Rapid Parmesan Classification using Automated Static Headspace-SIFT-MS

Mark J. Perkins[1], Diandree Padayachee[2], Vaughan S. Langford[2]
1 Anatune Limited, Wellbrook Court, Girton Road, Cambridge CB3 0NA, United Kingdom
2 Syft Technologies Limited, 68 Saint Asaph St, Christchurch 8011, New Zealand

Automated, direct headspace analysis using selected ion flow tube mass spectrometry (SIFT-MS) provides rapid and economic screening of food products and ingredients. This application note describes how SIFT-MS coupled with multivariate statistical analysis rapidly classifies genuine Italian Parmesan cheeses by product and manufacturer via the most important odor-impact compounds.

Introduction

Selected ion flow tube mass spectrometry (SIFT-MS) has been shown previously to readily discriminate genuine Italian Parmesan cheeses from imitation New Zealand variants,[1] including when narrowing the target compound list to the most significant odor-active species identified by Qian and Reineccius.[2-5]

This application note revisits Parmesan cheese analysis using SIFT-MS, but applies the more recent automated headspace variant. Since our previous study demonstrated that foreign imitations were poor, in this study the ability of SIFT-MS to differentiate Parmesan products from three Italian manufacturers by targeting the odor-active compounds is examined instead. Automated headspace-SIFT-MS analysis determines factory of origin at throughputs of 12 samples per hour, offering great potential for rapid product screening.

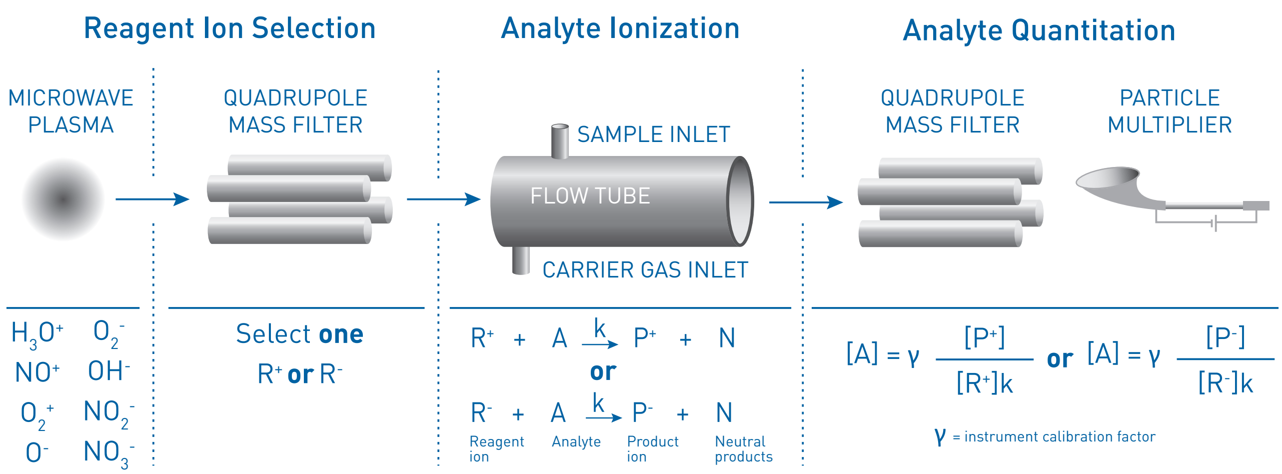
Method

1. The SIFT-MS technique

SIFT-MS[6-8] (Figure 1) uses soft chemical ionization (CI) to generate mass-selected reagent ions that can rapidly quantify volatile compounds to low parts-per-trillion concentrations (by volume, pptv). Eight reagent ions (H_3O^+, NO^+, O_2^+, O^-, OH^-, O_2^-, NO_2^- and NO_3^-) obtained from a microwave discharge of moist or dry air, are now applied in commercial SIFT-MS instruments. These eight reagent ions react with volatile compounds in well-controlled ion-molecule reactions, but they do not react with the major components of air (N_2, O_2 and Ar). This allows for real-time analysis of air samples at trace and ultra-trace levels without pre-concentration, and results compare well with gas chromatography mass spectrometry (GC-MS).[9]

Rapid switching between reagent ions provides high selectivity, because the multiple reaction mechanisms provide additional independent measurements of each analyte. The multiple reagent ions also help to remove uncertainty from isobaric overlaps in mixtures containing multiple analytes.

