The Oxygenated Sesquiterpenoid Fraction of Hops in Relation to the Spicy Hop Character of Beer

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ABSTRACT

J. Inst. Brew. 108(1):86–93 2002

Hop-derived sesquiterpenoid-type oxidation products have been associated with a spicy or herbal hoppy beer character. However, the flavour threshold values of hitherto identified oxygenated sesquiterpenes are generally much higher than their estimated levels in beer. By applying two-step supercritical fluid extraction of hop pellets using carbon dioxide, followed by chromatographic purification of the enriched sesquiterpenoid fraction, highly specific varietal hop oil essences containing all main oxygenated sesquiterpenes were obtained. Post-fermentation addition (at ppb levels) of these purified sesquiterpenoid essences from various European aroma hops led to distinctive spicy or herbal flavour notes, reminiscent of typical 'noble' hop aroma. It is concluded that a spicy hop flavour impression in beer depends significantly on minor constituents of the natural sesquiterpenoid hop oil fraction.

Key words: Beer, hops, supercritical fluid extraction, hop aroma, spicy flavour, oxygenated sesquiterpenoids.

INTRODUCTION

Although, some time ago, the contribution of hop essential oils to beer aroma was questioned[23,32], current views denote that numerous essential oil components or derivatives thereof impart to beer one of its most complex and unique sensory attributes, i.e. hoppy aroma and flavour[1,14,16,25]. The importance of essential hop oils to beer aroma was recognized by the identification of about 50 hop oil-derived components in a German kettle-hopped lager[27]. Since then, a number of studies have aimed at relating hop oil constituents or derivatives, formed during the brewing process, and beer hoppy character[2,4–6,8,10,14,16,18–21,25,28,36,37]. It is now generally taken for granted that hoppy aroma and flavour result from the intricate interactions of many different flavour-active compounds. However, in practice, hoppy character is difficult to control as important flavour contributors still remain to be identified.

Organoleptic impressions derived from the essential hop oils are usually described in terms of floral, citrus, and spicy or herbal flavour notes. While there is general agreement on the nature of particular compounds associated with floral and citrus scents, the spicy or herbal aspects of hop character are ill defined. Mainly monoterpene alcohols including linalool, geraniol, and citronellol are key elements of the floral and citrus bouquet of hop aroma[6,8–10,14,17–19]. In addition, several esters of linalool and geraniol, and some uncommon cyclic ethers, such as karahana ether, are often mentioned as contributors to a floral hop aroma in beer. On the other hand, the nature of a spicy hop note, which is believed to be associated with a desirable noble or kettle hop aroma[26], has not been fully identified.

Oxygenated hop sesquiterpenes, in particular humulene epoxides, have been related to a spicy character[10,18–20]. Although this view is largely accepted in the brewing world, the flavour threshold values of hitherto identified oxygenated sesquiterpenes are generally much higher than their estimated levels in beer[4,8]. Thus, their presence in beer could only account for the sensory impression of a spicy hop character if some synergistic action occurs[4,8,36,37]. This holds also for hydrolysis and isomerisation products of caryophyllene and humulene epoxides, formed during hop storage and beer preparation. Most importantly, sensory data available today show that the flavour of the oxygenated hop sesquiterpenes and derivatives does not reflect hoppy character[4,5,8,33]. It appears that the key components of spicy or herbal hop flavours in beer have yet to be identified[15,30].

In this paper, we report on the isolation of highly enriched sesquiterpenoid hop oil fractions through a combination of supercritical fluid extraction using carbon dioxide and solid phase extraction using octadecylsilica. The remarkable organoleptic properties of these varietal hop essences are associated to current knowledge of the spicy or herbal hop character of beer.

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Publication no. G-2002-0321-03R

MATERIALS AND METHODS

Hop samples

Type 90 hop pellets (crop 1999) of the varieties Hersbrucker, Perle, Saaz, Spalter Select, and Tettnanger, and Type 45 hop pellets of the cultivar Saaz (crop 2000) were purchased from Joh. Barth & Sohn (Nürnberg, Germany). The samples were stored under vacuum at 4°C.

