Use of GC−Olfactometry to Identify the Hop Aromatic Compounds in Beer

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This paper describes a sensorial aroma extract dilution analysis (AEDA) approach to the analysis of beer aromas derived from hops. To obtain an extract with an odor representative of the original product, the XAD extraction procedure was applied and the experimental conditions were optimized. The aromagrams of three beers were compared: one brewed without hops, one brewed with Saaz hop pellets, and one brewed with Challenger hop pellets. One spicy/hoppy compound, unmodified from hop to beer, proved responsible for the most intense odor in both hopped beer extracts. Another flavoring compound in hops, linalool, also survives through the process to the final beer. Other compounds such as γ-nonalactone and humuladienone, although not found in our extracts of hop, significantly modify beer aromagrams after hopping. Sulfur compounds characteristic of Challenger hops proved to be at least partially responsible for the unpleasant flavor found in the corresponding beer.

Keywords: *Hop; beer flavor; XAD; sensorial analyses; AEDA*

INTRODUCTION

For many years, investigators have attempted to determine which hop-derived compounds influence the organoleptic properties of beer (*1*−*5*). To assess the impact of hops on beer flavor, most authors have used methods based on the quantitative analysis (GC−FID and GC−MS) of identifiable compounds present in hopped beers and absent from unhopped beers.

In the case of kettle hopping, major components of essential oils, such as terpenes and sesquiterpenes, are rarely found in beers (*1*, *2*) and are not considered responsible for their hoppy aroma. Nevertheless, oxidation products of these hydrocarbons can be present (*3*). According to Murakami et al. (*6*), oxygenated compounds derived from hops and synthesized during the boiling step may contribute to the hoppy aroma of beer. Moreover, hydrolysis of humulene epoxides in the kettle is an important source of alcohols in fresh beer (*7*). α-Terpineol, caryophyllene oxide, geraniol, humuladienone, humulene epoxides, humulenol II, humulol, linalool, linalool oxide, and terpinen-4-ol have been identified in beer by Tressl et al. (*1*), and citronellol and geranyl acetate were identified by Moir (*4*). However, the impact of these compounds on beer aroma remains unclear. It is also obvious that these compounds are not the only hop-derived flavoring agents that influence beer aroma.

In this work we have used an alternative approach. Instead of determining the chemical composition of hopped beers by the usual chromatographic methods, we have constructed their aromatic profiles (GC−Sniffing) and determined which compounds in hops are most important for beer character, in terms of odor and intensity.

The extraction method was thus very important, as it was essential to ensure that it yielded extracts with an odor representative of the original product. In 1993, Abbott et al. (*8*) described a method using a mixture of three Amberlite resins (XAD-2, XAD-7, XAD-16) as a good way to obtain beer extracts with sensory characteristics representative of the beers from which they were obtained. Other authors (*9*, *10*) also proposed using an Amberlite resin, XAD-2, to extract the most active flavoring compounds known in beer. Absolute recoveries were almost all above 50% and close to 100% for some esters. More recently, Guyot et al. (*11*) confirmed the high efficiency of XAD-2 resin for extracting hydrophobic flavors from complex media such as worts or beers. Therefore, we optimized extraction with XAD-2 resin alone to draw the aromagrams of three beers: one brewed without hops, one brewed with Saaz hop pellets, and one brewed with Challenger hop pellets. We then compared the results with the chromatographic profiles of the corresponding hops.

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MATERIALS AND METHODS

Reagents. Diethyl ether (99.9% under nitrogen) and dodecane 99% (external standard) were purchased from Aldrich Chemical Co. (Milwaukee, WI), and dichloromethane and methanol, both HPLC grade, were purchased from Romil (Cambridge, UK). Amberlite XAD-2 resins, obtained from Supelco Inc. (Bellefonte, PA), were sequentially washed with methanol (4 h) and diethyl ether (4 h) in a 250-mL Soxhlet. Flavor chemicals, which ranged from 95% to 99% purity, were purchased from either Aldrich Chemical Co. (Milwaukee, WI), Sigma (St Louis, MO), or Janssens-Chimica (Geel, Belgium). The liquid chromatography columns with coarse frit and Teflon stopcock (300 mm × 10.5 mm i.d. × 13 mm o.d. column, 415 mm overall) were also obtained from Supelco Inc. Finally, Na_2SO_4 was from Janssens-Chimica, NaCl p.a. was from Merck (Darmstadt, Germany), and 37% hydrochloric acid was purchased from Vel (Leuven, Belgium).

