Identification of Hop Varieties by Gas Chromatographic Analysis of Their Essential Oils

Capillary Gas Chromatography Patterns and Analyses of Hop Oils from American Grown Varieties

RON G. BUTTERY AND LOUISA C. LING

Capillary gas chromatography patterns and tables of percentage composition are given for hop oils from the American grown hop varieties Cluster (early, late, seeded, seedless); Fuggle (seeded, seedless); Bullion (seeded and seedless); Brewers Gold (seeded, seedless), and Talisman (seedless). A scheme is described whereby each variety can be readily distinguished from the others by its capillary gas chromatography pattern and by the relative percentages of certain components.

Different hop varieties are rather difficult to distinguish by physical appearance especially after the hops have been picked and baled. There had been no reliable method available to identify the varietal origin of picked hops or hop concentrates until the recent publication of Likens and Nickerson (*4*), who were able to characterize the different American varieties by the packed column gas-liquid chromatography patterns of the volatile hop oil. The present work is an extension of the method of Likens and Nickerson, but using capillary column gas chromatography to differentiate the varieties. Capillary gas chromatography with its higher resolution over packed column gas chromatography separates more components for any particular hop oil and thus gives a greater number of differentiating factors.

Experimental

Hop Oil Samples. These were received from Likens and Nickerson in sealed tubes and were kept stored at −30° F. until analysis. The sources and isolation of the oils are as described by Likens and Nickerson (*4*.)

Capillary Gas-Liquid Chromatography. The capillary columns were 150-foot long by 0.01-inch i.d. stainless steel coated with Silicone SF-96(100) containing the tail reducing agent Igepal CO-880 (5% by weight of the silicone); the carrier gas was helium at a flow rate of 11 cm. per second and an inlet pressure of 10 p.s.i.; sample size was 3 μl., injector temperature 200° C. with a split of 1/300; column oven was programmed at 50° to 160° C. at 0.5° per minute and held at the upper limit; detection was by flame ionization detector.

Peak areas were automatically measured using a Perkin-Elmer Model D-2 digital integrator and printer. All components were assumed to have the same detector response, and the percentages in Table I are calculated on this basis.

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Albany, Calif. 94710.

Results and Discussion

Figure 1 shows typical curves for the oils from the different American varieties. Table I lists the relative percentages of the components of each oil. The identification of hop oil components has been outlined in previous publications (*1–3*). With seedless *vs.* seeded, late *vs.* early, and different growing areas there are minor differences in certain components and some quantitative differences especially with the ratio of the C_{15} components (humulene, etc.) to C_{10} components (myrcene, etc.). However, the main character of the pattern remains essentially the same with the same variety regardless of these above variables. A scheme for differentiating the varieties without regard to the growing variables is outlined below and shown diagrammatically in Figure 2.

A Scheme for Differentiating American Hop Varieties According to the Capillary GLC Pattern of the Steam Volatile Oil. The American oils can be put first into two main groups, which for convenience will be referred to as groups I and II.

Group I contains the varieties Bullion and Brewers Gold. The requirements for group I are as follows: peak 39 (geranyl acetate) greater than or approximately equal to peak 41 (copaene); peak 25 (methyl nonanoate) greater or approximately equal to peak 31 (2-undecanone); peak 52 (Santalene-type sesquiterpene) greater than peak 56a (2-tridecanone and others).

The Bullion variety may be distinguished from the Brewers Gold by the following conditions: Bullion has peak 34 (methyl deca-4,8-dienoate) considerably greater than peak 35 (methyl geranate); peak 50b (geranyl propionate) greater or approximately equal to peak 52 (Santalene type sesquiterpene); peak 39 (geranyl acetate) greater or approximately equal to peak 34 (methyl deca-4,8-dienoate).

