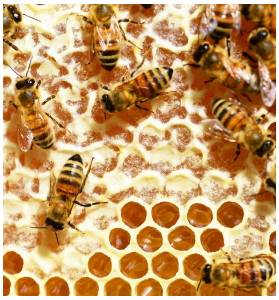

Application Note 275

Authentic or synthetic? Discovering authenticity markers in luxury to low-cost honey varieties using a high-capacity sorptive extraction technique (HiSorb™)

This study demonstrates how sorptive extraction using high-capacity HiSorb probes was used to extract aroma compounds spanning a wide volatility range from different honey samples. Automated statistical analysis was used to uncover subtle differences between the honey samples to determine possible markers of authenticity.

Introduction

Honey is a natural aromatic sweetener comprising over 95% water and sugars. The remainder is made up of compounds that include flavonoids, proteins, vitamins, free amino acids and volatile organic compounds (VOCs), which give different honey varieties their distinctive characterstics.[1] Unfortunately, honey is prone to food fraud, where either a less desirable honey is misrepresented as a more desirable one or honey substitutes are used to add bulk to the original product. The most common substitutes are cheap sweeteners, such as high fructose/maltose syrups, cane and refined beet sugar.[2] Traditional authentication techniques are becoming obsolete because they involve time-consuming sample preparation and pollen analysis by specially trained analysts. As a result, a new technique is being sought.[3]

In this study, we demonstrate HiSorb, a high-capacity sorptive extraction technique, to sample key aroma compounds present in different varieties of honey. High sensitivity was achieved by using robust, inert metal probes with a high volume of divinylbenzene/carbon wide range/polydimethylsiloxane (DVB/CWR/PDMS) sorptive phase. Sample extraction and enrichment were automated with the use of the Centri® platform coupled with gas chromatography–mass spectrometry (GC–MS) (Figure 1). The wide analyte range of VOCs extracted allowed a unique chemical profile to be determined for each honey sample. ChromCompare+ – easy-to-use chemometrics software – was used to carry out automated data mining to distinguish between honey varieties based on a statistical analysis of the differing features (i.e., compounds) between each sample. We show how this streamlined workflow can be used for the untargeted 'discovery' of authenticity markers and fast characterisation of honey varieties, which could help to combat food fraud and place less demands on analysts.

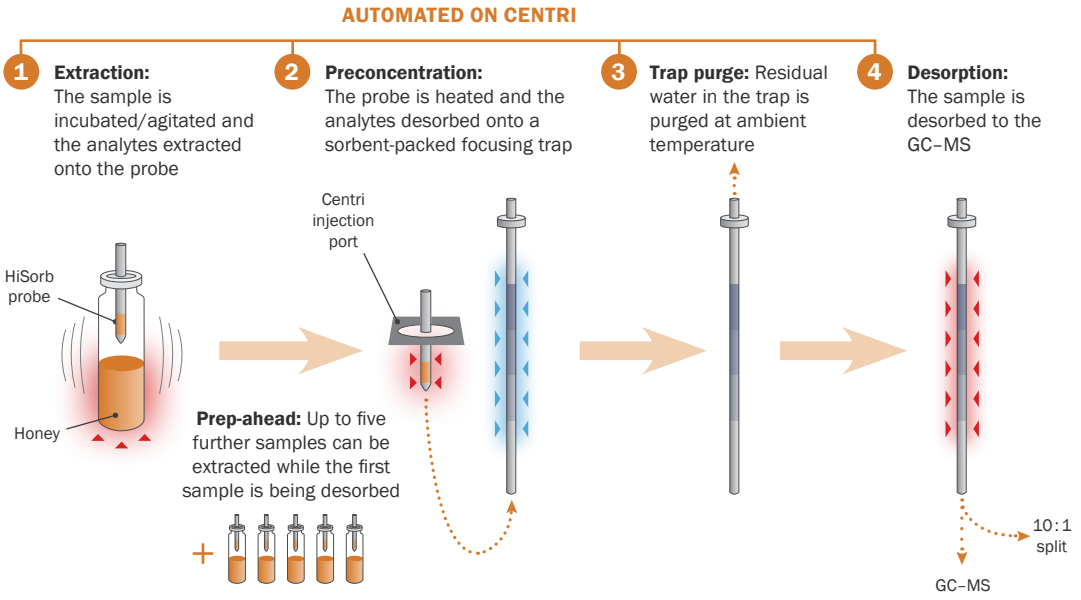

Figure 1: Automated HiSorb sample extraction workflow on the Centri platform.

