THE USE OF SIFT-MS IN THE ASSESSMENT OF FOOD QUALITY: SMOKED SALMON

APPLICATION NOTE AS-245

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Abstract

The measurement of volatile compounds in food can be critical in understanding the flavour profile of a product and ensuring the quality and authenticity for the consumer. The complexity of the matrix can sometimes present a challenge and can often require considerable sample preparation protocols to enable detection of analytes at low levels. Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is a real-time volatile organic compounds (VOC) analyser with an ability to detect analytes simultaneously at low concentrations with little or no sample preparation. This application note demonstrates the use of SIFT-MS to compare the volatile profiles of smoked salmon samples and illustrates the potential of this approach for both targeted and untargeted analysis.

Introduction

Food wastage is a major concern in the modern world with many countries not having enough to feed their people. Food wastage also costs manufacturers millions of pounds each year with many tonnes going to landfill. Part of the determination of food quality is done by assessing the aroma to determine freshness or quality. Understanding the aroma and flavour profile of food stuffs and the affect cooking and processing methods have on these foods is a major part of food research today.

The determination of volatile organic compounds (VOC's) in food is performed for a range of purposes and using a number of analytical methods. The range of compounds that may be monitored for quality, safety, flavour or authenticity are many and varied meaning that no one method can be used for all. The aim of this study was to demonstrate the potential of SIFT-MS to complement, or even replace traditional analysis techniques.

For example, volatile oxidation products like aldehydes are traditionally analysed by liquid chromatography using ultraviolet wavelength detectors (HPLC-UV), following derivatisation [1]. Sulphur compounds, such as methyl mercaptan, dimethyl sulphide and hydrogen sulphide, which can indicate spoilage can also be a challenge to analyse by traditional chromatographic techniques and have very low sensory thresholds. The monitoring of the production of biogenic amines during smoking of fish is also of interest due to concerns of potential hazard to health Microbiological approaches have been used for this, which are time consuming or HPLC methods on the finished products [2].

Although many laboratories have GC and LC capabilities, these can struggle to detect and quantify small polar molecules without the need for complex derivatisation. SIFT-MS has the capability to analyse these compounds as well as a wide range of other target compounds simultaneously (with very little sample prep requirement) and enables real time analysis to be performed to give rapid results or enable an entire process to be monitored [3].

SIFT-MS has been extensively used to monitor VOCs in food products, including detection of fish oil oxidation [4] Beef aroma [5] and sulphur compounds in Cheese [6]. In this study we chose to compare volatile profiles, in both a targeted and untargeted analysis for different samples of smoked salmon, due to the numerous analytical studies in literature using a range of techniques. The samples were initially analysed using headspace solid phase microextraction (HS-SPME) coupled to Gas Chromatography-Quadrupole Time-of-flight Mass Spectrometry (GC/Q-TOF) to identify the target compounds for the targeted SIFT-MS analysis.

Experimental

Instrumentation & Materials

For both the GC/Q-TOF and SIFT-MS, all sample preparations and injections were carried out using GERSTEL MPS Robotic. Figure 1 shows the automated SIFT-MS.

Figure 1: *Automated SIFT-MS for high throughput headspace analysis*

The GERSTEL Maestro software features the PrepAhead function, which increases sample throughput by intelligently preparing samples whilst others are being analysed. This results in very fast sample preparation and analysis time. Figure 2 shows the sequencing of 50 samples for headspace SIFT-MS. It is important to note that the incubation time for the first sample (30 min) is the rate-limiting step, but after that, a data file is generated every 4-5 mins.