The Brewers Gold variety may be distinguished from the Bullion by the following conditions: peak 35 (methyl geranate) greater or approximately equal to peak 34 (methyl deca-4,8-dienoate); peak 52 (Santalene type sesquiterpene) usually considerably greater than peak 50b (geranyl propionate); peak 34 (methyl deca-4,8-dienoate) considerably greater than peak 39 (geranyl acetate).

Table I. Relative Percentages of Components Found in the Steam Volatile Oil from American Grown Hop Varieties

Peak No.	Identity of Constituent	Early Cluster			Late Cluster			Brewers Gold			Bullion		Fuggle			Talisman 151
		Seedless Idaho	Seedless Wash.	Seeded Oregon	Seedless Idaho	Seedless Wash.	Seeded Oregon	Seedless Wash.	Seedless Oregon	Seeded Oregon	Seedless Oregon	Seeded Oregon	Seedless Wash.	Seedless Oregon	Seeded Oregon	
1	2-Methylpropyl isobutyrate	0.92	0.31	0.8	0.83	0.41	0.16	0.35	0.29	0.46	0.47	0.5	0.36	0.34	0.47	0.21
2	α-Pinene	0.17	0.09	0.1	0.11	0.07	0.04	0.18	0.13	0.2	0.21	0.17	0.02	0.05	0.1	0.15
3	2-Methylbutyl propionate	1	0.47	0.51	0.67	0.39	0.15	0.19	0.29	0.3	0.47	0.43	0.13	0.12	0.3	0.4
4	β-Pinene	1.2	0.74	1.1	0.94	0.86	0.65	0.99	1	1.2	0.89	1.1	0.51	0.68	0.67	1.2
6	Myrcene	58	41	62	59	51	28	61	62	72	61	69	44	58	47	59
7	3-Methylbutyl isobutyrate	0.4	0.30	0.71	0.32	0.46	0.57	0.18	0.18	0.22	0.25	0.26	0.14	0.09	0.15	0.19
8	2-Methylbutyl isobutyrate	4.4	1.9	3.5	3.7	2.6	1.4	1.4	2	2.3	1.5	1.9	1.3	0.95	1.1	2.6
9	Methyl heptanoate + methyl 4-methylhex-2-enoate	0.55	0.39	0.48	0.39	0.46	0.48	0.42	0.32	0.33	0.5	0.5	0.38	0.48	0.47	0.45
10	Limonene	0.23	0.16	0.33	0.27	0.21	0.1	0.31	0.29	0.33	0.27	0.31	0.33	0.44	0.41	0.23
10a	Terpene[a]	0.41	0.42	0.39	0.38	0.31	0.28	0.37	0.33	0.42	0.28	0.39				0.43
11	Terpene[a]	0.08	0.04	0.09	0.03	0.03		0.08	0.05	0.12	0.04	0.09	0.005	0.02	0.05	0 07
