The Benefits of GC/MS Coupled with a Headspace Trap to Monitor Volatile Organic Compounds in the Production of Beer

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Beer is a very popular beverage consumed all over the world. It is estimated that in 2015, the total sales of beer worldwide were approximately $522 billion [1]. There are many variations in the types, styles and flavours of beer, but the production process is very similar involving the fermentation of malted extracts from barley, and other grains together with the addition of various flavouring agents such as hops, fruits, honey, herbs and spices. However, even though the end products are very different, they are all highly complex mixtures of many compounds including sugars, proteins, alcohols, esters, acids, ketones, and terpenes. For anyone who really appreciates beer, flavour and aroma are two extremely important qualities, which are directly impacted by its chemical content. For that reason, there is a strong interest by brewers in quantifying the volatile organic compounds (VOCs) in a beer that gives rise to its unique flavour and aroma. In the brewing industry, it is well recognised that some VOCs have a positive effect (known as attributes) on the taste and smell of a beer, while others (known as defects) have a negative effect. Therefore, the ability to characterise these components in beer products before, during and after fermentation is critically important in the product development, process control, and quality control of the entire brewing process.

The traditional approach for the characterisation of VOCs in beer has been to use classical headspace coupled with gas chromatography using multiple detection devices such as electron capture (ECD), flame ionisation (FID) or thermal conductivity (TCD) detectors, depending on the analytes of interest [2]. These detectors work very well, but analysis using this kind of configuration is limiting because it lacks identifying capability, and also may require more than one analytical system and sample preparation [3]. Also, if low-level sulphur compounds are suspected, it would necessitate the use of a highly specific sulphur chemiluminescence detector which is expensive and limiting in its flexibility and range. Additionally, these specific analyte detectors will not fully characterise the beer, compare competitive beer products or identify other flavour components in the brew. The purpose of this research was to determine if a single system consisting of a headspace trap (HST), gas chromatograph (GC) and mass spectrometer (MS) detector can serve the needs of the brewing industry, by generating more useful information about the beer as well as offering time and cost savings when compared to the established detection principles mentioned.

This study will therefore describe- the use of the TurboMatrix™ HSTrap, and Clarus SQ 8 GC/MS system, (both PerkinElmer, Shelton, CT) to extract and concentrate volatile species from a beer sample for the purpose of separating, identifying and quantifying the VOCs. In addition, the investigation will demonstrate that the compounds responsible for both the attributes and defects can all be accomplished in a single injection using one system and one detector, which results in a faster analysis time, enhanced productivity, more cost effective, and a quicker return on investment. Additionally, this analytical approach reveals important information which can be used for fermentation and production troubleshooting purposes.

