

Occurrence of Odorant Polyfunctional Thiols in the Super Alpha Tomahawk Hop Cultivar. Comparison with the Thiol-rich Nelson Sauvin Bitter Variety

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ABSTRACT: Tomahawk hop (*Humulus lupulus*) is a recently developed Super Alpha cultivar (14–18% α -acids w/w), already widely used by brewers to impart bitterness and a citrus-like aroma to beer. By comparison with two bitter varieties (Nelson Sauvin and Nugget) and two aromatic ones (Cascade and Saaz), the Tomahawk cultivar showed a very particular terpenoid profile, rich in both α - and β -selinenes (>600 mg/kg IST equiv in total), methyl geranate (>40 mg/kg IST equiv), and geraniol (>200 mg/kg). Tomahawk also proved to contain a wide variety of odorant polyfunctional thiols. The major β -sulfanyl acetate, 3-sulfanyl-2-ethylpropyl acetate, newly identified here, was found at similar levels in the famous Sauvignon-like Nelson Sauvin and Tomahawk varieties (15–44 μ g/kg IST equiv). On the other hand, lower levels of total β -sulfanyl alcohols were measured in Tomahawk, although 3-sulfanylhexan-1-ol was found at a similar level and the 3-sulfanyl-4-methylpentan-1-ol previously claimed to be specific to the Nelson Sauvin variety was also evidenced in the Super Alpha cultivar (9–13 μ g/kg IST equiv). As revealed by boiling and fermentation, Tomahawk hop also contains very interesting bound polyfunctional thiols that should be investigated for better use by brewers.

KEYWORDS: hop (*Humulus lupulus*), essential oil, flavor, polyfunctional thiols, cysteine adducts, Super Alpha variety

INTRODUCTION

Only female inflorescences of the hop plant (*Humulus lupulus*) are used in the brewing industry. Because hop varieties contain bitter compounds in variable amounts and proportions, hops are usually classified into high bitter and low bitter/aromatic hops on the basis of their α -acid content (> or <7%, respectively; Table 1). After harvest in the autumn, the hop is dried (from 75–80% moisture to below 10%) before storage between -2 and 4 °C for up to one year. To avoid a strong decrease in α -acid content and flavors, it is quite usual to pelletize dried milled hop before it is sold to brewers.^{1–6} Mainly used in the brewing industry for bitterness and flavor, hop is also known to exhibit high bacteriostatic activity and to contribute to beer foam stability.^{7–10}

At the end of the 20th century, hop breeders focused selection on high α -acid content. The Tomahawk cultivar, also known as Columbus, emerged in the Yakima Valley as the first Super Alpha variety commercially available (α -acids, 14.0–18.0% with 30–35% cohumulone; β -acids, 4.5–5.8%). In 1998, Tomahawk already represented 11% of the American hop farming area. As Tomahawk and the Zeus variety are considered to be similar, they are often grouped together under the CTZ denomination (Super Alpha Columbus/Tomahawk and Zeus), accounting for 30% of the U.S. acreage in 2009. Bravo, Warrior, Millenium, and Apollo from the United States and Hallertau Herkules from Germany are other new Super Alpha varieties.

To reach good yields, heavy spray treatments are required on Tomahawk fields. Hop is usually considered to be an exceptional source of polyphenols, well-known phytoalexins, mainly procyanidins, chalcones, and flavonols.¹¹ Recently, the phytoalexins *trans*-resveratrol, *trans*-piceid, and *cis*-piceid were evidenced in

Tomahawk hop pellets.¹² Yet as previously shown for total polyphenols and flavanoids, high α -acid contents proved inconsistent with high amounts of stilbenes.^{13–15} Low levels of polyphenols may thus explain part of the susceptibility to powdery mildew.

The composition of hop oil and the hop flavor derived therefrom in beer depend on the hop variety. Essential oil analysis can differentiate most hop cultivars.¹⁶ Discrimination flowcharts have been published, but none of them takes Super Alpha varieties into account.^{6,17} The Tomahawk variety is claimed to display a high total oil ratio (2.0–3.5 g/100 g hop pellets) with up to 12% β -caryophyllene, 22% α -humulene, and 45% β -myrcene. Tomahawk is sometimes considered to be a dual variety and has even been recommended for late hopping, as it can impart very pleasant fresh notes to beer, some of which are similar to those imparted by the famous Nelson Sauvin. In the latter case, five polyfunctional thiols were recently evidenced as responsible for Sauvignon-like descriptors: 4-sulfanyl-4-methylpentan-2-one (**29** in Figure 1), 3-sulfanylhexan-1-ol (**23**), 3-sulfanylpentan-1-ol (**21**), 3-sulfanyl-4-methylpentan-1-ol (**26**), and its acetate (**12**).¹⁸

The aim of the present work was to investigate which terpenoid and sulfur compounds significantly distinguish the Super Alpha Tomahawk variety. The composition of hopped beer was compared to that of hop to check if the main free polyfunctional thiols found in the hop survive the boiling and fermentation steps. Concentrations were compared to assess the

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Table 1. Composition of Hop Samples, As Given by the Suppliers

type	variety	growing country	α-acids		total oils (g/100 g of hop)	aroma
			total % (w/w)	cohumulone (% of α-acids)		
low bitter/aromatic	Saaz	Czech Republic	3–4.5	24–26	0.8	mild hoppy, slightly spicy
low bitter	Cascade	USA	4.5–7	33–40	0.8–1.5	pleasant, floral, and spicy
high bitter	Nugget	USA	11–14	24–30	1.5–2.3	strong and herbal
	Nelson Sauvin	New Zealand	12–13	25	1.1	grape-like
high bitter/Super Alpha	Tomahawk	USA	14–18	30–35	2–3.5	intense, citrus-like

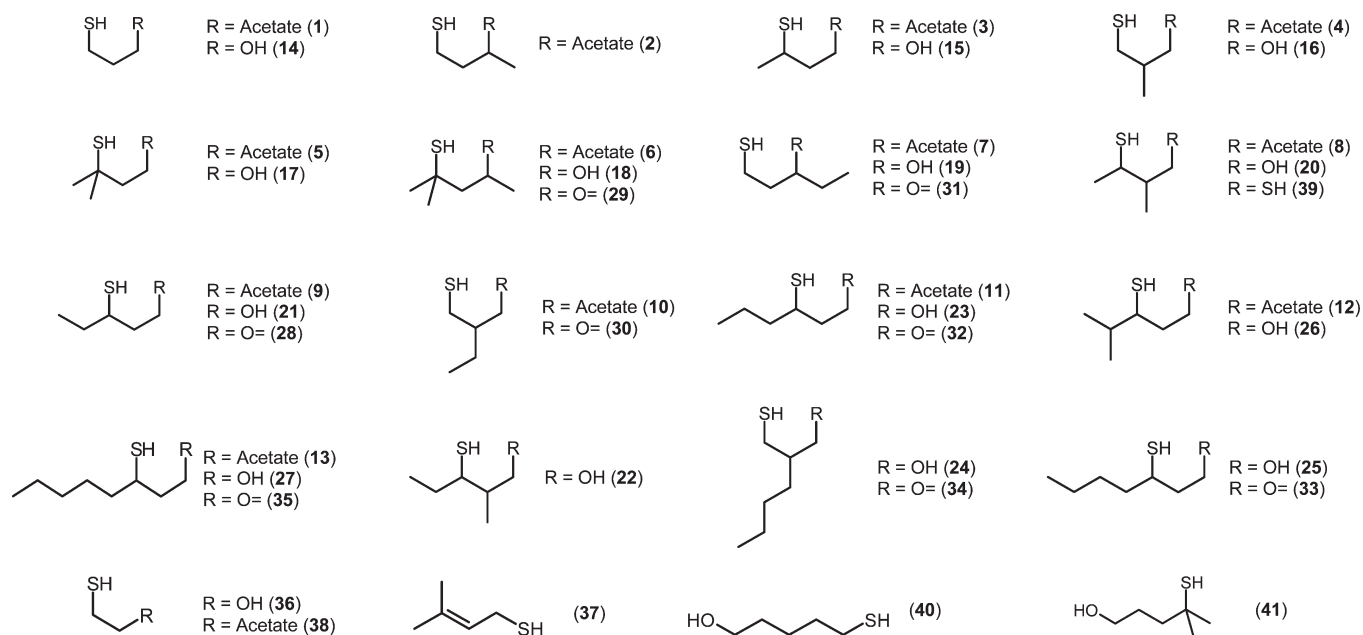


Figure 1. Chemical structures and numbering of thiols.

relative contributions of hop free polyfunctional thiols and suspected precursors.

