Application Note 279

HiSorb™ high-capacity sorptive extraction phase evaluation for aroma and flavour profiling of honey

HiSorb high-capacity sorptive extraction probes provide a highly sensitive, robust and fully automatable means of extracting organoleptic compounds from foodstuffs such as honey. The different properties of these compounds mean that sorptive phase combinations must be selected carefully to maximise uptake and achieve the most comprehensive profile. We report the evaluation of three HiSorb phase combinations – polydimethylsiloxane (PDMS) alone, carbon wide range (CWR) with PDMS and the triple phase combination of divinylbenzene (DVB) with CWR and PDMS – for aroma profiling of honey. The range of compounds extracted by each phase combination differed, as did the relative abundances of compounds that were common to each of the phase combinations. Importantly, the inclusion of additional phases alongside PDMS led to the extraction of substantially more compounds, including those that are important to the perception of flavour.

Introduction

Analysis of the volatile organic compound (VOC) content of foodstuffs is an important tool in the food and beverage industry, with applications in research and development, quality control, shelf-life assessment and even detection of food fraud. A common technique in food VOC profiling is sorptive extraction, wherein compounds partition from food onto a sorptive phase and are subsequently transferred to analytical instrumentation such as a gas chromatography–mass spectrometry (GC–MS) system for separation and detection. HiSorb high-capacity sorptive extraction probes (Figure 1) offer a highly sensitive, robust and fully automatable extraction solution, and have provided excellent results in GC–MS-based analysis of foodstuffs.[1,2,3]

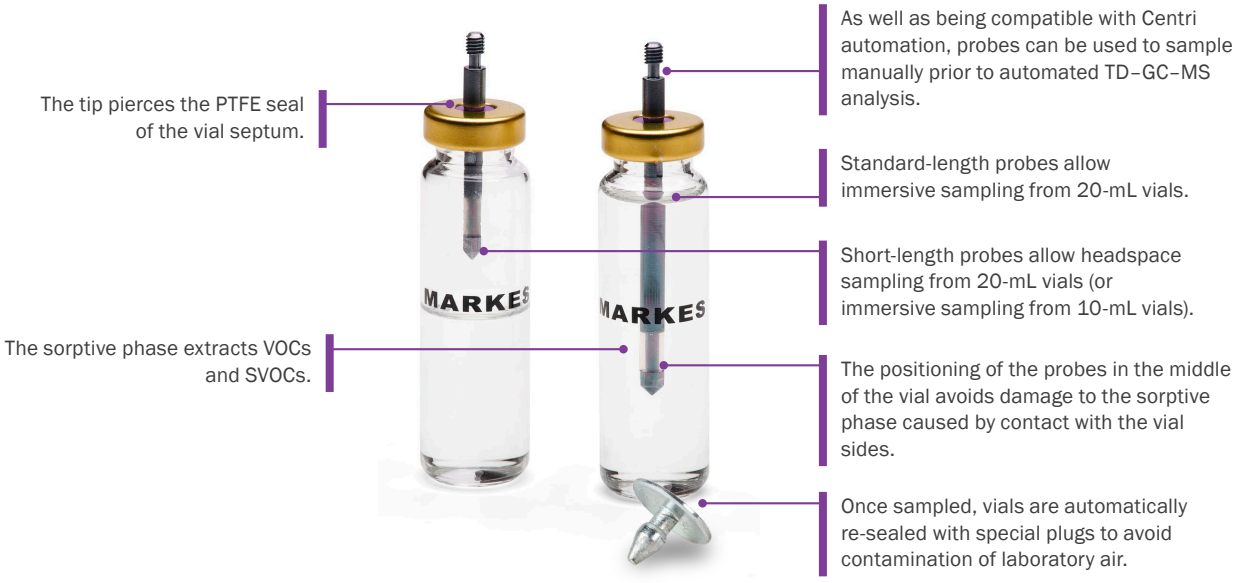

Figure 1: Headspace (left) and immersive (right) sampling with HiSorb probes.

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

A key consideration when using sorptive extraction is the choice of sorptive phase. Compounds are absorbed or adsorbed by a sorptive phase according to their partition coefficient, indicated by their log $K_{(O/W)}$[4] value, and failure to account for this during method development can lead to incomplete or biased results during VOC profiling. Use of multiple sorptive phases, each with distinct chemical properties, can broaden the range of VOCs extracted and so ensure a more comprehensive VOC profile is generated. However, each additional phase reduces the volume of existing phases. This means that extraction of compounds with high affinity for an existing phase may become less efficient when another is added and must also be considered during method development.