Markes International Ltd
T: +44 (0)1443 230935 **F:** +44 (0)1443 231531 **E:** enquiries@markes.com

Experimental

Samples: Five different varieties of honey were investigated and labelled as:

- **Mass market:** A low-cost product acquired at a local supermarket.
- **Hobbyist:** Produced by a hobbyist beekeeper in Wales in 2018.
- **Forest:** A specialist honey acquired from a wellness shop.
- **Manuka:** A manuka honey acquired from a wellness shop.
- **Welsh:** Acquired from a local farm shop in Wales.

Golden syrup (labelled as Syrup from here on) was acquired from a local supermarket as a sixth sample. The samples were prepared in a 20 mL headspace vial containing 1 g of sample and 1 mL of water, which was sonicated to ensure sample homogeneity. Five replicates of each sample were prepared to confirm method reproducibility.

Instrument: Centri (Markes International)

High-capacity sorptive extraction (HiSorb):

Sampling mode:	Headspace
Probe:	DVB/CWR/PDMS, inert-coated (H4-AXAAC)
Flow path:	150°C
Pre-incubation:	37°C for 15 mins at 300 rpm
Sample extraction:	37°C for 30 mins at 300 rpm
Probe desorption:	240°C for 7 mins

Preconcentration:

Focusing trap:	Material emissions (U-T12ME-2S)
Trap low:	20°C
Trap purge:	50 mL/min for 1 min
Trap desorption:	MAX heating rate (>100°C/s) to 300°C, 3 min
Split ratio:	10:1

GC–MS:

Column:	DB-Wax-UI, 60 m x 0.25 mm x 0.25 µm
Constant flow:	1 mL/min
Inlet:	200°C
Oven program:	45°C (2 min), 4°C/min to 190°C (10 min)
Transfer line:	200°C
Ion source:	280°C
Quad:	200°C
Scan range:	35–450 m/z

Software:

Data mining and chemometrics in ChromCompare+ (SepSolve Analytical).

Results and discussion

The aroma profiles generated for each sample (Figure 2) show that some compound classes are common to all the honey samples, but others differ between samples. Each class contributes characteristic aromas; for example, ketones, common in all samples, confer buttery and nutty odours.

Background to Centri®

Markes International's Centri system for GC–MS is the first sample extraction and enrichment platform to offer high-sensitivity unattended sampling and preconcentration of VOCs and SVOCs in solid, liquid and gaseous samples.

Centri allows full automation of sampling using HiSorb™ high-capacity sorptive extraction, headspace(–trap), SPME(–trap), and tube-based thermal desorption. Leading robotics and analyte-trapping technologies are used to improve sample throughput and maximise sensitivity for a range of applications – including profiling of foods, beverages and fragranced products, environmental monitoring, clinical investigations and forensic analysis.

In addition, Centri allows samples from any injection mode to be split and re-collected onto clean sorbent tubes, avoiding the need to repeat lengthy sample extraction procedures and improving security for valuable samples, amongst many other benefits.

For more on Centri, visit www.markes.com.

Multiple esters were discovered in all samples, which was not unexpected, given that this group of compounds provides sweet and fruity aromas. Aldehydes were prominent, bestowing fresh, green and herbal notes. Alcohols, which add fresh flavours to honey, were a large component. They can occur naturally or as a result of heat treatment during processing.[4]

Furans, known for their sweet woody notes, are also common in honey (Table A1 – see Appendix) and are typically present as a result of the dehydration of reducing sugars in the matrix. Their presence can also indicate that thermal processes and storage after collection from hives have degraded the sugars in the honey.[2]

Overall, the honey profiles have common descriptors of fruity and sweet notes; however, naphthalene, an aromatic hydrocarbon commonly derived from coal, was also highlighted as an odour in one honey sample. This off-odour may have come from the smoke used by beekeepers to calm bees before removal of honey from hives.[5]

To distinguish the key variances between samples, a custom library of VOCs in each sample was created using ChromSpace software, which provides streamlined quantitative and qualitative analysis of 1D and 2D GC–MS data.

