Gas Chromatography/ Mass Spectrometry

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Characterization of Hop Aroma Using GC/MS, Headspace Trap and Olfactory Port

Introduction

Hops are a critical ingredient in beer. They provide an important balance to the malt in the taste of many beers. They also aid the brewing process in precipitating out proteins, etc. during the boil. Hops also have preservative properties that help keep beer fresh and free from bacteriological attack.

Hops contribute to the taste of beer in three ways:

- Bittering – hops contain compounds such a humulones that are very insoluble in water but isomerize on boiling to form isohumulones, which are partially soluble and impart the bitter flavor to beer.

- Flavoring – compounds such as terpenes and esters provide the fruity, citrus, earthy, resiny flavors to many beers.

- Aroma compounds – these are the volatile organic compounds that migrate into the vapor above the head of beer and gives the beer its characteristic smell. This can be flowery, citrusy, fruity, etc. They form a very important part of the overall flavor of beer.

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For the Better

There are many types of hops that deliver a very wide range of flavors. Hops need to be stored carefully and be used when fresh since the flavor will degrade as they age. Consequently there is a need to characterize the quality of hops so that the brewer can develop and deliver the required product.

Aroma characterization of hops is complex; there are many compounds in hops that contribute to flavor. Table 1 lists the composition of typical hops and Table 2 lists some of the key aroma compounds. The traditional way to evaluate hop quality is to use an experienced brewer to assess the hops organoleptically by crushing a few of the hops in their fingers and smelling the released aroma. This is effective but not objective and lacks the quantitative information needed to make correct decisions on how to utilize the hops.

Table 1. Composition of typical hops.

Component	%
Vegetative material (cellulose, lignin, etc.)	40
Proteins	15
Total resins (bittering compounds)	15
Water	10
Ash	8
Lipids, wax, pectin	5
Tannins	4
Monosaccharides	2
Essential oils (flavor/aroma compounds)	0.5 to 2

Table 2. Key hop aroma compounds.

Component	Comment
Myrcene	Pungent flavor; normally oxidized during the boil into other flavor compounds such as linalool and geraniol and their oxides
Humulene	Delicate and refined flavor characteristic of noble hops; broken down by boiling into oxidative flavors
Caryophyllene and farnesene	Herbal spicy character -- not well characterized

This application note describes a system that is able to provide both an objective chemical analysis of hop aroma using gas chromatography/mass spectrometry and, at the same time, provide the means for the user to monitor the olfactory character of each component as it elutes from the chromatographic column. Such an approach allows the user to gain a fuller characterization of a particular hop sample.

Analytical System

The analytical system comprises five main components:

HS Trap

Static headspace (HS) sampling is very suited for extracting aroma compounds out of hops. A weighed amount of hops (pellets or leaves) is placed in a glass vial and sealed as shown in Figure 1. This vial is then heated in an oven at a set fixed temperature and for a set fixed time period. A portion of the vapor is then extracted from the vial by the headspace sampling system and introduced into the GC column for separation and analysis.
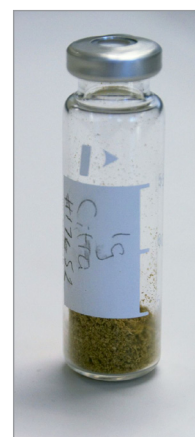

Figure 1. Hops inside a headspace vial awaiting analysis.

While extremely convenient, static headspace sampling only delivers a very small fraction of the headspace vapor into the GC column and so it is really best suited to high concentrations of compounds. In the analysis of complex samples, it is often found that low levels of some components are critical to the overall aroma of that sample. To increase the amount of sample value introduced into the GC column, a headspace trap system was used.

Using this technology, most or even the entire headspace vapor is passed through an adsorbent trap to collect and focus the VOCs. The trap is then rapidly heated and the desorbed components are transferred to the GC column. In this way, the amount of sample vapor entering the GC column can be increased by a factor of up to 100x. This technique is ideally suited for hop aroma analysis.