This publication describes the assessment of different HiSorb sorptive phase combinations in the analysis of honey aroma. Honey is a challenging matrix owing to its high variability and complexity, thus the phase combination used must be capable of extracting VOCs with a wide range of chemical properties. We evaluated three phase combinations – polydimethylsiloxane (PDMS) alone, carbon wide range (CWR) with PDMS and the triple phase combination of divinylbenzene (DVB) with CWR and PDMS – on a single honey variety to identify the most appropriate combination for this sample type. We demonstrate that additional compounds are recovered when multiple phases are used compared with the use of PDMS alone.

Following sorptive extraction, consideration must be given to how analytes are transferred from the sorptive phase to the analytical system. The Centri® extraction and enrichment platform is the ideal option for coupling HiSorb extraction with

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.

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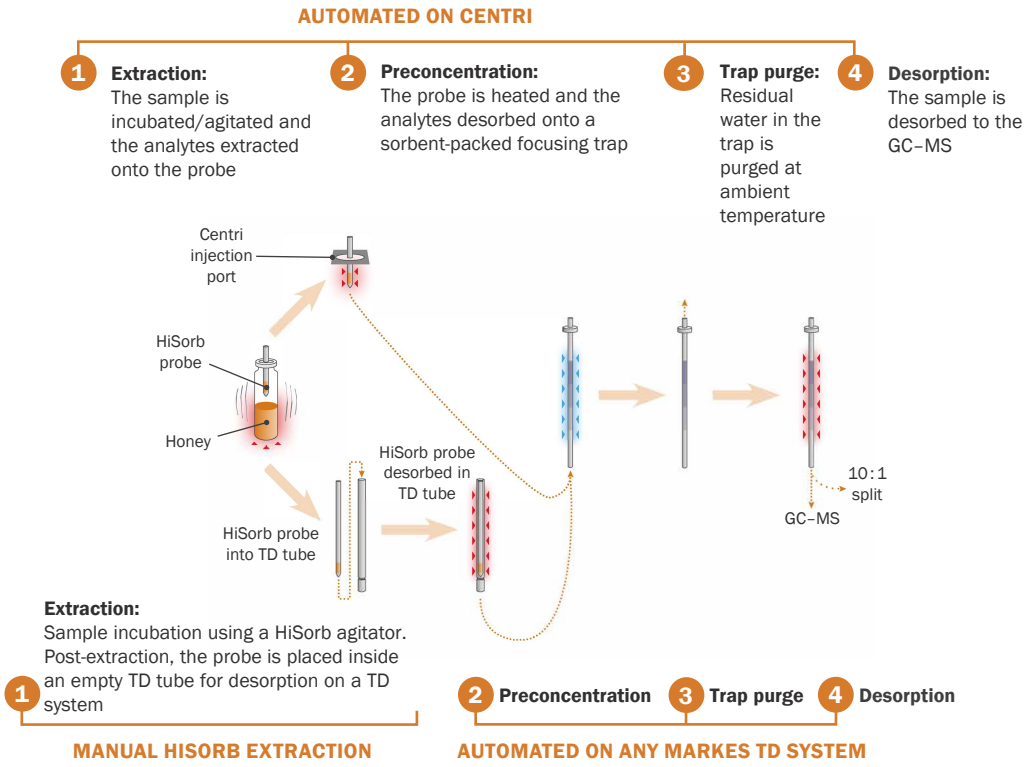

Figure 2: HiSorb extraction workflows using Markes' thermal desorbers. Bottom: offline, manual sample extraction using a HiSorb agitator, followed by probe desorption inside an empty TD tube. Top: fully automated workflow on Centri, from sampling to GC injection.

GC analysis, fully automating all aspects of sampling and incorporating Markes' leading focusing trap technology (Figure 2). Together, these features allow for hands-free high-throughput analysis of large sample batches with optimum chromatographic performance. Alternatively, HiSorb probes fit inside industry-standard thermal desorption tubes and can be desorbed with dedicated thermal desorption instruments such as TD100-xr™.