Brewing Process. Three beers were brewed: one without hops and two with hop pellets (either Saaz (2.9% α-acids) or Challenger (6.25% α-acids)) at a hopping rate of 1.8 g/L. A 15-L

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portion of a 12 °P (density equivalent to 12 g of sucrose per 100 g of wort) industrial wort (90% malt, 10% corn) was boiled. The boiling time was 75 min, and the hop was added 7 min before the end. After a 20-minute clarification period, the precipitated hot trub was removed and the wort was cooled. Fermentations and maturations were conducted in 3-L EBC-tubes with a lager yeast (15×10^6 cells/mL at pitching) at 12 °C for 5 days, 13 °C for 1 day, 14 °C for 1 day, 15 °C for 3 days, 7 °C for 3 days, and 0 °C for 24 h. After Kieselguhr filtration, the beer was bottled under CO_2 and stored at 3 °C until analyzed.

Beer Sensorial Analyses. Triangular tests (*12*) were conducted with the 3 beers described above. The panel was asked to smell only the headspace in order to avoid any interaction of aroma with the bitter taste imparted to beer by isohumulones also derived from hops. The tests were carried out in 3 sessions with a panel of 12 assessors, each session comparing 2 beers (unhopped vs Saaz; unhopped vs Challenger; Saaz vs Challenger). For each session, the panel was asked to describe the aroma of the product.

Beer Extraction Procedure and Sniffing Analysis. An optimized extraction procedure based on that of Hawthorne et al. (*10*) was used to recover hop aroma compounds from beer. Four grams of XAD-2 resin and 2 mL of 3.2% hydrochloric acid were added to 50 mL of beer in a wide-mouthed bottle. The bottle was sealed with a Teflon-lined cap and shaken for 2 h at 200 rpm. After extraction, the contents were poured into a liquid chromatography column with a coarse frit and Teflon stopcock, and the liquid was drained off, leaving a small bed of resin which was further rinsed with 40 mL of distilled water. Aroma compounds were then eluted with 25 mL of diethyl ether (5×5 mL), allowing a 5-min contact after each addition. The ether was dried with Na_2SO_4 and concentrated to 0.2 mL in a Danish Kuderna evaporator. The external standard, 5 mL of dodecane (2 mg/L in diethyl ether), was added before concentration (concentration factor = 250). Saturating the beer with NaCl as recommended by Hawthorne et al. (*10*) and Abbott et al. (*8*) to increase the salting-out properties (*13*−*16*), proved ineffective on hop aroma compounds and even very detrimental to the recovery of monoterpenes and sesquiterpenes (data not shown). On the other hand, adjusting the pH to below 2 to maximize the number of ionizable molecules present in a neutral state (*17*−*20*) had a small but always beneficial impact.

Hop Extraction Procedure. Hop pellets were extracted according to the Likens-Nickerson method described by Perpète et al. (*21*).

GC−Sniffing Analytical Conditions. For the sniffing analyses, we used a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250 °C, and the split vent was opened 0.5 min postinjection. Compounds were separated using a 50 m × 0.32 mm, wall-coated open-tubular (WCOT) apolar CP SIL5 CB capillary column (1.2 μm film thickness) connected to a flame ionization detector. The oven temperature was programmed from 36 to 120 °C at 20 °C/min, to remain constant at 120 °C for 20 min, to 250 °C at 2 °C/min, and then to remain constant at 250 °C for 30 min. To assess the olfactory potential of the compounds, a T-junction was used at the end of the capillary column. 50% of the eluent was sent to a FID detector maintained at 280 °C and connected to a Shimadzu CR6-A integrator, while the other part was directed to a GC-odor port at 250 °C. In the latter case, the eluent was diluted with a large volume of air (20 mL/min) previously humidified using an aqueous copper (II) sulfate solution. A 2-μL portion of the beer extract was injected.

In the case of sniffing analyses on the FFAP chromatographic column (WCOT, 25 m × 0.32 mm, 0.3 μm film thickness), the oven temperature was programmed from 36 °C to 85 °C at 20 °C/min, to 145 °C at 1 °C/min, to 220 °C at 3 °C/min, and then to remain constant at 220 °C for 40 min.

GC−SCD Analytical Conditions. The column was directly connected to a Sievers 355 SCD (sulfur chemiluminescence detector). In the 800 °C combustion chamber of the detector, the air and hydrogen flows were maintained at 40 and 100 mL/min, respectively. A 6-psi airflow was supplied to the ozone generator under a vacuum (150−275 Torr) obtained by an Edwards oil-sealed RV5 pump.

GC−MS Analytical Conditions. MS analyses were carried out with an HP5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV (2.45 scan per second). Spectral recording throughout elution was automatically performed with the HP59970 C software.