MATERIALS AND METHODS

Materials. *p*-Hydroxymercuribenzoic acid (*p*HMB), HCl 37%, carvone, myrcene, linalool, geraniol, β -caryophyllene, α -humulene, β -farnesene, undecan-2-one, 3-sulfanylpropyl acetate (1), 3-sulfanylpropan-1-ol (14), 3-sulfanyl-3-methylbutan-1-ol (17), 2-sulfanylethan-1-ol (36), and 2-sulfanylethyl acetate (38) were purchased from Sigma-Aldrich (Bornem, Belgium) (full chemical structures for thiols are given in Figure 1). 3-Methylbutyl isobutyrate, 4-methoxy-2-methylbutane-2-thiol, 3-sulfanylhexan-1-ol (23), and 3-methyl-2-butene-1-thiol (37) were obtained from Oxford Chemicals (Oxford, U.K.). 4-Sulfanyl-4-methylpentan-2-one (29) was from Frutarom (Hartlepool, U.K.). Sulfanylethyl thioacetate was purchased from Alfa Aesar (Heysham, Lancashire, U.K.). Dichloromethane (99.9%) obtained from Romil (Cambridge, U.K.) was distilled before use. Milli-Q water was used (Millipore, Bedford, MA). NaOH and Na₂SO₄ 99% were supplied by Janssen (Geel, Belgium). A strongly basic Dowex resin IX2, Cl⁻ form (Sigma-Aldrich) was stored in hydrogen chloride (0.1 M). Anhydrous

sodium sulfate was obtained from Merck (Darmstadt, Germany) and tris (hydroxymethyl)aminomethane (Tris) from USB (Cleveland, OH).

Reference Compounds Synthesized in Our Laboratory.

Sulfanylmethyl thioacetate, sulfanylmethyl thiopropionate, sulfanylmethyl thioisovalerate, sulfanylmethyl thiohexanoate, and sulfanylmethyl thiooctanoate have been previously obtained (reagents and complete procedure in ref 19). 1-Sulfanyl-3-butyl acetate (2) 3-sulfanylbutyl acetate (3), 3-sulfanyl-2-methylpropyl acetate (4), 3-sulfanyl-3-methylbutyl acetate (5), 4-sulfanyl-4-methyl-2-pentyl acetate (6), 1-sulfanyl-3-pentyl acetate (7), 3-sulfanyl-2-methylbutyl acetate (8), 3-sulfanyl-pentyl acetate (9), 3-sulfanyl-2-ethylpropyl acetate (10), 3-sulfanylhexyl acetate (11), and 3-sulfanyloctyl acetate (13) have been also synthesized prior to that work (reagents and complete procedure in ref 20). 3-Sulfanylbutan-1-ol (15), 3-sulfanyl-2-methylpropan-1-ol (16), 1-sulfanylpentan-3-ol (19), 3-sulfanyl-2-methylbutan-1-ol (20), 3-sulfanylpentan-1-ol (21), 3-sulfanyl-2-methylpentan-1-ol (22), 3-sulfanyl-2-butylpropan-1-ol (24), 3-sulfanylheptan-1-ol (25), 3-sulfanyloctan-1-ol (27), and 3-sulfanyl-2-methylbutane-1-thiol (39) have been obtained with the reagents and procedure disclosed in ref 21. Materials and methods to obtain 4-sulfanyl-4-methylpentan-2-ol (18) are detailed in ref 22. 3-Sulfanylpentanal (28), 3-sulfanyl-2-ethylpropanal (30), 3-sulfanylhexanal (32), 3-sulfanylheptanal (33), 3-sulfanyl-2-butylpropanal (34), and

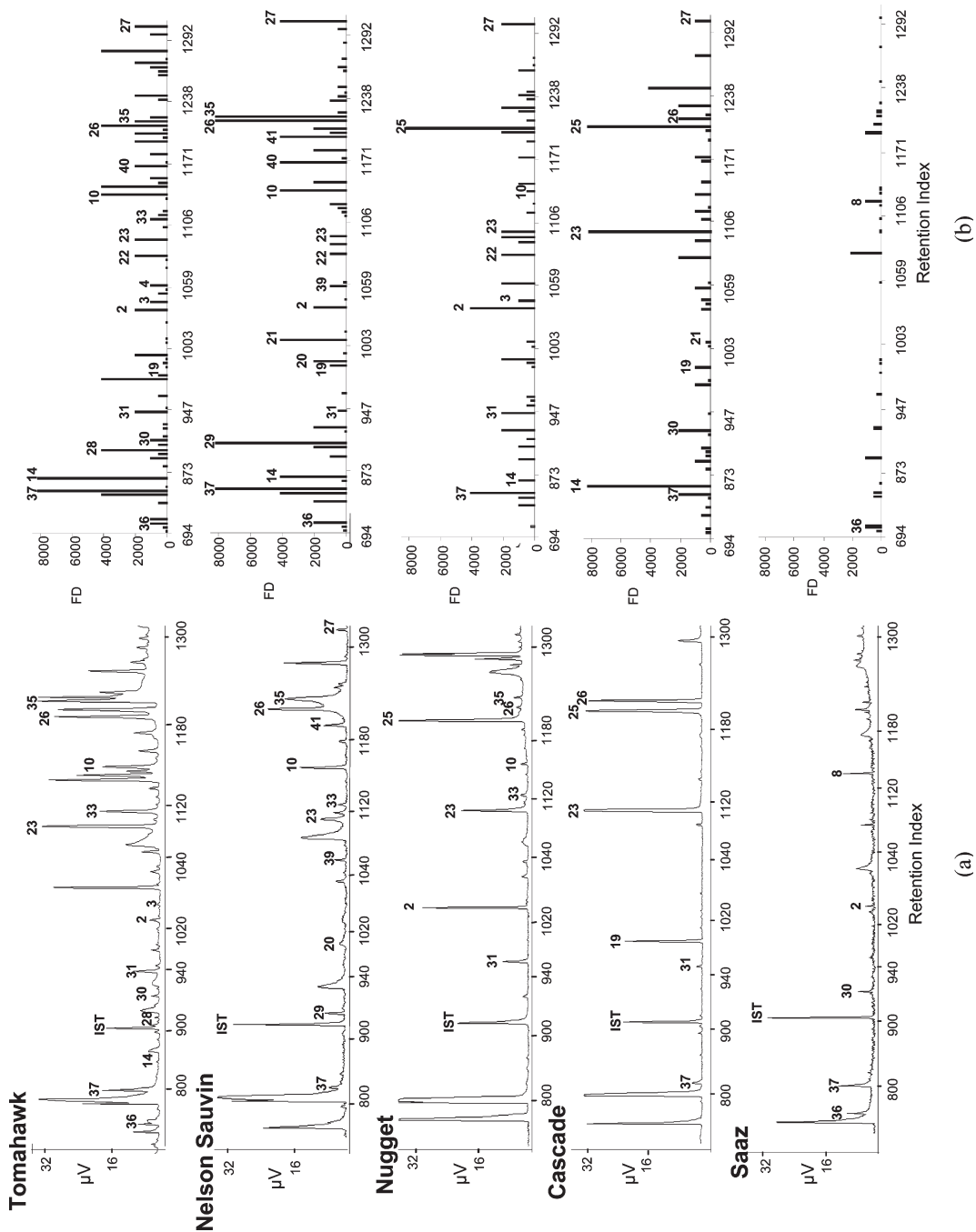


Figure 2. (a) GC-PPPD chromatograms of 2008 hop pellets *p*HMB extracts (IST at 33.5 $\mu\text{g}/\text{kg}$). (b) Aromagrams: flavor dilution of the corresponding odorants (FD = 2^n with $n + 1 =$ number of dilutions applied on the extract until no odor was perceived; precision: $n \pm 1$ or a factor 2 between FD).