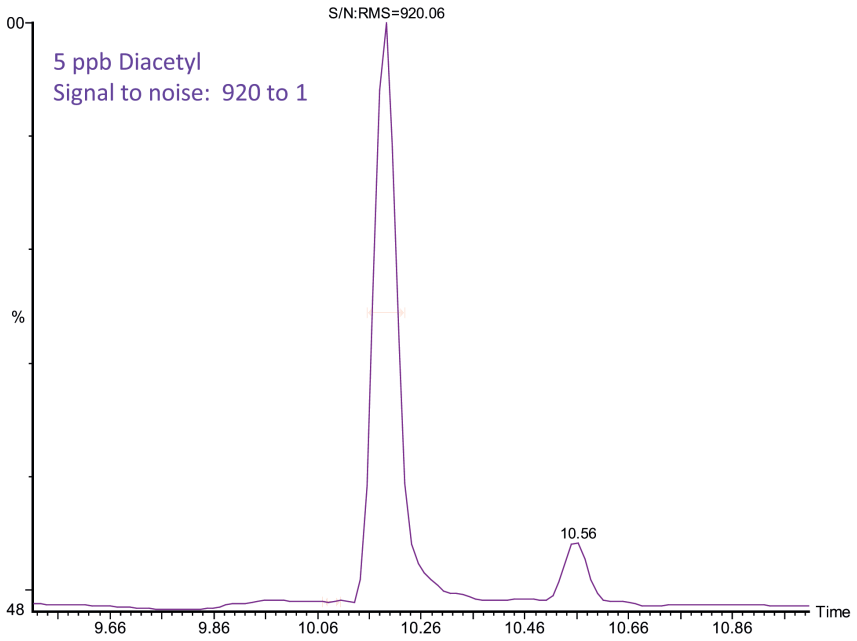

Figure 1: SIM chromatogram of Diacetyl peak at 5.0 ppb

Investigation

Several experiments were performed that were considered important to the brewing

Table 1: Headspace Trap Conditions

Headspace System	TurboMatrix (40 or 110) HS Trap
Vial Equilibration	80°C for 20 minutes
Needle	120°C
Transfer Line	140°C, 0.25mm id fused silica
Dry Purge	7 min
Trap	Beer: Low 30°C: High 260oC; hold 7 min
Extraction Cycles	1 cycle

Table 2: Gas Chromatographic Conditions

Gas Chromatograph	Clarus 680
Column	Elite-5MS 60m x 0.25mm x 1.0µm or Elite 624 Sil 60m x 0.25mm x 1.4µm
Oven	35°C for 5min, then 6°C/min to 245°C
Injector	Capillary Split/Splitless, 180°C, Split off
Inlet Mode	HS Mode

Table 3: Mass Spectrometer Conditions

Mass Spectrometer	Clarus SQ 8
Scan Range	35 to 350 amu
Scan Time	0.1 sec
Interscan Delay	0.06 sec
Source Temp	180°C
Inlet Line Temp	200°C

Table 4: Calibration for the five compounds

	SIM results from Simultaneous Full Scan/SIM Acquisition	
Component Name	Signal to Noise Ratio @ 5ng/mL	r^2 range from 5 to 1000 ng/mL
Acetaldhyde*	553 to 1	0.9990
Dimethyl Sulfide** (FS)	7081 to 1	0.9998
2, 3- Butanedione	358 to 1	0.9992
2, 3-Pentanedione	470 to 1	0.9991
trans-2-Nonenal	516 to 1	0.9993
*Acetaldhyde concentration were 8 times higher		
** For DMS, the Full Scan data was used for signal to noise and Calibration		

industry. For example, it is very important to monitor vicinal diketones (VDK), specifically 2, 3-butanedione (diacetyl) and 2, 3-pentanedione in the beer, because they are known to affect its taste. These components produce a butter-like flavour and are considered detrimental at high levels, especially in lighter style beers.

It is also critical to identify sulphur compounds in beer, such as sulphur dioxide and dimethyl sulphide (DMS). DMS in particular has the taste and aroma of sweet corn, which either comes from not boiling the malted wort long enough, or chilling the wort too slowly, resulting in bacterial contamination. When present in beer at low ppb quantities, sulphur components are considered acceptable, but at higher levels they give off an unpleasant taste and smell of rotten eggs.

In addition, the monitoring of unsaturated aldehydes like trans 2-nonenal is important, because they are reduced to ethanol by yeast during secondary fermentation. However, oxidation of the finished beer may reverse this process, converting ethanol back to an aldehyde. This is considered a defect, because (t) 2-nonenal in particular has been likened to the taste and aroma of cucumbers and in high concentrations, has been compared with wet cardboard or body odour.

Therefore, with these kinds of demands, plus other relevant testing procedures to ensure the quality of the brewing process, the following investigations were carried out:

- Quantitation of five VOC compounds:
  - o Acetaldehyde
  - o 2,3-butanedione (diacetyl)
  - o 2,3-pentandione
  - o dimethyl sulphide (DMS)
  - o trans (t) 2-nonenal
- Characterisation of flavour components of several types of beers
- Profiling the fermentation process
- Analysis of raw materials
- Aging studies

Experimental

For this analysis, a headspace trap sample introduction system was utilised which ensures that non-volatile components of the beer, such as sugars, remain in the headspace vial preventing contamination of the analytical system. This reduces maintenance and optimises productivity. In addition, headspace is a component concentration technique, and combined with a trap, allows the focusing of larger volumes of the sample to be analysed, enabling lower detection limits required for many attribute and defect compounds. This concentration step is required to compliment the sensitivity threshold of the skilled beer tasters, who are still a very important aspect of the quality assurance of the brewing process.

