

HOP AROMA; Fate of humulene epoxides and caryophyllene oxide in the brewing process

ABSTRACT

L. Mélotte¹, T. Van Havre, S. Collin² and S. Dupire¹.

A method adapted from the one established by Likens and Nickerson was set up for the extraction of hop oils. It allowed a reproducible quantification of the aroma components of hops and was applied to pellets samples from various aroma and bitter varieties. The aroma hops were overall characterized by their low oil content, what questioned about the importance for beer aroma of the high oil content of bitter hops. The terpene profile was used to distinguish varieties, while the oxidated sesquiterpenes reflected the freshness of the samples. The contribution of these compounds, mainly humulene oxides and caryophyllene oxide, to beer aroma was investigated. The analytical data showed that some hydrolysis products of the sesquiterpenes oxides from humulene and caryophyllene could be produced during wort boiling and could survive in the final beer. The tasting results showed that they were potentially aroma active and that they could participate to the aroma of the beer.

¹ Interbrew, Belgium

² Université Catholique de Louvain-la-Neuve, Belgium

1. INTRODUCTION

For a number of aromatic plants or spices, the aroma can be described by a single aromatic compound. Eugenol is responsible for the odor of clove oil, menthol is the main aromatic compound of mint, as anethole is the top note of anise oil.

In the case of hop, not any of the 300 identified compounds actually match the typical aroma of hop and the major hop compounds (mainly terpenes) are almost not found back in a kettle hopped beer.

If the compounds responsible for the hop aroma character are not yet really known, it is also difficult to define it accurately. The main descriptors are floral, citrus, spicy, grassy or even fruity which are already more useful than common terms such as "european hop" aroma or "noble hop" aroma. A distinction can also be made between the aroma arising from the addition of hop begin boiling (the kettle-hop aroma) and when aroma hop is added at the end of boiling (late-hop aroma). The first one should be more grassy or spicy whereas the second has normally more floral or citrus notes.

The hop aroma depends on the variety, on the growing and harvest conditions, and it is changing during storage. In beer, it is also the result of the quantity added, of the moment of addition or even of the fermentation conditions. But most of the time, the brewer does not have any analytical indications for the utilisation of the expensive aromatic hops and has to trust its own savoir faire.

2. OBJECTIVES

As far as hop aroma in beer is concerned, the long term goal of this project is to find some parameters that could govern the choice of the hop varieties and their utilisation.

In the first part of this work, we have set up a reproducible method allowing the extraction and the quantification of some aroma compounds of hop oil. We have applied it to different hop varieties and from these preliminary results, we have tried to point out some differences between them. Among them, the influence of the terpene profile and the fate of the related oxides were studied. The possible contribution of these compounds to beer aroma is discussed

2. METHODS

Different commercial samples from different hop varieties were chosen for this study. All hops from crop 93 were received as pellets in vacuum-sealed bags and were stored at 4°C. Both aroma and non-aroma hops were analyzed; for the aroma hops, 2 samples of Saaz (P45), one of Lublin (P 45) and one of Styrian Goldings were chosen. The non-aroma hops which were analyzed were 3 samples of Hallertau Northern Brewer (HNB), one sample of Target and one Pride of Ringwood. Two alpha-rich hops varieties were also included in the study: Galena and Nugget.

The extraction of the oil from hops was adapted from the method established by Likens and Nickerson¹. Using their apparatus, the hop oil components were simultaneously extracted and distilled in methylene chloride (Figure 1). The extraction was performed under a gentle stream of nitrogen and the cooling was set at -10°C to avoid oxidation phenomena and the loss of aromatic compounds². The methylene chloride extract was then concentrated to 1 ml in a Kuderna-Danish apparatus.

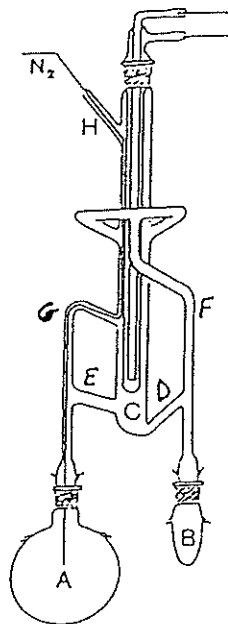


Figure 1: Micro-extractor for simultaneous steam distillation-solvent extraction.

