Hop Flavor Constituents in Beer by Headspace Analysis[1]

Aki Murakami, Etzer Chicoye, and Henry Goldstein, *Miller Brewing Company, Technical Center, 3939 West Highland Boulevard, Milwaukee, WI 53201*

ABSTRACT

The purpose of this research was to identify important hop-derived flavor compounds in beer. Unlike much previous work in this area, we used a recently developed headspace analysis method. Briefly, the method involves dynamic headspace displacement of beer volatiles onto a porous polymer trap, reconcentration in a cold trap, and direct transfer to a gas chromatographic capillary column for separation. Detection was by flame ionization detector (FID), flame photometric detector (FPD), and mass spectrometry (MS). Striking differences were found in chromatograms of hopped and unhopped beers. Approximately 72 peaks were present in the chromatogram of hopped beer that were absent in the chromatogram of unhopped beer. These differences between hopped and unhopped beers were observed by FID, FPD, and MS. The selective detection of FPD, particularly, revealed the differences in sulfur-containing volatile compounds that otherwise would be obscured by interfering peaks in the FID and MS detections. The described technique promises to be extremely useful for identifying hop-derived compounds that could be important to hop aroma in beer.

Key words: Headspace analysis, Hop aroma, Hopped beer, Unhopped beer

Hop aroma found in beer is a unique flavor; it makes a most important contribution to beer flavor and can often be used to distinguish one beer from another. The hop aroma in beer is derived from hops, but for the most part its flavor is not produced directly from the unaltered hop flavoring ingredients. For example, unlike vanilla flavored ice cream where the flavor is obtained directly from the added vanilla, hop-derived aroma is the result of a complex combination involving various reaction products derived from the hops and is influenced by the method of hopping, fermentation, and other brewing processes.

Extensive research done in the field of hop aroma is well documented in recent reviews (13,16). However, in spite of numerous studies, hop aroma in beer has not been completely defined chemically indicating the elusiveness and complexity of this aroma. Sample preparation techniques for most previous studies have used traditional analytical methods such as vacuum distillation, liquid column chromatography (4), distillation and extraction (6), or combinations of these (1,2,7,9,10,15).

In this work, we took a completely different approach for investigating hop aroma in beer, using a recently developed, highly sensitive and reproducible headspace analysis method described in our earlier work (8). This method allowed us to analyze both hopped and unhopped beers to determine compounds uniquely present in the hopped beer. Previously, this kind of comparison study was almost impossible using traditional analytical methods. An earlier study (14) using a liquid extraction method showed only one compound unique to hopped beer. There are two principal advantages to the method used in the present study: first, unlike traditional analytical methods, the headspace method can be used to analyze volatile flavor compounds with very little compound alteration or degradation. The method minimizes changes in composition of volatiles and does not introduce solvent interferences. The beer volatiles collected by the headspace method are in a state closely resembling the condition in which one would experience the aroma and taste of beer, which is very important in any flavor research on beer. Secondly, comparative analysis of gas chromatographic (GC) profiles of hopped and unhopped beer is a more direct approach to obtain the information on hop aroma constituents when compared to studying hop oils or hopped beers. This is only possible because of the sensitivity and reproducibility of the analytical method (8).

In this study, headspace volatiles of specially brewed hopped and unhopped beers were analyzed by GC with a flame ionization detector (FID) to monitor the overall beer volatile profile. A flame photometric detector (FPD) was used to monitor the volatile sulfur compounds. A gas chromatograph/mass spectrometer/data system (GC/MS/DS) was used for compound identification.

EXPERIMENTAL

The headspace method we recently developed (8) was employed to analyze the hopped and unhopped beers. Briefly, the method consists of dynamic headspace collection of beer into a Tenax trap, transfer of collected volatiles from the Tenax trap to the head of a capillary column, followed by gas chromatographic separation.

Beer Samples

The hopped beer was a pilot-brewed lager. It was kettle hopped with a mixture of aroma hop pellets; the finished beer had a bitterness level of 23 BU. The unhopped beer was similarly prepared without any hopping. Each beer was brewed in a 38-L lot. Both beers were packaged in 12-oz. bottles and stored at 5° C until analyzed. The hopped beer had a hoppy aroma and taste, while the unhopped beer had neither of these characteristics.