A volume of beer is dispensed into a vial and sealed, so the subsequent analysis can be fully automated. A slightly-polar 60m x 0.25 mm x 1.0 µm Elite 5 (5% phenyl-silicone) column was used for the separation. This column provided both sufficient retention to separate the most volatile and early-eluting components and the dynamic range necessary to separate both high level and low level components in the beer.

Sample Preparation and Chromatographic Conditions

5mL of each sample of beer was pipetted and sealed into a standard 22-mL sample vial with an aluminium crimped cap and PTFE lined silicone septum. The instrumental conditions for this analysis are given in Tables 1-3.

Results

A seven-concentration level calibration up to 1000 ng/mL (ppb) was prepared for

the five target components: acetaldehyde, dimethyl sulphide, 2,3-butanedione, 2,3-pentandione, and t-2-nonenal. The detection limit goal was 40 parts per billion (ppb) for acetaldehyde and 5.0 ppb for the remaining targets, which was made at the request of several breweries. The standards were analysed in Simultaneous Full Scan (FS) and Single Ion Monitoring (SIM) acquisition modes. An example of the SIM chromatogram of Diacetyl at the 5.0 ppb concentration is displayed in Figure 1.

The calibration data for all five compounds are shown in Table 4. Considering the extremely low levels in a highly complex matrix, it can be seen that these data demonstrate acceptable linearity. In addition, it should be pointed out that these results were acquired using a small turbomolecular pump; it can be assumed enhancements can be achieved using the recommended larger turbomolecular pump. The signal to noise for Dimethyl Sulphide at the 5 ppb concentration was 80,000 to 1, and the 1000 ppb concentration saturated; therefore, the results from full scan acquisition are used for this target.

Characterisation of Beer

One of the benefits of mass spectrometry is that it enables the identification of a large suite of volatile flavour compounds in beer, without having to change or use another detector. Figure 2 is an example of such a characterisation that was carried out on American pale ale, while Figure 3 shows a comparison of the flavour compound responses found in an alternative beer product. Note: For comparison purposes, three different batches of the pale ale are shown (red, yellow, light blue) with the other beer product (grey).

Monitoring the Fermentation Process

This approach also provides the ability to obtain analytical data during the entire fermentation process.

For this part of the investigation, an experimental batch of American pale ale was brewed and fermentation initiated. A sample was analysed every eight hours for the eight days of the fermentation process. Specific gravity is typically used as an indicator of the fermentation progress and is shown for this beer in Figure 5. It can be clearly seen that the final gravity of this beer was 1.012, which was achieved in about 100 hours.