Figures 2 to 4 are simplified representations of the HS trap operation – there are other valves and plumbing needed to ensure that sample vapor goes where it should and not anywhere else. Essentially, the principle is very similar to classical static headspace but at the end of the vial equilibration step, after the vapor is pressurized, it is fully vented through an adsorbent trap. This process may be repeated to effectively vent the entire headspace vapor through the adsorbent trap. Once the trap is loaded, it is rapidly heated and the desorbed VOCs are transferred to the GC column.
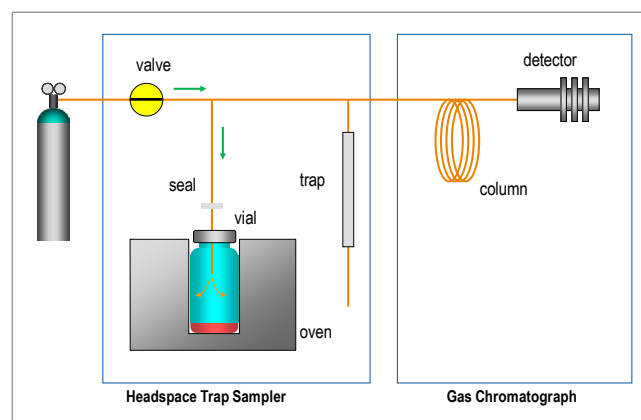

Figure 2. Schematic diagram of the HS trap system showing the equilibrated vial being pressurized with carrier gas.
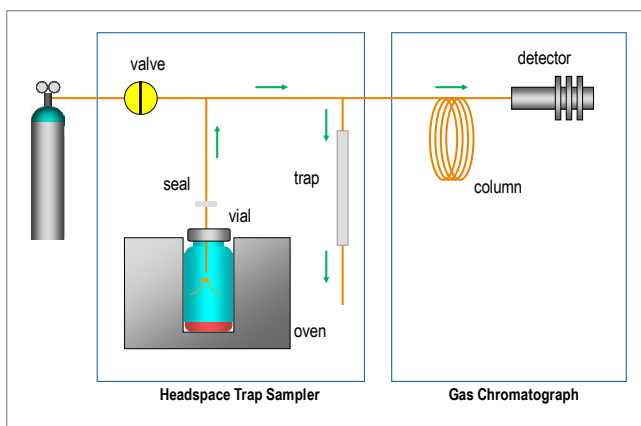
Figure 3. Schematic diagram of the HS trap system showing the pressurized headspace being released from the vial into the adsorbent trap.
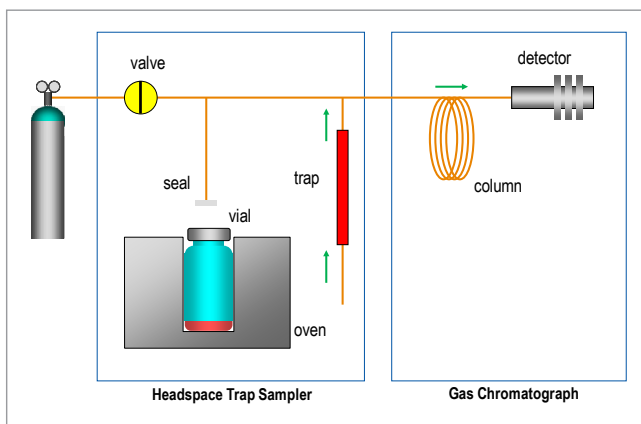

Figure 4. Schematic diagram of the HS trap system showing the VOCs collected in the adsorbent trap being thermally desorbed and introduced into the GC column.

Clarus 680 GC

The workhorse Clarus® 680 GC is an ideal complement to the rest of the system. The chromatography is undemanding so simple methods may be used. For olfactory monitoring, it is important to have sufficient time between adjacent peaks for the user to discern them from each other. It is also beneficial to load the column with as much sample as possible without overload to provide the best opportunity for the user's nose to detect them. For this reason, a long column with a thick stationary phase is used. Because many of the components in

Figure 5. The Clarus 680 SQ 8 GC/MS system.

hops are highly polar (acids, esters, ketones, etc.) a very polar Carbowax®-type stationary phase is used for the separation.