A: Flask with hop and deoxygenated water at 140 °C

B: Flask with methylene chloride at 90°C

C: Steam condensation

D: Return for methylene chloride

E: Return for water

F: Evaporation of the methylene chloride

G: Evaporation of water

H: Stream of nitrogen.

Carvone was the internal standard, its absence in the hop oil was previously confirmed for the different varieties. After some tests on a standard mixture and on hop samples, a quantity of an half gram extracted during 45 minutes was chosen as it gave the best recoveries.

The recovery and the reproducibility of the method was evaluated. A mixture of different compounds was extracted and the concentrations were compared to the original mixture. Recoveries higher than 95 % were recorded except for the humulene diepoxides. The reproducibility was tested on a sample of HNB, extracted five times. The variation factor was calculated for different compounds: terpenes, terpenes oxides, linalool, and geranyl acetate. It was lower than 10 % (from 1 to 10 %) for all the compounds excepted for the humulene diepoxides for which it varied from 1 to about 25 %

The extracts were analyzed on a gaz chromatograph HP 5890 equiped with a FID detector. 1 μ l of the extract was injected on column and the compounds were separated on a fifty meter long capillary column CP-SIL 5CB allowing the separation in one run of about 250 peaks.

The results presented hereafter for each varieties are the mean values for 3 different extractions. Some compounds which were commercially available were calibrated: limonene, myrcene, β -caryophyllene, α -humulene, linalool, geranyl acetate, caryophyllene oxide, 2-decanone and 2-undecanone. The terpenes and the terpenes oxides were expressed as caryophyllene equivalent whereas all the other concentrations were calculated as carvone equivalent.

3. RESULTS

3.1. Total hop oil

The total oil content was evaluated by the sum of all the peaks in carvone equivalent (Table 1). The first comment on this table is that aroma hops contained generally less oil than non-aromatic hops even for pellets 45. Aroma hops are thus not "oil rich" hops as it is still sometimes believed³. The hop oil content can be very different from one variety to another; from 4220 ppm for the Styrie sample to 13900 ppm for a sample of HNB, that is to say 3 times more for a non-aromatic hop than for an aromatic hop.

Table 1: Alpha-acid and essential oil content of aromatic and non-aromatic hop samples.

Variety	Type	α -Acid content (%)	Essential oil content (ppm)
Saaz (1)	Aromatic (P45)	6.5	5230
Saaz (2)	Aromatic	6.9	5175
Lublin	Aromatic	6.5	7680
Styrian	Aromatic	4.7	4220
HNB (1)	Bitter	10.3	11385
HNB (2)	Bitter	10.3	8110
HNB (3)	Bitter	9.5	13900
Target	Bitter	9.9	6500
Pride of Ring.	Bitter	9.5	6900
Nugget	α -rich	12.5	11800
Galena	α -rich	11.7	9750

Beer is generally bittered with a non-aromatic hop which is added begin boiling, whereas the hop character is hoped when an aromatic one is added for instance 30 minutes before the end of boiling. If 70% of the alpha acids are given by the non-aromatic hop and 30 % by the aromatic hop, we can easily calculate, according to the alpha acid and oil content, the respective contribution of each hop to the quantity of aroma compounds. In our example, the mean values for Saaz and HNB were taken. It appears that the non-aromatic hop could bring at least 3.4 times more essential oils to the wort than the aromatic hop did. So the question arise, do all those non requested volatiles added begin boiling have any consequence on the aroma. As it is known that hop aroma compounds are not only volatilized during boiling but also oxydized, thus more soluble, and hydrolized, it should be interesting to answer this question. The importance of the aroma of bitter hops has also been pointed out by other authors who advised the choice of varieties with a neutral aroma to bitter the beer⁴.

3.2. Terpenes

The concentration in hop of some important monoterpenes and sesquiterpenes is presented in the next table (Table 2). Terpenes and especially sesquiterpenes can be used for hop variety identification. The main difference between the aroma and non-aroma hops tested in this study was the presence of farnesene together with bergamotene in aroma hops. Farnesene is known to be present in varieties genetically linked to Saaz. Their contribution to beer aroma as precursors should be studied.

The two alpha-rich varieties (Galena and Nugget) had high levels of limonene which is considered by some authors as responsible or precursor of a citrus aroma in beer⁵.