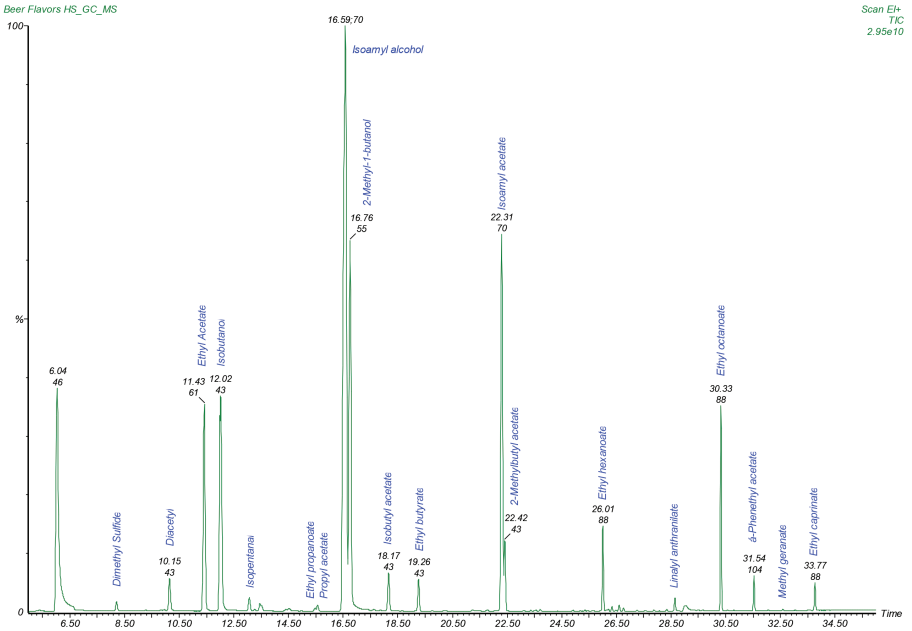

Figure 2: Typical chromatographic profile of volatile flavour compounds in an American pale ale

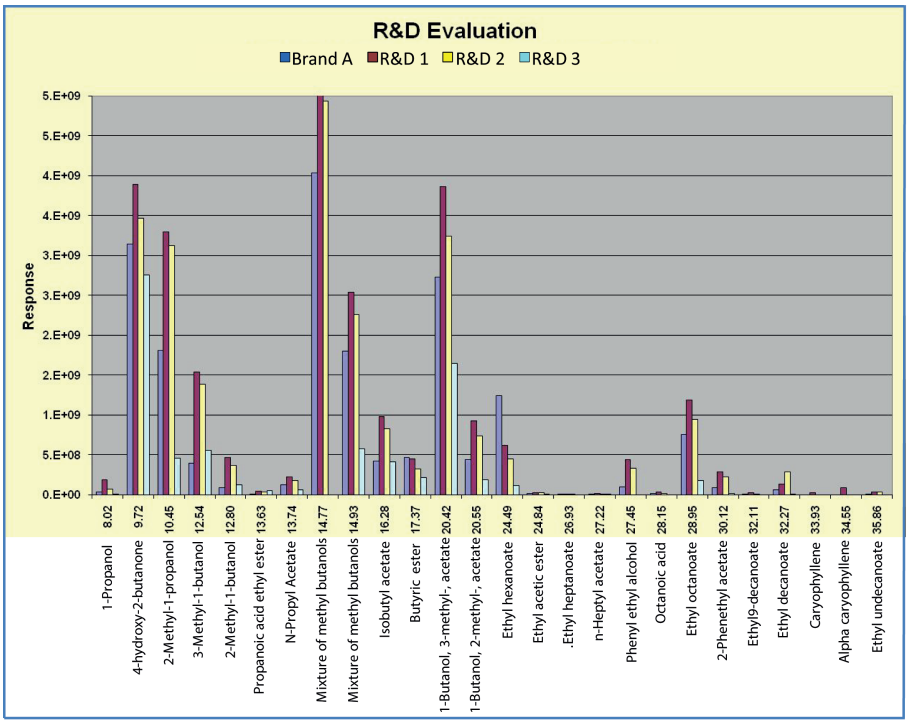

Figure 3: Comparison of chromatographic responses for a suite of volatile flavour compounds between two brands of beer (data courtesy of the Long Trail Brewing Company, Vermont). Note: The competitive beer sample is shown in grey

To get a better understanding of the characterisation of the favour compounds during the fermentation process, a comparison was made of the flavour profiles of the same beer from five different fermentation trials. This comparison is exemplified in Figure 4.

The concentrations of key components in the beer were also monitored during the fermentation process. The profiles of two key flavour 'defects', diacetyl and dimethyl sulphide, are shown in Figures 6 and 7, respectively. It can be seen that the concentration of the diacetyl was reduced to a negligible amount in about 80 hours while the dimethyl sulphide only took about 30 hours to get down to trace levels. This is an analytical approach of monitoring when the brewing process has been completed. Using an analytical technique to determine when the brew is ready can provide enhanced productivity of up to 40%.