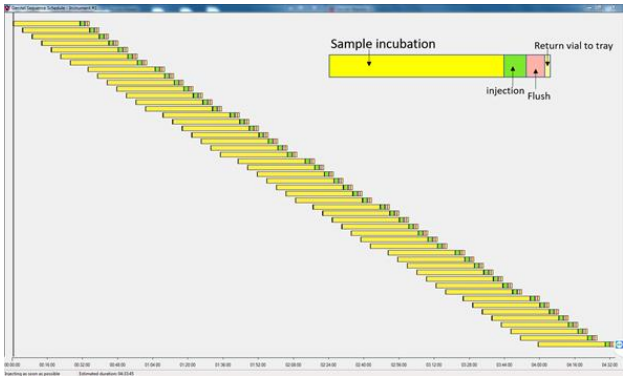


Figure 2: *Sequence scheduler from GERSTEL Maestro software for 50 samples by SIFT-MS taking 4.5 hours.*

If real time analysis is required, the SIFT-MS can be directly coupled to exhaust points in a production process.

METHOD

Packets of three different smoked salmon were purchased. Two were produced using industrial liquid smoking and one used traditional wood. For all analyses, 1 g of sample was weighed into 20 mL headspace (or SPME) vials.

HS-SPME on GC/Q-TOF

Samples were incubated at 40 °C and the volatiles collected on a PDMS SPME fibre prior to desorption in the hot inlet of the GC/Q-TOF. The resultant chromatograms were processed using Agilent Unknowns Analysis, to determine the compounds, present in the samples.

Static headspace on SIFT-MS

Targeted SIFT-MS analysis:

Samples were analysed by SIFT-MS, analysing for the compounds determined from the GC/Q-TOF analysis. Three replicates of each sample (1 g) were weighed into headspace vials. Samples were incubated for 30 minutes at 40 °C. An aliquot (2.5 mL) of the headspace was then injected into the instrument at a rate of 50 µL/s, giving a sampling time of just 50 seconds.

Untargeted SIFT-MS analysis:

The SIFT-MS was also set up to conduct an untargeted analysis of the samples, with 10 repeats of each sample, to enable a statistical analysis on the data using JMP. The SIFT-MS was set up to run in scan mode (mass range: 15-250) on each of the three positive reagent ions. This gives a 'fingerprint' of the volatile profile of each sample, as illustrated in Figure 3. Each bar represents a response for that product ion (m/z).

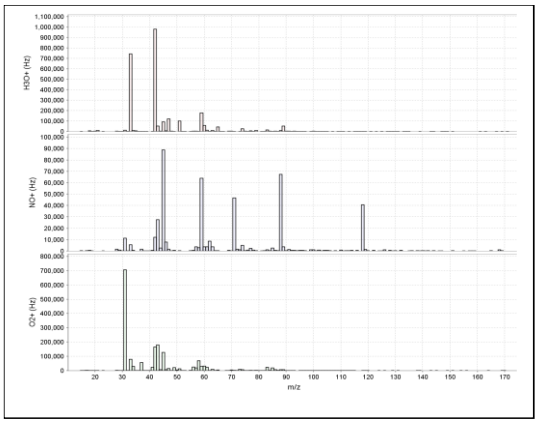


Figure 3*: SIFT-MS 'fingerprint' – example mass scan*

RESULTS AND DISCUSSION

GC/Q-TOF:

The chromatograms were then deconvoluted using Agilent Masshunter Unknowns Analysis, in order to obtain clean spectra to identify components and the spectra were then compared to the NIST library, in order to provide identification. An example of this process is shown in Figure 4.

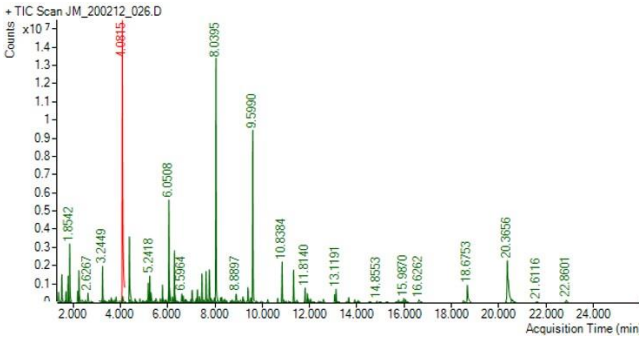


Figure 4: *Deconvoluted chromatogram (sample Type 1)*

Between 97 and 263 components were identified by the library search of the deconvoluted chromatograms. Table 1, a screenshot from the unknowns analysis software, shows a subset of those identified, of which a selection were chosen as targeted compounds for the SIFT-MS. The compounds analysed consisted of those relating to smoke flavour, some usually present in fish (e.g. triethylamine) and spoilage compounds (putrescine).

