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The use of GC-olfactometry to assess hop aromatic quality

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SUMMARY
For many years, research on hop aromatic compounds has focused on the qualitative analysis of markers allowing discrimination between hopped and unhopped beers. Unfortunately, the usual techniques such as GC-FID or GC-MS yield poor information. Our strategy was therefore to use GC-olfactometry to pinpoint hop derived compounds that really influence beer flavour. This technique alllowed us to identify new compounds of interest. Comparisons with hop dichloromethane extracts enabled us to evidence very active compounds occurring at diverse concentrations according to the cultivar. On the basis of our results, we propose a new explanation for the quality of the Saaz variety.

Die Verwendung der GC-Olfaktometrie zur Beurteilung der Qualität des Hopfenaromas

Deskriptoren
Aromatische Verbindung, Bieraroma, Ergebnis von sensorischen Analysen, Hopfenbestandteil

ZUSAMMENFASSUNG
Für viele Jahre hat die Forschung auf dem Gebiet der Hopfenaromen ihr Augenmerk auf die qualitative Analyse von Markern gerichtet, mit denen man den Unterschied zwischen gehopftem und ungehopftem Bier untersuchen konnte. Leider liefern die üblichen Methoden wie GC-FID oder GC-MS nur wenige Informationen. Unsere Strategie war es, mit Hilfe der GC-Olfaktometrie genau zu bestimmen, welche Hopfenbestandteile wirklich Einfluss auf das Bieraroma haben. Vergleiche mit Dichloromethanextrakten ermöglichten uns, über den Nachweis der Konzentration sehr aktiver Substanzen die einzelnen Hopfensorten zu unterscheiden. Aufgrund unserer Ergebnisse schlagen wir eine neue Qualitätsbestimmung der Saazer Sorten vor.

INTRODUCTION

Aromatic hops are usually defined only according to their low α-acid content (< 5%), as opposed to bitter varieties. The brewer's experience is therefore crucial to selecting the best cultivars taste-wise. To give brewers indicators of a more quantitative nature, we have attempted to determine what, exactly, an aromatic hop is[4].

Many compounds found in hops are known to survive up to the final beer. This is the case of α-terpineol, caryophyllene oxide, geraniol, humuladienone, humulene epoxides, humulenol II, humulol, linalool, linalool oxide, citronellol, geranyl acetate and terpinen-4-ol [6,9]. Yet who could say which of these are both organolepically active and desirable? The list, furthermore is probably incomplete, as extremely low concentrations of certain compounds, undetectable by standard chromatographic detectors, are often sufficient to modify beer's aromatic features.

Most published work to date has focused on the complete identification of volatiles by gas chromatography-coupled with FID or mass spectrometry. Unfortunately, the human detector, i.e. the nose, is often much more sensitive than the most advanced physico-chemical techniques. For this reason, we have preferred the opposite approach: instead of determining the chemical composition of hopped beer, we have used AEDA-GC-Olfactometry[10] to construct their aroma profiles and have evidenced which hop compounds are the most important for beer character in terms of odour and intensity. The next step was obviously to determine the structures hidden behind these odours on the basis of their retention times on two chromatography columns, the SCD detector responses, and their mass spectra.

BREWING, EXTRACTION AND ANALYTICAL PROCEDURE

Three lagers were brewed, one without hop and two with hop pellets, either Saaz or Challenger, at a hopping rate of 1.8 g/l. 15 Litres of a 12°P industrial wort were boiled 1h15 and the hop was added 7 min before the end. Fermentations and maturations were conducted in 3-l EBC-tubes at 12°C for 5 days, 13°C for one day, 14°C for one day, 15°C for three days, 7°C for 3 days and 0°C for 24 hours.

Triangular tests were conducted with the three beers described above. The panel was asked to smell only the glass headspace in order to avoid any interaction of aroma with the bitter taste imparted to beer by isohumulones. The tests were carried out in three sessions with a panel of 12 assessors, each session comparing 2 beers. For each session, the panel was also asked to describe the aroma of the product.

An optimised XAD-2 extraction procedure[2,3,5] was used to recover beer aromatic compounds.