Supercritical fluid extraction (SFE)

Prior to extraction, hop pellets were disrupted using a pestle and a mortar to facilitate extraction. Ground pellets were extracted using a Dionex SFE-703 supercritical fluid extractor (Dionex, Sunnyvale, California, USA). Carbon dioxide (SFE/SFC grade) was from Air Liquide Benelux, Liège, Belgium. The equipment consists of a thermostatted sample oven containing up to 8 extraction cells, a flow restrictor at the end of each extraction line, and a cooled cryo rack (approximately 5°C) holding the collection vials. These are screw-capped glass containers wherein a central inner glass tube is suspended to the septum. Collection of the extracted analytes is essentially based on cold solvent trapping, although instant condensation and enrichment of less volatile hop oil constituents invariably occurred at the cold surface of the inner glass tube. Ethanol (LC-grade; Merck, Darmstadt, Germany) was used as trapping solvent to ensure compatibility with the beer matrix. Stainless steel extraction cells (10 mL) were filled with ground pellets (approximately 5 g) and placed in the sample oven at 50°C. The restrictors (flow size: 500 mL) were set at 175°C to prevent plugging. SFE extraction was then carried out in two sequential stages. The first step was performed at a CO_2 pressure of 80 bar and 50°C during about 2.5 h to remove the most volatile hop oil constituents. After changing the collection vial, the hop residue was extracted at 110 bar and 50°C for about 2.5 h. During this step, the extracted oxygenated sesquiterpenoids and part of the sesquiterpene hydrocarbons condensed at the surface of the central glass tube. After the second extraction, the inner tube was carefully loosened from the septum and the enriched sesquiterpenoid hop oil fraction was dissolved in ethanol (3 mL).

Solid phase extraction (SPE)

Solid phase extraction of the sesquiterpenoid fractions was carried out to remove hydrocarbons and traces of other hop oil constituents. Varian Bond Elut C18 cartridges (500 mg) (Varian, Harbor City, California, USA) were used. The SPE columns were pre-conditioned with HPLC-grade ethanol (10 mL), followed by ethanol/water (1/1; v/v; 10 mL) (both HPLC-grade). The sesquiterpenoid SFE-extracts were adsorbed on the column and separated into 6 fractions (3 mL each) by gradually increasing the ethanol concentration from 50% to 100%. The fraction eluting with 70% ethanol contained the oxygenated hop sesquiterpenoids.

Capillary gas chromatography (CGC)

Capillary gas chromatography of hop oils or hop oil fractions was performed using a ThermoQuest CE Trace GC 2000 Series, equipped with a flame ionisation detector (FID), a cold-on-column injector, and an AS-2000 liquid autosampler (all from ThermoQuest Italia, Rodano, Italy). Data acquisition and processing were carried out with the software package 'Chrom-Card A/D' (version 1.00). A WCOT fused silica CP-Sil 5 CB column (60 m x 0.25 mm i.d.) with a film thickness of 1.0 µm was used (Chrompack, Middelburg, The Netherlands). Hop oil samples (1 µL) with *n*-nonadecane (Acros, New Jersey, USA) as internal standard were injected cold on-column. Helium was the carrier gas (1 mL/min) and the oven temperature was programmed from 40°C to 260°C at 10°C/min (16 min at the final temperature). The FID detector temperature was set at 290°C.

Brewing trials

Pilsner beers were prepared at our pilot scale brewery (2 hL) from one and the same brew. Bittering was achieved by addition of isomerised hop extract (English Hop Products, Tonbridge, England) at the end of wort boiling. Isomerised hop extract was used rather than hop pellets to prevent introduction of hop aroma. At the end of primary fermentation, the fermented wort was divided into several casks (25 L) and the extracted and fractionated hop oil products were added at estimated levels of 20 µg/L. This allowed sensory comparison of oxygenated sesquiterpenoids from various hop varieties in beers with a similar matrix.

Brewing was done as follows: grist: pilsner malt (34 kg) and maize flakes (6 kg); brewing water: reverse osmosis (1.7 hL) with addition of $CaCl_2$ (40 mg/L); brewing scheme: 36°C (15 min), 49°C (30 min), 63°C (20 min), 72°C (30 min), 78°C (120 min, including wort filtration with lauter tun); wort boiling: 75 min (evaporation: about 8%); wort clarification: whirlpool; original gravity: 12.2°P; pitching rate: 10^7 cells/mL; fermentation temperature and duration: 12°C, 8 days; bittering: end of kettle boil with isomerised hop extract (3.85 g iso-α-acids/hL; utilisation: 65%); post-fermentation addition of varietal oxygenated sesquiterpenoid fractions: approximately 20 ppb (µg/L) at transfer to cask (25 L); lagering: in cask (10 days at 2°C); beer filtration: kieselguhr/cellulose sheets (1 µm).