Table 1: *A cross section of identified compounds in smoked salmon (Type 1)*

Component RT	Compound Name	Match Factor	Formula	CAS#
7.7619	p-Cresol	98.6	C7H8O	106-44-5
9.5990	2-Methoxy-5-methylphenol	98.5	C8H10O2	1195-09-1
1.7849	Acetic acid	98.5	C2H4O2	64-19-7
5.2418	Ethanone, 1-(2-furanyl)-	98.4	C6H6O2	1192-62-7
8.0395	Phenol, 2-methoxy-	98.4	C7H8O2	90-05-1
6.2836	Phenol	98.2	C6H6O	108-95-2
1.8542	2-Butanone	98.2	C4H8O	78-93-3
3.2449	Toluene	98.1	C7H8	108-88-3
4.0815	3-Furaldehyde	97.8	C5H4O2	498-60-2
7.4477	Phenol, 2-methyl-	97.5	C7H8O	95-48-7
10.8384	Phenol, 4-ethyl-2-methoxy-	97.4	C9H12O2	2785-89-9
13.1191	trans-Isoeugenol	97.3	C10H12O2	5932-68-3
11.8140	Phenol, 2,6-dimethoxy-	97.2	C8H10O3	91-10-1
6.0508	2-Furancarboxaldehyde, 5-...	96.6	C6H6O2	620-02-0
11.9105	Eugenol	96.4	C10H12O2	97-53-0
7.2095	2-Acetyl-5-methylfuran	96.4	C7H8O2	1193-79-9
4.3793	3-Furanmethanol	96.1	C5H6O2	4412-91-3
18.6753	n-Hexadecanoic acid	96.0	C16H32O2	57-10-3
9.5299	Naphthalene	95.9	C10H8	91-20-3

Due to the SIFT-MS' rapid scan time, an analysis of a suite of compounds can be carried out very quickly. Figure 5 shows an analysis of 15 compounds, analysed in approximately 2 minutes.

The profile shows real time acquisition with an 'injection' in the inlet occurring, between 20 and 80 seconds.

Data was corrected for the mass of sample taken and any response in blanks subtracted. The averaged data was then compared between the three sample types as shown in Figure 6. This shows the variety of compounds the instrument can measure, as well as the levels of the compounds detected.

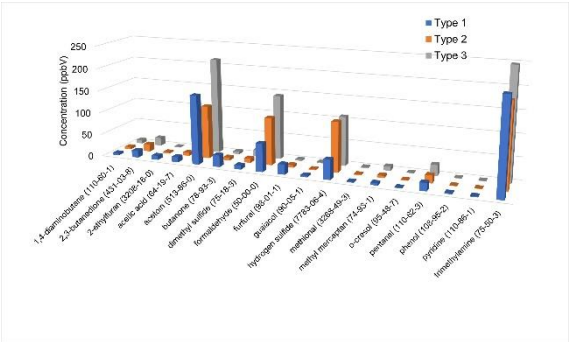


Figure 6: *Concentration of compounds analysed using SIFT-MS*

Figure 7 shows the same plot, with the largest peaks removed, to show differences even at very low levels (<5 ppbV).

