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Comparative Study of the Essential Oils from Hops of Various *Humulus lupulus* L. Cultivars

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Cultivars of the hop (*Humulus lupulus* L.) are of great interest for breweries. The lupulin glands of the hop cones contain a resinous fraction and an essential oil which are responsible for the bitter taste of beer and contribute to the flavour of it, respectively. The essential oils isolated by hydrodistillation from cones of ten hop cultivars were analysed by chromatographic methods. Two oils contained α-humulene as main component (ca 40%), in seven oils β-myrcene (42–58%) clearly predominated, and in one oil almost equal amounts of β-myrcene (25%) and α-humulene (27%) were present. The percentages of the quantitatively most important components of the oils are presented, while the composition of the oil of the cultivar 'Hallertau mittelfrüh' is given in more detail.

KEY WORDS Essential oil Hop cones *Humulus lupulus* L. Hop cultivars GC GC–MS

INTRODUCTION

Hop, *Humulus lupulus* L., belongs to the order Urticales and, depending on the plant taxonomist, to the family Moraceae or Cannabaceae (= Cannabinaceae).[1] Various cultivars of the plant are of economic importance, since the glandular hairs (lupulin) of the hop cones (hops) contain a resinous fraction and a volatile fraction which are used in brewing. The resinous fraction of the hops is responsible for the bitter taste of beer and the essential oil contributes to the flavour of it.[1,2] The flavour is imparted to beer either by late hopping or by dry hopping, i.e. a portion of hops is added towards the end of boiling ('lager beers') or after fermentation, respectively.[3]

The contribution of the essential oil of hops to the flavour of beer is not totally clear. In spite of numerous studies, the aroma of beer has not completely been determined chemically, probably because of its complexity.[4–7] Although more than 200 constituents have already been identified, many questions about the hop aroma still exist.[8,9]

Our aim was to analyse and compare the essential oils from cones of ten hop cultivars: 'Brewers Gold', 'Eroica', 'Hallertau mittelfrüh', 'Hersbrücker spät', 'Hugo de Groot', 'Olympic', 'Wye Challenger', 'Wye Northdown', 'Wye Target' and 'Yeoman'.

EXPERIMENTAL

Plant Material

Nine *Humulus lupulus* L. cultivars, viz. 'Brewers Gold', 'Eroica', 'Hersbrücker spät', 'Hugo de Groot', 'Olympic', 'Wye Challenger', 'Wye Northdown', 'Wye Target' and 'Yeoman', were grown in the pharmacognostical garden near the Gorlaeus Laboratories, Leiden University. In this way hop cones of cultivars grown under the same conditions were obtained. The origin of the cultivars, except 'Hugo de Groot' (unknown parentage), was the Land- en Tuinbouwschool, Vrij Technisch Instituut, Poperinge, Belgium, where hops of 'Hallertau mittelfrüh', 'Wye Challenger', 'Wye Northdown', and 'Wye Target' were also collected. The hops were collected in the first half of September, 1988, and dried at room temperature without air circulation, until crisp.

Voucher specimens of the plants grown in Leiden have been deposited in the Herbarium

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Isolation and Fractionation of the Essential Oils

The dried hops of each cultivar were cut coarsely and subsequently submitted to hydrodistillation using the apparatus described in the *European Pharmacopoeia*.[10] The distillations were performed with 20 g of cones in 500 ml of demineralized water to which *ca* 5 g of calcium carbonate was added.[11] The distillation rate was 2.5 ml/min, the distillation period 2 h.

Since 'Hallertau mittelfrüh' is known as an aromatic cultivar and preliminary GC analyses showed that its oil consisted of more components than the other oils, this oil was chosen for a complete analysis by liquid–solid chromatography (LSC), GLC and GC–MS. Therefore, 30 µl of the 'Hallertau mittelfrüh' oil were fractionated by LSC using a 3 ml Baker-10 SPE (solid phase extraction) silica gel column (J.T. Baker Chemicals b.v., Deventer, The Netherlands). Prior to application of the oil sample, a small amount of eluent was applied to the column. The elution was started with redistilled *n*-pentane (10 ml) yielding the hydrocarbons, and continued by redistilled diethyl ether (10 ml) yielding the oxygen-containing components of the oil. Both eluates were concentrated under reduced pressure in a rotatory evaporator at *ca* 0°C to 0.5–1 ml, and subsequently analysed by GLC.