Sensory evaluation of sesquiterpenoid fractions

Sensory attributes of the novel Hersbrucker sesquiterpenoid fraction were first evaluated by way of preliminary tasting sessions at KaHo St.-Lieven (5 experienced panellists). In these initial tests, varying levels of the hop essence (i.e., 20, 50, and 100 ppb, respectively) were added to a non-aromatised pilot lager, bittered exclusively with isomerised hop extract. The samples with the added fraction were organoleptically compared with the non-flavoured reference beer. Panellists were asked to describe their impressions, indicate preference(s), if any, and, likewise, preferred addition rate(s). In a separate tasting session, the approximate flavour threshold of the Hersbrucker fraction was determined according to the 'Ascending Method of Limits'[11]. Five sets of three glasses were presented to the individual assessors. Of each set, only one beer sample contained the added fraction at levels of 1, 2, 5, 10, and 20 ppb, respectively. Sensory analysis started with the lowest concentration set. The instruction was to select the sample with the addition for each triangular test. An approximate flavour threshold of the Hersbrucker sesquiterpenoid fraction was calculated as the geometric mean of the individual 'best estimate thresholds', in accordance with the Meilgaard's procedure[11].

Sensory comparison of sesquiterpenoid fractions extracted from pellets of different hop varieties was performed in pilot pilsners derived from the same primary fermentation. Thus, influences by variations in the beer matrix were minimized. At first, all finished beers were tasted by the trained panel of KaHo St.-Lieven. Off-flavours were not perceived and, consequently, a scheme to allow effective sensory evaluation by an external panel was established. This external panel, composed of 15 professional Flemish brewers, was installed while executing a research project on hop oils (HOBU IWT-Fund; see: Acknowledgements). Although the panellists were experienced beer tasters, the majority of them were not familiar with the sensory attributes of advanced hop oil preparations. Thus, at the onset of the project, three separate training sessions were organised. By adding varying levels (ranging from 50 to 200 ppb) of several commercially available 'late' hop essences (English Hop Products, Paddock Wood, Kent, England) to a non-aromatised pilot pilsner, the panellists were gradually acquainted with the unique properties of these products. Sensory analysis of our own sesquiterpenoid hop oil preparations by the external panel was then carried out as follows. Six beer samples were served simultaneously, i.e. a non-aromatised reference pilot pilsner and five beers with added sesquiterpenoid fractions (20 ppb) from Saaz, Spalter Select, Tettnanger, Hersbrucker, and Perle, respectively. The panellists were aware of the test set-up in that spicy hop oil preparations were to be evaluated. However, the varietal origin of the added fractions was not previously revealed. The instructions were: 'Describe sensory impressions. Are there differences between the samples? Give preference(s), if any.'

RESULTS AND DISCUSSION

At the EBC Symposium on Hops in 1994, Meilgaard[12] stated: "A fine hop aroma, especially the so-called noble hop aroma, is still an elusive quality. Despite 40 years of research, we still do not know what compounds cause it." Notwithstanding more recent investigations[9], Meilgaard's statement is still valid today. Indeed, in his excellent review on hops, Moir[15] concluded that our current understanding of hoppy aromatic characters in beer is still sadly incomplete.

One of the most controversial issues in respect to the contribution of hops to beer flavour has been, and still is, the spicy aspect of hop aroma. It appears that established key elements of spicy hop impressions in beer, i.e. sesquiterpene epoxides and their hydrolysis products, cannot account for the desired flavour effect[4,5,8,33,36,37]. On the other hand, a statistical correlation between the presence of hop sesquiterpene mono- and diepoxides and spicy hop flavour has been established[21]. To gain further insight into these rather contradictory observations, we aimed at obtaining highly purified natural hop sesquiterpenoid fractions to evaluate their flavour impact by post-fermentation addition to pilot lager beers bittered exclusively with iso-α-acids. In accordance with clean-label technology, an important objective was selective isolation of the hop sesquiterpenoid fraction without using harmful organic solvents. Thus, as in the modern hop processing industry[1], supercritical fluid extraction (SFE) of hop pellets with carbon dioxide was selected as the technique of choice.

Under particular conditions of pressure and temperature, SFE allows fractionated extraction of the essential oil of hops and the hop acids[3,31]. The enrichment and fractionation process is essentially based on density programming, thereby exploiting unique properties of supercritical fluids. For instance, De Keukeleire *et al*.[3] reported on the selective isolation of total hop oils from dried hops or hop extracts by applying a carbon dioxide density of 0.25 g/mL (85 bar, 50°C). Subsequent extraction of the same hop sample at a density of 0.75 g/mL (176 bar, 50°C) afforded the hop acids (humulones and lupulones). Next to the pressure, the temperature, and the extraction time, the extraction process is dependent on a number of other variables including the nature and the size of the sample matrix, and several parameters associated with experimental features of the SFE equipment (e.g. the restrictor type, the flow rate, the trapping mechanism). Thus, selective SFE extraction of hop oils and hop acids can be attained by density programming under optimised conditions.