Equipment and Procedure

The headspace equipment and procedure were described elsewhere in detail (8). This procedure was followed with several changes: A 4-L vessel (Tekmar Inc., Cincinnati, OH) was fitted with a cap assembly similar to the one used in the 500-ml Pyrex gas washing bottle (Corning no. 31770C). The cap assembly combined the gas inlet and outlet in one ground glass fitting instead of the original separate inlet and outlet configuration. This design eliminated possible gas leaks during purging. In addition to other vessels used in this study, a 1-L version (Tekmar), similarly modified, was used for headspace collection.

The secondary trap heater on the model 1000 Capillary Interface Instrument (Tekmar), which automatically activates after the primary heater to condition the trap, was modified to heat for a shorter time at higher temperature by making internal adjustments to the interface's control module. Also, the primary heater time was reduced from 17 to 13 sec. This modification allowed the cold trap to be heated from liquid nitrogen temperature to ~300° C in 13 sec (primary heater); the trap was then maintained at ~240–280° C for 3 min (secondary heater). Before modification, the cold trap was heated from liquid nitrogen temperature to ~300° C in 17 sec and maintained at ~100–200° C for 10 min. This modification gave much better recovery of compounds of low volatility.

Conditions for GC/FID analysis were identical to those described in earlier work (8). Two different conditions were used for the GC/FPD organo-sulfur compound analysis. To collect highly volatile sulfur compounds, 355 ml of beer was purged in the 1-L vessel for 5 min; to collect sulfur compounds of mid to low volatility, the beer was purged for 20 min. A Tenax trap was used for both collections.

GC/MS Analysis

Headspace equipment similar but not identical to that described (8) for the GC was built for the Finnigan 4021 GC/MS/DS. For electron impact GC/MS analysis, the beer volatiles were collected

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on the Tenax trap by purging 355 ml of beer in the 1-L vessel for 40 min. For chemical ionization GC/MS analysis, the volatiles were similarly collected for 80 min from 700 ml of beer in the 4-L vessel.

The volatiles were separated on an 89-m long, wide-bore, DB-1 fused silica capillary column (J&W Scientific, Inc., Rancho Cordova, CA). This column was made by connecting one 60-m and one 29-m column with a 1/32-in. Valco capillary column union (Valco Instruments, Houston, TX). Helium GC carrier gas was used with an average linear flow velocity of ~35 cm/sec. The GC oven temperature was programmed after 4 min at 40° C to 132° C at 2° C/min, then to 200° C at 20° C/min, and held at 200° C for 20 min.

For electron impact analysis, the electron multiplier voltage was −1,200 V, the electron energy was 72.5 eV, and emission current was −0.35 mA. The mass spectrometer was set to scan from m/z 35 to 350 in 1.95 sec with an 0.05-sec rest. For chemical ionization conditions, isobutane reagent gas was used. Under these conditions the mass spectrometer was set to scan from m/z 60 to 370 in 1.95 sec with a 0.05-sec rest. The ionizer temperature was 80° C, and pressure was 1.2×10^{-5} torr.

Compounds were identified by matching their spectra with the National Bureau of Standards Mass Spectra Library using the INCOS data system. Compound identification was also facilitated by use of a special mass spectra library containing hop and hop-derived compounds obtained from K. C. Lam of Oregon State University, Corvallis.

