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VARIETAL DIFFERENCES IN VOLATILE WATER-SOLUBLE FRACTIONS OF HOPS (*HUMULUS LUPULUS* L.)

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Abstract—A distillation–extraction technique was employed to obtain volatile water-soluble fractions from samples of six hop varieties within 10 weeks of harvest. Extracts were examined by gas chromatography and compared. Two of the six varieties were re-examined after storage for 15 months. It was shown that most of the volatile water-soluble compounds were formed in storage. The aqueous extract from stored hops of the variety Bullion contained about forty times as much 2-methylpropanoic and 3-methylbutanoic acids as found in stored Goldings. These fatty acids might well contribute to off-flavour which prevents many brewers using Bullion.

INTRODUCTION

VARIETAL differences in the compositions of steam-distilled hop oils are well established[1] although detailed results have only been presented for hops grown in America and Australasia.[2–4] In previous communications[5, 6] it has been shown that several volatile water-soluble compounds are not recovered by this method but can be isolated by a technique in which the steam distillate is continuously extracted by a suitable solvent. These volatile water-soluble compounds, nine of which have been identified,[6] may be of considerable importance in beer flavour and aroma since some of them can survive boiling in the brewing process.[7] Earlier work[6] has shown that these compounds are wholly or partly formed during storage of hops.

In the present work gas–liquid chromatography (GLC) was used to compare six varieties by examination of: (1) essential oils obtained by normal steam-distillation; (2) fractions obtained by the distillation–extraction technique soon after harvest; (3) water-soluble fractions obtained by extraction of (2); and (4) water-soluble fractions as in (3) but from hops stored for 15 months.

The varieties studied were Bullion, Fuggle, Golding, Northern Brewer, Early Promise and Sunshine. The first four are important commercially, while the last two are known to differ considerably from the others in essential oil consitution.

RESULTS AND DISCUSSION

Chromatograms of fractions obtained from the above six varieties by distillation–ether extraction within 10 weeks of harvest showed over sixty-five peaks, twenty-three of which corresponded with compounds known to be present[2, 6] (Table 1). The chromatograms are

[1] R. STEVENS, *Chem. Rev.* **67**, 19 (1967).
[2] R. G. BUTTERY and L. C. LING, *J. Agri. Food Chem.* **15**, 531 (1967).
[3] S. T. LIKENS and G. B. NICKERSON, *J. Agri. Food Chem.* **15**, 525 (1967).
[4] J. R. L. WALKER, *N.Z. J. Sci.* **10**, 476 (1967).
[5] R. D. HARTLEY and C. H. FAWCETT, *Chem. Ind.* 1601 (1967).
[6] R. D. HARTLEY and C. H. FAWCETT, *Phytochem.* **7**, 1395 (1968).
[7] R. D. HARTLEY, to be published.

TABLE 1. COMPOUNDS PRESENT IN ETHER EXTRACTS OF HOP VARIETIES

Peak No.†	Compound corresponding to retention time*
7	3-Methylbutan-2-one
11	2-Methylbut-3-en-2-ol
13	α-Pinene
18	2-Methylpropyl isobutyrate
20	β-Pinene
22	Myrcene
23	2-Methylbutyl propionate
24	2-Methylbutyl isobutyrate + *n*-Pentyl isobutyrate
25	Limonene
29	Ocimene
31	Methyl heptanoate + 2-Methylbutyl 2-methylbutyrate
35	*n*-Hexyl propionate + *n*-Hexyl isobutyrate
38	Methyl octanoate + Nonan-2-one
45	Methyl nonanoate
47	Linalool + Copaene
52	Undecan-2-one + Methyl decanoate
54	Methyl dec-4-enoate + Caryophyllene
57	Farnesene
59	Methyl-deca-4,8-dienoate + Methyl geranate
60	Humulene
63	Geranyl acetate
64	α- and β-Selinene
65	δ-Cadinene

* From compounds known to be present.[2, 6]
† Retention times of peaks 31 and 63 were 62 min and 120 min respectively.

very similar to those of the oils obtained by normal steam distillation except that peak Nos. 1–11 are comparatively very large.

The water-soluble extracts from these ether-soluble fractions were then examined and showed the presence of twenty-three peaks only. The two varieties Bullion and Golding were re-examined after 15 months' storage. The amounts of volatile water-soluble constituents present in the aqueous extracts are shown in Table 2. There were considerable differences between the six varieties when examined within 10 weeks of harvest and Bullion contained the greatest amount of these compounds. After storage, the aqueous extracts from Bullion and Golding contained many times as much of these compounds. The extract from stored Bullion contained about three times as much of the water-soluble compounds as stored Golding. There was about forty times as much 2-methylpropanoic and 3-methylbutanoic acids in the extract of stored Bullion as in Golding.

The above findings strongly suggest that the volatile water-soluble compounds result from oxidation in storage. The possibility that the results could be explained by a seasonal effect as the hops analysed after storage were from the 1966 harvest and the samples analysed near harvest were from the 1967 crop, is considered unlikely. All the nine compounds so far identified contain the *gem*-dimethyl group and six of these have the isoprene skeleton. It is likely therefore that they are mainly derived from terpenoid compounds, for example from the resins which account for about 20 per cent of dry matter of hops.

Two of the compounds found, 3-methylbutan-2-one and 2-methylbut-3-en-2-ol, have very recently been identified in hop aroma fractions.[8] Comparison of gas chromatographic retention times suggested that both of these constituents were formed when one of the

[8] M. DE METS and M. VERZELE, *J. Inst. Brewing* **74**, 74 (1968).