The terpenes profile of Pride of Ringwood was very different from the other samples; it had a very high level of α -selinene and almost no α -humulene.

But terpenes are generally not found back in the beer except if it is dry-hopped. It is thus thought that they should not be directly important for the aroma of beer. However, they are used to breed the best aroma varieties. Myrcene, one of the main terpenes, is generally associated with less desirable aromatic characteristics. As myrcene competes with humulene during hop oil synthesis, hops with higher humulene content are thought to give a better hop character to the beer. Hence, some brewers insist to buy varieties with a high humulene/myrcene ratio or a low (less than 30%) myrcene content. The same holds true for caryophyllene which is also generally regarded as a less desirable component.

Table 2: Terpenes content (in mg/kg hop) of aromatic and non-aromatic hop samples

Variety	Limonene	Myrcene	Humulene	Caryophyllene	Farnesene	α -Selinene
Saaz (1)	-*	205	2131	435	406	31
Saaz (2)	17	196	1422	277	100	26
Lublin	26	2902	2030	497	1180	45
Styrian	-	168	1390	299	183	37
HNB (1)	28	3272	4996	1204	10	71
HNB (2)	33	2566	3357	798	10	44
HNB (3)	24	3384	5899	1249	8	94
Target	-	198	1470	443	8	98
P. of R.	-	836	134	648	8	787
Nugget	102	4320	3268	1223	21	173
Galena	331	2890	1849	611	-	148

* - < 5ppm

However, the results showed that the level of myrcene in pellets was very different for aromatic hops (from 196 to 2909 ppm) as it was for non-aromatic hops (from 198 to 4320 ppm) (Table 2). Thus, what could be true for freshly harvested hop cones is certainly different for hop pellets. Indeed, as myrcene is very volatile, a low level could also be attributed to drying temperatures, to pelletization conditions, or to the hop storage. Thus, a bad handled pellet sample would have a high humulene/myrcene ratio and its quality would be overestimated. That could be true for our sample of Target which had a high humulene on myrcene ratio (Table 3), similar to aroma varieties. In fact, this ratio was probably due to the disparition of myrcene. Indeed, this sample was probably more oxidated than the others as the ratio between caryophyllene oxide and caryophyllene was higher. On the other hand, the Lublin sample should be classified as a bad aromatic variety because of its very low humulene on myrcene ratio. In this case, a good handling of the batch has probably avoided myrcene volatilisation as it has limited caryophyllene oxidation. Indeed, the oxidation index was also very low, less than 5 percent.

The ratio between humulene and caryophyllene is also sometimes used to evaluate the quality of hop. If it seems to be correct from these data that it is higher in european hops than in american hops (Galena and Nugget) and australian hops (Pride of Ringwood), it cannot be used to distinguish aroma and non-aroma hops.

Table 3: Indicative parameters for aromatic and non-aromatic hop samples
 H/M: ratio between humulene and myrcene content
 H/C: ratio between humulene and caryophyllene content
 $(Cox/C+Co_x) \times 100$: ratio between caryophyllene oxide and caryophyllene + caryophyllene oxide

Variety	H/M	H/C	$(Cox/(C+Co_x)) \times 100$
Saaz (1)	10.4	4.9	14.9
Saaz (2)	7.2	5.1	32.3
Lublin	0.7	4.1	4.4
Styrian	7.1	4.7	15.8
HNB (1)	1.5	4.2	9.7
HNB (2)	1.3	4.2	8.1
HNB (3)	1.8	4.7	12.7
Target	7.5	3.3	25.4
P. of Ringwood	0.2	0.2	10.6
Nugget	0.8	2.7	4.8
Galena	0.6	3.0	11.2

3.3. Oxygenated terpenes

Among the oxygenated terpenes, one has to consider the terpenes coming from the synthesis of the essential oils (linalool, geranyl acetate, ...) and those resulting from the oxydation of terpenes (caryophyllene oxide, humulene epoxides, humulene diepoxides, ...).

Linalool - Geranyl acetate

According to the literature¹⁰, linalool correlates with late-hop aroma and geranyl acetate contributes to it, even if it could also appear from the acetylation of geraniol during fermentation. However, they are not specific to aroma varieties, and their quantity were often higher in non-aroma hops (Table 4). For instance, Nugget should have a very good aroma as it contained high levels of linalool and geranyl acetate; but the raw aroma of Nugget was not as good as the Saaz samples. That could be explained notably if the aroma of late-hop compounds are masked by undesirable components or if other unknown compounds are not present the Nugget oil.