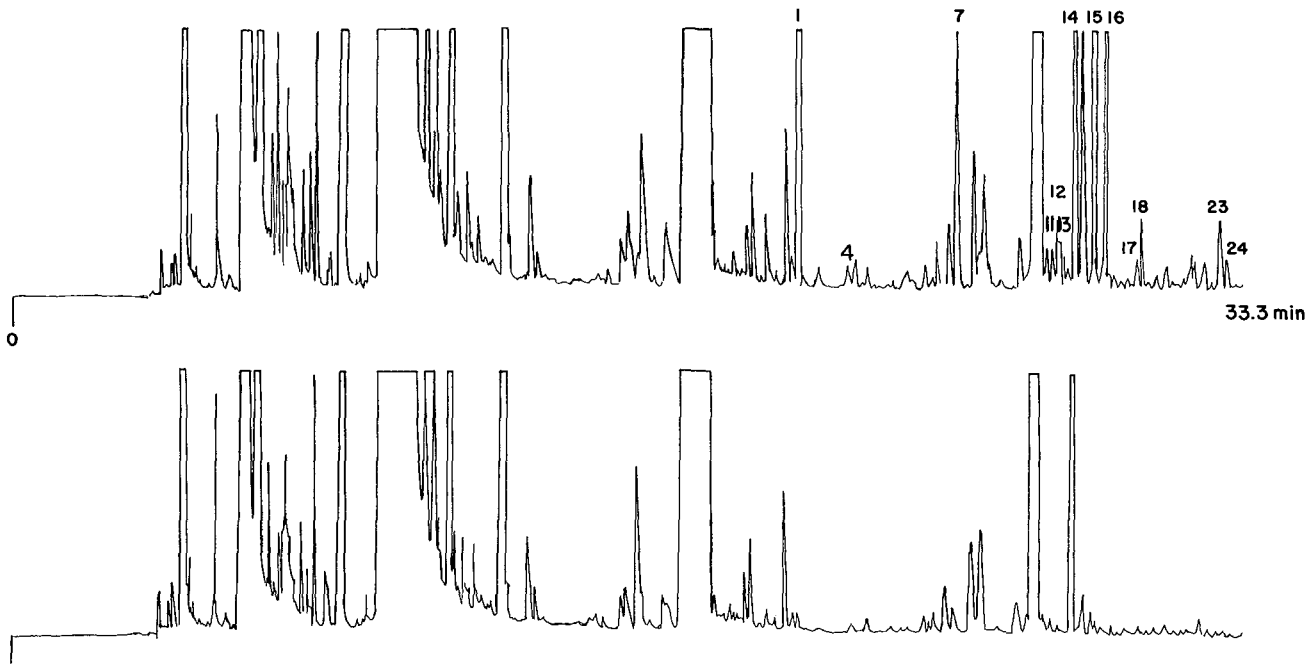

Fig. 1. Flame ionization detector headspace chromatograms of hopped beer (top) and unhopped beer (bottom) from 0 to 33.3 min.

