Varietal Discrimination of Hop Pellets by Essential Oil Analysis I. Comparison of Fresh Samples

Philippe Perpète, Laurent Mélotte, Stéphane Dupire & Sonia Collin

To cite this article: Philippe Perpète, Laurent Mélotte, Stéphane Dupire & Sonia Collin (1998) Varietal Discrimination of Hop Pellets by Essential Oil Analysis I. Comparison of Fresh Samples, Journal of the American Society of Brewing Chemists, 56:3, 104-108

To link to this article: https://doi.org/10.1094/ASBCJ-56-0104

Published online: 06 Feb 2018.

Submit your article to this journal

Article views: 2

View related articles

Citing articles: 12
Variatel Discrimination of Hop Pellets by Essential Oil Analysis
I. Comparison of Fresh Samples

Philippe Perpète, Laboratoire de Brasserie et des Industries Alimentaires, Université Catholique de Louvain, Place Croix du Sud, 2 bte 7, B-1348 Louvain-la-Neuve, Belgium; Laurent Mélotte and Stéphane Dupire, INTERBREW, Vaarstraat 137, B-3000 Leuven, Belgium; and Sonia Collin, Laboratoire de Brasserie et des Industries Alimentaires, Louvain-la-Neuve

ABSTRACT


The aim of this study was to differentiate hop pellets by essential oil analysis. Volatile compounds of five aromatic cultivars (Styrie, Saaz, Lublin, Mount Hood, and Hallertau) and seven bitter cultivars (Northern Brewer, Nugget, Pride of Ringwood, Northdown, Galena, Target, and Challenger) were extracted with a Likens-Nickerson simultaneous solvent extractor. The extracts had a strong hop aroma that varied according to the type of hop. Approximately 100 compounds were separated by gas chromatography (GC) and identified by GC-mass spectrometry. An identification flowchart including seven terpenic compounds, four esters, and one methyl ketone was established to discriminate between fresh samples of the 12 investigated cultivars. High amounts of bergamotene and farnesene were found only in Saaz, Lublin, and Styrie samples. Quantification of 4-decenoic acid methyl ester and 3-methyl butyl isobutyrate proved a quick means of distinguishing non-European and European bitter hops from aromatic cultivars.

Keywords: 3-Methyl butyl isobutyrate, Bergamotene, Flavor, Hop aroma, Hop cultivars

RESUMEN

El propósito de este estudio fue diferenciar lúpulo en pellets por análisis de aceites esenciales. Compuestos volátiles de 5 variedades aromáticas (Styrie, Saaz, Lublin, Mount Hood, y Hallertau) y siete variedades amargas (Northern Brewer, Nugget, Pride of Ringwood, Northdown, Galena, Target, y Challenger) fueron extraídas con el extractor simultáneo de solventes Likens-Nickerson. Los extractos tuvieron un fuerte aroma a lúpulo, variando de acuerdo al tipo de lúpulo. Casi cien compuestos fueron separados por cromatografía de gases e identificados por espectrometría de masas. Un diagrama de flujo de identificación incluyendo 7 compuestos terpénicos, 4 ésteres, y una metilcetona fueron establecidas para discriminar entre muestras frescas de las 12 variedades investigadas. Altas cantidades de bergamoteno y farneseno fueron encontradas solamente en muestras de Saaz, Lublin, y Styrie. La cuantificación del éster metilico del ácido 4-decenico y del isobutirato de 3-metilbutil proveen un medio rápido para distinguir lúpulos amargos europeos y no-europeos de variedades aromáticas.