12	Ocimene + pentyl isobutyrate	0.35	0.23	0.66	0.50	0.38	0.15	0.80	0.55	1.1	0.95	1.2	0.3	0.2	0.27	1.6
13 14	Methyl thiohexanoate Methyl isooctanoate	0.46	0.53	0.72	0.52	0.67	0.75	0.32	0.27	0.33	0.42	0.27	0.31	0.2	0.27	0.54
16	Hexyl propionate	0.2	0.13	0.25	0.19	0.15	0.38	0.15	0.16	0.2	0.2	0.21	0.25	0.15	0.17	0.25
18a	Linalool	0.4	0.18	0.38	0.23	0.21	0.15	0.45	0.4	0.6	0.48	0.65	0.34	0.53	0.5	0.23
19	Methyl octanoate	0.81	0.67	1.2	0.61	1.1	0.97	0.33	0.35	0.45	0.28	0.3	0.26	0.18	0.27	0.9
20	Hexyl isobutyrate	0.01	0.03	0.01	0.02	0.01	0.01	0.09	0.01		0.08	0.08	0.03	0.03	0.02	
21	Methyl thioheptanoate	0.12	0.2	0.15	0.09	0.15	0.25									
22	Oxygenated terpene[a]	0.04	0.04	0.09	0.02	0.05	0.06				0.03	0.04	0.22	0.06	0.1	0.2
23	Heptyl propionate	0.08			0.02	0.05	0.05	0.09			0.04	0.09	0.01	0.04	0.04	
24	Octyl acetate + methyl nonenoate[b]	0.13	0.15	0.15	0.1	0.1	0.22	0.08	0.01		0.07	0.08	0.2	0.12	0.12	0.12
25	Methyl nonanoate	0.32	0.34	0.35	0.3	0.27	0.34	0.17	0.2	0.11	0.28	0.23	0.19	0.12	0.15	0.48
26	Heptyl isobutyrate												0.05	0.1		
28	2-Undecanone (branched)	0.04	0.19	0.11	0.04	0.07	0.2	0.03				0.04	0.25	0.02	0.06	0.1
29	M.W. 204[a]		0.21	0.08	0.05	0.09	0.26	0.04				0.04	0.24	0.04	0.08	0.1
30	Methyl decanoate (branched)[b]	0.28	0.25	0.39	0.23	0.34	0.49	0.04	0.06	0.02	0.08	0.09	0.01		0.01	0.21
31	2-Undecanone	0.6	0.64	0.55	0.46	0.74	1.1	0.14	0.08	0.02	0.12	0.13	0.68	0.3	0.31	0.63
33	Methyl dec-4-enoate	2.0	2.0	2.5	1.9	4.5	4.5	0.68	0.7	0.59	0.77	0.63	0.72	0.51	0.51	1.9
34	Methyl deca-4,8-dienoate	1.8	1.3	1.9	1.9	2.1	2.4	0.68	0.85	0.68	0.59	0.43	0.76	0.75	0.61	1.5
35	Methyl geranate							0.58	1.5	1.2	0.12	0.15	0.2	0.2	0.33	0.12
36	Methyl decanoate	1.2	0.7	1.3	1.1	1.4	1.4	0.25			0.14	0.18				1.05