With the SFE equipment used in this work, an additional and remarkably efficient fractionation of the extracted hop oil components occurs during the collection step, which should be ascribed to the typical configuration of the trapping device as explained in the Materials and Methods. In our search for optimum SFE-parameters to extract hop oils, it was noticed that part of the sesquiterpene hydrocarbons and the fully oxygenated sesquiterpenoid fraction were selectively condensed at the cold surface of the inner glass tube. More volatile constituents such as monoterpenes and monoterpenoid alcohols were entrained with the carbon dioxide flow through the inner tube and subsequently trapped in the solvent. This particular fractionation during the collection stage, in combination with an optimised SFE procedure and further chromatographic purification, allowed the preparation of the highly enriched sesquiterpenoid hop oil fractions. The optimised SFE conditions and subsequent solid phase extraction (SPE) are described in the Materials and Methods. The SFE method comprises a sequential two-step procedure starting at 80 bar and 50°C to extract the most volatile hop oil constituents from the hop sample. Present in this fraction are mainly monoterpenes and derivatives, as well as part of the sesquiterpene hydrocarbons. In contrast to the results described by Verschuere *et al*.[31], oxygenated sesquiterpenoids were not extracted from the hop matrix under the above conditions. After changing the collection vial, the hop residue was extracted using carbon dioxide at 110 bar and 50°C, whereby all oxygenated sesquiterpenoids were collected at the surface of the inner tube, together with about one third of the extractable sesquiterpene hydrocarbons. Subsequent purification of this hop oil fraction by SPE yielded the enriched sesquiterpenoid essence.

Fig. 1 shows the GC profile of the sesquiterpenoid hop fraction from the Hersbrucker variety in comparison with its total hop oil trace. Identification of marker components, i.e. myrcene, linalool, β-caryophyllene, α-humulene, caryophyllene epoxide, humulene epoxide I, humulene epoxide II, and humulenol II, is based on comparison with published data[4,10,18,29]. Clearly, the combined SFE/SPE methodology furnishes a sharply defined hop oil fraction only consisting of sesquiterpenoids. Inspection of part of the chromatogram between retention times of 26.5 min and 31.5 min in Fig. 1 shows that the profile of the purified essence is remarkably similar to the sesquiterpenoid pattern in the total hop oil. Thus, it appears that all oxygenated sesquiterpenoids were obtained as they are originally present in hops.

Sensory evaluation of this sesquiterpenoid hop essence was most revealing. When added to a pilot lager, bittered exclusively with iso-α-acids, a typical spicy hop flavour was clearly introduced. The approximate flavour threshold in beer, determined by the 'Ascending Method of Limits'[11], was around 5 ppb. Taking into account that the sesquiterpenoid preparation from cv. Hersbrucker is a relatively complex mixture, see Fig. 1, the estimated threshold value is surprisingly low as it refers to the total concentration of the added fraction. According to our taste panellists (see: Materials and Methods), levels of the Hersbrucker essence of approximately 20 ppb in a pilot lager were preferred. There was general agreement that such an addition