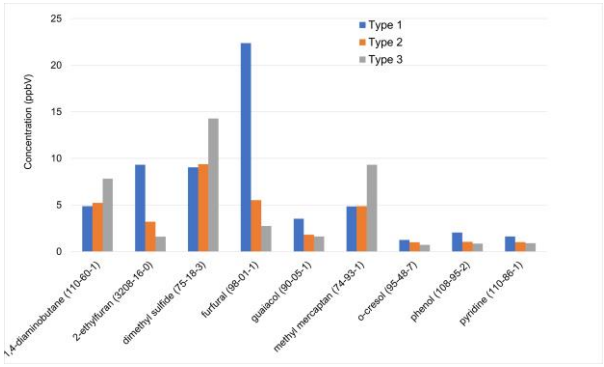
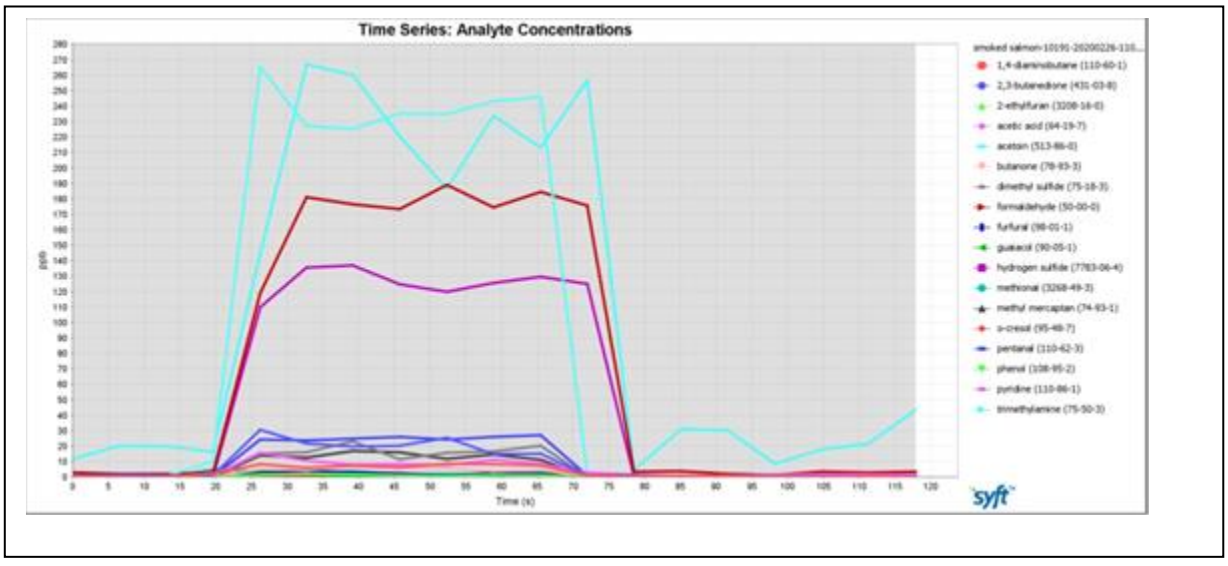


Figure 7: *Concentration of compounds analysed using SIFT-MS*

Figure 5: *Time series of compounds analysed by automated SIFT-MS showing the injection period*



UNTARGETED SIFT-MS RESULTS

Due to the speed of SIFT-MS analyses, more samples can be analysed for a number of compounds simultaneously, therefore data can be generated in much larger quantities than for traditional techniques. Therefore, this makes SIFT-MS a prime candidate for large data sets, and in-depth statistical analyses.

Principal Component Analysis (PCA) is a common statistical technique that allows for the observation of potentially correlated variables from large datasets by using an orthogonal transformation. Sample types can then be plotted in distinct groups, indicating statistical significance based on one, or more components.

Subsequently, SIFT-MS can be used as a tool for very quick screening of samples, to determine differences and similarities between samples.

The data from 30 samples was processed and corrected for MS sensitivity drifts, and for variations in sample mass. It was then imported into JMP (SAS, USA), a statistical analysis platform.

First, the Predictor Screening script was run on the whole dataset. This tool screens the data to find parameters that are most statistically relevant. It uses bootstrap forest partitioning to evaluate the contribution of predictors on affect responses. It then ranks the variables (in this case, the mass intensity) from highest to lowest.