Table 2. Non-Sulfur Flavors in Hop Pellets (Two Successive Harvest Years) Determined by GC-MS (Concentrations in mg/kg of Hop, IST Equivalents for Compounds Carrying the IST Superscript), after Likens–Nickerson Extractions (Mean of Duplicates, CV < 6%)^{17, a}

RI CP-SiIs	substance	odor (GCO)	Tomahawk		Nelson Sauvin		Nugget		Cascade		Saaz		identification reliability ^b (three main <i>m/z</i> ions in parentheses)
			2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	
982	myrcene	lime	3521.0 a	3117.1 b	1484.7 f	1616.0 e	1188.4 h	1274.5 g	2289.2 d	2565.2 c	619.1 i	481.8 j	I ⁺ (41, 93, 69)
1086	linalool	floral	153.6 c	168.1 c	278.1 b	311.9 a	56.3 d	41.6 de	36.2 de	41.9 de	19.8 e	32.7 de	I ⁺ (71, 93, 41)
1212	β -citronellol ^{IST}	lemon	0.2 c	0.2 c	0.6 b	0.5 b	0.2 c	0.2 c	1.0 a	1.2 a	1.1 a	1.0 a	I ⁺ (41, 69, 55)
1236	geraniol	floral	246.5 a	211.3 b	35.3 ef	38.4 de	38.9 de	22.1 ef	58.9 c	47.3 cd	11.3 f	14.5 ef	I ⁺ (69, 41, 68)
1313	methyl geranate ^{IST}	greenery	46.9 b	55.8 b	18.8 cd	14.1 d	18.8 cd	14.8 d	69.2 a	47.1 b	8.9 d	7.2 d	I ⁺ (69, 41, 114)
1435	β -caryophyllene	spicy	1737.1 ab	1889.6 a	1816.4 ab	1675.6 b	1211.1 c	1298.6 c	1208.2 c	1167.4 c	259.8 d	289.4 d	I ⁺ (41, 93, 69)
1469	α -humulene ^c	resine	1258.2 d	1489.1 c	719.1 g	908.2 ef	2221.1 b	3159.2 a	953.2 ef	802.4 fg	1029.7 e	1265.3 d	I ⁺ (93, 41, 121)
1475	bergamotene ^{e,IST}	tea	— e	— e	— e	— e	— e	— e	22.3 c	17.1 d	48.9 b	60.1 a	I ⁺ (119, 93, 41)
1486	β -farnesene ^e	woody	0.2 c	— c	1.0 c	1.1 c	2.2 c	2.4 c	6.8 c	5.1 c	825.6 a	761.2 b	I ⁺ (93, 41, 69)
1499	α -amorphene ^{e,IST}	woody	57.3 a	66.7 a	64.6 a	32.2 b	12.6 cd	16.4 cd	16.3 cd	21.3 c	5.9 d	8.3 d	I ⁺ (105, 161, 94)
1548	α -selinene ^{IST}	woody	341.2 b	459.6 a	137.4 c	129.4 c	82.3 de	96.2 d	78.2 de	62.9 ef	49.1 fg	39.5 g	I ⁺ (189, 93, 107)
1538	β -selinene ^{IST}	herbs	338.5 a	300.6 a	168.2 b	137.5 bc	94.7 cd	79.9 cde	31.5 e	42.9 de	27.2 e	25.9 e	I ⁺ (41, 105, 204)
1074	3-methylbutyl isobutyrate ^c	sweet	187.4 a	167.2 b	108.1 c	139.6 c	57.3 d	69.3 d	20.0 e	17.8 e	— f	— f	I ⁺ (43, 71, 70)
1279	undecan-2-one ^c	citrus	17.5 f	24.6 f	104.8 ab	77.7 de	94.3 bc	109.6 a	14.4 f	12.9 f	75.2 e	89.6 cd	I ⁺ (43, 58, 71)

^a —, below the detection limit (0.1 mg/kg). All samples that do not share a common letter are significantly different ($p < 0.05$) according to Tukey's test. ^b I⁺, compound identified by mass spectrometry (full scan monitoring) and coincidence with the retention index. ^c Compounds previously selected for discriminating Cascade, Challenger, Hallertau, Lublin, Mount Hood, Northdown, Northern Brewer, Nugget, Saaz, Styrie, and Target. ^{d,17}

Table 3. Thioesters in Hop Pellets Determined by GC-PFPD (Concentrations in mg/kg of Hop) and GC-O AEDA Analyses (FD in Parentheses) on Likens–Nickerson Extracts Assay in Duplicate^a

RI CP-SiIs	substance	odor (GC-O)	PFPD concentrations in mg/kg of hop, sulfanylethyl thioacetate equivalents (FD AEDA given in parentheses)												identification reliability ^b (three main <i>m/z</i> ions in parentheses)
			Tomahawk		Nelson Sauvin		Nugget		Cascade		Saaz				
			2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	
688	sulfanylmethyl thioacetate	food, cabbage	0.13 b (32)	0.10 bc (32)	0.53 a (512)	0.63 a (512)	0.08 bc (32)	0.10 bc (32)	– c (8)	– c (8)	– c (8)	0.10 bc (32)	I		
785	sulfanylmethyl thiopropionate	sprout, meaty	1.78 b (4096)	2.60 a (4096)	0.98 c (512)	1.11 c (512)	0.74 d (512)	0.62 d (512)	+ e (16)	+ e (32)	– f (2)	– f (4)	I* (57, 104, 45)		
923	sulfanylmethyl thioisovalerate	soup, cheese	+ a (4)	+ a (4)	+ a (8)	+ a (8)	+ a (8)	+ a (8)	+ a (8)	+ a (8)	– b (2)	– b (2)	I* (57, 85, 51)		
1073	sulfanylmethyl thiohexanoate	cabbage	– b (2)	– b (4)	+ a (8)	+ a (8)	– b (8)	– b (16)	– b (2)	– b (2)	– b (2)	– b (2)	I* (43, 99, 71)		
1293	sulfanylmethyl thiooctanoate	cabbage, cheese	0.80 ab (16)	0.70 c (16)	0.87 a (32)	0.78 bc (32)	0.23 e (8)	0.15 e (8)	– f (2)	0.53 d (16)	– f (2)	– f (2)	I* (57, 127, 109)		
	sum of concentrations		2.71 b	3.41 a	2.38 c	2.52 bc	1.05 d	0.87 d	+ e	0.53 e	– e	0.10 e			

^a +, detected under the quantification limit (0.09 mg/kg); –, not detected (<0.03 mg/kg). All samples that do not share a common letter are significantly different ($p < 0.05$) according to Tukey's test. ^b I, compound identified by coincidence with the GC-PFPD retention index and odor descriptor of the pure or synthesized compound on a CP-SiIs-CB capillary column; I*, additional confirmation by mass spectrometry (full scan monitoring).

3-sulfanyloctanal (35) have been previously produced according to ref 23. 1-Sulfanylpentan-3-one (31) was obtained with reference to ref 21 and 6-sulfanylhexan-1-ol (40) as described in ref 24.

3-Sulfanyl-4-methylpentyl acetate (12), 3-sulfanyl-4-methylpentan-1-ol (26), and 4-sulfanyl-4-methylpentan-1-ol (41) have been here synthesized, according to the method of Takoi et al.,¹⁸ from 4-methyl-3-penten-1-ol, thioacetic acid, sodium, and methanol provided by Sigma-Aldrich.

Hop Samples. Nugget, bred in the United States, and Saaz, bred in Czech Republic, were provided by Hopsteiner (Mainburg, Hallertau, Germany). Tomahawk and Cascade, bred in the United States, and Tomahawk CO₂ extract were provided by Yakima Chief (Louvain-la-Neuve, Belgium). Nelson Sauvin, bred in New Zealand, was provided by Hops Limited (Richmond, Nelson, New Zealand).