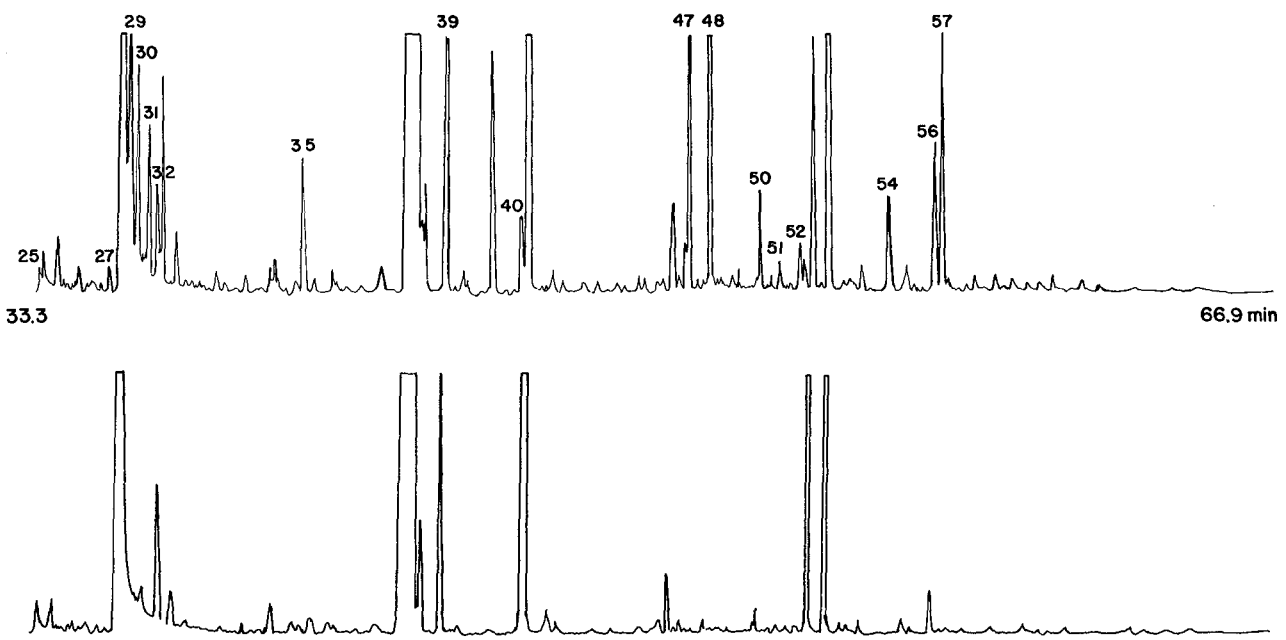

Fig. 2. Flame ionization detector headspace chromatograms of hopped beer (top) and unhopped beer (bottom) from 33.3 to 66.9 min.

RESULTS AND DISCUSSION

Chromatographic profiles of the hopped and unhopped beers were significantly different. A large number of peaks present in the hopped beer were not found in the unhopped beer. Very few peaks were found in unhopped beer only. This can be seen in Figures 1 and 2 in which the GC/FID chromatograms of hopped and unhopped beers were compared. No significant reproducible differences were observed between the two chromatograms during the first 20 min. The presence of large and medium size peaks unique to the hopped beer were determined from the GC/FID chromatograms; smaller peaks were determined by the GC/MS if their complete mass spectra were obtainable. Compounds of low volatility were more efficiently recovered when the GC/MS equipment was used; this resulted from differences in the headspace equipment.

A total of 72 peaks found exclusively in the hopped beer is compiled in Table I. Peak identifications are tentative, because no confirmations have been accomplished with authentic compounds. The same compound identification was assigned to more than one peak when the identification confidence levels were equally high for all applicable peaks identified by the GC/MS data system. This multiple identification might be caused by the presence of positional or stereoisomers of the same compound. Molecular weights are provided for most of the unidentified peaks. The compounds are listed in the order of elution. The GC/FID retention times are not listed for some of the smaller peaks whose presence was determined by the GC/MS analysis. Relative peak size information is provided. Compounds we believe are being reported for the first time in beer are marked with an asterisk. The GC/MS analysis revealed that for almost all large or medium size peaks unique to the hopped beer there were traces of corresponding peaks in the unhopped beer. This was probably caused by carryover of these compounds from the pitching yeast.

Differences in the beer volatile constituents should result from hopping. We propose that those compounds unique to the hopped beer may be partially if not entirely responsible for hop aroma formation. For example, nearly one third of the compounds in Table I are esters. Some of these shown as prominent peaks are isobutyl isobutyrate, amyl propionate, amyl butyrate, methyl 3-methyl-3-hexenoate, methyl geranate, and citronellyl acetate. Individually, these esters can provide fruity flavor notes that may not be related to hop aroma. Although their concentrations are yet to be determined, the esters, together, should contribute importantly to beer hop aroma, primarily because of their relatively high volatility. Other examples of compounds that may contribute to hop aroma are linalool and myrcene. Depending on their concentrations, these two compounds are capable of imparting flavor because of their low sensory threshold values (6). The flavor activities of some of the aforementioned esters were also reported by others. Moir et al (7) claimed that isobutyl isobutyrate and isoamyl isobutyrate, along with other compounds, are possible