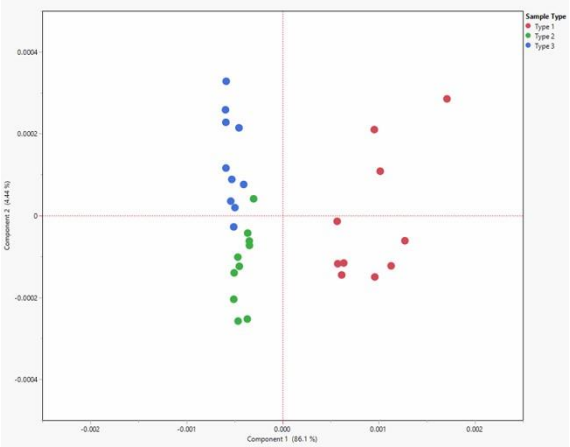
The top 30 predictors were used, as this gives a comprehensive understanding of the separation, whilst not introducing noise. These 30 predictors were then used to inform the PCA.

Figure 8 shows the PCA of the three samples. Type 1 is significantly separated from the other two samples along component 1 (x-axis).

There is some spread in the data, which could be due to differences in surface area of the samples. As this was a preliminary study, this was not accounted for, but potentially could lead to less spread within groups.

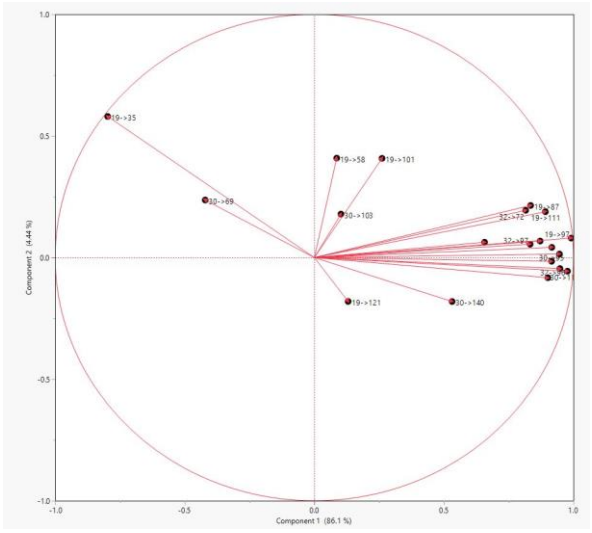
Types 2 and 3 are not separated at all by component 1, however, there is some indication of separation from component 2 (y-axis). Again, if corrected for surface area, these groups could be more distinct.

Figure 8: *Principal component Analysis of the three sample types*



Additional information can be obtained by interrogating the Loadings plot. This is shown in Figure 9.

Figure 9: *Loadings Plot showing the ions responsible for the separation seen in the PCA*



The Loadings plot shows the extent to which the different ions seen from the SIFT-MS analysis are influencing the separation of the groups (reagent ion>product ion). Those on the right are responsible for the separation of Type 1 sample (for example 19>97 could be attributed to furfural and 19>111 methyl furfuryl). These compounds could be linked to the wood smoking process that was used for sample Type1.

CONCLUSIONS

SIFT-MS can be used for direct analysis or real time monitoring of a wide range of compounds, including flavour, malodour and compounds related to spoilage. This relatively brief study determined clear differences between samples, in this case most likely linked to the smoking process employed as compounds determined are related to pyrolysis of wood and thermally induced Maillard reaction compounds. This demonstrates how easily and rapidly a large number of samples can be analysed for multiple analytes using the SIFT-MS.

The application demonstrated in this study shows how SIFT-MS can complement other more traditional techniques used in the standard food laboratory. It can capture suites of volatile compounds with one analysis this presents a much faster wat to identify differences in volatile profiles to classify or identify sample adulteration. Although this work was performed in the laboratory, previous work has also shown it can operate outside of the laboratory environment. This means that with a model built from the large data sets that can be obtained, a SIFT-MS could potentially be used in a factory or smokehouse site for quality control. It is able to target, quantify and screen in a fraction of the time it would traditionally take or can help monitor and control production processes by analysis in real time.

REFERENCES

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[6] Analysis of Volatile Sulfur Compounds in Swiss Cheese Using Selected Ion Flow Tube Mass Spectrometry (SIFT-MS), January 2011ACS Symposium Series 1068:153-181

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To discuss the potential of SIFT-MS for your analytical solution please contact us and we will be delighted to work with you from conception to method transfer into your laboratory or process environment.