SMPA from recombination beer

Conclusion

- ✓ We developed a new thiol-specific extraction method using an silver ion fixed SPE cartridge. We employed this and GC/MS/MS with SRM mode for quantitation of polyfunctional thiols in hops and beers. The method has sufficient sensitivity and also good apparent recovery and repeatability.
- ✓ We investigated six polyfunctional thiols in 50 varieties of hops. The result showed that 4MSP and 3SMP are contained in many varieties and considered as important thiols.
- ✓ We evaluated the contribution of these thiols to the aroma of beers brewed with Citra and Nelson Sauvin by a recombination test and an omission test. The results revealed that these thiols are very important for the characteristic aroma derived from these “flavor hop” varieties.

References 1. Kishimoto et al., *J. Agric. Food Chem*. 2006, 54, 8855–8861. ; 2. Takoi et al., *J. Agric. Food Chem*., 2009, 57, 2493–2502 . ; 3. Kishimoto et al., *J. Am. Soc. Brew. Chem*. 2008, 66, 192-196. ; 4. Tominaga et al., *J. Agric. Food Chem*., 1998, 46, 1044-1048. ; 5. Tokita et al., *J. Am. Soc. Brew. Chem*. 2014, 72, 154-161.