Table 1. Rate (%) of Correct Answers in Triangular Tests and Beer Odor Description

comparison	correct answers
unhopped beer vs Saaz beer	67%[a]
unhopped beer vs Challenger beer	92%[a]
Saaz beer vs Challenger beer	82%[a]

Beer Odor Description

unhopped beer	Saaz beer	Challenger beer
fruity (banana)	fruity (citrus)	hoppy
flowery	flowery	sulfury
fresh	spicy	cheesy
sweet	fresh	aggressive
cider-like	beer-like	heavy
pleasant	pleasant	unpleasant

[a] Significant with a 5% threshold (*12*).

Table 2. Recovery Yields and Coefficient of Variation of 50 mL of Beer after Addition of Fourteen Commercial Hop Compounds (1 ppm) Extracted with XAD-2 Resin in the Presence of 3.2% HCl for 2 Hours. Elution Was with 25 mL of Diethyl Ether

compound	recovery yield (%)	coefficient of variation[a] (%)
limonene	61	13
γ-terpinene	63	13
linalool	87	4
α-terpineol	84	5
geraniol	79	3
nerol	55	9
geranyl acetate	61	4
β-caryophyllene	44	9
α-humulene	47	9
farnesol	89	4
trans-2-nonenal	71	5
2-decanone	97	4
2-undecanone	88	4

[a] Four extractions.

RESULTS AND DISCUSSION

To determine aromatic changes caused by late hopping, we produced three beers with a malt−corn (90/10) mixed grist and lager yeast. One beer was unhopped, while for the other two the last seven minutes of boiling was in the presence of either Saaz or Challenger hop pellets.

Triangular Tests. Initial triangular tests were carried out with a 12-member panel so as to obtain a general description of the beer odor. The results depicted in Table 1 clearly show significant differences between the three beers. The odor of the unhopped beer was described as fruity, flowery, and cider-like. Beer brewed with a late kettle addition of Saaz hops was described as more pleasant and citrus- or beer-like, whereas the beer brewed with Challenger pellets was described as more aggressive, with cheesy or sulfury odors. Head-space GC analyses (*22*) confirmed that differences in fusel alcohol or ester concentrations could not explain these differences (data not shown). On the basis of this first experiment, the influence of late hopping (180 g/hL) on beer odor was thus obvious.

Table 3. Aromatic Compounds in Unhopped and Hopped Beers[a]

		n (FD)			
RI	odor description	unhopped beer	Challenger beer	Saaz beer	tentative identification (on the basis of odor and RI)
711	fusel oil	8 (128)	7 (64)	7 (64)	isoamyl alcohol[c1]
722	citrus	*d*	5 (16)	5 (16)	unknown
734	cheesy/glue	–	6 (32)	–	dimethyl disulfide
736	apple/citrus	*d*	–	5 (16)	unknown
774	apple/jonquil	7 (64)	7 (64)	6 (64)	ethyl butanoate
803	cheesy	7 (64)	7 (64)	8 (128)	2-and 3-methylbutanoic acids[b]
810	spicy/hoppy	–	9 (256)	8 (128)	unknown[c2]
849	fruit/sweet	7 (64)	7 (64)	7 (64)	isoamyl acetate[b]
855	roasted meat	–	*d*	7 (64)	2-methyl, 3-furanethiol[c3]
872	cooked potato	6 (32)	7 (64)	7 (64)	methional[b]
913	sweat/rubber	–	5 (16)	*d*	diethyl disulfide/S-methylthioisovalerate
938	sweat/fruity	–	6 (32)	6 (32)	*N*-(methyl)mercaptoacetamide
953	sweat/grapefruit	–	6 (32)	6 (32)	unknown
961	onion soup	–	7 (64)	7 (64)	dimethyltrisulfide[b]
974	fruity/rum	5 (16)	6 (32)	6 (32)	ethyl caproate[b]
1017	lily/rose	3	5 (16)	5 (16)	phenylacetaldehyde[b]
1024	strawberry	6 (32)	7 (64)	7 (64)	furaneol[b]
1032	grilled nut	*d*	*d*	5 (16)	acetylpyrazine
1042	woody/flowery	–	5 (16)	–	unknown
1050	cooked vegetable	*d*	5 (16)	5 (16)	unknown
1062	roasted meat	*d*	5 (16)	*d*	unknown
1066	clove/rum	5 (16)	5 (16)	7 (64)	dihydromaltol[b]/guaiacol[c4]
1070	greenery/geranium	–	5 (16)	–	unknown
1083	coriander	–	5 (16)	6 (32)	linalool[b]
1092	lily	7 (64)	7 (64)	8 (128)	β-phenylethanol[b]
1107	pineapple/strawberry	5 (16)	5 (16)	5 (16)	methyl octanoate[c5]
1126	plastic	6 (32)	7 (64)	6 (32)	unknown
1144	plastic/watermelon	–	5 (16)	5 (16)	unknown
1165	burned plastic	–	6 (32)	–	unknown
1218	greenery/lily	5 (16)	–		unknown
1231	fruity/geranium	5 (16)	*d*	5 (16)	β-phenylethyl acetate[b]
1233	lily/rose	5 (16)	–	5 (16)	phenylacetic acid[b]
1274	hay-like/coumarin	6 (32)	6 (32)	5 (16)	2-aminoacetophenone[c6]
1282	boiled wort	–	5 (16)	–	unknown
1289	dentist	6 (32)	6 (32)	7 (64)	4-vinyl guaiacol[b]
1309	cherry	*d*	*d*	5 (16)	unknown
1323	exotic wood	*d*	5 (16)	*d*	unknown
1325	fruity/sweet	*d*	6 (32)	6 (32)	γ-nonalactone[b]
1357	unpleasant	–	5 (16)	–	unknown
1360	perfumed-pine	–	*d*	5 (16)	geranyl acetate
1372	apple/peach	*d*	7 (64)	7 (64)	β-damascenone
1391	phenol	–	6 (32)	5 (16)	unknown
1393	cheesy	–	5 (16)	–	unknown
1421	flowery	–	–	5 (16)	unknown
1426	flowery/pine	*d*	5 (16)	*d*	unknown
1434	fruity	–	*d*	5 (16)	unknown
1441	strawberry/sweet	–	5 (16)	6 (32)	ethyl cinnamate
1537	pine	–	–	6 (32)	unknown
1569	terpene	–	–	6 (32)	unknown
1580	flowery/fresh	–	c	5 (16)	humuladienone[b]