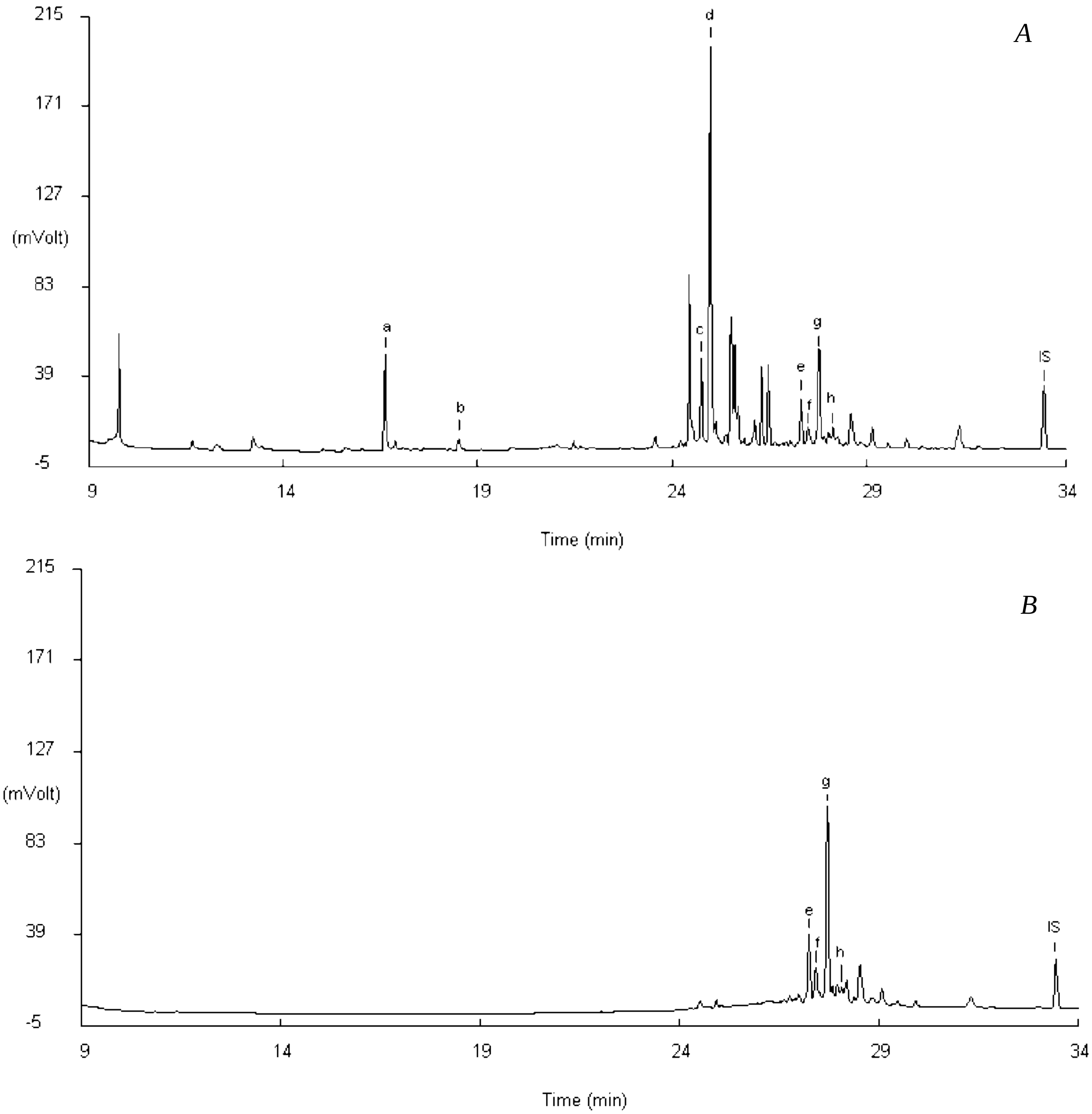

FIG. 1. GC analyses of the total hop oil (A) and the enriched sesquiterpenoid hop oil fraction (B) of cv. Hersbrucker. Peak identification: **a**: myrcene; **b**: linalool; **c**: β-caryophyllene; **d**: α-humulene; **e**: caryophyllene epoxide; **f**: humulene epoxide I; **g**: humulene epoxide II; **h**: humulenol II. For conditions of SFE/SPE and of chromatographic analyses, see Materials and Methods.

rate not only introduces a pleasant spicy hop flavour, but also imparts enhanced mouthfeel, fullness, and bitterness perception. When organoleptic comparison was made to a non-aromatised reference beer derived from the same brew, a strong preference was apparent for the beer with the post-fermentation dosage of purified sesquiterpenoids. However, higher addition rates, i.e. 50 ppb and 100 ppb, were described as overwhelming for pilsner type beers.