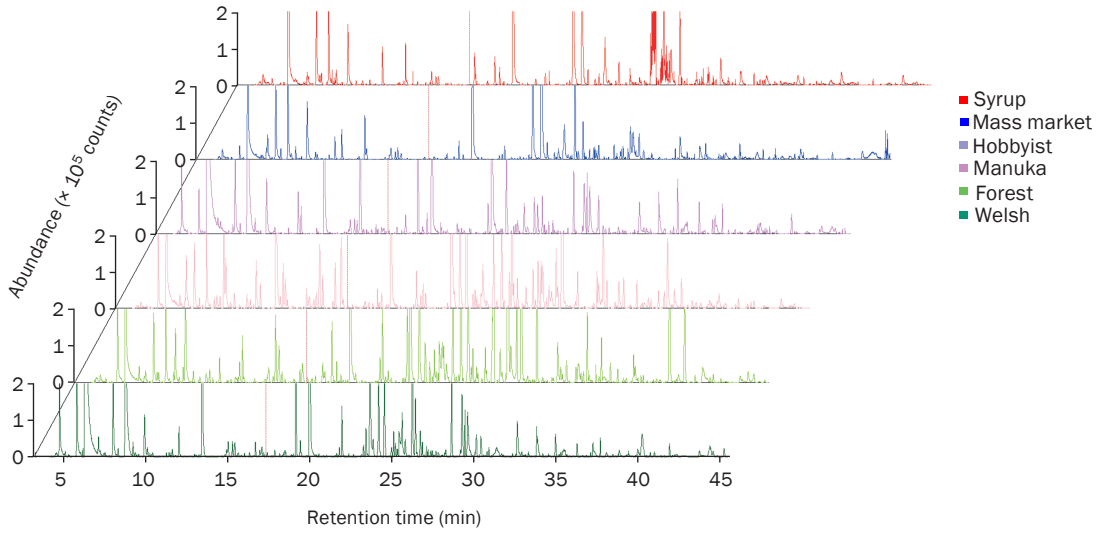

Figure 2: Aroma profiles of Syrup (red), Mass market (blue), Hobbyist (purple), Manuka (pink), Forest (light green) and Welsh honey (dark green).

A total of 79 compounds with match factors above 850 and probabilities above 85% (Table A1 – see Appendix) were used to generate the library and were chosen for further data mining analyses. ChromCompare+ enabled alignment and comparison of the chromatographic data shown in Figure 2 to form a principal components analysis score plot (Figure 3).

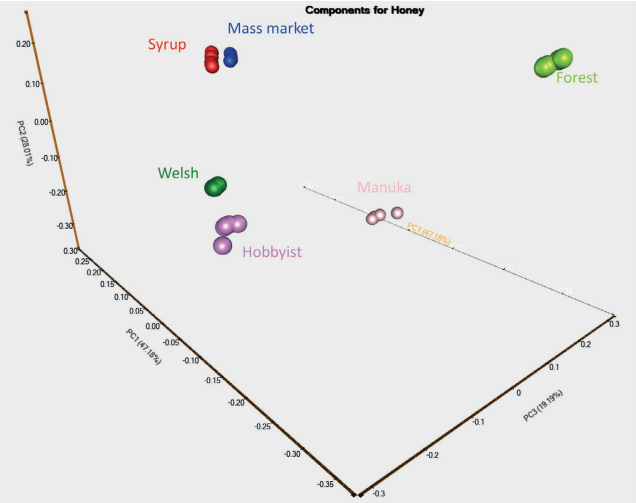

Figure 3: Principal components analysis score plot in ChromCompare+ software for the comparison of honey varieties extracted using DVB/CWR/PDMS HiSorb.

Close clustering of the replicates (n = 5 for each sample) indicated good reproducibility of the entire workflow, from sample extraction to analysis. The 'Mass market' honey appears very similar to the Syrup sample as they cluster closely in the PCA plot, indicating the possibility that additional sugars may have been added to bulk the honey product. The Welsh and Hobbyist honeys also seemed to have similarities, which could suggest that they have a similar geographical origin. The 'Manuka' and 'Forest' samples were the most distinctive varieties, which can be seen by the distance in these clusterings compared to the other honey samples.

20 key aroma compounds were determined as differentiating significantly between the samples (Table A2 – see Appendix). Figure 4 demonstrates the abundance of these compounds by sample type. Compounds that are specific to a particular sample have the potential to be used as markers for declarations of sample origin.

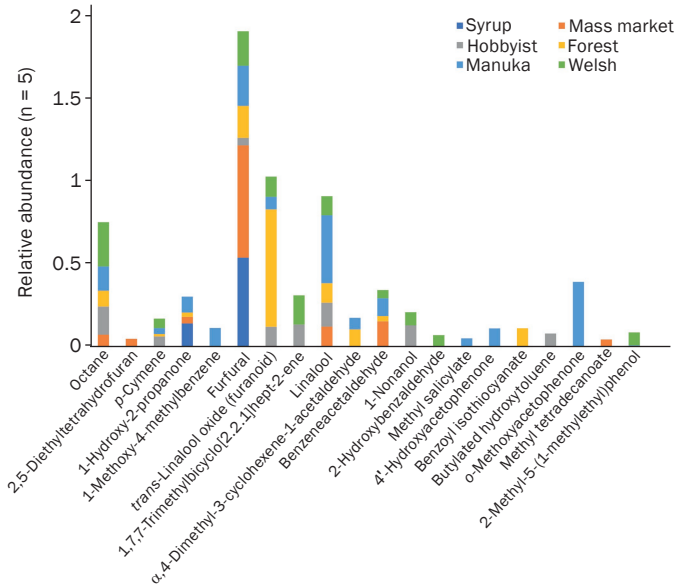

Figure 4: Comparison of relative abundance for the top 20 differentiating compounds identified in five honey varieties and a golden syrup.