S-Swafer System

Because the column effluent needs to supply both the MS and the olfactory port, some form of splitting device is required. This should not affect the integrity of the chromatography in any way and so should be highly inert and have low-volume internal geometry. The use of a make-up gas in the splitter provides additional control and stability of the split flow rates.

S-Swafer™ is an excellent active splitting device and well suited to this purpose. Figure 6 shows the S-Swafer configured to split the column effluent between the MS detector and the SNFR olfactory port. The split ratio between the detector and the olfactory port is defined by the choice of restrictor tubes connected between the Swafer outlets and the MS and SNFR.
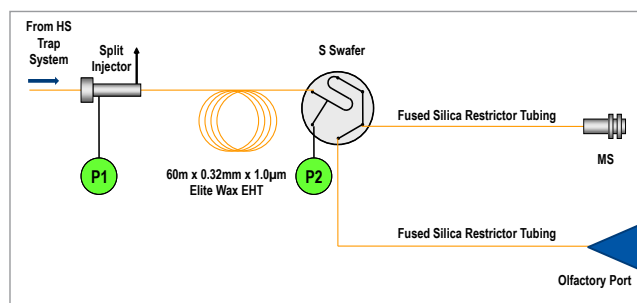

Figure 6. S-Swafer configured for use with the Clarus SQ 8 GC/MS and the SNFR.

The Swafer utility software, which is included with the Swafer system, may be used to calculate this split ratio. Figure 7 shows how this calculator was used to establish the operating conditions for the S-Swafer for this application.
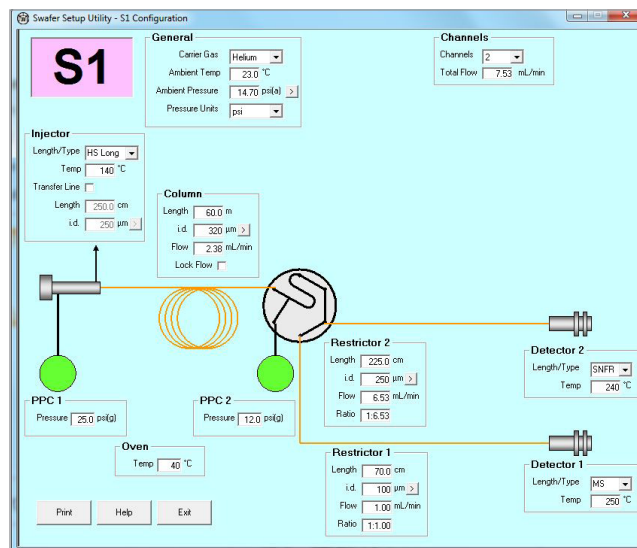

Figure 7. The Swafer utility software showing the settings used for this hop aroma characterization work.

Clarus SQ 8 Mass Spectrometer

A mass spectrometer is an important part of an aroma characterization system. It's important not only to detect and describe the aromas of the various components eluting from the GC column but to also to identify what those components are and possibly what their levels in the hops are.

The Clarus SQ 8 quadrupole mass spectrometer is ideally suited for this purpose and will quickly identify and quantify components using classical spectra in the supplied NIST library. This software is also able to interact with the olfactory information as described later in this document.

GC SNFR Accessory

Figure 8 shows a picture of the SNFR accessory. This is connected to the GC via a flexible heated transfer line. The split column effluent travels to the glass nose-piece through deactivated fused silica tubing.

While monitoring the aroma compounds eluting from the GC column, the user is able to capture vocal narration via a built-in microphone and aroma intensity by adjustment of a joystick.

Figure 8. The SNFR olfactory port accessory.

Analytical Conditions

Table 3. HS Trap conditions.

Headspace system	PerkinElmer® TurboMatrix™ 110 HS Trap
Vial equilibration	80 °C for 15 minutes
Needle	120 °C
Transfer line	140 °C, column connected directly to HS trap
Carrier gas	Helium at 25 psig
Dry purge	5 min
Trap	Air toxics, 30 °C to 300 °C, hold for 5 min
Extraction cycles	1 with 40 psig extraction pressure

Table 4. GC conditions.