The results, obtained with the Hersbrucker preparation, prompted us to conduct a comparative study involving economically important hop varieties, i.e. the European aroma hops Saaz, Spalter Select, Tettnanger, and Hersbrucker, and the dual-purpose hop Perle. Oxygenated sesquiterpenoid fractions were prepared from Type 90 pellets via the optimised SFE/SPE method and analysed by capillary GC. For comparison purposes, 21 peaks in the varietal sesquiterpenoid chromatograms were chosen as relevant components and the added integration values were set at 100%. The relative content of each compound was then calculated to describe the profiles of the respective hop oil fractions. The results, represented in Table I, show significant variations in the composition of the sesquiterpenoid preparations obtained from pellets of different hop varieties. Although ageing of hops during storage and concomitant oxidation of sesquiterpene hydrocarbons cannot be excluded[4,18,25], maximum precautions were taken to prevent possible ageing effects. All pellets were from the same crop year (1999) and, in order to limit oxidative transformations of important brewing constituents, pellets were stored for 2 months at maximum and under recommended storage conditions[1]. Thus, in agreement with earlier observations[6], our findings support the view that the profiles of oxygenated sesquiterpenes reflect varietal dependences. Compared to the other preparations, the sesquiterpenoid fraction of cv. Hersbrucker shows high values for caryophyllene epoxide and for peak numbers 14, 15, and 16. Conversely, the Saaz preparation is particularly rich in a compound with peak number 5, which is present at a 10-fold higher relative concentration than in the Hersbrucker and Spalter Select fractions. Humulene epoxide II is the major constituent in all oils.

The GC traces of the enriched hop oil products of the varieties Hersbrucker and Saaz are displayed in Fig. 2. The Hersbrucker fraction appears to be considerably more complex than the Saaz fraction, as was expected from a previous investigation[6]. Most striking is the high contribution of the compound with peak number 5, which is the second most important constituent of the Saaz oil. We could confirm this interesting finding by optimised SFE extractions of Type 45 pellets. In fact, the full sesquiterpenoid pattern of Saaz Type 45 pellets was similar to the profile represented in Fig. 2 (data not shown).

Comparison of the sensory impact of the various varietal fractions was pursued through the preparation of five individually flavoured pilot lagers with a similar beer matrix (see Materials and Methods). The panellists invariably found that addition of 20 ppb of any sesquiterpenoid extract imparted spicy or herbal flavour impressions, regardless of the varietal origin. When the sesquiterpenoid beers were compared to the non-aromatised reference sample, the beers with the additions were unequivocally preferred. In addition to pleasant spicy hop flavours, improved mouthfeel and fullness of beers, synergistic effects with respect to bitterness were clearly noticed. Of a total of 15 panellists, 8 persons preferred the beer sample with the sesquiterpenoid fraction derived from Hersbrucker. The Spalter Select sample was preferred 3 times and the Saaz sample twice, while the Tettnanger and Perle samples were both selected by only 1 panellist. Nevertheless, the taste panel agreed on the existence of organoleptic differences among the varietal preparations and it was concluded that oxygenated ses-

TABLE I. Relative composition of the enriched sesquiterpenoid hop oil fractions of the varieties Tettnanger, Hersbrucker, Saaz, Spalter Select and Perle.

		Relative amounts (%)[1]				
Peak no.	**Identification**	**Tettnanger**	**Hersbrucker**	**Saaz**	**Spalter Select**	**Perle**
1		0.7	0.9	0.1	0.8	0.4
2		2.1	1.3	0.1	1.8	1.1
3	Caryophyllene epoxide	6.6	15.0	9.5	13.3	8.7
4	Humulene epoxide I	6.6	6.6	5.5	7.9	8.5
5		n.d.	2.5	25.4	2.5	n.d
6		4.4	n.d.	n.d.	1.3	5.2
7	Humulene epoxide II	45.0	34.3	45.6	40.7	48.0
8		5.8	2.6	1.5	3.1	4.5
9		4.5	3.7	2.5	4.6	7.8
10	Humulenol II	7.5	3.8	1.7	3.9	5.3
11		7.1	3.3	3.9	4.6	6.2
12		1.3	0.2	0.4	1.0	1.8
13		2.6	0.6	0.7	1.8	1.1
14		1.8	13.9	0.2	7.7	0.3
15		tr.	3.6	0.6	n.d.	0.5
16		tr.	5.2	2.1	1.7	tr.
17		n.d.	0.2	tr.	0.3	n.d.
18		4.0	1.3	n.d.	1.9	0.4
19		n.d.	0.1	0.1	0.3	n.d.
20		n.d.	1.0	0.3	0.8	n.d.
21		n.d.	0.1	n.d.	0.3	n.d.

[1] The added integration values of the 21 peaks are set at 100%.
tr. traces, n.d. not detected.

quiterpenoid fractions from hops contribute to a well-balanced beer flavour, in particular for lagers.