The unique marker compounds found in the luxury 'Manuka' honey are 1-methoxy-4-methylbenzene, which has a phenolic, minty aroma, 4'-hydroxyacetophenone, which has a floral, sweet aroma, methyl salicylate, which has a wintergreen, mint aroma and *o*-methoxyacetophenone, providing an anisic, almond and cherry aroma. As these compounds are only present in the 'Manuka' honey, they could be used as confirmatory markers when classifying samples of unknown origin that are labelled as 'Manuka'.

Conclusions

In this study, HiSorb sorptive extraction using the DVB/CWR/PDMS phase combination proved to be a highly efficient technique for the VOC profiling of honey samples. The technique was used to extract a wide range of compounds, in particular those with key aroma and flavour properties. The robustness of the probes combined with a high-capacity phase provided consistent and highly sensitive extractions and analyses for determining authenticity markers in honey.

Using prediction models with ChromCompare+ chemometrics software, identification of differentials was easy in each of the honey matrices. The models allowed rapid classification of the honey samples, demonstrated by tight, reproducible clustering of the replicates and no cross-over of these clusters with other samples in the statistical plot.

This end-to-end workflow, from automated HiSorb extraction through to GC injection on the Centri platform, combined with fast, confident identification of key authenticity markers using untargeted data analysis in ChromCompare+, provides comprehensive profiling to aid quality evaluation for combatting food fraud.

References

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Trademarks

Centri®, ChromCompare®, ChromSpace® and HiSorb™ are trademarks of Markes International.

Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.

Appendix

Compound name	RT	MF	Probability
Cyclohexane	5.2125	915	86.57
Octane	5.7134	852	97.89
Methyl acetate	6.0741	881	98.99
Nonane	7.0279	889	97.92
2-Methylbutanal	7.3504	953	98.89
3-Methylbutanal	7.4237	918	98.78
3-Methyl-2-butanone	7.6876	862	98.95
2,5-Dimethylfuran	8.1325	863	98.81
2,3-Butanedione	8.6479	908	91.73
Methyl butanoate	8.8752	873	98.86
2,4-Dimethyl-3-pentanone	9.2497	918	89.49
Methyl 2-methyl butanoate	9.4854	873	94.22
2,5-Diethyltetrahydrofuran	10.4527	907	98.09
2,3-Pentanedione	10.7398	917	98.6
Ethyl 3-methyl-butanoate	11.1281	888	93.73
Dimethyl disulfide	11.3422	936	97.93
Methyl 4-methylpentanoate	13.4845	903	93.73
2-Heptanone	14.7739	903	93.73
Methyl hexanoate	14.9066	888	98.97
3-Methyl-2-butenal	15.3322	887	91.18
D-Limonene	15.3718	896	84.84
Isoamyl cyanide	16.9017	877	89.46
γ-Terpinene	16.9583	858	88.54
Dihydro-2-methyl-3(2H)-furanone	17.5847	854	92.81
p-Cymene	17.8043	877	93.41
Octanal	18.3832	915	92.29
3-Methylbutyl 3-methylbutanoate	18.5907	905	86.58
1-Hydroxy-2-propanone	18.8156	801	93.42
3-Methyl-1-Pentanol	19.6456	907	94.93
1-Hexanol	20.5001	959	97.66
Methyl octanoate	21.7493	844	89.36
Nonanal	21.8998	912	90.02
1-Methoxy-4-methylbenzene	23.4240	963	93.98
Furfural	24.1495	943	90.04
trans-Linalool oxide (furanoid)	24.3584	934	92.58
Methyl nonanoate	25.0649	914	86.78
1-(2-Furanyl)-ethanone	25.5426	953	90.34
Benzofuran	25.6303	946	94.18
1,7,7-Trimethylbicyclo[2.2.1]hept-2-ene	26.3940	964	98.84
Linalool	26.6902	902	94.44
2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-6-ene	26.7922	856	91.56
2,2,4,4-Tetramethyl-1,3-cyclobutanedione	27.1190	883	96.8
Lilac aldehyde C	27.1720	895	88.55
Lilac aldehyde B	27.2259	874	88.32
5-Methyl-2-furancarboxaldehyde	27.6532	929	94.87
Lilac aldehyde D	28.1752	874	93.63
Methyl decanoate	28.2469	927	98.92
3,7-Dimethyl-1,5,7-octatrien-3-ol	28.6082	894	87.02