37	Octyl isobutyrate			0.09								0.01	0.02			
38	Neryl acetate		0.06				0.15									
39	Geranyl acetate		0.02			0.02		0.03	0.01	0.08	0.93	1.4				
40	Sesquiterpene	0.01	0.07	0.03			0.07	0.16	0.07		0.02	0.03	0.05	0.03	0.1	0.1
41	Copaene	0.12	0.34	0.12	0.11	0.16	0.31	0.7	0.2	0.1	0.14	0.13	0.35	0.15	0.35	0.44
42	Methyl 9-methyldecanoate[b]	0.22	0.38	0.39	0.17	0.27	0.42				0.03	0.03		0.02		
43	.....	0.09	0.05	0.1	0.06	0.15	0.12	0.03				0.07				
													0.1			0.3
45	Methyl undecenoate[b]		0.05	0.03									0.01	0.02		0.03
47	Caryophyllene	3.6	8.4	4	4.4	5.1	8.9	6.2	6.5	3.9	6.7	4.5	8.2	6.2	8.2	2.
48	Sesquiterpene[a]	0.26	0.4	0.29	0.37	0.45	0.76	0.5	0.55	0.09	0.2	0.2	0.3	0.2	0.5	0.65
50	Humulene	8.8	21	9.8	11	12.5	24	10.3	11	6.2	12	7.2	30	22	26	3.6
50a	Farnesene	0.15		0.29	0.3	0.43	0.88	0.57	0.37				0.25	0.2	0.38	0.24
50b	Geranyl propionate		0.5			0.15				0.21	0.54	0.74				
51	Neryl isobutyrate															
52	Sesquiterpene (Santalene type)	0.2	0.85	0.42	0.45	0.42	0.76	0.64	0.64	0.3	0.6	0.44	0.74	0.5	0.85	1.5
54	β-Selinene	0.32	0.9	0.49	0.49	0.55	0.86	0.58	0.66	0.24	0.57	0.51	0.25	0.15	0.29	0.1
56	α-Selinene	0.35	1	0.38	0.33	0.51	0.98	0.7	1.2	0.32	0.6	0.55	0.2	0.25	0.48	0.55
56a	2-Tridecanone	1	1.7	1.5	1.2	1.6	1.5	0.4		0.2	0.48	0.24	1	0.6	1.3	1.8
57	Geranyl isobutyrate	1	1.4	1	1.2	1	1.2	1.1	0.89	0.32	0.88	1.4	1.2	1.0	1.5	1
58	γ-Cadinene									0.24	0.6					1.5
60	δ-Cadinene	0.67	1.6	0.7	0.9	0.95	1.4	0.98	0.92	0.52	0.8	0.7	1.3	1.1	1.5	2.4
61	Selinene No. 3	0.02	0.21	0.1	0.05	0.13	0.2	0.17	0.15	0.03	0.12	0.05	0.13	0.08	0.23	0.31
62	Selinene No. 4	0.06	0.25	0.13	0.06	0.2	0.3	0.3	0.19	0.03	0.1	0.12	0.18	0.1	0.3	0.5
63	2-Tetradecanone (branched)[b]	0.09	0.28	0.2	0.07	0.14	0.3	0.04				0.05		0.02		0.15
64	Tetradec-9-en-2-one[b]	0.1	0.9	0.3	0.08	0.22	0.03				0.28	0.46				0.26
64a	Sesquiterpene	0.65			0.03	0.2	0.74	0.88	0.7	0.26	0.4	0.3	0.32	0.15	0.4	0.4
65	Terpene ester[a]	0.2	0.03	0.04	0.04	0.22	0.4									
70	Humulene epoxide + 2-tetradecanone	1.5	0.47	0.11	0.09	0.38	2.0	0.05	0.1	0.03	0.28	0.28	0.28	0.08	0.2	0.8
70a	Methyl tridecenoate		0.4		0.1	0.25		0.3	0.4	0.13	0.32	0.18	0.25	0.13	0.3	0.1
71a	Oxy. sesquiterpene M.W. 222[a]		0.05													
73	.....	0.09	0.31	0.06		0.23	0.4	0.11	0.2		0.2	0.12	0.2	0.09	0.3	0.45
74	Pentadeca-6,9-dien-2-one + others	0.43	0.95	0.54	0.42	0.74	1.4	0.45	0.3	0.05	0.35	0.31	0.39	0.14	0.32	0.94
75		0.3	0.52	0.42	0.36	0.57	0.83	0.14		0.01	0.25	0.11	0.23	0.12	0.22	0.72
76	.....	0.15	0.3	0.15	0.1	0.25	0.5	0.15	0.18	0.02	0.1	0.11	0.24	0.05	0.14	0.4