From these results, it is clear that if linalool really correlates with hop aroma, this could only be true for a same hopping method using always the same varieties and where only the quantity of the aroma hop is changed (and certainly not the time of addition).

Table 4: Linalool and geranyl acetate content of aromatic and non-aromatic hop samples

Variety	Linalool		Geranyl acetate	
	mg/ kg hop	(%hop oil)	mg/kg hop	(%hop oil)
Saaz (1)	61	(1.16)	-*	(-)
Saaz (2)	71	(1.37)	-	(-)
Lublin	38	(0.49)	-	(-)
Styrian	55	(1.30)	-	(-)
HNB (1)	43	(0.38)	-	(-)
HNB (2)	25	(0.31)	-	(-)
HNB (3)	119	(0.86)	-	(-)
Target	63	(0.97)	-	(-)
P.of R.	49	(0.71)	7	(0.10)
Nugget	201	(1.70)	18	(0.15)
Galena	66	(0.68)	19	(0.19)

* - < 5 ppm

Oxidated sesquiterpenes

Oxidated sesquiterpenes are already present in hop oil. As we have seen before, their quantification could be used to estimate the freshness of a sample. In this study, it was evaluated by the ratio between caryophyllene oxide on caryophyllene ratio; but the same holds true for humulene and its oxidation products.

In hop, the level of the oxidated terpenes will depend on the freshness of the sample but also on the terpene profile of the variety.