TABLE I
Compounds Found Unique to Hopped Beer

Compound (tentative identification)[a]	Retention Time[b] (min)	Peak Size	Compound (tentative identification)[a]	Retention Time[b] (min)	Peak Size
1. Isobutyl isobutyrate	21.34	large	37. No id, MW = 180	···	very small
2. Myrcene	···	small	38. Citronellol	···	small
3. Methyl 3-methyl-2-methylene butanoate[c]	···	small	39. 1-*tert*-Butyl-4-ethoxybenzene[c]	45.74	medium
4. S-propyl pentanethioate[c]	22.71	small	40. 1-Methyl-2-pentylcyclopropane[c]	46.56	small
5. 1,4-Dimethyl-5-isopropyl-1-cyclopentene[c]	···	small	41. Ethyl nonanoate	···	very small
6. 2,4-Dimethyl-2,4-heptadienal[c]	···	small	42. Ethyl nonanoate	···	very small
7. Amyl propionate	25.63	large	43. 2-Undecanol	···	very small
8. Myrcene	···	small	44. Methyl geranate	···	very small
9. 7-Octen-4-ol	···	very small	45. No id, MW = 184	···	small
10. Myrcene	27.77	large	46. 2-Undecanone	···	very small
11. No id, MW = ?	28.08	small	47. No id, MW = 192	51.10	large
12. No id, MW = 158	28.37	small	48. Methyl geranate	51.69	large
13. Isobutyl 3-methylbutanoate	28.55	small	49. No id, MW = ?	···	very small
14. Amyl butyrate	29.06	large	50. Citronellyl acetate	53.03	small
15. Isoamyl isobutyrate	29.38	large	51. Neryl acetate	53.60	small
16. Methyl 3-methyl-3-hexenoate[c]	29.63	large	52. No id, MW = 198	54.15	small
17. β-Phellandrene[c]	30.54	small	53. No id, MW = 212	···	medium
18. Bornylene[c]	30.68	small	54. β-Caryophyllene	56.55	small
19. Pentyl isobutyrate	···	small	55. *sec*-Octyl acetate	···	small
20. No id, MW = 156	···	very small	56. 2,6-di-*tert*-Butylbenzoquinone[c]	57.80	medium
21. β-Ocimene[c]	···	small	57. α-Humulene	58.00	large
22. 5-Pentyloxy-1-pentene	···	small	58. γ-Muurolene[c]	···	medium
23. Ethyl heptanoate	32.81	small	59. No id, MW = 164	···	small
24. Methyl 2-methyl heptanoate[c]	33.01	small	60. Selinene[c]	···	small
25. Ethyl heptanoate	33.44	small	61. α-Copaene[c]	···	small
26. γ-terpinene[c]	···	small	62. Aromadendrene[c]	···	small
27. Ethyl heptanoate	35.33	small	63. γ-Muurolene[c]	···	small
28. Hop ether	···	small	64. No id, MW = 202	···	very small
29. Linalool	35.86	large	65. δ-Cadinene[c]	···	medium
30. Perillene[c]	36.05	medium	66. β-Guaiene[c]	···	very small
31. 2-Methylbutyl 2-methylbutanoate	36.37	medium	67. 1,2,3,4,6,8a-Hexahydro-1-isopropyl-4, 7-dimethyl-naphthalene[c]	···	very small
32. 2-Methylbutyl 3-methylbutanoate	36.62	small	68. Allo-aromadendrene[c]	···	small
33. Methyl 2-methyloctanoate[c]	···	very small	69. No id, MW = 200	···	very small
34. No id, MW = 170	···	very small	70. Eudesma-3,7(11)-diene[c]	···	small
35. No id, MW = 172, ethyl ester	40.61	medium	71. No id, MW = 204	···	very small
36. No id, MW = ?	···	small	72. No id, MW = 224	···	small

[a] The same compound identification was assigned to more than one peak when the identification confidence levels were equally high for all applicable peaks identified by the gas chromatography/mass spectrometry/data system. MW = molecular weight.
[b] Retention times were from the gas chromatography/flame ionization detector (GC/FID) chromatogram. No retention times were assigned for smaller peaks identified by GC/mass spectometry, but could not be matched with corresponding peaks on the GC/FID chromatogram with certainty.
[c] Compound may be reported for the first time in beer.

contributors to dry hop aroma in beer. Several researchers have investigated linalool (1,3,6,7,9–11) and myrcene (6,7,17) in beer.

The remaining compounds listed in Table I do not include many of the suspected hop aroma compounds reported by others (1,3,9–11,14,15). This does not mean that those compounds (reported by others but not by us) were not present in the beer we studied. Their absence is explained by their low volatilities. They could not be detected by the method employed. For example, several humulene epoxides, reported (1,11) as being of great importance to hop aroma, were not detected by the headspace method. They were detected in the beer we studied by other means (S. Rader, *personal communication*). On the other hand, the headspace method recovered other interesting aroma components. Preliminary GC sniffing work indicated that beer concentrate-like odors eluted intermittently in the area of 56–59 min on the chromatogram. We do not know if the aroma was caused by the compounds listed in the table, by trace quantities of compounds detected only by GC sniffing, or by interactions between detected and undetected compounds. The compounds eluting in this area, e.g., β-caryophyllene (5) and α-humulene (12), have relatively high sensory threshold values. Thus, individually, their aroma contributions may be insignificant, depending on their concentrations in the beer. If there are synergistic effects among these compounds, their flavor impacts could be greater.