[a] Identity more tentative or unknown.
[b] Exact identity tentative.

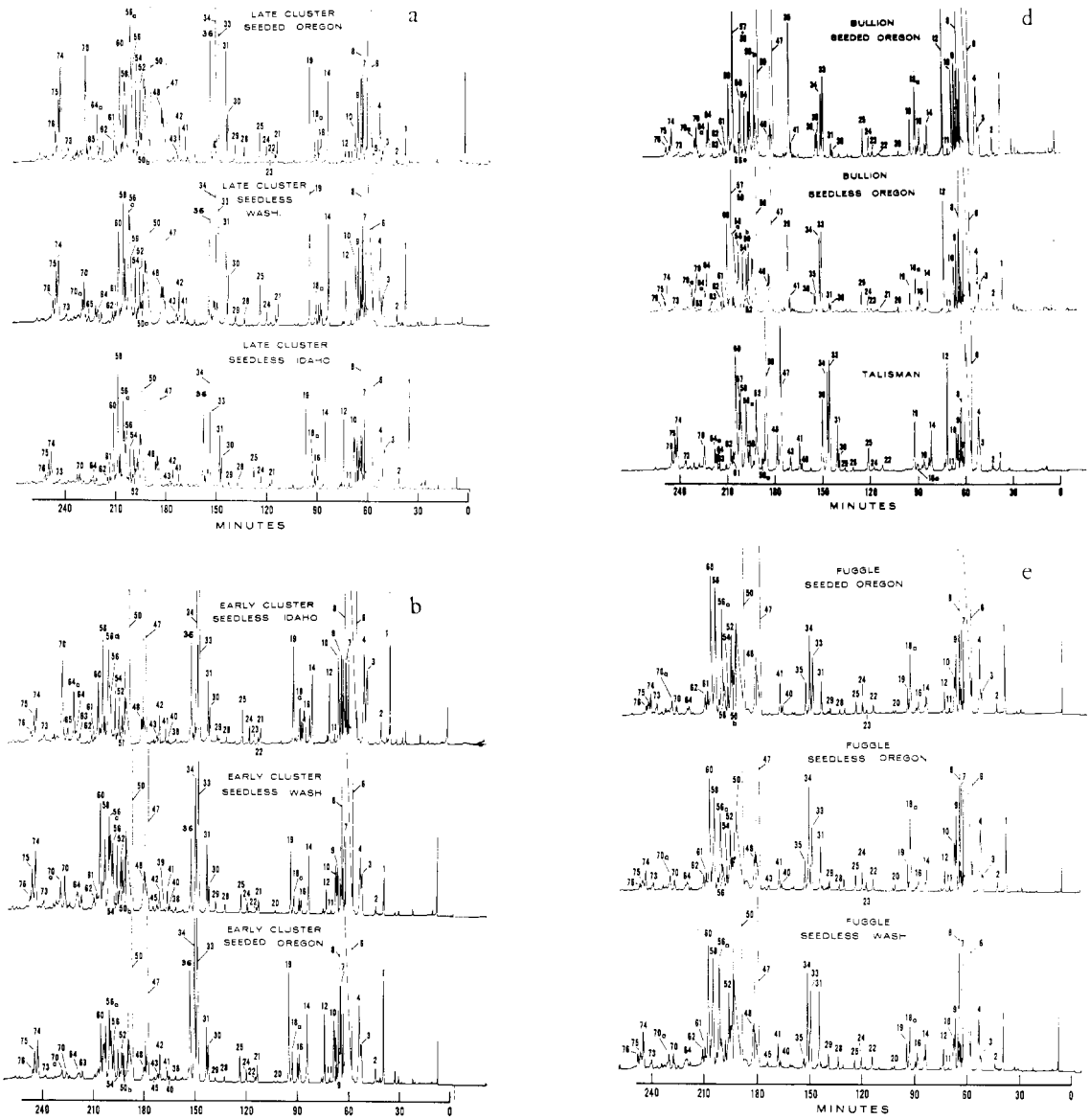

Figure 1. Capillary gas-liquid chromatography patterns of samples of hop oils from varieties indicated in the figures

Capillary gas-liquid chromatography conditions used are described in the text

Group II contained the varieties Cluster (Early and Late), Fuggle, and Talisman. The requirements for group II are as follows: peak 41 (copaene) considerably greater than peak 39 (geranyl acetate); peak 31 (2-undecanone) greater than peak 25 (methyl nonanoate); peak 56a (2-tridecanone plus others) greater or approximately equal to peak 52 (Santalene-type sesquiterpene).

The Cluster variety may be distinguished from the Fuggle and Talisman by the following conditions: peak 30 (methyl branched decanoate) is greater or approximately equal to peak 41 (copaene); peak 42 (methyl 9-methyldecanoate) is greater or approximately equal to peak 41 (copaene); peak 54 (β-selinene) is approximately equal to peak 52 (Santalene-type sesquiterpene).