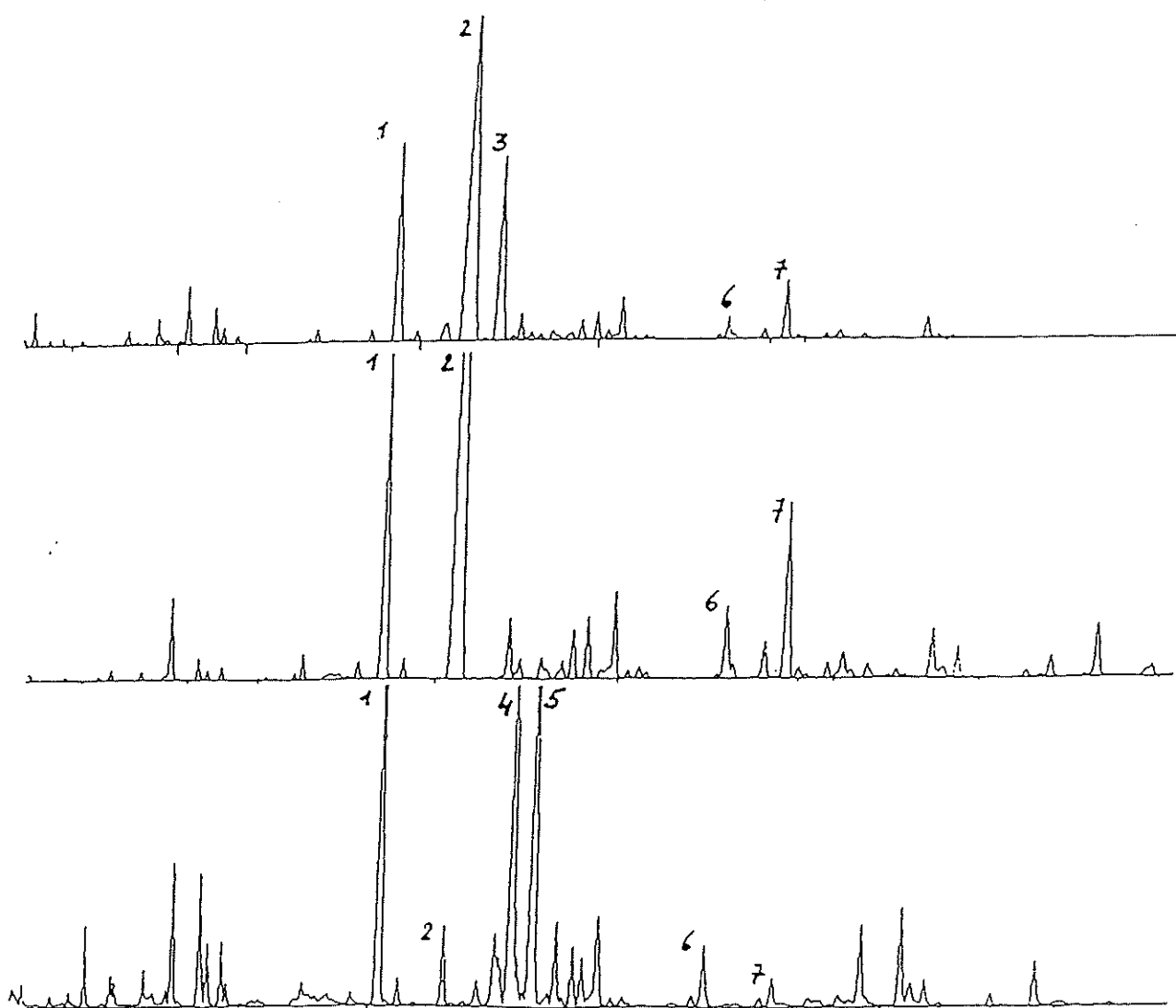


Figure 2: Part of the chromatogram of Saaz (2a), HNB (2b) and Pride of Ringwood (2c).

1: Caryophyllene; 2: Humulene, 3: Farnesene, 4: β -Selinene; 5: α -Selinene; 6: Caryophyllene oxide; 7: Humulene epoxide II.

For instance, Pride of ringwood will always contain very low quantities of humulene epoxides (Figure 2). On the other hand, the presence of unknown peaks in the chromatogram could be attributed to the oxidation of α - and β -selinene. It should also be very interesting to study the potential oxidation products of farnesene and bergamotene as they are characteristic of our aroma varieties (Figure 2).

The oxidation products of humulene and caryophyllene and their hydrolysis compounds have been studied extensively by Yang and his coworkers^{6,7,8}. They have shown that a great number of hydrolysis products can be produced under acidic conditions from caryophyllene oxide or humulene epoxides. It was also thought that the strong reducing power of the yeast can modify these compounds during the fermentation. However, the contribution of these compounds to the beer aroma is not yet well understood.

Fate of caryophyllene and humulene oxides during brewing process

In this part of the study, we have synthesized, without purification, the oxidation products of humulene. This was done according to the procedure of Lam and Deinzer by oxidation of humulene with *m*-chloroperbenzoic acid⁹. The main compounds were those found in hops what means humulene epoxides I, II, III. The five humulene diepoxides were also present. Then the mixture was hydrolyzed as described by Yang *et al.*⁶, and the mass spectra of the different peaks (about 30) produced were recorded. Caryophyllene oxide is the main oxidation product from caryophyllene which is found in hop and it is commercially available. It was also hydrolyzed and yielded about 20 compounds among them clovanediol was identified⁸. The mass spectra of the different compounds were also recorded.

The same hydrolysis was simulated during wort boiling. 3 beers were brewed and fermented according to the same procedure without any hop addition. In the first one, that we will call humulene beer, a mixture of humulene and humulene epoxides was added begin boiling. In the second one, caryophyllene oxide was added at a concentration of 16.7 ppm and we called it caryophyllene beer. In both cases, the concentrations represented about ten times more than what should normally be brought by hop. The last one was a key beer without any addition. At boiling, the two worts with addition of terpenes oxides had a typical aroma of sharpened pencil.

After fermentation, the beers were degustated; the descriptors used by the panelists were very different from beer to beer. The blank beer had a normal floral and fruity aroma whereas the humulene and caryophyllene beers were mainly described as herbaceous or grassy. The intensity of the aroma was much stronger in the humulene beer and descriptors such as hay-like, wet hay or asparagus were also cited. If the humulene beer was diluted 20 times with the key beer, the aroma was still perceptible in a triangular test.

The beers were analysed on GC/MS after extraction on a XAD-2 resin and the chromatograms were compared (Figure 3). Some peaks were present either in the humulene beer or the caryophyllene beer and not in the key beer.