GC-Olfactometry analytical conditions

For the sniffing analyses, we used a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250°C and opened after 0.5 min. Compounds were separated using a 50 m x 0.32 mm, wall coated open tubular (WCOT) apolar CP SIL5 CB capillary column (1.2 µm film thickness) connected to an FID detector. The oven temperature was set to rise from 36 to 120°C at 20°C.min^{-1}, to 250°C at 2°C.min^{-1}, 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 the FID detector maintained at 280°C and connected to a Shimazu CR6-A integrator, while the other part was directed to a GC-odour port at 250°C. In the latter case, the eluent was diluted with a large volume of air (20 $ml.min^{-1}$) previously humidified using an aqueous copper (II) sulphate solution. 2 µl of the beer extract were injected.

GC-Sievers analytical conditions

The column was directly connected to a Sievers 355 SCD (sulphur chemiluminescence detector). In the 800°C combustion room of the detector, the air and hydrogen flows were maintained at 40 and 100 $ml.min^{-1}$, respectively. A 6-psi airflow was applied in the ozone generator under a vacuum (150-275 Torr) obtained by an Edwards oil-sealed RV5 pump.

SENSORIAL ANALYSES

Three beers were compared, one brewed without hop, one with Saaz, and one with Challenger. In the latter two cases, only late hopping was investigated.

As depicted in table 1, the three beers were recognized to a significant extent in triangular tests, although panellists found likeness between the unhopped and the Saaz beer.

Comparison	Detection rate
Beer without hop >< Beer + Saaz	67%*
Beer without hop >< Beer + Challenger	92%*
Beer + Saaz >< Beer + Challenger	82%*

Table 1: Rate (%) of correct answers in triangular tests (* significant with a 5% threshold[7]).

The beer brewed without hop had a strong fruity flavour quite similar to that of cider. The Saaz-hopped beer was characterized by a "beer-like" flavour with fresh and pleasant spicy notes, while the Challenger-hopped beer exhibited cheese-like heavy flavours (table 2).

Beer brewed without hop	Beer + Saaz	Beer + Challenger
Fruity (banana)	fruity (citrus)	hop
Flowery	Flowery	sulfur
Fresh	Fresh	heavy
Sweet	Spicy	cheesy
Cider-like	Beer-like	aggressive
Pleasant	Pleasant	unpleasant

Table 2: Odour description of the three beers.

Figure 1 shows the aromagrams drawn for our three beers. More than forty odours were perceived in the beer brewed without hop (figure 1a). The horizontal line represents the threshold above which all the odours should be perceived even without synergy (calculated on the basis of isoamyl acetate concentration compared to its flavour threshold[5]). The other aromas may also interact but in synergy rather than alone. When hop was added at the end of boiling (figures 1b & c), the number of peaks almost doubled. A few odours appeared as determinants of beer flavour even though most were characterized by an "n" value below 5 (n = number of GC-O detections through the dilutions).

In all three beers we found, as expected, esters and fusel alcohols such as isoamyl alcohol, β-phenylethanol, isoamyl acetate, ethyl caproate, and ethyl caprylate (table 3). 4-Vinyl guaiacol and 2-aminoacetophenone appeared as two important additional flavouring compounds. Among the odours also present in the unhopped beer, two appeared more intense when hop was added. The former was a fruity/sweet aroma identified as γ-nonalactone while the latter revealed to be β-damascenone (table 3).

Odours (R.I.)		N			Compounds
		Beer without hop	**Beer + Challenger**	**Beer + Saaz**	
Solvent	(711)	8	7	7	isoamyl alcohol
Sweet	(849)	7	7	7	isoamyl acetate
fruity/rum	(974)	5	6	6	ethyl caproate
Lily	(1092)	7	7	8	β-phenylethanol
fruity/flowery	(1178)	4	4	4	ethyl caprylate
Honey	(1274)	6	6	5	2-aminoacetophenone
Dentist	(1289)	6	6	7	4-vinyl guaiacol
Fruity	(1325)	4	6	6	γ-nonalactone
apple/peach	(1372)	4	7	7	β-damascenone

Table 3: Odours common to extracts of all three beers (n = number of times the odours were perceived through the dilutions). R.I. = Retention Index calculated on the basis of alcanes on a CP-Sil 5 CB chromatography column.