Experimental

Samples: A speciality honey, described on the packaging as originating from African forests, was acquired from a local wellness shop. Samples were prepared by placing 1 g of honey and 1 mL of distilled water in a standard 20-mL vial. Vials were subsequently crimp-capped and sonicated to ensure sample homogeneity. The low volume of sample used ensured that the sorptive phase did not come into direct contact with the sample when inserted into the vial, instead extracting analytes from the headspace.

Instrument: Centri sample extraction and enrichment platform

Headspace high-capacity sorptive extraction:
Probe: Three phase combinations were assessed:

- Polydimethylsiloxane (PDMS) (H1-AXAAC)
- PDMS with carbon wide range (PDMS/CWR) (H2-AXAAC)
- Divinylbenzene with CWR and PDMS (DVB/CWR/PDMS) (H4-AXAAC)

All HiSorb probes were standard length and inert-coated. Each phase combination was evaluated in triplicate.

Sampling mode:	Headspace
Flow path:	150°C
Pre-incubation:	37°C for 15 min at 300 rpm
Sample extraction:	37°C for 30 min at 300 rpm
Probe desorption:	240°C for 7 min

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 processing using ChromSpace® (SepSolve Analytical).

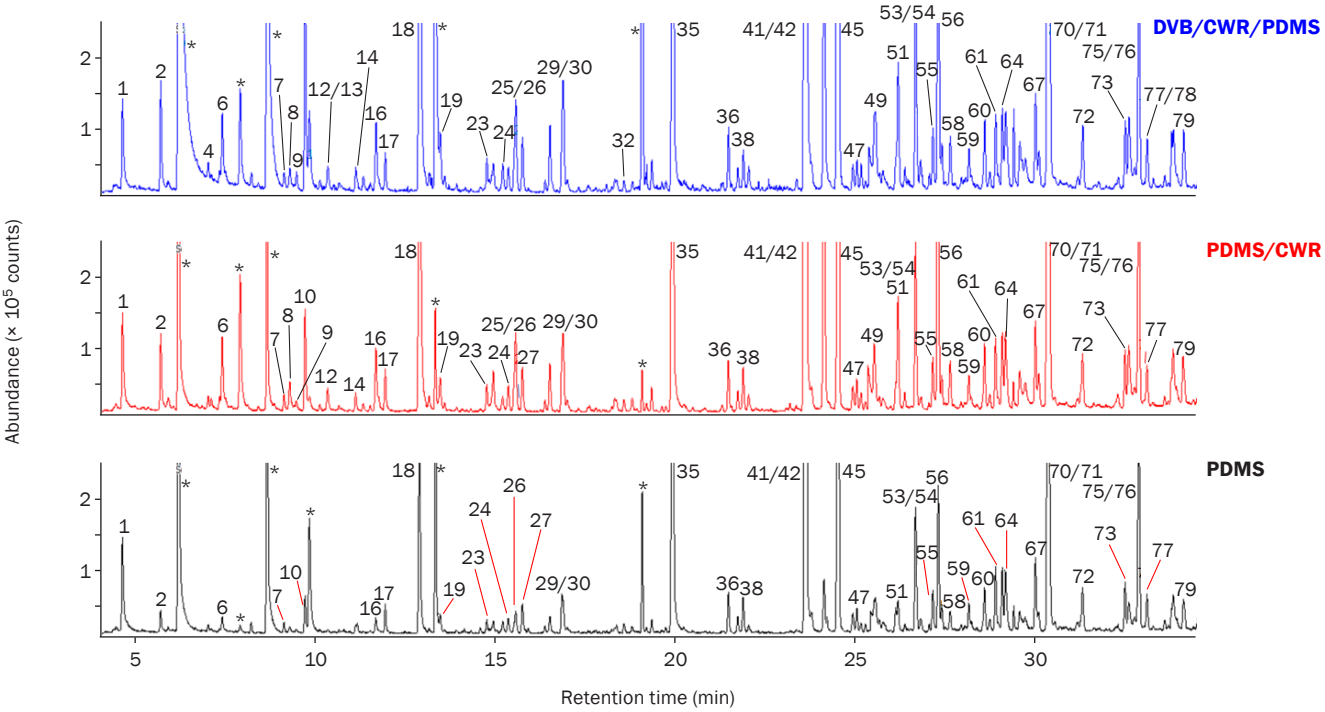

Figure 3: Total ion chromatograms (TICs) produced by sorptive extraction of honey by each of three HiSorb phase combinations as shown. Peak identifications are given in Table 1. Spectral deconvolution indicated that some peaks represent two co-eluting compounds. These peaks are therefore labelled with two numbers. Asterisks (*) indicate silica-based compounds derived from the PDMS phase.