Figure 1. *Schematic diagram of SIFT-MS – a direct chemical-ionization analytical technique.*



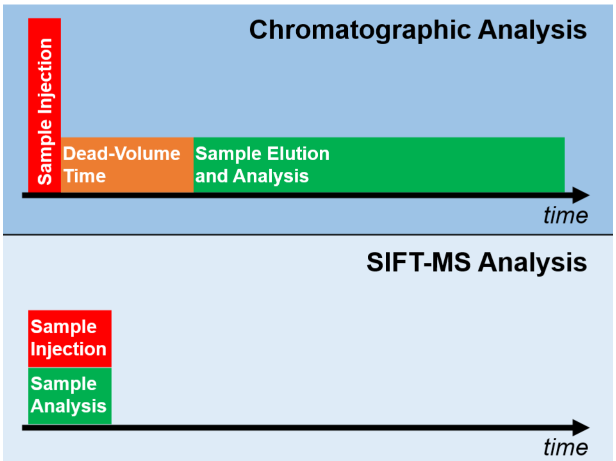
In this study, full mass scan data were obtained using a Voice200*ultra* SIFT-MS instrument (Syft Technologies, Christchurch, New Zealand). Data in positive and negative ion modes (m/z 15 – 250) were obtained for each sample, in a manner similar to that described elsewhere.[10] In brief, two samples of headspace were taken; the first for the positive-ion (H_3O^+, NO^+ and O_2^+) analysis, and the second for negative ions (OH^-, O_2^-). In this application note, only positive ion mode data were utilized. The target compounds (odor-active volatiles identified by Qian and Reineccius[2-5] and investigated in our previous study[1]) were extracted from the positive ion scans. The target compounds, and the selected reagent and product ions, are summarized in Table 1. Later work will examine the potential for classification of Parmesan cheeses using untargeted analysis (from SCAN data obtained using both positive and negative ionization).

2. Automated SIFT-MS analysis

In SIFT-MS, the capability for rapid direct analysis of a sample provides unique opportunities for high-throughput headspace analysis, irrespective of whether the task is routine VOC monitoring or the analysis of chromatographically-challenging species, such as the very polar small aldehydes and volatile fatty acids. In contrast to chromatographic techniques that require rapid injection to achieve good peak shapes and temporal separation, SIFT-MS simply requires steady sample injection for the duration of the analysis – that is, sample injection and analysis occur simultaneously (Figure 2).

Automated headspace analysis was carried out using a SIFT-MS instrument coupled with a multipurpose autosampler (MPS Robotic Pro, GERSTEL, Mülheim an der Ruhr, Germany). Samples were first incubated in a GERSTEL agitator prior to sampling of the headspace and subsequent injection into the SIFT-MS instrument through a GERSTEL septumless sampling head. A make-up gas flow was also introduced through the sampling head to maintain the standard, nominally 25 standard cubic centimeter per minute (sccm) sample gas flow into the SIFT-MS instrument.

Figure 2. *Graphical representation of the different sample-injection and analysis requirements of chromatographic techniques and SIFT-MS.*



The GERSTEL Robotic Pro MPS autosampler was controlled using GERSTEL's Maestro software. In addition to controlling the injection into the SIFT-MS instrument, the Maestro software's PrepAhead function allows for optimal scheduling of pre-injection preparation steps, such as syringe flush or incubation. This ensures that the highest sample throughput is achieved.