Hop Moisture Determination. Hop moisture was determined according to the EBC analytical method.²⁵ A portion of 3–5 g of milled pellets was put in an oven for 1 h at 103 ± 1 °C and weighed before and after drying. Because all of the samples exhibited moistures ranging between 6 and 9%, no corrective factor was applied to the quantifications.

Pilot Beer Production. Beer was produced in a 60 L microbrewery (Coenco, Belgium). In the brewing process, 12 kg of Pilsen Malt of spring (2 rows, Malterie du Château, Belgium) was brewed in 60 L according to the following mashing program: 30 min at 50 °C, 30 min at 63 °C, and 30 min at 72 °C. The wort was then heated to 82 °C and filtered through the lauter tun at a 0.3 L/min flow. The 11 °Plato wort thus obtained was boiled with 33 mg/L Tomahawk CO₂ extract for 75 min (10% evaporation). Ten minutes before the end, Tomahawk pellets were added at 1.78 g/L. The fermentation was conducted in cylindrical fermenting tanks with an ale type yeast (INBR Bras268) previously selected for high H₂S excretion.²⁶ This strain was pitched at 7.5 × 10⁶ cells/mL. The fermentation, carried out at 22 °C for 4 days, was followed by maturation for 7 days at 2 °C (until 3 × 10⁶ cells/mL). After filtration on plates (0.5 μM pores, Buon Vino, Cambridge, Canada), the beer was stored under carbon dioxide until extraction the next day.

Total Flavor Extraction by the Likens–Nickerson Method. Steam distillation–solvent extraction was carried out in a microextractor (Alltech 8910) according to the method of Bouseta and Collin.²⁷ Hop pellets (0.5 g) were milled and mixed with 50 mL of deoxygenated ultrapure water and 1.5 mL of a carvone solution at 20 mg/L (IST) in flask A. Dichloromethane and ultrapure, deoxygenated water (1.5 mL each) were introduced into the liquid/liquid extraction area. Dichloromethane (1.5 mL) was introduced in the organic phase vessel (B). A few clean grains of carborundum were added into flasks A and B. Prior to the procedure, the entire system was purged with nitrogen (2–3 mL/min) for 5 min. Flask A was then heated in a 140 °C oil bath and flask B in a 70 °C water bath. The vapors were condensed by means of a coldfinger maintained at –10 °C by a cryostat. The entire steam distillation–solvent extraction procedure was carried out under a 2 mL/min nitrogen flow. The steam distillation was stopped after 45 min. The dichloromethane extract was then concentrated to 0.5 mL at 45 °C with a Kuderna–Danish concentrator. The method allows most of the terpenic compounds to be recovered with factors of >90% and variation coefficients of <8%.

Extraction of Polyfunctional Thiols by pHMB.²⁸ Beer, wort (500 mL), or milled pellets (10 g) were stirred with distilled CH₂Cl₂ (200 mL) for 30 min. 4-Methoxy-2-methylbutane-2-thiol was added as internal standard (IST, at 33.5 μg/kg in hop and at 0.67 μg/L in beer or wort). After decantation (±15 min), the lower phase and the interfacial emulsion were centrifuged for 20 min at 4000 rpm. The organic phase was then extracted by 2 × 20 mL of a pHMB solution (360 mg of pHMB, 24.6 g of Tris in 1 L of Millipore water) for 5 and 10 min, respectively. The combined aqueous phases were loaded into a strongly basic anion

Table 4. Sulfanylalkyls Carrying an Acetate Function at the β -Position^a

RI	CP-Sil5	FFAP	substance	odor (GCO)	PFPD concentrations in $\mu\text{g}/\text{kg}$ of hop (FD AEDA given in parentheses)																identification reliability ^b (three main m/z ions in parentheses)
					Tomahawk			Nelson Sauvin			Nugget			Cascade			Saaz				
					hop	wort	beer	hop	hop	hop	hop	hop	hop	hop	hop	hop	hop	hop	hop		
1	1000	1579	3-sulfanylpropyl acetate	grilled	—	—	—	64.6 (20480)	—	—	—	—	—	—	—	—	—	I ⁺ (74, 43, 47)			
2	1024	1542	1-sulfanyl-3-butyl acetate ^{IST}	plastic, sprout	14.5 a (8192)	4.2 d (2048)	8.4 (5120)	6.1 (2560)	7.9 b	5.6 c	4.2 d	7.3 b	0.7 e	0.3 e	+ e	0.4 e	I ⁺ (88, 55, 60)				
3	1033	1536	3-sulfanylbutyl acetate ^{IST}	cheese, onion	3.4 c (512)	3.9 b (1024)	10.8 (10240)	32.4 (40960)	+ f	+ f	5.0 a	3.6 bc	2.2 e	2.8 d	- f	- f	I				
4	1059	1613	3-sulfanyl-2-methylpropyl acetate ^{IST}	popcorn, grilled nut	+ f (512)	0.8 c (1024)	- (5120)	3.9 (20480)	+ f	+ f	2.7 a	1.4 b	0.3 e	0.4 d	- f	- f	I ⁺ (88, 55, 47)				
5	1082	1576	3-sulfanyl-3-methylbutyl acetate ^{IST}	pepper, plastic	+ e (128)	- e (32)	1.9 (128)	40.4 (20480)	- e	- e	1.7 a	0.9 b	0.4 d	0.5 c	- e	- e	I ⁺ (69, 102, 87)				
6	1096	1506	4-sulfanyl-4-methyl-2-pentyl acetate ^{IST}	grilled nut	0.7 c (512)	0.6 c (256)	1.7 (1280)	3.6 (10240)	1.2 b	2.1 a	0.3 d	0.7 c	+ e	+ e	- e	- e	I ⁺ (101, 110, 83)				
7	1115	1627	1-sulfanyl-3-pentyl acetate ^{IST}	cheese, thyme	+ (1024)	+ (512)	- (2560)	- (1280)	+ +	+ +	- -	- -	- -	- -	- -	- -	I				
8	1119	1612	3-sulfanyl-2-methylbutyl acetate ^{IST}	cooked meat	2.3 c (128)	1.6 d (256)	-	37.3 (10240)	4.6 a	3.3 b	0.9 e	1.0 de	1.4 d	0.9 e	2.2 c	4.2 a	I ⁺ (102, 87, 74)				
9	1126	1604	3-sulfanylpentyl acetate ^{IST}	peach, passion	- (128)	- (64)	- (160)	- (2560)	-	-	-	-	-	-	-	-	I ⁻				
10	1147	1656	3-sulfanyl-2-ethylpropyl acetate ^{IST}	floral, vinegar	34.8 b (8192)	21.6 c (4096)	95.2 (10240)	183.1 (40960)	44.3 a	14.8 d	4.9 e	6.5 e	3.1 ef	0.3 f	+ f	0.5 f	I ⁺ (102, 60, 69)				
11	1215	1717	3-sulfanylhexyl acetate ^{IST}	candy, pumpkin	+ (512)	+ (1024)	+ (20480)	+ (40960)	+ +	+ +	- -	- -	- -	- -	+ +	- -	I ⁻				
12	1238	1708	3-sulfanyl-4-methylpentyl acetate ^{IST}	grapefruit	+ (1024)	0.2 a (512)	8.3 (2560)	48.2 (10240)	0.3 a	+b	- b	- b	- b	- b	- b	0.4 a	I ⁺ (83, 116, 73)				
13	1440	1920	3-sulfanyloctyl acetate ^{IST}	catty, lemon	+ (128)	+ (256)	- (320)	- (640)	+ +	+ +	- -	- -	- -	- -	- -	- -	I				
			sum of concentrations		55.6 a	33.0 b	126.3	419.6	58.4 a	25.8 c	19.7 d	21.5 d	8.1e	5.2 ef	2.2 f	5.5 ef					

^a Amounts determined by GC-PFPD in hop pellets ($\mu\text{g}/\text{kg}$ of hop, IST equivalents for compounds carrying the IST superscript). Comparison with Tomahawk 2008 pilot wort and beer, for which concentrations have been converted into hop equivalents (given in $\mu\text{g}/561\text{ L}$ of beer or wort = $\mu\text{g}/\text{kg}$ of hop used for production). GC-O AEDA analyses (FD in parentheses) on the pHMB extracts. Assay in duplicate. All samples that do not share a common letter are significantly different ($p < 0.05$) according to Tukey's test. +, detected under the quantification limit ($0.1\ \mu\text{g}/\text{kg}$); -, not detected ($< 0.03\ \mu\text{g}/\text{kg}$). ^b I⁺, compound identified by coincidence with the GC-PFPD retention indices and odor descriptor of the pure or synthesized compound on two capillary columns (CP-Sil5-CB and FFAP); I⁻, additional confirmation by mass spectrometry (full scan monitoring). I⁻, compound tentatively identified by coincidence with the GC-O retention indices and odors of pure or synthesized compound on two capillary columns (CP-Sil5-CB and FFAP).