It is now well established that the composition of hop oil, and the hop flavor derived therefrom, in beer depend on the hop cultivar. Many works authenticated hop oils by their flavor constituents (2, 4–6), but rarely by using cones (11) or pellet samples (14), the methods used in the present work. Several hop cultivars were compared: three European aroma hops, usually selected to impart the “noble” hop aroma; two other low-bitterness cultivars; plus four European and three non-European bitter cultivars. Three consecutive crops (1994–1996) were investigated for most of the cultivars (Table I), even though many authors (17) indicate good varietal uniformity of composition over a range of environmental conditions. The relationship between hop aroma and the aromatic compounds organoleptically active in beer is not known. Most hop oil constituents are not recovered in beer due to oxidation, hydrolysis, transesterification, and reduction during boiling and fermentation. The “kettle hop” aroma is therefore quite different from the aroma of hop itself. Hydrolysis of humulene and caryophyllene epoxides (7,13) produces organoleptically active, though not too pleasant, products in beer (9). Other flavoring compounds are probably required to impart the pleasant “kettle hop” aroma. The lack of both qualitative and quantitative knowledge makes it hard to choose which hop to use. For this reason, our identification flowchart was established both to highlight the distinctive features of each hop cultivar and to point out any common features between the aromatic hops or the various bitter hops.

EXPERIMENTAL

Hop Samples

A total of 32 commercial samples were collected, including 12 cultivars. Samples of the cultivars Saaz, Lublin, and Hallertau were in T45 pellet form, while the other cultivars were in T90 pellet form. For a better comparison, all our data were calculated for T90 pellet conditioning. All samples (5 kg) were stored under nitrogen at 4°C in hermetic dark packing.

Hop Aroma Extraction

An optimized version (1) of the Likens and Nickerson (12) extraction procedure was used, allowing high recovery of most essential oils (>90%, except for humulene diepoxides, for which recovery was only 50%) and high reproducibility (variation coefficients ≤10%). The results presented here for each cultivar are the means of two different extractions.

Steam-distillation solvent-extraction was conducted in a microextractor to remove hop oil components. Ground pellets (0.5 g) were mixed with 1.5 ml of internal standard solution (20 ppm of carvone) in 50 ml of ultrapure deoxygenated water (Milli-Q water purifier, Millipore, Bedford, MA) and transferred to flask A (Fig. 1). Dichloromethane (1.5 ml) was transferred to flask B. Dichloromethane and ultrapure deoxygenated water (1.5 ml each) were introduced into area C using arm H. A few clean carbonum grains were successively introduced into flasks A and B. Prior to the procedure, the entire system was purged with nitrogen (2–3 ml/min) for 5 min. Flask A was then heated in a 140°C oil bath. After 3 min, flask B was heated in a 70°C water bath. The vapors were then condensed in area C by means of a cold finger maintained at –15°C by a cryostat. The entire steam-distillation solvent-extraction procedure was conducted under a 2 ml/min nitrogen flow. The steam-distillation was stopped after 45 min and 2 ml of dichloromethane extract was removed from flask B. The dichloromethane layer in area C was then collected in flask B; flask B was finally washed with 1 ml of 4x dichloromethane. The extract was then concentrated to 1 ml in a Danish-Kuderna column, and 1 ml was analyzed by gas chromatography (GC) and GC-mass spectroscopy (MS).

1 Corresponding author.

Publication no. J-1998-0921-05R.
© 1998 American Society of Brewing Chemists, Inc.
GC Analytical Conditions

For GC, we used an HP5890 gas chromatograph equipped with an HP7673 automatic sampler, a flame-ionization detector, and a Shimadzu CR4-A integrator. Hop oil components were analyzed on a wall-coated, open tubular CP-SIL5 CB capillary column (50 m × 0.32 mm; 1.2-µm film thickness) allowing separation in one run of ≈250 peaks. The oven temperature was set to rise from 36 to 85°C at 50°C/min, to 145°C at 1°C/min, to 250°C at 3°C/min, and then to remain constant at 250°C for 30 min. The injector temperature was set at 280°C. The detector temperature was 280°C. The carrier gas was helium at a flow rate of 1.1 ml/min.

GC-MS Analytical Conditions

The column was directly connected to an HP5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV. Spectral recording throughout elution was automatically performed with the HP59970C software. Peaks were identified by their enhancement after co-injection of standard compounds and with the help of the NBS/EPA/NIH mass spectra library.