3. Samples and analysis conditions

Table 2 summarizes the Italian Parmesan samples obtained from various supermarkets (Aldi, Sainsbury's, Tesco) in Cambridge, UK. For each product, ten replicates were prepared (3 g each) in 20-mL headspace vials. The headspace was purged with 200 mL of zero air prior to incubation at 60 °C for 20 minutes. The autosampler then sampled the headspace using a 2.5-mL headspace syringe and injected it steadily (at 50 μL s^{-1}) into a flow of nitrogen make-up gas (11-fold dilution

Table 1. Odor-active compounds in Parmesan cheese (plus other major matrix species in italics) and their detection by the SIFT-MS instrument (shown by reagent ion with primary product ion m/z and branching ratio shown as percentage). Where secondary products are formed with water, a 'W' indicates additional measurement of the secondary product ion.

COMPOUND	H_3O^+	NO^+	O_2^+
acetaldehyde	45 + W (100%)	43 + W (100%)	
2-methylpropanal	73 + W (100%)	71 (100%)	
methylbutanal isomers	87 (94%)	87 (98%)	
hexanal	83 (50%) 101 + W (50%)	99 (100%)	
phenylacetaldehyde	121 + W (100%)	120 (60%) 150 (25%)	
2,3-butanedione (diacetyl)	87 + W (100%)	86 (75%)	
acetic acid	61 + W (100%)	90 (100%)	
butanoic acid	89 + W (95%)	118 (80%)	
hexanoic acid	99 (25%) 117 (75%)	146 (90%)	
ethyl butanoate	117 + W (100%)	146 (30%)	
ethyl hexanoate (incl. octanoic acid)	145 + W (100%)	174 (95%)	
ethyl octanoate	173 + W (90%)	202 (60%)	
2,3-dimethylpyrazine	109 + W (100%)	108 (100%)	108 (100%)
methional	105 (100%)	104 (95%)	104 (75%)
dimethyl trisulfide	127 + W (100%)	126 (100%)	126 (45%)
acetone	*59 + W (100%)*	*88 (100%)*	
ethanol	*47 + W (100%)*	*45 + W (100%)*	

in the inlet) for immediate, continuous analysis by the SIFT-MS instrument. The analysis times were less than 40 s and 30 s for positive and negative SCAN acquisition, respectively.

Samples were randomized and six blanks were analyzed at regular intervals through the sequence schedule. The average of the six blanks was subtracted from sample measurements.

4. Multivariate statistical analysis

Multivariate statistical analysis was utilized to determine the ability of SIFT-MS to discriminate between the Parmesan samples. The Soft Independent Modelling by Class Analogy (SIMCA) algorithm, developed by Wold in the 1970s,[11] was selected as this has proven well suited to SIFT-MS application.[12] SIMCA applies principal component analysis (PCA) to the whole dataset and to each of the classes with the end goal of creating a model that discriminates each class from the others. The Infometrix® Inc. (Bothell, WA) implementation of the SIMCA algorithm in the Pirouette software package was employed here.

Three types of output from the SIMCA analysis are presented in this application note:

1. **Class projections:** These three-dimensional plots show how each sample falls with respect to the three most important principal components derived from PCA on the entire data set. Each user-defined class shows the sample with the same color and a 'cloud' representing the calculated space in which all samples of the class are expected to lie. Better class separations lead to more confident assignment of unknown samples to a predefined class, if a suitable one exists.
2. **Interclass distances:** These are a measure of the separation between classes. A value of three (3) is usually considered acceptable for class separation.[13] Sometimes the class separability indicated by these distances is not apparent in the three-dimensional class projection plot.
3. **Discriminating power:** This parameter helps variables to be identified that provide the most discrimination between the classes. A variable with larger discriminating power has greater influence on separating the classes than one with a small discriminating power. There does not appear to be a set threshold value above which a discriminating power is considered "good", because these values vary strongly with interclass distance.