Table 5. Corresponding β -Sulfanyl Alcohols^a

RI	PFPD concentrations in $\mu\text{g}/\text{kg}$ of hop (FD AEDA given in parentheses)												identification reliability ^b (three main m/z ions in parentheses)							
	Tomahawk		Nelson Sauvin		Nugget		Cascade		Saaz											
	hop	wort	hop	beer	hop	hop	hop	hop	hop	hop										
CP-Si5	FFAP	substance	odor (GCO)	2007	2008	2008	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	
14	849	3-sulfanylpropan-1-ol	potatoes, pop corn	+ b (4096)	1.1 a (8192)	+ (10240)	10.3 (163840)	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	I ⁺ (57, 58, 45)
15	903	3-sulfanylbutan-1-ol ^{IST}	perspiration, catty	2.5 b (128)	3.6 a (512)	57.5 (10240)	12.3 (2560)	0.4 e	+ f	+ f	+ f	+ f	+ f	+ f	1.7 c	1.2 d	+ f		I ⁺ (106, 72, 61)	
16	922	3-sulfanyl-2-methylpropan-1-ol ^{IST}	broth, leek	+ b (128)	1.0 a (256)	5.6 (640)	23.9 (2560)	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	+ b	I ⁺ (106, 72, 57)
17	938	3-sulfanyl-3-methylbutan-1-ol	sulfur, soup	0.7 b (128)	1.0 a (256)	3.4 (1280)	165.9 (20480)	0.4 c	0.6 b	- d	- d	- d	- d	- d	- d	- d	- d	- d	- d	I ⁺ (86, 69, 75)
18	947	4-sulfanyl-4-methylpentan-2-ol ^{IST}	fuel, catty	- e (64)	0.8 b (128)	3.4 (2560)	5.6 (10240)	1.2 a	0.6 c	- e	- e	- e	- e	- e	+ e	- e	+ d		I ⁺ (57, 85, 100)	
19	977	1-sulfanylpentan-3-ol ^{IST}	mushroom, nettle	+ d (256)	+ d (512)	- (80)	178 (40960)	1.9 b	1.7 b	1.2 c	0.8 c	2.0 b	4.2 a	- d	- d	- d	- d	- d	- d	I
20	989	3-sulfanyl-2-methylbutan-1-ol ^{IST}	leek, hop	- c (256)	- c (128)	4.5 (20480)	+ (10240)	2.0 a	1.6 b	- c	- c	- c	- c	- c	- c	- c	- c	- c	- c	I ⁺ (120, 86, 60)
21	1009	3-sulfanylpentan-1-ol ^{IST}	catty, citrus	+ e (64)	0.4 d (256)	- (80)	3.4 (1280)	3.2 a	1.8 b	0.9 c	+ e	0.8 c	- e	- e	+ e	+ e	+ e	+ e	+ e	I ⁺ (86, 69, 57)
22	1080	3-sulfanyl-2-methylpentan-1-ol ^{IST}	gravy	1.1 c (2048)	1.6 a (2048)	10.6 (10240)	8.1 (5120)	+ e	0.7 d	+ 1.3 b	+ e	+ e	+ e	+ e	+ e	+ e	+ e	+ e	+ e	I ⁺ (74, 100, 83)
23	1094	3-sulfanylhexan-1-ol	grapefruit	6.6 de (256)	12.2 cd (512)	38.3 (10240)	115.1 (81920)	16.8 c	6.1 de	3.4 e	14.2 cd	72.6 b	117.1 a	2.9 e	1.9 e	1.9 e	1.9 e	1.9 e	1.9 e	I ⁺ (57, 41, 61)
24	1191	3-sulfanyl-2-butylpropan-1-ol ^{IST}	plastic	- (1024)	- (256)	- (1280)	- (2560)	+ 2.0 b	+ 2.0 b	+ 2.0 b	+ 2.0 b	+ 2.0 b	+ 2.0 b	+ 2.0 b	+ 2.0 b	+ 2.0 b	+ 2.0 b	+ 2.0 b	+ 2.0 b	I
25	1199	3-sulfanylheptan-1-ol ^{IST}	lemon, hoppy	+ e (512)	0.4 e (2048)	2.3 (2560)	54.0 (163840)	1.2 e	2.0 e	14.1 d	21.2 c	52.3 a	25.5 b	- e	- e	- e	- e	- e	- e	I ⁺ (114, 81, 55)
26	1208	3-sulfanyl-4-methylpentan-1-ol ^{IST}	rhubarb, grapefruit	8.8 de (2048)	13.3 c (4096)	15.3 (20480)	48.2 (40960)	27.0 b	31.5 a	4.4 fg	2.1 gh	6.6 ef	9.8 d	+ h	- h	- h	- h	- h	- h	I ⁺ (100, 55, 73)
27	1308	3-sulfanyloctan-1-ol ^{IST}	catty, grapefruit	6.9 b (2048)	3.3 e (2048)	17.2 (20480)	78.8 (655360)	4.7 d	8.1 a	4.6 d	1.7 f	1.6 f	1.2 f	+ g	+ g	+ g	+ g	+ g	+ g	I
sum of concentrations				26.4 f	39.7 e	158.1	703.6	59.2 c	55.2 c	28.6 f	41.2 e	136.1 b	160.6 a	4.1 g	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g	

^a Amounts determined by GC-PPFD in hop pellets ($\mu\text{g}/\text{kg}$ of hop, IST equivalents for compounds carrying the IST superscript). Comparison with Tomahawk 2008 pilot wort and beer, for which concentrations have been converted into hop equivalents (given in $\mu\text{g}/\text{kg}$ of hop used for production). GC-O AEDA analyses (FD in parentheses) on the pHMB extracts. Assay in duplicate. All samples that do not share a common letter are significantly different ($p < 0.05$) according to Tukey's test. +, detected under the quantification limit ($0.1 \mu\text{g}/\text{kg}$); -, not detected ($<0.03 \mu\text{g}/\text{kg}$).

^b I, compound identified by coincidence with the GC-PPFD retention indices and odor descriptor of the pure or synthesized compound on two capillary columns (CP-Si5-CB and FFAP); I⁺, additional confirmation by mass spectrometry (full scan monitoring); I⁻, compound tentatively identified by coincidence with the GC-O retention indices and odors of pure or synthesized compound on two capillary columns (CP-Si5-CB and FFAP).