The mass spectra of these peaks were compared with the mass spectra of the hydrolysis product of humulene epoxides and caryophyllene oxides. In the humulene beer, some peaks were already present in the mixture added (humulene epoxide III, diepoxide B and C) but most of them were hydrolysis products of these sesquiterpenes oxides. Among them, humulenol II was identified in the humulene beer. There were also 2 peaks which were not recorded either in the mixture or in the hydrolysis products; one was tentatively identified as humulol. They were probably formed under the reducing power of yeast during the fermentation. In the caryophyllene beer, 4 additional compounds were found: caryophyllene oxide itself, clovanediol and another hydrolysis product, and at last, an unknown peak also probably arising from the reducing conditions of the fermentation.

A few conclusions can be drawn from these preliminary results. The analytical data showed that some hydrolysis products of the sesquiterpenes oxides from humulene and caryophyllene can be produced during wort boiling and survive in the final beer. The degustation results showed that they were potentially aroma active and that they could participate to the aroma of the beer.

In our hopping process, it is probably not a top note but it could be a background flavor whose importance has still to be evaluated. Lastly, the intensity and the quality of the aroma coming from the humulene oxides mixture was very different compared to the aroma arising from caryophyllene oxide. That suggest that the terpenes profile and in consequence the hop variety could also be important for the aroma of beer.