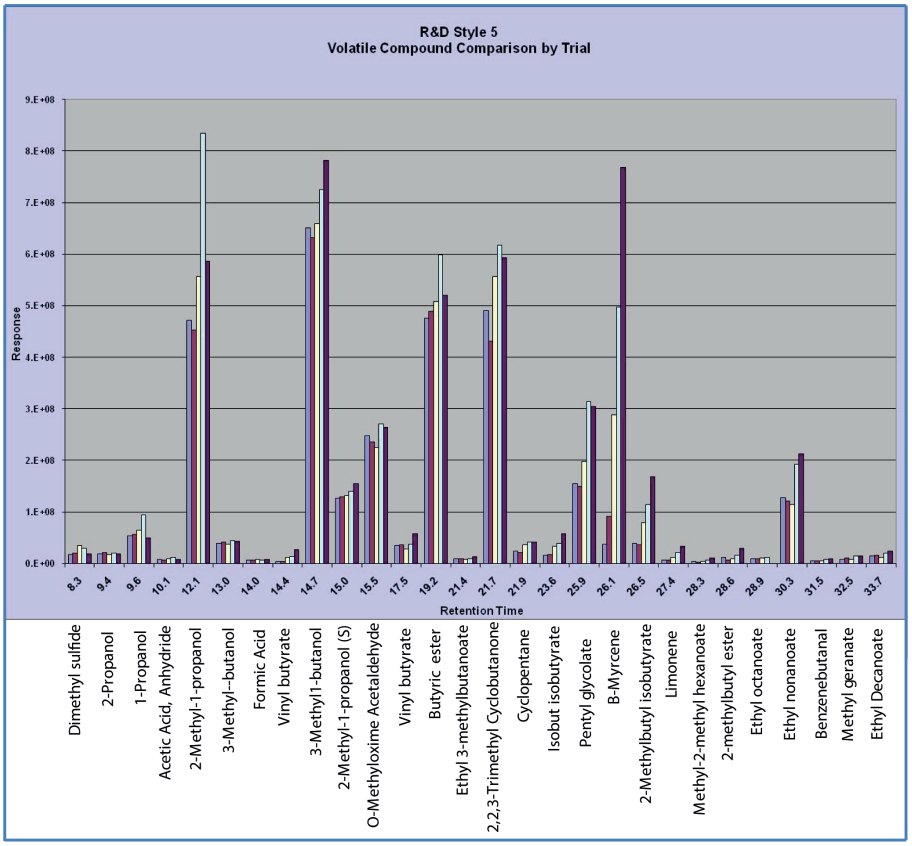

Figure 4: Comparison of flavour compounds between five different fermentation trials of the same beer type (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, VT)

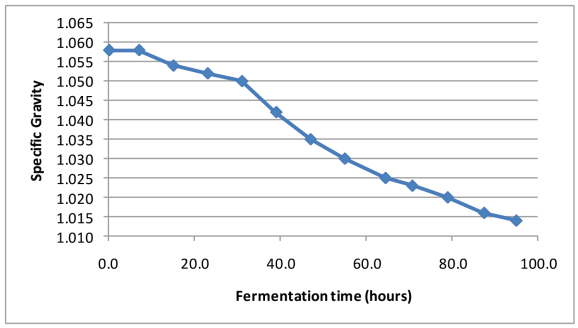

Figure 5: Specific gravity profile for the experimental beer, showing that the fermentation process was completed in about 100 hours

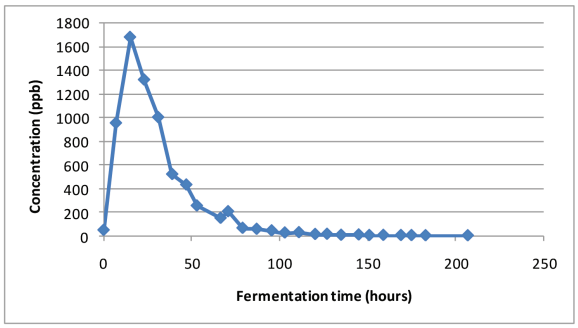

Figure 6: Concentration profile of diacetyl for the experimental beer during the fermentation process