Table 6. Sulfanylalkyls Carrying a Carbonyl Function at the β -Position^a

RI	CP-Si15	FFAP	substance	odor (GCC)	PPFD concentrations in $\mu\text{g}/\text{kg}$ of hop (FD AEDA given in parentheses)												identification reliability ^b (three main m/z ions in parentheses)		
					Tomahawk		Nelson Sauvin		Nugget		Cascade		Saaz						
					2007	2008	2008	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	
28	907	1454	3-sulfanylpentanal ^{1ST}	hoppy, flower	6.4 a (8192) + f (64)	3.9 b (4096) 1.4 d (512)	— (160) 5.2 (10240)	2.3 d 8.3 a	2.9 c 6.7 b	1.5 e 0.9 e	1.1 f 0.7 e	1.0 f 1.2 de	0.8 f 0.8 e	— g — f	0.1 g — f	— f	— f	1 ⁺ (85, 100, 118) 1 ⁺ (132, 75, 55)	
29	915	1381	4-sulfanyl-4-methyl- pentan-2-one	catty, blackcurrant															
30	931	1470	3-sulfanyl-2-ethyl- propanal ^{1ST}	skunky, plastic	6.2 a (2048)	4.7 b (1024)	— (80)	0.7 f	1.2 e	+	+	2.0 d	2.6 c	+	+	+	+	+	+
31	947	1521	1-sulfanylpentan-3-one ^{1ST}	green, mineral	2.7 c (1024)	4.9 b (2048)	+	1.7 d	0.7 e	7.2 a	4.1 b	0.6 ef	0.3 fg	+	+	+	+	+	+
32	1013	1544	3-sulfanylhexanal ^{1ST}	flowery, lemon	+	+	6.1 (1280)	+	+	+	+	+	+	+	+	+	+	+	+
33	1113	1665	3-sulfanylheptanal ^{1ST}	lemon, candy	4.6 b (256)	7.1 a (1024)	33.8 (10240)	2.3 c	1.7 de	0.9 f	1.8 d	1.4 e	+	+	+	+	+	+	+
34	1133	1659	3-sulfanyl-2-butyl- propanal ^{1ST}	chicken soup, plastic	+	+	6.8 (2560)	+	+	+	+	+	+	+	+	+	+	+	+
35	1214	1764	3-sulfanyloctanal ^{1ST}	citrus, peach	28.2 c (2048)	26.5 c (2048)	15.3 (5120)	43.2 a	32.8 b	8.5 d	2.7 ef	3.5 e	1.2 f	+	+	+	+	+	+
			sum of concentrations		48.1 b	44.5 b	67.6	94.1	58.6 a	46.0 b	19.1 c	10.3 d	9.7 d	5.7 de	+	+	+	+	+

^a Amounts determined by GC-PPFD in hop pellets ($\mu\text{g}/\text{kg}$ of hop, IST equivalents for compounds carrying the IST superscript). Comparison with Tomahawk 2008 pilot wort and beer, for which concentrations have been converted into hop equivalents (given in $\mu\text{g}/\text{kg}$ of beer or wort = $\mu\text{g}/\text{kg}$ of hop used for [production]). GC-O AEDA analyses (FD in parentheses) on the pHMB extracts. Assay in duplicate. All samples that do not share a common letter are significantly different ($p < 0.05$) according to Tukey's test. +, detected under the quantification limit ($0.1 \mu\text{g}/\text{kg}$); —, not detected ($< 0.03 \mu\text{g}/\text{kg}$). ^b I, compound identified by coincidence with GC-PPFD the retention indices and odor descriptor of the pure or synthesized compound on two capillary columns (CP-Si15-CB and FFAP); 1⁺, additional confirmation by mass spectrometry (full scan monitoring).

Table 7. Other Polyfunctional Thiols Found in Hop Pellets^a

RI	CP-SIS	FFAP	substance	odor (GCO)	PPPD concentrations in $\mu\text{g}/\text{kg}$ of hop (FD AEDA given in parentheses)												identification reliability ^b (three main m/z ions in parentheses)	
					Tomahawk		Nelson Sauvin		Nugget		Cascade		Saaz					
					hop	wort	beer	hop	hop	hop	hop	hop	hop	hop	hop			
36	722	1538	2-sulfanyethan-1-ol	soup, grilled,	2007	2008	2008	2007	2008	2007	2008	2007	2008	2007	2008	2008	5.2 e	Γ^+ (47, 60, 78)
				gas	4.9 e (1024)	8.1 c (1024)	9.6 (320)	32.5 (5120)	15.4 a	12.4 b	2.1 g	2.2 g	1.8 g	6.7 cd	5.2 e			
37	810	1003	3-methyl-2-buten-1-thiol	coffee, skunky	4.9 d (4096)	11 a (8192)	+ (163840)	327.6 (655360)	10.7 b	7.6 c	+ h	0.8 g	3.6 e	1.4 f	1.4 f	0.8 g		Γ^+ (69, 53, 102)
38	880	1454	2-sulfanyethyl acetate	burnt, grill	— (4)	— (8)	13.4 (2560)	5609 (40960)	—	—	—	—	—	—	—	—		Γ^+ (43, 60, 61)
39	1051	1780	3-sulfanyl-2-methylbutan-1-thiol ^{1ST}	mushroom,	3.8 c (512)	+ g (256)	1.2 (1280)	8.4 (20480)	11.5 a	9.7 b	3.2 d	1.4 e	0.9 ef	0.4 fg	— g	— g		Γ^+ (61, 136, 69)
				white fruits														
40	1170	2100	6-sulfanyhexan-1-ol ^{1ST}	mushroom,	+ e (128)	5.6 c (2048)	+ (10240)	15.1 (40960)	8.5 b	12.2 a	1.0 d	0.4 de	1.0 d	0.7 d	— e	— e		Γ^+ (42, 60, 82)
				flowers, gaz														
41	1185	1838	4-sulfanyl-4-methylpentan-1-ol ^{1ST}	curry, celery	6.8 c (2048)	5.2 d (2048)	— (10240)	+ (163840)	21.5 a	17.4 b	— e	+ e	— e	— e	+ e	— e		I
				sum of concentrations	20.5 d	30.0 c	23.2	5984.2	67.6 a	59.3 b	7.8 ef	4.6 g	7.8 ef	4.3 g	8.1 e	6.0 fg		

^a Amounts determined by GC-PPPD in hop pellets ($\mu\text{g}/\text{kg}$ of hop, IST equivalents for compounds carrying the IST superscript). Comparison with Tomahawk 2008 pilot wort and beer, for which concentrations have been converted into hop equivalents (given in $\mu\text{g}/561$ L of beer or wort = $\mu\text{g}/\text{kg}$ of hop used for production). GC-O AEDA analyses (FD in parentheses) on the pHMB extracts. Assay in duplicate. All samples that do not share a common letter are significantly different ($p < 0.05$) according to Tukey's test. +, detected under the quantification limit ($0.1 \mu\text{g}/\text{kg}$); —, not detected ($< 0.03 \mu\text{g}/\text{kg}$).
^b I, compound identified by coincidence with the GC-PPPD retention indices and odor descriptor of the pure or synthesized compound on two capillary columns (CP-Si5-CB and FFAP); Γ^+ , additional confirmation by mass spectrometry (full scan monitoring).

exchanger column (Dowex 1WX2-100 resin from Aldrich Chemicals), washed beforehand by 2 M NaOH and 2 M HCl and in between rinsed by ultrapure water. Then 50 mL of sodium acetate buffer (0.1 M, pH 6) was poured on the resin to remove impurities. Volatile thiols were released by percolating a purified cysteine solution (640 mg of hydrochloride L-cysteine monohydrated in 60 mL of Millipore water; this solution was washed with 2×5 mL of distilled CH_2Cl_2 before use). The eluate containing the volatile thiols was collected and extracted by 4 and then 3 mL of distilled CH_2Cl_2 using magnetic stirring (5 min). The organic phases were pooled, dried on anhydrous Na_2SO_4 , and finally concentrated in a Kuderna to 250 μL and under nitrogen to 70 μL to be stored at -80°C .

Gas Chromatography Hyphenated to Olfactometric Detection (GC-O) or to a Flame Ionization Detector (GC-FID).