Results and Discussion

The SIFT-MS concentration data for the odor-active compounds are summarized in Table 3. These data are the mean of the ten replicate analyses, whereas the individual replicates are utilized for the subsequent statistical analyses. Significant differences are apparent (e.g., relative concentrations of butanoic acid and ethyl butanoate) between products, even without the aid of statistical analysis.

Table 2. Italian Parmesan cheese samples analyzed – and labels used – in this application note.

SUPPLIER AND PRODUCT NAME	MANUFACTURER CODE	SAMPLE FORMAT	PRICE (GBP) PER KG	LABEL
Sainsbury's	08 158	Pre-grated	22.50	P1
Tesco	08 039	Wedge	15.50	P2
Aldi "Specially Selected"	08 621	Wedge	16.00	P3
Sainsbury's "Taste the Difference"	08 158	Wedge	20.00	P4
Sainsbury's	08 158	Wedge	16.50	P5
Sainsbury's "SO Organic"	08 158	Wedge	20.00	P6

Table 3. Concentrations (in ppbV) of odor-active compounds in the headspace of Parmesan cheese samples (averaged across replicates; average blank subtracted; corrected for dilution in autosampler inlet). Matrix compounds are shown, but not included in statistical analysis.

SAMPLE	P1	P2	P3	P4	P5	P6
acetaldehyde	10700	9150	63200	869	2070	14500
2-methylpropanal	1860	1370	2550	1760	1070	1140
methylbutanal isomers	1130	900	708	1190	914	687
hexanal	239	139	226	103	56.6	87.2
phenylacetaldehyde	3.5	9.1	12.4	6.7	5.3	n.d.
2,3-butanedione (diacetyl))	469	369	357	438	250	179
acetic acid	834	1130	1410	841	743	844
butanoic acid	1570	2330	3830	1440	858	1060
hexanoic acid	777	523	780	365	244	356
ethyl butanoate	1490	1520	2830	843	608	1230
ethyl hexanoate (incl. octanoic acid)	292	264	530	130	103	315
ethyl octanoate	14.5	24.5	36.3	23.3	12.3	27.0
2,3-dimethylpyrazine	0.9	1.7	1.8	1.7	1.7	3.0
methional	18.0	12.2	43.4	10.8	9.9	15.3
dimethyl trisulfide	37.7	26.1	25.9	9.4	2.9	11.4
acetone	*3590*	*3860*	*4970*	*10100*	*3950*	*4090*
ethanol	*64300*	*40700*	*53100*	*13700*	*36700*	*110000*

1. Evaluation of ability to discriminate different products

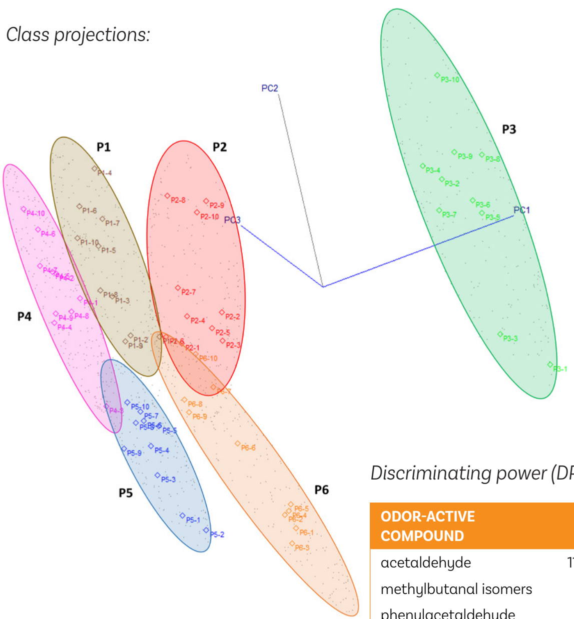
Figure 3 summarizes the results obtained when the odor-active compounds determined using SIFT-MS were used to classify the six products (Table 2). As measured by interclass distances, all products are readily distinguished using this approach, even when utilizing lower precision concentration data post-processed from rapid SCAN data.