Gas Chromatograph/ Mass Spectrometer	PerkinElmer Clarus 680 SQ 8
Column	60 m x 0.32 mm x 1.0µm Elite-5MS connected directly to the HS trap
Oven	40 °C for 2 min, then 4 °C/min to 240 °C for 8 min
Carrier gas	13 psig at Swafer
Injector	PSS at 300 °C, carrier gas off

Table 5. MS conditions.

Scan range	m/z 35 to 350
Scan time	0.8 s
Interscan delay	0.1 s
Source temp	250 °C
Inlet line temp	250 °C

Table 6. Olfactory port conditions.

Olfactory port	PerkinElmer SNFR
Transfer line	225 cm x 0.250 mm at 240 °C
Humidified air	500 mL/min with jar set to 37 °C

Table 7. Swafer conditions.

Swafer	PerkinElmer S-Swafer in the S1 configuration
Settings	Developed using the Swafer utility software – see Figure 7

Table 8. Sample details.

Sample preparation	Hops (leaves or pellets) were ground with a rotary coffee grinder and 1 g was weighed into a sample vial and sealed
Vial	Standard 22-mL vial with aluminum crimped cap with PTFE lined silicone septum

Typical Chromatography

Figure 9 shows total ion chromatograms (TIC) of four typical hops from different countries. Part of the German Hallertau is highlighted and is expanded in Figure 10. The power of the MS enables a particular peak to be identified from its mass spectrum (as shown in Figure 11) by searching the NIST spectral library supplied with the Clarus SQ 8 system. The results of this search are given in Figure 12. Results of this search very strongly indicate that the peak eluting at 36.72 minutes is 3,7-dimethyl-1,6-octadien-3-ol, otherwise known as linalool. Linalool is a very important aroma compound and will provide a delicate flowery aroma to the beer. The amount of linalool (or any other compound once identified) may be quantified by calibrating the GC/MS with standard mixtures of this compound.
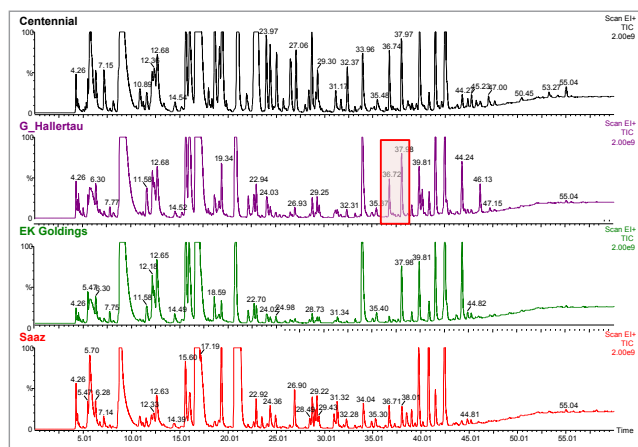

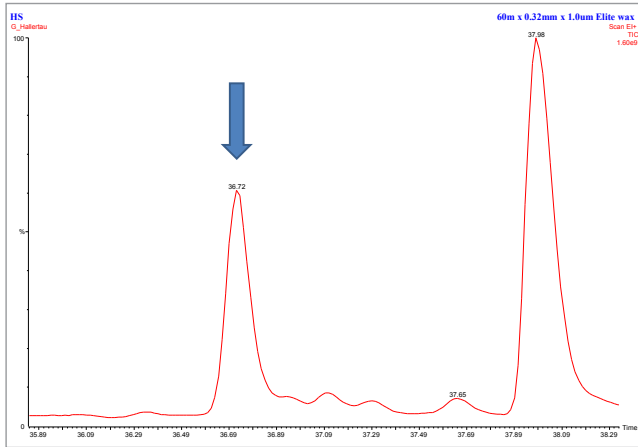*Figure 9.* Typical TIC chromatograms of four hop samples.