Results and discussion

Figure 3 shows the total ion chromatograms (TICs) produced when three HiSorb phase combinations are used for sorptive extraction of analytes from honey. The same sampling and desorption conditions were used for all probes. A subset of peaks is labelled, with identifications given in Table A1 in the Appendix. Release of silica-based PDMS derivatives during probe desorption are expected and these peaks are marked with asterisks (*). Visual inspection of the chromatograms reveals that the number and relative abundance of analytes detected varies among phase combinations. Notably, probes bearing only PDMS produce fewer and smaller peaks than PDMS/CWR and DVB/CWR/PDMS probes.

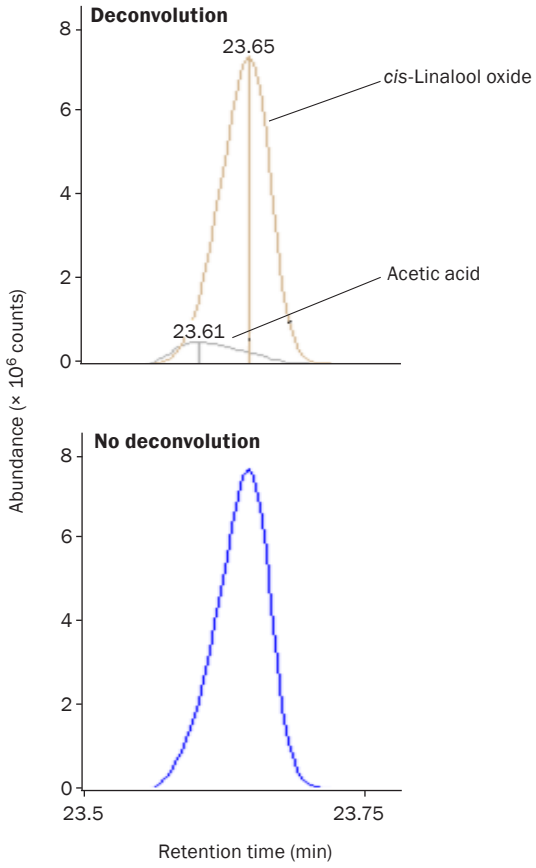

Figure 4: Spectral deconvolution with ChromSpace. In this honey sample, extracted with a DVB/CWR/PDMS HiSorb probe, acetic acid and *cis*-linalool oxide co-elute. Only with ChromSpace's proprietary spectral deconvolution are the peaks resolved and confidently identified, with NIST match factors of 917 and 947, respectively.

Honey is a complex matrix bearing a wide and highly variable range of compounds. With such samples, there is a high probability of analytes sharing retention times (co-eluting), making spectrum-based identification difficult. We used the proprietary spectral deconvolution feature in ChromSpace software to resolve overlapping peaks, enabling confident identification of co-eluting compounds. An example of the power of spectral deconvolution is given in Figure 4.

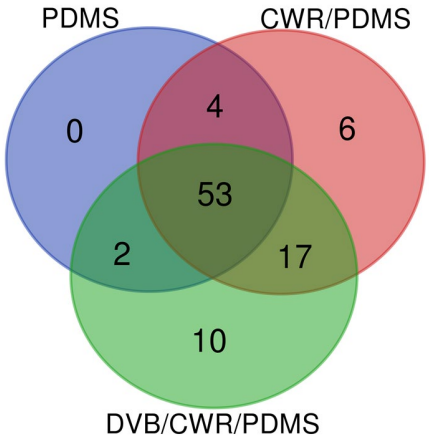

Figure 5: Number of compounds detected by HiSorb probes with the phase combinations indicated.

Table A1 details compounds recovered from honey by HiSorb probes. Compounds were included if they were identified in all three replicates of each phase combination and with NIST match factors > 750 following spectral deconvolution. In total, 92 compounds met these criteria; however, phase combinations differed in the particular compounds detected (Figure 5). PDMS/CWR and DVB/CWR/PDMS probes recovered similar numbers of compounds, at 80 and 82 respectively, but PDMS-only probes detected only 59 compounds.

Further, all compounds detected by PDMS-only probes were detected by at least one of the other two phase combinations. PDMS is a widely used sorptive phase owing to the broad range of compounds it extracts, but it is less efficient at extracting more volatile and/or polar compounds. Inclusion of additional sorptive phases with different molecular properties facilitates analysis of these otherwise challenging compounds, while simultaneously analysing compounds that are extracted well by PDMS.