Some new flavours were found in hopped beers only (table 4): a typical spicy/hoppy odour at R.I. = 810 with an "n" value of 8-9. With such an intensity, it clearly influences the beer's organoleptic quality. Until now this key compound has never been mentioned in the literature because no peak is detected by usual detectors. Dimethyltrisulfide was another important aroma in fresh hopped beers, with its well-known "onion" odour usually associated with staling[1].

Table 4 shows six other flavours found only in both hopped beers. Among them, only linalool had been mentioned previously as contributing to the aroma of hopped beer.

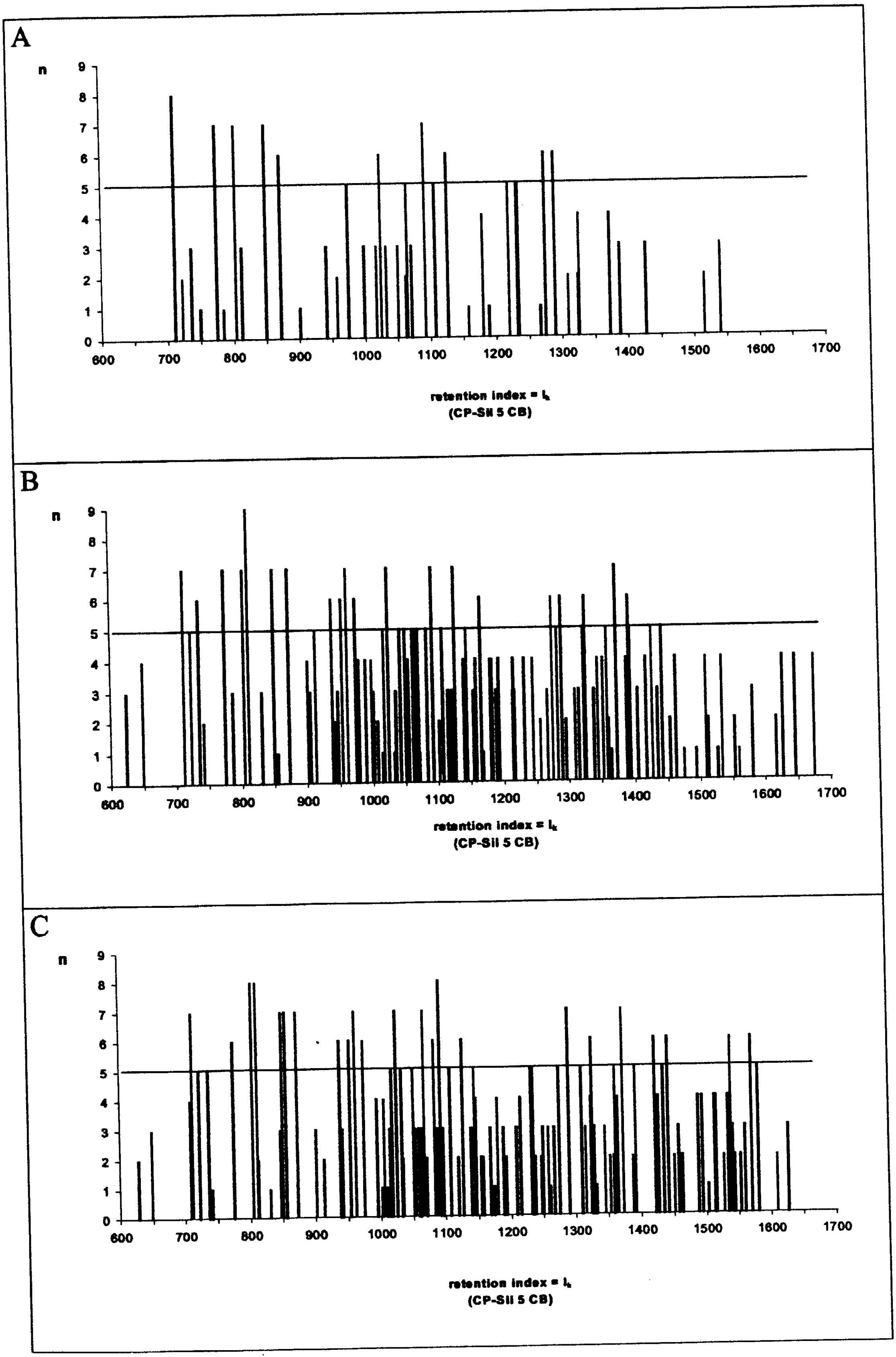

Figure 1: Aromagrams of the unhopped (a), Challenger (b) and Saaz (c) beers.