[a] RI, retention index (CP-SIL 5 CB); *n*, number of times the odor was detected along the dilutions (factor 2); (FD), dilution factor when ≥ 16. The beer concentration factor was 250. [b] MS confirmation. [c] Confirmation by co-injection on the FFAP chromatographic column: $RI_{c1} = 1218$; $RI_{c2} = 1131$; $RI_{c3} = 1329$; $RI_{c4} = 1867$; $RI_{c5} = 1414$; $RI_{c6} = 2233$. [d] Present but FD < 16.

GC–Sniffing Analyses. Prior to GC–sniffing analyses, compounds of the three beers were extracted with Amberlite XAD-2 resin (*8*–*11*). An optimized procedure was applied, allowing recovery factors above 50% for the chemical classes expected to be of interest, except for sesquiterpenes (Table 2). The recoveries and coefficients of variation obtained in a beer medium are also presented in Table 2.

Beer odor intensities were determined by the AEDA strategy proposed by Ullrich and Grosch, (*23*, *24*). The dilution factor (FD) was calculated as 2^{n-1}, with n being the number of dilutions (factor 2) required for no odor to be perceived. To identify the highly flavor-active compounds in our extracts, we compared the dilution factors of all compounds with that of isoamyl acetate. This ester occurred in all beers at 3.5–4.2 ppm, i.e., at about twice its threshold concentration established as 1.6 ppm by Meilgaard (*25*). As its FD value was 64 for all three extracts, we considered that flavor-active compounds in beer had to have an FD value above or equal to 32 in the absence of synergistic interactions. To be sure not to neglect some important odors, all FD values above or equal to 16 were investigated (Table 3).

Forty-five odors were detected in the unhopped beer (Figure 1). Only the most intense (FD ≥ 16) are depicted in Table 3. Most of these odors were also found in hopped beers, with similar FD values. Among the odor-responsible compounds, esters (isoamyl acetate, ethyl butanoate, ethyl caproate, and phenylethyl acetate), fusel alcohols (isoamyl alcohol and β-phenylethanol), and 4-vinylguaiacol are well-known secondary metabo-