A complete sensory study is needed to determine the significance of the analytical results, i.e., compounds unique to the hopped beer, obtained by the headspace method. A sensory study done in conjunction with the headspace analysis should provide new information previously unobtainable using extraction methods. This is because our technique recovers a different and broader group of compounds than the other methods; compounds that may be more closely correlated to human perception of hop aroma than the compounds recovered by traditional methods.

The GC/FPD analysis of hopped beer also revealed differences in chromatographic profiles between hopped and unhopped beer This can be seen in Figure 3. Two different headspace collectic parameters were used: one was optimal for the compounds of hig volatility, and the other was optimal for the compounds of mid low volatility. The major differences between the hopped ar unhopped beers are summarized in Table II. The peak are differences are not proportional to the concentration differenc because the FPD response is not linear with respect i concentration. Dimethyl sulfide, dimethyl disulfide, and dimeth trisulfide were found in larger quantities in the hopped beer. Oi unidentified peak at the retention time of 8.36 min was lower in tl hopped beer. The quantity of methionol remained the same in bo beers. S-propyl pentanethioate (tentative identification) was foun exclusively in the hopped beer. Again, differences should be result of hopping. Differences in sulfur compounds shou definitely have an impact on the flavor of the hopped beer becau of their generally lower sensory threshold values as compared nonsulfur compounds.

We suspect hop aroma is the result of a complex interactic between numerous compounds present in various concentratio in beer. Besides the differences already mentioned between t hopped and unhopped beers, other quantitative differences we noted. There were peaks that were found in both beers but we larger in the hopped beer. A few reverse cases, where peaks we larger in the unhopped beer, were also observed. All of the differences, not only the compounds uniquely found in the hopp beer, will have to be taken into consideration in future senso investigation of hop aroma in beer.

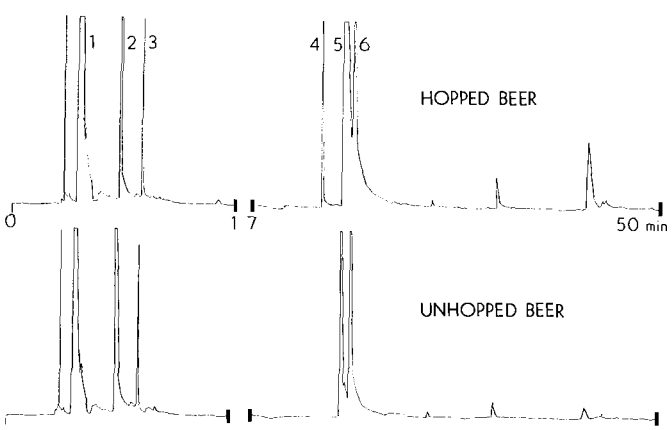

Fig. 3. Flame photometric detector headspace chromatograms of hopped and unhopped beers. The compounds of high volatility are shown from 0 to 17 min on the chromatogram (left), and the compounds of mid to low volatility are shown from 17 to 50 min on the chromatogram (right).

TABLE II
Comparison of Major Flame Photometric Detector Peak Areas for Hopped and Unhopped Beers

Retention Time (min)	Compound	Peak Area Difference
5.09	Dimethylsulfide	~×2 in hopped
8.36	No identification	~×2 in unhopped
10.20	Dimethyldisulfide	~×5 in hopped
23.08	S-propyl pentanethioate	Only in hopped
24.68	Dimethyltrisulfide	~×8 in hopped
25.40	Methionol	None

CONCLUSION

The goal of this study was to identify differences in volat constituents from hopped and unhopped beers by the headspa analysis method. This was accomplished by detection of numero peaks unique to the hopped beer and differences in t chromatographic profiles of sulfur-containing compounds. T headspace method was found to be very useful in detection of the analytical differences, all of which are a result of hopping. T compounds found in the hopped beer may be partially if n entirely responsible for hop aroma formation. The study w continue in an attempt to determine the significance of the compounds to hop aroma and flavor in beer.

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