As explained above, the true origin of spicy hop character is as yet unclear. It has been suggested that the organoleptically important principles might be hop-derived compounds, formed during wort boiling via conversions (oxidation, hydrolysis) of hop sesquiterpenoids, in particular caryophyllene and humulene epoxides[13]. Our results, obtained by post-fermentation addition of sesquiterpenoid fractions from hops, suggest that modification of hop oil components is not necessarily required to impart pleasant spicy hop flavours to finished beer. In other words, it appears that at least parts of the organoleptically active spicy compounds are already present in raw hops. This view is supported by the sensory properties of commercially available spicy 'Late Hop Essence'[1,2,6,7,34,35], although the commercial hop product does not show the same degree of enrichment in oxygenated sesquiterpenoids when compared with our preparations. In the case of traditional kettle hopping, there is no doubt that the brewing process leads to flavour compounds that are not originally present in hops[8]. On the other hand, spicy constituents of hop oils could be extracted into wort and directly contribute to the spicy aspect of hop character by direct transfer to finished beer. Indeed, in his study on varietal dependence of hop flavour

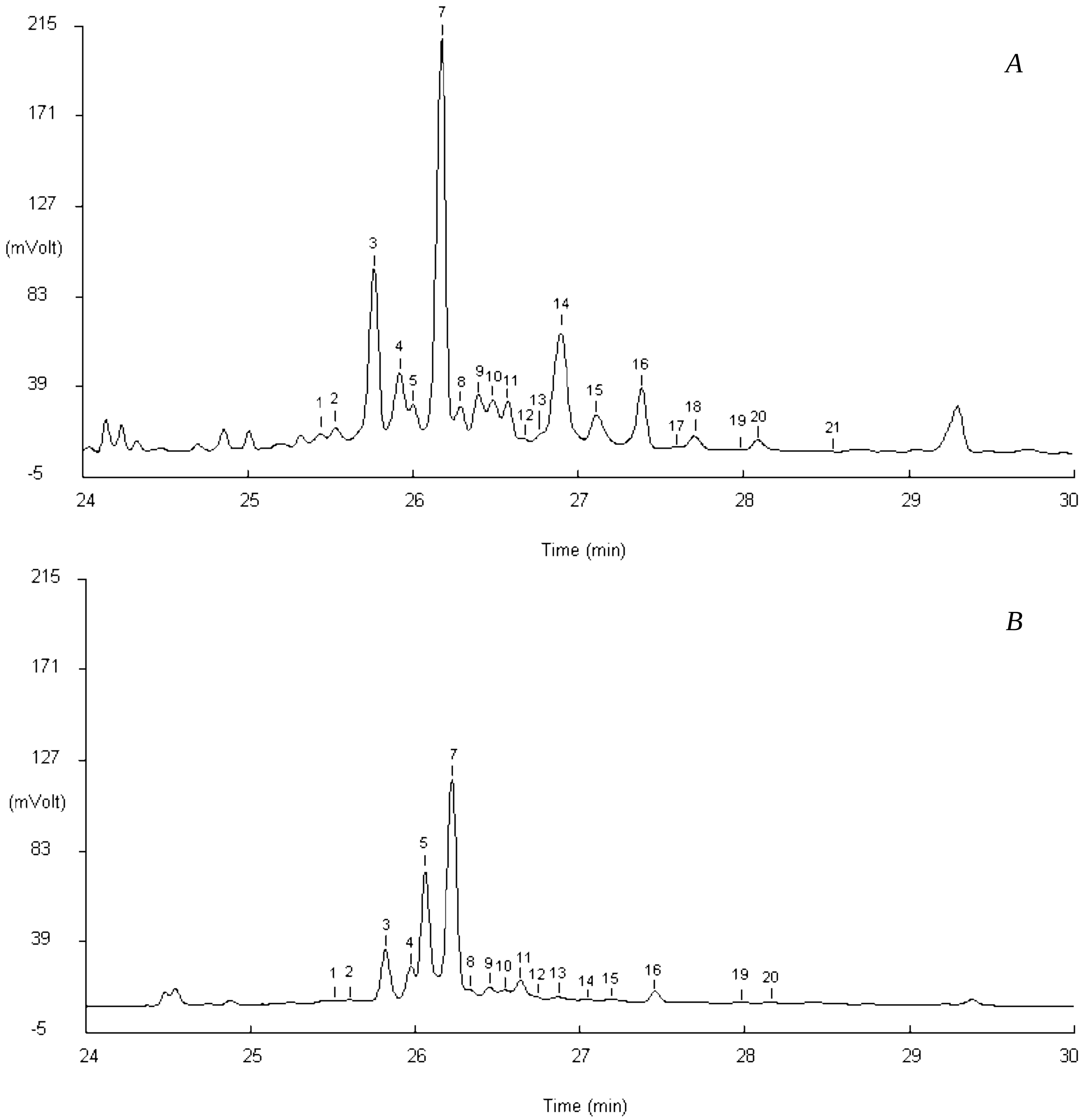

FIG. 2. GC analyses of the enriched sesquiterpenoid hop oil fractions of cv. Hersbrucker (A) and cv. Saaz (B). Peak identification: **3**: caryophyllene epoxide; **4**: humulene epoxide I; **7**: humulene epoxide II; **10**: humulenol II. For conditions of SFE/SPE and of chromatographic analyses, see Materials and Methods.