GC Analysis

GC analyses were carried out on a dual channel gas chromatograph, Packard model 439 (Chrompack b.v., Delft, The Netherlands), equipped with FIDs and connected with two chromatographic data processors, Chromatopac C-R3A (Shimadzu Corp., Kyoto, Japan). GC conditions were as follows: columns: fused silica, 50 m × 0.23 mm i.d. and 60 m × 0.25 mm i.d., coated with CP-Wax 52cb, film thickness 0.22 µm (Chrompack b.v., Middelburg, The Netherlands), and Durabond DB-1, film thickness 0.25 µm (J & W Scientific Inc., Rancho Cordova, CA, USA), respectively; oven temperature: programmed, 45–240°C at 3°C/min, and subsequently isothermal at 240°C for 15 min; injector and detector: 200°C; carrier gas: nitrogen; split sampling technique: ratio 1 : 100; sample size: 1 µl of LSC fractions or of oil dilutions in *n*-pentane. The percentage composition of the oil samples was computed from the GC peak areas without using correction factors.

GC–MS

GC–MS data were obtained on a gas chromatograph, Packard model 438 A, equipped with a fused silica column, 50 m × 0.22 mm i.d., coated with CP-Sil 5cb, film thickness 0.13 µm (Chrompack) and interfaced with a Finnigan MAT 700 ion trap detector (ITD; software version 3.0; Finnigan MAT, San Jose, CA, USA). Conditions were as follows: GC oven temperature: as above; transfer line: 250°C; carrier gas: helium, 150 kPa; split sampling technique: ratio 1:40; scan range: 50–249 u; scan time: 1 s.

The identity of most of the components of the 'Hallertau mittelfrüh' oil was assigned by comparison of their mass spectra and retention times, on the two columns of different polarities mentioned under 'GC Analysis', with those of components of reference oils and authentic compounds. Some components were identified by matching their spectra with those of the Mass Spectra Library of the US National Institute of Standards and Technology using the INCOS data system, because authentic compounds were not available.

RESULTS AND DISCUSSION

The yields of the essential oils isolated by hydrodistillation from the cones of the various hop cultivars studied, are given in Table 1. The

Table 1. The yields, based on dry weight, of the essential oils isolated by hydrodistillation from cones of hop, *Humulus lupulus* L., cultivars

Cultivar	Yield (%)
'Brewers Gold'	0.8
'Eroica'	0.3
'Hallertau mittelfrüh'	2.0
'Hersbrücker spät'	0.4
'Hugo de Groot'	< 0.1
'Olympic'	0.2
'Wye Challenger'	0.8
'Wye Challenger'[a]	1.0
'Wye Northdown'	0.8
'Wye Northdown'[a]	1.8
'Wye Target'	1.0
'Wye Target'[a]	1.0
'Yeoman'	1.9

[a]Collected in Belgium.

Table 2. Identified components of the essential oil from cones of *Humulus lupulus* L. 'Hallertau mittelfrüh'