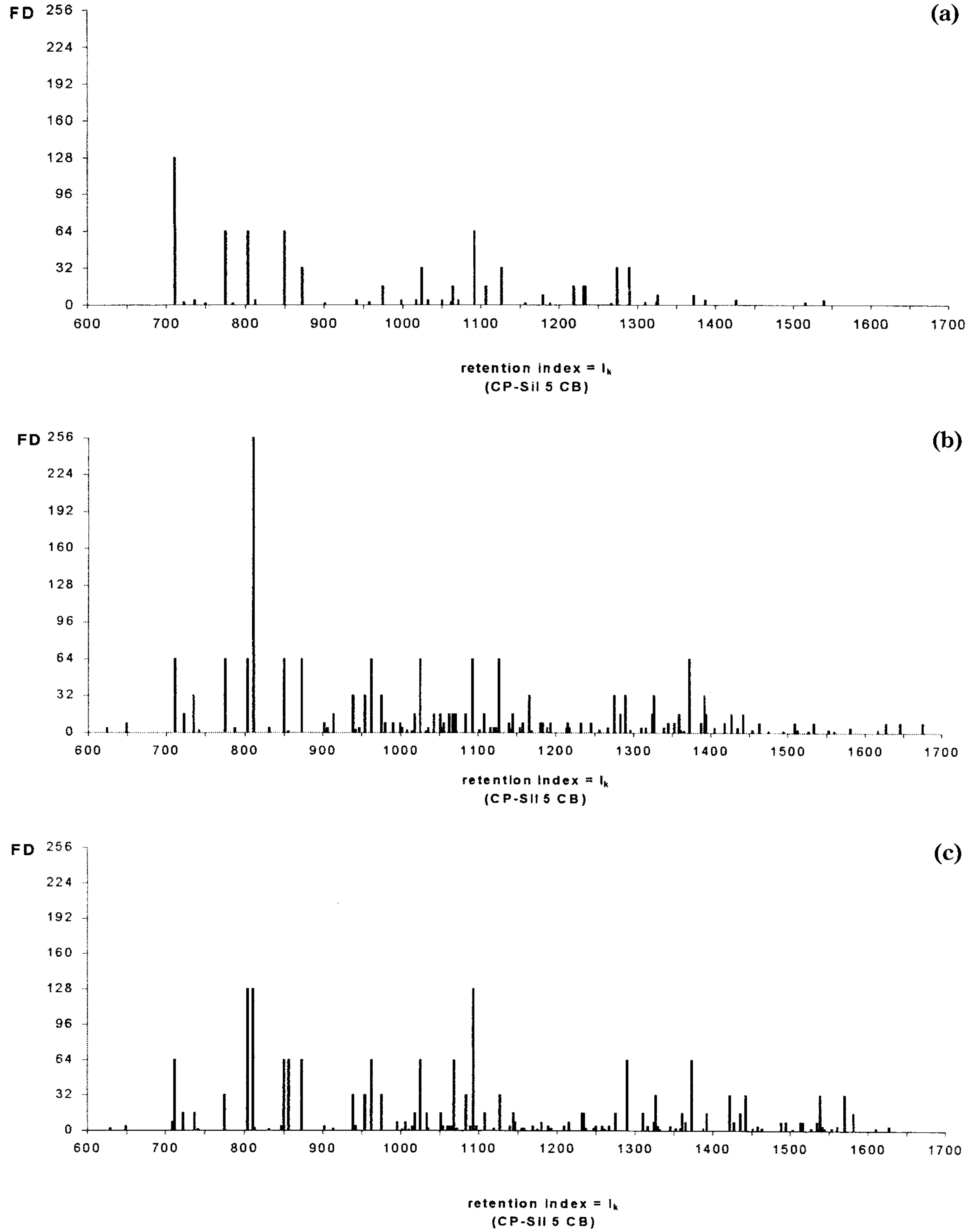

Figure 1. Aromagrams of the unhopped (a), Challenger (b), and Saaz (c) beers.

lites of yeast, whereas furaneol, methional, and phenylacetaldehyde derive partially from Maillard reactions during malt kilning and wort boiling (*26*−*31*). 4-Vinylguaiacol may also derive partially from malt (ferulic acid thermal degradation) or even from hops (*32*). Maltol was not well perceived because of coelution of a large β-phenylethanol peak masking its aroma, whereas dihydromaltol, first characterized in beer by Fickert and Schieberle (*33*), was well detected in our beer samples, coeluting with guaiacol. Although often described as degradation products of hop α- and β-acids, 2- and 3-methylbutyric acids with a characteristic cheesy aroma were found with similar odor intensities in all three beers. Finally, 2-aminoacetophenone (RI = 1274) and an unidentified compound (RI = 1126, plastic) also proved important to the aroma of even unhopped beer.

Several new odors were clearly perceived after hopping, but the corresponding FD values were usually low, falling between 1 and 8. Only 15 of them in the Saaz beer and 16 in the Challenger beer were characterized by an FD $\geq$ 16.