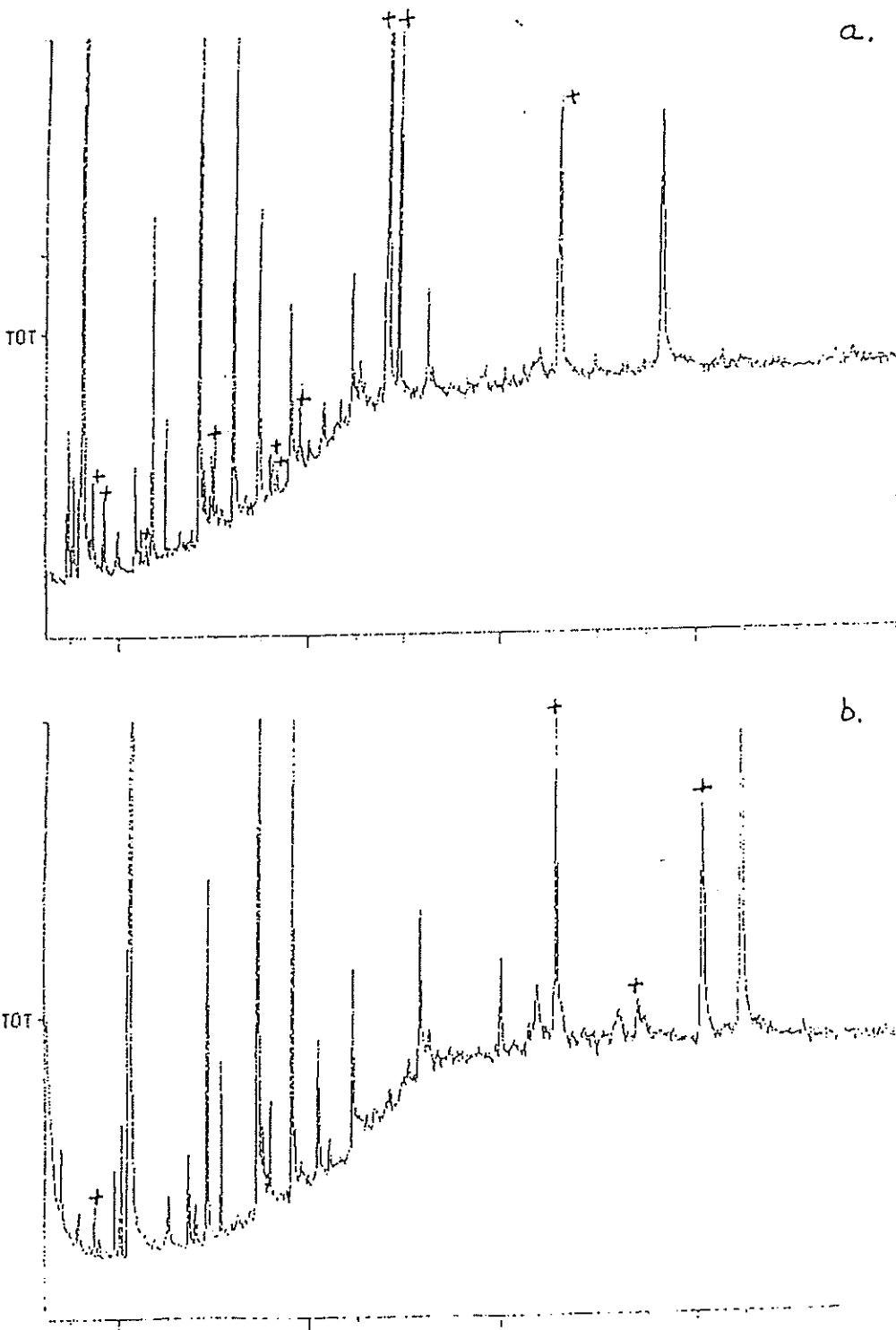


Figure 3: Chromatogram of beer extract from humulene beer (3a) and caryophyllene beer (3b)
+: peak which was not present in the blank beer

Comparison of beer aroma from HNB, Saaz and Pride of Ringwood

To confirm this, a comparison between HNB, Pride of Ringwood, and Saaz was performed at pilot scale. The aim of this trial was to point out if the aroma of bitter hop could be detected in the beer and if the description of this aroma was different of the Saaz aroma.

The following table represents the hopping conditions of the 3 beers together with their bitterness. Compared to a normal beer, a double dosis (about 15 g alpha/hl) of bitter hops (HNB and Pride of Ringwood) was added begin boiling to facilitate aroma recognition. A whole dosis of hop (about 7 g alpha/hl) was replaced by Saaz only and was added 30 minutes before the end of boiling.

Table 5: Indicative parameters of the hooped beers

Hop Variety	HNB	P.of Ringwood	Saaz
g alpha/hl	15	15	7.5
Addition	Begin boiling	Begin boiling	30 min. (before end boiling)
Boiling time	90 min	90 min	90 min
Final bitterness	38.1 BU	44.4 BU	14.6 BU
High Alcohols	89 ppm	92 ppm	88 ppm
Esters	16 ppm	16 ppm	17 ppm
Ratio A/E	5.6	5.8	5.2
Diacetylye	14 ppb	15 ppb	12 ppb
DMS	36 ppb	54 ppb	46 ppb

The characteristics of the beers were analysed and were found to be identical except, of course, for what concerned the bitterness. The quantification of different aroma compounds (higher alcohols, esters, DMS and diacetylye) showed well the weak differences existing between the beers.

It was asked to our panel (17 trained members) to taste the beers and to describe the hop aroma according to the perceived intensity, the quality, (Table 6) and the following descriptors (citrus, fruity, floral, spicy, grassy, hay-like, resinous and raw hop) (Figure 4). If these results have only an indicative value, it can be pointed out that hopping begin boiling with a double hop dosis can give an intense hop aroma. Indeed, its intensity was higher than the beer hopped with a whole dosis of Saaz at the end of boiling. Secondly, the quality of this aroma would be less good than the "late hop" aroma coming from Saaz addition. Indeed, the descriptors used for the Saaz beer were mainly citrus, and than spicy, fruity and floral, whereas those choosen to describe the bitter varieties were grassy, hay-like, resinous or even raw hop. These kind of aroma could come from the terpene oxides and their hydrolysis products and from the differences existing between HNB and P.of Ringwood.