Component	%	Identification[a]	Component	%	Identification[a]
α-Pinene	0.02	MS, RT	Methyl dec-4-enoate	1.02	MS
Camphene	0.02	MS, RT	*allo*-Aromadendrene	0.09	MS, RT
β-Pinene	0.36	MS, RT	Undecan-2-one	0.96	MS
β-Myrcene	27.32	MS, RT	α-Humulene	41.29	MS, RT
α-Phellandrene	0.01	MS, RT	Geranial	0.01	MS, RT
Propyl 2,2-dimethylpropanoate	0.12	MS	Germacrene-D	0.28	MS, RT
2-Methylheptyl propanoate	0.47	MS	α-Muurolene	0.09	MS, RT
Limonene	0.08	MS, RT	β-Selinene	0.51	MS, RT
β-Phellandrene	0.02	MS, RT	α-Selinene	0.68	MS, RT
cis-β-Ocimene	0.02	MS, RT	Citronellol	0.02	MS, RT
γ-Terpinene	0.38	MS, RT	δ-Cadinene	2.06	MS, RT
trans-β-Ocimene	0.03	MS, RT	γ-Cadinene	1.21	MS, RT
Methyl heptanoate	0.19	MS	Cadina-1,4-diene	0.48	MS, RT
Terpinolene	0.01	MS, RT	α-Cadinene	0.61	MS, RT
Methyl octanoate	0.08	MS	Tridecan-2-one	0.70	MS
Nonan-1,9-diol	0.12	MS	Cyclodecanol	0.02	MS
α-Cubebene	0.09	MS, RT	Geraniol	0.01	MS, RT
Methyl nonanoate	0.18	MS	Calamenene	0.13	MS, RT
Decan-2-one	0.02	MS	Heptadeca-5,8,11-trien-1-ol	0.85	MS
Dodecan-2-ol	0.04	MS	α-Calacorene	0.21	MS, RT
α-Ylangene	0.11	MS, RT	β-Caryophyllene epoxide	0.31	MS, RT
α-Copaene	0.32	MS, RT	(*E*)-Nerolidol	0.23	MS, RT
Methyl non-5-enoate	0.02	MS, RT	Cubenol	0.07	MS, RT
Linalol	0.41	MS, RT	*epi*-Cubenol	0.15	MS, RT
6,10-Dimethylundecan-2-one	0.09	MS	Methyl hexadecatrienoate	0.64	MS
β-Cubebene	0.02	MS, RT	α-Bisabolol oxide	0.34	MS, RT
9-Hydroxynonan-2-one	0.99	MS	T-Cadinol	0.25	MS, RT
β-Elemene	0.02	MS, RT	T-Muurolol	0.20	MS, RT
Calorene	0.01	MS, RT	δ-Cadinol	0.07	MS, RT
β-Caryophyllene	11.33	MS, RT	α-Cadinol	0.02	MS, RT

[a]MS = identification by comparison of mass spectra; RT = identification by comparison of retention times on two columns (see 'Experimental').

yields varied from <0.1% to 2.0% (volume/dry weight). Since the hops collected in the garden in Leiden were submitted to hydrodistillation three months later than the hops collected in Belgium, i.e. hops of 'Hallertau mittelfrüh', 'Wye Challenger', 'Wye Northdown' and 'Wye Target' which were submitted to distillation within a period of four to five weeks after collection, some lower yields might be partly due to the longer period of storage of the Leiden samples, although other factors might also play a role. The yields of the oils from 'Wye Northdown' cones collected in Leiden and in Belgium were found to be 0.6% and 1.1% respectively, after a storage of almost seven months at room temperature. On the other hand, the 'Wye Target' cones yielded 1.0% (Leiden) and 0.9% (Belgium) after almost seven months, which shows that a longer period of storage does not always markedly affect the yields. However, a comparison of the composition of the oils from the hops of 'Wye Challenger', 'Wye Northdown' and 'Wye Target' grown in Belgium with that of the corresponding oils from the hops grown in Leiden did not show marked differences.

As mentioned under 'Experimental', the oil of the cultivar 'Hallertau mittelfrüh' was analysed by LSC, GLC and GC–MS. Of the many components detected in this oil, 62 were identified which represented more than 95% of the oil. The results of the analysis in question are summarized in Table 2, which also shows the method of identification.