As suspected, the presence of linalool and humuladienone in hopped beers only was confirmed by GC−MS. Though free linalool is present in hop oils, its concentra-

Table 4. Aromatic Compounds in Fresh Saaz and Challenger Pellets[a]

RI	odor description	Challenger[b]	Saaz[b]	suspected compound
660	solvent	+	+	unknown
675	cooked vegetable	+[b1]	−	*S*-methylthioacetate
695	catty/black-current	+[b]	−	unknown
728	solvent	−	+	unknown
735	solvent	+[b2]	−	dimethyl disulfide
751	?	+	−	unknown
759	?	+	−	unknown
777	greenery	−	+	n-hexanal
794	spicy	+[b]	−	unknown
797	greenery	+[b]	−	unknown
801	cheesy	+	−	2 and 3-methylbutyric acids[c]
810	spicy	+	+	unknown
815	caramel	+	−	unknown
854	sweat	+	−	unknown
870	sweat	+[b3]	+	methional or S-methylthiobutanoate
880	cheesy	+[b]	+	unknown
902	?	+[b4]	−	4-methoxy-2-methylbutanethiol-2
912	burnt plastic	+	−	diethyl disulfide
921	catty/garlic	+[b5]	−	*S*-methylthioisovalerate
956	terpene	+	−	unknown
959	greenery	+[b6]	−	dimethyltrisulfide
982	citrus/greenery	+	+	myrcene[c]
992	spicy	+	−	3-methyl butyl isobutyrate[c]
996	cheesy	−	+	unknown
1002	cooked vegetable	+	−	2-methyl butyl isobutyrate[c]
1009	catty/black-current	+[b]	−	unknown
1018	greenery	+	−	unknown
1049	sweat	+[b]	−	unknown
1083	citrus/fruity	+	+	linalool
1098	?	+[b]	−	unknown
1104	cabbage	+[b7]	−	bis(methylthio)methane
1132	?	+	−	unknown
1149	flowery/greenery	+	−	unknown
1182	pine	+	−	myrtenal
1203	cabbage	+[b8]	−	dimethyltetrasulfide
1206	citrus	−	+	methyl nonanoate[c]
1213	?	−	+	unknown
1248	catty/black-current	+[b]	−	unknown
1249	pine	−	+	unknown
1256	sulfury	−	+	unknown
1267	catty/black-current	+[b]	−	unknown
1275	fusel oils	−	+	unknown
1294	greenery	+	−	unknown
1347	grilled nut	−	+	unknown
1372	flowery/fruity	+	+	β-damascenone
1415	catty/black-current	+[b]	−	unknown
1474	mushroom	+	−	unknown
1483	sulfury	+[b]	−	unknown
1486	catty/black-current	+[b9]	−	*S*-methylthiodecanoate
1500	pungent	+	−	α and β-selinene[c]
1572	spicy	+[b]	−	unknown
1633	mushroom	+[b]	−	unknown
1644	?	−	+	unknown
1654	pine	−	+	unknown
1674	woody/mushroom	+	+	unknown
1709	?	+	−	unknown

[a] RI, retention index (CP-SIL 5 CB). The last column represents the suspected compounds according to their odor and their RI (CP-Sil 5 CB). [b] Odor corresponding to the presence of an SCD-peak at the same RI (b1−b9 presented in Figure 2). [c] + means odor present in the sample and − means odor absent from the sample. [d] MS confirmation.

tion in beer can depend on the amount of linalool glycosides hydrolyzed during fermentation by β-glucosidases (*34*), linalool oxide, and linalyl acetate. Humuladienone more likely derives from oxidation of humulene and byproducts, occurring either during hop storage or in the kettle.

In hopped beers, an FD ≥ 16 was noted for dimethyltrisulfide, γ-nonalactone, and components with RI = 938, RI = 1372, and RI = 1441, suspected to be *N*-methylmercaptoacetamide, β-damascenone, and ethyl cinnamate, respectively. The postulated potential precursor of dimethyltrisulfide is *S*-methylcysteine sulfoxide (*31*, *35*). γ-Nonalactone might derive from yeast metabolization of nonanoic acid byproducts such as 4-keto-nonanoic acid or 4-hydroxy-nonanoic acid (*36*). An increase in the concentration of γ-nonalactone, reaching 20 ppb in hopped beers, was confirmed by GC−FID and GC−MS. Finally, as proposed by Enzell (*37*), grasshopper ketone is most likely the source of β-damascenone in hopped products.