TABLE 2. AMOUNTS OF VOLATILE COMPOUNDS PRESENT IN THE AQUEOUS EXTRACTS FROM DIFFERENT HOP VARIETIES

Peak No.	Compound (as indicated by retention time)†	Wt. of volatile water-soluble compounds (mg/100 g kiln-dried hops)*							
		Varieties analysed within 10 weeks of harvest						Varieties analysed after 15 months' storage	
		Bullion	Early Promise	Fuggle	Golding	Northern Brewer	Sunshine	Bullion	Golding
1	—	0·560	0·144	0·289	0·131	0·293	0·261	0·683	0·423
2	—	0·014	0·016	0·023	0·036	0·036	0·026	0·011	0·009
3	—	0·036	0·003	0·010	0·007	0·014	0·013	0·059	0·078
4	—	0·024	0·005	0·025	0·016	0·055	0·020	0·012	0·060
5	3-Methylbutan-2-one	0·140	0·015	0·042	0·040	0·071	0·037	0·321	0·167
6	—	0·013	zero	0·007	0·003	0·007	0·005	0·015	0·015
7	—	0·050	0·035	0·062	0·042	0·052	0·036	0·015	0·025
8	2-Methylbut-3-en-2-ol	1·694	0·210	0·724	0·503	0·826	0·496	14·697	5·264
9	2-Methylpropan-1-ol	0·021	0·012	0·014	0·008	0·017	0·025	0·119	0·042
10	—	0·016	0·019	0·014	0·008	0·026	0·024	zero	zero
11	—	0·016	0·013	0·015	0·008	0·025	0·013	0·006	0·004
12	3-Methylbutan-1-ol	0·020	0·011	0·020	0·008	0·013	0·020	0·026	zero
13	3-Methylbut-2-en-1-al	0·139	0·074	0·137	0·060	0·172	0·093	0·285	0·208
14	—	zero	zero	zero	zero	zero	zero	0·009	0·002
15	3-Methylbut-2-en-1-ol	0·191	0·018	0·039	0·031	0·050	0·026	0·553	0·161
16	—	0·012	0·050	0·003	0·006	0·012	0·010	0·007	0·002
17	—	0·015	0·002	0·006	0·006	0·004	0·002	0·036	0·015
18	—	zero	zero	zero	zero	zero	zero	0.021	0·006
19	—	0·012	0·002	0·013	0·018	0·018	0·003	0·012	0·016
20	—	zero	zero	zero	zero	zero	zero	0·023	0·012
21	2-Methylpropanoic acid	zero	zero	zero	zero	zero	zero	0·122	0·002
22	2,2-Dimethyl-5-oxo-2,5-dihydrofuran	0·013	0·002	0·004	0·006	0·002	zero	0·085	0·042
23	3-Methylbutanoic acid	0·004	zero	zero	0·002	0·002	zero	0·042	0·002
Essential oil content (ml/100 g kiln-dried hops)‡		0·55	0·40	0·60	0·50	0·80	0·85	0·75	0·45

* Estimated (area method) by comparison with aqueous solutions of 2-methylbut-3-en-2-ol as reference. Moisture content of hops approximately 10%.
† Retention time of 3-methylbutan-1-ol reference was 47 min.
‡ Estimated by normal steam-distillation technique.

constituents of hop resin, colupulone, was oxidized. It appears that 3-methylbutan-2-one arises from the acyl side-chain and that 2-methylbut-3-en-2-ol is formed from the isopentenyl side-chain. By a similar mechanism a second resin constituent, humulone, apparently gave the same alcohol.

It has been assumed that the essential oil of hops is mainly responsible for the aromatic odour of beers, but it now seems possible that this aroma is largely derived from oxidation of hop resin. Furthermore the production of aromatic odour may depend on transformations taking place during the fermentation stage. The high content of 2-methylpropanoic and 3-methylbutanoic acids in stored Bullion might well contribute to off-flavour which prevents many brewers using this variety.

EXPERIMENTAL

Hop Material

Samples examined within 10 weeks of harvest were grown at Wye (1967 crop) and stored at 5° before analysis. The Bullion and Golding hops examined after 15 months' storage at room temperature were commercial samples from the 1966 Kent crop.

Isolation of Fractions from Hops for Examination by GLC

Steam distilled oils. A method similar to that of Wright and Connery[9] was employed. For each variety, water (1 l.) was added to ground hops (100 g) and the slurry was boiled for 4 hr. The distillate was collected in an oil trap, the aqueous portion being returned continuously to the boiler.

Ether fractions from distillation–extraction. A modified method to that previously described[5] was employed using ground hops (100 g), water (5 l.) and a distillation time of 4 hr for each variety. Redistilled ether was used as solvent, the final volume of the extract being adjusted with fresh solvent to 5·0 ml.

Water-soluble fractions. For each variety, the ethereal solution (5·0 ml) from above was extracted with an equal volume of water.

Conditions of Analytical GLC

These were as previously described[6] using an 18 ft glass column, Carbowax 20M as stationary phase and a flame ionization detector. Sample sizes and amplifier attenuations, given in parenthesis, were as follows:

Steam-distilled oils. 0·4 μl (2×10^{-9}).

Ether fractions from distillation–extraction. Bullion, Fuggle, Golding, Northern Brewer varieties 10 μl each time (5×10^{-9}); Early Promise variety 14 μl (5×10^{-9}); Sunshine variety 6 μl (5×10^{-9}).

Water-soluble fractions. 30 μl (10^{-9}, except for Bullion examined within 10 weeks of harvest 2×10^{-9}).

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[9] R. G. WRIGHT and F. E. CONNERY, *Am. Soc. Brewing Chem. Proc.* 87 (1951).