One microliter of the *p*HMB extracts (thiol-specific) or LNEs (total flavor Likens–Nickerson extract) was analyzed with a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250°C ; the split vent was opened 0.5 min postinjection. Compounds were analyzed with a wall-coated open tubular (WCOT) apolar CP-Sil5-CB (50 m \times 0.32 mm i.d., 1.2 μm film thickness) and a polar FFAP (25 m \times 0.32 mm i.d., 0.3 μm film thickness) capillary column. The carrier gas was nitrogen, and the pressure was set at 50 kPa (CP-Sil5-CB) or 30 kPa (FFAP). The oven temperature was programmed to rise from 36 to 85°C at $20^\circ\text{C}/\text{min}$, then to 145°C at $1^\circ\text{C}/\text{min}$, and finally to 250°C at $3^\circ\text{C}/\text{min}$ and held for 30 min. The FID was set at 250°C . To assess the olfactory potential of the extract, the column was connected to a GC-O port (Chrompack) maintained at 250°C . The effluent was diluted with a large volume of air (20 mL/min) prehumidified with an aqueous copper(II) sulfate solution. All extracts were analyzed immediately after extraction by two trained panelists. Complete aroma extract dilution analysis (AEDA)²⁹ was performed on *p*HMB extracts and LNEs by one operator, with the CP-Sil5-CB column. The extracts were diluted stepwise with dichloromethane (1 + 1 by volume). Flavor dilution (FD) is defined as the highest dilution at which the compound could still be detected ($\text{FD} = 2^n$ with $n + 1 =$ number of dilutions applied on the extract until no odor was perceived). The precision of this AEDA is $n \pm 1$ (factor 2 between FD values).

Gas Chromatography Hyphenated to an Electronic Impact Mass Spectrometer (GC-MS). Mass spectra (m/z 40–380) were recorded at 70 eV on a ThermoFinnigan Trace MS mass spectrometer connected to a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector and an apolar CP-Sil5-CB MS capillary column (50 m \times 0.32 mm i.d., 1.2 μm film thickness). The carrier gas was helium, and the pressure was set at 100 kPa. The oven temperature program was the same as that described for GC-O. Spectral recording was automatic throughout elution; Xcalibur software was used.

Gas Chromatography Hyphenated to a Pulsed-Flame Photometric Detector (PFPD). Two microliters of the *p*HMB extracts or LNEs was analyzed on a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector maintained at 250°C and connected to the O.I. Analytical PFPD, model 5380. The injections were carried out in the splitless mode at 250°C , the split being turned on after 0.5 min. The carrier gas was helium at a pressure of 90 kPa. At the detector, the following parameters were selected: 250°C as the temperature, 600 V as the voltage, 18 ms as the gate width, 6 ms as the gate delay, 580 mV as the trigger level, and 3.70 Hz as the pulse frequency. The oven temperature program and the column were the same as described for GC-O.

Identifications. For all non-sulfur compounds, 4 sulfanylmethyl thioesters and 25 thiols, MS identifications were done by comparing the mass spectra obtained from each sample with those obtained with pure or synthesized compounds injected under the same conditions and/or present in the NIST library. The retention indices (retention times

normalized with respect to adjacently eluting *n*-alkanes; decimal numeral system) were determined by injection onto two capillary columns (CP-Sil5-CB and FFAP-CB) connected to the FID or the olfactometric detector (identification checked by co-injection). In the case of PFPD detections (interesting for traces giving no GS-MS peak), injection of thioesters allowed translation into the alkane-related decimal numeral system.

Quantifications. For commercially available terpenoids, full scan MS calibration curves (areas relative to carvone) were used. Sulfanylmethyl thioester molarities were calculated with the calibration curve of sulfanylethyl thioacetate (the equimolar response of the PFPD for thioesters has been previously checked; a correction according to the molecular weight ratio was applied to obtain real concentrations in mg/kg). For commercially available thiols, complete calibration curves relative to the IST were used. For commercially unavailable terpenoids and thiols, quantifications are given in IST equivalents. To better compare the concentrations with hop, all data in wort and beer were converted into hop equivalents (values multiplied by 561 because 1.78 g of hop was used per liter of beer). Their experimental FD values (AEDA) were multiplied by 10 to consider, in all cases, an undiluted extract with 10 g of hop.

Statistical Analyses. All analyses were carried out in duplicate. Multiple comparisons of means were performed by means of Tukey's test with SAS software version 9.2 (SAS Institute, Inc., Cary, NC). Values that do not share a common letter are significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

GC-MS analyses of hop LNEs representing two successive crops (2007 and 2008) confirmed major differences between cultivars (Tables 2 and 3).

As expected for a nonaromatic variety, Tomahawk did not contain significant concentrations of β -farnesene or bergamotene (respectively <0.5 and <0.1 mg/kg IST equiv in Tomahawk versus respectively >150 and >10 mg/kg in Saaz).^{6,17} By comparison with both bitter and low bitter here investigated varieties (Table 2), Tomahawk emerged as richer in myrcene (>3000 mg/kg) and α - and β -selinenes together (>600 mg/kg IST equiv). Relatively high amounts of selinenes together (>200 mg/kg IST equiv) combined with an α -amorphene level above 25 mg/kg IST equiv are also characteristic of Nelson Sauvin and two other bitter cultivars previously investigated: Challenger and Northdown.¹⁷

Also worth stressing, as for Cascade, are the higher levels of two terpenoids in the Super Alpha Tomahawk cultivar: methyl geranate (>40 mg/kg IST equiv) and geraniol (>180 and 45 mg/kg for Tomahawk and Cascade, respectively). Compared to mono- and sesquiterpenes, these terpenoids can probably be more solubilized in wort and partially transferred into the beer.^{2,17,30–37}

Derived from humulone degradation, 3-methylbutyl isobutyrate was revealed to be in higher concentration in Tomahawk and Nelson Sauvin (>100 mg/kg) than in the other here-investigated cultivars.

GC-PFPD and GC-O (AEDA methodology²⁹) applied to the same hop LNEs also evidenced big variations in sulfanylmethyl thioester concentrations between Super Alpha/high bitter (Tomahawk and Nelson Sauvin, total >2 mg/kg IST equiv; Nugget, total around 1 mg/kg IST equiv) and low bitter cultivars (Cascade and Saaz, total <0.6 mg/kg IST equiv) (Table 3). In Tomahawk, the sulfanylmethyl thiopropionate level accounted for $>65\%$ of the total. The high volatility of this major sulfanylmethyl

thioester, as compared to sulfanylmethyl thiohexanoate or octanoate, is stressed. Its sprout/meaty odor should therefore be strongly lost during essential oil extraction or beer production.^{38,39}

In the hop LNEs, some powerful aromas detected at trace level were identified as polyfunctional thiols according to their retention times and odors (data not shown). However, due to possible thiol oxidation (or degradation of thiol precursors) in the LNE apparatus, a more selective and gentle extraction procedure (*p*HMB) was conducted for thiols. As depicted in Figure 2 (crop 2008), the GC-PFPD and GC-O (AEDA) analyses applied to these *p*HMB extracts confirmed the key role of polyfunctional thiols in hop flavor.¹⁸ With respectively 16 and 18 compounds quantified above 2 $\mu\text{g}/\text{kg}$, the Nelson Sauvin and Tomahawk varieties proved richer in thiols than the Saaz cultivar (only 3 polyfunctional thiols above this level). The aromagrams showed the same trends, with 18 and 22 flavor dilutions above 512 in Nelson Sauvin and Tomahawk, respectively (versus only 3 for Saaz).

Thirteen odorants carrying an acetate moiety in the β -position of the sulfanyl group were identified by comparison with commercial standards and references issued from combinatorial syntheses (Table 4 and Figure 1).^{18,20} Among them, 3-sulfanyl-2-methylpropyl acetate (**4**, grilled nut descriptor), 3-sulfanyl-2-ethylpropyl acetate (**10**, floral/vinegar), 3-sulfanylbutyl acetate (**3**, cheese), and its isomer, 1-sulfanyl-3-butyl acetate (**2**, plastic/sprout), were identified for the first time in hop. With regard to the other varieties, Tomahawk and Nelson Sauvin were particularly rich in **10** (22–35 $\mu\text{g}/\text{kg}$ IST equiv in Tomahawk, 15–44 $\mu\text{g}/\text{kg}$ in Nelson Sauvin, although not reported by Takoi et al. in their paper dedicated to this cultivar).¹⁸ A total β -sulfanyl acetate range of 26–58 $\mu\text{g}/\text{kg}$ characterized these two cultivars, whereas <22 $\mu\text{g}/\text{kg}$ was found in the three others, with values below 6 $\mu\text{g}/\text{kg}$ for Saaz.