Several unknown compounds also proved very important for the aroma of hopped beer, such as the "spicy/hoppy" aroma perceived at RI = 810 which gave the highest FD value of our aromagrams (256 in Challenger beer). Initially suspected to be 3-mercapto butan-2-ol with an identical RI on CP-SIL 5 CB, it was found to

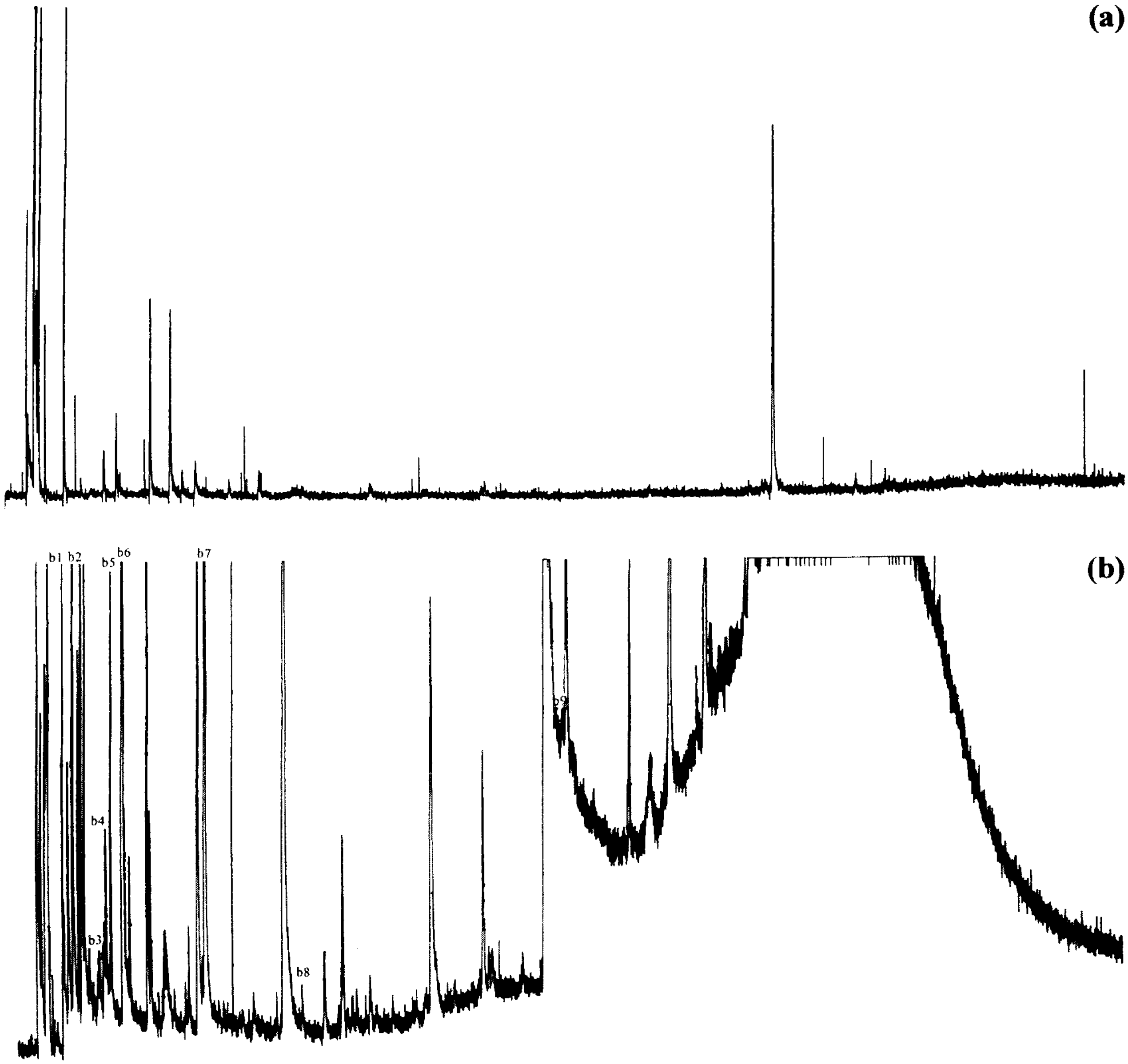

Figure 2. SCD Chromatograms of Hop Likens-Nickerson extracts: Saaz (a) and Challenger (b). Peaks designated b1−b9 represent the known sulfur compounds identified in Table 4. Extractions and analyses were performed two times for each variety.

be less polar according to its retention index on the FFAP column (RI_{FFAP} = 1131 versus 1446 and 1485 for both 3-mercapto butan-2-ol diastereoisomers). Although probably significant, its concentration is most likely below 5 ppb as no FID or MS peaks were found even after 250-fold concentration. Unknowns at RI = 953 (sweat, rubber), 1144 (plastic, watermelon), and 1391 (phenol) are also worth pointing out in both hopped beers.