RESULTS AND DISCUSSION

Gas chromatography of 32 hop samples (in duplicate) yielded typical fingerprints for each cultivar, although unexplained varia-

<table>
<thead>
<tr>
<th>Cultivar, Year</th>
<th>Compounds*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-European bitter hops</td>
<td></td>
</tr>
<tr>
<td>Pride of Ringwood (T90) 94</td>
<td>9.5...[h] 129 144 1 155 59 ... 8 134 186 65 787 823 16</td>
</tr>
<tr>
<td>Pride of Ringwood (T90) 95</td>
<td>9.2 3 16 173 205 14 1 35 85 105 38 669 669 13</td>
</tr>
<tr>
<td>Pride of Ringwood (T90) 96</td>
<td>7.6 9 49 150 ... 204 24 ... 18 81 178 723 721 13</td>
</tr>
<tr>
<td>Nugget (T90) 94</td>
<td>12.5 83 176 71 77 244 27 ... 21 3,268 86 73 173 170 143</td>
</tr>
<tr>
<td>Nugget (T90) 95</td>
<td>12.6 135 297 78 171 510 23 ... 75 3,858 128 97 273 294 88</td>
</tr>
<tr>
<td>Nugget (T90) 96</td>
<td>11.5 55 108 72 72 211 13 ... 20 2,378 156 60 246 263 32</td>
</tr>
<tr>
<td>Galena (T90) 94</td>
<td>11.7 97 407 72 4 447 17 ... ... 1,849 80 392 148 100 133</td>
</tr>
</tbody>
</table>

European bitter hops

Northern Brewer (T90) 94 | 10.3 24 62 69 2 58 21 ... ... 10 4,996 27 39 71 ... 368 |
| Northern Brewer (T90) 94 | 10.3 15 75 76 2 45 19 ... ... 10 3,357 41 38 44 ... 203 |
| Northern Brewer (T90) 95 | 5.9 30 188 ... 43 ... 8 3,296 96 23 70 70 187 |
| Northern Brewer (T90) 96 | 5.2 11 58 65 ... 11 29 ... 9 2,248 90 9 56 63 265 |
| Northdown (T90) 95 | 6.2 50 180 79 ... 20 76 1 119 3,508 184 7 551 584 257 |
| Northdown (T90) 96 | 6.4 42 150 65 2 19 65 ... 132 2,094 184 7 519 563 208 |
| Target (T90) 94 | 9.9 14 58 153 4 113 16 ... 8 1,470 110 54 114 114 113 |
| Target (T90) 95 | 10.3 40 233 147 12 28 53 ... 19 1,394 110 54 114 114 113 |
| Target (T90) 96 | 8.3 19 91 162 7 43 25 ... 26 1,439 148 27 129 125 101 |
| Challenger (T90) 95 | 6.2 30 120 127 ... 45 13 4 85 1,605 84 25 379 399 184 |
| Challenger (T90) 96 | 5.4 22 92 98 ... 26 10 ... 52 1,332 96 16 356 380 240 |

Low-bitter hops

Saaz (T45) 94 | 3.2 ... ... 41 2 47 7 38 203 1,066 28 21 16 ... 128 |
| Saaz (T45) 94 | 3.4 ... 38 5 11 11 40 150 711 13 13 13 ... 241 |
| Saaz (T45) 95 | 2.6 ... 53 2 61 4 49 861 990 26 24 15 20 81 |
| Saaz (T45) 96 | 2.6 ... 45 3 36 4 54 727 833 28 20 12 18 87 |
| Lublin (T45) 94 | 3.2 ... 30 2 47 5 42 590 1,015 29 19 23 ... 42 |
| Lublin (T45) 95 | 3.2 2 18 43 3 31 14 50 1,055 1,176 39 23 39 42 53 |
| Lublin (T45) 96 | 3.2 ... 3 41 ... 23 9 74 688 976 40 16 37 48 129 |
| Styrie (T90) 94 | 4.7 ... 15 53 2 23 40 25 183 1,390 21 18 37 ... 123 |
| Styrie (T90) 95 | 4.9 9 79 133 1 40 95 45 618 1,626 54 30 114 110 171 |
| Styrie (T90) 96 | 4.0 7 87 97 9 23 98 53 980 1,414 48 22 25 14 167 |
| Mount Hood (T90) 94 | 4.2 10 28 55 2 139 32 ... 76 3,589 239 22 83 96 332 |
| Mount Hood (T90) 95 | 4.3 10 30 58 ... 97 30 ... 28 2,677 202 17 63 95 703 |
| Hallertau (T45) 94 | 3.1 4 45 65 6 44 7 ... 10 2,101 81 12 58 72 366 |
| Hallertau (T45) 95 | 2.8 4 29 39 6 24 6 ... 18 1,218 72 9 48 55 206 |