Table 6: Tasting results

Aroma	HNB	P. of Ring.	Saaz
Intensity	4.4	4.2	3.9
Quality	5.12	4.76	6.47

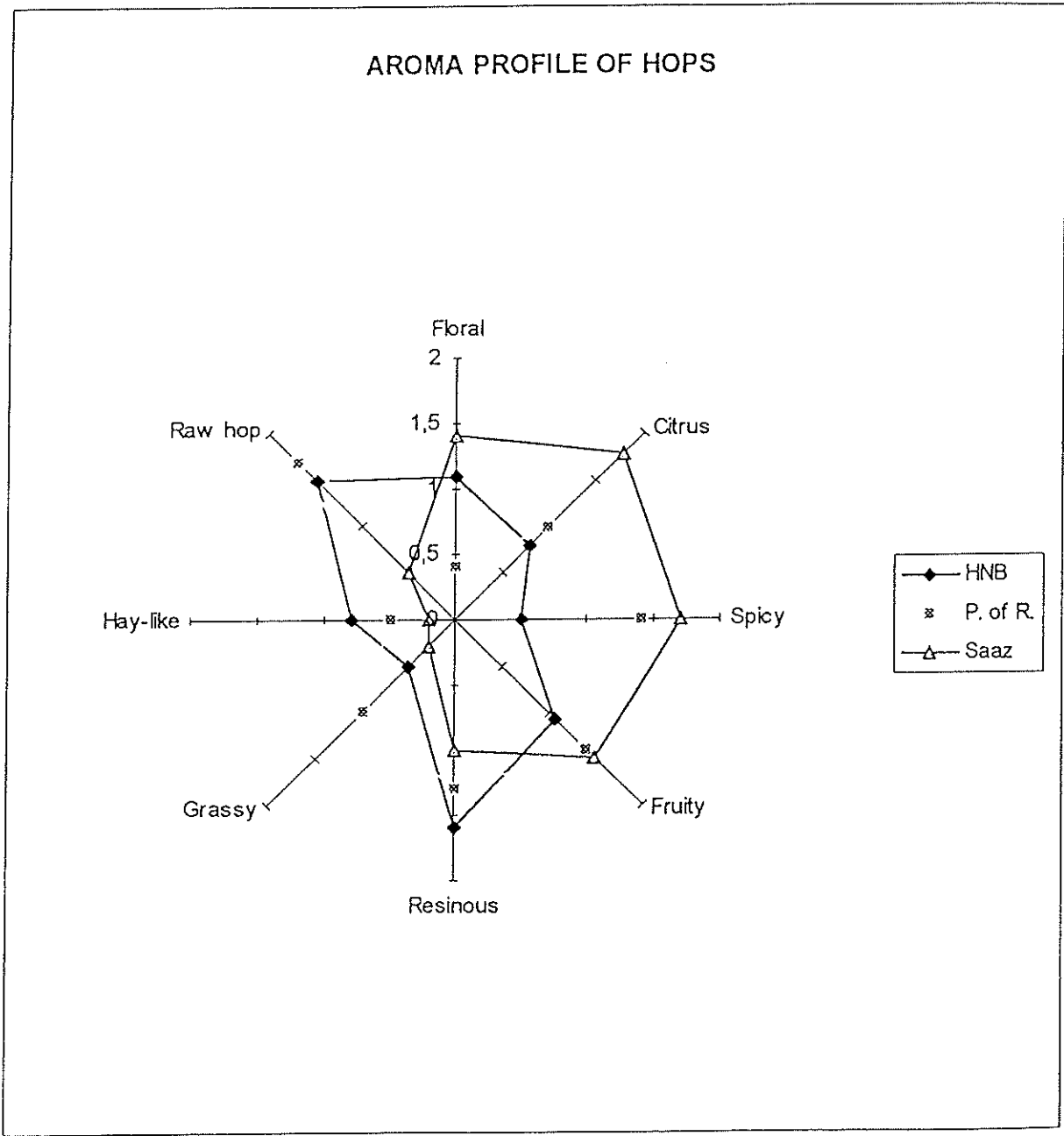


Figure 4: Aroma profile of hopped beers

4. CONCLUSIONS

A method adapted from the one established by Likens and Nickerson was set up for the extraction of hop oils. It allowed a reproducible quantification of the aroma components of hops and was applied to pellets samples from various aroma and bitter varieties. The aroma hops were overall characterized by their low oil content, what questioned about the importance for beer aroma of the high oil content of bitter hops. The terpene profile was used to distinguish varieties, while the oxidated sesquiterpenes reflected the freshness of the samples. On the other hand, the quantification of "late-hop" markers showed that they were sometimes in higher concentrations in bitter hops. The contribution of oxidated sesquiterpenes, mainly humulene oxides and caryophyllene oxide, to beer aroma was investigated. The analytical data showed that some hydrolysis products could be produced during wort boiling. Among them, clovanediol and humulenol II were identified in the final beer and could be used as marker of this phenomenon. Some of these compounds were partly reduced during the fermentation. The tasting results showed that they were potentially aroma active and that they could participate to the aroma of the beer. The aroma arising from bitter hops (HNB and Pride of Ringwood) added begin boiling was really different from the one arising from the addition of Saaz at the end of boiling. Moreover, differences were found between the aroma of HNB and Pride of Ringwood beers. So, the kettle hop aroma could be influenced by the terpene profile of the bitter varieties.

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