Surprisingly, most of the hop-derived compounds mentioned in the literature as possible beer flavoring agents were not perceived in our experiments, meaning that they probably do not influence the beer hoppy character (*2, 3, 6, 7, 38−42*). Such was the case for terpinen-4-ol (RI = 1172), α-terpineol (RI = 1182), citronellol (RI = 1224), geraniol (RI = 1226), humulenol II (RI = 1657), humulene epoxide I (RI = 1606), humulene epoxide II (RI = 1639), linalool oxide (RI = 1078)· and *β*-ionone (RI = 1475) (*1*).

The following descriptors were found mainly in the Challenger beer: cheesy/glue (RI = 734 may be due to dimethyl disulfide), sweat/rubber (913; suspected to be diethyl disulfide followed by *S*-methylthioisovalerate), woody (1042), greenery (1070), burned plastic (1165), unpleasant (1357), and cheesy (1393). Some of these might impart the characteristic unpleasant aroma reported by the panelists.

On the other hand, odors such as flowery (1421), fruity (1434), pine (1537), and terpene (1569) characterized the pleasant Saaz beer. A higher perception of dihydromaltol and/or guaiacol and, to a lesser extent, linalool, humuladienone, RI = 1360 and RI = 1441, suspected to be ethyl cinnamate and geranyl acetate, respectively, was also noted in this case. The apparent high perception of 2-methyl-3-furanethiol, not related to a higher concentration in that beer, was probably due to the proximity of isoamyl acetate in the aromagram.

GC−sniffing analyses of undiluted extracts were also performed on both types of fresh hop pellets. Forty-five odors were detected for Challenger hop and only 19 were detected for Saaz (Table 4). Few odors were common to both hops. Among them, probably the most interesting was the spicy odor (RI = 810) discussed above for hopped beers. This clearly confirms that hops bring this odor directly to the boiling kettle without subsequent yeast metabolization. Unfortunately, as in the case of beers, the chromatographic peak corresponding to this odor was too small to allow confirmation of a structure by mass spectrometry. Myrcene, linalool, an odor possibly due to β-damascenone, and two unknowns (RI = 660, solvent, and RI = 880, cheese) are the other flavoring compounds found in both hop varieties. Only linalool and β-damascenone were able to partially resist boiling and fermentation. In fact, 50−60 ppb linalool was added to the boiling wort (29−34 ppm in fresh hop pellets), but only 20−40 ppb was quantified in the final beers. Surprisingly, neither RI = 1441 (probably ethyl cinnamate) or γ-nonalactone (RI = 1325) were detected in hop extracts at the sniffing port, indicating that yeast is most probably involved in the biosynthesis of these compounds.

As depicted in Table 4, the Challenger hop variety is clearly distinguishable from Saaz pellets by a large number of unpleasant odors distributed across the chromatogram (cabbage, catty, sweat, soup, cheese, etc.). Sulfur compounds are in most cases responsible for these kinds of odors (Table 4). The sulfur chemiluminescence detector (SCD) chromatograms of both hop extracts (Figure 2) clearly corroborate this hypothesis. The presence in Challenger of dimethyl disulfide and diethyl disulfide, identified by co-injection and odor only, are worth pointing out, both being suspected to be present in beer hopped with Challenger. Although not found in the corresponding beer extract, α- and β-selinenes, previously proposed by Perpète et al. (*21*) and Lermusieau et al. (*43*) to authenticate Challenger hops, were perceived as expected in the Challenger hop extract. Concentrations of 378 and 393 ppm, respectively, were measured by FID in the Challenger sample, but only 32 and 29 ppm were found in Saaz. The ability of terpenes to be oxidized and hydrolyzed readily explains the absence of their odor in beer.

No odor distinctive of Saaz pellets (*21*, *43*) proved to correspond with odors found in beer, indicating that the kettle hop aroma is much more than a simple dissolution of hop flavors in wort.

GC−Olfactometry applied to XAD-2 beer extracts confirmed that most of the hoppy odors found in beer, such as γ-nonalactone, ethyl cinnamate, humuladienone, and at least partially dimethyltrisulfide, are produced during the brewing process of boiling and fermentation. Few of the compounds smelled in fresh hopped beers proved identical to compounds found in fresh hop cones. Among them, β-damascenone, linalool, and the intense spicy/hoppy odor at RI = 810 are probably the most interesting. Other typical compounds such as dimethyl disulfide and diethyl disulfide are perceived in the beer extracts only when a sulfur-rich hop, in this case the Challenger variety, is used in the kettle. We thus suspect that the high quality of Saaz hop is due to both the presence of very specific pleasant aromas and to low levels of sulfur compounds.

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