* All concentrations are calculated for T90 conditioning; * = carvone equivalent for quantification; ** = caryophyllene equivalent for quantification.

a-β-Acids.

3-Methyl butyl isobutyrate; numbering, 12; identification, gas chromatography (GC)-mass spectroscopy (MS); ret. index (CPSil5- CB) 973.

2-Methyl butyl isobutyrate; 13; GC-MS; index 977.

2-Undecanone; 45; GC-MS; index 1301.

Unknown 46; 46/mz 57, 75, 83, 69; index 1317.

4-Decenoic acid, methyl ester; 47; MS; index 1322.

Methyl geranate; 79; GC-MS; index 1337.

Bergamotene; 64; MS; index 1337.

β-Farnesene; 65; MS; index 1463.

α-Humulene; 67; GC-MS; index 1480.

α-Amorphene; 69; MS; index 1497.

3,6-Dodecanoic acid, methyl ester; 70; MS; index 1505.

β-Selinene; 71; MS; index 1515.

α-Selinene; 74; MS; index 1528.

Humulene epoxyde II; 85; GC-MS; index 1639.

Not detected or detected but not quantified.
tions are measured in a few cases between successive crops (e.g., farnesene, 3-methyl butyl isobutyrate, 2-methyl butyl isobutyrate). As previously shown by Krajl et al (6), the variability of the aroma composition among samples, as well as years, can be large; therefore, an analysis based on only one year’s production seems insufficient. In order to facilitate the authentication of aged samples, additional information about the stability of all our markers will be soon published. Figure 2 shows the chromatograms obtained for cvs. Saaz 95 and Nugget 95 hops. Peak identification and concentration values of the relevant compounds are summarized in Table I.

Only a few peaks required quantification to authenticate our 12 hop cultivars (Fig. 3). However, all discriminant compounds could be helpful in detecting when hop cultivars have been mixed prior to pelleting. Three major groups can be defined depending on whether the 4-decenoic acid methyl ester concentration is above or below 150 ppm (carvone equivalent) and the 3-methyl butyl isobutyrate concentration above or below 11 ppm (carvone equivalent). Surprisingly, factor analysis of sensory data also led Peppard et al (15) to group hops into three broad categories: the first including cvs. Hallertau and Saaz, the second cv. Northern Brewer, and the third cv. Galena.

The three non-European bitter cultivars (Pride of Ringwood, Nugget, and Galena) are characterized by higher concentrations of 4-decenoic acid methyl ester (>150 ppm, carvone equivalent). Relatively high concentrations (97–139 ppm) are also measured in cv. Mount Hood, the sole non-European aromatic cultivar investigated here. Nickerson and Likens (12) suggest that this compound is transesterified to the corresponding ethyl ester during fermentation. The three Pride of Ringwood samples are easily distinguishable from all nine other cultivars by their very low concentrations (<150 ppm) of α-humulene and its main epoxide, humulene epoxide II (<100 ppm, caryophyllene equivalent). High concentrations of α and β selenes (>650 ppm, caryophyllene equivalent) further characterize Pride of Ringwood hop. Identification of these two selinadienes was based on the mass spectra published by Davies and Menary (3). These authors also detected higher levels of these compounds in Pride of Ringwood samples than in cvs. Northern Brewer, Brewers’ Gold, Bullion, and Cluster. More than 25 ppm (carvone equivalent) of an unidentified compound (retention index = 1317) distinguished our three Nugget samples. Although only one Galena hop was analyzed, our data suggest that high levels of 3,6-dodecadienoic acid methyl ester (392 ppm as opposed to <100 ppm, carvone equivalent) could help to recognize this cultivar.