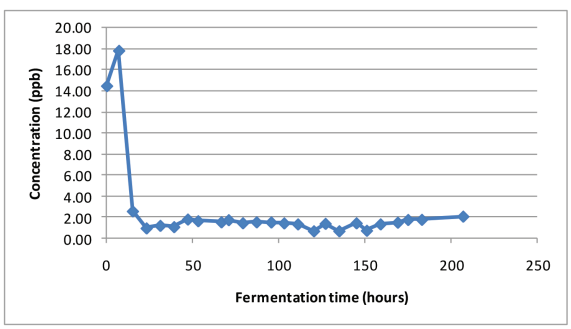

Figure 7: Concentration profile of dimethyl sulphide for the experimental beer during the fermentation process

Analysis of Raw Materials

This methodology can also be utilised to characterise the raw materials used in the production of beer. The resulting data could be utilised to assess what components will provide the desired flavour of the finished product. By monitoring the compounds in the raw materials 'up front', and not at the end of the fermentation process, it can save the brewer a significant amount of time and expense by increasing the chances of producing a consistent beer with the desirable flavour and aroma characteristics.

This is exemplified in Figure 8, which displays the results of a study comparing the VOC components of two different types of hops in order to understand and improve their characteristic bitterness on the flavour of the brew. Additionally, some beers use adjuncts to produce unique flavours, such as coffee, honey and fruits. Therefore, the same approach may be used to characterise these compounds. Figure 9 displays the results of a comparison between orange peel from different suppliers for use in Belgian style beers.

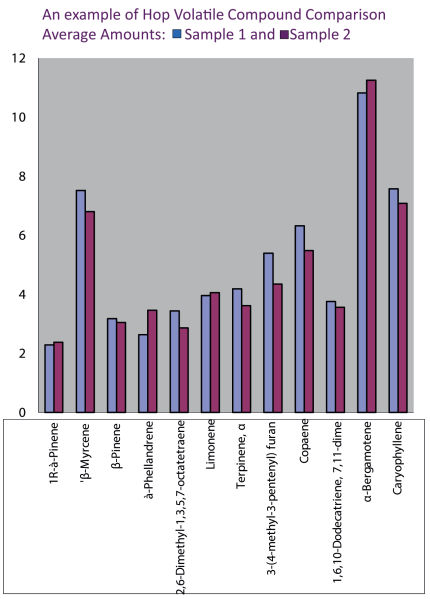

Figure 8: VOC profiles of two different types of hops (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, VT)

Aging Studies

Beer is a very complex matrix, which ages over time due to chemical and biological activity. For that reason, storage conditions are critical to its quality. Exposure to air promotes the formation of aldehydes, acetic acid and other undesirable compounds that can impair the flavour of a beer.