volatiles in lager beers, Irwin[8] noticed that, of 13 oxygenated sesquiterpenes that were unique to Hersbrucker beers, 12 were already present in the Hersbrucker pellets used. Thus, further chromatographic fractionation of the sesquiterpenoid hop fractions, combined with sensory assessment and profound identification studies, could be the appropriate way to unravel the chemical nature of spicy hop character.

As described by Irwin[8], common hop sesquiterpenoids, such as humulene monoepoxides and diepoxides or hydrolysis products thereof are unlikely to be significant contributors to hop character. Their flavour thresholds are generally much too high in comparison with estimated levels in beer and, above all, they do not exhibit hoppy aroma or flavour. Irwin[8] stated that the organoleptically important constituents have yet to be found and evaluated for flavour. Other researchers arrived at similar conclusions[4,12,22,25,30]. Table II represents a selection of previously published flavour threshold values of hop sesquiterpenoids and, in particular, hydrolysis mixtures. These numbers (100 ppb up to several ppm) contrast sharply with our estimated threshold value of 5 ppb and the preferred addition rate of 20 ppb of the enriched sesquiterpenoid hop oil fractions. We, therefore, suspect the presence of hitherto unknown hop oil constituents with an exceptional flavour activity in our enriched preparations. In relation to our findings, Fukuoka and Kowaka[5] concluded, based on sensory analysis of synthesised oxygenated humulenes (added to beer at levels of 500 ppb and 2 ppm), that none of the tested compounds were contributors to herbal hoppy flavour of beer. However, by applying GC-olfactometry and GC-MS of a herbal hop oil-derived fraction, they were able to pinpoint two minor constituents with a strong herbal or spicy hop flavour. The elemental composition of these compounds ($C_{15}H_{24}O$ and $C_{15}H_{26}O$) suggests a sesquiterpenoid nature.

CONCLUSIONS

An optimised clean-label-type methodology based on supercritical fluid extraction with carbon dioxide and solid-phase extraction on octadecylsilica using ethanol and water has been elaborated to isolate an enriched hop oil fraction containing oxygenated sesquiterpenoids. The composition of this essence is identical as in raw hops unlike the oxygenated sesquiterpenoids occurring in beer, the nature of which deviates from the original composition as a result of oxidative and hydrolytic transformations during brewing. Taste trials using the oxygenated sesquiterpenoid fractions of 5 hop varieties - added post-fermentation – undoubtedly demonstrated the spicy or herbal properties of the essences regardless of their varietal origin. Subtle organoleptic differences among the varietal preparations were noted and it was concluded that oxygenated sesquiterpenoids contribute significantly to a well-balanced flavour, particularly in lagers. The generally held view that hoppy character in beer requires transformation of hop sesquiterpenoids during wort boiling may prove futile. It is remarkable that published values for the flavour thresholds of known oxygenated sesquiterpenoids are much too elevated in regard to the observations by the taste panellists in this study. Hence, it is suggested that very flavour-active constituents, of as yet undefined nature, are present in the oxygenated terpenoid fraction. As to applications in the brewing industry, spicy hop flavour impressions in beer can readily be obtained by direct addition of an oxygenated sesquiterpenoid hop oil fraction to the finished product.

TABLE II. Flavour thresholds of some hop sesquiterpenoids and mixtures of hydrolysis products.

Compound or mixture of compounds	Flavour threshold in beer (ppb)	Reference
Humuladienone	100	24
Humulene epoxide II	450	18
Humulenol II	2500	18
	500	14
Humulol	>2000	8
Hydrolysis mixture of caryophyllene epoxide	3400	37
Hydrolysis mixture of Humulene epoxide II	2300	36

ACKNOWLEDGEMENTS

The authors wish to thank the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT-Vlaanderen, Brussels, Belgium) for financial support (HOBU IWT-Fund, project number 990070). We are grateful to Mrs. Brenda Van Hijfte and Mrs. Ilse De Pril for technical assistance.

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(Manuscript accepted for publication December 2001)