Higher amounts of 3-methyl butyl isobutyrate are found in almost all European bitter hops, while concentrations of <11 ppm are found in all five low-bitterness cultivars. Except in two samples of cv. Styrie, a 2-methyl butyl isobutyrate level of <50 ppm could provide additional proof. Particularly high amounts of both isobutyrates are evidenced in the two α-rich cultivars, Nugget and Galena, suggesting that such compounds could be from α-acids. Seaton et al (16) have shown that the hop fraction containing myrcene and 2-methyl butyl isobutyrate possess the major part of the “resinous” and “fruity/estery,” citrus-like character of the “hop loft” aroma. However, such compounds could be unstable during boiling and fermentation. According to Seaton et al (16), 2-methyl butyl isobutyrate was identified in a pilot-scale pale ale when late hopping was carried out with Aurora liquid CO2 extract. Murakami et al (10) also detected higher levels of isoamyl isobutyrate in Galena-brewed than in Hallertau-brewed beers. Experiments in our laboratory have confirmed that residual 2-methyl and 3-methyl butyl isobutyrate are present in beers when late hopping is carried out with Northern Brewer or Nugget hops, but absent when the Saaz cultivar or no hop is used.

In the five low-bitterness cultivars, bergamotene (>10 ppm, caryophyllene equivalent) and farnesene (>150 ppm, caryophyllene equivalent) appear as key markers for identifying the most pleasant aromatic cultivars: Saaz, Lublin, and Styrie. Peacock and McCarty (14) also categorized hops in two groups according to the farnesene content: high-farnesene cultivars included Saaz, Willamette, and Cascade; low-farnesene cultivars included Hersbrucker, Cluster, Hallertau, and most of the high α-acid cultivars. Due to its higher stability, bergamotene quantification should be preferred when hop freshness is unknown. As previously shown for humulenes (13), sesquiterpenes are oxidized and hydrolyzed during boiling. This suggests that a sensory investigation of bergamotene and farnesene degradation products should be conducted soon.

Methyl geranate emerges here as a reliable indicator for distinguishing Styrie (>20 ppm, caryophyllene equivalent) from Saaz and Lublin. Of all the relevant esters, methyl geranate is probably the most stable due to the presence of an α,β-unsaturation. No significant difference has been found between Lublin and Saaz, which are in fact the same cultivar grown at different geographical sites (14).

In the “European bitter hops” group, Northdown and Challenger are characterized by large amounts of α and β selenes (>200 ppm, caryophyllene equivalent), as compared to Target and Northern Brewer. Northdown is distinguishable from Challenger by the concentration of a sesquiterpene, which could be α-amor-

---

**Fig. 1.** Microextractor used for simultaneous steam-distillation solvent-extraction.
phene (>125 ppm, caryophyllene equivalent). The same terpene also occurs as a significant constituent distinguishing Mount Hood (>125 ppm, caryophyllene equivalent) from Hallertau in the “low bitterness” group. Perhaps the most interesting marker of Northern Brewer hops emerging from our study is the humulene-to-farnesene ratio (>249); all other cultivars show a ratio <220. The comparison between Northern Brewer and the low-humulene Target samples is even more relevant. An additional marker for distinguishing Target (>100 ppm) from Northern Brewer hops is 2-undecanone. High 2-undecanone concentrations can also occur in Pride of Ringwood samples. This methylketone could give rise to an organoleptically active reduced compound in beer, the 2-undecanol flavor threshold being estimated at 70 ppb in beer (8).

Obviously, a great deal of research is still required to obtain the full picture of hop-derived compounds. As cultivar markers emerge, this should encourage researchers to focus on the fate of such compounds through boiling and fermentation.

ACKNOWLEDGMENTS
We thank the Interbrew Baillet Latour Foundation for financial support.

LITERATURE CITED

8. Meilgaard, M. C. Flavor chemistry of beer. II: Flavor and threshold

[Received February 12, 1998. Accepted June 3, 1998.]