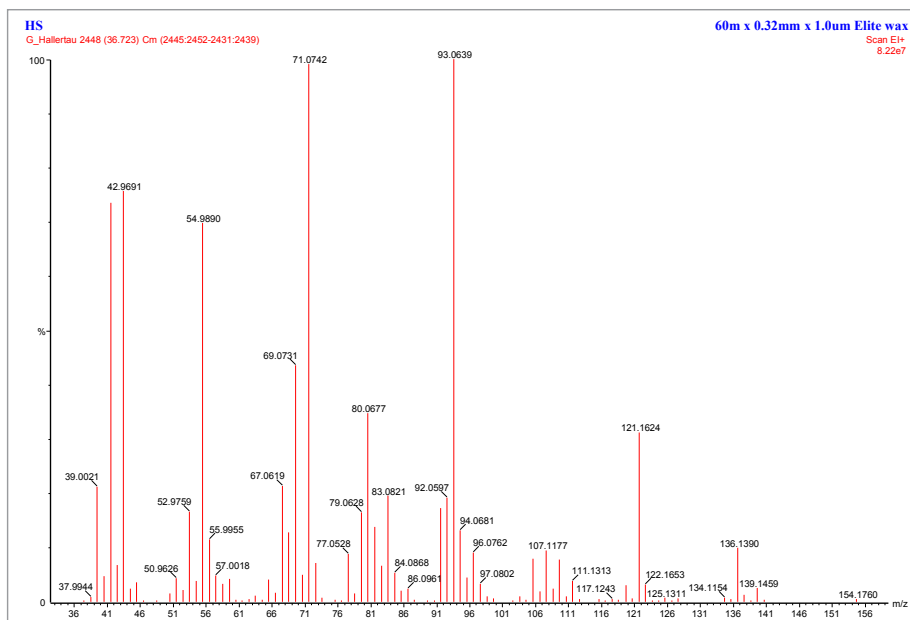*Figure 10.* Highlighted detail from Figure 9.

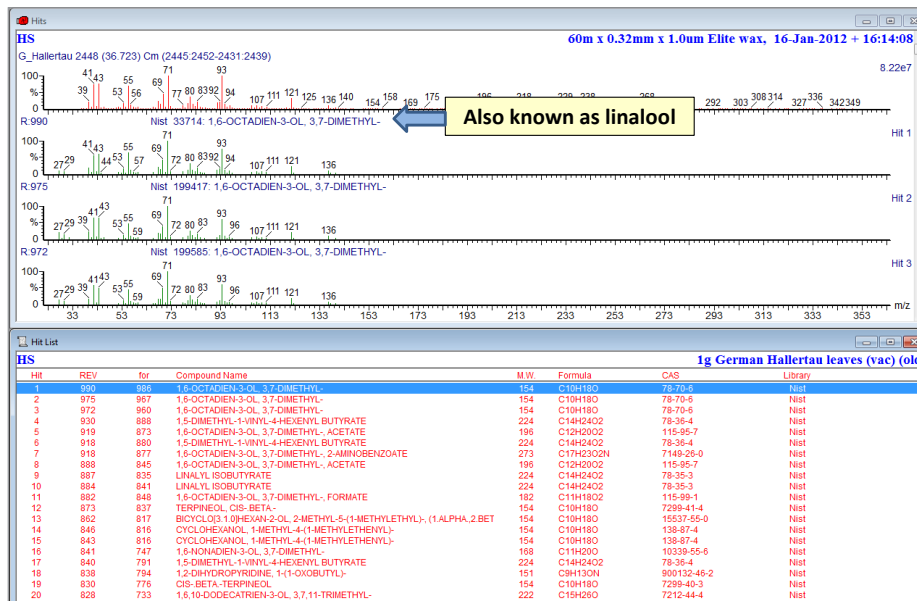*Figure 11.* Mass spectrum from peak highlighted in Figure 10.

Figure 12. Results from library search on mass spectrum shown in Figure 11.

By performing further identifications of the chromatographic peaks, a profile of the hop character may be established. Figure 13 shows further peaks identified in the German Hallertau chromatogram previously shown in Figure 9. Annotated peaks are mainly aliphatic acids which indicate a degree of oxidation in the hops in this particular sample. The strongly flavored myrcene peak is rather smaller than expected. These observations indicate that this particular sample is rather old (which was true – this was a really old sample that had been poorly stored).