Odours (R.I.)	N			Compounds
	Beer without hop	Beer + Challenger	Beer + Saaz	
spicy/hop/leek (810)	-	9	8	unknown
sweat/fruity (938)	-	6	6	unknown
sweat/grapefruit (953)	-	6	6	unknown
onion soup (961)	-	7	7	dimethyltrisulfide
Coriander (1083)	-	5	6	linalool
Plastic (1126)	-	5	5	unknown
Phenol (1391)	-	6	5	unknown
Strawberry (1441)	-	5	6	ethyl cinnamate

Table 4: Odours common to extracts of both hopped beer but absent from the beer brewed without hop.

As described in table 5, many unpleasant odours were perceptible only when the beer was hopped with Challenger. This probably explains the heavier flavour of this beer. On the other hand, few typical Saaz beer aromas were detected, most of them being, however, fresh and pleasant. Such is humuladienone previously mentioned in the literature as a determining hop compound[8].

Odours (R.I.)	n			Compounds
	Beer without hop	Beer + Challenger	Beer + Saaz	
Cheesy (734)	-	6	-	dimethyldisulfide
sweat/rubber (913)	-	5	-	diethyldisulfide
woody/flowery (1050)	-	5	-	unknown
Geranium (1070)	-	5	-	unknown
burned plastic (1165)	-	6	-	unknown
Unpleasant (1357)	-	5	-	unknown
Perfume/pine (1360)	-	x	5	geranyl acetate
Cheesy (1393)	-	5	-	unknown
Flowery (1421)	-	-	5	unknown
Pine (1537)	-	-	6	unknown
Terpene (1569)	-	-	6	unknown
flowery/fresh (1580)	-	x	5	humuladienone

Table 5: Odours found only in one type of hopped beer.

Both fresh hops were further analysed by GC-olfactometry. 45 GC-O peaks, mainly described as corresponding with cooked vegetable, cabbage, cheesy, catty, and sweaty odours, were detected in the Challenger variety. On the other hand, only 19 odours were found in the Saaz variety, all of which were attributed much more pleasant descriptors such as fruity, greenery, or pine. Most of the hop flavours found in fresh beers resulted from transformations occurring during boiling and fermentation. Only 2 intense odours found in beer proved to have derived directly from hop: the famous "spicy/hoppy" odour at R.I. = 810 and the "coriander" aroma from linalool.

Since the main aromatic differences between the two cultivars appeared to be due to sulfur-like flavours, we analysed the two samples by GC-SCD (Sulphur Chemiluminescence Detection) (figure 2). As foreseeable, the Challenger variety was characterized by huge amounts of sulfur compounds (82), while the Saaz variety contained almost none. Among the six hop varieties tested, the Saaz cultivar yielded the lowest number of SCD peaks (table 6). On this basis, a "non-quality" classification of hops, taking into account the increasing number of sulfur peaks, has been proposed; it seems to correspond quite well with the brewers' experience.