Subsequently, the oils isolated from the other cultivars were compared with the 'Hallertau mittelfrüh' oil, using the two GC columns of different polarities. Since the oils from the cultivars grown in Belgium as well as in Leiden did not show marked differences, and the nine cultivars were grown in Leiden under the same

Table 3. Some components of the essential oils from cones of various cultivars of hop, *Humulus lupulus* L., and their relative amounts

Component	'Brewers Gold'	'Eroica'	'Hallertau mittelfrüh'[a]	'Hersbrücker spät'	'Hugo de Groot'	'Olympic'	'Wye Challenger'	'Wye Northdown'	'Wye Target'	'Yeoman'
β-Pinene	0.8	0.7	0.4	0.6	0.3	0.5	0.6	0.7	0.7	0.7
β-Myrcene	51.4	41.6	27.3	50.9	17.2	25.0	47.5	41.9	57.9	52.2
2-Methylheptyl propanoate	1.6	0.1	0.5	0.1	0.2	0.3	1.3	2.5	1.1	2.8
γ-Terpinene	0.5	1.5	0.4	0.2	0.2	0.3	0.7	0.2	0.4	0.5
Linalol	0.4	0.2	0.4	0.3	0.2	0.3	0.4	0.2	0.5	0.3
9-Hydroxy nonan-2-one	0.3	4.6	1.0	1.9	1.3	0.4	2.3	0.3	2.5	0.7
β-Caryophyllene	7.1	11.4	11.3	5.6	11.6	10.3	4.7	9.3	4.1	4.5
Methyl dec-4-enoate	1.8	5.9	1.0	2.3	0.8	0.2	1.5	1.4	1.8	1.4
α-Humulene	18.9	1.9	41.3	23.6	37.9	27.4	18.5	22.0	11.1	17.4
β-Selinene	0.8	1.9	0.5	0.1	0.1	0.2	3.2	1.0	0.1	0.2
α-Selinene	0.7	2.0	0.7	0.2	0.3	0.3	3.5	0.9	0.2	0.2
δ-Cadinene	0.9	0.3	2.1	0.9	1.9	0.1	0.1	1.2	1.3	2.9
γ-Cadinene	0.5	0.3	1.2	0.5	1.2	0.2	0.3	0.6	0.6	3.1
Tridecan-2-one	0.3	4.0	0.7	0.7	0.7	0.2	1.1	0.3	1.2	0.2
Methyl hexadecatrienoate	1.5	6.3	0.6	1.0	0.3	2.3	0.3	2.0	0.8	0.3

[a]Cones collected in Belgium.

conditions, a comparative study of the essential oils of the various cultivars was made possible. In all oil samples, which showed to be rather complex, mono- and sesqui-terpene hydrocarbons clearly predominated. β-Myrcene, α-humulene and β-caryophyllene were characteristic main components present in all samples, although variations in their relative amounts did exist. Table 3 shows the percentages of the quantitatively most important components of the oils of the hop cultivars.

'Hallertau mittelfrüh' is known because of its good aroma, and because it yields an α-humulene-rich oil, which was confirmed by our investigation. The oil of one other cultivar, 'Hugo de Groot', also consisted much more of α-humulene than of β-myrcene. In one sample, 'Olympic', almost equal amounts of these compounds were found, whereas in the other samples the latter compound predominated. The amount of β-caryophyllene varied from 4.1% to 11.6%. In general, the total content of oxygen-containing components was below 10%, only the 'Eroica' oil consisted of more than 20% of such components. The composition of the mixture of these compounds was complex in all oil samples, and various compounds such as alcohols, aldehydes, ketones, epoxides and esters were present. Besides the important hop flavour components β-myrcene, α-humulene and linalol,[12] the esters are supposed to contribute to the hop flavour of beer to a large extent, in spite of their low concentrations.

In conclusion it can be stated that the oils isolated from various hop cultivars represented different genotypes: in two oils ('Hallertau mittelfrüh' and 'Hugo de Groot') α-humulene was found as main component (*ca* 40%), in seven oils β-myrcene (42–58%) clearly predominated, whereas in one oil ('Olympic') almost equal amounts of β-myrcene (25%) and α-humulene (27%) were found. Because of the various genotypes found in the study described here, the plant material will be suitable for our investigations on the formation of hop flavour components by means of plant cell cultures.

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