Figure 14 shows chromatography of four additional hop samples.
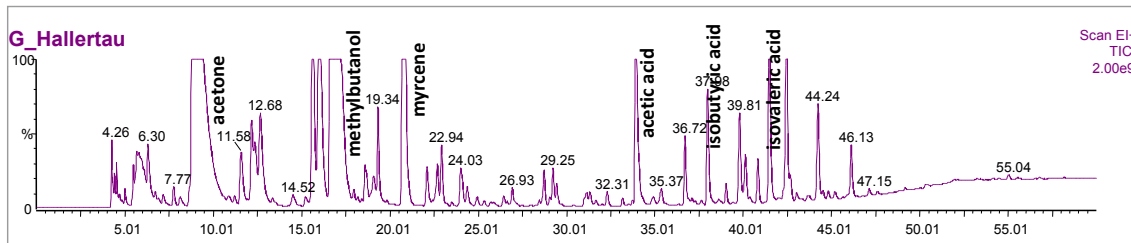

Figure 13. Typical TIC chromatograms of four hop samples.
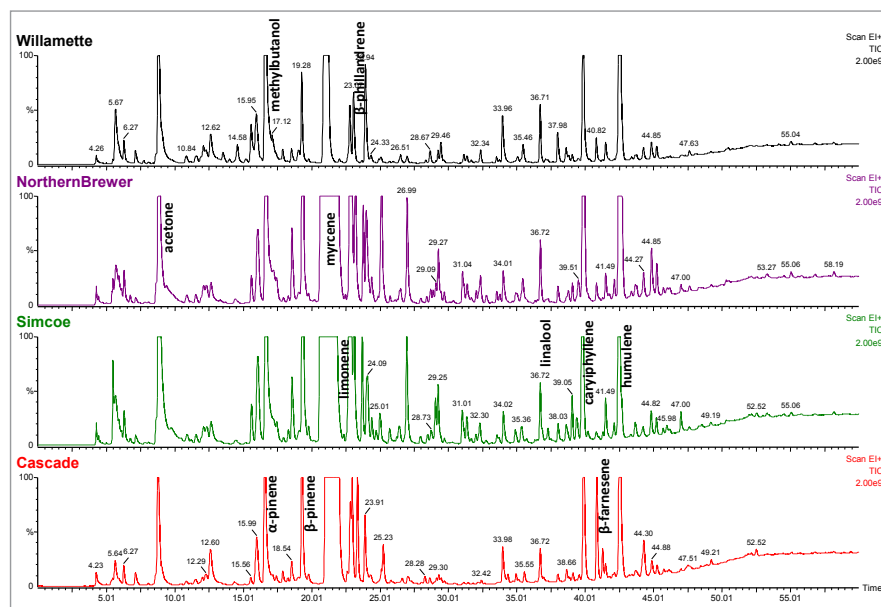

Figure 14. TIC chromatograms of a further four hop sample.
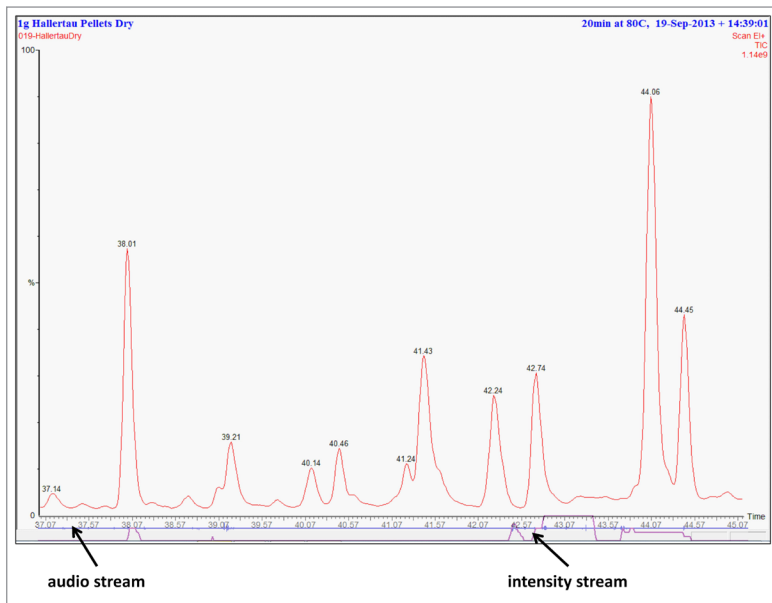

Figure 15. Example of a hop chromatogram being reviewed within the TurboMass™ software with the audio narration and aroma intensity graphically overlaid.