For most of the sulfanyl acetates reported in Table 4, the corresponding sulfanyl alcohols were identified (**14–21**, **23**, **26**, and **27** in Table 5). The well-known grapefruit-like 3-sulfanylhexan-1-ol (**23**) was found in all cultivars (7–12 $\mu\text{g}/\text{kg}$ in Tomahawk, FD = 256–512), yet in the Cascade cultivar it reached higher values (73–117 $\mu\text{g}/\text{kg}$), in the same range as those reported for Simcoe (U.S.), Topaz (Australia), and Fuggle (U.K.) pellets.⁴⁰ 3-Sulfanyl-2-methylbutan-1-ol (**20**, leek/hop) was smelled in Tomahawk but proved to be quantifiable only in the Nelson Sauvin cultivar (2 $\mu\text{g}/\text{kg}$). 3-Sulfanyl-4-methylpentan-1-ol (**26**), previously claimed by Takoi et al. to be unique to Nelson Sauvin,¹⁸ was also detected in Tomahawk, Nugget, and Cascade, albeit at lower levels (2–13 $\mu\text{g}/\text{kg}$ IST equiv, against >20 $\mu\text{g}/\text{kg}$ in Nelson Sauvin).

Surprisingly, the major sulfanyl acetate identified (**10**) was not found in its alcohol form (expected at RI CP-Sil5 = 1020). Likewise, the acetates derived from **22**, **24**, and **25** (expected RI CP-Sil5 = 1218, 1207, and 1324, respectively) were not detected at the sniffing port in our hop extracts.

On the other hand, the aldehyde corresponding to **10**, 3-sulfanyl-2-ethylpropanal (**30**), was found, along with five others: 3-sulfanylpentanal (**28**), 3-sulfanylhexanal (**32**), 3-sulfanylheptanal (**33**), 3-sulfanyl-2-butylpropanal (**34**), and 3-sulfanyloctanal (**35**) (Table 6). Two ketones, the famous 4-sulfanyl-4-methylpentan-2-one (**29**, black currant) and 1-sulfanylpentan-3-one (**31**, green), also proved to coexist with their corresponding alcohols and acetates. In line with previous results,^{18,41} **29** was found at levels above 5 $\mu\text{g}/\text{kg}$ in Nelson Sauvin but was not detected at all in the European Saaz variety, probably because of copper spraying before harvest.

1,4-Addition of hydrogen sulfide (or of cysteine if β -lyase activity can regenerate the free thiol) onto α,β -unsaturated carbonyls,^{42,43} followed by reduction and/or esterification, might explain the presence of all the β -sulfanyl backbones described above (Tables 4–6). Yet the balances between the carbonyl and alcohol forms were very different in each variety. For instance, 1-sulfanylpentan-3-one (**31**) was present at higher levels in Tomahawk and Nugget (3–7 $\mu\text{g}/\text{kg}$ IST equiv), whereas 1-sulfanylpentan-3-ol (**19**) concentrations were higher in Nelson Sauvin and Cascade (2–4 $\mu\text{g}/\text{kg}$ IST equiv). More generally, the Nelson Sauvin cultivar was characterized by higher levels of β -sulfanylalcohols (total = 55–59 versus 26–40 $\mu\text{g}/\text{kg}$ in Tomahawk, Table 5).

A few of the thiols found lack the β -sulfanyl acetate, alcohol, ketone, or aldehyde moiety: 2-sulfanylethan-1-ol (**36**), 2-sulfanylethyl acetate (**38**), 6-sulfanylhexan-1-ol (**40**), 4-sulfanyl-4-methylpentan-1-ol (**41**), the well-known skunky flavor 3-methyl-2-buten-1-thiol (**37**), and a dithiol, the 3-sulfanyl-2-methylbutan-1-thiol (**39**) (Table 7). Worth stressing is the predominance of **39** in all five cultivars (up to 10–11 $\mu\text{g}/\text{kg}$ IST equiv in Nelson Sauvin), as compared to 3-sulfanyl-2-methylbutan-1-ol (**20**), although only the latter has been mentioned previously.³¹

In the brewing process, β -sulfanyl acetates are suspected of being still formed by yeast during fermentation, whereas sulfanyl carbonyls should be reduced to sulfanyl alcohols.^{44–46} Another polyfunctional thiol profile is therefore expected in hopped beers. Although an exhaustive comparison of beers brewed with different hop cultivars will be addressed in another paper, a pilot beer produced with the addition of Tomahawk hop pellets (1.78 g/L) at the late stage of the boiling step was investigated in the present work. The *p*HMB extracts of beer and wort were analyzed by GC-PFPD and GC-O. All quantifications are given in hop weight equivalents in Tables 4–7 (italicized entries) for better comparison with the hop potential.

In the boiled wort, many hop polyfunctional thiols were found at higher levels than expected, although volatiles are known to be lost by steam distillation.⁴⁷ For instance, 3-sulfanyl-2-methylbutan-1-ol (**20**) was 160-fold more odorant in wort than expected, whereas a FD increment by 10–40 was observed for 3-sulfanylbutyl acetate (**3**), 3-sulfanylhexan-1-ol (**23**), 3-sulfanylhexanal (**32**), and 3-methyl-2-buten-1-thiol (**37**) (Tables 4–7). In line with the results of Takoi and Kishimoto, it can be concluded that boiling can most probably release thiols from bound forms.^{18,40} According to Takoi et al., the higher the extraction temperature, the higher the recovery.¹⁸ After 45 min, they observed a 10-fold gain for **23** if the temperature was increased from 25 to 90 °C. Similarly, Kishimoto reports 45% more after 1 h of hop boiling at 100 °C (Simcoe hop cultivar).⁴⁰

Through fermentation, strong FD increases were still measured in the Tomahawk hopped wort, especially for 1-sulfanylpentan-3-ol (**19**, 512-fold), 3-sulfanylhexanal (**32**, 16-fold), 3-sulfanylpentanal (**28**, 64-fold), and 3-sulfanyloctan-1-ol (**27**, 32-fold) (Tables 5 and 6). In the case of 3-sulfanyl-3-methylbutan-1-ol (**17**), only traces or low amounts were detectable by PFPD in wort, whereas 166 $\mu\text{g}/\text{kg}$ (hop weight equivalents) were quantified in beer (Table 5). Sulfanyl alcohols could arise partially through yeast aldehyde reduction (e.g., **27** from **35**),^{44,45} yet most sulfanyl aldehydes also proved to be produced through the fermenting process (Table 6). As previously suggested for wines, bound precursors (cysteine adducts in the case of grapes) present in raw materials most probably release free thiols at the late stages of fermentation, thanks to yeast β -lyase

activity.^{48,49} Another possible route is hydrogen sulfide addition onto α,β -unsaturated carbonyls, as previously proposed for onion defects in some beers and for **23** and **29** in wine.^{42,50}

It is interesting to note that the amounts of 3-sulfanylhexan-1-ol (**23**) (205 ng/L; values in beer), 4-sulfanyl-4-methylpentan-2-one (**29**) (40 ng/L), and 3-sulfanyl-4-methylpentan-1-ol (**26**) (86 ng/L) are significant in terms of their contribution to beer aroma as the thresholds of these compounds are 55,³¹ 1.5,³¹ and 70,¹⁸ respectively. As expected, the pilot Tomahawk beer exhibited strong black currant and passion-like flavors.

In conclusion, our data clearly show that the Tomahawk and Nelson Sauvin cultivars contain more odorant polyfunctional thiols than aromatic varieties. However, as free thiols found in hop only partially contribute to the final content in beer, complementary investigations are needed to determine the total polyfunctional thiol potential of each hop variety.

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ABBREVIATIONS USED

FD, flavor dilution; *p*HMB, *p*-hydroxymercuribenzoic acid; PFPD, pulse flame photometric detector; RI, retention index; AEDA, aroma extract dilution analysis; LNEs, Likens–Nickerson extracts; IST, internal standard.

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