For example, one flavour concern is that bittering components, such as

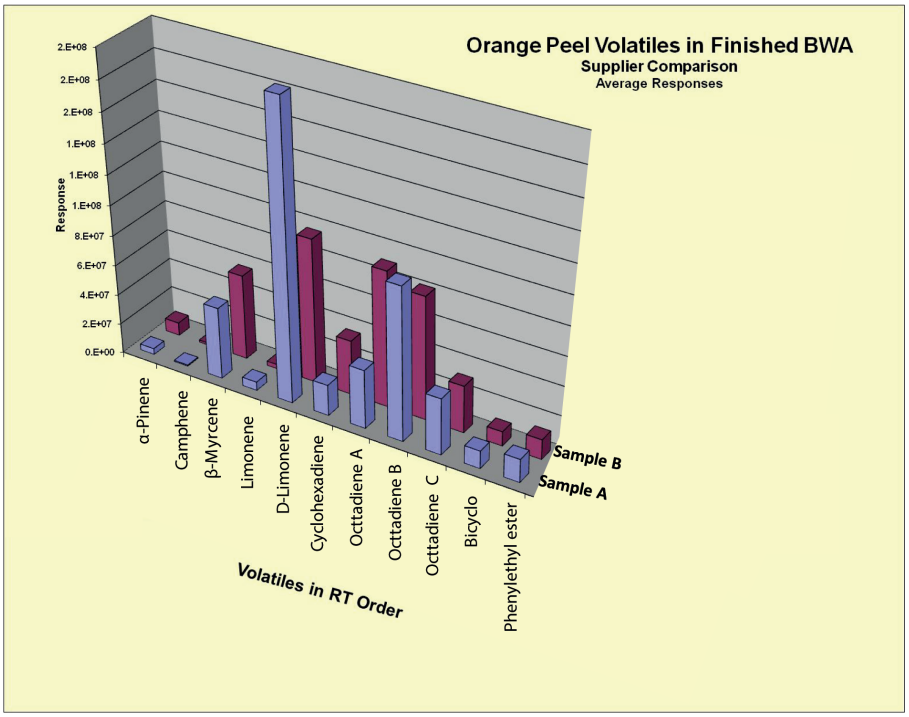

Figure 9: Flavour profiles in orange peel used in Belgian-style beers (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, VT)

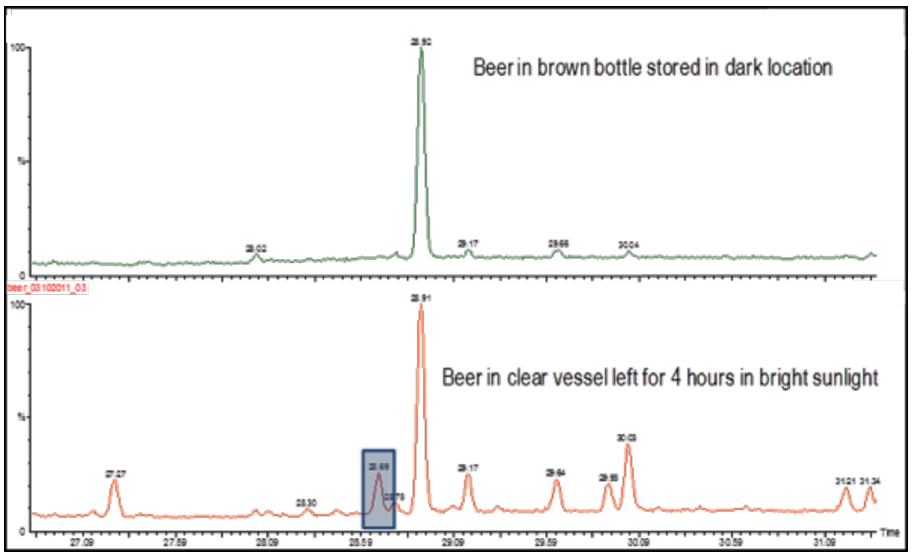

Figure 10: Effect of sunlight on beer volatile species. Note: Chromatogram on the top is the beer kept in the dark, while the one on the bottom is after 4 hours in bright sunlight

iso-humolones react to light and produce mercaptans and other volatile sulphur compounds giving a 'skunky' flavour to the beer. This is demonstrated in Figure 10 which shows chromatograms of the same beer kept in the dark (top chromatogram) and also in bright sunlight (bottom chromatogram). It can be seen very clearly there are major differences in the composition of the beer VOCs. One compound in particular (in the blue box) was identified as an olefinic thiophene which was confirmed by a search for the unknown compound using the on-board reference library.

Conclusion

It has been demonstrated that the combination of headspace trap concentration with GC/MS is a very powerful and easy to use tool to investigate many aspects of the beer production process. Many volatile organic compounds responsible for both positive attributes and negative defects can be monitored in the beer using a single column, single detector, under one set of optimised conditions. This technology can be utilised for checking raw materials, monitoring the fermentation process, quality control testing of the final product, product development, carrying out aging studies and for general troubleshooting purposes. Traditionally, this work would have been performed by skilled tasters which are still going to be an important part of any brewing process. However, the opportunity to compliment taste and odour assessments with objective analytical data can only enhance the art of making high quality beer.

Acknowledgements

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