PDMS/CWR and DVB/CWR/PDMS produced broadly similar compound profiles, with 10 compounds only detected by PDMS/CWR but not DVB/CWR/PDMS, and 12 compounds only detected in DVB/CWR/PDMS but not CWR/PDMS. In principle, the inclusion of DVB widens the range of compounds that can be extracted efficiently; however, it is important to note that by adding the DVB phase, there is a reduction in the volume of PDMS and CWR available for analyte extraction. Thus, analysis of compounds that are readily extracted by PDMS and/or CWR, but not DVB, may be made less efficient by the inclusion of an additional phase. This is demonstrated by compounds 26, 44 and 71 (among others) in Table A1, for which extraction was made much more efficient by the addition of CWR versus with a PDMS-only probe, but which were extracted less efficiently with the further addition of DVB.

Lastly, flavour descriptions are given for each compound in the same table. An abundance of aldehydes, ketones and esters with fruity and green flavour characteristics were identified, such that botanical notes dominated the flavour profile. This is to be expected given that honey is derived from plant nectars. These results demonstrate the exceptional ability of HiSorb to extract important aroma and flavour components, providing a more comprehensive profile of honey using sorptive extraction.

Conclusions

We have demonstrated that HiSorb is an effective extraction technique for identifying key aroma and flavour compounds in honey, with botanical flavours being highly prominent. Evaluation of how phase combination influences extraction of VOCs from honey – a complex and variable food matrix – found that the inclusion of CWR, or CWR with DVB, along with PDMS was highly beneficial for this application.

During method development, analysts must always consider that as each additional phase reduces the volume of all others, the effect of adding more phase is not always cumulative. For example, if compounds of interest are readily adsorbed by PDMS from the matrix under investigation, then a PDMS-only probe may be most effective.

References

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Appendix

Peak area average (n = 3) x10^5							
No.	**Compound**	**RT**	**PDMS**	**PDMS/ CWR**	**DVB/ CWR/ PDMS**	**logk$_{(o/w)}$**	**Flavour description[8]**
Acids							
41	Acetic acid	23.61	59.00	102.00	121.00	-0.17	Pungent sour fruit overripe fruit acetic
48	Formic acid	25.4	2.69	12.30	–	-0.3	Acetic astringent fruity mustard bready
52	Propanoic acid	26.41	–	1.82	2.47	0.48	Acidic dairy fruity
56	2-Methylpropanoic acid	27.32	21.10	59.00	73.20	0.73	Acidic sour cheesy cheesy limburger cheese dairy creamy
63	Butanoic acid	29.14	–	–	1.56	0.87	Acidic sour cheesy dairy creamy fruity
70	3-Methylbutanoic acid	30.36	107.00	247.00	283.00	1.12	Cheesy dairy creamy fermented sweet waxy berry
71	2-Methylbutanoic acid	30.41	23.20	55.60	51.10	1.12	Fruity dirty acidic dairy buttery cheesy
79	4-Methylpentanoic acid	34.13	3.71	7.51	6.44	1.51	Cheesy
82	Pentanoic acid	35.26	–	1.41	–	1.26	Acidic dairy milky cheesy
Aldehydes							
6	3-Methylbutanal	7.42	2.34	12.90	10.10	1.23	Fruity dry green chocolate nutty leafy cocoa
11	3-Methylpentanal	10.14	–	–	1.24	1.62	
38	Nonanal	21.91	4.59	5.94	5.14	2.94	Aldehydic citrus cucumber melon rindy potato raw potato oily nutty coconut
44	Furfural	24.15	11.50	97.80	77.00	1.09	Brown sweet woody bready nutty caramellic burnt astringent
51	Benzaldehyde	26.21	5.15	20.10	20.20	1.5	Sweet oily almond cherry nutty woody
54	Lilac aldehyde*	26.72	–	6.87	7.22	1.95	
55	Lilac aldehyde*	27.16	7.86	9.76	8.66	1.95	
57	Lilac aldehyde*	27.42	3.79	4.32	18.20	1.95	
59	Lilac aldehyde*	28.17	5.21	6.09	5.03	1.95	
58	5-Methyl-2-furancarboxaldehyde	27.66	3.77	9.10	7.83	1.4	Sweet brown caramellic grain maple
66	Benzeneacetaldehyde	29.75	1.69	3.71	3.47	1.43	Honey sweet floral chocolate cocoa spicy
67	Safranal	30.02	12.80	15.60	14.00	2.49	Woody medicinal phenolic spicy camphoreous fruity herbal
Alcohols							
25	2-Methylbutan-1-ol	15.53	–	5.93	5.30	1.29	Ethereal alcoholic fatty greasy cocoa whiskey fusel leathery
26	3-Methylbutan-1-ol	15.58	3.99	14.30	12.70	1.16	Fusel fermented fruity banana ethereal cognac
60	Hotrienol	28.6	8.43	13.00	10.10	2.45	Fresh floral woody
Alkanes							
1	n-Hexane	4.67	15.20	5560.00	15.80	3.9	
2	Octane	5.72	2.53	10.50	16.20	5.18	
4	Nonane	7.02	–	1.57	1.97	5.65	
7	Decane	9.14	1.12	2.34	2.14	5.01	
13	3-Methylpentane	10.37	–	–	1.50	3.6	
17	Undecane	11.95	3.70	5.95	4.72	4.54	
86	Hexadecane	36.61	2.50	2.69	2.99	6.49	
92	2,7,10-Trimethyldodecane	42.1	–	–	1.66	–	
34	2,2,3-Trimethylbutane	19.21	–	1.21	–	2.69	