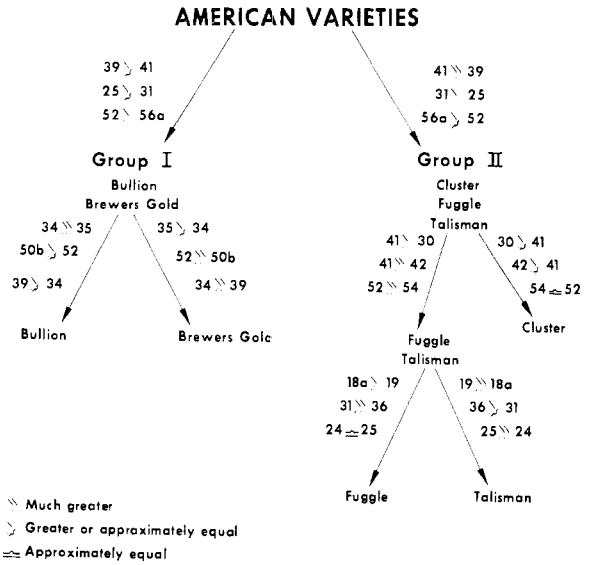

Figure 2. A scheme for differentiating American hop varieties according to the capillary GLC pattern of the hop oil shown diagrammatically

The Fuggle and Talisman varieties may be distinguished from the Cluster by the following conditions: Peak 41 (copaene) is greater than peak 30 (methyl branched decanoate); peak 41 (copaene) is considerably greater than peak 42 (methyl 9-methyldecanoate); peak 52 (Santalene-type sesquiterpene) is considerably greater than peak 54 (β-selinene).

The Fuggle variety may be distinguished from Talisman by the following conditions; Peak 18a (linalool) greater than peak 19 (methyloctanoate); peak 31 (2-undecanone) much greater than peak 36 (methyldecanoate); peak 24 (octyl acetate and methyl nonenoate) approximately equal to peak 25 (methyl nonanoate).

The Talisman variety may be distinguished from the Fuggle by the following conditions: Peak 19 (methyl octanoate) considerably greater than peak 18a (linalool); peak 36 (methyl decanoate) greater or approximately equal to peak 31 (2-undecanone); peak 25 (methyl nonanoate) considerably greater than peak 24 (octyl acetate and methyl nonenoate).

The capillary GLC analyses of hop oils could generally be carried out on a relatively routine daily basis. However, the capillary columns usually deteriorated giving lower efficiencies after about three months of continual use. This deterioration probably results from the fact that hop oil usually contains about 1% of the high boiling bitter constituents of hops which steam distill to some extent with the oil. The normal injection of hop oil naturally carries some of the high boiling material onto the capillary resulting in its eventual deterioration. The capillary columns therefore usually required replacement or recoating about once every three months of continued use.

A preliminary study of some samples of European varieties (Hallertau, Spalt, Tettnang, Styrian, Gebirg), of Australian varieties (Ringwood Special, Golden Cluster, Late Grape), and of a Japanese variety (Shinshuwase) indicates that these can also be readily identified and classified according to the capillary GLC pattern of their steam volatile oils.

Acknowledgment

We thank the U. S. Brewers Association Hop Committee for their financial support and guidance under U.S.B.A. Project No. 14. We also thank Sam T. Likens and Gail B. Nickerson for hop oil samples, Stan Brooks, H. K. Burr, and Robert G. Wright for helpful advice.

Literature Cited

(1) Buttery, R. G., Black, D. R., Guadagni, D. G., Kealy, M. P., *Am. Soc. Brewing Chemists Proc.* **1965,** pp. 103–11.
(2) Buttery, R. G., Black, D. R., Kealy, M. P., *Chromatog.* **18,** 399 (1965).
(3) Buttery, R. G., Lundin, R. E., Ling, L. C., *Chem. & Ind. (London)* **1966,** p. 1225.
(4) Likens, S. T., Nickerson, G. B., J. AGR. FOOD CHEM. **15,** 525 (1967).

Received for review October 6, 1966. Accepted January 13, 1967. Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.