Table A1: Custom-made library of compounds with match factor >850 and probability >85%. (*Continued on next page.*)

Compound name	RT	MF	Probability
2-Methylbenzofuran	28.6756	925	88.19
3,5,6,8α-Tetrahydro-2β,5,5,8α-tetramethyl-2H-1-benzopyran	28.9130	936	98.24
1,2-Dihydronaphthalene	28.9974	961	96.07
α,4-Dimethyl-3-cyclohexene-1-acetaldehyde	29.1447	895	89.85
Methyl benzoate	29.2372	936	94.41
Benzeneacetaldehyde	29.7405	898	89.07
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	30.0230	959	98.8
1-Nonanol	30.1298	910	97.17
2-Hydroxybenzaldehyde	30.8848	972	98.87
2,6,6-Trimethyl-2-cyclohexene-1,4-dione	31.3308	917	86.86
Terpineol	31.3532	883	92.17
(1*S*-*endo*)1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol	31.5931	891	98.9
Naphthalene	32.7802	931	88.25
(3*R*,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	32.8165	904	98.44
Methyl phenylacetate	33.1361	945	98.59
Methyl salicylate	33.7144	874	94.65
Methyl dodecanoate	34.1718	869	94.3
4'-Hydroxyacetophenone	34.4054	928	86.56
Hexanoic acid	35.2694	902	98.48
Benzoyl isothiocyanate	36.8282	946	98.94
Butylated hydroxytoluene	37.0933	948	97.46
Phenylethyl alcohol	37.2382	895	91.44
2,5-Furandicarboxaldehyde	38.8478	900	88.54
o-Methoxyacetophenone	39.4434	955	88.87
Methyl tetradecanoate	39.6241	895	98.49
Methyl 3-furoate	39.8588	952	98.72
Octanoic acid	40.9754	921	97.51
Heneicosane	42.0996	919	98.72
Ethyl 3-phenylprop-2-enoate	43.6254	885	98.6
Nonanoic acid	44.5567	929	98.22
2-Methyl-5-(1-methylethyl)-phenol	45.2398	961	95.94

Table A1: Custom-made library of compounds with match factor >850 and probability >85%. (*Continued from previous page.*)

Compound name	log $K_{O/W}$[(a)]	Odour/flavour notes[(b)]
Octane	3.9	Gasoline
2,5-Diethyltetrahydrofuran	2.5	Fruity, sweet, green, nutty, cherry
p-Cymene	4.1	Fresh, citrus, terpenic, woody, spicy
1-Hydroxy-2-propanone	-0.7	Caramellic, sweet, green, burnt
1-Methoxy-4-methylbenzene	2.7	Naphthyl, phenolic, ylang, minty
Furfural	0.4	Bready, brown, sweet, woody
trans-Linalool oxide (furanoid)	1.4	Floral
1,7,7-Trimethylbicyclo[2.2.1]hept-2-ene	3.6	
Linalool	2.7	Floral, citrus, orange, lemon
α,4-Dimethyl-3-cyclohexene-1-acetaldehyde	2.57[(c)]	Spicy, herbal
Benzeneacetaldehyde	1.8	Green, honey, sweet, floral
1-Nonanol	4.3	Floral, waxy, citrus, clean
2-Hydroxybenzaldehyde	1.8	Medicinal, spicy, cinnamon, wintergreen
Methyl salicylate	2.3	Minty, wintergreen, sweet, aromatic
4'-Hydroxyacetophenone	1.4	Floral, sweet
Benzoyl isothiocyanate	3.2	
Butylated hydroxytoluene	5.3	Phenolic, camphoreous
o-Methoxyacetophenone	1.8	Anisic, powdery, almond, cherry
Methyl tetradecanoate	6.8	Waxy, fatty, petal
2-Methyl-5-(1-methylethyl)-phenol	3.1	Spicy, woody, herbal, thyme

Table A2: The top 20 key differing aroma features (compounds) identified through HiSorb extraction across all honey samples with their characteristic aroma and flavour notes. [(a)]https://pubchem.ncbi.nlm.nih.gov/. [(b)]http://www.thegoodscentscompany.com. [(c)]https://www.chemeo.com/.