Table A1: Abundance of compounds extracted from honey by sorptive extraction with HiSorb using three sorptive phase combinations, with log k$_{(o/w)}$ values where available.[6,7] †Lilac aldehydes A, B, C and D could not be distinguished spectrally. (*Continued on next page.*)

Peak area average (n = 3) x10^5							
No.	Compound	RT	PDMS	PDMS/ CWR	DVB/ CWR/ PDMS	log$k_{(o/w)}$	Flavour description[8]
Aromatics							
12	Toluene	10.35	–	3.41	2.75	2.73	
20	*m*-Xylene	13.45	–	1.35	–	3.2	
74	Naphthalene	32.78	–	1.57	1.38	2.84	
76	1,1,5-Trimethyl-1,2-dihydronaphthalene	32.89	43.40	39.20	37.20	–	
Esters							
9	2-Methylmethylbutanoate	9.49	–	1.46	2.23	1.21	Ethereal estery fruity apple green pear tropical floral
10	Methyl isovalerate	9.72	6.27	20.10	30.00	1.82	Fruity pineapple apple juicy juicy fruit tropical
14	3-Methylethylbutanoate	11.13	–	3.32	3.27	1.6	Sweet fruity spicy metallic green pineapple apple
21	Methyl 4-methylpentanoate	13.48	2.12	5.14	5.60	1.6	Fruity sweet banana pineapple apple ripe candy
32	Isopentyl isovalerate	18.59	–	–	1.63	2.62	
37	Methyl octanoate	21.74	1.95	2.48	2.49	2.52	Green fruity waxy citrus aldehydic fatty
39	Methyl 4-oxovalerate	22.05	–	2.58	3.07	–	
47	Methyl nonanoate	25.06	2.92	2.92	3.65	3.48	Winey waxy green celery pear fruity
65	Methyl benzoate	29.23	2.10	2.10	3.92	4.47	Phenolic cherry camphoreous
78	Methyl benzeneacetate	33.13	–	–	1.73	1.4	Floral honey spicy waxy sweet
80	Methyl dodecanoate	34.18	2.35	2.22	–	4.08	Waxy creamy fatty soapy coconut
Furans and derivatives							
27	(2*R*,5*R*)-2-Methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran	15.76	5.11	8.46	8.46	–	
28	2-n-Pentylfuran	15.39	–	1.75	1.60	3.01	Green waxy musty cooked caramellic
29	Anhydrolinalool oxide	16.86	4.10	6.58	6.66	2.69	Woody pine spicy minty green citrus
69	Furfuryl alcohol	30.13	–	3.69	2.98	0.77	Burnt sweet caramellic brown
Ketones							
3	Acetone	5.93	–	1.55	–	-2.04	
5	2-Butanone	7.12	–	1.50	–	0.29	Chemical fruity green
19	4-Methyl-3-penten-2-one	13.17	–	2.16	1.72	1.54	Potato raw potato baked potato raw vegetable nutty dirty
22	Acetyl valeryl	13.6	–	–	1.39	1.33	Dairy buttery butterscotch rummy caramellic
23	2-Heptanone	14.78	2.14	4.90	4.43	2.16	Cheesy fruity coconut waxy green
31	Acetoin	18.33	–	2.67	–	-0.36	Creamy dairy sweet oily milky buttery yogurt
33	Acetol	18.82	–	2.57	1.48	-0.43	Sweet green burnt
49	1-(2-Furanyl)-ethanone	25.55	–	11.00	10.40	1.48	Sweet nutty roasted sweet baked
68	Acetophenone	30.11	1.57	2.34	7.01	1.89	Powdery bitter almond cherry coumarinic fruity
72	4-Oxoisophorone	31.33	8.45	10.40	9.27	1.5	Citrus floral musty tea green sweet fruity
81	β-Damascenone	34.95	6.63	7.66	6.90	3.43	Woody floral herbal green fruity spicy tobacco
83	6,10-Dimethyl-5,9-undecadien-2-one	35.65	1.64	–	1.57	4.05	Floral fruity tropical green pear apple banana citrus