2. Evaluation of ability to discriminate between manufacturers

The results obtained for Parmesans grouped according to manufacturer are summarized in Figure 4. Again, classification utilized the odor-active compounds. In this instance, it is evident – both from the class projection plot and the interclass distance table – that classification is not fully accomplished. Product from the manufacturer of P2 is similar to several replicates analyzed from P6 (c.f. Figure 3), leading to incomplete classification of the manufacturer's products. It is possible that a SIM method targeting the odor-active compounds would be more effective due to the higher measurement precision that it confers compared to the present approach that post-processes compound concentrations from SCAN data.

Figure 3. Evaluation of the ability of automated SIFT-MS analysis coupled with SIMCA multivariate statistical analysis to classify genuine Parmesan cheeses by individual products. Class projections, interclass distances, and discriminating powers of the 15 odor-impact compounds are shown.

Class projections:



Interclass distances:

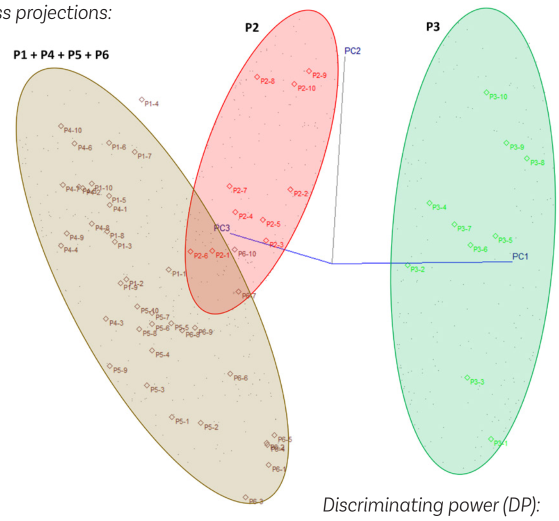
PRODUCT	P2	P3	P4	P5	P6
P1	4.7	35.1	33.1	13.3	4.8
P2		18.1	23.8	8.7	3.0
P3			172	58.2	20.7
P4				5.1	41.1
P5					13.8

Discriminating power (DP):

ODOR-ACTIVE COMPOUND	DP
acetaldehyde	116193
methylbutanal isomers	4382
phenylacetaldehyde	4293
butanoic acid	3048
ethyl butanoate	2322
dimethyl trisulfide	2071
hexanal	1772
2-methylpropanal	1227
ethyl octanoate	1014
acetic acid	994
hexanoic acid	967
2,3-butanedione	817
2,3-dimethylpyrazine	685
methional	113
ethyl hexanoate	86

Figure 4. Evaluation of the ability of automated SIFT-MS analysis coupled with SIMCA multivariate statistical analysis to classify genuine Parmesan cheeses by product manufacturer. Class projections, interclass distances, and discriminating powers of the 15 odor-impact compounds are shown.

Class projections:



Discriminating power (DP):

ODOR-ACTIVE COMPOUND	DP
acetaldehyde	1047
ethyl butanoate	788
2-methylpropanal	777
acetic acid	374
butanoic acid	325
2,3-butanedione	295
methylbutanal isomers	193
hexanal	131
ethyl hexanoate	117
dimethyl trisulfide	99
2,3-dimethylpyrazine	85
hexanoic acid	76
ethyl octanoate	21
phenylacetaldehyde	18
methional	9

Interclass distances:

PRODUCT	P2	P3
P1, P4, P5, P6	2.6	11.1
P2		18.1

Conclusions

This study demonstrates that automated SIFT-MS coupled with multivariate statistical analysis can rapidly analyze and classify genuine Parmesan cheese products based on the most significant odor-active volatiles. There is also potential for classification of products according to the manufacturer. The combined instrumental and statistical approach utilized here facilitates enhanced origin and quality control screening of Parmesan.

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