Olfactory Characterization

Figure 15 shows an example of a hop chromatogram with the audio narration and intensity recordings graphically overlaid. Audio narration is stored in a standard WAV file format that may be replayed from this screen to the operator from any point in the displayed chromatogram by means of a simple mouse-click. The narration WAV file may also be played back from most media applications including the Microsoft® Media Player, which is included with the Windows® operating systems. The audio data may be transcribed into text at the time of the recording. The Nuance® Dragon® Naturally Speaking software performs this function. It is included in the SNFR product. Table 9 shows a typical report from a hop analysis showing the user's transcribed narration and the recorded aroma intensity from the joystick. This report is formatted as a comma-separated value (CSV) file suitable for direct importation into Microsoft® Excel® or other application software.

Table 9. Typical output report showing text transcribed from the audio narration and the corresponding aroma intensity data.

Project Name	OKTOBERFEST.PRO		Start Time	9/19/2013 2:39:02 PM	
Sample Name	019-HallertauDry		Duration	60.00	

Time Stamp	Spoken text	Intensity	Time Stamp	Spoken text	Intensity
1.05	coming up on a minute	0	23.02	almost like a match	1
2.13	two minutes	0	23.07	a sulfur smell	0
5.15	a sweet smell	0	25.18	subtle	2
5.20	very faint	0	25.22	subtle	0
6.07	nothing there	0	25.33	not quite sure what that was	0
6.65	very very faint smell	2	25.70	nothing there	0
6.88	off order	3	30.70	little off odor	1
7.12	like sour milk	2	33.67	foul smell	2
7.25	sour milk	4	36.23	smell of cardboard must	0
7.30	was a very good banana smell	5	36.35	bananas	2
7.35	fruity smell	4	36.82	almost mint	2
8.18	like a sour milk	4	38.08	that was a nice fruit	3
8.23	sour milk	4	38.20	very citrus	0
9.17	fruit there	2	42.47	hot	4
10.02	nothing there	0	42.50	pepper	2
10.10	large peak and I smell nothing	0	42.70	again	3
11.52	burning smell	2	42.82	it's an off odor	6
11.58	almost woody	0	42.85	are very bad off order	6
12.00	little sweet	1	43.08	a sweaty socks smell	6
12.45	almost a hint of coffee	0	43.72	that's a fruity smell	2
13.22	that's an off smell	3	43.73	very pleasing	2
13.25	a rancid smell	3	45.78	floral	2
13.82	something	3	46.30	a burning smell	2
13.88	almost	0	46.37	burning match almost	2
13.90	medical	0	47.02	pepper smell	1
15.43	medical smell	2	47.95	pepper	1
15.47	is almost toffee like	2	48.93	sweet	1
15.57	very pleasing	4	49.13	a sweet smell	3
16.43	off order	0	49.88	interesting smell	1
17.92	slight sweet	0	49.92	can't describe it	0
18.58	bubblegum	0	50.32	ah	3
19.88	hint of something sweet	0	50.35	medical smell again	4
21.00	off order of skunk	3	50.40	medicinal	4
21.08	definite skunk	5	54.08	solvent	1
22.90	something	3			

Conclusions

The addition of an olfactory port to a HS GC/MS system extends its application for aroma characterization of samples such as hops. The ability to directly correlate organoleptic perception against hard analytical data provides insights difficult to obtain otherwise.

This system should be of interest to brewers and researchers involved in the following:

• Quality control of raw hops

• Product development

• Trouble shooting of off-flavors

• Storage/aging studies

• Comparison studies

• Aroma analysis of finished beer

• Reverse engineering of competitive products

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