Table A1: Abundance of compounds extracted from honey by sorptive extraction with HiSorb using three sorptive phase combinations, with log $k_{(o/w)}$ values where available.[6,7] †Lilac aldehydes A, B, C and D could not be distinguished spectrally. (*Continued from previous page and continued on next page.*)

Peak area average (n = 3) x10^5							
No.	Compound	RT	PDMS	PDMS/ CWR	DVB/ CWR/ PDMS	log$k_{(o/w)}$	Flavour description[8]
Nitriles							
8	Isobutyronitrile	9.3	–	6.16	3.08	0.46	
16	2-Methyl butanenitrile	11.69	2.93	12.60	11.30	1.56	
18	3-Methyl butanenitrile	12.9	49.20	203.00	182.00	1.56	
			0.00	0.00	0.00		
30	4-Methyl pentanenitrile	16.9	5.70	15.10	17.30	1.95	
75	2-Pyridinecarbonitrile	32.87	6.89	9.39	7.85	0.95	
90	2-Methylbenzonitrile	37.67	5.09	–	6.98	1.87	
Sulfurous							
15	Dimethyldisulfide	11.34	–	1.61	2.47	1.77	Sulfurous cabbage malty creamy
Terpenes and terpenoids							
24	D-Limonene	15.37	1.80	3.60	3.31	3.31	Sweet orange citrus terpenic
36	*cis*-(-)-1,2-Epoxy-*p*-menth-8-ene	21.49	7.06	9.42	9.05	2.52	
42	*cis*-Linalool oxide	23.65	729.00	981.00	909.00	1.88	
45	*trans*-Linalool oxide	24.53	172.00	224.00	201.00	1.88	
43	α-Ionene	23.8	1.66	1.98	1.76	3.61	
53	Linalool	26.7	21.60	30.10	29.80	2.67	Citrus orange lemon floral waxy aldehydic woody
64	Geijerene	29.19	–	–	7.07	3.72	
73	*trans*-Pyranoid linalool oxide	32.51	9.13	10.30	8.41	1.88	Fresh cooling
Miscellaneous							
35	N,N-Dimethylformamide	19.95	123.00	180.00	147.00	-0.3	
40	*p*-(1-Propenyl)-toluene	23.39	–	–	1.65	–	
46	Edulan II	24.96	1.21	1.76	1.88	3.47	
50	Dill ether	26.14	4.33	4.63	3.93	2.38	
61	Edulan I	28.9	9.67	12.80	10.60	3.47	
62	*p*-Menth-1-en-9-al	29.1	8.35	10.50	8.42	2.57	
77	Epoxylinalol	33.12	5.76	6.84	5.55	1.88	
84	2-Methoxyphenol	35.82	–	2.35	2.04	1.4	Woody phenolic bacon savory smoky medicinal
85	Benzyl alcohol	36.26	2.76	4.94	4.71	1.18	Chemical fruity cherry almond balsamic bitter
87	Methyl benzoylformate	36.82	20.30	26.70	–	1.04	
88	2-Oxo-2-phenylethyl formate	36.82	–	–	21.80	–	
89	Phenylethyl alcohol	37.23	3.93	5.60	4.79	1.36	Floral sweet rose bready
91	Furyl hydroxymethyl ketone	39.86	2.27	2.87	–	0.45	

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