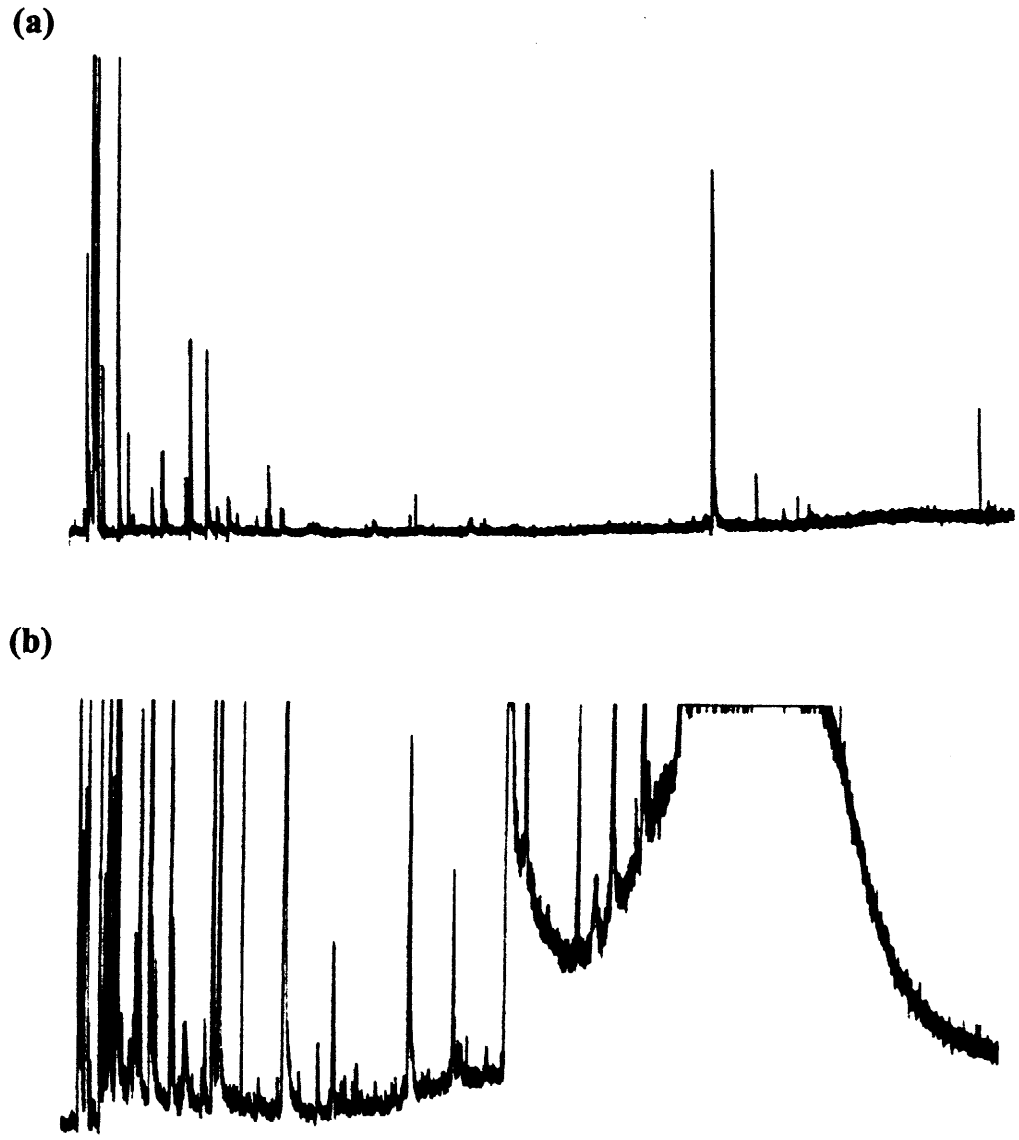

Figure 2: SCD-chromatograms of (a) Saaz and (b) Challenger hops.

Variety	Number of SCD peaks *(area > 5000 $\mu V.sec$)*
Saaz	10
Tettnang	19
Nugget	19
Hallertau Hersbrücker	20
Styrian Goldings	37
Challenger	82

Table 6: Comparison of the amount of sulfur compounds in 6 hop varieties.

CONCLUSION

The Challenger variety is characterized by large amounts of sulfur compounds leading to a beer with heavy unpleasant flavours. On the other hand, Saaz hop exhibits only a few peaks with the SC-detector while some delicate specific odours are detected by GC-olfactometry in the resulting beer.

Among the hop compounds previously mentioned in the literature, only linalool and humuladienone seem to have a determining influence on the hoppy character of beer. On the other hand, new compounds were evidenced, among which a typical spicy/hoppy flavour, found as one of the most intense peaks in both hop and beer aromagrams. Yet a large number of hop-related compounds found in beer seem to be derived from hop modifications occurring during boiling and/or fermentation.

As a general conclusion we advice the growers, sellers and brewers to perform SCD analyses of hops to assess the real aromatic potential of each cultivar.

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